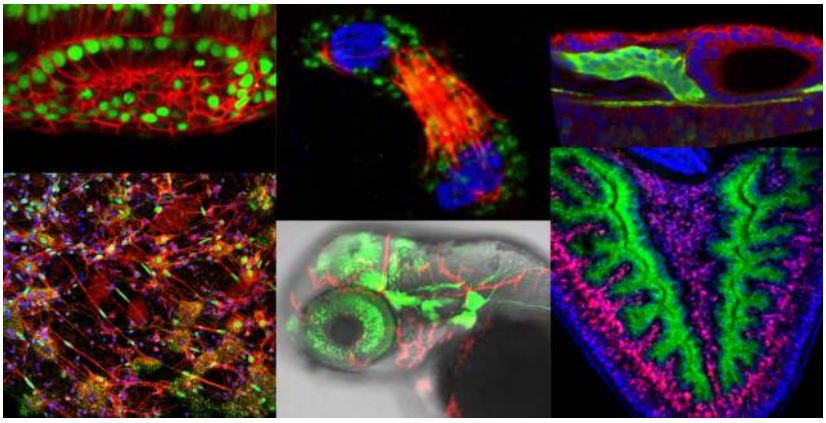

XXXI DEVELOPMENTAL BIOLOGY MEETING

Date: November 6th 2019

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

Organized by the Section of Developmental Biology of the SCB

Coordinator:
Francesc Cebrià (UB)

Collaborators:
Berta Alsina (UPF)
Marta Morey (UB)

Secretariat of the SCB:
Mariàngels Gallego
Maite Sánchez

Sponsored by:

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Societat Catalana de BIOLOGIA

XXXI JORNADA DE BIOLOGIA DEL DESENVOLUPAMENT
SOCIETAT CATALANA DE BIOLOGIA

Wednesday November 6th 2019

8:30-9.00 Arrival and Registration at Sala Prat de la Riba
9:00 Welcome by the Coordinator of the Section of Developmental Biology

9.10-10.00 **Miki Ebisuya (EMBL-Barcelona)** ***"Human time vs. mouse time: in vitro segmentation clock as a model system"***

Selected talks
10.00-10:20 Lara Barrio *"Regulation of anisotropic growth by two orthogonal signaling centers"*
10.20-10:40 Eudald Pascual *"ATACseq and RNAseq analysis reveal new elements of planarian posterior organizer"*
10:40-11:00 Carlos Herrera *"Microsyntenic Clusters Reveal Conservation of lncRNAs in Chordates Despite Absence of Sequence Conservation"*
11:00-11:30 Coffee break

Selected talks
11.30-11:50 Aitor Bañón *"Cellular and molecular mechanisms of the Development of the Statoacoustic Ganglion (SAG)"*
11:50 -12:10 Alba Ventos *"DNA methylation is crucial in cockroach early embryogenesis"*
12:10-12:30 Bastian-Jesper Klußmann-Fricke *"The influence of the basement membrane on cell morphology during the morphogenesis of the embryonic tracheal system in Drosophila"*

12:30-13:20 **Iñaki Ruiz-Trillo (IBE-CSIC-UPF-UB)** ***"A new perspective into the origin of animals"***

13:20-14:45 Lunch

Selected talks
14.45-15:05 James Cotterell *"Endogenous CRISPR/Cas9 arrays for scalable whole organism lineage tracing"*
15:05-15:25 Marta Morey *"Glial cells in the stem cell niche are required for proper neurogenesis and wiring of neural circuits"*
15:25-15:45 Juan Jose Fraire *"The role of ECM and Yki inactivation in the disassembly of a functional organ"*

15:45-17:45 Coffee break and Poster session

17:45 Concluding remarks and awards

INVITED TALKS

Miki Ebisuya (EMBL-BARCELONA)

Human time vs. Mouse time: in vitro segmentation clock as a model system

Different species have different tempos of development: larger animals tend to grow more slowly than smaller animals. My group has been trying to understand the molecular basis of this interspecies difference in developmental time, using the segmentation clock as a model system.

