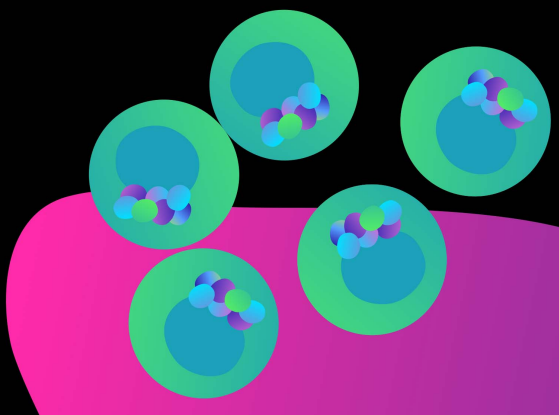
Joint Meeting of the Portuguese, Spanish and French Societies for Developmental Biology

4th Meeting of the Portuguese Society for Developmental Biology

7-10 NOVEMBER 2018
PORTO, PORTUGAL

Abstract Book



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PROGRAMME

WEDNESDAY, 7TH NOVEMBER

16:00 - 18:30 REGISTRATION and POSTER setup

18:30 - 18:45 OPENING SESSION

18:45 - 19:45 OPENING KEYNOTE LECTURE: ISDB-MOD LECTURE

Chair: Diogo Castro, Oeiras, PT

Jean-François Brunet, Paris, FR

A transcriptional view of the autonomic nervous system.

19:45 - 21:30 WELCOME RECEPTION

THURSDAY, 8TH NOVEMBER

SESSION 1_STEM CELLS / REGENERATION / ORGANOIDS

Chair: Miguel Manzanares, Madrid, ES

09:30 - 10:00 **Bernhard Payer**, Barcelona, ES

Epigenetic reprogramming in the female mouse germ line.

10:00 - 10:15 **Pedro Rifes**, Copenhagen, DK

Modelling human rostro-caudal neural tube patterning with a microfluidic morphogenic gradient.

10:15 - 10:30 **Inês Sequeira**, London, UK

The dynamics of hair follicle stem cells elucidated by clonal analysis and quantitative modelling.

10:30 - 11:00 **Anne Grapin-Botton**, Copenhagen, DK

Pancreas organoids to deconstruct developmental mechanisms.

11:00 - 11:30 COFFEE BREAK

11:30 - 12:00 **Lino Ferreira**, Cantanhede, PT

Induced pluripotent stem cell-derived tissues for drug screening and disease modeling.

12:00 - 12:15 **Hadi Boukhatmi**, Cambridge, UK

A population of adult satellite-like cells in Drosophila is maintained through a switch in RNA-isoforms.

12:15 - 12:30 **Covadonga Diaz-Diaz**, Madrid, ES

Analysis of cell-to-cell communication during cell competition in mammals.

12:30 - 13:00 **Stéphane Nedelec**, Paris, FR

Decoding rostro-caudal patterning mechanisms with human pluripotent stem cells.

13:00 - 14:00 LUNCH

14:00 - 16:00 POSTER SESSION 1 with coffee

SESSION 2_ EVO-DEVO / EMERGING MODELS

Chair: Moisés Mallo, Oeiras, PT

16:00 - 16:30 **Sylvie Rétaux**, Paris, FR

Eye morphogenesis in the blind Mexican cavefish.

16:30 - 17:00 **Patricia Beldade**, Toulouse, FR

Developmental plasticity: ExE and GxE effects in insect body size and pigmentation.

17:00 - 17:15 **Marco António Campinho**, Faro, PT

Asymmetric ossification drives eye migration during flatfish metamorphosis in a thyroid hormone-dependent manner.

17:15 - 17:30 **Élio Sucena**, Oeiras, PT

Chasing the elusive origins of novel enhancers: one step to novelty.

17:30 - 18:00 **Isaac Salazar-Ciudad**, Helsinki, FI

EmbryoMaker a general computational model for pattern formation, morphogenesis and evolution.

18:00 - 19:00 MEETINGS OF THE SOCIETIES

FRIDAY 9TH NOVEMBER

SESSION 3_NUCLEAR ARCHITECTURE / EPIGENETICS / TRANSCRIPTION / GRN

Chair: Domingos Henrique, Lisboa, PT

09:30 - 10:00 **Peter Askjaer**, Sevilla, ES

The nuclear envelope in gene expression and genome organisation.

10:00 - 10:15 **Samir Merabet**, Lyon, FR

Hox-dependent regulation of autophagy at the nuclear periphery.

10:15 - 10:30 **Paulo Navarro Costa**, Faro, PT

The Trithorax group protein dMLL3/4 instructs the assembly of the zygotic genome at fertilization.

10:30 - 11:00 **Ana Pombo**, Berlin, DE

In situ genome architecture mapping of rare cell types in the brain.

11:00 - 11:30 COFFEE BREAK

SESSION 4_NEURODEVELOPMENT: ISDN-SPONSORED SESSION

Chair: Fabienne Pituello, Toulouse, FR

11:30 - 12:00 **Patrick Blader**, Toulouse, FR

Coupling neural fate determination with morphogenetic movements during olfactory placode development.

12:00 - 12:15 **Xavier Morin**, Paris, FR

Asymmetric division, Notch signalling and neurogenesis in the embryonic spinal cord.

12:15 - 12:30 **Jonathan Enriquez**, Lyon, FR

Motoneurons and neuropil glia: Common origins yet divergent modes of development.

12:30 - 13:00 **Diogo Castro**, Oeiras, PT

Transcriptional control of glioblastoma cell invasiveness.

13:00 - 13:30 **Eloisa Herrera**, Alicante, ES

Building up bilateral circuits.

13:30 - 14:30 LUNCH

14:30 - 16:30 POSTER SESSION 2 with coffee

SESSION 5_SIGNALLING AND METABOLISM

Chair: António Jacinto, Lisboa, PT

16:30 - 17:00 **Alisson Gontijo**, Lisbon, PT

Dilp8-Lgr3 signaling facilitates cuticle remodeling during pupariation.

17:00 - 17:30 **Hitoyoshi Yasuo**, Villefranche-sur-Mer, FR

Distinct developmental routes of neural tissue formation in ascidian embryos.

17:30 - 17:45 **João Pedro Amorim**, Porto, PT

Impairment of a noggin2 notochord enhancer disrupts proper pancreas development.

17:45 - 18:00 **Marta Portela Esteban**, Madrid, ES

Active wingless vampirization by glioblastoma network leads to brain tumour growth and neurodegeneration.

18:00 - 18:30 **Marcos Gonzalez-Gaitan**, Geneva, CH

Scaling of morphogen gradients.

18:30 - 24:00 SOCIAL EVENT | MEETING DINNER

SATURDAY 10TH NOVEMBER

SESSION 6_MORPHOGENESIS / BIOMECHANICS / BIOPHYSICS

Chair: Alice Davy, Toulouse, FR

09:30 - 10:00 **Susana Lopes**, Lisbon, PT

The role of timely fluid-flow in the embryonic left-right organizer.

10:00 - 10:30 **Olivier Hamant**, Lyon, FR

Buffering intrinsic variability in development.

10:30 - 10:45 **Andrieu Cyril**, Toulouse, FR

Basolateral localization of MMP14/MT1-MMP drives cell polarity change during neural crest EMT independently of its catalytic activity.

10:45 - 11:00 **Marcelo Boareto**, Zürich, CH

Positional information encoded in the dynamic differences between neighbouring cellular oscillators.

11:00 - 11:30 COFFEE BREAK

11:30 - 12:00 **Verena Ruprecht**, Barcelona, ES

Adaptive protrusion and migration dynamics under tissue stress in early development.

12:00 - 12:15 Best SEBD Communication: **Maribel Franco**, Alicante, ES

Eph signalling controls mitotic spindle orientation and cell proliferation in the Drosophila optic lobe neuroepithelium.

12:15 - 12:30 SFBD Best PhD Thesis (sponsored by ZEISS)

12:30 – 12:45 SPBD Best PhD Thesis: **Rita Aires**, Oeiras, PT

Oct4 is a key regulator of vertebrate trunk length diversity

12:45 - 13:45 CLOSING KEYNOTE LECTURE: EMBO LECTURE

Chair: Miguel Torres, Madrid, ES

Angela Nieto, Alicante, ES

The complexity of the EMT: beyond cell migration in development and disease.

13:45 – 14:00 AWARD CEREMONY & CONCLUDING REMARKS

INTERNATIONAL SOCIETY of DEVELOPMENTAL BIOLOGISTS

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OPENING KEYNOTE LECTURE:

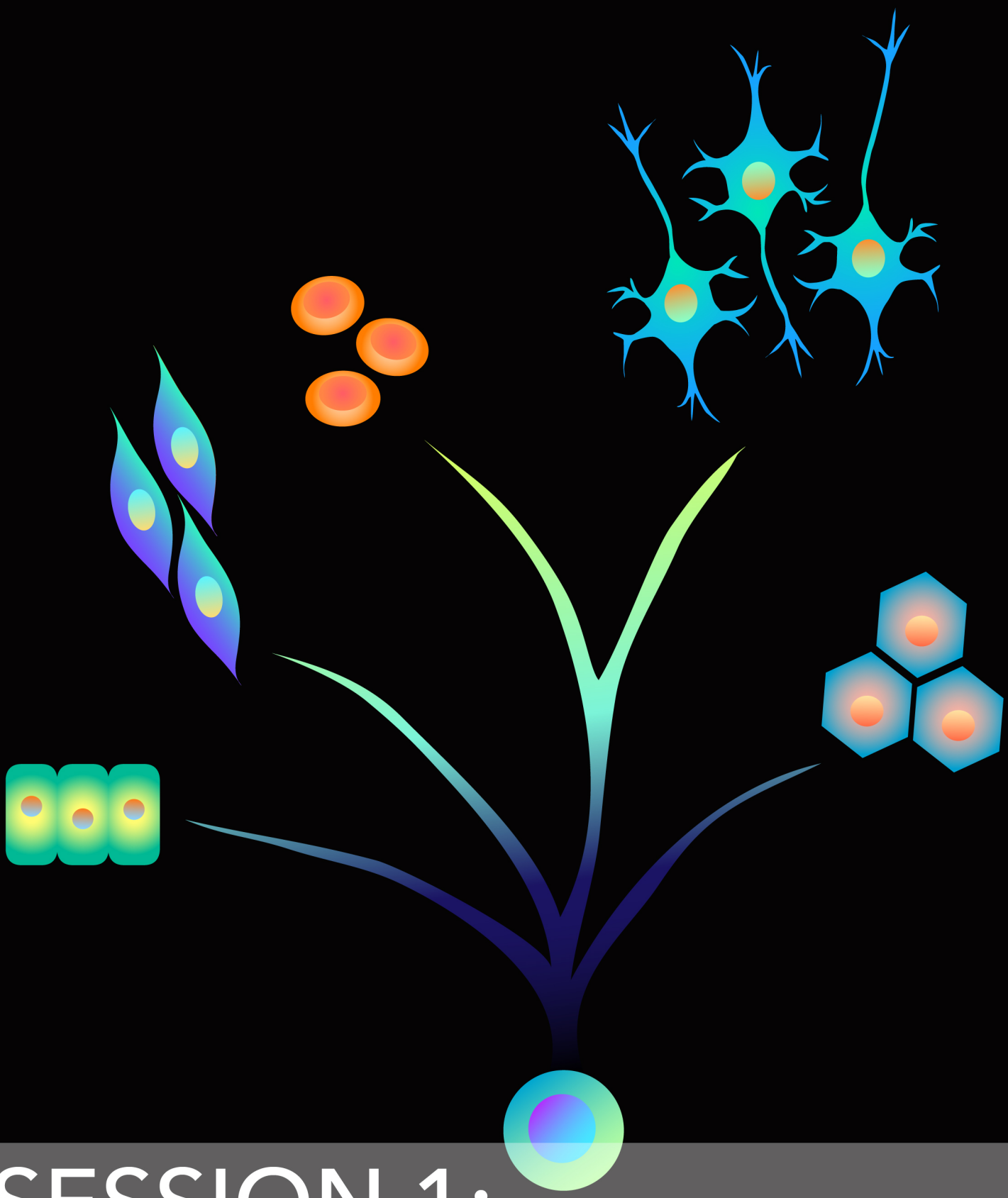
ISBD-MOD LECTURE

A TRANSCRIPTIONAL VIEW OF THE AUTONOMIC NERVOUS SYSTEM

Jean-François Brunet

École Normale Supérieure, Paris, FR

At the interface of developmental process and developmental outcome transcription factors are powerful descriptors of neuron types, the building blocks of neurophysiology. They are often viewed as making no sense individually, but only through their massively combinatorial action, from which neuronal identities and connections emerge. At variance with this general picture, I will discuss how a homeobox gene, singlehandedly and almost exhaustively, delineates a physiologically defined and evolutionarily ancient part of the vertebrate nervous system: the autonomic reflex circuits, in charge of bodily homeostasis; and how its expression status reveals the sacral autonomic outflow (to the pelvic organs) to be sympathetic, not parasympathetic, contrary to a century-old dogma.



SESSION 1:

STEM CELLS / REGENERATION / ORGANOIDS

EPIGENETIC REPROGRAMMING IN THE FEMALE MOUSE GERM LINE

Bernhard Payer

Centre for Genomic Regulation (CRG), Barcelona, ES

The germ cell lineage serves the unique function in transmitting genetic and epigenetic information from one generation to the next. In order to do so correctly, it has to undergo stepwise reprogramming events, which erase and establish epigenetic marks. In mammalian female germ cells the X-chromosome is an example for such changes as it switches from a previously inactive to an active state. Reactivation of the X-chromosome occurs during the migratory phase of primordial germ cells and during the colonization of the gonads.

In this talk, I will present our latest work on the X-reactivation process in mouse embryos and stem cell-derived germ cells. In particular I will focus on germ-cell intrinsic and extrinsic factors, which play a role in X-chromosomal epigenetic changes in germ cells. A key molecule we thereby identified is the transcriptional regulator PRDM14, which not only is important for germ cell specification, but which also regulates reprogramming of the repressive Histone H3 lysine 27 trimethylation (H3K27me3) mark. We found that PRDM14 plays a dual role in genome-wide and X-chromosomal H3K27me3 reprogramming and that these changes appear to be mechanistically separable.

Modelling human rostro-caudal neural tube patterning with a microfluidic morphogenic gradient

Pedro Rifes

University of Copenhagen - DanStem, Copenhagen, DK

Fundamental principles of embryonic development derived from animal models have been pivotal in understanding human development. However, only a fraction of these developmental findings has been validated in a human context due to limited availability of human fetal tissue.

Here, we designed an in vitro model of human brain development, based on pluripotent stem cells (hPSCs) and microfluidic culturing techniques, termed MISTR. In this system, we exposed differentiating hPSCs to a controlled gradient of WNT signalling to mimic early rostro-caudal neural patterning. We consistently obtained a coherent tissue, progressively caudalised from forebrain over midbrain to hindbrain, resembling the early regionalised human neural tube. Thus, by providing a pre-designed cue, we observe the autonomous recapitulation of neural tube patterning emerging in vitro, providing a glimpse into the primeval human neurodevelopment.

Using this model, we look into the of overexpression of LMX1A, a gene essential for the ventral midbrain, and into earlier MISTR tissues that depict the early steps of human neural patterning in search of putative defects arising from LMX1A overexpression. Therefore, MISTR represents a novel model of human early neurogenesis with controlled and predictable regionalisation for studying human brain development.

The dynamics of hair follicle stem cells elucidated by clonal analysis and quantitative modelling

Inês Sequeira

King's College London, London, UK

Adult stem cells (SC) reside in specialised compartmentalised niches. It is important to understand how SC self-renew to maintain their pool. Hair follicle SCs periodically self-renew to produce new hair. Their location has been identified in each phase of the hair follicle cycle, but the cellular relationships and the mode of self-renewal between these phases remain unclear. We combined extensive clonal analysis and quantitative modelling to gain insight into the process that ensures the coordination of SC loss and replacement. We show that the niche geometry together with coherent oriented growth are key features for stemness and lead to a uniform and stochastic replacement of SCs. Modelling allows us to estimate the division rate and to relate the SC position to the cell fate in the new follicle. We propose a novel mechanism based on the peculiar architecture of the hair follicle bulge organized in clonal columns. Also, we predict the degree of heterogeneity of the SC pool, the position of the population of cells, their proliferation rate and cellular behaviour throughout hair follicle cycles. These findings challenge the concept of the SC as slow-cycling and strengthens the idea of the cell heterogeneity and their hierarchical organization in the bulge/hair germ region based on their position in the niche.

PANCREAS ORGANOIDS TO DECONSTRUCT DEVELOPMENTAL MECHANISMS

Anne Grapin-Botton

DanStem, University of Copenhagen, Copenhagen, DK and Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, DE

Organoids representing a diversity of tissues have recently flourished, bridging the gap between cell lines or primary cells grown on the bottom of culture plates and experiments performed *in vivo*. Their ability to self-organize, that is to differentiate and organize cells in space, calls for the identification of the simple rules that underlie this capacity.

We established 3D culture conditions that enable the efficient expansion of dissociated mouse embryonic pancreatic progenitors. Two media compositions were established that unfold different responses in progenitors, either balancing progenitor expansion and endocrine cell production in spheres or balancing progenitor expansion and acinar cell production and the formation of a branched network of ducts. Focusing on the initial conditions leading to these organoids, we observed that the organoids formed if enough cells are clustered and identified a cooperative community effect. Assembling defined numbers of Notch active and inactive cells shows that their interaction is needed to initiate organoid formation and fuel growth. We also used this model to investigate how the branched structure of the pancreatic ducts emerges from the initial small cell aggregates.

Developing the model to study human development and model disease, we will also present recent data showing the robust expansion, differentiation and morphogenesis of human pancreatic organoids derived from embryonic stem cells.

INDUCED PLURIPOTENT STEM CELL-DERIVED TISSUES FOR DRUG SCREENING AND DISEASE MODELING

Lino Ferreira

CNBC, Cantanhede, PT

Human pluripotent stem cells (hPSCs) represent a potential source of cells for drug screening and disease modeling. In the last years, my group had a special interest in the derivation and maturation of vascular cells from hPSCs [1-3]. The group has done contributions in the identification and characterization of embryonic endothelial cells leading to the identification of a set of specific gene markers [2], in the derivation of brain-like endothelial cells and in the study of the role of the extracellular matrix as well as soluble factors in their specification, in the derivation of arterial and venous endothelial cells and in the functional characterization of these cells, and in the derivation of smooth muscle cells with an ageing phenotype. For the maturation process we have used biophysical forces and synthetic matrices with specific elastic moduli. During my talk I will give some examples about the use of these vascular tissues for drug screening and disease modeling.

References:

[1] H. Vazão, R.P. das Neves, M. Grãos, L. Ferreira, Towards the maturation and characterization of smooth muscle cells derived from human embryonic stem cells, PLoS One, 6 (2011) e17771.

[2] H. Vazão, S. Rosa, T. Barata, R. Costa, P.R. Pitrez, I. Honório, M.R. de Vries, D. Papatsenko, R. Benedito, D. Saris, A. Khademhosseini, P.H. Quax, C.F. Pereira, N. Mercader, H. Fernandes, L. Ferreira, High-throughput identification of small molecules that affect human embryonic vascular development, Proc Natl Acad Sci U S A, 114 (2017) E3022-E3031.

[3] L.S. Ferreira, S. Gerecht, H.F. Shieh, N. Watson, M.A. Rupnick, S.M. Dallabrida, G. Vunjak-Novakovic, R. Langer, Vascular progenitor cells isolated from human embryonic stem cells give rise to endothelial and smooth muscle like cells and form vascular networks in vivo, Circ Res, 101 (2007) 286-294.

A population of adult satellite-like cells in *Drosophila* is maintained through a switch in RNA-isoforms.

Hadi Boukhatmi; Sarah Bray

University of Cambridge, UK

Adult stem cells are important for tissue maintenance and repair. One key question is how such cells are specified and then protected from differentiation for a prolonged period. Investigating the maintenance of *Drosophila* muscle progenitors (MPs) we demonstrate that it involves a switch in zfh1/ZEB1 RNA-isoforms. Differentiation into functional muscles is accompanied by expression of *miR-8/miR-200*, which targets the major zfh1-long RNA isoform and decreases Zfh1 protein. Through activity of the Notch pathway, subsets of MPs produce an alternate *zfh1-short* isoform, which lacks the *miR-8* seed site. Zfh1 protein is thus maintained in these cells, enabling them to escape differentiation and persist as MPs in the adult. There, like mammalian satellite cells, they contribute to muscle homeostasis. Such preferential regulation of a specific RNA isoform, with differential sensitivity to miRs, is a powerful mechanism for maintaining a population of poised progenitors and may be of widespread significance.

Analysis of cell-to-cell communication during cell competition in mammals

Covadonga Díaz-Díaz; Rocío Sierra; Giovanna Giovinazzo; Miguel Torres

CNIC, Madrid, ES

In the mammalian epiblast and in embryonic stem cells (ESCs), Myc is heterogeneously expressed and Myc-low cells in confrontation with Myc-high cells are eliminated through cell competition. However, the mechanism by which cells compare each other and how cell interactions lead to Myc-low cells outcompetition remain unknown.

Identification of the cellular and molecular mechanisms specifically involved in the comparison of cell fitness remains a challenge. This question needs to be addressed to expand our knowledge on how multicellular organisms control cell quality.

Gap junctions are intercellular membrane channels that link cells for the exchange of ions and small molecules. In the *Drosophila* wing imaginal disc, cell communication is restricted at a cryptic boundary that divides the disk into cell units called compartments. Across these boundaries gap junctional exchange is lower than between cells of the same compartment. Interestingly, cell competition does not take place across compartment boundaries, suggesting limited communication between cells of different compartments prevents competition.

Here we develop strategies in the mouse model to restrict cell communication and assess the effect on competitive interactions. Preliminary results in ESCs suggest that blockade of gap junctional exchange partially inhibits competition. In order to study this *in vivo*, we are generating and analysing Gap junctions-null embryos for their ability to elicit Myc induced cell competition.

APPROACHING THE TEMPORALITY OF NEURONAL DIVERSIFICATION WITH HUMAN PLURIPOTENT STEM CELLS

Stéphane Nedelec

Institut du Fer à Moulin, Paris, FR

The vertebrate nervous system is composed of thousands of neuronal subtypes generated from limited pools of progenitors that sequentially give rise to different cell types. One striking example of temporal neuronal diversification is the progressive generation, from a pool of self-renewing spinal progenitors, of hundreds of motor neuron subtypes arrayed along the rostro-caudal axis of the spinal cord. The mechanisms regulating the temporality of this process remain unclear. To tackle this question, we have developed 3 dimensional-differentiation of human pluripotent stem cells that recapitulate many aspects of human spinal cord development. Using this approach, we are investigating the molecular mechanisms that control the patterning of spinal progenitors over time and underlie the rostro-caudal diversification of motor neuron subtypes. The elucidation of these developmental principles allows the engineering of spinal neuronal diversity with an unprecedented precision for future basic and translational studies.

SESSION 2:

EVO-DEVO / EMERGING MODELS

EYE MORPHOGENESIS IN THE BLIND MEXICAN CAVEFISH

Sylvie Rétaux[1]; Lucie Devos[1]; Joanne Edouard[2]; Victor Simon[1]; François Agnès[1]

[1]Paris-Saclay Institute of Neuroscience, Paris, FR; [2]Amagen Platform, FR

The fish *Astyanax mexicanus* comes in two forms: a normal river-dwelling form, and a blind cave-dwelling morph. Although the adult cavefish is completely eyeless, eyes first develop in embryos. The embryonic cavefish eyes suffer several molecular and morphogenesis defects: the lens undergoes apoptosis around 24 hours post-fertilization, and the retina shows a coloboma phenotype with an apparent lack of optic fissure closure. Through the analysis of regionalization and cell specification markers gene expression patterns and through 3D+time live imaging of the morphogenesis of the lens and the optic vesicle in the two morphs, we have examined the comparative development of the cavefish and surface fish eyes. We found that the early eye field and the early lens placode are reduced in size in cavefish, and that cell movements responsible for the invagination of the optic vesicle to form the optic cup are impaired. Potential molecular and cellular mechanisms will be discussed.

