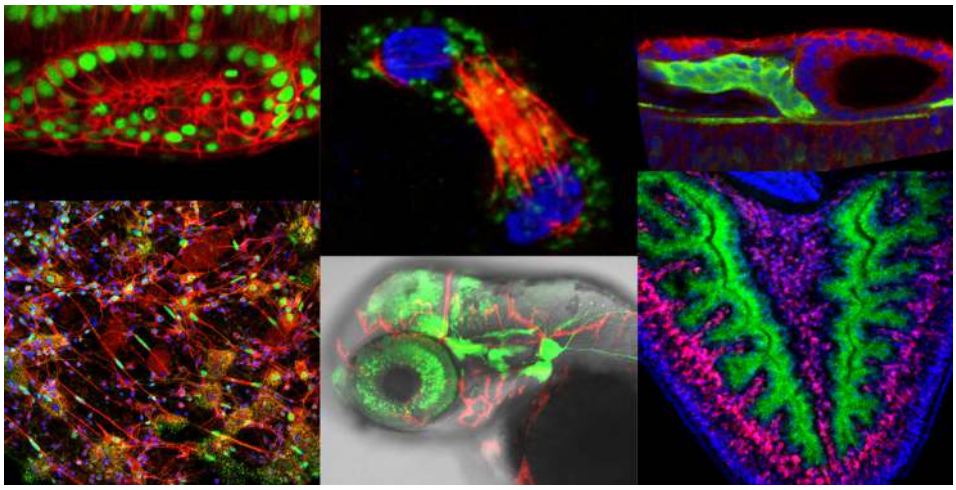
Institut d'Estudis Catalans

Societat Catalana de BIOLOGIA



XXXIII DEVELOPMENTAL BIOLOGY MEETING

Date: February 2nd 2022

Location: Sala Prat de la Riba, Institut d'Estudis Catalans,
C/ Carme 47, Barcelona

Organizers:
Marta Morey
Francesc Cebrià
(Section of Developmental Biology of the SCB)

XXXIII JORNADA DE BIOLOGIA DEL DESENVOLUPAMENT
SOCIETAT CATALANA DE BIOLOGIA

Wednesday, February 2nd 2022

8:45-9:15 Registration
9:15 Welcome by the Coordinator of the Section of Developmental Biology
9:20-9:45 **Valeria Venturini "The nucleus as a mechano-controller of migration plasticity "**
9:45-10:10 **Esteban Hoijman "Protecting the early embryo using cooperative protrusions"**
10.10-10.25 José Esteban "Contribution of TNFa as an early signal to stress driven regeneration"
10.25-10:40 Mª Dolores Molina "Planarian *cbp-2* and *cbp-3* play complementary roles during stem cell maintenance and differentiation"
10:40-10:55 Pablo Coronel-Córdoba "FoxK1 and FoxG are key transcription factors for cell differentiation and patterning during planarian regeneration"

11:00-12:00 Coffee break and poster session I

12.00-12:15 Marina Ruiz "A transcriptional program conserved across metazoans guides tissue differentiation in development"
12:15-12:30 Sandra Acosta "Enhancers with tissue-specific activity are enriched in intronic regions "
12:30-12:45 Jacqueline Severino "A temporally controlled sequence of X-chromosome inactivation and reactivation defines female mouse in vitro germ cells with meiotic potential"
12:45-13:35 **Cristian Cañestro "Matters of the heart, deconstruction and ancestral tunicate sessility"**

13:35-14:35 Lunch

14:35-14:50 Samuel Ojosnegros "Mechanotransduction of human and mouse embryo implantation"
14:50-15.05 Nerea Montedeoca "Elucidation of the role of sall1a during hair cell development in a zebrafish model using the CRISPR/Cas9 and CRISPR/Cas13d systems"
15:05-15.20 Ettore de Giorgio "Chitin deposition in the apical extracellular matrices of *Drosophila melanogaster*: a focus on Expansion, Rebuf and Chitin Synthases"
15:20-16:10 **Alfonso Martínez-Arias "Embryos, gastruloids and the Turing conjecture"**

16:10-17:10 Coffee break and poster session II
17:15 Concluding remarks and awards

Use the hashtag #DevBioSCB2022 @ Instagram and Twitter

INVITED SPEAKERS

The nucleus as a mechano-controller of migration plasticity

Valeria Venturini

Cell and Tissue Dynamics Group, Centre for Genomic Regulation (CRG) and Barcelona Institute of Science and Technology

Single cells in living tissues undergo shape deformations due to crowding, tissue-scale stresses or during cell migration. Cell compression, as occurring in confined microenvironments, induces myosin II activation and associated with an increase in cell contractility and can transform non-motile cells into a fast-amoeboid migration mode in various cell types. How single cells decode shape deformations and respond to shape changes by regulating their contractility setpoint remained unclear. We show that the cell nucleus functions as an elastic mechano-gauge that allows singe cells to autonomously measure shape changes. Nuclear stretch upon compression induces inner nuclear membrane unfolding that activates cytosolic phospholipase A2 (cPLA2), triggering a calcium-dependent mechanosensing pathway that regulates myosin II activity via arachidonic acid (AA) release. Highly contractile cells spontaneously acquire a stable-bleb phenotype and rapidly escape from the confined environment, reminiscent of an escape-reflex mechanism. Furthermore, nuclear stretch combined with intracellular nucleus positioning enables cells to distinguish different types of shape deformations, exemplified by anisotropic cell deformation in confinement and isotropic cell swelling. Our data support that the nucleus establishes a functional module for cellular proprioception, enabling cells to sense and interpret shape changes and to dynamically adapt their behavior to the 3D microenvironment.

Protecting the early embryo using cooperative protrusions

Esteban Hoijman

Facultat de Medicina I Ciències de la Salut, Universitat de Barcelona

Errors in early development are frequent and considered a main cause of human preimplantation failures. We found a phagocytic system present in the early embryo able to remove defective stem cells dying by apoptosis. Our quantitative live imaging approach allowed us to reveal the phagocytic character of the first tissue formed during vertebrate development, the superficial epithelium of the blastula. Using the zebrafish and mouse models, we found that apoptotic clearance relies on the mechanical cooperation between different types of epithelial protrusions, optimizing the tissue clearance efficiency. This protective tissue establishes the earliest immune functions operating during vertebrate ontogeny.

Matters of the heart, deconstruction and ancestral tunicate sessility

Cristian Cañestro

Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (Spain)

A central question in chordate evolution is the origin of sessility in adult ascidians, and whether the appendicularian complete free-living style represents a primitive or derived condition among tunicates. According to the “a new heart for a new head” hypothesis, the evolution of the cardiopharyngeal gene regulatory network (GRN) appears as a pivotal aspect to understand the evolution of the lifestyles of chordates. During our studies to investigate gene loss as an evolutionary force, we have discovered that appendicularians suffered the “deconstruction” of cardiopharyngeal GRN due to massive ancestral losses of cardiopharyngeal genes and subfunctions. Using *Oikopleura dioica* as a model to understand heart development in appendicularians, our results show that these losses can be related to the loss of cardiopharyngeal multipotency. Our findings suggest an evolutionary scenario in which the deconstruction of the cardiopharyngeal GRN can be related to processes of regressive and adaptive evolution during the transition from an ancestral tunicate with an ascidian-like sessile life style to a free-living style in appendicularians. Our results, moreover, highlights the relevance of also studying gene loss and the deconstruction of GRNs to better understand the evolution and diversification of life.

Embryos, gastruloids and the Turing conjecture

Alfonso Martínez Arias

Systems Bioengineering, DCEXS, Universitat Pompeu Fabra

More than one hundred years before genomic conservation revealed the relatedness between different animals, the work of KE von Baer and E. Haeckel had already suggested such relationships. Furthermore, this work has suggested that gastrulation results in a common body plan that then is elaborated in a species-specific manner. This idea has been later elaborated upon by D. Duboule with the notion of the evolutionary hourglass according to which, early in development embryos from different organisms are very different, as are at the end but go through a transition period in which are very similar: the phylotypic stage that would correspond to the root similarity suggested by von Baer and Haeckel.

In the course of our work with Embryonic Stem cells, we have created a model of early embryonic development that mimic many aspects of mammalian gastrulation. We call the structures that we create 'gastruloids". We can create similar structures from animal caps from zebra fish and, surprisingly, the resulting structures are more similar to mouse gastruloids than the embryos from the different species are to each other. This observation has led us to revisit the hourglass model and to a number of considerations about the relationship between cells and genes in the laying down of the body plan that I shall discuss in my talk.

