XXXII JORNADA DE BIOLOGIA DEL DESENVOLUPAMENT

SOCIETAT CATALANA DE BIOLOGIA

Monday October 19th 2020

9:00 Welcome by the Coordinator of the Section of Developmental Biology

9.10-10.00 **Emili Saló (UB): "Planarian regeneration, a system of extreme biological plasticity"**

10.00-10:20 Elena Gracia Latorre: *"Development, Regeneration & Tumorigenesis: multi-tasking of a single Wg enhancer"*

10.20-10:40 Daniel Font: *"Inhibition of the Hippo pathway in planarians impairs tissue renewal, associated to cell cycle and cellular architecture defects"*

10:40-11:00 Break

11:00-12:00 Poster session (Flash talks)

12:00 -12:20 Aleksandra Kozyczkowska: *"Establishment of genetic tools was vital in discovering alternative developmental pathways in Corallochytrium limacisporum"*

12:20-12:40 Sofia J. Araújo: *"The Spectraplakin Short-Stop promotes subcellular branching by mediating the crosstalk between microtubules and actin"*

12:40-13:30 **Elisa Martí (IBMB-CSIC): "Cell signalling in secondary neurulation"**

13:30-15:00 Lunch

15:00-15:20 Britta A. Kühne: *"Evaluation of the impact of fetal growth restriction on the developing brain using an in vitro neurosphere model"*

15:20-15.40 Carolyn Engel-Pizcueta: *"Role of her9 and Notch pathway in the maintenance of stemness of the rhombomeric boundary cell population"*

15:40-16:00 Maria Rosselló: *"Mitochondrial could have a role in overgrowth formation after hippo parthway inhibition"*

16:00 -16.50 **Núria Montserrat (IBEC): "Kidney organoids from human pluripotent stem cells: how to model development and disease in the Petri dish"**

16:50 Concluding remarks and awards

Use the hashtag #DevBioSCB2020 @ Facebook and Twitter

Us recordem que podeu seguir la Jornada, així com fer presentacions de les vostres comunicacions, clicant l'enllaç de Zoom següent:

https://us02web.zoom.us/j/88128194007?pwd=MTN1ZnZ3YzhROFlxelBxV0haOC9MZz09
ID de reunió: 881 2819 4007
Codi d'accés: rcydk4

INVITED SPEAKERS

Planarian regeneration, a system of extreme biological plasticity

Emili Saló

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

Planarians are flatworms (*Platyhelminthes*) with a remarkable ability to regenerate any missing body region; they can also grow and degrow according to environmental conditions. This capacity is mediated by the presence, in the adult planarian, of neoblasts, a proliferative cell population that contains pluripotent stem cells and the continuous presence of morphogenetic mechanisms as an embryo like.

In this presentation I will try to summarize the main events of a long period (1975-2020) working in planarian. We will jump from the initial tissue transplantation experiments to the last ATAC Seq and ChipSeq analysis that we run studying planarian regeneration, the main research topic of different research groups in the Department of Genetics of UB founded in the 60's by Professor Jaume Baguñà. Although there was considerable interest in planarian regeneration during the twentieth century, a change in research priorities around the middle of the century, typified by the search for organisms that were more amenable to classical genetic analysis, saw a decline in the use of planarians as a model system in research projects. Thus, research in this field became restricted to just a few laboratories around the world. Fortunately, over the new century, the development of omics and most specifically: RNAi methodologies, democratizes the functional analysis in the animal kingdom, opening the incorporation of a new generation of scientists that has revitalised the worldwide network of planarian regeneration laboratories.