Work supported by ANR, Equipe FRM, and AVIESAN/INSERM grants

DEVELOPMENTAL PLASTICITY: EXE AND GXE EFFECTS IN INSECT BODY SIZE AND PIGMENTATION

Patrícia Beldade

CNRS - UMR 5174, EDB, Université Paul Sabatier, Toulouse 3, FR

External environmental cues can influence developmental rates and trajectories and lead to the production of different phenotypes from the same genotype. This developmental plasticity can result in a better match between adult phenotype and its environment, and help organisms cope with environmental heterogeneity. While such plasticity is prevalent in natural populations, the effects of the interaction between environmental factors (ExE) and of those with genetic variants (GxE) have been under-investigated experimentally. We know that plasticity can be triggered by different environmental factors that may be redundant, additive, or synergistic, but we know very little about how complex environmental information impacts plastic phenotypes. We also know that developmental plasticity is heritable and subject to selection, but we do not have much information about what loci contribute to the natural variation in plasticity that can feed its evolution. I will present work on ExE and GxE focusing on insect body size and pigmentation.

Asymmetric ossification drives eye migration during flatfish metamorphosis in a thyroid hormone-dependent manner

Marco António Campinho

CCMAR, University of Algarve, Faro, PT

Flatfish metamorphosis is a unique thyroid hormone (TH)-dependent post-embryonic developmental event where the symmetric pelagic larva develops into an asymmetric benthic juvenile. During this transition one of the eyes migrates to the opposite side of the head so that in the post-metamorphic juvenile the blind lateral side becomes ventral and the ocular lateral side becomes dorsal. Although TH have been shown to be the necessary and sufficient factors to this developmental transition to occur, the developmental mechanisms at the basis of flatfish morphological asymmetry acquisition remain an open question.

Here using sole (*Solea senegalensis*) as experimental model, we demonstrate that asymmetric deiodinase 2 expression ventrally juxtaposed to the migrating eye gives rise to asymmetric TH-dependent signalling and ossification that in turn drives eye migration to the opposite side of the head. Although flatfish are considered to be asymmetric our data shows that only the most anterior head region delimited by the eyes becomes asymmetric whereas the remainder of the head and organs therein stay symmetric.

We propose that this newly discovered mechanism is universal and drives eye migration given that sub-ocular ossification is universal in all flatfish analysed to date.

Chasing the elusive origins of novel enhancers: one step to novelty

Élio Sucena[1,2]; Kohtaro Tanaka[1]

[1]Instituto Gulbenkian de Ciência, Oeiras, PT; [2]Faculdade de Ciências da Universidade de Lisboa, Lisboa, PT

The origin of novelties has been at the heart of evolutionary thought since its inception. We approach this concept at the transcriptional regulation level, that is, simply as the acquisition by a gene of a novel expression pattern without any other necessary functional or evolutionary criterion. Over the past decades, it has become well established that pairs of duplicated genes generally show both overlapping and divergent expression domains. However, important questions regarding the mechanisms underlying this pattern remain: what are the evolutionary trajectories leading to divergent gene expression and what are the underlying cis-regulatory changes? The increasing number of genome sequences from closely related species allows detailed studies of recent duplication events opening the door to address these issues. We have been examining the gene-regulatory evolution of two tandem duplicates, the *Drosophila* Ly6 genes *CG9336* and *CG9338*. Despite their relatively recent origin, the paralogs in *D. melanogaster* have diverged in embryonic tissue-specificities from each other. We show that a single mutational event in the regulatory region of the *CG9338* gene of *D. melanogaster h*as endowed an enhancer with a novel capacity to drive *CG9338*gene expression. With this example we show that novelty at the transcriptional level may occur with surprising ease and reinforce the idea that cis-regulatory evolution may contribute to the high evolvability to biological systems.

EMBRYOMAKER A GENERAL COMPUTATIONAL MODEL FOR PATTERN FORMATION, MORPHOGENESIS AND EVOLUTION

Isaac Salazar-Ciudad

University of Helsinki, FI

The transformation of the embryo during development requires complex gene networks, cell signaling and gene-regulated cell behaviors (division, adhesion, polarization, apoptosis, contraction, extracellular matrix secretion, signal secretion and reception, etc.). No current general mathematical model of development implements all these processes in a unified framework. Here, we present a new computational model and accompanying open-source software, EmbryoMaker, that allows the user to simulate custom developmental processes by designing custom gene networks capable of regulating cell signaling and all animal basic cell behaviors. We also include an editor to implement different initial conditions, mutations and experimental manipulations.

We show the applicability of the model by simulating early tooth development and the evolution of developmental stability in embryoids. For the former we contrast our tooth model predictions against cell-tracking experiments, mechanical relaxation experiments and the observed tooth shape changes over developmental time. We found that two biomechanical processes, differential tissue growth and differential cell adhesion, were enough, in the model, for the development of the 3D morphology of the early tooth germ. For the latter we show that developmental stability against noise requires embryonic fields to be always partitioned in relatively small fields of gene expression.

SESSION 3:

NUCLEAR ARCHITECTURE / EPIGENETICS / TRANSCRIPTION / GRN

THE NUCLEAR ENVELOPE IN GENE EXPRESSION AND GENOME ORGANISATION

Peter Askjaer; Agnieszka Dobrzynska; Celia Muñoz-Jiménez; Raquel Romero-Bueno; Cristina Ayuso

Andalusian Center for Developmental Biology, Sevilla, ES

The nuclear envelope (NE) is an essential component of eukaryotic cells and regulates the genome and gene expression in multiple ways. Communication between the cytoplasm and the nucleus relies on active transport of specific macromolecules through nuclear pore complexes (NPCs) in the NE. Moreover, large proportions of the genome are anchored at the NE, which has important consequences on DNA replication, repair and transcription.

In addition to NPCs, the NE consists of the inner and outer nuclear membranes (INM and ONM) and the nuclear lamina lining the INM. The INM is characterised by a large number of integral membrane proteins that interact with the nuclear lamina and with chromatin. Strikingly, mutations in ubiquitously expressed NE proteins are causatively linked to a wide variety of human diseases.

To study NE biogenesis and function we combine genetics, live imaging and functional genomics in the nematode *Caenorhabditis elegans*. Using tissue-specific DamID and *in vivo* bimolecular complementation assays, we analyse the intricate networks of protein-protein and protein-chromatin interactions at the NE across different cell types and during development and ageing. This has revealed an unexpected role of the INM protein emerin in neuromuscular junction activity. Moreover, we have identified novel interactions between emerin, the protein kinase VRK1 and several chromatin factors. We will discuss the implications of these findings in gene expression and cell differentiation.

Hox-dependent regulation of autophagy at the nuclear periphery

Samir Merabet

CNRS-ENS, Lyon, FR

Gene regulation is not occurring randomly within the nucleus, but is under the control of nuclear matrix and chromatin-associated proteins that participate to the genome-wide compartmentalization of transcriptionally active and inactive regions. Surprisingly, how the specific regulatory activity of transcription factors (TFs) could be influenced by the spatial localization within the nucleus has rarely been considered. As a consequence, whether and how nuclear matrix components could participate to gene-specific regulatory complexes with TFs is not known.

Here we used Bimolecular Fluorescence Complementation (BiFC), Fluorescent In Situ Hybridization (FISH) and super resolution microscopy to describe a novel partnership involving Hox and LaminC (LamC) proteins in the context of autophagy regulation in the *Drosophila* fat body. We found that Hox DNA-binding on one side, and Hox-LamC interaction on the other side, is necessary for triggering autophagy related genes (*atg* genes) in a repressive environment at the nuclear periphery. Later on, active nuclear export of Hox proteins liberates *atg* loci for transcriptional activation, leading to massive autophagy of the fat body and metamorphosis. Our results demonstrate that transcriptional specificity of Hox proteins is relying on the control of their subnuclear localization through interactions with nuclear matrix components.

The Trithorax group protein dMLL3/4 instructs the assembly of the zygotic genome at fertilization

Pedro Prudêncio[1]; Gastón Guilgur[2]; João Sobral[2]; Jörg Becker[2]; Rui Martinho[3]; **Paulo Navarro-Costa**[1]

[1]Instituto de Medicina Molecular, Universidade de Lisboa, PT; [2]Instituto Gulbenkian de Ciência, Oeiras, PT; [3]Center for Biomedical Research, Universidade do Algarve, Faro, PT

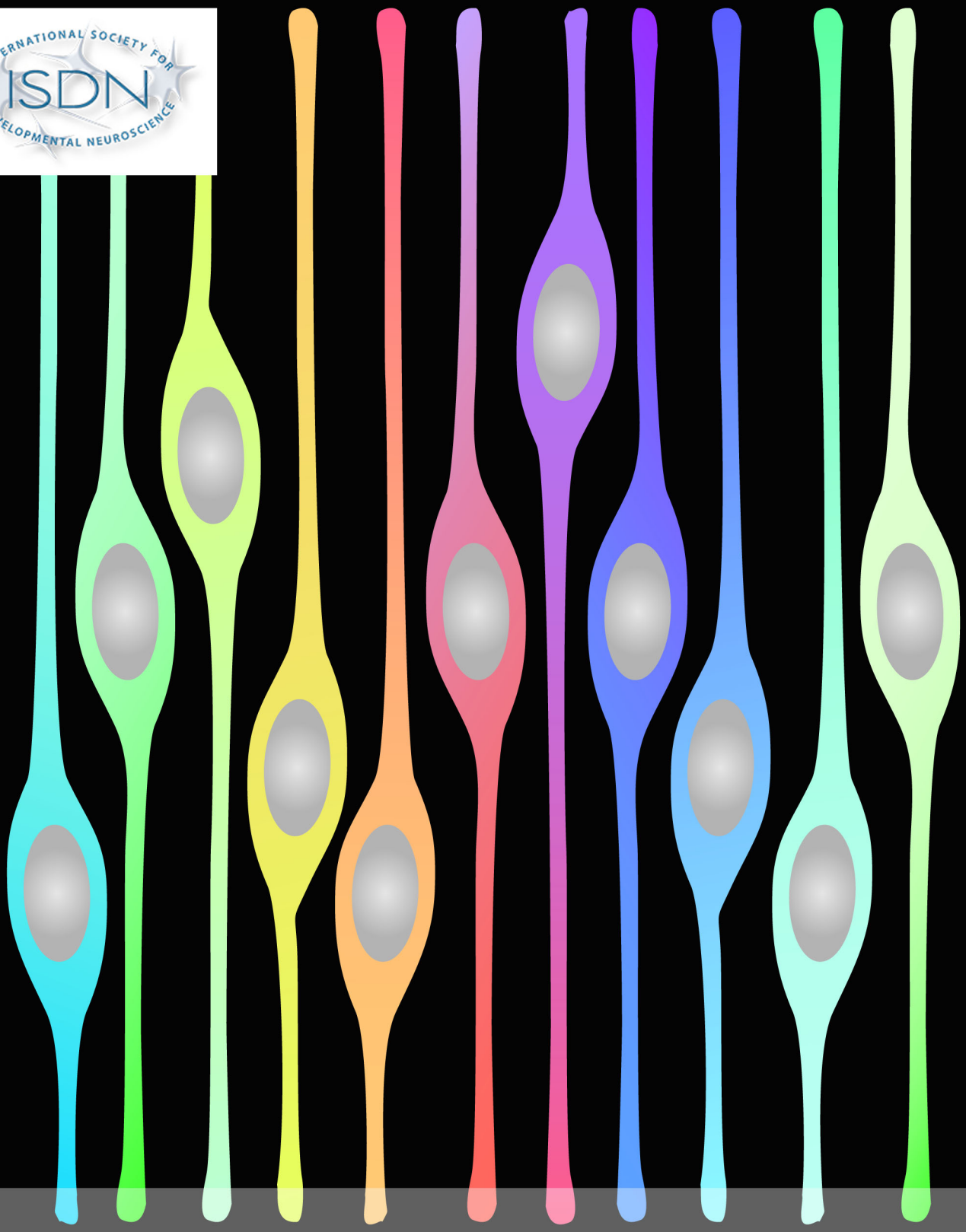
The transition from fertilized oocyte to totipotent embryo relies on maternal factors that are synthetized and accumulated during oocyte development. Yet, it is unclear how oocytes regulate the expression of maternal genes within the transcriptional program of oogenesis. Here we report that the *Drosophila* Trithorax group protein dMLL3/4 (also known as Trr) is essential for the transition to embryo fate at fertilization. In the absence of dMLL3/4, oocytes develop normally but fail to initiate the embryo mitotic divisions after fertilization. This incapability results from defects in paternal genome reprogramming and maternal meiotic completion. The methyltransferase activity of dMLL3/4 is dispensable for both these processes. We further show that dMLL3/4 promotes the expression of a functionally coherent gene subset that is required for the initiation of post-fertilization development. Accordingly, we identify the evolutionarily-conserved IDGF4 glycoprotein (known as oviductin in mammals) as a new oocyte-to-embryo transition gene under direct dMLL3/4 transcriptional control. Based on these observations, we propose that dMLL3/4 plays an instructive role in the oocyte-to-embryo transition that is functionally uncoupled from the requirements of oogenesis.

IN SITU GENOME ARCHITECTURE MAPPING OF RARE CELL TYPES IN THE BRAIN

Ana Pombo

Max Delbrück Center for Molecular Medicine, Berlin, DE

The folding of chromosomes and the structural organization of the genome impacts human health and disease. Gene expression is controlled by long-range chromatin contacts between non-coding regulatory regions and their target genes. Disruption of chromatin contacts due to disease-associated structural changes in the linear genome can result in altered patterns of gene expression. Chromatin contacts have so far only been mapped in cultured neuronal cell lines or in dissociated neural tissue. To overcome these disadvantages, we developed Genome Architecture Mapping (GAM) a novel in-situ approach that maps chromatin contacts by sequencing the DNA content of single nuclear sections from a population of individual cells. GAM is ideally suited to map contacts directly within a tissue of interest and with selective recovery of rare cell populations or states. Thus, we have developed the application of GAM in mouse brain tissues to identify in-situ chromatin contacts in rare neuronal populations. We have generated the first in-situ chromatin contact maps directly from dopaminergic neurons (DNs) in the ventral tegmental area and from pyramidal neurons (PNs) from the CA1 region of the hippocampus. We detect cell-type specific chromatin contacts between biologically important loci (e.g. neurodevelopment, synaptic plasticity) which can be separated by tens of megabases, revealing new aspects of 3D genome structure in neuronal function.



SESSION 4:

NEURODEVELOPMENT
ISDN SPONSORED SESSION

COUPLING NEURAL FATE DETERMINATION WITH MORPHOGENETIC MOVEMENTS DURING OLFACTORY PLACODE DEVELOPMENT

Patrick Blader

Centre for Integrative Biology, Centre for Developmental Biology, University of Toulouse, Toulouse, FR

The morphology of peripheral sensory organs is exquisitely adapted for detecting specific stimuli. While morphogenetic movements shape these organs, cell types are specified that will participate in the transmission of the sensory information. It remains an open question, however, as to whether morphogenesis and fate specification are molecularly coupled. We have shown that neurogenesis in the zebrafish olfactory placode requires the Neurog1. Concomitant with the earliest wave of olfactory neurogenesis, morphogenetic movements shape the placode into a rudimentary cup. We have used time-lapse confocal microscopy to characterize the morphogenetic behaviour of olfactory neural progenitors. Our results indicate that the oriented cell movements required for proper placode morphogenesis are affected in *neurog1* mutants. Morphogenesis is similarly affected by mutations in the chemokine receptor, *cxcr4b*, suggesting that it could be a Neurog1 target gene. Using a combination of promoter analysis, Chromatin IP and Crispr/Cas9 genome editing we show that Neurog1 directly regulates *cxcr4b* transcription via E-boxes located just upstream of the *cxcr4b* transcription start site. We conclude that proneural transcription factors, such as Neurog1, couple distinct aspects of nervous system development. Datasets generated in this study have been used to create a simple mathematical model of olfactory placode morphogenesis that we hope will further our understanding of this process in the future.

Asymmetric division, Notch signaling and neurogenesis in the embryonic spinal cord

Xavier Morin; Samuel Tozer; Chooyoung Baek

Institut de Biologie de l'École Normale Supérieure, Paris, FR

During vertebrate neurogenesis, progenitor cells undergo asymmetric divisions that maintain a pool of progenitors while producing differentiating neurons. Progenitors display high Notch activity, whereas neurons switch it off. This relies on the asymmetric partitioning of the Notch pathway regulator Mindbomb1 (Mib1). We have shown that the intrinsic asymmetry of centrosomes controls the asymmetric recruitment of Mib1 on spindle poles, priming daughter cells for unequal Notch activity.

Integrity of the neuroepithelium relies on subapical junctions while neurons lose this architecture. Newborn “prospective” neurons delaminate via a sequence of basal nuclear translocation, apical surface constriction and N-cadherin down-regulation, allowing the loss of adhesion before the cell differentiates. Surprisingly, Notch signaling remains active in prospective neurons during this transition. Upon precocious Notch blockade, nascent neurons disassemble their junctions but fail to reduce their apical surface. This weakens the junctional network and leads to breaches in the ventricular wall. We found that the Dll1 ligand promotes differentiation by reducing Notch signaling through cis-inhibition. However cis-inhibition is blocked by Mib1 during delamination. This transiently sustains high Notch activity and defers differentiation. A fine-tuned balance between trans-activation and cis-inhibition allows cells to seamlessly delaminate from the ventricular wall as they commit to differentiation.

Motoneurons and neuropil glia: common origins yet divergent modes of development

Jonathan Enriquez

Institut de génomique fonctionnelle de Lyon - CNRS - UMR5242, Lyon, FR

Neurons and glia are always associated with each other and exist in almost all bilateria, even in basal phyla such as flatworms (Hartline, 2011). As nervous system complexity increases during evolution, there is an increase in both neuronal and glia diversity (Paredes et al., 2016). In our lab we try to understand the development logic used by these two types of cells which lead to morphological diversity. We have shown that three of the stem cells that produced leg motor neurons in *Drosophila* also generate a specialized subset of glia, the neuropil glia, which wrap and send processes into the neuropil where motor neuron dendrites arborize. While the MNs use a code of TFs to create diversity and generate individual MNs with a very stereotyped morphology (Enriquez et al., 2015), the post-mitotic glia born from these lineages have not been observed to have a unique molecular code. Contrary to Neurons, the gliogenesis phase of these lineages is plastic and highly adaptable: when gliogenesis in one lineage is compromised, other lineages compensate to maintain the correct number of NG and the overall shape of the glial tissue (Enriquez et al., 2018). Thus, even though NG and MNs come from the same stem cells and generate adult neuromeres with highly stereotyped structures, there are fundamental differences in how these two cell types are morphologically specified.

TRANSCRIPTIONAL CONTROL OF GLIOBLASTOMA CELL INVASIVENESS

Pedro Rosmaninho[1]; Susanne Mükusch[2]; Stefan Momma[2], **Diogo S. Castro**[1]

[1]Instituto Gulbenkian de Ciência, Oeiras, PT; [2]Edinger Institute of Neurology, Frankfurt Medical School, DE

The importance of classical inducers of EMT such as ZEB1 in metastatic growth of a variety of cancers has been well documented, but how these transcription factors orchestrate such a complex genetic program remains poorly understood. This is due, at least in part, to the lack of a global view of the transcriptional programs regulated by EMT inducers. While Glioblastoma Multiforme (GBM), the most common and aggressive brain tumour in adults is not a classical model for EMT, recent studies have highlighted the importance of ZEB1 for tumour growth and invasion in this cancer type.

Here we combined genomics, functional and transcriptional studies in cellular models of GBM, with correlative analyses in patient tumour samples, to investigate ZEB1´s role in this brain tumour. We show that ZEB1 binding associates with either activation or repression of gene expression, depending on how it is recruited to regulatory regions. While direct binding results in transcriptional repression, indirect recruitment via LEF1 results in widespread gene activation, by a mechanism that is independent of the Wnt signalling. ZEB1 activated genes include predicted mediators of cell migration and invasion, such as the guanine nucleotide exchange factor PREX1.

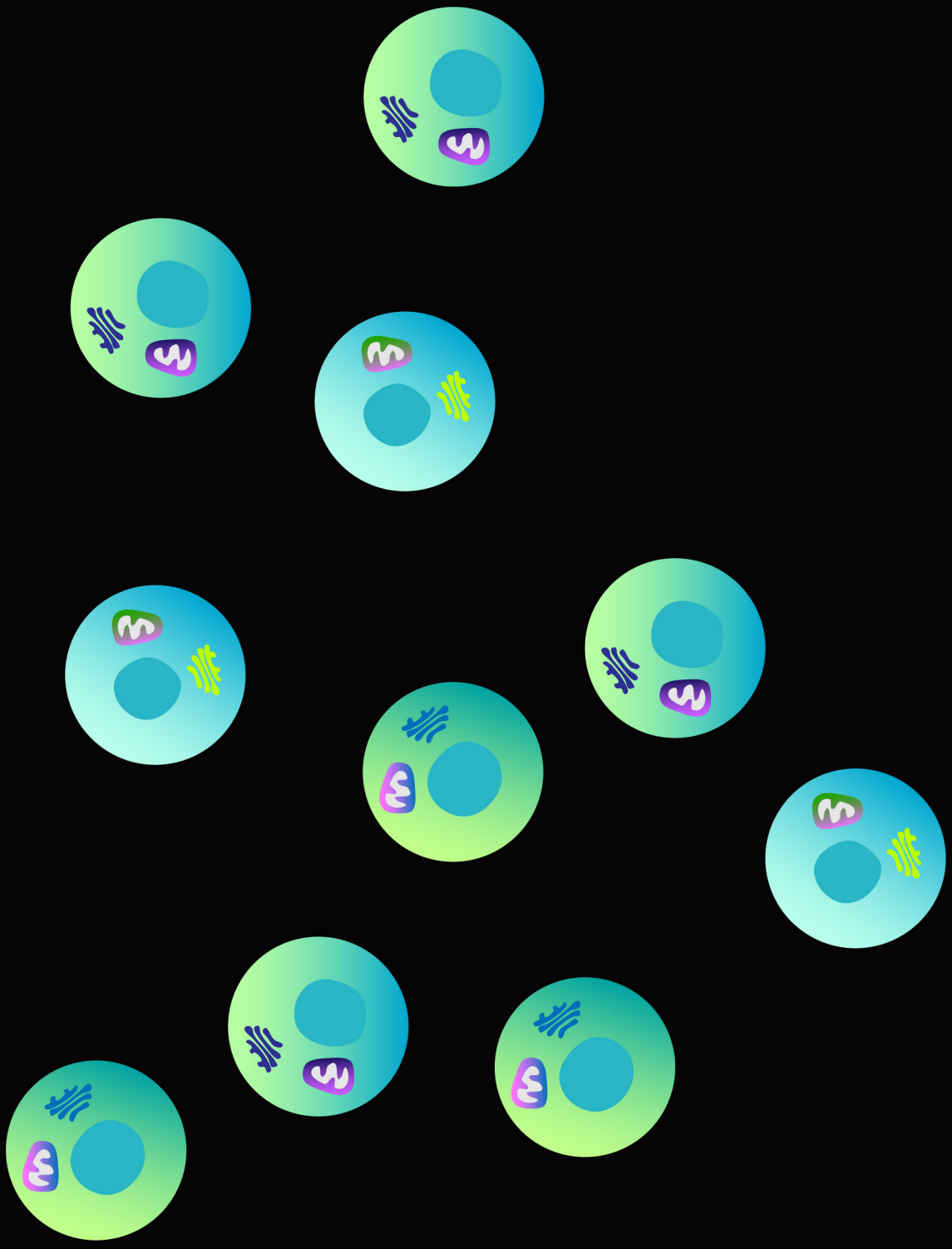
While providing important insights into the molecular mechanisms underlying GBM invasiveness, our work shows how a single EMT factor can coordinate a complex program of gene expression by simultaneously promoting gene activation and repression.

BUILDING UP BILATERAL CIRCUITS

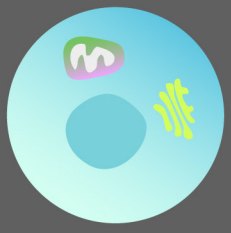
Eloisa Herrera

Instituto de Neurociencias, Alicante, ES

In humans and other species with bilateral symmetry, many features of the mature neural function depend on the coherent integration of sensory inputs coming from both sides of the body. The integration of sensory information and the communication between hemispheres both relay on the existence of bilateral circuits that contain neurons whose axons cross or avoid the midline to project contra- or ipsi-laterally. I will discuss the latest advances obtained in my laboratory that have contributed to identify some of the molecular mechanisms underlying the formation of bilateral circuits.