The segmentation clock is the oscillatory gene expressions that regulate the timing of somite formation from presomitic mesoderm (PSM) during embryogenesis. We have recently succeeded in inducing PSM from both human iPS cells and mouse ES cells, detecting the oscillation and traveling wave of segmentation clock in vitro. Interestingly, the oscillation period of human segmentation clock was 5-6 hours while that of mouse was 2-3 hours. Taking advantage of our in vitro system and simple mathematical models, we have been comparing the genome sequences and molecular processes of the segmentation clock between human and mouse to explain the interspecies difference in the oscillation period.

Iñaki Ruiz-Trillo (IBE-UPF-CSIC-UB)

A new perspective into the origin of animals

How animals emerged from their unicellular ancestors remains a mystery. To address this question, we first obtained genome data from the closest unicellular relatives of animals. Comparative genomic analyses demonstrated that the unicellular ancestor of animals was relatively quite complex, with genes involved in cell-adhesion, cell communication and cell differentiation. Moreover, we showed as well that many animal-like features of genomic regulation and spatial cell differentiation were also present in the unicellular ancestor. How those processes were co-opted and expanded, and how different cell types evolved remains unclear, and is the core of our current work. Overall, all the data generated from the closest unicellular relatives of animals allow us to challenge previous views of animal origins and propose a new perspective on how animals evolved.

SELECTED TALKS

ST1

Regulation of anisotropic growth by two orthogonal signaling centers

Lara Barrio and Marco Milán

IRBBarcelona

The primordium of the *Drosophila* wing has served as a paradigm to mechanistically characterize the role of morphogens in promoting patterning activities and growth from the signaling centers. Wingless (Wg) and Decapentaplegic (Dpp) morphogens are expressed in two orthogonal stripes that correspond to the compartment boundaries. While their gradients organize patterning by regulating the expression of well-defined target genes, the graded activity of these two morphogens is not an absolute requirement for wing growth. Despite the permissive growth-promoting role of Wg and Dpp, here we present evidence that these morphogens are utilized in a non-interchangeable manner by the two existing orthogonal signaling centers to promote preferential growth along the anterior-posterior and proximal-distal axes of the developing wing, respectively.

ST2

ATACseq and RNAseq analysis reveal new elements of planarian posterior organizer

E. Pascual-Carreras[1], M. Marín[1], S. Castillo-Lara[1], P. Coronel-Córdoba[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

Organizers or signaling centers are a group of cells with the ability to specify the fate of adjacent cells, allowing a patterned growth. Although organizers are mainly studied during embryogenesis, their function is also required in adults, for instance during regeneration. To better understand the formation and function of adult organizers, we study planarians, flatworms that are able to regenerate any missing body part. In planarians the anterior and posterior tips of the body behave as organizers, being defined by the expression of *notum* (a secreted Wnt inhibitor) and *wnt1*, respectively. The inhibition of any of those elements leads to a shift in polarity. Interestingly, during the first hours of regeneration both *notum* and *wnt1* are expressed in both poles, and it's around 36 hours that their expression becomes restricted to their respective tip. To decipher the molecular interactions that restrict the expression of *wnt1* to the posterior tip and confer the organizing activity we used genome wide approaches. ATACseq and RNAseq analysis of regenerating wild-type and *wnt1* (RNAi) planarians allowed the identification of specific Cis-Regulatory Elements (CREs) of posterior regeneration. We found that already at 12 hours of regeneration the accessible CREs in posterior and anterior blastemas have essentially changed, indicating that specific posterior chromatin changes induced by amputation occur much earlier than the formation of the organizers. Furthermore, we have identified specific transcription factors of the Otx and Fox families, which are enriched in posterior CREs and are essential for the specification of the posterior *wnt1*+ cells.

