Societat Catalana de Biologia 1912-2012

I CIBICAT

CONGRÉS INTERNACIONAL DE BIOLOGIA DE CATALUNYA

Global Questions on Advanced Biology

An international conference on interdisciplinary frontiers in biology
As part of the first Centenary of the Societat Catalana de Biologia

9th – 12th of July 2012

Barcelona

With the support of

Generalitat de Catalunya
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Secretaria d'Universitats i Recerca

Gobierno de España
Ministerio de Economía y Competitividad

Universitat de Barcelona

CREAF

CRG Centre de Regulació Genòmica

IRB Lleida Institut de Recerca Biomèdica

ibmb Institut de Biologia Molecular de Barcelona CSIC

IDIBGi Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta

XRB Xarxa de Referència en Biotecnologia de la Generalitat de Catalunya

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Benvinguts a la festa

Benvinguts al I Congrés Internacional de Biologia de Catalunya (CIBICAT), enguany titulat «Global Questions on Advanced Biology».

El CIBICAT vol ser un exercici de reflexió sobre les actuals fronteres de la biologia i sobre els nous reptes apareguts en aquest camp. Amb aquest plantejament, hem volgut fer un congrés atractiu, sobretot per als científics joves que es troben en la fase de definir la seva línia de recerca i que necessiten ser exposats a una visió àmplia dels problemes de la biologia. També hem volgut que científics reconeguts reflexionin sobre aquests temes i facin un exercici de pensament crític sobre el present i el futur de la biologia des d'una perspectiva al més agosarada possible. En definitiva, esperem que pugueu trobar un espai per a fer el que més ens apassiona: parlar de ciència.

El Congrés també vol ser una festa, ja que la Societat Catalana de Biologia (SCB) celebra els centenari de la seva fundació. Efectivament, ara fa cent anys, un grup de metges catalans van intuir que el futur de la medicina passava per la recerca profunda en els coneixements bàsics que aportava la biologia. Aquesta intuïció continua vigent després de cent anys, i no solament per al camp de la medicina: les enginyeries ambientals, la tecnologia alimentària, les aplicacions biotecnològiques i un llarg etcètera tenen el fonament i la capacitat de progressió en les dades que aporta la recerca biològica bàsica. Aquests metges també van intuir que per a progressar cal fer xarxa. Avui, i en part gràcies a aquests visionaris, som molts els científics catalans que ens apleguem en aquest congrés: us animem a fer un gran nombre de contactes tot parlant amb gent de fora els vostres grups.

En aquest context de crisi hem proposat quotes d'inscripció força econòmiques perquè hi pugui assistir la majoria de socis de la SCB. Aquest fet, junt amb l'èxit de participació (més de set-cents inscrits, quan escric aquestes ratlles) podria fer que en algun moment aparegués alguna disfunció. Us agraïm la comprensió.

Amb aquests horitzons de transversalitat, commemoració i col·laboració, el Comitè Organitzador desitja que gaudiu d'un alt nivell científic i un càlid ambient entre els participants. Si això s'aconsegueix serà perquè vosaltres, els socis de la SCB i els participants en general, ho heu fet possible. Gràcies a tots per endavant i benvinguts a la festa!

Josep Clotet

President del Comitè Organitzador del Primer CIBICAT

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- Núria Casals
- Josep Maria Espelta
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Session I:
Chromatin and Epigenetics
Genomics and Proteomics
Molecular Biology
Molecular Biology of cancer
Reproductive Biology

Session II:
Biophysics
Molecular Biology
Neurobiology
Systems Biology

Session III:
Cell Signalling
Developmental Biology
Evolutive Biology
Neurobiology

Session IV:
Aquaculture
Ecology
Microbiology
Virology

	Monday 9	**Tuesday 10**	**Wednesday 11**	**Thursday 12**
	Chromatin and Epigenetics, Genomics and Proteomics, Molecular Biology, Molecular Biology of cancer and Reproductive Biology	Biophysics, Molecular Biology, Neurobiology and Systems Biology	Cell Siganlling, Developmental Biology, Evolutive Biology and Neurobiology	Aquaculture, Ecology, Microbiology and Virology
8.30	Registration IEC	Registration IEC	Registration IEC	Registration IEC
9.15	Symposia 1 - 4	Symposia 9 - 12	Symposia 17 - 19	Symposia 21 - 24
11.15	Coffee-break	Coffee-break	Coffee-break	Coffee-break
11.45	Small piano recital			
12.00	Institutional Parliaments. Inaugural plenary lecture	Plenary lecture	Plenary lecture	Plenary lectures
13.30	Lunch	Lunch	Lunch	
14.15				Lunch
15.00	Workshop, Round- tables	Workshop, Round- tables	Round- tables	Round- tables (Symposia 25 - Virology)
15.30				Symposia 26 - 28
16.15	Symposia 5 - 8	Symposia 13 - 16	Symposia 20	
18.00	Beer & Posters	Beer & Posters	Beer & Posters	Beer & Posters
19.30				Closing plenary lecture. Paranimf UB
21.30				Gala dinner and dancing

MONDAY 9 TH OF JULY — SESSION I

	Room	
8.30		Registration IEC
9.15		**Parallel sessions**
	Prat de la Riba	**S 1- Epigenetic mechanisms in health and disease**
	Pere Coromines	**S 2- Biomedical proteomics and transcriptomics**
	Sala d'Actes del CSIC	**S 3- Gametes, stem cells and differentiation**
	Nicolau d'Olwer i Pi i Sunyer	**S 4- Computational and structural biology**
11.15		Coffee-break
	Conservatori de Música del Liceu	
11.45		**Small piano recital** Ilya Maximov (Alumne de Perfeccionament del Conservatori Superior del Liceu de Barcelona)
12.00		**Institutional Parliaments** Sr. Antoni Castellà (secretari d'Universitats i Recerca. Generalitat de Catalunya), Sr. Salvador Giner (president de l'IEC), Sr. Lluis Tort (president SCB), Sra. Maria Serrat (directora general del Conservatori).
		Inaugural Plenary lecture **Epigenetics: from biology to disease** Manel Esteller (IDIBELL)
13.30		Free time for Lunch
14.45	Pi i Sunyer	**Workshop on conference presentation skills** Elinor Thompson (PRBB)
15.00		**Round tables**
	Pere Coromines	**Frontiers of biology: 10 years after the human genome sequencing** Moderador: Josep Corbella (La Vanguardia). Participants: Xavier Estivill (CRG), Manel Esteller (IDIBELL), Manuel Perucho (IMPPC) and Ivo Gut (CNAG)
	Prat de la Riba	**"So now you got your PhD, what's next …?"** ***A first hand review on unexpected professional paths-1*** Organized by Luis Ruiz (Janus Developments S.L). Participants: Joan Roig Amorós (IRB), Petraki Munujos (Biosystems S.A), Ramon Bosser (Janus Developments S.L)
16.15		**Parallel sessions**
	Pere Coromines	**S 5- Regulation of chromatin functions**
	Prat de la Riba	**S 6- Genes and Genomes**
	Nicolau d'0lwer i Pi i Sunyer	**S 7- Biology of reproduction**
	Sala d'Actes CSIC	**S 8- Proliferation, angiogenesis and metastasis**
18.00		Beer & Posters

TUESDAY 10 TH OF JULY — SESSION II

	Room	
8.30		Registration IEC
9.15		**Parallel sessions**
	Sala d'Actes del CSIC	**S 9- Cell damage and cell death**
	Prat de la Riba	**S 10- From genotype to phenotype: where are we now?**
	Pere Coromines	**S 11- Traffic and signalling in health ans disease**
	Nicolau d'Olwer	**S 12- Receptors, channels and transporters**
11.15		Coffee-break
12.00		**Plenary lecture**
	Conservatori de Música del Liceu	**The evolution of language** Tekumseh Fitch (University of Vienna, Austria)
13.30		Free time for Lunch
14.45	Pi i Sunyer	**Workshop on conference presentation skills** Elinor Thompson (PRBB)
15.00		**Round tables**
	Pere Coromines	**Following the track of DNA: from crime to evolution** Participants: Jose A. Lorente (Univ. Granada) , Assumpció Malgosa (UAB), Tomàs Marqués (IBE, CSIC-UPF), Alicia Bofarull (Institute of Toxicology), Andrés Moya (UV)
	Prat de la Riba	**"So now you got your PhD, what's next …?"** ***A first-hand review on unexpected professional paths-2*** Organized by Luis Ruiz (Janus Developments S.L). Participants: Cristina Malagelada (UB), Montse Vendrell (Biocat), Ana Kosoy (Janus Developments S.L)
16.15		**Parallel sessions**
	Prat de la Riba	**S 13- Cellular and molecular neurobiology**
	Nicolau d'0lwer i Pi i Sunyer	**S 14- Systems biology**
	Pere Coromines	**S 15- Molecular biology in model organisms**
	Puig i Cadafalch	**S 16- Biophysics**
18.00		Beer & Posters
19.00	Prat de la Riba	**Assemblea general ordinària de la SCB**

WEDNESDAY 11 TH OF JULY		SESSION III
	Room	
8.30		Registration IEC
9.15		**Parallel sessions**
	Prat de la Riba	**S 17- Cell signalling in the nervous system**
	Sala d'Actes del CSIC	**S 18- Evolution**
	Pere Coromines	**S 19- Development**
11.15		Coffee-break
12.00		**Plenary lecture**
	Conservatori de Música del Liceu	**Huntingtin from evolution to pathology via the embryonic stem cells** Elena Cattaneo (University of Milan, Italy)
13.30		Free time for Lunch
15.00		**Round tables**
	Pere Coromines	**Research in Catalonia: University or Research Institutes?** Participants: Marta Aymerich (Generalitat de Catalunya), Jordi Alberch (UB), Joan Comella (IRVH), Ramon Gomis (IDIBAPS), Albert Sorribas (UdL)
	Prat de la Riba	**"So now you got your PhD, what's next …?"** ***A first-hand review on unexpected professional paths-3*** Organized by Luis Ruiz (Janus Developments S.L). Participants: Silvia Giner (UB), Raúl Martín Ruíz (Ysios Capital), Ramon Bosser (Janus Developments S.L)
16.15	Conservatori de Música del Liceu	**S 20- Evolution and development of the nervous System**
18.00		Beer & Posters

THURSDAY 12 TH OF JULY — SESSION IV

	Room	
8.30		Registration IEC
9.15		**Parallel sessions**
	Prat de la Riba	**S 21- Virology**
	Pere Coromines	**S 22- Microbiology**
	Nicolau d'Olwer	**S 23- Ecology**
	Pi i Sunyer	**S 24- Aquaculture**
11.30		Coffee-break
	Conservatori de Música del Liceu	**Plenary lectures**
12.00		**The origins of AIDS virus** Miguel A. Martínez (IRSICAIXA)
12.45		**Symbiotic planet** Ricard Guerrero (IEC)
13.30		**Epigenetics in ecological research and animal production** Francesc Piferrer (ICM-CSIC)
14.15		Free time for Lunch
		Parallel sessions
15.00	Prat de la Riba	**S 25- Virology**
15.30	Pere Coromines	**S 26- Microbiology**
	Nicolau d'Olwer	**S 27- Ecology**
	Pi i Sunyer	**S 28- Aquaculture**
18.00		Beer & Posters

THURSDAY 12 TH OF JULY
CLOSING CEREMONY
Paranimf de la Universitat de Barcelona

19.30	**Closing Plenary lecture** **Plant-Animal Mutualistic networks: the Architecture of Biodiversity** Jordi Bascompte (Doñana Biological Station, CSIC)
20.30	Official Closing of the Conference Sr. Dídac Ramírez (rector de la UB) Sr. Ricard Guerrero (secretari científic de l'IEC) Sr. Lluis Tort (president SCB) Sr. Josep Clotet (president del comitè organitzador del congrés)
21.30	Dinner in the gardens of the Universitat de Barcelona
23.00	Dancing

MONDAY 9 TH OF JULY
S 1 - EPIGENETIC MECHANISMS IN HEALTH AND DISEASE

Room: Prat de la Riba — Moderator: Marian Martínez-Balbas (IBMB)

9.15 **Diabetes and the genomic dark matter of pancreatic beta cells**
Speaker invited: Jorge Ferrer (UB, IDIBAPS)

9.45 **Overcoming the epigenetic barrier during reprogramming to pluripotency**
María José Barrero Núñez (CMRB)

10.00 **Linking ZRF1 with retinoic acid pathway in the regulation of transcription and differentiation of leukemic cells**
Santiago Demajo Meseguer (CRG)

10.15 **MacroH2A1 in myogenic differentiation and muscle regeneration – an interplay of metabolism and epigenetics**
Melanija Posavec (IPPMC)

10.30 **The histone demethylase PHF8 is essential for cytoskeleton dynamics**
Elena Asensio Juan (IBMB-CSIC)

10.45 **Snail1 regulates heterochromatin transcription**
Alba Millanes Romero (IMIM)

11.00 **Proteomic distribution in the human sperm chromatin**
Judit Castillo Corullón (UB)

MONDAY 9 TH OF JULY
S 2 - BIOMEDICAL PROTEOMICS AND TRANSCRIPTOMICS

Room: Pere Coromines — Moderator: Francesc Xavier Avilés (UAB)

9.15 **High-throughput biomedical transcriptomics and proteomics: their relevance in the health system**
Speaker invited: Jaume Reventós (IRVH)

9.45 **The proteome of isolated human sperm tail suggests new metabolic pathways**
Alexandra Amaral (UB)

10.00 **Urine Proteomic Analysis for the identification of Prostate Cancer biomarkers**
Marina Rigau (IRVH)

10.15 **Differential RNAs in the sperm cells of asthenozoospermic patients.**
Meritxell Jodar Bifet (UB)

10.30 **miRNA profile in "elite controllers": a pilot study**
Mireia Arnedo (IDIBAPS)

10.45 **Mitochondrial DNA expression and content in postmortem brain tissue of schizophrenia patients and control subjects**
Helena Torrell Galceran (URV)

11.00 **Molecular diagnosis of glioblastoma based on discriminant equations: objective recognition of primary and secondary cases**
Xavier Castells (UAB)

MONDAY 9 TH OF JULY
S 3 - GAMETES, STEM CELLS AND DIFFERENTATION

Room: Sala d'Actes del CSIC — Moderators: Mercè Durfort (UB), Francesca Vidal (UAB)

9.15 **Reprogramming the potency of somatic cells: how and what for?**
Speaker invited: Angel Raya (IBEC)

9.45 **Chromosome size and morphology determine bivalent positioning in human spermatocytes**
Laia Vergés (UAB)

10.00 **Aneuploid and diploid spermatozoa from reciprocal translocation carriers exhibit an altered segregation pattern**
Anna Godo (UAB)

10.15 **Treatment of mouse somatic cell nuclear transfer embryos with psammaplin a improves *in vitro* development and quality.**
Anna Mallol (UAB)

10.30 **Complete meiosis from human induced pluripotent stem cells**
Cristina Eguizabal (CRMB)

10.45 **The influence of E-cadherin on Embryonic Stem Cell derivation from mammalian isolated blastomeres**
Josep Santaló (UAB)

MONDAY 9 TH OF JULY
S 4 - COMPUTATIONAL AND STRUCTURAL BIOLOGY

Room: Nicolau d'Olwer i Pi i Sunyer — Moderator: Roderic Guigó (CRG)

9.15 **In Silico biology**
Speaker invited: Modesto Orozco (IRB, UB)

9.45 **The role of structural disorder in the rewiring of protein interactions through evolution**
Roberto Mosca (IRB)

10.00 **IntOGen: Large scale analysis and integration of cancer genomics data**
David Tamborero (UPF)

10.15 **Alternative splicing and stochastic gene expression**
Xavier De La Cruz (IBMB-CSIC)

10.30 **Mapping the Dark Side of the Human Genome: Long Non-Coding RNAs**
Rory Johnson (CRG)

10.45 **Somatic structural mosaicism as an early genetic marker of late-onset diseases**
Juan R González (CREAL)

11.00 **Accurate prediction of inversions in the human genome from paired-end mapping data with the GRIAL algorithm**
Alexander Martinez Fundichely (IBB)

MONDAY 9 TH OF JULY
S 5 - REGULATION OF CHROMATIN FUNCTIONS

Room: Pere Coromines — Modeerator: Marcus Buschbeck (IMPPC)

16.15 **Dealing with chromatin constrains in gene regulation**
Speaker invited: Miguel Beato (CRG)

16.45 E**pigenetic regulation of centromere function**
Olga Moreno (IBMB))

17.00 **The MyoD-BAF60c complex poises chromatin for rapid transcription**
Sònia Forcales (IMPPC)

17.15 **SIRT2 regulates genomic stability and cell cycle progression through the control of H4K16Ac and H4K20me1 levels**
Paloma Martínez (IDIBELL)

17.30 **Human histone H1 variants: knock-down and occupancy in the genome**
Lluís Millán Ariño (IBMB-CSIC)

17.45 **Contribution of hydrophobic interactions to the folding and fibrillation of the C-terminal domain of histone H1**
Alicia Roque (UAB)

MONDAY 9 TH OF JULY
S 6 – GENES AND GENOMES

Room: Prat de la Riba — Moderator: Xavier Estivill (CRG)

16.15 **The Mutational Landscape of Chronic Lymphocytic Leukemia**
Speaker invited: Elias Campo (Hospital Clínic)

16.45 **When nature decides: one gene, two DNA repair pathways and three human diseases**
Jordi Surralles (UAB)

17.00 **Molecular diagnosis of rare Mendelian diseases using whole exome sequencing**
Benjamín Rodríguez-Santiago (Quantitative Genomic Medicine Laboratories S.L)

17.15 **The PLAU P141L single nucleotide polymorphism is a potential genetic predictor of the arteriogenic response in coronary artery disease**
Joan Duran (UB)

17.30 **Study of genetic association in 1q21-23 locus, a candidate region for psychosis**
Marta De Castro i Català (UB)

17.45 **Large-scale validation and genotyping of inversions in the human genome by inverse PCR**
Cristina Aguado Esteban (UAB)

MONDAY 9 TH OF JULY
S 7 - BIOLOGY OF REPRODUCTION

Room: Nicolau d'0lwer i Pi i Sunyer — Moderators: Joaquima Navarro (UAB), Rafael Oliva (IDIBAPS, UB)

16.15 **Current knowledge of the proteomics of human spermatozoa**
Speaker invited: Rafael Oliva (UB)

16.45 **Gonadal transcriptome analysis of the effects of temperature on European sea bass (*Dicentrarchus labrax*) sex ratios**
Noelia Díaz (CSIC)

17.00 **Sperm nucleoprotein structure is more resistant to sustain cryopreservation procedures in good than in poor freezeability boar ejaculates**
Efren Estrada (UAB)

17.15 **An assessment of telomeric repeat-containing RNA (TERRA) and telomerase in human fetal oocytes**
Rita Reig-Viader (UAB)

17.30 **Comprehensive analysis of sperm DNA fragmentation through alkaline and neutral Comet assay in clinical groups of infertile patients**
Jordi Ribas-Maynou (UAB)

17.45 **Evidences that ATR is involved in DSB repair during meiotic prophase**
Sarai Pacheco (UAB)

MONDAY 9 TH OF JULY
S 8 - PROLIFERATION, ANGIOGENESIS AND METASTASIS

Room: Sala d'Actes del CSIC — Moderator: Gabriel Capellà (Institut català d'oncologia)

16.15 **Mechanism of Resistance to Anti-Angiogenic Therapies**
Speaker invited: Oriol Casanovas (IDIBELL)

16.45 **Identification of Sp1 targets involved in proliferation and cancer**
Veronica Noé (UB)

17.00 **Characterization of Prostate Cancer bone metastasis process by a highly bone metastatic cell line generated in vivo**
Marta Garcia (IRVH)

17.15 **Cancer and arsenic: dedifferentiation and effects on CSCs**
Anna Pastoret (UAB)

17.30 **Role of MSK1 in steroid hormone-induced breast cancer cell proliferation**
Diana Reyes Garau (CRG)

17.45 **PFKFB3 regulation by p38 MAPK pathway**
Laura Novellasdemunt Vilaseca (UB)

TUESDAY 10 TH OF JULY
S 9 - CELL DAMAGE AND CELL DEATH

Room: Sala d'Actes del CSIC — Moderator: Josefa Sabrià (UAB)

9.15 **Age-dependent decline of motor cortex but not hippocampal performance in heterozygous BDNF mice correlates with a decrease of cortical PSD-95 but an increase of hippocampal TrkB levels**
Albert Giralt (UB)

9.30 **Decreased PKC-delta protein levels as a neuroprotective mechanism in cells expressing mutant huntingtin**
Laura Rué (UB)

9.45 **Phenotypic and functional characterization of antigen-specific myeloid-derived suppressor cells generated during retroviral transduction of murine bone marrow**
Sílvia Casacuberta Serra (IRVH)

10.00 **Unique role of Bcl-xL regulating the antiapoptotic role of NF-kB**
Elisenda Casanelles Abella (UAB)

10.15 **Role of CDK11 in the β cell mass apoptosis in type I diabetes**
Ester Sala Soler (UdL, Lleida)

10.30 **Failure of caspase-dependent cell death to reach the classical apoptotic phenotype in SH-SY5Y human neuroblastoma derived cells**
Mercè Garcia i Belinchón (UAB)

10.45 **Development of a network to provide fresh human tissue for research**
Estephan Arredondo (PCB)

11.00 **Mitochondrial toxicity of carbon monoxide from tobacco in smoking pregnant women: reduced intrauterine growth**
Marc Catalán (UB, Hospital Clínic, IDIBAPS, CIBERER)

TUESDAY 10 TH OF JULY
S 10 - FROM GENOTYPE TO PHENOTYPE: WHERE ARE WE NOW?

Room: Prat de la riba Moderator:

9.15 **Systems biology challenges**
Speaker invited: Luis Serrano (CRG)

9.45 **Debate: From genotype to phenotype**
Roderic Guigó (CRG) -- RNA as a first phenotype (molecular) of a cell
Ricard Solé (UPF)
Roger Guimerà (URV, Tarragona) -- Complex biological networks: Challenges and opportunities
Juli Peretó (UV, València) -- Metabolic networks and the evolution of metabolisme

TUESDAY 10 TH OF JULY
S 11 - TRAFFIC AND SIGNALLING IN HEALTH AND DISEASE

Room: Pere Coromines Moderator: Víctor J. Yuste (UAB)

9.15 **Biogenesis of carriers for secreting bulky cargoes and proteins that cannot enter the endoplasmic reticulum**
Speaker invited: Vivek Malhotra (CRG)

9.45 **Apoptosis, immunogenicity and stability properties of PPRHs directed against survivin in mammalian cancer cell lines**
Laura Rodriguez (UB)

10.00 **Contribution of rare and common variants of PTCHD1 gene in autism**
Bàrbara Torrico Avilés (UB)

10.15 **Mitochondrial implication in adverse outcomes of HIV pregnàncies**
Glòria Garrabou Tornos (UB, Hospital Clínic, IDIBAPS, CIBERER)

10.30 **Gene Therapy for Diabetes: Moving to Clinic?**
David Callejas Castiñeiras (UAB)

10.45 **Mitochondrial implication in sepsis**
Ester Tobias (UB, Hospital Clínic, IDIBAPS, CIBERER)

11.00 **El rol del gen CNR1 (receptor cannabinoide tipus I) en la resposta clínica i la remissió al tractament amb citalopram (ISRS) en Depressió Major: estudi de seguiment a 12 setmanes**
Marina Mitjans (UB)

TUESDAY 10 TH OF JULY
S 12 - RECEPTORS, CHANNELS AND TRANSPORTERS

Room: Nicolau d'Olwer Moderator: Francesc Sepulcre Sánchez (UPC)

9.15 **New paradigms in GPCR signaling: G proteins at the mitochondria**
Speaker invited: Anna Aragay (IBMB-CSIC)

9.45 **Molecular Details of the Apolipoprotein E and the Amyloid Beta Peptide Interaction: Analysis of a Potential Binding Site Responsible for ApoE4 Misfolding**
Alex Perálvarez-Marín (UAB)

10.00 **In Silico analysis of neurokynin-1 at the sequence level: a preface to structural studies**
Danial Afsharzadeh (UPC)

10.15 **The voltage-dependent K+ channel Kv1.3 in adipocytes**
Mireia Pérez Verdaguer (UB)

10.30 **Assessment of the conformation profile of bombesin,neuromedin B and neuromedin C by computational methods**
Juan Jesús Pérez González (UPC)

10.45 **Visual phototransduction: from rhodopsin and cone opsin mutations to visual disease**
Eva Ramon Portés (UPC)

11.00 **The voltage-dependent K+ channel Kv1.5 in B lymphocytes**
Albert Vallejo (UB)

TUESDAY 10 TH OF JULY
S 13 - CELLULAR AND MOLECULAR NEUROBIOLOGY

Room: Prat de la Riba — Moderator: Teresa Vilaró (Iibb-csic, idibaps, ciberned)

16.15 **Normalization of P75 levels prevents cognitive decline in a knock-in mouse model of Huntington disease**
Veronica Brito (UB)

16.30 **Oxygen tension modulates glial cell lineage commitment through modifications on bmp7 expression.**
Juan Alberto Ortega Cano (UB)

16.45 **Activity-dependent gene transcription and memory in Alzheimer´s disease**
Carlos Saura (UAB)

17.00 **Disrupció de les oscil·lacions corticals de baixa freqüència per fenciclidina: un model vàlid per al cribatge de nous fàrmacs antipsicòtics**
Eva Troyano Rodríuez (IIBB-CSIC)

17.15 **Neurophysiological alterations in a mouse model of proliferative retinopathy**
Pilar Villacampa Alcubierre (UAB)

17.30 **SC-51089 chronic treatment decreases motor and cognitive deficits in mouse model of huntington's disease**
Marta Anglada Huguet (UB)

17.45 **Parkin loss of function leads to RTP801 accumulation and neurodegeneration in parkinson's disease**
Joan Romaní Aumedes (UB)

18.00 **Effect of acute exposure to cocaine in a dopaminergic neuronal model: a gene expression study**
Noèlia Fernàndez Castillo (UB)

TUESDAY 10 TH OF JULY
S 14 - SYSTEMS BIOLOGY

Room: Nicolau d'Olwer i Pi i Sunyer — Moderator:

16.15 **Organization Principles in Biology**
Speaker invited: Rui Alves (UdL, Lleida)

16.35 **Boundary Formation in Cell Populations: from Gene Regulation to Tissue Mechanics**
Speaker invited: Javier Buceta (PCB-UB)

16.55 **Integration of proteomic and genome-wide data to understand Polycomb function on mouse embryonic stem cells differentiation**
Speaker invited: Luciano di Croce (CRG)

17.15 **Ligand expression ahead of neurogenic wavefronts: a new design principle?**
Speaker invited: Marta Ibañes (UB)

17.35 **Defining the DNA interactome in a minimal bacteria**
Eva Yus (CRG)

17.45 **Precision of the Quorum Sensing Switch: Stochastic and Non-equilibrium Effects**
Marc Weber (PCB)

17.55 **Using Systems Biology to Learn About the Synapse Role In Neurologic and Psychiatric Disorders**
Àlex Bayés (Sant Pau BRI)

TUESDAY 10 TH OF JULY
S 15 - MOLECULAR BIOLOGY IN MODEL ORGANISMS

Room: Pere Coromines — Moderator: Carles Ciudad (UB)

16.15 **Models diversos en Genètica Molecular de Plantes: Arabidopsis thaliana, blat de moro, meló.**
Speaker invited: Pere Puigdomènech (CRAG)

16.45 **Dma1: E3 ligase cell cycle regulated?**
Natalia Ricco Pacheco (UIC)

17.00 **Protection of diet-induced obesity and insulin resistance in transgenic mice overexpressing HMGA1 in adipose tissue**
Efrén Riu (CBATEG)

17.15 **Deficiency of cyclin D3 contributes to the apoptosis of beta cell and development of type 1 diabetes in nod mice**
Alejandra Saavedra Avila (UdL, Lleida)

17.30 **Hematopoietic stem cell gene therapy corrects biochemical imbalances in a murine model of MNGIE**
Javier Torres-Torronteras (IRVH)

17.45 **AAV9-sulfamidase vector delivery to the Cerebrospinal Fluid corrects BRAIN and somatic pathology in MPSIIIA mice and results in detectable enzyme levels in dogs**
Virginia Haurigot (CBATEG, UAB)

TUESDAY 10 TH OF JULY
S 16 - BIOPHYSICS

Room: Puig i Cadafalch — Moderator: Esteve Padrós Morell (UAB)

16.15 **Mechanobiology of lung cells**
Speaker invited: Daniel Navajas (UB)

16.45 **Direct observation of stalled fork restart and lesion bypass via fork regression in T4 replication System**
Maria Manosas (UB)

17.00 **Probing G-protein-coupled receptor dimerization and its role in depression**
Mercè Tena Campos (UPC)

17.15 **Unraveling nucleic acids and proteins using single molecule methods**
Felix Ritort (UB)

17.30 **UB behaviour in biomimetic membranes of DPPC and MGDG**
Javier Hoyo (UPC)

17.45 **Biochemical changes in a rat model of stroke assessed by in vivo Magnetic Resonance Spectroscopy (MRS) and ex vivo High Resolution Magic-Angle Spinning (HRMAS)**
Myriam Davila (UAB)

18.00 **Structural and biochemical insights into the human mitochondrial transcription factor A with the light strand promoter**
Anna Rubio Cosials (IBMB-CSIC)

WEDNESDAY 11 TH OF JULY
S 17 – CELL SIGNALLING IN THE NERVOUS SYSTEM

Room: Prat de la Riba — Moderator: Francesc Vinyals (UB)

9.15 **Role of hippocampal nNOS/cGMP pathway in cognitive impairment in Huntington's disease**
Ana Saavedra (UB)

9.30 **Cox-2 regulation by IL-1β through MAPKs: A comparison of nasal mucosa and nasal polyps fibroblasts from AIA patients**
Francesc Josep Garcia i Garcia (IDIBAPS)

9.45 **The role of PDK1 beyond PKB/AKT in regulating neuronal survival analysed by knock-in mutation**
Jose R. Bayascas (UAB)

10.00 **A circadian controlled G-protein coupled receptor heterodimer modulates melatonin production**
Peter Mccormick (UB)

10.15 **A functional A2RE sequence is responsible for transport of the DDR1 mRNA to oligodendrocyte processes**
Nerea Abasolo Zabalo (URV)

10.30 **Hypothalamic ceramide levels regulated by cpt1c are involved in orexigenic effects of ghrelin**
Sara Ramírez Flores (UIC)

10.45 **A potential role of Kalirin-7 in cortico-striatal learning deficits in HD**
Mar Puigdellívol Cañadell (UB)

11.00 **The Persistence of Memory: Two-Photon Imaging Reveals How Synapses Learn and Remember in Real Time**
Miquel Bosch (MIT)

WEDNESDAY 11 TH OF JULY
S 18 - EVOLUTION

Room: Sala d'Actes del CSIC — Moderators: Montserat Papaceit (UB)

9.15 **Genomic shuffling affects recombination rates during mammalian diversification**
Aurora Ruiz-herrera (UAB)

9.30 **Evolution of recent rodent gene duplicates**
Cinta Pegueroles (IMIM)

9.45 **Biosphere's phylogenetic structure**
Cristian R. Altaba (UIB, Balears)

10.00 **Orígen i evolució del sistema quimiosensorial i desenvolupament de nous marcadors moleculars en aràcnids**
Cristina Frias (UB)

10.15 **EBV strain variation in different lymphoblastoid cell lines derived from 1000 Genomes Project individuals**
Gabriel Santpere (IBE-UPC)

10.30 **Identification of selective sweeps in the genome of the Boxer breed**
Javier Quilez Oliete (CRAG)

10.45 **Molecular analysis of the mechanisms involved in THBS4 differential gene-expression in the human brain**
Raquel Rubio Acero (UAB)

11.00 **Two new plant cytogenetic online resources: the GSAD and Plant rDNA databases**
Sònia Garcia (UB)

WEDNESDAY 11 TH OF JULY
S 19 - DEVELOPMENT

Room: Pere Coromines Moderators: Jordi Garcia (UB)

9.15 **Gene Loss Impact on EVO-DEVO: Dismantling the Retinoic Acid Pathway in the Chordate *Oikopleura dioica***
Cristian Cañestro (UB)

9.30 **Helios, a new transcription factor involved in the determination of striatal medium spiny neurons**
Mònica Pardo Muñoz (UB)

9.45 **Evolutionary conserved role of the beta-catenin/Wnt signalling throughout planarian life cycle**
Teresa Adell (UB)

10.00 **Functional characterization of neoblast specific transcription factors during planarian regeneration**
Alejandro González-Sastre (IBUB-UB)

10.15 **The role of the microRNA trio let-7, miR-100 and miR-125 in insect hemimetabolous metamorphosis**
Mercedes Rubio (UPF-CSIC)

10.30 **Ovarian follicle development in primitive insects**
Paula Irles (IBE-UPF)

10.45 **Regeneration in Isodiametra pulchra (Acoela, Acoelomorpha)**
Elena Perea Atienza (UB)

11.00 **How often does natural selection targets multiple, interacting genes? The prevalence of epistasis in recent human evolution**
Natalia Petit (IBE-UPF)

WEDNESDAY 11 TH OF JULY
S 20 - EVOLUTION AND DEVELOPMENT OF THE NERVOUS SYSTEM

Room: Conservatori de Música del Liceu Moderators:

16.15 **20 years of ephithelial to mesenchymal transition**
Speaker invited: Angela Nieto (UMH, Alacant)

16.35 **No place like home: stem cells and the neurovascular niche**
Speaker invited: Isabel Fariñas (UV, Valencia)

16.55 **The regulation of neural stem and progenitor cell fate by the activity of transcription factors**
Speaker invited: Carlos Vicario-Abejon (Cajal Institute, Madrid)

17.15 **An evo-devo approach for understanding the amygdala, a key forebrain center for control of emotions and social behavior**
Speaker invited: Loreta Medina (IRB, Lleida)

THURSDAY 12 TH OF JULY
S 21 - VIROLOGY

Room: Prat de la Riba

9.15 **Presentation**
Miguel Angel Martinez (IRSICAIXA)

9.20 **Quasispecies dynamics and the control of viral infections**
Speaker invited: Esteban Domingo (CBMSO)

9.45 **Deep sequencing of naturally evolving arbovirus populations in the mosquito vector identifies new variants with epidemic potential**
Speaker invited: Marco Vignuzzi (Institut Pasteur, Paris)

10.10 **mRNA Archaeology**
Speaker invited: Jordi Gomez (CSIC, Granada)

10.35 **Hepatitis A virus, a very special picornavirus**
Speaker invited: Albert Bosch (UB)

11.00 **Norovirus diversity in relation to population impact**
Speaker invited: Marion Koopmans (RIVM, The Netherlands)

THURSDAY 12 TH OF JULY
S 22 - MICROBIOLOGY

Room: Pere Coromines — Presented: Teresa Vinuesa (UB)

9.15 **Epigenetics and evolution in bacteria**
Speaker invited: Miquel Viñas (UB)

10.00 **Patogènesi bacteriana com a simbiosi imperfecta**
Speaker invited: Josep Casadesús (Universidad de Sevilla)

10.45 **Endosimbiosi i evolució**
Speaker invited: Juli Peretó (UV, Valencia)

THURSDAY 12 TH OF JULY
S 23 - ECOLOGY

Room: Nicolau d'Olwer

9.15 **Stequiometry and global metabolism**
Speaker invited: Josep Peñuelas (CREAF-CSIC)

10.00 **Phylogenetics, genomics and global change:understanding the past to enlighten the future**
Speaker invited: Vincent Savolainen (Imperial College, UK)

10.45 **Biodiversity: portfolio of responses to global change**
Speaker invited: Fernando Valladares (CCMA-CSIC, Madrid)

THURSDAY 12 TH OF JULY
S 24 - AQUACULTURE

Room: Pi i Sunyer

Challenges of aquaculture in the twenty-first century

9.15 **Aquaculture today, a look at challenges ahead: health management**
Speaker invited: Dolors Furones (IRTA)

10.00 **Molecular basis of the making of eggs in marine fish: the role of oocyte aquaporin**
Speaker invited: Joan Cerdá (CSIC-IRTA)

10.45 **Present and future of nutrition in fish. The need for sustainable feeds**
Speaker invited: Miguel Jover (Instituto de Ciencia y Tecnología Animal, UPV)

THURSDAY 12 TH OF JULY
S 25 - VIROLOGY

Room: Prat de la Riba

15.00 ® **Round tables**
"So now you got your PhD, what's next …?" ***A first-hand review on unexpected professional paths-4***
Organized by Luis Ruiz (Janus Developments S.L)
Participants: Meritxell Genesca (IGTP), Albert Pol (UB), Clara Campàs (Advancell), Ana Kosoy (Janus Developments S.L)

16.30 **Evolution of HIV-1 Protease over 14 Years: Fitness Loss or Robustness Gain**
Elena Capel (IRSICAIXA)

16.45 **Antiretroviral agents effectively block HIV replication after cell to cell transfer**
Marc Permanyer (IRSICAIXA)

17.00 **HIV maturation: a new RNA-supported paradigm of spatiotemporal nucleoprotein remodeling catalysed by a protease**
Gilles Mirambeau (IDIBAPS & Université Pierre et Marie Curie - Sorbonne)

17.15 **Transmissibility of two ipomoviruses by whitefly vectors under experimental conditions**
Lluisa Vilaplana (CRAG)

17.35 **The Movement Protein of *Cucumber mosaic virus* (CMV) determines the virulence in the melon accession PI161375**
Cèlia Guiu-Aragonés (CRAG)

17.45 **Canvis en l'evolució dels patògens sexualment transmisibles. De l'examen directe a la biologia molecular**
Martí Vall Mayans (ICS)

18.00 **Estudio de la infección del virus de la hepatitis C utilizando un sistema celular basado en la secreción de *Gaussia luciferasa***
George Koutsoudakis (Hospital Clínic)

18.15 **Caracterización de UL8, un nuevo miembro de la familia multigénica RL11 del HCMV**
Natàlia Pérez-Carmona (IDIBAPS)

THURSDAY 12 TH OF JULY
S 26 - MICROBIOLOGY

Room: Pere Coromines — Presented: Montserrat Agut (IQS)

15.30 **Bioremediation and cooperation**
Speaker invited: Balbina Nogales (UIB, Balears)

16.15 **Extremophile microorganisms and life beyond**
Speaker invited: Ricard Amils (UAM, Madrid)

17.00 **The biosphere of rare bacteria: the largest and oldest cooperative in the world**
Speaker invited: Carles Pedrós-Alió (ICM-CSIC)

17.45 ® **Round tables**
The 21st century microbiologies: prospects for the future
Isabel Esteve (UAB)

THURSDAY 12 TH OF JULY
S 27 - ECOLOGY

Room: Nicolau d'Olwer

15.30 **Phytoplankton as victims and players in the global change**
Speaker invited: Adriana Zingone (Anton Dohrn Zoological Station, UK)

16.15 **The threat of Earth's oceans becoming more acidic**
Speaker invited: Carles Pelejero (ICM-CSIC)

17.00 **Marine invasions: a future nuisance that is already present**
Speaker invited: Enric Ballesteros (CEAB-CSIC)

17.45 **Small closing**
Could global change finally merge terrestrial and aquatic ecologists?

THURSDAY 12 TH OF JULY
S 28 - AQUACULTURE

Room: Pi i Sunyer

Challenges of aquaculture in the twenty-first century II

15.30 **Endocrine regulation of lipid metabolism markers in fish**
Speaker invited: Isabel Navarro (UB)

16.15 **Diseases in fish: what do we have to know and what can we learn?**
Speaker invited: Francesc Padrós (UAB)

17.00 **The stress response in fish and the interaction between regulatory systems**
Speaker invited: Lluis Tort (UAB)

MONDAY 9 TH OF JULY

P1 - Perinatal Hypoxia and Psychotic Experiences in Adulthood. Putative Moderation effects of the Histone deactylases (HDAC) genes

A. Córdova-Palomera[1,2], X. Goldberg[1,2], M. Fatjó-Vilas[1,2], S. Alemany[1,2], A. Valldeperas[1,2], O. Kebir[3], M. O. Krebs[3], I. Nenadic[4], L. Fañanás[1,2]

[1] Unitat d´Antropologia, Departament de Biologia Animal, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona.

[2] Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III.

[3] University Paris Descartes, Sorbonne Paris Cité, Paris, France and INSERM, U894, Laboratory "Pathophysiology of Psychiatric Disorders", Centre of psychiatry and neurosciences, Paris, France.

[4] Department of Psychiatry and Psychotherapy, Friedrich-Schiller-University of Jena, Jena, Germany.

P2 - A polymorphic human inversion exchanges the first exon between two chymotrypsinogen genes

David Izquierdo[1], Marta Puig[1], Mario Cáceres[1,2]

[1] Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

[2] Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain.

P3 - Analysis, breakpoint definition and validation of inversions between assembled human genomes

David Vicente[1], Cristina Aguado[1], David Izquierdo[1], Alexander Martínez-Fundichely[1], Marta Puig[1], Mario Cáceres[1,2]

[1] Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain.

[2] Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

P4 - Polymorphism, recombination and selection efficiency along the complete genome of *Drosophila Melanogaster*

Maite Barrón, David Castellano, Miquel Ràmia, Antonio Barbadilla

Genomics, Bioinformatics and Evolution Group, Institut de Biotecnologia i de Biomedicina - IBB/Department of Genetics and Microbiology, Campus Universitat Autònoma de Barcelona.

P5 - Calling inversions from Next-Generation Sequencing Paired-End Mapping data with GRIAL

Sònia Casillas[1,2], Can Alkan[2,3], Francesca Antonacci[2], Peter H. Sudmant[2], Evan E. Eichler[2,4], Mario Cáceres[1,5]

[1] Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.

[2] Department of Genome Sciences, University of Washington School of Medicine, Seattle, Washington, USA.

[3] Current address: Department of Computer Engineering, Bilkent University, Ankara, Turkey.

[4] Howard Hughes Medical Institute, Seattle, Washington, USA.

[5] Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

P6 - Chronic treatment with a putative TrkB agonist, 7, 8-Dihydroxyflavone ameliorates motor and cognitive deficits in a Huntington's disease mouse model

García Díaz-Barriga A. Gerardo, Giralt, Albert, Anglada-Huguet Marta, Canals JM, Alberch Jordi

Departament Biologia Cel·lular, Immunologia i Neurociències Facultat de Medicina, Universitat de Barcelona-IDIBAPS.

P7 - Biochemical and biophysical parameters influencing macromolecular crystallization and X-ray diffraction quality

Cuppari A, Rubio Cosials A, Fernandez Millan P, Silva Espiña C, Solà M.

Department of Structural Biology, Molecular Biology Institute of Barcelona (CSIC), Barcelona, Spain.

P8 - Study of the molecular mechanisms that regulate *SCN5A* expression

Anna Tarradas, Pedro Beltran-Alvarez, Ramon Brugada, Sara Pagans

Centre de Genètica Cardiovascular, UdG-IDIBGI.

P9 - Apaf-1 es una diana farmacológica eficaz y segura para la prevención de la apoptosis no deseada

Carmen Herrero[1], Sandra Marchán[1], Mar Orzáez[2], Mónica Sancho[2], Enrique Pérez-Payá[2], Carmen Lagunas[1]

[1]Centro de Investigación´ Laboratorios SALVAT, S.A. Esplugues de Llobregat, Barcelona, España.

[2]Laboratorio de Química de Péptidos y Proteínas, Centro de Investigación Príncipe Felipe, Valencia, España.

P10 - Mutation spectrum in the CACNA1A gene in 49 patients with episodic ataxia type 2

Cèlia Sintas[1,2,3], Oriel Carreño[1,2,3], Roser Corominas[1,3,4], Claudio Toma[1,2,3], Marta Vila[4], Noèlia Fernández-Castillo[1,2,3], Ester Cuenca[4], Alfons Macaya[4], Bru Cormand[1,2,3]

[1] Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Spain.
[2] Institut de Biomedicina de la Universitat de Barcelona (IBUB), Spain.
[3] Center for Biomedical Network Research on Rare Diseases (CIBERER)-Institute of Health Carlos III, Spain.
[4] Pediatric Neurology Research Group, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Spain.

P11 - Significación clínica del genotipo interleuquina-I positivo en pacientes fumadores como factor pronóstico de la periimplantitis

García Delaney, Cristina; Sánchez Garcés, MºÁngeles; Figueiredo, Rui; Gay Escoda, Cosme

Máster de Cirugía Bucal e Implantología Bucofacial de la Universidad de Barcelona.

P12 - Characterization of mechanisms involved in cell specific expression of TREX2 exonuclease

Diana Gómez[1], Neus Serrat[1], Joan Manils[1], Alex Moreno[1], Monika Kucharewicz[1], Laura Marruecos[1], Carmen Benito[1], Antonio Felipe[2], Sònia-Vanina Forcales[3], Concepció Soler[1]

[1] Department of Pathology and Experimental Therapeutics, Faculty of Medicine, Universitat de Barcelona, Hospitalet de Llobregat,Spain
[2] Department of Biochemistry and Molecular Biology, Faculty of Biology, Universitat de Barcelona, Spain.
[3] Institute for Predictive and Personalized Medicine of Cancer, Badalona, Spain.

P13 -Diet-induced obesity SIRT1-mediated histone modifications in mouse liver

Altamira Arce-Cerezo[1], Enrique Blanco[2], Miquel García[1], Daniel Herranz[3], Albert Peró[1], Aida Rodríguez[1], Manuel Serrano[3], Montserrat Corominas[2], Fàtima Bosch[1], Efrén Riu[1]

[1] Center of Animal Biotechnology and Gene Therapy (CBATEG) and Dept Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona; 08193-Bellaterra and CIBER of Diabetes and Associated Metabolic diseases (CIBERDEM), Spain.
[2] Departament de Genètica, Universitat de Barcelona, Spain.
[3] Tumor Suppression Group, Spanish National Cancer Research Center, Madrid, Spain.

P14 - Structural determination of a complex of an AT-hook of human HMGA1a with AT-rich DNA

Elsa Fonfría-Subirós[1], Francisco Acosta-Reyes[1], Núria Saperas[1], Joan Pous[2], Raquel Sánchez-Giraldo[1], Juan A Subirana[1], Lourdes Campos[1]

[1] Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, Barcelona, Spain.
[2] Plataforma Automatitzada de Cristal·lografia, IRB-PCB-CSIC, Barcelona, Spain.

P15 - La inhibición de Apaf-1 como estrategia terapéutica en la prevención de fallo renal agudo

Estefanía Traver[1], Sandra Marchán[1], Mónica Sancho[2], Mar Orzáez[2], Enrique Pérez-Payá[2], Carmen Herrero[1], Carmen Lagunas[1]

[1] Laboratorios SALVAT, S.A. Barcelona.
[2] Centro de Investigación Principe Felipe (CIPF). Valencia.

P16 - Restoration of thymidine phosphorylase activity confers sensitivity to 5' fluoro-5' deoxyuridine (5'-dFUrd): towards a novel suicide gene strategy for cancer-associated gene therapy in MNGIE

Gemma Ferrer, Javier Torres-Torronteras[1], Sergio López, Sílvia Casacuberta, María José Mansilla, Lluís Martorell, Ramon Martí[1], Jordi Barquinero

Gene and Cell Therapy Laboratory.
[1] Neuromuscular and Mitochondrial Pathology Laboratory. Vall d'Hebron Research Institute (VHIR).

P17 - Involvement of the non-rgs rhogef proteins, P190RHOGEF and GEF-H1, in the G12 family signaling pathways

Georgina Garrido, Míriam Masià, Anna Aragay.

Institut de Biologia Molecular de Barcelona, CSIC, Barcelona, Spain.

P18 - Analysis of gene expression regulated by the ETV5 transcription factor in OV90 ovarian cancer cells identifies FoxM1 over-expression in ovarian cancer

Marta Llauradó, Blanca Majem, Josep Castellví, Sílvia Cabrera, Antonio Gil-Moreno, Jaume Reventós, Anna Ruiz.

Research Unit in Biomedicine and Translational and Pediatrics Oncology, Hospital Universitari Vall d'Hebron Institut de Recerca (VHIR), Universitat Autònoma de Barcelona, Spain.

P19 - Expression pattern of Epithelial-Mesenchymal transition (EMT) factors: Zeb1, Snail1, Twist, E-Cadherin, and their relationship with the adult stem cell marker ABCG2/BCRP1 transporter in epithelial *thyroid tumour*

E. Mato[1], C. González [2,1], A. Aulinas[2], O. Bell[1], A.Moral [4], JL Pérez [4], E. Lerma [3], A. de Leiva [2,1]
[1] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Hospital Santa Creu i Sant Pau, Autonomous University, Barcelona.
[2] Endocrinology Department, Hospital Sant Pau (HSCP), Autonomous University, Barcelona, Spain.
[3] Department of Pathology, (HSCP).
[4] General Surgery, (HSCP).

P20 - ETV5 and LPP, promoting EMT in endometrial carcinoma

Eva Colas[1,9], Laura Muinelo[2], Marta Llaurado[1], Marta Monge[1], Lorena Alonso[2], Marta Gonzalez[1], Marie Schoumacher[3], Nuria Pedrola[1], Tugçe Ertekin[1], Jorge Barbazan[2], Anna Ruiz[1], Josep Castellvi[4], Andreas Doll[1], Antonio Gil-Moreno[5], Jordi Xercavins[5], Rafael Lopez-Lopez[6], Sylvie Robine[3], Evelyn Friederich[7], Marian Castro[8], Jaume Reventos[1,9], Danijela Vignjevic[3], Miguel Abal[1,2]
[1] Research Institute Vall d'Hebron University Hospital.
[2] Complexo Hospitalario Universitario de Santiago de Compostela.
[3] Institut Curie.
[4] Department of Pathology, Vall d'Hebron University Hospital.
[5] Department of Ginecological Oncology, Vall d'Hebron University Hospital.
[6] Complexo Hospitalario Universitario de Santiago de Compostela.
[7] University of Luxembourg.
[8] Department of Pharmacology, University of Santiago de Compostela.
[9] Universitat Autònoma de Barcelona.

P21 - Molecular pathways regulated by ETV5 transcription factor in the invasion of endometrial carcinoma

Núria Pedrola[1], Irene Campoy[1], Eva Colas[1], Josep Castellvi[2], Antonio Gil-Moreno[3], Jordi Xercavins[3], Jaume Reventos[1], Anna Ruiz[1]
[1] Biomedical Research Unit, Research Institute Vall d'Hebron University Hospital, Barcelona.
[2] Department of Pathology, Vall d'Hebron University Hospital, Barcelona.
[3] Department of Ginecological Oncology, Vall d'Hebron University Hospital, Barcelona.

P22 - ETV5 regulates IgCAMs superfamily during myometrial invasion

Laura Devis[1], Irene Campoy[1], Nuria Pedrola[1], Lorena Alonso-Alconada[2], Josep Castellví[3], Miguel Abal[2], Jaume Reventos[1, 4], Eva Colas[1]
[1] Institut de Recerca del Hospital Universitari Vall d'Hebron and Universitat Autònoma de Barcelona, Barcelona.
[2] Laboratorio de Oncologia Traslacional, Complexo Hospitalario Universitario de Santiago/SERGAS, Santiago de Compostela.
[3] Departament de Patologia, Hospital Universitari Vall d'Hebron, Barcelona.
[4] Department de Ciències Bàsiques, Universitat Internacional de Catalunya.

P23 - Generation and characterization of orthotopic murine models for endometrial cancer

Silvia Cabrera & Marta Llauradó, Josep Castellví, Yolanda Fernández-Amurgo, Francesc Alameda, Anna Ruiz, Andreas Doll, Jordi Xercavins, Miguel Abal, Antonio Gil-Moreno, Jaume Reventós
Research Unit in Biomedicine and Translational and Pediatrics Oncology, Hospital Universitari Vall d'Hebron Institut de Recerca (VHIR), Universitat Autònoma de Barcelona, Spain.

P24 - MicroRNA expression profiles in urine as diagnostic biomarkers for prostate cancer

Melània Montes, Mireia Olivan, Tamara Sequeiros, Marta Garcia, Marina Rigau, Juan Morote, Jaume Reventós, Andreas Doll
Biomedical Research Unit, Vall d'Hebron Research Institute and University Hospital, Barcelona.

P25 - A Double Cysteine Opsin Mutant to Study the Stability and Structural Behavior of Green Cone Opsin

Sundaramoorthy Srinivasan, Maria Jesús Sánchez Martín, Eva Ramon, Pere Garriga
Group of Molecular and Industrial Biotechnology, Centre de Biotecnologia Molecular, Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, Terrassa, Catalonia.

P26 - Urinary exosomes as source of prostate cancer biomarkers

T. Sequeiros[1], M. Montes[1], N. Colome[2], F. Canals[2], I. de Torres[3], J. Morote[4], M. Olivan[1], A. Doll[1*], M. Rigau[1*], J. Reventos[1*].
[1] Biomedical Research Unit, Research Institute.
[2] Proteomics Laboratory, Vall d'Hebron Institute of Oncology.
[3] Department of Pathology; [4]Department of Urology; - All of them at Vall d'Hebron Universitari Hospital, Barcelona, Spain.
* Equally contributing

P27 - Gene expression profile associated with ovarian cancer dissemination. A comparative study of ovarian primary tumors, ascites and metastases

Marta Llauradó, Josep Castellví, Sílvia Cabrera, Blanca Majem, Dámaso-José Gallardo, Antonio Gil-Moreno, Jordi Xercavins, Anna Ruiz, Jaume Reventós
Research Unit in Biomedicine and Translational and Pediatrics Oncology, Hospital Universitari Vall d'Hebron Institut de Recerca (VHIR), Universitat Autònoma de Barcelona, Spain.

P28 - Human sperm likes sugars: the importance of glycolysis in male gamete functionality

Carla Paiva[1], Alexandra Amaral[2,3], João Ramalho-Santos[2,4]
[1] PhD Programme in Experimental Biology and Biomedicine (PDBEB), Center for Neuroscience and Cell Biology, University of Coimbra, Portugal.
[2] Center for Neuroscience and Cell Biology, University of Coimbra, Portugal;
[3] Human Genetics Group, IDIBAPS, Faculty of Medicine, University of Barcelona, Spain.
[4] Department of Life Sciences, University of Coimbra, Coimbra, Portugal. Center for Neuroscience and Cell Biology and Department of Life Sciences, University of Coimbra.

P29 - Estudi mutacional dels gens *HSPA2* i *SPAG16* en pacients estèrils i en controls

Rubén Azpiazu[1], Meritxell Jodar[1], José Luís Ballescà[2], Rafael Oliva[1]
[1] Grup de Genètica Humana, IDIBAPS, Facultat de Medicina, Universitat de Barcelona i Hospital Clínic.
[2] Institut Clínic de Ginecologia, Obstetrícia i Neonatologia. Hospital Clínic.

P30 - Detection of *Serratia marcescens* in boar semen samples by PCR

Eva Bussalleu[1], Sergi Bonet[1], Gary C. Althouse[2]
[1] Biotecnologia de la Reproducció Porcina, Departament de Biologia, Facultat de Ciències, Universitat de Girona, Catalonia.
[2] Department of Clinical Studies, New Bolton Center, University of Pennsylvania, 382 West Street Road, Kennett Square, USA.

P31 - Searching for biomarkers of boar sperm freezability in order to predict ejaculate susceptibility to the freezing-thawing protocol

Ingrid Vilagran[1], Judit Castillo[2], Sergi Bonet[1], Sílvia Sancho[1], Josep Maria Estanyol[3], Rafael Oliva[2]
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[3]Unitat de Proteòmica, Serveis Cientificotècnics, University of Barcelona.

P32 - Effect of different concentrations of *Clostridium perfringens* on sperm quality of boar seminal doses kept at 15ºC

L. Sepúlveda [1], E. Bussalleu [1], M. Yeste [2], E. Torner [1], S. Bonet [1]
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[2] Unit of Animal Reproduction, Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Autonomous University of Barcelona, Barcelona, Catalonia, Spain.

P33 - Pro/acrosin Activity and Expression Analysis Along Epididymis

M Puigmule, A Fabrega, M Yeste, S Bonet, E Pinart
Biotechnology of Animal and Human Reproduction, Faculty of Science, University of Girona, Spain.

P34 - Hyaluronic acid may improve the porcine embryo quality when added to *in vitro* culture medium depending on energy substrate

Eva Torner[1], Eva Bussalleu[1], Mailo Briz[1], Marc Yeste[2], Sergi Bonet[1]
[1] TechnoSperm, Departament de Biologia, Universitat de Girona, Girona. Espanya.
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P35 - Effects of adding L-ascorbic acid into *in vitro* culture media NCSU23 on porcine embryo development and quality

Castillo-Martín M[1], Yeste M[2], Torner E[1], Bonet S[1]
[1] TechnoSperm, Department of Biology, University of Girona, Girona, Spain.
[2] Department of Animal Medicine and Surgery, Autonomous University of Barcelona, Bellaterra, Spain

P36 - Identificació d'embrions bovins cultivats en grup mitjançant l'adhesió de codis a la zona pel·lúcida

Sergi Novo[1], Roser Morató[2], Oriol Penon[3,4], Sara Duran[5], Leonardo Barrios[1], Carme Nogués[1], Marta Duch[5], Lluïsa Pérez-García[3,4], Teresa Mogas[2], Elena Ibáñez[1]
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P37 - Genetic and environmental influences on zebrafish sex ratios

Laia Ribas, Noelia Diaz, Dafni Anastasiadi, Francesc Piferrer
Institut de Ciències del Mar (CSIC).

P38 - Comparison of the female reproductive system of two marine gastropods (Neogastropoda: Muricidae) with different reproductive biology

Alexandra Richter[1], María José Amor[2], Mercè Durfort[2]
[1] Department of Biology of Organisms and Systems, University of Oviedo, Oviedo, Spain
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P39 - Spermatogenesis of *Pomacea insularum* (Caenogastropoda: Ampullariidae): Eupyrene and Apyrene Sperm Ultrastructural Study

Enric Ribes, Maria Gràcia Bozzo, Mercè Durfort
Departament de Biologia Cel·lular, Universitat de Barcelona.
Xarxa de Referència en Aqüicultura de la Generalitat de Catalunya (XRAQ).

P40 - The spermatozoon of brachylaima mascomai. ultrastructural study

J. Ferrer[1], O. González-Moreno[2], M. Gracenea[2]
[1] Department of Cell Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain.
[2] Laboratory of Parasitology, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain.

P41 - Validation of a ngs strategy for genetic diagnosis of sudden cardiac death

Catarina Allegue[1], Óscar Campuzano[1], Lucía Quintana[2], Carles Ferrer-Costa[2], Sergio Castillo[2], Eduardo Salas[2], Mónica Bayés[3], Simon Heath[3], Mònica Coll[1], Anna M. Iglesias[1], Ramon Brugada[1]
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[3] CNAG, Barcelona (Spain.)

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P42 - The proton transport by bacteriorhodopsin depends on the mobility of transmembrane helices

Guillem Marco[1], Tzvetana Lazarova[1], Esteve Padrós[1]
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P43 - Fluctuation Relations and Entropy production in a Dual Trap Optical Tweezers Setup

Marco Ribezzi-Crivellari[1], Felix Ritort[1,2]
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P44 - Characterizing the E. Coli RecQ helicase using optical tweezers

M. Martí-Prieto[1], M. Manosas[1], M. Ribezzi[1], F. Ritort[1,2]
[1] Departament de Física Fonamental, Facultat de Física, Universitat de Barcelona, Barcelona, Spain.
[2] CIBER de Bioingeniería, Biomateriales y Nanomedicina, Instituto de Sanidad Carlos III, Madrid, Spain.

P45 - Control of the mitotic cyclin dependent kinase and anaphase in response to genotoxic stress

Gloria Palou[*], Roger Palou[*], David G. Quintana ([*]equal contribution)
Cell Cycle Lab, Unitat de Biofísica, Facultat de Medicina, Departament de Bioquímica i Biologia Molecular
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P46 - Cell model for Sanfilippo C syndrome using iPS cells from patients' fibroblasts

Isaac Canals[1,2,3,6], Yvonne Richaud[3,4], Senda Jiménez[3,4], Roger Torrent[6], Daniel Grinberg[1,2,6], Lluïsa Vilageliu[1,2,6], Angel Raya[3,4,5]
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P47 - Efecte xaperona de compostos glicomimètics sobre glucocerebrosidases mutades en fibroblasts de pacients Gaucher

Jenny Serra[3], Lucía Díaz[1,2], Gessamí Sánchez-Ollé[3], Daniel Grinberg[3], Helen Michelakakis[4], Antonio Delgado[1,2], Jordi Bujons[2], Josefina Casas[2], Lluïsa Vilageliu[3]
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[3] Departament de Genètica, Universitat de Barcelona; IBUB; CIBERER, Barcelona.
[4] Department of Enzymology and Cellular Function, Institute of Child Health, Athens, Greece.

P48 - Role of TREX2 exonuclease in the DNA damage response to UVB

Joan Manils, Diana Gómez, Laura Marruecos, Carmen Benito, Concepció Soler
Departament de Patologia i Terapèutica Experimental, Facultat de Medicina Universitat de Barcelona, Hospitalet de Llobregat, Spain

P49 - Dysregulation of CK2 subunits alters proliferation and migration in 786-O cells

Jordi Vilardell, Estefania Alcaraz, Eduard Sarro, Thaïs Cuadros, Anna Meseguer , Emilio Itarte
Departament de Bioquímica i Biologia Molecular, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, and Fisiopatologia Renal, CIBBIM-Nanomedicina, Institut de Recerca Hospital Universitari Vall d´Hebron, Barcelona.

P50 - Expression pattern and map of sumo and ubiquitin pathway enzymes in the mouse retina

Mariona Esquerdo, Víctor Abad, Erica Millo, Sílvia Garcia-Monclús, Vasileios Toulis, Maria José Iniesta, Alejandro Garanto, Gemma Marfany
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P51 - Epithelial -mesenquimal transition in Head and Neck Carcinoma cell lines

M.Téllez, L.C.Navas, X.León, A.Gallardo, R. Mangues, M.Pavón
Grup d'Oncogènesi i Antitumorals / CIBER-BBN. Institut d'Investigacions Biomèdiques Sant.Pau (IIB-Sant Pau).

P52 - Involvement of the NON-RGS RhoGEF proteins, p190RhoGEF and GEF-H1, in the G12 family signaling pathways

Georgina Garrido, Míriam Masià, Anna Aragay
Institut de Biologia Molecular de Barcelona, CSIC, Barcelona, Spain.

P53 - On the nature of the genetic bases of the high bone mass phenotype in Spanish postmenopausal women

Patricia Sarrión[1,2], Leonardo Mellibovsky[3], Roser Urreizti[1,2], Maria Soler-Sala[1,2], Neus Cols[1,2], Natàlia García-Giralt[3], Guy Yoskovitz[3], Alvaro Aranguren[1,2], Roberto Güerri[3], Xavier Nogués[3], Adolfo Diez-Perez[3], Daniel Grinberg[1,2], Susana Balcells[1,2]
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P54 - Determinants of the specificity of human histone H1 subtypes

Lluis Millan-Ariño[1], Jean-Michel Terme[1], Regina Mayor[1], Borja Sesé[2], María José Barrero[2], Albert Jordan[1]
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P55 - La inhibición de Apaf-1 tiene un efecto protector en la ototoxicidad inducida por Cisplatino

Marchán S[1], Traver E[1], Casares C[2], García-Berrocal JR[2], Trinidad A[2], Ramírez-Camacho R[2], García N[1], Sanagustin J[1], Herrero C[1], Lagunas C[1]
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P56 - Phosphate-sensing CDK stabilizes G1 cyclin to trigger cell cycle entry

Menoyo, S[1]; Ricco, N[1]; Bru, S[1]; Hernández-Ortega, S[1]; Escoté, X[2]; Aldea, M[3], Clotet, J[1]
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[2] Endocrinology and Diabetes Unit. Joan XXXIII University Hospital, IISPV, Universitat Rovira i Virgili.
[3] Institut de Biologia Molecular de Barcelona. CSIC.

P57 - Novel mechanism of degradation of G_1 cyclins.

Hernández-Ortega S.; Ricco N.; Bru S.; Menoyo S.; Mirallas O., Clotet J
Departament de Ciències Bàsiques. Universitat Internacional de Catalunya. Sant Cugat del Vallès, Barcelona

P58 - C/EBPδ plays a key role in glial activation

Tony Valente[1], Marco Straccia[1,2], Nuria Gresa-Arribas[1,2], Guido Dentesano[1,2], Josep M Tusell[2], Joan Serratosa[2], Carme Solà[2], Josep Saura[1]
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[2] Department of Brain Ischemia and Neurodegeneration, IIBB-CSIC-IDIBAPS, Barcelona, Spain.

P59 - Cyclin D3 and CDK11 partnership in the apoptosis of the pancreatic Beta cell in TYPE 1 diabetes

Upasana Sen Gupta[1]; Noemí Alejandra Saavedra[1]; Ester Sala[1]; Jill Lahti[2]; Peter Sicinski[3]; Martine Roussel[2]; Joan Verdaguer[1]; Dídac Mauricio[4], Conchi Mora[1]
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P60- DFF40/CAD-mediated nuclear morphology does not require oligonucleosomal DNA fragmentation during apoptotic cell death

Victoria Iglesias-Guimarais[1,2,3], Gisela Gabernet[1], Estel Gil-Guiñon, Mercè Garcia-Belinchón[1], María Sánchez-Osuna[1], Elisenda Casanelles[1,3], Joan X. Comella[2,3], Victor J. Yuste[1,3]
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[2] Cell Signaling and Apoptosis group, Institut de Recerca del HU Vall d'Hebron, Barcelona, Spain.
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P61 - Lack of the transcription repressor histone deacetylase 7 induces lymphocyte oncogenic transformation

Bruna Barneda-Zahonero[1], Olga Collazo[1], Abul B.M.M.K. Islam[2], Antonio Gómez-Moruno[1], Lidia Roman-Gonzalez[1], Núria López-Bigas[2,3], Manel Esteller[1], Maribel Parra[1]
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P62 - Mitochondrial function in healthy infants in utero exposed to HIV and antiretrovirals

Morén C[1], Garrabou G[1], Catalán M[1], Tobías E[1], Bañó[1] M, Rovira N[2], Noguera-Julian A[2], Nicolàs M[1], Grau JM[1], Cardellach F[1], Miró Ò[1], Fortuny C[2]
[1] IDIBAPS- Hospital Clínic, Universitat de Barcelona- CIBER de Enfermedades Raras (CIBERER).
[2] Hospital Sant Joan de Déu, Universitat de Barcelona; Barcelona (Spain).

P63 - Mitochondrial toxicity of highly active antiretroviral therapy (Haart) in HIV uninfected pacients

Bañó M[1], Morén C[1], Catalán M[1], Tobias E[1], Pedrol E[2], Grau JM[1], Cardellach F[1], Miró Ó[1], Garrabou G[1]
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P64 - Asymmetric stochastic switching driven by intrinsic molecular noise

David Frigola[1], Laura Casanellas[1], J.M. Sancho[1], Marta Ibañes[1]
[1] Dept. Structure and Constituents of Matter, Faculty of Physics, University of Barcelona.

P65 - Reversibility and memory in cellular decision making

David Palau-Ortin[1] , Marta Ibañes[1]
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P66 - Regulation of neurogenic wavefronts: a modeling perspective

Pau Formosa-Jordan[1,2], Marta Ibañes[1], Saúl Ares[2,3], José María Frade[4]
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P67 - Functional variants of the CX3CR1 gene in Alzheimer Disease and Amiotrophic Lateral Sclerosis

López-López A[1], Syriani E[2], Lopategui D[1], Morales M[2], Gamez J[3], Rodríguez MJ[1], Mahy N[1], Vidal-Taboada JM[1]
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[3] Neurology Department, Hospital Universitari Vall d'Hebron, Institut de Recerca, Autonomous University of Barcelona, Spain.

P68 - Treatment with a serotonin 5-HT$_4$-receptor agonist ameliorates cognitive deficits and amyloid pathology in the 3xTg-AD model of Alzheimer's disease

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P69 - Neuroinflammation in APP/PS1 mouse model of Alzheimer´s disease

Dmitry Petrov[1], Felix Junyent[1,3], Carme Auladell[2], Mercè Pallàs[1,3], Antoni Camins[1,3], Ester Verdaguer[2]
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P70- Motor deficits and neuromuscular junction alterations following chronic 3,3'-iminodipropionitrile exposure in the rat

Carla Soler-Martín[1], Sandra Saldaña-Ruíz[1], Neus Garcia[2], Jordi Llorens[1]

P71 - Secretory sorting receptors carboxypeptidase e and secretogranin iii in amyloid β-associated neural degeneration in alzheimer's disease

Virginia Pla[1], Gregory Ghezali[1], Sonia Paco[1], Victor Ciria[1], Isidro Ferrer[2], Fernando Aguado[1]
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P72 - Glycogen, a key player for astrocytes and neurons in the functioning of the brain

Jordi Duran[1,2], Isabel Saez[1,2], Agnès Gruart[3], José María Delgado[3], Joan J. Guinovart[1,2]
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[2] Department of Biochemistry and Molecular Biology, University of Barcelona (Barcelona, Spain).
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P73 - Anàlisi quantitativa de l'expressió d'isoformes de tau 3R i 4R a les malalties d'Alzheimer i dels grànuls argiròfils

Cortés R[1], Reyes-Irisarri E[1], Serrano-Acedo S[1], Ferrer I.[2], Mengod G.[1], Vilaró MT[1]
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P74 - Loss of vestibular function associates with afferent pathology during chronic ototoxicity in the rat
Lara Sedó Cabezon

P75 - The resistance to apoptotic stimuli is a common trait in human glioblastoma multiforme-derived cells
María Sánchez-Osuna, Mercè Garcia-Belinchón, Victoria Iglesias-Guimarais, Elisenda Casanelles, Victor J. Yuste
From the Cell Death, Senescence and Survival group, Departament de Bioquímica i Biologia Molecular & Institut de Neurociències, Facultat de Medicina, Universitat Autònoma de Barcelona, Barcelona, Spain.

P76 - Mitochondrial dysfunction in huntington's disease: role of CDK5
Cherubini M[1,2]., Puigdellívol M[1,2]., Alberch J[1,2], Gines S[1,2]
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P77 - Generation of LyzM-Cre x C/EBPβ$^{fl/fl}$ mice to obtain selective depletion of the transcription factor C/EBPβ in microglia
Pulido-Salgado M[1,2], Straccia M[1,2], Sterneck E[3], Solà C[2], Saura J[1]
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P78 - Study of the role of neurotrophins and their receptors in Autism Spectrum Disorders
Segura M., Martiañez T., Lamarca A., Pàmies M., Autet A., Mestres M., Romnos M., Renner T., Taurines R., Gella A.
Universitat Internacional de Catalunya.

P79 - CREB-regulated transcription coactivator-1 (CRTC1)-dependent gene expression in APP transgenic mice
Arnaldo J. Parra, Meng Chen, Jorge Valero, Elsa Martín, José Rodríguez-Alvarez, Carlos A. Saura
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P80 - Presenilin-1 regulates axonal growth through RhoA in hippocampal neurons
Sergi Marco, Carlos A. Saura
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P81 - New mouse models for vestibular hair cell degeneration
S. Saldaña-Ruíz[1], C. Soler-Marín, G. Hernández-Mir[1], C. Chabbert[2], J. Llorens[1]
[1] D. Ciències Fisiològiques II. Universitat de Barcelona, Hospitalet de Llobregat, Barcelona, Spain.
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P82 - The neurotrophin receptor TrkB as a target for tetanus toxin
Candalija A[1], Rummel A[2], Limón Pérez de León D[3], Scior T[3], Aguilera J[1]
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P83 - Efecte antihiperalgèsic del tramadol en el postoperatori immediat i tardà en el ratolí
E. Romero-Alejo, A. Romero, M.M. Puig
Unitat de Recerca en Dolor, Departament d'Anestesiologia, IMIM-Hospital del Mar. Universitat Autònoma de Barcelona.
Parc de Recerca Biomèdica de Barcelona, Barcelona

P84 - mTOR activity is deregulated in Huntington's disease striatum

Jordi Creus[1,2,3], Laura Rué[1,2,3], Joan Romaní[4], Jordi Alberch[1,2,3] Cristina Malagelada[4], Esther Pérez-Navarro[1,2,3]

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P85 - Histamine H3 receptor agonists decrease cocaine seeking

Santi Rosell-Vilar, Marta Gonzalez-Sepulveda, Silvia Fuentes, Noemi Robles, David Moreno-Delgado, Roser Nadal, Josefa Sabria, David Self[1], Jordi Ortiz

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P86 - Ethanol changes BDNF mRNA expression and BDNF alters dopamine synthesis in rat brain. Is BDNF a mediator of the effects of ethanol on dopaminergic neurons?

Noora Raivio [1, 2], Ettore Tiraboschi [3], Sirkku T. Saarikoski [2], Eero Castrén[3], Kalervo Kiianmaa [2]; Jordi Ortiz[1]; Josefa Sabrià [1]

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[2] National Institute for Health and Welfare, Helsinki, Finland.
[3] University of Helsinki, Neuroscience Center, Helsinki, Finland.