SESSION 5:



SIGNALLING AND METABOLISM

DILP8-LGR3 SIGNALING FACILITATES CUTICLE REMODELING DURING PUPARIATION

Andres Garelli[1,2]; Fabiana Heredia[1]; Yanel Volonte[1,2]; Joana Pereirinha[1]; Andreia Casimiro[1]; Filipe Viegas[1]; Kohtaro Tanaka[3]; Gisele Cardoso[1,4]; André Macedo[1]; Ana Leal[1]; Facundo Spalm[2]; **Alisson Gontijo**[1]

[1]CEDOC - NMS/FCM, Universidade Nova de Lisboa, PT; [2]INIBIBB, CONICET, AR; [3]Instituto Gulbenkian de Ciências, Oeiras, PT; [4]LGEA, DGBE, USP, São Paulo, BR

Higher dipterans undergo metamorphosis within a puparium, a protective capsule made up of the reshaped and hardened cuticle of the last larval instar. Puparium morphogenesis requires coordinated neuromuscular behaviors and chemical changes to the cuticle, all of which are poorly understood at the molecular and cellular level. Here, we find that loss-of-function mutations in the *Drosophila insulin-like peptide 8* (*dilp8*) and the *Leucine-rich repeat-containing G protein coupled receptor 3* (*Lgr3*) genes lead to an aberrantly elongated puparium. Tissue-specific genetic manipulations and gene expression studies show that puparium morphogenesis is facilitated by a developmentally-triggered 1-2h peak of *dilp8* expression that occurs in the cuticle epidermis at the onset of pupariation. Knock-down of *Lgr3* in a subset of neurons previously shown to require *Lgr3* to promote developmental stability of imaginal discs (adult appendage precursors) by controlling the timing of the onset of pupariation, had no effect on final puparium shape. In contrast, we find that *Lgr3* is required in a new subpopulation of neurons whose genetic synaptic silencing specifically disrupts puparium morphogenesis without affecting developmental timing. These results demonstrate that, apart from promoting developmental stability during larval development, the relaxin-like Dilp8-Lgr3 pathway mediates a new transient epidermis to neuron signaling event that facilitates cuticle remodeling during pupariation.

DISTINCT DEVELOPMENTAL ROUTES OF NEURAL TISSUE FORMATION IN ASCIDIAN EMBRYOS

Hitoyoshi Yasuo; Clare Hudson; Richard Copley; Cathy Sirour; Géraldine Williaume

CNRS, Villefranche-sur-Mer, FR

In the classical view of "neural induction" in chordates, neural fate is induced within embryonic ectoderm, which otherwise gives rise to epidermal fate. However, this view captures only part of the process and it now becomes clear that bi-potent neuromesodermal progenitors also make an important contribution to the nervous system. Thus, the chordate central nervous system is comprised of cells arising from distinct embryonic origins and developmental trajectories. It is thus important to determine how neural lineages are segregated via these distinct routes and to what extent the neural progenitors converge towards "generic" neural fate. Our research group has been addressing these questions using ascidian embryos, which develop into swimming larvae with a typical chordate body plan. Uniquely among chordates, ascidian embryogenesis proceeds with an invariant cell division pattern, such that cellular configurations and cell cycle progression are quasi-invariant. The cell lineages of two distinct neural lineages, one associated with ectoderm and the other with mesoderm, are documented with a resolution of individual cell divisions. In this talk, I will describe signalling mechanisms that progressively segregate these neural lineages and our recent single-cell RNA-seq analysis addressing the convergence of transcriptional trajectories of the two neural lineages.

Impairment of a *noggin2* notochord enhancer disrupts proper pancreas development

João Pedro Amorim[1]*; Ana Gali-Macedo[1]*; Hugo Marcelino[1]; Renata Carriço[1]; Silvia Naranjo[2]; Solangel Rivero-Gil[2]; Joana Teixeira[1]; Mafalda Galhardo[1]; Joana Marques[1]; Tania Medeiros[1]; Yolanda Roncero[3]; Jose Luis Gómez-Skarmeta[3]; José Bessa[1]

[1]i3S, Universidade do Porto, PT; [2]Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, ES; [3]CIBIO, Universidade do Porto, Vairão, PT
** Equal contribution*

During development, proper signaling cues are required for correct organ formation. In zebrafish, Bmp blocks the induction of dorsal bud-derived beta-cells. We have generated an insulator integration in the zebrafish genome that shows impaired beta-cell differentiation. This integration was mapped downstream of *noggin2* (*nog2*), that encodes for a diffusible antagonist of Bmp signaling. Embryos that carry this integration show decreased levels of *nog2* expression, suggesting that cis-regulatory enhancers of *nog2* are disconnected by the insulator. Accordingly, we found that knockdown of *nog2* results in a decreased number of beta-cells, most likely through the increase of Bmp signaling in pancreatic progenitor cells. Furthermore, analyzing the regulatory landscape of *nog2*, we have identified a downstream notochord enhancer, compatible with the *nog2* pattern of expression. Preliminary assays by Circular Chromosome Conformation Capture (4C-seq) have shown that this enhancer is indeed interacting with the promoter of *nog2*. In the hope to translate this important information to humans we have screened the genomic landscape of *NOG*, having found an enhancer that recapitulates similar regulatory functions. With these results we propose that Bmp from the lateral plate mesoderm must be counteracted by *nog2*, controlled by a notochord specific enhancer, to efficiently establish the correct differentiation of endocrine pancreatic cells.

Active wingless vampirization by glioblastoma network leads to brain tumor growth and neurodegeneration

Marta Portela Esteban[1]; Varun Ventakaramani[2]; Natasha Fahey-Lozano[1]; Esther Seco[1]; Maria Losada-Perez[1]; Frank Winkler[3]; Sergio Casas-Tinto[1]

[1]Cajal Institute, Madrid, ES; [2]Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg, DE; [3]Clinical Cooperation Unit Neurooncology, German Cancer Consortium (DKTK), DE

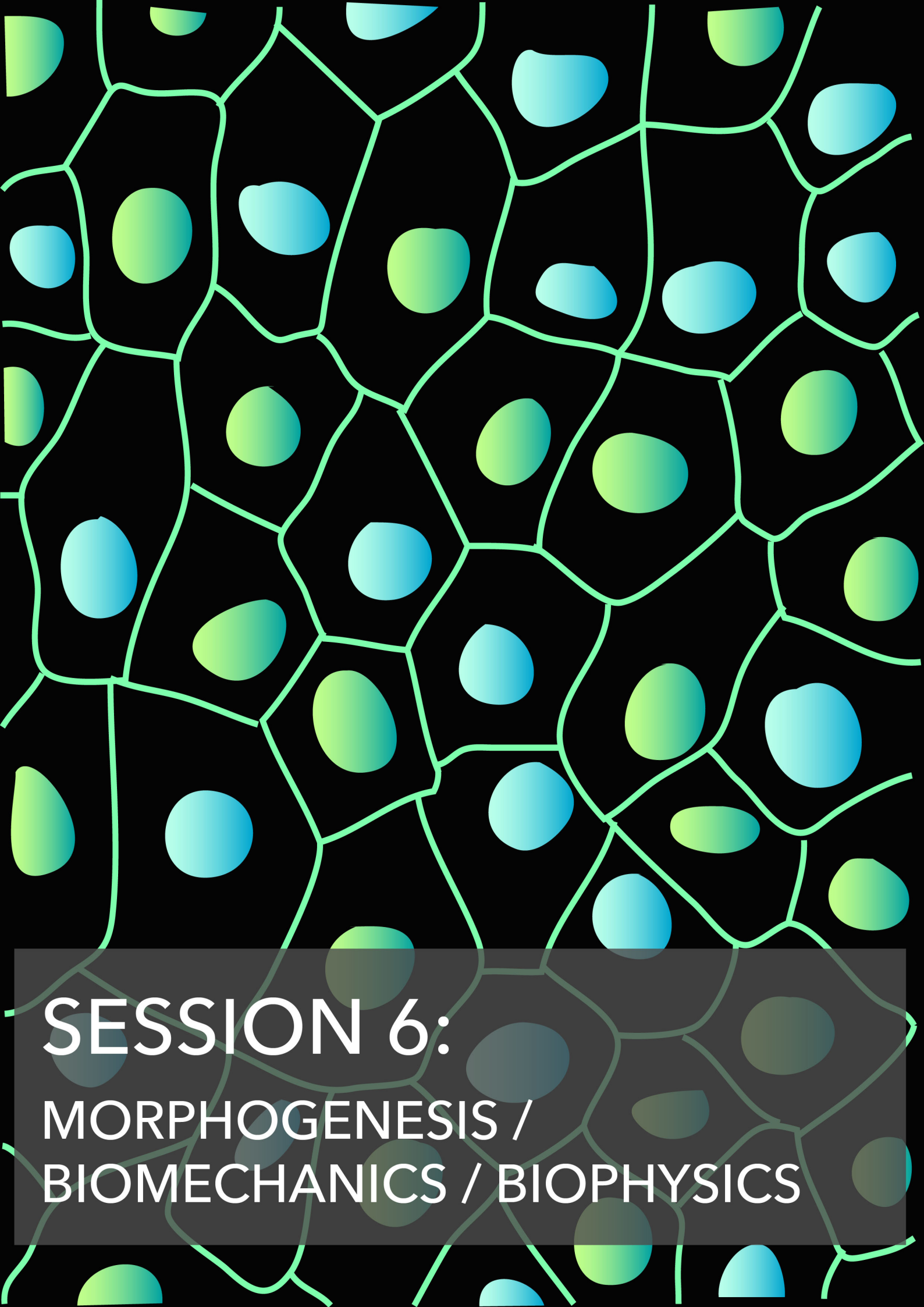
Glioblastoma (GB) is the most lethal brain tumor due to its high proliferation, aggressiveness, infiltration capacity and resilience to current treatments. Activation of the WNT pathway is a bad prognostic hallmark. Using a *Drosophila* and a primary xenograft model of human GB, we describe a mechanism that leads to the activation of WNT signaling (Wingless (Wg) in *Drosophila*) in tumor cells. GB cells display a cytoneme-like network of tumor microtubes (TMs) which enwraps neurons, accumulates Wg receptor Frizzled1 (Fz1), and thereby actively depletes Wg from neurons. Consequently, GB cells proliferate due to ß-catenin activation, and neurons loose synapses and degenerate due to Wg signaling extinction. This novel view explains both neurondependent tumor progression, and also the neural decay associated with GB.

SCALING OF MORPHOGEN GRADIENTS

Marcos Gonzalez-Gaitan

University of Geneva, Geneva, CH

Pattern formation and growth of developing tissues involve the graded distribution of morphogens. Scaling of the morphogen gradient ensures proportioned morphological patterning of tissues with different sizes. On the other hand, we showed that growth of the *Drosophila* wing disc is mediated by a mechanism in which cells compute the relative time derivative of the concentration of a morphogen: Dpp. In this context, scaling drives a homogenous increase of the morphogen concentration throughout the tissue and thereby can fuel homogeneous growth. A key question is how does the morphogen gradient scale? Scaling of the gradient implicates changes in endocytic trafficking of the morphogen and the rates of its lysosomal degradation. Effective changes of endocytic rates is fine tuned by the trafficking of the morphogen through two pathways: clathrin-mediated endocytosis and clic/geec. The molecular machinery controlling these endocytic events involve two extracellular factors, Pentagone and the HSPG Dally. We propose a mechanism by which these molecules can control scaling. The key observation is that Pentagone scale itself. We show that Pentagone scaling can drive the scaling of Dpp.



SESSION 6:

MORPHOGENESIS / BIOMECHANICS / BIOPHYSICS

THE ROLE OF TIMELY FLUID-FLOW IN THE EMBRYONIC LEFT-RIGHT ORGANIZER

Susana Lopes; Pedro Sampaio; Sara Pestana

CEDOC - NMS/FCM, Universidade Nova de Lisboa, PT

We are investigating the role of time in the left-right organizer (LRO). This transient embryonic organ is crucial for determining the LR identity of the vertebrate body-plan. Using micromanipulation to directly extract cilia-driven fluid-flow we now have narrowed the time-window for LR establishment to 1.5 h interval. We designed a new hypothesis, whereby laterality is initiated by a hybrid mechanism compatible with the new time-window. We argue that flow dynamics is the key driving force that sets the stage for the cellular trafficking events that will follow. This narrowed time-window presents biophysical properties that will challenge our understanding of LR patterning of asymmetric organs such as heart, liver and pancreas. The molecular mechanisms involved remain unknown and need to be uncovered to foster basic developmental biology and for understanding the causes of laterality disorders, such as *situs inversus*, i.e. internal organs placed in mirror image, or heterotaxy, i.e. uncoupling of the heart location compared to the liver, and vice-versa. Laterality diseases are highly enriched in patients with primary ciliary dyskinesia (PCD) because motile cilia are at its genesis.

BUFFERING INTRINSIC VARIABILITY IN DEVELOPMENT

Olivier Hamant

ENS Lyon, FR

Multicellular organisms exhibit reproducible shapes, yet at the cell level, growth can be extremely heterogeneous and variable. What are the buffering mechanisms that filter such heterogeneitity and variability? Here we take the example of plant organs where final shape only depends on cell division and cell elongation. We and others showed that shape- and growth-derived forces act as signals that orient microtubules and cellulose microfibrils in the cell walls. This response channels key biological features, such as cell shape or cell division plane orientation. We found that such mechanical feedback contributes to organ shape reproducibility. In parallel, we also identified molecular regulators that contribute to developmental robustness. Altogether, this work reveals the role of mechanical forces in the robustness of organ shapes and raises the novel question of the interactions between mechanotransduction pathways and the activity of molecular dampeners.

Basolateral localization of MMP14/MT1-MMP drives cell polarity change during neural crest EMT independently of its catalytic activity.

Andrieu Cyril

CBD, Toulouse, FR

The transmembrane Matrix Metalloproteinase MMP14/MT1-MMP is known to promote cell migration by cleavage of the extracellular matrix. To initiate migration, epithelial cells need to gain mesenchymal attributes. They lose cell-cell junctions and apicobasal polarity and gain migratory capabilities. This process is named epithelial-mesenchymal transition (EMT). MMP14's implication in EMT is still ill-defined. We used chick neural crest (NC) cells as a model to explore the function of MMP14 in physiological EMT. Our results show that MMP14 is expressed by chick NC cells. However, it is its subcellular localization, rather than its expression, that correlates with EMT. MMP14 is first apical and switches to basolateral domains during EMT. Loss of function and rescue experiments show that MMP14 is involved in EMT independently of its catalytic activity. It lies downstream of pro-EMT genes and upstream of cell polarity. We found that inhibiting its expression in cells that do not undergo EMT has no effect on cell polarity indicating that its apical localization prior to EMT has no obvious function. However, we found that basolateral localization of MMP14 is required and sufficient to induce polarity change in NC cells and neuroepithelial cells, respectively. These effects on polarity occur without impact on cell-cell adhesion or the extracellular matrix. Overall, our data points to a new function of MMP14 in EMT that will need to be further explored in other systems such cancer cells.

Positional information encoded in the dynamic differences between neighbouring cellular oscillators.

Marcelo Boareto

ETH Zurich, CH

How cells track their position during the segmentation of the vertebrate body remains elusive. For decades, this process has been interpreted according to the clock-and-wavefront model, where molecular oscillators set the frequency of somite formation while the positional information is encoded in signaling gradients. Recent experiments using ex vivo explants challenge this interpretation, suggesting that positional information is encoded in the properties of the oscillators. Here, we propose that positional information is encoded in the difference in the levels of neighboring oscillators. The differences gradually increase because both the oscillator amplitude and the period increase with time. When this difference exceeds a certain threshold, the segmentation program starts. Using this framework, we quantitatively fit experimental data from in vivo and ex vivo mouse segmentation, and propose mechanisms of somite scaling. Our results suggest a novel mechanism of spatial pattern formation based on the local interactions between dynamic molecular oscillators.

ADAPTIVE PROTRUSION AND MIGRATION DYNAMICS UNDER TISSUE STRESS IN EARLY DEVELOPMENT

Verena Ruprecht

CRG Barcelona, ES

Insight into the dynamics of single cellular building blocks is key to our understanding of tissue development, homeostasis and disease: shape change, active motility and collective cell behavior establish the architecture of complex multi-cellular tissue patterns in early morphogenesis and similar processes are activated during tissue homeostasis and pathological conditions.

But how are single cell dynamics regulated at specific spatial and temporal time-points of development and which mechanisms guarantee the robustness of dynamic tissue formation in the presence of stress and errors?

I will discuss the influence of the mechanical cellular microenvironment and tissue stress on cytoskeletal dynamics, motility and tissue clearance. We use a combination of simplistic 3D in-vitro assays and advanced live-cell ex-vivo and in-vivo imaging to study control mechanisms of cellular dynamics and motile cell transformation during early development. This experimental framework allowed us to uncover a contractility-based polarization mechanism that drives the rapid transformation of cells into a fast-amoeboid migration mode. Tissue stress promotes amoeboid cell transformation, providing a multi-scale feedback coupling between cellular and tissue-scale dynamics.

I will further discuss ongoing work on adaptive protrusion formation in specific tissue types during stress responses in early embryonic development that guarantee robustness against errors via rapid apoptotic cell clearance.

Eph signaling controls mitotic spindle orientation and cell proliferation in the *Drosophila* optic lobe neuroepithelium

Maribel Franco; Ana Carmena

Instituto de Neurociencias (CSIC-UMH), Alicante, ES

A tight regulation of mitotic spindle orientation is crucial during development and adult tissue homeostasis. It determines cell fate specification and tissue architecture in the context of asymmetric and symmetric cell division, respectively. Two major mechanisms, autonomous and non-autonomous, have been implicated in positioning the spindle during cell division. However, while the intrinsic factors that control spindle orientation have been extensively studied over the past decades, our knowledge about the extrinsic signals that modulate this process is much more limited. Here, we uncover a novel function of the Ephrin-Eph intercellular signaling, in *Drosophila* optic lobe neuroepithelial cells, in controlling mitotic spindle alignment through aPKC activity-dependent myosin II regulation. Moreover, core components of the mitotic spindle orientation machinery mislocalize in dividing *Eph* neuroepithelial cells and show spindle alignment defects in these cells when they are downregulated. Additionally, Eph loss leads to a Rho signaling-dependent activation of the PI3K/Akt1 pathway and a consequent increase of cell proliferation within this neuroepithelium. Hence, Eph signaling is a novel non-autonomous mechanism that regulates both spindle orientation and cell proliferation in the *Drosophila* optic lobe neuroepithelium. A similar mechanism could be operating in other *Drosophila* and vertebrate epithelia.

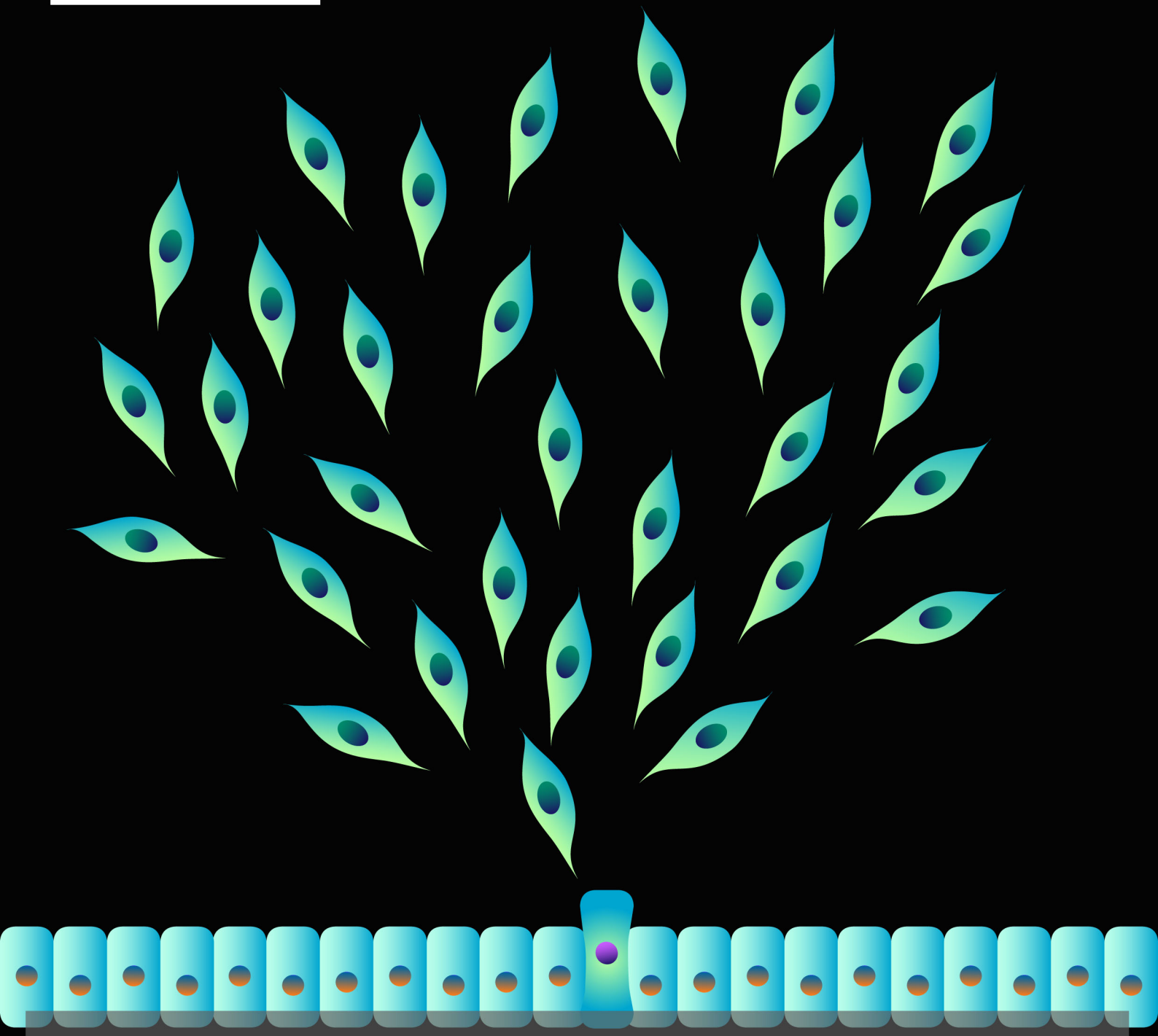
Oct4 is a key regulator of vertebrate trunk length diversity

Rita Aires

Instituto Gulbenkian de Ciência, Oeiras, PT

Vertebrates exhibit a remarkably broad variation in trunk and tail lengths. However, the evolutionary and developmental origins of this diversity remain largely unknown. Posterior Hox genes were proposed to be major players in trunk length diversification in vertebrates, but functional studies have so far failed to support this view. Here we identify the pluripotency factor Oct4 as a key regulator of trunk length in vertebrate embryos. Maintaining high Oct4 levels in axial progenitors throughout development was sufficient to extend trunk length in mouse embryos. Oct4 also shifted posterior Hox gene-expression boundaries in the extended trunks, thus providing a link between activation of these genes and the transition to tail development. Furthermore, we show that the exceptionally long trunks of snakes are likely to result from heterochronic changes in Oct4 activity during body axis extension, which may have derived from differential genomic rearrangements at the Oct4 locus during vertebrate evolution.