SHORT TALKS

Contribution of TNFα as an early signal to stress driven regeneration

José Esteban-Collado[1], Mar Fernández-Mañas[1,2], Paula Santabárbara[3], Montserrat Corominas[1] and Florenci Serras[1]

[1]Universitat de Barcelona, IBUB
[2]Hospital Clínic
[3]Institut Pasteur

Drosophila imaginal discs are able to undergo wound healing and regenerative growth after ablation. Several signals that participate in regeneration, such as reactive oxygen species (ROS), calcium and nutrient availability are sensed by the MAP3 kinase Ask1, which in turn activates downstream by phosphorylation the MAP kinases p38 and JNK. Apoptosis can occur as the result of sustained or high activation of these kinases, whereas short or low activation can promote regeneration. Therefore, the regulation of Ask1 is pivotal for driving regeneration and it is imperative to identify the molecules involved in this regulation. The Tumour Necrosis Factor (TNF) is a good candidate to participate in the Ask1-driven regeneration as is known to operate upstream JNK. *Drosophila* has a single orthologue of the TNF, called eiger (egr). Egr is a membrane protein that needs to be cleaved and binds to its receptors Wengen (Wgn) and/or Grindelwald (Grnd). We found that Eiger activates a survival pathway through the receptor Wengen and that this Wgn protective role is mediated by Traf1, Ask1 and p38 signalling. Furthermore, in the regeneration context Egr is expressed after tissue damage and acts as a survival signal. Wgn but not Grnd is necessary for regeneration and this Egr-Wgn survival pathway is mediated by p38 activation. This strengthens the evidence that the TNF pathway is not only involved in JNK activation but also in p38 signalling. In addition, we describe a new role of the receptor Wgn, under stress conditions, necessary for the activation of the p38 signalling pathway.

ST2

Planarian *cbp-2* and *cbp-3* play complementary roles during stem cell maintenance and differentiation

Mª Dolores Molina[1,2], Susanna Fraguas[1,2], Anna Guixeras[1], Carlotta Viana[1], Luisa Riedel[1], Marta Marín[1,2], Sergio Castillo-Lara[1,2], Josep Francesc Abril[1,2], Francesc Cebrià[1,2]

[1] Department of Genetics, Microbiology and Statistics. Faculty of Biology, University of Barcelona

[2]Institute of Biomedicine of the University of Barcelona (IBUB)

Characterizing how stem cells self-renew and differentiate is pivotal to understand how tissue renewal and regeneration occurs naturally, as well as to push forward the field of regenerative medicine in its search to use stem cell-based therapies. Freshwater planarians maintain an adult population of pluripotent stem cells, called neoblasts, and have become an attractive model to study stem cell-based regeneration. However, despite recent major advances, still little is known about the role that post-translational modifications and epigenetics play in the regulation of neoblast biology. Our laboratory is currently investigating the function of the conserved gene family of CBP/p300 acetyl transferases in the planarian *Schmidtea mediterranea*. RNAi-based functional experiments have uncovered distinct roles for two planarian *cbp* orthologs: *Smed-cbp-2* seems essential for stem cell maintenance and cell survival, while the silencing of *Smed-cbp-3* impairs neoblast differentiation and results in the growth of blastemas that remain largely undifferentiated, even after one month of regeneration. As a further step to better understand the function of these genes, we have recently performed high-throughput experiments based on ATACseq and RNAseq. Bioinformatic analyses support the hypothesis of the complementary roles of *cbp-2* and *cbp-3* on stem cell maintenance and differentiation, and suggest that planarian *cbp* genes might impact neoblast biology through their functions in the progression of the cell cycle and the composition of the neoblast niche.

ST3

FoxK1 and FoxG are key transcription factors for cell differentiation and patterning during planarian regeneration

P. Coronel-Córdoba, M. Dolores Molina, E. Pascual-Carreras, G. Cardona, S. Fraguas, F. Cebrià, E. Saló, T. Adell

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

Forkhead box (Fox) genes belong to the 'winged helix' transcription factor superfamily which encode for transcription factors that control key aspects of development. Few Fox members have been studied from the Lophotrochozoa clade, and specifically from planarians, which are a unique model for understanding development and regeneration, due to the striking plasticity of the adult. A prior study of our lab identified 27 fox genes in the planarian *Schmidtea mediterranea* (*Smed*), which were classified in 12 families. In the present study we characterize the role of two Fox families: *FoxK* and *FoxG*. The foxK family comprises 3 *foxK* paralogs (*foxK1-3*). Through the analysis of the regeneration of *foxk1* RNAi animals, which shows the strongest phenotype, we demonstrate that *foxK1* is required for regeneration of ectodermal tissues, including the nervous system and the epidermis. The FoxG family, presents a unique paralog in the *Smed* genome. Here we show that *foxG* inhibition prevents planarian posterior regeneration by inhibiting the expression of *wnt1*, the canonical WNT responsible to form the posterior organizer during regeneration. Altogether, these results show that *foxK* and *foxG* families have an essential role during planarian regeneration, possibly acting as a pioneer factors during cell determination and differentiation.

A transcriptional program conserved across metazoans guides tissue differentiation in development

Marina Ruiz-Romero[1], Cecilia C. Klein[1,4], Sílvia Pérez-Lluch[1], Amaya Abad[1,$], Alessandra Breschi[1,#], Roderic Guigó[1,2].

[1]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88, Barcelona 08003, Spain
[2]Universitat Pompeu Fabra (UPF), Barcelona, Spain
[4] Departament de Genètica, Microbiologia i Estadística and Institute of Biomedicine (IBUB), Universitat de Barcelona. Barcelona, Catalonia, Spain
[#] Current address: Tempus Labs, Inc.
$ Current address: Center for Applied Medical Research (CIMA), University of Navarra and Institute of Health Research of Navarra (IdiSNA), Pamplona, Spain.

During development, most cells undergo striking changes in order to develop into functional tissues. All along this process, the identity of each tissue arises from the particular combination of regulatory transcription factors that specifically control the expression of relevant genes for growth, pattern formation and differentiation. In this scenario, regulation of gene expression turns out to be essential to determine cell fate and tissue specificity. Although many studies aim to decipher tissue signature through the analysis of their transcriptome profiles, most lack temporal information during development and, in consequence, many differentiation events are poorly understood. To characterize dynamic transcriptional profiles during differentiation, we tracked down the transcriptome of committed cells throughout differentiation of eye, leg and wing of *Drosophila melanogaster*. We found that during fly development, temporal transcriptional changes shared across lineages are much larger than spatial lineage-specific transcriptional changes, and that cellular differentiation is dominated by a transcriptional program, common to multiple lineages, that governs the transition from undifferentiated to fully differentiated cells independently from the differentiation end-point. The program is under weak epigenetic regulation, and it is characterized by downregulation of genes associated with cell cycle, and concomitant activation of genes involved in oxidative metabolism. Transcriptome comparisons with worm, mouse and human, reveal that this transcriptional differentiation program is broadly conserved within metazoans.

Enhancers with tissue-specific activity are enriched in intronic regions

Beatrice Borsari[1,6], Pablo Villegas-Mirón[2,6], Sílvia Pérez-Lluch[1], Isabel Turpin[2], Hafid Laayouni[2,3], Alba Segarra-Casas[2], Jaume Bertranpetit[2], Roderic Guigó[1,4], Sandra Acosta[2,5]

[1]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona 08003, Catalonia, Spain
[2]Institut de Biologia Evolutiva (UPF-CSIC), Universitat Pompeu Fabra, Barcelona 08003, Catalonia, Spain
[3]Bioinformatic Studies, ESCI-UPF, 08003, Barcelona, Spain
[4]Universitat Pompeu Fabra (UPF), Barcelona 08003, Catalonia, Spain
[5]Department of Pathology and Experimental Therapeutics, Medical School, University of Barcelona, 08907, L'Hospitalet de Llobregat, Catalonia, Spain

Tissue function and homeostasis reflect the gene expression signature by which the combination of ubiquitous and tissue- specific genes contribute to the tissue maintenance and stimuli-responsive function. Enhancers are central to control this tissue-specific gene expression pattern. Here, we explore the correlation between the genomic location of enhancers and their role in tissue-specific gene expression. We find that enhancers showing tissue-specific activity are highly enriched in intronic regions and regulate the expression of genes involved in tissue-specific functions, whereas housekeeping genes are more often controlled by intergenic enhancers, common to many tissues. Notably, an intergenic-to-intronic active en- hancers continuum is observed in the transition from developmental to adult stages: the most differentiated tissues present higher rates of intronic enhancers, whereas the lowest rates are observed in embryonic stem cells. Altogether, our results suggest that the genomic location of active enhancers is key for the tissue-specific control of gene expression.

