Societat Catalana de BIOLOGIA

Jornades conjuntes de Biologia Molecular i del Desenvolupament

Organitzades per

Secció de Biologia Molecular
Coordinador: Bernat Crosas

Secció de Biologia del Desenvolupament
Coordinador: Francesc Cebrià

Col·laboradors: Teresa Adell, Marta Morey i Berta Alsina

PROGRAMA I RESUMS DE LES COMUNICACIONS

INSTITUT D´ESTUDIS CATALANS

Sala Prat de la Riba, Institut d'Estudis Catalans
Carrer del Carme, 47. Barcelona

Carrer del Carme 47

Barcelona

4 de maig de 2018

Programa

8:30-9:00 Arrival and Registration

9:00 Welcome by the Coordinators of the Sections of Molecular Biology and Developmental Biology

9.10-9:40 Origin of the sections and the joint meetings by Jaume Baguñà, Jordi Domingo and Pere Puigdomènech

Scientific sessions

Chair: TBA

9:40-10:20 Benoît Kornmann (ETH, Zurich)
"Membrane contacts, what are they, what are they good for and how can they be bad?"

10:20-11:00 Anna Rubio-Cosials (EMBL, Heidelberg)
"Transferring antibiotic resistance: structural insights into the mechanism of a conjugative transposon"

11:00-11:15 Damià Garriga (ALBA Synchrotron, Barcelona)
"Molecular basis for the inhibition of poxvirus assembly by the antibiotic rifampicin"

11:15-11:30 Andrea Izquierdo-Bouldstridge (IBMB-CSIC, Barcelona)
"Histone H1 depletion triggers an interferon response in cancer cells"

11:30-12:00 Coffee and Posters

12:00-12:15 Cinthia Raquel Millan (UPC, Barcelona)
"New drugs complexed with AT-rich DNA accumulate in kinetoplast DNA: a promising treatment against Sleeping Sickness"

12:15-12:30 Albert Torra (VHIR-CIBERNED, Barcelona)
"Activation of Transcription Factor EB as a neuroprotective strategy for Parkinson's disease"

12:30-12:45 Sílvia Pérez-Lluch (CRG, UPF, Barcelona)
"Natural no-coding antisense transcription along development and evolution"

12:45-13:00 Alba Ventós-Alfonso (CSIC-UPF, Barcelona)
"Role of Zelda in the hemimetabolan insect *Blattella germánica"*

13:00-13:40 Volker Hartenstein (UCLA, USA)
"Structure and development of neural circuits of the Drosophila brain: a lineage-centered approach"

13:40-15:00 Lunch and Posters

Afternoon scientific sessions

Chair: TBA

15:00-15:40 Fàtima Gebauer (CRG, Barcelona)
"RNA binding proteins in cancer progression"

15:40-16:20 Salvador Aznar-Benitah (IRB Barcelona)
“Epigenetic mechanisms in adult stem cells, and their possible impact over mutational burden of cancer stem cells”

16:20-16:35 Juan J. Fraire-Zamora (CRG, Barcelona)
“Dorsal closure in dipterans: epithelial rupture, contraction and seaming without genetic changes in the scuttle fly Megaselia abdita”6

16:35-16:50 Brenda Gavilán (UB, Barcelona)
“Serial section Transmission Electron Microscopy (ssTEM) analysis of the acoel *Symsagittifera roscoffensis”*

16:50-17:05 Eudald Pascual-Carreras (UB, Barcelona)
“Smed-BS is a novel peptide which inhibition produces bigger planarians or overgrowths depending on the nutritional status”

17:05-18:30 Drinks and Posters

18:30 Awards and concluding remarks

Invited Speakers

Membrane contacts, what are they, what are they good for and how can they be bad?

Benoît Kornmann

ETH- Zurich, Switzerland

Intracellular organelles constitute dense and branched membrane networks that are under constant remodeling. My lab is interested in how these organelle networks are generated, distributed and regulated. We also investigate how this networked morphology is related to the organelle's activity. These highly extensive and dynamic networks cohabit in the extremely crowded cytoplasmic space. This situation leads to unwanted collisions and entanglements that needs to be resolved. We show that, in the case of mitochondria, these collisions and entanglements can be resolved by mitochondrial fission. Mechanical forces applied to mitochondrial tubules lead to the recruitment and activation of the mitochondrial fission machinery, leading to the resolution of entanglements. These results imply that a biochemical response can be triggered by a mechanical stimulus and that forces within the cells participate in the shaping of organelles. The extended morphology of several organelles might allow them to contact each other to exchange lipid molecules. Because most of the factors involved in lipid exchange are unclear or unknown, we developed a novel method that uses transposons and next-generation sequencing to interrogate the yeast genome and map in a single step all proteins and protein domains necessary for growth in a given condition. We use it to identify redundancies in lipid exchange routes, but the power of the method finds myriad of applications far beyond our usage.

Transferring antibiotic resistance: structural insights into the mechanism of a conjugative transposon

Anna Rubio-Cosials, Eike C. Schulz, Lotte Lambertsen, Georgy Smyshlyaev, Carlos Rojas-Cordova, Kristoffer Forslund, Ezgi Karaca, Aleksandra Bebel, Peer Bork, Orsolya Barabas

Structural and Computational Biology Unit, European Molecular Biology Laboratory (EMBL), 69117 Heidelberg, Germany

anna.rubcos@gmail.com

Conjugative transposition drives the emergence of multidrug-resistance in diverse bacterial pathogens, yet the mechanisms are poorly characterized. The Tn1549 conjugative transposon propagates resistance to the antibiotic vancomycin used for severe drug-resistant infections. Here, we present four high-resolution structures of the conserved Y-transposase of Tn1549 complexed with circular transposon DNA intermediates. The structures reveal individual transposition steps and explain how specific DNA distortion and cleavage mechanisms enable DNA strand exchange with an absolute minimum homology requirement. This appears to uniquely allow Tn916-like conjugative transposons to bypass DNA homology and insert into diverse genomic sites, expanding gene transfer. We further uncover a structural regulatory mechanism that prevents premature cleavage of the transposon DNA before a suitable target DNA is found, and generate a peptide antagonist that interferes with the transposase-DNA structure to block transposition. Our results reveal mechanistic principles of conjugative transposition, which could help control the spread of antibiotic resistance genes.

Structure and development of neuronal circuits of the *Drosophila* brain: A lineage-centered approach

Volker Hartenstein

Department of Molecular Cell and Developmental Biology, University of California Los Angeles, Los Angeles, CA 90095

Common features of the central nervous system encountered in all bilaterian animals are the relatively high number of cells, diversity in neuronal cell types, and specificity in neuronal connections. This puts a heavy burden on the developmental process generating the central nervous system. In Drosophila, a fixed lineage mechanism plays a pivotal role in controlling neuronal diversity and connectivity. The fly brain is composed of a relatively small number of stereotyped neuronal lineages, groups of neurons descended from individual embryonic stem cells, called neuroblasts. During the course of its proliferation, each neuroblast expresses characteristic sets of regulatory genes. These genes control the differentiation of the neurons born from that particular neuroblast during a particular time interval. Through this mechanism, a lineage, or smaller subdivision of a lineage called sublineage, develops into a specific class of neurons which share common wiring properties, including the projection of their axons, branching pattern, and placement of synapses. Several discrete neuronal classes/lineages are put together into a neuronal circuit. In the first part of my talk I will delineate the lineage mechanism, concentrating on the example of a particular circuit, the anterior visual pathway (AVP). The AVP conducts input form the optic lobe to the central complex, a brain center known to process and store visual information in order to control fly locomotion. The central part of the AVP is formed by three lineages, whose neurons form several classes of highly ordered parallel and sequential elements.In the second part of my talk I will provide a progress report of our ongoing studies of neuronal wiring at the single synapse level of resolution. We are following the hypothesis that the wiring properties and choice of synaptic partners of a neuron is strictly correlated to its lineage association. In collaboration with numerous other groups we use a dataset consisting of a complete series of more than 5000 registered electron microscopic sections of an entire early larval brain (“L1 EM stack”). The reconstructed neurons are morphologically complex 3D graphs whose nodes are annotated with labels representing different types of synapses. By cross-referencing the L1 EM stack with similarly oriented stacks of confocal sections we could identify lineage identity of most neurons. The data allow one to study quantitatively the spatial relationship between synapses with different partners, and formulate hypotheses regarding neural function at the microcircuit level. We have designed software that generates 2D renderings of 3D neurons in order to help biologists analyze the wealth of data that is now available. The renderings are dendrograms that capture a neuron’s tree-like structure, and they realistically encode morphological features, such as relative length and branching depth of a side branch, and synapse locations. We hence refer to these neuron sketches as "morphological feature dendrograms" (MFDs).

RNA binding proteins in cancer progression

Fátima Gebauer

CRG Barcelona

RNA binding proteins (RBPs) are gaining attention in the oncology field for their potential to regulate essentially every hallmark of tumor development. However, to date only a few RBPs have been shown to play roles in cancer progression, in large part because RNA metabolism has been a poorly investigated aspect of cancer research. Fueled by our initial discovery that the conserved RBP UNR/CSDE1 has dedicated roles in melanoma metastasis, we have launched an unbiased genome-wide screen of RBPs for which cancer metastatic cells show specific dependencies. My talk will focus on our efforts to untangle the RBP diversity of metastatic cells.

Epigenetic mechanisms in adult stem cells, and their possible impact over mutational burden of cancer stem cells

Salvador Aznar Benitah

ICREA Professor; IRB Barcelona

Mutations are not evenly distributed throughout the genome. However, what causes the disparate genomic mutational distribution is still under debate. It has been proposed that a factor contributing to the uneven distribution of cancer mutations is the open versus closed chromatin distribution of their cell-of-origin. Importantly, mutations and expression changes of epigenetic modifiers are pervasive in human tumours, making epigenetic factors attractive as anti-tumour targets. However, if epigenetic alterations affect mutational burden, this raises the concern that targeting epigenetic factors in cancer patients might alter mutability and possibly aggravate disease progression in the long-term. Yet, a causal link between changes in chromatin accessibility in tissues and the mutational landscape of their cognate tumours has not yet been established. I will present functional data showing how altering chromatin accessibility severely affects tumorigenesis *in vivo*. I will comment the effects that changing chromatin openness has over tumor mutability. I will also discuss the implications of our results on the effect that our lifestyle (*i.e.* diet) could have on the epigenetic landscape of adult stem cells which might then influence the aggressiveness of their cognate tumors.

Selected Oral Presentations

Molecular basis for the inhibition of poxvirus assembly by the antibiotic rifampicin

Damià Garriga[1,2], Cathy Accurso[2], Stephen Headey[3], Melissa Germany-Partarrieu[2], Martin Scanlon[3] and Fasséli Coulibaly[2]

[1]ALBA Synchrotron, c/ de la Llum 2, Cerdanyola del Vallès, 08290.

[2]Biomedicine Discovery Institute and [3]Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Victoria (Australia)

dgarriga@cells.es

In contrast to most enveloped viruses, poxviruses produce infectious particles that do not acquire their internal lipid membrane by budding through cellular compartments. Instead, poxvirus immature particles are generated from atypical crescent-shaped precursors derived from host ER membranes. Two key viral proteins participate to this process: A17 inserts in the membrane and is proposed to induce curvature, while D13 forms a scaffold that remodels membranes into a closed, spherical particle. The formation of these viral crescents can be inhibited by the antibiotic rifampicin, although the mechanisms underlying such inhibition remain unknown. Using a combination of X-ray crystallography, surface plasmon resonance and CPMG, a ligand-detected NMR technique, we showed that rifampicin directly binds D13 and identified its binding site. We further proved that this inhibitor directly competes with A17, evidencing an overlap of binding sites. We then used a classical fragment-based drug design approach to target the A17/rifampicin binding site in D13, screening a library of 1137 compounds by STD and CPMG NMR. This allowed the identification of 25 fragments that bind D13. Out of these, 2 molecules with unrelated structures were found to compete with both rifampicin and A17. These two lead compounds were taken for optimisation towards the design of assembly inhibitors against poxviruses.