Cell signalling in secondary neurulation

Elisa Martí

Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Barcelona, Spain

Body axis elongation is a hallmark of the vertebrate embryo, involving the architectural remodelling of the tail bud. Although it is clear how bi-potential neuro-mesodermal progenitors (NMPs) contribute to embryo elongation, the dynamic events that lead to *de novo* lumen formation and that culminate in the formation of a 3-dimensional, secondary neural tube from NMPs, are poorly understood. Here, we used in vivo imaging of the chicken embryo to show that cell intercalation downstream of TGF-beta/SMAD3 signalling is required for secondary neural tube formation. Our analysis describes the initial events in embryo elongation including lineage restriction, the epithelial-to-mesenchymal transition of NMPs, and the initiation of lumen formation. Importantly, we show that the resolution of a single, centrally positioned continuous lumen, which occurs through the intercalation of central cells, requires SMAD3/YAP transcriptional activity. We anticipate that these findings will be relevant to understand caudal, skin-covered neural tube defects, amongst the most frequent birth defects detected in humans.

Kidney organoids from human pluripotent stem cells: how to model development and disease in the Petri dish

Nuria Montserrat[1,2,3]

[1]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain
[2]Pluripotency for Organ Regeneration, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Technology (BIST), Barcelona, Spain.
[3]Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina, Madrid, Spain.

The generation of human pluripotent stem cells (hPSCs) derived organoids is one of the biggest scientific advances in regenerative medicine. Recently, we have demonstrated that by lengthening the time that hPSCs are exposed to a three-dimensional microenvironment in the presence of defined renal inductive signals we are able to generate kidney organoids that transcriptomically match second-trimester human fetal kidneys. Furthermore, we have developed a transplantation method that exploits the intrinsic properties of the chick chorioallantoic membrane (CAM) to recreate a soft in vivo microenvironment for organoid growth and differentiation, including vascularization in vivo. Next, through bioengineering we have mimicked the stiffness of the chick CAM by fabricating compliant hydrogels. To exploit these systems to model renal disease we will also discuss our recent findings on the use of the CRISPR/Cas9 to target genes determinant for kidney development and disease.

Finally, we will further discuss how these findings have resulted in the application of our procedures for kidney organoid derivation in the study of SARS-CoV-2 infection and the identification of soluble recombinant ACE2 as a putative treatment to reduce SARS-CoV-2 infection in human engineered microtissues.

SHORT TALKS

Development, Regeneration & Tumorigenesis: multi-tasking of a single Wg enhancer

Elena Gracia-Latorre[1], Lidia Pérez[2], Mariana Muzzopappa[1], Marco Milán[1,3]

1. Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain
2. Francis Crick Institute, London, United Kingdom
3. ICREA, Catalan Institution for Research and Advanced Studies, Barcelona, Spain

Wnt1 is a member of the Wnt family present in almost all organisms. Wnt1 is involved in the development, regenerative capacity and tumorigenic growth of many organs and tissues. In *Drosophila*, Wingless (Wg, *Drosophila* Wnt1) not only triggers the specification of the wing during normal development but it also contributes to the regenerative capacity of the wing and to the emergence of wing-derived epithelial tumours. Interestingly, a single *wingless* enhancer mediates these radically different tasks. Using a combination of reporter assays and CRISPR/Cas9-driven defined deletions, we have identified the logic behind the context-dependent use of this enhancer by developmental and stress-induced signalling pathways. Our work will certainly contribute to our better understanding of the use of the same signalling molecules during development and disease.

Inhibition of the Hippo pathway in planarians impairs tissue renewal, associated to cell cycle and cellular architecture defects

Daniel Font[1,2], Emili Saló[1,2] and 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

Tissue renewal is a fine regulated process in adult homeostasis which defects may lead to neoplasia. The Hippo signaling pathway has been targeted as a key hub in the control of cellular renewal found systematically deregulated in tumoral processes. Hippo controls cell proliferation, cell death and cell differentiation in different model organisms. However, its specific cellular functions and molecular targets remain poorly understood. In the present study we characterize the function of 5 putative *hippo* target genes (*syne1*, *med17*, *trrap*,*ccdc175* and *sspo1*) found deregulated in a transcriptomic analysis of *hippo* RNAi planarians, flatworms that endow a continuous tissue renewal while changing their size according to nutrients availability. These candidates are related to different biological processes and cellular components ranging from transcription to cytoskeletal architecture but none of their roles has been properly characterized. Our team had observed that in planarians *hippo* inhibition produces overgrowths coupled with the inability of cells to maintain the differentiated structures, to properly cycle and to die when required. Here we report that RNAi inhibition of each of the selected putative effectors partially phenocopies the *hippo* RNAi phenotype. To further investigate their role in maintenance of the cytoskeleton we compared their phenotype with the one obtained after inhibiting lgl2, an upstream modulator of Hippo. We observed that cell polarity and nuclei morphometry were also impaired in RNAi of two candidates (*syne1* and *ccdc175*).