A temporally controlled sequence of X-chromosome inactivation and reactivation defines female mouse in vitro germ cells with meiotic potential

Jacqueline Severino[1], Moritz Bauer[1,9], Tom Mattimoe[1], Niccolò Arecco[1], Luca Cozzuto[1], Patricia Lorden[2], Norio Hamada[3], Yoshiaki Nosaka[4,5,6], So Nagaoka[4,5,6], Holger Heyn[2], Katsuhiko Hayashi[7], Mitinori Saitou[4,5,6] ,Bernhard Payer[1,8]

[1]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain

[2]CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

[3]Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Kyushu University, Maidashi, Higashi-ku, Fukuoka, Japan

[4]Institute for the Advanced Study of Human Biology (ASHBi), Kyoto University, Kyoto, Japan

[5]Department of Anatomy and Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[6]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

[7]Department of Stem Cell Biology and Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

[8]Universitat Pompeu Fabra (UPF), Barcelona, Spain

[9]Present address: Oncode Institute, Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, the Netherlands

The early mammalian germ cell lineage is characterized by extensive epigenetic reprogramming, which is required for the maturation into functional eggs and sperm. In particular, the epigenome needs to be reset before parental marks can be established and then transmitted to the next generation. In the female germ line, reactivation of the inactive X-chromosome is one of the most prominent epigenetic reprogramming events, and despite its scale involving an entire chromosome affecting hundreds of genes, very little is known about its kinetics and biological function. Here we investigate X-chromosome inactivation and reactivation dynamics by employing a tailor-made in vitro system to visualize the X-status during differentiation of primordial germ cell-like cells (PGCLCs) from female mouse embryonic stem cells (ESCs). We find that the degree of X-inactivation in PGCLCs is moderate when compared to somatic cells and characterized by a large number of genes escaping full inactivation. Nevertheless, PGCLCs that fail to undergo X-inactivation show an abnormal gene expression signature and deficiencies in meiotic entry. Subsequent to X-inactivation we observe gradual step-wise X-reactivation, which is mostly completed by the end of meiotic prophase I. Cells deviating from these progressive kinetics and undergoing X-reactivation too rapidly fail to enter a meiotic trajectory. Our data reveals that a fine-tuned X-inactivation and -reactivation cycle is a critical feature of female germ cell developmental competence towards meiosis and oogenesis.

Mechanotransduction Of Human and Mouse Embryo Implantation

Anna Seriola[1], Amélie Godeau[1], Marc Casals[1], Ester Aroca[1], Oren Tchaicheeyan[2,3], Shahar Goren[2,3], Anna Veiga[4], Montse Boada[5], Miquel Sole[5], Mónica Parriego[5], Ayelet Lesman[2,3], Samuel Ojosnegros[1]

[1]Bioengineering in Reproductive Health, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), 08028 Barcelona, Spain.
[2]School of Mechanical Engineering, Faculty of Engineering, Tel-Aviv University, Tel-Aviv, 55 Chaim Levanon St., Ramat Aviv 69978, Israel.
[3]Center for Physics and Chemistry of Living Systems, Tel Aviv University, Tel-Aviv, 55 Chaim Levanon St., Ramat Aviv 69978, Israel.
[4]Barcelona Stem Cell Bank, Institut d'Investigació Biomèdica de Bellvitge, IDIBELL, 08908, L'Hospitalet del Llobregat, Spain
[5]Dexeus Mujer, Gran Vía Carles III, 08028 Barcelona, Spain

The mammalian embryos initiate their development autonomously until they reach blastocyst stage soon after fertilization. At that point, the embryo attaches to the endometrium, the tissue lining the mother uterus. Upon attachment, the blastocyst invades the thick maternal tissue and elongates, transitioning from a radial to a bilateral symmetry. We hypothesize that mechanical forces may be involved in both the trophoblast invasion and axis elongation. However, due to the inaccessibility of implantation, we have very limited insight on the mechanisms allowing the maternal tissue invasion and symmetry breaking.

To overcome that roadblock, we developed a novel *ex-vivo* method which allows post-implantation embryo development and is amenable to light microscopy. We combine this method wit a Digital Volume Correlation algorithm (DVC) so we can perform traction force microscopy on live embryos. Mouse transgenic embryos were imaged using confocal microscopy and human embryos were imaged in a label-free set up using multiphoton illumination of autofluorescent signals. The DVC quantifications unveiled the strong tension generated by mammalian embryos during implantation and matrix invasion. The mouse embryo applied tension on the matrix anisotropically, typically through 1-to-3 axes and in a pulsatile fashion. The tension creates a rim of collagen around the embryo and remodels the orientation of the surrounding fibres. The human embryos instead, exert forces isotropically and embed themselves in the collagen matrix by sinking in. Escaping cells can be observed in the trophoblast of the human embryo.

Our results indicate that implantation is a remarkably mechanosensitive process. The embryos align their implantation axis in the direction of external forces exerted by a variety of samples such as neighbouring embryos, cell spheroids or a pulling microneedle. Furthermore, the direction of the external force also determined the formation of the proximo-distal axis of the embryo, thus contributing to the symmetry breaking and the formation of the first developmental axis.

Altogether, we have observed the process of implantation of human and mouse embryos in 4D (x,y,z, and t) at unprecedented level of detail. Our results indicate a key role of mechanical forces during implantation, not only in guiding the invasion of the extracellular matrix, but also during patterning. Our work sheds light onto a classical unsolved question in developmental biology, which is the transition from radial to bilateral symmetry in the mammalian embryo.

Elucidation of the role of sall1a during hair cell development in a zebrafish model using the CRISPR/Cas9 and CRISPR/Cas13d systems

Nerea Montedeoca, Aitor Bañón, Laura Fargas, Berta Alsina

Universitat Pompeu Fabra

Townes-Brocks syndrome is an autosomal dominant disorder characterized by mutations in the Spalt-like family transcription factor SALL1. Its clinical presentation involves the impairment of multiple organs. These include malformation of the limbs and ears, cardiomyopathy, renal dysfunction, imperforation of the anus and high-frequency sensorineural hearing loss. Most mutations described in this type of hearing loss have been linked to genes that are key in hair cell development. In Drosophila, the developmental role of sall1 in some organs has been previously described. Nevertheless, the role of Sall1 in the vertebrate inner ear and how it might be causing hearing loss remain elusive. We established that sall1a, one of the two zebrafish paralogues of the human SALL1 gene transcription factor, is expressed in differentiated hair cells in the inner ear from 48 hpf onwards after the expression of brn3c, suggesting that it might be involved in hair cell maturation. As a means of assessing the involvement of sall1a in hair cell maturation, we performed two different gene-editing and gene-modulating strategies in parallel in a brn3c:GFP zebrafish transgenic line. In our first approach, we co-injected two CRISPR/Cas9 guides flanking the sall1a zinc-finger domains in 1-cell stage embryos in order to generate a knockout. As for our second approach, we designed two CRISPR/Cas13 guides targeting the mRNA and co-injected them to generate a knockdown in sall1a expression. The injected embryos were stained with the vital dye DIASP, which is uptaken by functional and mature hair cells, and performed confocal in vivo imaging at different timepoints. Later on, we compared the numbers of both GFP+ and DIASP+ hair cells between injected and control embryos. Both approaches showed a significant decrease in GFP+ and DIASP+ hair cells in comparison to controls, which was more pronounced in the case of the CRISPR/Cas13 injection. This might be indicative of an earlier action of this system, which, in contrast to CRISPR/Cas9, can also target maternal mRNA, which is essential during the first hours of embryo development. Moreover, it hints to sall1a holding a role in the maturation and maintenance of hair cells in the auditory and vestibular systems. We are currently performing transcriptomic analysis of purified hair cells in control and sall1a knockout embryos to uncover the target genes that play a role in hair cell differentiation and function. In addition, we are establishing different sall1a KO/Tg(Brn3c:GFP) zebrafish lines displaying either large sall1a deletions or frameshift mutations upstream of its zinc finger domains. Altogether, our study highlights the relevance of using zebrafish for modelling complex diseases in the development of sensory systems, the broad availability of genetic tools for editing and transcriptional modulation and the ease and convenience for performing functional testing in vivo.

