Index

01. Program ........................................................................................................................................... 5

02. Oral sessions .................................................................................................................................. 9

The EMBO Keynote Lecture: .................................................................................................................. 10
Claude Desplan, New York University, US

The Developmental Dynamics Opening Symposium: Modelling Cell Behaviour and Morphogenesis ........ 10
Michel Milinkovitch, Université de Genève, Switzerland ................................................................. 10
Roberto Mayor, University College London, UK .................................................................................. 11
Richard Smith, MPI for Plant Breeding Research, Germany ............................................................ 11
Philipp Germann, Centre de Regulació Genòmica (CRG), Spain ...................................................... 11
Luis M. Escudero, Instituto Biomedicina de Sevilla, Spain ............................................................... 12

Symposium: Evo-Devo and Genomics ................................................................................................. 12
Héctor Escrivá, Observatoire Océanologique de Banyuls, France .................................................. 12
Benjamin Prud’homme, Institut de Biologie du Développement de Marseille, France .................... 13
Juan Pablo Couso, University of Sussex, UK ....................................................................................... 13
Isabel Almudi, CABB, Spain ................................................................................................................ 14
Cristian Cañestro, Universitat de Barcelona, Spain ............................................................................ 14

Symposium: Biological Oscillators ....................................................................................................... 15
Paloma Mas, CRAG, Spain .................................................................................................................... 15
Jordi García-Ojalvo, Universitat Pompeu Fabra, Spain ........................................................................ 15
Alexander Aulehla, EMBL, Germany ................................................................................................... 15

Postdoc Symposium: SEBD, SPBP and SEBC .................................................................................... 16
Joaquín Letelier, Centro Andaluz de Biología del Desarrollo, Spain .................................................. 16
Miguel Moreno, Center for Plant Biotechnology and Genomics, Universidad Politécnica de Madrid, Spain 16
Miguel Murillo, The Cick Institute / Institute of Cancer Research, UK ........................................... 17
Paulo Navarro-Costa, Instituto Gulbenkian de Ciência, Portugal ....................................................... 17
Oscar Ocaña, Instituto de Neurociencias de Alicante, Spain ............................................................. 18
Ligia Tavares, I3S/IBMC, Portugal ....................................................................................................... 18

The ISDB-MOD Keynote Lecture ........................................................................................................... 19
Magdalena Götz, Munich Center for Neurosciences, Germany

Symposium: Cell Biology ....................................................................................................................... 19
Fernando Martin-Belmonte, Centro de Biología Molecular, Spain ...................................................... 19
Helder Maiato, Instituto de Biología Molecular e Cellular, Portugal ................................................... 20
Rosa Ríos, CABIMER, Spain ................................................................................................................ 20
Sofía Araujo, IBMB-CSIC, Spain ......................................................................................................... 21
Sol Sotillos, CABB, Spain ..................................................................................................................... 21

Symposium: Signalling in Development and Disease ........................................................................... 22
Isabel Guerrero, Centro de Biología Molecular, Spain ........................................................................ 22
Isabel Fabregat, IDIBELL, Spain ......................................................................................................... 22
Marco Milan, IRB, Spain ..................................................................................................................... 23
Pablo Menendez, Josep Carretas Leukaemia Research Institute, Spain ............................................. 23
**Symposium: Neural Development**

Domingos Henrique, Instituto de Medicina Molecular, Portugal ................................................................. 24
Nuria Flames, Instituto de Biomedicina de Valencia, Spain ..................................................................................... 24
Victor Borrell, Instituto de Neurociencias, Spain ............................................................................................. 25
Francisca Vasconcelos, Instituto Gulbenkian Ciencia, Portugal ................................................................. 25
Marta Nieto, CNB-CSIC, Spain ......................................................................................................................... 26

**Keynote Lecture:** Rainer Friedrich, FMI, Switzerland .................................................................................... 26

03. **Posters sessions**

- Modelling Cell Behaviour and Morphogenesis .................................................................................. 28
- Evo-Devo and Genomics .......................................................................................................................... 35
- Cell Biology ................................................................................................................................................ 42
- Signalling in Development & Disease .................................................................................................. 53
- Neural Development ............................................................................................................................... 68

04. **List of Authors** ......................................................................................................................................... 79

05. **Committees** ............................................................................................................................................. 85

06. **Sponsors** .................................................................................................................................................. 87
Program
### WEDNESDAY 19th October

**9.30am–8.00pm** Registration

**Workshops**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Organizers</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.00am–1.00pm</td>
<td>Genome editing: CRISPR and beyond</td>
<td>Lluís Montoliu and Joan Galceran</td>
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<tr>
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<td>Single cell omics</td>
<td>Manuel Irimia and Ignacio Maeso</td>
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<td>Cell dynamics and nanobiology</td>
<td>Maria García-Parajo and Xavier Trepat</td>
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</tbody>
</table>

**SEBD Meeting**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Organizers</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.45pm–4.00pm</td>
<td>Welcome</td>
<td>CHAIR: ÁNGELA NIETO</td>
</tr>
<tr>
<td>4.00pm–5.00pm</td>
<td>The EMBO Keynote Lecture: Claude Desplan, New York University, US.</td>
<td>Generation of neuronal diversity through temporal and spatial patterning</td>
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<td></td>
<td>CHAIR: ÁNGELA NIETO</td>
<td></td>
</tr>
<tr>
<td>5.00pm–7.00pm</td>
<td>The Developmental Dynamics Opening Symposium</td>
<td>CHAIR: MIGUEL MANZANARES</td>
</tr>
<tr>
<td></td>
<td>Modelling cell behaviour and morphogenesis</td>
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<tr>
<td>5.00pm</td>
<td>Michel Milinkovitch, Université de Genève, Switzerland.</td>
<td>The EvoDevo &amp; physics of skin appendage and skin colour patterning in vertebrates</td>
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<tr>
<td>5.30pm</td>
<td>Roberto Mayor, University College London, UK.</td>
<td>The molecular basis of gastrulation: a novel role of complement factors</td>
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<td>6.00pm</td>
<td>Richard Smith, MPI for Plant Breeding Research, Germany.</td>
<td>Towards an integrated framework for computational MorphoDynamX</td>
</tr>
<tr>
<td>6.30pm</td>
<td>Philipp Germann, Centre de Regulació Genòmica (CRG), Spain.</td>
<td>Simulating large-scale epithelium-mesenchyme interactions</td>
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<tr>
<td>6.45pm</td>
<td>Luis M. Escudero, Instituto Biomedicina de Sevilla, Spain.</td>
<td>Fundamental physical cellular constraints drive self-organization of tissues</td>
</tr>
</tbody>
</table>

**7.00pm–8.30pm** Welcome Reception

### THURSDAY 20th October

**9.00am–11.00am** Symposium: Evo-Devo and Genomics  
CHAIR: JORDI GARCÍA-fernández

<table>
<thead>
<tr>
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<th>Organizers</th>
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<tr>
<td>9.00am</td>
<td>Héctor Escrivá, Observatoire Océanologique de Banyuls, France.</td>
<td>Amphioxus illuminates the origin of the vertebrates’ head</td>
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<tr>
<td>9.30am</td>
<td>Benjamin Prud’homme, Institut de Biologie du Développement de Marseille, France.</td>
<td>Evolution and development of wing pigmentation patterns in flies and beyond</td>
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<tr>
<td>10.00am</td>
<td>Juan Pablo Couso, University of Sussex, UK.</td>
<td>Small Open-Reading Frames include two molecular classes of metazoan protein-coding genes</td>
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<tr>
<td>10.30am</td>
<td>Isabel Almudi, CABD, Spain.</td>
<td>Understanding the origin of an evolutionary novelty: the male-specific turbanate eyes of the mayfly Cloeon dipterum</td>
</tr>
<tr>
<td>10.45am</td>
<td>Cristian Cañestro, Universitat de Barcelona, Spain.</td>
<td>Gene loss, pushing the limits of chordate Evo-Devo</td>
</tr>
</tbody>
</table>
11.00am–11.30am  **Coffee break**

**11.30am–1.00pm  Symposium: Biological Oscillators**  
**CHAIR: FERNANDO CASARES**

11.30am  **Paloma Mas**, CRAG, Spain,  
*Organ specification at the core of the Arabidopsis circadian clock*

12.00pm  **Jordi Garcia-Ojalvo**, Universitat Pompeu Fabra, Spain,  
*Metabolic oscillations in proliferating cellular populations*

12.30pm  **Alexander Aulehla**, EMBL, Germany,  
*Oscillatory signaling dynamics controlling mouse embryo patterning*

1.00pm–1.30pm  **Lunch box**

1.30pm–3.00pm  **Poster Session I**: even poster numbers

3.00pm–4.30pm  **Postdoc Symposium: SEBD, SPBP and SEBC**  
**CHAIR: PILAR CUBAS**

3.00pm  **Joaquín Letelier**, Centro Andaluz de Biología del Desarrollo, Spain,  
*The emergence of the rac3β/rfng/scca synexpression group in the Ostariophysi superorder refined the mechanisms responsible for rhombomere segregation during hindbrain development*

3.15pm  **Miguel Moreno**, Center for Plant Biotechnology and Genomics, Universidad Politécnica de Madrid, Spain,  
*Positioning new root organs through oscillating gene expression: new factors integrate auxin hormone signaling and specification of cell identity*

3.30pm  **Miguel Murillo**, The Crick Institute / Institute of Cancer Research, UK,  
*Disruption of the interaction of RAS with PI 3-kinase induces regression of EGFR-driven lung cancer*

3.45pm  **Paulo Navarro-Costa**, Instituto Gulbenkian de Ciência, Portugal,  
*Early programming of the oocyte epigenome temporally controls late prophase I transcription and chromatin remodeling*

4.00pm  **Óscar Ocaña**, Instituto de Neurociencias de Alicante, Spain,  
*A left-right differential cell migration drives heart bending in vertebrates*

4.15pm  **Lígia Tavares**, I3S/IBMC, Portugal,  
*Drosophila PS2 and PS3 integrins play distinct roles in retinal photoreceptors-glial interactions*

4.30pm–5.00pm  **Coffee break**

5.00pm–6.00pm  **The ISDB-MOD Keynote Lecture: Magdalena Götz**, Munich Center for Neurosciences, Germany,  
*Mechanisms of neurogenesis and repair*  
**CHAIR: VICTOR BORRELL**

6.30pm–7.30pm  **SEBD general assembly** at Sala Cambra  
**SPBD general assembly** at Sala Premsa

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**FRIDAY 21th October**

9.00am–11.00am  **Symposium: Cell Biology**  
**CHAIR: MARIAN ROS**

9.00am  **Fernando Martín-Belmonte**, Centro de Biología Molecular, Spain,  
*Signaling pathways in epithelial morphogenesis and patterning in tubular organs*

9.30am  **Helder Maiato**, Instituto de Biología Molecular e Celular, Portugal,  
*Cracking the mitotic code*

10.00am  **Rosa Ríos**, CABIMER, Spain,  
*Microtubule assembly in centrosome-less mammalian cells*
<table>
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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>10.30am</td>
<td>Sofia Araujo, IBMB-CSIC, Spain.</td>
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<td>Centrosome amplification increases single-cell branching in post-mitotic cells</td>
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<td>10.45am</td>
<td>Sol Sotillos, CABD, Spain.</td>
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<td>A novel function of aPKC controlling apical cell trafficking</td>
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<td>11.00am–11.30am</td>
<td>Coffee break</td>
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<tr>
<td>11.30am–1.00pm</td>
<td>Symposium: Signalling in Development and Disease</td>
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<td>CHAIR: PAOLA BOVOLENTA</td>
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<td>11.30am</td>
<td>Isabel Guerrero, Centro de Biología Molecular, Spain.</td>
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<td>Cellular synapses for Hedgehog signaling</td>
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<td>12.00pm</td>
<td>Isabel Fabregat, IDIBELL, Spain.</td>
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<td>TGF-beta: a targetable pathway in liver diseases?</td>
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<td>12.30am</td>
<td>Marco Milán, IRB, Spain.</td>
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<td>JAK/STAT controls organ size and fate specification by regulating morphogen production and signalling</td>
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<td>12.45am</td>
<td>Pablo Menéndez, Josep Carreras Leukaemia Research Institute, Spain.</td>
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<td>Proinflammatory signaling is dispensable for in vitro human hematopoietic specification from hPSCs</td>
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<td>1.00pm–1.30pm</td>
<td>Lunch box</td>
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<tr>
<td>1.30pm–3.00pm</td>
<td>Poster Session II: odd poster numbers</td>
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<tr>
<td>3.00pm–5.00pm</td>
<td>Symposium: Neural Development</td>
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<td>CHAIR: BERTA ALSINA</td>
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<td>3.00pm</td>
<td>Domingos Henrique, Instituto de Medicina Molecular, Portugal.</td>
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<td>The Notch ligands Dll1 and Dll4 have non-redundant functions in the developing mouse neural retina</td>
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<tr>
<td>3.30pm</td>
<td>Nuria Flames, Instituto de Biomedicina de Valencia, Spain.</td>
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<td>Deep homology of the serotonergic transcriptional code in nematodes and mammals</td>
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<td>4.00pm</td>
<td>Víctor Borrell, Instituto de Neurociencias, Spain.</td>
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<td>Genetic control of cerebral cortex expansion</td>
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<td>4.30pm</td>
<td>Francisca Vasconcelos, Instituto Gulbenkian Ciencia, Portugal.</td>
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<td>MyT1 counteracts the neural progenitor program to promote vertebrate neurogenesis</td>
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<tr>
<td>4.45pm</td>
<td>Marta Nieto, CNB-CSIC, Madrid.</td>
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<td>Cux1 enables inter-hemispheric connections of layer II-III neurons by regulating Kv1-dependent firing</td>
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<td>5.00pm–5.30pm</td>
<td>Coffee break</td>
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<tr>
<td>5.30m–6.30pm</td>
<td>Keynote Lecture: Rainer Friedrich, FMI, Switzerland.</td>
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<td>Deconstruction and reconstruction of olfactory neuronal circuits in zebrafish</td>
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<td>CHAIR: BERTA ALSINA</td>
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<tr>
<td>6.30pm</td>
<td>Concluding remarks and farewell</td>
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<td></td>
<td>CHAIR: BERTA ALSINA AND ÁNGELA NIETO</td>
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<tr>
<td>8.30pm</td>
<td>Conference Dinner</td>
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</tbody>
</table>
Oral sessions
THE EMBO KEYNOTE LECTURE

Generation of neuronal diversity through temporal and spatial patterning

Claude Desplan, Center for Developmental Genetics, Department of Biology, New York University, New York

The Drosophila visual system is composed of the retina and the optic lobes, lamina, medulla, and lobula complex. These structures receive retinotopic inputs from photoreceptors specialized in motion vision (lamina), or color and polarized light vision (medulla). At least 100 types of neurons in the optic lobes process these inputs for extracting visual information.

The medulla contains 40,000 neurons of more than 80 cell types that are organized in 800 columns corresponding to the 800 unit eyes in the retina (ommatidia). How is this variety of neurons generated and how is retinotopy established? Medulla neurons are born from 800 neural stem cells that sequentially express five transcription factors in a temporal manner, similar to the sequence of transcription factors observed in embryonic neural stem cells. Different neurons emerge in each temporal window, therefore generating a series of 800 neurons of each type: These ‘Uni-columnar neurons’ are generated throughout the neuroepithelium and have a 1:1 stoichiometry with the photoreceptors that innervate the medulla. We will describe the mechanisms controlling the transition from one neural stem cell stage to the next.

How are the less numerous ‘multi-columnar’ neurons that have larger receptor fields and are present at a ~1:10 stoichiometry with photoreceptors generated? We will show that these subtypes emerge from the same neural stem cells that also produce uni-columnar neurons, but differ in different regions of the medulla neuroepithelium, which is highly patterned with each region contributing to producing different multi-columnar neurons. In spite of their restricted origins, these neurons still contribute to the entire retinotopic map through migration of their cell bodies.

Therefore, the generation of 80 cell types involves the integration of temporal and spatial patterning that preserves retinotopy of neurons present at different stoichiometry.