Histone H1 depletion triggers an interferon response in cancer cells

Andrea Izquierdo-Bouldstridge[1,#], Alberto Bustillos[1,#], Carles Bonet-Costa[1], Daniel García-Gomis[1], Albert Jordan[1]

[1] Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Barcelona, Catalonia, 08028 Spain

[#] These two authors contributed equally to this work.

Seven linker histone H1 variants exist in human somatic cells with distinct prevalence depending on the cell type and along differentiation. H1 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. We have shown that H1 variants are not distributed uniformly along the genome and there are differences between variants, H1.2 being the one showing the most specific pattern. We have explored functions of H1 variants by inducible shRNA-mediated knock-down of each of the variants. Knock-down of each H1 variant alters expression of a different, reduced subset of genes. Combined depletion of H1.2 and H1.4 has a strong deleterious effect in the cancer cells examined, and induces a strong interferon (IFN) response with up-regulation of many IFN-stimulated genes (ISGs). Although H1 participates to repress ISG promoters, its activation upon H1 KD is mainly generated by the expression of noncoding RNA generated from heterochromatic repeats including satellites. In conclusion, redundant H1-mediated silencing of heterochromatin is important to maintain genome stability and to avoid an unspecific growth-inhibiting IFN response.

New drugs complexed with AT-rich DNA accumulate in kinetoplast DNA: a promising treatment against Sleeping Sickness

C.R. Millan[a], F.J. Acosta-Reyes[ab], L. Lagartera[c], N. Saperas[a], C. Dardonvillec, H. DeKoning[d] & J.L. Campos[a]

[a]Department of Chemical Engeniering, Universitat Politecnica de Catalunya, E08019 Barcelona (Spain); Lourdes.campos@upc.edu

[b]Howard Hughes Medical Institute, Columbia University, New York, NY 10032, USA

[c]Instituto de Química Médica, IQM–CSIC, 28006 Madrid, Spain; dardonville@iqm.csic.es

[d]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK; Harry.De-Koning@glasgow.ac.uk

Approximately 70 million people distributed over a surface of 1.55 million km2 are estimated to be at different levels of risk of contracting sleeping sickness. *Trypanosoma brucei* accounts for 82.2% of the population at risk. About 6 million to 7 million people worldwide, mostly in Latin America, are estimated to be infected with *Trypansosoma cruzi*, the parasite that causes Chagas disease. We study drugs interacting with minor groove of DNA, such as the N-phenylbenzamide bis(2-aminoimidazoline) derivatives 1 The main objective was to identify their cellular target inside the parasite. We were able to demonstrate that the drugs have a clear effect on the S-phase of T. brucei cell cycle by inflicting specific damage on the mitochondrial DNA, a unique and complex structure called kinetoplast. The kinetoplast has more than 70% of AT-DNA.

C.R. Millan, *et al*. "Functional and structural analysis of AT-specific minor groove binders that disrupt DNA-protein interactions and cause disintegration of the *Trypanosoma brucei* kinetoplas" *Nucleic Acid Research* (2017) vol.45, pag.8378-8391 Doi: 10.1093/nar/gkx521

Acknowledgements. We thank all the Staff for valuable assistance at the ALBA BL13-Xaloc beamline and to Dr. Leandro Lemgruber for the SEM images.

Activation of Transcription Factor EB as a neuroprotective strategy for Parkinson's disease

Albert Torra1, Jordi Galiano1, Annabelle Parent1, Thais Cuadros1, Beatriz Rodríguez-Galván1, Esther Ruiz-Bronchal2, Analía Bortolozzi2, Miquel Vila1,3,4 and Jordi Bové1

1Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute (VHIR)–Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Barcelona, Spain

2Department of Neurochemistry and Neuropharmacology, IIBB–CSIC, August Pi i Sunyer Biomedical Research Institute (IDIBAPS)-Center for Networked Biomedical Research on Mental Health (CIBERSAM), Barcelona, Spain

3Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona, Barcelona, Spain

4Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

Postal address: Edifici Mediterrània, Hospital Vall d'Hebron, Passeig de la Vall d'Hebron, 119-129, 08035 Barcelona. Phone number: +3493 274 6899. Electronic address: albert.torra@vhir.org

Neurotrophic factor-based therapy stands as one of the most promising disease-modifying therapeutic approach for Parkinson's disease (PD). However, axonal impairment and downregulation of their receptors may account for the lack of therapeutic success in clinical trials. An alternative to delivering neurotrophic factors to overcome these hurdles is to directly activate the intracellular signaling pathways responsible for their effect. We demonstrate that Transcription Factor EB (TFEB) overexpression in mice drives a previously unknown bona fide neurotrophic effect that involves the activation of the MAPK1/3 and AKT pro-survival pathways, giving rise to cell growth, and increased dopamine release. We also demonstrate that TFEB overexpression prevents neuronal death, increases dopaminergic function and counteracts atrophy and the associated protein synthesis decline in the MPTP mouse model of PD. It has been suggested that TFEB neuroprotective effect may be due to its capacity to boost the autophagy-lysosomal system for the clearance of protein aggregates. However, we show that knocking down the master transcriptional repressor of autophagy ZKSCAN3 is not sufficient to protect dopaminergic neurons in this model. Overall, our results suggest that TFEB activation is an alternative neuroprotective/neurorestorative strategy to neurotrophic factor-based therapies for PD and related disorders.

Natural no-coding antisense transcription along development and evolution

Sílvia Pérez-Lluch[1,2], Alessandra Breschi[1,2], Amaya Abad[1,2], Cecilia Klein[1,2], Marina Ruiz-Romero[1,2], Emilio Palumbo[1,2], Carme Arnan[1,2] and Roderic Guigó[1,2,3].

1. Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88, Barcelona 08003, Catalonia, Spain
2. Universitat Pompeu Fabra (UPF), Barcelona, Catalonia, Spain
3. Institut Hospital del Mar d'Investigacions Mediques (IMIM), Barcelona 08003, Catalonia, Spain

e-mail: silvia.perez@crg.eu

The genome of *Drosophila melanogaster* is estimated to encode over two thousand lncRNAs; however, only few of them have a characterized function. Natural antisense transcripts (NATs) are fully processed lncRNAs which overlap protein coding genes on the opposite strand with or without exonic complementarity. Several roles in genomic regulation are reported for NATs in metazoa, including gene expression regulation of the overlapping protein coding gene, DNA methylation, chromatin modifications and RNA editing. Here, we have identified 855 lncRNAs overlapping 873 protein coding genes in antisense orientation, forming 953 sense-antisense (SA) pairs in the fruit fly genome. By analysing the transcriptome of different imaginal tissues at 3rd instar larvae, we have explored the relationship between NATs expression and alternative transcript usage across fly larval samples. Of the 376 SA expressed pairs involving a protein coding gene with multiple isoforms, *blistered*/*CR44811* is the one showing a highest correlation between changes in coding gene isoform usage and NAT expression. *blistered* (*bs*) gene encodes for two main isoforms: a short one, expressed mainly in the wing where the NAT *CR44811* is also expressed, and a long one, expressed in the other tissues where the NAT is silent. *CR44811* CRISPR mutant flies show a dramatic change in the *bs* isoform usage in larval and pupal wings, as well as a strong phenotype in the adult, indicating impairment of the proper wing development. Manual annotation of the *bs* locus using available RNAseq data from other species has allowed us to align both isoforms of the coding gene along development, suggesting a role of both isoforms in further species and its possible regulation through the action of the lncRNA.

Role of Zelda in the hemimetabolan insect *Blattella germanica*

Alba Ventos-Alfonso, Xavier Belles

Institute of Evolutionary Biology (UPF-CSIC)

Passeig Marítim, 47, 08003 Barcelona, Tel. 93 230 95 00,

alba.ventos@ibe.upf-csic.es

There are two modes of metamorphosis in insects: holometabolan and hemimetabolan. In the hemimetabolan (which is the ancestral mode), the embryo develops the basic adult body structure, whereas in the holometabolan, the adult body structure becomes completed in the pupal stage. Therefore, to better understand the evolution of metamorphosis it is important to study the differences in the mechanisms regulating embryo developmental processes in hemimetabolan and holometabolan species. One of those processes is the maternal-to-zygotic transition (MZT), where the maternal mRNAs are eliminated and the zygotic genome starts to be transcribed. Zelda has been described as a key protein in the MZT in holometabolan insects, such as *Drosophila melanogaster*, being involved in both maternal mRNA cleavage and activation of the zygotic genome. Conversely, information about the role of Zelda in hemimetabolan insects is very limited. In this work, we carried out a functional analysis of Zelda in the embryo of the hemimetabolan insect *Blattella germanica*, using maternal RNAi. We found that Zelda regulates the expression of early zygotic genes (involved in abdomen formation and dorso-ventral patterning), and that of miR-309, a microRNA that plays a key role in eliminating maternal mRNAs during the MZT. We have also observed that Zelda regulates the expression of epigenetic factors, like DNMT1 or Nejire. The whole results confirm that Zelda is a key protein in MZT in both hemimetabolan and in holometabolan insects. However, a key difference between both metamorphosis modes is that Zelda is expressed only in early embryogenesis in *B.germanica*, whereas in *D. melanogaster* it is expressed all along the embryogenesis. This difference could explain the different output of both types of embryo development.

Dorsal closure in dipterans: epithelial rupture, contraction and seaming without genetic changes in the scuttle fly Megaselia abdita

Juan J. Fraire-Zamora, Johannes Jaeger and Jérôme Solon

Cell and Developmental Biology Program, Centre for Genomic Regulation (CRG).

Dr. Aiguader 88, 08003, Barcelona, Spain. juanjose.fraire@crg.eu

The evolution of morphogenesis is generally assumed to be associated with changes in genetic patterns that lead to the spatial reorganization of tissues. I will present a case study where rearrangements of epithelial organization occur without major changes in genetic patterning. In Drosophila melanogaster, the process of dorsal closure consists of the fusion of opposing epithelial sheets where a contractile extraembryonic amnioserosa and a JNK/Dpp-dependent epidermal actomyosin cable result in a microtubule-dependent seaming of the epidermis. In the scuttle fly Megaselia abdita, dorsal closure must occur in the presence of a separate serosa, amnion and epidermis. It differs from Drosophila in morphogenetic rearrangements despite conservation of the JNK/Dpp signaling pathway. Using a quantitative approach in a non-model organism, we show that dorsal closure in Megaselia is driven by the rupture and contraction of the serosa, an epidermal actomyosin cable and the consecutive microtubule-dependent seaming of amnion and epidermis. Upon rupture, serosa cells retract and reduce their size through actomyosin apical accumulation. This process promotes the internalization of serosa cells bringing together the opposing amniotic flanks, followed by the contractile activity of an epidermal actomyosin cable that depends on JNK/Dpp signaling. The final process of dorsal closure in Megaselia depends on two sequential microtubule-dependent seaming events. First the amniotic flanks must seam at the dorsal midline, followed by the seaming of the opposing epidermal flanks. Microtubule depolymerization prevents amniotic seaming and rescue experiments resume Megaselia dorsal closure. Using high-resolution time-lapse imaging, immunostaining and molecular tools, we obtained evidence indicating that the evolutionary transition to a reduced system of dorsal closure involves the simplification of the seaming process without changing the signaling pathways of closure progression.