Chitin deposition in the apical extracellular matrices of *Drosophila melanogaster*: a focus on Expansion, Rebuf and Chitin Synthases

Ettore De Giorgio, Marta Llimargas

Institut de Biologia Molecular de Barcelona (IBMB), CSIC, Parc Científic de Barcelona, C/ Baldiri Reixac, 10-12, 08028, Barcelona, Spain

Chitin has a recognised importance in physiology but also as a biomaterial. In insects, it is the principal component of the apical extracellular matrix of epidermis, tracheae, foregut and hindgut. In these compartments, chitin is synthesised by the family A of Chitin Synthases, which in *Drosophila* is encoded by the gene *kkv*. Kkv alone is not sufficient to deposit chitin, and two interchangeable MH2-containing proteins, namely Expansion (Exp) and Rebuf (Reb), are equally required for chitin deposition. Kkv and Exp/Reb constitute the minimal genetic program necessary and sufficient for chitin deposition. In this work, we investigated the molecular mechanisms of activity of Exp, Reb and Kkv in the tracheal system. Through a structure-function analysis approach, we focused our attention on domains that we speculated could be involved in putative protein-protein interactions between these factors. We found that the Nα-MH2 domain of Exp and Reb is necessary for the extracellular chitin deposition, but it is not involved in the subcellular localisation of the chitin machinery. We identified a new highly conserved region of Exp/Reb that may be relevant for Exp/Reb localisation but it does not seem to be involved in chitin deposition. On the other hand, our results suggest that Exp/Reb proteins appear to regulate the organised distribution of Kkv at the apical membrane. From the structure-function analysis of Kkv, we found out that the conserved motif WGTRE is involved in Kkv subcellular localisation and that mis-localised Kkv is not able to deposit chitin, indicating that the localisation of the protein is important for its function. Instead, the coiled-coil domain of Kkv is dispensable for the chitin deposition activity of Kkv.

POSTERS

Session I **P1**

Development and patterning of tail muscle cells in appendicularian tunicates

Marc Fabregà-Torrus, Beatriz Iralde Cárdenas, Marcos Plana-Carmona, Alba Almazán, Anna Moncusí Moix, Gaspar Sánchez-Serna, Alfonso Ferrández-Roldán, Ricard Albalat, Cristian Cañestro

Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (Spain)

Understanding how ancestors of each chordate subphyla were is essential for understanding our own origins. Tunicates are considered the sister group of vertebrates. A recent study from our lab of the cardiopharyngeal gene regulatory network suggested that the last common ancestor of tunicates had an ascidian-like sessile lifestyle, and the fully free-living style of appendicularians was an innovation of this lineage upon the invention of the house. To better understand the transition from the sessile to the free-living style, we are now focusing our investigation on the development of axial muscle along the tail, since this feature is key for the evolution of the new lifestyle. Our analyses reveal independent duplications and expansions of muscle genes in different tunicate lineages, and complex patterns of expression pattern that illuminate a complex patterning of different cell identities along the anterior posterior axis.

Mechanosensing cation channels and calcium signaling involvement in mechanical-driven induction of RG cells

Soriano-Esqué JP[1], Borau C[2], Asín J[3], García-Aznar JM[2] and Alcántara S[1]

[1]NDlab, Department of Pathology and Experimental Therapeutics, Institute of Neurosciences, Universitat de Barcelona, IDIBELL
[2]Multiscale in Mechanical and Biological Engineering (M2BE), Department of Mechanical Engineering, University of Zaragoza
[3]Department of Statistical Methods, University of Zaragoza

Mechanobiology afford the interaction between physical stimuli and cell behaviour in a wide range of scales. At cellular level, mechanobiology contributes to explain cell proliferation, migration and cell fate. During brain development, radial glia (RG), the principal embryonic neural stem cell, form a radial palisade spanning the entire neuroepithelia. We previously described an RG-niche mimetic material (poly (methyl methacrylate) (PMMA) with 2µm linear topographies (ln2PMMA)) that mimics RG surface properties and topography. Ln2PMMA physical signals induce the conversion of cultured astrocytes into functional RG-like cells. However, little is known about the molecular mechanisms by which RG-linage cells sense and respond to these mechanical signals.

Here we used cerebral cortex primary astrocyte enriched cultures from newborn mice, grown for 3DIV in control (glass) and Ln2PMMA substrates. RG-like cells were identified by simultaneous expression of nestin and Pax6. We have developed a custom-made MATLAB algorithm, similar to high-throughput image analysis, to correlate cell and nuclear morphology with specific cell-lineage markers (GFAP, nestin, Pax6, NG2 and Sox2).

Ln2PMMA significantly reduce GFAP+ astrocytes with concomitant increase in double positive nestin/Pax6 RG-like cells. Then, we analyze by WB and RT-PCR the expression of a subset of mechanosensing channels (transient receptor potential and acid-sensing ion channels) in both substrates. We found a significant protein reduction in TRPA1 together with a significant increase in TRPC1 and, at the limit of significance, ASIC1 in ln2PMMA. All of them are non-selective mechanosensing cation channels. Then, we used a mechanosensitive and stretch-activated ion channel inhibitor (GsMTx-4), and a specific CaMKII inhibitor (KN93). Pharmacological inhibition of mechanosensitive cation channels or calcium signaling prevents the increase of double positive nestin/Pax6 cells, indicating their involvement in ln2PMMA induction of RG-like cells. In summary, our data point to an essential role of mechanosensing cation channels and calcium signaling in mechanical-driven induction of RG cells.

DYRK1A kinase is necessary to sustain neurogenesis in the developing dorsal and ventral telencephalon

Alejandro Trujillano[1], María José Barallobre[1], Mariona Arbonés[1]

[1]Instituto de Biología Molecular de Barcelona (IBMB), CSIC, 08028 Barcelona, Spain

DYRK1A (Dual specificity Tyrosine Phosphorylation Regulated Kinase 1A) is a dosage-dependent protein kinase that has a conserved role in brain growth across evolution. The overexpression of DYRK1A contributes to some of the neurodevelopmental features presented in Down Syndrome while its haploinsufficiency causes a neurodevelopmental syndrome characterized by autistic traits and microcephaly. *Dyrk1a* null mutant mice die at the onset of neurogenesis. Here we report the early brain development of a Nestin-Cre: *Dyrk1a*$^{Fl/Fl}$ conditional knockout mouse model (c*Dyrk1a*-KO) in which the expression of DYRK1A is abrogated in all neural progenitors at the onset of neurogenesis. c*Dyrk1a*-KO mice die at birth. At the end of embryonic development, subcortical structures in c*Dyrk1a*-KO mutant brains are absent or severely affected. The neocortex of these mutants also shows alterations in laminar organization and a deficit of neurons. Studies performed along neurogenesis, from E11 to E17, indicates that the deficit of neurons in both ventral and dorsal c*Dyrk1a*-KO brain regions results from an exacerbated DNA damage response leading to an apoptotic cell death. This cell death seems to be mediated by p53 (TP53) activation and is more prominent and earlier in time in the ventral brain regions than in the dorsal, where most neocortical neurons are generated. At the onset of neurogenesis, the levels of the DYRK1A substrate Cyclin D1 in dorsal neural stem cells (NSC) is significantly higher in c*Dyrk1a*-KO embryos than in the controls. However, c*Dyrk1a*-KO NSCs proliferate at slower rates mainly due to a significant lengthening of the S phase. Moreover, c*Dyrk1a*-KO embryos show defects in early NSC differentiation. In summary, these results indicate that DYRK1A is necessary to cope with replication-derived DNA damage in neurogenic NSCs and confirm the essential role of the kinase in NSC cell decisions. The severity of the ventral phenotype observed in c*Dyrk1a*-KO brains suggest that alterations in the number of ventral-generating neurons are contributing to neocortical circuit formation in patients with an imbalance dosage of DYRK1A.

scRNAseq of patient derived ventral midbrain organoids shed light on mechanisms of a-syn accumulation in Parkinson's Disease

Veronica Testa[1], Franz Ake[2], Arianna Colini Baldeschi[1], Merixtell Pons Espinales[1], David Piñol[1], Irene Fernandez Carasa[1], Giulia Carola[1], Yvonne Richaud[2,3], Angel Raya[2,3,4], Alysson Muotri, Mireya Plass[2], Antonella Consiglio[1]