THE DEVELOPMENTAL DYNAMICS OPENING SYMPOSIUM

MODELLING CELL BEHAVIOUR AND MORPHOGENESIS

The EvoDevo & physics of skin appendage and skin colour patterning in vertebrates

Michel Milinkovitch, Laboratory of Artificial & Natural Evolution (LANE). Dept of Genetics & Evolution & Swiss Institute of Bioinformatics (SIB). University of Geneva, Switzerland

Combining evolutionary developmental biology, physics and computer science, my research group investigates the emergence of complexity and diversity of integumentary traits in vertebrates. More specifically, we perform descriptive and mechanistic analyses of morphogenesis and patterning of skin colour and skin appendages in reptiles and mammals. Using as showcases some of our recent results in snakes and lizards, I will argue that it becomes possible to understand, in nonmodel species, the genetic and physical determinisms of developmental processes that generate both intra- and inter-specific variation of skin traits.

First, I will show that the scales on the face and jaws of crocodilians are not genetically-controlled developmental units and that their spatial patterning is generated through physical cracking of the skin. Second, I will show that rapid skin colour changes in chameleons are not caused by dispersion/aggregation of pigment-containing organelles but by the active tuning of an intracellular 3D photonic structure. Third, I will discuss our analyses of skin patterning in snakes and lizards, with special emphasis on our gene mapping program in corn snakes for the identification of mutations affecting colour traits.
The molecular basis of gastrulation: a novel role of complement factors
Roberto Mayor, University College London, UK

Gastrulation is a key process in embryonic development that leads to the formation of a three layered embryo and is based in complex and coordinated cell rearrangements. One of these cell rearrangements corresponds to radial intercalation, which is responsible for the thinning of multi-layered tissues during large-scale morphogenesis; however, its molecular mechanism has remained elusive. Using amphibian epiboly, the thinning and spreading of the animal hemisphere during gastrulation, here we provide evidence that radial intercalation is driven by chemotaxis of cells toward the external layer of the tissue. This role of chemotaxis in tissue spreading and thinning is unlike its typical role associated with large-distance directional movement of cells. We identify the chemoattractant as the complement component C3a, a factor normally linked with the immune system. The mechanism is explored by computational modeling and tested in vivo, ex vivo, and in vitro. This mechanism is robust against fluctuations of chemoattractant levels and expression patterns and explains expansion during epiboly. This study provides insight into the fundamental process of radial intercalation and could be applied to a wide range of morphogenetic events.

Towards an integrated framework for computational MorphoDynamX
Richard Smith, MPI for Plant Breeding Research, Germany

Computational morphodynamics is an emerging new field that proposes a close integration between live imaging experiments, image quantification, and computer simulation modeling. The study of morphogenesis requires the analysis of interactions between genetic and mechanical processes that occur in vivo over time. The complexity of the processes involved and their often non-intuitive behavior, have promoted the use of computer simulation models to aid our understanding. Models of morphogenesis are quantitative descriptions of development, and are ideally informed by quantitative information on shape change, cell growth and proliferation, and gene expression over time. Current techniques for live imaging and microscopy have enabled the study of development at increasingly higher spatial and temporal resolution, but equally important has been the development of specialized softwares to quantify these data. Here I will present an image processing software we have developed called MorphoGraphX that is targeted to the quantification of time lapse data on plant development. The software can be used to analyze cell growth and organ shape change, lineage tracking, and gene expression in developing plant organs. I will then present our recent work on combining our softwares for plant modeling and image processing, representing a step towards an integrated framework for computational MorphoDynamX.

Simulating large-scale epithelium-mesenchyme interactions
Philipp Germann 1, James Sharpe 1

1EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), The Barcelona Institute, Spain

Morphogenesis involves interactions and transitions between hundreds of thousands of mesenchymal and epithelial cells. To capture such large-scale behaviors at the cellular level we build on the efficient and flexible spheroid model where cells are described by points in space which attract and repel each other. To simulate cellular rearrangement we extend the mesenchyme by protrusions, which we model as forces between temporarily linked cells. This allows us to simulate cell sorting and convergent extension. To enforce lateral adhesion in epithelia we add polarity to the cell centers and introduce forces that push the polarities and the centers towards a state with the polarities orthogonal to the pairwise connections. Such epithelial cells self-organize into layers in three dimensional space without imposed geometry or topology. Restricting ourselves to pairwise interactions allows efficient implementations running on graphics cards and scaling linearly in the number of cells.
Fundamental physical cellular constraints drive self-organization of tissues

Sánchez-Gutiérrez Daniel 1, Tozluoglu Melda 2, Barry Joseph D 3, Pascual Alberto 4, Mao Yanlan 2, Escudero Luis M 1

1Departamento de Biología Celular, Universidad de Sevilla and Instituto de Biomedicina de Sevilla (IB)
2MRC Laboratory for Molecular Cell Biology, University College London, London, UK
3EMBL Heidelberg, Heidelberg, Germany
4Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad

Morphogenesis is driven by small cell shape changes that modulate tissue organization. Apical surfaces of proliferating epithelial sheets have been particularly well studied. It has been accepted that a stereotyped distribution of cellular polygons is conserved in proliferating tissues among metazoans. In this work, we challenge these previous findings showing that diverse natural packed tissues have very different polygon distributions. We use Voronoi tessellations as a mathematical framework that predicts this diversity. We demonstrate that Voronoi tessellations and the very different tissues analysed share an overriding restriction: the frequency of polygon types correlates with the distribution of cell areas. By altering the balance of tensions and pressures within the packed tissues using disease, genetic or computer model perturbations, we show that as long as packed cells present a balance of forces within tissue, they will be under a physical constraint that limits its organization. Our discoveries establish a new framework to understand tissue architecture in development and disease. We will also show how this new approach can be used for the complex analysis of other packed tissues where more than one cell type co-exists.

SYMPOSIUM

EVO-DEVO AND GENOMICS

Amphioxus illuminates the origin of the vertebrates’ head

Héctor Escrivà, Observatoire Océanologique de Banyuls, France

A central question in Evo-Devo is to understand the origin of the vertebrates’ head. It is generally admitted that on the contrary to vertebrates where head mesoderm is unsegmented, the ancestor of all chordates was completely segmented, from its most anterior to its most posterior part of the body in a similar way to extant cephalochordates (i.e. amphioxus). Interestingly, we recently showed that the FGF signal plays a central role in the formation of the anterior-most somites of amphioxus. Thus, inhibition of FGF during early development impede the formation of the three most anterior somites. This suggests that functional evolution of FGF signaling played an important role in the evolution of the vertebrate’s head. Hence, in order to understand the implication of the FGF signaling in the control of anterior somitogenesis in amphioxus, we studied the function of genes under regulated by the FGF in amphioxus early embryos. These studies revealed that the regulatory cascade controlling anterior somitogenesis in amphioxus resembles that for the control of trunk somitogenesis in vertebrates and diverges from the gene cascades controlling the formation of the vertebrate head muscles. Altogether, our results strengthen the hypothesis that changes in the FGF function during early development were instrumental for the loss of anterior somites, releasing developmental constraints in the anterior part of the embryo and allowing a secondary acquisition of head muscles in the ancestor of vertebrates.
Evolution and development of wing pigmentation patterns in flies and beyond

Benjamin Prud’homme, Institut de Biologie du Développement de Marseille, France

Most animal species are decorated with coloration patterns that can give them very different appearances on otherwise similar body forms. These coloration patterns are good models to explore how morphological patterns form during development, and how they change between species. We are studying the evolution of a particular wing pigmentation pattern (a male wing spot) in Drosophila species. The evolutionary history of this trait is such that we can address different questions. First we are tracing the evolutionary origin of the wing spot. Second, we are exploring how the wing spot has evolved different forms in various species. Last, we are studying how a similar wing spot has evolved repeatedly in independent species. Our goal is to identify the genetic changes underlying these different evolutionary transitions, and to understand how these genetic changes translated into phenotypic modifications.

Small Open-Reading Frames include two molecular classes of metazoan protein-coding genes

Juan-Pablo Couso, Centro Andaluz de Biología del Desarrollo, UPO, Seville, Spain; Brighton and Sussex Medical School, Brighton, United Kingdom

Studies into the coding content and its functional outcome in animal genomes have uncovered the reciprocal annotation problems of long noncoding RNAs (lncRNAs) and small open reading frames (smORFs). While open-reading frames are formally defined as DNA sequences with the potential to encode proteins, smORFs are usually excluded in practice due to a useful-if arbitrary-lower cutoff of 100 codons in the majority of currently annotated metazoan genomes. Despite this, the human genome has been found to contain millions of small ORFs (smORFs), defined, by extension, as encoding for proteins of less than 100 amino acids in length. Many of these sequences occur in transcripts currently annotated as lncRNAs, but have been experimentally found to both associate with ribosomes and undergo active translation. The Drosophila melanogaster genome contains thousands of smORFs, hundreds of which have recently been shown by us to undergo active translation [1]. Interestingly, the translated smORF population had a partially overlapping but significant distinction into two molecular classes; the first class includes longer smORFs around 80 amino acids in length, which encode for strongly translated proteins with canonical amino acid frequencies and a tendency to allocate and function in cellular membranes. A second category of translated smORFs, dwarf smORFs, has around 20 codons in length, with amino acid usage, protein secondary structure and translational profiles that do not resemble those of canonical proteins.

We have extended the aforementioned observations to vertebrate smORFs, showing that the distinction between longer and dwarf smORFs is a general feature of small protein-coding genes in Metazoans. Further observations of the peptides encoded by smORFs confirm that these constitute distinct categories of molecular actors in animal cells, and introduce an experimentally-based (and thus biological) distinction between the protein and peptide-coding complements of metazoan genomes.

Understanding the origin of an evolutionary novelty: the male-specific turbanate eyes of the mayfly Cloeon dipterum

Almudi Isabel 1, Martín-Blanco Carlos 1, Kalender Zeynep 2, Davie Kristofer 2, Aerts Stein 2, Alba-Tercedor Javier 3, Casares Fernando 4

1Centro Andaluz Biología del Desarrollo
2University of Leuven, School of Medicine
3Dept. of Zoology, Univ. Granada
4Centro Andaluz Biología Desarrollo

Evolutionary innovations are biological revolutions: new organs are critically associated with the emergence of new species and their exploitation of new niches. Despite their importance in the history of life, how morphological novelty arises and evolves is a long-standing question in Evolutionary Biology. How the genetic network associated to the new structure appears? How this new structure is functionally and anatomically integrated into the pre-existing body plan? One of the most striking examples of a sexually dimorphic novel structure occurs in males of the mayfly species Cloeon dipterum. Cloeon males develop, in addition to the compound eyes (shared by males and females), an extra pair of extremely large dorsal, turban-shaped eyes. Thus, by comparing males versus females, this mayfly species provides a privileged system to understand the origin and integration of new structures. To answer these questions, first, we have successfully established a C. dipterum culture in the lab. Next, we describe the development of the eye and its integration with the optic lobes of male and female Cloeon nymphs using X-ray microtomography (micro-CT). Furthermore, we compare sex-specific gene expression in nymphal heads, with a special focus on genes of the highly conserved Retinal Determination Network (RDN), to show how RDN elements could have played a role in the origin of this novel sexually dimorphic visual organ.

Gene loss, pushing the limits of chordate Evo-Devo

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The bloom of Genomics is revealing a new perspective of gene loss as a pervasive evolutionary source of genetic variation that can influence the evolution of the mechanism of development of animal species. Our group, using the dismantling of developmental gene networks in the chordate Oikopleura dioica as a case study, investigates how the dismantling of gene signaling pathways such as Retinoic Acid, Fgf and Wnt has impacted on the evolution of the development of this chordate. Our work illustrates how the identification of patterns of gene co-elimination can be a useful strategy to recognize developmental gene network modules associated to distinct embryonic functions, and how the identification of survival genes can help to recognize neofunctionalization events and ancestral functions. Our work also reveals examples of the inverse paradox of Evo-Devo –i.e. how similar structures at the morphological level are build despite important differences in their developmental genetic toolkits–, including cases of genetic convergence to maintain developmental ancestral conditions.
SYMPOSIUM
BIOLOGICAL OSCILLATORS

Organ specificity at the core of the Arabidopsis circadian clock
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The circadian clock is a timing mechanism that coordinates biological rhythms with a period of 24 hours. The rhythms are synchronized every day by the environmental changes (mostly changes in light and temperature) that occur during the day-night cycle. In plants, the circadian clock controls many developmental transitions including germination, hypocotyl growth and the photoperiodic regulation of flowering time. However, much of what we know about the Arabidopsis inner clockwork comes from studies using the whole plant as experimental system so that information is rather scarce about the function of the clock in specific cells, tissues or organs. In our studies, we have discovered that the clocks at the shoot apex of the plant are highly synchronized due to their strong circadian coupling or communication. The high coupling provides specific properties for resynchronization and improved robustness against mutations and pharmacological perturbations. Furthermore, micrografting experiments have shown that rhythms at the shoot apex influence the rhythms in other parts of the plant such as roots. Altogether, our results show that the circadian system in plants is hierarchical, with the shoot apex clocks functioning as a Master Clock, with a similar organization to that described for the suprachiasmatic nucleus (SCN) in the mammalian circadian system.

Metabolic oscillations in proliferating cellular populations
Jordi Garcia-Ojalvo, Department of Experimental and Health Sciences. Universitat Pompeu Fabra, Barcelona, Spain

Multicellular organisms rely on an exquisite coordination of cellular behavior in time and space. Expanding aggregates of undifferentiated cells offer the opportunity of uncovering the fundamental principles of self-organization underlying the early stages of this coordination. In this talk I will use bacterial biofilms as model systems for this purpose, discussing in particular how metabolic constraints regulate the growth of these cell communities. Our results show that these constraints lead to growth oscillations that help the bacterial community cope with conflicting demands of protection and nutrient availability. Furthermore, we observe that cells within these structured populations communicate their state of stress via electrical signals similar to those found in more complex cells such as neurons.

Oscillatory signaling dynamics controlling mouse embryo patterning
Alexander Aulehla, EMBL, Germany

We are studying the temporal aspect, or timing, of embryonic development and in particular, investigate the role of embryonic clocks, or oscillators. Oscillations in Notch-, Wnt and Fgf -signaling pathway activity (period ~2hours) have been identified during mesoderm segmentation in mouse embryos and are linked to the periodic formation of pre-vertebrae, the somites. Most strikingly, oscillations occur phase-shifted between neighboring cells, producing spatio-temporal wave patterns within the embryonic mesoderm. Combining real-time imaging of customized dynamic reporter mouse lines with functional perturbations and also novel ex vivo models for mesoderm patterning, we will present our latest findings addressing the role of spatiotemporal signaling oscillations during mesoderm patterning and cellular differentiation.
The emergence of the rac3b/rfng/lgca synexpression group in the ostariophysi superorder refi-
ned the mechanisms responsible for rhombomere segregation during hindbrain development.

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Compartmentalization of cells during development is a fundamental process that allows the segregation of precursor populations into distinct tissue domains. The vertebrate hindbrain (HB) is subdivided along the antero-posterior axis in 7 rhombome-
res (Rs) emerging during development through a finely tuned cascade of TFs that confer identity to each R. In zebrafish, each rhombomere is flanked by actomyosin cables that prevent cells to intermingle. These cables form physical boundaries that keep neuroblasts confined to their precise location within the HB. Here we found that the small GTPase Rac3b is expressed at the HB boundaries and regulate actomyosin cables assembly. In addition, Rac3b was found to be part of a synexpression group of genes only found in teleost fish from the Ostariophysi superorder, a group that comprise 68% of fresh water species. Comparative genomics and chromosomal conformation capture analysis suggest that after the teleost WGD, the novel expression pattern emerged in the Ostariophysi lineage by a chromosomal inversion causing the fusion of two highly conserved TADs. Using an enhancer reporter vector we found two CREs that drive expression to R boundaries. A full deletion (∆2,5kb) of both enhancers was generated using the CRISPR/Cas9 system. Phenotypic analysis of this mutant points to the existence of additional CREs (i.e. shadow enhancers) within the novel TAD directing the expression of these genes to the boundaries. The observed redundancy stresses the relevance of this novel mechanism for rhombomere segregation during development.