Fraire-Zamora JJ, Jaeger J and Solon J. (2018) Two consecutive microtubule-based epithelial seaming events mediate dorsal closure in the scuttle fly Megaselia abdita. eLife

Serial section Transmission Electron Microscopy (ssTEM) analysis of the acoel *Symsagittifera roscoffensis*

Brenda Gavilán[1], Pedro Martínez [1,2] and Volker Hartenstein[3].

[1]Universitat de Barcelona (UB), Departament de Genètica, Microbiologia i Estadística.

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

[3]University of California, Los Angeles (UCLA).

In collaboration with S. Sprecher and V. Hartenstein laboratories from University of Fribourg (Switzerland) and University of California, Los Angeles (USA), respectively, we have undertaken a thorough study of the acoel cellular organization leading towards the 3D reconstruction of structures of major interest. Transmission Electron Microscopy serial sections (ssTEM) of the most anterior part of a *Symsagittifera roscofensis* juvenile body were taken, covering approximately the first half of the body, where the brain and beginning of the nerve cords are located. These sections were aligned in the correct order forming a stack using the ImageJ plugin TrakEM2 (developed by Albert Cardona, HHMI, Janelia Farm, USA). The software allows us the 3D reconstruction of cells and cell groups. My work in this project consists in analyzing the structure and organization of different cell types present, and how they connect with each other. This is particularly challenging in this group of animals, since the acoel body is not organized as well-delimited organs; the different cell types appear intermingled and their membranes highly folded. As acoels (together with their entire group, the phylum Xenacoelomorpha) are considered the sister group of the remaining bilaterians, this study can help us to shed some light in the evolution of organs' architectures. Our main aim is to see if there are patterns of distribution of these cellular types, how they are connected to each other and very especially to the central nervous system. We also took particular interest in the different sensory receptors and gland necks located in the epidermis, with a focus on their subtype classification, distribution and innervation. This systematic approach gives us a unique opportunity of studying the nervous system in much more detail, and aiming at a complete 3D reconstruction of the brain, nerve chords and main peripheral nerve tracks.

Smed-BS is a novel peptide which inhibition produces bigger planarians or overgrowths depending on the nutritional status

Pascual-Carreras, E[1], de Sousa, N[1], Marín, M[2], Eckelt, K[1], Saló, E[1] and Adell, T[1]

[1] Department of Genetics, Microbiology and Statistics, Universitat de Barcelona (UB) & Institute of Biomedicine of Universitat de Barcelona (IBUB), Barcelona, Spain.

[2] Norwich Research Park, Norwich NR4 7UH, UK.

Department of Genetics, Microbiology and Statistics, University of Barcelona

The control of cell number is crucial to define the final body size during animal development, and also to restrict tumoral transformation in adult organisms. The cell number of any organism results from the balance between cell proliferation and cell death. Although a large number of genes are known to be involved in the control of both processes, the molecular mechanisms relating cell number and body size remain poorly understood. To further understand this relationship we study planarians, flatworms that continuously change their body size depending of food viability. In this study, we present a novel secreted peptide, Smed-Blitzschnell (Smed-BS), which inhibition produces an increase of proliferation and a decrease in cell death, thus leading to an increase in cell number. Interestingly, *Smed-bs* RNAi inhibition in starved planarians results in animals with more cells than controls but with the same body size; thus, showing a higher density of cells that are smaller. Eventually, this increase in cell density leads to overgrowths. In contrast, *Smed-bs* RNAi fed planarians growth faster than controls, since they have more cells, but those cells are of the same size than the control ones. Thus, the increase in cell number after *Smed-bs* RNAi is translated to: 1) a tumoral transformation in starved conditions, and 2) an increase in body size in animals with a rich nutritional status. Thus, the impact of an increase in cell number depends on the energetic state of cells

Posters

P1. Analysis of Nse1 function in maintenance of genomic stability in human cells

Sonia Apostolova, Marta Rafel, Jordi Torres-Rosell, Neus Colomina

Departament de Ciències Mèdiques Bàsiques

Universitat de Lleida/IRBLleida

Edifici Biomedicina. Av. Alcalde Rovira Roure 80, 25198 Lleida

sonia.apostolova@cmb.udl.cat

During cell division multiple proteins are involved in replication and segregation of chromosomes to ensure an accurate transmission and distribution of genetic material to daughter cells. Failure in these key processes leads to the occurrence of aneuploidy and genomic instability, a hallmark of cancer cells. The Smc5/6 protein complex is conserved from yeast to humans and plays a crucial role in chromosome segregation and DNA repair. One of the subunits of the complex - Nse1 - contains a RING domain with ubiquitin ligase activity. Mutations in this domain impair DNA repair in budding yeast, causing hypersensitivity to genotoxic drugs. To reveal the function of Nse1 in mammalian cells we have created human cell lines carrying a deletion or a point mutation in the RING domain of Nse1 by CRISPR/Cas9 system. Both type of mutants express extremely low levels of the Nse1 protein, show slow growth and a higher death rate. Our preliminary results indicate that there is an increase in gamma-H2AX phosphorylation, a slight increase in anaphase bridges and micronuclei and a significantly increased number of BLM foci, compared to wild type cells. Besides, FACS analysis shows a higher number of cells with less than G1 DNA content in the Nse1 mutant population, suggesting a chromosome segregation defect.

Overall, we conclude that the integrity of the RING domain in the human Nse1 is important for Nse1 protein stability and for the maintenance of genomic integrity.

P2. Tissue Engineering Unit at CRG. Services for Stem Cell and Developmental Biology

Laura Batlle Morera, Martin Gigirey, Marta Vila

CRG, Carrer Dr Aiguader n88, 08003, Barcelona. SPAIN

Laura.batlle@crg.eu

The goal for the Tissue Engineering Platform is to provide services to researchers in the latests technologies used in the fields of stem cell biology, stem cell differentiation, organoid formation and induced pluripotent stem cells (iPSCs). The Unit is constantly setting up new technologies that are emerging in the above fields. The Tissue Engineering platform works in collaboration with the Biomolecular Screening & Protein Technologies Unit at the CRG to provide CRISPR/Cas9 genome editing technology service. We provide the following services for stem cell and developmental biology researchers:

-Stem cells and iPS cells
-CRISPR/Cas9 gene editing to cell lines and directly to embryos
-Embryo micromanipulation
-2D and 3D (organoids) specific stem cell differentiation

P3. D-GADD45 as putative modulator of JNK pathway in regeneration

Carlos Camilleri[1], Elena Vizcaya[1], Florenci Serras[1] and Montserrat Corominas[1]

[1]Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona. Barcelona, Catalonia, Spain

Av. Diagonal 643 (08028), Barcelona. carloscamilleri@ub.edu

Drosophila imaginal discs are a well established model system to study regeneration, both after physical damage or cell-death induction. Although little is known about the early signals driving the regenerative process, the activation of the Jun N-terminal kinase (JNK) pathway is likely to play a decisive role. Studies on the gene expression profiles of imaginal discs at different time points after cell death reveal several genes showing an expression burst right after damage and returning to normal levels early in the process. We focus on the *Drosophila* Growth arrest and DNA damage-inducible gene 45 (D-GADD45), which is a stress sensor involved in DNA repair, apoptosis and cell cycle control. Downregulation of D-GADD45 after cell death blocks the activation of the JNK pathway and severely compromises the regeneration process. We suggest that D-GADD45 activates the JNK pathway upstream of basket, promoting the signaling cascade that activates the expression of key genes involved in regeneration.

P4. How centrosome number influences collective cell migration

Rita Capella and Sofia J. Araújo

Department of Genetics, Microbiology and Statistics, University of Barcelona, Spain

Cell migration is a complex process that plays an important role both during the biological development of organisms and in disease conditions. During cell migration, cell movement is driven by the continuous reorganization and turnover of microtubules and the actin cytoskeleton. The centrosome is the major microtubule-organizing center (MTOC) in mammalian cells and centrosome abnormalities are associated with human tumours. However, the role of centrosomes in cell migration and invasiveness is still not well understood. To study cell migration during development, we use *Drosophila melanogaster* as a model organism, focusing on the embryonic development of the tracheal system, an organ whose development relies heavily in cell migration. In this study, we compare the migration patterns of the tracheal cells in wild type flies to tracheal cell migration in different mutants that have alterations in centrosome number. To approach this, we use confocal microscopy both in fixed and *in vivo* embryos.

P5. *Smed-cbp* regulates stem cells commitment and differentiation in planarians

Sheila Cárcel[1], Susanna Fraguas[1], Thileepan Sekaran[2], Kerstin Bartscherer[2] and Francesc Cebrià[1]

[1]Departament de Genètica, Microbiologia i Estadística, Universitat de Barcelona

[2]Max Planck Institute for Molecular Biomedicine, Münster, Germany

The plasticity and differentiation of stem cells into multiple specific lineages is an open basic question in developmental biology. CBP (CREB-binding protein) is a conserved gene that functions as transcriptional co-activator and histone acetyl transferase. In different organisms, CBP plays an important role in wide range of cellular processes, including cell proliferation and differentiation, cell death, DNA damage response and tumorigenesis. Here, we have studied the function of a CBP homologue in planarians, which have astonishing cellular plasticity thanks to their neoblasts (adult pluripotent stem cells). Because planarians have remarkable regenerative abilities they provide us an ideal scenario to understand the molecular mechanism underlying stem cell differentiation in vivo. Our data show that the silencing of *Smed-cbp* in planarians results in apparently proper blastema formation but severely impairs tissue differentiation. Analyses with specific molecular markers for neural and eye lineage-committed progenitors indicate that neoblast specialization into these cell types is largely blocked, which results in the absence of neural regeneration. In contrast, for the epidermal lineage it seems that *Smed-cbp* is not necessary for the specialization of epidermal progenitors, and opposite to what it is seen for neural lineages there is an increase in the number of differentiated epidermal cells. Overall, our results suggest that *Smed-cbp* could have a multiple function in regulating neoblast commitment and differentiation.

P6. Functional characterization of a mutation identified in an Opitz C syndrome patient

L. Castilla-Vallmanya, R. Urreizti, H. Franco-Valls, G. Cunill, A. Cueto-González, E. Tizzano, J.M. Opitz, G. Neri, D. Grinberg, S. Balcells

Department of Genetics, Microbiology and Statistics, Faculty of Biology (UB)

Avinguda Diagonal, 643 Edifici Prevosti 08028 (Barcelona)

934021794

lcastilla30@gmail.com

Opitz C syndrome (OTCS, MIM #211750) is an extremely rare genetic disorder characterized by multiple malformations (e.g. trigonocephaly, congenital heart defects), contractures, variable intellectual and psychomotor delay and a high mortality rate. In a cohort of 11 patients clinically diagnosed as OTCS, mutations in 9 different genes were identified as disease-causing by whole exome sequencing (WES). Thus, OTCS could be causally heterogeneous phenotype instead of a specific entity. In this project, we aim to functionally characterize a *de novo* heterozygous missense variant found in a gene of the TRAF family in one of the patients and to assess its pathogenicity. The protein encoded by this gene has a role as E3 ubiquitin ligase in different signalling pathways mediated by Tumor Necrosis Factor (TNF) family ligands, such as the NF-κB pathway.