[1]Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital-IDIBELL, 08908 Hospitalet de Llobregat, Spain; Institute of Biomedicine (IBUB) of theUniversity of Barcelona (UB), Barcelona, Spain
[2]Regenerative Medicine Program, Bellvitge Biomedical Research Institute (IDIBELL), andProgram for Clinical Translation of Regenerative Medicine in Catalonia (P-CMRC), Hospital Duran i Reynals, Hospitalet de Llobregat, 08908 Barcelona, Spain
[3]Centre for Networked Biomedical Research on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain
[4]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Parkinson's disease (PD) is an incurable disorder of old age characterized by the selective loss of dopaminergic neurons (DA) within the ventral midbrain (VM) and the presence of α-synucleinprotein (α-Syn) in intracytoplasmic inclusions termed Lewy bodies. Despite decades of intense research, the mechanisms underlying PD onset and progression are for the most part still unknown. Understanding how genes and environment come together to increase our propensity to the development of PD is crucial to identify better ways to prevent the disease or to find a cure. Here, we have used patient induced pluripotent stem cells (hiPSC) harbouring LRRK2 PDcausing mutation and its respective isogenic control, to develop human midbrain organoids (mOs). These organoids show dynamic changes in cell populations during their development and maturation over several months and recapitulate several disease hallmarks such as DAn neurodegeneration. Taking advantage of single-cell transcriptomics, we have characterized the cell populations present in the organoid before DA neurodegeneration starts. These analyses identify a dopaminergic neural population that shows clear changes in gene expression in the PD samples compared to the isogenic controls, shedding light into the mechanisms of DA neurodegeneration in PD. Together, our analyses elucidate the role of LRKK2 in α-Syn accumulation and DA neurodegeneration in PD. These findings contribute to the understanding of the early molecular and cellular pathogenic mechanisms underlying PD onset and progression, and identify new targets for future development of early diagnostic tests and therapeutic interventions.

Modelling metastasis in adult Drosophila

Patricia Montes[1], Jamie Adams[2], Alba Vicario[1], Kyra Campbell[2], Andreu Casali[1]

[1]Institut de Recerca Biomèdica de Lleida Fundació Dr. Pifarré (IRBLleida), 25198, Lleida, Spain
[2]Bateson Centre, Department of Biomedical Science, University of Sheffield, Western Bank, S10 2TN, Sheffield, UK

The process of metastasis, or how the cells spread outside the primary tumor and colonize distant organs, remains poorly understood despite is key importance as it's responsible for more than 90% of cancer-related deaths. Our laboratory developed two colorectal cancer (CRC) models in adult Drosophila: one non-metastatic (with mutant clones for Apc and Ras) and one metastatic (with mutant clones for Apc, Ras and Snail). Snail overexpression eventually leads to dissemination of primary tumor cells, survival in the hemolymph and growth onto secondary macroscopic metastatic tumors. We are able to track the tumors because they also express GFP, and we can quantify the tumor burden as they express Luciferase. It's a powerful *in vivo* system genetically tractable and amenable to high-throughput analysis. Our laboratory is currently using both models to perform a genetic screening aimed to identify genes and cellular mechanisms underlying successful cell migration, in which the metastatic cascade begins. We aim to assess their potential as therapeutic compounds targets. It recently came out an interesting gene called Baiser, whose human homolog is TMED10, involved in vesicular protein trafficking. Reduction of Baiser in Apc-Ras clones causes a significant increase in the number of circulating tumor cells (CTCs), suggesting a putative role as a metastatic suppressor, and becoming a new model of metastatic CRC model in Drosophila.

Oxidative stress and tumour suppressor Pten in tissue regeneration

Iván Sopena-Majós, José Esteban-Collado, Montserrat Corominas, Florenci Serras

Department of Genetics, Microbiology and Statistics, School of Biology, University of Barcelona and Institute of Biomedicine of the University of Barcelona (IBUB), Diagonal 643, 08028 Barcelona, Spain

Regenerative biology pursues to unveil the genetic and signalling networks triggered by tissue damage. After injury, apoptotic or damaged cells produce reactive oxygen species (ROS), which signal neighbouring living cells. In these cells, ROS activate the Insulin pathway which is necessary for tissue recovery. Akt is the kinase downstream the Insulin pathway that phosphorylates Ask1's Ser83, a key event for p38 signalling activation during regeneration. We have previously demonstrated that upon damage, Akt is activated in a ROS dependent manner. Whether ROS target Akt or any other gene product of the insulin pathway is unknown. Phosphatase and tensin homolog (Pten) antagonises Pi3K-Akt, as *Pten* overexpression results in a drop of Akt activity, and loss-of-function results in high activation of Akt. It has been suggested that Pten is susceptible to ROS oxidation by inducing the formation of a disulphide bond between two cysteine residues and in turn results in Pten inactivation. To explore if Pten is the ROS sensor upstream of Akt in the Insulin pathway, we used *Drosophila* imaginal discs and exposed them to oxidants. Our data proved this experimental system sensitive, showing a significant increase in p-Akt levels after oxidant exposure. Hence, so far we demonstrated that Insulin pathway can be activated by H2O2-induced oxidative stress.

Development of a targeted integration approach by CRISPR-Cas9 in O. dioica in the absence of NHEJ

Rodríguez, Eva; Ferrández Roldán, Alfonso; Cañestro, Cristian

Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (Spain)

CRISPR-Cas has become an essential technique to determine gene's function by eliminating or silencing genes or even to achieve gene integration in many model and non-model organisms. In Oikopleura dioica, an emergent tunicate model to study gene loss in chordates, it has been recently described that CRISPR-Cas functions with fidelity and efficiency in O. dioica. The exceptional absence of the canonical nonhomologous end joining repair system (c-NHEJ) in O. dioica4 makes CRISPR-Cas technique to generate high rates of indels, especially in regions with 4-5 microhomology repeats present near both fragment ends. However, CRISPR-Cas has never been used to integrate genes in the genome of O. dioica. We are currently developing a a transgene targeted integration approach using the CRISPR-Cas system. We aim to use a fluorescent reporter gene under a strong promoter to target genes of interest. On our screenings, we expect the presence of fluorescence when the integration is successful. When the fluorescence is accompanied by knock-out phenotype may indicate a biallelic integration. To achieve this aim we are using the novel homology-mediated end joining (HMEJ)-based strategy that uses CRISPR-Cas9-mediated cleavage of both transgene donor that contains guide RNA target sites and around 800 bp of homology arms, and the targeted genome due to this system has been described to have a much greater efficiency than homologous recombination (HR), microhomology-mediated end joining (MMEJ) or non-homologous end joining (NHEJ)-based strategies. By using different guide RNAs (gRNAs) and transgenes with different homology lengths we want to analyze the efficiency of this technique in O. dioica which will improve to increase the molecular tools for gene manipulation available for this organism.

Neural plasticity during tissue remodeling

Marina Recatalà, Marta Morey

Departament de Genètica Microbiologia i Estadística, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona

Over the past decades we have learned a great deal about how the nervous system assembles into functional circuits during development. Much less is known about the plasticity capacity of established adult circuits. Neural plasticity includes axonal regeneration when a neuron is damaged, but also the reshaping of surrounding intact nervous tissue. Hence, understanding neural plasticity and how to modulate it will be essential to restore the functionality of circuits. We use Drosophila melanogaster digestive tract as a model system. It has a relatively simple innervation, mostly from the peripheral nervous system, which is known for its plasticity capacity in many organisms including the fly. The fly gut also has an extraordinary tissue remodeling ability, responding with changes in organ size to metabolic events, tissue damage and cancer. We will characterize the neural plasticity response of intact nervous tissue to: 1) epithelial damage induced by controlled cell death and regeneration (reduction of organ size and recovery to normal size), and 2) the presence of a tumor in a model of colorectal cancer (increase in organ size). Our final goal is to investigate the signals emanating from the remodeling tissue that shape neural plasticity, and signals from the nervous system shaping tissue remodeling. This inter-organ communication has been documented in vertebrates during regeneration and cancer but is still poorly understood.

ATAC-seq analysis identifies possible *Smed-cbp3* target genes required for neoblast differentiation

Anna Guixeras[1*], Susanna Fraguas[1,2*], Ma Dolores Molina[1,2], Sergio Castillo-Lara[1,2], Marta Marín[1], Josep Francesc Abril[1,2], Francesc Cebrià[1,2]
*Both authors share first authorship

[1]Department of Genetics, Microbiology and Statistics. Faculty of Biology, University of Barcelona
[2]Institute of Biomedicine of the University of Barcelona (IBUB)

Planarians are used as a model organism because of their remarkable regenerative capabilities. They are able to regenerate any part of their whole body thanks to the presence of pluripotent stem cells: the neoblasts. How the differentiation of the neoblasts in all cellular lineages occurs remains still unclear. CBP (CREB- binding protein) proteins belong to a conserved gene family which functions as a transcriptional co-activator and shows an important role in a wide range of cellular processes, such as stem cell proliferation and differentiation, cell death, DNA damage response and tumorigenesis, in many organisms. CBPs have an acetyl transferase activity that is relevant for histone and non-histone acetylation, including a high number of transcription factors, producing epigenetic marks and changing chromatin architecture that affects gene expression. In our laboratory, we have characterized the function of Smed-cbp-3, which seems to regulate stem cell differentiation. In order to gain insights into the function of cbp-3 we have recently carried out ATAC-seq experiments to identify changes in chromatin architecture after silencing cbp-3. Here, we characterize the expression pattern and function of a set of genes located in chromatin regions differentially open in controls and cbp-3 RNAi animals. These results open the door to further investigate the epigenetic regulation of planarian stem cells.