These results set these candidate genes as potential effectors of the Hippo pathway, and highlight the diversity of processes controlled by hippo and deregulated in tumoral processes. They also put planarians on the spot as a dynamic model to understand the cellular and molecular processes that fine tune cell renewal in adult organisms.

Establishment of genetic tools was vital in discovering alternative developmental pathways in *Corallochytrium limacisporum*

Aleksandra Kożyczkowska[a], Sebastián R. Najle [a], Iñaki Ruiz-Trillo[a,b,c], Elena Casacuberta[a]

[a] *Institut de Biologia Evolutiva (CSIC-UPF), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, , Spain E-mail: aleksandra.kozyczkowska@gmail.com*
[b] *Departament de Genètica, Microbiologia i Estadística, Universitat de Barcelona, Avinguda Diagonal 643, 08028 Barcelona,*
[c] *ICREA, Passeig Lluís Companys 23, 08010 Barcelona, Spain.*

The Holozoa clade emerges as an important group for comparative cell and developmental biology studies among eukaryotes (Figure 1). In addition to the well-studied Metazoa group, it includes four different unicellular lineages (Choanoflagellata, Filasterea, Ichthyosporea and Corallochytrea) (Figure 1). Interestingly, these unicellular lineages are highly heterogeneous in their ecological distribution and have diverse developmental modes, cell morphologies, and life stages. Importantly, some species in each of the unicellular holozoan clades can transiently form multicellular structures. For instance, *Salpingoeca rosetta* (Choanoflagellata) develops through clonal division forming colonies, *Capsaspora owczarzaki* (Filasterea) goes through an aggregative stage, and *Creolimax fragrantissima* (Ichthyosporea) forms a multinucleated cell (coenocyte) that resembles the syncytium of the embryonic stage of *Drosophila melanogaster*. So far, the developmental modes of the fourth remaining lineage, the Corallochytrea has not been described. To have a complete understanding of developmental modes and their origin among Holozoa, we need to study the Corallochytrea.

Therefore, we here report the development of stable transfection as well as the use of this technique to unravel previously unknown cellular and developmental features. Our findings show that *C. limacisporum* can grow through two developmental pathways: binary fission and coenocytic growth, demonstrating that *C. limacisporum* life cycle is non-linear. We discovered that unlike in most studied eukaryotes, nuclear division in the binary fission is decoupled from the cellular division, resulting in cells that are bi-nucleate for most of the life cycle of this organism. Moreover, we also detected that during the coenocytic growth nuclei can divide in asynchronously, also a rare phenomenon in eukaryotes. The existence of these two different developmental patterns in one organism is a unique opportunity to study the "decision-making" process by which the organism follows one pathway or the other. Studying *C. limacisporum* with the newly developed genetic tools can bring new clues to better understand the origin of multicellularity and shed light on the origin of different developmental modes in Metazoa. We wish to present and further discuss the potential implications of these peculiar findings.

Role of *her9* and Notch pathway in the maintenance of stemness of the rhombomeric boundary cell population

Carolyn Engel-Pizcueta, Covadonga F. Hevia, Cristina Pujades

Department of Experimental and Health Sciences, Universitat Pompeu Fabra, 08003 Barcelona, Spain