EMBO
excellence in life sciences



CLOSING KEYNOTE LECTURE:

EMBO LECTURE

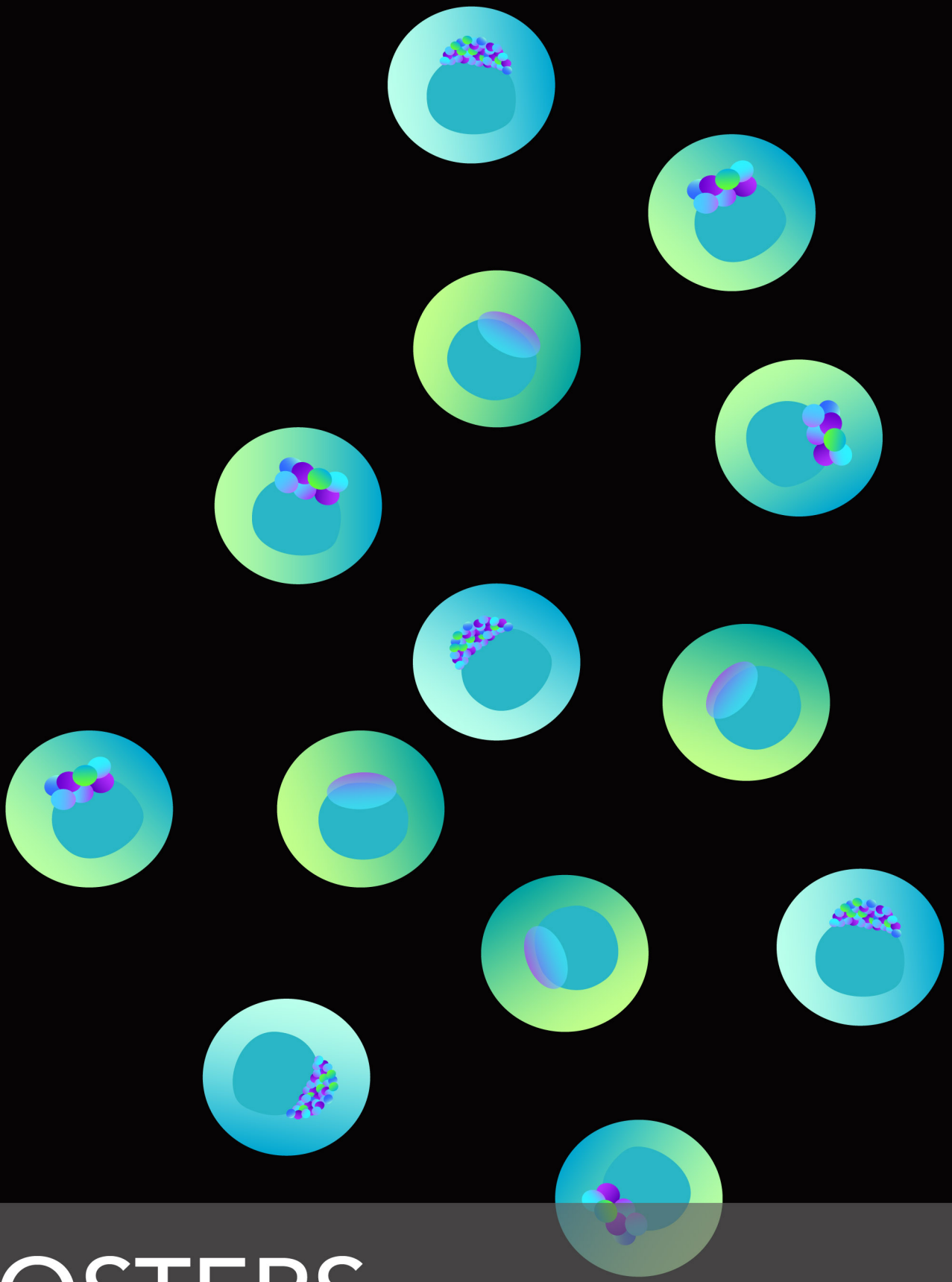
THE COMPLEXITY OF THE EMT: BEYOND CELL MIGRATION IN DEVELOPMENT AND DISEASE

Sonia Vega; Cristina López-Blau; **M. Ángela Nieto**

Instituto de Neurociencias (CSIC-UMH), Alicante, ES

The epithelial to mesenchymal transition (EMT) was implemented and fixed in evolution for the formation of tissues which cells originate far from their final destination, as this program endows cells with migratory properties. Interestingly, the EMT is reactivated in several pathologies including the delamination of cancer cells from the primary tumor in their way to form metastasis. Importantly, although the triggering of the EMT involves a handful of transcription factors (EMT-TFs), the complexity of the program is extremely high due to the activation of different combinations of them in different cell contexts leading to a new concept of EMT-TF code and the plasticity of the process.

The EMT program in embryonic and cancer cells usually involves not only the transition from epithelial towards a mesenchymal migratory phenotype but also the activation of associated programs that contribute to the fitness of these migratory cells. These programs include invasion, control of cell proliferation, resistance to cell death and stem cell-like properties. However, cell context also impinges on the behavior of the responding cells and for instance, the reactivation of EMT during organ degeneration and fibrosis fails to activate the invasion subprogram. Thus, the question is how all the different subprograms are regulated and implemented in different cell contexts. I will discuss some of the differences between the response of embryonic and cancer cells versus that of non-transformed adult epithelial cells and the role of the EMT-TF Snail in developing bones, a context in which the main subprogram implemented is the control of cell proliferation and differentiation, with putative key implications in treating achondroplasia, the most common form of human dwarfism.



POSTERS

(IN ALPHABETICAL ORDER)

1• Tail bud progenitor activity relies on a network comprising Gdf11, Lin28a and Hox13 genes

Rita Aires

Instituto Gulbenkian Ciência, Oeiras, PT

In the course of embryonic development, the entire post-cranial body is gradually extended through the progressive addition of tissue to the embryo's caudal end. This process of axial elongation is driven by the axial progenitors, which contribute with many types of derivatives to generate all axial structures in the vertebrate body. Here we show that, during the final stages of axial extension, Gdf11 signalling restricts neuronal tissue growth, down-regulates *Lin28a* and *Lin28b* activity and promotes expression of genes of the *Hox13* group, particularly *Hoxb13* and *Hoxc13*. Additionally, we demonstrate the *Lin28* genes are necessary for extension through the tail. Conversely, *Hoxb13* and *Hoxc13* both arrest axial progenitor proliferation and trigger cell death specifically at tail levels, thus contributing to end axial elongation. As such, the last stages of axial extension are regulated by a network comprising Gdf11, and genes of the *Lin28* and *Hox13* families, which contrasts with the *Oct4*-dependent type of extension throughout the trunk.

2• Fetal muscle stem cells interact with and contribute to their niche but fail to expand normally in the absence of laminin-211.

Inês Antunes; Ricardo Andrade; Gabriela Rodrigues; Sólveig Thorsteinsdóttir

cE3c - Faculdade de Ciências, Universidade de Lisboa, Lisboa, PT

Merosin-deficient congenital muscular dystrophy is a severe neuromuscular disease caused by the absence of laminins 211/221. In the dyW mouse model for the disease, there is an impairment in fetal muscle growth at E18.5, which correlates with a reduction in the number of Pax7+ muscle stem cells (MuSCs) and Myogenin+ myoblasts. MuSCs normally enter their niche containing laminin 211 at E16.5-E17.5. Since dyW muscles did not show increased apoptosis, we asked whether proliferation levels were altered. Quantification of EdU incorporation in muscles at E15.5, E17.5 and E18.5 showed that proliferation is highest at E15.5, becoming 4 times lower at E18.5. Interestingly, dyW muscles had lower proliferation levels than controls at E17.5 and the number of Pax7+ cells was significantly lower at E17.5 and E18.5 in dyW versus controls, suggesting that these cells are reacting differently to their abnormal niche. We next asked how normal MuSCs interact with their niche. Pax7+ cells are negative for integrin alpha7 at E14.5 but are positive for this laminin receptor at E17.5. Culture of myogenic cells isolated from E18.5 fetuses showed the presence of Pax7+, Myf5+, MyoD+ and Myogenin+ cells in these cultures, which all express integrin alpha7 and produce laminin 211 and 511. We conclude that at late fetal stages, MuSCs and myoblasts can interact with and contribute to their niche. Future studies aim to characterize the transcriptional response of the dyW cells to their abnormal niche.

3• The Spectraplakin Short-Stop is an essential microtubule regulator mediating subcellular branching

Sofia Araújo; Delia Ricolo; Miquel Lledós

University of Barcelona, ES

Branching networks are a very common feature of multicellular animals and underlie the formation and function of numerous organs including the nervous system, the respiratory system, the vasculature and many internal glands. 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) (Ricolo, et al. Cur. Biol. 2016). However, not much is known on the events that trigger the formation of these subcellular branches or what makes them choose a particular trajectory within the cytoplasm of the TC. We have identified that the spectraplakin *Shortstop* (*Shot*) is involved in the microtubule stabilisation events that lead to the formation and extension of the subcellular lumen. We observed that an excess of Shot induces more branching points in the embryonic tracheal TC leading to cells with extra subcellular lumina and that a *shot* loss-of-function leads to cells deficient in *de novo* subcellular lumen formation.

4• Cellular events leading to otic neuroblast delamination and communication

Aitor Bañón González; Berta Alsina

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The generation of the otic sensory neurons during development is a fascinating process in which distinct events such as neuronal specification, epithelial-mesenchymal transitions (EMT), cell communication, migration and proliferation are tightly coordinated at the molecular and cellular level. After the activation of the proneural gene *neurod*, the otic neuroblasts exit the epithelium. However, the relationship between proneural genes and delamination is not well understood. Making use of high spatiotemporal imaging of labelled neuroblast and intracellular proteins in the zebrafish embryo, we have been able to investigate the dynamics of cells during delamination. 3D reconstructions of single labeled neuroblasts with polarity proteins have allowed us to reconstruct the process. Our results indicate that as the cells delaminate, the apical membrane starts thinning, losing *pard3* from the apical side but carrying the primary cilia while delaminating, suggesting that polarity is not completely lost. Interestingly, we have also observed dynamic and directed filopodia extending between predelaminating and delaminated neuroblasts, suggesting that they could be involved in cell delamination, communication and/or migration. Currently, we are investigating the role of these signaling filopodia (Cytonemes) making use of Tol2 gateway-based transgenesis. Moreover, we are testing through CRISPR knock-out the possible effect of morphogens such as Fgfs in the development of the SAG.

5• Impact of Hippo signaling in chick lung branching morphogenesis

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Hippo signaling is involved in diverse cellular events, including organ growth control. YAP phosphorylation status impact transcription of target genes (*ctgf*) and pathway activity. Hippo is studied in various organ systems, yet, information regarding developing lung is limited. Therefore, our study aims to determine Hippo role during pulmonary branching in avian animal model.

Spatial distribution of Hippo machinery was assessed by *in situ* hybridization, in embryonic chick lung. Also, *in vitro* lung explants were cultured in plain medium for 48h, and protein levels of phosphorylated-YAP(pYAP)/YAP were assessed by Western blot at two time-points (0 and 48h). After, lung explants were cultured with YAP-TEAD inhibitor verteporfin (VP) (5 or 7.5 μM) and DMSO (control) supplemented media, followed by *in situ* hybridization for *ctgf* and morphometric analysis.

In situ hybridization and western blot revealed presence of Hippo signaling members and confirmed pathway activity in early chick lung branching stages. Protein analysis showed similar expression levels of YAP and pYAP at 0h, which declined after 48h of culture, even so pYAP/YAP ratio was maintained. Lung explants treated with 7.5 μM VP showed statistically significant reduction in lung size and branching, and decreased expression of *ctgf* when compared to control.

Overall, our data indicate that Hippo machinery is present and active in early stages of avian pulmonary branching and possibly involved in the regulation of lung growth.

6• LUZP1 is a novel centrosomal protein underlying primary cilia and actin cytoskeleton defects in Townes-Brocks Syndrome

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Townes-Brocks Syndrome (TBS) is a rare disease characterized by a spectrum of malformations in digits, ears, heart and kidneys, overlapping symptoms with human ciliopathies. In fact, TBS patient-derived fibroblasts display longer and more frequent cilia than controls. TBS is caused by mutations in *SALL1* gene, leading to the formation of a truncated protein that interferes with the normal function of the cell. We found that truncated SALL1 interacts with the leucine-zipper containing protein LUZP1, which plays important roles during development. Loss-of-function mouse models of LUZP1 phenocopy some features associated with human ciliopathies, such as neural tube defects and cardiac malformations. Our findings show that LUZP1 localizes to the centrosome and actin cytoskeleton. Loss of LUZP1 in CRISPR-Cas9-derived cells alters actin cytoskeleton, cell division and facilitates ciliogenesis. These findings support the hypothesis that LUZP1 is a novel negative regulator of ciliogenesis, acting through the modulation of actin dynamics and centrosomal function. Given that cilia assembly and disassembly are coupled to actin dynamics, we propose that LUZP1 might be involved in the integration of these two crucial processes during development.

7• Role of LRRFIP2 in mouse heart development

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Heart is the first functional organ in the early mouse embryo. Its growth and development are controlled by several signaling pathways, like those of canonical and non-canonical Wnt which regulate proliferation, polarity and actin cytoskeleton organization.

LRRFIP2 (Leucine Rich Repeat in Flightless Interacting Protein 2) is known to interact with different partners like Disheveled and Flightless, involving LRRFIP2 into several signaling networks.

We generated a *LRRFIP2* floxed allele and have shown that the absence of *LRRFIP2* leads to embryonic lethality around E13.5, due to severe cardiac malformations. *LRRFIP2* mutant embryos have a reduced cardiomyocyte (CM) number from E10.5 and a decreased pHH3 positive CM, suggesting a cell cycle alterations.

We also investigated the actin cytoskeleton organization and the state of the sarcomeres in these embryonic mutant CM. We found precocious sarcomerisation of *LRRFIP2* mutant CM, suggesting their premature maturation.

Our results pinpoint a role of *LRRFIP2* in heart development, which precise mode of action will be discussed.

8• Identifying how Integrins promote intestinal stem cell proliferation in *Drosophila*

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Throughout adult life, somatic stem cells (SC) constantly renew damaged and aging cells to maintain organ size and function. SC integrate numerous signals from the surrounding cells and environment, including the extracellular matrix (ECM) to decide whether to proliferate or differentiate. Varying physiological conditions (e.g. inflammation, tumours) cause dynamic changes in ECM composition and properties, resulting in mechanical signals activating SC proliferation. This mechanotransduction is mostly achieved by integrins, resulting in the regulation of gene expression and cell behaviour. Specific integrin subunits are enriched in many mammalian adult stem cells, but functional characterization remains complicated due to genetic redundancy. In the *Drosophila* gut, ablation of the ubiquitous ßPS integrin prevents SC maintenance and proliferation, but how integrins can control proliferation is still unknown.

Here we use a genetic tool to uncouple Integrin adhesive and signalling functions in the *Drosophila* intestine. We show that the decreased proliferation observed upon integrin loss of function can be rescued when integrin signalling is forced, indicating that integrin role is not restricted to its adhesive properties. We use this genetic construct to identify the transcriptional output of integrin signalling. We thus hope to identify factors modulated following mechanosensing and able to regulate SC proliferation in different conditions such as regeneration and tumour growth.

9• Asymmetric cell migratory behaviors near the zebrafish left-right organizer

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Correct establishment of left-right (LR) patterning during vertebrate development is fundamental for internal organ position. In most vertebrates, symmetry breaking occurs in a structure called the LR organizer (LRO). In zebrafish, the LRO is called Kupffer's Vesicle (KV) where *dand5* - the first asymmetric gene is expressed in a flow-dependent manner. Asymmetry is established with the expression of *nodal/spaw* only in the left Lateral Plate Mesoderm. This left-sided *spaw* expression results from the earlier Dand5-Spaw inhibition near the KV.

We have recently identified a novel group of migratory mesendodermal cells expressing *sox17* in the vicinity of the KV. Using *Tg(sox17:GFP)* reporter line for live-imaging we observed that these cells migrate further on the left side of the KV towards the anterior. Complemented with fixed samples, we also observed left-sided asymmetries regarding the number of cells, distance to KV and distance between cells. The relevance of these cells in LR patterning is still unknown. However, since Nodal is known to affect cell migratory speed, we hypothesize that these asymmetries are induced by the asymmetric signals from the KV. Being adjacent to the KV these cells are more exposed to Nodal/Spaw on the left-side since Spaw is inhibited by Dand5 on the right-side.

We are currently testing if the observed asymmetries are originated from asymmetric signals from the KV and trying to assess the fate of this population of migratory mesendodermal cells.

10• RNA-mediated regulation of embryo clock expression dynamics

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An embryonic clock (EC) has been described to underlie both somitogenesis and limb development in the chicken embryo. However, the periodicity of EC oscillations in these two tissues is strikingly different: 90 minutes in the presomitic mesoderm (PSM) and 6 hours in the distal limb. The mechanisms that dictate the pace of the EC are poorly understood. Additionally, the dynamics of expression of individual EC genes also varies between these developmental processes. We are analyzing the mRNA molecules produced by EC genes in different tissues to assess if the production of alternative transcripts could underlie different paces of the EC. We found that *hairy1* encodes multiple transcripts, which are differentially expressed in the PSM and forelimb. These arise from alternative start sites, as well as polyadenylation sites. Functional assays for these transcripts are underway in order to assess the functional relevance of each transcript for EC oscillations. Besides alternative transcription, the pace of the EC depends on post-transcriptional regulation of mRNA stability. To test the impact of this factor in EC clock periodicity microRNA sequencing (miRNA-Seq) was performed to identify differentially expressed miRNAs in the PSM and distal forelimb capable of targeting EC core components. Our dataset represents a valuable resource to further understand the role of miRNA-mediated regulation of EC periodicity.

11 • The role of telomeres and telomerase in stem cells driven extreme regeneration in the sea anemone *Nematostella vectensis*

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Telomeres and the telomerase reverse transcriptase (TERT) play crucial roles in stem cell maintenance, regeneration and aging. TERT modulates embryonic and organ stem cell behavior by its capacity to regulate signaling pathways critical for proliferation and differentiation (e.g. Wnt, MYC). Using the emerging, powerful new model for regenerative biology *Nematostella vectensis* (Nv) – Anthozoa, Cnidaria – we aim to clarify the implications of telomeres and TERT during the process of whole body regeneration, i.e. in the regulation of stem cells.

By studying the limits of the regenerative potential of Nv, we have identified specific structures that are required to induce the regenerative response that requires the activation of two distinct populations of potential stem cells. Further, we have performed a screen of stem cell markers and characterized Nv telomeres and their associated shelterin proteins. Finally, we developed CRISPR/Cas9 based knock-in tags and KO's for key stem cell markers and TERT itself.

Our results show a surprising strong evolutionary conservation of the Nv telomere complex and that cnidarian shelterin proteins are able to interact with the mammalian ones. Interestingly, in addition to the overall presence of TERT activity our results suggest the presence of an alternative mechanism involved in telomere length regulation that may explain the amazing stem cell-dependent regenerative and longevity capacity of Nv.

12• Functional characterization of iPSC-derived arterial- and venous-like endothelial cells

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The aim of this work was to functionally characterize human induced pluripotent stem cells (iPSCs)- arterial and venous-like endothelial cells (ECs), derived in chemically defined conditions, either in monoculture or seeded in a scaffold with mechanical properties similar to blood vessels. iPSC-derived arterial- and venous-like ECs were obtained in two steps: differentiation of iPSCs into ECs precursor cells ($CD31^{pos}$/KDR^{pos}/$VE\text{-}Cad^{med}$/$EphrB2^{neg}$/$COUP\text{-}TF^{neg}$) followed by their differentiation into arterial and venous-like ECs using a gradient of vascular endothelial growth factor (VEGF). Functionally, both arterial and venous-like ECs responded to vasoactive agonists such as thrombin and prostaglandin E2 (PGE2), similar to somatic ECs, by increasing the intracellular levels of Ca^{2+}. Both cell monolayers showed a decrease or increase in basal trans-endothelial electrical resistance (TEER) by stimulation with thrombin or PGE2, respectively. They also adhered, proliferated and prevented platelet activation when seeded in poly(caprolactone) scaffolds. Interestingly, both iPSC-derived ECs cultured in monoculture or in a scaffold showed a lower inflammatory response to TNF-a than somatic ECs. We have characterized at functional level iPSCs-derived arterial and venous-like ECs, our results show that both cells have discrete differences in their functional properties.

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13• Oncogenic addiction of glioma cells to Kish/TMEM167A regulation of vesicular trafficking

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Genetic lesions in glioblastoma (GB) include constitutive activation of PI3K and EGFR pathways to drive cellular proliferation and tumor malignancy. An RNAi genetic screen, performed in *Drosophila melanogaster* to discover new modulators of GB development, identified a member of the secretory pathway: *kish/TMEM167A*. Downregulation of *kish/TMEM167A* impaired fly and human glioma formation and growth, with no effect on normal glia. Glioma cells increased the number of recycling endosomes, and reduced the number of lysosomes. In addition, EGFR vesicular localization was primed towards recycling in glioma cells. *kish/TMEM167A* downregulation in gliomas restored endosomal system to a physiological state and altered lysosomal function, fueling EGFR towards degradation by the proteasome. These endosomal effects mirrored the endo/lysosomal response of glioma cells to BrefeldinA (BFA), but not the Golgi disruption and the ER collapse, which are associated with the undesirable toxicity of BFA in other cancers. Our results suggest a novel oncogene addiction for gliomas, which depends on modifications of the vesicle transport system reliant on *kish/TMEM167A*. Non-canonical genes in GB could be a key for future therapeutic strategies targeting EGFR-dependent gliomas.

14• *prrx1a* in the morphogenesis of the zebrafish heart

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Most animals exhibit an external bilateral symmetry. Nevertheless, internally there is a left-right (L/R) asymmetry in the position of the visceral organs, which is fundamental for their proper function[1]. Regarding heart laterality, published data from the laboratory indicate that Prrx1, an EMT inducer, presents a transient L/R asymmetric expression in the lateral plate mesoderm (LPM) with higher levels on the right-hand side. This gives rise to a differential EMT and an asymmetric contribution of cells migrating towards the posterior pole, leading to its leftward displacement from the midline, a mechanism that is conserved in vertebrates[2].

Interestingly, Prrx1 is not only fundamental for proper heart laterality but also for its morphogenesis, as *prrx1a* loss of function causes a reduction in the atrium[2] as well as in the ventricle, indicating that Prrx1a$^+$ cells may also migrate towards and contribute to the anterior pole. Once Prrx1a$^+$ cells are incorporated into the heart they do not longer express this EMT inducer[2], as expected from their commitment to differentiate into cardiomyocytes. We have generated a *prrx1a* expression reporter line in zebrafish which contains a stabilized version of EGFP and allows us to observe the contribution of Prrx1a$^+$ cells to the developing heart.

1. Raya, A. & Izpisúa Belmonte, J. C. Nat. Rev. Genet. **7**, 283–293 (2006)

2. Ocaña, O. H. *et. al*,. Nature. **549**, 86-90 (2017)

15• Embryonic hematopoiesis modulates the inflammatory response and larval hematopoiesis in *Drosophila*

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Recent lineage tracing analyses have significantly improved our understanding of immune system development and highlighted the importance of the different hematopoietic waves. The current challenge is to understand whether these waves interact and whether this affects the function of the immune system. Here we report a molecular pathway regulating the immune response and involving the communication between embryonic and larval hematopoietic waves in *Drosophila*. Down-regulating the transcription factor Gcm specific to embryonic hematopoiesis enhances the larval phenotypes induced by over expressing the pro-inflammatory Jak/Stat pathway or by wasp infestation. Gcm works by modulating the transduction of the Upd cytokines to the site of larval hematopoiesis and hence the response to chronic (Jak/Stat over-expression) and acute (wasp infestation) immune challenges. Thus, homeostatic interactions control the function of the immune system in physiology and pathology. Our data also indicate that a transiently expressed developmental pathway has a long-lasting effect on the immune response.

16• Genetic dissection of Eph/Ephrin signalling during hindbrain segmentation

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The establishment of sharp borders between tissue interfaces is critical for the correct organization of organs and can underlie the formation of signalling centres between different territories.

During embryogenesis, the hindbrain is subdivided in rhombomeres along its anterior-posterior axis. This becomes apparent by the segmentally restricted expression of transcription factors, which is initially fuzzy and subsequently refined. Following this, the distinct boundary cells appear at the borders between rhombomeres and act as signalling centres.

Multiple Eph receptors and Ephrins are expressed in segmentally restricted patterns in the hindbrain and the perturbation of Eph/Ephrin signalling is sufficient to disrupt border sharpening and the formation of boundary cells. However, the individual contribution and the genetic interactions between different Ephs and Ephrins, as well as the intracellular signalling pathway mediating cell sorting and boundary cell formation remains elusive.

Using CRISPR/Cas9 we generated zebrafish null mutants for different Ephs and Ephrins as well as truncations of their intracellular domains to assess their individual relevance. Finally, by coinjecting CRISPR/Cas9 with exogenous donor sequences we introduced mutations affecting specific Eph and Ephrin signalling motifs. Analysis of single and combined Eph/Ephrin mutants is starting to shed light into the molecular mechanisms controlling cell segregation during hindbrain segmentation.

17• Pattern formation through cell movement in *Xenopus* embryonic skin

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During *Xenopus* mucociliary epidermis development, multiciliated cells (MCCs) intercalate radially from the mesenchymal inner layer where they are born, into the outer epithelial layer. Remarkably, MCCs emerge at the surface of the outer layer only at vertices, and respect a strict rule of non-contiguity, resulting in a regular pattern of distribution.

We found that before they initiate apical migration, MCCs are distributed in irregular clumps within the inner layer and progressively disperse. Live-imaging shows that MCCs first separate from each other through planar migration powered by actin rich protrusions; in a second step, MCCs stabilize beneath vertices by remodeling actin cytoskeleton along extracellular junctions. We show that the signaling pathway dependent on the tyrosine kinase receptor c-KIT and its cognate ligand SCF is required for the proper spatial distribution of MCCs. SCF is expressed by outer layer epithelial cells, while expression of c-KIT is restricted to MCCs. The disruption of the SCF/c-KIT pathway results in severe abnormalities in the distribution and intercalation of MCCs, which stems from loss of mutual repulsion, impaired actin reorganization and reduced affinity for vertices. We propose a model in which the SCF/c-KIT pathway promotes MCC dispersion and stabilizes their association with outer layer vertices. Mathematical modelling confirmed that integration of these two functions is sufficient to recapitulate a regular pattern of distribution.