Novel genetic players in subcellular branching and cell guidance in *D.melanogaster*

Laia De La Torre, Sofia J. Araújo

Department of Genetics, Microbiology and Statistics of the University of Barcelona and Insititute of Biomedicine of the University of Barcelona (IBUB)

Embryonic development is the joint of processes, which implies a lot of morphogenetic changes, that leads to the complete formation of an individual from a fertilized egg. Each of its organs are formed throughout these stages by different cellular processes. Migration and branching processes are important processes involved in the nervous system and the tracheal system formation in *Drosophila melanogaster*. How cells branch or migrate at the correct time and place and what are the molecular mechanisms implicated are essential for the modulation of this cell behavior during development and regeneration, as branching and cell guidance processes require a precise temporal and spatial coordination. The aim of this project is the understanding of different cell branching and migration phenotypes in the tracheal and nervous systems respectively, in order to unveil new molecules involved in them during development. Therefore, we used *D. melanogaster* mutants – GA582 and GA833 – that displayed phenotypes in the nervous and tracheal systems. We did a characterization of the mutant phenotype and a variety of experiments – crosses and complementation tests among others. Moreover, we analyzed *teiresias gene (tei)* as it is located in the mutated region. Thus, we might answer which are the molecular and cellular mechanisms involved and the genes mutated.

Novel mutants involved in nervous and tracheal development in Drosophila melanogaster

Núria Corbella[1], Sofia J. Araújo[1]

[1]Department of Genetics, Microbiology and Statistics, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain
[2]Institute of Biomedicine of the University of Barcelona (IBUB)

The *Drosophila melanogaster* embryonic tracheal system is formed by a network of interconnected epithelial tubes that branch inside the body to allow the distribution of oxygen to all tissues. We focused on the tracheal terminal cells (TCs), which are specialized cells that form unicellular branches with a cytoplasmatic fine tube called subcellular lumen. This lumen is produced by an extensive reorganization of the cytoskeleton and the growth of a new membrane which invaginates from the adjacent stalk cell while the TC is elongating. Aimed at finding novel genes involved in subcellular lumen formation, we analysed mutants from a screen with phenotypes in tracheal and nervous system development. From this, we selected one mutant that displayed a phenotype in subcellular lumen formation and in the development of the nervous system. This mutant presented extra subcellular lumina (ESL) in the TCs at embryonic stages and a strong nervous system phenotype. We mapped the mutation to chromosome 2 with the Bloomington Deficiency Kit. We confirmed the position of the mutation with a complementation test and identified a previously unidentified gene in *Drosophila*, whose human ortholog is part of a complex involved in ribosome biogenesis and connected with a group of diseases called ribosomopathies. We named this gene *kid kazoom* (*kkz*). We characterized the role of *kkz* in the embryonic development of the nervous and tracheal systems by classifying several *kkz* mutant alleles according to the phenotype they presented in different genetic situations. *kkz* mutant alleles did not show defects in embryonic nervous system development, but instead they displayed a severe phenotype of the tracheal system. Together, these results provide the first evidence of a direct link between *kkz* and the development of the tracheal system in *Drosophila melanogaster*.

Inhibition of the Hippo pathway in planarians impairs tissue renewal through controling cellular architecture and transcription

Daniel Font[1,2], Emili Saló[1,2], Teresa Adell[1,2]

[1]Department of Genetics, Microbiology and Statistics and Institute of Biomedicine, Universitat de Barcelona, Barcelona, Catalunya, Spain
[2]Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, Barcelona, Catalunya, Spain

The Hippo signaling pathway has been found systematically deregulated in tumoral processes. However, its role in the transformation process remains unclear. Although in the firsts studies the Hippo signaling was related with the control of cell proliferation and growth, nowadays it is seen as a complex mechanism that controls cellular behavior in response to the physical environment. To further understand the cellular role of the Hippo signaling we study its function in planarians, flatworms that endow a continuous tissue renewal fueled by the presence of totipotent adult stem cells. In a previous study we demonstrated that Hippo inhibition prevents tissue maintenance and produces cell dedifferentiation and overgrowths in planarians, associated to a decrease in cell death, an arrest in mitosis and the increase in cell plasticity. In the present work we study the function of 3 putative *hippo* target genes (*syne1*, *ccdc175* and *med17*) found deregulated in the transcriptome of *hippo* RNAi planarians. Our results show that RNAi inhibition of the three of them phenocopies the dedifferentiation and overgrowths found in *hippo* RNAi. *syne1* and *ccdc175* RNAi could lead to this phenotype through modulating the cytoskeleton, whereas *med17* would produce dedifferentiation through controlling RNApolII activity. These results set these genes as potential effectors of the Hippo pathway, and highlight the diversity of processes controlled by *hippo* and deregulated in dedifferentiation processes associated with tumorigenesis. They also put planarians on the spot as a model of choice to understand the cellular and molecular processes that fine tune cell renewal in adult organisms.

Comparison of two neurobehavioural tests in zebrafish embryos to assess developmental neurotoxicity

Noelia G. Romero[1], Gerard Gutierrez [1] Laura Guzmán[1], Elisabet Teixido[1], Jesús Gómez-Catalán[1,2], Marta Barenys [1,2]

[1] GRET and Toxicology Unit, Department of Pharmacology, Toxicology and Therapeutic Chemistry, Faculty of Pharmacy and Food Sciences, University of Barcelona, Av. Joan XXIII 27-31, Barcelona, Spain

[2] Institute of Nutrition Research and Food Safety (INSA-UB), University of Barcelona, Barcelona, Spain

The zebrafish embryo model is increasingly used to investigate neurodevelopmental biology and neurodevelopmental alterations. In our work we evaluate the suitability of the zebrafish embryo model to assess the developmental neurotoxicity of several products. A combination of two neurobehavioural tests, Touch evoked Response and Locomotor Activity Response, always performed before 5 days post-fertilization (dpf), together with general developmental toxicity test and total morphological score evaluation were performed. A free analysis workflow in KNIME for Locomotor Activity Response was developed and two analysis methods of Touch evoked Response were compared. In case of significant adverse neurodevelopmental effects detected in a mixture product, single compounds present in them and described in the literature to be proposed for its use during pregnancy were further tested. Different time-exposure approaches were performed to mimic the described human pattern of consumption of the different products during pregnancy. Locomotor Activity Response showed to be more sensitive than Touch evoked Response for the purpose. The combination of assays proposed during 5 dpf in zebrafish embryo is a promising strategy to screen for developmental neurotoxicity effects of several products in the future.

FGF evolution in the appendicularian *Oikopleura dioica*, a chordate evolutionary knockout of the retinoic acid signalling

Gaspar Sánchez-Serna, Paula Bujosa, Jordi Badia Ramentol, Eva Rodríguez Quintana, Marc Fabregà-Torrus, Alfonso Ferrández-Roldán, Ricard Albalat, Cristian Cañestro

Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (Spain)

The extensive family of Fibroblast Growth Factors (FGFs) and their receptor tyrosine kinases (FGFRs) constitute a conserved cellular communication system that is widely spread throughout ParaHoxozoa. In chordates, the FGF gene content varies from 7 in the tunicate *Ciona robusta*, or 8 in cephalochordates, to 19-27 in vertebrates, many of them being involved in developmental events including axial organization and mesodermal patterning. Although the orthology of the FGF repertoire in the chordate phylum is not completely resolved, six subfamilies with at least one representative in each of the three subphyla have been identified, rising an interest in exploring the association between the evolution of FGF subfamilies and the evolution of chordate developmental and morphological traits. In this project, we study the evolution of the FGF-FGFR system in one of the classes within the tunicate subphylum, the Appendicularians, and use the emerging model *Oikopleura dioica* to study the evolutionary impact of gene loss. The loss of RA-signaling in appendicularians provides a unique opportunity to explore its impact in the evolution of FGF-signaling, since the counteracting effects of these two pathways are highly conserved in all other olfactores. Our phylogenetic approaches reveal that appendicularians have lost all the chordate FGF subfamilies but two, and that they have amplified the members of these remaining families in a species-specific manner. As for the FGFRs, the appendicularians have also amplified the only gene present in the basal tunicate in a species-specific manner. We have identified 7 FGF genes and 3 FGFR genes in *Oikopleura dioica*, and their expression analyses point to some degree of subfunctionalization. Functional approaches using molecular inhibitors of the pathway reveal that some of the classical functions of FGF signaling in chordate development, such as notochord or neural induction, have been conserved in *O. dioica*, while others such as heart induction have been lost.