ST3

Microsyntenic Clusters Reveal Conservation of lncRNAs in Chordates Despite Absence of Sequence Conservation

Carlos Herrera-Úbeda[1], Marta Marín-Barba[2], Enrique Navas-Pérez[1], Jan Gravemeyer[3], Beatriz Albuixech-Crespo[1], Grant N. Wheeler[2] and Jordi Garcia-Fernández[1]

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[2]School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TU, UK
[3]German Cancer Research Center, 69120 Heidelberg, Germany

Homologous long non-coding RNAs (lncRNAs) are elusive to identify by sequence similarity due to their fast-evolutionary rate. Here we develop LincOFinder, a pipeline that finds conserved intergenic lncRNAs (lincRNAs) between distant related species by means of microsynteny analyses. Using this tool, we have identified 16 bona fide homologous lincRNAs between the amphioxus and human genomes. We characterized and compared in amphioxus and *Xenopus* the expression domain of one of them, *Hotairm1*, located in the anterior part of the Hox cluster. In addition, we analyzed the function of this lincRNA in *Xenopus*, showing that its disruption produces a severe headless phenotype, most probably by interfering with the regulation of the Hox cluster. Our results strongly suggest that this lincRNA has probably been regulating the Hox cluster since the early origin of chordates. Our work pioneers the use of syntenic searches to identify non-coding genes over long evolutionary distances and helps to further understand lncRNA evolution.

ST4

Cellular and molecular mechanisms of the Development of the Statoacoustic Ganglion (SAG)

Aitor Bañón and Berta Alsina

Laboratory of Developmental Biology, UPF-PRBB

The development of the statoacoustic ganglion (SAG) is a fascinating process in which distinct events such as neuronal specification, delamination, cell communication, migration and proliferation are tightly coordinated. However, it is little known how neuroblasts regulate their cellular behavior within the SAG. We make use of high spatiotemporal imaging of mosaic labeled neuroblasts in zebrafish to track and perform 4D cell reconstructions at the single cell level and investigate the dynamics of movements and cell shape changes. We also make use of CRISPR and Tol2 transgenesis techniques for missexpression and loss-of-function experiments of candidate genes implicated in SAG development. At early stages, neuroblasts display directional movements through active membrane protrusions towards a medial position where they coalesce to form the SAG. Neuroblasts also show dynamic and directed filopodia making contacts between them, suggesting that they could be involved in cell-cell communication. Currently, we are investigating the role of these signaling filopodia (cytonemes) by inhibiting filopodia exclusively in neuroblasts through the expression of the dominant-negative form of Irsp53, a protein needed for filopodia nucleation. At later stages, neuroblasts start the process of axogenesis. We have uncovered that these cells do not only innervate in a bipolar manner, but at early stages are multipolar, and display different shapes according to their location. Moreover, we are testing the role of FGF13a through CRISPR knock-out, a candidate gene implicated in sensory neuronal shape. Altogether, our results will shed light onto how sensory neurons communicate and arrange properly within the SAG.

ST5

DNA methylation is crucial in cockroach early embryogenesis

Alba Ventos-Alfonso, José Carlos Montañés and Xavier Belles

Institute of Evolutionary Biology (UPF-CSIC)

DNA methylation is a widespread epigenetic mechanism involved in the regulation of gene expression in eukaryotes. It is catalyzed by DNA-methyltransferases (DNMTs). In mammals, DNMTs are classified as *de novo* methylase (DNMT3) and as maintenance methylase (DNMT1). Studies in insects show the existence of functional DNMTs in different orders, although, in general,, DNA methylation is associated with the presence of DNMT1 rather than DNMT3. Hemimetabolan insect orders, like Hemiptera and Blattodea, show high levels of CpG methylation, whereas it is excepcional in holometabolans. We hypothesize that this difference in DNA methylation could be instrumental in the type of embryo development, andin the mode of metamorphosis. In the genome of the German cockroach, *Blattella germanica,* we found DNMT1*,* we studied its expression pattern and we observed that it is expressed in a narrow temporal window during early embryogenesis. Functional studies using maternal RNAi, showed that DNMT1-depleted embryos were unable to progress beyond day 3 (16% of development). Moreover, the analysis of DNA methylation status in CG rich regions by using Reduced Representation Bisulfite Sequencing (RRBS), indicated that DNMT1-depleted embryos had significantly lower levels of DNA-methylation. Regarding the possible relationship between DNA methylation and gene expression, we found that the expression of methylated genes is higher, in general, and more stable, than that of non-methylated genes, considering the early embryogenesis of *B. germanica.*

ST6

The influence of the basement membrane on cell morphology during the morphogenesis of the embryonic tracheal system in *Drosophila*

Klußmann-Fricke, B.-J. and Llimargas, M.