18• Bisphenol-A induces overexpression of Oct4, Sox2, and Nanog in rabbit gonocytes

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The primordial germ cells (PGCs) are bipotential. They differentiate either into oocytes or spermatozoa, depending upon the somatic cell context of the fetal gonad. The oogonia and spermatogonia express hallmarks of somatic embryonic stem cells (ESC) like *Oct4, Sox2*, and *Nanog*. The PGCs arrive at the genital ridge; here, they are called germ cells (GCs). As gametogenesis proceeds, GCs repress the hallmark genes and follow the genetic pathway leading to gamete formation. However, *Oct4, Sox2*, and *Nanog* can be expressed again in adults under experimental and pathological conditions, including cancer. Bisphenol-A (BPA) is an environmental pollutant used in the production of epoxy resins for canned food products. Thus, we are exposed daily to BPA. It is important to point out that BPA can cross the placental barrier of rodents and humans and reach the fetus disturbing some processes during development. Despite multiple studies in somatic tissues, it is not clear whether BPA disturbs the expression profiles of *Oct4, Sox2*, and *Nanog* of fetal GCs and, modify their normal differentiation trajectory up to the adult testis. Results: The GCs of rabbit fetal testis treated with BPA, overexpress the three pluripotency genes markers at early stages of developing testis. This altered state may be due to an increased proliferation of some germ cell lineages leading to a delayed commitment of the gonocytes to become differentiated as spermatogonia.

19• Variations in neural progenitors lineages in the fast growing killifish *Nothobranchius furzeri*

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The turquoise killifish *Nothobranchius furzeri* is a naturally short-lived vertebrate that lives in ephemeral water pools of East Africa. Its peculiar life cycle is notably characterized by an explosive growth during the first weeks post-hatching. We wish to decipher whether the acceleration of larval growth is associated with alterations in the identity and lineages of neural progenitors. In the killifish pallium, we could identify two types of dividing progenitors. Both divide apically and express the master neural stem cell regulator Sox2. The first type is defined by a radial morphology and the expression of astroglial markers (GS, GFAP, BLBP). These radial glia cells (RGCs) are intermingled with a second type of apical progenitors (APs). While the proliferation rate of RGCs drops down to very low levels at larval stages, APs are actively dividing up to adult stages and likely sustain the fast growth of the tissue. We also show that RGCs and APs respond differentially to Notch inhibition. This situation is strikingly different from the one described in zebrafish, in which RGCs constitute the vast majority of pallial neural progenitors. While in zebrafish RGCs enter quiescence only at adult stages, our data also suggests that killifish RGCs do so in a very different context, at a time when the brain is still actively growing and the sexual maturity has not been reached. Our work thus reveals unexpected variations in neural progenitors lineages between fish species.

20• A transcriptomic snapshot of turtle shell morphogenesis

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Phenotypic innovation is predicted to originate via major departures from an ancestral developmental state. For instance, the development of the atypical turtle body plan is hypothesized to be guided by the molecular signaling capacity of a unique and ephemeral embryonic structure known as the carapacial ridge (CR). The CR is a mesenchymal protrusion, on the lateral flank of early turtle embryos, that exhibits similar epithelial-mesenchymal interactions and molecular signaling patterns as in feather and limb morphogenesis. If so, then early shell morphogenesis is likely orchestrated by signaling pathways that have also been co-opted to give rise to novel structures in other tetrapod groups. To address this expectation, I examined transcriptomes of the AER and the CR at stage 16 in turtle (before ribs contact the CR mesenchyme). Differentially expressed genes (DEGs) of the AER versus CR were congruent with the expected signaling profile of mesenchymal tissue and contributed to the enrichment of the 'skeletal system development' GO term. Homeobox-containing genes, as well as predicted protein interactions of PBX3 and MEIS1 transcription factors, were overrepresented in DEGs. I discuss the relevance of these preliminary findings to further clarifying turtle shell morphogenesis using standard tools of developmental biology and within a broad evolutionary (evo-devo) framework.

21• Oligodendrocyte precursor survival and differentiation requires chromatin remodeling by Chd7 and Chd8

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Oligodendrocyte precursor cells (OPCs) constitute the main proliferative cells in the adult brain, and deregulation of OPC proliferation-differentiation balance results in either glioma formation or defective (re)myelination. OPC differentiation requires significant genetic reprogramming implicating chromatin remodeling. Here, we report on uncharacterized functions of the chromatin remodelers *Chd7* and *Chd8* in OPCs. Their OPC-chromatin-binding profile combined with transcriptome and chromatin accessibility analyses of *Chd7*-deleted OPCs, demonstrates that Chd7 protects non-proliferative OPCs from apoptosis by chromatin-closing and transcriptional repression of *p53*. Furthermore, Chd7 controls OPC differentiation through chromatin-opening and transcriptional activation of key regulators, including *Sox10*, *Nkx2.2* and *Gpr17*. Chd7 is however dispensable for oligodendrocyte stage progression, consistent with Chd8 compensatory function, as suggested by their common chromatin binding profiles and genetic interaction. Mutations in *CHD7* and *CHD8* are associated with developmental disorders, such as CHARGE syndrome and autism respectively, and our results offer new avenues to understand and modulate their functions in disease.

22• Asymmetric Left/Right Prrx1 expression drives endodermal organ laterality in vertebrates

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Most vertebrates show external bilateral symmetry. However, there are numerous interior left–right (L/R) asymmetries, including the morphology and position of several internal organs. The digestive system is also subjected to L/R positional cues, resulting in the asymmetrical positioning of liver, pancreas and gut. Displacement of the gut in zebrafish is driven by asymmetric migration of the lateral plate mesoderm (LPM). However, the molecular mechanism behind this behaviour of LMP is poorly understood. We have recently showed that asymmetric activation of Prrx1 in the LPM produces an asymmetric contribution of right and left cells to the posterior pole of the primary heart tube and the heart tube loops toward the right side. Here, we examined whether this L/R asymmetric expression of Prrx1 in the LMP could also play a role in positioning the endodermal organs. We show here that *prrx1a* downregulation also affects gut looping and impairs the asymmetric positioning of the liver and pancreas in zebrafish, all remaining aligned at the midline. During development, LPM cells descendants of Prrx1+ cells migrate and surround the dorsal part of the gut whereas a similar population from the right LPM moves more ventrally pushing the gut to left at 30 hpf. Loss of prrx1a function not only disturbs gut displacement from the midline but also affects the cellular organisation and morphological asymmetry of the LPM. We also show that this mechanism seems to be conserved in the chick embryo.

23• Role of Meis transcription factors in limb development

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Meis 1 and *2* are members of the TALE homeobox Transcription factor (TF) family. These TFs are expressed in the lateral plate mesoderm in the early embryo and then, as the limb bud grows, their expression becomes restricted to a proximal domain. *Meis* genes have been shown to regulate proximo-distal patterning along the limb bud and have been traditionally proposed as proximalizing factors. Here, by analysing GOF and LOF mutants, we show that they are not only needed for the correct pattern of the most proximal skeletal element but also for zeugopod development and determinant for limb initiation and antero-posterior pre-patterning.

Moreover, and concerning proximo-distal patterning, MEIS would be preventing the distal programme of the limb by binding to the same sites as HOXA13. This is suggested by a significant overlap of peaks when comparing HOXA13 and MEIS ChIPseq data. The premature and expanded *Hoxa13* expression in *Meis* KO embryos also support this idea.

24• Two different functional types of EMT are required during vertebrate axial growth

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The vertebrate body is laid down progressively in an anterior to posterior sequence by dedicated axial progenitor cells with potential to produce neural and mesodermal tissues. During primary body formation (head, neck and trunk), axial progenitors are located in the epiblast (which is an epithelium) and generate mesoderm through gastrulation (a process involving an EMT). However, as the embryo engages in secondary body formation (tail), axial progenitors are relocated to the tailbud and mesoderm formation no longer requires an EMT, as the progenitors are now thought to be mesenchymal. The transition from primary to secondary body formation remains a mystery since tailbud axial progenitors are thought to derive from their epiblast counterparts, but the mechanisms involved in this process and their regulation are still unknown. Here, we will show that tailbud progenitors indeed derive from epiblast progenitors and that their movement occurs through a process involving activation of a functionally different EMT in these progenitors that, instead of promoting mesodermal fates, keep their full activity as progenitors but changing their functional properties, so that they now extend the body axis by generating secondary body structures. We will also show that Snai1 is the key regulator of this process as it is blocked in the absence of this gene and premature Snai1 activation, generates ectopic tailbud-like axial progenitors able to produce ectopic neural and mesodermal tissues.

25• Developmental characterization of neuronal populations involved in visually guided behaviours

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The Zebrafish (*Danio rerio*) larva is an ideal model for identification of neuronal circuits such as those associated with visually guided behaviors like the optomotor and the optokinetic responses. The use of transgenic lines expressing the calcium level indicator GCaMP in particular neuronal subpopulations allows monitoring of the activity of those neurons and facilitates their anatomical and functional characterization.

Our goal, in this context, is to characterize some of these subpopulations taking advantage of the expression of GFP using the Gal4-UAS system during the first 6 days of development. To examine and characterize the GFP expression in zebrafish larvae, we have applied an immunofluorescence protocol and recorded anatomical stacks using confocal imaging. This information gathered from several individuals and registered to a reference brain may be then used to establish anatomical atlases at these early stages of development that will complement those already developed for the 6 dpf (days post fertilization) larvae. Characterization of the transgenic lines at the early stages will help us to locate the functional clusters identified at 6 dpf with greater anatomical accuracy and precision.

In addition, we are using time lapse imaging with light sheet microscopy to follow the dynamics of neuronal differentiation and extension of projections during the 20-48 hours post fertilization period.

26• A microRNA OMICS approach to understand the temporal control of the embryo molecular clock

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Vertebrate segmentation occurs progressively along the anterior-posterior body axis of the early embryo. An Embryo Molecular Clock (EC), evidenced by cycles of gene expression in the presomitic mesoderm (PSM), underlies temporal control of somite formation. An EC has been described in other tissues, such as the limb bud, with strikingly different periodicity. To assess if microRNA-mediated regulation underlies EC periodicity, we performed miRNA-sequencing of chick embryo tissues presenting different EC dynamics: distal limb bud, undetermined PSM, and determined PSM (N=3/each). We found 927 known Gallus gallus' miRNAs targeting 4441 different genes, and identified over 120 high-confidence novel miRNAs, mostly located in chromosomes 1, 4, 5, and 12. Functional analysis of predicted target genes for miRNAs differentially expressed between the three embryo tissues, revealed biological processes related to primary metabolism, cell differentiation and adhesion, apoptosis, and general developmental processes. Enriched molecular functions pertain to the extracellular matrix, transcription regulation, signalling, and catalysis. Finally, the most targeted signalling pathways are Laminin and Integrin, NODAL, WNT, TGFß, and GnRHR. This approach identified critical processes which may be differentially regulated in each tissue, potentially regulating the EC. Our dataset represents a valuable resource to further prioritize candidate miRNA-gene pairs for experimental validation.

27• The role of histones chaperones Hira and Daxx during muscle development

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The regulation of chromatin organization is required for appropriate gene expression in various lineages. Hira and Daxx are two histone chaperones required for the deposition of H3.3 histone in different regions of the genome.

We have investigated the role of Hira and Daxx during C2C12 myoblast proliferation and differentiation by generating Crispr/Cas9-mediated KO lines for Hira or Daxx. These clones show similar phenotypes: decreased Pax7 expression and impaired differentiation and fusion index.

We next investigated the consequences of ablating Hira for muscle development using a *Hira*^{flox} conditional allele combined with *Pax3*^{Cre} mice. Our results show that in E15.5 *Pax3*^{Cre}*;Hira*$^{fl/fl}$ fetuses muscles display a severe reduction in the number of Pax7+ cells while the number of Myogenin+ cells and muscle size are not affected. The phenotype severity is increased at E17.5 with a decrease in the total number of myogenic cells and muscle size. In addition, we observed that Pax7+ cells proliferate less in *Pax3*^{Cre}*; Hira*$^{fl/fl}$ embryos.

In order to determine if these phenotypes are due to changes in the incorporation of H3.3 into regulatory regions of myogenic genes we plan to perform RNA-seq and ChIP-seq in these various mutants. Our goal is to identify the role of Hira and Daxx in the chromatin organization of myogenic cells and its consequences for myogenic gene expression and cell fate.

28• Analysis of the role of Nidogen/entactin in basement membrane assembly and morphogenesis in *Drosophila*

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Basement membranes (BMs) are thin sheet-like specialized extracellular matrices found at the basal surface of epithelia and endothelial tissues. They are required for proper tissue growth, differentiation and maintenance. The major constituents of BMs are two independent networks of Laminin and Type IV Collagen interlinked by the proteoglycan Perlecan and the glycoprotein Nidogen/entactin (Ndg). The ability of Ndg to bind in vitro Collagen IV and Laminin, required during embryogenesis, anticipated an essential role for Ndg on morphogenesis linking the Laminin and Collagen IV networks. Here, we have isolated mutations in the only Ndg gene present in *Drosophila*. We find that while, similar to *C.elegans* and mice, *Ndg* is not essential for overall organogenesis or viability, it is required for appropriate fertility. We also find tissue-specific requirements of *Ndg* for proper assembly and maintenance of certain BMs, namely those of the adipose tissue and flight muscles. We have performed a thorough functional analysis of the different Ndg domains in vivo supporting an essential requirement of the G3 domain for Ndg function and unravel a new key role for the Rod domain in regulating Ndg incorporation into BMs. Furthermore, uncoupling of the Laminin and Collagen IV networks in the absence of Ndg supports a linking role. We propose that BM assembly and/or maintenance is tissue-specific, which could explain the diverse requirements of a ubiquitous conserved BM component like Nidogen.

29• miR-15 family coordinates EMT transcription factor switch in development and disease

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Epithelial to Mesenchymal Transition (EMT) plays pivotal roles during development and disease through the activation of several EMT transcription factor families (EMT-TFs), including Snail, Zeb, Twist and Prrx1. We previously showed that patterns of *PRRX1* and *SNAIL1* expression are complementary in chicken embryos and cancer cells, and that their functions in EMT subprograms such as the regulation of stemness also seem to be distinct. Here, we find that this complementary expression of *Snail1* and *Prrx1* is conserved during vertebrate development, and also in pathological EMTs, after examining zebrafish, chicken and mouse embryos, and public databases of single-cell RNA sequencing data from patients of breast cancer, head-and-neck cancer and mouse pulmonary fibrosis. After studying the transcriptome of cancer cells and performing biochemical assays, we describe here a gene regulatory network (GRN) in which Snail1 and Prrx1 form a double-negative feedback loop, involving the miR-15 family. After EMT induction by BMP administration in chicken embryos or TGFβ administration in MDCK cells, we show that this GRN coordinates an expression switch between Snail1 and Prrx1, with *Snail1* being an early-response gene to EMT inducing signals followed by the activation of *Prrx1*, which in turn, attenuates Snail1 expression. This GRN, coordinated by miR-15 family, mediates a switch between the expression of Snail1 and Prrx1 EMT-TFs with important implications in development and disease.

30• Lung branching morphogenesis is accompanied by a metabolic Warburg-like profile in the chicken model

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Lung branching morphogenesis is an intricate process governed by epithelial-mesenchymal interactions and dependent on a complex signaling. To sustain high proliferative rates, free energy and building blocks are necessary. However, the metabolic requirements/changes that occur during early lung branching morphogenesis are unknown. In this work, we characterized the metabolic profile of early stages of chick lung branching: b1, b2, and b3. *Ex vivo* lung explant culture was performed, and the medium collected to analyze the production/consumption of metabolic intermediates associated with glucose catabolism (lactate, acetate, alanine) by ^{1}H-NMR. *In situ* hybridization and qPCR were performed to assess the expression patterns/levels of key enzymes and transporters from the correspondent metabolic pathways. Lactate Dehydrogenase protein expression levels were evaluated by Western blot. Results revealed an increase in lactate and acetate production, in the b3 stage. Still, glucose consumption is maintained with a concurrent decrease of *glut3* transcript levels. *ldha* is mainly expressed in the proximal region of the lung whereas *ldhb* is restricted to the growing tips. LDH protein levels increased in the three stages. This study describes, for the first time, the temporal metabolic changes associated with early chick pulmonary branching. Results revealed lactate and acetate as potential developmental biomarkers, and branching morphogenesis dependent on a glycolytic-lactate metabolism.

31• HoxD13 and its targets and the evolution of vertebrate limbs

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Limbs evolved from fish fins, which suffered profound changes distally, with addition of novel endoskeleton units and reduction of the apical ectodermal finfold. In the past, we have shown that *hoxd13a* overexpression during zebrafish fin development leads to distal expansion of chondrogenic tissue and finfold reduction, a phenotype that reflects the morphological changes expected during the fin-to-limb transition. In order to understand how *hoxd13* contributed to this morphological evolution, we characterized the expression of fin/limb developmental genes, including 10 putative *hoxd13a* downstream targets identified in a Chip-to-Chip assay, after causing overexpression of *hoxd13a*, using the transgenic zebrafish line *hsp70:hoxd13a*. Our results suggest that the overexpression of *hoxd13* 1) promotes cell proliferation maintaining *fgf10* and *shh* signaling activity for longer and in higher levels; 2) promotes skeletogenic fate in the most distal cells by overexpressing *fbn1, dachA* and *dachB*; and leads to a finfold reduction with the down-regulation of the *and1* gene and up-regulation of *bmp2b* and *bmp4*. In order to evaluate the impact of *bmp2b* upregulation, potentially mediated by *hoxd13a,* on the morphology of the fins, we conducted assays and found that the overexpression of this gene leads to finfold reduction, which might have been key for the morphological transformation from fins to limbs during evolution.

32• An endocrine pancreas phenotype caused by the disruption of developmental cis-regulatory elements in the *nog2* locus

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** Equal contribution*

Cell therapy for diabetes requires a deep knowledge of the pathways underlying normal and disrupted pancreas development. Considering that, we have performed a genetic screen in zebrafish using the Expression Disruption (ED) transposon, an enhancer trap with strong mutagenic capacity that disconnects cis-regulatory elements (CREs) through the presence of an insulator. One of the ED insertions was mapped downstream of *noggin2,* recapitulating its expression pattern including the notochord, an embryonic signaling center adjacent to the pancreas. *nog2* encodes for a diffusible protein that functions as a negative regulator of BMP signaling, which have been shown to repress pancreatic fate. Accordingly, we found that a knockdown of *nog2* impairs beta-cell differentiation. In addition, homozygous ED embryos show a decreased area of insulin expression, a phenotype rescued by the deletion of the ED`s insulator, suggesting that it is able to disrupt *nog2* CREs essential for proper beta-cell development. By analyzing the *nog2* regulatory landscape, we found a notochord enhancer that interacts with the promoter of *nog2* and is located downstream of the *ED* insertion. Hoping to translate this important information to humans, we have screened putative CREs in the human NOG locus and found a functional orthologous CRE that drives expression in the same tissue, the notochord. In summary, our work shows that the disruption of CREs might impact the proper development of the endocrine pancreas.

33• Isoforms of the EMT inducer Prrx1 in development and disease

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The epithelial-mesenchymal transition (EMT) endows cells with migratory and invasive properties and it is crucial for the formation of many tissues and organs during embryonic development. This cellular program is triggered after the activation of transcription factors, referred to as EMT-TFs. In the adult, the reactivation of the EMT program contributes to the progression of diseases like fibrosis and cancer (Nieto et al., 2016). Prrx1, identified as a novel EMT-TF in our lab, produces three different splice isoforms, whose functions remain to be characterized. We have tested the ability of each of these isoforms to induce EMT in vitro, and only the longest isoform (Prrx1L) is able to do so. To test the role of the different isoforms *in vivo*, we have generated isoform-specific mutant mouse lines that we are characterizing using the phenotype of the Prrx1 null mice in the skeleton and the vasculature as a reference (Martin et al. 1995, K. Ihida-Stansbury et al 2004). In addition, as Prrx1 is reactivated during cancer progression, we are also addressing the role of each of the isoforms in MMTV-PyMT breast cancer model that we are combining with the Prrx1 isoform mutants.

34• Dissecting the mechanisms of developmental signals Notch and Akt in oncogenesis

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The building of an organism is the result of coordinated gene action and cellular signals that directs the developmental fate of individual cells. Often, the same signalling mechanisms that nourish normal tissue growth, patterning, and maintenance during development, are also involved in tumour formation when derails from normal activity. Notch and PI3K/Akt signalling pathways play fundamental roles in metazoan development through the execution of differentiation, proliferation and apoptotic programs. Nevertheless, simultaneous aberrant activation of these two signals is causative of several aggressive cancers, although the mechanism behind is poorly understood. Here, we used *Drosophila* to investigate molecular aspects of this interaction using a model entailing the simultaneous over-activation of both Notch and Akt signalling pathways (N^+Akt^+) in the developing fly eye. By employing phospho-proteomic analysis on N^+Akt^+ induced tumours we have identified a specific downstream target belonging to the mitochondrial electron transport chain (ETC). Genetic inactivation of ETC components provokes defects on development, but also induces the generation of reactive oxygen species, which in turn cooperates with Notch signal to fuel tumorigenesis. Interestingly, the oxidative stress response signal JNK is increased in N^+Akt^+ tumours although with a tumour suppressor role. Altogether, our data define molecular events involved in the N^+Akt^+ oncogenic cooperation.

35• The role of *cis*-regulatory elements in morphological adaptation to cave environment in *Astyanax mexicanus*

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Cis-regulatory elements and regulatory landscapes are essential for evolution, defining how and where the genes should be expressed. Despite their obvious importance, they have been the least understood DNA regulatory elements, until recently. One of our main questions is how *cis*-regulatory elements appear and are modified during evolution. Here we aim to elucidate how the regulatory information impacts on the phenotypic adaptation to new environments at a micro evolutionary level.

For that purpose, we will use *Astyanax mexicanus* as a model organism. This fish presents two well morphologically differentiated populations, Surface Fish and Cavefish. For instance, Cavefish has lost pigmentation, the eyes, and has developed an increased number and size of neuromasts. Therefore, the comparison of the activity of *cis*-regulatory elements in both surface and cavefish could provide us with new insights about the relationship between a certain phenotype and the underlying gene-regulatory network. Also, this approach could be fundamental for modeling some human diseases in cavefish, like age-related macular degeneration or Retinitis pigmentosa.

In order to assess the activity of *cis*-regulatory elements, we have used ATAC-seq at different development stages in both populations. This approach has allowed to successfully identify *cis*-regulatory elements that show differential activity near genes that are important for eye development and other cave specific traits.

36• Fibronectin extracellular matrix: a link between the segmentation clock and morphological somite formation?

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The mechanical environment within tissues plays a major role in morphogenesis. As cells bind to each other and to the extracellular matrix (ECM), mechanical cues are transduced into intracellular signaling pathways, thus regulating cell behavior. One of the major morphogenetic events during vertebrate development is somitogenesis, which occurs in the rostral presomitic mesoderm (PSM). Here, a fibronectin-rich ECM supports the epithelialization of PSM cells to form a new somite. A segmentation clock is proposed to underlie this periodic morphological event, as cyclic waves of gene expression travel from the posterior-most PSM and stabilize in the rostral PSM, defining the next somitic border. An open question in the field is how the segmentation clock and morphological somite formation are linked. Here we asked whether the mechanical tension provided by the fibronectin ECM interacts with the segmentation clock. We found that interfering with fibronectin (1) assembly, (2) its binding to integrins or (3) its downstream tension-response pathway in the chick PSM impair somite cleft formation and epithelialization to different extents. Importantly, all treatments perturb segmentation clock dynamics and the molecular specification of the presumptive somite border. We conclude that mechanical tension provided by the gradient of fibronectin matrix in the PSM regulates segmentation clock oscillations along the PSM, coupling dynamic gene expression with epithelial somite formation.

37• Myf5/Mrf4-dependent myotome provides essential cues for the maintenance of Pax7+ cells during epaxial myogenesis.

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In amniotes, all skeletal muscles of the body derive from the dermomyotome, a reservoir of muscle stem cells (MuSCs) marked by the expression of Pax3 and/or Pax7. MuSCs delaminate from the dermomyotome to form the underlying myotome. The myotome is a transient segmented muscle which later transforms into the epaxial (deep back) muscles. The myotome is not only a mere archaic muscle but it is also a signalling centre important for the formation of axial bones and tendons.