Positioning new root organs through oscillating gene expression: new factors integrate auxin hor-
mone signaling and specification of cell identity

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Plants form new organs postembryonically as part of their development programs providing tractable systems, while this is nor-
mally restricted to the embryo in animals. Plant postembryonic organogenesis requires new organs to be positioned at specific loca-
tions. In Arabidopsis thaliana, we have found that (lateral) postembryonic root positioning is dependent on oscillating gene
expression, which is part of a developmental clock, the Lateral Root Clock. Gene expression oscillations at the root tip precede
a static site of expression from where a new root will be form (Moreno-Risueno et al. (2010) Science, 329: 1306) through the
specification of organ founder cells. Subsequently, founder cells undergo a morphogenic to generate new tissues arranged in a
precise pattern. To gain further insight into this morphogenetic mechanism we have generated plants carrying specific cell iden-
tity markers and have performed a mutagenesis screen to identify mutants with altered postembryonic organogenesis. In one of
these mutants, which we named potent, many pericycle cells change their identity becoming organ founder cells, which results
in overproduction of lateral roots. Our results indicate that POTENT integrates auxin signaling with oscillating factors. Specifi-
cally POTENT interacts with an AUXIN RESPONSE FACTOR oscillating in antiphase that is required for correct positioning of
founder cells. POTENT is a transcriptional repressor and therefore we propose that a double repression mechanism is required
to set the pace of organ founder cell positioning. In addition, POTENT is also involved in root morphogenesis by regulating auxin
signaling required for asymmetric divisions of founder cells to generate tissue stem cells.
Disruption of the interaction of RAS with PI 3-kinase induces regression of egfr-driven lung cancer

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Type I phosphoinositide 3-kinases (PI3Ks) are regulated by RAS family GTPases via a RAS Binding Domain (RBD) in the catalytic p110 subunits. We have previously shown that disruption of this domain impairs mutant RAS oncogene driven tumor formation and maintenance through cell autonomous and non-autonomous effects, such as impaired tumor-induced angiogenesis and alterations in the immune compartment of the host. Here we have investigated the effect of disrupting the RBD of p110α on the development and maintenance of mutant EGFR driven lung tumors, both in a genetically engineered mouse model and in non-small cell lung cancer (NSCLC) transplanted human cells. Disrupting the interaction of endogenous wild type RAS proteins with p110α blocks mutant EGFR induced tumor onset and promotes a major regression of tumors that have already been established, through induction of tumor cell apoptosis and growth arrest, without any observed systemic toxicity. Disrupting the RAS-PI3K interaction causes defective EGFR signalling to PI3K leading to impaired activation of both AKT and the small GTPase RAC1. These effects lead to reduced growth of EGFR-mutant lung cancer cells. We also provide proof of concept that disrupting the RAS-PI3K interaction in vivo might be exploited clinically in EGFR mutant lung cancer.

Early programming of the oocyte epigenome temporally controls late prophase in transcription and chromatin remodeling

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Oocytes are arrested for long periods of time in the prophase of the first meiotic division (prophase I). As chromosome condensation poses significant constraints to gene expression, the mechanisms regulating prophase I transcription are still poorly understood. We hypothesized that gene expression during the prophase I arrest is primarily epigenetically-regulated. To test this we have established what is currently the most comprehensive map of the Drosophila oocyte epigenome, via the analysis of 21 histone post-translation modifications. We found that prophase I-arrested oocytes have a unique, dynamic and remarkably diversified epigenome with both euchromatic and heterochromatic marks. To understand the functional significance of this epigenome, we conducted a germ line-specific in vivo RNAi screen against 50 chromatin-remodeling enzymes. This approach identified the histone demethylase dKDM5 as a major remodeler of oocyte H3K4me3 levels. The germ line-specific knockdown of dKDM5 resulted in severely reduced female fertility and significant defects in meiotic completion. Furthermore, we observed that dKDM5 temporally regulates meiotic transcription by determining the levels of RNA polymerase II in the chromatin of prophase I-arrested oocytes and by temporally regulating the onset of chromatin remodeling. This regulation is dependent on the catalytic activity of dKDM5. Surprisingly, we observed that dKDM5 was evicted from the oocyte’s chromatin at the initial stages of oogenesis. Based on these observations we propose a novel epigenetic mechanism in the female germ line, whereby the dKDM5-dependent early programming of the oocyte epigenome primes meiotic chromatin for transcriptional regulation and chromosome remodeling in late oogenesis. * - Co-first authors.
A left-right differential cell migration drives heart bending in vertebrates

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The establishment of a left-right asymmetric pathway is a central event during embryo development for proper positioning, morphogenesis and function of internal organs. Activation of Nodal-Pitx2 axis specifically within the left lateral plate mesoderm confers left identity during organ positioning and differentiation. The epithelial-mesenchymal transition (EMT) inducer Snail represses Pitx2 on the right. Whether in addition to the repression of the left cascade an informative right-derived information operates in the embryo has remained elusive. Here we show that in vertebrates, BMP signaling activates EMT inducers preferentially on the right that promote differential L/R cell movements and heart bending through an actomyosin-dependent mechanism. Downregulation of EMT prevents heart looping leading to mesocardia, one of the most severe congenital heart defects. This indicates that a right-handed informative cascade also exists in vertebrates and therefore, that two parallel left and right pathways, respectively driven by Nodal and BMP integrate left and right information to govern the morphogenesis and positioning of the heart.

Drosophila PS2 and PS3 integrins play distinct roles in retinal photoreceptors-glia interactions

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1 IBMC/I3S

Cellular migration and differentiation are important developmental processes that require cellular adhesiveness malleability. Integrins are heterodimeric transmembrane receptors important for cellular adhesion and migration but also for signal transduction. Integrins and their ligands play key roles in immune responses, leukocyte trafficking, haemostasis, and EMT transition and are at the heart of many human diseases. They are also important for nervous system development, as they regulate neural precursor cell migration, proliferation and survival, and glia myelination. We explore the developing visual system of Drosophila to study the roles of integrin heterodimers in glia development. Our data show that αPS2 is essential for retinal glia migration from the brain into the eye disc and that glial cells have a role in the maintenance of the fenestrated membrane (Laminin-rich ECM layer) in the disc. Surprisingly, the absence of glia cells in the eye disc did not affect the targeting of retinal axons to the optic stalk, challenging the commonly held view that retinal glia act as guidepost cells in axon guidance. On the other hand, αPS3 is not required for retinal glia migration, but together with Talin, it functions in glial cells to allow photoreceptor axons to target to the optic stalk. Thus, we present evidence that αPS2 and αPS3 integrin heterodimers have different and specific functions in the development of retinal glia.
THE ISBD-MOD KEYNOTE LECTURE

Mechanisms of neurogenesis and repair
Magdalena Götz, Munich Center for Neurosciences, Germany

A main question in neural repair is to which extent new neurons can integrate into circuits that normally do not undergo adult neurogenesis and hence do not integrate new neurons. I will show data monosynaptic tracing of the full input connectome of new neurons transplanted into the visual cortex. This reveals a striking and quantitative similarity of the input connectome of layer 2/3 pyramidal neurons in this region mapped with the same technique, monosynaptic Rabies virus tracing. I will then move on to compare the input connectivity in different injury paradigms and discuss how scar formation influences the functional integration of new neurons into the murine cerebral cortex. I will then move to comparison of reprogrammed neurons using novel reprogramming strategies allowing neuronal subtype specification. Thus, comparison of distinct modes of generating new neurons after brain injury and comparison of different lesion paradigm will allow determining best strategies for repair.

SYMPOSIUM

CELL BIOLOGY

Signaling pathways in Epithelial Morphogenesis and Patterning in Tubular Organs
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Epithelial organs require an exquisite control of cell proliferation and differentiation in order to achieve their final form and function during development, which depends on the regulation of polarity proteins and signaling receptors such as Crumbs, Frizzled and Notch. The general aim of our research is to characterize new genes involved in epithelial morphogenesis, patterning and regeneration, and to further understand their function and molecular mechanism using a combination of epithelial organoids, and in vivo models of epithelia morphogenesis. Our previous studies have shown that transcriptional promotion and repression control the expression of multiple genes involved in apical polarity and luminal development, including membrane trafficking pathways, control of the mitotic spindle orientation, polarity complex, etc. In summary, we are using cutting-edge methodologies such as super-resolution microscopy and organotypic 3-dimensional micropatterned cell cultures, combined with genomic and bioinformatic tools, to unravel the process of epithelial morphogenesis and the acquisition of cell polarity. We expect to obtain key information on the machinery and molecular mechanisms that regulate these processes. Indeed, this subject is central to human health. Many human diseases, such as cancer, initiate or proceed with defects in epithelial organization machineries. A molecular understanding of tube morphogenesis will lead to new ways of preventing and treating these conditions, and is therefore, a major challenge for the future.
Cracking the mitotic code

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During the human lifetime it is estimated that 10 000 trillion cell divisions take place to ensure tissue homeostasis, the renewal of epithelia, an efficient immune response against pathogens and sexual reproduction. Mitosis is the process that ensures that dividing cells preserve the chromosome number of their progenitors, while deviation from this, a condition known as aneuploidy, represents the most common feature in human cancers. In our laboratory we are currently testing the “tubulin code” hypothesis in mitosis, which predicts that mitotic motors “read” tubulin post-translational modifications on spindle microtubules. Our proof-of-concept experiments demonstrate that tubulin detyrosination works as a navigation system that guides chromosomes towards the cell equator and are currently expanding our comprehension of the role played by the tubulin code during mitosis. Thus, in addition to regulating the motors required for chromosome motion, the cell appears to regulate the tracks in which they move on. Overall, this work will contribute to our understanding of how spatial information is conveyed to faithfully segregate chromosomes during mitosis.

Microtubule assembly in centrosome-less mammalian cells

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In most mammalian cells, microtubules (MTs) are nucleated at the centrosome (CTR) and the Golgi Apparatus (GA) during interphase. Several components of the MT nucleation machinery are common to both organelles, suggesting a co-regulation of MT assembly. In order to investigate the mechanisms of such a possible co-regulation, we used the PLK4 inhibitor centrinone, which induces loss of CTRs, and CRISPr/Cas9 mutagenesis of AKAP450 and CDK5Rap2 that are candidates for participating in MT nucleation at both organelles. We show that MTOC activity of the GA is regulated by the number of CTRs whereas centrosomal MT nucleation is independent of GA activity. We also show that blocking GA-associated MT nucleation in CTR-less cells induces MT growth from cytoplasmic foci that contain AKAP450, CDK5Rap2, pericentrin and γ-tubulin but lack CEP192 and centriolar markers. Either GA or cytoplasmic MTOCs are fully competent for organizing a cell-wide, although perturbed, MT network in the absence of CTR. Strikingly, centrosome-less cells contain almost twice more MTs than non-treated cells, suggesting that the CTR control the total number of MTs by acting as a negative regulator of MT nucleation at other subcellular locations. We also identify AKAP450 and pericentrin as critical regulators of GA- or cytoplasmic-MT nucleation respectively, whereas CDK5Rap2 is apparently unnecessary for any MT nucleation event during interphase. Finally, we found that the GA is able to self-organize as a ribbon with proper cis-trans polarity independently of where MTs are being nucleated. Our data reveals a hierarchical regulation of the MT nucleation process and unveils mechanisms that might be relevant for understanding how specific MT arrays form during cell differentiation.
Centrosome amplification increases single-cell branching in post-mitotic cells
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Centrosome amplification is a hallmark of cancer although we are still far from understanding how this process affects tumorigenesis. Besides the contribution of supernumerary centrosomes to mitotic defects, their biological effects in the post-mitotic cell are not well-known. Here we exploit the effects of centrosome amplification in post-mitotic cells during single cell branching. We show that Drosophila tracheal cells with extra centrosomes have a higher branching capacity than wild-type cells. We found that mutations in Rca1 and CycA affect subcellular branching, causing tracheal tip cells to form more than one subcellular lumen. We show that Rca1 and CycA post-mitotic cells have supernumerary centrosomes and that other mutant conditions that increase centrosome number also show excess of subcellular lumen branching. Furthermore, we show that de novo lumen formation is impaired in mutant embryos with less centrioles. The data presented here define a requirement for the centrosome as a microtubule-organizing center (MTOC) for the initiation of subcellular lumen formation. We propose that centrosomes are necessary to drive subcellular lumen formation. In addition, centrosome amplification increases single-cell branching, a process parallel to capillary sprouting in blood vessels. These results shed new light on how centrosomes can contribute to pathology independently of mitotic defects.

A novel function of aPKC controlling apical cell trafficking
Sotillos Martín Sol, Centro Andaluz de Biología del Desarrollo/CSIC/Universidad Pablo de Olavide

Cell polarity is a basic characteristic of any cell, required for their proper physiological function. To establish cell polarity a number of determinants are required (reviewed in [1]) to restrict the different functional domains in the cell. When establish and for maintenance of cell polarity a proper trafficking to and recycling from the membrane is required. And, vice versa, once established cell polarity is necessary to define the cell domains for trafficking delivery. Thus, an intermingled between cell polarity and cell trafficking is essential to maintain each other and for the proper cell physiology. Recently we have shown a new interacting point between cell polarity and trafficking at the level of the polarity determinant aPKC and the Rab11-adaptor protein Nuclear fallout (Nuf, [2]). aPKC is a Ser/Thr kinase of the Par complex essential in most of the polarity processes. Nuf belongs to the Rab11-family interacting proteins (Rab11-FIP [3]) that function as an adaptor of Rab11 to the microtubule motor protein kinesin and the dynein complex [2, 4]. We have demonstrated that active aPKC interacts directly with and phosphorylates Nuf, modifying its cellular distribution. Furthermore, aPKC is a cargo of the Nuf-Rab11 recycling endosomes and it seems to be regulating its own recycling in the cell by phosphorylation and displacement of Nuf from the apico-lateral cortex. I will present new data showing how Nuf-Rab11-RE are also required during embryogenesis to sustain the apical cell polarity, affecting mainly to aPKC membrane maintenance. However, in contrast with data from vertebrates [5] Rab11-REs are not required to establish cell polarity during early embryogenesis.

Cellular synapses for Hedgehog signaling

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Hedgehog (Hh) morphogen acts at a distance and in a concentration-dependent manner, controlling the differential activation of target genes according to the distance from the source of production. However, Hh lipid modifications anchor Hh molecule to the membrane, which is an obstacle for Hh free extracellular movement. We have proposed that filopodia-like structures or cytonemes arising at the basolateral side of Drosophila epithelia act as mediators of Hh dispersion. To determine the factors that regulate the dynamic of cytonemes our studies are based on life imaging in Drosophila histoblasts, the precursor cells of the adult abdomen. We observed that the extension of cytonemes correlates spatially and temporally with the formation of the Hh morphogenetic gradient (1), and that Hh transport via exovesicles along cytonemes emanating from Hh producing cells is essential for the restricted distribution of Hh (2). We have also observed that Ihog, one of the Hh receptors, has an essential role for the regulation of the dynamic behavior of cytonemes. To investigate which are the extracellular and intracellular regulators of Hh cytoneme dynamics we are characterizing both in vivo and biochemically the interactions of the Ihog functional domains with the ECM components and with the actin dynamic regulators. Currently, we are focusing on the study of the cytoneme-mediated communication between Hh producing and receiving cells. By expressing some of the components of the Hh reception complex, such as the Hh coreceptors Ihog and Boi and the glypicans Dally and Dlp in either A or P compartment, we observe an interaction between Hh sending and receiving cytonemes at discrete sites along A and P compartment cytonemes. We envision the reception process as a “synapses” where probably cytoneme-cytoneme interaction drives exosome liberation. To visualize this process we are using the GRASP technique of GFP fluorescence reconstitution when two complementary fragments of GFP (sp:GFP1-10 and sp:GFP11) get in contact. Thus, using tagged forms of CD4, SNARE complex components with complementary fragments of GFP we analyze the GRASP reconstitution sites for reception along cytonemes.


TGF-β: a targeteable pathway in liver diseases?