The hindbrain is the most posterior embryonic brain vesicle and it is subdivided into a series of seven segments called rhombomeres. The rhombomeric Boundary Cell Population (rBCP) is generated at the interface between rhombomeres. During the early wave of neurogenesis in the hindbrain, the rBCP is non-neurogenic, whereas the adjacent domains are actively engaged in neurogenesis. Also, the rBCP displays a specific pattern of gene expression, including the enriched expression of the *Enhancer of Split* gene, *her9,* an ortholog of *Hes1*. This gene has a role in the maintenance and proliferation of neural progenitor cells. Thus, our aim is to understand the mechanism regulating the maintenance of the stemness and proliferative properties of the rBCP, focusing on the role of *her9*. In order to answer it, we combined genetic approaches with high-resolution *in vivo* imaging in zebrafish. At early stages of development, the rBCP does not display Notch activity, coinciding with the enrichment of *her9* expression in the rBCP. At later stages, the enrichment of *her9* expression is lost and the rBCP starts expressing Notch receptors and ligands. This results in a significant increase of rBCP cells displaying Notch activity and in the appearance of *her4,* a classical Notch target, in the rBCP. As a consequence, some derivatives from the rBCP undergo neurogenesis. Altogether, our results indicate the potential role of *her9* in maintaining the progenitor capacity of the rBCP at early stages before the onset of Notch activity.

Evaluation of the impact of fetal growth restriction on the developing brain using an *in vitro* neurosphere model

Britta A. Kühne[1,2*], Paula Vázquez[1,2], Fuentes-Amell Mercè[1], Carla Loreiro[2], Fatima Crispi[2], Eduard Gratacós[2], Jesús Gómez-Catalán[1], Ellen Fritsche[3], Miriam Illa[2], Marta Barenys[1,2]

1. GRET, INSA-UB and Toxicology Unit, Pharmacology and Therapeutical Chemistry Department, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.
2. BCNatal-Barcelona Center for Maternal-Fetal and Neonatal Medicine (Hospital Clínic and Hospital Sant Joan de Déu), Fetal i+D Fetal Medicine Research Center, IDIBAPS, Barcelona, Spain.
3. IUF – Leibniz Research Institute for Environmental Medicine. Auf'm Hennekamp 50, 40225 Düsseldorf, Germany.

*brkuhnek24@alumnes.ub.edu

Intrauterine growth restriction (IUGR) is defined as a significant reduction of fetal growth rate leading to a birth weight below the 10th percentile for the corresponding gestational age. The prevalence accounts for 5-10% of all pregnancies, approximately 600.000 cases in Europe. Placental insufficiency reduces placentary blood flow leading to fetal development under chronic hypoxia which is associated with neurodevelopmental damage, cognitive dysfunctions and cardiovascular adverse outcomes. The characterization of neurostructural changes in fetus with IUGR is essential to design therapeutic strategies directed to prevent or revert its deleterious effects. We have established for the very first time an *in vitro* model based on primary rabbit neuronal progenitor cells (NPCs) cultured as three-dimensional cell aggregates called neurospheres. Neurospheres are able to mimic basic processes of fetal brain development like proliferation, migration and differentiation into neurons, oligodendrocytes and astrocytes. We successfully evaluated other relevant endpoints like neurite outgrowth, branching and synaptogenesis. By comparing the functionality of control and IUGR neurospheres we identified that rabbit NPCs from IUGR individuals have a significantly reduced ability to form oligodendrocytes. To find a neuroprotective therapy preventing/reverting adverse effects of IUGR we tested six different compounds at increasing concentrations (Docosahexaenoic acid (DHA), choline, lactoferrin, melatonin, zinc, and 3_,_3_',_5_-Triiodo-L-thyronine (T3)) in the neurosphere model. Basic processes of neurogenesis were assessed to determine the maximum tolerated concentration (MTC) and effective concentration (EC). DHA (MTC=10µM; EC=1µM), melatonin (MTC=3µM; EC=1µM) and T3 (MTC=30nM; EC=0,1nM) have been selected as the most promising therapies due to their oligodendrogenesis promoting effects in this culture.
The *in vitro* model allows us to evaluate different processes of the developing brain in a fast, economic and ethical way and contributes to a better understanding of IUGR induced neurodevelopmental damage and to the selection of new neuroprotective therapies.

This research is supported by Fondo de Investigaciones Sanitarias (FIS) del Instituto de Salud Carlos III (project number: PI18/01763). B.A.K. received a scholarship from Fundació Bosch i Gimpera (project number: 300155) and C.L. received the support of the Health Department of the Catalan Government (Grant nº SLT006/17/00325).