Here, we address the importance of the myotome for epaxial myogenesis itself using a Myf5^{nlacZ} knockout line in which myotome formation is delayed (Tajbakhsh et al., 1996). Our data reveal that only one group of epaxial muscles develops in these mutants showing the importance of the myotome for epaxial myogenesis. Also, Pax7 expression is gradually downregulated at the time of the dissociation of the dermomyotome, suggesting the myotome plays a role in maintaining MuSC identity.

To test if the myotome is involved in the maintenance of Pax7 expression in MuSCs, the signalling pathway of factors specifically secreted by the myotome (Fgf and Pdgf) were inhibited in wildtype embryo explants. The expression of Pax3 and Pax7 is affected in explants where Fgfr's are blocked showing that the lack of myotomal Fgf in the Myf5nlacZ mutant embryos could be the reason of the Pax7 downregulation and impaired epaxial myogenesis.

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38• Robo/Slit signaling as mediator of lung branching morphogenesis via epithelial progenitor's cells

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Fetal lung development is dependent on a finely regulated network of cellular and molecular events. Indicated by recent evidence that Robo/Slit signaling has a critical effect on neurogenesis and mammary development, we analyzed the potential role of Robo/Slit along fetal lung development. Thus, Robo1 and Robo2 receptors, and proximal (Sox2) and distal (Sox9) epithelial progenitors were evaluated during normal fetal lung development, in terms of spatial distribution (by immunohistochemistry) and expression levels (by western blot). Recombinant proteins for Robo1 or Robo2 functional impairment were used to reveal morphological and cellular *in vitro* dynamics.

Our results demonstrate distinct profiles for spatial distribution with a broad Robo1 and restricted Robo2 epithelial dynamics; and expression levels with significant decrease and a constitutive increase of Robo1 and Robo2, respectively. Additionally, slight Sox2 decreasing with unchanged Sox9 expression characterize the pseudoglandular and canalicular phases of fetal lung development. Finally, functional *in vitro* studies show a critical increase on number of peripheral airway buds after Robo2 inhibition with significant Sox2 depletion and Sox9 overexpression, whereas no significant effects were detected after Robo1 functional impairment.

These findings suggest a complementary/differential function for Robo1 and Robo2 signaling as mediators of proximal and distal epithelial progenitor function along fetal lung development.

39• Intron retention regulates Scratch1 expression during adult neurogenesis

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In mammals, adult neurogenesis is restricted to few niches in the central nervous system, being the subependymal zone (SEZ) the largest germinal region of the adult mammalian brain. In rodents, the neural stem cells (NSCs) that reside in this region give rise to neuroblasts that migrate and integrate in the olfactory bulb (OB), where they contribute to the neural plasticity of olfactory information processing. A group of transcription factors that might regulate this process is the Scratch family, which belongs to the Snail superfamily and has been shown to promote neuronal differentiation in different species. We show that *Scratch1* is expressed in the SEZ both in NSCs and in neuroblasts, although its transcripts present different subcellular localizations in these two cell types. In NSCs, *Scratch1* mRNA accumulates in the nucleus, due to intron retention, which in turn affects mRNA export to the cytoplasm. During differentiation, RNA methylation promotes the splicing and export of *Scratch1* mRNA. Moreover, this regulatory mechanism is not implemented in zebrafish or in the subgranular zone (SGZ) of the mouse hippocampus, but seems specific to the SEZ. In addition, we have found that the expression of other genes implicated in NSCs differentiation might be also regulated by mRNA nuclear retention. Therefore, we propose that intron retention can operate as a mechanism to tightly control the timing on neural differentiation specifically in the adult SEZ.

40• Paracrine activation of EMT genes by MMP28

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The Matrix metalloproteinase MMP28 is a secreted protease activated via cleavage of its pro-domain by Furin. MMP28 is involved in wound repair, central nervous system development, and macrophage maturation and recruitment in lung tissues. In addition, MMP28 misregulation has been linked to several cancers, and MMP28 can contribute to TGF-ß-dependent epithelial-mesenchymal-transition (EMT) in lung carcinoma cells. However, the molecular mechanisms underlying its activity are not well understood and little is known about MMP28 targets or its regulation. In *Xenopus laevis* embryos, MMP28 is expressed in the pre-placodal region (PPR) and medial neural crest (NC) at the end of gastrulation (st 12.5/13), and persists until the onset of NC cells migration (st 19). MMP28 knockdown in the embryo caused a strong reduction of expression of several transcription factors regulating NC cells EMT, and later these cells failed to properly migrate into the branchial arches. NC cells cultured in vitro indicate that MMP28 is required for NC cell dispersion. Preliminary results show that secreted MMP28 is internalized by neighboring cells suggesting that it acts non-cell autonomously, consistent with its expression in the PPR. Furthermore we demonstrate that MMP28 function is dependent on its catalytic activity and its localization to the nucleus. All together these results suggest a paracrine activation of EMT transcription factors by MMP28 to regulate NC development.

41• Rho1 regulates ploidy and cell size in *Drosophila* retinal glia

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Glial cells and glia-neuron interactions are of utmost importance for nervous system normal development, namely for neuronal function and scaffolding, trophic support of neurons, neuronal migration and synapse formation. Previous work from the group, successfully used the eye imaginal disc of *Drosophila melanogaster* as a model to investigate the role of glia cells in neurodevelopment. Here, we employ this model to study the role of Rho1, a small GTPase implicated in cytoskeleton dynamics impacting glial migration, axonal ensheathment and appropriate glial scaffolding.

First, we characterized Rho1 distribution in the eye imaginal disc by immunohistochemistry. Rho1 is expressed both in photoreceptors and glia, but particularly in the subperineurial glia. To uncover a possible biological relevance, we carried out glia-specific Rho1 knockdown, through RNA interference. Eye imaginal discs with glial Rho1 knockdown present a much reduced number of glia cells however, these cells show a marked increase in nuclear area. This might be part of a compensatory mechanisms since, not only is the overall area occupied by glia in the optic disc comparable to controls but also, glia seems to be able to wrap the axons, which can project onto the optic lobe.

In the future, we will investigate potential functional consequences of Rho1 knockdown in retinal glia to gain some insight into the role of Rho1 in physiological features as blood-brain barrier integrity, ensheathment and axonal targeting.

42• Delayed aneuploidy stress response of Neural Stem Cells impairs adult lifespan in flies

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Studying aneuploidy during organism development has strong limitations, as chronic mitotic perturbations used to generate aneuploidy result in lethality. We developed a genetic tool to induce aneuploidy in an acute and time controlled manner during *Drosophila* development. This is achieved by reversibly depleting cohesin, a key molecule controlling mitotic fidelity.

Larvae challenged with aneuploidy hatch into adults with severe motor defects shortening their lifespan. Despite being aneuploid, neural stem cells keep dividing, resulting in the quick appearance of chromosomal instability, complex array of karyotypes and cellular abnormalities.

Notably, when cells are forced to do self-renewal, the aneuploidy-associated stress response is significantly delayed; indicating that stemness state confers resistance to aneuploidy. If only the brain is spared from induced aneuploidy, all motor defects are rescued as well as the adult lifespan, suggesting that neural tissue is the most ill-equipped to deal with developmental aneuploidy.

43• Rab23-/- mice exhibit lambdoid suture craniosynostosis through aberrant Fgf signaling

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Rab23 is a GTPase protein and proposed to regulate endocytosis. Mutations in *Rab23* causes Carpenter Syndrome; heart defect, polysyndactyly, obesity and craniosynostosis. Craniosynostosis is a premature fusion of calvarial sutures that largely affecting brain & craniofacial development. Here we show, in *Rab23*$^{-/-}$ mice *Fgf10* is overexpressed in the lambdoid suture (LS). Upregulation of Fgf10 enhances the expression of its receptor *Fgfr1b* and *Fgfr2b* in the mutant LS that subsequently activate MAPK signaling and enhance pErk levels. *Pitx2,* is known as an upstream regulator of Fgf10 expression. Our study shows that Rab23 deficiency causes LS craniosynostosis through a positive regulatory axis of Pitx2-Fgf10-MAPK signaling. Moreover, overall upregulation of *Gli* transcription factors and *Hh* giving further evidence of MAPK pathway over activation, which results in higher cell proliferation in the mutant LS and leading to premature suture fusion due to an imbalance between osteoblast proliferation and differentiation. We further validated our hypothesis by in vitro LS tissue culture in presence or absence of Mapk signaling antagonist U0126. Our result suggests that inhibiting Mapk signaling by U0126 can rescue mutant LS from premature fusion in *Rab23*$^{-/-}$ mice. These findings suggest a novel role of Rab23 during LS development through Pitx2-Fgf10-MAPK signaling. Targeting Fgf10 or inhibiting MAPK signaling might be a potential tool to treating patients with LS craniosynostosis.

44• Unique Dll4/Notch function in regulating the Horizontal cell fate in the developing mouse neural retina

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The Notch pathway functions to ensure that neighbouring cells adopt distinct decisions, creating cellular diversity. Two Delta-like ligands, Dll1 and Dll4, are expressed in the developing mammalian neural retina, with Dll1 being expressed in a larger number of cells and earlier than Dll4. Using transgenic mice carrying conditional alleles of *Dll1* or *Dll4*, and a retina specific Cre-driver, we are addressing the function of Dll1 and Dll4 in the developing neural retina, aiming to unveil at which step of the differentiation cascade each ligand acts, and what consequences result from activation of Notch receptors by each ligand in neighboring cells. Our results show that Dll1 and Dll4 exhibit non redundant roles in retinal neurogenesis. Dll1 is involved in the control of RGC generation, whereas Dll4 regulates the generation of Cone photoreceptors and Horizontal cells. Our data supports a model in which both Dll1 and Dll4 control the pool of multipotent RPCs, while Dll4 acts upon a subsequent stage of RPC competence to regulate the acquisition of Cone and Horizontal fates. Surprisingly, our results show that Dll4 acts through different mechanisms to control these two fates: it inhibits the transition from multipotent RPCs to bipotent RPCs, and promotes the Horizontal fate by activating an RBPj/Ptf1A auto-regulatory loop, necessary to impose this fate on RPCs.

45• Epithelial apical vertices are important for morphogenesis

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During animal development, many epithelial tissues extend in one orientation while narrowing in the orthogonal axis. This process is called convergence and extension.Cell rearrangement, which results from the remodelling of cell-cell contacts is a key driving force in this process. In epithelia, cell-cell contacts are connected via vertices, where 3 or more cells meet. Whereas several recent studies have suggested that cell vertices are important sites for sensing and regulating tissue tension, it is unknown whether cell vertices play an active role in regulating cell rearrangement. Using as an entry point a newly discovered marker of epithelial vertices, the Immunoglobulin-superfamily domain protein Sidekick, we investigate the behaviour and role of vertices in epithelial morphogenesis in the *Drosophila* model. Using automated cell tracking, large-scale quantitative analysis and modelling we reveal that apical vertices are important sites in regulating epithelial shape and remodelling.

46• Investigating the importance of mitotic bookmarking by RBPJ/Notch signaling in vertebrate neurogenesis

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Maintenance of cell identity must face the challenges that occur during mitosis, when DNA becomes highly condensed, nuclear transcription is arrested and most of transcriptional machinery disengages from chromatin. The ability of some transcription factors (TFs) to associate with mitotic chromosomes suggests a role in propagating cell identity across cell divisions (mitotic bookmarking), by promoting a quick reactivation of target genes upon mitotic exit. Progress has been hampered however, by the difficulty in inactivating TFs specifically during mitosis.

The Notch pathway is a major regulator of neural stem cell maintenance and identity. Strikingly, its downstream effector RBPJ was shown to interact with chromatin throughout mitosis. Here we propose to investigate a possible mitotic bookmarking function of RBPJ/Notch in neural stem/progenitor cells. Live-imaging of cells expressing tagged versions of RBPJ and its cofactors is being used to study the dynamics of interaction with condensed chromosomes, while genome-wide mapping of RBPJ binding to mitotic chromatin is being performed using ChIP-seq in synchronized cultures of neural stem cells. To knock-down the expression of RBPJ protein specifically during mitosis, we are using the Auxin-inducible degron system in mitotic synchronized NS cells. We will evaluate how RBPJ binding impacts on accessibility and architecture of mitotic chromatin, and how this may correlate with the kinetics of transcriptional reactivation.

47• Dilp8-Lgr3 neural circuit couple metabolism and developmental timing

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Animal size influences nearly every aspect of the biology of an organism, including number and size of offspring, reproductive success, fitness and survival. As such developing animals must employ homeostatic mechanisms to adjust their growth and maturation time in other to compensate and withdraw size variations arising from environmental changes and genetic noise, thereby ensuring the correct final body size, proportions and perfect bilateral symmetry are attained. Recently we have shown that such plasticity entails communication between peripheral organs and the brain via the relaxin hormone Dilp8 binding to its receptor Lgr3 in two pair of brain neurons. Flies deficient for the hormone *dilp8*, or its receptor *lgr3*, are incapable to maintain the correct size, and bilateral symmetry. Intriguingly, we found that Lgr3 neurons have extensive axonal arborisations that connect the PTTH-producing neurons and the insulin-producing cells among others neuronal population. Consistently, activation of Lgr3 neurons by Dilp8 has an impact on PTTH activity, and insulin expression, which may explain the extended larval period and slow growth rate of Dilp8-overexpresing flies (Vallejo *et al*. 2015). Moreover, Dilp8-overexpresing flies are of correct size but overweight (obese), suggesting that during the extended larval period animals continue feeding and accumulative body mass. We found that this extra-weight has a positive impact on fitness in later adult life.

48• Decoding the partial Epithelial to Mesenchymal (EMT) programme

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Epithelial-to-Mesenchymal transcription factors (EMT-TFs) are fundamental for the development of multiple tissues and organs. They can be reactivated in pathological conditions resulting in a profound remodeling and repression of epithelial organization, leading to cancer cell dissemination or functional loss in organ fibrosis (1). While different EMT-TFs can promote EMT, it is still unclear how EMT-TFs are coordinated to orchestrate heterogeneous EMT programmes, especially the highly plastic and reversible intermediate or partial EMT states. Here, we used multiple approaches to decipher the relationship between the re-activation of different EMT-TFs and the changes in epithelial and mesenchymal phenotypes: (i) Using *in silico* analysis, we identify a stereotypic EMT-TFs expression-code accompanying the epithelial, hybrid epithelial/mesenchymal (E-M) and mesenchymal (M) phenotypes. (ii) Analysis of TGF-ß-induced EMT in MDCK epithelial kidney cells associate partial and full EMT programmes with a stereotypic EMT-TFs expression code. (iii) In renal fibrosis, damaged tubular epithelial cells acquire a stable partial EMT phenotype upon the reactivation of specific EMT-TFs, reminiscent of E-M hybrid phenotype profile identified in our *in silico* and *in vitro* analyses. In summary, this work presents strong evidence for the association between EMT modalities and specific combinatorial codes of EMT-TFs, leading to different pathological outcomes.

1- Nieto et al. EMT 2016. *Cell* (2016).

49• Kruppel-like factor dar1 is required for proper musculoskeletal architecture of the *Drosophila* leg

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Musculoskeletal development is a coordinated process that requires integration of multiple cues including interactions between muscle and connective tissue (CT). However, despite the critical impact on skeletal muscles, the developmental CT program is poorly understood. Based on gene expression and developmental convergences between flies and vertebrates we hypothesized that homologous molecular players ensure specification of CT and applied *Drosophila* model to identify new CT genes. We focused on leg tendon precursors, which in fly develop into tube-like CT structures.

We developed a cell-specific approach to isolate tendon precursors and performed RNAseq analysis. This has led to identification around 900 genes whose transcripts are CT enriched including 67 transcription factors (TF). Amongst them, tendon targeted RNAi knockdown of the Kruppel-like factor dar1, known to regulate the microtubule dynamics, resulted in severely affected fly mobility. Tissue section of fly legs with attenuated dar1 revealed highly affected musculoskeletal architecture with loss of internal appendicular CT structures and aberrant leg muscle attachments suggesting that dar1 plays a key role in CT formation. Interestingly, dar1 orthologue KLF-5 is also expressed in mouse tendon precursors and studies conducted on chicken explants suggest that it could impact CT development.

Thus our work contributes to a better understanding of CT development and its impact on muscle structure and function.

50• *mastermind* regulates niche ageing independently of the Notch pathway in the *Drosophila* ovary

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Proper stem cell activity in tissues ensures the correct balance between proliferation and differentiation. This is often achieved by the homing of stem cells in specific microenvironments or niches. The *Drosophila* ovary develops well-defined niches that contain on average 2-4 germline stem cells (GSCs), whose maintenance depends on both systemic and local factors. A known player in the decline of tissue homeostasis is ageing, which correlates with the waning of resident stem cell populations. In fact, ovaries from old females contain fewer GSCs than young flies. In an attempt to understand how ageing affects stem cell maintenance, we isolated niche cells of progressively older ovaries and compared their transcriptomic profile to younger controls. Our analysis has identified *mastermind (mam)* as an essential factor for stem cell maintenance.

Mam is a canonical coactivator of the Notch pathway in many *Drosophila* tissues. However, its role in the adult GSC niche is unknown. Our studies have revealed that *mam* is up-regulated in aged niches and that we can induce premature GSC loss by overexpressing *mam* in otherwise young niches. High *mam* levels in young niche cells induce a reduction in the cadherin-mediated adhesion of GSCs to the niche, an increase in reactive oxygen species in niche cells and defective *hedgehog* signalling in the niche, three scenarios known to provoke GSC loss. Finally, we will present evidence to support a Notch-independent role for *mam* in the ovarian niche.

51 • Dynamics of early chick embryo body elongation

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Vertebrate embryo body elongation involves strict spatial and temporal control. Little is known about the rate of early embryo elongation and the relative contribution of each tissue for overall growth. With the advent of live-imaging, visualization of embryo morphogenesis with high temporal and spatial resolution is now attainable, allowing for new experimental approaches to unveil how these processes are regulated. We performed time-lapse imaging of HH4 chicken embryos for up to 24h (N=13) in *New* and *EC* culture. The embryos elongated at an overall rate of 160 µm/h from HH4-HH10, independently of the culture system used. The rates of different body portions were assessed, and the paraxial mesoderm was found to contribute the most to total elongation. Surprisingly, although head morphology changes significantly, the distance from the head tip to the second somite is overall maintained. Further measurements revealed that the head fold length directly correlates with time, thus providing an excellent internal reference when experimentally addressing paraxial mesoderm-driven embryo elongation. We present a detailed quantitative framework of early chicken embryo elongation with high temporal and spatial resolution. This is a particularly useful staging tool when 1) analyzing morphological events or perturbations which are transitory in time and/or 2) when a specific portion of the embryo is experimentally manipulated, and an internal reference of developmental time is required.

52• Integrin-mediated mechano-transduction: a key regulator of size

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Size control during development remains a fascinating problem. The size of a developing tissue is a function of the number and size of its constituent cells and their packing. However, while the contribution of cell proliferation to tissue growth has been well studied, cell growth has received less attention. Cell culture experiments have shown that adhesion to the extracellular matrix (ECM) promotes cell growth. Still the significance of this for development is unclear. We use the follicular epithelium of the *Drosophila* ovary as a model system to address this. By combining genetics with live imaging, 4D image analysis, biophysics and super-resolution microscopy, we show that integrins, the main cell-ECM receptors, are required for proper growth of follicle cells. Contractile actomyosin fibers are anchored to the ECM by integrins. *In vivo* analysis of actomyosin dynamics reveals that integrin elimination releases these fibers that now contract freely in the cell center. We believe this increases cortical contractility that in turn inhibits cell growth. This is supported by our laser ablation and atomic force microscopy analysis showing that integrin mutant cells display increased basal cortical tension. Our results also show that integrin mutant cells are mechanically outcompeted by wild type cells. Based on these results, we propose a model in which epithelia cell growth requires a balance between actomyosin contraction forces and opposing adhesion forces mediated integrins.

53• Analysis of YAP/TAZ-dependent transcriptional response during early morphogenesis in teleost embryos

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The transcriptional co-regulators Yap and Taz are the main vertebrate effectors of the Hippo pathway: a well-known cascade that controls cell homeostasis, organ size, and tissue morphogenesis. Here we show that Yap family's paralogs composition is variable among vertebrates. Whereas many of the vertebrate branches maintained functional copies of both *Yap* and *Taz* (such as in tetrapods, spotted gar or zebrafish), *taz* is not present in Acanthomorpha, the largest group of teleost fishes (e.g. cod, tilapia, platyfish, stickleback, or medakafish). In contrast, a second copy of *yap1*, here referred as *yap1b*, appears conserved in their genomes. This closer paralog encodes for a protein with a divergent c-terminal transactivation domain. Interestingly, comparative analysis of zebrafish and medaka mutants shows that the mutation of *yap1* in medaka (generated by CRISPR-Cas) results in a strong morphogenetic phenotype early during development, which is similar to the simultaneous mutation of *yap1* and *taz* in zebrafish. We use iDamID-seq to identify the potential targets of these transcriptional regulators during gastrulation in medaka embryos. Our results indicate that, despite yap1b divergent transactivation domain, both medaka paralogs target a largely overlapping set of genes. Furthermore, by analysing CRISPR-CAS9-induced *yap1* and *yap1b* double mutants, we show that that both proteins critically cooperate during morphogenesis at early developmental stages.

54• Genome-wide analysis of the nascent RNApolII transcriptome during *Drosophila* development

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Processing of eukaryotic mRNAs is a complex and highly regulated process that occurs mostly co-transcriptionally, being our aim to better understand the way its genome-wide regulation is important for development of multicellular organisms. Previously, we proposed that splicing efficiency is rate limiting for gene expression during *Drosophila* early embryogenesis. In order to address this and other questions, we optimized a next generation sequencing technique to capture and analyse native elongation transcripts (NET-Seq) in developing *Drosophila* embryos. Nascent transcripts were IPed using specific antibodies to map RNA polymerase II position during transcription with single nucleotide resolution. Nascent transcripts reads were largely mapped in the gene body of known transcriptionally active genes. Remarkably, NET-Seq identified previously described as well as new recursive splice sites. Moreover, NET-Seq detected nascent spliced products corresponding to approximately 10% of all splicing events. This shows that splicing in *Drosophila* can occur shortly after 3' splice site synthesis, as reported in yeast.

In conclusion, we will describe for the first time, and with single nucleotide resolution, the nascent transcriptome of a developing multicellular organism, and we will propose hypotheses regarding the way its regulation is relevant for development.

55• Embryo clock dynamic expression in early chick development

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Segmentation is a key process in vertebrate embryo development. An embryo segmentation clock (EC) was first described as gene expression oscillations in the presomitic mesoderm (PSM) of chick embryos with 15-20 somites. Here, the EC displays the same periodicity as somite formation: 90 min. Both somitogenesis and EC oscillations are significantly delayed in later stages, further supporting a role for the EC in timing segmentation. Early somites are formed concomitantly with gastrulation. However, the time required to form these somites is unknown, and it is unclear how the EC operates in these developmental stages. We have characterized the expression pattern of the EC gene *hairy1* in gastrulation and early somitogenesis. *hairy1* displays a dynamic pattern of expression along both the primitive streak and PSM. Using half-embryo explant cultures we found that the time period of *hairy1* expression oscillation displayed variability among early gastrulating embryos. To address *hairy1* expression dynamics during early somitogenesis, early somite formation rate was first determined using live-imaging techniques. We found that the first somites are each formed consistently in under 90 min, with significant variability. From somite 8 onwards, somite formation time stabilizes at 90 min per somite. Accordingly, *hairy1* clock oscillations were faster in early somite stages. Altogether, our data suggests a correlation between the differentiation stage of a tissue and the EC dynamics therein.

56• Scaling of BMP signalling gradients in the zebrafish pectoral fin

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Appropriate growth control is critical for correct achievement of organ size during morphogenesis. In particular, during zebrafish pectoral fin development, proportional growth and patterning are tightly controlled by morphogens coordination. Here, we have systematically characterized pectoral fin growth along time through quantitative live imaging, scanning electron microscopy and mitosis markers. We report the identification of two BMP signalling gradients as crucial growth effectors to achieve pectoral fin proportionality. These are exponential concentration gradients that scale with the proximal-distal length of the growing fin. Importantly, manipulation of the gradients leads to fin growth defects. Additionally, we have found a specific BMP gradient modulator, *smoc1*. Smoc1 acts specifically on the anterior BMP signalling gradient, depending on concentration levels – CRISPR mutants and morpholino knock-down of *smoc1* cause anterior steeper gradients, which in turn lead to no gradient scaling with tissue size. As a result, *smoc1* deficient fins have growth and patterning defects, presenting smaller and rounder fins. Interestingly, gradient length scaling has been observed in the fly wing disc, where it represents one of the key signatures of growth control by time derivatives of morphogenetic signalling. Now, we propose that gradient scaling is a conserved fundamental mechanism also in vertebrate organ morphogenesis.