We have analyzed cell viability by MTT assay and while a slight increase in the patient's cell viability could be observed, there is no significant difference. *In vitro* analysis of fibroblasts from 3 patients bearing similar mutations were performed at mRNA level by qPCR and mRNASeq. Compared to control fibroblasts, patient's cells showed an altered basal expression of several genes of the NFKB pathway and gene expression patterns in response to TNFα stimulation. Results so far strongly suggest a pathogenic role of the mutation, however they do not clarify if the effect is a loss or a gain of function of the protein. Future experiments, such as co-immunoprecipitation assays with the major partner of the protein and further analysis of the mRNASeq data, may help to elucidate the impact of these mutations.

P7. EGFR and ecdysone in *Blattella germanica* oogenesis.

Fleur Chelemen and Maria-Dolors Piulachs

Institut de Biologia Evolutiva - IBE- (CSIC-UPF)

Pg. Marítim de la Barceloneta 37-49. 08003 Barcelona. Tel. 932309648.

fleurchelemen@hotmail.com

Oogenesis is a crucial process to ensure continuity of the species and, therefore, it needs to be finely regulated. Even the regulation of this process is a complex network where different genes and pathways participate, it is important to know each piece of the puzzle and its function, and one of the main pieces of this puzzle is ecdysone that is already known in *Drosophila melanogaster* for stimulating the germ stem cell proliferation. Our work is focused on the epidermal growth factor receptor (EGFR) using as a model the cockroach *Blattella germanica*, a species with panoistic ovaries, the most primitive ovary type. In previous work, we found that the depletion of EGFR determines an increase in the number of germinal cells and our objective is to unveil a possible interaction between EGFR signaling and ecdysone, an interaction that must be regulating ecdysone biosynthesis or its signaling gene cascade. To measure the activity of the ecdysone biosynthesis, we pay special attention to the expression of Shadow an enzyme that triggers the final step in the biosynthesis of ecdysone. To unveil these interactions, we use the RNAi methodologies to deplete the expression levels of EGFR and Shadow, observing at microscopic levels how this depletion could affect the differentiation of the germinal cells in the germarium and by qRT-PCR quantifying the expression of those genes that can be implicated in this process.

P8. Proteasomal degradation of naturally occurring glutamine-rich peptides

B. Coll[1], A. Zuin[1], Y. Palau[1], B. Crosas[1]

1. Molecular Biology Institute of Barcelona, CSIC, Barcelona, Spain

The ubiquitin proteasome system (UPS) is a central player in eukaryotic protein homeostasis. In a cellular context, ubiquitin activating, conjugating and ligating enzymes act sequentially marking the substrates for their degradation in the proteasome. In this way, ubiquitin serves as a common recognition tag among most of the proteasome substrates. As part of the UPS system, the proteasome acts as a protein degrading hub for the cell, integrating degradative signals from multiple ubiquitin ligase enzymes. This tag, however, is not imperative for protein degradation. Instead, it has been described that location to the proteasome is sufficient for protein degradation, given that the substrate features an unstructured region able to interact with the inner ATPases of the proteasome. When protein homeostasis fails, the whole cell functioning is impaired. This is often associated with cell stress, toxicity and death. Here we investigate the interaction between proteasome and disease related proteins such as huntingtin or ataxin-3. These proteins have a common trait; they all feature glutamine rich regions. In this project we propose that these characteristic domains can serve as signal sequences able to direct the proteins to the proteasome in an ubiquitin independent way. Direct targeting of toxic proteins to the proteasome serves as a model to study how aggregation prone proteins affect the proteasome system. Additionally, it is to be studied how replicating these proteasome modifications in eukaryotic cells could shed some light into new treatment for the above-mentioned neurodegenerative diseases.

P9. *In vivo* and *in vitro* models in the study of the FGF23 regulation

Nuria Doladé[1]; Sandra Rayego-Mateos[1]; Jose Manuel Valdivielso[1]

[1]Institut de Recerca Biomèdica de Lleida (IRBLleida), Av. Alcalde Rovira Roure, 80, Lab. 1.10, Lleida, 25198, Spain. 973702952. nuriadolade@irblleida.udl.cat, srayego@irblleida.cat, valdivielso@medicina.udl.cat

Introduction: Chronic kidney disease (CKD) is a major public health problem. The fibroblastic growth factor 23 (FGF23) is a fosfaturic protein that is expressed predominantly in osteocytes and whose levels are increased in patients with CKD. The possible regulation of FGF23 by calcium and phosphorus independently remains unknown. The objective of this study is to establish the models that allow us to study it. **Materials and methods:** A. Para-thyroidectomy model (T-PTX): The animals underwent a para-thyroidectomy (T-PTX) and a tyrosine hormone replacement therapy (T4). B. Hypercalcemia model: Intraperitoneal injection of calcium gluconate monohydrate (250 mg/kg every 2 hours during 8h). C. Hypocalcemia model**:** Intramuscular injection of EDTA (150 mg/kg). The animals were sacrificed 6 hours later. *In vitro*: Primary cultures of bone marrow mesenchymal stem cells (MSCs), differentiated by a conditioned medium. **Results and conclusion:** Mice subjected to T-PTX showed a reduction in calcium levels, as well as an increase in serum phosphorus levels. In mice undergoing hypercalcemia, a significant increase in the excretion of calcium in urine was observed without changes in serum calcium levels. In mice subjected to hypocalcemia, a decrease in calcium levels was observed in both serum and urine. Finally, in *in vitro* differentiated cells (MSCs differentiated to osteocytes-like) there was an increase in gene expression of mineral matrix markers such as OSC, OSX, RUNX2, OPN and FGF23, as well as a decrease in mesenchymal markers such as COL I. We can conclude from the results obtained that the T-PTX was successful and can be used for the study of the regulation of FGF23 independently of PTH. On the other hand, models of hyper-and hypocalcemia in the doses studied exert the desired effects on the regulation of calcium levels. The individual combination of these two models with the T-PTX will allow us to study the role of calcium independently of PTH in the regulation of FGF23.

P10. The effect of stress in the retinal cells of a *Cerkl* mouse model generated by CRISPR/Cas9

Domènech, E. B.[1, 2], Serra-Pascual, C.[1, 2], Gonzàlez-Duarte, R.[1, 2, 3], Marfany, G.[1, 2, 3]

[1]Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona, Avinguda Diagonal 643, 08028 Barcelona, Spain.

[2]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Barcelona, Spain.

[3]Institut de Biomedicina de la Universitat de Barcelona (IBUB-IRSJD), Barcelona, Spain.

elenabdomenech@ub.edu

Retinal neurodegeneration, characterized by the apoptosis of photoreceptor cells, is the major cause of genetic blindness. So far, mutations in over 200 genes are associated with inherited monogenic retinal diseases (1:3000 worldwide), but we are still far from completely understanding their ethiopathology. Therefore, animal models and *in vitro* cell culture assays are essential to characterize the precise role of *CERKL*, a causative gene of retinal dystrophies. The physiological function of CERKL is yet to be determined, but it is related to stress cell resilience since its overexpression protects cells from the apoptosis triggered by oxidative stress. We generated a mouse model by causing the full deletion of the *Cerkl* gene using CRISPR/Cas9. in order to investigate the retinal effects in the oxidative/light stress response. Unexpectedly, complete ablation of *Cerkl* causes perinatal lethality in homozygosity. Therefore, to approach the CERKL function in the retina, we have generated a heterozygous knockdown/knockout mouse model ($Cerkl^{KD/KO}$) in an albino background to perform *in vivo* light stress experiments. This model is viable and fertile, and the expression of *Cerkl* has been reduced to 18%. Our results showed that CERKL localized to the stress granules formed the retina in response to stress. Moreover, the number of stress granules was notably higher in the retinas with reduced CERKL expression compared to those of wild type mice, thus supporting the CERKL role in the protection and maintenance of retinal cells.

P11. The role of ASK1 in Drosophila's wing disc regeneration

José Esteban Collado, Giacomo Viola, Montserrat Corominas and Florenci Serras

Department of Genetics, Microbiology and Statistics, University of Barcelona, Barcelona

Recent work has strengthened Drosophila imaginal discs as a model system for regeneration studies. Evidence is accumulating that oxidative stress drives the cellular responses for repair and regeneration. Apoptotic cells result in a burst of reactive oxygen species (ROS) that can propagate to neighboring cells. These ROS are known to activate JNK and p38 kinases for regenerative growth.Two key issues arise from these observations. The first is how the ROS are propagated. The second, what is the link between ROS and the stress activated protein kinases p38 and JNK. Our results reveal that Ask1 senses ROS differently in apoptotic cells and living surrounding cells. High levels of ROS are produced in apoptotic cells, which in turn generates high activity of Ask1 that turns on JNK, which is known to enhance apoptosis. Neighboring undamaged cells show low ROS levels, which are beneficial for the regenerating tissue. In these, Ask1 is activated but its activity is attenuated by Pi3K dependent Akt1 phosphorylation, as a survival signal that results in beneficial levels of p38 and JNK. Our data reveal a non-autonomously activated ROS sensing mechanism driven by Ask1 and Akt to drive regeneration in the neighboring unstressed cells.

P12. Gene-Repair of point mutations at the endogenous locus using PolyPurine Reverse Hoogsteen hairpins in mammalian cells

Alex J. Félix, Anna Solé, Véronique Noé and Carlos J. Ciudad

Departament of Biochemistry and Physiology, School of Pharmacy, University of Barcelona. Av. Juan XXIII #27, cciudad@ub.edu

Repair of point mutations in the DNA is important for the correction of monogenic diseases. Repair-PPRHs are a new powerful tool within this field. A Repair-PPRH consists of a PPRH hairpin core bearing an extension sequence at one end, which is homologous to the DNA sequence to be repaired but containing the wild type nucleotide instead of the mutated one. Previously, we designed Repair-PPRHs to correct deletions, insertions, substitutions and a double substitution present in a collection of Chinese hamster ovary (CHO) cell mutants in the endogenous *locus* of the *dhfr* gene. Surviving colonies in a DHFR selective medium (lacking glycine-hypoxanthine-thymidine) were analyzed by DNA sequencing, mRNA and protein levels and enzymatic measurements, confirming that all the *dhfr* mutants had been corrected. To explore the generality of this methodology, we attempted to repair point mutations in a different gene, that coding for adenosyl phosphoribosyl transferase (*aprt*). By using different Repair-PPRHs we were able to correct nonsense mutations caused by a single substitution. In this case, surviving colonies were obtained by applying the AAT (adenine-aminoptherine-thymidine) selection and were analyzed by DNA sequencing and by mRNA expression. No off-target effects were detected when comparing the S23 mutant and the S23 repaired with HpS23E1rep-L after sequencing with a mean coverage of 26x. These results correspond to the set of 3158 contigs longer than 100Kb totaling around 90% of the genomic sequence (2.2 Gb), indicating no major mutational differences between the two samples. We did not see either any major bias when looking at the indels (insertion and deletions together) or only at the insertions in the treated cells. Moreover, any of the insertions detected within the variation set had similarity to the sequence present in the Repair-PPRH used for the treatment. These results demonstrate that Repair-PPRHs are able to achieve a permanent correction of point mutations in the DNA sequence of mammalian cells.

Acknowledgments: Work supported by grant SAF2014-51825-R

P13. Early *hippo* target genes in planarians

Daniel Font, Nídia da Sousa, Emili Saló and Teresa Adell

Department of Genetics, Microbiology and Statistics, University of Barcelona, Barcelona, Spain

Regeneration and tissue renewal are essential processes of adult homeostasis that must be tightly controlled, since its dysfunction may lead to cancer. The Hippo signaling pathway has recently emerged as a key hub in the control of cellular renewal being found systematically deregulated in tumoral processes. Several studies in animal models demonstrate the essential role of the Hippo pathway in the control of cell proliferarion, cell death and cell differentiation. However, the specific cellular function and molecular targets of the pathway remain poorly understood. Recent studies in our lab have demonstrated that *hippo* inhibition produces tumoral overgrowths in planarians, flatworms that endow a continuous tissue renewal while changing their size according to nutrients availability. In this *in vivo* context, we demonstrated that overgrowths are caused by the inability of *hippo*knockdown cells to maintain the differentiated fate, to properly cycle and to die when required. In the present study, we characterize the function of putative *hippo* target genes found deregulated in a transcriptomic analysis of *hippo* RNAi animals. The RNAi inhibition of some of these early target genes produced overgrowths similar to the ones observed after *hippo* inhibition. This connection hinters potential effectors contributing to the tumorigenic mechanisms upon *hippo* inhibition in planarians and, probably, in humans.