Modeling Tyrosine Hydroxylase Deficiency (THD) development and disease with human brain organoids

Tristán-Noguero A.[1], David Piñol[2,3], Fernández-Carasa I.[2,3], Testa V.[2,3], Lazzarano S.[4], Domingo-Jiménez R.[5], Pineda M.[6], Alcántara S.[2], Raya Á.[4,7,8], Artuch R.[9,10], García-Cazorla A.[1,10], Consiglio A.[2,3]

[1]Neurometabolic Unit and Synaptic Metabolism Lab, Neurology Department, Institut Pediàtric de Recerca, HospitalSant Joan de Déu, Barcelona, Spain
[2]Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital-IDIBELL, 08908 Hospitaletde Llobregat, Spain
[3]Institute of Biomedicine of the University of Barcelona (IBUB), Barcelona, Spain.
[4]Center of Regenerative Medicine in Barcelona (CMRB), Hospital Duran i Reynals, Hospitalet de Llobregat, 08908 Barcelona, Spain
[5]Departamento de Neuropediatria, Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Murcia, Spain
[6]Fundació Sant Joan de Déu (FSJD), Hospital Sant Joan de Déu (HSJD), Barcelona, Spain
[7]Centre for Networked Biomedical Research on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), 28029 Madrid, Spain
[8]Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain.
[9]Metabolic Unit, Departments of Neurology, Nutrition Biochemistry and Genetics, Institut Pediàtric de Recerca, Hospital San Joan de Déu, Barcelona, Spain
[10]CIBERER (Centro de Investigación Biomédica En Red Enfermedades Raras) Instituto de Salud Carlos III, Barcelona, Spain

Tyrosine Hydroxylase Deficiency (THD) is an inherited metabolic disorder caused by a defect in the TH enzyme, which catalyses the rate-limiting step in the biosynthesis of dopamine. Two clinical phenotypes have been described: i) “Type A” which refers to a progressive hypokineticrigid syndrome and dystonia with an onset in infancy or childhood and L-Dopa responsiveness; ii) “Type B” which produces a severe early-onset encephalopathy, mental retardation, oculogyric crises and parkinsonism with sub-optimal L-Dopa response. We have recently studied the emergence of THD-related phenotypes in dopamine neurons (DAns) derived from induced pluripotent stem cells (iPSCs) generated from THDA and THDB patients and showed that these models recapitulated THD disease phenotypes and response to treatment. An early L-Dopa treatment at the stage of neuronal precursors prevented the described alterations in THD-B iPSCDAn, thus suggesting the existence of a critical developmental window when new therapies could be successfully employed in THD. To test this hypothesis, we are now establishing cortical organoid (CO) and midbrain organoids (mOs) culture systems from THDB patients, healthy donors and isogenic controls. Consistent with findings in patients, THDB mOs displayed less TH+ neurons compared to controls, thus reproducing the disease. Interestingly, at earlier stages THD COs show more proliferating (Ki67+) NSC pool that may indicate an altered molecular regulation of neurogenesis that may have a role in the development of the THD disease. Analysis of TH and dopamine genes expression levels at later stages are ongoing. In conclusion,

we hope that the new THD-3D cell model will help us to investigate neurodevelopmental aspects in THD that have not been studied until now and to test different therapeutic approaches in a three-dimensional environment.

Role of cellular senescence during planarian regeneration

Daniel Moreno-Blas, Emili Saló, Teresa Adell

Department of Genetics, Microbiology and Statistics and Institute of Biomedicine (IBUB), Universitat de Barcelona, Barcelona, Catalunya, Spain

Regeneration is one of the major enigmas of biology, and how it occurs is only partially understood. Novel insights indicate that cellular senescence, a cell response commonly viewed as an important contributor to ageing and degenerative diseases, also contributes to tissue regeneration in vertebrates. Planarians offer an ideal model to investigate the link between senescence and regeneration. These fascinating animals possess an unlimited regenerative potential, as they can regenerate any missing or damaged body tissue dependent upon a population of self-renewing adult stem cells, called neoblasts. The goal of this work is to study whether cellular senescence occurs during the normal physiology of these immortal animals and whether it participates in planarian regeneration. To investigate the potential induction of senescent cells during planarian regeneration, we are analyzing well-known markers of the senescent phenotype in the planarian *Schmidtea mediterranea* at different timepoints after amputation. Afterwards, we will explore whether senescence dysregulation in planarians contribute to regeneration defects. We speculate that senescent cells cooperate with stem cells, either by secreting signals that attract them to the wound site or that promote their entrance in the cell cycle.

Differential autophagy promotes elimination of specific tracheal segments in Drosophila metamorphosis

V. Beatriz Pino, Panagiotis Giannios, Jordi Casanova

IRBBarcelona

Autophagy is a lysosome-mediated cellular degradation process that can either contribute to cell survival by recycling its own constituents or to cell death at elevated levels. This process has been showing their implications in multiple roles as tissue degradation, renovation and development among others. During the metamorphosis of Drosophila melanogaster, driven by the moulting hormone Ecdysone, the juvenile tissues have to be removed before the adult structures are formed. To carry out this process, most of larval cells die along this stage, while others, the Adult Progenitor Cells (APCs), survive and give rise to the adult organism. It has already been shown that larval cells fated to die use autophagy as a preparative phase of programmed cell death to free up stored biomass that is utilized by the APCs as a nutrient and energy source during metamorphosis (Lörincz et al. 2017). In this stage of Drosophila's development, autophagy has been found to have a role in the degradation of structures such as the larval midgut, the fat body and the salivary glands (Berry and Baehrecke 2007, Denton et al. 2009). In this project, we study the specific role of autophagy in the Drosophila larval tracheal system. This organ is particularly interesting to examine this process, because it´s compose of some different kind of cells, which have to react in a different way to the metamorphosis signals, as developing or dying. There are polyploid cells, fated to die at metamorphosis, and diploid ones, that are progenitors of the adult tracheal system and therefore, have to survive. In fact, our results show that autophagy is differentially active in the tracheal segments made up of polyploid cells versus the one that consists of APCs. Now, we are working in analyse how the perturbation of this differential expression can affect the trachea degeneration in the pupal stage, and if this is affecting also the viability of those animals.

Characterization of *Smed-cbp2* and *Smed-cbp3* function in neoblast biology and lineage commitment

Luisa Riedel[*1], Anna Guixeras[*1], Ma Dolores Molina[1,2], Susanna Fraguas[1,2], Francesc Cebrià[1,2]

*Both authors share first authorship
[1]Department of Genetics, Microbiology and Statistics. Faculty of Biology, University of Barcelona
[2]Institute of Biomedicine of the University of Barcelona (IBUB)

Planarians are flatworms capable to regenerate their whole body from tiny fragments of them, a process requiring a population of adult pluripotent stem cells called neoblasts. Little is known about how neoblasts are directed to differentiate into the multiple cellular lineages. Regarding genes that are involved in neoblast biology, our laboratory has suggested candidates of planarian CBP (CREB-binding protein) as important regulators. The conserved CBP/p300 gene family functions as a transcriptional co-activator and plays important roles in a wide range of cellular processes. The acetyl transferase activity of CBPs is particularly important, as histone and non-histone acetylation affect gene expression. Previous functional analyses in our laboratory have suggested that the planarian cbp homologues Smed-cbp-2 and Smed-cbp-3 are involved in maintenance and differentiation of neoblasts, respectively. Currently, we aim to investigate the role of these genes on neoblasts turnover, lineage specification and asymmetric division. We have set up different doses of ionizing radiation to reduce the quantity of neoblasts in animals to a minimum, in order to be able to study how these remaining neoblasts proliferate and produce new neoblasts that eventually differentiate into neuronal, intestinal, and other known post-mitotic cell types. This approach will allow us to gain insights into the role that Smed-cbp-2 and Smed-cbp-3 may have in the specification of lineage-specific progenitor cell types from the pluripotent neoblasts, as well as their ability to go through symmetric or asymmetric cell divisions.