IBMB-CSIC

During development, different tissues and organs grow at different rates relative to each other, suggesting that tissue specific growth mechanisms control the morphogenesis of these structures. It has been shown that the extracellular matrix (ECM) in general, and the basement membrane (BM) in particular, plays a crucial role during those processes by being involved in cell migration and tissue-differentiation. The BM is formed by a network of proteins, which provides tissue and organstability and directly influences the development and maintenance of organ shape via its mechanical properties and composition. Recent studies demonstrated that, for example, induced erroneous signals from the BM promote localized changes in cell behaviour like altering patterns of cell motility, morphology, and adhesion. One of the best established model systems to study specific aspects of organ morphogenesis is the tracheal system of *Drosophila melanogaster*. It has been shown that the BM undergoes various steps of remodelling and degradation during the process of post-embryonic development and that Matrix Metalloproteases (MMPs) play a central role in the degradation of the BM.We have recently found that during embryonic development defects in the organisation of the BM leads to defects in the size and shape of the tracheal tubes. To characterize the cellular mechanisms underlying these defects and understand the contribution and influence of the BM on the morphogenesis and cellular architecture of the tracheal system, we misexpressed certain MMPs during embryonic development to degrade the BM. By using IHC and confocal microscopy in combination with deconvolution and 3D-reconstruction we determined the cell morphology and volume of the tracheal cells at different stages of embryonic development.

ST7

Endogenous CRISPR/Cas9 arrays for scalable whole organism lineage tracing.

James Cotterell and James Sharpe

EMBL-Barcelona

The last decade has seen a renewed appreciation of the central importance of cellular lineages to many questions in biology (especially organogenesis, stem cells and tumor biology). This has been driven in part by a renaissance in genetic clonal-labeling techniques. Recent approaches are based on accelerated mutation of DNA sequences, which can then be sequenced from individual cells to re-create a “phylogenetic” tree of cell lineage. However, current approaches depend on making transgenic alterations to the genome in question, which limit their application. Here, we introduce a new method which completely avoids the need for prior genetic engineering, by identifying endogenous CRISPR target arrays suitable for lineage analysis. In both mouse and zebrafish we identify the highest quality compact arrays as judged by equal base composition, 5’ G sequence, minimal likelihood of residing in the functional genome, minimal off targets and ease of amplification. We validate multiple high quality endogenous CRISPR arrays, demonstrating their utility for lineage tracing.

ST8

Glial cells in the stem cell niche are required for proper neurogenesis and wiring of neural circuits

Haritz Plazaola-Sasieta[1], Qi Zhu[1], Héctor Gaitán-Peñas[2,3], Martín Rios[1], Raúl Estévez[2,3], Marta Morey[1,4 *]

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Glial cells form part of the neural stem cell niche and express a wide variety of ion channels; however, the contribution of these channels to nervous system development is poorly understood. We explored the function of the *Drosophila ClC-a* chloride channel, since its mammalian ortholog *CLCN2* is expressed in glial cells, and defective channel function results in leukodystrophies, which in humans are accompanied by cognitive impairment. We found that *ClC-a* was expressed in the niche in cortex glia, which are closely associated with neurogenic tissues. Characterization of loss-of-function *ClC-a* mutants revealed that these animals had smaller brains and widespread wiring defects. We showed that *ClC-a* is required in cortex glia for neurogenesis in neuroepithelia and neuroblasts, and identified defects in a neuroblast lineage that generates guidepost glial cells essential for photoreceptor axon guidance. We propose that glia-mediated ionic homeostasis could non-autonomously affect neurogenesis, and consequently, the correct assembly of neural circuits.