57• Uncovering alternative Hedgehog trafficking routes

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Hedgehog (Hh) is a classical secreted morphogen, which acts both at short and long distances. It has multiple well characterized roles: it directs development, shapes metabolism and controls cancer progression in a wide range of models studied up to now. However how this very important molecule is secreted from the producing cells, and/or how it is transported to its place of action is still not well understood.

According to the current available scientific evidence, Hh is first secreted to the plasma membrane, then reinternalized in a Dynamin, Rab5 and Dispatched dependent manner. Following this step it is likely recycled back to the plasma membrane and then extracted on various carriers including lipoprotein particles, multimers or exovesicles. The main question concerns as to what extent intracellular trafficking influences the final packaging of Hh into its carriers, and directs the molecule to the final place of secretion.

On my poster I will introduce the above described classical secretory mechanisms as well as experimental evidence for possible new, alternative Hh endocytic routes using the *Drosophila* wing imaginal disc as a model system.

58• Evolutionary conservation of mechanosensitive cues involved in endomesoderm formation

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The emergence of the mesoderm is an important transition of evolution but still poorly understood. Building upon the previous work of the team [1], we are working on *Nematostella Vectensis*, a sea anemone with a "primitive" mesoderm : the endomesoderm. We tested for the mechanical specification of the endomesoderm through the activation of the mechanosensitive ß-catenin (ß-cat) pathway at the onset of gastrulation.

At the beginning of the gastrula stage, blastopore cells apically constrict and invaginate. With immunofluorescence imaging (IF), we showed that ß-cat is phosphorylated in the constricted future endomesodermal cells. We observed that blocking the gastrulation movements upon morpholino injections inhibits the phospho-ß-cat (p-ß-cat) activation. We demonstrated that this phosphorylation is mechanosensitive as we rescued p-ß-cat levels up to normal by applying mechanical stresses on the inhibited embryos. Using programming to analyse IF images, we revealed that the junctional actomyosin meshwork and the cytoplasmic p-ß-cat signals are positively correlated. We postulate that the tensions induced by the actomyosin meshwork needed to the blastopore invagination trigger the mechanosensitive ß-cat pathway leading to endomesodermal gene expression.

These results indicate that the mechanosensitivity of ß-cat is conserved and could be at the origin of the mesoderm emergence.

[1] Brunet & Bouclet et al., Nat Com, 2013

59• Lgl phosphoregulation couples apico-basal polarization with cell division

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Epithelial tissue organization and function are tightly dependent on the asymmetric distribution of polarity complexes and intercellular junctions along the apico-basal axis, a feature termed apico-basal cell polarity. Several studies have focused on how these complexes antagonistically control each other localization during interphase, but knowledge about how epithelia proliferate while maintaining their polarity and integrity is still limited. Recent studies have shown that activation of Aurora A kinase induces phosphorylation and consequent cortical release of the basolateral polarity protein Lgl. However, how Lgl cortical localization is restored to polarize the cortex of the new daughter cells is unknown. Combining live imaging in the *Drosophila* follicular epithelium with photoconversion experiments in S2 cells, we show that Lgl is fully reloaded from the cytoplasm to the cortex at mitotic exit. Moreover, we found that cortical reloading is controlled by a serine/threonine phosphatase of the PP1-family that directly dephosphorylates Lgl on the serines phosphorylated by aPKC/AurA kinases. This work therefore shed light on how cell-cycle-dependent regulation of kinase and phosphatase function controls the localization of a key apico-basal polarity determinant, Lgl, ensuring the polarization of the new daughter cells during proliferation.

60• Impact of signaling pathways in the cis-regulation during embryonic development

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The animal body plan formation takes place during the embryonic development and it results from the precise spatio-temporal regulation of the gene expression. The animal form evolves by altering the expression of functionally conserved developmental genes and the vast networks they control. Most of the genomic signatures underlying key evolutionary processes can be found in the cis-regulatory elements (CREs) that control when, where and how much the genes are transcribed.

We have started to identify genome-wide CREs acting during early development in zebrafish. To do that, we performed both ATAC-seq and RNA-seq, which allowed us to link the identified CREs to their target developmental genes and determine how their activity change in different developmental stages. The regulatory DNA can act at long distances, allowing the contact with promoters of target genes. That occurs in a highly regulated manner, showing the importance of a precise regulation of the gene expression.

To identify activated or repressed enhancers upon modulation of the genetic pathways, embryos have been treated with different drugs during the gastrulation period, when body plan basic layout is generated. At this point the open chromatin profiling have been done at two different stages: the beginning and the end of the neurulation period. The goal of this project is to understand how signaling pathways control CREs activity and regulate gene expression over different stages during the embryonic development.

61• Extraembryonic tissue spreading requires decoupling from yolk sac in scuttle fly *Megaselia abdita*

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Extraembryonic tissues contribute to organismal development from the outside of the growing embryo proper. Throughout the animal kingdom, this often entails differentiation into a squamous epithelium that spreads over embryo proper or yolk sac. Apart from as changes in cell shape, the requirements for this tissue spreading are not well understood. Here we analyze spreading of the extraembryonic serosa in the scuttle fly *Megaselia abdita*. The serosa forms from a columnar blastoderm anlage, differentiates into a squamous epithelium, spreads over the embryo, and eventually forms an envelope around the developing embryo proper. We describe the dynamics of this process in long-term, whole-embryo time lapse recordings, which demonstrate that free serosa spreading is preceded by a prolonged pause in tissue expansion. Closer examination of this pause reveals a mechanical coupling to the underlying yolk sac, which is released during subsequent serosa spreading. We find mechanical coupling prolonged and serosa spreading impaired after knockdown of *Megaselia Matrix metalloprotease 1* (*Mab-Mmp1*) in the yolk sac. Our results indicate a critical role of the yolk sac in the regulation of extraembryonic tissue spreading in *Megaselia*. In an evolutionary context, we propose that the loss of mechanical de-coupling between yolk sac and serosa could provide a plausible mechanism to explain the origin of the non-spreading extraembryonic tissue of *Drosophila melanogaster*, the amnioserosa.

62• Co-option in *Drosophila*: metamorphosis and the immune system

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In Insecta, different postembryonic development strategies, have evolved namely Holometabola (complete metamorphosis) and Hemimetabola (incomplete metamorphosis). The regulation of metamorphosis relies on the secretion and action of two peripheral hormones: 20-hydroxyecdysone and juvenile hormone that regulate timing and nature of molt, respectively.

The evolution of metamorphosis entailed new challenges, including the novel gateways for exposure to infection. Interestingly, in *Drosophila*, the expression of antimicrobial peptides increases at pupariation coinciding with an ecdysone peak. Larvae purge gut contents shortly before pupariation but this process does not eliminate gut microbiota completely. We hypothesize this constitutes a selective pressure that, through co-option of immune system activity at pupariation, led to the evolution of a pre-emptive mechanism to ensure successful metamorphosis. We have shown that the immune enhancement at pupariation is dependent on ecdysone but not on the presence of microbiota. In parallel, we have also shown that flies that do not produce the anti-microbial peptide Drosomycin have higher bacterial proliferation during metamorphosis and are more likely to fail pupariation. Ultimately, we will use different species to test the predicted correlation between the action of this mechanism and the evolution of complete metamorphosis.

63• Insulin signalling and maternal age impact recovery after prolonged quiescence in *C. elegans*

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Cell quiescence and return to proliferation are crucial for maintenance of stem cell pools, which hold the potential to support tissue homeostasis or replace dead cells after injury. Prolonged quiescence comes with a cost, reducing proliferation potential and survival. Developmental arrest of *C. elegans* at the L1 stage is an emerging model for the study of cellular quiescence and reactivation. During arrest, L1 larvae undergo a process that shares phenotypic hallmarks with the ageing of the adult. Interestingly, insulin signalling, a prominent pathway in the regulation of ageing, also balances cell proliferation and activation of stress resistance pathways during quiescence, becoming a candidate regulator of proliferation potential. Here we report that prolonged L1 quiescence delays reactivation of stem cell-like divisions in *C. elegans*. We propose that the delay in cell division results from the decline that animals suffer during L1 arrest. To that end, we show that insulin signalling modulates the rate of L1 ageing, affecting proliferative potential after prolonged quiescence. Furthermore, we show that variable yolk provisioning to the embryos, as a consequence of maternal age, is one of the sources of inter-individual variability in recovery after prolonged quiescence of genetically identical animals. Taken together, our results support the relevance of L1 arrest as a model to study *in vivo* proliferation after quiescence.

64• Vertebral body formation: A cell fate decision story

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The amniote vertebral body is formed when the most-medially located sclerotome cells (MMS) respond to signals from the notochord. While in amniotes the MMS cells are described to contain the signal to form segmented vertebrae, in anamniotes like the zebrafish, it was shown that mineralization of the notochordal sheath in specific places establishes the segmented pattern of the vertebral body. Since both the notochord and MMS cells are of mesodermal origin and arise from the same embryonic location, we hypothesize that this seemingly notochord-to-MMS shift of segmental information might be due to changes in the expression territories of genes conferring tissue identity fate. These genes would be expressed in the presumptive territories of both structures and should have mutations that cause changes in their respective structure's size. The *flh* mutant in zebrafish has no notochord and somites expand to fill its territory, while, *spt* mutants show enlarged tail notochords and no somites are formed. Studying the expression patterns of the chicken orthologues of *fll* and *spt*, *CNOT2* and *TBX6L* respectively, we found that both genes have complementary expression patterns, with a small overlap area corresponding to the presumptive territories for MMS and notochord. In order to test the role of each gene in tissue fate decision of either notochord or MMS, we produced expression constructs of these genes and electroporated each one on the presumptive territory of the other and present the results.

65• Function of a transcriptional Mediator complex subunit in tissue proliferation

Adeline Payet

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The correct development of complex organism requires an accurate regulation of gene transcription. A major actor in this regulation is the Mediator complex, serving as a bridge between DNA-bound transcription factors and RNA polymerase II. Whereas the Mediator complex has a global role in Pol II dependent transcription, it has been shown that some mediator subunits display striking specificity. For example, Med19 has the unique property to be required or not for cell proliferation depending on the cellular context, suggesting a Med19 involvement in cell competition. This process results in the elimination, by apoptosis, of developmentally competent Loser cells, through interaction with neighboring Winner cells. I have shown that blocking cell competition sensor expression or apoptotic pathway in Med19 mutant cells partially rescue their lethality. Furthermore, a loss of function of Med19 leads to a decreased expression of the Myc proto-oncogene, a known cell competition inductor, suggesting an early role of Med19 in this process. In accordance with a role in Myc regulation, I showed that Med19 depletion leads to a proliferation decrease and a cell death increase, independently of cell competition. Deciphering how Med19 controls cell proliferation and death should give us novel insights into the role of Med19 human counterpart in tumor development.

66• The role of PIL neurons in the Dilp8- and Lgr3-dependent developmental stability pathway

Joana Pereirinha, Andreia Casimiro, Andres Garelli, Mariana Pinho, André Macedo, Fabiana Herédia, Alisson Gontijo

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Animals coordinate their growth through autonomous and non-autonomous programs, which produce individuals with organs of a size and proportion typical of species. This ability to achieve the developmental program even in the face of severe developmental and/or environmental perturbations is termed developmental stability. In *Drosophila*, an insulin-like peptide Dilp8 mediates interorgan growth coordination during larval development. Dilp8 is produced whenever abnormal growth occurs in peripheral epithelial tissues of larvae and induces a delay in the onset of metamorphosis, by antagonizing the metamorphosis-inducing ecdysone signalling pathway. This way Dilp8 activity gives more time for the larvae to compensate for the growth of abnormally growing tissues, promoting developmental stability. Dilp8 has been found to act in the CNS via a neuronal circuitry that includes two bilateral interneurons (PIL neurons) expressing the conserved relaxin receptor-like protein, Lgr3. Lgr3 is also critical for preventing developmental variability. PIL neurons have been hypothesized to be the critical cells mediating the Lgr3-dependent response to the Dilp8 signal. The model is that the peripheral Dilp8 signal somehow activates Lgr3 expressed in the PIL neurons inside of the CNS, increasing the cAMP signalling and PIL neuron activity. Our main aim is to test this model by generating PIL neuron-specific drivers and to test if Lgr3 is indeed required in PIL neurons for the Dilp8-dependent delay.

67• Testing the role of extracellular vesicles in early Left-Right patterning

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Almost every cell in the human body has at least one cilium which can perform diverse biological roles such as cell motility, movement of fluid and reproduction, or as sensors. Recently, the cilium also seems to release extracellular vesicles (EVs) or ectosomes, suggesting a conserved intercellular communication, important in signaling. During development, motile cilia have a role in embryonic symmetry breaking within the left-right organizer (LRO) by generating a leftward fluid flow that triggers a genetic signaling cascade to the lateral plate mesoderm, ultimately, leading to the correct laterality placement of organs. However, how ciliary motility is perceived and transduced into an intracellular signal at the LRO cells remains controversial. Using the zebrafish, we have observed EVs inside the lumen of the LRO, by electron microscopy. We also manage to label a group of these vesicles by overexpressing mCherry tagged CD63, a protein enriched in exosomes. We are now trying to establish a specific assay for *in vivo* tracking of those particles. In addition, we have evidence based on two LRO-specific transcriptomic studies that secretion and uptake of exosomes may occur in the apical membrane of the LRO. These observations support the morphogen model which proposes that putative secreted factor is transported and accumulated by flow towards the left side of the LRO. Therefore, we want to further test if EVs are responsible for the transportation of that sidedness factor.

68• Brain-like endothelial cells derived from hiPSCs to study BBB development

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Blood brain barrier (BBB) is a selective barrier formed by brain endothelial cell, that separates the bloodstream from the brain, assuring its homeostasis and protection. *In vitro* BBB models are important to understand the steps that occur during BBB development and maintenance. Here, we describe a differentiation protocol to derive brain-like endothelial cells (BLECs) from hiPSCs. Initially, hiPSCs were differentiated into endothelial progenitor cells followed by the induction of BBB properties combining soluble factors and extracellular matrices. Our results show that VEGF, Wnt3a and RA are crucial for the maintenance of the endothelial markers and induction of the BBB properties. Along the maturation process, there is an increase in the co-localization of the CD31 marker with claudin-5, ZO-1 and Pgp, suggesting a specification for the BBB phenotype. Functionally, these cells are able to generate a continuous monolayer with ZO-1 and claudin-5 in the cell membrane, relatively low permeability to Lucifer yellow ($1.2\pm0.1\times10^{-3}$cm/min), high transendothelial electrical resistance (55 ± 0.6Ocm^2) and, when exposed to an inflammatory stimulus they up-regulate the expression of ICAM-1 and ICAM-2. BLECs when co-cultivated with pericytes present an active Pgp, as demonstrated by the accumulation of Rhodamine123 in the presence of a Pgp inhibitor. In conclusion, we were able to generate BLECs which have the potential to be further used in the generation of human *in vitro* BBB models.

69• Pax3 and Pax7 are evolutionary conserved regulators of myogenic stem cells

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Pax3 and Pax7 are paired-homeobox transcription factors regulating muscle progenitors and stem cells functions during development and postnatal myogenesis. Over the last 15 years, we have identified Pax3 and Pax7 regulated genes involved in the control of the myogenic programme. We used gene targeting to evaluate the conserved and diversified functions of Pax3 and Pax7 transcription factors via gene replacement. While Pax7 was able to replace most Pax3 functions during development, adult myogenesis was impaired. In order to evaluate more distant-family evolution, we have also replaced *Pax3* by *Pax8* and demonstrated that Pax8 is unable to rescue any of the Pax3 functions. To further analyse Pax3/7 conservation during evolution, we have also replaced Pax3 by *AmphioxusPax3/7* and *LampreyPax3/7*. Our results show that *Amphioxus* and *LampreyPax3/7*, similar to mouse *Pax7*, can compensate for *Pax3* deficiency in dorsal neural tube and somite development. Strikingly, muscle progenitor cells migration and neural crest cells migration are also restored, despite lampreys and amphioxus do not form limbs and lack migratory neural crest cells. Our results will be discussed in the context of the evolution of this subfamily of transcription factors.

70• Vascular repair after spinal cord injury in zebrafish

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Spinal cord injuries have dramatic and irreversible effects on motor and sensory functions in mammals. By contrast, zebrafish are able to repair the spinal cord and restore motility and are increasingly used to study successful strategies of regeneration. In this study we investigate if the vascular system is reestablished after spinal cord injury in zebrafish and whether the vasculature is important for the efficient recovery of spinal cord function.

We show that the zebrafish spinal cord has a similar organization and specialised blood-spinal cord barrier modifications as observed in mammals. We followed the vascular response over the course of spinal cord regeneration and confirmed that zebrafish, unlike mammals, are able to restore the vascular network. The repair of the damaged blood vessels occurs through the activation of angiogenesis. The new blood vessels are also able to rapidly recruit pericytes, thus contributing to the reestablishment of the blood-spinal cord barrier.

To address the role of the vasculature during spinal cord regeneration we are inhibiting the formation of new blood vessels using a genetic approach. Our preliminary results show that interfering with the spinal cord re-vascularisation results in impaired functional recovery.

This work reveals the enhanced capacity of zebrafish to repair the spinal cord vasculature when compared to mammals and highlights the importance of tissue re-vascularisation during regeneration.

71 • BMPs, key signals to promote the lineage bifurcation between relay and associating spinal sensory interneurons during targeted differentiation of pluripotent stem cells

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Mastering the manipulation of morphogenetic signals in order to generate specific cell types from pluripotent stem cells (PSC) stand as a seminal step for the development of autologous cell-based therapies. Here, we tackle this challenge by proposing robust protocols to generate human and mouse PSC derived organoids composed of specific subclasses of spinal sensory neurons. Neuralising conditions using the dual Smad inhibition and/or the FGF activation associated to the combined exposure of cells to RA and Wnt signaling activators is sufficient to drive PSC towards a brachial spinal progenitor state, that with time give rise to associating sensory interneurons. The fate of these progenitors can be switch towards a relay interneuron fate, upon the exposure to BMP4. By manipulating the timing of exposure, the duration and the concentration of BMP4, organoids can be enriched in specific relay interneuron populations, supporting, against actual models, *bona fide* morphogenic patterning properties of BMP signaling (Gupta et al., 2018). Furthermore, we showed that the intracellular activation of BMP4 signaling is heterogeneous and biased by the apico-basal polarity of the epithelia composing the organoids and that BMP4 inhibits the terminal differentiation of relay interneuron progenitors, which can be compensated by a triggered inhibition of Notch signaling. All these effects underpin the challenge of making organoids composed of pure populations of BMP4 dependent neuronal subtypes.

72• A natural transdifferentiation event involving mitosis is empowered by integrating signaling inputs with conserved plasticity factors

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Transdifferentiation (Td) is the direct conversion of one differentiated cell type into another, a key process in some regeneration contexts. Td occurs naturally in the worm and the ability to follow single cells in live animals makes *C .elegans* a great model to study Td. The lab has first focused on how the Y rectal cell becomes PDA neuron (1). Here we investigated whether and what Td core mechanisms and factors exist across Td events.

We selected other putative *C. elegans* plasticity events using the known somatic cell lineage (2) as a guide. We focused on the formation of a neuron from the rectal cell K, the formation of two neurons from the pore cell and Y-to-PDA in males. We explored the role of Y-to-PDA factors and cell division in these contexts.

We confirmed transdifferentiation in all these cases by expression of marker genes and cellular morphology. Our data point to the existence of a "plasticity cassette", important for all Td events, and event-specific factors. Excitingly, focusing on K, we found that both oriented cell division and activity of the Td cassette are crucial. The latter initiates the erasure of the initial mother ID in one daughter, while the former requiring Wnt, acts as an environmental re-differentiation cue leading to expression of a terminal selector TF that instructs the final ID. Thus, our data suggest that two parallel and necessary processes are at play, one to erase the initial ID and the other to subsequently superimpose the final one.

73• Identification of the SP8 regulatory network in the limb ectoderm

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SP6 and SP8 are two members of the SP family of transcription factors that are conjointly required in the limb bud ectoderm for correct proximo-distal and dorso-ventral patterning (Talamillo et al., 2010; Haro et al., 2014).

To identify the SP8 regulatory network in the limb ectoderm, we performed ChIPmentation and RNA-seq experiments in the E10.5 limb bud ectoderm. The genome-wide binding pattern of SP8 yielded 1,451 conserved and significantly enriched regions while the comparison between the transcriptional signature of WT and *Sp8*-null mutant embryos found 892 differentially expressed genes (DEG). The combination of the ChIPmentation and DEG datasets identified 183 direct SP8 target genes, 30% repressed and 70% activated by SP8. Further analyses revealed that SP8 acted on these direct targets mainly from distally located binding sites. We also found that AT-rich motifs, described as the preferential motif of SP7 to indirectly bind the DNA through DLX5, were predominant when SP8 functions as an activator. Accordingly, Co-Immunoprecipitation (CoIP) experiments showed binding between SP8 and DLX5. Interestingly, SP1 like CG-rich motifs were more frequent when SP8 acts as a repressor. Finally, CoIP and Bimolecular Fluorescence Complementation assays also showed that SP8 and SP6 form homo and heterodimers providing some insights into their functional redundancy.

Haro *et al.* (2014) *PLoS Genet*. 10(8):e1004468.

Talamillo *et al.* (2010) *Dev. Biol*. 337, 363–374.

74• Countershading in zebrafish results from an Asip1 controlled asymmetric pigment cell differentiation

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Pigment pattern formation is a classic problem in developmental biology and has been the subject of extensive experimental investigation and mathematical modelling. Vertebrate pigment patterns frequently show a clear distinction between a pale ventral area and a darker dorsal area. We have known for some time that in mammals this pattern results from spatially-regulated expression of agouti-signaling protein (Asip). The molecular basis of pigment pattern has also been widely studied in zebrafish, but to date the emphasis has been almost exclusively on the mechanisms controlling striped and spotted patterns in zebrafish and its sister species. However, zebrafish also show a pronounced dorso-ventral pigment pattern gradient. Here, we show that this dorso-ventral patterning in zebrafish is also Agouti-signaling protein-dependent. Our loss-of-function experiments identify *asip1* as a key gene required for the establishment of the dorso-ventral countershading pattern,showing the evolutionary conservation of the role for Asip in generating dorso-ventral countershading between fish and mammals. Our conclusion is particularly interesting because the cellular bases for the patterns in mammals (and birds) and in fish appear very different.

75• Developmental expression of Membrane type 1-matrix metalloproteinase (Mt1-mmp/Mmp14) during mouse embryonic development

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MT1-MMP is a member of a subgroup of membrane-anchored proteinases that belong to the matrix metalloproteinase family involved in the degradation and remodeling of the extracellular matrix during embryonic development, tumor metastasis and vasculogenesis. Deficient mice for this enzyme result in early postnatal death due to severe defects in skeletal development and angiogenesis. Here we report the expression pattern of this proteinase during embryonic development by using a mutant mouse strain expressing the LacZ reporter under the control of the Mt1-mmp promoter. Thus, Mt1-mmp expression was first detected in the endocardium of the heart and the arterious trunk by E8.5. Strong expression was also detected during vascular development including the dorsal aorta, the umbilical artery and vein or the perineural vascular plexus. In the brain, LacZ reporter expression was detected in the olfactory bulb and the rostral cerebral cortex by E11.5. A strong expression localized in the caudal mesencephalic tectum from E10.5 to postnatal stages in the superior colliculus. In addition, LacZ-positive cells were observed in neural progenitors of the spinal cord, neural crest cells and the intersomitic region. In the limb, Mt1-mmp expression was restricted to blood vessels, cartilage primordium and muscles. This expression pattern was confirmed by western blot and immunohistochemical analysis. From these results we suggest that Mt1-mmp contributes to cardiovascular and brain development.

76• Genetic control of skeletal muscle fiber type

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Skeletal muscles are composed of slow and fast fibers. There are 3 fast fiber subtypes, each expressing a different isoform of myosin heavy chain (Myh) coded by 3 different genes located at the same locus. The spatio-temporal control of the expression of these genes is not known.

Our goal is to study the link between the 3D chromatin organization of the Myh locus and the expression of these genes in adult fibers in order to understand how the hundreds of nuclei of a myofiber activate a single fast Myh gene and repress the adjacent Myh genes.

We created a transgenic mouse model of the fast Myh locus with a BAC possessing Myh2, Myh1 and Myh4 linked with different fluorescent reporter. In transgenic mice possessing the full length BAC transgenes expression recapitulates that of the endogenous genes proving that the BAC possesses all the regulatory elements required for correct gene expression.