P14. Organ remodeling through the actomyosin cytoskeleton and programmed cell death: the trachea of Drosophila melanogaster as a case study

Juan J. Fraire-Zamora, Jérôme Solon and Jordi Casanova

Cell and Developmental Biology Programs, Centre for Genomic Regulation (CRG) and Institute for Research in Biomedicine (IRB), Barcelona. Dr. Aiguader 88, 08003 and Baldiri Reixac 10, 080028, Barcelona, Spain. juanjose.fraire@irbbarcelona.org

Many cellular processes are in play during organ remodeling. Most of the attention has focused on the role of progenitor cells and their transformation into adult tissues and organ growth, however, little is known about the mechanisms of organ regression. An example of this process is the removal of epithelial cells during mammary gland involution at weaning. The fruit fly Drosophila melanogaster offers an advantageous system to study organ remodeling at the cellular and molecular level. The larval trachea, a functional respiratory organ, is a modular network of tubes that must remodel during metamorphosis to give rise to the adult trachea. Our current work focuses on the cellular and molecular mechanisms that orchestrate the reduction in size of the main tracheal tube, the dorsal trunk. Concomitant to the migration of progenitor cells, the modules (i.e. metameres) of the dorsal trunk undergo a sequential reduction in length that correlates with a reduction in apical cell area and the appearance of actomyosin filaments along the longitudinal axis of the tube. Upon maximum reduction in tracheal length, we observe caspase activation and a consequent loss of posterior metameres of the dorsal trunk. These events result in a considerable reduction in size of the larval trachea at the same time that progenitors migrate to form the abdominal adult trachea. We are currently exploring the interplay between cell size reduction through actomyosin contractility and the activation of programmed cell death through apoptosis. We believe that such cellular and molecular interplay leads to the regression of the tubular dorsal trunk during metamorphosis of Drosophila melanogaster, as it occurs in other flat epithelial monolayers.

P15. Generation of Sanfilippo C syndrome cellular models using CRISPR/Cas9 system

Edgar Creus-Bachiller (1)(*), Noelia Benetó (1)(*), María García-Morant (1), Daniel Grinberg (1), Lluïsa Vilageliu (1), Isaac Canals (2)

(1)Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, CIBERER, IBUB, IRSJD, Barcelona, Spain; (2) Stem Cells, Aging and Neurodegeneration Group, Lund Stem Cell Center, University Hospital, Lund, Sweden.(*) Equal contributors.

Sección Biologia Molecular i Biologia del Desenvolupament. Facultat Biologia(UB): Avinguda Diagonal, 643, 08028 Barcelona.

mg_morant@hotmail.com

Sanfilippo syndrome is a rare lysosomal storage disorder (LSD) caused by mutations in genes that encode enzymes involved in heparan sulfate (HS) degradation. The accumulation of HS, due to mutations in these genes, causes a progressive and severe neurodegeneration in patients, leading to an early death. There is currently no cure for this disease. The main objective of this project is to generate cellular models for Sanfilippo C syndrome using induced pluripotent stem cells (iPSC). For that purpose, we used CRISPR/Cas9 system to knock-out (KO) the *HGSNAT* gene in iPSC cells, whose mutations cause Sanfilippo C syndrome. Moreover, we optimized a protocol to differentiate iPSC into neurons, the most affected cell type in this syndrome. Combining these two novel technologies, we will be able to obtain KO and WT differentiated neurons in order to analyze differences between these isogenic lines. With this approach, possible variations due to differences in the genetic backgroundWe aim to compare levels of HS storage between lines by immunocytochemistry. Furthermore, branching and spines measurements will be performed in GFP+ isolated neurons. To refine the analysis, synapses will be quantified combining specific antibodies for pre- and post-synaptic markers. To confirm that the phenotypes observed in the KO neurons are only caused by *HGSNAT* disruption, we will carry out a rescue experiment transfecting KO cells with a plasmid bearing the *HGSNAT*-WT cDNA. After transfection, we will perform all the assays in order to test whether or not the WT phenotype has been restored. We are confident that once this cellular model is validated, it will be valuable to test potential treatments such as the use of shRNAs as a substrate reduction therapy.

P16. Role of Aquaporins in ROS diffusion following damage

Viola Giacomo, Prieto Cristina, Corominas Montserrat and Serras Florenci
Departament de Genètica and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain

Drosophila wing imaginal discs are able to regenerate following different types of injury, which leads to the reconstruction of normal adult wings. Recent studies pointed out the importance of oxidative stress in driving the cellular response for wound repair and regeneration. Upon apoptotic stimuli or physical injuries, a burst of reactive oxygen species (ROS) is generated in the wing disc epithelium. This results in the activation of different signalling pathways (like JNK and p38) that are required for regeneration. A key issue is to unveil how reactive oxygen species (H_2O_2 in particular) spread from cells that are committed to die to the surrounding ones, however the mechanism of ROS propagation is poorly understood. In this study, we focus on two different *Drosophila* aquaporins: AQP and Drip as putative mechanisms of oxidative stress propagation. Our results indicate that, in stressing conditions, AQP is a key element of cell-to-cell communication facilitating ROS diffusion across membranes, thus allowing the onset of the regenerative stimulus. Interestingly, while AQP loss of function impairs the regenerative process, no significant defects were observed with Drip, suggesting that the function in regeneration is not shared among all aquaporins.

P17. New Cyclin D1 cytoplasmic interactors

Marta Guasch, Tània Cemeli, Noel P. Fusté, Francisco Ferrezuelo, Neus Pedraza, Eloi Garí.

IRB Lleida, Universitat de Lleida, Avinguda Alcalde Rovira Roure 80, 25198, Lleida

martaguasch@cmb.udl.cat

The cell cycle is a set of well-ordered events that allows the cell to divide into two cells genetically identical. The different stages of this cycle depends on the activity regulation of cyclins and cyclin dependent kinases (Cdk) complexes. For instance, cyclin D1 (Ccnd1)-Cdk4 complex accumulates into the cell nucleus and phosphorylates and inactivates the Retinoblastoma (RB1) transcriptional repressor. This allows the induction of a group of genes that promote progression from G1 into S phase. For many years, export of Ccnd1 to the cytoplasm was viewed as a mechanism to prevent the cell cycle entry and to reduce cellular proliferation. More recently it has been described that Ccnd1 physically interacts with proteins associated to the cell membrane, such as filamin A, PACSIN2, Ral GTPases and paxillin. Furthermore, our group has described that Ccnd1-Cdk4 complex modulates cell adhesion, migration and invasion through the regulation of some of those cytoplasmic proteins. In our lab, we have recently carried out an iTRAQ-based protein analysis that has produced a set of cytoplasmic proteins associated to the cell membrane that are new candidate interactors of Ccnd1. Among these there are a number of proteins involved in cell signalling. Here we report the validation of some of these candidates (PGRMC1, Dab2IP and Plekhh2) by co-immunoprecipitation with Ccnd1. Specifically, PGRMC1 protein (Progesterone Receptor Membrane Component 1) interacts with the N-terminal region of Ccnd1 and promotes its stabilization. In experiments in HEK 293T cells with the protein synthesis inhibitor cyclohexmide, we have observed that expressing PGRMC1 increases Ccnd1 half-life. In conclusion, we show preliminary data pointing out to a possible function of cytoplasmic Cyclin D1 in the regulation of cell signalling.

P18. Identifying wiring specificity mechanisms: what's up with the mTOR pathway?

Camille Guillard Sirieix, Qi Zhu, Marta Morey

Section of Genetics, Faculty of Biology, and Institute of Biomedicine of the University of Barcelona (IBUB)

A fundamental requirement in the assembly of neural circuits is that neurons establish synaptic connections with their appropriate partners. In many systems, this involves extension into a particular synaptic layer, and selection of the appropriate partner among all the cells in the layer. Virtually nothing is known about the mechanisms governing the establishment of specific connections in any system. Our hypothesis is that the molecular differences that exist between neuronal subtypes, with similar developmental origin and function, contribute to their distinct connectivity. We study the differential layer selection of the closely related R7 and R8 photoreceptors. Each eye contains 750 R7 and 750 R8 cells, and the entire population of each subtype proceeds synchronously to their respective final synaptic layer during pupal development. Taking advantage of such precise coordination, we have profiled the R7 and R8 transcriptomes right before this final extension. Our bioinformatic analysis has identified differentially expressed genes between the R7 and the R8. We have focused on 229 R8 enriched genes and performed an RNAi screen. Out of 186 genes analyzed we have identified 44 candidate genes showing layer selection defects. One of them is 4E-BP, an inhibitor of translation best known for its role in the mTOR pathway. Interestingly, the mTOR pathway has been shown to have a neurogenic role uncoupled from its well-known control of cell proliferation and growth. Following this lead we present future work exploring the role of 4E-BP in wiring specificity through detailed characterization of the mutant phenotype and genetic interactions with other members of the pathway.

P19. Study of sumoylation of *TRIM28* variants associated with intellectual disability

Isabel Hinarejos, Roser Urreizti, MI Isabel Alvarez-Mora, Laura Domènech, Irene Madrigal, Montserrat Milà, Xavier Estivill, Gemma Marfany, Daniel Grinberg, Raquel Rabionet

Universitat de Barcelona (Facultat de Biologia) Avinguda Diagonal, 643, 08028 Barcelona

isabelhinmar@gmail.com

Intellectual disability (ID) is characterized by high clinical and genetic heterogeneity. Sporadic cases of ID are frequently related to *de novo* mutations in neurodevelopmental genes. Our group has identified a *de novo* variant (p.P654L) in the *TRIM28* gene in a sporadic case of ID as the most likely causative variant; two additional ID patients harboring *TRIM28* mutations (p.L708P and p.A180V) have been identified through *Genematcher*. *TRIM28* is a nuclear corepressor for KRAB domain-containing zinc finger proteins (KRAB-ZFPs) expressed in several tissues, including the brain. TRIM28 mediates gene silencing by recruiting CHD3, a subunit of the nucleosome remodeling and deacetylation (NuRD) complex, and SETDB1, which specifically methylates histone H3 at 'Lys-9' (H3K9me), to the promoter regions of KRAB target genes. Sumoylation/desumoylation events regulate *TRIM28*-mediated transcriptional repression, as sumoylation is required for interaction with CHD3 and SETDB1 and the corepressor activity. The mutation we detected in this patient is located in the PHD-type zinc finger domain, which directs the sumoylation of the adjacent bromodomain required for gene silencing. Two different mutations in the same domain (p.C651A and p.L709A) have previously been shown to affect *TRIM28* sumoylation. We hypothesize that the mutations detected in the ID patients will affect *TRIM28* sumoylation, and thus its repression of gene expression. In order to prove this hypothesis, we are performing directed mutagenesis and sumoylation analysis of the mutant forms of TRIM28.