Isabel Fabregat, Bellvitge Biomedical Research Institute (IDIBELL), Department of Physiological Sciences II, School of Medicine, University of Barcelona, Spain

The Transforming growth factor-beta (TGF-β) family regulates cell proliferation, differentiation, migration and death, playing relevant roles in the homeostasis of tissues and organs. Due to the diverse and pleiotropic TGF-β functions, de-regulation of its pathways contributes to human diseases. In the case of the liver, TGF-β signaling participates in all stages of disease progression, from initial liver injury through inflammation and fibrosis, to cirrhosis and cancer. TGF-β inhibits growth and induces apoptosis in hepatocytes, promoting liver differentiation during embryogenesis and physiological liver regeneration. However, high levels of TGF-β, as a consequence of chronic liver damage, produce activation of stellate cells to myofibroblats and massive hepatocyte cell death, which contributes to the promotion of liver fibrosis and later cirrhosis. During liver tumorigenesis, TGF-β plays a dual role. It may behave as a suppressor factor at early stages, but overactivation of its signaling could later contribute to tumour progression, once cells escape from its cytostatic effects. For all these reasons, targeting the TGF-β signaling pathway is being explored to counteract liver disease progression.

JAK/STAT controls organ size and fate specification by regulating morphogen production and signalling

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The developing limbs of Drosophila have proved a valuable model system to genetically and molecularly identify morphogens and members of their corresponding signaling pathways. They have also been instrumental to functionally dissect the interplay between morphogens and their biological functions and to unravel the genetic logic of pattern formation. In Drosophila, the Unpaired cytokines are Interleukin-6 (IL-6)-like secreted proteins produced and released from a localized source that spread along the tissue to activate the conserved JAK/STAT signaling pathway. Here we will present evidence that the JAK/STAT pathway is required in a sequential manner in the development of the Drosophila wing to guarantee the correct fate- and growth-promoting activities of Wingless, Hedgehog and Dpp morphogens. Interestingly, JAK/STAT mediates these activities by three distinct mechanisms. Overall, our findings add a new member to the ample repertoire of signalling molecules and corresponding pathways involved in limb development and unveil an unprecedented role of pro-survival cues and mitogenic signals in limb development.

Proinflammatory signaling is dispensable for in vitro human hematopoietic specification from hPSCs

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Recent studies in zebrafish and mice demonstrated that proinflammatory signaling is a positive regulator of definitive hematopoietic development. Whether proinflammatory signaling regulates also human hematopoietic specification remains unknown. Here, we explored the impact of the proinflammatory cytokines tumor necrosis factor-α (TNFα), interferon-γ (IFNg) and interleukin-1β (IL1β) on in vitro hematopoietic differentiation using human pluripotent stem cells (hPSCs). Gene expression analysis and ELISA revealed the absence of a proinflammatory signature during hematopoietic development of hPSCs. Functionally, the emergence of hemogenic endothelial progenitors (HEPs; CD31+CD34+CD45- or CD34+CD43-CD45-) and hematopoietic cells (CD43+CD45+) was not affected by treatment with increasing doses of TNFα, IFNg and IL1β irrespective of the developmental window or the differentiation protocol used (embryoid body- or OP9 coculture-based). Similarly, knock-down of endogenous NF-κB signaling had no impact on hematopoietic differentiation of hPSCs. This study serves as a demonstration that TNFα, IFNg and IL1β signals do not improve existing protocols for hematopoietic differentiation of hPSCs and suggest that proinflammatory signaling is dispensable for in vitro human hematopoietic development.
**SYMPOSIUM**

**NEURAL DEVELOPMENT**

**The Notch ligands Dll1 and Dll4 have non-redundant functions in the developing mouse neural retina**

Claudia Gaspar, Alexandra Rosa, Domingos Henrique

*Instituto Medicina Molecular, Faculdade de Medicina Lisboa, Portugal*

The Notch pathway functions to ensure that two neighbouring cells adopt distinct developmental 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. Approximately 30% of the Dll1-expressing cells show also Dll4 expression, which is consistent with a sequential expression of the 2 ligands during neuronal differentiation. However, fate-mapping shows that retinal cells expressing these genes differentiate into any of the retina neuronal types without any particular bias. What roles might then these two Notch ligands play during retinal neurogenesis?

Using transgenic mice carrying conditional alleles of Dll1 (Dll1flox) or Dll4 (Dll4flox), and a retina specific Cre-driver, we are addressing the function of Dll1 and Dll4 in the developing neural retina. Our results show that Dll1 and Dll4 exhibit non redundant roles in retinal neurogenesis. Dll1 is mainly involved in the control of RGC generation, whereas Dll4 regulates the generation of Cones, Amacrines and Horizontal cells. Our data supports a model in which Dll1 acts upstream of Dll4 to inhibit RGC fate and to control the pool of multipotent RPCs, and Dll4 acts upon a subsequent stage of RPC competence to regulate the acquisition of Cone and Horizontal cell 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 acts positively to promote the Horizontal cell fate by activating the RBPj/Ptf1A auto-regulatory loop, necessary to impose this fate on RPCs.

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**Deep homology of the serotonergic transcriptional code in nematodes and mammals**

Nuria Flames, *Instituto de Biomedicina de Valencia, Spain*

The identification of rules underlying the transcriptional cis-regulatory code remains a daunting task. cis-regulatory codes directing neuron subtype specification are particularly challenging as the expression of a few hundreds of genes (neuronal genome) is distributed in thousands of different combinations to generate neuronal diversity. We have identified a combinatorial code of six transcription factors (TFs) that coordinately regulates C.elegans HSN serotonergic neuron differentiation. Clusters of TF binding sites for these six factors configure a regulatory signature that can be applied to de novo identify HSN expressed genes and enhancers. We find that mouse orthologs of the HSN regulatory code are known regulators of mammalian serotonergic differentiation and are able to functionally substitute their worm counterparts. Finally, expression profile comparison shows that, from all C.elegans neurons, HSN is molecularly the closest to the mouse Raphe serotonergic neurons. Our results show a remarkable extent of homology in the specification and molecular profile of a critically important neuronal cell type in two species separated by over a billion years of evolution.
Genetic control of cerebral cortex expansion
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One of the most prominent features of the human brain is the fabulous size of the cerebral cortex and its intricate folding, both of which emerge during development. Cortical size is determined by the balance between progenitor cell self-renewal and neurogenesis. Cortical folding depends on the abundance of a particular type of basal progenitor, basal Radial Glia Cells (bRGCs), which abundantly populate a unique germinal layer, the Outer Subventricular Zone (OSVZ). Here I will discuss recent findings from my laboratory revealing novel cellular and genetic mechanisms that regulate cortical expansion and folding. In particular, we have identified an unprecedented mechanism of cortical development that underlies the embryonic emergence of the OSVZ. During a brief developmental period, apical progenitors in the ventricular zone generate a burst of bRGCs that become founders of the OSVZ. After closure of this period and for the remaining development, progenitors in the OSVZ follow a lineage completely independent from the other germinal layers. This brief time window is confined by the dynamic temporal regulation of genes key for bRGC formation, which hence determine the emergence of the OSVZ. Once the OSVZ is formed, the cortex folds in highly stereotyped patterns. We have identified unique transcriptional signatures along cortical germinal layers that map the prospective location of folds and fissures, including genes mutated in human cortical malformations. This map reflects mosaic expression patterns across the developing gyrencephalic cortex of ferret and human, but not the lissencephalic mouse, and may contribute to define cortical folds in gyrencephalic species.

MyT1 counteracts the neural progenitor program to promote vertebrate neurogenesis
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The generation of neurons from neural stem cells requires global changes in gene expression. These are controlled to a large extent by proneural transcription factors such as Ascl1, and include the repression of the Notch transcriptional program characteristic of neural progenitors. While recent studies have focused on characterizing the differentiation genes activated by proneural factors, less is known on the mechanisms that suppress progenitor cell identity. Here, we show that Ascl1 induces the transcription factor MyT1 while promoting neuronal differentiation. We combined functional studies of MyT1 during neurogenesis, with the characterization of its transcriptional program. MyT1 binding is associated with repression of gene transcription in neural progenitor cells. It promotes neuronal differentiation by counteracting Notch signaling at multiple levels, targeting Notch pathway components and many of its downstream targets. These include regulators of the neural progenitor program such as Hes1, Sox2, Id3 and Olig1. Thus, Ascl1 suppresses Notch signaling cell-autonomously via MyT1, coupling neuronal differentiation with repression of the progenitor program.
Cux1 enables inter-hemispheric connections of layer ii-iii neurons by regulating kv1-dependent firing
Nieto Marta, CNB-CSIC

Neuronal subtype specific transcription factors (TF) instruct key features of neuronal function and connectivity. Activity-dependent mechanisms also contribute to wiring and circuit assembly, but whether and how they relate to TF-directed neuronal differentiation is poorly investigated. Here we demonstrate that the TF Cux1 controls the formation of the layer II-III corpus callosum (CC) projections through the developmental transcriptional regulation of Kv1 voltage-dependent potassium channels and the resulting postnatal switch to a Kv1-dependent firing mode. Loss of Cux1 function led to a decrease in the expression of Kv1 transcripts, aberrant firing responses and selective loss of CC contralateral innervation. Firing and innervation were rescued by re-expression of Kv1 or postnatal reactivation of Cux1. Knocking-down Kv1 mimicked Cux1-mediated CC axonal loss. These findings reveal that activity-dependent processes are central bona fide components of neuronal TF-differentiation programs, and establish the importance of intrinsic firing modes in circuit assembly within the neocortex.

KEYNOTE LECTURE
Deconstruction and reconstruction of olfactory neuronal circuits in zebrafish
Rainer Friedrich, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

We use the olfactory system of zebrafish as a model to understand the organization and function of neuronal circuits involved in higher brain functions such as pattern classification. Exploiting the small size of the zebrafish brain, we measure neuronal activity patterns using multiphoton calcium imaging, manipulate neuronal activity using optogenetics, and reconstruct neuronal circuits using serial block face scanning electron microscopy (SBEM). Recently, we reconstructed all 1047 neurons in the olfactory bulb (OB) of a zebrafish larva, annotated most of their synapses and identified two new rare cell types. Interneurons form a network that is similar to the insect antennal lobe and corresponds to the juxtaglomerular network in adults while granule cells were essentially absent. Many interneurons had widespread projections that innervated multiple glomeruli. Inter-glomerular projections were neither random nor organized by an obvious topographical principle but depended on glomerular identity. In order to examine the function of this network we analyzed odor responses across the same neurons that were recorded by multiphoton calcium imaging before circuit reconstruction. Results provide strong evidence that specific inter-glomerular projections optimize representations of natural odorants and support their classification. Hence, the larval OB contains a “core circuitry” that appears to be established by innate mechanisms and performs important computations. These results show that “functional connectomics” is a powerful approach to obtain mechanistic insights into the development and function of complex neuronal circuits. Supported by the Novartis Research Foundation, Swiss Nationalfonds (SNF) and Human Frontiers Science Program (HFSP).
Poster sessions
Cytoskeletal rearrangements driven by a Notch-BMP2 signalling cascade underlie the emergence of the proepicardium in the zebrafish

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The proepicardium (PE) gives rise to the epicardial layer of the heart, and contributes to progenitor cells for the coronary vasculature and intracardiac fibroblasts. We analyze PE formation in the zebrafish to study this process in vivo with cellular resolution. During zebrafish embryonic development, PE cells arise as two groups of cells emerging from the dorsal pe- ricardium, one close to the venous pole of the embryonic heart tube and a second at the atrioventricular canal boundary region. It has been previously shown that PE formation is driven by BMP and Notch signaling, but the relation between both pathways has not been addressed yet. Moreover, we recently found that PE cell release and attachment to the myocardium is dependent on a beating heart: genetic inhibition of cardiac contraction or the addition of myosin inhibitors abrogates PE release. Interestingly, we found that the myosin inhibitor 2,3-Butanedione monoxime (BDM) reverted PE formation, in a process whereby PE cells get flattened and regress. Here we have studied how BMP and Notch signaling interact during PE formation and how these pathways relate to the effect of mechanical forces during PE formation. For this, we combined the use of genetic tools, drug treatments and advanced in vivo imaging to manipulate these signaling pathways, the heartbeat and the actomyosin cytoskeleton. We developed a method to quantify pericardial cell movements occurring prior to PE formation. Our results propose a model in which the coordinated action of BMP2 and Notch control pericardial cell movements, which are dependent on actomyosin rearrangements and allow PE emergence.

Genetic control of cell geometry in epithelia: the morphogenesis of the vertebrate optic cup as experimental paradigm

Buono Lorena1, Naranjo Silvia2, Moreno -Mármol Tania3, Bovolenta Paola1, Martinez-Morales Juan Ramon2

1CABD/CBMSO
2CABD
3CBMSO

The orchestration of the morphogenetic mechanisms involved in organ formation during embryogenesis and tissue homeostasis entails a precise genetic control of cellular shape. The development of complex organs requires coordination among molecular mechanisms specifying the identity of each cellular domain and downstream effectors. Using available tissue models, only few effector molecules have been identified and the role of many morphogenetic genes has not been explored. Furthermore, little is known about how these effector molecules integrate into broader developmental gene networks. Here we focus on the development of the optic cup as a model to identify key effector genes determining cell and tissue architecture in zebrafish. During development an undifferentiated mass of precursor cells from the neural plate, morphologically and molecularly indistinguishable, differentiates into the neural retina (NR) and the retinal-pigmented epithelium (RPE). In the frame of a few hours, the eye specification network bifurcates into mutually exclusive developmental programs for the NR and RPE and each domain displays a distinctive morphology. These differential cell geometries will condition the optic cup invagination. We have isolated these populations by FACS, to perform an RNAseq analysis of the bifurcating gene networks (GRNs) that establish the identity of these domains. By interrogating eye GRNs, we aim to identify downstream genes operating directly on basic cell morphological properties. This work will contribute to the identification and analysis of novel components of the machinery driving optic cup morphogenesis, many of which will be causative genes for the most common hereditary malformations of the eye.
4 Live analysis of MYC-regulated spontaneous cell competition in mouse ESCs
Díaz-Díaz Covadonga¹, Fernández-de-Manuel Laura¹, Jiménez-Carretero Daniel¹, Montoya María, Torres Miguel ¹

Cell competition is a cell-cell interaction in which cells compare each other’s anabolic ability and the less fit ones are eliminated from the tissue (loser) by the fitter cells that proliferate at their expense (winner). This mechanism is conserved in metazoans and has been proposed to maintain tissue quality and to promote organ regeneration through the elimination of suboptimal cells. During early mouse development pluripotent epiblast cells show heterogeneous MYC levels and anabolic activity, which gives rise to endogenous cell competition selecting for high-MYC cells. Mosaic MYC overexpression produces induced cell competition by generating “supercompetitor” cells able to outcompete their wild type neighbours. We are currently aiming to define the factors that determine MYC heterogeneity and the mechanisms leading to cell competition, both aspects essential to explore the role of cell competition during development and regeneration. Interestingly, we have characterized cell competition in cultured mES cells as a similar process to that described in epiblast cells and we are exploiting this culture system by using live imaging techniques to explore cell competition mechanisms and dynamics. On one hand, clonal analysis experiments in ESC showed that MYC is not only regulated by extracellular signals but also by intrinsic heritable determinants. In order to study deeper this MYC regulation and its role in cell competition we are currently using a mouse ESC line in which GFP reports MYC and the membrane is labelled with tdTomato. This tool allows us to make 24 hours time-lapse experiments and to process these 4D images by segmenting and tracking the cells during time. We have developed computer methods for the 4D tracking and multiparametric quantitative analysis of single cells and their neighbourhood. We verified the heritable condition of MYC and we were able to analyse cell size and MYC level fluctuations during cell cycle. Additionally, we are studying the interactions and MYC-level history of loser cells and its neighbours to understand how MYC level heterogeneity leads to cell competition. This powerful in vitro model gives us the opportunity to unravel MYC-dependent cell competition dynamics and can shed light on the factors that control this phenomenon in vivo.