ST9

The role of aECM and Yki inactivation in the disassembly of a functional organ

Juan J. Fraire-Zamora[1,2,3], Jérôme Solon[1,2] and Jordi Casanova[3]

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Epithelial integrity is a homeostatic condition necessary to maintain organ functions where epithelial cells must receive information from their environment, transduce it into the nucleus and take cellular decisions leading to survival or death. Very little is known on the cellular and molecular mechanisms leading to organ degeneration or disassembly. In the fruit fly *Drosophila melanogaster*, the larval respiratory organ (trachea) must disassemble during metamorphosis. Here I will present our results on how organ disassembly is achieved in *Drosophila* through cell area reduction, trachea shortening and activation of programmed cell death. During the initiation of metamorphosis, remodeling of the tracheal apical extracellular matrix (aECM), through matrix metalloprotease 1 (MMP1), results in apical cell area reduction and the initial shortening of the trachea. This length reduction is concomitant with i) a nuclear-to-cytoplasmic relocalization of the transcriptional coactivator Yorkie (Yki), ii) a decrease in transcription of the Death-associated inhibitor of apoptosis (diap1), iii) caspase activation and iv) initiation of apoptosis. Downregulation of both MMP1 and the Ste-20-like kinase Hippo (Hpo) prevents trachea shortening and cell death. Our results are in agreement with work in cell culture systems where ECM properties can modulate the nuclear localization of the Yes-associated protein (YAP, the mammalian orthologue of Yki). They also suggest that, in *Drosophila*, aECM remodeling through MMP1 activity and Yki inactivation via the Hippo signaling pathway lead to tracheal shortening and the initiation of apoptosis. These conserved cellular and molecular events could represent the general basis of organ disassembly in both invertebrates and vertebrates.

POSTERS

P1

Micromass culture to study the rolse of reaction-diffusion and active cell forces as mechanisms of self-organization in skeletal patterning.

Heura Cardona, Andrea Malandrino, Xavier Diego, Xavier Trepat, James Sharpe

EMBL-Barcelona

During limb development, skeletal patterning requires mesenchymal cells to form some of the most intricate structures observed during development. For the digits, it has been proposed that a reaction-diffusion mechanism triggers the breaking of symmetry that precedes the formation of skeletal condensations. However, as in other developmental processes, active cell forces and cell movement could also be a key mechanism to trigger symmetry-breaking.

To assess the relative contributions of these two mechanisms, we have studied the patterns formed by cells from limbs of mouse embryos in micromass cultures on substrates of different stiffness. This experimental setup has allowed us to identify the symmetry breaking moment by analysing the dynamic expression of Sox9 and the local changes in cell density and velocity, confirming that stiffness changes the timing and features of the patterns in a consistent way.

We have used Traction Force Microscopy to quantify the evolution of cell forces during the formation of the patterns and their variation with stiffness. In addition, we have analysed the transcriptome of the different cell populations performing RNA-seq at different time-points and studied the effects of stiffness on gene expression. These observations point to a possible coupling of mechanics and reaction-diffusion as the mechanism of cellular self-organization in skeletal patterning.

P2

The role of centrosomes in dendrite branching

Judith Castro and Sofia J. Araújo

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Institut de Biomedicina de la UB, IBUB

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.

P3

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 production of branched structures by single cells involves complex cytoskeletal remodelling events. In *Drosophila*, tracheal system terminal cells (TCs) and nervous system dendrites are models for these subcellular branching processes. During tracheal embryonic development, the generation of subcellular branches is characterized by extensive remodelling of the microtubule network and actin cytoskeleton, followed by vesicular transport and membrane dynamics. We have recently shown that centrosomes are key players in the initiation of subcellular lumen formation where they act as microtubule organizing centres (MTOCs). However, not much is known on the events that lead to the growth of these subcellular branches or what makes them progress through a particular trajectory within the cytoplasm of the TC. We have identified that the spectraplakin *Short-stop* (*Shot*) promotes the crosstalk between microtubules 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 signalling and an excess of Shot induces ectopic acentrosomal branching points in the embryonic and larval tracheal TC leading to cells with extra subcellular lumina.

P4

Role of Sema3E/PlexinD1 in the developing and adult hippocampal formation.

V. Gil[1,2,3,4], A. Mata[1,2,3,4], J. Pérez-Clausell[2], E. Soriano[2,3,4,5,6] and J.A. del Río[1,2,3,4]

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Introduction: The hippocampal formation is a well-organized architecture: principal cells are located in single layers and their afferents (entorhinal and commissural/associational axons) are distributed in well-defined lamina. The development and axonal wiring of the hippocampal formation depends on the action of numerous axon guidance cues. Among others, Class III Semaphorins are difussible molecules with crucial roles during hippocampal and neocortical development. Although the role of several members of the family is well described, little is known about the roles of Sema3E and its high affinity receptor, PlexinD1, during the development of the hippocampal formation and in the adult.