P87 - Calcium channel subunit α2δ1 is regulated by BDNF. A new insight in BDNF synaptogenic function?

Castells Santamaria, A. [1], Ortega, J.A. [1], Alcántara, S.[1]

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P88 - Evidence of dopaminergic and histaminergic regulation of cell viability via D_1-H_3 heteromers in striatal tissue

Moreno-Delgado D, Moreno E, Mayol J, Casadó V, Cortés A, Lluís C, McCornmick PJ

P89 - Is aripiprazole a partial agonist on brain dopamine D_2 receptors?

Guo Fen Ma, Santi Rosell, Josefa Sabriá, Jordi Ortiz

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P90 - The new multi-target directed ligand ASS234 reduces Ab fibrillogenesis and protects neuroblastoma cells from Ab-induced toxicity

Irene Bolea, Alejandro Gella, Abdelouahid Samadi, Jose Luis Marco, Mercedes Unzeta

P91 - Dual action of dopamine on striatal tyrosine hydroxylase activity

Josefa Sabriá, Marta González-Sepúlveda, Santi Rosell-Vilar, Carlos Ruiz-Arenas, Guo Fen Ma, Noora Raivio, Jordi Ortiz, David Moreno-Delgado

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P92- Antioxidant effect of LMN diet enriched in polyphenols and polyunsaturated fatty acids, vía Nrf2 pathway in SH-SY5Y neuroblastome cell line

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P93 - A quantitative metabolomics study of the oxygen availability impact on recombinant *Pichia pastoris* central carbon metabolism

Carnicer M., [1], Baumann K.[1], ten Pierick A.[2], Zeng Z.[2], Seifar R.[2], van Dam J.[2], Heijnen J.[2], Albiol J.[1], Van Gulik W.[2], Ferrer P.[1]
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P94 - PDE4 inhibition in EAE mice: cAMP-PDEs and inflammatory cytokines mRNA expression analyses in spinal cord

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P95 - DNA-binding proteins analysed by SAXS

Pablo Fernández-Millán, Anna Rubio-Cosials, Anna Cuppari, Cristina Silva Espiña, Pau Bernado, Maria Solà
Institut de Biologia Molecular de Barcelona (IBMB-CSIC);*Centre de Biochimie Structurale (CBS).

P96 - Regulation of striatal-enriched protein tyrosine phosphatase (STEP) levels by BDNF *in vivo* and *in vitro*

Shiraz Tyebji[1,2,3], Ana Saavedra[1,2,3], Albert Giralt[1,2,3], Jordi Alberch[1,2,3] and Esther Pérez-Navarro[1,2,3]
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P97 - Characterization of brain-specific PDK1 conditional knock-in mice expressing the L155E mutation in the nervous system

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P98 - Utp promotes schwann cell wound repair through P2Y activation

Lamarca A, Gella A, Martiañez T, Segura M and Casals, N .

P99 - Unraveling the kinetics of aggregation of single peptide-DNA complexes using force spectroscopy

J. Camunas-Soler[1,2], S. Frutos[1,2], C.V. Bizarro[1,2], S. de Lorenzo[1,2], M.A. Fuentes-Perez[3], R. Ramsch[4,2], S.Vilchez[4,2], C. Solans[4,2], F. Moreno-Herrero[3],F. Albericio[5,2], R. Eritja,[4,5,2], E. Giralt[5, 2], S.B. Dev[5], F. Ritort[1,2]
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P100 - Functional characterization of cancer-related genes during planarian regeneration

José Ignacio Rojo-Laguna, Alejandro González-Sastre, Gustavo Rodríguez-Esteban, Emili Saló
Departament de Genètica, Facultat de Biologia and IBUB, Universitat de Barcelona

P101 - Oikopleura dioica as a Model Animal in Evo-Devo for studying Genetic Loss

Josep Martí-Solans, Cristian Cañestro, Ricard Albalat
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P102 - Dynamics of mammalian meiosis in distantly mammalian species

Joana Segura[1], Laia Capilla[1], Fernanda Reis[2], Hugo Fernández[3], Montserrat Garcia-Caldés[1,2], Aurora Ruiz-Herrera[1,2]
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P103 - NEDD4-1 regulation of the pro-apoptotic protein RTP801 in *in vitro* models of parkinson's disease

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Department of Pharmacology, Faculty of Medicine, University of Barcelona, Barcelona, Catalonia (Spain).

P104 - Serotonin transporter knockdown by siRNA induces fast adult neurogenesis and antidepressant like effects

Albert Ferrés-Coy[1,3], Fuencisla Pilar-Cuéllar[2,3], Rebeca Vidal[2,3], Verónica Paz[1,3], Mercè Masana[1,3], Roser Cortés[1,4], Leticia Campa[1,3], Ángel Pazos[2,3], Elsa M Valdizán[2,3], Francesc Artigas[1,3], Analía Bortolozzi[1,3]
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P105 - Lack of Helios causes long-term outcome cognitive deficits and impairs hippocampal plasticity

A. Giralt[1], R. Martín-Ibáñez[1], A. Zamora-Moratalla[2], E. Blasco[3], S. Chan[4], P. Kastner[4], M. Pumarola[3], M.Dierssen[5], E.D. Martín, J. Alberch[1], J.M. Canals[1]
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P106 - Effect of rock inhibiton on human embryonic stem cells when passaging from feeder to feeder-free conditions

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P107 - Effect of Dyrk1a dose-reduction on self-renewal and differentiation potentials of cortical embryonic progenitors

Elisa Balducci[1], María José Barallobre[1], Sonia Najas[1], Agustín Fernández[2], Mario F. Fraga[2], Maria L. Arbonés[1]
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P108 - Dyrk1a overexpression alters the development of specific type of cortical interneurons

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P109 - Human fetal neural stem cells: are there differences depending on the developmental age and region of origin?

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P110 - 3D electrospun Polylactic acid nanofibers induce radial glia like cells and neurons migrating phenotypes

Zaida Alvarez.[1,2], Oscar Castaño[1],Josep Planell[1,3,4], Elisabeth Engel[1,3,4], Soledad Alcántara[2]
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P111 - Molecular changes underlying oocyte resorption derived from water stress in the cockroach *Blattella germanica*

Alba Herraiz, Anibal de Horna, Xavier Belles, Maria-Dolors Piulachs
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P112 - Are plants ready for mountains without snow? Adaptation to snow scarcity in early life stages of *Silene ciliata*

Garcia-Fernandez, A.[1]; Lara-Romero, C.[2] and Iriondo J.M.[2]
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P113 - Whole genome comparison between human and macaque genomes: defining homologous synteny blocks and evolutionary breakpoint regions

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P114 - Accelerated exon evolution in duplicated regions in hominids

Belen Lorente-Galdos[1,2], Jonathan Bleyhl[3], Laura Vives[3], Gregory Cooper[3], Arcadi Navarro[1,4], Evan Eichler[3,5], Tomas Marques-Bonet[1]
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P115 - Evolució dels llocs d'unió del factor de transcripció dFOXO en el gen "*Insuline-like Receptor*" (*InR*) de Drosophila

Orengo, D. J., Aguadé, M., Juan, E.
Departament de Genètica i Institut de Recerca de la Biodiversitat. Universitat de Barcelona, Barcelona. Espanya.

P116 - CpG DNA methylation in *Culex pipiens*: Insights into the role of epigenetics in vector-borne parasite systems

E. Gómez-Díaz[1], M. Jordà[2], A. Rivero[3] and M. A. Peinado[2]
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P117 - Cloning and Expression of Rainbow trout (*Oncorhynchus mykiss*) and Gilthead sea bream (*Sparus aurata*) Interleukin-6 (IL-6)

Vallejos-Vidal, E.[1], Reyes-López, FE.[1], Planas, J.[2], MacKenzie, S.[1]
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P118 – A genome-wide comparative study of DNA methylation in great apes

Irene Hernando[1], Paras Garg[2], Holger Heyn [3], Javier Diez[3], Manel Esteller[3], Andrew Sharp[2], Tomas Marques-Bonet[1,4]
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P119 - microRNA regulation of Krüppel homolog 1 gene by the miR-2 family in the context of German cockroach metamorphosis

Jesús Lozano, Raúl Montañez, Xavier Belles
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P120 - Great ape versus human genetic diversity: the great ape genome diversity project
Javier Prado-Martinez[1], Peter H Sudmant[2], Great Ape Genome Diversity Project[1], Evan E Eichler[2,3], Tomas Marques-Bonet [1,4]
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P121 - Adaptive transposable element insertions in Drosophila: NATs, miRNAs and piRNAs
Lídia Mateo, Raúl Montáñez, Josefa González
Institut de Biologia Evolutiva (CSIC-UPF).

P122 - Fascin in *Drosophila* tracheal system development.
Pilar Okenve Ramos, Marta Llimargas.
Instituto de Biologia Molecular de Barcelona – CSIC.

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P123 - Use of liposomes as immunostimulant encapsulation agents in aquaculture
Àngels Ruyra[1,2], Mary Cano[2], Simon MacKenzie[1], Daniel Maspoch[2], Nerea Roher[1]
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P124 - Global DNA methylation patterns in the European sea bass exposed to different temperature profiles during embryogenesis and early larval development
Dafni Anastasiadi, Noelia Díaz, Francesc Piferrer
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P125 - Ontogeny of the digestive system in the brachyuran crab *Maja brachydactyla* (Malacostracea, Decapoda)
Guillermo Guerao[1], Enric Ribes[2], Mercé Durfort[2], Olga Bellot[1] and Guiomar Rotllant[1]
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P126 - Decreases in condition and fecundity of freshwater fishes in a highly polluted reservoir
Lluís Benejam, Josep Benito & Emili García-Berthou
Institute of Aquatic Ecology, University of Girona, Girona, Catalonia, Spain.

P127 - Fast Real-Time PCR assay for detection of *Tetramicra brevifilum* in cultured turbot
Mercedes Alonso[1], Fátima C. Lago[1], María Gómez-Reino[2], Jacobo Fernández Casal[3], Iris Martín Varela[3], Juan M. Vieites[1], Montserrat Espiñeira[1]
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P128 - Fast Real-Time PCR assay for detection of the enteric parasite *Enteromyxum scophthalmi* in cultured turbot *(Scophthalmus maximus* L.)
Mercedes Alonso[1], Fátima C. Lago[1], María Gómez-Reino[2], Jacobo Fernández Casal[3], Iris Martín Varela[3], Juan M. Vieites[1], Montserrat Espiñeira[1]
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P129 - Stress effect on the interrenal cells function in rainbow trout (*Oncorhynchus Mykiss*)
C. Fierro-Castro, M.C. Santa-Cruz, R. Tridico, L. Martin, M. Teles, L. Tort
Department of Cell Biology, Physiology and Immunology. Universitat Autónoma de Barcelona, Barcelona, Spain.

P130 - Stress and immune response in sea bream (*Sparus Aurata*) after experimental treatment with lps of *a. salmonicida* and *l. anguillarum*

Tridico, R.[1], Boltaña, S.[1,2], Fierro-Castro, C.[1], Teles, M.[2], Cortes, R.[1], MacKenzie S.[2], Tort, L.[1]
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P131 - Cortisol implants induce changes in complement and lysozyme levels in plasma, as well as in the transcription of immune-related genes in rainbow trout

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P132 - ¿Puede la Acuicultura ayudar a recuperar una especie en peligro de extinción? Estudio con *Epinephelus marginatus* (Lowe, 1834)

M. Ureta[1,2], C. Durruty[3,2], R. Seoane[2], C. Hispano[4], A. Mechaly[5,6], N. Díaz[5,6] F.Piferrer[5,6], C. Arnabat[7,6], P. Maíllo[7,6], H. Salvadó[7,6], F. Chauvigné[8,6], J. Cerdà[8,6], A.Velez[1,2],M.A. Bruzón[9], B. Peleteiro[10], P. Bulto[4], F.Castelló[7], P. Bou[6], A. Nebot[6], J. Gutierrez[11,6]
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P133 - Comparison of the lipolytic activity in guts of two sparid species; gilthead sea bream (*sparus aurata*) and red porgy (*pagrus pagrus*)

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P134 - Les Col·leccions Biològiques de Referència de l'Institut de Ciències del Mar: un recurs per a la recerca interdisciplinària de la biodiversitat marina

Alícia Duró, Félix Pérez, Francisco J. Olivas, Pere Abelló, Antoni Lombarte, Roger Villanueva
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P135 - Biometric tools in biodiversity and global change: the case of Juniperus phoenicea L. (Cupressaceae) from Andorra

Małgorzata Mazur[1], Angel Romo[2], Karolina Sobierajska[3], Adam Boratyński[3]
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P136 - Community structure and biodiversity patterns in coralligenous communities over spatial and temporal scales

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P137 - Pautes de biodiversitat al llarg de gradients altitudinals

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P138 - Trichomycetes: an understudied group with scientific potentialities

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P139 - Reflexió ètica sobre el valor i la conservació de la biodiversitat. Eina per al desenvolupament de competències transversals en sostenibilitat a la universitat

S. Albareda, M. Fernández, A. Alferez, J. Puig i S. Vidal
Departament de Didàctica de les Ciències Experimentals. Facultat d'Educació. Universitat Internacional de Catalunya.

P140 - Plant warning signals in a global warming scenario can help to combat climate change

Joaquín Azcón-Bieto i Salvador Nogués
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P141 - Use of high resolution microscopy techniques for determining the effect of Copper in phototrophic microorganisms and its metal sequestration capacity

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P142 - NrdR modulate differentially the expression of *Pseudomonas aeruginosa* ribonucleotide reductase genes

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P143 - Cord formation in *Mycobacterium brumae* and *Mycobacterium fallax*

Cecilia Toledo Brambilla, Alejandro Sánchez-Chardi, Esther Julián Gómez y Marina Luquin Fernández
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P144 - Mecanismes de resistència a rifaximina en soques d'*Escherichia coli* comensals i diarrogèniques de nens de Lima, Perú

C. Gomes[1], M. J. Pons[1], L. Ruiz[1], E. H. Mercado[2], T. J. Ochoa[2], J. Ruiz[1]
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P145 - H-NS as a novel transcriptional modulator for the ribonucleotide reductase genes in *Escherichia coli*

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P146 - Encapsulation of ciprofloxacin within poly-(lactic-co-glycolic acid) (PLGA) nanoparticles enhances efficacy against bacterial pathogens in biofilm

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P147 - Chromosomal and metabolic stasis in *Blattabacterium cuenoti*, the ancient primary endosymbiont of cockroaches

Rafael Patiño-Navarrete[1], Miguel Ponce de León[2], Francisco Montero[2], Andrés Moya[1,3,4] Juli Peretó[1,5], Amparo Latorre[1,3,4]
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P148 - Technological applications of the yeast *Hanseniaspora*

López, S., Madrigal, T., López, C., Lilao, J., Mateo, J.J., Maicas, S.
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P149 - Desenvolupament i anàlisi de mutants de *Escherichia coli* i *Shigella spp.* resistents a furazolidona

S. Martínez-Puchol[1], C. Gomes[1], M.J. Pons[1], T.J. Ochoa[2], J. Ruiz[1]
[1] Centre de Recerca en Salut Internacional de Barcelona (CRESIB), Hospital Clinic, Universitat de Barcelona. Barcelona
[2] Universidad Peruana Cayetano Heredia, Lima, Perú.

P150 - Direct antitumor effect of heat-killed and irradiated *Mycobacterium bovis* BCG in superficial bladder cancer

Silvia Secanella Fandos, Estela Noguera Ortega, Marina Luquin Fernández, Esther Julián Gómez
Universitat Autònoma de Barcelona. Departament de. Genètica i Microbiologia, Facultat de Biociències, Bellaterra, Barcelona, Spain.

P151 - Indirect antitumor activity of irradiated *Mycobacterium bovis* BCG in non-invasive bladder cancer

Silvia Secanella Fandos, Hasier Eraña Lasagabaster, Marina Luquin Fernández, Esther Julián Gómez
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Els Congressos de Metges i Biòlegs de Llengua Catalana: passat, present i futur

L'acte se celebrarà el dilluns 9 de juliol de 2012, a les 18 hores, a l'antic amfiteatre anatòmic,

la Sala Gimbernat, de la Reial Acadèmia de Medicina de Barcelona

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Sessió oberta al públic

Barcelona, juny del 2012

PROGRAMA

Presentació
RICARD GUERRERO
President de la Fundació Alsina i Bofill (FAB)

Els homes dels Congressos i les humanitats en els Congressos
ORIOL CASASSAS
President del 13è Congrés de Metges i Biólegs de Llengua Catalana (CMBLC), Andorra, 1988

Les publicacions dels Congressos
ÀLVAR MARTÍNEZ VIDAL
Universitat de València

El llegat dels Congressos: biomedicina per al s. XXI
RAMON GOMIS
Director de l'Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona

El futur dels Congressos: cap a on poden anar
JACINT CORBELLA
President de la Reial Acadèmia de Medicina de Catalunya (RAMC)

Cloenda
MIQUEL VILARDELL
President del Col·legi Oficial de Metges de Barcelona (COMB)

ORAL COMUNICATIONS

MONDAY 9 TH OF JULY
S 1 - EPIGENETIC MECHANISMS IN HEALTH AND DISEASE

Overcoming the epigenetic barrier during reprogramming to pluripotency

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Chromatin structure plays fundamental roles in the regulation of gene expression during development and contributes to define and preserve cell identity. However, adult somatic cells can be reprogrammed to pluripotency by the overexpression of critical transcription factors. This process entails overcoming the epigenetic barriers that prevent the reactivation of the endogenous pluripotency genes. In order to identity critical players that participate in the maintenance of these barriers, we compiled genome wide expression data from several independent reprogramming experiments and extracted a list of chromatin related candidates that showed differential expression between human induced pluripotent (iPS) cells and somatic cells. Among them, we identified the histone variant macro H2A as highly expressed in somatic cells and downregulated after reprogramming to pluripotency. The knock down of macro H2A in human keratinocytes increased the efficiency of reprogramming to pluripotency partially due to the acceleration of the cell cycle. Moreover, genome-wide occupancy profiles show that macro H2A1 preferentially occupies genes that are not transcribed and are marked with H3K27me3 in somatic cells. These include pluripotency genes and also bivalent genes, for which the presence of macro H2A1 adds an additional layer of regulation that maintains them repressed in somatic cells. Altogether, our results suggest that this histone variant plays essential roles in preserving the identity of somatic cells.

Linking ZRF1 with retinoic acid pathway in the regulation of transcription and differentiation of leukemic cells

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Cellular differentiation is a highly complex process that is controlled by the activation of diverse transcriptional programs. The maintenance of such programs is regulated mainly by transcription factors and epigenetic modifications. We recently identified the protein ZRF1, which specifically binds one of these modifications, the histone H2A-ubiquitin, and displaces the Polycomb repressive complex 1 from chromatin, thus facilitating the transcriptional activation of Polycomb target genes. Using acute myeloid leukemia cells, we show here that ZRF1 regulates retinoic acid (RA) induced differentiation. Depletion of ZRF1 give rise to an increase in the differentiation status in basal conditions but reduces the potential of the cells to fully differentiate upon RA administration. Consistently, its overexpression enhances the differentiation potential upon RA treatment. On the other hand, depletion of ZRF1 notably reduces proliferation and enhances apoptosis in these cells. At the molecular level, we show that ZRF1 binds to the retinoic acid receptor α (RARα), both *in vitro* and *in vivo*, and may modulate its transcriptional activity. A microarray study shows that ZRF1 regulates the expression of a highly significant proportion of the RA-target genes; in basal conditions, depletion of ZRF1 leads to the upregulation of about 30% of RA-targets and, interestingly, the full activation of about 45% of the RA-targets upon RA treatment is not achieved in the absence of ZRF1. Our results suggest that ZRF1 works both as a transcriptional repressor and a transcriptional activator of retinoic acid target genes depending on the context, and it plays an important role in the regulation of RA-induced differentiation of leukemic cells.

MacroH2A1 in myogenic differentiation and muscle regeneration – an interplay of metabolism and epigenetics

Melanija Posavec*, Elvira Carrio, Vanesa Valero, Yaiza Nuñez, Hélene Delage, Philippe Bouvet, Monica Suelves and Marcus Buschbeck*

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The macroH2A histone variants are important epigenetic regulators of embryonic and adult stem cell function (Creppe et al, 2012). In a set of proof-of principle experiments it could further be shown that one of the two splicing variants of macroH2A1 binds NAD+ derived-metabolites *in vitro* and *in vivo* (Kustatscher et al, 2005; Timinszky et al, 2009). The functional relevance of this observation remains elusive. Our preliminary results show that the expression of macroH2A1 in muscle precursor cells switches from the metabolite-binding to the non-binding form during muscle stem cell activation and in the inverse direction during differentiation.

The metabolic requirements of a muscle precursor cell dramatically change during the induction of muscle regeneration. Epigenetic mechanisms further guarantee the unidirectionality of the regeneration process by maintaining and regulating cellular states (Perdiguero et al, 2009). However, the exact link of metabolic and chromatin states is still poorly understood. Therefore we want to test the intriguing possibility that macroH2A1 could be an integration point of metabolic and epigenetic regulation. Our specific objectives include the analysis of the relative levels of macroH2A forms and relevant metabolites in muscle precursor cells during different stages of the myogenic differentiation. Furthermore we plan to study in details the macroH2A1 loss-of-function phenotype in primary muscle precursor cells *in vitro* and during injury-induced skeletal muscle regeneration *in vivo*. We also plan to analyze the genome-wide distribution, target gene regulation and the physiological relevance of metabolite-binding and non-binding isoforms of macroH2A1. Finally we will utilize a state-of-the-art liquid chromatography-coupled mass spectrometry for the analysis of metabolites to test *in vitro* and *in vivo* whether myogenesis can be metabolically modulated in a macroH2A1-dependant manner.

The histone demethylase PHF8 is essential for cytoskeleton dynamics

Elena Asensio Juan

PHF8 is a histone demethylase associated with X-linked mental retardation (XLMR). It has been described as a transcriptional coactivator involved in cell cycle progression, but its physiological role is still poorly understood. Here we show that PHF8 controls the expression of genes involved in cell adhesion and cytoskeleton organization such as RhoA, Rac1 and GSK3β. A lack of PHF8 not only results in a cell cycle delay but also in a disorganized actin cytoskeleton and impaired cell adhesion. Our data demonstrate that PHF8 directly regulates the expression of these genes by demethylating H4K20me1 at promoters. Moreover, c-Myc transcription factor cooperates with PHF8 to regulate the analyzed promoters. Further analysis in neurons show that depletion of PHF8 results in down regulation of cytoskeleton genes and leads to a deficient neurite outgrowth. Overall, our results suggest that the mental retardation phenotype associated with loss of function of PHF8 could be due to abnormal neuronal connections as a result of alterations in cytoskeleton function.

Snail1 regulates heterochromatin transcription

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Pericentromeric heterochromatin is highly variable between species in size and repetitive DNA sequence composition, but conserved in chromatin protein composition and structure from yeast to human. Characterized by the presence of HP1 family proteins, hypoacetylated histones, H3K9me3 and H4K20me3, it is actively transcribed, and the transcripts generated are responsible of its compacted and silenced status. However, the mechanism by which the transcription of these regions is regulated is still under study.

Here we show that Snail1, a transcription factor implicated in epithelial to mesenchymal transition (EMT), is able to bind pericentromeric regions of several human chromosomes and mouse major satellites. We also demonstrate that in the absence of Snail1, major satellite transcription is upregulated and reorganization of chromocenters occurs. During the EMT process, specific chromatin domains are reprogrammed and also the acquisition of new features suggests important chromatin reorganization. We propose that Snail1 regulates major satellite transcription during EMT and this regulation is essential for heterochromatin reorganization. Disruption of this regulatory process leads to genome instability.

Proteomic distribution in the human sperm chromatin

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The spermatogenic cell experiences an extremely marked chromatin transition during the last stage of spermatogenesis. Histones are disassembled and replaced by protamines, which are highly positive charged proteins that form tight toroidal complexes. However, this structural change of the sperm chromatin is not complete and, at the end, 85-95% of human sperm DNA is packaged by protamines (NP), while 5-15% remains associated with histones (NH). It is known that the differential chromatin distribution in the protamine and histone-associated regions is non-random. The NH domain is significantly enriched in important developmental genes, indicating a potential epigenetic role during the formation of the embryo. In a previous study from our laboratory, we have performed a proteomic analysis of the human sperm nucleus. Among the proteins characterized, zinc fingers proteins, transcription factors and several novel histones variants have been described. These results were unexpected in a cell supposed to be transcriptionally inactive. The aim of the present study is to contribute to a more detailed characterization of the human sperm nucleus establishing the distribution of the proteins in the NH and NP domains. Purified sperm nuclei were digested using endonucleases to obtain two sperm chromatin fractions: NH domain (endonuclease-sensitive) and NP domain (endonuclease-resistant). The proteins extracted from each fraction have been analysed by LC-MS/MS. The preliminary results show an enrichment of nuclear proteins (24% of proteins not previously characterized) and a differential protein pattern between the two domains of the sperm chromatin (4 proteins specific for NH domain, including a histone variant). These results open the possibility to further characterize these specific proteins and the DNA associated to study a potential epigenetic function. Supported by the Ministerio de Economía y Competitividad (BFU2009-07718) and University of Barcelona (APIF fellowship).

MONDAY 9 TH OF JULY
S 2 - BIOMEDICAL PROTEOMICS AND TRANSCRIPTOMICS

High-throughput biomedical transcriptomics and proteomics: their relevance in the health system

Rigau M, Garcia M, Pedrola N, Sequeiros T, Devis L, Montes M, Majem B, Gallardo D, Campoy I, Martínez E, Fité L, Vidal I, Altadill T, Abal M, Olivan M, Colás E, Llauradó M, Doll A and Reventós J.

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Major investments in high-throughput techniques, such as transcriptomics and proteomics, have created an opportunity for significant progress in biomedical research and, in particular, in oncology. During the last years, researchers have identified hundreds of genes and proteins that harbor variations contributing to cancer development and also can serve as biomarkers for screening, diagnosis and prognosis. Moreover, the recent improvement of sensitivity and dynamic range for those techniques, has led to the possibility for the analysis of complex biological samples, such as body fluids (blood, urine, etc.), which are considered non-invasive methods and provide an easier methodology for the detection of the diseases.

Our main aim is to improve the early detection of hormo-dependent cancers, including prostate (PC), endometrial (EC) and ovarian cancer (OC). For all those cancers, poor sensitivity, failure rate and invasiveness of the current diagnosis methods limit the success to early detect them and consequently advanced disease is often encountered. We have analyzed urine (for PC), uterine aspirates (for EC) and ascites (for OC) by using high-throughput techniques to pursue our goal.

Our data demonstrated that both, transcriptomic and proteomic approaches are able to reveal novel biomarkers for these cancers. This constitutes an important step towards advancing accurate non-invasive diagnosis and prognosis, which currently represents a setback in our ability to cure patients.

The proteome of isolated human sperm tail suggests new metabolic pathways

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Although a lot have been done in the sperm proteomics field, more detailed descriptions are expected to clarify additional cellular and molecular sperm attributes. The aim of this study was to characterize the subcellular proteome of human sperm tail, and hopefully identify less concentrated proteins (not found in whole cell proteome studies). Specifically, we were interested in characterizing the sperm metabolic proteome and add new insights to the sperm metabolism issue. Sperm were isolated from normozoospermic semen samples and tail fractions were obtained by sonication and sucrose-gradient ultracentrifugation (and their purity was confirmed by various techniques). Liquid chromatography and tandem mass spectrometry of isolated sperm tail peptides resulted in the identification of 1049 proteins, more than half of which had not been previously described in human sperm. The categorization of proteins according to their function revealed two main groups: proteins related with metabolism and energy production (26%), and proteins related with sperm tail structure and motility (11%). Interestingly, a great proportion of the metabolic proteome (24%) were constituted by enzymes involved in lipidic metabolism, including enzymes for the mitochondrial beta-oxidation of saturated and unsaturated fatty acids, and for the utilization of ketone bodies. Unexpectedly, we have also identified various peroxisomal proteins, some of which known to be involved in the beta-oxidation of very long chain fatty acids. Analysis of our data using *Reactome* suggests that both mitochondrial and peroxisomal pathways might indeed be active in sperm, and that the use of fatty acids as fuel may be more preponderant than previously thought. Notably, and contradicting a common concept in the literature, we suggest that the male gamete may have the capacity to obtain energy from endogenous pools, and thus to adapt to putative exogenous fluctuations. Supported by a grant from the “Ministerio de Ciencia e Innovación” (BFU2009-07718) to RO and a postdoctoral fellowship from the “Fundação para a Ciência e a Tecnologia” (SFRH/BPD/63120/2009) to AA.

Urine Proteomic Analysis for the identification of Prostate Cancer biomarkers

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Background: The discovery of biomarkers is a main focus in the early detection of prostate cancer (PCa). All currently known biomarkers are secreted or shed proteins, which make cancer secretome analysis quite promising in biomarker discovery. As the secreted products from prostate epithelial cells can be detected in the urine, their use as a proximal body-fluid to detect PCa is very attractive. Clinical proteomics is one of the recent approaches used to identify biomarkers in biological fluids. The main aim of this work is to provide a protein-non-invasive early diagnosis of PCa.

Methods: We used a combination of proteomic technologies, i.e., 2D-DIGE and MALDI-TOF-MS, in 30 aged matched post-prostate massages (PM) urine specimen to identify differentially expressed proteins for patients with PCa. The resulting identified proteins were verified using selected reaction monitoring (SRM) assays on an independent cohort of 51 PM-urine samples. Two-three proteotypic peptides were targeted per protein candidates. After logistic regression analysis, the best candidate biomarkers were selected. This candidate biomarker list and other interesting secreted proteins from PCa secretome studies were validated using SRM-based absolute quantification assay on a total cohort of 107 PM-urine samples.

Results: A proteomic profile of 24 potential biomarkers for the detection of PCa were identified in PM-urine samples. Biological pathway analysis showed that the majority of these proteins were secreted and related to several well-known functional cancer pathways. After SRM-based relative quantification assay we selected the most promising proteins together with other proteins already described for PCa secretome. At the end, 42 proteins were quantified in a cohort of 107 urine-PM samples by SRM-based absolute quantification assay.

Conclusions: Biomarker discovery in human body-fluids is clearly one of the areas with enormous potential. New proteomics technologies, such as SRM-based assays, are promising for clinical application. This study demonstrate that proteomics analyses are able to reveal novel biomarkers/diagnostic profiles for PCa in urine secretome. This constitutes an important step towards advancing its accurate diagnosis, which currently represents a setback in the ability to cure patients of PCa.

Differential RNAs in the sperm cells of asthenozoospermic patients

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The evaluation of seminal parameters is useful in the diagnosis of male infertility. However these parameters have important limitations since not always good seminal parameters are indicative of a good fertilization potential. Therefore there is a need for additional markers useful in the assessment of the sperm fertility potential. It is well known, that the mature human sperm cell contains RNA. Independently of the functions of the sperm RNA, the differences in RNA amounts between infertile patients and controls provide a means to assess the fidelity of past events of spermatogenesis. Therefore the analysis of the sperm RNA has the potential to be used as a marker to assess the fertility status. It has been demonstrated the presence of an altered amount of a subset of the sperm RNAs in infertile teratozoospermic patients. However, so far there were no published studies in the sperm RNA content in asthenozoospermic patients, as analyzed using a microarray based strategy. Therefore, we started the present project with the goal to characterize the RNA expression in asthenozoospermic patients as compared to controls. To reach this objective we initially selected four normal fertile donors and four asthenozoospermic infertile patients. Equal amounts of RNA were extracted from the sperm samples, subjected to different quality controls and hybridized to the Affymetrix U133 Plus version 2 arrays. Several differential transcripts present in patients as compared to controls were identified. The differential expression of three of the detected transcripts, which are related in spermatogenesis or sperm motility (ANXA2, BRD2 and OAZ3), were validated using real-time PCR in a larger set of samples. These results open up the possibility to investigate the implication of these genes in the pathogenic mechanisms in asthenozoospermia and to consider their potential utility as infertility biomarkers.

Supported by the Ministerio de Economia y Competitividad (BFU2009-07718).

miRNA profile in "elite controllers": a pilot study

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Background: Host genome plays an important role in the control of viremia. The emerging relationship between microRNA (miRNA) and viral control becomes topic of interest in the field of HIV. We hypothesize that part of viremia control in HIV elite controllers (EC) may be carried-out through those miRNA.

Objective: to study the specific profile of miRNAs that can contribute to viremia control in EC.

Subjects and Methods: we have compared 8 HIV EC (VL<200cp/ml without HAART treatment) with 8 chronic HIV infected patients (VP) (VL >5000 cp/ml without treatment), 8 HIV treated patients (ART) (VL<200cp/ml with HAART treatment) and 5 HIV negative individuals (HIV-). miRNAs were extracted from frozen PBMCs using mirVana kit (Ambion). The expression level of 745 human miRNAs was assessed using quantitative PCR assay (TaqMan human micro RNA array, Applied Biosystems). Analysis was performed using SDS software (Applied Biosystems) and StatMiner (Integromics). Ct data was normalized and non parametric Mann-Whitney U test with an estimated false discovery rate (FDR) of 5% was run to compare the difference between EC and VC. A hierarchical clustering of candidate miRNAs was done. Results were validated using specific probes for each miRNA by RT-qPCR.

Results: 45% of miRNA were significantly expressed in all samples of the three groups. A differential expression pattern of two fold was detected at least in 23 candidate miRNAs. The full population was segregated in two groups (group A included EC and HIV-; group B included VP and ART). Two miRNA expression patterns were segregated between those two groups. A set of 4 miRNAs (hsa-miR-221, 27a,27b and 29b) were overexpressed only in group A and 13 miRNA were overexpressed in group B.

Conclusion: PBMC cells exhibit a differential miRNAs expression in EC compared to VP. Expression level of 4 miRNA among EC group were similar to HIV-. Since miR-27a, miR-27b and miR-29 have been implicated in HIV replication control and miR-221 seems involved in the regulation of the IFN-γ pathway, so they may also play a role among HIV control in EC. These results allow us to initiate more focused functional studies on enriched monocyte and T lymphocyte subsets from HIV controllers.

Mitochondrial DNA expression and content in postmortem brain tissue of schizophrenia patients and control subjects

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The aetiology of schizophrenia is known to involve a major genetic contribution that interacts with environmental factors. Most genetic studies have focused on the nuclear genome; however, several lines of evidence support a role of mitochondrial DNA (mtDNA) in this illness. The mtDNA is a maternally inherited 16.6-Kb molecule crucial for energy production that is implicated in numerous human traits and disorders. In schizophrenia, alterations in mitochondrial morphometry, brain energy metabolism, and enzymatic activity in the mitochondrial respiratory chain suggest a mitochondrial dysfunction in schizophrenia that could be related to the genetic characteristics of mtDNA. The objective of this study was to determine whether mitochondrial DNA (mtDNA) expression or content were implicated in schizophrenia (SCH). MtDNA gene expression and mtDNA content (including the MT-ND4 deletion) were measured by RT-qPCR and qPCR, respectively. Post-mortem brain tissue from 15 SCH subjects and 15 normal controls (C), donated by the Neuropathology Consortium of the Stanley Brain Collection (Stanley Medical Research Institute, MD, USA), was analyzed. We did not find a significant difference in the mtDNA content or mtDNA common deletion or mtDNA expression between controls and patients. However, SCH patients showed a different pattern of mtDNA expression compared to C subjects. Similarly, a larger number of SCH patients tended to have the MT-ND4 gene deleted compared with C subjects. Notably, high variability was observed in the mtDNA gene expression and content in each group.

Previous studies and the present work provide evidence for a role of mtDNA in SCH. However, further studies conducted with larger patient and control groups are needed to elucidate the role of mtDNA in major psychiatric disorders.

Molecular diagnosis of glioblastoma based on discriminant equations: objective recognition of primary and secondary cases

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5 Departament de Radiologia, Centre Bellvitge, Institut de Diagnòstic per la Imatge, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat.

6 Servei d'Anatomia Patològica, Institut de Neuropatologia, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat.

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Background: In the preceding decade, various studies on glioblastoma (Gb) demonstrated that signatures obtained from gene expression microarrays correlate better with survival than with histopathological classification. However, there is not a universal consensus formula to predict patient survival.

Methods: We developed a gene signature using the expression profile of 47 Gbs through an unsupervised procedure and two groups were obtained. Subsequent to a training procedure through leave-one-out cross-validation, we fitted a discriminant (linear discriminant analysis (LDA)) equation using the four most discriminant probesets. This was repeated for two other published signatures and the performance of LDA equations was evaluated on two independent test sets, which contained status of IDH1 mutation, *EGFR* amplification, MGMT methylation and gene *VEGF* expression, among other clinical and molecular information.

Results: The unsupervised local signature was composed of 69 probesets and clearly defined two Gb groups, which would agree with primary and secondary Gbs. This hypothesis was confirmed by predicting cases from one independent data set using the equations developed by us. The high survival group predicted by equations based on our local and one of the published signatures contained a significantly higher percentage of cases displaying *IDH1* mutation and non-amplification of *EGFR*. In contrast, only the equation based on the published signature showed in the poor survival group a significant high percentage of cases displaying a hypothesised methylation of *MGMT* gene promoter, overexpression of gene *VEGF* and low percentage of proneural cases.

Conclusion: We have produced a robust equation to confidently discriminate Gb subtypes based in the normalised expression level of only four genes. Considering the features of each group, we suggest that this equation distinguishes in an objective way the classical primary (low survival group) and secondary (high survival group) Gbs. Moreover, the validation of gene expression values using RT-PCR could lead to a future implementation of this equation in routine clinical practice as a diagnosis tool.

MONDAY 9 TH OF JULY
S 3 - GAMETES, STEM CELLS AND DIFFERENTATION

Reprogramming the potency of somatic cells: How and what for?

Ángel Raya

ICREA Research Professor at the Institute for Bioengineering of Catalonia (IBEC)

The derivation of embryonic stem cells (ESCs) from early-stage embryos created an unprecedented interest among scientists and the general public. These cells have the abilities to self-renew indefinitely in vitro and to differentiate into any cell type of the organism. However, both practical and ethical concerns hampered the research and application of human ESCs, which stimulated researchers worldwide to find new ways to produce ESC-like cells in vitro from somatic cells. Takahashi and Yamanaka were the first to generate induced pluripotent stem cells (iPSCs) by nuclear reprogramming of somatic cells with defined combinations of transcription factors. Ever since, a large number of laboratories worldwide have validated the technique, and in fact iPSCs can now be produced routinely from multiple species (including humans) and using multiple methods. The implications of reprogramming in general and of human iPSCs in particular are vast. On the one hand, the technique can be used to understand epigenetic modifications that underlie the acquisition or maintenance of developmental (pluri)potency of cells. On the other hand, human iPSCs are providing outstanding experimental platforms to model human disease, as well as to carry out drug/toxicity screening in relevant target cell types of human origin. Human iPSC also hold promise for future cell-based therapies once the technology progresses further and current safety concerns are overcome.

Chromosome size and morphology determine bivalent positioning in human spermatocytes

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The chromosomal arrangement in the interphase nuclei is determined by chromosomal intrinsic factors such us chromosome size or gene density. However, how the chromosomes are organized during meiotic metaphase I is not well established. The aim of this work was to determine if there is a preferential bivalent distribution pattern in human spermatocytes and to evaluate how chromosome size, gene density, acrocentric chromosomes and heterochromatic blocks influenced in this positioning. We also assessed whether the presence of unpaired XY at metaphase I or the feature of an abnormal meiosis in the patient interfered in the bivalent positioning.

This study was undertaken on 30 testicular biopsies from men consulting for infertility. A total of 253 metaphases I were analysed by contrasting the images obtained after Leishman staining with those captured after Multiplex-FISH protocol in order to identify all chromosomes. For each metaphase and each bivalent a proximity analysis was performed, considering as nearby bivalents those that were part of the “first ring” around the bivalent studied. Statistical analysis was performed using the SCHIP programme.

Results demonstrated that some bivalents have a preferential relative positioning. Significant associations among bivalents related to chromosome size and acrocentric morphology were observed. Bivalent distribution changed by the presence of unpaired X and Y, and remained independent of an abnormal meiosis diagnostic result in the patient. Finally, our study supports that some features defining chromosome territories are maintained along meiosis.

Key words: bivalent, chromosome territories, metaphase I, SCHIP, spermatocytes

Aneuploid and diploid spermatozoa from reciprocal translocation carriers exhibit an altered segregation pattern

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In reciprocal translocation carriers, the segregation of the reorganized chromosomes and the interchromosomal effect (ICE) phenomenon produce a significant amount of unbalanced gametes. Sperm fluorescent *in situ* hybridization is a useful tool to estimate the frequency of chromosome anomalies. Usually, segregation and ICE analyses are performed independently, thus the anomalies produced from both events cannot be directly related. The aim of this work was to find out if there is a relationship between the production of numerical abnormalities and the occurrence of specific segregation modes in spermatozoa from reciprocal translocation carriers.

Semen samples of eight reciprocal translocations carriers were analyzed by a sequential fluorescent *in situ* hybridization protocol based on two successive hybridization rounds. Spermatozoa with numerical chromosome abnormalities for chromosomes 13, 18, 21, X and Y were detected in the first round. The spatial position of each one of them was recorded using their specific coordinates. A second hybridization round was performed to analyze the segregation content in the same nuclei, as well as in a non-selected population of spermatozoa using a specific combination of probes for each reorganization.

In the aneuploid and diploid sperm population, a significant increase of unbalanced segregation modes was detected when compared to non-selected spermatozoa (87% vs. 56.2%) whereas the alternate balanced mode was drastically reduced (13% vs. 43.8%). This deviation might be related to an increased activation of the spindle assembly checkpoint at metaphase I. The intrinsic failure rate of this mechanism would allow the progression of spermatocytes with an abnormal chromosomal content. These cells would accumulate anomalies originated both, from unbalanced segregation modes and the occurrence of ICE.

Keywords: Chromosome numerical abnormalities, FISH, ICE, reciprocal translocation, segregation pattern, spermatozoa.

Treatment of mouse somatic cell nuclear transfer embryos with psammaplin a improves *in vitro* development and quality.

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The low efficiency of the somatic cell nuclear transfer (SCNT) technique is in part due to an incomplete or incorrect reprogramming of the differentiated somatic nucleus to the totipotent embryonic state. Treatment of SCNT embryos with chromatin-modifying agents such as valproic acid (VPA), a histone deacetylase inhibitor (HDACi), has been proved to increase embryonic development and cloning efficiency. Psammaplin A (PsA) is a natural and potent DNA methyltransferase inhibitor and HDACi that has never been used in nuclear reprogramming studies. The purpose of our study was to determine the effect of PsA on the *in vitro* development and quality of mouse SCNT embryos and to compare it with that of VPA.

Mechanically enucleated oocytes from B6CBAF1 (C57BL/6JxCBA/J) female mice were reconstructed with a cumulus cell nucleus and parthenogenetically activated. After culture in KSOM medium (37ºC, 5% CO_2), embryos that reached the blastocyst stage were differentially stained for counting of inner cell mass (ICM) and trophectoderm cells at 96 h post-activation. In a first set of experiments, embryos were exposed to different concentrations of VPA (2 and 4 mM) or PsA (5, 10 and 20 µM) during 1-2 h after reconstruction and 6 h of activation (total 8-9 h). We found that only VPA 2 mM and PsA 10 µM significantly increased blastocyst rates (31 and 37.3 *vs.* 23.3 for the control group), although no differences were found in blastocyst quality (10.4-13.6 ICM cells). In a second set of experiments, we studied the effect of treatment duration by incubating the embryos in VPA 2 mM or PsA 10 µM during 8-9 h, 16 h or 24 h after reconstruction. With VPA, treatments for 8-9 h and 16 h were equivalent in terms of blastocyst rates (34.0 and 32.5%) and higher than the control group (20%), but only VPA 16 h yielded blastocysts with a higher number of ICM cells (15.6 *vs*. 10 for the control group). With PsA, all treatments showed equivalent blastocyst rates (35.2-43.3%), which were higher than in the control group, but only treatments for 16 h and 24 h yielded blastocysts with higher numbers of ICM cells (16.3 and 18.5).

To sum up, PsA not only enhances *in vitro* development and quality of mouse SCNT embryos, but it is also more powerful than VPA.

Studies are currently being performed to determine whether this improvement in blastocyst rates by PsA treatment correlates with an increased development to term. So far, two live cloned pups have been obtained from PsA-treated embryos.

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Complete meiosis from human induced pluripotent stem cells

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Gamete failure derived infertility affects millions of people worldwide and for many patients, gamete donation by unrelated donors is the only available treatment. Embryonic stem (ES) cells can differentiate *in vitro* to germ-like cells, but they are genetically unrelated to the patient. Using an *in vitro* protocol that aims at recapitulating development, we have achieved, for the first time, complete differentiation of human induced pluripotent stem (iPS) cells to post meiotic cells. Unlike previous reports using embryonic stem (ES) cells, post-meiotic cells arose without the over-expression of germline related transcription factors. Moreover, we consistently obtained haploid cells from human iPS cells of different origin (keratinocytes and cord blood), produced with a different number of transcription factors, and of both genetic sexes, suggesting the independence of our approach from the epigenetic memory of the reprogrammed somatic cell. Our work brings us closer to the production of personalized human gametes *in vitro*.

The influence of E-cadherin on Embryonic Stem Cell derivation from mammalian isolated blastomeres

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Efforts to optimize embryonic stem cells (ESC) derivation from isolated blastomeres have been done both to minimize ethical concerns about human embryo destruction and to increase global derivation efficiency. Previous studies in our laboratory indicated a poor derivation efficiency of mouse ESC lines from isolated blastomeres at the 8-cell stage (1/8 blastomeres) due, in part, to a low division rate of the single blastomeres in comparison to their counterparts with a higher number of blastomeres (2/8, 3/8 and 4/8 blastomeres). Communication and adhesion between blastomeres from which the derivation process begins could be important aspects to efficiently derive ESC lines. In the present study, an approach consisting in the adhesion of a chimeric E-cadherin (E-cad-Fc) to the blastomere surface was devised to recreate the signalling produced by native E-cadherin between neighbouring blastomeres inside the embryo. These experiments have been done both in mouse and human embryos. Referring to the mouse embryos, the division rate of 1/8 blastomeres increased from 44.6% to 88.8% and a short exposure of 24 h to the E-cad-Fc produced an ESC derivation efficiency of 33.6% with regards to the 2.2% obtained from the control group without E-cad-Fc. By contrast, in human embryos, the same exposition to E-cad-Fc induced a slight increase in the division rate, from 21.4% to 28.9% in control and exposed groups, respectively, whereas derivation efficiency increased from 0% to 2,2%, although these differences were not statistically significant. Thus, we establish an important role of E-cadherin-mediated adherens junctions both in promoting the division of single 1/8 blastomeres and in the beginning of the ESC derivation process in mouse. However, this effect was not observed in human embryos confirming once again the intrinsic differences in the ESC derivation process between both species.

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MONDAY 9 TH OF JULY
S 4 - COMPUTATIONAL AND STRUCTURAL BIOLOGY

"In Silico biology".

Modesto Orozco

IRB, UB

Biology is moving in a deluge of data that is expected to be more and more dramatic in the near future, as experimental techniques are increasing continuously their throughput. In this scenario, Computational Biology emerges as the only possibility to transform data into information. Particularly impressive is the amount of information being collected regarding chromatin structure and gene regulation mechanisms. However, despite all the information available, complete rationalization on the general mechanisms connecting chromatin structure with gene regulation. During my talk I will show how, physical models, which deep roots in the basic formalisms of quantum chemistry can help us to establish links between chromatin structure, epigenetic signals and gene regulation mechanisms.

The role of structural disorder in the rewiring of protein interactions through evolution

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Structurally disordered regions play a key role in protein-protein interaction networks and the evolution of highly connected proteins, enabling the molecular mechanisms for multiple binding. However, the role of protein disorder in the evolution of interaction networks has been only investigated through the analysis of individual proteins, being impossible to distinguish its specific impact in the (re) shaping of their interaction environments. Now, the availability of large interactomes for several model organisms permits to explore the role of disorder in protein interaction networks not only at the level of the interacting proteins, but of the interactions themselves.

By comparing the interactomes of human, fly and yeast, we discovered that, despite being much more abundant, disordered interactions are significantly less conserved than their ordered counterparts. Furthermore, our analyses provide evidence that this happens not only because disordered proteins are less conserved, but also because they display a higher capacity to rewire their interaction neighborhood through evolution.

Overall, our results support the hypothesis that conservation of disorder gives a clear evolutionary advantage, facilitating the change of interaction partners during evolution. Moreover, this mechanism is not exclusive of a few anecdotal cases but a global feature present in the interactome networks of entire organisms.

IntOGen: Large scale analysis and integration of cancer genomics data

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With the accummulation of large amount of cancer genomics data, an important issue that arises is how to analyze, integrate and access this data in a meaningful way, aimed, among other things at identifying the entire catalog of cancer genes. We have previously developed IntOGen, a discovery tool for cancer researchers (Gundem et al., Nature Methods 2010). IntOGen provides the results of the analysis of more than 300 microarray experiments, including expression and DNA copy number arrays, from about 40 cancer types using a statistical framework specifically designed to identify genes and groups of genes significantly altered in a cohort of tumors. A unique feature of IntOGen is the structured clinical annotation of each tumor sample (using International Classification of Disease Oncology terms), which allows the combination of results from independent experiments that analyze the same tumor type.

With the advent of next generation sequencing, large numbers of tumor genomes are being sequenced, leading to an expansion of the catalogs of somatic alterations in cancer. One of the key challenges posed by this growth is the identification of cancer driver genes and pathways. We have developed new methodologies and a complete pipeline to assess the functional impact of somatic variants and identify driver genes and pathways. We have applied this pipeline to more than a dozen cancer somatic mutations datasets. We found that a large number of tumor mutations are predicted to be deleterious, and that very different pathways to tumorigenesis prevail in each cancer type. All these results can be accessed through IntOGen web interface (www.intogen.org), through its biomart portal (biomart.intogen.org) and through Gitools (www.gitools.org).

Alternative splicing and stochastic gene expression

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Alternative splicing is an important regulator of gene expression. As any molecular-level process, alternative splicing displays cell-to-cell variations, which result from the small number of macromolecules present in cells. We know, from recent experimental and computational studies, that cell-to-cell variability in gene products can play an important role in biological processes such as development or stress response. However, we still lack a quantitative view of the contribution of alternative splicing to this variability and of the corresponding functional impact. Addressing this problem from an experimental point of view is quite complex, but computational methods provide reasonable approaches to its solution, and can be used as hypothesis generators.

Our results show that we can integrate alternative splicing within the framework of stochastic gene expression with relative simplicity. Using this model we can provide a coarse-grained, but clear description of the main features of cell-to-cell variations in protein isoform amounts and their ratios. Our first results show some interesting features: for example, even when alternative splicing reaches levels over 20%, there is always a non-negligible amount of cells with normal levels of the main isoform. We also find that alternative splicing variability critically depends on isoform properties, such as mRNA and protein degradation rates, etc. I will discuss how our results may help clarifying the role of alternative splicing in tissue differentiation and the evoluationary mechanisms underlying the appearance of alternative splicing.

Mapping the Dark Side of the Human Genome: Long Non-Coding RNAs

Rory Johnson, Thomas Derrien, Carme Arnan, Cinzia de Bendictis, Chiara Medoro, Andrea Tanzer, Giovanni Bussotti, Hagen Tilgner, Cedric Notredame, Roderic Guigo.

One of the biggest surprises since the publication of the human genome, has been the discovery that it harbours thousands of long non-coding RNAs (lncRNAs). Of the >10,000 lncRNA loci, only ~100 have been investigated with even the simplest experimental techniques. From this tiny sample set, we tentatively infer that many lncRNAs function as gene regulators, although the mechanisms remain controversal and are probably diverse. There are now two main challenges: (1) defining high quality annotations of lncRNAs, which is a necessary prerequisite for (2) systematic screens for functional lncRNAs in human biological and disease processes. I will present our work within the ENCODE consortium to analyse the biggest, highest quality lncRNA collection to date: the GENCODE version 7 catalogue, produced by the HAVANA team using manual annotation. Using various bioinformatic and deep sequencing analyses, we have comprehensively defined these genes' structures, expression in the human body and brain, evolution, and both genomic and coexpression relationship to protein-coding genes. In particular we show that lncRNAs have strikingly different subcellular localisation within the compartments of the cell. Our focus is now turning to the in vivo role of these molecules. We are developing various custom tools and reagents based on GENCODE 7 annotation, which forms the starting point for experimental programmes to discover novel functional lncRNAs that are ongoing in our laboratory. The annotation can not only be used for new experiments, but also employed to reinterpret many existing datasets to discover candidate disease-associated lncRNAs.

Somatic structural mosaicism as an early genetic marker of late-onset diseases

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Structural variations are one of the main sources of inter-individual genetic differences in the huma genome. However, the impact of *de novo* structural variants in somatic cells has not been fully explored given the difficulty to analyze single or small groups of cells by using high-thorughput technologies. Recent studies have addressed the analysis of somatic mosaicm (the coexistence of cells of distinct genetic composition within and organism) by inferring mosaic paterns from DNA obtained from a pool of cells. In this talk, we will present an algorithm and a bioinformatic tool to detect mosaic alterations using existing data belonging to SNP array data. By analyzing SNP array data from over 50,000 subjects recruited for GWAS studies in cancer, we may conclude that many of detected mosaic anomalies are associated with hematological cancers and that its frequency rises in the elderly (Jacobs, et al., Nat Genet, 2012). Our studies in a Estonian general population-based cohort, including more than 8,000 individuals, also demonstrate that these alterations can be used as an early marker of some late-onset diseases, including cancer.

Accurate prediction of inversions in the human genome from paired-end mapping data with the GRIAL algorithm

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During the last years there has been a great interest in the characterization of genomic structural variation, and paired-end mapping (PEM) is the most widely used method to detect different types of these variants. However, compared to insertions and deletions, inversion prediction presents unique challenges. GRIAL is a new algorithm developed specifically to detect and map accurately inversions. It is based on geometrical rules derived from expected inversion PEM patterns to cluster individual mappings belonging to each breakpoint locus, merging clusters into inversions, and refine breakpoint location. Using available fosmid PEM data from 9 different individuals, we have been able to predict 636 inversions in the human genome and have compared the performance of other currently used methods with that of GRIAL, which shows higher sensitivity and precision in breakpoint location. In addition, by creating different quality scores to asses the reliability of the predictions according to the ratio of discordant and concordant mappings and the support for the breakpoints, we have been able to identify misleading PEM patterns and their causes, and to determine that a big fraction of the predicted inversions are false positives. Therefore, GRIAL improves significantly the inversion prediction and the value of the biological information obtained.

MONDAY 9 TH OF JULY
S 5 - REGULATION OF CHROMATIN FUNCTIONS

Dealing with chromatin constrains in gene regulation

Miguel Beato, Guillermo Vicent, Cecilia Ballaré, Roni Wright, Francois LeDily, Roser Zaurin, Diana Reyes and Jofre Font

CRG & UPF, Barcelona

How eukaryotic transcription factors negotiate access to genetic information stored in chromatin and how chromatin structure is used for orchestrating the transcriptional response are central questions, which relate to the specific ways in which different genomic regions are epigenetically organized during cell differentiation. We have explored these questions at different levels of chromatin structure using as a model the response of breast cancer cells to the steroid hormones estrogens and progestins.

At the first level of DNA compaction in chromatin, the nucleosome, we have found that core histones and linker histones contribute to setting up the appropriate platform for hormone receptor docking and recruitment of chromatin remodeling enzymes. Binding of hormone receptors to their genomic target sites is favored by their organization in nucleosomes, which also interact with the receptor-associated chromatin modifying enzymes. These include multiple protein kinases - activated by crosstalk with membrane-anchored hormone receptors -, histone modifying enzymes, poly(ADP)-ribose polymerases and glycohydrolases, and ATP-dependent chromatin remodeling complexes. Within one minute of hormone addition the receptor-associated kinase MSK1 catalyzes the phosphorylation of H3 at S10 and the removal of a repressive complex containing HP1γ, COREST, LSD1, HDACs and the non-coding RNA SRA. The repressive complex is targeted to hormone inducible genes by non-liganded hormone receptor and stabilized by an interaction of HP1γ with H3K9me3. Upon removal of the repressive complex, a combination of at least 6 receptor-associated enzymes (CDK2, PARP1, PARG, NURF, MLL2/3, PLU1) catalyze the trimethylation oh H3 at K4 - which anchors the NURF complex -, and the phosphorylation and parylation of linker histone H1 leading to its displacement. In a subsequent cycle another combination of receptor-associated enzymes including BAF and PCAF, catalyze acetylation of H3 at K14 and the displacement o dimers of histone H2A and H2B. This leaves the nucleosomal DNA accessible for binding of additional transcription factors, co-regulators and eventually the basal transcriptional machinery. Steroid hormones also repress numerous target genes using the same repressive complex that silences inducible genes prior to hormone addition.

To study further levels of DNA compaction in chromatin we use 3C-derived methods and high-resolution microscopy to study how the chromatin fiber is folded in topological domains and chromosomal compartments genome wide. The 3D organization of chromatin could determine the space relationship of similarly regulated target genes and enable their coordinated expression. Our final goal is to use this information for a better classification and management of breast cancers.

Epigenetic regulation of centromere function

Olga Moreno

IBMB

Centromere identity and propagation are regulated epigenetically. Active centromeres contain a centromere-specific histone H3-variant (CenH3/CENP-A) that, being essential for centromere function and cell viability, is required for kinetochore assembly. The last years has seen an explosion of information concerning our understanding of the regulatory mechanisms responsible for centromere and kinetochore assembly and function, which are critical for genome stability. Several crucial aspects are, however, not well understood. On one hand, though CenH3/CENP-A is exclusively found at centromeres, the precise molecular mechanisms accounting for its specific deposition at centromeres are not yet fully understood. In this context, we have unveiled the essential contribution of proteolysis to centromeric localisation of CenH3/CENP-A^{CID} in *Drosophila*. To define the pathway(s) of kinetochore assembly is another main task in the field. Kinetochores are large macromolecular entities that, depending on the organisms, are composed by dozens to more than a hundred different protein components. Kinetochore architecture is well understood only in *S. cerevisae*, where formation of a relatively simple kinetochore requires co-operation of six different protein complexes resulting in more than 500 protein molecules participating in binding of a single microtubule. In the rest of eukaryotes, kinetochore composition remains poorly understood. We are addressing this question in *Drosophila*. In this particular, we address characterisation of the molecular/structural determinants of the contribution(s) of CenH3/CENP-A^{CID} to kinetochore assembly, as well as identification of new centromere/kinetochore components. In this presentation, I will address our recent work on these topics.

Running title: The MyoD-BAF60c complex poises chromatin for rapid transcription.

Sonia-Vanina Forcales

Institute of Predictive and Personalized Medicine of Cancer

Chromatin remodeling by the SWI/SNF complex is required to activate the transcription of myogenic-specific genes. Our work addressed the details of how SWI/SNF is recruited to myogenic regulatory regions in response to differentiation signals. Surprisingly, the muscle determination factor MyoD and the SWI/SNF subunit BAF60c form a complex on the regulatory elements of MyoD-targeted genes in myogenic precursor cells. This Brg1-devoid MyoD-BAF60c complex flags the chromatin of myogenic-differentiation genes before transcription is activated. On differentiation, BAF60c phosphorylation on a conserved threonine by p38 alpha kinase promotes the incorporation of MyoD-BAF60c into a Brg1-based SWI/SNF complex, which remodels the chromatin and activates transcription of MyoD-target genes. Downregulation of BAF60c expression prevents MyoD access to the chromatin and the proper loading of an active myogenic transcriptosome preventing the expression of hundreds of myogenic genes. Our data support an unprecedented two-step model by which 1) pre-assembled MyoD-BAF60c complex poises the chromatin of myogenic genes for rapid transcription; 2) chromatin-bound BAF60c “senses” the myogenic differentiation cues and recruits an active SWI/SNF complex to remodel the chromatin allowing transcriptional activation.

SIRT2 regulates genomic stability and cell cycle progression through the control of H4K16Ac and H4K20me1 levels

Paloma Martínez

Evidence suggests that the members of the sirtuin family coordinate multiple biological processes geared to chromatin. Sirtuins are evolutionarily conserved NAD-dependent deacetylases and ADP-ribosyltransferases of a myriad of substrates, including histone and non-histone proteins, involved in the regulation of cell cycle, apoptosis, DNA damage repair, life-span and genomic silencing. Among them, acetylation of lysine 16 in the N-terminal tail of histone H4 (H4K16Ac) stands out for its unique role in chromatin structure, expression and epigenetic phenomena through evolution.

Of all the seven mammalian sirtuins (1-7), SirT1 and SirT2 have been related to H4K16Ac deacetylation. However, the implication of SirT2 in this process is quite intriguing due to its general citoplasmic localization. Interestingly, SIRT2 shuttles to the nucleus in G2/M transition when H4K16Ac levels drop dramatically. Indeed, loss of SIRT2 correlates with hyperacetylation of H4K16Ac during mitosis but doesn't seem to alter mitosis progression, and leads to a more extensive G1-phase and a shorter S-phase. Therefore, the implications of SirT2 in mitosis and cell cycle progression are still not well understood.

Our work shows how the *in vivo* loss of SirT2 in mice causes increased H4K16Ac levels, particularly during mitosis, involving important defects in genome instability, heterochromatin structure and DNA replication. Interestingly, our studies suggest that SIRT2 deacetylation of H4K16Ac regulates another epigenetic mark also influenced by the cell cycle, H4K20me1. This important histone modification is implicated in cell cycle progression, DNA replication, DNA repair and chromatin organization. In agreement with these findings, these mice not only show chromosomal aberrations, including pericentromeric and proximal telomere defects, but also are more prone to develop tumors. Supporting the role of SIRT2 in regulation of H4K20me1 deposition, its loss correlates with a significant decrease in H4K20me2 and H4K20me3 levels, that associates with several defects already related with H4K20me2-3 deficiency. Overall, our results support a key role for SirT2 in the control of genomic integrity maintenance along cell cycle progression.

Human histone H1 variants: knock-down and occupancy in the genome

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Seven linker histone H1 variants exist in human somatic cells (H1.0, H1.1 to H1.5, and H1X), with distinct prevalence depending on the cell type analyzed and along differentiation, that bind to linker DNA contributing to higher order chromatin compaction. In addition, H1 seems to be actively involved in the regulation of gene expression. It is not well known whether the different variants have specific roles or regulate specific promoters. We have explored this by inducible shRNA-mediated knock-down of each of the H1 variants in a human breast cancer cell line. Rapid inhibition of each H1 variant was not compensated by changes of expression of other variants. Thus, specific phenotypes are observed in breast cancer cells depleted of individual histone H1 variants. On another hand, by taking advantage of specific antibodies for H1 variants and HA-tagged recombinant H1 variants-expressing cell lines, we have investigated the distribution in particular promoters and genome-wide of the different H1 variants.

Contribution of hydrophobic interactions to the folding and fibrillation of the C-terminal domain of histone H1

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H1 linker histones are involved in chromatin structure and gene regulation. In addition, histone H1 performs functions outside cell nuclei, which may depend on its properties as a lipid-binding protein. The carboxy-terminal domain (CTD) of histone H1 is very basic with ~40% Lys residues. The CTD has little structure in diluted solution, but becomes cooperatively folded upon interaction with DNA. The DNA-bound CTD contains α-helix, β-structure, turns and flexible regions. We studied the folding of the CTD in the presence of neutral detergents Brij 35 and Triton X-100. Neutral detergents were able to induce the folding of the CTD with proportions of secondary structure similar to those observed in the DNA complexes. These results thus identify a folding pathway for the CTD based on hydrophobic interactions, and independent of charge compensation. The CTD is phosphorylated by cyclin-dependent kinases (CDKs) in a cell-cycle dependent manner. The general effect of phosphorylation in the presence of detergents was a decrease in the α-helix and an increase in the β-structure. The greatest effect was found with the fully phosphorylated CTD (3 phosphate groups) in the presence of SDS at a detergent/CTD molar ratio of 7:1; in these conditions, the CTD became an all-β protein with 83% β-structure and no α-helix. The CTD in all-β conformation readily formed ribbon-like fibres. Fibres were of the amyloid type, as judged by strong birefringence in the presence of Congo red and thioflavin fluorescence enhancement. Amyloid fibre formation was only observed in SDS, suggesting that it requires the joint effects of charge neutralisation and hydrophobic interactions, together with the all-β potential provided by full phosphorylation.

MONDAY 9 TH OF JULY
S 6 – GENES AND GENOMES

The Mutational Landscape of Chronic Lymphocytc Leukemia

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults in western countries with a marked clinical heterogeneity that has been related in part to two major molecular subtypes defined by the extent to which somatic hypermutations occur in the variable regions of the immunoglobulin genes. Genetic studies have identified a number of chromosomal aberrations that are also related to behavior of the disease. However, the molecular alterations and mutated genes responsible for its clinical and biological heterogeneity are still poorly understood. Recent studies have sequenced the whole genome and exome of more than 200 patients providing the first comprehensive view of somatic mutations in CLL. Subsequent functional and clinical studies in expanded series of patients have demonstrated the oncogenic potential and clinical implications of a number of these mutations.

The global number of somatic mutations is lower than those described in solid tumors but in agreement with previous estimates of less than one mutation per megabase in acute myeloid leukemias. The number and pattern of somatic mutations differ in tumors with unmutated and mutated *IGHV* extending at the genomic level the clinical differences observed in these two CLL subtypes. One of the striking views of these studies has been the marked genetic heterogeneity of the disease with a relative large number of genes recurrently mutated at low frequency and only few genes mutated in up to 10-15% of the patients. Although some mutations are distributed equally among the *IGHV*-mutated and unmutated CLL, other genes appear preferentially mutated in one of the two subtypes. The mutated genes tend to cluster in different pathways. *NOTCH1* is mutated in around 10% of the cases. The mutations stabilized the protein, activate the pathway and confer a more aggressive behavior to the disease and higher risk of transformation to diffuse large B-cell lymphoma. *SF3B1*, an element of the spliceosome complex, is mutated in around 10% of the CLL. The mutations influence the splicing pattern of selected genes and also confer a worse outcome to the patients. Other genes of the RNA splicing and processing machinery are also mutated in a higher number of patients indicating that this pathway may have a relevant role in the pathogenesis of the disease. *MYD88* mutations identify a subgroup of young patients with mutated *IGHV* and enhance the TLR response of the tumor cells with increasing production of different chemokines involved in the pathogenesis of the disease. These results highlight the molecular heterogeneity of CLL and may provide new biomarkers and potential therapeutic targets for the diagnosis and management of the disease.

When nature decides: one gene, two DNA repair pathways and three human diseases

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Fanconi anemia (FA) is a rare chromosome fragility syndrome that uncovered a novel repair mechanism against stalled replication forks. Fifteen FA genes have been identified but the genetic basis in some FA patients still remains unresolved. Whole exome sequencing was used to identify two unclassified FA patients with biallelic mutations in *XPF*, a nuclease previously connected to xeroderma pigmentosum and segmental progeria. Further genetic, biochemical and functional analysis suggest that the newly identified *XPF* mutations specifically disrupt the function of XPF in interstrand-cross link repair without severely compromising nucleotide excision repair. Our data thus show that depending on the type of *XPF* mutation patients present with three clinically distinct genome instability disorders. A proper genetic characterization of genetic diseases allows not only a better diagnosis and genetic counseling but also will foster the application of novel therapeutic strategies including gene therapy and regenerative medicine based on pluripotent stem cells. Our steps forward these novel applications of advanced biotechnology to cure human genetic diseases will be shown.

Molecular diagnosis of rare Mendelian diseases using whole exome sequencing

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The development in the recent years of the so-called next generation sequencing technologies based on massive parallel methods currently allows the production of millions of DNA sequences at an unprecedented speed with an increasingly reduced cost per nucleotide. The potential of this technology is being used to create new applications and biological tests that are soon going to revolutionise the pre- and postnatal diagnosis of genetic disorders. In order to assess the utility of this technology in molecular diagnostics we have applied whole-exome sequencing to two groups of patients with normal aCGH results. Group 1 included patients diagnosed with rare Mendelian disorders with genetic heterogeneity and well defined phenotype whereas group 2 was formed by patients with severe phenotypes but no specific diagnosis. Among group 1, patients with primary autosomal recessive microcephaly (7 causative genes known), Bardet-Biedl syndrome (15 genes), Joubert syndrome (10 genes), microcephalic dwarfism (4 genes) and Walker-Warburg syndrome (6 genes) were analysed. Potential causative biallelic mutations were identified and validated in parental samples in 4 out of 6 cases (66%) from group 1. Candidate mutations putatively related to the phenotype were also detected in all group 2 patients, but follow-up studies are required to define their pathogenic role. Our data indicates that exome sequencing in a single patient is highly effective (and likely cost-effective) as the initial diagnostics tool to identify causative pathogenic mutations in rare Mendelian disorders while in phenotypes without specific diagnosis the technology is a research tool that provides new candidate pathogenic variants.

The PLAU P141L single nucleotide polymorphism is a potential genetic predictor of the arteriogenic response in coronary artery disease

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Growth of coronary collateral arteries (coronary arteriogenesis) is an alternative source of blood supply to the ischaemic myocardium in coronary artery disease (CAD). These conducting vessels develop from pre-existing coronary arterioles, and their growth and distribution varies largely, presumably being under genetic control. Complete arterial wall remodelling, including the reorganization of extracellular matrix (ECM), occurs during arteriogenesis, in which urokinase-type plasminogen activator (u-PA) has a prominent role. This enzyme converts plasminogen to plasmin, which in turn activates growth factors and matrix metalloproteinases, which participate in the extracellular matrix degradation during collateral growth. The aim of this work is to evaluate the association of the single nucleotide polymorphism (SNP) P141L in the *PLAU* gene, which causes a missense mutation in the kringle domain of uPA protein, with coronary collateral artery growth. Although this polymorphism has been associated with asthma, Alzheimer's disease and colorectal cancer, its association with coronary arteriogenesis has not been addressed to date. We hypothesized that *PLAU* L141 variant is associated with a reduced coronary collateral growth in CAD patients.

We designed a study to evaluate the association of *PLAU* P141L polymorphism with coronary collateral circulation (CCC) in CAD. We enrolled a cohort of 676 CAD patients with severe stenoses. Blood samples were used for SNP genotyping. CCC was assessed angiographically (Rentrop method). CAD patients were classified into two groups according to the grade of CCC: poor CCC (Rentrop 0-1) (n=547) and good CCC (Rentrop 2-3) (n=129).

Analysis of the *PLAU* P141L (C>T) polymorphism showed an association between genotype distribution and the grade of CCC (p=0.020). The frequency of the allele encoding for the L141 (T) variant in homozygosis (TT) was higher in CAD patients with poor CCC than in those with good CCC. Moreover, the allelic T variant was also more common in patients with poor CCC (p=0.006). The relative risks of having poor CCC in patients bearing the T allele encoding the L141 form (adjusted by clinically relevant variables) under the dominant model (CT+TT vs CC) (OR=1.83 [1.16-2.90; p=0.010]) or the additive model (OR=1.73 [1.14 - 2.62; p=0.009]) were statistically significant. Although the results presented herein must be validated in additional cohorts, the *PLAU* P141L SNP emerges as a potential genetic tool to predict the arteriogenic response in CAD patients.

Study of genetic association in 1q21-23 locus, a candidate region for psychosis.

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INTRODUCTION: Schizophrenia (SZ) is a complex and disabling brain disorder with a prevalence of 1%. Several linkage and association studies have implicated a number of genomic regions potentially involved in disease susceptibility (Craddock et al. 2005), including 1q21-23 (Brzustowicz et al. 2000 and 2002, Gurling et al. 2001). In a previous association study by our team we pointed out a positive allelic association with marker D1S1679, which lies in 1q21-23, between candidate genes for schizophrenia (CAPON, SH2D1B, UHMK1 and RGS4) (Rosa et al. 2002).
The aim of our study was to explore more thoroughly this region for disease-associated SNPs in candidate genes with a family-based association study.

METHODOLOGY: The sample comprised 185 nuclear families, including a patient with functional psychosis. We genotyped 25 SNPs in the 1q21-23 region using the Sequenom iPLEX MassARRAY® technology. The Transmission Disequilibrium Test (TDT) was used to study preferential transmission of alleles from parents to the affected offspring using SNPator (Morcillo-Suárez et al. 2008). Haploview was used for the analysis of haplotypic associations (Barrett et al. 2005).

RESULTS: When we examined the transmission of the alleles from the parents to the affected offspring, we found a significant association for rs6694863 ($p = 0.03$), within the UHMK1 gene. Tests of haplotypic association showed trends toward association for one haplotype in the UHMK1 gene ($p = 0.05$). The haplotypic analysis also showed association with one haplotype in the SH2D1B gene ($p = 0.04$).

DISCUSSION: Our results showed a trend towards positive association between functional psychosis and two haplotypes in UHMK1 and SH2D1B genes at the 1q21-23 region, confirming previous results. These genes encode for proteins expressed in different regions of the brain that could be involved in the etiology of psychosis. These results should be further analyzed in terms of statistical power using bigger samples and specific phenotypes of the disease to fully evaluate the validity of these conclusions.