5 A mathematical model of tissue growth based on progenitor-to-differentiated state transition
Sánchez-Aragón Máximo¹, Ramaekers Arianne², Almudi Isabel¹, Martín-Blanco Carlos¹, Hassan Bassem², Casares Fernando¹

Developing tissues are composed of cells that undergo an irreversible transition of gene regulatory states, from unspecified progenitors to precursors of final functional fates. This process is marked by a change in the cells’ proliferation potential which is tightly controlled by transcription factors belonging to specific gene regulatory networks (GRN), therefore determining the size of the mature organ. Here we present a simple mathematical analysis of tissue growth that allows to predict final organ size based on the speed of the cell state transition and on the cell proliferation rate. The model also predicts properties of the system, such as a) sensitivity to initial conditions of the parameters and b) the parameter subspace that ensures the successful termination without overgrowth. We tested the model by predicting the changes in parameters that would explain the size difference of the eyes of two Drosophila species, its predictions on the natural eye variations that occur between two species of Drosophila, D. melanogaster and D. pseudoobscura. Even though the initial size of the eye primordia in these two species differ, the model predicts that these size differences alone cannot explain the final sizes of the eyes without the readjustment of differentiation rates, something that we verified experimentally. This would indicate that quantitative differences in pathways involved in controlling the differentiation rate, such as those of Dpp/BMP2 or Hh would be involved in these inter-specific eye size differences. With this framework at hand we expect to identify quantitative effects on cellular parameters for several loss-of-function mutants affecting eye size, and from other dipteran species (i.e. Episyrphus balteatus) with varying eye sizes in the hope to restrict the scope of solutions for molecular models of gene regulation.
6  Does tension keep you in shape? Relevance of Drosophila melanogaster peripodial epithelium on final organ size
Villa-Fombuena Gema¹, Gómez-Gálvez Pedro², Escudero Luis M.², Casares Fernando¹
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How animal organs sense and control that they have reached their species specific target size is a longstanding biological question that hasn’t been fully addressed yet. Possible mechanisms have been proposed in which molecular concentrations vary throughout organ development and regulate the termination of the process. But during growth, organs not only experience chemical variations but also undergo mechanical stress. We are currently exploring the role tension may play regulating organ growth. Most of what is currently known about growth control mechanisms has been deciphered using the primordia of the adult organs in Drosophila, called imaginal discs. Drosophila imaginal discs are flat sacs of monolayered epithelium. Of the two epithelial sheets, the Peripodial epithelium (PE) plays an ancillary role, not giving rise to adult structures, but participating in the final eversion of the discs during metamorphosis. Therefore, the PE has a mechanical role. We have shown that PE cells accumulate thick acto-myosin bundles with an anterior-posterior orientation, indicative of a strong polarized tension. Therefore, the PE could exert some mechanical influence during disc growth. We will present data on the quantitative characterization of cell and tissue parameters (size, polarity, acto-myosin accumulation, neighbors distribution, cell connectivity, etc) of the PE during disc development, with the aim to infer global properties of the tissue. To relate cell/tissue properties with tension sensing, we will analyze how the subcellular localization of Yki, the co-transcriptional activator of the Hippo pathway and tension sensor, correlates with those properties.

7  Regulation of expression and functions of Hox complex by Meis transcription factors
Lopez-Delgado Alejandra Cristina¹, Cadenas-Rodriguez Vanessa C.¹, Torres Miguel¹
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Hox genes are key regulators of regional pattern formation along the antero-posterior and other embryonic axes. In most of the vertebrates, Hox genes are organized in four clusters and present spatial and temporal collinearity. The precise localization of Hox gene products is essential for the correct patterning of tissues; therefore the tight transcriptional regulation of the Hox genes is essential to the proper orchestration of embryonic morphogenesis. The activation of Hox genes starts in the posterior primitive streak during gastrulation. However, the regulation of this initiation is not very well known. We have observed that the TALE homeodomain transcription factor Meis2 is expressed in this region from embryonic day 7 (E7) up to E8. Moreover, previous results of the group indicate that Meis binding sites are abundant in Hox clusters, especially in HoxA cluster. Taken into account these results, Meis could be involved in the first activation of Hox genes during gastrulation. After that, we compared the pattern of Meis with different Hox genes and we observed that Meis2 coincides in space and time with HoxA1-6 genes during early gastrulation. In addition, Hox mutants present homeotic transformations that lead to a change in the vertebrate axial skeleton. The analysis of the axial skeleton in Meis mutants revealed a phenotype similar to HoxA4, HoxA5 and HoxA6. Meis mutant phenotype and the colocalization of Meis and Hox genes during their activation lead us to think in a possible role of Meis in the regulation of the expression of Hox genes by Meis.

8  Neurovascular interaction in the vertebrate inner ear and brain
Taberner Laura¹, Alsina Berta¹
¹Universitat Pompeu Fabra

The inner ear is one of the main neurosensory organs of the head, responsible for the sensing of hearing and balance. The Statoacoustic ganglion (SAG) is composed by bipolar afferent neurons that transmit acoustic and vestibular information acquired by mechanosensory hair cells in the inner ear to their corresponding nuclei in the hindbrain. Until now, efforts have focused primarily on the autonomous and intrinsic signals produced by the inner ear and the SAG itself to give rise to the proper generation, organization and innervation of this ganglion. However, it has poorly been considered the putative influence by other organs to the neuronal otic development. Our working hypothesis is that signals for the neuronal growth, differentiation, proliferation and migration could come from the adjacent vascular network. These two systems are found in close proximity in the inner ear, besides, there is a growing evidence of vessels to neurons signalling phenomenon in other systems’ development. First, the relation between the neurovascular system around the ear will be described in detail, then we will elucidate the influence of the vascular system in the inner ear and hindbrain neurogenesis. Preliminary results point to a role of vessels on neuron differentiation and axonal patterning.
9 **Mutations of genes involved in the early-eye morphogenesis in zebrafish**  
Undurraga Cristian¹, Letelier Joaquin¹, Vazquez-Marin Javier¹, Martinez-Morales Juan¹  
¹Centro Andaluz de Biología del Desarrollo, CABD/UPO/CSIC. Sevilla, Spain

The organogenesis of vertebrate eye is a process that requires a complex choreography between inductive signals, intrinsic genetic programs and morphogenetic movements. All these processes finally result in a three-dimensional organ comprising functionally specialized tissues. Two of these tissues are the neural retina (NR) and the retinal pigmented epithelium (RPE), which are specified in the developmental window comprised between the establishment of the eye field and the onset of neuronal differentiation. The vertebrate fish zebrafish (Danio rerio) and medaka (Oryzias latipes) are excellent models to observe the eye development at early stages also to do reverse genetic to investigate gene function. We performed on zebrafish a RNA-seq assay to identify genes that can act as direct morphogenetic effectors during early eye development. Using the latest technology CRISPR/Cas9, we selected 3 genes to generate loss of function models in zebrafish: rac3b, rab32a and lamtor3, and 2 genes to generate loss of function alleles both in zebrafish and medaka: supervillin-a and supervillin-b. For all these genes we have generated Indels mutations, and homozygous mutants lines are now available for rac3b, supervillin-a and supervillin-b. We did not observe any evident embryonic phenotype in these homozygous mutants. We are currently investigating which compensatory mechanisms may account for the apparently normal phenotypes observed.

10 **Analysis of YAP/TAZ-dependent transcriptional response during epithelial morphogenesis in teleost embryos**  
Vázquez-Marín Javier¹, Gutiérrez-Triana José Arturo², Letelier Joaquín¹, Buono Lorena¹, Miesfeld Joel B.³, Link Brian A.³, Mateo Juan L.², Wittbrodt Joachim², Martínez-Morales Juan Ramón¹  
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The Hippo signaling pathway is a genetic regulatory cascade that controls tissue homeostasis and organ size by coordinating the expression of proliferation, differentiation and apoptotic genes during development. The main constituent of this pathway is an effector complex, composed of paralog proteins YAP and TAZ. When YAP/TAZ is active, it gets into the cell nucleus and regulates the expression of its target genes interacting physically with TEAD, among other transcription factors. YAP/TAZ activity is regulated by many external factors, among which stand out mechanical cues. When the cell senses these signals, YAP/TAZ protein complex gets translocated into the nucleus where it plays its transcriptional role. During the gastrulation of teleosts, several morphogenetic movements including convergent-extension and cell involution direct the formation of the basic body plan. This complex choreography entitles the generation and transmission of mechanical tensions. In fact, our imaging results suggest a link between morphogenetic tension and nuclear translocation of YAP in gastrulating cells in zebrafish embryos. The aim of this project is to investigate the role that those mechanical cues play on the YAP/TAZ complex and to analyse its transcriptional outcome during gastrulation. To understand YAP/TAZ-dependent transcriptional response, we have analysed the binding of these factors to the genome using an iDamID-seq approach both in zebrafish and medaka embryos. The computational analysis of this data set, in combination with a RNA-seq transcriptomic profiling of mutant embryos for YAP/TAZ, will allow defining downstream targets controlled by the Hippo pathway in the morphogenetic context of a gastrulating embryo.
HoxD13 and its targets and the evolution of vertebrate limbs
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Fossil data suggest that limbs evolved from fish fins by sequential elaboration of the distal endoskeleton, giving rise to the autopod close to the tetrapod origin, with a simultaneous reduction of the distal ectodermal fold of fish fins. Interestingly we have shown that overexpression of hoxd13a during zebrafish fin development causes increased proliferation and distal expansion of chondrogenic tissue expressing markers characteristic of autopod development. Simultaneously it occurs a finfold reduction, a phenotype that reflects the morphological changes expected during fin evolution. In order to understand how hoxd13 contributed to the fin-to-limb formation in vertebrates it is important to understand its impact as a transcription factor in its downstream targets. Here 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. We are now conducting functional assays directed to bmp2b and its inhibitor nog3, which suggest that these genes were key for the diversification of fish fins.

Unraveling RPE morphogenesis and its influence in optic cup folding
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During eye morphogenesis the optic vesicle folds over itself and originates the optic cup. Concomitant to this process, eye progenitors become specified and differentiate into three cell subpopulations: the neural retina (NR), the optic stalk and the retinal pigment epithelium (RPE). In zebrafish, the RPE develops from a discrete group of cells in the dorso/proximal region of the optic vesicle. These cells, initially organized as a pseudostratified epithelium, undergo a dramatic change in shape, forming a flat epithelial monolayer that spreads over the apical surface of the NR. Besides for their different shape, RPE cells can be distinguished from NR cells by the expression of a different set of regulatory genes. Furthermore, RPE cells are thought to have a differential proliferation rate and to acquire specific biomechanical properties as compared to the NR. Here we will present the results of our current studies aimed at unraveling RPE morphogenesis and the influence that this tissue has on the folding of the optic cup. To this end, we have generated two transgenic lines that direct the expression of GFP or GAL4 specifically in the RPE under the regulation of a specific enhancer of the transcription factor bhlhe40. Using these lines and in vivo imaging, we followed RPE morphogenesis since its initial specification and altered the fate of RPE cells to assess the impact that these manipulations have on optic cup formation. In addition, we have knocked-down bhlhe40 expression in order to study its potential role in the eye morphogenesis.
13 Molecular and cellular mechanisms controlling cell rearrangement during development
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The process of organogenesis involves several morphogenetic mechanisms that are coordinated by gene networks controlling the different cell behaviours during development. Due to the complexity of this process we concentrate on the study of a simple epithelial organ, the posterior spiracles, of Drosophila melanogaster. This model allows us to study the cell shape changes and cell rearrangements that occur in its formation. The HOX gene Abd-B induces a gene network that coordinates in the 8th abdominal segment (A8) the formation of the two structures forming the posterior spiracles: the spiracular chamber and the stigmatophore. The stigmatophore is the only engrailed-expressing circumferential structure of the embryo. Here we studied how the early segmentation pattern that subdivides the embryo in identical stripes of cells evolves into a circumferential pattern mediated by Abd-B activation of the Spalt gene. Spalt re-specifies anterior compartment cells in the A8 segment, activating the polarity gene engrailed to create the circumferential structure. We have found this is achieved through several spiracle specific spalt and engrailed enhancers that respond to different signals transforming the stripe information into circumferential information. We propose that Spalt homogenizes the stigmatophore positional information by activating engrailed in both anterior and posterior compartment cells, providing all stigmatophore cells with a similar positional and morphogenetic behaviour. Therefore, this work is focused on finding out how spalt expression in the stigmatophore is activated and how spalt directs the cell rearrangement to create a circumferential structure.

14 Cell number and body size regulation
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The size of adult animals and their organs depends on the number and size of their cells. Cell number relies on the tight balance between cell death and cell proliferation. The control of cell size remains more controversial, but seems largely dependent on nutrient conditions. The molecular mechanisms underlying both processes and their tight relationship during adult development remain largely unknown, although its malfunction causes multiple human disorders as cancer. A reason for this gap is the lack of simple and suitable adult animal models. In contrast to most animal species, in which growth and nutritional intake becomes uncoupled when adult size is achieved, adult planarians continuously regulate body size in a nutrition-dependent manner, offering an ideal scenario to approach this question. Here we present two signaling molecules essential for the control of cell number and body size in planarians, which inhibition produces variations in cell number and, eventually, overgrowths. The first consists in a non-evolutionary-conserved peptide (Smed-bs) that attenuates cell proliferation and promotes cell death, with no effect on cell differentiation. Its inhibition results in planarians with an increase in the total cell number, although body size is normal because cell size is reduced. We are currently exploring whether nutrition supply could deactivate the mechanism that so tightly adjust body size. Finally, we have found that an evolutionary conserved pathway that controls growth in mammals also influences cell proliferation and death in planarians, although its main function is to enable cell differentiation.
Tracing the fate of yolk sac hemangioblasts in the chick embryo

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During vertebrate embryogenesis, there is a close developmental relationship between hematopoiesis and vasculogenesis. This observation led to the hypothesis that blood and endothelial lineages derive from a common precursor, known as the hemangioblast. More recently, hemangioblasts were shown to give rise to hematopoietic stem cells through a hemogenic endothelium intermediate. At early developmental stages, hemangioblasts are found in yolk sac blood islands, where primitive hematopoiesis takes place. In addition, yolk sac hemangioblasts give rise to a definitive population of tissue-resident macrophages as well as to the vast majority of adult microglia. Although still controversial, it has been proposed that yolk sac-derived cells may also seed other intraembryonic hematopoietic tissues, such as the hemogenic endothelium of the dorsal aorta. However, these studies had several limitations, such as the lack of a specific marker of yolk sac hematopoietic lineage or the lack of live imaging data. We have established a method for labeling and tracing the progeny of yolk sac hematopoietic precursors in living chick embryos using a novel hemangioblast-specific reporter. Using this lineage-tracing system, we can follow the migration of yolk sac hemangioblasts, identify their homing tissues, and reveal their contribution to intraembryonic hematopoiesis. Moreover, we are able to live image the generation of hematopoietic stem cells via endothelial-to-hematopoietic transition. Ultimately, these insights will not only further our knowledge on hematovascular development, but can potentially be applied toward the isolation and in vitro generation of hematopoietic progenitor cells for clinical purposes.

STAGER, staging tool for the analysis of expression results

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STAGER, Staging Tool for the Analysis of Expression Results: a supervised classifier based on Principal Component Analysis. We have developed a computational method that classifies samples based on gene expression data. Our method allows to pinpoint the molecular signature of new samples. In addition, allows for staging samples within developmental windows where morphological staging could not resolve. Our method has been proving to be an important tool in the context of developmental biology and chronobiology, where the assessment of developmental time of samples is crucial.