The Spectraplakin Short-Stop promotes subcellular branching by mediating the crosstalk between microtubules and actin

Delia Ricolo and Sofia J. Araújo

Department of Genetics, Microbiology and Statistics, University of Barcelona, Spain
Institut de Biomedicina de la UB, IBUB

The establishment of branched structures by single cells involves complex cytoskeletal remodeling events. In *Drosophila*, epithelial tracheal system terminal cells (TCs) and dendritic arborisation neurons are models for these subcellular branching processes. During tracheal embryonic development, the generation of subcellular branches is characterized by extensive remodeling of the microtubule (MT) network and actin cytoskeleton, followed by vesicular transport and membrane dynamics. We have previously shown that centrosomes are key players in the initiation of subcellular lumen formation where they act as microtubule organizing centres (MTOCs). However, not much is known on the events that lead to the growth of these subcellular luminal branches or what makes them progress through a particular trajectory within the cytoplasm of the TC. Here, we have identified that the spectraplakin *Short-stop* (*Shot*) promotes the crosstalk between MTs and actin, which leads to the extension and guidance of the subcellular lumen within the TC cytoplasm. Shot is enriched in cells undergoing the initial steps of subcellular branching as a direct response to FGF signaling. An excess of Shot induces ectopic acentrosomal luminal branching points in the embryonic and larval tracheal TC leading to cells with extra subcellular lumina. These data provide the first evidence for a role for spectraplakins in subcellular lumen formation and branching.

Mitochondrial could have a role in overgrowth formation after hippo pathway inhibition

Maria Rosselló, Teresa Adell & Emili Saló

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

Understanding how tissue homeostasis is maintained is an essential question in developmental biology, since its malfunction leads to common diseases as cancer. One mechanism important for tissue maintenance is the hippo pathway, a conserved pathway that modulates cell proliferation, cell death and cell differentiation, responding to extracellular cues. Planarians are flatworms that continuously and completely renew all their tissues, since they do not only regenerate any missing body part but also continuously change their size according to nutrients. Inhibition of *hippo* in planarians leads to the formation of overgrowths, which is the same phenotype observed after *hippo* inhibition in all animals studied. We recently demonstrated that the formation of these overgrowths is not caused by over proliferation of the stem cells but by dedifferentiation of committed cells. The transcriptomic analysis of those *hippo* knockdown animals revealed that changes in the mitochondrial activity could be related to the formation of the overgrowths. Mitochondria are organelles that haver a central role in controlling metabolism. Changes in mitochondrial function and structure give rise to severe changes in cellular function and homeostasis. Thus, deregulation of mitochondrial function could be an underlaying cause of the *hippo* phenotype. Inhibition of several genes with a mitochondrial function, altered after *hippo* RNAi, show a phenotype similar to the one previously described in hippo. We developed some tools in planarians to analyze mitochondrial structure after hippo inhibition and its impact in the overgrowth formation.

FLASH TALKS

An animal model to study the association between the accumulation of DNA damage and the incidence of ataxia in patients with SCAN1

Delia Ricolo[1,2] and Sofia J. Araújo[1,2]

[1]Department of Genetics, Microbiology and Statistics, School of Biology, University of Barcelona, 08028 Barcelona, Spain