By Circular Chromatin Conformation Capture (4C-seq) experiments we identified a region at the fast Myh locus that could act as a locus control region and activate the expression of only one Myh gene.

77• Uncovering p63 targets involved in hereditary malformations

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p63 is a transcription factor that plays a major role during ectoderm development, promoting the formation of epidermis, limbs and neural crest derivatives. Mutations affecting its coding sequence or its target genes lead to malformations characterized by split hand/foot malformation, orofacial cleft and ectodermal dysplasia. The search of genetic events underlying p63-related malformations has been mainly focused so far on transcription factor binding studies, in which binding sites falling into non-coding regions are commonly associated to the nearest genes. This might lead to wrong assignments, since *cis*-regulatory elements can control the expression of genes located at longer distances and their impairment could lead to similar phenotypes than those of knocking out the regulated genes. In this work, we study the dynamics of gene regulation orchestrated by p63 during ectoderm development in zebrafish embryos. We use a combination of genomic, epigenomic and transcriptomic data, as well as *p63* mutants generated by CRISPR/Cas9, to get new clues about the p63 genetic network and its mechanisms of target gene regulation. In addition, we used HiChIP to detect enhancer-promoter interactions and properly associate *cis*-regulatory elements bound by p63 with their target genes. This approach allowed us to identify genes that could be missassigned to p63-related phenotypes in humans and to explore the possible contribution of neighbor genes and p63 binding sites to the phenotype.

78• Sox2+ cells in the incisor labial cervical loop express the transcription factor MEIS1

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Dental epithelial stem cells within the labial cervical loop contribute to the continuous growth of the murine incisor. A number of markers for these stem cells have been identified, including transcription factor SOX2. In this study, we describe the expression of Meis1 in the dental epithelial stem cell niche, at the gene and protein levels. MEIS1 is a regulator of organ development and marks stem cells in various tissues. Meis1 expression arises in the incisor labial cervical loop during development, and in the adult stages it is specifically expressed by the Sox2+ cells. Meis1 in the incisor is coexpressed with potential binding partner Pbx1 during embryonic and adult stages. Using Meis1-null allele mice we show that MEIS1 is not essential for tooth initiation. Additionally, we have compared the expression patterns of Meis1 and Meis2 during tooth, tongue and palate formation. Their differential spatial and temporal expression patterns suggest different roles for these two genes in the oral cavity.

79• Maternal thyroid hormones are essential for neurogenesis and gliogenesis in the zebrafish embryo

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Thyroid hormones (THs) are essential for proper embryonic brain development. During this period maternal THs are the sole supply to the embryo and even mild deficiency levels in the mothers have been associated with unfavourable neurological outcomes in the offspring. MCT8 is the main thyroid hormone transporter present in brain and vascular tissue during embryonic stages and MCT8 mutations in humans give rise to the Allan-Herndon-Dudley syndrome (AHDS). In this study we employed as strategy the knockdown of MCT8, blocking maternal thyroid hormones (MTHs) entry to target cells, and in this way elucidate the molecular mechanisms underlying their action. Knockdown of MCT8 leads to cytoarquitectural disorganization of brain and spinal cord, including loss of neural progenitor cells and a pronounced effect over the expression domains of radial glia and astrocyte-like cells. qPCR analysis of selected genes showed MTHs are involved in the regulation of NOTCH pathway components such as dla, dld, her2 and her4 during early neurogenesis whilst SoxB1 neuroectodermal specification genes were not disturbed. Overall these results stress the involvement of MTHs in the early stages of neurogenesis by promoting the maintenance of specific neural progenitor populations and generating glial cell type diversity. These results, beside establishing a developmental time window for MTHs action may contribute to clarify the phenotype observed in ADHS patients.

80• Electronic cigarette aerosol effects in chick lung development

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Smoking is a major public health problem responsible for 700 000 deaths/year in Europe. Recently, electronic cigarettes (e-cig) emerged as an alternative to conventional cigarettes (c-cig). Previous studies revealed that c-cig exposure impairs lung development and triggers inflammation. However, nothing is known regarding the impact of e-cig aerosol during pulmonary development.

In this work, we evaluated the effect of e-cig aerosol and c-cig smoke in the early chick embryonic lung. *Ex vivo* lung explants were cultured in smoke/aerosol medium or unexposed medium (control) for 48 hours. Explants were assessed for total, epithelial and mesenchymal area and perimeter. Additionally, TNF-a levels were evaluated by ELISA assay.

When compared to controls c-cig treated explants disclosed a significant decrease, between 15 to 30%, in all the morphometric parameters. E-cig treated explants displayed a significant reduction just in lung total area and mesenchymal perimeter (roughly 10%). Lastly, c-cig explants presented a decrease in all the morphometric parameters, between 11 to 26%, when compared to e-cig treated explants. Additionally, e-cig and c-cig treatment induced similar TNF-a release, nearly 7 times higher than control.

In conclusion, this study describes, for the first time, the impact of e-cigs on early lung development. Results revealed that e-cig aerosol promotes lung inflammation, however, its impact on early lung growth is less detrimental than conventional cigarette smoke.

81• Investigating a potential mitotic bookmarking function of the proneural factor Ascl1/Mash1 in vertebrate neurogenesis

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Recent studies have identified several sequence-specific transcription factors (TFs) with the ability to interact with condensed chromatin during mitosis. This suggests some TFs may play a role as mitotic bookmarkers (MB), conveying transcriptional identity during cell division to the newly formed daughter cells. Although the structural determinants that mediate such interactions remain largely unknown, both sequence-specific binding and non-specific electrostatic interactions have been invoked. In addition, a strong association has been established between MB function and "pioneer" TF activity.

Ascl1 (Mash1) is a proneural bHLH TF expressed in proliferating neural stem and progenitor cells. We have previously shown that Ascl1 functions as pioneer TF while coordinating many components of the differentiation program along the neuronal lineage. Here we investigate a putative mitotic bookmarking function of Ascl1, by assessing the ability of this TF, and various mutant derivatives, to associate with mitotic chromatin using a live imaging approach. Our results strongly suggest Ascl1 does not display the "chromosome coating" ability characteristic of MBs. Similar results were obtained when using fluorescent protein reporters, and a 12 residue long tetracystein-tagged live imaging protocol. Importantly, our studies show how pioneer and bookmarking activities are separate features of TFs.

82• Pou3F transcription factors are enriched in mitotic chromatin in neural stem/progenitor cells: potential function in early G1

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Maintaining transcriptional identity in dividing cells is thought to rely on "bookmarking" of genes and regulatory regions by histone and DNA modifications that can be propagated throughout mitosis, when transcription is shut down. In addition, the ability of some transcription factors (TFs) to associate with mitotic chromosomes suggests a possible role in the mitotic inheritance process.

Here we investigate the importance of mitotic bookmarking by sequence-specific TFs in dividing neural stem/progenitor cells. Using a live imaging approach, we identified members of the POU3f of TFs (e.g. Brn2) as mitotic bookmarkers, as they associate with condensed chromosomes during cell division. We used site-directed mutagenesis, FRAP studies and ChIP-seq in synchronized neural stem cell cultures, in order to characterize the structural determinants, and the dynamics of interaction of Brn2 with mitotic chromatin. By contrast, we found the pioneer TF Ascl1 not to associate with mitotic chromosomes, contradicting a general assumed link between pioneer and mitotic bookmarking activities. Importantly, we observe the association with condensed chromosomes to result in the presence of Brn2 earlier in the G1 nucleus, in a nuclear-import independent manner. We suggest this may contribute to the temporal patterning of transcriptional reactivation, by promoting the presence of a large pool of TF inside the newly formed nuclear envelope, which becomes available immediately after mitotic exit.

83• From plankton to benthos: epigenetic regulation could be one contributing factor

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Most marine organisms have a biphasic life-cycle dependent on metamorphosis and settlement. These critical events need that developmentally competent larvae experience morphological/physiological changes in synchrony with the ecological transition from a pelagic to benthonic lifestyle. Pelagic-benthic transition requires multiples adaptations however how/why they occur still remains unclear. Epigenetic regulation specifically DNA methylation has been suggested to be important for organisms to adapt to varying environments. Seahorses are a fascinating group of fish distinguished by their anatomical features, reproductive strategy and behavior. They are unique due to their male pregnancy, where males nourish developing embryo/larvae in a brood-pouch until hatching/parturition occurs. After birth, free-swimming offspring are pelagic and then they change into a demersal lifestyle. To address the question if epigenetics could be involved in the plankton-benthos transition in seahorses, we studied DNA methylation in *Hippocampus reidi*. We performed MSAP and qPCR for genes involved in the methylation machinery at 1, 5, 10, 20, 30 and 40 day post-birth. Results revealed that *H. reidi* genome have different methylation profile during metamorphosis and settlement. Also, upregulation of DNMTs gene expression was found. Our data show that the differences in DNA methylation among developmental stages during pelagic-demersal transition suggest a potential for epigenetic regulation in this species

84• The nuclear receptor Ftz-f1 controls lipid storage in the fat body and organism systemic growth in *Drosophila melanogaster*

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In *Drosophila*, nutrients are stored primarily in the fat body, which corresponds to the vertebrate adipose tissue and liver, during the larval stages. The fat body functions as a nutrient sensor that links nutrition with systemic growth and developmental timing. We show that the nuclear receptor of the NR5 subfamily, Ftz-f1, is required in the fat body for larval systemic growth. RNAi-mediated knockdown of *ftz-f1* in this tissue leads to abnormal autophagic flux and to a severe defect in the development of the fat body. In addition, triacylglycerol storage and mobilization is also altered. Signaling from the fat body to the brain is disrupted affecting the systemic ecdysone levels. Strikingly, attainment of the critical weight at early L3 stage is impeded, placing Ftzf-f1 as a key factor necessary for this developmental decision.

85• Insights into the role of a novel hemangioblast gene

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During early vertebrate embryogenesis, the first hematopoietic and endothelial cells arise in the extraembryonic yolk sac blood islands from a common precursor known as the hemangioblast. In addition to primitive hematopoietic cells, yolk sac hemangioblasts also give rise to erythromyeloid progenitors, that originate tissue-resident macrophages and microglial cells, and to definitive hematopoietic stem/progenitor cells, that originate all definitive hematopoietic lineages. In our previous work, we used a novel hemangioblast-specific reporter to isolate the population of chick yolk sac hemangioblasts and characterize its gene expression profile by microarray analysis. In addition to known hemangioblast markers, this analysis led to the identification of previously uncharacterized genes that may be involved in hemangioblast differentiation. One of the genes most highly expressed in the hemangioblast transcriptome was *cXorf36* or *DIA1R* (*Deleted in Autism-1 Related*), a gene implicated in autism spectrum disorders and X-linked mental retardation. Here, we present our preliminary results on the expression patterns and function of *DIA1R* genes in early zebrafish and chick embryos. Our observations suggest that DIA1R may restrict the population size of early hematopoietic progenitors and promote their differentiation towards the myeloid lineage. Ultimately, we propose that the potential role of DIA1R in microglia ontogeny may justify its implication in neurodevelopmental disorders.

86• The influence of the niche fitness in glia (over)migration

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In the nervous system, glial cells provide crucial insulation and trophic support to neurons and are important for neuronal survival. In reaction to a wide variety of insults, glial cells respond with changes in cell morphology and metabolism to allow repair. Additionally, these cells can acquire migratory and proliferative potential. In particular, after axonal damage or pruning the clearance of axonal debris by glial cells is key for a healthy nervous system. Thus, bidirectional neuron-glial interactions are crucial in development, but little is known about the cellular sensors and signalling pathways involved. In here, we show that decreased cellular fitness in retinal progenitors caused by reduced *Drosophila Myc* expression triggers non cell autonomous activation of retinal glia proliferation and overmigration. Glia migration occurs beyond its normal limit near the boundary between differentiated photoreceptors and precursor cells, extending into the progenitor domain. This overmigration is stimulated by JNK activation (and the function of its target Mmp1), while proliferative responses are mediated by Dpp/TGF-ß signalling activation.

87• CRISPR/Cas9 tools in *Drosophila*: pitfalls and success cases

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Since the discovery of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) locus in the 1990s, much has been published on CRISPR/CRISPR-associated protein (Cas)9 system and its applications on genome editing. The ability to precisely edit the genome of a living cell holds enormous potential to accelerate life science research, improve biotechnology, and even treat disease.

Despite the broad list of advantages and applications, the CRISPR/Cas9 system has its limitations. There are several aspects affecting both efficiency and specificity, including Cas9 activity, target site selection and short guide RNA design, delivery methods, off-target effects and the incidence of homology-directed repair.

The Champalimaud Center for the Unknown (CCU) Molecular and Transgenic Tools Platform (MTTP) supports the researchers at Champalimaud Research (CR) on molecular biology techniques, from basic services such as the production of competent bacterial cells, or primer design, to complex clonings of knock-out and knock-in constructs to be delivered into cells/tissues in culture or into one-cell stage embryos of model animals such as zebrafish, fly or mouse, with the purpose of creating transgenic animals. For this purpose we work in close combination with the CCU animal platforms (Fish, Fly and Rodent).

Here we present some CRISPR case studies in *Drosophila melanogaster* and go through the difficulties and troubleshooting at two levels: the design and at the bench.

88• Maternal thyroid hormones regulate vegfaa signalling during zebrafish blood-hindbrain barrier development

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Maternal thyroid hormones (MTHs) are essential for the development of the brain in vertebrates. Zebrafish embryos with impaired MTH transport, by knock-down of the T3-specific transporter MCT8, lose specific central arteries (CtAs) in the developing blood-hindbrain barrier (BHB). In this study, we looked into the role of MTH on vegfaa signalling during BHB development. We hypothesize that MTH is responsible for neural-derived angiogenic cues responsible for the chemoattraction of particular CtAs into the hindbrain (hb).

Transcriptomic analysis of 25hpf MCT8 morphant (Mo) and control embryos revealed that the vegf-signalling pathway was significantly affected. In situ hybridization analysis shows expression of vegfaa occurs juxtaposed to the developing CtAs. In MCT8Mo from 32-48hpf vegfaa expression is lost where CtAs 2, 3, 5 and 6 are to develop. Lost CtAs in the MCT8Mo are rescued by co-injecting vegfaa mRNA. Afterwards we investigated the identity of the neural cells responsible for vegfaa origin. Several neural hb cells are lost in MCT8Mo. A transgenic GFP line under the influence of copine4 lose specific hb cells juxtaposing MTH-dependent CtAs in MCT8Mo. Some of these GFP cells are positive for glial cells and for pax6a indicating that these cells might be the mediators of CtAs ingression. Functional assays will be done to confirm these findings.

In sum, MTHs play a role in BHB development by regulating the expression of vegfaa in a subpopulation of hb ventral glial cells.

89• Co-option of a single pathway is at the origin of embryonic colour diversification in water striders

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While the variety of colours existing in animals is considerable, little is known about the genetic basis of this diversity. In addition, most efforts to understand the function and molecular mechanism of colouration have been done on adults. We discovered striking extra-ocular colouration in the embryos of semi-aquatic insects (Gerromorpha: Hemiptera). Our hypothesis is that the extra-ocular colouration is due to the activation of eye pigmentation genes in embryonic legs and antennae. Using RNAi and *in situ* hybridisation on homologous genes from the pteridin and ommochrome biosynthesis pathways, we found that both classes of pigment are involved in the colouration of eyes in the embryos of *Limnogonus franciscanus*. By contrast, the extra-ocular colouration is solely due to the presence of pteridins. Moreover, analysis of key genes in a sample of species of semi-aquatic insects revealed that the same gene network controls the extra-ocular pigmentation across the entire group despite the large variation in the colour and colour patterns across species. Altogether, our results show how the recruitment of a single multigenic pigmentation pathway can fuel the impressive diversification of colours and colour patterns in a group of animals.

90• The *Drosophila* Alary Muscles. A keystone of the heart

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A specific set of muscles, called alary muscles (AMs), due to their delta wing shape, was described as early as 1950 in *Drosophila*, as connecting the heart to the lateral skeleton in each abdominal segment. Since that time, AMs largely escaped attention, however. Based on morphological descriptions in various adult arthropods, a role of AMs in controlling heart beating amplitude was proposed, but not assessed. A first embryonic/larval function of *Drosophila* AMs was described in 2013, in maintaining the anterior malphigian tubules in proper position during organogenesis (Weawers and Skaer, 2013). In parallel, our lab discovered the existence of AM lineage-related muscles in the thorax, which we named Thoracic Alary Related Muscles (TARMs) (Boukhatmi, et al., 2014). TARMs connect the exoskeleton to different regions of the gut. Both AMs and TARMs circle specific branches of the respiratory, tracheal system. This led us to postulate that AMs and TARMs are a novel type of flexible muscles acting as "abseiling ropes" in maintaining the internal anatomy of the moving larva. At SFBD 2018, we will report the live-imaging of AMs and TARMs during larval locomotion, and the key role of AMs in maintaining the heart/aorta in proper position. Homeotic transformations of AMs into TARMs, and reciprocally, raise the question of the evolutionary history of these atypical muscles.

91• How to regenerate stem cells?

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Regeneration is a widespread phenomenon in animals with many animals able to regenerate, upon injury, complex body structures. Despite long-lasting interest for this process, we still lack a general view of the evolution of animal regeneration. We study regeneration of the annelid *Platynereis dumerilii*, as it constitutes, due to its phylogenetic position, its belonging to a slow-evolving lineage, and the available tools, an outstanding model to address fundamental questions about the evolution of animal regeneration. After amputation of the posterior part of their body, *P. dumerilii* worms regenerate both the differentiated posteriormost part of the body and a stem cell-rich growth zone responsible for the addition of segments. To characterize posterior regeneration, we used a combination of morphological, cellular, molecular, transcriptomic and functional approaches. We first defined stages of the process and identified parameters that affect its timing. Various labellings and *in situ* hybridizations for tissue patterning, cell cycle, and stem cell genes were performed to further characterize the process and indicated that regeneration is a rapid process in *P. duremilii*. Wound healing is achieved by one day post-amputation and a regeneration blastema forms one day later. At this time point, some tissue specification already occurs, and a functional posterior growth zone is re-established as early as three days after amputation. Using EdU incorporations, labellings for cell cycle markers, and inhibitors such as Hydroxyurea, we showed that cell proliferation is required for posterior regeneration. We also investigated the origin of the cells of the regenerating structures, providing evidence for a local origin of the blastema, whose constituting cells mostly derive from the segment immediately abutting the amputation plane. We also conducted RNA-seq experiments at different stages of the process to characterize the transcriptional landscape of the posterior part of the worm's body during regeneration. Our data provide a thorough characterization of *P. duremilii* posterior regeneration, and pave the way, through comparative analyses, for a better understanding of the evolution of regeneration in animals.

92• Ontogeny of the gastrointestinal tract in the shark *Scyliorhinus canicula*

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The gastrointestinal tract (GIT) develops from a simple tube into highly differentiated regions. Each region has a specific structure and gene expression signature enabling different functions. The origin of jawed vertebrates coincides with the appearance of a novel GIT region, the stomach, characterized by the presence of gastric glands. The regionalization of the GIT and the differentiation of the gastric glands have been studied during the development of traditional model organisms. However, no comparable information exists in the living representatives of basal jawed vertebrates, such are the chondrichthyans. Here we describe GIT development in a chondrichthyan representative, catshark *Scyliorhinus canicula*. We identify a clear molecular regionalization of the embryonic gut assigned by the expression of *Barx1* and *Sox2* in the anterior portion of the digestive tract and by the expression of Cdx2 in its posterior portion. Moreover, we show that these expression domains relate with the formation of the stomach anteriorly and the intestine posteriorly. Finally, we provide evidences of gastric gland development close to hatching and accompanied by the beginning of the gastric proton pump H+/K+ ATPase expression. Our findings suggest that regionalization of the gut and differentiation of structures specialized in distinct stages of the digestion, involved developmental networks that appear to be clearly active at the root of divergence of gnathostomes.

93• Establishing a mechanistic relationship between neuronal stem cell identity and progeny motor neurons morphologies

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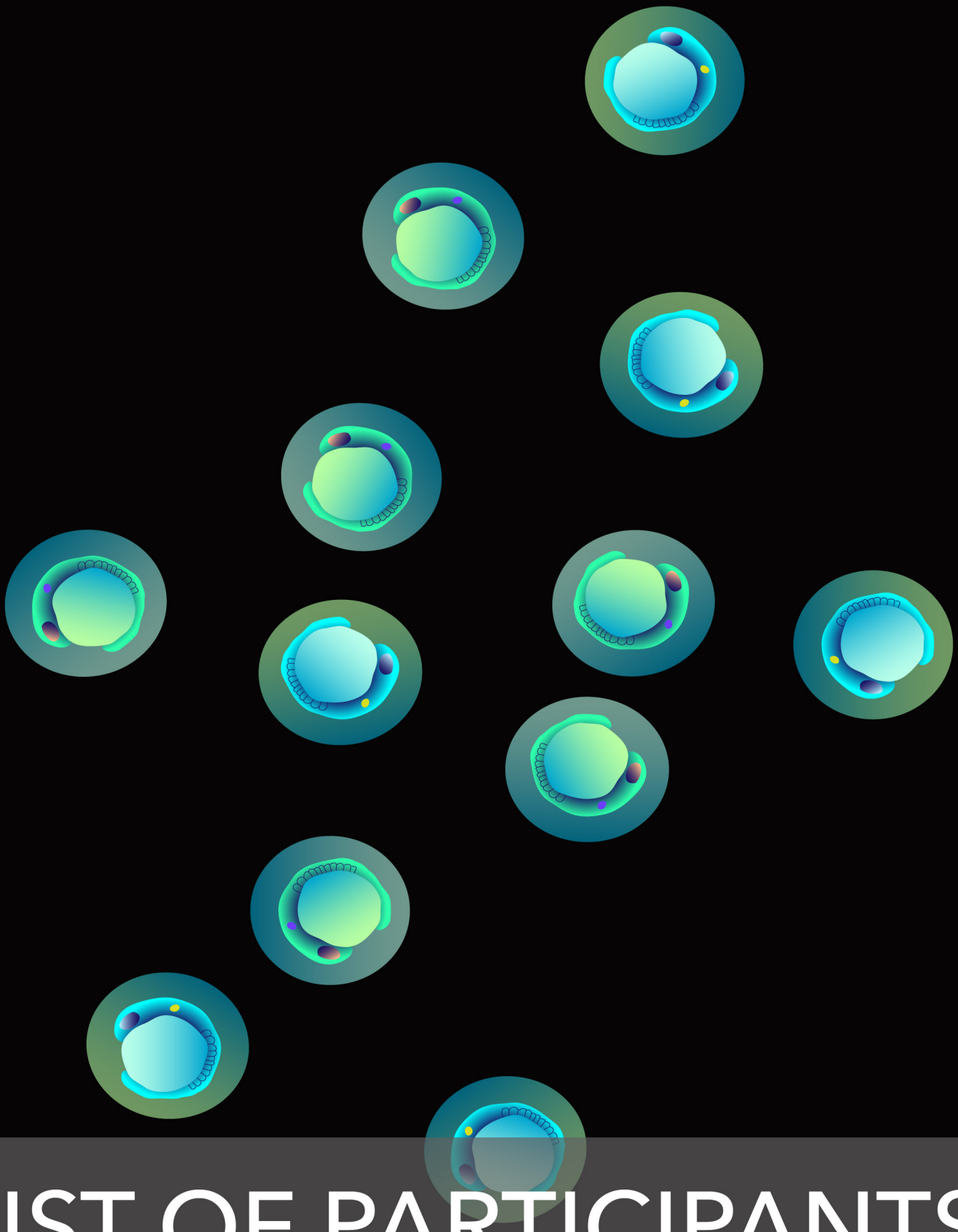
Locomotion is essential for animal survival. *Drosophila* locomotion requires the coordinated excitation of muscles by motor neurons (MNs). In *Drosophila*, each of the six legs is innervated by 50 MNs having a unique morphology defined by a specific dendritic arborization and axon target. Here, we propose to understand how single MNs acquires their morphologies by dissecting their transcriptional program. Previous study of our team has provided evidence that each MN expresses a combinatorial code of morphological transcription factors (mTFs) specifying their unique morphology. This raises a new question: what are the upstream regulators of these mTF codes. In *Drosophila*, neurons are derived from neural stem cells, called neuroblast (NBs). Each NB gives rise to a unique set of progeny. Two groups of TFs identified in NBs, termed spatial and temporal selectors, defining their spatial and temporal identities, contribute to changes in progeny identity, and generate neuronal diversity. We hypothesize that spatial/temporal selectors control the expression of mTF codes, which in turn control individual motor neuron morphology.



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