Materials and methods: We have been able to analyze the expression patterns of Sema3E and PlexinD1 in the developing and adult hippocampal formation of wild-type mice. We developed co-culture experiments in vitro to study how the entorhinal axons of the developing hippocampal formation behave in presence of Sema3E. In addition, we analyzed the development of the entorhino-hippocampal connection at early postnatal stages in presence or absence of Sema3E or PlexinD1, by using KO and lox mice respectively. Lastly we have analysed the citoarchitecture and alterations of the hippocampus in their adult stages.

Results and conclusions: Our results demonstrate that:

1) Sema3E/PlexinD1 play a role in the development of the entorrino-hippocampal connection by inhibiting entorhinal axons.

2) Absence of Sema3E/PlexinD1 signalling triggers the aberrant layering of entorhinal axons in the hippocampus.

3) Absence of Sema3E/PlexinD1 signalling in the adult misorganizes mossy fibers with relevant ectopic fibers in the molecular layer of the dentate gyrus.

4) In addition, the granule cell layer of the dentate gyrus displayed a “wavy” organization due to alteration in the proliferation of dentate precursors and altered position of postmitotic granule cells.

P5

The *Smed-cbp* family regulates stem cell biology in the planarian *Schmidtea mediterranea*

Susanna Fraguas[1], Coral Vivancos[1], Sheila Cárcel[1], Marta Marín[1], Jordi Ginés[1], Thileepan Sekaran[2], Kerstin Bartscherer[2], Rafael Romero[1] and Francesc Cebrià[1]
[1]Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona and Institute of Biomedicine of the University of Barcelona (IBUB)
[2]Hubrecht Institute, The Netherlands

Stem cells plasticity and differentiation is still an open question in developmental biology. Recently, it has been shown the importance of epigenetics in the regulation of the behaviour of stem cells. CBP (CREB- binding protein) is a conserved gene familiy, which functions as a transcriptional co-activator and shows an important role in a wide range of cellular processes, cell death, DNA damage response and tumorogenesis, in many organisms. Moreover, CBPs have an acetyl transferase activity that is relevant as histone acetylation results in changes in chromatin architecture that affects gene expression. 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 have identified five different *Smed-cbp* genes in *S. mediterranea* that show different expression patterns. Functional analyses indicate that *Smed-cbp-2* plays an important role in blastema formation. Moreover, this gene seems to be essential for stem cell survival which results in many defects on neural and eye differentiation after its silencing. On the other hand, the inhibition of *Smed-cbp-3* by RNAi results in the growth of apparently normal blastema; however, these remain largely depigmented and undifferentiated, even after two weeks of regeneration. *Smed-cbp-3* silencing affects the differentiation of several cell lineages including neural, digestive and excretory cell types. Recently, ATACseq experiments have uncovered differences in the chromatin architecture between controls and *Smed-cbp-3* RNAi animals. These results open the door to further investigate the epigenetic regulation of planarian stem cells proliferation and differentiation.

P6

Early *hippo* downstream effector genes in planarians homeostatic tissue renewal

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

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

Regeneration and tissue renewal are key tightly controlled processes in adult homeostasis which dysfunction may lead to neoplasia. The Hippo signaling pathway acts as a key hub in the control of cellular renewal found systematically deregulated in tumoral processes controlling cell proliferation, cell death and cell differentiation in different animal model organisms. However, its specific cellular functions and molecular targets remain poorly understood. Our team has observed that *hippo* inhibition produces tumoral overgrowths in planarians, flatworms that endow a continuous tissue renewal while changing their size according to nutrients availability. In this *in vivo* context, overgrowths are caused by the inability of *hippo* knockdown cells to maintain the differentiated fate, to properly cycle and die when required. In the present study we characterize the function of putative *hippo* target genes found deregulated in a transcriptomic analysis of *hippo* RNAi animals. These candidates are related to different biological processes and cellular components ranging from transcription to the cytoskeletal architecture but none of their roles have been properly characterized. The RNAi inhibition of these target genes produces overgrowths coupled in most of the cases with alterations in the cell cycle and disruption of the nervous system like in an *hippo* inhibition context. This connection sets them as potential effectors upon inhibition of the pathway in planarians and, probably, in humans. It also puts planarians on the spot as dynamic model to analyze the molecular effects of the Hippo pathway inhibition in relation to cell renewal through the study of novel putative effectors.