[2]Institute of Biomedicine University of Barcelona (IBUB), Barcelona, Spain

Spinocerebelar ataxias (SCAs) are a group of progressive and irreversible neurodegenerative diseases affecting movement coordination. Molecular mechanisms for this pathology include polyglutamine tract expansion (SCA1, SCA2, etc), defects in basal transcription (SCA17) and defective DNA repair (ataxia telangiactasia (AT), spinocerebellar ataxia with axonal neuropathy type 1 (SCAN1) and ataxia with oculomotor apraxia type 1 (AOA1). By focusing on SCAN1, we are studying the connection between defects in DNA repair, developmental abnormalities and the incidence of ataxic phenotypes in adults. Spinocerebellar ataxia with axonal neuropathy type 1 (SCAN1) is a neurodegenerative disorder targeting almost exclusively the nervous system and is characterized by cerebellar ataxia and axonal sensorimotor neuropathy. The disease is linked to mutations in TDP1, encoding tyrosyl-DNA phosphodiesterase 1, a member of the phospholipase D superfamily involved in repairing covalent topoisomerase I-DNA adducts from abortive topoisomerase I reactions. In human cells, TDP1 is required for repair of chromosomal single-strand breaks (SSBs) arising independently of DNA replication. Since human protein sequences associated with disease have highly related sequences in *Drosophila melanogaster* and a number of inherited human neurodegenerative diseases have been successfully modelled in the fly, we aim to create *Drosophila* models of SCAN1. We have started studying the phenotypes of *Drosophila melanogaster* embryos bearing both deletions and point mutations in TDP1. We are analysing the nervous and tracheal system phenotypes of *Drosophila* embryos mutated in the *Drosophila* homologue of TDP1, during the course of embryonic development, in order to investigate the molecular mechanisms associated with the phenotypes in human patients.

Planarian CREB-binding protein (CBP) gene family regulates stem cell maintenance and differentiation

Susanna Fraguas[1,2], Mª Dolores Molina[1,2], Sheila Cárcel[1], Coral Vivancos[1], Judith Mazariegos[1], Jordi Ginés[1], Rafael Romero[1] and 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)

Stem cells plasticity and differentiation is still an open question in developmental biology. Many studies have shown the importance of post-translational modifications and epigenetics in the regulation of the behavior of stem cells. CBP (CREB- binding protein) is 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 tumorogenesis, 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. In our lab, we work with the planarian *Schmidtea mediterranea*, an excellent model to study in vivo the molecular mechanism underlying stem cell differentiation during regeneration. We identified 5 distinct *Smed-cbp* genes in *S. mediterranea* that show different expression patterns. Functional analyses revealed that *Smed-cbp-2* appears to be essential for stem cell maintenance and cell survival. On the other hand, the silencing of *Smed-cbp-3* resulted in the growth of blastemas that were apparently normal, but remained largely depigmented and undifferentiated, even after one month of regeneration. The knockdown of *Smed-cbp-3* affects the differentiation of several cell lineages including neural, epidermal, digestive and excretory cells during regeneration. Finally, we analysed the predicted interactomes of CBP-2 and CBP-3 as an initial step to better understand their functions in planarian stem cell biology. These results open the door to further investigate the epigenetic regulation of planarian stem cells.

Unveiling radial glial heterogeneity in the embryonic hindbrain

Carla Belmonte-Mateos and Cristina Pujades

Universitat Pompeu Fabra – Department of Experimental and Health Sciences

One of the transversal questions in developmental neurobiology is how the enormous cellular diversity in the Central Nervous System (CNS) is generated. Neurons arise first by asymmetric division from Radial glial cells (RGc) and later in development, these progenitors give rise to glial cells. All at the same time, the tissue undergoes extensive proliferation and morphogenesis. Therefore, the balance proliferation vs. differentiation must be tightly coordinated and regulated over time. My project aims to tackle these questions using the zebrafish hindbrain since it is one of the mostconserved structures in the embryonic vertebrate CNS. The hindbrain is transiently segmented, along the anteroposterior axis, in seven rhombomeres separated by a boundary cell population that displays different features from the rest of the rhombomeric cells. However, rhombomeric progenitor cells are not a homogeneous population either. The center of the rhombomeres harbors RGc that display glial features in a spatiotemporal restricted manner, while the rest of the rhombomeric progenitors are engaged in neurogenesis and do not express glial markers until later in development. We are trying to unveil what might orchestrate this RGc heterogenicity, as well as the functional relevance of specifying different RG populations from an early developmental stage. Several studies have demonstrated the specification of progenitor cells within the center of the rhombomeres is regulated by FGF signaling. Our results suggest they also depend on the Notch signaling pathway as they express high levels of Notch activity and restricted expression of the Notch direct target and effector hey1. We are also generating transgenic tools to study thE behavior of this RGc population and to unveil its derivatives.