P20. DNA activates the Nse2/Mms21 SUMO E3 ligase in the Smc5/6 complex

Eva Ibars, Nathalia Varejão, Neus Colomina, David Reverter, Jordi Torres-Rosell

Institut de Recerca Biomèdica de Lleida-Universitat de Lleida

IRBLLEIDA, Dept. Ciències Mèdiques Bàsiques, Universitat de Lleida. 973702414, evaibars@cmb.udl.cat

Modification of chromosomal proteins by conjugation to SUMO is a key step to cope with DNA damage and to maintain the integrity of the genome. The recruitment of SUMO E3 ligases to chromatin may represent one layer of control on protein sumoylation. However, we currently do not understand how cells upregulate the activity of E3 ligases on chromatin. Here we show that the Nse2 SUMO E3 ligase in the Smc5/6 complex, a critical player during recombinational DNA repair, is directly stimulated by binding to DNA. We have identified a cluster of lysine residues in the coiled coil of Smc5 able to promote DNA-dependent upregulation of the Nse2 SUMO ligase activity. To test the relevance of these residues in budding yeast, we introduced different combinations of lysine to glutamic acid mutants to countercharge binding to phosphate groups of DNA. Using these *smc5-KE* mutants we show that compromising DNA binding to Smc5 sensitizes yeast cells to MMS-induced DNA damage. Accordingly, sumoylation of Smc5 itself and the Nse2-target Sgs1 protein (a homologue of the Bloom's and Werner's syndrome genes and a member of the STR complex) is impaired in *smc5-KE* cells. These defects are not due to defective binding of Nse2 to Smc5 or to impaired loading of Smc5/6 onto chromatin in *smc5-KE* alleles. Overall, we conclude that a positively charged patch in the Smc5 molecule acts as a DNA sensor in yeast, able to interact with DNA and to promote the activity of the Nse2 SUMO ligase thus ensuing repair of DNA damage. We propose that this mechanism restricts sumoylation in the vicinity of those Smc5/6-Nse2 molecules directly engaged on DNA.

P21. Role of Myoglianin in metamorphosis of *Blattella germanica*

Orathai Kamsoi, Xavier Belles

Institute of Evolutionary Biology (UPF-CSIC)

Passeig Marítim 47, 08003 Barcelona, Spain,

orathai.kamsoi@ibe.upf-csic.es, xavier.belles@ibe.upf-csic.es

The onset of insect metamorphosis occurs when juvenile hormone (JH) titer decrease in the pre-adult stage. In *Blattella germanica*, this is determined in the last nymphal instar (N6). At the beginning of N6, JH production declines, the expression of Kr-h1 (a transducer of the JH anti-metamorphic action) is concomitantly reduced, and that of E93 (an adult specifier factor) starts increasing. However, it remains unclear what determines the initial decline of JH production. In the cricket *Gryllus bimaculatus*, Ishimaru and coworkers (2016) demonstrated that Myoglianin (*myo*), a homolog of *Drosophila* Myoglianin/vertebrate GDF8/11, suppresses the expression of *jhamt*. *jhamt* is a gene that encodes the enzyme JH acid *O*-methyltransferase (JHAMT), which catalyzes the last step of JH synthesis in the corpora allata (CA), which are the JH producing glands. When *myo* expression is suppressed in last nymphal instar of the cricket, expression of *jhamt* became activated and metamorphosis is inhibited. In *B. germanica*, high expression levels of *myo* were observed in the CA of the penultimate nymphal instar (N5). Moreover, using RNAi approaches, we showed that *myo* depletion in N5 up-regulated *jhamt* expression in N6, and metamorphosis became inhibited. The *myo*-depleted insects molted to a supernumerary nymph (N7) instead to molt into adults. The results suggest that high *myo* expression in N5 promotes the initiation of metamorphosis in N6 through the cessation of JH synthesis. In addition, our results also suggest a conserved role of *myo* in regulating JH production and metamorphosis, at least in hemimetabolan insects.

P22. The Spectraplakin Short-Stop is an essential microtubule regulator mediating subcellular branching

Delia Ricolo, Miquel Lledós and Sofia J. Araújo

Department of Genetics, Microbiology and Statistics, University of Barcelona, Spain

Branching networks are a very common feature of multicellular animals and underlie the formation and function of numerous organs including the nervous system, the respiratory system, the vasculature and many internal glands. These networks vary from subcellular structures such as dendritic trees to large multicellular tissues such as the lungs. The production of branched structures by single cells, which has been better described in neurons and in cells of the respiratory and vascular systems, involves complex cytoskeletal remodelling events. In *Drosophila*, tracheal system terminal cells (TCs) and nervous system dendrites are models for these subcellular branching processes. During tracheal embryonic development, the generation of subcellular branches is characterized by extensive remodelling of the microtubule network and actin cytoskeleton, followed by vesicular transport and membrane dynamics. We have recently shown that centrosomes are key players in the initiation of subcellular lumen formation where they act as microtubule organizing centres (MTOCs) (Ricolo, et al. Cur. Biol. 2016). However, not much is known on the events that trigger the formation of these subcellular branches or what makes them choose a particular trajectory within the cytoplasm of the TC. We have identified that the spectraplakin *Shortstop* (s*hot*) is involved in the microtubule stabilisation events that lead to the formation and extension of the subcellular lumen. We observed that an excess of s*hot* induces more branching points in the embryonic tracheal TC leading to cells with extra subcellular lumina and that a *shot* loss-of-function leads to cells deficient in *de novo* subcellular lumen formation. In addition, we show that *shot* expression is intimately linked to the tip-cell fate, being modulated by the transcription factor DSRF.

P23. Differential expression of piRNAs during the ontogeny of Blattella germanica, a short germ-band, hemimetabolan insect

Natalia Llonga, Guillem Ylla, Josep Bau, Xavier Belles, Maria-Dolors Piulachs

Institute of Evolutionary Biology (CSIC-Universitat Pompeu Fabra).

Passeig Maritim de la Barceloneta, 37. 08003 Barcelona, Spain.

natalia.llonga@uvic.cat

Genomic stability is especially important in germ-line cells and in embryo stages for the insect development. For this reason the insect needs to control transposable elements (TEs). There is an evolutionary conserved mechanism, called piwi-interacting RNA (piRNA) system to control TEs. This system is common within species among metazoans and insects. To study piRNA system it is more suitable to use a long genome
with many TEs. Thus, we use the hemimetabolan insect Blattella germanica as a reference model. In the present work we have identified and studied the expressed piRNA in B. germanica across 11 developmental stages, with a stringent process that includes non-fertilized egg, embryo, nymphs and adult females. We have been able to classify the piRNAs from B. germanica based in their biogenesis (primary and secondary pathways). We found that the majority piRNAs identified are generated from the primary pathway, although there are a small but highly expressed set of piRNAs participating in the secondary ("Ping-Pong") reamplification pathway. Furthermore, we have analysed the expression pattern of all these piRNAs identified, observing that the expression of piRNA generated in the "Ping-Pong" pathway is quite restricted to early embryo stages. In addition, an important number of piRNA clusters are exclusively expressed in late embryo and nymphal stages. This study contributes to confirm the role
of the piRNA system controlling TEs in early embryogenesis, but also to show that the function of piRNAs are wider than previously thought, as we found different expression of piRNAs in different stages of development.

P24. Involvement of AGT1 in the cystinuria mouse model Slc7a9

Clara Mayayo-Vallverdú[1], Miguel López de Heredia[1], Isabel Cano-Morey[1], Laura González[1], Esther Prat[1,2], Virginia Nunes[1,2]

(1) U730, Human Molecular Genetics Laboratory, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain. (2) Genetic Unit, Physiological Sciences Departament, Health Science and Medicine Faculty, University of Barcelona, Spain.

Cystinuria (OMIM #220100) is a rare inherited aminoaciduria characterized by urine hyperexcretion of cystine and dibasic amino acids (lysine, arginine and ornithine). Its clinical manifestation is cystine lithiasis in the urinary system due to the low solubility of cystine at physiological urine pH that induces its precipitation and, as a consequence, stone formation. Cystinuria, with a prevalence of 1:7000 births, is the most common primary inherited aminoaciduria and, to date, genetic alterations in the renal amino acid transporters, *SLC7A9* ($b^{0,+}$AT) and *SLC3A1* (rBAT) have been identified as responsible for this manifestation. However, the lack of genotype/phenotype correlation in cystinuria patients justifies the search for modulating genes. AGT1, the second renal cystine transporter encoded by *SLC7A13*, heterodimerizes with rBAT in the renal apical membrane where mediates efflux of anionic amino acids in exchange for cystine. Urine aminograms, renal expression of rBAT and AGT1 analyzed by western blot, analysis of *Slc7a13* mRNA by RT-PCR in WT and mutant (*Slc7a9*$^{-/-}$) mice of both sexes were compared to study AGT1 contribution in amino acid reabsorption. Significant differences were observed in CssC, Glu, Asp urine concentration among genders. Male mice have 30-40 times more rBAT protein than females and, as previously shown, female mice showed no AGT1 protein in kidney BBMs although *Slc7a13* mRNA was detected in kidney preparations.

Financed by: FIS (PI13/00121 and PI16/00267)

P25. Wiring specificity and cell type specific signaling downstream of widely expressed cell surface molecules: the cytoplasmic molecule Espinas

Martí Monge Asensio, Alejandra Fernandez Pineda, Marta Morey

Section of Genetics, Faculty of Biology, and Institute of Biomedicine of the University of Barcelona (IBUB)

The exquisite precision with which neurons assemble into neural circuits has fascinated neuroscientists since Ramon y Cajal described how different types of neurons established connections with specific synaptic partners. Both biochemical and genetic approaches have revealed the significant role of cell surface molecules (CSM) in wiring specificity since they are final effectors of cell-cell contact events. However, while most CSM are widely expressed, they have specific roles in some neurons and not others. Some of these situations have been explained by CSM working in distinct combinations. Alternatively, specificity could come from differential expression of other types of molecules with the potential to regulate the function and/or signaling of CSM, such as for example intracellular cytoplasmic molecules. To address wiring specificity we use as a model system the Drosophila R7 and R8 photoreceptors, closely related neurons with differential layer selection. Through RNAseq transcriptional profiling comparisons of these two cell types we have identified the cytoplasmic molecule Espinas (Esn) significantly enriched in R8 versus R7 and characterized R8 targeting defects in *esn* mutants. Esn has been shown to physically and genetically interact with the atypical cadherin CSM Flamingo (Fmi) in Da neurons were it regulates dendritic self-avoidance. Interesting, Fmi is widely expressed in the fly visual system where *fmi* mutations result in R8 targeting phenotypes while R7 targeting is not affected. Our results suggest that Fmi and Esn also work together in R8 targeting. Our working hypothesis is that Esn cytoplasmic signaling could explain the differential functional outcome of R8 versus R7 Fmi expression, highlighting the importance of cytoplasmic molecules in wiring specificity.

P26. Identification and molecular characterization of adenosine A_2A—cannabinoid CB_1 receptor heteromers in the dorsal striatum as targets for Hungtinton disease

Moreno E[1,2], Chiarlone A[1,3], Galve-Roperh I[1,3], Ciruela, F[4], Beat Lutz[5], Krisztina Monory[5], Lluís C[1,2], Casadó V[1,2], McCormick, P.J[1,2,6*,] Guzmán, M[1,3*,] Canela, E.I[1,2*]. Estefaniamoreno@ub.edu.

[1]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas; [2]Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain; [3]Department of Biochemistry and Molecular Biology, Complutense University, Madrid, Spain; [4]Department of Pathology and Experimental Therapeutics, IDIBELL, UB, Spain; [5]Institute of Physiological Chemistry, University Mainz, Germany; [6]School of Pharmacy, University of East Anglia, Norwich, UK. *Co-last authors.