Large-scale validation and genotyping of inversions in the human genome by inverse PCR

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In the last years, different types of structural variants (SVs), have been discovered in the human genome and their importance to human health has become increasingly clear. Typically arrays have been used to characterize unbalanced changes. Inversions, however, are more difficult to study and less known. In this study we investigate the general applicability of inverse PCR (iPCR) for the analysis of inversions. We have tested different reagents and conditions to optimize the iPCR method and designed a high-throughput iPCR protocol to genotype inversions in a large number of individuals in just one day and with a small amount of DNA (10 ng for each inversion). As an example of the potential use of this method, we have analyzed 19 inversions predicted in humans with a size between 8 kb to 200 kb and mediated by inverted repeat sequences of 1.5-25 kb. First, we validated 17 of the 19 inversions in a panel of 9 Hapmap individuals (Yoruba, European, and Asian). Then, we genotyped these inversions in >60 additional European individuals and found total frequencies for the inverted allele between 1.5% and 62%. For these inversions we also checked the genetic transmission in ~10 mother-father-child european trios. Finally, we have determined the possible gene effects of the validated inversions, with around half of them changing the orientation of genes or exchanging the 3´or 5´ regions. In conclusion, the iPCR is a powerful, simple and fast method for high-throughput validation and genotyping of a wide range of inversions.

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S 7 - BIOLOGY OF REPRODUCTION

Current knowledge of the proteomics of human spermatozoa.

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The spermatozoon is an accessible cell that is particularly well suited for proteomic analysis. Catalogs of thousands of spermatozoan proteins in human and in model species are becoming available setting up the basis for subsequent research, diagnostic applications and eventually the development of specific treatments for infertile patients. In addition to the overall proteomic composition of the spermatozoa, the sperm cell nuclei, deserves a special attention as it has the important function to transmit to the next generation the paternal genetic message. While the majority of the human sperm genome (about 90%) is tightly packaged by protamines, approximately 10% of the sperm DNA is organized by histones, many of which are sperm specific variants. Of relevance, retained nucleosomes in human sperm chromatin are significantly enriched at loci of developmental importance, including imprinted gene clusters, microRNA clusters and HOX gene clusters, and developmental promoters are generally DNA hypomethylated in sperm (Arpanahi et al., Genome Res. 2009; 19:1338-49. Hammoud et al., 2009 Nature 460:473-8). But despite these observations, little attention has been devoted so far to the complete identification the sperm chromatin protein cargo providing additional layers of epigenetic information delivered by the father. Different groups had previously applied mass spectrometry contributing to the identification of over 1000 proteins in the human sperm cell using a whole sperm proteomic approaches (Oliva et al., 2009, Protemics 9: 1004-1017). In our present project we have focused on the proteomic analysis of the sperm chromatin using isolated human sperm nuclei. This approach has resulted in the identification of 408 proteins from the sperm head and over 1100 proteins from the tail, many of which are novel proteins not previously detected in whole sperm proteomic analysis (unpublished data). Differential proteomics is also leading to the identification of proteins and chromatin domains altered in the sperm chromatin of infertile patients with implications for understanding the essential conserved mechanisms and their alterations in infertile patients and related pathological imprinting defects. Supported by Ministerio de Ciencia e Innovación, Spain (BFU2009-07118) to RO.

Gonadal transcriptome analysis of the effects of temperature on European sea bass (Dicentrarchus labrax) sex ratios

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The European sea bass (*Dicentrarchus labrax*) is a gonochoristic species with a polygenic sex determination system with environmental influences. Thus, elevated temperatures can alter population sex ratios by masculinizing fish that otherwise would have developed as females. In the present study, sea bass larvae were exposed to either natural or elevated temperature, the latter expected to induce the male pathway in some of the animals, followed by facultative Estradiol treatment to induce feminization. Growth and sex ratios were determined and samples taken for gene expression analysis. A custom-made microarray was used to determine profiles of gene expression in the gonads of putative females, which were identified as the individuals with the highest *cyp19a* expression, a marker of female differentiation. Sex ratio analysis of eleven-month-old sea bass showed twice as males in the high temperature group as compared to the low temperature group, while Estradiol treated fish were essentially all females. Microarray analysis of females from these groups, showed 589 differentially expressed genes when comparing high versus low temperature groups (461 up regulated and 128 down-regulated) including genes important for reproduction such as the anti-Müllerian hormone, the transcription factor dmrt1 or the gonadotropin-releasing hormone. Together, these results help to understand the changes brought by elevated temperatures and their connection with resulting sex ratios, a relevant issue under both an aquaculture context and in a global change scenario. *Supported by grants CSD2007-0002 (“Aquagenomics”) and AGL2010-15939 (“Epigen-Aqua”) to FP.*

Sperm nucleoprotein structure is more resistant to sustain cryopreservation procedures in good than in poor freezeability boar ejaculates

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The freezability of boar ejaculates seems to be more dependent on the individual features than on the cryopreservation process itself, so that the ability of a given boar to produce cold-shock resistant sperm has several implications on its own ejaculate characteristics. Previous reports have classified boar ejaculates into good (GFE) and poor (PFE) freezability ejaculates according to their resistance to sustain cryopreservation, which has been related to sperm functional parameters. Since destabilisation of nucleoprotein structure due to a disruption of disulphide bonds between cysteines is one important alteration of the boar sperm-head during freezing/thawing procedures, the present study sought to determine whether such levels of disulphide bonds in sperm nucleoproteins differed between GFE and PFE. Other sperm functional parameters, such as sperm motility, and membrane and acrosome integrity, were also assessed. Twenty ejaculates coming from twenty healthy post-pubertal boars were frozen/thawed and subsequently classified as GFE and PFE on the basis of sperm functional parameters and hierarchical cluster analyses. In all the twenty ejaculates, the levels of cysteine-free residues (FCR) in sperm head proteins and the sperm chromatin integrity using chromatin dispersion test were assessed before cryopreservation and 30 and 240 min after thawing. Data obtained for GFE and PFE were compared following a general linear model for repeated measures. After 30 min post-thawing, the levels of FCR were significantly lower ($P<0.05$) in GFE (4.9 ± 0.5) than in PFE (7.6 ± 0.8). In addition, the percentages of spermatozoa with fragmented DNA were also significantly higher ($P<0.05$) in PFE (11.2% ± 0.9) than in GFE (5.5% ± 0.5) after 240 min post-thawing. Therefore, we can conclude that the chromatin integrity of boar spermatozoa, especially the stability of nucleoprotein structure assessed by the non-disrupted disulphide bonds, is higher in GFE than in PFE. This may be likely involved in the higher degree of resistance to cryopreservation in the formers than in the latters.

An assessment of telomeric repeat-containing RNA (TERRA) and telomerase in human fetal oocytes

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Mammalian telomeres play an important role throughout meiotic chromosome events since they tether telomeres together promoting alignment, synapsis and recombination between homologs. Telomeric repeat-containing RNA (TERRA), a recently discovered structural constituent of the telomeric complex, has been initially described in somatic mammalian cells cultivated *in vitro,* but its presence and distribution in the germ line remains elusive. Given the importance of telomeres during meiotic progression and regarding the fact that some studies relate telomeres with reproductive senescence, we studied the presence of TERRA and telomerase in human fetal oocytes.

We analyzed human oocytes and stroma cells obtained from ovaries of four euploid fetuses of 22 gestational weeks. The co-localization of TERRA, telomerase and telomeres was performed optimizing a combination of immunofluorescence (IF) and RNA-fluorescent in situ hybridization (RNA-FISH) techniques. We show for the first time the presence and nuclear distribution of TERRA and TERT in human fetal ovarian cells. TERRA forms discrete foci at telomeres of ovarian tissue cells and is mostly localized at telomeres. TERRA is present at ~23% of the telomeres of human oocytes showing co-localization with TRF2. Likewise, we show how TERRA and TRF2, separately, co-localize with ~22% of the molecules of the protein component of telomerase (TERT) in all the ovarian tissue cells. Finally, TERRA levels are higher in oocytes when compared with ovarian stroma cells, whereas they do not vary along the progression of the prophase I stage.

The presence of TERRA at the telomeres of human oocytes all through the meiotic prophase I, together with its co-localization with the telomerase protein component also at telomeres suggest that this RNA might participate in the regulation of telomere structure stability and/or function all through the long process of human meiosis, opening new avenues for the study of telomere homeostasis in the germ line.

Comprehensive analysis of sperm DNA fragmentation through alkaline and neutral Comet assay in clinical groups of infertile patients

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Many reports have shown that sperm DNA fragmentation (SDF) is a limiting factor in male fertility and is related to poor rates of fertilization, implantation, miscarriage and birth defects. SDF can occur through reactive oxygen species and nuclease activity, producing single-stranded (ssDNA) and double-stranded (dsDNA) DNA breaks, respectively. Among the available techniques to assess SDF, the Comet assay has the advantage of evaluating both ssDNA and dsDNA breaks, which could be related to different clinical outcomes depending on the DNA damage profile shown. The aim of this work is to perform an extensive analysis of SDF by using alkaline and neutral Comet assay in 196 semen samples, classified in 10 groups, attending their clinical status. SDF results obtained using alkaline and neutral Comet assay for the different clinical groups were (mean ± SD): 23.53±10.79 and 44.00±30.18, respectively, for fertile donors; 45.84±8.79 and 53.32±15.38, respectively, for normal semenogram infertile patients; 60.74±8.92 and 69.37±10.21 for ATZ patients; 62.12±11.92 and 62.85±16.46 for OATZ; 52.54±13.71 and 57.94±14.63 for TZ; 56.42±11.42 and 58.69±8.43 for AsZ; 69.90±13.82 and 73.51±15.26 for varicocele patients; 53.96±4.99 and 68.73±10.78 for operated varicocele patients; 71.32±27.95 and 69.55±27.02 for chromosomal reorganization carriers; and 32.22±12.77 and 83.34±16.30 for recurrent pregnancy loss patients. The differential profile of SDF is associated with different clinical features, where a low-equivalent profile with low values for both ss and dsDNA SDF would be the best prognosis to achieve pregnancy outcome, and the high-equivalent profile with high values of both ss and dsDNA breaks would be indicative of a bad prognosis for achieving a pregnancy. Interestingly, the non-equivalent profile, characterized by low values of ssDNA SDF and high values of dsDNA breaks, is associated with the risk of undergoing spontaneous miscarriage.

Evidences that ATR is involved in DSB repair during meiotic prophase

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Meiosis is a special cell division that generates haploid gametes. During meiotic prophase, programmed double-strand breaks (DSBs) are formed and their subsequent repair promotes synapsis and recombination of homologous chromosomes. ATR is a kinase that belongs to the so-called DNA damage response (DDR) and it is implicated in the DSBs repair in somatic cells. (Cimprich and Cortez, 2008). In the last years cytological studies have revealed that ATR is present on paired cores chromosomes and co-localize with other DDR proteins during meiotic prophase (Moens et al., 1999). In addition, I t accumulates on unsynapsed chromosomes axes, resulting in transcriptional silencing of those regions (Turner et al., 2005). These facts suggest that ATR may be involved in meiotic recombination. To study the role of ATR during meiosis, we have used a mouse model containing a hypomorphic ATR mutation that is responsible for the Seckel syndrome ($ATR^{S/S}$) (Murga et al., 2009). This mutation reduces ATR expression in most tissues. Nevertheless, $ATR^{S/S}$ mice have standard-size testis presenting all stages of spermatogenesis (judged by histological procedures) and are fertile. However, our studies have provided evidences that some pachytene-stage cells present unsynapsed sex chromosomes, though they are always incorporated in a sex body. Moreover, $ATR^{S/S}$ mice show an increased number of meiocytes with defective DSB repair in late-pachynema. These data suggest that ATR might be involved in the repair of DSBs during meiotic prophase.

MONDAY 9 TH OF JULY
S 8 - PROLIFERATION, ANGIOGENESIS AND METASTASIS

Mechanism of Resistance to Anti-Angiogenic Therapies

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Angiogenesis inhibitors targeting the VEGF signaling pathway have proven to be efficacious in preclinical cancer models and in clinical trials and multiple angiogenesis inhibitors have been therapeutically validated in several in clinical trials. While anti-tumor effects and survival benefit are often evident, relapse to progressive tumor growth typically ensues, reflecting multiple mechanisms of adaptation to anti-angiogenic therapies.

More recently, in several animal models of cancer tumor adaptation to anti-VEGF/VEGFR therapies has been described concomitant with progression to stages of greater malignancy, with heightened invasiveness into surrounding tissue and in some cases increased lymphatic and distant metastasis to the liver. Thus, anti-angiogenic therapy that effectively inhibits neovascularization and produces anti-tumor effects and survival benefit can additionally alter the tumor phenotype and promote tumor progression to stages of greater malignancy. The causes of this adaptive evasive resistance to anti-angiogenic therapies include emerging tumor hypoxia due to vascular trimming. Consequently, the potency of the anti-angiogenic drug used seems to directly correlate with its pro-invasive and malignant effects.

A current unanswered question is how to maximize the significant anti-tumor effects but avoid initiating this adaptive evasive resistance. Several hypothesis together with results from some experimental approaches will be presented in this meeting.

Identification of Sp1 targets involved in proliferation and cancer

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Sp1 is a transcription factor able to regulate many genes through its DNA binding domain, containing three zinc fingers. Previously, we cloned the promoter of Sp1 and studied its regulatory transcriptions factors, mainly NF-Y, E2F and Sp1 itself. Now, we were interested in identifying target genes regulated by Sp1, with a special emphasis to those involved in proliferation and cancer. Our approach was to treat HeLa cells with a siRNA directed against Sp1 mRNA to decrease the expression of Sp1 and, in turn, the genes activated by this transcription factor. Sp1-siRNA treatment led to a great number of differentially expressed genes as determined by whole genome cDNA microarray analysis. Underexpressed genes were selected since they represent putative genes activated by Sp1. These underexpressed genes were classified in six Gene Onthology categories, namely proliferation and cancer, mRNA processing, lipidic metabolism, glucidic metabolism, transcription and translation. Putative Sp1 binding sites were found in the promoters of the selected genes using the Match^{TM} software. After literature mining, 11 genes were selected for further validation of their expression levels using RT-real time PCR. Underexpression was confirmed for the 11 genes plus Sp1 in HeLa cells after siSp1 treatment. Additionally, EMSA and chromatin immunoprecipitation assays were performed to test for binding of Sp1 to the promoters of these genes. We observed binding of Sp1 to the promoters of RAB20, FGF21, IHPK2, ARHGAP18, NPM3, SRSF7, CALM3, PGD and Sp1 itself. Finally, the mRNA levels of RAB20, FGF21 and IHPK2, three genes related with proliferation and cancer, were determined after overexpression of Sp1 in HeLa cells, to confirm their relationship with Sp1.

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Characterization of Prostate Cancer bone metastasis process by a highly bone metastatic cell line generated *in vivo*

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Prostate cancer (PCa) is the most common neoplasia in men and the second leading cause of death in males along Europe and USA. When the disease is detected in its early stages, it is still treatable by various therapies, but once disseminated at distance, there is no curative therapy.
Bone is the most common site of this metastatic spread, where up to 80% of the metastases are found. Skeletal metastasis in PCa patients result in significant complications that diminish the quality of life. Half of the patients with metastases die within 30 months after the bone metastasis diagnosis because of a lack of an effective therapy.

Objectives:
a) to generate an animal model of bone metastasis in order to select a highly bone metastatic cell line which allows monitoring the process of metastasis in vivo, b) to identify the molecules responsible for the formation of bone metastasis by comparing the bone metastatic PCa cells to the parental PCa cells by genomics and proteomics.

Methodology:
We injected intracardiacally a human PCa cell line (PC3) overexpressing green fluorescence protein/luciferase (GFPluc) into Balb/c nude and NOD SCID mice. We used the IVIS 200 System to monitor tumor growth and detect metastases in the living animal.
Cells from bone metastasis were isolated, expanded in culture and re-injected again into another group of mice to obtain a highly bone metastatic PCa cell line.

Results:
After three rounds of in vivo cell selection, the highly bone metastatic PCa cell population showed a more aggressive phenotype compared to the parental PCa cell line. We observed a similar bone metastasis pattern of distribution as in humans, where the main mice bone affected was the femur.

Conclusions:
It is possible to use an immunocomprised animal as an in vivo sorter for cancer cells in order to generate a highly bone metastatic cell population.
The next step now is the identification of bone metastasis specific signatures by comparing expression profiles from the highly bone metastatic cell line with the parental one.

Cancer and arsenic: dedifferentiation and effects on CSCs

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Human chronic exposure to the world-widely spread environmental toxicant arsenic represents an important health concern due to its demonstrated ability to induce several types of cancer, as liver cancer among other pathologies. Moreover, arsenic is used as antitumor treatment for acute promyelocytic leukemia (APL) since 2009 and nowadays, its application against other kind of cancer has been studied. Although arsenic has been studied for a long time, the exact action mechanism of arsenic is not well known. The study of the mechanism implicated in arsenic carcinogenesis would be very useful to make progress arsenic use as antitumor drug and to prevent health effects in people chronically exposed to environments with arsenic content.

In order to identify the mechanism(s) implicated in arsenic carcinogenesis, the expression of a group of hepatic transcription factors involved in differentiation processes has been studied in a HepG2 cell line and hamsters chronically treated with sub-toxic doses of arsenite. Thus, arsenic-inhibition expression of HNF1α, HNF4α, HNF6α, FOXM1 and PXR was determined by real-Time PCR and Western-Blot. Some cancer characteristics were studied such as differentiation status, epithelial-to-mesenchymal transition (EMT) and activity of secreted invasivity markers metalloproteinases. The effect of arsenic on stem cells population was also studied by quantification of several stemness markers.

Results indicate how arsenic induces loss of differentiation, EMT, degradation of extracellular matrix and it has an effect on stem cells population. In this work, we propose that these arsenic effects may be responsible for the de-regulation of the hepatic transcription factors studied, mainly HNF1α and HNF4α.

Role of MSK1 in Steroid Hormone-induced breast cancer cell proliferation

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The steroid hormones Progestins (Pg) and Estrogens (E2) promote cell proliferation in those breast cancer cell lines expressing the Progesterone receptor (PR) or the Estradiol receptor (ER).
The Mitogen and Stress-activated kinase 1 (MSK1) has a key role in mediating Progesterone and Estrogens-induced cell proliferation in cultured cells and in T47D xenografts in mice. A lack of MSK1 activity blocks specifically hormone-dependent G1-S phase transition, resulting in an abrogation of cell proliferation and tumor growth. Neither cell viability nor proliferation in response to other stimuli is compromised when inhibiting this protein kinase.

Expression Array experiments performed in T47D cells have revealed that both steroid hormones activate transcription of a subset of positive regulators of cell cycle progression (fc >1,4, p-value <0.05) such as EGF, its receptor (EGFR), or the Cyclins D1 and E2 in response to Progestin; or the Trefoil Factor 1 (TFF1), WISP2 and the mitogen-activated protein kinase 9 (MAPK9) in response to Estrogens. Inhibition of MSK1 blocks hormone-dependent induction of these genes.

Progestin-mediated MSK1 activation occurs in response to Pg that activates PR-dependent signaling trough the MAPK pathway resulting in MSK1 phosphorylation. Active MSK1 interacts with PR and the complex is recruited to Progesterone-Responsive Elements (PREs) in DNA as demonstrated by ChIP-seq. Gene promoters, regions surrounding the Transcription Termination Site (TTS) and coding exons are significantly enriched in Progestin-dependent MSK1 occupancy. PR binding sites enriched in MSK1 exhibit a higher degree of chromatin remodeling upon hormone treatment.

In conclusion, MSK1 is involved in the hormone regulation of a significant fraction of target genes, particularly those involved in cell proliferation. This effect is likely mediated by a contribution of MSK1 to the hormone induced chromatin remodeling via phosphorylation of histone tail targets.

PFKFB3 regulation by p38 MAPK pathway

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Cellular stress activates multiple mitogen-activated protein kinase (MAPK) cascades and the transcription of immediate-early genes. Mitogen-activated protein kinase (MAPK) signalling occurs in response to almost any change in the extracellular or intracellular milieu that affects the metabolism of the cell, organ or the entire organism.

Glycolytic flux is mainly controlled by 6-phosphofructo-1-kinase, with fructose-2,6-bisphosphate (Fru-2,6-P_2) being its most powerful allosteric activator. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB) catalyzes the synthesis and degradation of Fru-2,6-P_2 and hence critically regulates carbohydrate metabolism. *PFKFB3* gene codifies for PFKFB3 isoenzyme which has been found overexpressed in proliferating cells and tumors. Here we analyse its mechanism of regulation by cellular stress in different types of cancer cells. We report that exposure of HeLa and T98G cells to anisomycin (a drug that activates p38 pathway), UV radiation, hydrogen peroxide and osmotic shock lead to a rapid increase in Fru-2,6-P_2 concentration and *PFKFB3* mRNA levels. Western blot results showed a short-term activation due to PFKFB3 isoenzyme phosphorylation. Transient transfection of HeLa cells with deleted gene promoter constructs allowed us to identify a serum response element (SRE) through which p38-MK2 pathway transactivates *PFKFB3* gene transcription. A dual mechanism affecting PFKFB3 protein and gene regulation operates in order to assure glycolysis in these cell types. An immediate early response through MAPK phosphorylation of PFKFB3 protein is followed by activation of mRNA transcription via *cis*-acting sequences on *PFKFB3* promoter.

TUESDAY 10 TH OF JULY
S 9 - CELL DAMAGE AND CELL DEATH

Age-dependent decline of motor cortex but not hippocampal performance in heterozygous BDNF mice correlates with a decrease of cortical PSD-95 but an increase of hippocampal TrkB levels

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Brain-derived neurotrophic factor (BDNF) is a key player in synaptic plasticity and directly contributes to learning and memory processes. However, little is known about brain area-specific functions of this neurotrophin. In this study we investigated whether BDNF could differently affect cortical and hippocampal-related cognitive and plastic morphologic changes in young (12-week-old) and middle-aged (30-week-old) BDNF heterozygous (BDNF+/-) and wild type (wt) mice. We found that at 30 weeks of age, BDNF+/- mice showed impaired performance in accelerating rotarod and grasping tests while preserved spatial learning in a T-maze and recognition memory in an object recognition task compared with age-matted wild type mice suggesting a specific cortical dysfunction. Accordingly, a significant cortical but not hippocampal reduction of dendritic spine-like structures and synaptic markers (PSD-95 and GluR1) was observed in BDNF+/- mice. Interestingly, 30-week-old BDNF+/- mice displayed increased TrkB levels in the hippocampus but not in the motor cortex, which suggests a specific compensatory mechanism in the hippocampus as a consequence of BDNF decrease. In conclusion, our data indicates that BDNF could differentially regulate micro-structural plasticity and cognition in a region-specific and in an age-dependent manner.

Funding

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Decreased PKCδ protein levels as a neuroprotective mechanism in cells expressing mutant huntingtin

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A balance between cell survival and apoptosis is crucial to avoid neurodegeneration. Here, we analysed the regulation of the novel pro-apoptotic PKC, PKCδ, in several brain regions of the R6/1 mouse model of Huntington's disease (HD) during the disease progression. We detected a dramatic decrease of PKCδ protein levels in all the regions examined (striatum, cortex and hippocampus) beginning at early stages of the disease and reaching more than 80% reduction at late stages. Moreover, we also found a reduction of PKCδ protein levels in post-mortem human putamen. The decrease in PKCδ protein levels was not due to modified expression of the gene, nor to changes in intracellular distribution, since mRNA levels were not altered and reduced protein levels were detected in both nuclear and cytoplasmic enriched fractions. Although PKCδ was detected in neuronal nuclei it did not colocalize with mutant huntingtin intranuclear inclusions. Phosphorylation of PKCδ at Thr505 leads to its degradation. Our results indicate that phosphorylation of PKCδ is increased in R6/1 mice striatum, cortex and hippocampus suggesting that decreased levels of PKCδ in the presence of mutant huntingtin could be the result of increased degradation. Since PKCδ is a pro-apoptotic protein, these results suggest that decreased PKCδ levels could be a pro-survival mechanism activated in HD. We confirmed this hypothesis by showing that over-expression of PKCδ in striatal cells expressing mutant huntingtin, but not wild-type huntingtin, increases cell death. In conclusion, our results suggest that in the presence of mutant huntingtin neurons increase the degradation of PKCδ as a compensatory response that can contribute to neuronal survival.

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Phenotypic and functional characterization of antigen-specific myeloid-derived suppressor cells generated during retroviral transduction of murine bone marrow

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Our previous work showed that transferring bone marrow cells transduced with the MOG_{40-55} autoantigen into non-myeloablated mice with experimental autoimmune encephalomyelitis (EAE) improved the disease evolution. To identify the population of cells primarily responsible for the observed therapeutic effect we performed a number of phenotypic and functional studies with the cells generated during the 4-day standard retroviral transduction of bone marrow cultures. Interestingly, we found that the majority of transduced cells expressed myeloid markers and consisted of two main cell subpopulations ($CD11b^+$ $Gr\text{-}1^{low}$) and ($CD11b^+$ $Gr\text{-}1^{high}$), which correspond, respectively, to the phenotypes reported for the monocyte-like and the granulocyte-like myeloid-derived suppressor cells (mMDSCs and gMDSCs). *In vitro*, mMDSCs displayed high levels of arginase-1 and NO synthase activities, while gMDSCs exhibited moderated levels of these enzymes. Moreover, both cell types produced reactive oxygen species. Besides these well known suppressive mechanisms, both MDSCs subtypes expressed PD-L1 (Programmed Death Ligand-1) and this expression was further increased by stimulation with interferon-γ and lipopolysaccharide. Both MDSC subtypes inhibited MOG-induced proliferation of splenocytes from mice with MOG-induced EAE, although mMDSC cells were more immunosuppressive than gMDSC. MOG-expressing MDSCs were more suppressive than their sham-transduced counterparts suggesting that both antigen-specific and nonspecific mechanisms play a role in mediating the therapeutic effect. Adoptive cell therapy using antigen-specific MDSCs may constitute a useful approach to induce tolerance in the context of autoimmune diseases and transplantation.

Unique role of Bcl-xL regulating the antiapoptotic role of NF-κB

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Apoptotic cell death is triggered by several different stimuli, among which the activation of Death Receptors by the so-called Death Ligands, such as TNFα. To induce apoptosis, TNFα needs the cooperation of RNA transcription or protein synthesis inhibitors, i.e. Actinomycin D (ActD) or Cyclocheximide, respectively. In this study we demonstrate that ActD renders HeLa cells susceptible to the cytotoxic effect of TNFα. Proteins involved in TNFα receptor signalling complex are not affected by TNFα/ActD co-treatment. However, the analysis of Bcl-2's family anti-apoptotic members demonstrates that Bcl-x_L protein levels decrease after treatment with ActD. To point out the relevance of Bcl-x_L in TNFα cytotoxic signalling pathway, the specific knock-down of Bcl-x_L, and not those of the main anti-apoptotic Bcl-2 family members, Bcl-2, Bcl-w or Mcl-1, renders cells sensitive to apoptosis induced by the cytokine. Moreover, the sensitization effect of Bcl-x_L knock-down does not alter the activation status of NF-κB induced by TNFα. Therefore, the results obtained demonstrate that Bcl-x_L is the most relevant Bcl-2-cytoprotective protein regulating TNFα cytotoxicity.

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Role of CDK11 in the β cell mass apoptosis in type I diabetes

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Cdk11 is downregulated in pancreatic islet endocrine cells during the autoimmune attack progression that leads to diabetes in NOD mice. CDK11, protein-kinase PITSLRE, has ubiquitous expression and exhibits two gene products: p58 and p110 (p130 in mouse) in humans. CDK11^{p110} regulates transcription and RNA splicing. CDK11^{p58} action is essential in mitosis and apoptosis. Both, upon a proapoptotic stimulus, are activated by caspase 3 mediated proteolysis, rendering p60 and p46, which seem to amplify apoptotic processes. In order to assess the involvement of CDK11 expression levels in pancreatic beta cell homeostasis, we have generated NOD mice heterozygous for CDK11 deficiency, (homozygosity in CDK11 is embryonic lethal), and studied spontaneous diabetes incidence. Although these are preliminary results, it seems that CDK11 hemi-deficiency renders NOD mice more resistant to diabetes onset, which would agree with the fact that, CDK11^{p110} and CDK11^{p58}, upon a proapoptotic stimulus, may amplify apoptotic processes, and the downregulation could be a protective mechanism against diabetes. On the other hand, we are performing adoptive transfer experiments, in which NOD/SCID mice hemi-deficient in CDK11 are transferred with diabetogenic lymphocytes to determine the islet cell susceptibility to transferred diabetes in CDK11-hemideficient recipients compared to CD11 wild type littermates. *In vitro* approach: NIT-1 NOD insulinoma cell lines are stably transfected with either CDK11^{p130} or CDK11^{p58}, and submitted to the apoptotic stimuli triggered by IL-1□ and/or IFN□. Therefore, the results obtained from our work will allow us to establish the role of CDK11 as molecular target in type I diabetes.

Failure of caspase-dependent cell death to reach the classical apoptotic phenotype in SH-SY5Y human neuroblastoma derived cells

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DNA degradation by intracellular DNAses is one of the biochemical hallmarks of apoptosis. This process is regulated by cysteine proteases called caspases, which activate the ICAD-CAD system to induce the DNA degradation through CAD endonuclease. DNA is degraded into internucleosomal DNA fragments, known also as low molecular weight (LMW) fragmentation. LMW degradation is usually concomitant to chromatin condensation and nuclear fragmentation, which confer the classical apoptotic nuclear morphology. Moreover, LMW degradation is normally subsequent to another kind of DNA fragmentation, known as high molecular weight (HMW), associated to initial nuclear morphological changes during the apoptotic process. Here, we show a type of cell death without the biochemical hallmarks of apoptosis but dependent of caspases activation in a human neuroblastoma cell line, SH-SY5Y, after challenge with a benzophenanthridine alkaloid, DMBP. We have found that after this treatment, SH-SY5Y cells display HMW but not LMW DNA degradation. In addition, neither chromatin condensation nor nuclear fragmentation has been detected in these cells when they are exposed to DMBP. Executioner caspases are activated and their substrates are properly processed upon this treatment, including ICAD, which is cleaved by caspase-3. However, caspases activation and substrates cleavage take place earlier in DMBP-treated SHY5Y cells than staurosporine (STP) apoptotic insult, which can induce the classical apoptotic phenotype in these same cells. Besides this, even higher STP doses do not reach to induce this early caspases activation in SH-SY5Ycells. On the other hand, the mitochondrial or intrinsic pathway is also properly triggered through the release of mitochondrial proapoptotic factors to the cytosol in DMBP-treated SH-SY5Y cells. Furthermore, the pan-caspase inhibitor Q-VD-OPh can prevent caspases activation and cell death induced by DMBP treatment. Regarding to CAD endonuclease, their levels are not deregulated during cell death induced by DMBP. More interestingly, CAD levels from the chromatin-enriched fraction required to induce the LMW DNA degradation, are similar in DMBP- and STP-treated SH-SY5Y cells. In addition, an oxidative stress process is demanded to induce the cell death of DMBP in SH-SY5Y cells. Indeed, DMBP-induced caspases activation and cell death can also be prevented by the addition of oxidative stress inhibitors in the culture media. Therefore, the modulation of an oxidative stress by DMBP allows cells to reach another type of apoptotic cell death. The classical nuclear morphology and late caspases activation are detected through the oxidative stress inhibitors modulation. Intriguingly, LMW DNA degradation reversion is not achieved, even when cells are co-treated with DMBP and oxidative stress inhibitors. Altogether, we present a kind of cell death dependent of caspases activation but different from the classical apoptosis. A higher knowledge on the intracellular pathways controlling this cell death could be of outstanding interest to understand malignant molecular processes related to classical apoptotic phenotype escape during chemotherapeutic treatment and the consequences derived from.

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Development of fresh human tissue recovery protocol for research: hepatocytes isolation experience

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Introduction

Human sample acquisition is now a critical step for research activities involved in target validation, drug discovery and clinical development. Using human tissues for research can provide key data from early stage discovery through pre-clinical research, drug safety evaluation, biomarker development, patient pre-selection and entry of novel compounds into the clinic. Researchers face many challenges in this area, not least of which being the legal and ethical issues around sourcing human tissue, but also the practical challenge of sourcing high quality, fully characterized specimens that are fit for use in a diverse range of analytical processes.

Objectives

To develop an international network to provide fresh human tissue with high quality and viability criteria to organizations involved in biomedical research.

Materials and Methods

Hospitals from Spain and Europe will be invited to participate in a network to provide organs, tissues and cells for research from living and deceased donors. The development of the network requires the support and involvement of health professionals in each of the processes involved in tissue procurement: donor detection and evaluation, tissue retrieval and processing. The tissue recovered will come from patients undergoing planned liver resection surgeries for known pathologies as a matter of standard procedure of care. The liver tissue sample collected will then be flushed with appropriate cold-preservation solution. Demographic information (i.e. age, height, weight, sex, race) pertaining to the subject will be provided to the project researchers. Cell isolation and cryopreservation will be conducted by the laboratory sponsor by the established in-house methods and others published in the literature.
All these processes must be performed following the legal Spanish and European requirements to ensure quality, safety and traceability of the generated material. (Ley 14/2007 de Investigación biomedica, 2004/23/EC, 2006/17/EC and 2006/86/EC).

Results

The "Program for the obtainment of liver resections for hepatocytes isolation for research" started in 2009 and linked 5 hospitals from Spain. So far, 91 donation offers have been reported and 63 resected livers have been obtained with an average of cold ischemia time of 16 hours. 44% of the tissues showed a high vascularization and perfusion. A total of 7468 million of hepatocytes (4,07 millions of hepatocytes per gram) with a viability average of 80,7% have been isolated.

Conclusions

The organization of a consolidated hospital network makes it possible to provide fresh tissue for research with high quality and viability criteria. Developing studies about the influence of donors' pre-, intra- and post-operative parameters could increase the outcome of isolated human cells.

Mitochondrial toxicity of carbon monoxide from tobacco in smoking pregnant women: reduced intrauterine growth

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Background: Maternal smoking is associated with reduced birth weight. Tobacco carbon monoxide (CO) is considered harmful due to its capacity to bind hemoglobine and mitochondrial complex IV (mCIV) leading to tissue and cell hypoxia. We wonder if mitochondrial dysfunction or hypoxic-response may underlie obstetric and perinatal adverse outcome of smoking pregnant women.

Methods: Homogenate placentas of 20 non-smokers and 30 smokers pregnant women at delivery were studied. Tobacco consumption was measured by plasmatic cotinine (units/ml). We analyzed: mCIV activity and oxidative stress lipid peroxidation spectrophotometrically, mitochondrial DNA (mtDNA) content by rtPCR, and mitochondrial number and tissue hypoxia by Western-Blot Normal distribution was ascertained (Kolmogorov-Smirnov), differences were assessed through Student's T-test and correlations by Pearson's analysis. Significance was set at 0.05 and results were expressed as mean values and standard error of the mean (SEM).

Results: In utero tobacco-exposed newborn showed reduced weight trends and increased intrauterine growth restriction (p=NS) by gestational age at delivery. Mean fetal weight at delivery was 350g lower in newborn of smoking mothers and their mean growth percentile was 16 units lower (p=0.05). Fetal cotinine positively correlated in the plasma of smoking mothers and newborn and negatively correlated with newborn weight at delivery ($p<0.001$). Mitochondrial parameters in placentas of smoking mothers trend to be preserved except for the number of mitochondria, which was significantly increased in placentas of smoking mothers ($p<0.05$). Placenta of smoking mothers showed no tissue response to hypoxia.

Conclusions: Tobacco consumption along gestation is associated with intrauterine growth restriction and their harmful compounds are present in exposed newborn. Placenta develops homeostatic mechanisms through increasing mitochondrial number to preserve mitochondrial function but no tissue hypoxic response.

TUESDAY 10 TH OF JULY
S 10 - FROM GENOTYPE TO PHENOTYPE: WHERE ARE WE NOW?

Complex biological networks: Challenges and opportunities

Roger Guimerà

URV, Trarragona

In biological systems, components (for example proteins or metabolites) interact with each other through intricate networks of interactions. This makes understanding biological systems challenging---just as we cannot understand the behavior of an ecosystem by simply listing all its species, or understand conscience by fully understanding the behavior of a single neuron, we probably should not expect to be able to understand how even the simplest organism works by knowing its genome. At the same time, biological networks are the outcome of evolutionary processes, which means that each network contains, hidden within its structure, important cues about how the system operates and evolves.

TUESDAY 10 TH OF JULY
S 11 - TRAFFIC AND SIGNALLING IN HEALTH AND DISEASE

Biogenesis of carriers for secreting bulky cargoes and proteins that cannot enter the endoplasmic reticulum

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Collagens are the most abundant secretory proteins and essential for tissue organization, skin biogenesis and bone mineralization. How are these bulky proteins secreted? We carried out a genome wide search for new components involved in protein secretion (Bard et al., Nature 2006). One of these components called TANGO for Transport ANd Golgi Organization is anchored at the ER exit site and required for collagen VII export in mammalian cells (Saito et al., Cell. 2009). Mice lacking TANGO1 are defective in the secretion of numerous collagens, from chondrocytes, fibroblasts, endothelial cells and mural cells. Collagen deposition by these cell types was abnormal, and extracellular matrix composition was compromised. Chondrocyte maturation and bone mineralization were severely compromised in TANGO1 null embryos, leading to dwarfism and neonatal lethality. TANGO1 thus appears essential for collagen secretion in vivo (Wilson et al., 2011). TANGO1 is also required for the secretion of collagens in Drosophila (Pastor-Pareja and Xu. 2011). TANGO1 binds another protein at the ER exit site called c-TAGE5, which is also required for collagen secretion (Saito et al., 2011;). We have now identified a new protein, which, like TANGO1, contains two large coiled-coiled domains and a proline rich domain anchored to the cytoplasmic face of the ER exit site. TALI, however, like c-TAGE5 lacks the lumenal coiled-coiled and SH3 like domain of TANGO1. TALI is also required for collagen secretion. I will present data on the TANGO1-cTAGE5-TALI complex and their role in collagen packing into transport carriers at the ER exit site.

The unconventional secretion of the Acyl-CoA binding protein of S. cerevisiae, Acb1, requires proteins involved in the formation of autophagosomes, in the fusion of membranes with the endosomes, proteins of the multivesicular body pathway, the cell surface t-SNARE Sso1 and the GRASP ortholog Grh1 (Kinseth et al, 2007, Duran et al, 2010). Our new findings reveal that upon nutrient starvation, Grh1 concentrates in a Phosphatidylinositol 3 phosphate (PI3P)-kinase dependent manner to unique membranes near the Sec13 containing ER exit sites. Furthermore, these membranes are enriched in PI3P and contain the ESCRT protein Vps23, and the autophagy-related gene products Atg8 and Atg9 thereby offering commonality between the different proteins required for secretion of Acb1. Electron microscopy revealed that these membranes are CUP shaped and we have therefore dubbed them CUPS for Compartment for Unconventional Protein Secretion. The biogenesis of CUPS is starvation specific but independent of the Rapamycin induced autophagy. A number of genes are involved in the biogenesis of CUPs including Grh1, Bug1, Vps34, and the ESCRT-II and III proteins Vsp25, 36, 20, and 2. Based on our findings we believe that CUPS serve as a station for the biogenesis of autophagosomes that contain Acb1 (Bruns et al., 2011). These secretory autophagosomes do not fuse with the vacuole and instead, in an Sso1 dependent reaction, fuse with the cell surface to release Acb1 into the extracellular space. We suggest that secretion of the cytokine IL-1ß and many other signal sequence lacking proteins is likely mediated by a similar process involving secretory autophagosomes. I will present our new findings on the biogenesis of CUPS and downstream events involved in Acb1 secretion.

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Apoptosis, immunogenicity and stability properties of PPRHs directed against *survivin* in mammalian cancer cell lines

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Polypurine Reverse-Hoogsteen Hairpins (PPRHs) are double-stranded DNA molecules formed by two antiparallel polypurine strands linked by a pentathymidine loop and bound by intramolecular Hoogsteen bonds that allow the formation of a hairpin structure. These hairpins have the ability to bind polypyrimidine stretches in the DNA via Watson-Crick bonds, thus causing the displacement of the polypurine strand of the dsDNA. Consequently, PPRHs knock down gene expression. To broaden the potential applications of this new gene-silencing tool, we compared the effect of coding- and template-PPRHs in several cell lines. We also tested the immunogenicity and stability of PPRHs in comparison with siRNAs.

PPRHs against different regions (exon, intron, promoter) of the anti-apoptotic *survivin* gene were compared in terms of cytotoxicity, mRNA levels and apoptosis in MiaPaCa 2 (pancreatic cancer), PC3 (prostate cancer) and HCT116 (colon cancer). We proved that some coding-PPRHs are more efficient than template-PPRHs in terms of cytotoxicity and apoptosis. Given that coding-PPRHs against promoter sequences were very effective, we are studying their mechanism of action using EMSA assays.

The immunogenicity of siRNA and PPRHs was determined in THP-1 cells. While no differences in IL-6 and IFN-α expression and IL-8 secretion were found, an induction in Caspase-1 cleavage, and IFN-β and TNF-α expression were observed by siRNA transfection but not by PPRH. Moreover, stability was assessed by incubating fluorescein-labeled PPRHs and siRNAs in 10% FCS at different time points. In these conditions, PPRHs were more stable than siRNAs and they are extremely resistant to degradation when bound to their target.

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Contribution of rare and common variants of the PTCHD1 gene to Autism Spectrum Disorder and Intellectual Disability

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Autism is a severe neurodevelopmental disorder, characterized by impaired verbal communication, limited reciprocal social interaction, restricted interests and repetitive behavior, often accompanied by intellectual disability (ID). Although it is one of the most heritable neuropsychiatric disorders, the underlying genetic factors remain largely unknown.

A recent study reported rare mutations in the X-linked gene *PTCHD1* (patched domain-containing protein 1) in patients with autism spectrum disorder (ASD) and ID, suggesting a possible role of this gene in cognitive development. The *PTCHD1* gene is highly expressed in brain regions and encodes a transmembrane protein containing a patched-related domain. It has been suggested that *PTCHD1* plays a role in the hedgehog signaling pathway.

In this study we aimed to investigate the possible contribution of *PTCHD1* common variants to ASD through a case-control association study. The study sample consisted of 595 Caucasian autistic patients (270 Spanish, 247 Dutch and 78 German) and 680 gender-matched controls (320 Spanish, 269 Dutch and 82 German). Twenty-eight tag SNPs were selected on the basis of linkage disequilibrium (LD) patterns. A significant association, that overcame the Bonferroni correction for multiple testing and permutations was obtained with the marker rs7052177 (p= 6.13e-4).

Furthermore, in order to evaluate the possible participation of *PTCHD1* rare variants in ASD and ID, we are currently performing a mutation screening in the Spanish ASD cohort, and in an additional sample of 200 individuals with ID. The preliminary results of this study support the involvement of this gene in autism and cognitive impairments.

Mitochondrial implication in adverse outcomes of hiv-pregnancies

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Background: HIV and antiretrovirals cause mitochondrial DNA (mtDNA) depletion which can lead to mitochondrial dysfunction and oxidative stress (OxS). Several secondary effects of HIV-infection have been associated to mitochondrial injury. We wonder if the aetiology of adverse obstetric and perinatal outcome characteristic of HIV-pregnancies may be due to mitochondrial disturbances.

Methods: We studied obstetric and perinatal results to correlate clinical findings and mitochondrial state in 5% (w/v) placenta homogenates of 23 HIV-treated and 32 uninfected pregnant women. MtDNA content was measured by rt-PCR (mtND2/nRNApolII genes) and OxS by the spectrophotometric quantification of lipid peroxidation (μM malondialdehyde and 4-hydroxyalkenal/mg prot). Results, expressed as mean±SEM, were non-parametrically analyzed and significance level was set at 0.05.

Results: Global adverse perinatal outcome, intrauterine growth restriction and preterm birth were increased in HIV-pregnancies (OR 11.5 [2.2-60.2], 24.1 [1.3-454.5] and 5.29 [0.95-29.20], $p<0.05$). In placentas of HIV-infected patients mtDNA content was reduced by 15% and OxS was increased by 25% with respect to controls (0.59±0.27 vs. 0.69±0.19 and 23.23±1.64 vs. 17.94±1.03, $p=0.005$ and $p=0.05$, respectively). Additionally, a trend towards increased OxS and decreased mtDNA content was observed in pregnancies with adverse perinatal outcome and higher viral load and a trend to negative correlation was found between OxS and mtDNA levels.

Conclusions: HIV and antiretrovirals cause placental mtDNA depletion and increased OxS that may lead to mitochondrial failure. Mitochondrial lesion could underlie adverse obstetric and perinatal outcome of HIV-pregnancies. Further studies should address whether these alterations are present in newborns of HIV-infected women.

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Gene Therapy for Diabetes: Moving to Clinic?

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Diabetes is a chronic disease for which there is currently no cure. Diabetes is associated with severe secondary complications, caused largely by poor glycemic control. Treatment with exogenous insulin fails to prevent these complications completely, leading to significant morbidity and mortality. We hypothesized that we could generate a "glucose sensor" in skeletal muscle through co-expression of glucokinase (Gck) and insulin (Ins) to increase glucose uptake and obtain correction of hyperglycemia in diabetic animals. In this study we demonstrate long-term efficacy of this approach in a large animal model of diabetes. A one-time intramuscular administration of adeno-associated viral vectors of serotype 1 (AAV1) encoding Gck and Ins in diabetic Beagle dogs resulted in normalization of fasting glycemia, accelerated disposal of glucose after an oral challenge, and no episodes of hypoglycemia during exercise for more than four years after gene transfer. This was associated with recovery of body weight, normalization of glycosylated plasma protein levels and lipid profile, and long-term survival without secondary complications. Moreover, the two transgenes act cooperatively since neither Ins nor Gck alone was sufficient to achieve the same tight control of glycemia obtained with their co-expression in muscle. This demonstration of long-term correction of diabetic hyperglycemia provides the first proof-of-concept in a large animal model for a gene transfer approach to treat diabetes. This work lays the foundations for the future translation of this approach to the clinic.

Mitochondrial implication in sepsis

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Background: Severe sepsis is associated with mitochondrial dysfunction, impaired oxygen consumption and energy production which may thereby condition clinical outcome, independently of tissue oxygenation. However, the role of mitochondrial dysfunction in sepsis severity remains unknown. We aimed to characterize mitochondrial lesion in human sepsis, establish its origin and cellular consequences and determine its correlation with clinical symptoms and outcome.

Methods: Different markers of mitochondrial function, nitrosative and oxidative stress, apoptosis and inflammation were measured in peripheral blood mononuclear cells (PBMC) and plasma of 19 septic patients and 20 controls. Additionally, plasma capacity to induce mitochondrial dysfunction was assessed in muscle mitochondria from 5 healthy individuals incubated with plasma of septic patients or controls.

Results: Despite unaltered mitochondrial mass and protein synthesis, enzymatic mitochondrial complexes I, III and IV and oxygen consumption were significantly inhibited in sepsis (32%, 42%, 23% and 19% decrease, respectively;$p<0.05$). Septic plasma tended to reduce oxygen consumption of healthy mitochondria and showed increased expression of the inflammatory cytokines V-CAM (46%;$p<0.05$), I-CAM (155%;$p<0.001$), IL-6 (1062%;p=NS), TNFα (98%;p=NS) and MCP-1 (494%;$p<0.05$), especially in patients presenting adverse outcome ($p<0.05$). Active NFKB was increased 68% in sepsis ($p<0.05$) together with nitric oxide (174%;$p<0.001$), oxidative stress (76%;$p<0.05$) and apoptosis (316%;$p<0.05$). Additionally, sepsis severity correlated with complex I inhibition, NFKB activation and I-CAM expression.

Conclusions: A plasmatic factor such as nitric oxide which is increased in inflammation and able to induce mitochondrial dysfunction, oxidative stress and trigger apoptosis may be responsible for cell damage in septic patients. Mitochondrial dysfunction and inflammation correlate with sepsis severity and outcome, becoming targets for supporting therapies.

El rol del gen *CNR1* (receptor cannabinoide tipus I) en la resposta clínica i la remissió al tractament amb citalopram (ISRS) en Depressió Major: estudi de seguiment a 12 setmanes

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Introducció: El tractament per la Depressió Major (DM) es basa principalment en el bloqueig de la neurotransmissió serotoninèrgica. No obstant, la resposta clínica és un fenomen complex on altres sistemes, com l'endocannabinoide, poden estar-hi involucrats. L'objectiu d'aquest estudi va ser analitzar el rol de variabilitat genètica al gen CNR1 en la resposta clínica i la remissió després del tractament amb citalopram.

Mètodes: 155 pacients amb DM van ser tractats amb citalopram i avaluats per la resposta (setmana 4) i la remissió (setmana 12). Es van genotipar 5 SNPs del gen CNR1 (rs1535255, rs806377, rs806371, rs1049353, rs806368) mitjançant la tecnologia Sequenom MassArray. Els anàlisis estadístics es van realitzar mitjançant els programes PASW v18.0, EpiInfo, Haploview 3.2 i 'R'.

Resultats: El nostre estudi va mostrar diferències significatives entre Remitents (Rm) i no Remitents (N-Rm) per les distribucions genotípiques i al·lèliques dels polimorfismes rs806371 i rs806368. Els individus rs806371-TT i els rs806368-TT presentaven 2,8 i 1.5 vegades, respectivament, més risc per a la N-Rm (P=.012, P=.008). L'haplotip T-C (rs1535255-rs806377) era més freqüent en els N-Rm (40.2%) que en els Rm (26.6%) (P=.04). L'estudi longitudinal pel polimorfisme rs806368 va mostrar efectes significatius de temps-sexe (P<.001), temps-genotip (P=.003) i temps-sexe-genotip (P=.026), mostrant que els homes portadors de l'al·lel C presentaven una millor resposta al tractament.

Discussió: La variabilitat genètica del gen CNR1 té un efecte en la remissió i en l'evolució de la resposta longitudinal. Recentment, s'ha associat aquest gen amb la resistència a antidepressius, particularment en els pacients amb DM del gènere femení (Domschke i cols., 2008). Més estudis enfocats en aquest sistema podrien ajudar a comprendre la resposta clínica als antidepressius.

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TUESDAY 10 TH OF JULY
S 12 - RECEPTORS, CHANNELS AND TRANSPORTERS

New paradigms in GPCR signaling: G proteins at the mitochondria

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$G\alpha_{q/11}$ proteins are members of the heterotrimeric G proteins known to be essential to transmit extracellular stimuli trough their receptors at the plasma membrane (GPCRs) controlling intracellular calcium signaling. $G\alpha_q$ is amply linked to normal cardiac physiology and pathophysiology, known to be one of the major players in hypertrophy and heart development. The classic view of GPCRs and G proteins has given emphasis in the signaling that occurs at the plasma membrane. But lately our view of the cellular location of these proteins is changing. Several studies have provided evidence of the presence of different components of the signaling machinery in other cellular compartments like Endoplasmic Reticulum, Golgi and mitochondria. We are the first to show the surprisingly new mitochondrial inner membrane location for $G\alpha_{q/11}$ proteins, showing that their presence is really important for normal mitochondrial fusion and energy production, linking the organelle dynamics and physiology.

Molecular Details of the Apolipoprotein E and the Amyloid Beta Peptide Interaction: Analysis of a Potential Binding Site Responsible for ApoE4 Misfolding

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The relationship between Apolipoprotein E (ApoE) and the aggregation processes of the amyloid b (Ab) peptide has been shown to be crucial for Alzheimer's disease (AD). ApoE4 is considered as a contributing risk factor for AD. Although various mechanisms have been proposed to explain the physiological and pathological role of this relationship, the detailed molecular properties of ApoE4 interacting with Abeta peptide are unknown. In our studies, a peptide-protein docking approach has been used to investigate the process of Ab interaction with the N-terminal domain of the human ApoE4 isoform. The use of molecular dynamics simulations (10 ns in water) has allowed studying the interaction mechanism between the protein and the peptide. Our results show that ApoE4 forms a partially unfolded intermediate (molten globule) stabilized by the interaction with Ab. The initial SDS-induced a-helix used as Ab peptide model, becomes unstructured due to the interaction with ApoE4. Peptide interaction with the different ApoE isoforms changed the pattern of the salt bridges network in ApoE4 compared to ApoE4 alone. By analysis and statistics of these electrostatic interaction patterns, we present a model for the salt bridge network in the ApoE4- Ab complex, crucial for the understanding of the interaction mechanism and relevant for potential drug design and therapeutics.

In-silico analysis of Neurokinin-1 at the sequence level: A preface for structural studies

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G protein-coupled receptors (GPCRs) are the largest family of transmembrane receptors in the mammalian genome and are involved in the regulation of a wide range of physiological processes through transmitting signals in response to different stimuli such as light, Ca^{2+} ions, nucleotides, odorants, amino acids, peptides or proteins to cells. GPCR super family is currently the largest class of therapeutic targets since they interact with more than 50% of prescription drugs and are still the best potential targets for drug design. The lack of high–resolution structures of GPCRs has limited the application of structure–based drug design on these targets. Neurokinin–1 receptor (NK1R) is a member of the family A of GPCR, which on modulation by substance P (SP) comprise a variety of physiological and pathophysiological conditions. Since during recent years we are involved in expression of NK-1 in different organisms, its further structural studies and crystalography, we are going to obtain 3D model structures of neurokinin receptor in more than 60 vertebrates in parallel. So far, a high–resolution structure of NK1R is not yet available and therefore alternative approaches must be used for building a model 3D–structure of the NK-1 receptor. As a preface, we used to evaluate the conservation of different domains, motifs and interacting sites of this protein in different organisms using Split of its amino acid sequence into known structural or functional domains. Consequently, we aligned each region of NK-1 considering all the vertebrates for which their genomic maps were already exposed in Ensemble genome database and accordingly the distances coefficients between them were obtained. Finally, we assessed distances and the corresponding coefficients to visualize the conservation and divergence ratios of NK-1 segments in the form of a plot using data analysis software. Moreover, the plot data confirmed the existence of high conservation of NK-1 among the phylogenetically nearer organisms. Obviously, different regions that are more conserved may be more important areas for both structure and function. Finally, it was concluded that the sequence of NK-1 is a highly conserved one. Therefore, both highly conserved regions and areas with higher rate of divergence were determined and are going to be assessed by means of NK-1 structural studies to determine their biological role.

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The voltage-dependent K^+ channel Kv1.3 in adipocytes

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The voltage-dependent K^+ channel Kv1.3 is involved in a myriad of physiological events. Thus, Kv1.3 functions in leukocytes, sensory neurons, vascular smooth muscle tone, insulin resistance and obesity. In most cases, Kv1.3 concentrates in specific membrane lipid microdomains, called lipid rafts. These domains are considered as signal platforms where signaling molecules and targets converge. Specialized forms of rafts are caveolae. These omega shaped structures present in multiple cell lines are highly abundant in adipocytes where they account for 30% of the plasma membrane surface. The structure is due to the presence of caveolin 1, which participates in the transport of cholesterol from endoplasmic reticulum to plasma membrane.

The role of Kv1.3 in adipocytes raises an important debate as it has been proposed that Kv1.3 could be a pharmacological target in obesity. Because the localization of the channel is important for its function, in the present work we studied the presence and localization of Kv1.3 in adipocytes. We have characterized the presence of Kv1.3 in rat and human adipocytes and before and after adipogenesis induction of 3T3-L1 cell line. Since adipogenesis induces caveolin 1 expression and the appearance of caveolae, we further analyzed the specific localization of Kv1.3. Our results indicate that adipogenesis triggers a relocalization of Kv1.3 in newly synthesized caveolae. Because most of insulin-dependent adipocyte signaling is located in these structures, our results bring light to the role of Kv1.3 and open new venues for therapeutic intervention aimed at controlling the channel expression level in obesity.

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Assessment of the conformational profile of bombesin, neuromedin B and neuromedin C by computational methods

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The family of bombesin-like peptides exhibit a wide spectrum of biological activities in the central nervous system. Most of the members, like bombesin (BN) are isolated from amphibian skin, whereas others, like neuromedin B (NMB) and C (NMC) are found in mammals [1]. Actions of the members of this family are mediated through at least three different G-protein coupled receptors: BB1, BB2 and BB3.

Whereas BN binds with high affinity to BB1 and BB2, NMB preferentially binds to the former and NMC to the latter [2]. Since the three peptides share high sequence identity at the C-terminus, the differential pharmacological behavior of NMB and NMC could be associated with differences in their respective conformational profiles, compared to that of BN. Accordingly, in the present work we report the results of a comparative analysis of the conformational profile of the three peptides, carried out using computational methods. Calculations were done in explicit solvent, using replica exchange molecular dynamics as a sampling technique [3]. Analysis of the respective conformational ensembles reveals that NMB and NMC attain conformations that also found in BN, but more importantly, we can identify a common conformations with BN not shared by between NMB and NMC, pointing them as putative bioactive forms, respectively.

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Visual phototransduction: from rhodopsin and cone opsin mutations to visual disease

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Rod and cone opsins are the visual pigments of the vertebrate retina belonging to the G-protein-coupled receptors superfamily, and characterized by having the 11-cis-retinal chromophore covalently bound to the opsin apoprotein. Upon photoactivation, these receptors bind and activate their specific G protein, transducin. Several mutations in rhodopsin and cone pigments have been associated with the retinal degenerative disease retinitis pigmentosa and with color blindness respectively. Our goal is to dissect the molecular mechanisms associated with different kinds of mutations underlying these visual alterations. For this purpose, Glu90Asp (1), Met39Arg, Val204Ile for rhodopsin and Cys203Arg for red and Trp177Arg green cone pigments, were constructed by site-directed mutagenesis, expressed in COS-1 cells, immunopurified in detergent solution and studied by UV-visible and fluorescence spectroscopies, and radioactive binding assays. All purified mutants were found to be correctly folded, with the exception of Cys203Arg and Trp177Arg cone pigments which showed no chromophore regeneration. Some mutants showed altered photobleaching and transducin activation. Furthermore, differences in the active conformation stability were observed between rhodopsin and the cone opsin pigments. Overall, these findings may clarify our understanding of the differences between rod and cone phototransduction and shed more light into the molecular mechanisms of inherited retinal disorders.

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The voltage-dependent K^+ channel Kv1.5 in B lymphocytes

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Voltage-dependent K^+ channels (Kv) play a pivotal role in the leukocyte physiology. Numerous Kv blockers have demonstrated that Kv participate in the cell cycle progression. Leukocytes have a limited repertoire of Kv. Although Kv1.3 is major in T-lymphocytes, macrophages also express Kv1.5, which controls many physiological processes. Concerning B-lymphocytes, scarce information is available. The aim of the present work was to analyze the Kv isoforms expressed in B-cells and to characterize any putative physiological role. By pharmacological treatment and electrophysiological recordings we report that, unlike Jurkat T cells, human Ramos and Raji B-lymphocytes express of both, Kv1.3 and Kv1.5 similarly to macrophages. Blockage of Kv channels by specific antagonists inhibited cell proliferation, which is of physiological relevance in lymphocytes. In addition, by lentivirical transduction of shKv1.5, which produced a knock-down of Kv1.5, we elucidate further the role of the channel in the B cell physiology by analyzing proliferation, migration and cell volume. Because Kv1.3 and Kv1.5 channels modulate proliferation and activation of different mammalian cells, these proteins have been analyzed by RT-PCR, immunoblotting and immunocytochemistry in a number of cell lines, lymphoid tissues, tumors and cancer cells from mouse and human samples. In most cancers, the expression patterns of Kv1.3 and Kv1.5 are remodeled. In addition, a correlation has been established between Kv1.5 abundance and grade of tumor malignancy (abundance of p53 and Ki67). Because potassium channels may play a pivotal role in tumor cell proliferation, these proteins should be taken into account when designing new cancer treatment strategies.

TUESDAY 10 TH OF JULY
S 13 - CELLULAR AND MOLECULAR NEUROBIOLOGY

Normalization of p75 levels prevents cognitive decline in a knock-in mouse model of Huntington disease

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Neuroprotective therapies based on BDNF administration have been proposed to treat Huntington´s disease pathology. However, reduction of TrkB levels in HD mouse models and HD human brain has been previously described by our group, suggesting that besides a decrease on BDNF levels a reduction of TrkB could also contribute to altered neurotrophic support in HD. In this study we have now analyzed the levels of the low affinity neurotrophin receptor p75NTR that it is well known to modulate Trk-mediated survival. We have shown an increase of p75NTR in the striatum and hippocampus of exon-1 (R6/1) and full-length (Hdh^{Q111}) HD mouse models. Importantly, in full-length heterozygous $Hdh^{Q7/Q111}$ mice and in exon-1 R6/1 mice we found that the increase on p75 expression was earlier than the decrease on TrkB levels. Notably, we have also confirmed an increase of p75 in caudate-putamen and hippocampus from human HD patients. Moreover, to analyze whether 75^{NTR} increase could underlie motor and cognitive deficits in HD, we have generated double mutant mice heterozygous for mutant huntingtin and p75NTR (HdhQ7/111:p75+/-). We cross-mated HdhQ7/111 mice with p75 NTRIII heterozygous to generate a double-mutant mouse with mutant huntingtin protein and reduced levels of p75. In these HdhQ7/111: p75 +/- animals, p75 levels are normalized in the striatum as well as in the hippocampus. Using different paradigms to evaluate cortico-striatal (strategy shiffting and accelerating rotarod) and hippocampal depending task (Novel Object recognition Test, T-maze spontaneous alternation test and Pasive Avoidance) , we detected that severe cognitive deficits in the HdhQ7/111 model were prevented in the double mutant mice. These findings demonstrate an important role for p75 in cognitive dysfunction in HD and suggest that modulation of p75 and TrkB expression or their signaling could become a therapeutic strategy for the treatment of memory deficits in this disease.

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Oxygen tension modulates glial cell lineage commitment through modifications on BMP7 expression

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Oxygen concentration is a vital parameter to control the differentiation of neural progenitors. Mild alterations of oxygen concentration during perinatal period can lead to cognitive and behavioural defects which are thought to be consequence of altered neural differentiation. Recent studies demonstrated that changes in neural cells differentiation produced by hypoxia are in part due to modifications in Bone Morphogenetic Proteins (BMP) signalling. We have focused in BMP7, because it is known to exert a neuroprotective action in response to hypoxia. We analyzed whether there are BMP7 expression changes induced by hypoxia and if these changes are associated with modifications in cortical progenitors. Our results showed that mild hypoxia provokes an initial decrease of BMP7 expression followed by an increase in the number of NG2-positive oligodendroglial progenitors in cortical cultures. We used the hypobaric chamber as a prenatal hypoxic model that allows analyzing deleterious effects on cognitive function associated to reduced oxygen availability. As happened in vitro, E16 animals subjected to hypobaric hypoxia showed an immediate decrease of BMP7 levels after hypoxic treatment, followed by an increased immunoreactivity for the oligodendroglial markers NG2 and Olig2. We corroborated these results using gain and lose of function models of BMP7 both in vivo and in vitro, through treatments with BMP7 and its inhibitor follistatin (FST), respectively. While FST treatments mimic the effects observed in hypoxic conditions, increasing the immunoreactivity for oligodendroglial markers, BMP7 treatments increase the expression of the astroglial marker GFAP, and decrease the levels of the oligodendroglial markers NG2 and Olig2. Thus, aberrant BMP7 expression modifies the differentiation of cortical progenitors during perinatal development that might lead to brain circuitry alterations underlying behavioural and cognitive disorders.

Activity-dependent gene transcription and memory in alzheimer´s disease

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Memory processing depends on synaptic plasticity changes mediated by expression of specific gene expression programs. Gene expression changes in the brain are associated with cognitive decline during aging, but the molecular mechanisms underlying deregulation of gene programs leading to memory loss in age-related memory disorders are largely unknown. We performed gene expression profile analysis to identify genes potentially linked to memory impairment in a transgenic mouse model of Alzheimer´s disease expressing a mutant β-amyloid precursor protein (APPSw,Ind). Gene-annotation enrichment analysis revealed a network of genes comprising biological pathways such as neurotransmission, learning, long-term potentiation and depression and oxidative phosphorylation differentially expressed in the hippocampus of APPSw,Ind mice. We specifically found a transcriptional program regulated by the cAMP-responsive element binding protein (CREB)-regulated transcription coactivator-1 (CRTC1) that is deregulated at early pathological and cognitive deficits in APPSw,Ind mice. Disruption of CRTC1-dependent transcription is mediated by reduced dephosphorylation and nuclear translocation of CRTC1 in the hippocampus of memory-impaired APPSw,Ind transgenic mice. Importantly, CRTC1-dependent transcription is specifically activated in response to neuronal activity and spatial memory training. Together, these findings suggest that CRTC1 plays a key role in coupling synaptic activity to gene transcription required for hippocampal-dependent memory.

Disrupció de les oscil·lacions corticals de baixa freqüència per fenciclidina: un model vàlid per al cribatge de nous fàrmacs antipsicòtics.

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Els antagonistes no competitius del receptor NMDA com la fenciclidina (PCP) són àmpliament utilitzats com a model farmacològic d'esquizofrènia. Dades prèvies del nostre laboratori mostren que la PCP disminueix les oscil·lacions corticals de baixa freqüència (OCBF, <4 Hz) en l'escorça prefrontal medial de rates i ratolins anestesiats[1,2]. Aquesta alteració és revertida pels antipsicòtics (APS) haloperidol i clozapina. L'objectiu d'aquest treball és 1) examinar la possible participació de la transmissió glutamatèrgica i/o gabaèrgica en l'efecte de la PCP 2) validar aquest model per al cribatge de fàrmacs APS e identificació de dianes terapèutiques.

Els fàrmacs avaluats són: 1) els agents glutamatèrgics NBQX i LY379268, 2) l'agent gabaèrgic muscimol, 3) APS atípics amb afinitat preferencial pels receptors 5-HT2A (olanzapina, ziprasidona, risperidona i quetiapina), 4) els APS clàssics amb alta afinitat pels receptors D2 (clorpromazina i perfenazina) i 5) controls negatius (l'ansiolític diazepam i l'antidepressiu citalopram).

Els APS, l'LY379268 reverteixen significativament la disrupció de les OCBF causada per la PCP. Per altra banda, l'antidepressiu citalopram no reverteix aquests efectes i el diazepam i el muscimol ho fan de forma parcial. Finalment, l'NBQX no reverteix l'efecte de la PCP sobre les esmentades oscil·lacions.

Els presents resultats suggereixen que una activació de la transmissió glutamatèrgica excessiva està implicada en els efectes de la PCP tenint en compte els efectes de LY379268 i el muscimol. Per altra banda, els APS de famílies diferents comparteixen la capacitat de restablir aquestes oscil·lacions malgrat les diferents dianes receptorials. En definitiva, aquest estudi recolza la utilització del model de supressió de les OCBF per la PCP per a l'avaluació de fàrmacs APS potencials.

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Ajudes: FIS PI 09/1245, EU grant NEWMEDS (IMI; Innovative Medicines Initiative).

Neurophysiological alterations in a mouse model of proliferative retinopathy

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Diabetic retinopathy is the leading cause of loss of visual acuity and blindness in adulthood and constitutes an unmet medical need. Most diabetic rodents do not develop a complete phenotype of proliferative retinopathy, but only mild vascular alterations. In contrast, transgenic mice overexpressing Insulin-like Growth Factor (Tg-IGF-I) in the retina have vascular alterations that evolve in a progressive manner through the animal's life, similar to those of the human disease. The aim of this work was to determine whether neuronal and glial function were also affected in these animals, as observed in human diabetic patients. To do so, neuronal functionality was analyzed by electroretinography in TgIGF-I at different ages. Neuronal populations were quantified by morphometric analysis. The expression of genes key to glial metabolism were studied by qRT-PCR or Western blot. No alterations were observed in the ERG of 3 months-old TgIGF-I mice. At 6 months of age, abnormal responses were detected in some but not all TgIGF-I, but it was not until 7.5 months of age that differences became statistically significant. Furthermore, all mice aged 9 months were completely blind. By histological analysis, decreased populations of bipolar, amacrine and ganglion neurons were found at 7.5 months of age. Moreover, rod photoreceptors showed decreased outer segment length. Gliosis and microgliosis were detected in transgenic mice at early ages. Transgenic mice showed alterations in the glutamate metabolism, signs of oxidative stress and impaired potassium buffering. Increased production of pro-inflammatory cytokines such as TNF-α and MCP-1 were also observed at this age. In summary, TgIGF-I mice presented a progressive decline in retinal neuronal function and reduced cellular populations. Glial dysfunction may underlie neuronal damage in transgenic retinas. Most of these glial alterations have also been reported in human diabetic retinas, making TgIGF-I a suitable model for the study of new therapeutic approaches for diabetic retinopathy.

SC-51089 chronic treatment decreases motor and cognitive deficits in mouse model of huntington's disease

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A large number of studies have demonstrated a neurotoxic function of the prostaglandin E_2 EP1 receptor in models of cerebral ischemia and inflammation. Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder caused by an expanded CAG/polyglutamine repeat in the coding region of the *huntingtin* (*htt*) gene that is characterized by psychiatric, motor and cognitive symptoms. In this study, we want to study the possible therapeutic role of prostaglandin E_2 EP1 receptor in Huntington's disease pathology using R6/1 model of HD that exhibits neuropathological features of HD. For this aim we administrated an EP1 receptor antagonist, the SC-51089, by intraperithoneal (i.p.) osmotic pump system implantation. First, to analyze whether i.p. osmotic pump administration of SC-51089 arrives to the central nervous system, we study the neuroprotective effect against excitotoxicity of SC-51089 in WT mice by intraestriatal quinolinic acid (QUIN) injection. We observed a protective effect of SC-51089 against QUIN-mediated cell death by Fluorojade staining 10 days after osmotic pump implantation. Then, to explore whether SC-51089 reduce neurological phenotype of HD, four-teen weeks-old R6/1 mice were chronically treated with SC-51089 during 28 days and motor and cognitive deficits were assessed by different paradigms. We demostrated that SC-51089 chronical administration reduce clasping in R6/1, a neurological characteristic phenotype of these mice. Motor coordination and balance were assessed between 10 and 18 weeks of ages and we shown significant improvements in the rotarod, the balance beam and the vertical pole task in SC-51089 R6/1-treated-mice. Additionally, chronic EP1 receptor antagonism reverses R6/1 long-term recognition memory deficits in the T-maze task and in the novel object recognition test at 15 and 17 weeks of age. In agreement with these behavioral improvements we observed increased expression in the striatum and hippocampus of some synaptic markers that are down-regulated in R6/1 mice. Thus, our results suggest that chronic administration of EP1 receptor antagonist attenuated motor and long-term spatial and recognition memory deficits in R6/1 mice. With these findings, we propose that antagonism of EP1 receptor could become a good therapeutic strategy for the treatment of motor and memory deficits in HD patients.

Financial support was obtained from Ministerio de Ciencia e Innovación (SAF2008-0436 and SAF2011-29507) Fondo de Investigaciones Sanitarias (Instituto de Salud Carlos III, PI10/01072; RETICS, R006/0010/0006) and Generalitat de Catalunya (2009SGR-00326).

Parkin loss of function leads to RTP801 accumulation and neurodegeneration in parkinson's disease

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RTP801/REDD1 protein is induced in cellular and animal models of Parkinson's disease (PD) and in SNpc (Substantia nigra pars compacta) degenerating neurons of PD patients. Elevation of RTP801 is necessary and sufficient to mediate neuron death in cellular and animal models of PD, by a sequential mechanism that inactivates key kinases mTOR and AKT. PARK2 gene encodes a RING domain E3 ubiquitin ligase named Parkin that targets proteins for signaling or proteasomal degradation. In humans, mutations in the PARK2 gene are associated to the appearance of an autosomal recessive juvenile form of PD (AR-JPD).

Here, we report that RTP801 is degraded by the ubiquitin proteasome system and that its ubiquitination is, at least in part, Parkin E3 ligase activity dependent. We confirmed that Parkin loss-of-function triggers a toxic accumulation of RTP801 in both *in vitro* and *in vivo* models of PD. Furthermore, we found that RTP801 protein levels were increased in Parkin knockout mice brains, in human postmortem SNpc neurons of PD and AR-JPD patients, and in cultured human fibroblasts derived from mutant Parkin patients.

Altogether, these results place RTP801 as a novel Parkin substrate and as a potential therapeutic target for both idiopathic and genetic forms of PD with impaired Parkin functions.

Effect of acute exposure to cocaine on gene expression in a dopaminergic neuronal model

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Cocaine reward and reinforcing effects are mediated mainly by dopaminergic neurotransmission. The aim of this study is to assess gene expression changes induced by an acute 30-minutes cocaine exposure in SH-SY5Y neuroblastoma cells, which have been widely used as a dopaminergic neuronal model after differentiation with retinoic acid. SH-SY5Y cells were successfully differentiated to dopaminergic neurons as shown by phenotypic changes compatible with neuronal-like morphology, expression of dopaminergic markers and decreased S and G2/M phases. Cytotoxic effects of cocaine were discarded under different cocaine concentrations up to 20 µM and during 48 hours. Evaluation of neuronal activity through calcium fluorescence imaging before and during the exposure to 1 and 5 µM cocaine showed that the cells became active immediately after the exposure to the drug. Gene expression data from Affymetrix microarrays were obtained at different cocaine concentrations (0, 1 and 5 µM) and two time-points after drug exposure (6 and 24 hours). As expected from the lack of cytotoxic effects in these cells, the expression levels of inflammatory or immune response genes did not change either. Gene expression changes were only observed at 6 hours after 5 µM cocaine exposure, involving genes related to regulation of transcription and gene expression, cellular movement and neuronal adaptations. Five of these genes, encoding proteins with functions such as neurite formation and outgrowth and axon guidance, were validated by qRT-PCR. No differences were observed after 24 hours of exposure. Our experiments in this dopaminergic model suggest that a single acute exposure to cocaine cause specific alterations in gene expression that could lead to neuroadaptive changes.