The role of centrosomes in dendrite branching

Judith Castro Ribera[1,2] and Sofia J. Araújo[1,2]

[1]Department of Genetics, Microbiology and Statistics, School of Biology, University of Barcelona, 08028 Barcelona, Spain

[2]Institute of Biomedicine University of Barcelona (IBUB), Barcelona, Spain

Dendrite branching plays an essential role to obtain a complex and functional nervous system. It is a process by which neurons achieve their arborized structure, which receives and integrates the electrochemical stimuli from other neural cells. The cytoskeleton provides the shape to the cell, therefore it is also involved in the ramification of dendrites. Branching development is driven by the assembly of the microtubule cytoskeleton from structures called Microtubule Organizing Centres (MTOCs). In many single cell branching processes, the centrosome is the major MTOC. Altered microtubule dynamics can be the origin of changes in neuronal branching. For example, it can lead to decreased branching, related to some neuropsychiatric and neurodevelopmental diseases such as mental retardation and Down's Syndrome. The full link between the number of centrosomes and the amount of dendrite branching is not yet known. To perform this study, we used specific neurons from the Peripheral Nervous system (PNS) of *Drosophila melanogaster*, called dendritic arborisation neurons. Specifically, we studied dendritic branching in one of these neurons, called v'pda. The number of centrosomes and the magnitude of dendrite branching is quantified in these cells during embryonic and larval dendritic development. To do so, we used and compared wild-type and mutant, with normal and altered number of centrosomes.

Genome wide approaches reveal a putative *wnt1* enhancer with a *foxG* motif as a key element to specify posterior fate in planarians

P. Coronel-Córdoba[1], E. Pascual-Carreras[1], M. Marín[1], S. Castillo-Lara[1], , M.S. Magri[2], J.F. Abril[1], J.L. Gomez-Skarmeta[2], E. Saló[1] and T. Adell[1]

[1]Department of Genetics, Microbiology and Statistics, Universitat de Barcelona (UB) & Institute of Biomedicine of Universitat de Barcelona (IBUB), Barcelona, Spain.
[2]Centro Andaluz de Biología del Desarollo (CABD), Universidad Pablo de Olavide, Sevilla

Planarians are tiny flatworms that can regenerate any missing part of their bodies. This huge capacity of regeneration is thanks to a large population of adult pluripotent stem cells combined with the signals that allow the proper pattern of the regenerated tissue. The signals responsible to regenerate anterior versus posterior structures according to the polarity of the regenerating fragment came from organizers (or organizing centers) that are established in any planarian wound. Previous studies show that the posterior organizer is defined by the expression of *wnt1* and the anterior by *notum* (*wnt1* inhibitor). Importantly, at 12h of regeneration both *wnt1* and *notum* are expressed in any wound, but by 36h *notum* becomes restricted to anterior and *wnt1* to posterior. To understand how *wnt1* is restricted to the posterior pole we performed genome wide approaches (ATAC-Seq and ChIP-Seq) and we found a foxG motif in an enhancer located in the first intron of *wnt1*. This data suggested that a context-specific regulation of this enhancer by foxG could be essential to restrict *wnt1* expression to the appropriate pole. Accordingly, we demonstrate that *foxG* (RNAi) animals do not express *wnt1* and lack a posterior organizer. At high doses of inhibition, *foxG* (RNAis) become two-headed (regenerates a head in posterior instead of a tail). We are preparing in vitro assays to demonstrate that planarian *foxG* can activate the *wnt1* enhancer.