Sculpting cavities: the relevance of Lumen Initiation Foci (LIF) for lumen shape

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Defects in lumen morphogenesis underlie diseases in organs as the brain and the kidney. How a lumen initiates and grows has been studied in the last years; however how it acquires a particular shape is just starting to be elucidated. We recently have introduced the zebrafish inner ear as a model to study lumen morphogenesis (Nature communications 2015), and identified fluid- and mechanic-driven mechanisms that mediate lumen expansion. Similar to most epithelial lumens, the otic lumen initiates from small apical foci that hollow, grow and fuse. Here we evaluate the specific contribution of these LIF to the shape of the fused lumen. We present data showing that the position, the number and the fusion of the foci determine not only the size but also the shape of the expanded lumen. Formation of these foci is promoted by different FGFs, which explain luminal phenotypes observed in mutant embryos. Our results suggest that the early geometry of the LIF is used to give shape to epithelial lumens. We propose that LIF work as lumen building blocks, constituting the earliest fundamental units for lumen shaping.
Development of a new micropattern-based platform to study the role of cell adhesion in tubulogenesis

Bosch Minerva¹, Rodríguez Fraticelli Alejo², Delgado Barea María¹, Hachimi Mariam¹, Martín Belmonte Fernando¹

¹Centro de Biología Molecular Severo Ochoa

Tubulogenesis can occur through a variety of different processes. Morphogenesis of some epithelial tubes requires a first step of de novo lumen initiation, this lumen eventually expands and fuses to adjacent lumens to form a unique cavity along the whole tubular structure. We have developed a micropattern-based device that allows renal epithelial cells to grow in 3D and form tubes in vitro through a process that closely resembles in vivo tubulogenesis. This device, with the potential to recapitulate tubulogenesis in vitro, would emerge as a powerful tool to study the cellular and molecular mechanisms involved in organ tube formation. Besides, it would also provide a new platform for drug discovery and nephrotoxicity assays.

EVO-DEVO AND GENOMICS

Two recently evolved isoforms of the BRANCHED1a gene fine-tune the development of aerial and subterranean architecture in Solanum tuberosum

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The morphological evolution of plants and animals is driven by amplification and diversification of developmental genes. In the plant species Arabidopsis thaliana, the BRANCHED1 (BRC1) gene encodes a transcription factor of the TCP family that controls lateral branching patterns. One of the BRC1 paralogs in Solanum tuberosum (potato), StBRANCHED1a (StBRC1a), controls lateral stolon branching and tuber apical dominance as well as aerial lateral shoot outgrowth. We have shown that a new alternative splice site has evolved in this gene in the Solanum genus, which generates two transcripts. The two BRC1a encoded proteins, BRC1aLong and BRC1aShort, differ in their C-terminal regions. The BRC1aLong C-terminal region is a strong transcriptional activation domain that allows the protein isoform to act as transcription factor. The C-terminal region of the BRC1aShort protein is predicted to form an amphipathic helix that prevents protein nuclear localization. Heterodimerization of BRC1aShort and BRC1aLong leads to increased levels of BRC1aLong in the cytoplasm, and to reduced activity of this protein as a transcription factor. Indeed, the phenotypes of the over-expression of BRC1aLong and BRC1aShort resemble, respectively, BRC1a gain and loss of function. Finally, the ratio between the two alternative transcripts is regulated by physiological and environmental cues that affect branch outgrowth. This intronization event has led to the evolution of a new post-transcriptional and post-translational mechanism that regulates BRC1a activity and potato architecture.
**20 miR-15/16 family, a regulatory node for cell plasticity in search for a revised nomenclature**

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MicroRNAs (miRNAs) are small noncoding RNA molecules that regulate gene expression by pairing with the 3’ UTR of target mRNAs. The miR15/16 family is involved in cell differentiation, development and disease. Unpublished data from the lab, indicates that members of this family are downstream targets of transcription factors that induce epithelial-to-mesenchymal transition (EMT), suggesting that they act as regulators of cell plasticity. The current annotation of the miR15/16 sequences suggests the existence of many subfamilies, the presence and absence of distinctive members in equivalent loci in different species, and some other not very parsimonious assignments. Our objective has been to study the emergence, evolution and phylogenetic relationships of the miR15/16 family to provide a robust nomenclature and evolutionary history that can help us correctly design functional studies of EMT and cell plasticity in embryos and cancer cells. Our results show that i) the miR15/16 family members classification is simpler than that suggested by the current annotation, ii) most clusters are widely conserved among vertebrates, iii) there are potential precursor miRNAs in organisms where miRNAs have not yet been identified and iv) synteny and phylogenetic information on stem-loop sequences should be used for the classification and nomenclature of miRNAs. We propose a revised nomenclature for the miR15/16 family that simplifies the existing one and truly reflects the phylogenetic relationships among the miRNAs, helping in the identification of orthologs and paralogs to better design and interpret intra- and interspecies functional studies.

**21 Regulation of early mammalian embryo development through alternative splicing**

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A number of crucial events take place during the early stages of mammalian development. These include embryonic genome activation, segregation of the extra-embryonic lineages that will support embryo development and formation of the three germ layers that will go on to form all embryo tissues. Understanding how these early events are regulated is a key question in the embryology and stem cell fields. Alternative splicing (AS) is the process by which different exons are selected in precursor mRNAs to generate multiple protein products. Recent studies have shown that 95% of human multiexonic genes undergo AS, however to date no study has addressed its role in modulating early embryo development. We have used ultra-deep coverage RNA-sequencing data from three mammalian species to identify more than a thousand alternative exons that show dynamic usage during early developmental stages. Many of these exons are located in genes with an important function during early development and/or pluripotency, highlighting the impact that AS can have at these early stages. Using the CRISPR-Cas9 technology we are currently generating exon specific knockouts both in stem cells and mouse embryos in order to assess the functional effect that isoform switching has during early embryo development. Altogether our results show for the first time the impact that AS has on early embryo transcriptome remodelling, and contribute to the understanding of the regulatory networks controlling mammalian development and pluripotency.
22 Characterization of potential sources of extrinsic influences on cortical progenitors in gyrencephalic brains

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Cerebral cortex development depends critically on the regulation of progenitor cell proliferation and output. Extrinsic factors have been proposed to play key roles in this regulation, but the timing and extent of interaction between extrinsic elements and each class of cortical progenitor remain undefined. Here we have searched for extrinsic elements that may modulate cortical progenitor cell proliferation by analyzing the spatial-temporal overlap of germinal layers and progenitor subtypes with early neuronal and axonal populations during ferret development. We show that multiple sets of migrating neurons and axon tracts, including thalamocortical and corticocortical, overlap extensively with subdivisions of Inner and Outer Subventricular Zones, in an exquisite lamina-specific pattern. Given that apical and basal Radial Glial Cells (RGCs) extend radial processes intersecting multiple cortical layers, we propose a bar code model for extrinsic progenitor cell regulation. According to this model, subpopulations of RGCs and Intermediate Progenitors may interact at close range with a variety of extrinsic cells and processes, depending on their germinal layer of residence and the span of their processes across cortical layers. Our findings provide a cellular substrate for the feed-back influence of both intra- and extra-cortical neurons onto progenitor cells to modulate their dynamics and fate decisions.

23 Oct4 is a key regulator of vertebrate trunk length diversity

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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 into 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.
24 Attenuation of Robo signaling promotes cerebral cortex expansion in evolution

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The olfactory bulb (OB) develops as a unique specialization of the rostral pallium (OB primordium) from early stages of development. Here we show that the distinction between OB and neocortical (NCx) development starts at the onset of neurogenesis, with changes in progenitor cell cycle parameters such as cycle lengthening and increased cycle exit, producing within a short developmental period a prominent amount of neurons. We find that these changes in progenitor cell dynamics involve mainly apical progenitors, and are linked to an equally important accumulation of newborn pallial neurons within the VZ of the OB primordium, virtually absent in the neocortex. Time-course and time-lapse analyses of progenitor cell lineages demonstrate that these differences between OB and NCx are preceded by significant changes in progenitor cell dynamics, mostly the abundant occurrence of direct neurogenesis from apical Radial Glia Cells (aRGCs). We show that direct neurogenesis is nearly anecdotic in the neocortex, where the majority of neurons are born from intermediate progenitor cells through indirect neurogenesis. We also identify Robo1 and Robo2 receptors as key molecules regulating the balance between direct and indirect neurogenesis. We show that Robos are highly expressed in aRGCs of the OB but not NCx, and in their absence OB neurogenesis and growth are impaired. Finally, we demonstrate that this regulation of direct vs indirect neurogenesis by Robo1/2 requires the cooperation with the Notch signaling pathway, and that this molecular mechanism of regulation is conserved along the evolution.

25 A comprehensive pipeline for identifying lincRNAs on the basal-branching chordate amphioxus

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Among the numerous classes of RNAs, long intergenic non coding RNAs (lncRNAs) are similar in terms of expression and gene structure to the mRNAs but lack the potential to encode proteins. Over the last years, lncRNAs have been proven to play important roles in gene regulation, and have shown to be involved in many key developmental processes. However, the low sequence conservation of lncRNAs has hindered the identification of deep orthologues among distantly related species, hence the evolutionary dynamics of lncRNAs has been scarcely studied, and few data are known at evolutionary key-nodes of animal evolution. We aim to identify the lncRNA complement in the cephalocordate amphioxus, the best proxy to the key evolutionary node of the origin of chordates and vertebrates. For this, we used strand specific RNA-seq data from several adult tissues and developmental stages of Branchiostoma lanceolatum. We used first the CPAT software in order to assess the coding potential of each canonical transcript, then a selection of probably non coding, multiexonic gene structures (at least 2 exons) and a minimum length of 300 nucleotides. Transcripts were blasted using blastx against the non-redundant protein database, and the ones that did not had a significant hit were filtered again with hmmmer searches to eliminate the ones with similarity to conserved protein domains upon 6-frame translation. This yielded around 1700 transcripts that were classified according to their relative position among coding genes into intergenic, antisense, intragenic or overlapping.
26 Evolution of developmental gene complexes in Drosophila: What can we learn?

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Developmental gene complexes are often conserved in metazoans. This conservation has been interpreted as necessary for the correct regulation of their genes. However, developmental gene complexes usually contain highly related genes originated by gene duplication. The question arises as to which of these two factors (functional requirement or origin by tandem duplication) is responsible for their existence. We previously showed that the Hox gene complex (HOM-C) in Drosophila is not necessary for proper function and only the result of phylogenetic inertia. But is this the rule or the exception? Here we reexamine this case and look into five more developmental gene complexes in Drosophila to shed light on this question. We conclude that two types of developmental gene complexes exist in Drosophila. The first, which includes HOM-C, IRO-C and NK-C, is formed by large genes regulated by polycomb. These genes do not need to be clustered but their regulation by polycomb favors their proximity in the nucleus and indirectly their proximity in the chromosome (clustering). The second, which includes AS-C, E(spl)-C and Brd-C, is formed by small, mostly intronless, genes involved in Notch signaling. The latter complexes include a large proportion of genes exclusive to the Brachycera. Its relative new origin may explain, in part, the high conservation of these complexes. Overall we think that the existence of gene complexes is due to their origin by tandem duplication. However, their highly regulated expression constraints the dispersal of the genes and makes it slower than for non-developmental genes.

27 Origins and regulation of an eutherian novelty: the BGW cluster

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Two related gene subfamilies known as BEX and TCEAL (also known as WEX) map to a genomic region specific to Eutheria (placental mammals), located on the X chromosome. These families are part of a gene cluster, named BGW cluster, together with the ARMCX family and HNRNPH2. Some of the BEX/TCEAL genes have been related to control the balance between proliferation and differentiation, while others promote apoptosis in a p75-dependent manner, but most of them remain poorly studied.

The ARMCX family and HNRNPH2 are derived from retrocopies of the ARMC10 and HNRNPH1 genes respectively conserved across bilateria, and located in autosomal chromosomes, whereas no orthologs have been found for the BEX/TCEAL family outside of Eutheria. However, all these genes share an intriguing feature: a sequence motif in their proximal promoter region that appears to be crucial for their expression, the BGW motif. To further understand the evolution of this gene cluster, we investigated the origin of the BEX/TCEAL genes and traced it to an atypical formation in the ancestor of eutherians. Furthermore, novel features associated with BEX/TCEAL suggest a more complete scenario for the origin of the cluster: the BGW motif was already present at the HNRNPH2 locus in the ancestor of therian mammals, being subsequently duplicated and coopted in the eutherian lineage by the BEX/TCEAL ancestor and, posteriorly, by the ARMCX ancestral gene. Finally, we also studied the expression of the BEX/TCEAL genes during mouse development using in situ hybridization. We found that they are highly expressed in the brain and placenta, which are structures that require a well-tuned control of cell cycle during their development in eutherian mammals.

Here we propose a scenario for the origin of the BEX/TCEAL family and for the formation of the BGW cluster where they belong. Their uncommon origin, their pattern of expression, and their putative biological function during development make these genes an interesting subject of study to understand how lineage-specific genes could contribute to mammalian evolution.
28 3D chromatin organisation and enhancer-promoter specificity

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Myogenic regulatory factors (MRFs) control the determination, specification and differentiation of skeletal muscle during development. Two of these factors, Myf5 and Mrf4, are closely linked in the genome of all vertebrates analyzed. Their transcriptional regulation relies on multiple enhancers that are intermingled and scattered throughout the locus. In this work, we try to unveil the mechanisms by which precise long-range interactions between enhancers and promoters are established; interactions that change during development and differentiation in order to allow the genes to be accurately expressed according to their highly specific spatiotemporal expression pattern. We hypothesize the existence of a novel type of regulatory element named TRABS (transcriptional balancing sequences) which, together with the promoters and enhancers of the locus, create a series of equilibria states that ensure the establishment of the correct enhancer-promoter regulatory interactions. To reveal the three-dimensional organization of the locus, 4C experiments were carried out in the muscle-derived cell line C2C12 both in growing and differentiating conditions, as well in embryos at different developmental stages and genotypes (including KOs for Mrf4 or Myf5 promoters and TRABS). The integration of all the generated datasets would improve our understanding of how this locus is organized and regulated during the formation of skeletal muscle throughout development.

29 The evolution of xenacoelmorph nervous systems (a case of evolutionary centralization)

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Xenacoelomorph is a monophyletic animal group that includes three clades: Acoela, Nemertodermatida and Xenoturbellida. The group still has contentious phylogenetic affinities; though most authors place it as the sister group of the remaining bilaterians, some would include it as a fourth phylum within the Deuterostomia. Over the last few years, our group, along with others, has undertaken a systematic study of the microscopic anatomy of these worms; our main aim is to understand the structure and development of the nervous system. This research plan has been aided by the use of molecular/developmental tools, the most important of which has been the sequencing of the complete genomes and transcriptomes of different members of these clades. A major focus of our research is the origin of “cephalized” (centralized) nervous systems. How complex brains (ours being just a particular example) are assembled from simpler neuronal arrays has been a matter of intense debate for at least a hundred years. We are now tackling this issue using Xenacoelomorpha models. These represent an ideal system for this work, since the members of the three clades have nervous systems showing different degrees of cephalization; from the relatively simple subepithelial net of Xenoturbella to the compact brain of acoels.
30 Deconstructing the genetic toolkit for heart development in a chordate
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Recent increase in genomic data reveals that gene losses are abundant in metazoans. Little is known, however, about how gene loss can impact the evolution of the mechanisms of development. As a case study, we investigate how gene losses affected the cardiogenic toolkit in the chordate Oikopleura dioica. After the first description of the heart in 1903 by Saliensky, our work provides the first modern atlas of its development and describes the cell lineage fate map of all cardiac progenitors up to tailbud stage. Our data reveals that cardiac precursor cells derive from the most anterior muscular cells and migrate from the tail into the trunk, very similar as in ascidians. In O. dioica, however, precursor cells finally migrate and fuse to form the heart primordium in the left side of the animal, rather than in the midline as in ascidians. Our exhaustive in silico survey for all cardiogenic factors conserved in other chordates reveals important differences in O. dioica regarding its early signaling pathways as well as cardiac transcription factors involved in migration, differentiation and cardiogenesis. Thus, our work reveals that despite the highly similar process of early heart development between O. dioica and ascidians, the former appears to have pushed its mechanisms of heart development to their functional limits, deconstructing its cardiac genetic toolkit with several gene losses, absence of cardiac expression and lack of action of developmental signaling pathways that are fundamental to make a heart in other chordates.