TUESDAY 10 TH OF JULY
S 14 - SYSTEMS BIOLOGY

Organization Principles in Biology

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This talk will start by discussing the importance of model organisms in biology and presenting a method to choose organisms that are more likely to be good models from which to extrapolate results about the system with want to study to other organisms.

Then, it will be argued that model organisms, together with other research, has led to the identification of regularities in molecular biology. Such regularities can be due to selective advantages and might constitute biological organization principles.

A method to analyze and identify such organization principles, that of Mathematically Controlled Comparisons, will be presented and an application to bacterial signal transduction will be discussed.

Boundary Formation in Cell Populations: from Gene Regulation to Tissue Mechanics

Javier Buceta

PCB - UB

In this talk I will review the mechanisms involved in the establishment of boundaries between cellular compartments using the dorsoventral boundary of the wing imaginal disc as a case study. Thus, I´ll show that a positive feedback-loop between boundary and non-boundary cells and mediated by the activities of Notch and Wingless leads to the establishment of a Notch dependent organizer at the boundary and that the complex interplay between the cytoskeleton mechanics, the cell cycle, the cell growth, and the cellular interactions is also key in order to shape an organizer as a robust source of positional information and a lineage controller.

Integration of proteomic and genome-wide data to understand Polycomb function on mouse embryonic stem cells differentiation

Luciano Di Croce

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Polycomb group proteins are essential regulators of cell fate decisions during embryogenesis. In mammals, at least five different Cbx proteins (Cbx2, -4, -6, -7, and -8) are known to associate with the core Polycomb Repressive Complex-1 (PRC1). I will present data showing that pluripotency and differentiation of mouse embryonic stem (ES) cells is regulated by different Cbx-associated PRC1 complexes with unique functions. Maintenance of pluripotency primarily depends on Cbx7, while lineage commitment is orchestrated by Cbx2 and Cbx4. At the molecular level, we have uncovered a Polycomb auto-regulatory loop in which Cbx7 represses the expression of pro-differentiation Cbx proteins, thereby maintaining the pluripotent state. I will additionally present data supporting that the occupancy of Cbx7 on promoters is completely dependent on PRC2 activity but only partially dependent on a functional PRC1 complex. Thus, Cbx proteins confer distinct target selectivity to the PRC1 complex, achieving a balance between the self-renewal and the differentiation of ES cells.

Ligand expression ahead of neurogenic wavefronts: a new design principle?

Pau Formosa-Jordan[1,2], Marta Ibañes[1], Saúl Ares[2,3] and José María Frade[4]

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Neurogenesis in metazoans is regulated by lateral inhibition dynamics of the Delta/Notch signaling system. These dynamics enable the singling out of precursors to become neurons from groups of equivalent cells. Frequently, neurogenesis is a dynamical process that involves a propagating neurogenic wavefront that spreads through non-neurogenic regions in response to specific morphogens. Since lateral inhibition involves signaling between cells, the question arises of how do non-neurogenic regions affect lateral inhibition at the neurogenic wavefront To explore this issue, we provide experimental evidence for generalized Notch-independent Delta ligand (*Dll1*) expression in non-neurogenic areas of the developing chick retina. We formulate a mathematical model for neurogenic wavefront propagation based on lateral inhibition dynamics induced by a diffusing morphogen. Through mathematical modeling we predict that the absence of ligand expression ahead of the neurogenic wavefront disrupts different aspects of the neurogenic process. These predictions are consistent with previous observations. We extend our computational study to morphogenetic furrow progression in the developing Drosophila eye and find equivalent predictions. Taken together, our results suggest that Notch-independent ligand expression ahead of the neurogenic wavefront is as a general developmental mechanism for robust neurogenesis [1].

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Defining the DNA interactome in a minimal bacteria

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Identifying the whole set of transcription factors in an organism is key to unveil its physiology and response to stimuli. So far, no such unbiased, systematic method has been developed for any species. Here we present the attempt to define all DNA interacting proteins, both regulators and structural proteins, in a very simple bacterial species, *Mycoplasma pneumoniae*. This organism has a handful of annotated transcription factors but however depicts a complex transcriptional regulation. It has the advantage of bearing less than 700 genes due to progressive genome reduction, therefore representing very likely, the minimal number of regulators necessary to sustain live. Several biochemical methods (DNA affinity columns, chromatin isolation , etc) were used to get a reliable set of DNA interacting proteins. This proteins were: produced recombinant (to validate the DNA binding), overexpressed *in vivo* (to get a phenotype), subjected to chromatin immunoprecipitation (to identify their target motifs) and in some cases, motifs were validated by *in vitro* binding analysis. Interestingly, many moonlighting proteins were found, that is, proteins such as enzymes, that had already a know function, indicating the cross-talk between gene expression and other cellular processes like metabolism.

Precision of the Quorum Sensing Switch: Stochastic and Non-equilibrium Effects

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We propose a model of the quorum sensing (QS) network in Vibrio fischeri that takes into account the key regulatory elements of the LuxR/LuxI signaling network, the autoinducer transport, and the cellular growth and division dynamics. By using both deterministic and stochastic approaches, we analyze the response and dynamics at the single-cell level and compare it to the global response at the population level. In this framework, we introduce the concept of precision and show that the response of the LuxR/LuxI system depends on the interplay between non-equilibrium and stochastic effects and that the burst size of the transcription/translation noise at the level of LuxR controls different features of the QS switch. All in all, our in silico experiments of the QS switch reveal a highly dynamic mechanism that allows for plasticity and suggest a trade-off between the activation onset of the operon and the collective response of the population

Using Systems Biology To Learn About The Synapse Role In Neurologic And Psychiatric Disorders

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Synapses are key to nervous system function. The long-term events behind cognitive abilities, such as learning and memory, occur mainly within the synapse. The protein machinery at the postsynaptic side is organised into a very large protein complex, called the postsynaptic density (PSD), containing hundreds of different proteins, including the neurotransmitter receptors and all the signalling machinery that ultimately encodes electrical activity into high brain functions.

We have isolated the postsynaptic density from the human cortex for the first time and, through a proteomics approach, have identified around 1500 different protein components. By integrating different sources of mammalian and human phenotypic information we have performed a study of the role of the PSD in brain diseases. This Systems Biology approach has allowed us to study the clinical relevance of this molecular machine as a whole system. From this analysis we have learned two main things. Firstly the PSD is a structure with a very large disease burden, containing many more proteins causing disease than anticipated. This is reflected by the fact that genes expressed in the PSD are responsible for more than 130 inherited neurological and psychiatric disorders. Furthermore, we have been able to elucidate to what disease types is the PSD most relevant, concluding that this structure is particularly important to Mental and Behavioural Disorders, such as Intellectual Disabilities, as well as Motor Disorders.

TUESDAY 10 TH OF JULY
S 15 - MOLECULAR BIOLOGY IN MODEL ORGANISMS

Models diversos en Genètica Molecular de Plantes: Arabidopsis thaliana, blat de moro, meló.

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La Genètica va tenir un dels seus inicis en l'anàlisi dels caràcters transmissibles entre generacions en les plantes. La importància de tenir una agricultura productiva ha portat des de sempre que es busquessin maneres d'assegurar que les llavors que es plantaven tinguessin les característiques que el pagès necessita. A partir del segle XVIII aquesta necessitat es va concretar en l'aparició de professionals de la producció de llavors i a partir del XIX es va anar desenvolupant la teoria de la transmissió de caràcters que va culminar amb els treballs de Mendel amb el pèsol. Les **Cucurbitàcies** van ser en aquesta època un dels sistemes més estudiats.

Amb la descoberta de les lleis de Mendel a principis del segle XX, la Genètica es va constituir com a ciència i ben aviat les particulars característiques del **blat de moro** i el seu interès com a planta cultivada van fer que aquesta espècie es convertís en la planta model per excel·lència per la Genètica. La producció d'híbrids, la definició dels anomenats caràcters quantitatius o la descoberta dels elements mòbils són algunes de les fites assolides en aquest estudi.

Quan les tècniques moleculars van estar disponibles va ser evident l'avantatge que oferia una planta amb un cicle de vida curt i un genoma petit com és ***Arabidopsis thaliana***. L'any 2001 es va publicar la seqüència del seu genoma, que és un dels de millor qualitat que estan disponibles. Però les plantes són un mon divers degut a la diversitat de la seva fisiologia, dels seus genomes i dels seus usos. Per això estem obtenint una multitud de genomes d'espècies vegetals, el darrer dels quals és el genoma del **meló**, una espècie de les més utilitzades en alimentació i en els estudis de la Biologia vegetal, sobre tot en els països mediterranis.

Dma1: E3 ligase cell cycle regulated?

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G1 progression in yeast is controlled by two CDK proteins: CDC28 and PHO85. CDC28 is associated with two G1 cyclins (CLN1 and CLN2), whereas PHO85 is associated with PCL1 and PCL2. Both teams of cyclins present high level of redundancy in their substrate specificity and for this reason it is though that both kinases have partially redundant functions. Our group is interested to uncover the specific physiological relevance of PHO85 in G1 progression. For this reason, we decided to find independent substrates of both kinases.

We performed pulldown experiments of PCL1-TAP and CLN2-TAP proteins from G1 synchronized cells and looked for their associated partners by mass-spect. Among others, we found that PCL1 associates with DMA1 while CLN2 does not. DMA1 is an E3-ubiquitin ligase that in mammals controls several aspects of cell cycle progression as well as is downregulated in many type of cancer and that in *S. cerevisiae* regulates spindle positioning and septin ring assembly. We demonstrate that DMA1 is phosphorylated *in vitro* by the complex PHO85-PCL1 in a specific serine and threonine residues, as well as *in vivo*. We also confirm, by CoIP experiments, that both proteins interacts *in vivo*. Moreover, *in vitro* assays of ubiquitin ligase activity seems to show that DMA1 activity is downregulated by PHO85 phosphorylation. These preliminary results suggest that during G1 phase PHO85 could be controlling DMA1 activity.

Our efforts are focused now in research the physiological relevance of these set of biochemical reactions.

Protection of diet-induced obesity and insulin resistance in transgenic mice overexpressing HMGA1 in adipose tissue

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Adipocyte functionality is lost during obesity and has been related to impaired transcriptional regulation of the key factors that control adipogenesis. Among them, the high mobility group A1 (HMGA1) proteins may play an important role in adipogenesis. To examine the effects of adipose tissue HMGA1 expression in the development of obesity and insulin resistance, we generated transgenic mice overexpressing HMGA1 specifically in adipose tissue (aP2-HMGA1). HMGA1 expression in fat tissues was markedly increased compared with control animals. Intriguingly, HMGA1 transgenic mice showed decreased white (WAT) and brown (BAT) adipose tissue weight and lower triglyceride content than control mice. These transgenic mice were normoglycemic and normoinsulinemic and no differences in glucose tolerance and insulin sensitivity were observed between groups when fed a standard diet. In contrast, under a high-fat diet (HFD), body weight gain was lower in transgenic than in control mice. This was parallel to lower WAT and BAT weight. Transgenic mice also showed increased glucose tolerance and whole body insulin sensitivity and decreased levels of serum free fatty acid, triglyceride and glycerol compared to controls. Strikingly, the overexpression of HMGA1 in adipose tissue of transgenic mice led to a marked decrease in the expression of genes involved in fatty acid metabolism and in mitochondrial biogenesis and function, suggesting mitochondrial dysfunction of the adipose tissue in these transgenic mice. We are further analysing the mechanisms by which HMGA1-mediated BAT dysfunction in transgenic mice protects them against high-fat diet-induced insulin resistance and obesity.

Supported by grants from the Ministry of Science and Innovation of Spain (SAF2008-03083 and RYC-2006-001955) and the European Union (MIRG-CT-2007-207745).

Deficiency of cyclin D3 contributes to the apoptosis of beta cell and development of TYPE 1 diabetes in NOD mice

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Using the Microarray technology we have identified genes that have downregulation in pancreatic islet endocrine cells during the autoimmune attack progression, one of which encodes for cyclin D3 that is a protein involved in cell cycle because its activity triggers cell cycle progression through G1 phase towards the S phase. Curiously, cyclin D3 has also been involved in cell cycle-independent processes, such as adipogenesis, the inhibition of granulocyte differentiation, and can also bind certain transcription factors and activate inflammation process and development of the T cells (NFκB, GATA). Hence there may exist for this protein unknown roles related to pancreatic beta cell, besides cell proliferation, that could explain why cyclin D3 transcript downregulation due to the autoimmune attack, leads to beta cell death and, then, to T1D. We have results concerning about the natural history of the RIPCcnD3 Tg mice which have less percentage and develop the disease late than a wild type and a cyclin D3 deficient NOD mice that develop exacerbated diabetes when compared to wild type littermates. Therefore cyclin D3 protects from the disease in a diabetic phenotype.

Hematopoietic stem cell gene therapy corrects biochemical imbalances in a murine model of MNGIE.

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Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a disorder caused by mutations in the *TYMP* gene that encodes the enzyme thymidine phosphorylase (TP). TP catalyzes the first step in the catabolism of the nucleosides thymidine (Thd) and deoxyuridine (dUrd). In MNGIE patients, TP dysfunction results in systemic Thd and dUrd overload, which selectively impair mitochondrial DNA replication. Patients develop initial symptoms at a mean age of 19 years with death occurring at a mean age of 37 years. The only treatment that has been shown to be effective for MNGIE at long term is the allogeneic hematopoietic stem cell transplantation; however there are high rates of mortality and morbidity related with this treatment.

We tested the feasibility of lentiviral gene therapy, as an alternative treatment, in preclinical studies with a human cell and a murine MNGIE model of the disease. In both cases the introduction of a functional copy of the human *TYMP* gene stably restored TP activity. In partially myeloablated mice, transplantation of hematopoietic stem cells transduced with the therapeutic lentiviral vector allowed TP expression in hematopoietic cells with a concomitant reduction of Thd and dUrd to levels similar to those observed in wt mice in plasma and different tissues. This biochemical pyrimidine homeostasis restoration was achieved with mild bone marrow molecular chimerism and was maintained at least for 18 months *in vivo*, the whole period monitored. Our results constitute a proof of concept of the feasibility of gene therapy for MNGIE, and support that this approach may be an alternative treatment for this disorder in the future.

AAV9-sulfamidase vector delivery to the cerebrospinal fluid corrects brain and somatic pathology in mpsiiia mice and results in detectable enzyme levels in dogs

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For most lysosomal storage diseases (LSD), particularly those affecting the central nervous system (CNS), there is currently no cure. The blood-brain barrier (BBB) that limits bioavailability of drugs administered systemically, combined with the short half-life of certain lysosomal enzymes, hamper the development of effective therapies for LSDs. Mucopolysaccharidosis Type IIIA (MPSIIIA) or Sanfilippo A syndrome is an autosomic recessive LSD presenting with CNS and peripheral disease caused by deficiency in sulfamidase, a sulfatase involved in the stepwise degradation of the glycosaminoglycan (GAG) heparan sulfate. Here we demonstrate that intra-CSF administration of a serotype of AAV vector with broad biodistribution profile, AAV9, encoding for the sulfamidase transgene mediates widespread correction of both CNS and peripheral pathology in a mouse model of MPSIIIA disease. Four months after intracisternal administration of AAV9-Sulfamidase, increased enzyme activity was detected throughout the brain of treated MPSIIIA mice, resulting in correction of GAG storage, lysosomal distention, cellular ultrastructure, and neuroinflammation. In the periphery, high sulfamidase activity was detectable in the liver and in the circulation with whole-body normalization of GAG accumulation and lysosomal pathology. Importantly, treated MPSIIIA mice had normal locomotor activity, showed correction of behavioral deficits, and had extended lifespan with a mean survival similar to that of healthy littermates. Scale up to healthy Beagle dogs was performed by both intracisternal and intracerebroventricular delivery, resulting in transgene expression throughout the CNS and liver, and increased sulfamidase activity detectable in the CSF. The results presented herein support the clinical translation of this approach for the treatment of MPSIIIA and other LSD with CNS involvement.

TUESDAY 10 TH OF JULY
S 16 - BIOPHYSICS

Direct observation of stalled fork restart and lesion bypass via fork regression in T4 replication system

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Restarting a stalled replication fork is a major challenge for DNA replication. Depending upon the nature of the damage, different repair processes might be triggered; one of which is bypass of a leading strand lesion via a chicken-foot structure formed by fork regression. Using micromanipulation techniques to study the T4 bacteriophage enzymes, we have reproduced in vitro the complete process of bypassing a leading strand lesion. We show that the UvsW DNA helicase in cooperation with the T4 holoenzyme can overcome such damage by a pseudo stochastic process periodically forming and migrating a four ways Holliday junction. We show that this process is regulated by the inhibition of UvsW by the replicative helicase and not by the polymerase or single-stranded binding protein, suggesting that helicase departure initiates the lesion bypass mediated by fork regression pathway.

Probing G-protein-coupled receptor dimerization and its role in depression

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G-protein-coupled receptor (GPCR) dimerization has been established as an important mechanism in GPCR function and signalling. Recently, galanin receptor 1 (GalR1) and serotonin receptor 1A (5-HT_{1A}) have been shown to form heterodimers in the brain which may play a role in depression. It is not clear how the dimerization process occurs, and which are the specific receptor motifs involved in this process. This structural information is needed for a better understanding of the dimerization process, in order to develop new therapeutic agents. We have used a novel bioinformatics approach to predict aminoacid triplets that would be involved in the dimer interface. We have used GalR1 and 5-HT_{1A} and site-directed mutagenesis to change some of these sequences. In addition, we have also used zinc as potential modulator of these interactions. HEK-293T cells co-transfected with GalR1 and 5-HT_{1A} have been studied by means of FRET spectroscopy. FRET has been used to track dimerization processes in wild-type and mutated receptors exposed to different experimental conditions. Mutation of LLG triplets to AAA (positions 153-155 and 380-382) in 5-HT_{1A} receptor produced an increase in the dimerization affinity between GalR1 and 5-HT_{1A}, whereas zinc exposure showed the opposite effect. Moreover, GalR1 homodimerization was not affected by zinc, suggesting that the zinc effect is mediated by 5-HT_{1A} receptors. In conclusion, we find that the mutants studied seem to enhance dimerization, and that zinc could be a potential dimerization modulator for 5-HT_{1A} receptors. This finding may provide the molecular basis for the therapeutic use of zinc in depression treatment.

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Unraveling nucleic acids and proteins using single molecule methods

Felix Ritort

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Recent developments in micro and nano-technologies allow for the controlled manipulation of individual molecules by exerting and detecting forces in the piconewton range [1]. Molecular unzipping is a force-induced reaction that makes possible to disrupt the bonds that hold molecular structures in nucleic acids and proteins. In this way, for example, a double stranded DNA molecule can be converted into two individual single strands by pulling apart the two strands (molecular unzipping) [2]. The capability of single molecule techniques of detecting weak forces together with the ability of measuring extensions with nanometer resolution allow scientists to monitor molecular reactions in real time (e.g. molecular folding). In this talk I will show some of the most recent applications of molecular unzipping in our lab that make possible to derive base-pair free energies in DNA and RNA with unprecedented accuracy and extract folding free energies of proteins with 1kcal/mol resolution [3,4].

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Uq behaviour in biomimetic membranes of dPPC and MGDG

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The light depending reactions of the photosynthetic process take place in the thylakoid membrane which is based on galactolipids being the most important monogalactosyldiacylglycerol, MGDG. One of the main electron and proton shuttle molecules in green plants is plastoquinone (PQ)1. On the other hand, ubiquinone (UQ) is present in eukaryotic cells at the hydrophobic core of the phospholipid bilayer, where plays the role of electron and proton shuttle2. Several attempts have been done to achieve artificial photosynthesis using several quinones3 for electron transport instead of using PQ which has an elevated isolation process cost. UQ and PQ are very close in size and shape, making UQ a suitable candidate for substituting PQ. In response of that, studies have been performed to understand PQ or UQ position and some other characteristics of these molecules1,4. Langmuir-Blodgett technique and supported planar bilayers have been revealed as successful techniques to prepare natural mimicking environment. So that, we have prepared Langmuir and Langmuir-Blodgett films to study UQ content influence on the lipid matrix using a model phospholipid (DPPC) and MGDG. The results show a similar behaviour for UQ in the natural mimicked conditions as PQ producing similar distortions on the lipid matrix, confirming these observations with AFM images. The electrochemistry of UQ in these conditions reveals the position of UQ headgroups in the lipid matrix and the candidature of this molecule for electron shuttle in artificial photosynthesis.

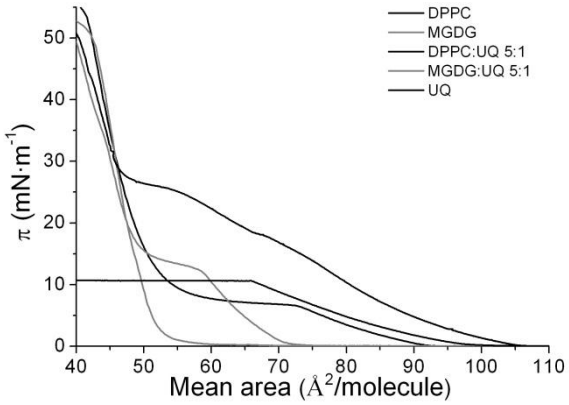

Figure 1. Isotherms for pure components DPPC,MGDG and UQ and for the mixtures DPPC:UQ 5:1 and MGDG:UQ 5:1.

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Biochemical changes in a rat model of stroke assessed by *in vivo* Magnetic Resonance Spectroscopy (MRS) and *ex vivo* High Resolution Magic-Angle Spinning (HRMAS).

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Background: Stroke is a disease caused by the interruption of blood flow causing changes in brain metabolic profile that can be detected *in vivo* with MRS. However, this technique has low spectral resolution. *Ex vivo* HRMAS has better resolution and detects more brain metabolites than MRS, but the spectroscopic pattern could change due to post-mortem metabolism. This can be avoided using focused microwave irradiation (FMW) for animal sacrifice, which arrests metabolic processes. The aim of this study has been to determine biochemical changes in rat brain after stroke using both MRS and HRMAS approaches.

Methods: Sprague-Dawley rats were subjected to 90-minute transient right middle cerebral artery occlusion. MRS at 7T was performed in controls (n=3) and in stroke animals at 1 (n=3) and 7 days (n=3) after the insult. Animals were sacrificed by FMW and brains were dissected in samples from stroke and contralateral regions. HRMAS at 9.4T and 37ºC was then performed. Unit length normalized peak heights were used for analysis. Statistical differences were assessed with Student's t-test.

Results: Metabolism was arrested with FMW as shown by the detectable phosphocreatine/creatine (3.95/3.93 ppm) ratio 0.80±0.19 in contralateral samples using HRMAS. Significant changes between stroke and contralateral regions were detected at the studied time points (1 and 7 days) with both techniques in several metabolites. Highest change (5.3 fold increase) for MRS and HRMAS was seen in apoptotic mobile lipids (2.80ppm) at 7 days. Additionally, HRMAS detects differences from choline (3.19ppm), phosphocholine (3.21ppm), glycerophosphocholine (3.23ppm) and phosphatidylcholine (3.25ppm) not resolvable from in vivo MRS. A maximal increase, up to 1.4 fold at 7 days was detected in phosphocholine (3.21ppm).

Conclusion: MRS and HRMAS techniques successfully detect changes in stroke and contralateral brain regions after the insult. Combining both techniques could significantly improve the metabolomic characterization of infarcted brain tissue in preclinical studies.

Structural and biochemical insights into the human mitochondrial transcription factor A with the light strand promoter

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Mitochondria contain their own DNA (mtDNA), which is expressed and replicated by nucleus-encoded factors imported into the organelle. Human mitochondrial transcription factor A, TFAM, is essential for mitochondrial DNA packaging and maintenance and also has a crucial role in transcription. Crystallographic analysis of TFAM in complex with an oligonucleotide containing the mitochondrial light strand promoter (LSP) revealed two high-mobility group (HMG) protein domains that, through different DNA recognition properties, intercalate residues at two inverted DNA motifs. This induced an overall DNA bend of ~180°, stabilized by the interdomain linker. The mode of DNA bending induced by TFAM shows a remarkable parallel with the HU protein family, which have analogous architectural roles in organizing nucleoids in bacteria. In TFAM, the U-turn allows the C-terminal tail, which recruits the transcription machinery, to approach the initiation site, despite contacting a distant DNA sequence. We also ascertained that structured protein regions contacting DNA in the crystal were highly flexible in solution in the absence of DNA. According to the SAXS experiments, TFAM has two highly disordered, non-structured regions in solution, the linker and the C-terminal tail suggesting structural reorganization and fitting upon DNA binding. Finally, our data suggest that TFAM bends LSP to create an optimal DNA arrangement for transcriptional initiation while facilitating DNA compaction elsewhere in the genome.

WEDNESDAY 11 TH OF JULY
S 17 – CELL SIGNALLING IN THE NERVOUS SYSTEM

Role of hippocampal nNOS/cGMP pathway in cognitive impairment in Huntington's disease

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The nNOS/cGMP pathway is implicated in synaptic plasticity, and in learning and memory processes. nNOS protein levels/activity are reduced in the striatum and cortex of Huntington's disease (HD) mouse models, and nNOS mRNA levels are decreased in the caudate of HD patients. Both patients and mouse models show learning and memory impairment even before the onset of motor symptoms. In this work we investigated the nNOS/cGMP pathway in the hippocampus of the R6/1 mouse model of HD to determine whether it can be a good therapeutic target for cognitive improvement in HD. We found that hippocampal nNOS levels in R6/1 mice at 8 weeks of age were unchanged compared with wild-type animals. In contrast, a severe down-regulation of nNOS was observed in R6/1 hippocampus at 12, 20 and 30 weeks of age. Consistent with nNOS levels, cGMP levels were unaltered in 8-week old R6/1 mice, but were 3-fold lower in 12 week-old mutant mice compared with control littermates. These findings suggested that the nNOS/cGMP pathway can be a good target to ameliorate cognitive impairment in HD. Thus, we next investigated whether a phosphodiesterase (PDE) 5 (cGMP-specific PDE) inhibitor could improve cognitive deficits in R6/1 mice. Twelve-week old mice received a single i.p. injection of sildenafil (3 mg/kg) immediately after training. Long-term memory was assessed using two tasks involving the hippocampal function, the novel object recognition test (NORT) and the passive avoidance test. Both tests indicated that PDE5 inhibition improves long-term memory in R6/1 mice. These results indicate that the nNOS/cGMP pathway can be a suitable target for cognitive improvement in HD.

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Cox-2 regulation by IL-1β through MAPKs: A comparison of nasal mucosa and nasal polyps fibroblasts from AIA patients

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Background. Asthma and nasal polyposis are inflammatory diseases in which cyclooxygenase-2 (Cox-2) downregulation has been demonstrated, especially in aspirin-intolerant asthmatic (AIA) patients. It has been suggested the involvement of mitogen-activated protein kinase (MAPK) in Cox regulation, but no works have reported their role in fibroblasts from AIA patients.

Objectives. To study the effect of IL-1β on JNK, ERK and p38 MAPKs phosphorylation and which of them are involved in Cox-2 expression in nasal polyp (NP) fibroblasts from AIA patients.

Methods. Fibroblasts were isolated from specimens of NM (N=3) and NP (N=3) obtained during nasal surgery. Cells were treated with 1 ng/ml IL-1β for 24 h. Cox-2 and phosphorylated forms of p38, ERK and JNK MAPKs were analysed by western blot. The role of MAPKs in IL-1β-induced Cox-2 expression was assessed by treating cells with ERK (PD98059), JNK (SP600125) and p38 MAPK (SB203580) inhibitors (0.1-10 µM) before being exposed to IL-1β.

Results. Phosphorylated forms of JNK and p38 MAPKs were detected at 5 min, with a maximum expression at 15 min. Phosphorylated ERK MAPK was detected in non-stimulated cells, and peaked within 5-10 min. There were no differences in phosphorylation time-course between NM and NP fibroblasts. IL-1β induced Cox-2 expression was significantly inhibited by p38 MAPK inhibitor, while ERK and JNK MAPKs inhibitors had no significant effect.

Conclusions. NM and NP fibroblasts have a similar MAPKs phosphorylation dynamics. p38 MAPK are involved in Cox-2 induced expression. The altered Cox-2 expression in AIA patients appears not to be caused by differences in MAPKs phosphorylation.

The role of PDK1 beyond PKB/akt in regulating neuronal survival analysed by knock-in mutation

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The PI 3-kinase signaling pathway plays a central role in promoting neuronal survival, neuritogenesis and plasticity. PDK1 is a crucial master kinase that, in response to PI 3-kinase activation, switches on a number of AGC-kinase family members, including PKB/Akt, which mediate many of the actions of neurotrophins, neurotransmitters and hormones. Activation of PKB by PDK1 relies on the binding of the PH domains present on both kinases with the PtdIns(3,4,5)P3 second messenger. By contrast, in order to activate S6K, RSK or SGK, PDK1 interacts with a phosphorylated docking site on these targets through the PIF-pocket. We exploited this exclusive mechanism of activation of PKB by employing two mice strains expressing the PDK1 knock-in allele Lys465Glu (K465E) disrupting the folding of the PH domain, and the Leu155Glu (L155E) mutation within the PIF pocket. These mutant forms of PDK1 were meant to affect the activation the PKB/akt, or substrates others than PKB (S6K, SGK, RSK), respectively. Stimulation of PDK1 K465E mutant neurons with BDNF resulted in reduced PKB activation as well as phosphorylation of the PKB substrates PRAS40 and TSC2, which translated onto reduced mTORC1 and S6K activities. Although PKB is considered to be one of the key enzymes driving cell survival, the survival responses of the PDK1 K465E neurons appeared to not to be affected, thereby suggesting that PKB-independent pathways might as well play crucial roles in controlling cell viability. In this regard, preliminary data shows that in conditional knock-in mice expressing the PDK1 L155E mutation only in neuronal tissues, the PDK1 PIF pocket mutation abolished the BDNF-mediated activation of S6K, RSK, and SGK, but not PKB, resulting in reduced survival of the mutant cortical cultures.

A circadian controlled G-protein coupled receptor heterodimer modulates melatonin production.

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Animals respond to cycles of light and dark with patterns in sleeping, feeding, body temperature alterations, and other biological functions. The pineal gland translates these light signals received from the retina into a language understandable to the rest of the body through the rhythmic synthesis and release of melatonin in response to the light and dark cycle. This process is controlled by adrenergic receptors. One impressive and mysterious aspect of the system is the rapid ability of rhythmic melatonin production and/or degradation to respond to changes in the cycle. In this study, we demonstrate that part of this response is due to the formation of receptor-receptor complexes (heteromers) between the adrenergic receptors α1B or β1 and the D4 dopamine receptor. Using both biochemical and biophysical methods in transfected cells and in ex vivo tissue we show that dopamine, a neurotransmitter, inhibits adrenergic receptor signaling through these heteromers. This inhibition causes a dramatic decrease in melatonin production of the pineal gland. We postulate that these heteromers provide a rapid feedback mechanism for the neuronal hormone system to modulate circadian-controlled outputs.

A functional A2RE sequence is responsible for transport of the DDR1 mRNA to oligodendrocyte processes.

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In the central nervous system, oligodendrocytes transport myelin components to their distal processes to form the myelin lamellae. The intracellular trafficking and the subsequent localized translation of mRNA molecules in subcellular compartments are important regulatory processes for gene expression. In humans, myelin basic protein (MBP) is a major structural component of myelin. The mRNA of MBP contains an hnRNP A2 response element (A2RE) sequence that is recognized by the heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 which is responsible for its transport to oligodendrocyte processes. In addition, hnRNP A2/B1 knockdown experiments suggest that this protein plays a direct role in the trafficking pathway. We have identified that discoidin domain receptor 1 (DDR1) is a transmembrane receptor expressed by oligodendrocyte cells, is present in myelin, and colocalyzed with MBP. We hypothesized that the mRNA of DDR1 has a similar trafficking transport like MBP has. The objectives of this study were: 1) to identify whether DDR1 mRNA has a functional A2RE sequence; 2) to determine whether DDR1 and hnRNP A2/B1 co-localize in oligodendrial HOG16 cells and in human oligodendrocytes; and 3) to determine whether the hnRNP A2/B1 knockdown with an hnRNP A2/B1 siRNA leads to differences in the pattern expression of mRNA levels. By in silico analysis, we identified an A2RE-like sequence in the human DDR1 mRNA. We also observed that the hnRNP A2/B1 and DDR1 signals were intensively co-localized in HOG 16 cells and in human brain. Silencing hnRNP A2/B1 showed a different pattern expression of DDR1 mRNA levels, which was accompanied by a decrease in DDR1 protein levels at the cytoplasmic edges. All these data suggest that the putative A2RE sequence identified in DDR1 functions as a true RNA trafficking sequence.

Hypothalamic ceramide levels regulated by CPT1C are involved in orexigenic effects of ghrelin

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Ceramides are part of a big group of lipids that have been related with obesity pathologies. In fact, we have recently demonstrated that ceramides are involved in the satiating response to leptin in the arcuate nucleus of hypothatalamus (ARC). We also have demonstrated that the brain specific carnitine palmitoyltrasefase 1 (CPT1c), which is located in the endoplasmic reticulum of neurons, enhances the synthesis of ceramides and blocks the anorexigenic response to leptin. Nevertheles, it is unknown whether CPT1c and ceramides are involved in the hypothalamic response to the orexigenic hormone ghelin.

To answer this question we performed intracerebroventricular (icv) administrations of ghrelin to WT and CPT1c KO mice and analyzed the food intake and the molecular signalling pathway in the medio basal hypothalamus (MBH).

Results show that the orexigenic effects of ghrelin, as well as associated changes in neuropeptides (NPY and AgRP) and their transcription factors (pCREB and FoxO1) are blocked by the lack of CPT1c. Then, we analyzed whether ceramide levels were regulated by ghrelin administration. Our findings show that icv ghrelin injection produced a rise in ceramide levels within 30 minutes in WT mice, an effect that was not observed in CPT1c KO mice, suggesting that CPt1c is regulating the increase in ceramide levels in response to ghrelin. Finally, we demonstrate that the inhibition of the de novo synthesis of ceramides by myriocin blocks the orexigenic effects of ghrelin and that the administration of C6-ceramide to CPT1c KO mice restores it in these mice.

Our data indicate that ghrelin increases hypothalamic ceramide levels through CPT1c and that this effect is necessary for its orexigenic action.

A potential role of Kalirin-7 in cortico-striatal learning deficits in HD

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Aberrant spine morphology and plasticity are remarkable features of many neurodevelopmental, neuropsychiatric and neurodegenerative disorders. Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder caused by an expanded CAG/polyglutamine repeat in the coding region of the huntingtin (htt) gene. Although HD is characterized by severe motor disturbances there is considerable evidence that early cognitive deficits appear in patients before the onset of motor symptoms. Kalirin-7 (Duo), a guanine-nucleotide exchange factor (GEF) for Rac1 and RhoG, was first identified as an interactor with Huntingtin-associated protein 1 (HAP1). Based on over-expression and knock-out studies, Kalirin-7, is emerging as an important regulator of spine plasticity. We explored the possibility that Kalirin-7 is involved in HD synaptic pathology. Here, we demonstrate an early impairment of cortico-striatal dependent learning associated with an alteration in Kalirin-7 protein and mRNA levels in HD mouse models and HD patients. Given that kalirin has been reported as crucial for synaptic structure and function through modulation of dendritic spine dynamics, density and morphology of dendritic spines have been analyzed in HD mice. Altogether our findings suggest that Kalirin-7 may be involved in the altered molecular control of structural plasticity in HD and underlie the cognitive deficits in this devastating disorder.

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The Persistence of Memory: Two-Photon Imaging Reveals How Synapses Learn and Remember in Real Time

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Subtle changes take place deep inside in our brain every instant we learn, store or retrieve information. The basic mechanism of memory persistence is explained by the ability of synaptic connections to modify their properties in response to neuronal activity. However, nobody has ever observed how these changes occur in a single synapse in real time.

I will present data showing the structural and molecular remodeling of dendritic spines during memory formation. We induced long-term potentiation (LTP) in individual synapses by two-photon glutamate uncaging and visualized protein dynamics using fluorescent and photoactivatable chimeras.

We discovered a unique protein that accumulates rapidly (seconds) and selectively in the potentiated spine, by forming a long-term persistent complex with F-actin. We propose this complex to play a crucial role in the process of memory tagging. On the contrary, the postsynaptic density exhibited the opposite behavior: an hour-delayed structural plasticity, totally independent of the spine morphology. We confirmed it by using a novel photo-labeling procedure that correlates two-photon and electron microscopy.

After a systematic study of the capture of multiple postsynaptic proteins into single potentiated spines, we propose a three-phase temporal model that provides the molecular explanation for some known phenomena of synaptic plasticity and metaplasticity.

WEDNESDAY 11 TH OF JULY
S 18 - EVOLUTION

Genomic shuffling affects recombination rates during mammalian diversification

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Reorganization (shuffling) of the genomic landscape plays an important role in the evolutionary processes as well as in the development of inherited diseases and carcinogenesis. Chromosomal rearrangements could reduce gene flow and potentially contribute to speciation by the suppression of recombination. According to this "suppressed recombination" model, genome shuffling could have a minimal influence on fitness, but would suppress recombination leading to the reduction of gene flow across genomic regions and to the accumulation of genetic incompatibilities. However, few empirical data are available that address the mechanisms by which new chromosomal variants are fixed in populations of mammalian species and how recombination influences chromosomal speciation and *vice versa*.

In this report we test whether the genomic shuffling had affected the distribution of recombination during two speciation events: human-chimp and rat-mouse. This end, we have constructed high-refined maps of the reorganizations and evolutionary breakpoint regions between human and chimpanzee, and mouse and rat genomes. We then analyzed the reorganized regions detected in relation to (i) a high-resolution genome-wide map of meiotic double strand breaks in the mouse, and (ii) recombination rates in the human genome. Our preliminary data reveal the existence of a reduction in recombination within genomic regions that have been implicated in the genome evolution during the speciation events when compared with collinear regions. Standardized recombination rate and the number of meiotic double strand breaks are significantly higher in collinear than in rearranged chromosomes. In the light of our results, we propose that genome reshuffling is affecting recombination rates genome-wide during different speciation nodes.

Evolution of recent rodent gene duplicates

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Gene duplication is a major mechanism modeling genomes, contributing to genome evolution and adaptation. Three main evolutionary models have been proposed to explain the preservation of both gene copies after a duplication event: neofunctionalization (gain of a new function in one of the copies), subfunctionalization (accumulation of degenerate mutations and split of functions) and, increased gene dosage advantage. Under the neofunctionalization model, daughter copies are expected to undergo positive selection and to evolve faster than the parental copy, whereas approximately symmetric rates among copies are consistent with the other scenarios. The importance of these mechanisms is still not clear.

We examined the evolutionary rates and the impact of positive selection of a large set of gene duplicates originated before or after the Mus musculus and Rattus norvegicus split. Evolutionary rate was estimated as the ratio of non-synonymous versus synonymous substitutions (dN/dS). In the post-speciation duplication set we have observed a general trend of asymmetry between the two duplicates, with the slow evolving copy having a similar rate to the single-copy ortholog gene. We have detected a negative relationship between the age of the duplication and evolutionary rate indicating that, after an initial burst of evolutionary rate acceleration, the rate progressively slow down. Positive selection, mainly detected in the faster copy, appears to be prevalent after a duplication event and the tissue-expression pattern observed in the single-copy ortholog is generally maintained by the slow evolving paralog copy. Thus, neofunctionalization mediated by adaptive selection in one of the copies seems the most likely scenario driving the fate of recently duplicated genes.

L'estructura filogenètica de la Biosfera

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La biologia actual ha incorporat explícitament la perspectiva comparada en totes les seves branques, amb un ús cada vegada més freqüent dels arbres filogenètics. Per això és rellevant preguntar-nos què és una filogènia i fins a quin punt hi podem confiar, com també de quina manera reflecteix l'estructura històrica de diversificació a la Biosfera.

Els arbres filogenètics són fonamentalment inferències de naturalesa estadística amb una geometria ultramètrica. Per això és important explorar les propietats de l'espai geomètric, i alhora copsar la prevalència d'artefactes difícils d'evitar, com són l'esbiaixament en el seu balanç o l'atracció entre branques llargues. Així es poden avaluar els mèrits i les febleses dels diferents mètodes d'inferència filogenètica. Ara bé, aquest apartat metodològic, per bé que críticament necessari, és tan sols la porta a plantejaments més pregons.

Tot i que el paradigma cladista ha permès desenvolupar eines poderoses, hi ha problemes que en són particularment resistents, com ara l'inevitable dilema de les espècies incipients. La introgressió, que resulta en evolució reticulada i difumina les cladogènesis, se sap que és molt més freqüent del que hom imaginava. La dimensió temporal que és pròpia de la paleontologia obliga a reconsiderar el significat dels grups parafilètics i, de retruc, a rellegir les filogènies. D'aquí es deriva una nova visió.

Els llinatges que sobreviuen i es diversifiquen ocupen una fracció menuda del que hauria estat possible, i això apunta al paper fonamental (i paradoxal) de l'extinció en la generació de biodiversitat. El progrés evolutiu (un fenomen distint i que no és pas teleològic) té el seu motor en la integració simbiogenètica. Sobre aquestes bases es proposa una metàfora fructífera de l'estructura filogenètica de la Biosfera.

Orígen i evolució del sistema quimiosensorial i desenvolupament de nous marcadors moleculars en aràcnids.

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Macrothele calpeiana (Aranae, Hexathelidae) és una espècie d'aranya migalomorfa singular, un endemisme ibèric que passa per ser l'única aranya protegida per la legislació europea. Malgrat això, disposem d'un escàs nombre de marcadors moleculars i d'una manca de coneixement sobre processos biològics fonamentals, com ara el del sistema quimiosensorial. Per avançar i aprofundir en el coneixement biològic d'aquesta espècie, estem aplicant tècniques de seqüenciació massiva (454 GS-FLX Titanium) amb la finalitat d'identificar marcadors genètics idonis per estudis filogenètics i filogeogràfics. Per altra banda, també estem caracterizant els gens del sistema quimiorreceptor.

Hem seqüenciat una fracció del genoma en 4 espècies d'aranyes corresponents a 2 famílies de migalomorfs Ctzenizidae i Nemesiidae, per identificar posicions variables que serveixen com a marcadors genètics. Vam obtindre un total de 442,735
reads (N50 de 440 bp) els quals van ser ensamblats amb el software CAP3. Amb eines bioinformàtiques desenvolupades en el nostre grup estem carateritzant les regions variables adients.

Per l'estudi de l'origen i l'evolució del sistema quimiosensorial hem seqüenciat el transcriptoma de diversos teixits de Macrothele calpeiana (Aranae, Hexathelidae). Hem realitzat 2 llibreries sustractives de cDNA dels potencials òrgans quimioreceptors, pota i palp. Hem obtingut uns 50,000 reads per teixit (N50 de 404 bp) que s'han ensamblat utilitzant els softwares Newbler 2.6 i CAP3. Les cerques amb BLAST mostren que aproximadament un 50 % de les seqüències presenten similaritat amb proteïnes de quelicerats, i un 65 % tenen un termeGO i/o domini InterPro descrit. Aquestes dades s'estan utilitzant per l'identificació i anotació dels gens relacionats amb el sistema quimiosensorial.

EBV strain variation in different lymphoblastoid cell lines derived from 1000 Genomes Project individuals

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Infection by Epstein-Barr virus (EBV) has been related to a number of diseases including different types of cancer (i.e. Burkitt's lymphoma, Hodgkins'lymphoma, or nasopharyngeal carcinoma), and also with multiple sclerosis. Such diseases show different incidences in different regions of the world, and thus, establishing phylogenetic relationships among EBV strains from different human populations might enlighten the current patterns of distribution of EBV and EBV-related diseases and help understanding co-evolution between the virus and its host. However, so far, only 4 complete EBV strains have been described.

Most people in the world (~90%) are infected by EBV, which establishes permanently in B-cells. Here, we have reconstructed EBV sequences extracted from B-cell-lines belonging to hundreds of different individuals from 14 different populations worldwide, which have been fully sequenced within the 1000 Genomes Project. We know for sure that at least one particular EBV strain (B95-8) must be present, since B-cells of subjects were transformed using this strain to obtain a steady source of DNA. However, B95-8 possesses a specific deletion in its genome, which may allow us to distinguish between natural and artificial EBV strains.

We have developed tools for viral DNA variant calling using EBV full-genome sequences obtained by means of next-generation sequencing data. Although we have observed very little genetic variation among individual B95-8 strains, we have determined the presence of natural EBV at least in 15 individuals (notably, 12 of them were Africans) and obtained a set of variants, which are specific of these natural strains. We have been able to reconstruct the complete natural EBV strain of 5 individuals, for which we observe enough proportion of the endogenous strain. With these sequences we are now able to conduct analyses exploring signatures of positive selection on viral genes and point to candidate genes subjected to human-EBV coevolution.

Identification of selective sweeps in the genome of the Boxer breed

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The generation of vast single nucleotide polymorphism (SNP) repertoires from genome sequencing projects together with rapid improvements in large-scale SNP genotyping allowed the development of high-density genome-wide SNP microarrays in many animal species. This work presents an example of the application of SNP arrays to understanding the evolutionary history of the domestic dog, which is marked by severe population bottlenecks during domestication from wolf and breeds creation and by strong artificial selection for traits favored by humans.

Specifically, we searched for genomic footprints of selection in the Boxer dog breed. A typical signature of selection is the sweep of genetic variability due to fixation of not only selectively advantageous genetic variants but also of nearby linked neutral variants.

We present a novel selective sweep of >8 Mb on chromosome 26 in the Boxer. Hinted by the presence of another selective sweep on chromosome 1 previously associated with canine brachycephaly, characterized by severe shortening of the muzzle and a breed-defining trait of the Boxer, we investigated on the relationship between the sweep on chromosome 26 and this trait. We tried to prove that the sweep is representative of the Boxer breed and that it is also present in other brachycephalic breeds but absent in non-brachycephalic breeds and wolf. Finally, we examined the genetic content of the sweep to find putative targets of selection and potential undesired health consequences for the breeds bearing the phenotype.

Keywords: dog, evolutionary history, selective sweep, brachycephaly

Molecular analysis of the mechanisms involved in *THBS4* differential gene-expression in the human brain

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The last decades have seen a growing interest in how the human brain differs from that of our closest relatives at the molecular level. Hundreds of genes with expression differences between human and non-human primates have been identified. However, it is important to study these genes in more detail to see if they are really involved in human brain characteristics. Thrombospondins (THBSs) are multimeric extracellular glycoproteins that modulate cell-cell and extracellular matrix interactions and have been implicated in synaptogenesis. Within the THBSs family, *THBS2* and *THBS4* show, respectively, a ~2-fold and ~6-fold upregulation in human cerebral cortex compared to chimpanzees and macaques. To analyze the causes of these expression differences, we have carried out a comparative and functional analysis of the *THBS4* promoter region in humans and chimpanzees. First, we have identified and validated an alternative transcription start site (TSS) for *THBS4* that is located ~44 kb upstream from the known TSS and generates a new mRNA isoform encoding a 91 amino acid shorter protein. Second, we used quantitative RT-PCR to compare expression levels of both *THBS4* mRNAs in different human tissues and cortical regions of 11 humans and 11 chimpanzees. Interestingly, the new isoform of *THBS4* is expressed mainly in brain tissues. Moreover, consistent with the observed differences for total *THBS4* expression, it shows ~6-fold higher expression in human than in chimpanzee cortex. Third, we have evaluated the activity of the two *THBS4* promoter sequences from humans and chimpanzees in different human cell lines using reporter assays, we have found significant differences between both promoters, but not between species. Finally, the comparison of the DNA methylation in a CpG island upstream the new isoform in 5 humans and 5 chimpanzees detected similar low methylation levels in all of them. Increased *THBS4* expression in the human brain therefore appears to be related to higher transcription from the alternative promoter and understanding its regulation could be relevant to the functional consequences of *THBS4* expression differences during human brain evolution.

Two new plant cytogenetic online resources: the GSAD and Plant rDNA databases

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Here we present two recently created electronic databases of interest for plant cytogenetic researchers. The first is the "GSAD: a genome size database in the Asteraceae"; launched in July 2010, it is the first to be focused on the nuclear DNA amounts in a specific botanical family, considered the largest plant family (≈23,000 species), with many of its representatives being of economic and ecological interest. The GSAD is available on the internet at www.asteraceaegenomesize.com. With the first release having 1,780 entries, the database deals with all the research done in this field, containing genome size information for 110 genera, 820 species and 185 infraspecific taxa, data coming from around 100 publications. The second is the "Plant rDNA database" a resource accessible at www.plantrdnadatabase.com and launched in February 2012, which compiles information on number, position and structure of the 5S and 18S-5.8S-26S ribosomal DNA (rDNA) loci in plants (angiosperms, gymnosperms, and few bryophytes). Current knowledge regarding chromosomal rDNA sites is provided for more than 1,000 plant species (including more than 1,400 different accessions). The data come from fluorescent in situ hybridisation experiments (FISH) reported in more than 300 publications. With a similar layout, both web pages are intuitive and user-friendly, including basic and advanced search options. We expect these databases to be used for comparative and evolutionary studies, by quickly supplying a type of information otherwise scattered in a variety of sources.

WEDNESDAY 11 TH OF JULY
S 19 - DEVELOPMENT

Gene Loss Impact on EVO-DEVO: Dismantling the Retinoic Acid Pathway in the Chordate *Oikopleura dioica*

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Since Susumo Ohno's work on the 70s, gene duplications have been considered the major force driving the Evolution of the mechanisms of Development (Evo-Devo) and generating innovations that promote animal diversification. Comparative genomics is revealing, however, extensive events of gene loss in many lineages, suggesting that gene loss might be also an important force driving animal evolution. Nevertheless, the actual impact of gene loss on Evo-Devo remains mostly unknown. For this reason, the principal aim of our research group is to provide experimental evidence to evaluate the impact of gene loss on Evo-Devo, taken the loss of the Retinoic Acid (RA) genetic machinery in the chordate *Oikopleura dioica* as a case study. RA, the bioactive form of vitamin A (retinol), plays a major role on the regulation of cell proliferation and differentiation, and has been recruited in many biological contexts such as axial patterning and organogenesis during development, and homeostasis of adult tissues, in all animal groups of our own phylum, the chordates. The main components of the "classical" genetic machinery that control RA activity (Aldh1a and Cyp26, which regulate the spatio-temporal distribution of RA, and RAR, which regulates the expression of RA-target genes) have been conserved in all chordates so far analyzed, with the striking exception of *Oikopleura dioica*, an urochordate specie that has lost these components. Our results reveal a correlation between the loss of the RA genetic machinery and the disintegration of the *Hox* cluster, the loss of temporal co-linear expression of *Hox* genes, and the innovation of a determinative mode of development in urochordates. Currently, we are analyzing the extension of gene losses in the RA pathway in *Oikopleura*, testing for the presence of "alternative" pathways by which RA could still alter organogenesis in *Oikopleura*, and evaluating the impact of the loss of the classical RA pathway in the evolution of the components of other morphogenetic pathways known to antagonize RA in chordates.

Helios, a new transcription factor involved in the determination of striatal medium spiny neurons

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During brain development progenitor cells from the lateral ganglionic eminence (LGE) give rise to medium spiny neurons (MSN). However, little is known about the factors involved in this process. Helios has been described recently as a member of the Ikaros family of transcription factors that is expressed from E14.5 until P15 within the LGE. In this area Helios colocalizes with MSN markers such as ctip2 and Foxp1.

To study the putative role of Helios during striatal development we characterized Helios knockout (He-/-) mice. Firstly, we performed birthdating experiments and observed that the lack of Helios induced an impairment of the second wave of striatal neurogenesis. This effect was accompanied by an increase in the number of proliferating neural progenitor cells (NPCs) in the subventricular zone, suggesting a role for Helios in cell cycle regulation. In agreement with this, in vitro studies over-expressing and knocking down Helios showed that it reduces NPC proliferation through the regulation of S phase entry and cell cycle length. Secondly, we analyzed cell death at embryonic and postnatal developmental stages in He-/- mice. Cleaved caspase 3 immunohistochemistry showed increased cell death at postnatal stages in the absence of Helios. Finally, to study if the blockade of striatal neurogenesis was affecting the differentiation of a specific cell type, neuronal populations were counted in adult He-/- mice. The Helios deficiency caused a specific reduction in the number of neurons positive for DARPP-32 and Calbindin (MSN markers), while interneuron number remained unaffected. Altogether our results show that Helios is involved in MSN development at three levels: proliferation, differentiation and cell survival.

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Evolutionary conserved role of the beta-catenin/Wnt signalling throughout planarian life cycle.

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Planarians are a classical model for regeneration studies due to their striking plasticity. They can regenerate a whole animal from any piece of their body and show a continuous remodeling, growing and shrinking according to the environment. These capabilities rely on the pluripotency of their adult stem cells, the neoblasts, and a remarkably high and continuous activation of major developmental signalling pathways. Despite its broad range of functions, the beta-catenin dependent Wnt signalling is a highly conserved mechanism to specify endomesoderm and pattern the antero-posterior axis during embryogenesis across metazoans. The functional study of several elements of the pathway, in particular of beta-catenin1, whose silencing leads to a striking fully anteriorized adult animal, has clearly demonstrated that the canonical Wnt signal is essential for posterior specification in adult planarians, during both regeneration and homeostasis. Using a specific antibody we have analyzed the beta-catenin1 subcellular localization throughout the planarian life cycle (embryogenesis, sexual maturation and regeneration). Our results support, first, its ancestral role in endomesoderm specification; and, second, its conserved function in antero-posterior patterning throughout embryonic development, adult homeostasis and regeneration. Overall, our study demonstrates the functional conservation of this master regulatory gene at an evolutionary and a developmental level, and sets planarians as a significant and appealing model to better understand and relate regeneration, homeostasis and embryogenesis.

Functional characterization of neoblast specific transcription factors during planarian regeneration

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The great plasticity of freshwater planarians have made them a classical model for regeneration, which has lately become even more informative due to the introduction of the new molecular tools available such as RNAi. Among them, Schmidtea mediterranea is one of the most used for regeneration studies.

This extraordinary regenerative capacity is based on the presence of a specific cell type, the neoblasts, which can give rise to any other cell type of the organism. In order to better characterize the neoblast biology, a comparative RNA-Seq (DGE) was performed between neoblasts and differentiated cells isolated by Fluorescence-Activated Cell Sorting (FACS).

Among those genes overexpressed in X1 subpopulation (dividing cells) compared to Xin subpopulation (differentiated cells), here we aim to characterize several transcription factors that in other organisms have been related to important processes such as stem cell maintenance, differentiation to a specific cell type, migration, etc. A better understanding of the transcription factors that are playing a role in neoblasts will be useful to be able to distinguish those that are important for stem cell maintenance from those responsible for differentiation into a specific cell type.

In addition to the expression pattern analysis of these transcription factors during regeneration, we also knocked them down using RNAi, both during regeneration and homeostasis. After that, we have analysed the phenotypes using different molecular markers for different structures and cell types.

Among the plethora of transcription factors upregulated in neoblasts, we show that RNAi knock-down of some of this genes such as homologues of the Serum Response Factor (Srf) and subunits of the NFY leads to a deficient regeneration, while others give rise also to an impairment of homeostasis in intact non-regenerating animals. However, additional analyses are essential to better understand the function of these transcription factors in neoblasts, which would be useful to make a more comprehensive picture of regeneration in S. mediterranea. Moreover, it would be also interesting to compare our results with the genes that are necessary to control stem cells in other organisms.

The role of the microRNA trio let-7, miR-100 and miR-125 in insect hemimetabolous metamorphosis

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In most insects, the miRNAs let-7, miR-100 and miR-125 cluster in the same primary transcript. The three miRNAs are involved in developmental timing in the nematode Caenorhabditis elegans and in the fly Drosophila melanogaster. In the cockroach Blattella germanica, the expression of these miRNAs peaks in the last instar nymph, before metamorphosis. When let-7 and miR-100 are depleted with specific anti-miRNAs, the resulting adult shows the wings malformed: either reduced in size (anti-miR-100) or with wrong vein patterning (anti-let-7). Depletion of miR-125 induces no apparent effects. In D. melanogaster, many are genes involved in wing maturation, and one of them is the Drosophila serum response factor (DSRF). Towards the end of the last instar larvae, previous to metamorphosis, DSRF expression is down-regulated in the longitudinal veins by Ras-mediated epidermal growth factor (Egfr) signaling. Reduction of DSRF expression seems necessary to reach a right proportion between wing veins and interveins. In B. germanica wing buds, DSRF is down-regulated during the last instar nymph, in parallel to the up-regulation experienced by let-7. We propose that let-7 plays a key role in vein-intervein patterning of B. germanica by targeting DSRF mRNA, thus helping to down-regulate the expression of this gene post-transcriptionally.

Ovarian follicle development in primitive insects

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Drosophila melanogaster, the classical insect model of meroistic ovaries, has been thoroughly studied in the past years. Hovewer, some information is available about the molecular mechanisms regulating oogenesis in panoistic ovaries. In an attempt to afford data on this primitive ovarian type, we currently work on *Blattella germanica*, a cockroach species having panoistic ovaries where only the basal oocyte is developed in each gonadotrophic cycle. In the long term, our interest is to understand the evolutionary transition between panoistic and meroistic ovary types. Ovarian transcriptomes obtained in our research group throws us most of the components that determines polarity and proliferation in meroistic ovaries, and we have studied the mRNA expression pattern, and the function of some of them in the regulation ovarian follicle development using RNAi techniques. Our results are consistent with a number of previous studies in the meroistic ovary of *D. melanogaster*, thus suggesting that some pathways leading to oocyte maturation are conserved in insect ovaries.

Regeneration in *Isodiametra pulchra* (Acoela, Acoelomorpha)

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Acoels are microscopic marine worms that recently have been placed outside the Platyhelminthes with which they share a very similar stem cell system and regeneration capacity. To date, *Isodiametra pulchra*, is the most promising "model acoel" as it can easily be cultured, reproduces year-round, and gene knock-down can be performed with double-stranded RNA. We have investigated the regeneration of the posterior part and some of its structures (the head does not regenerate) using histology, electron microscopy, fluorophore-tagged-phalloidin labeling, and staining of S-phase and mitotic cells to provide a basis for future molecular projects, comparisons with platyhelminths, and gene knock-down experiments on regenerants (the embryo is not yet accessible).

After amputation the wound is closed by contraction of circular muscles and an extension of the dorsal epidermis. Subsequently neoblasts in the vicinity of the wound start to proliferate and form an indefinite blastema. The so-called chordoid vacuoles move caudally and ventrally and consequently the female and male primordia, which are recognizable from day 2 on, shift rostrally. On day 3 the bursal nozzle forms and certain cells of the male primordium contain bundles of muscle fibers. On day four the male copulatory organ is clearly organized in a muscle layer forming the seminal vesicle and the penis tube. A day later the epithelial cells of the penis tube have the typical microvilli on the apical side and the bursa is distinct. On day seven the vagina and the bursal stalk are formed and the epidermis at the future gonopores becomes more glandular. The last distinct structures to form are the sphincter on day 9 and afterwards the gonopores.

Our results show that the cells, which form the copulatory organs, go through rapid proliferation within the first 24–48 hours and that the copulatory organs are restored from proximal to distal. Furthermore there is evidence of intercalary regeneration: first the distance between the male copulatory organ and the bursal nozzle grows continuously during regeneration, and second the swallow´s nest receptors are restored and positioned at the tip of the posterior end from where they are dispersed. Finally we found that "sick" animals need longer to regenerate and that the homeostasis of cell turn-over is disrupted, at least for the swallow´s nest receptors.

How often does natural selection targets multiple, interacting genes? The prevalence of epistasis in recent human evolution

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Epistasis in its broadest sense could be defined as the dependence of the outcome of a mutation on its genetic background. Mutations in functionally related genes could be selected jointly if they jointly affect the total fitness of individuals carrying them. Whenever this happens, the signature that selection may leave in patterns of genome variability might differ dramatically from the signature of selection when it acted upon a single gene. An excellent case-study is provided by ancestral and current human populations, which experienced significant changes in their selective pressures after leaving Africa to colonize the whole Planet. In this study, we studied derived human populations looking for evidence of recent positive selection acting upon pairs of protein-coding genes that are known interact within pathways ("interacting genes"). Evidence of recent positive selection events was investigated using Tajima's D and other frequency-spectrum statistics. We found an excess of interacting pairs of genes with evidence of recent positive selection in derived populations when compared with African populations. These observations cannot be accounted for neither by (1) the demographic history of populations nor by (2) an excess of outlier genes in derived relative to African populations. Further analyses showed that the pairs of outlier interacting genes tend to be more central in the networks than the rest of outliers genes, and that this trend is stronger in derived than in African populations. Taken together, our results suggest that changes in selective pressures in human derived populations, affects groups of interacting genes.

WEDNESDAY 11 TH OF JULY
S 20 - EVOLUTION AND DEVELOPMENT OF THE NERVOUS SYSTEM

No place like home: stem cells and the neurovascular niche

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A characteristic feature of all stem cell systems in long-lived metazoans is their capacity to produce stem cells and cells that are committed to terminal differentiation in a continuous way. Capacity for self-renewal endows stem cell populations with regenerative potential but also underlies susceptibility to neoplastic transformation. The adult brain subependymal zone (SEZ) is a very active neurogenic niche in which a relatively quiescent population of radial glia/astrocyte-like GFAP+ neural stem cells (NSC) continually produce new neurons and oligodendrocytes, via a population of rapidly-diving transit-amplifying progenitor cells. Stem cell expansion in response to increased cellular demand suggests that niche signals can modulate division mode, but very little is known about the molecular players involved. Therefore, one approach to the understanding of self-renewal is to analyze the mechanisms that regulate the maintenance of normal NSCs in their natural environment. Although some intrinsic determinants are known to regulate stem cell division, the observation that stem cells can respond to excessive cellular demand in pathological situations or after traumatic injury suggests that they have ways to increase their number in response to external signals. Within the specialized microenvironments in which stem cells reside, vascular elements appear to play an important role in the regulation of stem cell self-renewal vs. commitment, both under normal and pathological conditions but the signalling pathways involved are still under investigation. We will present our most recent data on intrinsic and extrinsic regulators of NSC maintenance and homing.

The regulation of neural stem and progenitor cell fate by the activity of transcription factors

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Following closure of the neural tube, neuroepithelial (NE) cells in the vertebrate central nervous system (CNS) divide symmetrically to expand the cell population. Although NE cells can directly differentiate to produce the first neurons in the CNS, the majority transform into radial glial (RG) cells that, following rounds of asymmetric division, generate intermediate progenitors that differentiate into neurons or glia. Accordingly, NE and RG cells in particular represent primary progenitors or neural stem cells (NSCs), and they are best distinguished by their self-renewal abilities, as well as their multipotent differentiation into neurons, astrocytes and oligodendrocytes. It has been proposed that the combinatorial activity of specific transcription factors can regulate the sequential generation of neurons and glia from NSCs. Accordingly, the temporal and spatial regulation of the expression of such transcription factors modulates NSC self-renewal, cell cycle exit, cell fate specification, and neuronal and glial differentiation. Using in vivo and ex vivo murine-based experimental models, our studies are focused on the role of specific transcription factors, namely, T-box brain 1 (Tbr1) and Genetic screened homeobox 2 (Gsx2), during the self-renewal of NSCs, cell proliferation as well as on the regulation of the generation of neurons and glia from these cells.

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An evo-devo approach for understanding the amygdala, a key forebrain center for control of emotions and social behavior

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The amygdala is a highly complex structure located in the telencephalic hemispheres, which is involved in a large variety of functions including the control of innate behaviors essential for individual and/or species survival such as fear responses, ingestion, reproduction and defense, as well as complex cognitive functions such as associative learning and memory formation of emotionally-relevant events. Understanding how the amygdala controls each distinct function and the neurons and pathways involved in each is extremely difficult, considering its mosaic-like structure, with multiple molecular subdomains and neuron subtypes, and its ample network of connections. In recent years, the evo-devo approach is offering new data that are becoming of extraordinary help for unravelling the complex organization of the amygdala. First, the expression patterns of developmental regulatory genes combined with fate mapping studies have shown that different neurons of each amygdalar subdivision originate in distinc progenitor domains of the embryonic forebrain (reviewed by Medina et al., 2011). The embryonic origin is directly correlated with the expression of specific transcription factors (or other regulatory proteins), the differentiation of specific neuron subtypes and the formation of specific connections (Medina et al., 2011; Sokolowski and Corbin, 2012). This is offering a new perspective for understanding the structural and functional organization of the amygdala. Second, the use of this approach is helping to understand the organization of the amygdala in other vertebrates, the evolution of each distinct neuron subset and functional pathway, and the possible developmental mechanisms involved in the evolutionary changes (Medina et al., 2011). One particularly interesting case is the possible implication of evolutionary changes in the expression of the transcription factor Nkx2.1 in the evolution of a part of the amygdala involved in social behavior.

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THURSDAY 12 TH OF JULY
S 21 - VIROLOGY

Quasispecies dynamics and the control of viral infections

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In recent years a lot has been learned about viral quasispecies, in addition to being reservoirs of genetic and phenotypic variants. Internal interactions are established among components of a mutant spectrum that can complement functionally defective or suboptimal mutants. Alternatively, a mutant spectrum can suppress specific variants despite the latter displaying higher fitness than the ensemble where they are immersed. The behavior of a quasispecies as a whole cannot be predicted from the behavior of its individual components. Mutual influences among genomes within a quasispecies are currently proving very relevant to design antiviral strategies, in particular combination therapies based on lethal mutagenesis (virus extinction by an excess of mutations). Use of inhibitors of viral replication, together with virus-specific mutagenic agents, must consider that the mutagen may increase the frequency of inhibitor-escape mutants while the inhibitor may impede replication of interfering mutants. These interactions between inhibitors and mutagens may result in sequential therapies being more effective than the corresponding combination therapies. Results with several viruses will be presented, and evaluated in connection with a possible clinical application.

Deep sequencing of naturally evolving arbovirus populations in the mosquito vector identifies new variants with epidemic potential

Marco Vignuzzi

A hallmark of RNA viruses is rampant replication and high mutation rates that result in the emergence of epidemic variants. Generally such variants are identified retrospective to epidemics, since in depth genetic monitoring of viruses is often not conducted and predicting virus evolutionary trajectories is difficult. Since surveillance currently relies on consensus sequencing, emerging adaptive mutations are only identified once they dominate the virus and host population. For arthopod-borne viruses such as chikungunya virus (CHIKV), consensus sequence changes are relatively infrequent despite high mutation frequencies. Nevertheless, variants with fitness advantages emerge that promote epidemics, as occurred in the recent Indian Ocean outbreak when a E1 glycoprotein mutation (A226V) enhanced infectivity of a primary mosquito vector. Given that emerging variants do not displace wild type until repeated infection and spread across hosts, we hypothesized that deep sequencing techniques would reveal virus mutant compositions in fewer infection cycles in a single host and could identify positively selected variants before rising to dominance. We repeatedly detected two new glycoprotein mutations emerging in virus subpopulations in mosquito saliva that confer fitness advantages in mosquitoes and mammals and augment virion stability, underscoring their potential as future epidemic strains. To validate the predictive power of our approach, we showed that the A226V mutation from the recent epidemic could also have been identified in this manner. Thus, metagenomics and experimental evolution of RNA virus subpopulations involved in host-to-host transmission can identify and perhaps predict strains with epidemic potential before they dominate a population.

mRNA Archaeology

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RNA transcripts corresponding to the 5' region of the hepatitis C virus, which covers approximately the first 600 nts, present highly stable secondary structures, numerous tertiary structures, and a generally highly complex folding pattern. Likewise, an alternative conformation mediated by interaction with the hepatic micro RNA miR-122 has been described *in vitro*. Our group has been able to identify a number of these elements using various biochemical and biophysical tools. Thus, we have shown that the internal ribosome entry site (IRES) of HCV contains cleavage sites for both human and bacterial RNase P, which is the activity that processes the tRNA precursor, thereby suggesting the presence of a tRNA-mimetic structure; other cleavage sites for RNase III in the two sequences neighbouring the IRES element, thus indicating the presence of RNA:RNA duplex structures; and a region that is able to promote a new UV light-mediated self-processing reaction that is indicative of a ribozyme-type structure. The cleavages mediated by RNAse P and UV light occur in regions required for binding to the ribosome, whereas those mediated by RNase III occur in regions neighbouring the IRES, which coincide with the miR-122 binding region, and mediate the conformational change that governs the presentation of a tRNA-type structure. We therefore wonder whether the tRNA-mimetic structure, and other specific structures that accompany it, form part of ordered series of structural motifs optimised in the depths of time, perhaps in the "RNA world".

Natural tools (RNase P, RNase III and UV light), which have proved to be useful for describing the RNA structures in HCV, have been tested *in vitro* in other RNA transcripts from related flaviviruses and pestiviruses, as well as in hepatic cell mRNAs, in order to detect specific recognition sites. The presence of these structural motifs in West Nile flavivirus, but in a different genomic location to those in HCV, as well as in closely related hepatic cell mRNAs, such as interferon alpha 5 (INFA) RNA, and others unrelated to viral infection, suggests the accumulation of structural elements in mRNAs which probably make up a new layer of communication and molecular interactions, the basis for the presence of RNA analogical-based codes. The identification of these possible codes, especially a common pathological code for some of the RNA viruses responsible for hepatitis A, C, E and delta, is therefore a long-term goal.

Hepatitis A virus, a very special picornavirus

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Hepatitis A is the most common infection of the liver worldwide and is fecal-orally transmitted. Its incidence tends to decrease thanks to improvements in the hygienic conditions but at the same time its severity increases. Hepatitis A virus is the causative agent of acute hepatitis in humans and belongs to the *Hepatovirus* genus in the *Picornaviridae* family, and it has very unique characteristics. HAV exhibits some molecular and biological properties which allow the virus to live in a very quiescent way and to build an extremely stable capsid able to persist in and out of the body. HAV as a RNA virus occurs as a swarm of mutants or quasispecies and shows a nucleotide diversity similar to that of other picornavirus, allowing its differentiation into six genotypes and several subgenotypes. However, despite this nucleotide variability at the amino acid level the diversity is limited and only few natural antigenic variants have been isolated. This suggests the occurrence of severe structural and biological constraints of the capsid that prevents the existence of more than a single serotype and concomitantly ensures a highly compact capsid that confers a high environmental persistence which is critical for a virus transmitted through the fecal–oral route with long extracorporeal periods. Large foodborne and waterborne outbreaks of hepatitis A have been reported worldwide. Foods of primary importance, susceptible to be contaminated at the pre-harvest stage, are bivalve mollusks, particularly oysters, clams and mussels, salad crops, as lettuce, green onions and other greens, and soft fruits, such as raspberries and strawberries. All these types of food have been implicated in foodborne viral outbreaks. Post-harvest contamination results most likely from poor hygiene practices during food-handling, and hence the foods most at risk are uncooked or lightly cooked foods. Apart from the expected structural constraints due to amino acid residues playing critical roles in capsid folding, a certain contribution of the codon usage to the low antigenic variability of the HAV capsid has also been suggested since 15% of the surface capsid residues are encoded by rare codons which are highly conserved among the different HAV strains and their substitution is negatively selected even under specific immune pressure. The emergence of a new serotype requires extensive substitutions in the capsid that seem quite unlikely to occur in a virus with such severe genomic, structural and biological constraints. However the isolation of several natural antigenic variants of the immunodominant site during an outbreak of hepatitis A in the MSM community of Barcelona has been recently reported.

THURSDAY 12 TH OF JULY
S 22 - MICROBIOLOGY

Epigenetics and evolution in bacteria

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The rapid evolution of microorganisms producing antibiotic-resistant orms is a cause of growing concern. Surprisingly, the comparison of the susceptibility of large old collections (isolated at the start of the antibiotic era) and of present ones does not show important differences. This has led us to see the resistance under the prism of the biology of populations and also to look for ways to go back to the susceptibility. Besides, adaptive or epigenètics mechanisms are becoming evident in prokaryotes. Finally, the phenomenon of heteroresistance is increasingly apparent. In this contribution, we analyze the experimental results of the exploration of resistances in a collection of antique and modern strains of *Serratia marcescens*. Few significant differences have been observed if we separate the resistant and sensitive isolates. However, it appears that when we analyze with detail —even in cases that 100 % are considered resistant (or sensitive)— the values have been increasing gradually. This contribution describes also mechanisms of resistance to imipenem, meropenem and colistin, which do not correspond to any of the commonly accepted mechanism to explain the resistance to antimicrobial agents. These other mechanisms of resistance range from changes in the morphology that allow the survival of the bacterium, to forms of lipopolysaccharide that escape the action of polications, such as colistin. The detection of integrons of class I using PCR, and the determination of sequences, have allowed to compare the genetic profiles of multiresistant strains of *Pseudomonas aeruginosa*. Otherwise, the reflux pumps and the study of the conditions under which old antibiotics, now discarded, can be the basis of combinations that allow us to treat multiresistant infections.