The dorsal striatum is a key node for many neurobiological processes such as motor activity, cognitive functions, and affective processes. The proper functioning of striatal neurons relies critically on metabotropic receptors. Specifically, the main adenosine and endocannabinoid receptors present in the striatum, ie, adenosine A_{2A} receptor ($A_{2A}R$) and cannabinoid CB_1 receptor (CB_1R), are of pivotal importance in the control of neuronal excitability. Facilitatory and inhibitory functional interactions between striatal $A_{2A}R$ and CB_1R have been reported, and evidence supports that this cross-talk may rely, at least in part, on the formation of $A_{2A}R$-CB_1R heteromeric complexes. However, the specific location and properties of these heteromers have remained largely unknown. Here, by using techniques that allowed a precise visualization of the heteromers in situ in combination with sophisticated genetically modified animal models, together with biochemical and pharmacological approaches, we provide a high-resolution expression map and a detailed functional characterization of $A_{2A}R$-CB_1R heteromers in the dorsal striatum. Specifically, our data unveil that the $A_{2A}R$-CB_1R heteromer (i) is essentially absent from corticostriatal projections and striatonigral neurons, and, instead, is largely present in striatopallidal neurons, (ii) displays a striking G protein-coupled signaling profile, where co-stimulation of both receptors leads to strongly reduced downstream signaling, and (iii) undergoes an unprecedented dysfunction in Huntington's disease, an archetypal disease that affects striatal neurons. Altogether, our findings may open a new conceptual framework to understand the role of coordinated adenosine endocannabinoid signaling in the indirect striatal pathway, which may be relevant in motor function and neurodegenerative diseases.

P27. Transcriptome analysis of Salmo trutta. Dating the whole genome and local duplication events in Salmonidae and identifying positive selected transcripts in salmo speciation

Mariona Pascual-Pons[1], Neus Oromi[1], José Luís Royo[1], Antoni Palau[2] and Joan Fibla[1]

[1]Institute of Biomedical Research of Lleida (IRBLleida). University of Lleida. 25196 Lleida. Spain. 973702299 (joan.fibla@cmb.udl.cat)

[2]ENDESA, 25005 Lleida. Spain.

Understanding animal adaptation and its underlying molecular basis is an important role in evolutionary biology. A prevailing hypothesis is that such adaptation has been favored by gene duplication events that provide large amounts of material for evolutionary adaptation. Evolution in vertebrates is marked by numerous duplication events, both whole-genome duplication (WGD) and local gene duplications (LGD) as well as gene losses. One example of recent whole-genome duplications in vertebrate is the salmonid-specific WGD, which occurred subsequent to the common teleost WGD event. It is assumed that WGD provided the teleost with diversification potential that can become effective much later, such as during phases of environmental change. In this study, genome-wide and local gene duplication events in the salmonids were investigated. A de novo transcriptome sequencing strategy was used to characterize the
transcriptomes of two key organs of Salmo trutta: telencephalon and muscle. We identified over 140000 sequences. Potential unique expressed transcripts were annotated
by sequence homology to databases and tissue expression was determined. An analytic workflow was designed to distinguish between orthologues and paralogues originated from LGD or from WGD events. The phylogenetic reconstruction and dating of these events was performed using the Ks ratio of neutral mutations between species. The identification of positive selected transcripts was performed using Ka/Ks ratio. Result allow dating both teleost's WGD and salmonid's WGD. In addition, we found evidence for LDG took place shortly after the speciation event. From Salmo trutta – Salmo salar speciation event a total of 158 positive selected transcripst (Ka/Ks>0.5) were characterized by anatomical structure (n=24), KEGG pathways (27) and GO orthology (101). Enrichment for Xenobiotics and Glycan metabolism pathways was detected among selected transcripts.

P28. Role of the transcription factor E93 in the oogenesis of *Blattella germanica*

Ariadna Pedraza and Maria-Dolors Piulachs

Institut de Biologia Evolutiva - IBE- (CSIC-UPF)

Pg. Marítim de la Barceloneta 37-49. 08003 Barcelona

ariadna.pedraza.12@gmail.com

E93, also known as "adult specifier", is the key gene for metamorphosis in both holometabolous and hemimetabolous insects. By using *Blattella germanica* as a model of hemimetablolous insect with the most ancient type of ovaries (panoistic type), our aim is to study the function this transcription factor has in both the capacitation and maturation of the ovaries, the main processes involved in the oogenesis. The expression of E93 is depleted by using interference RNA technique during the last nymphal stage. The depletion of E93 results in a change in the adult phenotype which makes us describe the adults obtained as intermediate adults (Ai), because they show both nymphal and adult structures. The ovarian changes due to the E93 depletion are also studied by immunofluorescence. We also study the changes that this depletion may cause to the other components of the Juvenile Hormone (JH) signalling pathway and the Ecdysone signalling pathway, in which E93 plays a regulation role. Ai, where JH is not produced, provides us with a unique animal model where we can study the effects E93 has in the ovary development in a JH free system.

P29. *tbx5a* in left/right asymmetry in zebrafish

Aina Pi-Roig[1] , Carolina Minguillón[2] and Jordi Garcia-Fernàndez[1]

[1] University of Barcelona, Biology Faculty: Genetics, Microbiology and Statistics Dpt

[2] Pasqual Maragall Foundation

Tbx5 is a transcription factor expressed in the developing heart, eyes and anterior appendages. Mutations in human TBX5 cause Holt-Oram syndrome, a condition characterized by heart and upper limb malformations. In our lab, we identified a novel *tbx5* gene in zebrafish (*tbx5b*) that is co-expressed with its paralogue (*tbx5a*). *tbx5* paralogues downregulation in zebrafish revealed that *tbx5* genes have essential roles in the establishment of cardiac laterality, dorsoventral retina axis organization and pectoral fin development. Our data show that distinct relationships between *tbx5* paralogues are required in a tissue-specific manner to ensure the proper morphogenesis of the three organs in which they are expressed. Additionally, we uncover a novel role for *tbx5* in the establishment of heart asymmetry in zebrafish embryos. Further analysis of *tbx5a* role during left-right (LR) asymmetry establishment show that the expression of left-side markers expressed in the lateral plate mesoderm (LPM) is also randomized. We also detected a randomization of *lefty1* expression in the dorsal diencephalon in *tbx5a* morphant embryos and that the display of the endodermal structures of the liver, pancreas and gut is also affected after *tbx5a* morpholino knock-down.

To our surprise when we specifically knocked-down tbx5a in the DFCs/KV (dorsal forerunner cells / Kupffer's vesicle) lineage, responsible for LR asymmetry generation, cardiac jogging was randomized. Interestingly, we observed a stronger phenotype with this DFC-targeted injection. Furthermore, we detected by ISH and RT-PCR early *tbx5a* expression during gastrulation. Finally, a reduction on BMP signalling levels in DFC-targeted *tbx5a* morphants was observed and consistently, a putative binding site for tbx5 was found in the *bmp4* regulatory region after an *in silico* analysis, pointing towards a regulatory mechanism that would at least partially rely on BMP signalling as a downstream effector.

P30. Glial ionic homeostasis in brain development

Haritz Plazaola-Sasieta, Qi Zhu , Marta Morey

Department of Genetics, Microbiology and Statistics, Faculty of Biology and Institute of Biomedicine of the University of Barcelona (IBUB)

Glia-neuron interactions are crucial during development and the correct function of the adult nervous system. The mammalian CLCN2 chloride channel is widely expressed in the brain. While its expression in oligodendrocytes and astrocytes is related to K+ buffering in myelinated processes, its physiological role in other glial types during development is unknown. In humans, in addition to vacuolization and edema of the brain due to disruption of the myelin sheath, patients with mutations in *ClCN2* have cognitive defects and *CLCN2* has been linked to autism spectrum disorders. Together these observations suggest that *CLCN2* could play a role in the assembly if neural circuits. We turned to the Drosophila visual system as a convenient structure to address glial cell biology and glia-neuron interactions. We have detected expression of the *CLCN2* Drosophila homolog gene *ClC-a* in cortex glia and several other glial types in the developing brain. Mutations in *ClC-a* result in brain compartimentalization defects due to cortex glia impaired ionic function. We focused on the *ClC-a* expressing glial barrier, which acts a landmark for photoreceptor axon guidance. The glia-photoreceptor interaction in early development of the visual system is mediated by Slit/Robo signaling. Slit secretion from glial cells is necessary for the correct guidance of photoreceptors. Similar to optic lobe specific *slit* mutations, *ClC-a* mutant animals showed defects in photoreceptor axon guidance. Through detailed developmental analysis we have characterized the formation of the barrier, the glial types contributing to the barrier and their Slit expression in wild type and mutant animals. Our findings indicate that ClC-a is required during development for the correct assembly of the glial barrier and Slit signaling. We propose that, in addition to its relevance in adult brain physiology, glial ionic homeostasis is an important aspect of brain development.

P31. Monoamine oxidase A (MAOA) interaction with parenting practices on Callous-Unemotional Traits in Preschoolers

Pueyo Ayhan, N., Blas Navarro, J., Fatjó-Vilas, M., de la Osa, N., Penelo, E., Fañanás, L., Ezpeleta, L.

Departament de Psicologia Clínica i de la Salut. Edifici B., Universitat Autònoma de Barcelona. 08193 Bellaterra (Barcelona) SPAIN

natalia.pueyo@e-campus.uab.cat

Purpose: Monoamine oxidase A (MAOA) is a mitochondrial enzyme that catalyzes the degradation of several biogenic amines. A polymorphism in the *MAOA* gene (*MAOA*-uVNTR) results in differential enzyme activity: high-activity alleles (*MAOA*-H) could result in reduced dopamine, serotonin and norepinephrine availability in comparison to low-activity allele (*MAOA*-L). *MAOA*-uVNTR has been described to moderate the relationship between childhood maltreatment and aggressive and antisocial behavior. This study hypothesized that in interaction with other environmental factors such as parenting practices, *MAOA* might also be associated with increased callous-unemotional traits (CU) in preschoolers. CU traits have been associated with more severe antisocial behavior.

Methods: In a longitudinal study, data was collected from a sample of preschoolers through diagnostic interviews and questionnaires answered by parents and teachers. *MAOA*-uVNTR was genotyped in 368 Caucasian participants (51.9% male). Multiple linear regression analyses were conducted to analyze the interaction effect of *MAOA* genotypes and both positive parenting and punitive parenting practices on CU traits at two different periods (3 and 5 years old) and separately by sex.

Results: No significant interactions were found for boys. Among girls, a significant interaction effect was found for *MAOA*-LL carriers, who showed higher CU traits at age 5 when exposed to higher positive or punitive parenting.

Conclusions: This study provides the first evidence for significant MAOA × parenting effects on CU traits in preschoolers, specifically among female MAOA-LL carriers.

P32. Comparison between the operation of different kinases in the development of *Drosophila melanogaster*'s embryos

Daniel Rius Carreras, Meritxell Chamayou and Enrique Martin-Blanco

Instituto de Biología Molecular de Barcelona (IBMB-CSIC)

Parc Cientific de Barcelona, Baldiri Reixac 10-12, 08028 Barcelona, Spain

The MAPK signalling cascades are key elements implementing cellular responses in numerous developmental and physiological processes. These cascades can act individually or in parallel and frequently display cross-regulatory interactions. Further, they implement negative and positive feedback loops that complicate the identification of their singular roles. Three main MAPK pathways, ERK, JNK and P38, have been identified characterized by the specificity of their phosphorylation targets. In order to obtain information on the functions of JNK, p38 and ERK, in the *Drosophila* embryo, we used kinase translocation reporters (KTRs). These reporters are peptides carrying a GFP fluorescent marker that when are phosphorylated by a specific kinase are exported out of the nuclei. Otherwise if this phosphorylation does not happen another a nuclear localization sequence is revealed and the fluorescent marker is translocated to the nuclei. We have generated transgenic animals carrying KTR reporters specific for JNK, ERK and P38 that can be expressed in different tissues and times using the Gal4/UAS system. Preliminary experiments targeting these sensors to the epithelia (Pannier-Gal4), the mesoderm (Mef2-Gal4) and both, the epidermis and the nervous system in restricted domains (En-Gal4) have let us to identify specific differences and dynamics in the activity of these three kinases in different tissues and processes. We are now in the way to characterize in more detail these differences. The use of these sensors will eventually allow us to infer cross-regulatory interactions between these pathways and with other signalling elements in epistatic analyses.