P7

Mitochondrial activity in hippo-related overgrowths

Rosselló, M., Sànchez, N., Saló, E. & Adell, T

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Understanding how tissue homeostasis is maintained is an essential question in developmental biology, since its malfunction leads to common diseases as cancer. The Hippo pathway is an evolutionary conserved cell-cell communication mechanism that controls the cell renewal underlying homeostasis. The Hippo pathway senses the contacts of the cellular membrane with the surrounding cells or extracellular matrix components and, according to it, regulates cell proliferation, cell death and cell differentiation. 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. This ability relies on a population of adult pluripotent stem cells located through their body. Inhibition of *hippo* in planarians leads to the formation of overgrowths, which is the same phenotype observed after *hippo* inhibition in all animals studied. Importantly, we recently demonstrated that the formation of this overgrowths is not caused by the over proliferation of the stem cells but by the dedifferentiation of committed cells. The transcriptomic analysis of those *hippo* knockdown animals revealed that changes in the mitochondrial activity could be directly linked to the formation of the overgrowths. The RNAi inhibition of candidate genes of the transcriptomic analysis with mitochondrial function caused a similar phenotype to the one observed after *hippo* inhibition. We are currently investigating the specific effect that *hippo* inhibition could have in the mitochondrial activity in different planarian cell types and its impact in the formation of the overgrowths.

P8

The cell adhesion protein Sidekick acts as a mechanosensor at tricellular adherens junctions to resolve epithelial cell rearrangements

Annalisa Letizia, DanQing He, Sergio Astigarraga, Colombelli Julien, Hatini Victor, Jessica Treisman and Marta Llimargas

IBMB-CSIC

Specialised types of cell junctions, tricellular junctions, are found at the points where three or more cells meet. tSJs (tricellular Septate Junctions) in Drosophila and tTJs (tricellular Tight Junctions) in vertebrates contain transmembrane proteins that are specifically localised at these tricellular vertices and are required to seal the epithelial barrier. tAJs (tricellular Adherens Junctions) are much less characterised and no specific components have been identified so far. tAJs have been postulated to represent points of high tension at which the ends of actin filaments must be anchored to the membrane. We have identified Sidekick, a cell adhesion protein of the immunoglobulin family, as a key component of tAJs. We find that Sidekick strongly accumulates at tAJs and that this localisation is modulated by tension. In turn, Sdk is required to maintain tension in remodelling epithelia, and its loss of function prevents normal cell rearrangements in tissues like the embryonic tracheal system or the pupal retina. Sidekick contains an intracellular PDZ-binding motif that is required for Sidekick function and that interacts with Polychaetoid. Polychaetoid and its partner protein Canoe are enriched at tricellular contacts in a Sidekick-dependent manner. We propose that Sidekick is a critical component of tAJs, that may sense tension and coordinate cells through homophilic interactions, and recruit, in response, an intracellular complex formed by actin, actin-binding proteins, adhesion proteins and actin-AJs crosslinkers that regulate the tension required to properly resolve cell rearrangements.

P9

DYRK1A kinase is essential to sustain neurogenesis in the developing brain

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Human DYRK1A (Dual specificity Tyrosine Phosphorylation Regulated Kinase 1A) is encoded by a dosage dependent gene. The overexpression of *DYRK1A*, which is on chromosome-21, causes some of the neurological alterations associated to Down syndrome while its haploinsufficiency results in a syndrome characterized by the presence of developmental delay, microcephaly and cognitive impairment. We previously showed that mice with one null *Dyrk1a* allele (*Dyrk1a*$^{+/-}$) phenocopy the symptoms of *DYRK1A* syndrome while the mutation of the two *Dyrk1a* alleles causes severe growth delay and lethality at midgestation.