Patogènesi bacteriana com a simbiosi imperfecta

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La selecció natural afavoreix els patògens que presenten una taxa elevada de reproducció, la qual cosa és producte de dos factors: la taxa en la qual s'infecten i el temps que aquests hostes sobreviuen després de ser infectats. Encara que una taxa d'infecció alta pot ser suficient per a l'èxit evolutiu, certs patògens atenuen la seva virulència, augmentant així la supervivència de l'hoste. Si la capacitat d'infecció és prou alta, l'augment de la supervivència de l'hoste augmentarà la taxa de reproducció del patogen. L'atenuació de virulència pot emmascarar la distinció entre un patogen i un comensal. En certs casos, una infecció atenuada pot beneficiar l'hoste en una mena de simbiosi imperfecta. Els mecanismes d'atenuació de virulència són ben coneguts a *Salmonella*, un patogen que infecta els éssers humans i els animals. Alguns serovars de *Salmonella* estan adaptats als hostes, mentre que d'altres no ho estan, i només certes combinacions de serotip-hoste causen malaltia (p.ex., gastroenteritis i febre tifoide en els éssers humans). L'atenuació de virulència en *Salmonella* durant la infecció dels animals implica una varietat de fenòmens (i la llista podria ser incompleta): (i) Heterogeneïtat fenotípica. Durant la infecció, poblacions clonals de *Salmonella* se separen en subpoblacions fenotípicament diferents de cèl·lules. Alguns d'aquests llinatges són virulentes, mentre que d'altres mostren una virulència reduïda. (ii) Atenuació del creixement intracel·lular. La proliferació intracel·lular *in vivo* de *Salmonella* està restringida als macròfags, i el creixement bacterià està estrictament controlat. Com a conseqüència, la colonització dels òrgans dels animals per *Salmonella* es limitada. D'altra banda, *Salmonella* persisteix en un estat de no-proliferació en certs tipus de cèl·lules no fagocitàries (p.ex., els fibroblasts). (iii) Infecció crònica. Certs serovars de *Salmonella* poden persistir en els animals sense causar símptomes de la malaltia. Per exemple, *Salmonella* Typhi pot persistir en la vesícula biliar humana de manera gairebé asimptomàtica, la qual cosa seria un exemple de simbiosi imperfecte: la vesícula biliar actuaria com a un reservori de *Salmonella* per a la infecció d'individus susceptibles a través de les femtes, mentre que l'hoste podria beneficiar-se de la modulació de la resposta immunitària, tant la innata com l'adaptativa.

Endosimbiosi i evolució

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Més de cent anys després dels debats clàssics sobre la individualitat dels líquens, el protagonisme de la simbiosi en la natura i la seua implicació en l'emergència d'innovacions evolutives ja forma part del cànon de la biologia contemporània. La teoria de la simbiogènesi, originalment formulada per Boris Mikhaylovich Kozo-Polyansky (1890-1957) i desplegada amb tota la seua potència explicativa per Lynn Margulis (1938-2011), permet l'estudi de l'aparició de noves estructures, metabolismes i comportaments a partir de l'associació d'espècies diferents. Així, tenim un suport empíric molt sòlid per saber que els orgànuls energètics de les cèl·lules eucariòtiques (mitocondris i cloroplasts) tenen avantpassats comuns amb bacteris actuals de vida lliure. L'anàlisi filogenòmica permet de suposar que els mitocondris podrien haver-se establert després de l'atenuació del parasitisme d'uns bacteris flagel·lats i amb capacitat de tolerar concentracions d'oxigen baixes amb uns hostes d'identitat encara boirosa. D'altra banda, els cloroplasts actuals semblen també monofilètics, excepte l'orgànul fotosintètic de Paulinella chromatophora, que provindria d'una endosimbiosi primària independent. Mentre que els mitocondris han originat una diversitat d'orgànuls adaptats a l'anaerobiosi presents en llinatges de fongs i protoctistes, els cloroplasts han fet gala d'una exuberància notable a través d'endosimbiosis secundàries i terciàries. Però el lligam entre endosimbiosi i evolució no s'acaba ací. Les associacions amb microorganismes procariòtics han estat presents i reiterades al llarg de tota la història evolutiva dels eucariotes. Un dels casos millor estudiats són les simbiogènesis metabòliques entre insectes i bacteris, que han ocorregut moltes vegades i de manera independent durant els darrers 300 milions d'anys, produint múltiples fusions de les branques de l'arbre de la vida. Els endosimbionts d'herència vertical i les microbiotes intestinals han esculpit les capacitats metabòliques del grup animal més nombrós de la natura. Si l'associació entre procariotes fou essencial durant l'emergència de la cèl·lula eucariòtica, també hem de reconèixer que les associacions simbiòtiques amb procariotes, explorades durant la diversificació eucariòtica, han estat cabdals en l'evolució del metabolisme eucariòtic. Els eucariotes són veritables mosaics metabòlics.

THURSDAY 12 TH OF JULY
S 23 - ECOLOGY

Stequiometry and global metabolism

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The availabilities of carbon (C) from rising atmospheric carbon dioxide levels and of nitrogen (N) from various human-induced inputs to ecosystems are continuously increasing. These increases are not paralleled by a similar increase in phosphorus (P) inputs. The inexorable change in the stoichiometry of C and N relative to P has no equivalent in Earth's history. We will report on the profound and yet uncertain consequences for the structure, functioning, and diversity of terrestrial and aquatic ecosystems and the subsequent socio-economic impacts on the Earth system including climate change and food security. If there is time left, I will try to frame these stoichiometric changes into local, regional and global responses of organisms, communities and ecosystems to global change, especially to climate change, at different temporal (mostly for the last, current and future decades) and spatial scales (from genes to the biosphere). Phenotypic, genotypic, migratory, die-back and extinction responses will be considered. The reciprocal effects of all these changes on atmosphere and climate through the consequent biophysical and biogeochemical changes will provide an overview of the importance of the biosphere and its stoichiometry and metabolism for the Earth system and climate.

"Biodiversity: portfolio of responses to global change"

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It has long been recognized that biodiversity involves not only different species but also different life forms and functional strategies. In a rapidly changing world, species respond differently to environmental changes, which prevents generalization and introduces uncertainties in community composition and dynamics in future scenarios. Species can thus be seen as alternative solutions to changing challenges. Biodiversity also encapsulates the notion of intraspecific variability and population differentiation so the portfolio of responses includes a range of alternatives on local adaptation and plasticity that we are just beginning to uncover.

THURSDAY 12 TH OF JULY
S 24 - AQUACULTURE

Molecular basis of egg formation in marine fish: the role of the oocyte aquaporin

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The pre-ovulatory hydration of the oocyte of marine teleosts that produce pelagic (buoyant) eggs occurs concomitantly with meiosis resumption (oocyte maturation), and results in a massive expansion and dilution of the egg cytoplasm. This mechanism is critical for the development of viable gametes as it assures a water reservoir when eggs are released into the hyperosmotic seawater, and facilitates egg buoyancy and dispersal. Therefore, egg hydration and buoyancy are the first markers of egg quality in the aquaculture of marine fish. While a number of studies have identified the major inorganic and organic osmolytes that create the intracellular osmotic driving force for oocyte hydration, water influx was thought to occur passively. However, the discovery of molecular water channels (aquaporins) in all kingdoms of life recently led to the investigation of the role of these membrane proteins during teleost oocyte hydration.

Initial studies in the gilthead seabream uncovered a novel water channel, now termed aquaporin-1ab (Aqp1ab), that mediates water permeation and resultant swelling of the oocytes. Further functional, genomic and phylogenetic analyses revealed that Aqp1ab belongs to a teleost-specific subfamily of water-selective aquaporins, which likely evolved by tandem duplication of a common ancestor. This gene is highly expressed in the ovary of marine and catadromous teleosts that produce hydrated eggs, including the Atlantic halibut, which spawns one of the largest pelagic eggs known. In this species, we recently obtained the first unequivocal functional evidence for the essential role of Aqp1ab during oocyte hydration. Interestingly, however, aqp1ab transcripts are also highly accumulated in the ovary of some freshwater species, such as the stinging catfish, in which oocytes partially hydrate during meiotic maturation, suggesting that Aqp1ab may also play a role in the oocyte of teleosts producing non-buoyant (benthic) eggs.

Using the seabream as the experimental model, we recently demonstrated that the oocyte Aqp1ab is tightly regulated at the transcriptional and post-translational levels. Thus, transcriptional activation of the aqp1ab promoter in oogonia and primary growth oocytes is dependent on the nuclear progestin receptor (nPgr) and regulated by Sry-related high mobility group [HMG]-box (sox) genes, with Sox3 and Sox8b acting synergistically with the nPgr and Sox9b acting as a repressor. Translation of aqp1ab mRNAs occurs in primary oocytes, and subsequently, throughout the period of oocyte growth, Aqp1ab-containing vesicles are transported towards the oocyte cortex. Structural analyses have revealed that the cytoplasmic tail of Aqp1ab is highly divergent in teleosts, but retains specific motifs that regulate vesicular trafficking and temporal insertion in the oocyte plasma membrane during hydration. These processes appear to involve alternative mechanisms of phosphorylation and/or dephosphorylation of specific C-terminal residues. Evidence for the rapid neofunctionalization of the C-terminus is found in the Atlantic halibut, which causes ex vivo loss of function of Aqp1ab when expressed in amphibian oocytes, but not in zebrafish or native oocytes.

These findings are revealing that the Aqp1ab-mediated mechanism of oocyte hydration in marine teleosts is a conserved, but divergently regulated process based on the interplay between osmolyte generation and the controlled synthesis and insertion of Aqp1ab at the oocyte surface. The discovery of Aqp1ab thus provides important new insight into the molecular basis of egg formation and quality in marine fish.

THURSDAY 12 TH OF JULY
S 25 - VIROLOGY

Evolution of HIV-1 Protease over 14 Years: Fitness Loss or Robustness Gain?

Elena Capel, Glòria Martrus, Mariona Parera, Bonaventura Clotet and Miguel Angel Martínez.

Background: The emergence of resistance mutations to protease inhibitors (PI) of the HIV-1 not only reduces the binding of the PIs to the protease but also decreases the viral replication capacity (VRC). Nevertheless, it remains to be elucidated the long-term effect of HIV-1 protease mutations on virus VRC. We observed a positive correlation between a protease sequence conservation, related to the ancestral subtype B sequence, and its *in vitro* viral fitness (r^2 = 0.1115, p = 0.0005). Here, we aim to explore the robustness of the HIV-1 Protease.

Methods: We have studied 3 groups of viral protease sequences, two of them obtained from *naive*-infected patients in two different periods and a third group from infected patients treated with one or more PIs. Firstly, sequence conservation of 139 *naive*-infected patients from our clinical unit, 89 obtained in 1993-1994 and 50 obtained in 2006-2007 was analyzed. Secondly, VRC of 33 recombinant viruses encoding plasma-derived proteases from 11 patients from each group was measured. We calculated VRC as the slope of the natural log of viral antigen p24 production in the cell culture supernatants between days 0 and 7 post-transfection. We also measured the VRC of these 3 groups of proteases after being hypermutated *in vitro* using an error-prone PCR (1.56×10^{-2} nucleotide $\pm$ 4.88×10^{-3}). The VRC from the hypermutated recombinant viruses was related to their *wt* recombinant virus. (Student t-test).

Results: The average conservation of the protease sequences from the *naive*-infected patients dropped significantly between the sequences isolated in 1993-1994 (98%, nt; 96.5%, aa) and the ones isolated in 2006-2007 (96%, nt; 95%, aa) ($p < 0.0001$, nt; $p < 0.0001$, aa). Recombinant viruses from 1993-1994 *naive*-infected patients had better VRC than the 2006-2007 ones. Similarly, both groups displayed a better viral fitness than the recombinant viruses carrying proteases with resistant substitutions to PIs. Nevertheless, these differences were not significantly different. For the *in vitro* hypermutated recombinant viruses, the VRC significantly diminished in all of the three groups. The hypermutated recombinant viruses from the PIs resistant patients suffered the highest VRC drop.

Conclusions: Viruses under selective pressure of PIs appear to be more vulnerable to the emergence of new mutations, whereas viruses from *naive*-infected patients seem to be more robust independently of their conservation to ancestral sequences.

Keywords: Viral Replication Capacity (VRC), protease, robustness, evolution.

Antiretroviral agents effectively block HIV replication after cell to cell transfer

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Cell to cell transmission of HIV has been proposed as a mechanism contributing to virus escape to the action of antiretrovirals and a mode of HIV persistence during antiretroviral therapy. Here, cocultures of infected HIV-1 cells with primary CD4+ T cells or lymphoid cell lines were used to evaluate virus transmission and the effect of known antiretrovirals. Transfer of HIV antigen from infected to uninfected cells was resistant to the reverse transcriptase inhibitors (AZT and tenofovir), but was blocked by the attachment inhibitor IgGb12. However, quantitative measurement of viral DNA production demonstrated that all anti-HIV agents blocked virus replication with similar potency compared to cell-free virus infections. Similarly, cell-free and cell-associated infections were equally sensitive to inhibition of viral replication when measuring HIV-1 LTR-driven GFP expression in target cells. However, detection of GFP by flow cytometry may incorrectly estimate the efficacy of antiretrovirals in cell-associated virus transmission, due to replication independent Tat-mediated LTR transactivation consequence of cell-to-cell events that did not occur in short-term (48h) cell-free virus infections. In conclusion, common markers of virus replication may not accurately correlate and measure infectivity or drug efficacy in cell to cell virus transmission. When accurately quantified, active drugs blocked proviral DNA and virus replication in cell to cell transmission, recapitulating the efficacy of antiretrovirals in cell-free virus infections and *in vivo*.

HIV maturation: a new RNA-supported paradigm of spatiotemporal nucleoprotein remodeling catalysed by a protease

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Extracellular maturation of a HIV-1 virion by its protease results in establishing a condensed ribonucleoproteic architecture, i. e. the nucleocapsid, that internalize the retroviral RNA associated to the mature nucleocapsid protein (NCp7) within its surrounding capsid. Up to now, it is still poorly documented how this critical process is controlled, as well as how protease acts within HIV-1 virions to sequentially produce NCp7 from its Gag substrate with as intermediate products NCp15 and NCp9. Here, we will see that protease-driven NCp15 to NCp9 processing provokes RNA condensation and that NC-RNA interactions provoke an allosteric switch of the NCp15 thus a severe activation of PR. These data drive us to a new vision of HIV-1 maturation with the impact as an upregulator of the intravirion RNA leading to its own condensation by directing NC processing. Such a model also leads to highlight new transactions between a RNA molecule and a protease.

Transmissibility of two ipomoviruses by whitefly vectors under experimental conditions

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Plant viruses are important pathogens of crops, disseminated usually by vector organisms. In the family *Potyviridae*, the existence of the viral auxiliary factor HCPro has been described as essential for aphid transmission of potyviruses, but little is known about the transmission by whiteflies of ipomoviruses, another genus in the family.

To clarify the mechanisms involved in dissemination of ipomoviruses, we have performed controlled transmission experiments using *Bemisia tabaci* whiteflies and two ipomoviruses: one isolate of the virus type Sweet potato mild mottle virus (SPMMV), and one isolate of Cucumber vein yellowing virus (CVYV). The genomic organization of these two viruses is different, with an HCPro region only present in SPMMV. Despite the absence of HCPro in its genome, the isolate of CVYV was readily transmitted from plant to plant using infected cucumbers and *B. tabaci* vectors, while in parallel experiments the isolate of SPMMV failed to be transmitted from tobacco to tobacco plants, or between different combinations of tobacco and Ipomoea plants (*I. nil* and *I. setosa*). To follow up viruses along the transmission process, we designed a highly sensitive real time RT-PCR assay in individual whiteflies. Similar frequencies of detection of CVYV and SPMMV amplicons were obtained in vectors after different periods of access on infected plants. The subsequent inoculation failure of SPMMV could not be explained by any divergences with respect to CVYV.

Mixed-infections of SPMMV and other viruses are very frequent in naturally infected plants, and often produce a synergistic effect resulting in higher titers of the virus in the double-infected plants. This increase in accumulation might facilitate transmission in natural conditions, providing an explanation for the observed lack of transmission in our experiments with single-infected plants. To test this hypothesis, we are carrying out co-infection assays of SPMMV with other viruses to reproduce the synergistic effect before testing vector transmission.

The Movement Protein of Cucumber mosaic virus (CMV) determines the virulence in the melon accession PI161375

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Cucumber Mosaic virus(CMV), the type member of the genus Cucumovirus, belonging to the family Bromoviridae, is one of the most widespread plant viruses, being able to infect more than 1000 species. Its genome consists of three single-stranded sense RNAs. There are only two accessions of melon reported to be resistant to CMV, Freeman's Cucumber and the exotic accession Sonwang Charmi, PI161375.

Using a collection of Near Isogenic Lines (NILs) between the Spanish variety "Piel de Sapo" and PI161375, we have described a recessive gene, *cmv1*, that confers total resistance to strains LS, P9 and P104.82, but not to FNY, TL or M6. The strains non-responsive to *cmv1* are probably not able to interact with this gene to complete their infectious cycle, whereas the responsive strains can. Therefore, both sets of strains are different in their virulence determinant. Reassortment studies between FNY and LS RNAs have shown that the determinant of the virulence that governs the response to *cmv1* is in RNA3. RNA3 is composed by three untranslated regions and two proteins, the movement and the coat protein. Chimaeras between FNY and LS showed that the determinant of the virulence is the movement protein (MP). Now we are trying to identify the aminoacid that confers virulence or avirulence against *cmv1*.

Current work is also aimed at analysing the mechanisms of the resistance provided by *cmv1*. We have showed that the strain LS is able to accumulate in the cotiledons of the resistant line. Additionally, we have started to study the cell-to-cell movement using printings of inoculated leaf, observing that the virus can move cell-to-cell until the veins. These data indicate that the lack of systemic infection of LS strain would be at the level of long distance movement.

Canvis en l'evolució dels patògens sexualment transmissibles. De l'examen directe a la biologia mol.lecular.

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Introducció

Les infeccions de transmissió sexual (ITS) han estat sempre presents entre els humans. Els seus canvis epidemiològics estan influenciats per l'evolució dels patògens, i pels comportaments sexuals i les intervencions en l'hoste. Els avenços en les tècniques de biologia mol.lecular també han influït en una major capacitat de detecció i caracterització dels agents implicats.

Mètodes

Revisió de dades microbiològiques i epidemiològiques sobre ITS de Catalunya, especialment de la Unitat d'ITS de Barcelona (UITSB), del període 2002-2012.

Resultats

Gonocòccia, clamídia i sífilis són infeccions tractables amb antibiòtics. A l'augment dels casos cal afegir-hi l'emergència de la resistència antibiòtica. De la simplesa de l'examen directe mitjançant la tinció de gram, el maneig de la gonocòccia és cada cop més complicat com es posa de manifest seqüènciant gens resistents als diferents antimicrobians (el primer cas ja resistent a ceftriaxona va ocórrer el 2011). La prevalença de clamídia arriba al 10% actualment, essent destacable l'aparició del genotip L (limfogranuloma veneri) des de l'any 2004, el qual mitjançant MLST ha permès definir la clonalitat de la soca europea. La sífilis ha reemergit amb força multiplicant-se per 10 el nombre de casos en aquest període. El nombre d'infeccions per l'HIV tendeix a incrementar-se novament, essent el 80% del total per transmissió sexual actualment. La prevalença del virus del papil.loma humà arriba al 14% de les dones, la disponibilitat d'una vacuna contra alguns dels seus genotips ha demostrat el seu impacte potencial en algunes de les malalties que causa. Les tècniques de PCR han permés conèixer l'epidemiologia del virus de l'herpes simple, un 30% del casos a nivell genital estarien relacionats amb el tipus 1.

Discussió

L'emergència i la reemergència de diverses ITS està relacionada amb la interacció dels patògens sexualment transmissibles amb l'hoste. El patró epidemiològic de les diferents ITS és dinàmic fruit de l'evolució d'aquesta interacció. El laboratori juga un paper imprescindible pel seu coneixement, sobretot amb la incorporació de tècniques mol.leculars.

Estudio de la infección del virus de la hepatitis C utilizando un sistema celular basado en la secreción de gaussia luciferasa

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Introducción y objetivo: El cultivo celular del virus de la hepatitis C (VHC) está prácticamente restringido al genoma JFH-1 (genotipo 2a).Dado que el VHC derivado de suero de pacientes replica a niveles bajos *in vitro*, es necesario el desarrollo de sistemas muy sensibles para evaluar su infectividad en cultivo. Un sistema así sería de gran utilidad para seleccionar aquellos sueros que contienen los virus más infecciosos *in vitro* y para clonar nuevos aislados.

Métodos: Se ha generado una línea celular (Huh7.5/EGFP-GL) que expresa de forma estable la proteína verde fluorescente (EGFP) fusionada con la *gaussia luciferasa,* mediante una secuencia de reconocimiento de la proteasa NS3/4A del VHC. Después de la infección por el VHC, la proteasa corta en su sitio de reconocimiento y la *gaussia luciferasa* se secreta al medio de cultivo; la intensidad de la actividad *luciferasa* se determina por luminometria y se correlaciona con la infectividad del virus.

Resultados: La línea celular Huh7.5/EGFP-GL permitió la cuantificación rápida y sensible de la infección por el VHC utilizando la cepa JFH-1. El tratamiento de las células con inhibidores de la entrada y de la replicación del virus produjo una disminución de la secreción de *gaussia luciferasa* de forma dosis-dependiente. Por otra parte, también se investigó la capacidad replicativa *in vitro* del VHC presente en los sueros de pacientes infectados (genotipo 1b y con elevada carga viral). De los 86 sueros analizados, 4 fueron capaces de infectar y replicar *in vitro*.

Conclusiones: La línea celular Huh7.5/EGFP-GL es un buen sistema para la identificación de sueros que contienen VHC capaz de replicar *in vitro.* Estos sueros podrían ser la base para clonar nuevos genomas del VHC. Además, podría ser una herramienta sensible para el cribado de inhibidores de la entrada viral y de otros compuestos antivirales.

Caracterización de UL8, un nuevo miembro de la familia multigénica RL11 del HCMV

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Una proporción significativa del complejo genoma del citomegalovirus humano (HCMV) está comprendida por 12 familias multigénicas. Una de ellas, la familia RL11, consta de 12 genes agrupados en el extremo izquierdo del genoma, los cuales en su mayoría permanecen inexplorados. Por su localización, características y el hecho de que no sean esenciales para el crecimiento del HCMV en cultivo de tejidos, se ha hipotetizado que estos genes podrían estar implicados en la interacción virus-huésped.

En este estudio, presentamos la caracterización de UL8, un nuevo miembro de la familia RL11 generado a partir de un proceso de *splicing* no predicho en la descripción original del genoma del HCMV. UL8 se encuentra altamente conservado entre las diferentes cepas del HCMV y posee un único ortólogo identificable en el citomegalovirus de chimpancé.

UL8 codifica para una proteína de 324 aminoácidos con un péptido señal, un dominio inmunoglobulina *v-like* extracelular, un tallo tipo mucina, una región transmembrana y una cola citoplasmática con un potencial motivo de internalización. El dominio inmunoglobulina, compartido con UL7, un producto viral previamente estudiado en nuestro grupo, presenta homología con el dominio N-terminal de CD229, un inmunoreceptor involucrado en la activación de los leucocitos. Consistente con esto, presentamos que el dominio inmunoglobulina de UL8 es capaz de mediar adhesión a células dendríticas y neutrófilos activados. Ensayos de mutagénesis dirigida han permitido determinar los aminoácidos críticos implicados en esta interacción. El tratamiento con endoglicosidasa F demuestra que UL8 es una proteína altamente glicosilada. Además, mediante la generación de anticuerpos específicos, hemos determinado que UL8 se localiza principalmente en la superficie celular y es liberada al espacio extracelular, identificando una región del tallo proximal a la membrana relevante en este proceso. Actualmente, mediante ensayos adicionales de mutagénesis dirigida y el uso de inhibidores farmacológicos, se está explorando la función de la cola citoplasmática de esta proteína.

THURSDAY 12 TH OF JULY
S 26 - MICROBIOLOGY

Remediation and cooperation

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Most chemical pollutants are transformed by microorganisms, that can either degrade the pollutants completely, or modify them, originating intermediates which might have higher toxicity than the original compounds. This ability for pollutant degradation is biotechnologically exploited in what we know as bioremediation. In the last decades, more than five hundred microbial pollutant degraders, more than two hundred pathways and more than fourteen hundred reactions have been discovered. However, we still do not understand the complexity of factors involved in bioremediation, which comprise characteristics of the pollutants, abiotic factors of polluted environment, as well as the response of microbial communities to pollution, including that of degraders which are part of those communities but not the only members. We have learnt that microbial communities in chronically-polluted environments are highly diverse. Besides, we know that the predominant microorganisms in these communities are not those that we consider typical degraders. We should consider these communities as well adapted to their particular environmental conditions, including the presence of pollutants. The process of adaptation of a microbial community to a pollution event comprises several degrees of complexity. Firstly, a cellular response, often taking place in the form of a stress response. Secondly, a population-level response, leading to the selection of clonal variants with higher fitness. Finally, a global response, originating changes in community structure and composition. The experience accumulated so far proves that pollutant degradation relies in the cooperation between microorganisms, and probably involves species which are not recognized as pollutant degraders so far. Thus, degradation would be the result of the activity of a complex metabolic network, integrating different catabolic abilities present in diverse microorganisms. The challenge now is to understand the integration of the different elements in order to develop more efficient bioremediation processes.

Extremophilic microorganisms, and life beyond

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One of the fundamental goals of biology is to understand the limits of life. The exploration of extreme environments has allowed the discovery of numerous habitats that were considered unsuitable for development of life only a few years ago. As a result, the interest in learning about diversity and ecology of extreme environments has grown exponentially, not only to deepen the knowledge of them but also to explore possible applications of microorganisms that inhabit them or their components. Extremophiles also have a role in the development of astrobiology. According to the roadmap of the NASA Astrobiology Institute (http://astrobiology.arc.nasa.gov), one of the main objectives of this area of transdisciplinary research is the characterization of extreme environments, microorganisms that live and the different mechanisms used to solve physiological problems created by the extreme conditions in which they develop. Research in extremofilia has increased the possibility of finding life in the Universe, as has demonstrated that life does not need for its development conditions requiring the complexity of eukaryotic ecosystems that we use for reference. Among extreme environments, the acidic habitats associated with mining activities deserve special attention, since they are not adapted to conditions imposed by the geophysics of the planet but are products of chemolithotroph metabolism performed by microorganisms capable of obtaining energy from reduced mineral, essentially metallic sulfides. The exploration of subsoil geomicrobiology of the Iberian Pyrite Belt allows us to understand the dynamics of chemolithoautotrophic ecosystems that are independent of the solar irradiation and are capable of generating extreme acidic environments of astrobiological interest, such as the Río Tinto Basin, a terrestrial geochemistry analogue of Mars.

The biosphere of rare bacteria: the largest and oldest cooperative in the world

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All communities are dominated by a few species that account for most of the biomass and carbon cycling. On the other hand, a large number of species are represented by only a few individuals. In the case of bacteria, these rare species were until recently invisible. Due to their low numbers, conventional molecular techniques could not retrieve them. Isolation in pure culture was the only way to identify some of them, but current culturing techniques are unable to isolate most of the bacteria in nature. The recent development of fast and cheap high throughput sequencing has begun to allow access to the rare species. In the case of bacteria, exploring this “rare biosphere” has several points of interest. First, it will eventually produce a reasonable estimate of the total number of bacterial taxa in the oceans. Right now we do not even know the right order of magnitude. Second, it will solve the question of whether everything is everywhere or not. Third, it will require hypothesizing and testing the ecological mechanisms that allow subsistence of many species in low numbers. And fourth, it will open an avenue of research into the immense reserve of genes with potential applications hidden in the rare biosphere. One of the most attractive perspectives is to consider this immense set of bacteria and genomes as a community that cooperates to maintain the cycles of elements and the flux of energy.

THURSDAY 12 TH OF JULY
S 27 - ECOLOGY

The threat of Earth's oceans becoming more acidic

Carles Pelejero

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Oceans, which cover over 70% of Earth's surface, contain about 97% of the Earth's surface water, represent over 99% of its living space, and provide approximately half of the oxygen that we breathe, are under pressure due to human activity. Ocean health is being disturbed by a range of stressors, three of them critical on a global scale. Global warming, now beyond doubt, was the first to be realized. Now, two additional stressors have been shown to be at play: Ocean acidification and ocean deoxygenation. In this talk, I will focus on the first, which is an insidious consequence of the anthropogenic rise in atmospheric CO2 due primarily to fossil fuel combustion. This phenomenon, often referred to as 'the other CO2 problem', is the consequence of the marine absorption of a large portion of the cumulative anthropogenic atmospheric CO2 emissions, which has already acidified the surface oceans by about 0.1 pH units since preindustrial times. Models project that surface ocean pH will further decrease 0.3 to 0.4 pH units by the end of this century. This change will represent a 150% increase in the concentration of protons and a 50% decrease in carbonate ions with respect to pre-industrial values.

In this presentation, we will take a tour through the different key aspects involved in this serious environmental problem, starting with a brief overview of the evolution of the CO2 levels in the air, the absorption of part of it by the oceans and the chemical changes that it causes, with examples on instrumentally measured data over the last decades in different parts of the ocean, which clearly reflect that the progressive acidification of the oceans can indeed already be detected. In addition, we will place into context these recent anthropogenic changes through an evaluation of paleoreconstructions at different temporal scales, where we will find that today surface oceans have already achieved levels of acidity without precedents over the last 20 million years and that the values that oceans will reach by the end of this century are unprecedented, at least, in 40 million years. More importantly, we will see how this rise in CO2 and lowering of pH is occurring at a rate not seen for hundreds of millions of years, so the oceans are entering an unknown territory possibly involving major ecosystem changes. We will discuss which are the consequences of acidification for marine organisms, with special emphasis on species that build skeletons of calcium carbonate such as corals, shellfish and important planktonic species. We will also list some examples where the existence of adverse effects on marine biota has already been detected in situ, and we will review some of the strategies of studying this phenomenon, with particular emphasis on pH-manipulation experiments in aquaria, instrumental measurements in cruises and at fixed stations, and paleoreconstructions.

Marine bioinvasions: a future nuisance that is already present

Enric Ballesteros,

CEAB-CSIC

Current knowledge on the effects of invasive species in the marine environment is delayed when compared to terrestrial ecosystems. However, there is already enough data to ascertain that their effects can be as important as those observed in terrestrial ecosystems. Here I will refer to which are the main vectors of introduction of alien species in marine ecosystems with comments on their most outstanding examples. I will also carry out a critical review of their effects and highlight its high unpredictability based on the biological characteristics of the species. Finally, I will focus on the possible role of climate change in accelerate or decelerate the introduction of species and their expansion.

THURSDAY 12 TH OF JULY
S 28 - AQUACULTURE

Endocrine regulation of lipid metabolism markers in fish

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Regulation of lipid metabolism in fish is a complex process controlled by multiple factors. In the last years, there has been an increased interest in the study of the mechanisms underlying the regulation of metabolism related to fatty acids (FA) in fish, due to their importance if final product quality and animal health. In sea bream and salmonids, adipose tissue and muscle are the main organs of fat depot. Therefore, the development of in vitro tools such as fresh isolated adipocytes, primary preadipocyte cultures and myocyte cultures in sea bream, trout and salmon has permitted to advance in the study of the regulation of lipid storage and mobilization in these species. Specifically, isolated adipocytes lipid metabolism and gene expression of enzymes such as hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) reflect the physiological stage of fish, which can be affected by vegetable diets. Control of glucose and FA uptake by insulin and insulin-like growth factor (IGF-I) and the involvement of novel signaling pathways (TOR) in the modulation of FA metabolization in fish muscle have been analyzed. In addition, the regulation of the expression of the recently identified fatty acid transporters CD36 and FATP1, as well as the translocation to the plasma membrane in response to insulin of the latter has been studied. Moreover, the transcription factor liver X receptor (LXR), recently described in salmonids, and some of its target genes, considered markers of FA and cholesterol metabolism, have been characterized in trout muscle and adipocyte cells, showing tissue specificity. Transcription of LXR and its target genes, i.e. peroxisome proliferator-activated receptors (PPARs), ATP-binding cassette transporter A1 (ABCA1), fatty acid synthase (FAS) and LPL is finely regulated by hormones such as insulin and growth hormone. Furthermore, the characterization of the expression of adiponectin and its receptors, a new regulatory system described in fish, opens new fields of study. Overall, these findings can contribute to the better understanding of the control of lipid metabolism in fish muscle and adipose tissue and may help identify gene markers for adiposity, potentially useful to obtain a good quality product for aquaculture.

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The response to stressors in fish. An interactive integration involving all regulatory systems

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Stress is an experience that all animals can be exposed and that generates a number of responses either at short term or at longer term that depends on the species, the stressor type and the exposure time. The stress response is characterized by a number of changes driven by the three regulatory systems, neural, endocrine and immune. The interaction between these three systems, the signaling pathways and in particular, the mediators of these interactions are relevant to characterize the overall response. Stressors may be deletereous under chronic situations but short term stressors may not be negative if they provide a life experience that prepares for a next stressor, therefore the characteristic behaviour of each species, the interaction with conspecifics when kept in a confined environment and moreover the coping strategy of individuals also influence the reponse.

PLENARY LECTURES

TUESDAY 10 TH OF JULY

The Evolution of Language: Case Studies in Cognitive Biology

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It is quite widely accepted that human language rests upon an evolved biological foundation, some components of which are unique to our species. There is considerable debate, however, about the precise nature of the cognitive prerequisites for language, the degree to which they are specific to language, and which components are or are not shared with other animals. The evolutionary basis of such components is also a topic of much disagreement. I will argue that resolution of such long-running debates requires a strongly comparative approach that adopts a Tinbergian framework treating mechanism, function, ontogeny and phylogeny as equal partners in an adequate biological explanation. Even though language, as a whole, appears unique to humans, many components of language can nonetheless be studied comparatively. I will illustrate this perspective with two case studies, focused on speech and syntax, respectively. In speech, recent data indicate that a long-standing focus on the speech periphery, and particularly the descended human larynx, has deflected attention away from more fundamental changes in the neural pathways involved in speech control. A surprisingly broad set of species, including monkeys, deer, songbirds, and seals, provide comparisons that are relevant to this conclusion. Turning to syntax, recent data examining pattern perception in both auditory and visual domains supports the contention that some aspects of linguistic syntax rest on a cognitive basis that, although unusual or perhaps unique to our species, seems to apply across cognitive domains including speech, music and visual pattern perception. This ability is characterized by a human propensity to attribute complex, hierarchically-embedded structures to visual or auditory inputs. I conclude that the broad comparative approach favored by cognitive biologists has much to teach us about the biology and evolution of language, and that future progress will require investigation of a much broader set of species than has typified past work.

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WEDNESDAY 11 TH OF JULY

Huntington's Disease: from evolution to pathology

Elena Cattaneo

Università degli studi di Milano

Huntingtin (htt) is the ~800 million-year old protein product of the Huntington's disease (HD) gene. The gene contains a polymorphic tri-nucleotide CAG repeat that is translated into polyglutamine amino acid (polyQ) residues in the protein. When this polyQ stretch at the 18 aminoacid (aa) position of the protein expands to over 39 residues, HD occurs, a fatal, genetically dominant, neurodegenerative disease. The CAG repeats are well conserved in deuterostomes, which suggests that they are an ancestral feature retained during the evolution of the protein. Htt carries a number of specific activities in the adult brain; for instance, it promotes transcription of neuronal genes among which is the BDNF, a neurotrophin critical for the survival and activity of cortical and striatal neurons that degenerate in HD.

This presentation will highlight the power of combining evolutionary and developmental approaches to the study of the biology of disease-genes and will review the more recent discovery of a function for htt in neuroepithelial stem cells.

THURSDAY 12 TH OF JULY

The origin of AIDS

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June 1981 is the official birth date of the acquired immunodeficiency syndrome (AIDS) epidemic. In a short article published in the Centers for Disease Control's *Morbility and Mortality Weekly Report*, American clinicians described a cluster of five cases of *Pneumocystis carinii* pneumonia, an infection of lungs hitherto seen only in patients with severe impairment of their immune system. Nobody could have imagined that, within tree decades, thirty millions individuals would have died of AIDS, living in the process sixteen millions orphans. By 2011, another thirty-four million were living with human immunodeficiency virus (HIV). Human AIDS is caused by two lentivirus, HIV type 1 and 2 (HIV-1 and HIV-2). Despite the scientific advances made since the birth of AIDS epidemic, questions of the pandemic's origin still trouble us. Some AIDS creation myths continue to have an allure, for example, that HIV came out of oral polio vaccines, or that the virus is a man-made germ-warfare agent that was deliberately released in Africa by the United States. Even so, today is irrefutable that HIV-1 and HIV-2 are the result of multiple cross-species transmissions of simian immunodeficiency viruses (SIVs) naturally infecting African primates. Most of these transfers resulted in viruses that spread in humans to only a limited extent. However, one transmission event, involving SIV from chimpanzees (SIVcpz) in west central Africa, gave rise to HIV-1 group M, the principal cause of the AIDS pandemic. The circumstances that enhanced HIV-1 group M human-to-human infections remain more controversial. It has been suggested that large scale medical injection campaigns conducted in west central Africa at the beginning of the twentieth century, together with the destabilization of social structures by colonisation, and an increased prevalence in sexually transmitted diseases may have facilitated the early dissemination and adaptation of HIV-1 and HIV-2. Although searching the past may seem irrelevant, understanding the origin of AIDS might help avoid future pandemics.

Symbiotic planet

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Throughout evolutionary time, microorganisms have been responsible for maintaining the biosphere. Despite the crucial role that they play in the cycling of nutrients, only rarely the microorganisms responsible for key processes have been identified. Obstacles that have traditionally impeded fundamental microbial ecology inquiries are now yielding to technical advancements. All current information about prokaryotes is based on measurements performed on less than 5000 isolated species, which represent ca. 0.1 % of the total estimated diversity of prokaryotes in the biosphere. The Earth's habitats present complex gradients of environmental conditions that include extreme variations in temperature, light, pH, pressure, salinity and both inorganic and organic compounds. Each geochemical setting features its own panoply of resources that can be physiologically exploited by microorganisms. Although small (10–7 to 10–6 m), they are abundant (1030 to 1032 individuals globally, including viruses). Their phylogenetic and physiological diversity is considerably greater than that of animals and plants, and their interactions with other life forms are correspondingly more complex. Microbes interact with the other forms of life (their descendants) in many forms, from the planetary scale to the cellular dimensions. In the planetary scale, microbes regulated essential processes in both continents and seas. Over geological time the ocean has evolved from being an anaerobic incubator of early cellular existence into a solar-powered emitter of molecular oxygen, a transformation that has been punctuated by catastrophic extinctions followed by the iterative re-emergence of biological diversity. Today, the ocean is becoming substantially warmer, more acidic and expansion of oxygen-starved regions, which causes changes in the cycling of trace gases such as methane, nitrous oxide and carbon dioxide, which are very significant for the global metabolism and can affect the climate change. Down to the cellular scale, microbes live on and inside (both peri- and intra-cellularly) all other organisms, affecting their metabolism and fitness. None a single species can evolve without the concomitant evolution of its accompanying microbes. Evolution is an integrative process in which organisms, populations and whole ecosystems adaptatively change following modifications in the environment according to the constrictions of natural selection.

Epigenetics in ecological research and animal production

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Epigenetics is an exciting and fast developing area of biology. It deals with the study of changes in gene expression and function contributing to the phenotype that, nevertheless, do not involve alterations of the genotype. These changes can be heritable through cell mitoses and even individual generations. They include three major mechanisms that can activate or suppress gene expression: DNA methylation, modification of chromatin structure through alterations in histone proteins, and regulatory processes mediated by small RNAs. Epigenetic changes are actively studied in developmental biology and for their implications in human health, notably in oncology. However, less attention has been paid in other fields, including evolutionary biology and ecology. Since epigenetic processes have been found to be responsible for the integration of both biotic and abiotic factors, epigenetics can help to understand how organisms respond to environmental change. In addition, epigenetic variation may explain phenotypic changes in ecologically important traits observed in natural populations that cannot be explained by genetic variation, and thus how phenotypic plasticity is brought about. Finally, epigenetic modifications can be considered in the light of selection programs to increase animal production. The link between conditions during early development, where many heritable epigenetic marks are established, and gene function later in life is particularly interesting. Thus, proper management of these early conditions may contribute to better growth and health of farmed animals. Because of their external fertilization, fish are excellent research subjects where to study epigenetic changes. Here, these various aspects of epigenetics will be discussed. Among others, an example will be provided on how modifications of a major environmental cue such as temperature is integrated through an epigenetic mechanism, ultimately determining phenotypic sex and growth in a production fish. Supported by grants CSD2007-0002 (Consolider “Aquagenomics”) and AGL2010-15939 (“Epigen-Aqua”).

THURSDAY 12 TH OF JULY
Closing Plenary lecture

Plant-Animal Mutualistic networks: the Architecture of Biodiversity

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The mutualistic interactions between plants and the animals that pollinate them or disperse their seeds can form complex networks involving hundreds of species. These coevolutionary networks are highly heterogeneous, nested, and built upon weak and asymmetric links among species. Such general architectural patterns increase network robustness to random extinctions and maximize the number of coexisting species. Therefore, mutualistic networks can be viewed as the architecture of biodiversity. However, because pylogenetically similar species tend to play similar roles in the network, extinction events trigger non-random coextinction cascades. This implies that taxonomic diversity is lost faster than expected if there was no relationship between phylogeny and network structure. I will conclude by exploring the trade-offs between a species' relative contribution to the above patterns of network architecture, and its own survival probability.

POSTERS

MONDAY 9 TH OF JULY

P1

Perinatal Hypoxia and Psychotic Experiences in Adulthood. Putative Moderation effects of the Histone deactylases (HDAC) genes.

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Obstetric complications (OCs), especially hypoxia, have been associated with schizophrenia in several studies (Cannon, Jones et al. 2002); however, their implication in the risk for Psychotic Experiences (PEs) in healthy people from the general population has been less analyzed (Zammit, Odd et al. 2009). On the other hand, the existence of gene-environment interactions (GxE) between hypoxia-related genes and schizophrenia has been proposed (Schmidt-Kastner, van Os et al. 2012). Histone deactylases (HDAC) are enzymes involved in the epigenetically-mediated response to hypoxia (Watson, Watson et al. 2010). Individual genotypic differences for the HDAC genes could also be related to epigenetic consequences after exposure to environmental stressors. This work aims to test the interaction between hypoxia and adult PEs and the possible modulating effect of HDAC genes in this risk.

The results shown here indicate that perinatal hypoxia is associated with an increased risk of having positive PEs (i.e., subclinical forms of positive psychotic symptoms) during adulthood, and that individuals both carrying the C allele at the rs7290710 (*TUBGCP6* gene, adjacent to *HDAC10*) and exposed to perinatal hypoxia are more prone to develop adult positive PEs. Even though the precise mechanism underlying the GxE is unclear, it is interesting that *TUBGCP6* gene, which encodes for gamma tubilin, is involved in neurodevelopment and *HDAC10* gene affects neuronal physiological processes.

Acknowledgements

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P2

A polymorphic human inversion exchanges the first exon between two chymotrypsinogen genes

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In the last years, we have seen an increasing interest on structural variants, including their effects and prevalence in the human genome. One of the most complicated rearrangements to study are chromosomal inversions, that have been relatively overlooked. We present a detailed analysis of a polymorphic inversion of 19.6 kb in chromosome 16 that had been previously predicted by different techniques. This inversion was generated by non-allelic homologous recombination between two segmental duplications of 1.5 kb and 99% identity that flank the inverted region, and causes the exchange of the first exon and promoter regions between genes *CTRB1* and *CTRB2*. These genes are expressed in pancreatic tissues and encode chymotrypsinogen, an inactive precursor of the enzyme chymotrypsin, which is involved in protein digestion in the gastrointestinal tract. The inversion has been genotyped by PCR in three HapMap populations (European, Asian and Yoruban) and its frequency ranges between 0% in the Asian population and 8% in Europeans. The analysis of this region has also allowed the detection of a 574-bp deletion associated to the ancestral standard allele that eliminates one exon of *CTRB2* and that could have functional consequences by inactivating one of the gene copies. This deleted allele is found in the three analyzed human populations but presents a higher frequency in Europeans where 6.5% of the genotyped alleles carry this deletion. Even though the effect of this polymorphic inversion on chymotrypsinogen genes remains still unknown, it provides an excellent opportunity to study the effects of inversions on the expression of neighboring genes and to explore the role of these rearrangements in human evolution.

P3

Analysis, breakpoint definition and validation of inversions between assembled human genomes

David Vicente[1], Cristina Aguado[1], David Izquierdo[1], Alexander Martínez-Fundichely[1], Marta Puig[1] and Mario Cáceres[1,2]

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Historically inversions were the first structural variants studied in different species using cytogenetic approaches. However, their difficult detection and study shifted the interest to other types of structural variants. In the last years detection methods have focused on sequence-based approaches and many inversions have been reported, although most have not been examined any further. Here, we have analyzed in detail the 90 inversions previously predicted from the comparison of two independently assembled human genomes: the human reference genome (NCBI36.1 or HG18) and the J. Craig Venter genome (HuRef). Our strategy consists of an initial bioinformatic sequence analysis and a posterior experimental validation by PCR. First, the putative inverted regions between these two genomes were aligned to check for the presence of inverted segments, and 31 regions were discarded due to alignment mistakes, gaps or assembly errors caused by duplicated sequences. Next, 45 of the remaining 59 inverted regions were tested using PCR and inverse PCR on a diversity panel formed by 9 HapMap individuals from European, Asian and Yoruban origin together with HuRef DNA. In 17 inverted regions standard and inverted alleles were detected and were classified as validated polymorphic inversions. For the 28 inverted regions that showed the same conformation in all analyzed individuals, the orientation of the source DNA where the alternative arrangement had been initially predicted (BACs from the human genome project or HuRef DNA) is currently being checked by PCR. So far all 8 regions tested have been discarded as errors in HG18 or HuRef genome assemblies. This validation process therefore discards most of the inversions predicted in the Venter genome as false, and highlights the need of careful analysis of the structural variants obtained from genome sequence comparisons. These results also contribute to generate a reliable catalog of human polymorphic inversions, which is essential to understand the functional effects of inversions and their contribution to human evolution.

P4

Polymorphism, recombination and selection efficiency along the complete genome of *Drosophila Melanogaster*.

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Whether diversity in the genomes are shaped mainly by neutral processes or selection is a recurrent theme in population genetics. Under neutrality, no relationship between levels of polymorphism and recombination is expected. However, the correlation between polymorphism and recombination appears to be one of the most consistent patterns of population genetics, with similar relationships found in every species examined. The neutral model predicts that if recombination itself is mutagenic then high recombination regions will exhibit higher levels of both polymorphism and divergence. The alternative hypothesis is that some form of linked selection is acting across the genome such that loci within higher recombination are more likely to escape from the effects of nearby selection, whether advantageous or deleterious.

We used the 158 DGRP Illumina sequence data and genome sequences from *D. simulans* and *D. yakuba* to perform genome wide analyses of polymorphism and divergence and assessed the association of these parameters with the recombination landscape on a much larger scale than had been previously done. We used two recombination estimates, recombination map estimates (in cM/Mb units) and the population recombination rate ($4N_er$) based on linkage disequilibrium in 50kbp non-overlapping windows.

Regions whose recombination is < 2cM/Mb there is a positive correlation between polymorphism and recombination rate, while divergence levels remain unaffected. Above this threshold, recombination and polymorphism behaves independently. We would expect to have a stronger correlation between polymorphism and recombination when selection efficiency increases. However, we find a greater adaptive propensity in genes whose recombination context is > 2cM/Mb. We explore this apparent contradiction establishing a specific threshold for each chromosome arm and analyzing different selection regimes above and below these thresholds. While in autosomes less than 1/3 of the chromosome is above this threshold, it spans more than 2/3 on the X chromosome.

We hypothesize that in genomics regions where polymorphism and recombination are uncorrelated, sites behaves as effectively independent between them. Thus, in those regions, the expectations of Neutral Theory are fulfilled even though the efficiency of selection is higher. Below this threshold, sites are linked and genetic hitchhiking and/or background selection reduce levels of polymorphism by an amount proportional to the strength of selection and the recombination rate. The independence between sites is achieve depending on the interactions of levels of polymorphism and recombination rate. These results throw new insight in the relationship between demographic changes and natural selection as well as in sex chromosome evolution.

P5

Calling inversions from Next-Generation Sequencing Paired-End Mapping data with GRIAL

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Over the past years there has been an increased interest to identify submicroscopic structural variation in genomes. However, studies on the characterization of inversions have been limited and underdeveloped. Paired-end mapping (PEM) sequence signatures allow for inversion detection in a genome compared to a reference genome. GRIAL is a novel algorithm for the prediction of inversions using paired-end (PE) sequence data that incorporates geometric rules to accurately locate the breakpoints and remove many typical cases of false positives. Here we use GRIAL to analyze next-generation sequencing (NGS) PEM data generated by mrFast (Alkan et al. 2009) and score the predicted inversions to discard false positives. The strategy has been applied to the detection of inversions in six human individuals with high-coverage NGS PEM data, and genetic transmission is analyzed in a family trio. The results have been compared to previous validated datasets obtained from fosmid PEM data. Our preliminary data suggest that the current strategy could be missing most long inversions in the human genome flanked by highly identical segmental duplications due to the short insert sizes of the libraries and the conservative criteria used for inversion prediction. With the exception of inversions mediated by complex duplication, our results suggest that we can accurately predict smaller inversions using this approach. In any case, the analysis of concordant mappings seems essential to get reliable inversion calls.

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P6

Chronic treatment with a putative TrkB agonist, 7, 8-Dihydroxyflavone ameliorates motor and cognitive deficits in a Huntington's disease mouse model

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Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by an expansion of the polyglutamine tract of huntingtin protein, which displays preferential degeneration of medium spiny neurons in the striatum. Various data suggest that the reduced Brain-derived neurotrophic factor (BDNF) levels and general loss of activity of its receptor trkB, play a crucial role in HD pathogenesis; accordingly, the reversal of these phenomena can delay the onset and ameliorate the cognitive and motor deficits in mouse models of HD. Thus BDNF/TrkB activation is a main candidate for neuroprotective therapeutic strategies in HD, however BDNF's poor pharmacological profile and potentially adverse local effects limit its direct use as a treatment.

We used a putative TrkB agonist: 7,8-Dihydroxyflavone chronically (5 mg/kg daily from 8 to 20 weeks of age) in R6/1 mice that express exon 1 of human huntingtin gene.

Functional motor evaluation showed significant improvement of treated R6/1 animals in the Rotarod task at 24 r.p.m. starting from week 16. Additionally, treated mice showed significant enhancement in the long term memory paradigm of Novel Object Recognition Test at 15 weeks .

Biochemical analyses show a significant recovery of encephalin levels in striatum of treated mice by western blot; additionally histological stereological analyses of DARPP32 immunohistochemistry showed a partial recovery of striatum volume. All together these results support the idea that the use of 7,8 dihydroxyflavone may have a therapeutic effect on HD

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P7

Biochemical and biophysical parameters influencing macromolecular crystallization and X-ray diffraction quality

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One way to investigate and properly understand the function of a protein and its interaction with partners is to know its three-dimensional structure. Macromolecular crystallography is a tool that provides the three-dimensional structure at atomic level of a protein that has been previously crystallized. A protein crystal consists of a very large number of repeating units where each individual unit is known as the unit cell, with no internal crystalline symmetry and which contains the crystallized sample. In general, crystallization starts with the formation of nuclei of protein molecules in supersaturated chemical conditions. There are several techniques available for bringing a pure protein solution gradually to a supersaturated state, such as batch, microbatch, vapour diffusion by hanging or sitting drops, and seeding. Once obtained a protein crystal, a potential bottleneck is to obtain a well-ordered crystal that will diffract X-rays strongly. Sometimes co-crystallization of a protein with a substrate may help the crystal quality, because the protein is structurally stabilized by the ligand, the crystal packing is more regular and this improves the X-ray diffraction pattern. In this case, a protein in complex with different substrates may result in different crystals that yield X-ray diffraction patterns of variable quality. We will present an example of crystal quality improvement of a protein/DNA complex in which we changed the design of the oligonucleotides harboring the DNA binding site, including the sequence, the length and the type of ends, blunt or cohesive. These changes modified the crystallization, as assessed by the macroscopic aspect of the crystals and the corresponding X-ray diffraction quality.

P8

Study of the molecular mechanisms that regulate *SCN5A* expression

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Brugada Syndrome (BrS) is a life-threating arrhythmogenic disease characterized with alterations of the sodium currents (I_{Na}) in the heart and associated with a high risk of Sudden Cardiac Death. Genetic alterations in *SCN5A*, which encodes the alpha subunit of the cardiac sodium channel ($Na_v1.5$), are the most common cause of BrS, although they only explain 20-25% of the patients with BrS. Therefore it has been suggested that a dysregulation in *SCN5A* expression levels could be a cause of BrS. However, little is known regarding the molecular mechanisms which control *SCN5A* gene expression. To better understand these mechanisms, we are studying the regulation of *SCN5A* gene at transcriptional and posttranscriptional levels. Using online prediction programs, we have identified binding sites for the transcriptor factor GATA-4 at the human *SCN5A* promoter. In order to examine the role of GATA-4 in *SCN5A* promoter transcriptional activity, we performed transient transfection experiments in H9c2 cells (a cardiac cell line derived from embryonic rat ventricle). We studied the effect of overexpressing or knockingdown GATA-4 on *SCN5A* promoter function in luciferase reporter experiments, and confirmed the results by real-time PCR. Our results show that the transcriptor factor GATA-4 acts as transcriptional activator of *SCN5A* promoter. We also observed that co-transfection of GATA-4 together with p300 acetyltransferases further increases *SCN5A* expression. This suggests that GATA-4 transcriptional activity on *SCN5A* promoter could be regulated by p300-mediated acetylation. To study the binding of GATA-4 to the *SCN5A* promoter *in vitro* and *in vivo* we are optimizing Electrophoretic Mobility Shift Assay (EMSA) and Chromatin immunoprecipitation (ChIP), respectively. Ultimately, we will demonstrate the role of GATA-4 in *SCN5A* expression by recording I_{Na} using patch-clamp techniques in GATA-4 knockdown cells.

In conclusion, our findings identify GATA-4 as a key regulator in *SCN5A* expression levels. This study will contribute to the understanding of a novel molecular biology-based mechanism of BrS, as well as, other arrhythmias and uncover new therapeutic targets for these heart disorders.

P9

Apaf-1 es una diana farmacológica eficaz y segura para la prevención de la apoptosis no deseada

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El exceso de apoptosis es la causa de algunas enfermedades y situaciones patológicas como pueden ser el daño tisular provocado por isquemia (trasplantes) o por determinados productos citotóxicos (ototoxicidad por CisPt) (Green, 2005). La inhibición de la apoptosis podría resultar en una estrategia terapéutica adecuada para estas situaciones. Los inhibidores de caspasas han resultado ser eficaces en fases clínicas (Pockros, 2007) pero han presentado problemas de seguridad (Burgess, 2008). Dado el papel clave que la proteína Apaf-1 tiene en la maquinaria apoptótica pensamos que su inhibición podría ser una alternativa competitiva.

Hemos caracterizado la actividad anti-apoptótica de inhibidores de Apaf-1 generados por nuestro equipo en modelos celulares de situaciones patológicas de interés como son la apoptosis inducida por CisPt en células de oído interno o por agentes de contraste en células renales. La inhibición de Apaf-1 ha resultado en la inhibición de caspasa 3 así como en la disminución de la liberación de citocromo c mitocondrial, punto de no retorno en la activación apoptótica. Este doble efecto inhibidor es una característica diferencial de la diana Apaf-1 respecto a otras como son la inhibición de la producción de radicales libres o de caspasas. Con tal de analizar la seguridad de la diana, se realizaron curvas de proliferación en diferentes líneas celulares en presencia de inhibidores de Apaf-1. En ningún caso el ritmo proliferativo se vio aumentado y por tanto, los tiempos de duplicación celular no se acortaron. Por otro lado, los diferentes tipos celulares tampoco sufrieron transformaciones en la cantidad de ADN y no se observaron fenómenos de poliploidas durante varios pases de cultivo celular. Finalmente destacar que en los modelos celulares estudiados, la expresión génica de Apaf-1 aumenta tras un estímulo apoptótico. Este hecho puede constituir una ventaja adicional para nuestra diana ya que las células que se estuvieran muriendo por apoptosis tendrían una expresión elevada de la diana favoreciendo por tanto, una inhibición más específica.

P10

Mutation spectrum in the *CACNA1A* gene in 49 patients with episodic ataxia type 2

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Episodic ataxia is an autosomal dominant ion channel disorder characterized by episodes of imbalance and incoordination. Episodic ataxia type 2 (EA2) features recurrent episodes of vertigo and cerebellar ataxia, lasting from minutes to a few days, with often interictal nystagmus.

Many EA2 patients harbor mutations in the CACNA1A gene, encoding the α1A subunit of the P/Q-type voltage-gated calcium channel Cav2.1. The vast majority of them are loss-of-function missense or nonsense mutations leading to decreased channel currents. Recently, CACNA1A exonic deletions were reported in EA2 using quantitative approaches, such as multiplex ligation dependent probe amplification (MLPA) and quantitative multiplex PCR of short fluorescent fragments (QMPSF).

We performed a mutational screening of the CACNA1A gene, including the promoter and 3'-UTR regions, in 49 unrelated patients diagnosed with EA2. When point or small indel mutations were not found, we performed MLPA and QMPSF assays to screen for large duplications or deletions. Overall, a mutational screening by PCR amplification and sequencing allowed identification of 6 point mutations in 7 patients (3 nonsense and 3 missense changes) and 2 small deletions (leading to frameshift and protein truncation), covering 18% of the patients. Subsequently, quantitative analysis identified a deletion of exon 35 as a result of a homologous recombination event between flanking Alu sequences. Our data suggest that these variations are disease-causing, although functional studies are warranted

P11

Significación clínica del genotipo interleuquina-I positivo en pacientes fumadores como factor pronóstico de la periimplantitis.

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Objetivos: Valorar la relación entre el polimorfismo de la interleuquina-1 (IL-1α, IL-1β e IL-1RN) y el desarrollo de periimplantitis en pacientes fumadores. Esta patología viene definida por una reacción inflamatoria que afecta a los tejidos circundantes a los implantes dentales endoóseos y que da como resultado la pérdida de hueso de soporte. Hipótesis de trabajo: La presencia de polimorfismos genéticos del complejo IL-1 (genotipo positivo) supone un factor de riesgo para desarrollar periimplantitis en pacientes fumadores.

Introducción: A pesar de que el tratamiento del edentulismo parcial o total con prótesis implantosoportada tiene unos resultados altamente predecibles, los implantes pueden presentar una periimplantitis y fracasar durante el periodo de mantenimiento. Entre los factores implicados en la aparición de este cuadro clínico se describe la predisposición genética del paciente.

Pacientes y Método: Se efectuó un estudio retrospectivo en una serie de 54 pacientes edéntulos totales o parciales que habían sido tratados en el Máster de Cirugía Bucal e Implantología Bucofacial (Universidad de Barcelona) para rehabilitarse con prótesis implantosoportada. Se establecieron 2 grupos: 27 pacientes fumadores con periimplantitis (grupo experimental) y 27 pacientes fumadores sin periimplantitis (grupo control). A ambos grupos se les realizó el estudio genético del polimorfismo de la IL-1 presente en la saliva y se relacionó con las siguientes variables: tipo de implante (superficie, longitud y diámetro), tipo de prótesis, nivel óseo perimplantario, antecedentes de periodontitis y hábito tabáquico (número de cigarros/día y años del hábito). El análisis estadístico se llevó a cabo mediante el software SPSS v15.0 para Windows (licencia de la U.B.), aplicando el test de chi-cuadrado de Pearson y ANOVA de medidas repetidas.

Resultados: En el grupo experimental la media de pérdida ósea fue de 4 mm. La reacción inflamatoria y por tanto el riesgo de desarrollo de una periimplantitis, mediada por la IL-1α, IL-1β, e IL-1RN resultó ser elevada en este grupo, aunque no se encontró una diferencia estadísticamente significativa con el grupo control.

Discusión y Conclusiones: Hasta el momento no existe consenso respecto al valor del polimorfismo de la IL-1 como factor de riesgo de la periimplantitis en pacientes fumadores. Sin embargo, esta relación sí está demostrada en pacientes con periodontitis. En el futuro serían necesarios más estudios para identificar los genes que controlan o modifican los diversos aspectos de la respuesta del hospedador para poder reconocer a aquellos individuos con una predisposición elevada a padecer periimplantitis, con la finalidad de valorar la indicación y extremar las pautas preventivas durante el periodo de mantenimiento de los implantes.

P12

Characterization of mechanisms involved in cell specific expression of TREX2 exonuclease

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TREX2 is a nonprocessive 3'-5' exonuclease predominantly expressed in tissues with stratified squamous epithelia, such as the skin, and specifically in keratinocytes. Loss of *Trex2* gene in mice increases susceptibility to carcinogen-induced skin tumorigenesis, highlighting the importance of this protein in the maintenance of keratinocyte genome integrity under conditions of genotoxic stress. In fact, keratinocytes are exposed to multiple DNA-damaging agents, including chemicals and ultraviolet radiation, which are the most common risk factors for skin carcinogenesis. To identify mechanisms responsible for the specific *Trex2* gene expression in keratinocytes, we have determined transcription start sites and promoter region of murine *Trex2* gene, and we are characterizing potential transcription factors required for cell-specificity. Data from deletion/mutation analysis of *Trex2* promoter revealed that a promoter region containing ets and AP1 transcription factor binding sites confers keratinocyte-type specificity. Also, we have evaluated the promoter DNA methylation state. Interestingly, we have found that pattern of DNA methylation of *Trex2* promoter strongly differs between cells expressing and non-expressing TREX2. Altogether, our findings suggest that cell specific pattern of TREX2 exonuclease expression might be regulated by the interplay between DNA methylation status and the binding of specific transcription factors to the promoter.

P13

Diet-induced obesity SIRT1-mediated histone modifications in mouse liver

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Obesity, whose incidence has increased dramatically in recent decades, is a major risk factor for other chronic diseases such as insulin resistance and type 2 diabetes. Because of the serious social problem posed, it is necessary to increase our knowledge of these diseases and to develop new and more effective therapeutic approaches. In addition to the identification of new genes involved in the pathogenesis of obesity, a better knowledge of the molecular regulation of obesogenic genes is necessary. Epigenetic changes associated with obesity are still poorly understood. Sirtuin 1 (SIRT1) links chromatin epigenetic changes and transcriptional regulation with energy metabolism. However, the genetic network/s that SIRT1 regulates at the level of histone deacetylation in metabolic tissues are unknown. In this work, we have performed genome-wide binding of SIRT1 and several histone modifications by using ChIP-Seq together with gene expression microarrays. These analysis were carried out in liver homogenates from standard and high fat diet (HFD) fed wild type, SIRT1 transgenic and SIRT1 knock-out mice. Preliminary results indicate a direct correlation between gene expression levels and SIRT1 genome-wide binding in the liver of mice fed a high-fat diet. Moreover, in the liver of HFD fed SIRT1 transgenic mice we have identified specific histone H4 lysine 16 acetylation (H4K16Ac) binding in genes involved in oxidation reduction, drug metabolism and PPAR signalling pathway. In contrast, enrichment of H4K16Ac in genes involved in transcription factor activity and RNA metabolic processing has been observed in HFD wild type. The results of this study may contribute to a better understanding of the mechanism of SIRT1 regulation both at a transcriptional and at an epigenetic level, which could lead us to the development of new therapies for obesity related diseases.

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P14

Structural determination of a complex of an AT-hook of human HMGA1a with AT-rich DNA

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HMGA (High Mobility Group A, formerly called HMG-I/Y) proteins are present in the cell nucleus of eukaryotic organisms. They are characterized by containing three DNA-binding domains, called AT-hooks, which preferentially bind to the minor groove of short stretches of AT-rich DNA (Reeves & Nissen, 1990). The consensus sequence of these AT-hooks is (P/G)RGRP. The HMGA proteins are involved in many cellular processes such as embryonic development, repair of DNA damage, growth, proliferation, differentiation and cellular death. They are also related to several pathological processes and metabolic disorders, like obesity or cancer. Details of the interaction between DNA and two AT-hooks have been determined previously by NMR (Huth et al., 1997).
Here we present for the first time the crystal structure of the complex of a DNA oligonucleotide ($CGA_2T_2A_2T_2CG$) with the third AT-hook (RKPRGRPKK) of the human HMGA1a protein. A new packing has been found in the crystal. Although this oligonucleotide has two potential AATT interacting regions, we have only found an interaction with one of them. The structure presents both analogies and significant differences with previous NMR studies: the AT-hook forms hydrogen bonds between main-chain NH groups and thymines in the minor groove, the DNA is bent and the minor groove is widened.

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P15

La inhibición de Apaf-1 como estrategia terapéutica en la prevención de fallo renal agudo

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La apoptosis juega un papel clave en el daño renal ocasionado por productos nefrotóxicos como agentes de contraste o en situaciones de isquemia renal como las que se producen durante intervenciones como la cirugía cardiovascular. Hemos estudiado la inhibición de Apaf-1 como estrategia terapéutica para la prevención del daño en ambas situaciones.

Hemos establecido un modelo celular *in vitro* en el que la línea de células tubulares renales de cerdo LLC-PK1 es sometida a condiciones de hipoxia. En esta situación se induce apoptosis mediante la activación de caspasa 3 y una pérdida de la viabilidad celular que es prevenible en presencia de los inhibidores de Apaf-1. Por otro lado, durante la hipoxia la expresión génica de Apaf-1 se induce y en presencia de los inhibidores, este efecto se revierte.

Se han establecido también dos modelos *in vivo* en rata, uno de nefrotoxicidad inducida por contraste y otro de isquemia y reperfusión renal (I/R), que simula el fallo renal agudo inducido durante la cirugía cardiovascular. En ambas situaciones se observa un aumento de marcadores de daño renal, como KIM-1, y de apoptosis, como caspasa 3. Los inhibidores de Apaf-1 revierten el aumento en la apoptosis, traduciéndose en ambos casos en una disminución del daño renal.

En conclusión, los inhibidores de Apaf-1 parecen constituir una buena estrategia terapéutica en situaciones patológicas donde la muerte celular por apoptosis contribuye al daño renal.

P16

Restoration of thymidine phosphorylase activity confers sensitivity to 5' fluoro-5' deoxyuridine (5'-dFUrd): towards a novel suicide gene strategy for cancer-associated gene therapy in MNGIE.

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Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) is a rare multisystemic disease caused by deleterious mutations in the Tymp gene, which encodes the enzyme thymidine phosphorylase (TP). This results in systemic accumulations of thymidine (Thd) and deoxyuridine (dUrd), which interferes the correct replication of the mitochondrial DNA. Allogeneic bone marrow transplantation results in clinical improvement, but the procedure is associated with a significant mortality. Using a double knockout murine model ($Tymp^{-/-}$ $Upp1^{-/-}$) we previously showed that partially myeloablative hematopoietic gene therapy is sufficient to restore the TP activity and to decrease the levels of Thd and dUrd to normal levels. However, one of the main risks of integrative vectors is insertional oncogenesis, as evidenced in some gene therapy clinical trials. In this work, we evaluate an original strategy aimed to eliminate potential tumors (necessarily derived from the corrected cells that express TP), which takes advantage of the fact that TP is the enzyme that converts 5'-dFUrd (a metabolite of the pro-drug capecitabine) into 5-fluorouracil (5-FU), a chemotherapeutic drug that inhibits DNA and RNA synthesis and causes cell death. To this aim, we used a cell line derived from a MNGIE patient ($TP^{-/-}$ B-LCL) which we transduced with a lentiviral vector encoding TP. We evaluated the sensitivity of both the transduced and the non-transduced cell lines to 5-FU, to 5'-dFUrd and we investigated whether there is a bystander effect using in vitro cytotoxicity assays. Both transduced cell lines were equally sensitive to 5-FU toxicity, which induced apoptosis in a dose-dependent manner. As for 5'-dFUrd, TP transduced B-LCL were also highly sensitive. In contrast, their non-transduced counterparts were extremely resistant to this pro-drug. Co-culture experiments and exposure of non-transduced B-LCL to supernatants of TP-transduced B-LCL previously exposed to 5-dFUrd, also resulted in significant levels of apoptosis, indicating a bystander effect. These results suggest that Tymp, the therapeutic gene for MNGIE, can also function as a suicide gene in individuals lacking the enzyme. This is highly relevant for gene therapy in these patients, as it provides a window of therapeutic opportunity in case of potential insertional oncogenesis associated with the therapy.

P17

Involvement of the non-rgs RhoGEF PROTEINS, p190RhoGEF and GEF-H1, in the G12 family signaling pathways

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Signaling via G protein-coupled receptors has been implicated in a myriad of physiological and pathological processes. The G12 and G13 comprise one of the families of the heterotrimeric G proteins and are known for their regulation of actin cytoskeleton and epithelial cell junctions, and have recently been implicated in the progression of tumor cell and cancer metastasis. The most extensively characterized downstream mediators of signaling through the G12 subfamily are members of the RhoA family. The members of this family of proteins are known mainly for their role in regulating the actin cytoskeleton, but they also play important roles in dictating cell polarity, microtubule dynamics, membrane transport pathways, transcription factor activity, and cell growth. G12 and G13 interact and stimulate the activity of a sub-family of Dbl family of guanine nucleotide exchange factors (GEFs) called RGS-RhoGEFs, which are characterized for their binding to G proteins through their RGS-like domain. Nevertheless, the RhoGEFs superfamily comprises a very extensive family of proteins and some of the ones lacking the RGS domain also participate downstream G protein signaling pathways.

p190RhoGEF is a non-RGS-RhoGEF protein known to regulate FAK signaling downstream of GPCRs and is involved in promoting tumor progression. FAK forms a complex with p190RhoGEF and promotes p190RhoGEF tyrosine phosphorylation, events associated with the enhanced activation of RhoA by p190RhoGEF. We have evidence for a novel interaction between G12/13 and p190RhoGEF. Our working hypothesis is that p190RhoGEF is a downstream effector of G12/13 and as a consequence they are also implicated in the progression of colon cancer.

GEF-H1 is a novel member of GEFs family with RhoA-specfic enzymatic activity and without a RGS-like domain. Subcellular localization analysis demonstrated that GEF-H1 is associated with microtubules and its depolymerization leads to GEF-H1 activation, accompanied by a RhoA-dependent reorganization of the actin cytoskeleton. Our results show that GEF-H1 can interact exclusively with G12 through the N-terminal domain that comprises the DH domain. G12 seems to negatively regulate the activity of GEF-H1.

P18

Analysis of gene expression regulated by the ETV5 transcription factor in OV90 ovarian cancer cells identifies FoxM1 over-expression in ovarian cancer

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Epithelial ovarian cancer is the most lethal gynecological malignancy and the fifth leading cause of cancer death in women in the Western world. ETS transcription factors have been implicated in the regulation of gene expression during a variety of biological processes including cell growth and differentiation. We recently examined the role of the ETS transcription factor ETV5 in epithelial ovarian cancer and described ETV5 as being upregulated in ovarian tumor samples as compared to ovarian tissue controls. In ovarian cancer cells, we showed that ETV5 regulated the expression of cell adhesion molecules, enhancing ovarian cancer cell survival in anchorage-independent conditions and suggesting that it plays a role in ovarian cancer cell dissemination and metastasis into the peritoneal cavity To understand the role of ETV5 transcription factor during ovarian cancer cell dissemination, we analyzed by gene expression microarray technology those genes whose expression was altered in an ovarian cancer cell line with a stable down-regulation of ETV5. The analysis of the genes and signaling pathways under the control of ETV5 in OV90 cells has unraveled new signaling pathways that interact with ETV5, among them the cell cycle progression and the TGFβ signaling pathway. In addition, we found that the down-regulation of ETV5 reduced the expression of the oncogenic transcription factor FoxM1. Consistently, FoxM1 was over-expressed in ovarian tumor samples, and its transcriptional levels increased with ETV5 transcription in ovarian tumor samples. Moreover, FoxM1 expression levels increased with tumor grade, suggesting a role in the progression of ovarian cancer.