31 Muscle development and tail elongation in Oikopleura dioica, a chordate evolutionary knockout for retinoic acid signaling
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A characteristic feature of vertebrates and cephalochordates is an obvious segmented body plan made of repetitive muscular units that develop by a complex process of somitogenesis. Retinoic acid (RA) and Fgf are two of the main signaling pathways that regulate the temporal and spatial formation of somites in an anterior-to-posterior direction. In urochordates, however, tail muscle consists of an array of muscle cells, but no obvious structural somites are observed. The evolutionary origin of somitogenesis remains unknown, and whether the formation of the array of muscle cells of urochordates shares some of the signaling pathways that underlie somitogenesis remains unclear. To address this issue, our group investigates the mechanisms of development of the tail muscle in Oikopleura dioica, a larvacean urochordate species that does not suffer a drastic metamorphosis as ascidians and preserve their tail throughout their entire life. We have performed an exhaustive in silico survey to identify several muscular gene markers, many of which have suffered extensive gene duplications during larvacean evolution. Comprehensive analyses of their expression patterns revealed an unexpected anterior-posterior molecular regionalization that correlated with their cell lineage origin. We are now investigating the role of Fgf signaling in muscle development and tail elongation in the absence of RA, a signaling pathway that has been lost during O. dioica evolution due to the high propensity of this species to lose genes.
Identification of pathogenic bacteria in pepper seedlings

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Capsicum annuum L. is considered as an important part of the diet of great part of the Latin-American population given its high nutritional value. The quality of this food is affected by the presence of pests and diseases, basically bacteria when this plant grows under greenhouse conditions. Based on the above, this research was conducted at the Universidad Autónoma de Chihuahua and the Center of Research in Nutrition and Development of Delicias, Chihuahua, Mexico in order to identify the pathogen causing this disorder. From diseased seedlings with leaf spots of jalapeño chili, ten bacteria were purified and submitted to the Koch postulates in order to verify their pathogenicity; only five of them resulted pathogenic which were characterized by biochemical methods and were identified later with genetic-molecular methods. The 16SrRNA gene was amplified through PCR using the specific 8F and 1492R primers. The PCR products were sequenced and compared through the Blast on the GeneBank of the National Center for Biotechnology Information (NCBI). A phylogenetic tree was constructed with the obtained sequence of 788bp and others deposited in the NCBI, which was possible to determine that Pseudomonas fluorescens is the bacterium responsible for the disease in the chili pepper plants established under greenhouse conditions in Delicias, Chihuahua, México. The sequence of this bacterium has been deposited in the GeneBank under the Access Number JF739251. Keywords: Phytopathogenic bacteria, jalapeño chili, sequencing, NCBI.

Influence of Notch on the development and fate of the hemogenic endothelium using mouse genetic mosaics

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Hematopoietic stem progenitor cells (HSPCs) arise from the hemogenic endothelium of the dorsal aorta (DA) between E9.5-E11.5 during mouse embryonic development. Through a process known as Endothelial-to-hematopoietic transition (EHT), some endothelial cells (ECs) will up-regulate markers such as ckit and bud into the dorsal lumen to then form hematopoietic clusters. The Notch pathway is proposed to regulate initiation of EHT and cell fate yet current studies lack single-cell resolution of HSPCs in vivo. Our aim is to study the role of Notch in mouse HSPC development without altering gene expression in the whole tissue of the embryo. By using a mosaic inducible mouse model, we can 1) visualize and track fate of HSPCs with different levels of Notch and 2) observe how single cells behave in the same environmental context. We show that up-regulation of Notch in dorsal ECs results in a decrease in total HSPC number. We found that HSPCs overexpressing NICD+ have less cells per cluster as compared to neighboring WT clusters. Furthermore, they exhibit decreased ckit levels compared to WT cells. This suggests that Notch blocks differentiation or proliferation of HSPCs in the DA.
Integrins positively regulate cell survival in the wing imaginal disc in Drosophila melanogaster

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Integrins are a widely expressed family of transmembrane receptors that binds preferentially extracellular matrix components. Integrins can provide mechanical links and activate different pathways, thus regulating various cellular processes, including cell survival. In fact, disruption of integrin function results in a class of apoptosis called anoikis. Although there is a lot of information about the role of integrins in promoting cell survival in cell culture experiments, little is known about its role during morphogenesis. In this study we aim to better understand the role of integrins in regulating cell survival during development using Drosophila wing imaginal disc as a model system. We show that loss of integrin function in the wing disc results in caspase dependent anoikis and cell extrusion. Removal of integrins results in ectopic activation of JNK pathway, which in turn leads to an increase in the expression of the proapoptotic gene hid. These results suggest that integrins regulate cell survival by inhibiting hid expression through downregulation of JNK pathway. In addition, loss of integrin function results in an increase in myosin tension which per se has shown to induce cell death and extrusion. Moreover, blocking cell death in a lack of integrin function context does not prevent cell extrusion. Given these results we would like to propose that elimination of integrin function results in an increase in tension and cellular extrusion that precludes cell death. Thus, in a physiological context integrins would act as mechanosensors and could regulate cell survival in part by controlling cellular tension.

Role of integrins in epithelial architecture in Drosophila ovary

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Tissue invasion could be caused by loss of proper epithelial architecture. When this happens, multi-layered epithelia are formed, increasing the invasive potential of a tissue and hyperplasia. Recently, mechanical properties of tumor cells have emerged as important factors during invasion. In our lab, we are interested in the processes that control epithelial formation and maintenance. We use the monolayer follicular epithelium (FE) of the Drosophila ovary as a model system. Integrins are main cell surface receptors connecting the extracellular matrix to the cell’s cytoskeleton. Elimination of integrins from the FE results in multi-layered epithelia. The aim of this study is to identify the mechanisms by which integrins maintain epithelial integrity. By laser-ablation experiments, STED and in vivo analysis, we show that integrin mutant cells exhibit an increase in cellular tension and F-actin levels. In addition, integrin mutant cells show ectopic localization of the myosin light chain (sqh) at their basal membrane. Furthermore, increasing one copy of sqh enhances the loss-of-integrin phenotype, creating malformations in the FE and invasion into the germline. The Hpo pathway has recently emerged as a novel mechanosensor and its elimination from the FE also causes multilayer. Interestingly, we find that in the FE loss of integrins or induced F-actin formation, by overactivation of actin nucleation factors, results in downregulation of the Hpo pathway. Our progress in trying to understand the concerted roles of F-actin regulators, Hippo and integrin signalling pathways in controlling cellular tension in the FE, critical to preserve its monolayered structure, will be discussed.
36 Coelomic epithelium-derived cells migrate into the developing pancreas and contribute to the stellate cell population in mice
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The embryonic mesothelium lining the visceral organs gives rise to mesenchymal cells through a localized epithelial-mesenchymal transition (EMT). This has been extensively studied in some cases, such as the heart, where the epicardium gives rise to epicardial-derived cells that contribute to the cardiac vascular and connective tissues. In other organs, such as the lungs, liver and gut, the developmental fate of the mesothelial-derived mesenchyme and their importance for visceral morphogenesis has also been demonstrated (reviewed in Ariza et al., Dev Dyn, 2016, 245:307-22). In normal adult pancreas, the pancreatic stellate cells (PSCs) are quiescent, star-shaped cells with a periacinar distribution. They contain vitamin A-containing lipid droplets in the cytoplasm and exhibit positive immunostaining for desmin and glial fibrillary acidic protein). When activated by profibrogenic stimuli such as inflammatory cytokines or oxidant stress, PSCs transform into myofibroblast-like cells. Thus, PSCs are the major source of extracellular matrix in the adult pancreas but their embryonic origin remains unknown. PSCs are similar to hepatic stellate cells (HSCs), located in the perisinusoidal space of the liver. These cells share many features with PSCs and they are also involved in the fibrotic process. It has been described that the liver mesothelium contributes to the HSCs and vascular endothelium during development (Jpennen et al. Dev Biol, 2007, 312: 157-170; Asahna et al., Hepatology, 2011, 53:983-95). Thus we checked was a similar developmental origin accounts for PSCs using Wt1 (Wilms’ tumor suppressor gene) as a coelomic mesothelium lineage marker. We have used two lines of transgenic mice for lineage tracing, systemic (Wt1Cre; R26REYFP) and tamoxifen-inducible (Wt1ERT2; R26REYFP). Our results confirm that WT1 protein is only expressed in the mesothelium of the developing pancreas, allowing for tracing of the mesothelial-derived cells with the Cre/LoxP system. During the early stages of pancreas morphogenesis, its mesothelium shows the typical features of EMT. Mesothelial-derived cells, identified by YFP expression, differentiate into a part of the PSCs and contribute to other connective and vascular cell types. Thus, mesothelial-derived cells originated by EMT seem to constitute an important subpopulation of mesodermal cells during pancreas development, contributing to its morphogenesis.

37 Expression of the Wilms tumor suppressor gene (Wt1) in a subpopulation of embryonic cardiomyocytes is required for cardiac development
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The Wt1 gene encodes a zinc-finger transcription factor that is expressed during development in the coelomic epithelium and other mesodermal domains. Wt1 is required for spleen, liver, and genitourinary development. In the heart, Wt1 plays a critical role in the development of the epicardium controlling the epithelial-mesenchymal transition that originates epicardial-derived cells. These cells contribute to the connective tissue and the vascularization of the heart, and in this way Wt1 is also involved in cardiac development. We have also checked if Wt1 is expressed in the embryonic myocardium. Using transgenic mice lines for both, Wt1 reporter expression and lineage tracing (Wt1tm1Nhsn and mWt1/IRES/GFPCre; ROSA26REYFP), we have detected a small population of cardiomyocytes expressing Wt1 in early developmental stages (E8.5-E9.5). These cardiomyocytes were mainly located in the inflow tract, but some of them were observed in the ventricles. We confirmed Wt1 expression by RT-PCR in hearts before the attachment of proepicardial cells, when the cardiac tube is only constituted of myocardium and endocardium. We also confirmed expression of Wt1 in ventricular cardiomyocytes from human fetuses by RT-PCR and Western Blot. The lineage of the Wt1-expressing myocardium expanded in later stages and formed patches of cardiomyocytes in the ventricles (interventricular septum and free walls). atrium and sinus venosus. The conditional deletion of Wt1 in cardiac troponin T-expressing cells (cTnTCre;Wt1f/f) was lethal for the embryos. The mutant hearts showed myocardial defects in the interventricular septum and sometimes also in the free ventricular walls. We have observed that part of the epicardial cells also expressed cardiac troponin-T, and this could explain part of the lethality of the conditional deletion of Wt1. However, we never found myocardial defects when Wt1 was conditionally deleted in the epicardium using an epicardial-specific driver. Thus, we conclude that WT1 is expressed in a subpopulation of early embryonic cardiomyocytes, and this expression seems to be essential for the correct development of the heart.
38 Overexpression of Hif2a in mouse hepatic stellate cells induces liver fibrosis
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Liver fibrosis is a disease with a high incidence and causes 2% of global mortality. Therefore, it is crucial to identify biomarkers and potential targets in order to improve the diagnosis and treatment of this disease. Hepatic stellate cells (HSCs) play a major role in the induction and progression of liver fibrosis. Upon liver damage, HSCs acquire a proliferative state and myofibroblastic morphology, which is accompanied by the expression of extracellular matrix proteins (ECM), such as laminin and collagen. The massive accumulation of these proteins disturbs the architecture of the liver, resulting in metabolic insufficiency. Previous studies have shown that the reversion of active HSCs to an inactive state is crucial to regress the progression of the fibrosis. Accordingly, it is important to elucidate the mechanisms leading the activation/inactivation of the HSCs. As a first approach, we are studying the role of hypoxia inducible transcription factor 2 (HIF-2) in the activation of HSCs and in liver fibrosis. Using Cre/loxP technology, we are evaluating the effect of Hif-2 overexpression in mouse HSCs. Our results show that the overexpression of Hif-2 in mouse HSCs results in embryonic lethality at midgestation, likely due to hematopoiesis defects. Histological and immunofluorescence analyses of the embryonic livers overexpressing Hif-2 show an activation of HSCs and accumulation of ECM. Conclusions: Our data indicates that Hif-2 induces HSCs activation and therefore the induction of liver fibrosis. Based on these results, Hif-2 might be a potential target for therapeutic strategies in liver fibrosis.

39 The role of SNBPs in the differential susceptibility to genotoxic damage within the sperm nucleus
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The chromatin of mammalian spermatozoa is configured in three structural domains: toroids (protamine-bounded DNA: PDNA), nucleosomes (DNA wrapping histones: HDNA) and MAR regions (nuclear matrix attached DNA). This conformation supports the hypothesis of a differential sensitivity to genotoxic damage according to the association to the sperm nuclear basic proteins (SNBPs). Our group analyzed species with different SNBPs, showing in trout, homogeneously compacted with protamines, a dissimilar vulnerability amongst genes which was dependent on the tested genotoxic agent, discarding the relation with SNBPs. To go further, we have analyzed in human and zebrafish sperm (exclusively with HDNA) the number of lesions in 7 genes located in the human PDNA or HDNA promoted by UV irradiation, H2O2 and cryopreservation. Global DNA fragmentation was analyzed by TUNEL and 8-OHdG, histone H3 and topoisomerase IIα (TOPOIIα) were visualized by immunocytochemistry. UV irradiation caused the higher number of lesions homogeneously distributed in both species, whereas H2O2 and cryopreservation promoted a differential sensitivity amongst genes. After oxidative stress, 8-OHdG remained in a peripheral region of the zebrafish nucleus, as described in trout spermatozoa, and was confined in a well-defined subequatorial ring of the human sperm nucleus. Moreover 8-OHdG did not co-localize either with TOPOIIα or with H3. Our results do not support the initial hypothesis, strengthening that positional effects are dependent on the genotoxic mechanisms, rather than on the DNA associated SNBPs. Supported by the Spanish Ministry of Economy and Competitiveness (AGL2014-53167-C3-3-R), Junta de Castilla y León (Spain) (EDU/1083/2013) and Fondo Social Europeo.
**40 Scratch factors in the neural stem cell niche**
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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 germinial 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 neural plasticity of olfactory information processing. The balance between self-renewal and differentiation of adult NSCs in this niche is tightly controlled. A group of transcription factors that might regulate this process in mammals is 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 expression level is higher in the latter cell type. In addition, Scratch1 mRNA presents different subcellular distributions between these cell types, showing a canonical cytoplasmic distribution in neuroblasts, whereas its mRNA seems to accumulate in specific regions of the nucleus in NSCs. This differential subcellular distribution suggests that Scratch1 mRNA export might be selectively regulated in this neurogenic niche. Considering the role of this gene in promoting neuronal differentiation, selective Scratch1 mRNA export might constitute a mechanism involved in the generation of new neurons in the adult brain.

**41 WIP (WASP Interacting Protein) drives tumor progression through YAP/TAZ-dependent autonomous cell growth**
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In cancer, the deregulation of growth signaling pathways drives changes in the cell’s architecture and its environment that allow autonomous growth of tumors. These cells then acquire a tumor-initiating “stemness” phenotype responsible for disease advancement to more aggressive stages. Here we show that levels of the actin cytoskeleton-associated protein WIP (WASP-interacting protein) correlate with tumor growth, both of which are linked to the tumor-initiating cell phenotype. We found that, via a mechanism mediated by the endocytic/endosomal system, WIP controls tumor growth by boosting signals that stabilize the YAP/TAZ complex. When WIP levels are high, the APC-axin-GSK3-beta-catenin destruction complex is sequestered to the multivesicular body compartment, where its capacity to degrade YAP/TAZ is inhibited. WIP maintenance of YAP/TAZ stability is Rac/PAK- and mDia-dependent, and Hippo-independent. This close biochemical relationship indicates an unusual oncogenic role for WIP in the physiology of cancer pathology by increasing YAP/TAZ stability.
Optimization of adoptive cell transfer therapy through the combination of magnetic targeting and p85 down-regulation
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Adoptive cell transfer therapy is currently one of the most promising approaches to treat cancer. This therapy uses cancer patients own lymphoid cells with anti-tumour activity, expanded in vitro and reinfused into the patient. One of the limitations in adoptive cell transfer therapies is the dispersion of in vivo-administered lymphoid cells such that only a small proportion reaches the tumour. There is a clear need to develop new strategies to promote specific cell accumulation and infiltration in the tumour microenvironment so that they can exert their function effectively. Nanotechnology has proven very useful in selectively directing drugs or molecules to a target site. Previous studies in our laboratory show that superparamagnetic iron oxide nanoparticles (SPIONs) loaded with biomolecules can be specifically targeted to tumours using an external magnetic field. Our goal is to analyse in mouse models whether lymphoid cells could be associated with SPIONs prior transfer therapy so that they could be directed and accumulated to the tumour through an external magnetic field. Furthermore, we have shown that p85 deficiency enhances NK cell activity through increased IFN production and more effective degranulation after NKG2D activation. These data suggest p85 as a therapeutic target to boost NK cell activity toward NKG2D ligand-expressing tumours. Combination of magnetic targeting and p85 down-regulation could ensure that a larger number of more active NK or T cells reaches the tumour, leading to more efficient treatment and a better clinical outcome.