To provide further evidence on the role of DYRK1A in brain development we have generated a conditional *Dyrk1a* knockout mice (DYRK1A-Nes) by crossing *Dyrk1a*$^{fl/fl}$ mice with a Nestin-Cre transgenic mouse in which Cre-mediated recombination takes place in the neural tube as early as embryonic day (E)8. DYRK1A-Nes mice die at birth likely as a result of a severe reduction of the brain parenchyma that is already notorious at mid neurogenesis, by (E)15. This reduction particularly affects the striatum and is caused by a massive apoptosis that stars at E12.5 and affects both progenitors and differentiating neurons of the ventral telencephalon. Apoptosis in the dorsal telencephalon is also prominent but later in development. E13.5 DYRK1A-Nes brains show an augmented number of ventral and dorsal SOX2 progenitors and decreased number of neurons. These features correlate with an aberrant accumulation of nuclear Cyclin D1 that may affect cell cycle progression. Preliminary studies indicate that apoptosis in DYRK1A-Nes brains is triggered by DNA damage and depends on p53 activation. Collectively, our results suggest that replicative stress could be the cause of the severe brain phenotype observed in DYRK1A-Nes embryos.

P10

Gene loss impact on the evolution of the heart gene regulatory network in the chordate *Oikopleura dioica*

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In the advent of the bloom of sequenced genomes, it is becoming clear that gene losses are prevalent over gene gains**.** Little is known, however, about how gene loss can impact the evolution of the mechanisms of development. As a case study, we investigate how gene losses affected the cardiogenetic toolkit in the chordate *Oikopleura dioica*. The heart of *O. dioica* is the simplest of all chordates with only two tissue layers, the pericardium and the myocardium that beats against the stomach wall. Our work provides the first modern developmental atlas of the heart of *O. dioica* and describes the cell lineage fate map of cardiac progenitors up to tailbud stage and reveals that cardiac precursors derive from the most anterior muscular cells and migrate from the anterior part of the tail into the trunk, very similar as in ascidians. However, our exhaustive *in silico* survey for all cardiogenic factors conserved in chordates reveals important differences in *O. dioica* regarding its early signaling pathways as well as cardiac transcription factors involved in migration and differentiation, and little is known about the genetic mechanisms driving these cellular processes in *O. dioica*. Recent approaches such as knockdown generation and double FISH provide further evidence that, despite the highly similar behavior of early heart progenitors between *O. dioica* and ascidians, the former appears to have drastically changed the genetic mechanisms of chordate heart development, deconstructing its cardiac genetic toolkit with several gene losses, absence of cardiac expression and lack of action of developmental signaling pathways that are fundamental to make a heart in other chordates.

P11

ROS activated AKT and ASK1 act synergistically to promote regeneration in Drosophila imaginal discs

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Recent work has strengthened Drosophila imaginal discs as a model system for regeneration studies. Evidence is accumulating that oxidative stress drives the cellular responses for repair and regeneration. Apoptotic cells result in a burst of reactive oxygen species (ROS) that can propagate to neighboring cells. These ROS are known to activate JNK and p38 kinases for regenerative growth. A key issues arise from these observations: What is the link between ROS and the stress activated protein kinases p38 and JNK. We present here the Apoptosis Signal-regulating Kinase 1 (ASK1), a serine/threonine kinase belonging to the MAPKKK family as an intracellular sensor that responds to various stresses by phosphorylation of the JNK and p38 MAK pathways during Drosophila imaginal disc regeneration.

We show that Drosophila Ask1 senses reactive oxygen species (ROS) differently in damaged and undamaged cells and is required for regeneration. Stressed apoptotic cells produce high levels of ROS and promote high Ask1 activity. Neighboring undamaged cells shown low levels of ROS and activate Ask1, but such activity is attenuated by Pi3K dependent Akt1 phosphorylation. This attenuated activity of Ask1 in undamaged cells is necessary to drive regeneration.

We demonstrate here that the oxidative stress produced by ROS in apoptotic cells is sensed in neighboring cells by Ask1 and Akt1. This indicates that ROS act as a true signaling mechanism for regeneration. These findings contribute to our understanding of the molecular mechanism of communication between dying and living cells to trigger regeneration.