P19

Expression pattern of Epithelial-Mesenchymal transition (EMT) factors: Zeb1, Snail1, Twist, E-Cadherin, and their relationship with the adult stem cell marker ABCG2/BCRP1 transporter in epithelial *thyroid tumour.*

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The Epithelia-Mesenchymal transition, or EMT, described during the regeneration of several tissues, it has been linked with the maintenance of stem cell phenotype, and in the development of cancer. Pathophysiological conditions, such as tumorigenesis, can influence the differentiated cells to acquire a multipotent stem cell-like phenotype through a Mesenchyma-Epithelial-Transition (MET) induction. The BCG2/BCRP1 gene is expressed in several adult stem or progenitors cells in human tissues. In spite of, the physiological function of them is unknown, it has been postulated that they confer protection against a number of xenobiotics. Moreover, the progenitor cells can lose their strict control and it may trigger the generation of tumours or become involved in the metastatic process. In the case of the thyroid gland, the molecular mechanisms that control the epithelial thyroid tumour progression, or the dedifferentiation process, is still poorly understood. One of the cellular mechanisms investigated in this dedifferentiation process is the EMT, which may involve the follicular cell population, and populations of stem cell remnants in the adult tissue. The identification of the Snail1, ZEB1, Twist genes transcription factors, as inducers of EMT, have been implicated in cancer progression and the metastasis in several types of solid tumours. However, little is known about the role of these markers regarding the epithelial thyroid tumours and their relationship with the thyroid stem cells. The aims were: 1.- Evaluate the expression of ABCG2/BCRP1 transporter in WRO follicular cell line and human thyroid tumours with different histological patterns. 2.- Investigate whether Zeb1, Snail1, Twist, E-Cadherin, and vimentin expression are correlated with ABCG2/BCRP1 expression in WRO cell line. 3.- Investigate the effect in the Snail1, ABCG2 and E-catherin genes expression in the WRO derived from ABCG2 resistant sublines after inhibition of the Zeb1 and Twist expressions by RNAs(siRNA) technology. In summary, we detect the expression of the ABCG2/BCRP1 transporter in both cell lines and thyroid tumours. The use of RNAs(siRNA) demonstrated that Zeb1 and Twist genes can regulate the expression of epithelial and stem cell genes, such as ABCG2, Snail1 and E-catherin, independent of their gene expression.

P20

ETV5 and LPP, promoting EMT in endometrial carcinoma

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Aim: We aim to characterize the mechanisms of invasion of the endometrial cancer (EC) by focusing on the role of ETV5, an ETS transcription factor, and LPP, a LIM domain protein. ETV5 and LPP were recently found to be upregulated in the invasive stage of the disease. Methods: Hec1a and/or Ishikawa cells and its GFP-ETV5 stable clones were used for *in vitro* studies. Epithelial-mesenchymal-transition (EMT) was characterized on ETV5 overexpressing cells using microarray data, expression assays, chromatin immunoprecipitation, focal adhesion turnover analysis, adhesion, migration and invasion assays. In human samples, ETV5 and LPP were examined at mRNA and protein level. Finally, we performed luciferase and invasion assays in presence/absence of stimuli to study the role of LPP knockdown on Hec1a-GFP-ETV5 cells.

Results: ETV5 overexpression promotes EMT at a molecular and functional level in Hec1a and Ishikawa cells, probably through its binding to ZEB1 promoter. Regarding LPP, we correlate ETV5 and LPP expression and localization in EC samples and *in vitro*, LPP relocalizes to focal adhesions upon ETV5 overexpression, which we associate with a probable function of LPP as an extracellular sensor to support ETV5 promoted invasion.

Conclusions: We have widely characterized the role of ETV5 on EMT during the initial steps of tumor invasion in EC. In addition, we have identified LPP as a novel protein involved in the EC invasive process correlating with ETV5 expression. Both proteins might participate in a communication pathway between membrane and nucleus, necessary to sustain invasion.

P21

Molecular pathways regulated by ETV5 transcription factor in the invasion of endometrial carcinoma

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Background and aim: Our group has been interested in the molecular pathology of myometrial infiltration that defines the initial steps of invasion in endometrial cancer (EC). As a result of a microarray study comparing EC tissues, we described the ETS transcription factor ETV5 specifically upregulated in endometrioid EC and associated with the early steps of invasion. In this study, we aim to identify downstream genes regulated by ETV5, which will be involved in myometrial infitration.

Methods: We have performed a microarray assay comparing Hec1a, a human endometrial cancer cell line, against its stable population overexpressing ETV5. Statistical mining and literature were done to select candidate target genes of ETV5 suggesting a putative role in cancer invasion. The differential expression of selected genes was validated by RTqPCR and immunobloting. All selected genes were analysed by chromatin immunoprecipitation (ChIP) to determine whether ETV5 interact with their promoters. At this moment, studies in human samples and functional analysis are being performed.

Results: The promoters of ten genes were selected for ChIP analysis. Among them, NID1 and NUPR1 were further validated at mRNA level. Those two genes resulted to be positive targets for ETV5 transcriptional regulation as ETV5 is able to interact with its promoter region.

Conclusions: We demonstrate that ETV5 can interact with the promoters of NID1 and NUPR1. NID1 is involved in tumor invasion and migration and NUPR1 is involved in oxidative stress suggesting a putative role of those two genes in EC invasion regulated by ETV5.

P22

ETV5 regulates IgCAMs superfamily during myometrial invasion

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Endometrial carcinoma (EC) is the most frequent among infiltrating tumors of the female genital tract, with myometrial invasion representing an increase in the rate of recurrences and a decrease in survival for the most common subtype, the endometrioid EC. We have previously identified an ETS transcription factor, ETV5, associated with myometrial infiltration in human ECs through the promotion of EMT, regulation of metalloproteinases and protection against the oxidative stress.

A cDNA microarray study, comparing Hec1a cells and its stable population overexpressing ETV5, analyzed gene expression patterns on a whole-genome scale. Bioinformatics analysis evidenced that ETV5 promotes EMT and pointed to cell adhesion, cell-cell contact and cellular junctions, and actin cytoskeleton reorganization. One of the main families of this molecular cells adhesions altered was the Immunoglobulin superfamily (IgCAMs). IgCAMs have been described as key mediators of cell-cell and cell-matrix adhesion. The differential expression of selected genes was validated by qRT-PCR, immunobloting and immunofluorescence when comparing Hec1a against its stable population overexpressing ETV5. We identified that ETV5 regulates the expression of the selected genes through ChIP and luciferase-reporter assays. We performed qRT-PCR and immunohistochemistry on tissues of eleven independent non invasive and invasive ECs to characterize Ig-CAMs expression and validate ETV5 regulation through Chip-on-Chip assay. We concluded that ETV5 overexpression modulates the IgCAMs profile at transcriptional and protein levels binding to ICAM2, NrCAM, and ALCAM promoters both in EC cells and tissues.

P23

Generation and characterization of orthotopic murine models for endometrial cancer

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Endometrial cancer (EC) is the most commonly diagnosed gynecologic malignancy in the western countries. EC is often diagnosed (≈75% cases) in early stages, when disease is still confined to the uterus, and 5-year survival rate is around 96%. However some patients have a more aggressive disease with myometrial and lymphovascular invasion, and a 5-year survival rate around 67% and 17% in regional and distant metastases, respectively. To understand the EC molecular mechanisms and to improve clinical treatment, the use of clinically relevant mouse models is an essential requirement.

Our aim is to present new orthotopic mouse models for EC, which are reproducible and imitate its infiltrative process and metastatic behavior. We generated an orthotopic murine model for EC from Hec-1A cancer cells by direct transmyometrial injection into the uterus of female mice, and we followed the tumor growth up by bioluminescence (BLI) imaging. We also describe a murine model derived from endometrioid human tissue. The first model generates abdominal dissemination and lymph node and hematogenous metastases, showing the same metastatic pattern than patients. In the second model, local and locally advanced endometrioid cancer develops with pelvic dissemination and lymph node metastasis.

The first model represents EC in advanced stage and it is easily monitored by BLI, so it is a useful tool in preclinical studies. The second model corresponds to the most frequent histological and clinical presentation of EC, so it might facilitate the study of the myometrial infiltrative process.

P24

MicroRNA expression profiles in urine as diagnostic biomarkers for prostate cancer

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Background and aims: Prostate cancer (PCa) is the most common neoplasia among males from developed countries and the second leading cause of death from cancer. During the last years, serum prostate specific antigen (PSA) has been used as biomarker for PCa diagnosis. Although PSA test has increased PCa detection, it has a low specificity and leads to over diagnosis and over treatment, therefore, it is necessary to improve the current diagnostic methods for PCa. In the past decade, urine has received more and more attention for its simplicity, as well as its potential use in identifying new biomarkers as non- or minimum invasive detection method. Deregulation of miRNAs in serum/plasma or tissue has been associated with many diseases including PCa, suggesting the possible use of miRNAs as diagnostic biomarkers also in other body fluids. For that reason the aim of this study was to evaluate if miRNAs known to be deregulated in blood or tissue could also be detected in urine and used as biomarkers for the detection of PCa.

Methods: Post-prostate massage urine samples were prospectively collected from 40 consecutive patients, who will undergo a prostatic biopsy as part of their routine medical care, due to the suspicion of PCa (PSA≥4.0 ng/mL and/or abnormal digital rectal examination). Total RNA was isolated of 20 PCa patients and 20 benign controls and differential expression levels of 21 selected miRNAs were analyzed by RT-qPCR.

Results: Data showed that a total of 7 miRNAs were differentially expressed, 5 of them were down- and 2 up-regulated significantly in the urine of PCa patients compared with the controls ($p < 0.05$).

Conclusion: The results suggest that changes in the miRNA profiles can be detected in urine samples and could be good candidates as biomarkers to improve PCa diagnosis.

P26

A Double Cysteine Opsin Mutant to Study the Stability and Structural Behavior of Green Cone Opsin

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Cone opsins are the visual pigments of the vertebrate retina belonging to the G-protein-coupled receptors superfamily, and characterized by having the 11-*cis*-retinal chromophore covalently bound to the opsin apoprotein. One of the problems that prevented a detailed study of these opsins is its relatively high instability in the purified state. This intrinsic instability could be related to the kinetics of their functionality which is different from that of rhodopsin. Introducing Cys residues in membrane proteins at positions which are structurally closer enough to form disulfide linkages is a good strategy to stabilize these membrane receptors and at the same time to gain further insights into their structure and function.

We have used green cone opsin as a model cone opsin system and generated a double cysteine mutant at positions 90 and 169 at the cytoplasmic domain of the receptor (G_SS). The double mutant containing W90C and A169C was constructed by site directed mutagenesis of the green cone opsin wildtype (G_WT) gene cloned into the pMT4 plasmid. Plasmids with G_WT and G_SS were chemically transfected and expressed in mammalian COS-1 cells. The opsins were regenerated with 11-*cis*-retinal and purified by immunoaffinity chromatography using 1D4 antibody coupled to a Sepharose matrix. Our results indicate that the double Cys mutant could successfully regenerate with 11-*cis*-retinal showing the typical absorbance band at about 530 nm. Treatment with a specific cysteine reagent confirms the formation of disulfide bond between the two newly introduced Cys residues. G_SS also showed better post-bleaching regeneration with 11-*cis*-retinal than G_WT. Further stability experiments would be needed to gain in-depth knowledge of the structure and function of green cone opsin by using this mutant.

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P26

Urinary exosomes as source of prostate cancer biomarkers

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Background: Rapid and reliable diagnosis of prostate cancer (PCa) is highly desirable. Sensitivity and failure rate of current methods for diagnosis limit the success to early detect this type of cancer and, consequently, advanced disease is often encountered. Due to the localization of the prostate in the body, its secreted products can be detected in urine. Many cancer-derived proteins are secreted through small vesicles known as exosomes, which could serve as novel platform for diagnosis. Our aim is to carry out a discovery phase, using a proteomic approach, to find new urinary exosome-related biomarkers for the diagnosis and prognosis of PCa.

Methods: We isolated exosomes from a pool of urine samples from PCa and benign patients by an ultracentrifugation protocol (with or without DTT treatment). We characterized these isolated vesicles by electronic microscopy (EM). The exosomal protein fraction was extracted and analyzed in the LC-MS/MS instrument. Finally, the results were verified by Western Blot (WB) and Immuno-Gold EM (IEM).

Results & Conclusions: We found that ultracentrifugation plus DTT treatment was the best method for isolating urinary exosomes, which can be observed as membranous vesicles by EM. A preliminary analysis of the proteomic content of the exosomes showed a list of 93 proteins, among which we find proteins related with PCa (PSA, PAP), and others described to be involved in the exosomes secretion pathway. WB analysis of the isolated exosomal fraction confirmed the presence of known exosome markers, such as Flotillin-1, Rab5 and CD81, and also PSA (known as PCa biomarker). Finally, we verified PSA expression in urinary exosomes by IEM. In summary, our data demonstrate that exosomes from body fluids are useful for extensive comparative proteomic studies and will allow us to discover new proteomic-candidate markers for PCa diagnosis.

P27

Gene expression profile associated with ovarian cancer dissemination. A comparative study of ovarian primary tumors, ascites and metastases

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Epithelial ovarian cancer is the most lethal gynecological malignancy and the fifth leading cause of cancer deaths in women in the Western world. Largely asymptomatic, ovarian cancer is frequently (75-80%) detected at late stage. Five year survival rate for women with advanced stage disease is less than 20%. In contrast, the cure rate is almost 90% when women are diagnosed at stage I. Epithelial ovarian cancer metastasizes either by direct extension from the ovarian tumor to neighboring organs or by cancer cells detachment from the primary tumor seeding in the peritoneal cavity. Gene expression profiling using microarray technology allows the detection of expression signatures that may underlie ovarian carcinogenesis.

Our aim is to study the ovarian cancer dissemination comparing gene expression profiles of human paired ovarian primary tumors, ascites and metastasis. To reach this proposal we collected ovarian primary tumor, ascites and peritoneal metastasis of five patients. Tumor cells from ascites were cultured in vitro for further analysis. We determined their global gene expression by microarray analysis and compared the expression profile between all paired samples. After data filtering, literature and database mining, we selected five differentially expressed genes which have been validated at the protein level by Western blot on the same samples. We have performed immunohistochemistry in a new set of paired primary tumor and metastasis samples. The present study highlights the role of previously unknown proteins in ovarian cancer dissemination that might be used as tumor biomarkers, to clinically monitor the progression of the disease.

P28

Human sperm likes sugars: the importance of glycolysis in male gamete functionality

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The sperm cell can survive for days in the female reproductive system requiring energy for several purposes, such as progressive motility that can be mainly obtained by glycolysis or oxidative phosphorylation (OXPHOS). The relative significance and differential input of these metabolic pathways remains controversial.

In this work human sperm samples were maintained at 37ºC (5% CO_2) in an aerobic PBS medium with 1) no exogenous substrates; 2) glycolysis and OXPHOS substrates. We then tested the effect of potassium cyanide (an OXPHOS inhibitor of complex IV of the mitochondrial electron transfer chain) and iodoacetic acid (a glycolysis inhibitor acting at the glyceraldehyde 3-phosphate dehydrogenase level) in sperm viability, motility, ATP levels and energetic charge.

We observed that the inhibitory effect of the mitochondrial poison potassium cyanide on sperm activity is completely rescued if glucose is present. Furthermore, the inhibition of glycolysis clearly affects sperm ATP content and progressive motility, even in the presence of exogenous substrates.

Our data clearly points to the importance of glycolysis for human sperm homeostasis.

P29

Estudi mutacional dels gens *HSPA2* i *SPAG16* en pacients estèrils i en controls.

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La majoria de casos d'esterilitat masculina en l'home segueixen sent de causa desconeguda. És molt probable que una proporció important d'aquests siguin deguts a herència recessiva. Diferents estudis han identificat possibles gens candidats de la infertilitat quan van generar ratolins genoanul·lats, com és en el cas dels gens *SPAG16* i *HSPA2*. Aquests genoanul·lats presentaven alteracions a la seva espermatogènesi. Per altra banda, se suposa que possibles mutacions en gens relacionats amb la fertilitat poden provocar canvis qualitatius o quantitatius en les proteïnes de l'espermatozoide. Per exemple, d'acord amb els resultats proteòmics previs del nostre grup vam trobar un augment significatiu de la proteïna HSPA2 en pacients amb major lesió germinal determinada per TUNEL i en pacients astenozoospèrmics.

En el present treball hem fet un estudi mutacional dels gens *HSPA2* i *SPAG16* en pacients infèrtils i controls fèrtils. Per l'estudi del gen *HSPA2* s'han inclòs 16 pacients azoospèrmics amb aturada madurativa, 10 pacients oligoastenoteratozoospèrmics severs, 10 pacients teratozoospèrmics, i 10 controls d'individus amb fertilitat provada. S'han aconseguit identificar 3 polimorfismes, un dels quals no estava descrit. Per l'estudi del gen *SPAG16* vam estudiar 80 mostres de pacients oligozoospèrmics i 46 mostres controls d'individus normozoospèrmics. Hem identificat 2 polimorfismes descrits prèviament i 3 variants noves identificades en pacients independents, una d'elles no sinònima en un aminoàcid que es troba dins de la seqüència consens i conservat evolutivament en diferents espècies. No s'han trobat diferències significatives entre la freqüència dels polimorfismes identificats al comparar pacients i controls. Aquests resultats indiquen que la possible presència de mutacions patogèniques dels gens *SPAG16* i *HSPA2* en cas d'existir, deuen ser una causa molt rara d'esterilitat masculina.

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P30

Detection of *Serratia marcescens* in boar semen samples by PCR

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The presence of *Serratia marcescens* in boar extended doses destined to artificial insemination (IA) produces acidification of the medium, sperm agglutination, decrease in the percentage of motile spermatozoa and acrosomal damage. Contamination of doses can be due to the extender, the environment or the preputial fluids. This bacterium is gentamincine-resistant, so it can survive in extended boar semen despite of the presence of certain antibiotics. Moreover, bacteria's isolation from semen using bacterial culture is a complex, laborious and time-consuming method that can be easily altered by the presence of antibiotics and inhibitors and it can also be affected by low levels of microbes. In this study we designed a conventional PCR for the detection of the conserved gen 16S ribosomal RNA from *Serratia marcescens*. DNA was extracted using two methodologies: 1) commercial kit (DNeasy, Blood and Tissue kit; QIAGEN, CA) and 2) a thermal shock. The designed primers were tested for specificity and PCR conditions were set up as follows: 5 minutes at 95ºC; 40 cycles of 30 seconds at 95ºC, 30 seconds at 59.5º and 15 seconds at 72ºC, and an extension step of 7 min at 72ºC. Once the PCR conditions set up, we artificially inoculated semen samples at the following ratios spermatozoa:bacteria: 100:1, 50:1, 20:1, 10:1, 8:1, 6:1, 4:1, 2:1, 1:1, 1:10 and PCR was performed to detect *S. marcescens*. Amplification was seen in all the ratios when the commercial kit for DNA extraction was used, but *Serratia marcescens* was only detected with thermal shock at ratios 1:1 and 1:10.

This PCR is a useful and quick tool for the detection of *Serratia marcescens* in boar semen samples and would avoid the use of contaminated doses with lower sperm quality due to the presence of *Serratia marcescens* in AI programs

P31

Searching for biomarkers of boar sperm freezability in order to predict ejaculate susceptibility to the freezing-thawing protocol

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Boar sperm cryopreservation offers several advantages that cannot allow other semen preservation systems, such as genetic material exchange over great distance, germoplasm banks creation and to improve management and organization of production centers. However, cryopreservation is still seldom used in the sector, in part due to the large variability in post-thaw semen viability that exists among individuals, as well as between ejaculates from the same boar. Therefore, the aim of this study is to find biomarkers of boar sperm freezability which will be able to be used to predict boar ejaculate susceptibility before starting a cryopreservation protocol. This way, it could be engineered pre-selection tests that would maximize the use of this advantageous technique in the pig industry worldwide. Ejaculates from a total of 33 boars were frozen and thawed in order to classify all the samples in two groups: freezing-resistant ejaculate (FRE) or freezing-sensitive ejaculate (FSE). Ten ejaculates from each group, which presented extreme values of semen quality post-thaw, were selected for the study. Sperm protein extraction and quantification were carried out and samples were analyzed using 2D-DIGE. The results showed 28 significant differential protein spots between FRE compared to FSE, of which, 20 were identified using LC-MS/MS. Each spot corresponded to more than one single protein and a same protein was identified in more than one spot. These findings suggest that these potential biomarkers maybe are subjected to post-translational modifications. We are currently validating the study of these proteins through Western blotting. This approach will allow a greater knowledge of molecular basis of boar sperm cryopreservation. Moreover, it would let the engineering of molecular markers based tests that would provide the standardization of this preservation system, avoiding the obstacles that still limit its expansion in the sector. Supported by Ministerio de Economia y Competitividad (RZ 2011-00001-00-00 to S.B. and BFU2009-07718 to R.O.), a APIF fellowship of the University of Barcelona to J.C. and BR fellowship of the University of Girona to I.V.

P32

Effect of different concentrations of *Clostridium perfringens* on sperm quality of boar seminal doses kept at 15ºC

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Bacteriospermia in boar fresh and extended semen is a frequent finding that produces alterations on sperm quality and, consequently, causes economic losses in artificial insemination (AI) centres. The present study sought to evaluate the effect of different infective concentrations of *Clostridium perfringens* on boar sperm quality, assessed as sperm motility (CASA), morphology and viability, through 11 days of storage at 15ºC. With this purpose, four commercial seminal doses coming from post-pubertal and healthy boars were artificially inoculated with different infective concentrations of *C.perfringens*, ranging from 10^8 to 10^2 cfu·mL^{-1}. The negative controls were non-inoculated doses. The sperm quality was checked after 1, 2, 3, 4, 7, 8, 9, 10 and 11 days of storage at 15ºC, and the presence/absence of bacteria was detected 24 h and 7 days after the inoculation at all infective concentrations using a PCR protocol with specific primers for this pathogen. Statistical analyses consisted of repeated measures ANOVA and post hoc Sidak's tests for multiple comparisons. Significant decrease in the percentages of both viable and progressive motile spermatozoa were observed after 1 day of inoculation at an infective concentration of 10^8 cfu·mL^{-1} and also after 3 days of storing at an infective concentration of 10^7 cfu·mL^{-1}, when compared to negative control. This tendency was maintained until the end of the experiment. However, no significant differences were observed regarding sperm morphology in any treatment when compared to the negative control. Thus, we can conclude that *Clostridium perfringens* cause deleterious effects on boar sperm quality during storage at 15ºC, and that PCR analysis of this bacterium in seminal doses can be used to minimize the use of doses with low sperm quality due to bacterial contamination and to avoid the potential spread of infective diseases.

P33

Pro/acrosin Activity and Expression Analysis along Epididymis

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Acrosin, a tripsin-like proteinase in the mammalian sperm acrosome, is present in spermatid cells and in epididymal and ejaculated spermatozoa as an inactive zymogen proacrosin (Phi-Van et al. 1983; Polakoski and Parrish 1977). It is synthesized during spermatogenesis and stored in the acrosome in its zymogen form, proacrosin. During capacitation, proacrosin is apparently converted into the mature enzyme being involved in sperm penetration through the zona pellucida. The aim of this experiment was to analyze pro/acrosin activity and expression during epididymal sperm maturation. Undiluted epididymal samples were obtained by cannulation of four epididymal regions of adult fertile boars (proximal and distal caput, corpus and cauda) and washed with PBS at 600x g for 10 min. Acrosin activity of epididymal fluid was determined spectrophotometrically after their incubation with a detergent-substrate solution mixture. Protein extracts from different epididymal regions were analysed by SDS-PAGE, transferred to PVDF membrane and incubated with mouse monoclonal anti-acrosin (1:4000). Membranes were incubated with secondary antibody (1:5000) and developed using an enhanced chemiluminescence kit. Results showed that acrosin activity (μUI acrosin/10^6 spz) of epididymal spermatozoa from proximal (403.7 ± 53.37) and distal (438.5 ± 3.15) caput, corpus (336.8 ± 142.26) and distal cauda (438.38 ± 11.9) did not differ significantly ($p \geq 0.05$). Westerns blots analyses demonstrated a very intense band corresponding with the immature isoform of proacrosin in the different regions of the epididymis studied. α- and β-acrosin isoforms were present in all the epididymal regions studied but their expression increased progressively reaching their maximum in the cauda. During epididymal maturation, α- and β-acrosin expression increases despite, acrosin activity is maintained high and constant throughout the epididymal regions. Further studies are needed to elucidate the enzymatic activity degree of proacrosin and their biological significance in sperm maturation throughout the epididimis.

P34

Hyaluronic acid may improve the porcine embryo quality when added to *in vitro* culture medium depending on energy substrate

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Addition of hyaluronic acid (HA) to culture media has been reported to be beneficial for the embryo development in bovine, murine and human species. However, the effects of HA in porcine *in vitro* produced (IVP) embryos seem to rely on the media and the conditions of maturation, fertilization and culture. Thus, the present work sought to determine how the addition of HA to the culture medium affects the porcine embryo development and whether these effects differ between the energy substrate added to the culture medium. With this aim, three physiological concentrations of HA (0, 0.5 and 1 $mg \cdot mL^{-1}$) were added to IVP embryos cultured: a) only with glucose for 168 h post-insemination (pi) (IVC-Glu) or b) with pyruvate-lactate for the first 48 h pi and glucose from 48 to 168 h pi (IVC-PL). The embryo developmental capacity was evaluated by cleavage and blastocyst rates at 48 and 168 h pi, respectively. Moreover, the quality of blastocysts was evaluated according to the IETS criteria. Addition of HA had no effect either on blastocyst or cleavage rates ($P>0.05$). Similarly, there was no interaction between energy substrate and HA concentration both on cleavage and blastocyst rates ($P>0.05$). In contrast, there was a significant two-way interaction (HA concentration × energy substrate) on the morphological quality of the blastocysts. Hyaluronic acid, mainly at a 0.5 $mg \cdot mL^{-1}$, significantly improved the quality of blastocysts cultured with glucose, but it did not affect the quality of those cultured with pyruvate-lactate. Percentages of blastocyst with excellent and good quality were 80.0±25.3 in IVC-Glu + 0.5 $mg \cdot mL^{-1}$ HA and 35.4±8.9 in IVC-Glu + 0 $mg \cdot mL^{-1}$ HA ($P<0.05$). In addition, the blastocyst rate was higher when the embryos were cultured in pyruvate-lactate for the first 48 h pi than when they were cultured in glucose (9.1±1.6 % *v.* 5.2±1.0 %; $P<0.05$), regardless of HA concentration. In conclusion, supplementing HA both in glucose and pyruvate-lactate culture medium had no effect on development of porcine IVP embryos. However, HA seems to improve the quality of the blastocysts cultured in glucose-based medium due to the suboptimal conditions that this energy substrate causes.

P35

Effects of adding L-ascorbic acid into *in vitro* culture media NCSU23 on porcine embryo development and quality

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Protecting embryos against oxidative stress during *in vitro* culture seems to be one of the keys to improve embryo development. Accordingly, previous reports have demonstrated the addition of antioxidants is effective for scavenging reactive oxygen species (ROS). Therefore, the present study was conducted to evaluate the effect of adding L-ascorbic acid (AC), an antioxidant, into the culture media NCSU 23 on the embryo development and the quality of *in vitro* produced (IVP) blastocysts. IVP blastocysts were derived from porcine fertilised oocytes cultured with 0.17mM pyruvate and 2.73mM lactate from Day 0 to 2 and then with 5.5mM glucose up to the blastocyst stage. In order to determine the effect of L-ascorbic acid, embryos were incubated in IVC-PL culture medium supplemented with 100 µM AC or non-supplemented (control). After 144h, blastocyst yield and development stage were assessed by stereomicroscopy. Total cell number and apoptosis index were evaluated using the TUNEL staining. Supplementing culture medium with 100 µM AC for the entire culture period of 144h did not affect either cleavage or the blastocyst rates. However, the percentage of hatching/hatched blastocysts was significantly higher (P<0.05) in control medium (3.65%±1.01%) than in medium supplemented 100 µM of AC (0.62%±0.24%). When embryos were cultured in the presence of 100 µM of AC, no beneficial effect was observed in terms of total cell number and percentage of apoptotic cells compared with the control. In conclusion, the addition of 100 µM L-acid ascorbic during *in vitro* culture does not enhance either the blastocyst yield or the embryo quality.

P36

Identificació d'embrions bovins cultivats en grup mitjançant l'adhesió de codis a la zona pel·lúcida

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L'obtenció d'oòcits per punció fol·licular en l'espècie bovina suposa la recuperació d'un baix nombre d'oòcits/femella. Un cop fecundats, són pocs els zigots/femella que es poden posar a cultivar *in vitro*, i està ben descrit a la literatura que el cultiu individual o en grups reduïts és menys eficient que el cultiu en grups més nombrosos. Per això, s'han dissenyat sistemes de cultiu on els embrions poden seguir-se individualment, tot i compartir el mateix medi. Aquests sistemes, però, impedeixen el moviment lliure dels embrions, interferint en la transmissió dels factors paracrins i en els canvis de gradient que generen els embrions en moure's. Aquest treball presenta un sistema alternatiu que permet cultivar conjuntament embrions de diferents orígens, sense restriccions de moviment i preservant el seu pedigrí, mitjançant el marcatge amb codis de polisilici biofuncionalitzats amb una lectina que permet la seva adhesió a la cara externa de la zona pel·lúcida (ZP).

Per tal de valorar l'eficàcia del sistema, presumptes zigots provinents d'oòcits de vaques d'escorxador madurats i fecundats *in vitro* van ser codificats, un cop decumulats, adherint 8 codis a la ZP de cada embrió. Es van emprar 4 tipus de codis, cadascun amb una codificació diferent, per codificar 25 embrions (6-7 embrions/tipus de codi) que llavors es van co-cultivar en la mateixa gota de medi. Es va valorar la taxa de divisió a les 48 h i les taxes de blastocist a dia 7 i dia 8 de cultiu i el nombre de codis que es mantenien adherits a la ZP (taxa de retenció). A més, els blastocists de dia 7 van ser vitrificats utilitzant el mètode del *cryotop* i es va calcular la supervivència post-escalfament com la taxa de re-expansió a les 3 i a les 24 h en cultiu.

Els resultats preliminars mostren que l'adhesió dels codis a la ZP dels embrions bovins no afecta el seu desenvolupament *in vitro*, mentre que la supervivència després de la vitrificació sembla veure's disminuïda. Tots els embrions van mantenir almenys un codi adherit a la ZP fins al dia 8 de cultiu (4,4±1,8 codis/embrió) i van poder ser identificats.

Grup	n	Desenvolupament, n (%)		n	Supervivència post-vitrificació, n (%)	
		Divisió 48 hpi	Blastocists		3 h	24 h
Control	70	51 (72,9%)	19 (27,1%)	13	13 (100%)	13 (100%)
Codificat	99	86 (86,9%)	39 (39,4%)	29	17 (58,6%)	19 (65,5%)

En conclusió, la identificació d'embrions cultivats en grup mitjançant l'adhesió de codis biofuncionalitzats a la ZP és possible i permet el co-cultiu d'embrions de diferents orígens en una mateixa gota de medi. No obstant, es requereixen més estudis per confirmar aquestes dades i, sobretot, per determinar l'efecte dels codis en la vitrificació dels embrions.

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P37

Genetic and environmental influences on zebrafish sex ratios

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Zebrafish has become a well established model in many areas of research. However, a basic aspect of its biology, i.e., how sex is determined, is still not known. In contrast to other vertebrates, fish exhibit a wide range of sex determining systems, and whether an animal develops as a male or a female depends on the interplay between genetic, physiological and environmental influences. Although it is well established that the zebrafish is an undifferentiated gonochorist, studies aimed at identifying its sex determining mechanism are equivocal, as are the gene pathways supposedly involved. Thus, sex determination and differentiation are far from being clear. Further, the presence of non-Fisherian sex ratios, usually male biased, in facilities all over the world has puzzled researchers. Here, we report experiments involving different zebrafish families, exposure to different temperatures, as well as rearing at different population densities. Our results show a wide interfamily variation in sex ratios under control conditions, conserved sex ratios in successive broods of the same parents, a strong masculinizing effect of high temperature, the existence of genetic x environment interactions, and a relationship between density, growth and sex ratios. Changes in phenotype were confirmed by changes in in the expression of genes related to sexual differentiation and DNA methylation. Together, these results support a polygenic sex determining system with environmental influences for the zebrafish, with the possibility that the phenotype is discordant with the genotype. Thus, more attention should be paid to these genetic and environmental components in studies where whatever is being investigated may be linked to the sexual phenotype. *Supported by grants CSD2007-0002 ("Aquagenomics") and AGL2010-15939 ("Epigen-Aqua") to FP.*

P38

Comparison of the female reproductive system of two marine gastropods (Neogastropoda: Muricidae) with different reproductive biology

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The present work undertakes a comparison of the female pallial reproductive system of *Coralliophila meyendroffii* (Calcara, 1845) and *Bolinus brandaris* (L., 1758), paying special attention to the ultrastructure of the capsule gland. Both species have contrasting reproductive modes: *C. meyendorffii* is protandric, females incubate thin egg-capsules inside the pallial cavity, a single female may copulate with several males and females provide fertile eggs with nurse eggs, yet development is planktrotrophic. *B. brandaris* have separate sexes and females aggregate to lay communal benthic egg-capsules. Females also provide fertile eggs with nurse eggs, but development is completely intracapsular. The pallial female reproductive system of both species has a similar organization. However, the capsule gland of *C. meyendorffii* is proportionally smaller than that of *B. brandaris*, has a reduced dorsal lobe and lacks an antero-ventral lobe. Its bursa copulatrix is hypertrophied when compared to *B. brandari*s, and the posterior sperm receptacle is not continuous with the lumen of the capsule and albumen gland but connected to the pallial oviduct through a lateral ciliated duct. Ultrastructurally, the capsule gland of *B. brandaris* differs from that of *C. meyendorffi*, in that their epithelial cells apparently contribute to the production of secretory granules for the formation of the capsule, a character that may be regarded as primitive. It is here suggested that in both species, sperm competition may occur inside the bursa copulatrix and that male lancet parasperm may act as mucous plugs or repellents of alien eusperm. The reduced capsule gland in *C. meyendorffi* may be an adaptation to its brooding behaviour.

P39

Spermatogenesis of *Pomacea insularum* (Caenogastropoda: Ampullariidae): Eupyrene and Apyrene Sperm Ultrastructural Study

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Eupyrene sperm are filiform (about 37 µm in length, by 0.5 µm in diameter at its widest point) with a conical acrosome, elongate nucleus, midpiece and principal piece. The conical acrosome differentiates from the proacrosomal granule which is derived from a Golgi apparatus. The chromatin is sequentially organized in granules, fibers and finally, in a very condensed structure. The mitochondria become spiraled around the axoneme and theirs membranes fuse to from the complex mitochondrial or *Nebenkerne*. They contain large store of glycogen in the principal piece. A distinct annular structure is located at the junction between midpiece and principal piece.

Apyrene parasperm result from a citodifferentiation process occurs in specific areas of the seminiferous tubules. Mature atypical spermatozoa have a vermiform shape, 45 µm long and 1.8 µm wide, are flagellated cells. The remnant chromatin condenses to form dense bodies. The mitochondria take up a position between the flagella and fuse forming an elongated complex mitochondrial. The glycogen deposits, clearly visible, fill up the cytoplasm. Numerous electron-dense granules located at the peripheral cytoplasm are observed. At a distance of about 14 µm from the apex of the apyrene spermatozoa emerges a tuft of four or five long flagella.

P40

The spermatozoon of brachylaima mascomai. ultrastructural study

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Despite of the phylogenetic interest of ultrastructural features of spermiogenesis and the spermatozoon in Digenea (Trematoda), in bachrylaimid species data are scarce. An analysis by Transmission Electron Microscope (TEM) of spermatozoon of *B. Mascomai* (Brachylaimidae) is carried out in this work. Mature spermatozoa from seminal vesicle of the adult have been studied. The material have been processed according with the conventional protocol for TEM. The nucleus defines a *nuclear region* and partially overlaps with one mitochondrion and two axonemes. One of the axonemes ends before the mitochondrion does which in turn ends before the nucleus does. The other axoneme runs until the distal tip of the spermatozoon. In the *non nuclear region* of the spermatozoon the proximal mitochondrial tip occupies a central position, liying between the two axonemes and two sets of cortical microtubules located between the axonemes. Glycogen inclusions fill the citosolic space between the mitochondrion and microtubular sets. Proximally the mitochondrion ends and the number of microtubules decreases. The proximal region of the spermatozoon is characterized by the existence of two axonemes, absence of glycogen and external ornamentation formed by membrane particles in T-shape with a regular distribution pattern. Lateral *cytoplasmic expansion* partyally lines longitudinally the non nuclear region. A set of cortical microtubules and membrane particles in T-shape also are present in the expansion. The TEM analysis demonstrate the presence of a *dense zone* of cytoplasm which also lines on the non nuclear region, in opposite side to the cytoplasmatic expansion. Seriate sections suggest a variable shape of this zone, from dumbell-shaped to acute eminence, and helical displacement forming, near the nucleus, a dense protrusion between two axonemes. This dense zone lacks of cortical microtubules and T-shape membrane particles. Tridimensional diagram of spermatozoon is propsed in this work.

P41

Validation of a NGS strategy for genetic diagnosis of sudden cardiac death

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Aim: Sudden cardiac death (SCD) is defined as the natural and unexpected death of cardiac origin in apparently healthy individuals. In many cases SCD is the consequence of inherited diseases, either structural cardiomyopathies or arrhythmogenic abnormalities. Heritability of the related diseases makes genetic screening indicate not only for affected individuals but also for their relatives, potentially at risk of a sudden death event. However, the large and increasing list of SCD related genes and the heterogeneity of the related pathologies, makes difficult to approach a complete genetic screening. Conventional Sanger sequencing, is being replaced by Next Generation Sequencing (NGS) as a more cost- effective strategy with higher throughput. NGS application in sudden cardiac death genetic screening allows a fast way to sequence the whole SCD related genes.

Methods: A new high capacity genetic screening method has been developed and validated by means of NGS platform HiSeq2000 (Illumina). It has been analyzed 1210 exons and exon-intron boundaries as well as 'UTR regions of 55 genes implicated in arrhythmogenic and structural diseases that causes SCD. The validation strategy was performed in Coriell samples and patient samples with known genetic variants (detected by conventional Sanger sequencing).

Results: The results showed a high call rate value exceeding 99.2% even at 100X of coverage. The accuracy against the reference sequence (NCBI) exceeds 99.5% from 1x to 100x. The average coverage was about 1587x, with a maximum of 2959x. Only 6 exons did not exceed the coverage limit of 95% at 20x. The accuracy results showed 98,9% of sensitivity and 100% specificity.

Conclusion: We have developed and validated a new genetic screening method which has proved to be of extremely high quality. This new method will have a beneficial impact both for patients and family members to confirm or not the clinical suspicious of being at high risk of SCD.

TUESDAY 10 TH OF JULY

P42

The proton transport by bacteriorhodopsin depends on the mobility of transmembrane helices

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Bacteriorhodopsin (BR) is a light-driven proton transporter that is found in the membrane of *Halobacterium salinarum*. Although the transport mechanism is one of the best understood, still some specific structural aspects remain unclear, as the implication of the mobility of transmembrane helices in the function. Conformational changes are needed to accomplish the translocation of protons in BR, but, do the BR helices follow an alternating mechanical rocker-switch mechanism or do they work more likely as soft structures? To address this question, we have designed, constructed and characterized three double mutants, in which two close side chains located in two contiguous α-helical ends are mutated to Cys and crosslinked. These are Val101/Met163 (cytoplasmic side of helices D and E); Thr157/Tyr172 and Ala160/Ala168 (cytoplasmic side of helices E and F). Several structural and functional characteristics of the reduced and the oxidized forms of these mutants were studied, with the goal to understand better whether and how the immobilization of the ends of helices D, E and F affect the protein function. Light-dark adaptation experiments show no difference between the reduced and the oxidized forms of the mutated BR compared to the wild type BR. However, the immobilization of the D and E helices in the oxidized mutant makes the protein more resistant to thermal denaturation compared to it reduced form. In general, the titration of Asp85 shows that the Asp85 environment is more solvent accessible in all Cys BR mutants, compared to the wild type. According to the functional studies, the proton transport of the Cys mutants incorporated into liposomes is decreased to about 50% in the oxidized forms with respect to the reduced ones. Finally, FTIR studies show that the photocycle's kinetics is affected when helices D and E are fixed. In summary, locking the cytoplasmatic ends of helices D and E affect more significantly the BR functioning than locking the cytoplasmatic ends of helices E and F.

P43

Fluctuation Relations and Entropy production in a Dual Trap Optical Tweezers Setup

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Recent theoretical developments in nonequilibrium statistical physics have shown how it is possible to recover free energy differences (FED) and free energy landscapes (FEL) from irreversible work measurements in thermodynamic transformations of small systems.

These developments go under the name of Fluctuation Relation (FR) and have found several applications in the field of single molecule manipulation [1],[2],[3].
A crucial concern in using FRs is the identification of the right observables which satisfy the relation: the use of the wrong observable can lead to a large error in the estimated FED or FEL [4].
In this communication we will discuss the applicability of FRs to non-equilibrium measurements obtained in Dual Trap Optical Tweezers setups (DTOTs), the standard high resolution tool in single molecule biophysics. We will show that in general three different observables satisfy a FR.
In particular our study proves that the differential work measurement, based on the differential coordinate [5] (which provides the highest resolution in DTOTs) satisfies a FR and can be used in FED or FEL estimates, independently of how the pulling is carried on.
The theoretical results will be confronted with experimental results obtained on several DNA tethers in a novel counter-propagating DTOT setup which directly measures forces by linear momentum conservation.

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Characterizing the E. Coli RecQ helicase using optical tweezers

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Helicases are ATPases that translocate and unwind dsDNA, providing the ssDNA substrate needed in many cellular processes such as DNA replication, recombination and repair. They can be considered as active or passive enzymes depending on the coupling between ssDNA translocation and unwinding activity. Whereas active helicases use the energy of ATP hydrolysis to move unidirectionally along ssDNA and also to actively destabilize the DNA fork and promote DNA unwinding, passive helicases rely on the thermal fluctuations that open the DNA fork to move forward thus unwinding the helix. Among DNA helicases, the RecQ family (conserved from bacteria to human) is essential for the maintenance of the genomic integrity. In the presented work we use optical tweezers to manipulate a DNA substrate (DNA hairpin) and monitor the E. Coli RecQ activity in real-time in order to characterize the dynamics of this molecular motor. We measure the unwinding velocity under different applied forces and observe that the unwinding rate depends only weakly on the force applied, revealing that RecQ behaves as an active helicase. We also estimate the step size of RecQ analyzing the power spectra of the activity signal. The results presented in this work are the first step towards measuring the mechanical work exerted by the enzyme. These experiments, which we plan to perform in the near future, will allow us to estimate the entropy production using the steady-state fluctuation theorem and finally to obtain valuable information regarding the mechano-chemical cycle of RecQ.

P45

Control of the mitotic cyclin dependent kinase and anaphase in response to genotoxic stress

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Eukaryotic cells rely on a crucial surveillance mechanism, the S phase checkpoint, to preserve genomic integrity in response to genotoxic insults during the critical DNA replication phase. The S phase checkpoint attenuates and protects DNA replication, triggers an ad hoc transcriptional response, and blocks cell cycle progression until the problem is solved. An impaired checkpoint response results in genomic instability, and promotes cancer in metazoan organisms.

Cell cycle control and the S phase checkpoint response are best understood in the eukaryotic model organisms Schizosaccharomyces pombe (fission yeast) and Saccharomyces cerevisiae (budding yeast). The factors and pathways first identified in yeasts are conserved in humans.

A conundrum in the budding yeast response to genotoxic stress is the control of Mitotic Cyclin Dependent Kinase (M-CDK) activity. CDKs are the engine of cell cycle progression, and they are activated by phase specific, highly unstable subunits known as cyclins to drive cells through the different cell cycle phases. It's been long known in fission yeast that in response to genotoxic stress the S phase checkpoint blocks M-CDK activity by regulating the balance of the wee1/cdc25activites. The wee1 kinase phosphorylates Tyr15 of the cdk1 catalytic subunit, which results in M-CDK inhibition and a block of mitosis. The cdc25 phosphatase is responsible to counteract wee1 and allow cells to enter M phase. Null wee1 mutants fail to block mitosis in the presence of genotoxic stress.

Such control is conserved from yeasts to humans, and similar experiments in budding yeast show that the wee1 ortholog kinase Swe1 phosphorylates the homologous Tyr19 residue in the Cdk1 catalytic subunit.

Puzzingly, non-phosphorytable Cdk1 mutants, or swe1Δ null strains altogether, fail to elude the checkpoint block of mitosis in response to of DNA damage or replication stress (genotoxic stress). We show here that Swe1 is indeed involved in the block of M-CDK. However, contrary to fission yeast, a second, redundant control operates. Both controls are under the checkpoint central kinase Mec1 (ATR/ATM in humans), although each of them appears to be regulated by different downstream effector pathways. We predict that this novel control, missing in fission yeast, is likely to be conserved in higher eukaryotes, and is therefore likely to be of relevance to cancer.

Lab recent publications:

Palou G, Palou R, Guerra-Moreno A, Duch A, Travesa A, Quintana DG (2010) Cyclin regulation by the S phase checkpoint. J Biol Chem 285:26431-40.

Duch A*, Palou G*, Jonsson ZO, Palou R, Calvo E, Wohlschlegel J, Quintana DG (2011) A Dbf4 Mutant Contributes to Bypassing the Rad53-mediated Block of Origins of Replication in Response to Genotoxic Stress. J Biol Chem 286:2486-91. (*equal contribution)

P46

Cell model for Sanfilippo C syndrome using iPS cells from patients' fibroblasts

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Introduction: Mucopolysaccharidosis III (MPS III), or Sanfilippo syndrome, includes four autosomal recessive diseases characterized by deficient heparin sulphate degradation. Clinical symptoms are similar for all types of MPS III, including progressive and severe deterioration of the central nervous system during childhood. HGSNAT is the gene responsible of MPS IIIC, which encodes the acetyl CoA:α-glucosaminide N-acetyltransferase, a lysosomal membrane protein. At present, no therapies are available for Sanfilippo syndrome.
Human induced pluripotent stem cells (iPSc) offer a unique opportunity to model human neurodegenerative diseases. Recently, several models have been obtained using that technology.

Objective: The aim of this study is to develop a neuronal model for Sanfilippo syndrome type C. We obtained skin fibroblasts from two unrelated patients carrying different mutations. Patient 1 was heterozygous for a missense (p.L445P) and a splicing (c.633+1G>A) mutation, while patient 2 was homozygous for a splicing mutation (c.372-2A>G) prevalent in Spanish patients.

Methods and results: Retroviral vectors encoding KLF4, OCT4, and SOX2, with or without one coding for c-MYC, were used to obtain at least 6 clones of iPSc from each patient, as well as from a control healthy individual. One clone of each line (control, patient 1 and patient 2) was propagated for more than 10 passages on HFF. Cells were positive for alkaline phosphatase, showed normal karyotypes, expressed markers of pluripotency (SSEA3, SSEA4, NANOG and TRA-1-81) and the genes encoding for the different factors were integrated in their genome. Quantitative PCR after reverse transcription was carried out to establish the efficient repression of the exogenously introduced genes, and the ability of the cells to differentiate to the 3 germ layers was assayed in vitro and in vivo. The cells are being differentiated to neurons in order to study the molecular basis of the disease into this cellular type and to assay different therapies for the Sanfilippo syndrome.

Conclusions: Different iPSc lines from Sanfilippo patients' fibroblasts have been generated. These model will be a useful tool to assay different therapeutic approaches.

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Efecte xaperona de compostos glicomimètics sobre glucocerebrosidases mutades en fibroblasts de pacients Gaucher

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La malaltia de Gaucher (GD, OMIM 230800) és una esfingolipidosi causada per l'acumulació de glucosilceramida (GlcCer) en els lisosomes dels macròfags com a conseqüència de l'alteració de l'enzim glucocerebrosidasa (GBA). De totes les aproximacions terapèutiques per el tractament de la malaltia, cal destacar que la teràpia de substitució enzimàtic és la principal, però darrerament ha adquirit importància la utilització de les denominades xaperones farmacològiques. Aquestes són generalment inhibidors competitius de l'enzim, que a concentracions sub-inhibitòries s'uneixin de forma específica amb aquest, facilitant el seu correcte plegament i transport al lisosoma. L'objectiu d'aquest treball ha estat estudiar l'efecte d'onze compostos com a xaperones farmacològiques sobre GBAs mutades, utilitzant fibroblasts de 7 pacients Gaucher portadors de diferents genotips i un control. Nou d'aquests onze compostos són aminociclitols, els quals van ser sintetitzats als nostres laboratoris i els altres dos compostos han estat la N-nonildeoxinojirimicina (NNDNJ) i la isofagomina (IFG).
solan
Els fibroblasts portadors dels distints genotips van ser incubats durant 6 dies amb els compostos problema i el sisè dia es va analitzar l'activitat GBA mitjançant un assaig fluoromètric. De tots ells, cal destacar l'efecte de la IFG i d'alguns compostos de tipus aminociclitol sobre la mutació G202R.

D'altra banda, es van determinar els nivells de GlcCer en aquelles condicions en què s'havia observat un increment de l'activitat enzimàtica en els fibroblasts portadors de la mutació G202R. Els resultats no foren els esperats, ja que els fibroblasts tractats amb els compostos van donar lloc a un increment de la concentració de GlcCer.

Aquests resultats semblen indicar que aquestes xaperones actuen de forma diferent sobre la GBA lisosomal i la citosòlica (GBA2). Així, podrien alhora activar la primera i inhibir la segona, fet que podria conduir a un augment net del contingut cel·lular de GlcCer i permetria explicar aquesta controvèrsia en els resultats. Actualment s'està treballant en aquesta hipòtesi.

P48

Role of TREX2 exonuclease in the DNA damage response to UVB

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TREX2 is an exonuclease that can play a role maintaining genome stability by processing DNA 3' termini in multiple processes of DNA metabolism, ranging from DNA synthesis and repair to DNA degradation. Interestingly, TREX2 is specifically expressed in keratinocytes, which are naturally exposed to multiple DNA-damaging agents, including virus, chemicals and ultraviolet radiation. In this context, TREX2 deficiency increases susceptibility to carcinogen-induced skin tumorigenesis that correlates with an impaired apoptosis. To determine the mechanisms by which TREX2 exonuclease promotes apoptosis, we have analyzed DNA damage response to UVB in wild-type and knockout keratinocytes. In agreement with *in vivo* data, reduced levels of UVB-induced apoptosis were observed in *Trex2*$^{-/-}$ compared with wild-type keratinocytes. Consistently, data from survival colony assays indicate that *Trex2*$^{-/-}$ keratinocytes are more resistant than *Trex2*$^{+/+}$ keratinocytes to UVB-genotoxicity. In the other hand, TREX2 is not recruited to sites of UVB-induced DNA lesions, and we have not seen differences in UVB-induced p53 and γH2AX phosphorylation between wt and *Trex2* knockout keratinocytes. Thus, analysis of DNA damage response to UVB radiation in wt and *Trex2*$^{-/-}$ keratinocytes suggest that loss of TREX2 exonuclease confers resistance to UVB-induced DNA lesions by compromising apoptosis execution, but not DNA damage sensing, signaling or DNA repair.

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Dysregulation of CK2 subunits alters proliferation and migration in 786-O cells

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Clear cell renal cell carcinoma (ccRCC) is characterized by a high incidence in renal tumours and poor prognosis. Protein kinase CK2 is an enzyme whose activity is increased in cancer cells. Tissue microarray analysis of ccRCC samples revealed a dysregulation between the levels of the catalytic (CK2α/α') and regulatory subunits (CK2β) of CK2 holoenzyme, due in part to decreased CK2β expression.

To study the functional consequences of the dysregulation between CK2 subunits we used the 786-O cell line (established from human ccRCC) and its derivatives generated by lentiviral transfection to obtain CK2α- or CK2β- silenced cell lines. CK2α-downregulated 786-O cells also showed a decrease in CK2β, but no decrease in CK2α was detected in CK2β-depleted cells. Downregulation of either CK2α or CK2β caused a decrease in 786-O cells proliferation, which was more marked in CK2β-depleted cells. Furthermore, CK2β-depleted cells migrated faster than control cells in wound healing assays. A slight increase in cell migration was also observed in CK2α-downregulated cells. CK2α-downregulation did not significantly alter the epithelial morphology of 786-O cells. In contrast, CK2β-depleted 786-O showed an elongated fibroblast-like phenotype, suggesting an epithelial to mesenchymal transition. In agreement with this, the levels of epithelial cell markers E-cadherin and Slug decreased in CK2β-depleted 786-O cells. Interestingly, E-cadherin and Slug levels also decreased in CK2α-downregulated cells. Moreover, downregulation of CK2α , but not of CK2β, caused a decrease in N-cadherin levels. In addition, ERK1/2 activation in response to HB-EGF is less sustained in CK2α-silenced 786-O cells than in control 786-O control cells, whereas CK2β-depletion did not affect ERK1/2 response to HB-EGF.

Summing up, both CK2 subunits contribute to ccRCC 786-O cells phenotype or their response to growth factors.

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P50

Expression pattern and map of sumo and ubiquitin pathway enzymes in the mouse retina

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Many transcription factors and other proteins that control crucial fate decisions, such as those occurring in the differentiation of specific retinal neuron types, undergo SUMO and/or ubiquitin post-translational modifications. The aim of our work is to analyze the expression of a battery of enzymes involved in the SUMO and ubiquitin conjugation/deconjugation pathways in the mouse retina.

After RNA purification and cDNA obtention from wild type mouse retinas, the expression of the battery of genes selected for the study has been analyzed by real-time RT-PCR, *in situ* hybridization and immunohistochemistry. The genes of the SUMO and ubiquitin pathways analyzed have been classified according to their level of transcription in the mouse retina. *In situ* hybridizations performed on retinal sections have shown whether the expression was basal and ubiquitous throughout the retinal cell layers, or whether a specific pattern of expression is indicative of specific neuronal type function for each gene. Those genes with a particularly interesting function or pattern of expression have been further characterized by immunohistochemistry.

CONCLUSION: An expression map for the battery of selected genes encoding SUMO and ubiquitin pathways has been drawn. A subset of genes show specific expression patterns, with higher transcriptional levels in specific cell types, such as the outer and inner plexiform, ganglion cell and photoreceptor cell layers. Future work will focus on the search for specific substrates for the most relevant genes of these two pathways.

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Epithelial -mesenquimal transition in Head and Neck Carcinoma cell lines

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In a previous microarray study, we identified different subtypes of HNSCC which gene expression was associated with clinical outcome. We observed that the tumor subtype with the poorest prognosis presented epithelial-mesenquimal transition (EMT) features. The purpose of this study was to determine the association between the EMT phenotype and the resistance/sensitivity to cisplatin treatment in a pannel of HNSCC cell lines. We assessed protein expression levels of EMT markers (E-cadherin, Vimentin, N-cadherin and Integrin β1) by Western-Blot and Inmunocytochemistry in six HNSCC cell lines. Cytotoxicity to cisplatin was performed with the XTT assay (Roche). We evaluated differences in the expression profiles of UM-SCC 22A, UM-SCC-22B, UM-SCC-74B and SCC9 cell lines by microarray technology. Finally, we validated an EMT gene expression signature, previously described by Chang et al.(2004) using GSEA computational method.

UM-SCC-74B and SCC9 cell lines showed a fibroblast like morphology and scattered growth, while UM-SCC-22A, UM-SCC-22B, SCC25 and Fadu grew forming cell groups and depicted epithelial features. Determination of protein expression levels showed a high Vimentin and N-Cadherin expression in UM-SCC-74B and SCC9 cell lines. In addition, both cell lines did not express E-Cadherin. This expression pattern was consistent with epithelial mesenquimal transition process. In contrast, UM-SCC22A, UM-SCC22B, SCC25 and Fadu cell lines maintained E-Cadherin as in most epithelial cells. We identified 353 genes differentially expressed by comparing the expression profiles between EMT (SCC9 and UM-SCC-74B) and no EMT (UM-SCC-22A and UM-SCC-22B) cell lines. We found a high number of genes related to EMT phenotype that were upregulated in SCC9 and in UM-SCC-74B. We validated, in these cell lines the EMT genetic signature previously described by Chang et al. The SCC9 cell line was the most resistant to cisplatin treatment.

In summary, we have showed the presence of the EMT-phenotype in some of the evaluated cell lines as we observed in HNSCC tumor biopsies. SCC9, with an EMT phenotype, presented a high resistance to cisplatin treatment. These results suggest that SCC9 is an appropiate cell line model to understand wich biological processes are impaired in poor prognosis HNSCC patients, as they present EMT features and are resistant to cisplatin based chemotherapy .

P52

Involvement of the NON-RGS RhoGEF PROTEINS, p190RhoGEF and GEF-H1, in the G12 family signaling pathways

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Signaling via G protein-coupled receptors has been implicated in a myriad of physiological and pathological processes. The $G\alpha_{12}$ and $G\alpha_{13}$ comprise one of the families of the heterotrimeric G proteins and are known for their regulation of actin cytoskeleton and epithelial cell junctions, and have recently been implicated in the progression of tumor cell and cancer metastasis. The most extensively characterized downstream mediators of signaling through the G12 subfamily are members of the RhoA family. The members of this family of proteins are known mainly for their role in regulating the actin cytoskeleton, but they also play important roles in dictating cell polarity, microtubule dynamics, membrane transport pathways, transcription factor activity, and cell growth. $G\alpha$12 and $G\alpha$13 interact and stimulate the activity of a sub-family of Dbl family of guanine nucleotide exchange factors (GEFs) called RGS-RhoGEFs, which are characterized for their binding to G proteins through their RGS-like domain. Nevertheless, the RhoGEFs superfamily comprises a very extensive family of proteins and some of the ones lacking the RGS domain also participate downstream G protein signaling pathways.

p190RhoGEF is a non-RGS-RhoGEF protein known to regulate FAK signaling downstream of GPCRs and is involved in promoting tumor progression. FAK forms a complex with p190RhoGEF and promotes p190RhoGEF tyrosine phosphorylation, events associated with the enhanced activation of RhoA by p190RhoGEF. We have evidence for a novel interaction between $G\alpha_{12/13}$ and p190RhoGEF. Our working hypothesis is that p190RhoGEF is a downstream effector of $G\alpha_{12/13}$ and as a consequence they are also implicated in the progression of colon cancer.

GEF-H1 is a novel member of GEFs family with RhoA-specfic enzymatic activity and without a RGS-like domain. Subcellular localization analysis demonstrated that GEF-H1 is associated with microtubules and its depolymerization leads to GEF-H1 activation, accompanied by a RhoA-dependent reorganization of the actin cytoskeleton. Our results show that GEF-H1 can interact exclusively with $G\alpha$12 through the N-terminal domain that comprises the DH domain. $G\alpha$12 seems to negatively regulate the activity of GEF-H1.

P53

On the nature of the genetic bases of the high bone mass phenotype in Spanish postmenopausal women

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The purposes of this study were: to establish the prevalence of the high bone mass (HBM) phenotype in the BARCOS cohort (n=1600); to determine whether any of the HMB cases carry LRP5 mutations that explain the phenotype; to characterize the expression pattern of osteoblast-specific and Wnt pathway genes in primary osteoblast RNA samples from two HBM cases; and to test the hypothesis of an inverse correlation between the number of common variant osteoporosis risk alleles and HBM.

Material and methods: HBM individuals within the BARCOS cohort were identified according to the criterion of a sum Zscore > 4 (total LS-Zscore + total Hip-Zscore). Relevant exons of LRP5 were PCR-amplified and sequenced. Cosegregation analysis of markers in the LRP5 gene region was performed in one family. Primary osteoblasts from two HBM and two control individuals were cultured and RNA was extracted. A Roche RealTime ready Custom Panel was used to analyse the expression of 88 osteoblast-specific and/or Wnt pathway genes. SNPs from previous GWA studies were genotyped in the HBM cases and relatives and a weighted score was obtained for each individual. These scores were plotted against BMD values.

Results: In the BARCOS cohort of postmenopausal women, 0.6% of individuals display BMD values in the HBM range. No mutations in the analysed exons of the LRP5 gene were found in these patients. Additionally, in one familiar case in which the mother and one of the two sibs had BMD values in the range of HBM, cosegregation analysis ruled out LRP5 involvement. Further cosegregation studies in this family allowed the exclusion of the following genes: DKK2, IL6R, RANK, BMP2, and KRM1. The only gene cosegregating was RANKL, but it was sequenced in the proband and no mutations were found. Regarding the expression analysis, five genes were found to be overexpressed in the two HBM samples: BMP4, COL10A1, RUNX2, FZD3 and SOX6, while four were underexpressed: DLX3, TWIST1, IL6R, and PPARG.

Finally, preliminary results point to an inverse correlation between risk alleles and BMD in this group of women, although two women with the highest BMD values presented with the highest risk score. A low frequency penetrant unknown genetic variant could be a possible explanation for these cases.

Conclusions: LRP5 is not the cause of the HBM phenotype in these cases from BARCOS cohort. The results of the expression study raise new hypotheses that should be further investigated. HBM maybe genetically heterogeneous.

P54

Determinants of the specificity of human histone H1 subtypes

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Seven linker histone H1 variants exist in human somatic cells (H1.0, H1.1 to H1.5, and H1X), with distinct prevalence depending on the cell type analyzed and along differentiation, that bind to linker DNA contributing to higher order chromatin compaction. In addition, H1 seems to be actively involved in the regulation of gene expression. It is not well known whether the different variants have specific roles or regulate specific promoters. We have explored this by inducible shRNA-mediated knock-down of each of the H1 variants in a human breast cancer cell line. Rapid inhibition of each H1 variant was not compensated by changes of expression of other variants. Thus, specific phenotypes are observed in breast cancer cells depleted of individual histone H1 variants. Moreover, knock-down of each H1 variant alters expression of a different, reduced subset of genes, with more genes being repressed than activated suggesting a local positive role of H1 on gene expression control. By taking advantage of specific antibodies for H1 variants and HA-tagged recombinant H1 variants-expressing cell lines, we have also investigated the role of some H1 post-translational modifications. Then, we have included in our studies H1x, which is the most unknown histone variant, observing it presents some interesting and specific features that differentiate it from the other variants. Finally, we have found changes on the histone H1 variants content during differentiation of human embryonic stem cells and reprogramming of keratinocytes, and these H1 repertoires appear critical to maintain the functionality of such cells.

P55

La inhibición de Apaf-1 tiene un efecto protector en la ototoxicidad inducida por Cisplatino

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Uno de los efectos adversos más significativos del tratamiento con el agente quimioterapéutico Cisplatino (CisPt) es la pérdida auditiva. Ésta se produce por la muerte celular por apoptosis de las células ciliadas de la cóclea debido a la producción masiva de radicales libres de oxígeno (ROS) que activan la vía mitocondrial o intrínseca de apoptosis. El objetivo de este trabajo es estudiar el efecto protector de los inhibidores de Apaf-1 en la ototoxicidad inducida por CisPt, tanto en modelos in vitro como in vivo.

El tratamiento con CisPt de una línea celular de oído interno derivada del órgano de Corti (HEI-OC1), induce su muerte celular por apoptosis. La presencia de los inhibidores de Apaf-1 previene esta muerte celular inhibiendo la liberación de citocromo c y la activación de caspasa 3. Por otro lado, el tratamiento con CisPt aumenta la expresión génica de Apaf-1 y ésta se ve disminuida por el tratamiento con los inhibidores. En un modelo de ototoxicidad en pez cebra, el CisPt induce la perdida de las células ciliadas de la línea lateral (otolitos). Este efecto es revertido por la presencia de los compuestos inhibidores de Apaf-1.

Finalmente, en un modelo in vivo de inducción de sordera por CisPt en ratas, la administración intratimpánica de un inhibidor de apoptosis disminuye la pérdida de audición. El incremento de la activación de caspasa 3 en cóclea y la inducción de la expresión de genes pro-apoptóticos tras el tratamiento con CisPt, se ven disminuidos en presencia del inhibidor de apoptosis.

Por todo ello, podemos concluir que el uso de los inhibidores de Apaf-1 puede ser una terapia efectiva para la prevención de la ototoxicidad generada por el tratamiento con CisPt.

P56

Phosphate-sensing CDK stabilizes G1 cyclin to trigger cell cycle entry

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Cell cycle control by trophic factors has a key role in regulation of cell proliferation in all organisms. The *S. cerevisiae* G1 cyclin Cln3 is one well demonstrated common effector of multiple nutrient-dependent signalling pathways and its regulation is crucial for coordinating progression through the cell cycle. However, mechanisms that link nutrient sensing to regulation Cln3 cyclin are still unknown. Pho85 CDK seems to be a perfect candidate that could run these two highly different tasks, because of its properties in phosphates sensing and cell cycle regulation. Here, we show that the lack of phosphates downregulates Cln3 levels and leads to G1 arrest through an inactivation of Pho85. Cln3 is an in vitro substrate of Pho85, and both interact in vivo. Moreover, phosphomimetic changes to specific Pho85 consensus sites in Cln3 protein leads to stabilization of this cyclin and to avoid G1 arrest in the absence of phosphates, decreasing cell viability. Finally, Pho85 activity is essential for sufficient Cln3 accumulation to triggers reentry to cell cycle after phosphate refeeding. Taken together, our data indicate that Cln3 is a molecular target of Pho85 kinase required to modulate cell-cycle entry in response to environmental changes in nutrient availability.

P57

Novel mechanism of degradation of G_1 cyclins. Molecular Biology

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In *S. cerevisiae* the progression through the cell cycle is controlled by two Cyclin Dependent Kinases (CDKs): Cdc28 and Pho85. During G_1, Cdc28 is associated to the cyclins Cln1 and Cln2 and Pho85 is associated to Pcl1 and Pcl2. Intriguingly, most of the Pho85 substrates that have a role in G_1 cell cycle progression are also substrates of the homologous CDK Cdc28. To explain this redundancy, some authors suggest that the phosphorylation by Pho85 could regulate proteins in the same way as the phosphorylation by Cdc28, but under different conditions. For example, one possibility could be that the different G_1 cyclins were destroyed by specific ubiquitin systems in determined conditions and not in others. In this regard, at the end of G_1, Cln2 is degraded by Grr1 E3 ubiquitin ligase, but it is unknown how Pcl1 is degraded.

In order to deepen on the specific G_1 roles of CLNs and PCLs we decided to investigate the profile of expression and destruction of Cln2 and Pcl1. Although, as described, both cyclins were expressed at the same time by the same transcription factor, we determined that the kinetics of disappearance were different. We determined that Pcl1 is degraded by proteasome and that this degradation is dependent on Dma1, Dma2 ubiquitin ligases and not on Grr1. Acordingly, Pcl1 is less ubiquitylated and notably more stable in *dma1*Δ *dma2*Δ strain. Finally, we also determined that Pho85 phosphorylates Pcl1 *in vivo* and *in vitro*, and we determined the Ser and Thr residues of Pcl1 necessaries to destabilize it.

All this results suggest that during G_1 the CLN family and the PCL family are degraded by different mechanisms, suggesting a possible point of regulation. Indeed, we determined physiological conditions in which Dma1 becomes active (and hence the cells progress through G_1 with low levels of Pcl1) and, on the contrary, conditions in which the cells progress with low levels of Cln2.

As a conclusion, we have described a novel mechanism of G_1 cyclins degradation that could help us to explain the specific roles of the different CDK/cyclin complexes in G_1.

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C/EBPδ PLAYS A KEY ROLE IN GLIAL ACTIVATION

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Glial activation plays a central role in CNS inflammatory processes and is mediated by a selected group of transcription factors. CCAAT/enhancer binding protein δ (C/EBPδ) is a transcription factor expressed in activated glial cells that is involved in the regulation of pro-inflammatory genes. C/EBPδ is upregulated in human neurological disorders (Alzheimer's disease, spinocerebellar ataxia type 3, ...) and in animal models (Alzheimer's disease and experimental autoimmune encephalomyelitis). The aim of this study was to investigate whether C/EBPδ regulates gene expression in glial activation. For in vitro studies, primary mixed glial cultures were prepared from wild type and C/EBPδ -/- cortices treated with LPS+IFNγ, while in vivo studies were carried out in adult male mice from both genotypes which received systemic injection of lipolysaccharide (LPS). Moreover, C/EBPδ expression was also studied in G93A-SOD1 mice and in human samples from amyotrophic lateral sclerosis (ALS) patients. The expression of the pro-inflammatory genes NO synthase-2, cyclooxygenase-2 and interleukin (IL)-6 induced by LPS+IFNγ was attenuated in mixed glial cultures prepared from C/EBPδ -/- mice. Also, in neuron-microglia co-cultures the neurotoxicity elicited by activated microglia was abolished when C/EBPδ was absent in microglia. Systemic injection of LPS in wild type mice induced C/EBPδ expression in activated glial cells and upregulation of pro-inflammatory genes in the CNS. In C/EBPδ -/- mice, LPS-induced brain expression of NO synthase-2, tumor necrosis factor-α, IL-1β and IL-6 was attenuated. Finally, C/EBPδ was upregulated in ALS and G93A-SOD1 spinal cord where it co-localized with microglial markers. Taken together, these data show that C/EBPδ regulates pro-inflammatory gene expression in glial activation and plays an important role in microglial-induced neurotoxicity. C/EBPδ inhibition could therefore be useful in the treatment of neurodegenerative disorders with a strong neuroinflammatory component such as ALS.

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Cyclin D3 and CDK11 partnership in the apoptosis of the pancreatic beta cell in TYPE 1 diabetes

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Cyclin D3 (CcnD3) and CDK11 are downregulated in pancreatic islet endocrine cells during the autoimmune attack progression in autoimmune-prone NOD mouse strain.D-type cyclins are crucial in order to connect mitogenic signals with the Rb/E2F pathway, which regulates transcription of factors involved in further cell cycle progression. CDK11**,** protein-kinase PITSLRE, exhibits two gene products: p58 and p110 (p130 in mouse) in humans. $CDK11^{p110}$ regulates transcription and RNA splicing. $CDK11^{p110}$ is expressed in all cell cycle phases, while $CDK11^{p58}$ is only expressed during mitosis (G2/M) and is essential in apoptosis. The interaction between $CDK11^{p58}$ and Cyclin D3 represses certain nuclear receptors action, as leading to negatively affect the transcriptional activity of androgen receptor. This observation may suggest that in pancreatic beta cells simultaneous downregulation of cyclin D3 and CDK11 may obey to a coordinated regulation of both molecules. At present we are studying whether there is a causal relationship between coordinated Cyclin D3 and CDK11 downregulation and diabetes onset in type 1 diabetes in vivo and in vitro. *In vivo* approach: We have generated NOD mice deficient in both, CcnD3 and CDK11, which are being monitored for spontaneous diabetes incidence and islet infiltration. *In vitro* approach: NIT-1 NOD insulinoma cell lines stably co-tranfected with CcnD3 and either $CDK11^{p130}$ or $CDK11^{p58}$, are being submitted to the apoptotic stimuli trigerred by IL-1β and IFNγ, and apoptosis susceptibility measured by Annexin V staining. The outcome of our research will allow us to establish Cyclin D3 and CDK11 as molecular targets in type 1 diabetes.

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DFF40/CAD-mediated nuclear morphology does not require oligonucleosomal DNA fragmentation during apoptotic cell death

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Apoptotic cell death is characterized by chromatin fragmentation and oligonucleosomal DNA degradation, mediated by the caspase-dependent specific activation of DFF40/CAD endonuclease. In this work we have performed an analysis of DNA fragmentation and nuclear morphology in different human-derived neuroblastoma cell lines upon staurosporine (STP) treatment. Among the cell lines characterized, we found that SK-N-AS cells display nuclear chromatin condensation without degrading DNA in low molecular weight fragments.Cytotoxicity afforded after staurosporine treatment is comparable with that obtained in SH-SY5Y cells, which exhibit a complete apoptotic phenotype upon STP treatment. SK-N-AS cell death is a caspase-dependent process that can be impaired by the pan-caspase inhibitor q-VD-OPh. The endogenous inhibitor of DFF40/CAD, ICAD, is correctly processed, and *dff40/cad* cDNA sequence does not reveal mutations altering its amino acid composition. Results obtained with cell-free systems suggest that cytosols from STP-treated SK-N-AS cells are not able to induce DNA laddering. In summary, we demonstrate that oligonucleosomal DNA fragmentation process can be dissociated from typical apoptotic caspase-dependent nuclear morphology. The existence of biological models like SK-N-AS cells, gives us an important tool to analyze the relevance of nuclear morphology *versus* DNA fragmentation in gene mutations, gene amplifications and chromosomal instability.

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Lack of the transcription repressor histone deacetylase 7 induces lymphocyte oncogenic transformation.

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The generation of B cells is the result of several cellular transitions that take place in a stepwise manner and comprise cell lineage choices, cell commitment and differentiation. Every differentiation step is characterized by the activation of a new, lineage-specific, genetic program and the extinction of the previous one. To date, the central role of specific transcription factors in positively regulating these distinct differentiation processes to acquire a B cell specific genetic program is well established. However, very little is known on the role of transcriptional repressors, such as histone deacetylases, in B lymphopoiesis. Importantly, deregulation of linage-specific transcriptional programs during B cell generation leads to the development of hematological malignancies such as leukemias and lymphomas. Our laboratory has found that the histone deacetylase HDAC7 is a key transcriptional repressor of lineage inappropriate genes in B lymphocytes. We have obtained promising data indicating that aberrant expression of HDAC7 could play a crucial role in leukemia and lymphoma development. We have found that HDAC7 expression is lost in some B lymphoblastic leukemia and lymphoma cell lines. To investigate the molecular basis for the involvement of HDAC7 in hematopoietic malignances development, we have ectopically reintroduced HDAC7 to these cell lines. HDAC7 reintroduction promoted apoptosis and abrogated the oncogenic capacities of these cell lines. Thus, we suggest that HDAC7 acts a tumor suppressor in B lymphocytes.