P33. Regulation of mitochondrial function through Hippo pathway signalling underlies tumoral transformation

Maria Rosselló, Nídia de Sousa, Emili Saló and Teresa Adell

Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona.

Avinguda Diagonal, 643, 08028 Barcelona.

Email: mrossexa7@alumnes.ub.edu

The Hippo pathway is a master regulatory network that regulates cell proliferation, cell death and cell differentiation in response to the environment. Its deregulation leads to the unbalance of this processes and to the establishment of a variety of diseases including neurodegeneration and cancer. Its targeting appears as a powerful medical tool to improve regeneration and to prevent tumoral transformation. However, its precise molecular outs and inputs remain poorly understood. Recent results from our lab demonstrate that inhibition of hippo in planarians, flatworms which continuously change their size and renew their tissues according to nutrition, leads to overgrowths. Those overgrowths are caused by a decrease in apoptosis, aberrant mitosis and the inability of cells to maintain the differentiated fate. With the aim to understand the hippo targets responsible for the tumoral transformation we performed a transcriptomic analysis of hippo RNAi animals. The results revealed an enrichment of differentially regulated genes with a mitochondrial function. RNAi inhibition of those genes produced overgrowths similar to the ones observed after hippo inhibition. We are currently analyzing whether inhibition of mitochondrial genes also deregulates apoptosis, mitosis and cell differentiation. Our findings support that mitochondrial function could be involved in the tumoral transformation that occurs after hippo inhibition.

P34. Analysis of ATPase-Dependent Binding of the Smc5/6 Complex onto Chromatin

Marc Tarrés, Jordi Torres-Rosell

Institut de Recerca Biomèdica de Lleida – UdL, Avda Alcalde Rovira Roure nº80, 25198, Lleida

marc.tarres@cmb.udl.cat

The three eukaryotic SMC complexes, cohesin, condensin and Smc5/6, play essential roles in genomic integrity. All SMC proteins display a rod-shaped structure with one ATPase domain at one end, connected to a hinge domain through a long coiled coil. Pairs of SMC proteins heterodimerize through their hinge domain. A kleisin subunit then connects the two ATPase heads at the other end, closing a ring-shaped structure able to entrap DNA. Previous work from our lab has shown that the Smc5/6 complex is essential to remove different type of sister chromatid junctions, including recombination and replication intermediates arising in response to replication fork damage. Their removal probably requires the engagement of Smc5/6 on chromatin. However, very little is known about what promotes Smc5/6 association with chromatin. Here we have analyzed the role of the ATPase and observed that binding of ATP is required for Smc5/6 loading onto chromatin. In fact, the ATPase activity is not only necessary to mediate the interaction with DNA but also to ensure its association with the kleisin subunit. Besides, we have observed that loading of Smc5/6 is maximal towards the end of the S phase, and can be induced by replication fork damage. Using various mutants affected in the metabolism of sister chromatid junctions, we show that binding of the complex onto chromatin is not triggered by the presence of junctions; in contrast, the complex seems to directly bind damaged forks, before they are channeled into different repair and bypass pathways. These findings suggest that the Smc5/6 complex binds early onto damaged forks in an ATPase-dependent manner, to promote the subsequent removal of junctions.

P35. Exploring the function of relevant retinal genes: animal and cellular models

Vasileios Toulis[1], Izarbe Aísa Marín[1], Carlos de la Peña Ramirez[1], Gemma Marfany[1,2,3]

[1]Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 643, 08028 Barcelona, Spain

[2]Institut de Biomedicina de la Universitat de Barcelona (IBUB-IRSJD), Barcelona, Spain

[3]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Barcelona, Spain

vtoulis@ub.edu

Cell fate decisions during the differentiation and maintenance of specific neuronal retinal cell types are controlled by transcriptional factors and other regulatory proteins. In order to shed light on the mechanisms regulating retinal development, we generated animal and cell models to study the role of two genes associated to human neurodegenerative disorders, namely *ATXN3*, which encodes a deubiquitinating enzyme involved in hereditary ataxia, as well as *NR2E3,* a retinal dystrophy gene that encodes a transcription factor required for cone and rod differentiation. The function of ATXN3 was analysed by observing the retinal phenotype of *Atxn3* knockout (KO) mice. A significant elongation of photoreceptor cilia and outer segments was observed in the KO retinas by immunofluorescence and transmission electron microscopy. These results were confirmed in an *in vitro* cell model, since silencing of endogenous *ATXN3* caused elongation of primary cilia. We are currently exploring the implication of ATXN3 in both cilia formation and control of phagocytosis, whose alteration is causative of retinal dystrophy. To address the function of NR2E3, we have generated several mutant alleles with small and large deletions of *Nr2e3* using the CRISPR/Cas9 system in mouse. The retinal phenotype of these mouse models is being currently analysed in wildtype, heterozygous and homozygous mouse littermates. Besides, in order to dissect the molecular function of the genome-edited alleles, *in vitro* cell models have been generated by transfection of minigenes designed to express the CRISPR-generated mutations. Our preliminary results show that both genes are relevant for retinal development and photoreceptor function.

P36. Injury, repair and regeneration of the Drosophila larval CNS

Víctor Valencia, Nuria Clotet, Giulia Mencattelli and Enrique Martin-Blanco

Instituto de Biología Molecular de Barcelona (CSIC)

Parc Cientific de Barcelona, Baldiri Reixac 10-12, 08028 Barcelona, Spain

vb_victor003@hotmail.com

The neuroblasts, the stem cells of Central Nervous System (CNS) of Drosophila undergo two waves of neurogenesis: the first one during embryogenesis, and the second one during the second larval stage L2. Those two waves are separated by a period of quiescence (a 24 hours window), during the first larval stage (L1) in which neuroblasts do not divide. This quiescent period gives us the opportunity of studying the reactivation of proliferative capabilities of stem-like cells during repair and regeneration. To target specific cells to necrotic death (mimicking traumatic injury), we employed the Gal4/Gal80ts system and overexpressed a mutated variant of the Glutamate Receptor (GluR1Lc), which directs the accumulation of intracellular calcium, the toxic death of cells and necrotic spreading. We alternatively employed Reaper, an apoptotic response inducer, to distinguish the behavior of the tissue to different types of insult. In first place, in search for Gal4 lines with distinct patterns of expression, we selected a set of lines (Flylight database (Janelia, HHMI)) and characterized their pattern of expression in first instar larvae. This let identifying lines active at this period with restricted patterns. We initiated our analyses employing the RN2 Gal4, which is just specifically expressed in the RP2, aCC, pCC and MP2 inter and motoneurons. Secondly, we set up genetic and dissecting protocols aimed to explore the response of the tissue. Death induction was temporally controlled and the tissue let to recover via temperature shifts. Activating expression during 12 hours at the beginning of the first instar larvae and letting them recover for another 16 hours we could monitor robust death responses. In third place, we initiated the characterization of the tissue recovery and potential regeneration by studying both cell proliferation and structural modifications at or around wounded areas. Interestingly, associated to local induced proliferation, we observed “scar-like” tubulin-rich structures. We are now set out to investigate what are those structures and how do they participate in wound recovery.

P37. The role of Wnt modulation in the derivation of mouse embryonic stem cell lines from single blastomeres isolated from 8-cell embryos

Marta Vila-Cejudo, Elena Ibáñez and Josep Santaló

Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biociències, Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain

marta.vila@crg.eu

Naïve pluripotency displayed by mouse embryonic stem cells (mESC) depends on the activation of the Wnt pathway and the inhibition of the MAPK pathway. On the other hand, epiblast stem cells, which show a primed pluripotency, depend on the inhibition of the Wnt pathway, suggesting that this pathway plays a critical role in maintaining both pluripotent states. In this study, the effect of signalling modulations on mESC derivation from isolated blastomeres from 8-cell embryos has been analysed. To do so, blastomeres were cultured with the Wnt activator CHIR99021 (CHIR), the Wnt inhibitor IWR-1-endo (IWR) and the MAPK inhibitor PD0325901 (PD), either alone or combined. Non-treated (NT) blastomeres were used as the control group. After the validation of the mESC established, Wnt transcriptional activation, assessed by AXIN2 levels, and the state of pluripotency of the mESC lines, assessed by FGF5 levels and alkaline phosphatase (AP) activity, were determined. Blastomeres from NT, IWR or CHIR groups resulted in the lowest derivation rates (1.4%-4.9%), whereas the combination of any two of the inhibitors increased the derivation rates (15%-24.7%). On the other hand, mESC lines derived from the NT, CHIR or CHIR-PD (2i) groups displayed high AXIN2 levels, corresponding to a high Wnt-transcriptional activity, whereas the lowest levels were displayed by mESC treated with IWR. Concerning FGF5 levels and AP activity, only mESC derived from CHIR- or 2i-treated blastomeres actually acquired the features of naïve pluripotency, showing basal levels of FGF5 and high AP activity. By contrast, IWR-CHIR and IWR-PD treatments induced the expression of features of the primed pluripotency state. Surprisingly, mESC derived from the NT, IWR or PD groups displayed features of both pluripotency states, suggesting that they are in an intermediate state between naïve and primed pluripotency. To conclude, the activity of Wnt pathway plays a key role in pluripotency maintenance in mESC lines established from single blastomeres, originating mESC lines displaying features of either naïve or primed pluripotent states by modulating this pathway.

P38. The regulatory genome of Drosophila regeneration

Elena Vizcaya[1], Cecilia C. Klein[2], Florenci Serras[1], Rakesh Mishra[3], Roderic Guigó[2] and Montserrat Corominas[1]

1. Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona. Barcelona, Catalonia, Spain

2. Centre for Genomic Regulation (CRG), Universitat Pompeu Fabra, Barcelona, Catalonia, Spain

3. The Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India.

elenavizcayamolina@gmail.com

The ability to regenerate varies greatly not only between species but also between tissues and organs or developmental stages of the same species. Differential activation of the genome, determined by a complex interplay of regulatory elements functioning at the level of chromatin, must be the initial mechanism behind these different regenerative capabilities. Resetting gene expression patterns during injury responses is, thus, shaped by the coordinated action of genomic regions that integrate the activity of multiple sequence specific DNA binding proteins. *Drosophila* imaginal discs, which show a high regenerative capacity after genetically induced cell death, are a great model to interrogate chromatin function trough the regeneration process. Using genome- wide approaches (RNA-seq and ATAC-seq) at different tissue time points after injury we have identified the regulatory elements and the expression profile dynamics governing the process. Our findings point to a global co-regulation of gene expression and provide evidence for a regeneration program driven by different types of Damage Responsive Regulatory Elements (DRRE). Among them, novel-DRRE are found acting exclusively in the damaged tissue, and cooperating with DRRE co-opted from other tissues and developmental stages. Altogether, our results decipher the regulome of regeneration and suggest the existence of a specific toolkit to drive the regenerative capacity.