Establishment of human embryonic lung organoids and morphological analysis by transmission electron microscopy

Melissa Acosta-Plasencia[1], Jordi Canals[1], Tania Díaz[1], Lina Berrada[1], Mariano Monzó[1,2], Alfons Navarro[1,2]

[1]Molecular Oncology and Embryology Laboratory, Human Anatomy Unit, Faculty of Medicine and Health Sciences, University of Barcelona, IDIBAPS, Barcelona, Spain
[2]Thoracic Oncology Unit, Hospital Clinic, Barcelona

Introduction: Until now, the study of human lung development has been limited by the lack of functional models to study the organogenesis process. However, organoids have emerged as an *in vitro* model able to reproduce the architecture of the embryonic tissue. Two different groups, Spence and Rawlins, have described different culture methods to growth human embryonic lung organoids. We aimed to establish organoids derived from the bronchial tree tips of human embryonic lungs comparing the efficiency of both methods and characterizing them by transmission electron microscopy (TEM).

Methods: Human embryonic lungs were obtained from aborted fetuses of 7-14 post-conception weeks. After lung mesenchymal disaggregation under the stereoscopic microscope, the epithelial tips (enriched in lung stem cells) were localized, dissected, cultured in Matrigel with two different methods and organoids were obtained. Morphological characterization was performed by brightfield and fluorescent microscopy, and by TEM.

Results: We observed that human lung organoids cultured with Rawlins' culture medium had a significant increase in the size and proliferation rate and were able to retain higher viability in long-term cultures (more than 3 months) than to those grown with Spence's medium. Morphological analysis by TEM of the obtained organoids showed a pseudostratified epithelium composed of ciliated cells, basement membrane and mesenchyme, reproducing the tissue characteristics of the human embryonic lung *in vitro.*

Conclusions: Human embryonic lung organoids reproduce the *in vivo* architecture of the human embryonic lung, can be maintained in long-term cultures, and have a great potential for performing functional studies of human organogenesis.

Interrogating the importance of X-chromosome inactivation and reactivation for meiotic potential

Tom Mattimoe[1,2], Jacqueline Severino[1,2], Moritz Bauer[1,3], Katsuhiko Hayashi[4], Mitinori Saitou[5], Bernhard Payer[1,2]

[1] Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain
[2] Universitat Pompeu Fabra (UPF), Barcelona, Spain
[3] Oncode Institute, Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, The Netherlands
[4] Institute for the Advanced Study of Human Biology (ASHBi), Kyoto University, Kyoto, Japan
[5] Department of Stem Cell Biology and Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

In order to achieve X-chromosome dosage parity between male and female mammals, one of the X chromosomes in the female must undergo large-scale epigenetic remodelling and silencing – a process known as X-chromosome inactivation. As the germ line is established in the embryo, this process is reversed, and the silenced X chromosome is reactivated in the developing primordial germ cells before entering prophase I of meiosis. In a recent study we visualise this process *in vitro*. Using a dual X-chromosome reporter mouse embryonic cell line, we identified a subpopulation of primordial germ cells that exhibit two active X chromosomes, rather than the expected single active X. These cells demonstrate aberrant behaviour and a reduced meiotic capability. RNA-seq analysis reveals an abnormal gene signature of these cells, and shows that the cell fate trajectory towards meiotic entry is not achieved when the X chromosome is reactivated too early. Furthermore, we look to investigate the relationship between X-chromosome kinetics and meiotic entry by functional interrogation.

We have developed two cell lines; a homozygous knockout of the long non-coding RNA *Xist* and a conditional *Xist* overexpression cell line, to prevent X-chromosome inactivation and reactivation, respectively. Using these tools, we aim to understand the necessary prerequisites of the X-chromosome epigenetic state for proper meiotic entry in female germ cells, and therefore achieve a greater understanding of the relationship between X-chromosome kinetics and meiosis.

Role of centrosomes in axon guidance of the motor neurons in Drosophila melanogaster

Júlia Sellés, Sofia J. Araújo

Department of Genetics, Microbiology and Statistics of the University of Barcelona and Insititute of Biomedicine of the University of Barcelona (IBUB)

Axon guidance plays and essential role to obtain a complex and functional nervous system. Is a mechanism that are part of the morphogenic process. The final morphology of neurons plays a key role in ensuring that the functional requirements are met. The formation, stabilization and remodelling of axons is achieved in response to extensive microtubule dynamics. Centrosomes are the main microtubule organizing centres (MTOC) and because microtubules regulate cell shape, centrosomes are involved in this subcellular branching process. The full link between the number and structure of centrosomes and the axon pathfinding is not well known yet. To perform this study, we used specific neurons from the Central Nervous System (CNS) of *Drosophila melanogaster*, called aCC motor neurons. We quantified axon pathfinding in this cell during late embryonic development. To do so, we compared control and mutant conditions, with altered number or structure of the centrosomes.

The Mayfly eyes: a new structure to study evolutionary innovations

Maria Rosselló[1], Helena García-Castro[2], Noelle Anderson[3], Scott Roy[3], Jordi Solana[2], Fernando Casares[4], Isabel Almudí[1]

[1]Dept Genètica, Microbiologia i Estadística, Universitat de Barcelona, Barcelona, Spain
[2]Dept of Health Sciences, Oxford Brookes University, Oxford, UK
[3]Department of Biology, San Francisco State University, San Francisco, California
[4]Centro Andaluz de Biología del Desarrollo, CSIC-UPO-JA, Seville, Spain

Innovations in evolution are related with the emerging of new species and the colonization of new ecosystems. Although we know their importance a little is know about the genetic mechanisms that give rise to these new structures. In the mayfly *Cloeon dipterum* we can find an interesting example of sexually dimorphic novel structure; males develop an additional pair of compound eyes in addition to the ones shared with the females. These new eyes are extremely large and have a characteristic turban-shape. The main question in our work is to fully understand how are this sex specific eyes developed and their specific function in the male mayfly. In this study we will combine confocal and electron microscopy techniques to fully characterize the development of the turbanate eye and intergrade this information with genomic data in a single cell level.

Human embryonic mesenchymal lung-conditioned medium promotes a loss of tumoral characteristics and differentiation to myofibroblast in A549 cell line

Jordi Canals[1], Alfons Navarro[1, 2], Cristina Vila[3, 4, 5], Josep M. Canals[3, 4, 5], Tania Díaz[1], Melissa Acosta-Plasencia[1], Coralí Cros-Font[1], Bing Han[1], Yangyi He[1, 6], Mariano Monzó[1,2,*]

[1]Molecular Oncology and Embryology Laboratory, Human Anatomy Unit, Faculty of Medicine and Health Sciences, University of Barcelona, IDIBAPS, Barcelona, Spain
[2]Thoracic Oncology Unit, Hospital Clinic, Barcelona
[3]Laboratory of Stem Cells and Regenerative Medicine, Department of Biomedical Sciences; Creatio, Production and Validation Center of Advanced Therapies, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, Barcelona, Spain
[4]August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain
[5]Networked Biomedical Research Centre for Neurodegenerative Disorders (CIBERNED), Spain
[6]University of Cheng Du, 610106, Cheng Du, China

Background: When genes responsible for normal embryonic development are abnormally expressed in adults, it can lead to tumor development. This can suggest that the same mechanism that controls embryonic differentiation can also control tumor differentiation. We hypothesize that the malignant phenotype of lung cancer cells could acquire benign characteristics when in contact with an embryonic lung microenvironment.
Methods: The human embryonic mesenchymal lung-conditioned medium (hEML-CM) was obtained by culturing lung cells from embryos in the pseudoglandular stage of development. The NSCLC cell line A549 we cultured in the hEML-CM and in a tumor-conditioned medium. Morphological changes were analyzed with optical microscopy. To evaluate the functional effect of conditioned medium in tumor cells, we analyzed cell proliferation, migration and *in vivo* tumor growth capacity. The expression of the pluripotency genes OSKM was analyzed with qRT-PCR. Transcriptomic analysis was performed using Affymetrix arrays.
Results: The A549 cells cultured in hEML-CM lost their epithelial morphology, acquired mesodermal characteristics, and decreased proliferation, migration and reduced its capacity to growth *in vivo*. In addition, the expression of OSKM genes decreased. Distant matrix analysis based on transcriptomic profile showed that conditioned cells were closer to myoblast and human lung fibroblast than to normal epithelial immortalized lung cells.
Conclusions: To the best of our knowledge, this is the first study to report that stimuli from the embryonic lung can modulate the malignant phenotype of lung cancer cells, control their growth capacity and activate their differentiation into myofibroblasts. These findings could lead to new strategies for lung cancer management.