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Mitochondrial function in healthy infants in utero exposed to hiv and antiretrovirals

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Background: Mitochondrial dysfunction of HIV-infection and antiretrovirals, especially nucleoside-reverse-transcriptse-inhibitors (NRTIs), has been widely associated to secondary effects in adults. Antiretrovirals are administered to HIV-infected pregnants to prevent vertical transmission. However, little is known about their safety in their *in utero*-exposed newborn.
Objectives: To characterize mitochondrial function in peripheral blood mononuclear cells from healthy infants born to HIV-infected mothers and who were perinatally exposed to NRTIs.
Methods: Cross-sectional and longitudinal case-control study in 2 groups of healthy children: those perinatally exposed to HIV and NRTIs during pregnancy and the neonatal period (Case Group, n= 132) and those born to HCV-infected mothers (Control Group, n=34). PBMC were obtained at 6 weeks, 3, 6 and 12 months of life by atemperate dextran method and prospectively cryopreserved. Mitochondrial content (citrate synthase activity) and mitochondrial respiratory chain enzymatic activity of CIV were assessed spectrophotometrically.

Results: Mitochondrial content remained similar in both groups along time. Compared to the control group, a decrease in CIV function was found in NRTIs and HIV-perinatally exposed infants at 6 weeks and 3 months of life (23.03±4.28 vs. 9.12±0.83 and 25.88±3.59 vs. 9.62; $p<0.005$ and $p<0.001$, respectively). An increase in CIV activity was found in cases from 6 weeks to 12 months of life (9.12±0.83, vs. 15.40±2.47; $p=0.022$).

Conclusions: CIV activity was reduced in NRTIs and HIV-perinatally exposed children compared to controls at 6 weeks and 3 months. Accordingly, a recovery in CIV function was found at 12 months compared to 6 weeks in this group, suggesting reversibility in mitochondrial toxicity.

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Mitochondrial toxicity of highly active antiretroviral therapy (haart) in HIV uninfected pacients

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Background: HIV-infection together with highly active antiretroviral (ARV) therapy (HAART), especially nucleoside-analogues such as zidovudine (AZT), induces mitochondrial (mt) DNA (mtDNA) depletion, responsible of clinical adverse effects. However, contribution of either HIV or ARV to the observed mt damage is difficult to elucidate in HIV-infected patients under HAART. We aimed to assess *in vivo* mt toxicity of HAART in HIV uninfected subjects.

Methods: We studied 12 healthy patients under 1 month of prophylactic HAART to prevent HIV-infection after risk exposure, 6 of them including AZT. Mitochondrial toxicity was determined by assessing mtDNA content using rtPCR in peripheral blood mononuclear cells (PBMCs), before and after HAART. We also included 16 uninfected controls, 17 HIV+ naive typical progressors (TP), 11 HIV+TP on HAART (Efavirenz/Tenofovir/Emtricitabine or EFV/TDF/FTC) and 18 HIV+ long term non progressors (LTNPs).

Results: After one month of HAART, all HIV-exposed subjects remained uninfected without significant differences in their mtDNA content, although AZT+ patients presented increased trends towards mtDNA depletion (67% vs. 17% of population). Levels of mtDNA were significantly higher in healthy controls vs. HIV+ naive TP, and in HIV+ LTNP vs. HIV+ naive TP (117.00±16.46 vs. 51.61±5.44 and 89.44±7.21 vs. 51.61±5.44, both $p<0.001$). HIV+TP subjects after one year on free-AZT HAART presented an increase in mtDNA levels (51.61±5.44 vs. 83.99±9.18, $p=0.002$).

Conclusions: One month of prophylactic HAART does not significantly reduce mtDNA in HIV exposed/uninfected patients, despite patients on AZT-therapy showed increased trends towards mtDNA depletion. It is confirmed mtDNA levels gradation (healthy controls>LTNP>naive TP). Naive TP patients after 1 year on the new AZT-free HAART (EFV/TDF/FTC) significantly recovered mtDNA content, which could prevent future symptomatology.

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Asymmetric stochastic switching driven by intrinsic molecular noise

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Stochastic fluctuations (or 'noise') are present in genetic circuits, with a relevant effect that can modify the behaviour and functionality of the networks[1]. To understand the effect of fluctuations on a bistable positive feedback loop, we present a simple autoactivating model and study through a theoretical and simulated analysis the differences in the system whether we consider stochastic fluctuations or not. We find that stochastic noise relatively increases the ON (high concentration) to OFF (low concentration) stochastic switching rates, by effectively stabilizing the OFF state. This is related to the OFF state being less noisy than the ON state. These results are in agreement with experimental data on noise and switching rates in the bistable galactose signalling network in yeast [2] and provides, up to our knowledge, its first explanation.

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Reversibility and memory in cellular decision making

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Developmental biology gives many examples of processes that can be understood in terms of bifurcations of nonlinear dynamical systems.

In some cell differentiation processes, a transient signal induces cells to change state. Whether this or which change takes place (cellular decision making) is stochastic [1,2]. From a theoretical point of view, these cellular differentiation processes involve multistable dynamics in which each cell type is associated to an attractor. Stochasticity in cellular decision making arises partially from the inherent randomness of biological reactions with low copy number of reactants. We have performed a theoretical and numerical analysis of a molecular circuit architecture of two genes interacting with a mutual inhibition and a positive auto-regulation [3]. Our study characterizes the role of intrinsic gene expression noise and the properties of reversibility and memory in different types of cell decisions [4].

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Regulation of neurogenic wavefronts: a modeling perspective

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In the retina of chick embryos, a salt-and-pepper pattern of neural and non-neural cells emerges first in the centre of this tissue and spreads out defining a differentiation wavefront which divides the retina in two areas: a differentiating inner area, which is the neurogenic domain, and a surrounding undifferentiated area, which corresponds to a non-neurogenic region.

Based on molecular evidences, we propose a mathematical model for neurogenic wavefront dynamics whose spreading depends on a morphogen. On the one hand, the morphogen enables lateral inhibition dynamics to select which precursors will become neurons. On the other hand, those cells committed to become neurons start to secrete the morphogen. This scenario is what we call a self-regulated wavefront.

We perform a quantitative analysis on the parameter space, characterizing the density of neurons in the neurogenic region, the velocity and the shape of the neurogenic wavefront. We predict that conditions ahead of the neurogenic wavefront are of paramout importance since their alteration can lead to neural overproduction, alteration of regular wavefront morphology, and speed up of the wavefront, disrupting neurogenesis. Our results are very robust to different aspects of the model, namely dynamical noise, cell morphology, initial morphogen sources and other model parameters.

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Functional variants of the *CX3CR1* gene in Alzheimer Disease and Amiotrophic Lateral Sclerosis

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Microglia, the resident immune cells of the CNS, is found in the reactive state in a high number of patients with chronic neurodegenerative diseases, including Alzheimer disease (AD) and Amyotrophic Lateral Sclerosis (ALS). Reactive microglia has been proposed as a contributing factor to neuroinflammation and chronic neurodegeneration in AD. In ALS, microglia cells are activated surrounding the motor neurons degenerating zone. The chemokine receptor CX3CR1 gene is critical in the microglia-neuron communication. In animal models for AD, the knockout of this gene protects against neuronal death. Functional CX3CR1 polymorphisms in human have been associated with the susceptibility to develop Crohn's inflammatory disease. Recently, haplotypes in the CX3CR1 gene have been associated with the disease course of multiple sclerosis, but neither their implication nor relationship with the risk of developing AD or ALS have been studied. We are carrying out a genetic association study of functional CX3CR1 SNPs and the risk and severity of suffering AD or ALS. We have recruited 530 AD patients, 330 ELA patients and 750 control subjects representative of the Spanish population. After obtention of DNA samples, their genotype analysis is being performed. Single polymorphisms and haplotypes using logistic regression adjusted for age, gender and severity will be identified. The results of this study together with our conclusions, will presented at the congress.

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Treatment with a serotonin 5-HT$_4$-receptor agonist ameliorates cognitive deficits and amyloid pathology in the 3xTg-AD model of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by extracellular neuritic plaques of amyloid-beta peptide (Aβ), non-fibrilar intraneuronal soluble forms of Aβ and intracellular neurofibrillary tangles of hyperphosphorylated tau protein. Aβ is generated from its precursor protein APP after cleavage by β- and γ-secretases. In contrast, cleavage of APP by α-secretase releases the soluble, non-amyloidogenic peptide sAPPα. Activation of serotonin 5-HT$_4$ receptors enhances APP processing by α-secretase, thus increasing sAPPα levels *in vitro* and *in vivo*. The triple transgenic mouse model of AD (3xTg-AD), harboring the APP_{Swe}, $PS1_{M146V}$ and tau_{P301L} mutant genes, mimics critical aspects of AD neuropathology including learning and memory deficits. We have studied in this model the effect of chronic stimulation of 5-HT$_4$ receptors on APP processing, Aβ pathology, cognitive deficits and other behavioral symptoms.

Six month-old male and 12 month-old female 3xTg-AD mice and age-matched wild-type (wt) animals were given the 5-HT$_4$ partial agonist RS67333 or its vehicle using osmotic minipumps. Spatial learning and memory in the Morris Water Maze (MWM) were unaltered in 6 months-old 3xTg-AD males but severely impaired in 12 month-old 3xTg-AD females compared with age- and sex-matched wt controls. These deficits were rescued by agonist administration: agonist-treated 3xTg-AD mice performed as well as vehicle-treated wt controls. The anxious profile of 3xTg-AD mice in the Open Field and Dark/Light Box tests at both ages, showed a tendency to recovery after treatment with RS67333. Immunohistochemical analysis revealed marked decreases of extracellular amyloid plaques in the subiculum of 12 month-old agonist-treated 3xTg-AD animals compared with vehicle-treated 3xTg-AD animals.

These results suggest that sustained activation of 5-HT$_4$ receptors may be beneficial to counteract the cognitive deficits and to ammeliorate Aβ pathology in this model of AD.

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Neuroinflammation in APP/PS1 mouse model of Alzheimer´s disease

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Innate immune responses, along with the accompanying pro-inflammatory phenotype, are thought to play a role in Alzheimer´s disease (AD) progression. We have studied the brains of double transgenic mice expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9), both directed to CNS neurons (APP/PS1 model). Cognitive deficits are observed at 6 months of age in this model. We have measured the expression of pro-inflammatory markers, both at mRNA and protein level, in brain homogenates of APP/PS1 mice. Results show significant activation of innate immunity pathways in the hippocampi of 6 months-old animals. Importantly, no neuronal cell-death is detected at this age. Our data highlight the importance of pro-inflammatory processes in the early, non-terminal stages of AD *in vivo*. The aim of these studies is to identify potential immunomodulatory strategies for the treatment of AD.

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Motor deficits and neuromuscular junction alterations following chronic 3,3'-iminodipropionitrile exposure in the rat

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Rodents exposed to 3,3'-iminodipropionitrile (IDPN) develop an axonopathy similar to that observed in amyotrophic lateral sclerosis motor neurons, in which neurofilaments accumulate in swollen proximal axon segments. Recent work has demonstrated that this proximal axonopathy associates with an early dramatic loss of neurofilaments in the neuromuscular junctions. This study addressed the hypotheses that this distal effect may be a cause for final motor deficits and neuromuscular degeneration. Adult male Long-Evans rats were exposed to 0 or 15 mM of IDPN in drinking water for 1 year, and their motor function studied by the vertical ladder test, analysis of walking patterns, and assessment of tail dragging. The neuromuscular junction organization was studied by immunohistochemistry and transmission electron microscopy at 3, 6, 9, or 12 months. Quantitative data were obtained by confocal microscopy on whole mounts of the *Levator auris longus* (LAL); the same muscle was used for ultrastructural observation. Rats exposed to IDPN showed impaired endurance in a vertical ladder grip test and significant tail dragging but normal walking stride parameters. The amount of neurofilament labeling in the neuromuscular junctions was progressively reduced by IDPN. The percent of junctions, defined by TRITC-BTX labeling, showing any evidence of neurofilament signal declined from 63% at three months of IDPN to 29% at 12 months, compared to 98% in control animals. TEM observations showed disappearance of terminal neurofilaments and limited but significant evidence of neuromuscular junction degeneration. Conclusion: The proximal neurofilamentous axonopathy induced by IDPN is chronically associated with a progressively decrease in neurofilament content in the motor terminals, with behavioral alterations suggesting motor weakness, and ultrastructural changes in the neuromuscular junction suggesting impaired synaptic function.

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Secretory sorting receptors carboxypeptidase e and secretogranin iii in amyloid β-associated neural degeneration in alzheimer's disease

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The sorting receptors Carboxypeptidase E (CPE) and Secretogranin III (SgIII) exert essential functions in the activation and targeting at the regulated secretory pathway of neuropeptides and trophic factors. Alzheimer's disease (AD) is typically characterized by the presence of senile plaques and neurofibrillary tangles, synaptic and neuronal loss and glia-mediated inflammation. The recent data showing decreased CPE and SgIII levels in the cerebrospinal fluid of AD patients together the alterations in BDNF and neuropeptide systems detected in this neurodegenerative disease suggest an impairment of the regulated secretion in AD. Although CPE and SgIII are abundant components of the secretory granules, they are poorly studied in the CNS. Here we show for first time the distribution of both proteins in the healthy human cerebral cortex and we analyse how they are affected in AD. In the control human cortex CPE was mainly observed in dendrites and perykarya, while SgIII was associated with axons and terminal-like buttons. Immunoreactivity for the two proteins was also found in astrocytes and their thin processes. Consistently, CPE and SgIII were aberrantly accumulated in dystrophic neurites and reactive astrocytes surrounding most beta-amyloid plaques in the cerebral cortex of AD patients and transgenic APPswe/PS1dE9 mice. Taken together these results show an implication of the secretory sorting receptors CPE and SgIII in Aβ-associated neural degeneration. Moreover, a role for these proteins in the pathological progression of AD is suggested.

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Glycogen, a key player for astrocytes and neurons in the functioning of the brain

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Glycogen is synthesized from glucose by glycogen synthase (GS) and is the only carbohydrate reserve of the brain, with a concentration three to four times higher than free cerebral glucose. Although it has been traditionally considered as an emergency energetic reservoir, increasing evidences point to a role of glycogen in the normal activity of the brain. Our aim is to investigate not only the physiological role of glycogen in the brain, but also pathological situations in which neurons accumulate glycogen. For that purpose we have generated several tissue-specific loss-of-function and gain-of-function animal models.

P73

Anàlisi quantitativa de l'expressió d'isoformes de tau 3R i 4R a les malalties d'Alzheimer i dels grànuls argiròfils.

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El procés de tall i unió (*splicing*) alternatiu de l'exó 10 de la proteïna associada a microtúbuls tau dóna lloc a isoformes amb 3 o 4 repeticions en el domini d'unió als microtúbuls. A les malalties neurodegeneratives anomenades taupaties aquestes isoformes de tau, anomenades tau 3R i 4R, respectivament, són hiperfosforilades i s'acumulen a les neurones i la glia d'una manera específica segons la malaltia. Així, les formes tau 3R són les que predominen a la malaltia de Pick, les tau 4R a la paràlisi supranuclear progressiva (PSP), la degeneració corticobasal (CBD) i a la malaltia amb grànuls argiròfils (*argyrophilic grain disease*, AGD), mentre que a la malaltia d'Alzheimer s'hi troben les dues isoformes 3R i 4R. Pel que fa a la proporció dels mRNAs de les isoformes de tau, s'ha demostrat que la relació 4R/3R està augmentada en algunes regions cerebrals a la PSP i la CBD, mentre que a les malalties d'Alzheimer i de Pick aquesta relació no és diferent de la dels controls. Per tal de determinar si es produeixen alteracions en l'expressió d'mRNA de tau 3R i 4R que puguin contribuir a l'acumulació de tau 4R en els grànuls argiròfils a l'AGD, hem examinat per RT-PCR quantitativa mostres de cervell de pacients amb AGD en comparació amb casos control i pacients en etapes inicials de la malaltia d'Alzheimer (Braak III-IV). Hem utilitzant sondes i primers específics per les variants 3R i 4R de tau. A les regions analitzades, que inclouen l'hippocamp anterior i posterior, escorça frontal i temporal, no hem trobat diferències significatives en la relació 4R/3R entre AGD, Alzheimer primerenc i casos control. Aquests resultats estan d'acord amb estudis anteriors en malalts d'Alzheimer. L'absència d'alteracions en l'expressió del mRNA de tau 3R i 4R suggereix que la composició d'isoformes de tau que s'agreguen tant en els cabdells neurofibril·lars a la malaltia d'Alzheimer com en els grànuls argiròfils a l'AGD no depèn de factors genètics sinó que estaria controlada principalment per esdeveniments post-translacionals.

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Paraules clau:
grànuls argiròfils, demència, taupaties, isoformes de tau, malaltia d'Alzheimer, hippocamp.
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P74

Loss of vestibular function associates with afferent pathology during chronic ototoxicity in the rat

Lara Sedó Cabezon

IDPN (3,3'-iminodipropionitrile) is an ototoxic compound causing necrotic and apoptotic degeneration of the inner ear hair cells following acute exposure. However, chronic exposure to IDPN causes loss by extrusion of the vestibular hair cells. In this study, adult male Long-Evans rats were administered IDPN at 20 mM in the drinking water for 4 weeks. Vestibular function was assessed at weekly intervals by a specific behavioral test battery. The vestibular sensory epithelia were examined by scanning electron microscopy (SEM), as well as light and transmission electron microscopy (TEM). Vestibular dysfunction started at 2 weeks and increased over time. By SEM analysis, no extensive hair bundle abnormalities were observed, with only a minute fraction of the hair cells showing initial ciliary coalescence. Light microscopy and TEM observations showed well preserved overall epithelial structure, with no evidence of hair cell degeneration, hair cell extrusion, or epithelial vacuolization. However, major changes were observed in the afferents to the type I hair cells (HCI). These afferents have a calyceal shape enwrapping most of cell body of the HCI. In about 2/3 of this contact, the calyx and the HCI form a septate junction, the invertebrate version of tight junctions that is found in vertebrates only in this synaptic contact and at the paranodal regions of myelinated axons. After 4 weeks of exposure to IDPN, the electrodense scaffolding material characterizing the septate junction was no longer present in most calyx endings. The terminals had retracted and only partially covered the HCI membrane. The retracted afferents were not swollen. We conclude that loss of septate junctions, synaptic uncoupling, and afferent retraction are the events associated with initial loss of vestibular function during chronic ototoxic exposure.

P75

The resistance to apoptotic stimuli is a common trait in human glioblastoma multiforme-derived cells.

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It is widely accepted that the hallmarks of apoptosis are the round-packaged chromatin condensation and the oligonucleosomal DNA degradation. In these work, we analyzed the effect of apoptotic drugs on human glioblastoma (GBM)-derived cells trough the observation of these two specific features. First, we determine that GBM-derived cells present different sensitivity to different apoptotic insults, being the kinase inhibitor staurosporine (STP) the most effective drug. Interestingly, although STP treatment is able to induce cell death, when regarding nuclear morphology and genomic DNA we observe the absence of the aforementioned apoptotic markers: chromatin condensation and nuclear fragmentation and oligonucleosomal DNA degradation. Second, we wanted to describe the biochemical effect of STP in LN-18 cells (a GBM-derived cell line). Intriguingly, we detect the activation of the executioner caspases-3, -6 and -7, and the consequent cleavage of their specific substrates fodrin, lamin A/C and chaperone p23, respectively. Third, we wanted to elucidate whether the activation of caspases could culminate with the activation of the DFF40/CAD endonuclease. To this purpose, we have determined that the endogenous inhibitor of DFF40/CAD endonuclease, ICAD, is correctly cleaved by caspase-3. Subsequently, we analyzed the activation of DFF40/CAD endonuclease by *in vitro* assays. By means of cell-free assays, we have determined that the cytoplasmic fraction is unable to degrade DNA into oligonucleosomal fragments, even with the addition of exogenous pre-activated caspase-3 to the *in vitro* reaction. Interestingly, the cellular ability to degrade chromatin into oligonucleosomal-size DNA pieces after STP treatment is completely restored by DFF40/CAD over-expression, by either transient or stable transfections in LN-18 cells. Altogether, our results point out that GBM cells present a widely resistance to undergo a canonical apoptotic cell death when they are challenged to apoptotic stimuli by affecting the activation of the apoptotic endonuclease DFF40/CAD. This fact could be of capital relevance to understand the huge resistance of this kind of tumors to the conventional anticancer therapies.

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P76

Mitochondrial dysfunction in Huntington's disease: ROLE OF CDK5

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Huntington's disease (HD) is characterized by severe motor disorders that are primarily associated with dysfunction and degeneration of striatal GABAergic neurons. While it is known that the disease is caused by a polyglutamine expansion in huntingtin protein, the underlying molecular mechanisms have not been fully elucidated. Cdk5 is a multifunctional protein that can participate in a wide range of neural functions from neurite outgrowth to synaptic plasticity and cell survival. We found that deregulation of this kinase induced by mutant huntingtin increases the susceptibility of striatal neurons to dopamine via D1 receptor activation and that this neuronal death can be widely prevented by roscovitine, a potent Cdk5 inhibitor. There is also evidence that an altered Cdk5 signaling could contribute to striatal neurodegeneration through the activation of mitochondrial dynamics processes. It has been already shown its implication in fission and fusion during neuronal apoptosis and its contribution in oxidative stress and mitochondrial dysfunction. Here, we tested whether a deregulation of Cdk5 after dopaminergic activation impairs balance of mitochondrial fission/fusion processes. We examined mitochondrial properties in cells and mouse models of huntington disease by confocal image analysis and we verified changes levels of proteins implicated in fusion and fission processes. In addition, we asserted the role of Cdk5 in mitochondrial alterations by direct inhibition of the kinase and we analized the presence of a preventive effect in Cdk5 knock-out mouse models. Our findings support the hypothesis that Cdk5 plays a crucial role in mitochondrial defects involved in the striatal neurodegeneration of HD.

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Generation of LyzM-Cre x C/EBPβ$^{fl/fl}$ mice to obtain selective depletion of the transcription factor C/EBPβ in microglia

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Complete deficiency of the transcription factor CCAAT/enhancer binding protein β (C/EBPβ) protects from excitotoxic and ischemic insults suggesting the involvement of C/EBPβ in neurotoxicity in these models. C/EBPβ can be expressed by numerous cell types and the cell types involved in the potential detrimental effects of C/EBPβ have not been determined. We have recently shown that C/EBPβ absence results in the attenuated expression of pro-inflammatory genes in primary mixed glial cultures and in the abrogation of the neurotoxicity elicited by activated microglia in neuron-microglia co-cultures. In order to determine the neuroprotective potential of C/EBPβ inhibition in microglia we have generated a mouse transgenic line in which C/EBPβ gene is flanked by LoxP sequences (C/EBPβ$^{fl/fl}$) and Cre-recombinase is expressed under the control of the microglia/macrophage promoter Lysozyme M.

In primary mixed glial cultures from wild-type or from the two parental lines, C/EBPβ was expressed both in astrocytes and microglia. Primary mixed glial cultures prepared from the cerebral cortex of neonatal LyzM-Cre x C/EBPβ$^{fl/fl}$ mice revealed that virtually all microglial cells, identified by CD11b or Iba1 immunostaining, lacked C/EBPβ expression whereas C/EBPβ expression in astrocytes, identified by GFAP immunostaining, was similar to control lines. Interestingly, LPS or LPS+IFNγ-induced NO production in mixed glial cultures, which is of microglial origin, was markedly attenuated in LyzM-Cre x C/EBPβ$^{fl/fl}$ cultures when compared to control lines and the same effect was observed in microglial-enriched cultures.

We are in the process of obtaining samples to analyze gene expression profile of control and activated microglial cultures from LyzM-Cre x C/EBPβ$^{fl/fl}$ and wild-type mice. In parallel, we are interested to determine whether the cell specific absence of C/EBPβ in microglia in LyzM-Cre x C/EBPβ$^{fl/fl}$ mice is also observed in vivo. In this case, LyzM-Cre x C/EBPβ$^{fl/fl}$ mice would be a useful tool to analyze the involvement of microglial C/EBPβ in neurodegeneration in vivo and to test the hypothesis that inhibition of microglial C/EBPβ could have potential for the treatment of neurological disorders in which neuroinflammation plays a pathogenic role.

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P78

Study of the role of neurotrophins and their receptors in Autism Spectrum Disorders

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Background

Scientific evidence supports the notion that autism spectrum disorders (ASD) are neurodevelopmental disorders with a strong and complex genetic influence. A broader clinical phenotype is subsumed as ASD, encompassing less severe forms of autistic disorders. Regarding cognitive functions "low functioning autism" (LFA) versus "high functioning autism" (HFA) are distinguished. Impairments in social interaction, communication and language are characteristic of ASD. For the underlying neurobiology of these symptoms, disturbances in neuronal development and synaptic plasticity have been discussed. Thereby, neurotrophins are good candidates for a pathophysiological involvement in ASD, because they have a fundamental role in guiding central nervous system development and cortical organization.

Objectives

This study aims to investigate a gene variant val66met (rs6265) of BDNF; to analyze the mRNA expression of neurotrophins (BDNF, NT-3 and NT-4/5) and their receptors (Trk and $p75^{NTR}$), as well as the protein levels in plasma and in peripheral blood mononuclear cells (PBMCs) samples of autistic patients with HFA or LFA, too.

Methods

A total of 349 Spanish and German subjects were included in case-control study. This representative population was divided into two important cohorts: controls and heterogenic ASD group. The ASD patients were diagnosed according to ICD 10 and DSM-IV criteria; diagnostics were confirmed by the ADI-R and ADOS. Control population was separated in two subgroups: adolescent plus adult subgroup (>16 years; 21.54 ± 5.67; n = 23) and children subgroup (<16 years; 11.38 ± 2.35 years; n = 142). ASD cohort was also divided in same subgroups according to the age of participants. Then, adults ASD were subdivided into ASD with mental retardation and HFA (19.79 ± 6.7 years; n = 26). On the other hand children with ASD were subdivided into HFA (10.47 ± 3.02 years; n = 55) and LFA (7.98 ± 2.22 years; n = 20). RFLP-PCR had been the method used to determine the genotype of subjects. To evaluate the mRNA expression, quantitative RT-PCR by SYBR Green had been used. Finally, to determine the protein levels in plasma western blot method had been employed.

Results & Conclusion

The results indicate no association between the *BDNF* rs6265 and the ASD phenotype ($p > 0.05$). However, it is evident an association between the mRNA expression of neurotrophins and LFA, but not with HFA. On the other hand, the protein expression of neurotrophins (BDNF and NT-3) and their receptors (TrkA, TrkC and $p75^{NTR}$) in plasma of adolescent and adult group are correlated with the mRNA expression results; meaning that there is no association between the levels of protein and pathology. However, levels of BDNF in peripheral blood mononuclear cells (PBMCs) are statistically different ($p = 0.0211$) between ASD and control groups.

The present study demonstrates a correlation between neurotrophins and their receptors mRNA expression and cognitive function of ASD; these results are correlated with plasma levels because no association between HFA Is reflected with no association of protein levels. Furthermore, it can be concluded that the val66met of BDNF is not associated with the pathology.

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CREB-regulated transcription coactivator-1 (CRTC1)-dependent gene expression in APP transgenic mice

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Gene expression changes in the brain are associated with cognitive dysfunction in normal and pathological aging, but the molecular mechanisms underlying deregulation of gene expression programs leading to memory loss in age-related memory disorders are largely unknown. Here we show that transcription mediated by the cAMP-responsive element binding protein (CREB)-regulated transcription coactivator-1 (CRTC1) is decreased in transgenic mice expressing a human mutant β-amyloid precursor protein ($APP_{Sw,Ind}$). Microarray analysis revealed altered CREB-regulated gene expression in the hippocampus of $APP_{Sw,Ind}$ mice after spatial memory training. A specific set of genes related to synaptic plasticity and memory regulated by CRTC1 was differentially expressed in $APP_{Sw,Ind}$ mice, suggesting that CRTC1 dysfunction induced by Aβ accumulation is associated with altered gene expression in these mice. At the molecular level, neuronal activity induced dephosphorylation and nuclear translocation of CRTC1 in cultured neurons in a time-dependent manner. By contrast, cultured neurons from $APP_{Sw,Ind}$ mice show decreased CRTC1-dependent transcription, which was associated with enhanced CRTC1 phosphorylation at Ser151 by a mechanism involving reduced calcineurin-dependent CRTC1 dephosphorylation and nuclear translocation. Taken together, our results suggest that CRTC1 deregulation affects transcriptional programs required for hippocampal-dependent memory in $APP_{Sw,Ind}$ mice.

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P80

Presenilin-1 regulates axonal growth through RhoA in hippocampal neurons

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Presenilins (PS) are the catalytic subunits of γ-secretase, an enzymatic complex that cleaves transmembrane domains of type I membrane proteins, including APP, Notch or ephrin receptors (Eph). Recent evidence indicates that familial AD-linked mutations in PS cause reduced γ-secretase cleavage of several transmembrane proteins, suggesting a loss-of-function mechanism. Indeed, genetic inactivation of both PS in the adult brain causes synaptic plasticity and memory impairments and neurodegeneration. In this study, we analyzed the biological role of PS on regulating molecular mechanisms mediating axon growth in developing hippocampal neurons. We performed immunofluorescence imaging analyses using neurofilament and dendritic/growth cone (β-actin) markers to quantify axonal growth in primary hippocampal neurons derived from control (WT) and PS1-/- knockout mouse embryos. Our results show a 50% decrease in axon length in hippocampal neurons from PS1-/- embryos. A similar result is achieved by the PS/ γ-secretase inhibitor DAPT, suggesting that the effect of PS on axonal morphology is mediated by γ-secretase activity. Due to the essential function of RhoGTPases in actin cytoskeleton reorganization during axon development, we examined whether this family of GTPases were involved on the PS-dependent regulation of axon growth. A dominant negative RhoA mutant and an inhibidor of ROCK, the effector of RhoA during actin cytoskeleton reorganization, reversed the axonal collapse in PS1-/- hippocampal neurons. In summary, our results suggest that PS mediates axon elongation in hippocampal neurons by inhibiting RhoA, whereas PS inactivation induces axon collapse.

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P81

New mouse models for vestibular hair cell degeneration

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Degeneration of the inner ear hair cells leads to irreversible hearing and balance deficits. Research in mammalian hair cell regeneration is hampered by the lack of good models for hair cell ablation in the mouse. Several nitriles, including 3,3'-iminodipropionitrile (IDPN), allylnitrile, cis-crotononitrile, and cis-2-pentenenitriles have been demonstrated to cause hair cell degeneration in both the auditory and the vestibular systems in several different species, including the mouse. However, IDPN has other toxic effects, while allylnitrile and cis-crotononitrile cause acute lethality. Recent data from our laboratory demonstrate that this acute toxicity depends on cyanide release by cytochrome P450-2E1 (CYP2E1)-mediated metabolism, while this metabolism is not involved in the ototoxic effect. We hypothesized that pharmacological inhibition of CYP2E1 simultaneously to nitrile administration would provide a model for hair cell degeneration with reduced acute toxicity. We used mice of the 129S1/SvImJ (129S1) strain, developed by Jackson Labs as an adequate control for many transgenic lines, and of the RjOrl:SWISS / CD-1 (Swiss) strain, which is widely used. Female 129S1 mice were administered *cis*-crotononitrile (0, 1.75, 2.25, 2.75, or 3.25 mmol/kg, oral), with or without co-administration with the CY2E1 inhibitor diallylsulfide. Male Swiss mice were administered allylnitrile (0, 1.0, 1.125, 1.25, or 1.5 mmol/kg, oral), with or without co-administration with the CY2E1 inhibitor trans-dichloro-ethylene. Vestibular function was assessed by a behavioral test battery at 0 (pre-test) 2-3, 7, and 21 days after administration. Hair cell loss was assessed by scanning electron microscopy. Both allylnitrile and cis-crotononitrile caused a dose-dependent loss of vestibular function. Co-administration with CYP2E1 inhibitors reduced mortality and systemic toxicity. Hair bundle counts correlated with the loss of vestibular function as assessed by behavioral testing. In conclusion, nitrile exposure with simultaneous CYP2E1 inhibition offers a robust model for hair cell ablation in the mouse vestibule.

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WEDNESDAY 11 TH OF JULY

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The neurotrophin receptor TrkB as a target for tetanus toxin

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Tetanus toxin (TeTx) is a potent neurotoxin, lethal in a subnanomolar range, which acts on peripheral and central nervous system, causing spastic paralysis and often leading to death. TeTx is internalized at presynaptic membrane of motorneuron in neuromuscular junctions and it is retroaxonally transported to the spinal cord where blockage of neurotransmitter release occurs in inhibitory interneurons, resulting in characteristic motor spasms. The C-terminal domain of the heavy chain of the toxin (Hc-TeTx) is the responsible for nerve cell recognition, binding and internalization via endocytosis. The specific mechanism by which the toxin binds and enters in the nervous cell has not been elucidated yet, although the role of gangliosides as high-affinity receptors for TeTx has been described. However, current research results support a dual-receptor mechanism where proteins of the cell surface may be involved in toxin binding, thus explaining its extremely high affinity and specificity. Based on previous studies of our group, we propose the family of Trk receptors as possible targets for TeTx cell-surface binding. Molecular modeling analysis allows us to study how Hc-TeTx mimics the binding of neurotrophin-4/5 to the d5 ectodomain of TrkB receptor. We predict certain sequences in Hc-TeTx responsible for its interaction with TrkB. Directed mutagenesis in 3 amino acidic positions of Hc-TeTx was performed to check the *in silico* results. The mutant protein tested in cerebellar granule neurons model shows a decrease in the TrkB activation when compared with the caused by Hc-TeTx *wild-type*, thus indicating the probable implication of this position in the interaction, although it seems that would be more residues involved in these interaction. The determination of the TeTx receptor will be a major breakthrough for the discovery of a gateway leading from the peripheral to the central nervous system. This will have important implications in designing new drugs capable of crossing the blood-brain barrier.

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Efecte antihiperalgèsic del tramadol en el postoperatori immediat i tardà en el ratolí

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Antecedents i Objectiu: En ratolí, la cirurgia indueix canvis neuroplàstics que es manifesten amb dolor i hiperalgèsia en el postoperatori immediat. Després de recuperada la lesió, els canvis romanen latents >150 dies (sensibilització latent al dolor, SLD), i l'administració de naloxona (NX) produeix hipersensibilitat (Campillo, 2011). L'objectiu del estudi es determinar si el tramadol (TRM), protegeix de la hiperalgèsia i la SLD post-quirúrgiques.

Material i mètodes: Ratolins mascle CD1 sotmesos a una incisió plantar sota sevoflurà, van rebre durant la intervenció salí (INC+SS), o dosis creixents de TRM (INC+TRM) subcutani (s.c). S'avaluà la hiperalgèsia mecànica (von Frey) a les 4h i 1, 2, 20 i 21 dies del postoperatori. El dia 21, van rebre 1 mg/kg de NX s.c. abans de l'avaluació. Els resultats s'expressen en grams(g)±E.E.M, percentatges(%)±E.E.M d'efecte i àrees sobre la corba (4h-20d, ASC). La DE_{50} (mg/kg) mostra la potencia antihiperalgèsica del TRM.

Resultats: La incisió induí descensos significatius dels llindars nociceptius respecte el basal (1,11±0,03g) a las 4h, 1d i 2d ($p<0,001$); el dia 20 els valors s'havien restablert (1,13±0,01g), i la administració de NX el dia 21 va induir novament hiperalgèsia *tardana* en ratolins INC+SS (0.51±0.03g). A las 4h, el TRM va induir antihiperalgèsia dosi-depenent ($p<0.001$ vs. INC+SS) amb DE_{50} de 42,97±2,41 mg/kg, i a dosis de 75 i 100mg/kg, va reduir significativament la hiperalgèsia *tardana* ($p<0.01$ vs INC+SS). Les ASC mostren que dosis altes de TRM redueixen significativament la hiperalgèsia postoperatòria immediata al comparar amb el grup INC+SS ($p<0.01$)

Conclusions: El TRM redueix la hiperalgesia postoperatoria i podria prevenir parcialment la SLD en el nostre model

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mTOR activity is deregulated in Huntington's disease striatum

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Huntington's disease (HD) is a hereditary neurodegenerative disease caused by a CAG repeat expansion in the exon-1 of the huntingtin gene that gives rise to a mutant huntingtin (mhtt) protein. Striatal projection neurons are the most affected, but altered molecular mechanisms accounting for this degeneration have not been elucidated yet. mTOR is a serine/threonine kinase, which is an energetic imbalance sensor and forms the catalytic core of two complexes, the mTOR complex 1 (mTORC1) and 2 (mTORC2). To form these complexes mTOR binds to different regulatory accessory proteins, raptor or rictor, respectively. Since two mechanisms regulated by mTOR, autophagy and the Akt-survival pathway are altered in HD striatum, our aim was to analyze whether these changes resulted from altered mTOR activity. For that, we analyzed protein levels of total and different phospho-mTOR forms, mTOR substrates, and the two different mTOR partners in the striatum of R6/1 mouse model of HD at different stages of disease. In addition, we analyzed the intracellular localization of mTOR and phospho-mTOR by immunohistochemistry. mTOR protein levels were not altered at any of the ages analyzed. In contrast, phosphor (Ser2448) mTOR and phosphor (Ser2481) mTOR protein levels were increased from 12 weeks of age until the last stage analyzed suggesting increased mTOR activity. However, mTOR has been previously shown to co-localize with mhtt inclusions in the brain of N171-82Q mouse and of HD patients. Thus, we analyzed by immunohistochemistry whether this also happens for phospho-mTOR in R6/1 mouse striatum. Similar intracellular distribution was detected in wild-type and in R6/1 mice striatum, with no colocalization of phospho-mTOR with nuclear mhtt aggregates. To determine whether increased phospho-mTOR correlated with an increased mTORC1 kinase activity, we analyzed phosphor (Ser757) ULK1/2 as a mTORC1 substrate regulating autophagy. PhosphoUlk1/2 protein levels were not altered in R6/1 mouse striatum at any of the ages analyzed. Moreover rictor, but not raptor, protein levels were increased in the striatum of HD mice at all ages analyzed, and in the putamen of HD patients. Our results showing increased phosphorylated forms of mTOR in R6/1 mouse striatum from 12 weeks of age onwards, suggest an overactivation of the mTOR pathway. However, the study of mTORC1 and mTORC2 substrates revealed no changes in phosphor (Ser757) ULK1/2 protein levels whereas previous results from our group showed increased levels of phosphor (Ser473) Akt. Thus these results suggest a possible specific up-regulation of the mTORC2 activity in the R6/1 mouse striatum, which could be due, at least in part, to increased levels of rictor.

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P85

Histamine H3 receptor agonists decrease cocaine seeking

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Cocaine dependence has no pharmacological treatment. Relapse models in animals are used as a screen for new medications. Histamine H3 receptors are relatively abundant in brain respect to other tissues, and particularly in the nucleus accumbens and striatum. Previous studies suggest that H3 antagonists/inverse agonists could facilitate dopamine release, reinforcement and/or the subjective effects of psychostimulants. Here we show that histamine H3 receptor agonists decrease cocaine seeking in tests of extinction and reinstatement of cocaine self-administration. Rats were subjected to cocaine self-administration under a FR5 schedule, followed by a reinforcement dose-response, progressive ratio, extinction and reinstatement tests elicited by two doses of cocaine priming. Pretreatment with the standard histamine H3 agonist imetit decreased responding in extinction and reinstatement tests. No effects of H3 agonists were found under cocaine reinforcement, except for a delay in starting lever press. The histamine precursor L-histidine (which is transformed into histamine in brain) mimcked most of imetit effects. Locomotion tests performed in naive animals showed a small, but significant decrease of cocaine-induced hyperlocomotion in imetit and L-histidine treated animals. We conclude that histamine H3 receptor agonists could be interesting cadidates for clinical trials where relapse into cocaine abuse is determined. Furthermore, the aminoacid L-histidine could also have beneficial effects [2].

[2] Ortiz J, Self DW- Use of H3 histaminergic agonists for the treatment of addiction to drugs of abuse. Patent application WO2011/064274. Supported by Spanish government grants SAF2006-08240, SAF2009-12510 and Red de Trastornos Adictivos RD06/0001/0015. M.G.S. has received a spanish government FPI fellowship.

P86

Ethanol changes BDNF mRNA expression and BDNF alters dopamine synthesis in rat brain. Is BDNF a mediator of the effects of ethanol on dopaminergic neurons?

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Earlier findings suggest that, in addition to its well-known neurotrophic role, brain-derived neurotrophic factor (BDNF) is also involved in the rewarding and reinforcing effects of drugs of abuse. In the present data we show that acute ethanol administration modulates the expression of BDNF mRNA in several regions of the brain associated with the development of addiction to ethanol and other drugs of abuse. The BDNF mRNA expression was determined with real time-PCR and SYBR Green detection in the frontal cortex, nucleus accumbens, amygdala, hippocampus and ventral tegmental area of Wistar rats as well as alcohol preferring AA and alcohol avoiding ANA rats after the ethanol injection. Acute ethanol administration decreased BDNF mRNA levels in several brain regions of all three rat lines. Conversely, an increase was detected in the ventral tegmental area of Wistar and AA rats, but not in ANA rats. These data suggest regional differences in the interaction between ethanol and the BDNF system and a role for BDNF in the acute effects of ethanol. Keeping in mind the role of dopaminergic system in drug addiction and previous data showing that BDNF increases dopamine release in striatal slices, we decided to examine the effect of BDNF on dopamine release and synthesis in rat brain striatal miniprisms by means of HPLC and radioactive detection of dopamine formed from ^{3}H-tyrosine. Surprisingly, our most consistent finding was that BDNF decreases dopamine synthesis in striatal miniprisms. Furthermore, it appears that the effect is not mediated by TrkB, the primary receptor of BDNF, since the tyrosine phosphatase inhibitor K252a could not modify this effect. The effects of BDNF on dopamine synthesis should be further explored to understand the role of BDNF in the effects of ethanol on the dopaminergic system.

P87

Calcium channel subunit α2δ1 is regulated by BDNF. A new insight in BDNF synaptogenic function?

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The Brain-Derived Neurotrophic Factor (BDNF) is known among other functions for supporting the formation of new synapses in response to activity. Regarding synaptogenesis, recent studies have shown that Cacna2d1, the α2δ1 subunit of the L-type calcium channel, interacts with thrombospondin, activating a synaptogenic signaling complex which is needed to form the synapse. In this study we wanted to investigate if BDNF could regulate the synaptogenesis through regulating Cacna2d1.

We initially performed a low density custom made microarray using cortical tissue from E18 mice embryos injected with BDNF in the lateral ventricle at E14. We observed that among the family of voltage dependent channels, only Cacna2d1 was highly regulated by BDNF *in vivo*. To corroborate these results, we performed RT-PCR analysis in the same model, which confirmed the increase of Cacna2d1 expression induced by BDNF. To determine if Cacna2d1 was expressed by neurons or glia we treated cultured cortical neurons or glial cells with BDNF at 100 ng/mL. After 6 hours of treatment, RT-PCR analysis identified the increase of Cacna2d1 in neurons, but not in glial cells.

To identify through which pathway BDNF regulates Cacna2d1, we treated cortical neurons with different inhibitors of different points of the TrkB pathway, such as K252a, which blocks TrkB receptors; Wortmannin, a specific, covalent inhibitor of PI3k; and UO126, a MAPK pathway inhibitor. The results obtained demonstrated that BDNF-dependent Cacna2d1 induction is mediated by direct activation of TrkB and MAPK signaling.

Once confirmed that BDNF induces Cacna2d1, ongoing studies are analyzing if Cacna2d1 is the main mediator of BDNF-induced synaptogenesis. We have developed both *in vitro* and *in vivo* models in which we analyze if the synaptogenesis caused by BDNF can be blocked by Gabapentin and Vigabratin, two antiepileptic drugs that inhibit Cacna2d1.

P88

Evidence of dopaminergic and histaminergic regulation of cell viability via D_1-H_3 heteromers in striatal tissue

Moreno-Delgado D, Moreno E, Mayol J, Casadó V, Cortés A, Lluís C, McCornmick PJ

Neuronal signalling constitutes a highly tangled machinery which includes not only up/downstream signalling but also horizontal signalling through protein-protein interactions at the plasma membrane level. In this sense, the ability of G protein coupled receptors (GPCRs) to interact between them, forming homo or heteromers, represents a new molecular entity entirely different from the monomers. One example of such an interaction is the D_1R-H_3R heteromer. Dopamine D_1 receptors (D_1Rs) and Histamine H_3 receptors (H_3R) are expressed throughout the striatonigral MSNs, the most populated neuronal type in the striatum. We have previously demonstrated that D_1R and H_3R form functional heteromers not only in cell cultures but also in the MSNs. An important feature of the D1R-H3R heteromer is that H3R agonists increase the agonist affinity for D1R and activates ERK 1/2 signalling in a receptor heteromer context, an effect that is blocked not only by H3R antagonist but also by D_1R antagonist in a cross-antagonism phenomenon. Altered dopaminergic signalling has been proposed as a contributing factor in striatal cell death observed in Huntington's disease. Here, we analyze the role of D_1R - H_3R in dopamine-mediated cell death. Using rat striatal organotypic cultures we have observed that SKF81297 a D_1R agonist increased cell death, but not imetit a H_3R agonist. However, imetit reverted SKF81297 induced cell death, suggesting a role of D_1R - H_3R in D_1R mediated cell death. In the same sense, thioperamide a H_3R antagonist also reverted SKF81297 mediated cell death. Moreover, we have recently described that cocaine can break D_1R -H3R heteromer functionality. Thus, we tested the effect of cocaine in striatal organotypic cultures. Cocaine did not increase cell death by itself but completely blocked the effect of the H_3R agonist and antagonist on D_1R-mediated cell death. These results suggest that histaminergic neurotransmission and H_3R may play an important role in impairing striatal specific cell death by forming heteromers with D_1R. In addition, the cocaine effect on D_1R - H_3R may partly explain the activation of a cell death mechanism described in long-term cocaine addicts.

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Is aripiprazole a partial agonist on brain dopamine D2 receptors?

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Aripiprazole is a new, effective, atypical antipsychotic with less side-effect which has very high affinity to $D_{2/3}$, 5-$HT_{1/2A}$ receptors, and it also has high affinity to D_4, 5-HT_{2C} and 5-HT_7 receptors. Due to the broad spectrum of receptors targeted by aripiprazole, there are many papers covering almost every aspect of it. And among them, the most controversial is the action of aripiprazole on the dopamine D_2 receptors. Aripiprazole is considered a partial agonist on D_2 receptors, however, its intrinsic efficacy as an agonist depends on where it is tested. The current work examines the effect of aripiprazole on D_2 receptors through directly measuring dopamine synthesis in rat brain striatal miniprisms incubated *in vitro*. The result we got is intriguing: aripiprazole neither acted as an antagonist nor a partial agonist, but as a full agonist, as compared to quinpirole. Sulpiride completely blocked the effect of 10nM aripiprazole indicating that it is an agonist of D_2 receptor at this concentration. But we also found that aripiprazole at 10nM has fully activated D_2 receptors. Further effects of higher concentrations of aripiprazole on dopamine synthesis should not be mediated only through D_2 receptors, and there must be some collaboration of D_2 receptor with others. The conclusion here is consistent with the hypothesis that aripiprazole is a functionally selective D_2 receptor ligand rather than a simple partial agonist.The opinion that aripiprazole's antipsychotic effect is due to its partial agonist effect of D_2 receptor may need more studies.

P90

The new multi-target directed ligand ASS234 reduces Aβ fibrillogenesis and protects neuroblastoma cells from Aβ-induced toxicity

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The cholinergic hypothesis of Alzheimer's disease (AD) suggests that a selective loss of cholinergic neurons results in a deficit of cholinergic transmission at specific brain regions that mediate learning and memory functions. However, alterations in other neurotransmitter systems, especially serotoninergic and dopaminergic, are also thought to be responsible for the behavioural disturbances observed in AD patients. Moreover, other aspects such as Aβ peptide aggregation and deposition and oxidative stress have also been reported to play a central role in the development of this neurodegenerative disorder. Thus, AD can be defined as a multifactorial disorder that might be more effectively addressed by using drugs able to bind to different type of targets. In this context our aim was to design and synthesize a novel hybrid molecule with a multipotent profile against cholinesterases (ChE) and monoamine oxidases (MAO) being also able to inhibit Aβ peptide aggregation.

The newly synthesized molecule, ASS234, showed a potent inhibitory profile able to inhibit both MAO and ChE enzymes (nM range). ASS234 was also able to prevent the $A\beta_{42}$ self-aggregation (47.8 ± 2.1%) and the AChE-dependent $A\beta_{40}$ aggregation (32.4 ± 7.0%). In addition, ASS234 significantly reduced the Aβ-induced apoptotic death in neuroblastoma cells by preventing caspase-9 and caspase-3 activation as well as blocking the cleavage of PARP. These results indicate that the mitochondrial pathway of apoptosis is involved in the anti-apoptotic action of this compound. Besides the anti-apoptotic properties, ASS234 was also able to restore the Aβ-induced depletion of SOD-1 and Catalase expression and thus showing an anti-oxidant effect. Finally, ASS234 prevented the formation of the toxic oligomeric species of Aβ, suggesting that this may be the mechanism by which it confers neuroprotection in neuroblastoma cells. All together these results demonstrate that ASS234 is a promising multi-target drug candidate with potential to modify the natural course of the disease.

P91

Dual action of dopamine on striatal tyrosine hydroxylase activity

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Dopaminergic neurons play an important role in Parkinson's disease, psychosis, and addiction. Dopamine biosynthesis is rate-limited by tyrosine hydroxylase (TH) activity, which can be modified by phosphorylation and/or by dopamine in a feedback inhibition process. In vitro studies have shown that tyrosine hydroxylase has two binding sites for dopamine inhibitory effects: a high affinity site (additionally regulated by phosphorylation) and a low affinity site. When studying dopamine biosynthesis in rat brain striatal miniprisms we observed that: a) tyrosine hydroxylase was strongly inhibited by newly formed dopamine, b) increased concentrations of extracellular or intracellular dopamine inhibited dopamine synthesis in a dose-dependent manner to an almost complete stop, c) tyrosine hydroxylase activation by phosphorylation appeared to be reduced at high dopamine concentrations, and d) as expected, the high affinity site seemed to be regulated by dopamine and also by phosphorylation through changes in the affinity for the tetrahydrobiopterin cofactor. These results suggest a dual action of dopamine at high- and low affinity sites, depending on the actual concentrations of dopamine. Thus, at low concentrations activation of TH by phosphorylation would relieve dopamine from the high-affinity site facilitating cofactor binding but this equilibrium would be altered by high dopamine concentrations. An interesting corollary is that physiological concentrations of endogenous dopamine can inhibit tyrosine hydroxylase independently of its phosphorylation state.

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Antioxidant effect of LMN diet enriched in polyphenols and polyunsaturated fatty acids, vía Nrf2 pathway in SH-SY5Y neuroblastome cell line

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Introduction: Oxidative stress plays an important role in Alzheimer's disease and other neurodegenerative disorders. LMN diet, previously reported by our group as inducer of neurogenesis in adult mice, also improves results in Morris Water Maze test in Tg2576 and Wt 18-month's old mice. The aims of this work are to elucidate the neuroprotect effect of LMN in the oxidative stress induced in human neuroblastome cells SH-SY5Y cells by hydrogen peroxide and to determine the molecular pathway involved.

Materials and Methods: SH-SY5Y cells treated with H_20_2 were used to explore the antioxidant effect of LMN. Cytotoxicity of H_20_2 was studied by measuring methylthiazol tetrazolium (MTT) and mitochondrial membrane potential by JC-1, a membrane-permeant dye. The fluorescent probe 2', 7'-dichlorofluorescein acetoxymethyl ester (DCF-AM) was used to measure the production of reactive species (ROS). Dihydroethidine (DHE) was used to detect intracellular superoxide and the expression of antioxidant enzymes was detected by western blot.

Results: LMN dose-dependently restored H_2O_2-induced ROS intracellular levels as well as the elevation of intracellular superoxide level. Furthermore, pre-treatment with LMN markedly attenuated H_2O_2-induced cell viability loss as well as the loss of mitochondrial membrane potential.
The mechanisms by which LMN protected neuroblastome cell line from oxidative stress included the induction of antioxidant enzymes, Cu-Zn-superoxide dismutase (SOD1) and gluthatione peroxidase (GPx) and the upregulation of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2).

Conclusions: These results confirm the neuroprotective role of LMN, describing its antioxidant capacity and suggesting the route Nrf2-ARE as implied in the mechanism of action.

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P93

A quantitative metabolomics study of the oxygen availability impact on recombinant *Pichia pastoris* central carbon metabolism

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Global quantitative metabolome analysis provides a unique platform to characterize in a systematic way the impact of environmental and genetic perturbations on the cell's metabolism, also enabling to dissect the hierarchical regulation of metabolic flux distributions into contributions by the different "omic" levels.

In previous studies, we identified a beneficial effect of low oxygen availability on recombinant protein production in *Pichia pastoris* [1]. In the present study, a quantitative metabolome analysis on a recombinant *P. pastoris* strain growing in glucose-limited chemostat cultures under different oxygen availability conditions has been performed, in combination with metabolic flux analysis. Furthermore, a thermodynamic pathway analysis was carried out on the acquired metabolome data with the aim to validate it, as well as to further extract relevant mechanistic relationships of the central carbon metabolism network. This lead to propose an alternative configuration of the pentose phosphate pathway allowing a better consistency of the calculated metabolic flux distribution and the thermodynamic constraints derived from the metabolite levels.

In addition, integration of transcriptional data from previous cultivation series performed under analogous conditions [2] allowed us to quantify the different contribution of each "omic" level to the regulation of carbon flux through the glycolysis, which, in contrast to *S. cerevisae*, showed an important transcriptional regulation of this pathway upon adaptation to hypoxia.

Overall, the results obtained by integrating different quantitative metabolomics analyses combined with transcriptional and metabolic flux analyses provide a metabolic fingerprint of the impact of oxygen availability and recombinant protein secretion, thus improving the knowledge base for metabolic engineering of *P. pastoris*.

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P94

PDE4 inhibition in EAE mice: cAMP-PDEs and inflammatory cytokines mRNA expression analyses in spinal cord

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Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis that courses with neuroinflammation, axonal damage and demyelination, characterized by T- and B-cell responses to myelin oligodendrocyte glycoprotein which produce a wide range of pro- and anti-inflammatory cytokines. PDE4B gene has been related to the inflammatory immune response in mice. We have previously shown that in the brain and spinal cord of EAE rats and mice there was a dramatic increase in the mRNA expression levels of the PDE4B2 mRNA splicing variant in infiltrating cells.

We analyzed the expression of mRNA coding for PDE4A, PDE4B, PDE4D, PDE7A, PDE7B, pro-inflammatory and anti-inflammatory cytokines and several cellular markers by semiquantitative real-time PCR in spinal cord from EAE mice and compared it with animals treated with a "classic" PDE4 inhibitor, roflumilast along the different stages of the disease. Animals were treated i.p. daily with 10 mg/kg roflumilast from day 5 after the onset of the disease, and sacrificed at 10, 17, 21 and 40 days p.i. Mice chronically treated with roflumilast showed a slight lessening of clinical score compared to control and vehicle treated animals. We observed that roflumilast treatment maintained elevated expression levels of PDE4 and PDE7 mRNAs analyzed along treatment, especially at day 40. TNFalpha, COX-2, TGFbeta, IL-6 and IL-1beta mRNA levels were high at day 40 after roflumilast treatment. Alterations on expression of Tbx21 and IFNgamma (proinflammatory Th1 cells); Rorc and IL-17 (proinflammatory Th17 cells); FoxP3 and IL-10 (Treg) and Gata3 and IL-4 (anti-inflammatory Th2 response) were observed at day 40 for Th1 and Treg response. We will discuss on the relevance of PDE4 isozymes as therapeutic targets for the treatment of some neuroinflammatory diseases.

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Neuroinflammatory disease, cAMP-PDE4 inhibition, EAE, cytokine mRNA expression

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DNA-binding proteins analysed by SAXS.

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Small Angle X-ray Scattering (SAXS) of macromolecules in solution is a technique that allows low-resolution structural analysis of proteins and their complexes, including protein-DNA complexes. The number of SAXS studies on macromolecular particles has increased significantly in the last years, due to improvement of algorithms and beamtime availability in synchrotron facilities. One advantage of this technique is that is no limited by factors like particle size or flexibility; on the contrary, it is possible to assess these or other features, like the coexistence of particles of different sizes in the same preparation. The experimental SAXS curve allows to fit against it a theoretical curve calculated using a crystallographic atomic model or an electron microscopy shape, which might be modified to optimise the curve fitting. Fitting optimisation can be attempted by exploring the conformational space of the particle on the basis of the available model, using techniques like simple manual displacement of domains or more sophisticated ones like normal mode analysis, and select those models that altogether yield a curve that fits best; such a methodology can be useful in determining the degree of flexibility of domains or interdomain segments. Macromolecular complexes are reconstructed using static three-dimensional models of the complexes or by docking the individual components; by playing with the proportion of non-interacting *vs* interacting partners, is possible to establish the dynamics of the complex. Furthermore, by introducing the conformational-space analysis algorithms within macromolecular complexes is possible to explore the conformational changes induced by ligands, substrates or interacting partners.

We are going to present a SAXS analysis of three phylogenetically-related proteins whose structure is based on homology models, and in one case involving a protein/DNA complex. The strategies applied will be analysed and the results discussed.

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Regulation of striatal-enriched protein tyrosine phosphatase (STEP) levels by BDNF *in vivo* and *in vitro*

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Striatal-enriched protein tyrosine phosphatase (STEP), a brain-specific phosphatase, has an important role in synaptic plasticity and neuronal function through the modulation of key signaling molecules (ERK1/2, p38, Fyn, *N*-methyl-D-aspartate and AMPA receptors). Here we analyzed STEP protein expression from postnatal day (P) 3 until 12 weeks of age in mouse striatum, cortex and hippocampus. In the striatum and cortex STEP expression increased substantially during the first 1 to 3 weeks of life, while in the hippocampus STEP expression peaked at P7 and remained stable until the adult age. Likewise, brain-derived neurotrophic factor (BDNF) expression also increases significantly during the first postnatal weeks, which lead us to investigate whether BDNF might regulate STEP expression. STEP protein levels were significantly reduced (40-60%) in the striatum and cortex of BDNF knockout mice at P7 and P14. In contrast, hippocampal STEP levels were not altered in BDNF-deficient animals at either P7 or P14. Analysis by real-time PCR at P7 revealed a significant reduction of STEP mRNA levels only in the striatum of BDNF knockout mice compared with their littermate controls. These findings indicate that BDNF regulates STEP levels through different mechanisms in distinct brain regions during postnatal development. Interestingly, we found that BDNF decreased STEP levels in cortical primary cultures. The mechanisms implicated in this effect are now being investigated, but calpain activation is likely to be involved. Given that STEP regulates several molecules with significant roles in synaptic plasticity, our result suggests that at least some effects of BDNF signaling might involve a reduction in STEP levels/activity.

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Characterization of brain-specific PDK1 conditional knock-in mice expressing the L155E mutation in the nervous system

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The phosphoinositide 3-kinase signaling pathway plays important roles in the central nervous system by mediating the effects of neurotrophic factors, neurotransmitters and hormones promoting neuronal survival, neuritogenesis and plasticity.

PDK1 is a crucial master kinase that, in response to PI 3-kinase activation, switches on a number of AGC-kinase family members including PKB/Akt. Activation of PKB by PDK1 relies on the interaction of the PH domains present on both kinases with PtdIns(3,4,5)P_3, the PI 3-kinase product. By contrast, in order to activate the rest of targets including S6K, RSK and SGK, PDK1 interacts with a phosphorylated hydrophobic motif on those substrates through the PIF-pocket docking site.

To dissect the contribution of the different PDK1 targets to PI 3-kinase actions, two knock-in mice expressing two specific single-aminoacid mutations of PDK1 were previously generated. The PDK1 K465E impairing the binding of the PH domain to PtdIns(3,4,5)P_3, and the PDK1 L155E that disrupts the PIF-pocket.

Since the PDK1 L155E mutation caused embryonic lethality, and to define in vivo the function of the PDK1 PIF-pocket dependent substrates in the central nervous system, we employed conditional knock-in strategies to direct the expression of the L155E mutant to neuronal tissues. Activation of PKB by BDNF reached normal levels in the PDK1 L155E primary cortical neurons, while activation of S6K and RSK was totally abolished. Phosphorylation of NDRG1, a specific SGK1 substrate, was only marginally affected. As a consequence, BDNF-mediated neuronal survival decreased, and apoptosis induced by serum withdrawal increased, in the PDK1 mutant cortical cultures when compared with control littermates. By contrast, in mice expressing the complementary PDK1 K465E mutation, neuronal viability was not compromised, thereby suggesting a prominent role of the PIF-pocket branch of the PDK1 pathway in controlling neuronal survival.

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P98

UTP promotes schwann cell wound repair through P2Y activation

Lamarca A, Gella A, Martiañez T, Segura M and Casals, N .

In peripheral nerve injury, Schwann cells undergo in a profound phenotypic modulation, adopting a migratory phenotype and remodeling the extracellular matrix making it permissive to axonal regrowth. It is known, that after nerve injury, UTP and other nucleotides activate purinergic receptors expressed on the Schwann cell surface, but the role of UTP and the molecular mechanisms involved in the peripheral glia repair remains unclear. The aim of this study was to determine if P2Y activation by UTP stimulates Schwann cell migration and to elucidate the intracellular signaling pathways involved in wound repair. Here, we report a critical role for UTP in Schwann cell migration and the involvement of purinergic signaling in this repair mechanism. This migratory effect was inhibited using specific kinase inhibitors suggesting an important role of mitogen-activated protein kinases (MAPKs) in this migratory mechanism. Moreover a prominent biphasic phosphorylation of MAPKs (early and late activation) including c-Jun N-terminal protein kinase (JNK), ERK1/2 and p38 was observed after UTP treatment. Finally, P2Y blockade with suramin (a P2Y inhibitor) and using a shRNA against P2Y2 leads to a drastically decreased Schwann cell migration. We conclude that UTP released after nerve injury activates Schwann P2Y receptors which consequently stimulates the MAPKs pathway that contributes to cell migration and wound repair. These results corroborate the hypothesis that UTP mediates the immediate response to injury and we propose a novel role for P2Y receptors on Schwann cells by supporting peripheral nerve recovery following injury.

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Keywords: Peripheral nerve, P2Y, UTP, Schwann cells, wound closure, MAPK

P99

Unraveling the kinetics of aggregation of single peptide-DNA complexes using force spectroscopy

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The knowledge of the mechanisms of interaction between hydrophobic molecules and essential cellular components is key to our understanding of many aggregation processes underlying several human diseases. Kahalalide F (KF) is an hydrophobic marine-derived peptide with a strong anticancer activity which contains a positively charged residue (L-Orn). KF is an ideal model to elucidate the mechanisms by which self-aggregation competes with binding to a strongly charged polyelectrolite such as DNA. Here we carry out mechanical stretching and unzipping experiments of single DNA molecules (in double and single stranded form) complexed with KF using optical tweezers. We show that KF and DNA interact forming large aggregate complexes promoted by the recruitment and wrapping of DNA around the aggregate which are further stabilized by hydrophobic interactions within the KF-DNA complex. These experiments reveal unique features of the aggregation process, and the proposed methodology might be useful to quantitatively characterize other compounds or proteins in which the formation of aggregates is of relevance.

P100

Functional characterization of cancer-related genes during planarian regeneration

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The great regenerative capacity of the freshwater planarian *Schmidtea mediterranea* makes it a valuable model for the study of the molecular basis of regeneration. Its extraordinary regeneration capacity relies on the presence of neoblasts, the only proliferative cell type of the animal, that can differentiate into any cell lineage of the organism.

Previous to this study, comparative RNA-Seq (DGE) was performed between neoblasts and differentiated cells isolated by Fluorescence-Activated Cell Sorting (FACS). Among the multiple genes that were overexpressed in neoblasts, we could detect an important number of cancer-related genes. In other species, it is known that mutation in genes involved in processes such as migration, differentiation, proliferation or apoptosis are usually linked to the development of tumors. Moreover, some of this genes are also involved in stem cell biology. Although planarians seldom develop tumors, this study reveals that cancer-related genes are a key element for a better understanding of the regenerative process.

The main objective of this work is to characterize the function of some cancer-related genes that are overexpressed in neoblasts, by means of expression patterning description and the analysis of the phenotypes obtained after RNAi knock-down.

Our results suggest that some of these cancer-related genes, apart from being overexpressed in the neoblast population, are important for both regeneration and homeostasis in *S. mediterranea* such as the homologues of Tumor Suppressor Gene 101 (TSG101), several retinoblastoma-binding proteins, NFY and hnRNP. Thus, the phenotypes obtained range from partial to total blocking of regeneration and also the apparition of necrotic spots through the body in intact non-regenerating animals, that finally leads to its disintegration after some days.

To sum up, the results obtained so far suggest that some cancer-related genes have an important function in neoblast regulation, both during regeneration and homeostasis. However, further analysis are necessary in order to better characterize the function of these genes and compare it with its role in other models.

P101

Oikopleura dioica as a Model Animal in Evo-Devo for studying Genetic Loss

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Comparative genomics has revealed extensive events of gene loss in many lineages, suggesting that these events may have been fundamental forces driving Evolution. The recent sequencing of the genome of *Oikopleura dioica* has become a revolution in our understanding of the limits to which a metazoan genome can evolve, revealing a huge genomic plasticity with high degree of architectural restructuring, low conservation of the intronic positions, extreme miniaturization of the genome, and extraordinarily high number of gene losses. Our group is developing a research project in *Oikopleura dioica* as an animal model to study the impact of gene loss in the Evolution of the mechanisms of Development (Evo-Devo). The *Oikopleura* is a free-living planktonic urochordate that has a key phylogenetic position as sister group of the vertebrates. A key feature of *Oikopleura* is that it maintains a typical chordate body plan throughout life, in contrast to the rest of urochordates, which suffer a drastic metamorphosis, losing the notochord, restructuring the central nervous system and becoming sedentary. *Oikopleura* is a cosmopolitan animal available in most oceans of the world, which despite their fragility can be cultured in the laboratory. In our laboratory, we have developed one of three *Oikopleura* culturing systems of the world, and we are now able to keep *Oikopleura* populations, and to generate hundreds of sexually mature animals and thousands of embryos per week. We are currently in preliminary stages for establishing reliably microinjection protocols to make genetic manipulation and generate *knockdown* animals.

P102

Dynamics of mammalian meiosis in distantly mammalian species

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Meiotic recombination is an important biological process that provides physical connections between homologues during the first meiotic division, contributing to correct chromosomal segregation. This exchange of genetic material increases genetic diversity and introduces inheritable new allelic variants that can become fixed with a probability that depends on various population parameters (i.e., frequency, effective population size, among others), contributing, in the long term, to the formation of new species.

The aim of this study is to analyze the dynamics of recombination among mammals by characterizing the distribution of crossover events through the immunolocalization of MLH1, a recombination protein. In doing so, we analyzed cells at pachytema stage in different species across the mammalian tree, such as Afrotheria (*Elephantulus edwardii*), Rodentia (*Rattus norvegicus*), Primates (*Lemur catta, Saguinus oedipus, Cebus libidinosus, Cebus nigritus* and *Aluatta caraya*) and Carnivora (*Panthera tigris).*

The results revealed a high variability in the number of crossover events per species, ranging from 27.19±3.36 MLH1 foci per cell in afrotherian species to 59.41±5.92 in the tiger. The analysis of different individuals belonging to the same species and individuals from related species (i.e. among primates) did not show significant differences in the recombination rate. However, the differences observed between orders were statistically significant (Kruskal-Wallis, $p<0.0001$), showing a negative correlation with the divergence rate ($p<0.0001$). Our results suggest the role of an evolutionary factor that is affecting the variation in the recombination rate among mammals.

P103

NEDD4-1 regulation of the Pro-Apoptotic protein RTP801 in *in vitro* models of parkinson's disease

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RTP801 is elevated in cell and animal models of Parkinson's disease (PD) and in affected neurons of PD patients. RTP801 over expression is sufficient to promote neuron death in cellular models of PD by a mechanism involving repression of mTOR and Akt kinase activity. NEDD4-1 belongs to a HECT family of E3 ligases that mediates the turnover of a number of membrane- and non membrane- associated proteins. Moreover, it has been found that NEDD4-1 is one of the most abundant E3 ligases in mammalian neurons and plays a key role in aspects of neuronal development in neurodegenerative diseases.

Here, we report that RTP801 is poly-ubiquitinated by NEDD4-1 and targeted to proteasome degradation. In NGF-differentiated PC12 cells we observed that PD toxins 6-OHDA and MPP+ decreased NEDD4-1 protein, levels along with an induction of RTP801. Furthermore, ectopic expression of NEDD4-1 protected NGF-differentiated cells from 6-OHDA. We also proved in a cell free *in vitro* assay that NEDD4 efficiently poly-ubiquitinated recombinant RTP801. In line with these results, in NGF-differentiated PC12 cells, ectopic NEDD4-1 diminished significantly the levels of over expressed RTP801 by promoting its degradation.

Collectively, our data suggest that NEDD4-1 can be deregulated in cellular models of PD, contributing to lethal accumulation of RTP801.

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P104

Serotonin transporter knockdown by siRNA induces fast adult neurogenesis and antidepressant like effects

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Identifying the factors contributing to the etiology of anxiety and depression is critical for the development of more efficacious therapies. Serotonin (5-HT) is linked to both disorders. Current antidepressants, which block the serotonin transporter (SERT), show limited efficacy and slow onset of action. Here, we used a small interference RNA (siRNA) to examine the biological consequences of reducing SERT expression. Adult mice were locally infused with vehicle, nonsense-siRNA and SERT-siRNA into dorsal raphe nucleus (DR). The functional effects of SERT-siRNA knockdown were compared with those following repeated fluoxetine treatment. Local SERT-siRNA infusion (4-days) suppressed DR SERT expression (40%). This was accompanied by a selective and widespread reduction of SERT-binding sites throughout the brain. Moreover, a 4-day regimen with SERT-siRNA modified brain mouse variables considered to be key markers of antidepressant action: *a)* reduced expression and sensitivity of 5-HT_{1A}-autoreceptors, *b)* augmented 5-HT extracellular in DR-projecting areas, *c)* increased hippocampal neurogenesis and d) increased plasticity-associated gene expression (BDNF, VEGF and ARC). In contrast, fluoxetine (4-days) did not alter any of these variables and only started to modify them after 15-day treatments. These findings highlight the critical role of SERT in the control of serotonergic function, including serotonin-mediated neural plasticity. They also support the use of siRNA targeting serotonergic genes (SERT, 5-HT_{1A}-autoreceptor) as a new generation of antidepressant therapies with a potential greater efficacy faster onset of action than current treatments.

P105

Lack of Helios causes long-term outcome cognitive deficits and impairs hippocampal plasticity

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We have recently reported that Helios is expressed in neural tissues; but its role in neurobehavioral function has yet to be elucidated. Here we perform an extended neurobehavioral characterization from neonatal to young stages of Helios knock-out (He-/-) mice. No gross behavioral or neurological alterations were detected in neonatal He-/- mice. However, we found that 5 to 7-week old He-/- mice displayed clear deficits in cognitive abilities. Firstly, He-/- mice displayed difficulties in acquiring new motor skills as measured by the rotarod and balance beam tests, although they displayed regular behavior after repeated training. These impairments in motor-skill learning could be due to striatal projection neuron dysfunction since Helios is highly expressed in these cells. Secondly, He-/- mice displayed a strong deficit in spatial learning as measured by spontaneous alternation in the T-maze task. Further behavioral assessment in the T-maze revealed that He-/- mice display response strategies when place strategies are required. This indicates an imbalance between striatum and hippocampus, pointing out the most severe alterations in the striatum. Interestingly, Helios is also expressed at high levels in a sub-population of CA1-pyramidal neurons. Electrophysiological recordings in hippocampal slices from He-/- mice corroborated the latter results by showing LTP impairment of synaptic transmission. In conclusion, Helios is essential for the development of brain structures involved in learning behavior, with Helios deletion causing a long-term detrimental effect in hippocampal and striatal function.