Long non-coding RNAs and the evolution of heart development in *Oikopleura dioica*

Beatriz Iralde-Cárdenas, Carlos Herrera-Úbeda, Cristina Frías-López, Alfonso Ferrández-Roldán, Jordi García-Fernàndez, Cristian Cañestro

Departament de Genètica, Microbiologia i Estadística and Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Barcelona (Spain)
Correspondence: canestro@ub.edu

Oikopleura dioica (O. dioica) is an emergent model organism in Evo-Devo to study the impact of gene loss in the evolution of the mechanisms of development. In this context, we study long non-coding RNAs (lncRNAs) in O. dioica, using the development of the heart as a case study. We focused our attention on lncRNAs that could be regulating myosins as has been described for myosin heavy chain 7 that has an associated lncRNA conserved in different vertebrates, MHRT. To accomplish the objectives of this project we aimed to characterize the first catalogue of lncRNAs in O. dioica and to analyze the role of lncRNAs in the regulation of cardiac myosins. After adapting a pipeline from other non-vertebrate chordates, we have identified 12,581 putative lncRNAs and made the first bioinformatic resource to navigate and test for the presence of lncRNAs throughout the genome of O. dioica. In our gene survey, we have identified the presence of 14 myosins of class II in O. dioica. Our phylogenetic analyses of 119 sequences, from 14 species representing all groups of chordates, reveals a duplication that originated two paralogous groups of 'striated' myosins within Urochordates: Myo-uroA and Myo-uroB. Mapping of all lncRNAs shows that 15 lncRNAs are intimately linked to 6 of the myosins in O. dioica. Comparisons across distant populations of O. dioica reveals two candidate lncRNAs with conserved positions and high sequence similarity as the most suitable candidates to be regulating myosins in O. dioica. Our results also reveal the presence, for the first time, of a 'non-striated' myosin in appendicularians, which could be useful to discover the presence of smooth muscle that have passed unnoticed until today.

Nutrition and Insulin signaling are required for p38-dependent regeneration

José Esteban-Collado, Mar Fernandez Mañas, Montserrat Corominas and 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

Regeneration upon tissue damage needs early signals to trigger the tissue repair machinery. Reactive oxygen species (ROS) are early signals released by damaged cells. In the living tissue ROS are sensed by the MAP3 kinase Ask1, which in turn activates the MAP kinases p38 and JNK by phosphorylation. Sustained or high levels of activation of those kinases can result in apoptosis, whereas short or low activation can promote regeneration. It is known that cells need to activate Ask1-dependent regeneration program. In this process PI3K/Akt signaling is necessary for Ask1 to activate p38 but not JNK. Nutrient restriction or mutations that target Ser83 of the *Drosophila* Ask1, a PI3K/Akt-sensitive residue, block regeneration. However, these effects can be rescued by ectopic activation of p38, but not of JNK. Our results demonstrate that Ask1 controls the function of p38 through Ser83 and that p38 is a nutrient-sensitive molecule necessary for regeneration. This mechanism is key to discriminate between p38 and JNK in cells involved in tissue repair and growth.

The cytoplasmic LIM domain protein Espinas/PRICKLE2 contributes to wiring specificity in the visual system

Fernández-Pineda, Alejandra*; Monge-Asensio*, Martí; Morey, Marta

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

During circuit assembly it is essential that neurons connect with their specific synaptic partners. To facilitate this process, a common strategy in many organisms is the organization of brain regions, including the fly visual system, in layers and columns. The atypical-cadherin Flamingo (Fmi) and the receptor Golden Goal (Gogo) were proposed to regulate the temporary- and final-target layer selection of the R8 photoreceptor, through the cytoplasmic domain of Gogo. Our data suggests that Fmi intracellular signaling is also relevant for R8 final-target layer selection. The LIM-domain cytoplasmic molecule Espinas (Esp) binds Fmi, and they cooperatively control dendritic self-avoidance in sensory neurons. We observed defects in R8 layer selection in *esn* mutants with axons extending past the final target layer, and we demonstrated that the LIM domain is necessary for layer selection. *fmi* knockdown in photoreceptors results in most R8 axons stalling at the temporary layer, however, we also detected R8 axons projecting past the final-target layer, and showed that *fmi* and *esn* genetically interact. Based on the previously described physical and genetic interactions between Fmi/Esn and the findings presented here, we propose that Esn signals downstream of Fmi to stabilize the R8 axon in its final target layer. The fact that Fmi and Esn, as well as their respective vertebrate homologs CELSR1/2 and PRICKLE2, are widely expressed in the brain, suggests that understanding their interactions can reveal conserved mechanisms and provide valuable insights into the development of the nervous system.