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P106

Effect of ROCK inhibiton on human embryonic stem cells when passaging from feeder to feeder-free conditions

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Human embryonic stem cells (hESC) hold great promise for treating diseases, understanding human development and for drug discovery. Different methods exist to generate neural cells from hESC requiring adaptation to feeder-free conditions. However, low proliferation- and attachment rates limit their availability after adapting to extracellular matrices. The Rho associated coiled protein kinase (ROCK) dependent signaling pathway plays important roles in physiological functions such as cell proliferation, adhesion or migration and its inhibition increases survival and attachment of hESCs after cryopreservation and single-cell passaging. In the present work we evaluated the influence of the ROCK inhibitor Y-27632 on stemness, proliferation and survival in 3 karyotypically normal hESC lines (HS181, HS293 and HS420) after clump passaging from human feeders to Matrigel in mTeSR1-medium. When hESCs were treated with 10 mM Y-27632, we observed a significantly increased number of attached cells without altering the presence of stemness or proliferation markers. However, Phalloidin staining revealed drastic changes in morphology of hESC treated with Y-27632. Because excessive attachment could affect cell behavior, we analyzed its effect on cell death, accumulated number of hESCs and marker expression at different days after seeding. Y-27632 treatment increased cell death 24 hours after seeding which lead to a decrease in cell-number 5 days later. These results question the use of the ROCK-Inhibitor Y-27632 in hESC culture when adapting to feeder-free cultures. Further studies are in process to understand how this alteration in morphology might change gene-expression patterns or neural differentiation propensities.

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P107

Effect of Dyrk1a dose-reduction on self-renewal and differentiation potentials of cortical embryonic progenitors.

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DYRK1A belongs to the DYRK family of protein kinase with roles related to growth and differentiation. *DYRK1A* is a dosage-sensitive gene involved in neurodevelopment with putative roles in corticogenesis. In this study we show that cortical progenitors of *Dyrk1a*$^{+/-}$ haploinsufficient mice have reduced capability to self-renew and to differentiate into oligodendroglial cells *in vitro.* We also show that the epigenetic state of some glial genes is altered at specific promoter positions in the cortex of *Dyrk1a*$^{+/-}$ embryos before the onset of gliogenesis. The epigenetic changes are in accordance with the *in vitro* results and suggest that *Dyrk1a*$^{+/-}$ neural progenitors may have an increased astrogliogenic and a decreased oligodendrogenic potential *in vivo.* Consistently, the number of oligodendroglial cells is reduced in the postnatal cortex of *Dyrk1a*$^{+/-}$ mutants whereas the number of Gfap^{+} astrocytes is increased in the adult brain. These results indicate that normal levels of Dyrk1a are needed to maintain the differentiation potential of embryonic cortical progenitors. The new role of Dyrk1a in glial development suggested here might have implications in human diseases involving changes in *DYRK1A* gene-dosage.

P108

Dyrk1a overexpression alters the development of specific type of cortical interneurons

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DYRK1A is a protein kinase involved in several neurodevelopmental functions and in adult brain physiology. Human DYRK1A is encoded by dosage-sensitive gene located within the Down Syndrome (DS) critical region. Triplication of this gene likely contributes to DS mental retardation (MR) because transgenic mice with moderate overexpression of *DYRK1A* present defects in learning and memory. Although the pathological bases underlying MR in DS are poorly understood, it is thought that alterations in the excitatory/inhibitory balance might contribute to DS cognitive deficits. The increased number of GABAergic interneurons recently observed in the cortex of Ts65Dn mouse, a trisomic DS model, strongly supports this notion.

In the present work we have used the BACtg*Dyrk1a* mouse (a model that bears 3 copies of *Dyrk1a*) to assess whether a 50% increase in *Dyrk1a* affects the numbers of cortical interneurons. Sterological quantifications performed in the somatosensory cotex (SSC) of adult mice showed a decreased neuronal density in BACtg*Dyrk1a* mouse. Transgenic adult mice also show increase density of somatostatin+ (SST) cells and decrease density of parvalbumin+ (PV) cells in the SSC. However, no changes in calretinin+ (CR) cell density were observed. The same alterations in SST+ and PV+ populations were observed in transgenic mice at postnatal (P) day 14, when the generation and migration of cortical interneurons has already ended. Importantly, both SST+ and PV+ interneurons are generated in the same region, the medial ganglionic eminence (MGE), while CR+ interneurons are generated in the caudal ganglionic eminence. Therefore, a defect in cell fate acquisition of interneurons generated in the MGE may explain the observed alterations. Because the Ts65Dn mice only show alterations in these two types of interneurons our data indicates that triplication of *Dyrk1a* likely contributes to the altered proportions of GABAergic interneurons in the Ts65Dn model.

P109

Human fetal neural stem cells: are there differences depending on the developmental age and region of origin?

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Human fetal neural stem cells (hfNSC) hold great potential for cell therapy strategies for neurodegenerative diseases. However, little is known about the characteristics of these cells obtained from different origins and developmental stages. In this work we characterized hfNSCs from cortex, whole ganglionic eminence (WGE), cerebellum and forebrain at several developmental stages (6-12 weeks post conception)

In order to explore the sensitivity to the dissociation process of primary tissue obtained at different developmental stages we analyzed cell death. Tissue obtained from younger fetuses was less affected by the trypsinization process than older samples. Cells obtained from younger stages also presented higher ratios of proliferation than older ones following the first passage. These results suggested the presence of more differentiated cells in samples obtained from older fetuses that might be more sensitive to dissociation. In agreement, gene expression analysis showed higher levels of mature markers in samples obtained at 12 weeks compared to younger ones.

Next we analyzed the expansion potential of hfNSC over 6 passages. A common feature was observed for all the cultures independently of the brain area and the developmental stage; cultures went through three initial critical passages characterized by high percentages of cell death and substantial variability in the ratios of proliferation. Following this phase the cultures stabilized, with reduced cell death and maintained a constant expansion potential. Forebrain hfNSC were the most readily expandable and cerebellar cells the ones that proliferated least. In conclusion, our results indicate the existence of differences in the expansion potential and gene expression between primary cells obtained at different developmental stages from different brain regions.

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P110

3D electrospun Polylactic acid nanofibers induce radial glia like cells and neurons migrating phenotypes.

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To develop tissue engineering strategies useful for repairing damage in the central nervous system it is essential to control and optimize neural cells growth and interactions with functional scaffolds. The aim of this study is to develop an artificial scaffold of polylactic acid (PLA) nanofibers to induce an environment that mimic embryonic radial glia organization and favors neuronal migration after a brain injury. For this purpose uncoated 3D electrospun PLA nanofibers were used to study the behavior of neural cells from mice cerebral cortices *in vitro*. Both, random and aligned fibers supported neural cells growth, but only aligned fibers permit neural cells invasion. Moreover, aligned fibers induce immature phenotypes in neuronal and glial cell cultures. Glial cells grown in aligned fibers showed bipolar shape and expressed the radial glia markers Nestin and BLbP, and the progenitor marker Pax6. On the other hand, neurons grown in aligned fibers were characterized by a decrease in the expression of β-III Tubullin, and an increase of neuron restricted progenitor marker, Tbr2.

On the other hand, lactate is the degradation product of biodegradable PLA scaffolds. The material degradation study showed a linear release of lactate to the medium in the physiological range of µM. In order to explain the role of lactate in influencing cell phenotype, cells were treated with Lactate. Glial cells culture showed no changes in their phenotype, while neuronal cells showed the same phenotype as neurons cultured in PLA, maintaining progenitor's populations and the immaturity of culture *in vitro*.

According to previous work, these results suggest that PLA properties might act synergistically with nanotopography in the modulation of the astrocytic phenotype, but probably is the lactate released by the scaffold which guides neuronal maturation and progenitor's selection.

P111

Molecular changes underlying oocyte resorption derived from water stress in the cockroach *Blattella germanica*

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Water stress impairs reproduction on insects, causing oocyte resorption in females. The present communication describes the molecular changes underlying oocyte resorption that occur in the German cockroach, *Blattella germanica*, after water deprivation. We suppressed drinking water to *B. germanica* females on day three of the adult life, and fluorescence microscopy showed that ovarian resorption started between 1 and 2 days later, as evidenced by a severe disorganization of the cytoskeleton. To identify genes that might be involved in oocyte resorption we carried out RNA ultrasequencing and obtained two respective transcriptomes from ovaries of five-day-old females: one from control specimens and the other from females that were water-deprived two days earlier. The comparative analysis of these two transcriptomes revealed that sequences that are differential expressed, with a p-value of 0.999, are mostly assigned to a Gene Ontology terms related with structural cell components. Transcripts related with processes of proteolysis and engulfment are up-regulated, whereas those involve in cell components organization are down-regulated in the ovaries from water-deprived females, which highlight the state of cellular disintegration involved in resorption. Further steps of this study would be to select the most significant transcripts, to validate the quantitative changes by qRT-PCR, and to carry out RNAi studies to assess their functions.

P112

Are plants ready for mountains without snow? Adaptation to snow scarcity in early life stages of Silene ciliata.

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A decrease in snow precipitation and early melting is one of the consequences of climate warming in temperate mountains. Snow cover is an essential factor that modulates germination and the early life stage development of high-mountain Mediterranean plants. Thus, high-mountain Mediterranean plants usually display seed dormancy to allow germination responses synchronized with snow melt. Furthermore, seedling development is dependent on water availability derived from snow melt to the point that summer drought is the first cause of seedling mortality. *Silene ciliata* Poiret is a mountain plant specialist which shows local adaptation patterns linked to water availability along an altitudinal gradient in its southern range of distribution (Central Spain). We evaluated the effect of different periods of cold humid stratification (simulated snow cover) in seed germination, seedling fitness and stomatal response with a common garden experiment, using seeds from populations at different elevations (Low, Intermediate, High). Seeds without stratification had lower but faster germination than those subject to stratification, while no effects were found in seedling growth and stomatal number or size. Seedlings from the Low population grew larger than those from higher elevations, although seedlings from the High population had greater number and larger stomata than those from the lower elevations. In conclusion, early life stages of *S. ciliata* show adaptation to snow cover – water availability that can be linked to the elevation of the population of origin.

P113

Whole genome comparison between human and macaque genomes: defining homologous synteny blocks and evolutionary breakpoint regions

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Comparative genomics makes possible to establish reliable comparisons among genomes, thus providing new insights into the driving forces that generate gene variation, adaptation and evolution. Specifically, the study of rearrangements among primate genomes allows for our better understanding of human genome organization and evolution. Our purpose is to characterize the genomic regions involved in rearrangements (Evolutionary Breakpoint Regions, EBR) between conserved blocks (Homologous Synteny Blocks, HSB) in the human and rhesus monkey genomes. To achieve this objective we applied two algorithms: Synteny Tracker and Cassis, which compare the position and orientation of orthologous markers.

A total of 16,133 orthologous genes were analyzed in our estimations of HSBs and EBRs. We identified 59 EBRs (median length 259 kb) in the human genome using Synteny Tracker, whereas Cassis detected 109 and 111 EBRs in human and rhesus genome, respectively (median length 51’3 kb and 26 kb, respectively). Merging the data from both algorithms and excluding those EBRs situated near telomeres, centromeres and gaps we obtained a total of 89 EBRs in the human genome and 87 EBRs in the macaque. Of these, 30 EBRs were implicated in the 19 macro rearrangements between human and rhesus monkey: 9 pericentric inversions, 5 paracentric inversions, 1 fusion and 3 fissions. Moreover, we detected new putative micro-inversions involving (39 and 41 EBRs in human and rhesus genome, respectively) and six indels (in both genomes).

Once the EBRs were defined in the macaque genome, we studied the pattern of gene and the tandem repeat distribution along macaque chromosomes by analyzing non-overlapping windows of 100kb (the average length of EBRs). We did not find an increment of tandem repeats in the EBR when compared to HSBs, as it has been described previously for great apes. However, preliminary results indicate a higher gene density in EBR (1.91 genes/100Kb) when compared with HSB (0.99 genes/100Kb) (T-Student, p-value<0.0001).

P114

Accelerated exon evolution in duplicated regions in hominids

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Identification of signatures of positive selection has long been a major issue for understanding the unique features of any given species. However, only a fraction of human genes have been interrogated. Genes within segmental duplications are usually omitted due to the limitations of draft nonhuman genome assemblies and the methodological reliance on accurate gene trees. In this work, we show the feasibility of a new method that does not need accurate gene trees or individual high-quality assemblies. We consider all high-quality nucleotide differences between shotgun sequencing reads from single human and macaque individuals relative to the human assembly. Comparing the observed rates of nucleotide differences between coding exons and their flanking intronic sequences with a likelihood ratio test, we identified 74 exons with evidence for rapid coding sequence evolution during human and Old World monkey evolution and validated five of them by means of experimental analysis. Strikingly, compared to only 6% of duplicated exons initially analyzed, 55% (41/74) of rapidly evolving exons were either partially or totally duplicated. Our results suggest abundant accelerated coding sequence evolution within these duplicated and highly dynamic regions of the genome and provide a more comprehensive view of the role of selection on human genome evolution.

P115

Evolució dels llocs d'unió del factor de transcripció dFOXO en el gen "*Insuline-like Receptor*" (*InR*) de Drosophila.

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El gen *InR* codifica el primer component de la via de senyalització de la insulina. Aquesta via juga un paper important en diferents processos i funcions biològiques tal com el creixement, el desenvolupament, la homeòstasi metabòlica, la reproducció, la durada de la vida i la mida corporal. S'ha descrit l'acció de la selecció positiva en la regió codificadora d'aquest gen, però queda per esbrinar si la regió reguladora també ha evolucionat adaptativament. Per respondre aquesta pregunta és necessari primer definir de forma precisa els llocs d'unió dels factors de transcripció. En condicions limitants de nutrients, la transcripció del gen *InR* és activada per la unió de dFOXO en la regió 5' del promotor P2. Donat que dFOXO té un domini d'unió altament conservat en les 12 espècies de Drosophila amb el genoma seqüenciat, s'ha usat dFOXO de *D. melanogaster* per a caracteritzar els llocs d'unió d'aquest factor de transcripció en un fragment de 1,4 kb a 5' del promotor P2 del gen *InR* en 5 espècies de Drosophila. S'han caracteritzat de forma precisa els llocs d'unió de dFOXO utilitzant la tècnica automàtica de *DNase I footprinting* i s'ha obtingut la *position weighted matrix* per dFOXO. A més, per tal de tenir una primera visió de les forces evolutives que modelen l'evolució dels llocs d'unió, també s'ha examinat la variabilitat nucleotídica tant en *D. melanogaster* com en *D. simulans*.

P116

CpG DNA methylation in *Culex pipiens*: Insights into the role of epigenetics in vector-borne parasite systems.

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A growing body of evidence suggests that epigenetics may play a role in host-parasite systems in providing a source of readily available adaptive non-genetic variation. Avian malaria provides an ideal opportunity to explore the epigenetic basis of host-parasite co-adaptations as well as its evolutionary significance. In this system, the *Plasmodium* life cycle in both (bird) host and the (mosquito) vector can be completed experimentally, thus allowing the investigation of epigenetic patterns along the course of the whole malaria infection. The first step of this project was to characterize the DNA methylation profile in the vector, the mosquito *Culex pipiens*. For this purpose we applied the amplification of inter-methylated sites (AIMS) approach, a method widely applied to study DNA methylation in cancer and aging, and more recently, to the discovery of DNA methylation in a social insect (*Apis mellifera*). The AIMS is based on the differential enzymatic digestion of genomic DNA with methylation-sensitive and methylation-insensitive isoschizomers followed by restrained PCR amplification of methylated sequences. Preliminary results show evidence of CpG DNA methylation in *Culex* mosquitoes. Validation by the bisulphite technique and methylated-site sequencing analyses, further confirm these findings. Our results are relevant in the context of current knowledge on the extent and pattern of DNA methylation in insects, and open up investigations to the role of epigenetics in vector-borne systems of important medical and economic concern.

P117

Cloning and Expression of Rainbow trout (Oncorhynchus mykiss) and Gilthead sea bream (Sparus aurata) Interleukin-6 (IL-6)

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Interleukin-6 (IL-6) is a cytokine involved in the regulation of crucial immune processes being the most described function its implication in the pro-inflammatory immune response. Actually little information is available about IL-6 in fish and specially in fish with relevance in aquaculture industry like Rainbow trout and Sea bream. Different efforts have been made in order to evaluate the characterization and expression of IL-6, however no research has conducted on the effects of is protein in fish. One reason is due to the lack of tools to evaluate the immune status of fish. Therefore it is necessary to develop biotechnological tools to assess the functional effects of key cytokines such as IL-6. To do this, we cloned, expressed and purified recombinant trout and sea bream IL-6 (IL-6r) from the soluble fraction of competent E. *coli* M15 [pREP4] cells using cDNA from cell cultured macrophages stimulated with 10µg/mL of LPS. The solubility of IL-6r was evaluated by growing the bacteria at different temperatures after induction with IPTG and we found a small amount of rainbow trout IL-6r (rtIL6r) and sea bream IL-6r (sbIL6r) in soluble fraction at 16°C and at 25ºC respectively. After purification we obtained both IL-6r in only one fraction visualizing a 25kDa band corresponding to IL-6r in SDS-PAGE and Western blot against poly-His tag. This approach is the first report of purifying this protein from the soluble cytoplasmic fraction of bacteria which represents the best alternative to obtain the protein in its native conformation, a clear advantage for antibody production and to evaluate the biological effect of these cytokines.

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P118

A genome-wide comparative study of DNA methylation in great apes

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DNA methylation is an epigenetic modification involved in regulatory processes such as cell differentiation during development, X-chromosome inactivation, genomic imprinting and susceptibility to complex diseases. These changes can be inherited through generations and likely have played an important role during human evolution. In this study, we performed a comparative analysis of CpG methylation patterns between humans and all four species of great apes (Chimpanzee, Bonobo, Gorilla and Orangutan). To characterize epigenetic differences that have occurred during primate evolution between these species we used Illumina Methylation450 BeadChips on a total of 33 individuals, including both male and female individuals, and different subspecies of Chimpanzee, Gorilla and Orangutan. We identified 19,521 CpG sites showing significantly different methylation between humans and chimpanzees. These positions are associated with a total of 1,284 genes, representing ~7% of Refseq annotations, suggesting that a significant fraction of genes have undergone altered epigenetic regulation during recent primate evolution. This list includes genes such as *HOXA5*, which is required for embryonic development, and *KCNAB3*, an ion channel protein expressed predominantly in the cerebellum. Even after accounting for the distribution of probes on the array, we observe that methylation differences occur preferentially at gene promoters, suggesting that these epigenetic differences are associated with altered transcriptional regulation. We also identify species-specific differences in methylation of the inactive X chromosome, and of imprinted genes. We have recently performed whole-genome bisulfite sequencing in one human, one chimpanzee, one bonobo, one gorilla and one orangutan, which will allow comprehensive assessment of epigenetic changes that have occurred during the last 15 million years of human and primate evolution.

P119

microRNA regulation of Krüppel homolog 1 gene by the miR-2 family in the context of German cockroach metamorphosis

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Insect metamorphosis is regulated by two main types of hormones: ecdysteroids, which induce molts, and juvenile hormones (JH), which repress metamorphic changes. From a molecular point of view, the action of ecdysteroids has been thoroughly studied in two species, in the highly derived fly *Drosophila melanogaster*, which is a holometabolous model, and in the less-derived cockroach *Blattella germanica*, a hemimetabolous species. Conversely, the molecular action of JH is poorly understood, with the bulk of data being available only for holometabolous models. Research on the molecular action of JH in hemimetabolous species is therefore needed if we aim at elucidating the evolution of insect metamorphosis from hemimetaboly to holometaboly. In light of this, we have studied the function of Krüppel homolog 1 (Kr-h1) in *B. germanica*. Kr-h1 is a Zn finger transcription factor whose function as transductor of the antimetamorphic action of JH has recently been demonstrated in *D. melanogaster* and in the beetle *Tribolium castaneum,* both being holometabolous species. RNAi experiments in *B. germanica* indicate that the antimetamorphic role of Kr-h1 is conserved among insects. The expression studies during the last nymphal instars show that Kr-h1 is suddenly down-regulated 24 h after the molt to the last instar nymph. Previous experiments showed that microRNAs play a key role in metamorphosis. In order to test if the decrease in levels of kr-h1 is regulated by microRNAs we performed anti-miR experiments, which suggested that miR-2/miR-13 family of microRNAs are involved in such down-regulation.

P120

Great ape versus human genetic diversity: the great ape genome diversity project

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Although human genome diversity has been the subject of extensive research in the last 30 years, the diversity of non-human Great Apes has been much less explored. We set out to understand the full spectrum of genetic diversity from single nucleotide to large structural variation using high-coverage next-generation sequencing of a diverse sample of 100 great apes. The set includes wild-born/unrelated specimens from all major subspecies including 28 chimpanzees (10 *Pan troglodytes ellioti*, 6 *Pan troglodytes verus*, 8 *Pan troglodytes schwenifurthii*, 4 *Pan troglodytes troglodytes*), 16 bonobos (*Pan paniscus*), 39 gorillas (35 *G.gorilla* and 4 *G. beringei graueri*) and 16 orangutans (8 *Pongo abelii* and 8 *Pongo pygmaeus*). We characterize the SNP diversity of each of these species and define lineage CNVs. Comparisons within species revealed unexpected differences in genetic diversity. Chimpanzee subspecies show the greatest population structure with heterozygosities ranging from approximately equal to twice that of humans. We find that G.gorilla individuals are almost twice as diverse at the SNP level than *G.beringei graeuri*, suggesting an ancient bifurcation and bottleneck similar to that of the two orangutan species. Overall, we predict almost 1000 genes have been lost within different ape lineages due to fixed disruptive mutations. While most forms of genetic diversity have behaved in a clock-like manner, both within and between species, copy-number variation especially that associated with segmental duplications appears more episodic. These data provide a rich resource to understand the biology of great apes, reconstruct their population history and improve conservation efforts for these remarkable species.

P121

Adaptive transposable element insertions in Drosophila: NATs, miRNAs and piRNAs

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In previous works, we identified a putatively adaptive transposable element (TE) inserted in the 3' UTR region of *Kmn1* gene. *Kmn1* plays a role in chromosome segregation and neurogenesis and its annotated transcript includes the TE insertion. However, there are some ESTs that do not contain the insertion or contain only a fragment. Additionally, the TE insertion could also affect the structure of *CG11699* since this gene overlaps in its 3' region with *Kmn1,* generating cis-natural antisense transcripts (cis-NATs).

In order to elucidate the molecular effects of this TE insertion, we performed 3'RACE and found that the TE insertion changes the length of the 3' UTR region not only of *Kmn1* but also of *CG11699*. In the case of *Kmn1,* flies without the insertion have a transcript with a short 3'UTR and another with a long 3'UTR. The TE insertion adds 186bp to the long transcript and it introduces piRNA binding sites. In the case of *CG11699,* when the TE is absent we find a transcript with a short 3' UTR and another transcript with a long 3' UTR. However, when the TE is present only the short transcript is produced because the presence of the TE affects the choice of polyA signal. Different 3' UTRs of *Kmn1* and *CG11699* have different secondary structures and miRNA binding sites, which could affect the expression of these two genes. Additionally, the length of the overlap between *Kmn1* and *CG11699* varies depending on the presence/absence of the TE potentially affecting the formation of endogenous siRNAs. Indeed, qRT-PCR experiments revealed that the TE insertion is associated with changes in the expression of both genes.

We conclude that TE insertions can affect the structure not only of the genes where they are inserted but also of nearby genes and that these structural changes can translate into expression changes potentially involving several molecular mechanisms.

P121

Fascin in *Drosophila* tracheal system development.

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One of the most important cellular processes in the formation of a respiratory organ that has to reach each cell of the organism is the directed cell migration. It has been known for years that tracheal cells in *Drosophila* migrate during embryogenesis towards the source of FGF/branchless (bnl), by the activation of the FGF/breathless pathway in the cells closest to the bnl source (called leading or tip cells). Even if it has been shown that this pathway regulates the cytoskeleton dynamics of the leading tracheal cells, which show extraordinary migratory features in spite of keeping intact adhesion with neighbouring stalk cells, it is still unclear which are the molecular mechanisms at play. We have focused our attention to the gene *singed*, which encodes for the fascin *Drosophila* homologue. Fascin organizes F-actin into tightly packed parallel bundles and its role is associated with the formation of cortical cell protrusions that regulate cell migration and adhesion. Interestingly, Singed is highly accumulated in tip tracheal cells during embryogenesis, and we demonstrate that this pattern is positively regulated by bnl through the positive transcription factor pointed (pnt) and the negative transcription factor yan. We detect several defects in tracheal formation affecting particularly the tip cells, which can be explained by its possible role in the regulation of the actin cytoskeleton. Importantly, these defects, which we quantified in fix tissue experiments, are observed in several loss of function conditions analysed. *In vivo* live imaging of singed mutant conditions has also revealed a delay in the migration of the dorsal branches (DBs) towards bnl source. Our results suggest that fascin is one of the players of the FGF pathway regulating tracheal system migration.

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P123

Use of liposomes as immunostimulant encapsulation agents in aquaculture

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Intensive aquaculture often involves high pathogenic burdens in farms that can provoke disease outbreaks accounting for immense economic losses being the development of protective/vaccination strategies a priority research area for aquaculture industry. Although there are a number of commercial finfish vaccines the initial expectations have not been fulfilled because the achieved protection levels are usually low, particularly viral vaccines. In this particular aspect nano-carriers could help to increase the fish immunisation levels by improving delivery of vaccines and other bioactive agents to specific immune actors. It can also be a useful key for a proper administration of the adequate doses in order not to over stimulate the immune system, avoiding in this way, the presence of unwanted side effects.

The current project has addressed the following fundamental goals: 1) we have systematically developed nanocarriers based on biocompatible and environmentally safe lipid formulations (Nanoliposomes, NLs); 2) we have loaded the NLs with immunological relevant molecules such as a cocktail of PAMPs (Pathogen-associated molecular patterns) that will stimulate the innate immune response protecting fish against a pathogenic challenge; 3) we have studied their *in vitro* uptake using NL formulations containing a fluorescent labels (Fluorophores). This labeled NLs will be used in the future to evaluate its biodistribution and portals of entry, that would allow for the design of rational immunisation protocols and the comparision of three different immunisation routes: injection, inmersion and oral in three different aquacultured fishes (trout, seabream and seabass).

P124

Global DNA methylation patterns in the European sea bass exposed to different temperature profiles during embryogenesis and early larval development

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Fish represent good animal models for the purposes of identifying persistent epigenetic marks which arise from different early developmental environments, and which correlate with relevant biological processes later in life. In adult European sea bass (*Dicentrarchus labrax*), the methylation levels of the promoter of the gonadal aromatase (*cyp19a*), which converts androgens into estrogens, has been shown to increase in females after exposure of larvae to high temperature. This epigenetic mechanism is involved in the masculinization of sea bass under farming conditions due to the use of elevated temperatures. In the present study, European sea bass were exposed to different combinations of temperatures during different periods covering embryogenesis or the early larval stages and were sampled at 15 days post fertilization. Global DNA methylation patterns are studied in these fish by applying a modification of the AFLP technique called Methylation Sensitive Amplified Polymorphism (MSAP). This technique allows the comparison and the detection of differences in the DNA methylation pattern profiles of groups of animals or treatments. The results contribute to better characterize the epigenetic mechanism linking temperature changes during the early developmental environment and DNA methylation of the European sea bass.

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P125

Ontogeny of the digestive system in the brachyuran crab *Maja brachydactyla* (Malacostracea, Decapoda)

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The spider crab Maja brachydactyla Balss, 1922 has recently been proposed as a potential candidate for aquaculture, due to its high fecundity and a relatively rapid larval development. The aim of the present study is to describe the digestive system of the larval stages. The larval development of M. brachydactyla comprises two zoeal stages (ZI, ZII) and a megalopa. M. brachydactyla larvae were reared at IRTA laboratory. The larvae in each larval stage were fixed in Davidson's solution; the sections (3 µm) were stained with hematoxylin and eosin. The digestive system of larvae is divided into three parts: foregut, midgut and hindgut. The most pronounced changes from ZI to ZII involved the posterior midgut caeca only present in ZII and, more developed in megalopal stage. The anterior midgut caeca is present in all larval stages, but in megalopa is longer and bilobed. The midgut of larvae was dominated by a simple columnar epithelium, and the hindgut by a simple squamous epithelium. The most pronounced changes from ZII to megalopa involved the foregut; the megalopa foregut makes an abrupt morphological change after metamorphosis. The hepatopancreas (midgut gland) is a bilobed gland located on both sides of the digestive tract, and morphologically similar in all larval stages, without significant structural changes. In newly hatched ZI, large lipid vacuoles with stored yolk material are the most prominent feature. The gross morphological features of external mouthparts and internal digestive tract structures of larvae at different developmental stages indicate that ingestion and digestive capacities are well developed already from newly hatched ZI. The histology of digestive system of larvae is in agreement with previous studies on digestive functional morphology of decapod brachyuran larvae.

P126

Decreases in condition and fecundity of freshwater fishes in a highly polluted reservoir

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Despite many efforts for pollution abatement in aquatic ecosystems, there are still some cases of high accumulation of industrial pollutants due to past activities. In Flix reservoir (Ebro River, Spain), there are around 200,000–360,000 tons of industrial pollutants with a high concentration of heavy metals and organochlorides due to the activity of an organochlorine industry during more than half a century. This exceptional amount of pollutants provides a good opportunity (and need) to analyse their effects on fish populations under natural conditions, which is rarely available to ecotoxicologists. We compared the reproductive traits and prevalence of diseases and parasites at this impacted area with a neighbouring upstream reservoir unaffected by the pollution (reference sites) and also to downstream sites. Deformity, eroded fin, lesion and tumour (DELT) anomalies and ectoparasites were clearly more frequent at the impacted area for several fish species (common carp, roach and pumpkinseed). A significant negative impact of Flix reservoir on condition (eviscerated and liver weights, adjusted for fish size with analysis of covariance) and reproductive traits (gonadal weight and number of mature eggs, adjusted for fish size) was also detected for several fish species. The responses to the pollutants were species-specific, and common carp (*Cyprinus carpio*) was the species with the clearest effects on fitness-related traits at the impacted area, despite also being among the most tolerant to pollution.

P127

Fast Real-Time PCR assay for detection of *Tetramicra brevifilum* in cultured turbot

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The global aquaculture production of turbot has rapidly increased in the last decade in the world and it is expected to have even bigger growth in the next years due to new farms operating. The losses caused by pathogen infections have grown at the same time than the production of this species. Parasitological infections are among the main relevant pathologies associated with the culture of turbot. Parasites produce serious losses in aquaculture, reduce the growth rate in the fish and may lead to unmarketable fish due to skeletal muscle abnormalities in cases with high intensity of infection. The microsporidian parasite *Tetramicra brevifilum* causes severe infections and generates major losses in farmed turbot. Infections by this parasite are difficult to control because of the spore longevity and its direct transmission. To facilitate the management of the infection, an effective tool for fast detection and identification of *T. brevifilum* is needed.

The result of this study provides the aquaculture industry with a molecular methodology of FAST Real-Time PCR for *T. brevifilum* detection, useful for routine control of *T. brevifilum* at turbot farms. The method is characterized by its high specificity and sensitivity and it can be applied on cultured turbot for the parasite detection despite the life cycle stage of the pathogen or the infection intensity.

KEYWORDS: Aquaculture; Fast Real-Time PCR; *Tetramicra brevifilum;* Microsporidian; Turbot disease; Detection

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Fast Real-Time PCR assay for detection of the enteric parasite *Enteromyxum scophthalmi* in cultured turbot *(Scophthalmus maximus* L.).

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Enteromyxum scophthalmi is a mixozoan parasite that affects farmed turbot *Scophthalmus maximus*. This pathogen is responsible for causing severe parasitic diseases in cultured turbot. The parasite causes enteric disease in turbot affecting mainly the intestine of the host. It is characterized by producing acute enteritis, starvation and eventually death.

Histological techniques are currently used to diagnose *E. scopthalmi* in Aquaculture and are based on the identification of the morphological structure of the parasite. The adoption of control measures might not be favored by the extended time these techniques take to be carried out. In addition they require experts at the farm for the identification of the parasite through the histological techniques.

This study develops a Fast Real-Time PCR molecular tool for the detection of *E. scophthalmi* in infected farmed turbot. The methodology developed is applicable in routine controls on the farm at every stage of the parasite infection. Obtained results have shown the robustness, specificity, efficiency and reliability of the technique.

KEYWORDS: Aquaculture; Fast Real-Time PCR; *Enteromyxum scophthalmi;* Myxozoa; Turbot disease; Detection; Fish parasites;

P129

Stress effect on the interrenal cells function in Rainbow trout (*Oncorhynchus Mykiss*)

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In the current study, the function of the rainbow trout interrenal cells, which are involved in the cortisol synthesis, was studied in response to stress. Fish were subjected to an acute handling stress for 1h, 6h and 24h and to a chronic crowding stress for 5 and 11 days.

The levels of cortisol, glucose, lactate and osmolality were measured in trout plasma. Changes in the mRNA abundance of selected genes involved in corticosteroidogenesis were also determined in the head kidney of fish using quantitative real-time PCR. These genes were melanocortin 2 receptor (mc2r), sterol transport protein (StAR), cytochrome P450 side-chain cleavage (p450scc), 3β-hydroxysteroid dehydrogenase (3β-hsd), and cytochrome P450 11B1 (11B-H).

The area of the interrenal cells was measured as a total surface occupied, by this specific cell type, in relation to the total sampling surface using histological techniques and stained with Trichrome Masson.

To verify the interrenal cells morphology and integrity, we complete our study with electron microscopy images.

Plasma levels of cortisol, glucose and lactate levels were significantly increased at 1h in stressed fish, when compared to the control group. On the other hand, the implicated genes were significantly up regulated afterward, at 6 and 24h.

The expression of genes involved in corticosteroidogenesis suggests that after the acute response at 1hour, a period of time is needed to activate the mechanisms for the further cortisol release.

The histological images also showed relevant changes in morphology and integrity at longer term.

P130

Stress and immune response in sea bream (*Sparus Aurata*) after experimental treatment with LPS of *a. Salmonicida* and *L. anguillarum*

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In fish, the defence system recognise pathogenic microorganisms via pathogen recognition receptors (PRRs) that sense particular structures as bacterial lipopolysaccharide (LPS) and initiate the immune response. *L. anguillarum* (La) and *A. salmonicida* (As) are G-negative bacteria that drives fish disease such as vibriosis and furunculosis.

In this study, seabream were injected intraperitoneally with LPS-La and LPS-As (10 mg * kg/fish) and with positive control group of *E. coli* LPS (Ec). Head-kidney, intestine, spleen, liver and blood samples were collected at 3-6-12-24 hours post-injection. Plasma levels of cortisol, prostaglandins and ROS were significant increased with LPS from fish pathogen a start contrast to LPS-Ec.

Gene expression of pro-inflammatory cytokines such as IL6, IL1, TNF and COX2 were highly induced by LPS-As and LPS-La and differentially expressed in different tissues. Although LPS is a structural component highly conserved among bacteria, fish pathogen LPS induce a differential-tissue regulation when compared with LPS-Ec.

The strong host-pathogen relationship between LPS-As-La and seabream suggest that defence response is related with structural LPS differences of pathogen.

These differences trigger a differential immune response that is tightly linked with the fish-pathogen relationship.

P131

Cortisol implants induce changes in complement and lysozyme levels in plasma, as well as in the transcription of immune-related genes in rainbow trout

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In the present research work we assessed the effects of artificially increased plasma cortisol levels induced by slow release cortisol implants on the activities of immune parameters of the rainbow trout (Oncorhynchus mykiss). The effects of the high plasma cortisol levels were also assessed at the transcript level using quantitative real-time PCR, by analysis of some relevant immune-related genes. These genes were: lysozyme, complement (C3, factor B and factor H) and several cytokines, namely tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6) and transforming growth factor-β (TGF-β).

Rainbow trout were implanted intraperitoneally with slow release implants of cortisol (50 µg cortisol/g body weight in coconut oil). Vehicle implants were used as control. At 1, 5 and 10 days after cortisol implantation, fishes were randomly sacrificed by over-anesthetization with MS222 and blood and organs (liver and head kidney) were sampled.

The cortisol implant caused an increase in the plasma levels of cortisol at the fifth day and returning to normal levels at the tenth day after cortisol implantation. Concerning complement and lysozyme plasma levels, we observed a decreasing trend at day 5 after cortisol implantation when compared to control. These tendencies become more evident at day 10 after cortisol implantation, when both plasma indicators were significantly decreased.

Concerning the transcription results, we observed a significant down-regulation of C3, factor B and factor H mRNA abundance in the liver at days 1 and 5 after cortisol implantation. Meanwhile in head kidney, we found a down-regulation in the levels of IL-1β at day 5 and TNF-α after 1 and 5 days. There were no differences in IL-6 and TGF-β.

P132

¿Puede la Acuicultura ayudar a recuperar una especie en peligro de extinción? Estudio con *Epinephelus marginatus* (Lowe, 1834)

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Dusky grouper (*Epinephelus marginatus*) is an economically important and emblematic species in the Mediterranean Sea, also gastronomically appreciated. However, this species has been suffering from an important fishing pressure to date and it is considered as an endangered species in the Red Book of Threatened Species (UICN). For these reasons, the Catalonian Aquaculture Network (XRAq) has put in contact some of the most important researchers in Spain related to this species to carry out an ambitious project. The goal of this project is to improve the reproduction techniques used for the dusky grouper to restock natural populations and to diversify the Spanish aquaculture market offer. Both actions are expected to benefit the natural populations. This project is being held in Barcelona and in Cadiz, working with *Epinephelus marginatus*, and in Vigo with another species of grouper (*Polyprion americanus*) also highly appreciated.

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Comparison of the lipolytic activity in guts of two sparid species; gilthead sea bream (*Sparus aurata*) and red porgy (*pagrus pagrus*).

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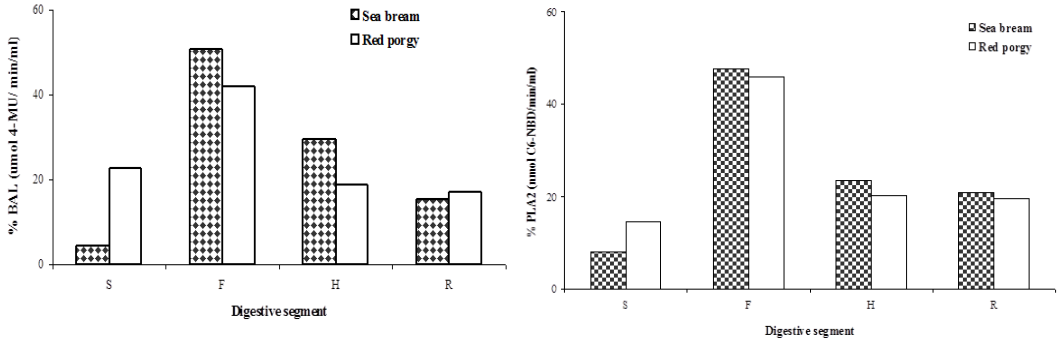
Gilthead sea bream (138.97 ± 19.78 g body weight, BW and 17.08 ± 0.1 cm furcal length, FL) and red porgy (121.79 ± 13.06 g BW and 17.0 ± 0.31 cm, FL) were kept in 500 l tanks with supply of water and aeration at around 20º C and fed with a 4mm grain size feedstuff commercially designed for gilthead sea bream which contained a raw composition of 44% proteins and 21% lipids (% dry diet). The total BAL and PLA_2 activities measured along their entire digestive tracts showed a twofold higher BAL activity in gilthead sea bream (136. 67 vs. 67.31 μmol 4-MU/min/ml) than in red porgy, while PLA_2 activities in guts of both fish species were very similar (133.55 vs. 147.82 nmol C_6-NBD/min/ml, in gilthead sea bream and red porgy, respectively). Both lipases were widely distributed and detectable along the entire digestive tract of both sparids, and no species specific differences were detected in the distribution patterns of BAL and PLA_2 activities. Significantly higher both BAL and PLA_2 activities found in foreguts of both gilthead sea bream and red porgy indicated a higher dietary lipid digestion in the anterior or piloric region of their digestive tracts, while both enzymes activities decreased progressively towards rectum. Generally, low %BAL and %PLA_2 activities were found in stomach, particularly in gilthead sea bream. The existence of a relationship between the length and the lipolytic activity found in each digestive segment was suggested in intestines of both sparids. Finally, it is suggested that a higher dietary lipid hidrolysing capacity of gilthead sea bream in comparison with red porgy could be related to the idoneity of the diet fed and the higher intestinal absorptive surface of the former species.



P134

Les Col·leccions Biològiques de Referència de l'Institut de Ciències del Mar: un recurs per a la recerca interdisciplinària de la biodiversitat marina

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Les Col·leccions Biològiques de Referència (CBR) de l'Institut de Ciències del Mar (ICM) del Consell Superior d'Investigacions Científiques (CSIC) comprenen més de 25.000 espècimens d'història natural. La seva funció és actuar com a referents científics dels resultats obtinguts en les investigacions i aportar coneixement sobre la biodiversitat marina. El seu fons actual és aproximadament d'unes 2.000 espècies catalogades, procedents majoritàriament del Mediterrani i de l'Atlàntic adjacent, però també d'altres àrees estudiades específicament per l'ICM tal com l'Oceà Antàrtic, Sudàfrica-Namíbia, Terra del Foc, Indo-Pacífic.El conjunt de les seves instal·lacions estan destinades a identificar, investigar, catalogar, dipositar i conservar organismes marins (peixos, crustacis, decàpodes i cefalòpodes, a l'actualitat), recol·lectats en els projectes científics i en les campanyes oceanogràfiques dutes a terme per als seus investigadors, així com per altres institucions nacionals i internacionals amb les quals col·labora l'ICM. La importància científica dels fons que contenen són una eina per a la comunitat científica nacional i internacional i les bases de dades es poden consultar al Web http://www.cmima.csic.es/cbr/usr/. A més les CBR participen en plataformes internacionals de biodiversitat tal com GBIF (Global Biodiversity Information Facility).

L'actual ampliació i adecuació de les CBR permetrà la realització d'estudis de biodiversitat, biologia evolutiva, taxonomia, filogenia, genètica de poblacions i biogeografia, així com l'optimització dels recursos de les mateixes en quant a plataforma oberta a la comunitat científica i social, com a infraestructura d'investigació i de difusió de les activitats i dels coneixements generats.

P135

Biometric tools in biodiversity and global change: the case of Juniperus phoenicea L. (Cupressaceae) from Andorra

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The isolated populations of *Juniperus phoenicea* found in mountain areas of the Pyrenees have traditionally been assigned to subsp. *phoenicea* However, the populations occurring in the southern limits of the taxon in the Maghreb have been assigned with little precision both to subsp. *phoenicea* and subsp. *turbinata*. In this context. subsp. *turbinata* has traditionally been considered as a taxon belonging to the plant communities found growing on coastal sands.

In order to verify the taxonomic status of the Pyrenean populations, and to better understand the distributión of subsp. *turbinata*, a biometric study based on morphological characteristics has been carried out. This enables us to clarify the taxonomic position of the Andorran populations, and to elucidate the taxonomy of the remaining Iberian populations situated in mountain and coastal regions, as well as the coastal populations from the Italian peninsula and the coastal and inland Moroccan populations.

The resulting conclusions indicate that the Andorran populations should be included within subsp. *phoenicea* along with the other inland Iberian populations. Subsp. *turbinata*, on the basis of the studied samples, colonizes the Iberian and Italian coastal regions. In Morocco, however, all the studied populations – both along the coast and in the Atlas mountains – correspond to subsp. *turbinata*.

The high degree of morphological variation of the Andorran populations suggest the relict character of those isolated Pyrenean valley stands.

The biogeographical interest of those fragmentary populations is commented on. They are plants that grow in the limits of the taxon's area, and should be included in the priority strategies for conservation of mountain biodiversity. Their dynamism in the global change scenario needs to taken into account.

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Community structure and biodiversity patterns in coralligenous communities over spatial and temporal scales

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Coralligenous communities are “hot spots” of Mediterranean marine biodiversity. Most of the benthic species constituting these communities are long-lived and slow-growing and, therefore especially vulnerable to global change (e.g. global warming, invasive species, and habitat loss). In fact, coralligenous communities in the North Western Mediterranean are already affected by several disturbances derived from global change, which may be accentuated if other disturbances act synergistically. However, assessing the scale and effects of these disturbances on coralligenous biodiversity may prove to be particularly difficult as very little baseline data are currently available. A necessary step in scaling up such information is to describe patterns at local and also at large spatial and temporal scales. To assess the natural variability of coralligenous biodiversity at both, spatial (3 regions and 2 sites within each region) and temporal scales (5 years), photo-quadrats of 25 x 25 cm were sampled and macro-species (> 1cm) were identified at each site. Significant differences among regions were found regarding the specific composition of coralligenous outcrops. However, no significant differences were found over the temporal scale. Regarding species growth forms they did not differ between regions and years being the encrusting the most common growth form observed. Similarly, biodiversity values of alfa and gamma did not differ across time and regions. Significative lineal relationship between local and regional species richness was found supporting the regional enrichment hypothesis in the coralligenous community. Under the climate change scenario, the final purpose of this study is provide baseline information on natural variability in order to manage future impacts and to identify sites with particularly high conservation value which may need specific protection.

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Pautes de biodiversitat al llarg de gradients altitudinals

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La biodiversitat al llarg d'un gradient d'altitud tendeix a ser màxima en el centre del gradient i més baixa en els dos extrems. Aquest patró unimodal s'ha observat per a un ampli ventall d'organismes, incloent-hi plantes, artròpodes i mamífers. I s'ha descrit en diferents indrets com ara les Muntanyes Rocalloses, les Himàlaies i els Pirineus.

Alguns autors apunten a la relació espècies-àrea com a causa explicativa d'aquest patró unimodal. La disminució de l'àrea colonitzable en altitud (i.e. la forma geomètrica d'una muntanya s'aproxima a un conus), sumada a una més elevada humanització de les zones baixes, amb la conseqüent pèrdua d'àrea potencial que això suposa per a moltes espècies, constituirien una explicació parsimoniosa. Altres estudis, però, indiquen que les espècies amb òptims altitudinals pròxims als extrems tendeixen a presentar rangs de tolerància altitudinal més estrets, el que dóna lloc a un màxim de biodiversitat al centre del gradient. Aquest patró es coneix com "l'efecte del domini intermedi" ("mid-domain effect") i s'ha observat no només en altitud sinó també en gradients latitudinals i de fondària en la mar.

La influència de la relació espècies-àrea en els patrons de biodiveristat i l'efecte del domini intermedi són fenòmens difícils de diferenciar. En principi, la relació espècies-área afecta a totes las espècies de la mateixa manera, tret de sistemes aïllats on els efectes són més forts en espècies de dispersió limitada, que molts cops també són de distribució restringida i relativament rares. En canvi, l'efecte del domini intermedi és més fort en espècies comunes i d'àmplia distribució. Tots dos fenòmens, però, comparteixen una capacitat explicativa basada en processos estocàstics, al marge de les relacions existents entre espècies i ambient. Per tant, i amb independència de quin dels dos fenòmens tingui una major influència sobre les pautes de biodiversitat observades, és del tot necessari considerar aquesta estocasticitat per arribar a comprendre i, eventualment, protegir aquesta biodiversitat.

A tall d'exemple, es presentarà l'anàlisi de la distribució de 195 tàxons de macroinvertebrats en 82 estanys de muntanya dels Pirineus, situats al llarg d'un gradient d'altitud de 1.370 m.

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Trichomycetes: an understudied group with scientific potentialities

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Arthropods can harbour a particular group of endobionts, formerly recognized as the Trichomycetes and included in the Zygomycota (Lichtwardt 1986). This is now recognized as an ecological group of fungi (Kickxellomy-cotina) and protzoa (Hibbett et al 2007). They are associated with a variety of hosts, from immature aquatic insects, to some Crustacea and Diplopoda (Benny and White 2001, White et al. 2001, White 2006). The trichomycetes, although scantly known, have high potentialities:

1) The most diverse order of gut symbionts, the Harpellales, live associated to arthropods in delicate fresh-water environments that need special attention from the scientific community. In these habitats, arthropods (with their symbionts) play an important role in countless organisms' maintenance, and thus in the ecosystem equilibrium.

2) Model organisms for the study of symbiosis processes. Symbiosis has been crucial in shaping life on earth and has allowed evolutionary novelties (Margulis and Fester 1991). The trichomycetes are no exception; they adapted to a new ecological niche and became endosymbionts, evolving unique morphological and physiological features. Their success is manifested by their wide distribution and wide range of hosts and habitats.

3) They are potential biocontrol agents. A few species of *Smittium* (Harpellales) have shown levels of pathogenicity against mosquito larvae and also can affect adult reproduction by forming ovarian cysts (Moss and Descals 1986).

4) They can provide information for understanding their hosts' evolution and dispersion. Harpellales are limited in their dispersions, and have a conservative morphology. Cosmopolitan species of gut symbionts can live inside diverse evolutionary-related species, and thus may be used to establish phyletic relations amongst diverse arthropods hosts.

5) They are potential source of bioactive metabolites. Their genetic potentiality has to be evaluated; some species of *Smittium* can produce vitamins (McCreadie et al 2005).

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Reflexió ètica sobre el valor i la conservació de la biodiversitat. Eina per al desenvolupament de competències transversals en sostenibilitat a la universitat

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Paraules Clau:
Biodiversitat, Competències en sostenibilitat, Transversalitat, Educació Superior, Conscienciació ètica.

Resum:
L'Informe Brundtland defineix el *desenvolupament sostenible* com «aquell desenvolupament que té en consideració les necessitats de les generacions actuals sense comprometre les generacions futures» (1987). Sembla que deixi d'haver-hi confrontació entre el desenvolupament humà i la conservació dels recursos naturals, però això no ha estat així. La destrucció de la biodiversitat en els darrers anys (Carson,1980; Delibes i Delibes, 2007; Duarte Santos, 2007; Bovet et al., 2008) ha provocat malalties, fam i desertització (WorldWatch Institute, 1984-2011; Comissió Mundial del Medi Ambient i del Desenvolupament, 1988; Lynas, 2004) i ha afectat la vida de milions de persones. Les conseqüències socials de la pèrdua de la biodiversitat fan que, des de l'àmbit educatiu, es consideri urgent educar la ciutadania per valorar i fer un ús responsable dels recursos naturals i afavorir la conservació de la biodiversitat.
En el marc de la Dècada de l'Educació per a la Sostenibilitat, des de la universitat s'han promogut iniciatives per conscienciar de la gravetat de la destrucció de la biodiversitat i reflexionar sobre les relacions entre biodiversitat i desenvolupament humà, com una crida ètica que convidi a un canvi en les pautes de consum cap a comportaments més sostenibles. A través d'un *Taller transversal en sostenibilitat i cooperació* s'han desenvolupat les següents competències en sostenibilitat: competència de pensament sistèmic, competència anticipatòria, competència normativa (ètica), competència estratègica i competència interpersonal (Wiek et al, 2011).

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P140

Plant warning signals in a global warming scenario can help to combat climate change

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The rapid increase in the atmospheric CO_2 concentration due to huge and continued anthropogenic emissions of this greenhouse gas is the main factor originating global climate change via increased temperature of the Earth's surface, according to conclusions of the IPCC in 2007. Precisely, both CO_2 and temperature have enormous effects on the physiology of plants and other photosynthetic organisms: CO_2 is the substrate of photosynthesis and a by-product of respiratory processes, and temperature strongly influences metabolic reactions. Both factors also greatly affect stomatal function and transpiration rates, among other processes. Therefore, plant performance will certainly be very much influenced by climate change. In this sense, the effects of elevated CO_2 on photosynthesis and respiration rates will surely improve the carbon balance of plants: diurnal carbon assimilation and plant growth and production will be increased, while leaf stomatal conductance, tissue nitrogen content and probably respiratory costs will be decreased under elevated CO_2. On the other hand, several studies raise alarm about the negative effects on plant and crop performance of temperature increases that are foreseen by the end of the century in IPCC projections. For example, Europe experienced an extreme climate anomaly during 2003, with July temperatures up to 6 ^{o}C above long-term means, and severe annual precipitation deficits, causing a 30% reduction in gross primary productivity, which in turn resulted in a strong net source of CO_2. Therefore, plant responses are warning about dangerous threats for plant ecosystems and food security if Earth's temperature is allowed to rise in the future. The scientific community has recognized that the increase in global temperature should be below 2 degrees Celsius in order to combat climate change. In summary, there is a considerable risk that food and feed supply can be very negatively affected by an excessive temperature increase in the future if CO_2 emissions are not sufficiently reduced through international cooperation efforts.

Is it possible to avoid global warming if the CO_2 emissions continue? The question is important because most negative effects of climate change, not only those affecting plants and ecosystems, are mostly related to the global temperature increase. We propose that the extent of global warming can be controlled directly through local or regional geo-engineering approaches, involving management of solar radiation (i.e. by increasing surface albedo) and also of terrestrial vegetation, which have proven to be successful in several recent studies. These solutions must have reasonable costs and impacts and could be implemented in parallel with programs of reduction of CO_2 emissions.

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Use of high resolution microscopy techniques for determining the effect of Copper in phototrophic microorganisms and its metal sequestration capacity.

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Confocal Laser Scanning Microscopy coupled to a spectrofluorometer (CLSM-*λscan*), which determines the effect of heavy metals *in vivo* and at the cellular level and Scanning Electron Microscopy (SEM) coupled to an Energy dispersive X-ray detector (EDX), wich determine the ability of the microorganisms to capture metals (1, 2) have been applied in this work. The current study determines the effect of copper on *Microcoleus* DE2006 and on the microalga DE2009, both isolated from Ebro Delta microbial mats, and also the capacity of these microorganisms to sequestrate this metal.

The results obtained demonstrated how the MFI (Mean Fluorescence Intensity) peak decreases in both microorganisms, in inverse proportion to the concentration of the essayed metal, from the control culture to the maximum dose assayed. Moreover, the MFI peak differences were statistically significant between control culture and 0,1 µM of Cu in *Microcoleus*, and between the control and 5 µM of Cu in the microalga, indicating that this microorganism is more resistant to the metal than *Microcoleus* sp. On the other hand, the analysis of the individual cells of both microorganisms by means SEM-EDX demonstrate their ability to accumulate copper in extrapolymeric substances (EPS).

In summary, both microorganisms have the capacity to remove copper, but given the results presented in this paper, we believe that the use of pesticides containing copper salts in agricultural areas close to microbial mats should be reviewed, due to the toxicity of copper, even at very low levels, which mainly affects the cyanobacteria that are responsible for the stability of these ecosystems.

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NrdR modulate differentially the expression of *Pseudomonas aeruginosa* ribonucleotide reductase genes

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Pseudomonas aeruginosa is a ubiquitous Gram-negative γ-proteobacterium capable of causing disease in plants, animals and humans. It is an opportunistic pathogen and the leading source of nosocomial infections, causing disease in a wide range of immunocompromised patients. It is also the common cause of chronic lung infections in individuals suffering from cystic fibrosis. P. aeruginosa is not only noted for its pathogenicity but for its environmental versatility because it is able to grow with very simple nutrient requirements and uses a huge number of different metabolic pathways. Although the bacterium is respiratory and never fermentative, it will grow in the absence of oxygen if nitrate is available as a respiratory electron acceptor.

Interestingly, P. aeruginosa is one of a few organisms to encode in its genome three different classes (Ia, II and III) of the enzyme ribonucleotide reductase (RNR). This essential enzyme catalyzes the reduction of ribonucleotides to the corresponding 2'-deoxyribonucleotides via a radical-dependent mechanism, thereby providing cells with the necessary building blocks for DNA synthesis. All known RNRs can be divided into three classes (I, II and III) based on structural differences, metallocofactor requirements, and mechanisms used for radical generation. Class I RNRs, encoded by the nrdA and nrdB genes, are found in both prokaryotic and eukaryotic organisms. The activity of class I RNR is restricted to aerobic conditions. Class II RNRs, encoded by the nrdJ gene, use adenosylcobalamin (AdoCbl) in the radical generation process, and operate both under aerobic and anaerobic conditions. This class has been found in archaea, eubacteria and some lower eukaryotes. Class III RNRs, encoded by the nrdD gene, can only operate under anaerobic conditions and has been found in archaea and eubacteria.

The recent discovery in Streptomyces and Escherichia coli of a novel regulatory protein NrdR that controls RNR gene expression prompted the studies reported here. The NrdR protein is a zinc-finger /ATP-cone transcriptional regulatory protein (COG1327). Abolishment of NrdR function resulted in a dramatic increase in transcription of all RNR genes in this two bacterium. Further studies revealed that NrdR binds to tandem repeat sequences, called NrdR boxes, located in or near to the promoter regions. NrdR-boxes are widespread in diverse bacterial genomes and are almost invariably located in the regulatory regions of different RNR genes.

In this paper we explore the role of the P. aeruginosa homolog of NrdR in regulating co-ordinately the transcription of the three RNR genes and its importance during infection and how the nrdR gene is regulated.

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Cord formation in Mycobacterium brumae and Mycobacterium fallax

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Summary

The cord formation (cording) of *Mycobacterium* genus has been related to their virulence. Cording consists in a particular arrangement of the bacilli in which the orientation of the long axis of each bacterium is parallel to the long axis of the cord. The compounds responsible for cording remain unknown, but this phenomenon has been recently related to the fine structure of α-mycolic acids. This investigation attributes to the proximal cyclopropane in α-mycolic acids cording in *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG. However, cording was also described in *Mycobacterium brumae* and *Mycobacterium fallax,* which present α-mycolic acids and are devoid of cyclopropane rings. In fact, cording was described in these species using light microscopy does not have sufficient resolution to distinguish between aggregates and the true cords.

Objective: To investigate the formation of true cords in *M. brumae* and *M. fallax* by high-resolutive scanning electron microscopy (SEM) to verify whether is necessary or not the presence of cyclopropane proximal in α-mycolic acids for cord formation.

Methods: *M. brumae* ATCC 51384^{T}, *M. fallax* ATCC 35219^{T}, *M. bovis* BCG ATCC 35737 ^{T} Japan were grown in Middlebrook 7H9 broth. Cultures were incubated for 4 weeks at 30ºC, except for BCG, which was incubated at 37ºC for 6 weeks. The Mycobacterium samples were e processed following conventional methods for SEM.

Results: All strains grew on 7H9 liquid medium forming spreading pellicles. Analysis by SEM showed microscopic cords formation in all cases. The ultrastructures of *M. brumae* and *M. fallax* showed similar cords than those formed by the positive control strain *M. bovis* BCG.

Conclusion:

1- *M. brumae* and *M. fallax* both devoid of cyclopropane rings in their α-mycolic acids can form true cords.

2- SEM is the best technique for studying microscopic cords in *Mycobacterium*.

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Mecanismes de resistència a rifaximina en soques d'*Escherichia coli* comensals i diarrogèniques de nens de Lima, Perú.

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Introducció: Les soques diarrogèniques d'*Escherichia coli* (DEC) són una de les principals causes de diarrea en menors de 5 any en països de baixa i mitjana renda, com Perú. En aquest context, l'aparició de soques resistents als antibiòtics emprats en llur tractament fa precís trobar alternatives, com la rifaximina (Rfx), un antibiòtic no absorbible. Aquest estudi avalua els nivells de resistència a Rfx, descrivint els mecanismes moleculars involucrats en llur resistència, en soques d'*E coli* obtingudes de femtes de nens menors de dos anys en zones periurbanes de Lima, Perú.

Mètodes: S'estudiaren 107 soques d'*E coli:* 79 DEC i 27 comensals (no DEC). El caràcter diarrogènic s'establí per Real-Time PCR. La Concentració Mínima Inhibitòria (CMI) es determiná per dilució en agar, en presència/absència de Phe-Arg-β-naphtylamide (PAβN), un inhibidor de bombes de flux. En les soques on la MIC amb PAβN era >8mg/L, es buscaren mutacions al gen *rpoB* i la presència del gen *arr-3* mitjançant PCR i una posterior seqüenciació.

Resultats: En tots els casos, el rang de MIC per Rfx oscil·là entre 32 i >256mg/L, disminuint en presència de PAβN a 2-128mg/L (DEC) i 2->256mg/L (no DEC). La MIC_{50} fou de 32mg/L per DEC i no DEC, disminuint a 4mg/L amb PAβN. La MIC_{90} fou de 64mg/L per DEC i de >256mg/L per no DEC, baixant a 4 i 128mg/L amb PAβN, respectivament. L'efecte de les bombes de flux permetré explicar la resistència al 74% (no DEC) i 96% (DEC) de les soques. No es trobaren mutacions al gen *rpoB*, i només una soca presentà el gen *arr-3*.

Conclusions: La resistència fou més elevada en soques no DEC que en DEC. El principal mecanisme involucrat foren les bombes de flux, mentre que mutacions en els gens *rpoB* i la presència del gen *arr-3* no foren freqüents. En 4 soques DEC i 6 no DEC, no s'identificà el mecanisme de resistència suggerint la presència d'altres mecanismes. Rfx no es una bona alternativa als tractaments en us al Perú.

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H-NS as a novel transcriptional modulator for the ribonucleotide reductase genes in *Escherichia coli.*

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Ribonucleotide reductases (RNR) are essential enzymes in all-living organisms because of its indispensable function of providing the four different deoxiribonucleotides: dATP, dTTP, dGTP and dCTP necessaries for the DNA synthesis and repair. Three different RNR classes (I, II and III) can be distinguished based on structural differences, metallo-cofactor requirements, mechanisms for radical generation, and responses to oxygen: class I RNR is subdivided in two subclasses: class Ia and Ib and are aerobic, class II RNR is an oxygen independent and class III RNR only is active under anaerobic conditions (1). Surprisingly eukaryotic organisms only encode for one type of RNR, the aerobic class Ia whereas in prokaryotes encode for any combination of RNR class, three different RNR classes (Ia, or Ib, or II or III), different RNR class combination (Ia + Ib, Ib+II, Ia+Ib+III) or in some species, all three RNR classes encoded in the chromosome (2)

The aim of our work is to study the transcriptional regulation of the different RNR classes in Escherichia coli. This bacterium encodes in its genome for three different RNR classes: Ia, Ib and III. In this study we have unravel the implication of the H-NS (nucleoid-associate protein), a transcriptional factor implicated in the condensation and supercoiling of DNA and which helps that the genome be correctly packed into de cell (3), to be one of the first repressor identified for the transcriptional regulation of some RNR genes in E. coli. Our results will indicate the action of H-NS in the down-regulation of class Ia RNR expression at stationary phase, where the cellular requirement of dNTPs decreases.

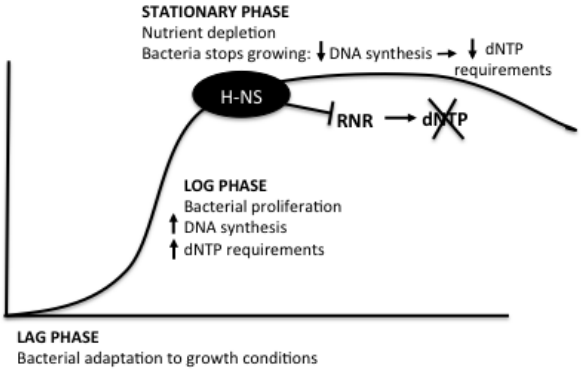

Figure 1: Effect of H-NS in the down-regulation of class Ia RNR at stationary phase where no dNTP are required for the bacterial growth. H-NS will be acting repressing the expression of class Ia RNR and promoting a decrease of the dNTP levels.

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P146

Encapsulation of ciprofloxacin within poly-(lactic-co-glycolic acid) (PLGA) nanoparticles enhances efficacy against bacterial pathogens in biofilm.

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In humans Pseudomonas aeruginosa and Staphylococcus aureus are the major cause of nosocomial infections and is frequently associated in chronic pulmonary infection like chronic obstructive pulmonary disease (COPD) or cystic fibrosis (CF) patients where it is the principal cause of morbidity and mortality [1]. The establishment of chronic Pseudomonas and Staphylococcus infections correlates with the formation of a biofilm structure with clusters of cells encapsulated in a polymeric matrix. It is very important the study of different ways of drug stabilization and delivery, since most of antibiotics did not reach optimal concentrations in some parts of the organism, for example, in biofilms, where almost all bacteria causing chronic infection can persist. Polymeric biodegradable nanoparticles (NPs) encapsulating antibacterial agents, including those made up of the biocompatible poly (lactic-co-glycolic acid) (PLGA), can be properly designed in terms of size to penetrate airway mucus, avoid steric inhibition by the dense mucin fibre meshes, and can hide chemical properties of the encapsulated drug (e.g., charge, degree of lipophilicity) in order to reduce its unspecific interactions with biofilms surrounding target bacteria [2]. Furthermore, NPs can provide a temporal control on release kinetics and enhanced efficacy of loaded drug.

The aim of this study is to assess the potential of drug loaded PLGA NPs in the treatment of P. aeruginosa and S. aureus infections. NPs were obtained and loaded with the fluorquinone antibiotic ciprofloxacin (CPX) through a fabrication method involving non-toxic chemicals.

Our results show a good in vitro antimicrobial activity against P. aeruginosa and S. aeruginosa planktonic cells. In addition its antimicrobial activity is demonstrated when both bacteria are establishing a mature biofilm suggesting a promising potential of using this NPs for the treatment of bacterial lung infections.

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Chromosomal and metabolic stasis in *Blattabacterium cuenoti*, the ancient primary endosymbiont of cockroaches.

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Long-term symbiotic associations between insects and intracellular bacteria are widespread in nature, actually it has been estimated that between 15 to 20% of insects had established such kind of relationship. Blattabacterium cuenoti is an obligate symbiont that has established a mutualistic relationship with cockroaches, and is one of the most ancient associations of this kind described up today. Here we present the genome of B. cuenoti endosymbiont from the cockroach Blatta orientalis (BBor) as well as the comparison of this genome with the previously sequenced Blattabacterium strains from the cockroaches Blattella germanica (BBge), Periplaneta americana (BPam), Cryptocercus punctulatus (BCpu) and the termite Mastotermes darwiniensis (BMda). The results of the comparison shows an extreme conservation of the genomic architecture and the gene content despite that these genomes are evolving separately since the apparition of the extant families of cockroaches around 140 Myr. Evolutionary analyses point that purifying selection is the main evolutive force operating in this system whit few genes accelerated. In spite of several enzymatic steps of the Krebs and urea cycles have been lost in the ancestors of BBor/BPam ans BMda/BCpu, the Stoichiometric analysis of the central metabolic pathways show no differences in terms of biosynthetic precursor production. Through these analyses we can assume that genomic and metabolic have been quickly established after the establishment of this association.

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Technological applications of the yeast Hanseniaspora

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Hanseniaspora species are yeasts mainly found in soil, on fruits and trees and in spoiled foods and beverages. Members of this genus are characterised by apiculate cells with vegetative reproduction by bipolar budding in basipetal succession. The six described species in the genus (H. uvarum, H. vineae, H. valbyensis, H. osmophila, H. occidentalis and H. guillermondi) are physiologically very similar as they ferment glucose, assimilate few carbon compounds (arbutin, cellobiose, glucose, glucono-δ-lactone and salicin), and require inositol for growth. However, they show marked differences in the shape and number of ascospores, a criterion used for species identification.

In this work, the identification of yeast species has been based on assimilation and fermentation tests and morphological traits (API 20C AUX and Rapid ID Yeast Plus System strips) and molecular biology based methods. For this latter purpose we have amplified the 5.8S-ITS region and subsequently digested it with several restriction enzymes to differentiate strains at the intraspecific level. Identification of yeast isolates at the species level was also carried out by sequencing of the D1/D2 region of the 26S rRNA gene. The D1/D2 sequences were used in a similarity search by means of the BLAST program in GenBank.

In order to characterize their enzymatic abilities, xylanase, □-glucosidase, lipase, esterase, protease, pectinase, polygalacturonase and peroxidase qualitative and quantitative assays were carried to determine the potentiality of Hanseniaspora species to be used as a source of enzymes in winemaking industry and also in other biotechnological processes.

P149

Desenvolupament i anàlisi de mutants de *Escherichia coli* i *Shigella spp.* resistents a furazolidona.

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Objectius: La diarrea infantil es una de les principals causes de mortalitat als països pobres, degut en part a problemes en el tractament per el gran increment en la resistència a antimicrobians que trobem en l'actualitat. L'objectiu d'aquest estudi és analitzar el comportament d'un antibiòtic, la furazolidona (Fur) i veure la seva capacitat de generar resistència *in vitro* amb soques d' *Escherichia coli* i *Shigella spp* aïllades en nens menors d'un any.

Materials i mètodes: S'empraren 4 soques de *E.coli* i 2 de *Shigella spp.* provinents de mostres de diarrea infantil de Lima, Perú. S'establí la concentració mínima inhibitòria (CMI) a Fur, tetraciclina, cloramfenicol, àcid nalidíxic i ampicil·lina, i per mitjà de creixement en concentracions seriades de Fur per sobre de la CMI s'obtingueren mutants resistents dels que es determinà llur freqüència de mutació, estabilitat, resistència creuada, mecanismes de resistència (rol de bombes d'expulsió, mutacions a *nfsA* i *nfsB)* i alteracions en la fitness.

Resultats: Les soques mutants exhibiren increments en la CMI d'entre 2 i 5 vegades, assolint nivells de fins a 16mg/l. Les freqüències de mutació oscil·laven de < $9{,}6\text{x}10^{-10}$ a $7{,}3\text{x}10^{-6}$.Romangueren estables després de 20 sembres consecutives. En cap cas s'observà resistència creuada amb els altres antimicrobians. No s'observà efecte de les bombes d'expulsió inhibides per Phenyl-Arginine-ß-naphtylamide en el desenvolupament de resistència. La resistència s'associà preferentment a canvis aminoacídics a *nfsB* (54.2%) i alteracions a *nfsA* (56.3%) incloent generació de codons STOP (44,4%) deleccions (7,4%), i canvis aminoacídics. En el 20,8% no es detectà cap mecanisme de resistència. No s'observaren diferències de creixement entre els mutants seleccionats i les soques mare.

Conclusions: No es seleccionaren soques amb CMI > 16mg/l. La resistència desenvolupada s'associà a les mutacions trobades en *nfsA* i *nfsB*. Seria necessari estudiar la presencia d'altres mecanismes de resistència, com possibles alteracions a l'expressió de porines, als mutants sense mecanisme de resistència identificat.

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Direct antitumor effect of heat-killed and irradiated *Mycobacterium bovis* BCG in superficial bladder cancer

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Mycobacterium bovis BCG is currently the most effective agent for therapy and prophylaxis of superficial bladder cancer. Following transurethral resection, intravesical chemotherapy with mitomycin C (MMC) and live BCG are administered in order to prevent recurrence, tumor progression and prolong survival. Although BCG treatment is highly effective side effects are common and, cases of disseminated BCG infection are described. To avoid these effects, the use of non-viable BCG could be advantageous. Although BCG extracts are now under evaluation to replace live BCG, some authors argue that its antitumor effect is only achieved when BCG is alive. This work aims to determine the direct anti-tumor capacity of heat-killed and irradiated BCG, and their possible synergistic effect with MMC. Human bladder cancer cell lines (T24, J82 and RT4) were infected with live or killed BCG, with or without MMC. Cell proliferation inhibition and the production of IL-6, IL-8, TNF-α and NO by infected cultures were analyzed. Furthermore, metabolic activity of killed BCG was also tested. Both heat-killed and irradiated BCG inhibited tumor proliferation similarly to live BCG. In all cases a synergistic effect with MMC was observed. Killed-BCGs induced lower cytokine levels in tumor cells than live BCG, but only irradiated BCG induced similar levels than live BCG in T24 cell line. This result corresponds with the observation that irradiated BCG retained some metabolic activity, thus “killed but metabolically active” concept could be applied to the bacilli. Finally, while both live and killed BCG together with MMC induced higher cytokine levels than BCG alone in J82 cell line, contrary live and killed BCG alone induced higher cytokine levels than in combination with MMC in T24 and RT4 cells. On conclusion, non-viable BCG retains its direct antitumor capacity and acts synergistically with MMC. However, non-viable BCG triggers lower levels of cytokines production than live BCG, which are important for antitumor response.

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Indirect antitumor activity of irradiated *Mycobacterium bovis* BCG in non-invasive bladder cancer

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Immunotherapy using live Mycobacterium bovis BCG is the most effective treatment in non-invasive superficial bladder cancer. BCG acts both by inducing a direct citotoxicity over tumoral cells and by triggering an immune response mediated by macrophages and dendritic cells, which includes the release of proinflammatory cytokines (indirect antitumor activity). We have demonstrated that irradiated BCG retains its direct antitumor capacity. Thus, we aimed in this study to determine whether irradiated BCG exerts also an indirect antitumor activity.

Firstly, the expression of activation markers in murine macrophages (J774) infected with live and irradiated BCG was analyzed. Secondly, the cytotoxic capacity of peripheral blood mononuclear cells (PBMC) stimulated with live and irradiated mycobacteria against bladder tumor cells were determined. In both cases, the cytokine production induced by mycobacteria infection was measured.

Our results showed that while live BCG induced an increased expression of CD40, CD80 and CD86, irradiated BCG was only able to trigger the expression of CD86. In the same manner, although irradiated BCG induced the production of proimflamatory cytokines in infected macrophages, the levels were lower than those induced by live BCG. PBMC stimulated with irradiated BCG retained the cytotoxic capacity against tumor cells, but only live BCG was able to induce the secretion of soluble factors by PBMC with cytotoxic capacity. In PBMC, live BCG triggered higher levels of IL-10, IL-12 and TNF-α, but lower levels of IFN-γ than irradiated BCG.

Altogether, our results suggest that viable BCG is needed for a complete cytotoxic activity against tumor cells.