SCOX partial loss of function causes mitochondrial disruption and skeletal muscle wasting in drosophila melanogaster
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Respiratory chain complex IV malfunction causes a broad spectrum of syndromes with several clinical presentations. They can affect single or multiple organs, being cardiac dysfunction one of the most frequent symptoms. Mutations in COX assembly factors are responsible for the majority of these rare human diseases. Pathogenic mutations in SCO1 and SCO2 COX assembly factors cause hypertrophic cardiomyopathy among other symptoms. Nevertheless, the molecular mechanisms underlying cardiac dysfunction remain virtually unknown. We have previously shown that cardiac-specific scox knockdown in Drosophila causes severe dilated cardiomyopathy, glycolysis up-regulation, increased ROS levels and dp53-dependent cell death as fatal outcome. Since different tissues rely on distinct responses to cope with mitochondrial dysfunction, in order to test the tissue-specificity of the cardiac response to loss of SCOX function, we have developed a flight muscle-specific scox knockdown Drosophila model. We show that IFM specific scox knockdown causes a reduction of COX activity and, as it occurs in the heart, muscle fibre disruption and degeneration. Besides, mitochondrial structure is disturbed, with the organelle losing its prismatic shape. However, overall phenotype is quite different; metabolic state does not display significant permanent changes and muscle degeneration occurs in a much slower manner than in the heart. Furthermore, vesicular-like structures, reminiscent of mitophagy vesicles, are observed in scox KD IFM. We speculate that, in response to mitochondrial dysfunction and in an attempt to maintain cellular homeostasis, mitophagy is upregulated together with mitochondrial biogenesis. However, the maintenance of mitochondrial dysfunction causes a mitophagy/mitogenesis imbalance that, in turn, leads to apoptosis and muscle wasting.
44 Autophagy and cathepsins are activated with programmed cell death in stress-induced microspore embryogenesis and pollen development

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Autophagy, the degradation pathway that recycles cell materials upon stress conditions or during development, can act with with a survival role and also in both developmental and stress-induced programmed cell death (PCD). In vivo, the microspore follows the gametophytic program to form the pollen grain, with the nursing function of the tapetum. In vitro, upon stress treatments, the microspore can be deviated towards embryogenesis to produce double-haploid plants for breeding; the efficiency of this process is limited by cell death. In this work we have studied the activation and possible involvement of autophagy in two PCD processes: PCD in stress-induced microspore embryogenesis and developmental PCD of tapetum, in Hordeum vulgare and Brassica napus. In tapetal cells at early PCD, Atg5 and Atg8 were up-regulated and localized in autophagic structures and vacuoles, cathepsins L, B, H and caspase 3 enzymatic activities highly increased. Ultrastructural analysis revealed numerous autophagosomes and vacuoles in cytoplasms. Microspore cultures, after the stress treatment to induce embryogenesis, showed high levels of cell death, ROS production and increased cathepsins L, B, H and caspase 3 activities; concomitantly, autophagosomes, also increased. Inhibition of autophagy by 3-MA reduced autophagosome formation, while proteases inhibition by E-64 leads autophagosomes to accumulate, both inhibitors reduced cell death and increased embryogenesis induction rate. Taken together, results indicate that autophagy is induced at early developmental PCD in tapetum and stress-treated microspores and suggest a role for autophagy in plant PCD. Supported by project AGL2014-52028-R funded by MINECO and the European Regional Development Fund (ERDF/FEDER).

45 Sea urchin sperm express cannabinoid receptors in mitochondrial region and flagellum

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Introduction. Cannabinoids exert their actions by binding to specific membrane receptors, cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2). CB1 and CB2 distribution are highly diverse in nature. In this sense, the non-mammalian models, like sea urchin (Arbacia lixula), have become of interest because they provide relevant data about the evolution of cannabinoid receptors and their role in the reproduction. In this sense, the cannabinoids effect in the fusion of sea urchin egg and sperm has been reported. However, topographical distribution of cannabinoid receptors is not known. Thus, the aim of this study was to determine the subcellular localization of CB1 and CB2 in sea urchin sperm. Methods. Sperm samples were collected by intracoelomic injection of KCl solution. Sperm suspensions were fixed and prepared for confocal microscopy, field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). The cannabinoid receptors immunolocalization was performed with the primary antibody directed against CB1 or CB2; and a fluorescent-conjugated secondary antibody to confocal microscopy, or gold-conjugated secondary antibody to FESEM and TEM. Results and Conclusions. Immunoreactivity study confirmed the presence of both cannabinoid receptors in sea urchin sperm. Confocal analysis revealed CB1 and CB2 an intense immunofluorescence labeling in the mitochondrial region and a faint labeling in the flagellum. This cannabinoid receptors distribution was confirmed through gold immunolabeling, which was analyzed by FESEM and TEM. These data suggest that sea urchin sperm express both types of cannabinoid receptors, and their subcellular localization is confined to the mitochondrial region and the sperm flagellum.
46 Functional requirements of Cubilin orthologues in Drosophila nephrocytes
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Drosophila nephrocytes are very specialized cells with a high endocytic activity which function is to filter and regulate the haemolymph composition. Their surface is completely covered by the labyrinthine channels, which are invaginations of the plasma membrane where most of the endocytic function of these cells occurs. The entrance of these channels is capped by filtration diaphragms that limit the entrance of molecules to the channels according to their size. It has been shown that these diaphragms are molecularly and functionally similar to the slit diaphragms of the vertebrate kidneys (¹). In addition, recently it has been proposed that nephrocytes might also fulfil some of the function of the renal proximal tubules, since they express and require two proteins that are orthologues of vertebrate Cubilin (Cubn) and Amnionless (Amn), involved in protein reabsorption at the proximal tubules of the kidney (²). We found that Drosophila has two cubn orthologues both expressed in nephrocytes. We have induced novel mutations for both genes and found a specific requirement in nephrocytes. In these mutants nephrocyte endocytosis is profoundly affected and the distribution of filtration diaphragms is aberrant. Similar phenotypes were obtained in amn mutants. Based in our data a possible function for these receptors in nephrocytes will be presented.


47 Silencing caveolin-1 or clathrin inhibits TGF-β-induced anti-apoptotic signals in liver cells
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In hepatocytes, the Transforming Growth Factor-beta (TGF-β) is an important tumour suppressor factor, inhibiting proliferation and inducing cell death. However, TGF-β also regulates other processes that contribute to the progression of fibrosis and cancer. In proliferative hepatocytes and HCC cells, TGF-β can act with a pro- and anti-apoptotic role. Through Smads-phosphorilation plays its pro-apoptotic role regulating growth inhibition and apoptosis. Nonetheless, TGF-β can also transactivate the EGFR pathway which through its signals is involved in the anti-apoptotic role regulating proliferation and survival. In these sense, it has been emphasized the essential role of the TGF-β-receptors’ trafficking in the control of the activity and termination of signalling events. However, little is known about whether alterations of these endocytic pathways occur in liver fibrosis or tumorigenesis. TGF-β-receptors can internalize via at least two distinct internalization routes: via cholesterol-rich membrane microdomain lipid rafts/caveolae, and via clathrin-coated vesicles. It is described that lipid rafts/caveolae are indicated to facilitate the degradation of TGF-β-receptors and therefore turn off signaling, whereas clathrin-mediated endocytosis does not play a significant role in the TGF-β-induced early signals. We describe that caveolin-1 regulates TGF-β-anti-apoptotic signals through EGFR transactivation. Caveolin-1 establishes a signaling platform that permits the activation of the metalloprotease TACE/ADAM-17, in charge of the EGFR ligands shedding. In the other hand, blocking clathrin-trafficking does not alter TGF-β-induced Smads-phosphorylation. However, in these conditions, EGFR signaling pathway is impaired and the pro-apoptotic pathways is induced. These results show the relevance of receptors’ trafficking in the control of signaling pathway induced by TGF-β and further analysis must be done.
Modeling coenzyme Q10 deficiency-associated phenotype using patient-specific IPS cells

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CoQ10 plays a crucial role in mitochondria as an electron shuttle within the mitochondrial respiratory chain (MRC) and is an essential antioxidant. Mutations in genes responsible for CoQ10 biosynthesis (COQ genes) cause primary CoQ10 deficiency which is a rare and heterogeneous mitochondrial disorder with no clear genotype-phenotype association, mainly affecting tissues with high-energy demand including brain and skeletal muscle (SKM). Here, a 4-year old girl with modest mental retardation and lethal rhabdomyolysis was diagnosed harboring a heterozygotic mutation (c.483G>C (E161D)) in COQ4. Patient’s fibroblasts are functionally impaired and showed a strong reduction in [CoQ10], CoQ10 biosynthesis and MRC dysfunction affecting complexes I/II+III. Bona fide iPSCs carrying the COQ4 mutation (CQ4-iPSC) were generated, characterized and genetically edited using CRISPR-Cas9 system (CQ4ed-iPSC). Extensive differentiation and metabolic assays comparing control-iPSCs, CQ4-iPSCs and CQ4ed-iPSCs evidenced a genotype-phenotype association, faithfully reproducing the disease features. Similar to patient’s symptoms, COQ4 mutation in iPSCs was associated with CoQ10 deficiency, metabolic dysfunction and impaired differentiation into SKM; however, COQ4 mutation did not impair iPSC differentiation into either dopaminergic or motor neurons. This study offers an unprecedented iPSC model recapitulating CoQ10 deficiency-associated functional and metabolic phenotypes caused by COQ4 mutation.

In microspore reprogramming to embryogenesis auxin biosynthesis and transport are induced while they decrease during gametophytic development

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Microspores follow the gametophytic pathway to form pollen grains. However, isolated microspores are reprogrammed in vitro by stress, becoming totipotent cells and producing doubled-haploid embryos and plants via microspore embryogenesis. Limited information is available regarding the dynamics of auxin during these two microspore pathways. This work involved the analysis of auxin concentration and cellular accumulation; expression of TAA1, NIT2 and PIN1-like auxin biosynthesis and efflux carrier genes during the two microspore developmental pathways in Brassica napus and Hordeum vulgare. Effects of inhibition of auxin transport and action by N-1-naphthylphthalamic acid (NPA) and α-(p-Chlorophenoxy) isobutyric acid (PCIB) in microspore embryogenesis were also analyzed. Results indicated de novo auxin synthesis after microspore reprogramming and embryogenesis initiation and its increase during embryogenesis, correlating with expression of TAA1, NIT2 and PIN1. Inhibition of polar auxin transport (PAT) and action, by NPA and PCIB, impaired embryo development, indicating that PAT and auxin action are required for microspore embryo progression. In contrast, auxin levels, TAA1 and PIN1 expression were high at early microspore development, in tetrads and tapetum, while they progressive decreased during development in both pollen and tapetum cells. Findings indicate different auxin dynamics in the two microspore pathways with different fates. Endogenous auxin biosynthesis, action and polar transport are required for microspore embryogenesis initiation and progression while auxin progressively diminishes during gametophytic development. Rodríguez-Sanz et al. (2015) Plant Cell Phys. 56, 1401-1417 Supported by project AGL2014-52028-R funded by Spanish MINECO and European Regional Development Fund (ERDF/FEDER).
50 TGF-β-induced partial EMT offers to hepatocellular carcinoma the highest advantage for a migratory stemness phenotype


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Objective: The Transforming Growth Factor-beta (TGF-β) induces epithelial-mesenchymal transition (EMT) in hepatocellular carcinoma (HCC) cells. Recent studies indicate that EMT and stemness are cross-related processes. Here we have analyzed whether TGF-β may regulate the stemness properties of HCC cells and which is its relation with the EMT phenotype.

Design: Two epithelial HCC cell lines were chronically treated with TGF-β. In vivo analysis was performed in a model of diethylnitrosamine (DEN)-induced hepatocarcinogenesis in mice. Samples from HCC patients (n=38, tumor and surrounding tissue) were collected. EMT and stem-related gene expression was analyzed by Real-Time PCR, immunocytochemistry and/or flow cytometry. Colony- and sphere-forming assays were performed to explore the stemness properties of the cells.

Results: Long-term treatment with TGF-β in the Hep3B cells (with a mixed epithelial-mesenchymal phenotype) induces EMT, which switches stem-cell gene expression from epithelial EpCAM and CD133, to mesenchymal CD44. Cells acquire slight advantage in stemness properties, but a much higher enhanced ability to migrate and invade. Long-term treatment with TGF-β in the epithelial PLC/PRF/5 cells induces a partial EMT, maintenance of EpCAM and CD133 expression, and increased expression of CD44. Although they don’t acquire advantage in stemness, which is maximal in these cells, they enhance their migratory potential. Finally, from the analysis of gene expression both in DEN-induced hepatocarcinogenesis in mice and in tumors from HCC patients the most frequent gene signature coincident with up-regulation of TGF-β expression is a partial EMT, with increased expression of mesenchymal genes, maintenance of EpCAM and CD133 and up-regulation of CD44 expression.

Conclusion: TGF-β may induce a partial EMT in HCC, which would allow the simultaneous expression of epithelial and mesenchymal stem-related genes, as well a higher migratory and invasive capacity.

51 Melatonin effects on glucose metabolism of cancer cells

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Melatonin is an indolamine mainly produced in the pineal gland, although it is synthesized locally in many organs. This hormone induces cytotoxicity or inhibits proliferation of different cancer cell types, and this is associated with an increase or a decrease in reactive oxygen species, respectively. Intracellular oxidants originate mainly from oxidative metabolism, and cancer cells frequently show alterations in this metabolic pathway, such as the Warburg effect. Thus, we hypothesize that differential effects of melatonin could be due to different actions on metabolism. Accordingly, we initially evaluate basal energy metabolism of different tumor types using phenotypic microarrays, noting that one of the cell lines (Ewing Sarcoma) shows a typical tumor metabolism (Warburg effect) while the other one (chondrosarcoma) obtains energy mainly through the mitochondrial pathway. Melatonin treatment induces a strong decrease in the ability of Ewing Sarcoma cells to use Krebs cycle compounds as energy source while in chondrosarcoma cells leads to the opposite effect. We show that melatonin treatment could be making a switch between Warburg effect and oxidative metabolism or vice versa. Focusing on chondrosarcoma cells, this possible switch was confirmed though the activation of the master glycolysis regulator, HIF-1α after melatonin treatment. We observed a reduction of mitochondrial function. Parallel to these results, we also observed a decrease in intracellular calcium levels that could also be involved in the reduction of mitochondrial function. These data correspond well to the increase of cells in G0 / G1 phase of the cell cycle after melatonin treatment.
**52 BMP9: A novel regulator of oval cell function during chronic liver injury. Crosstalk with the HGF/c-Met pathway**

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Oval cells (OCs) constitute a bi-potential progenitor cell population from adult liver. Under chronic liver disease (CLD), they proliferate and differentiate into mature parenchymal cells to compensate for the cellular loss contributing to maintain liver homeostasis. BMP9 has recently emerged as a critical regulator of liver pathology therefore we decided to explore its potential role in the regulation of OC function. For this, WT and BMP9-KO mice were submitted to 0.1% DDC-supplemented diet as a model of liver damage associated with OC expansion. We found a greater expansion of the oval cell population in BMP9-KO mice respect to WT in response to the DDC diet, which suggests that BMP9 plays a negative role in OC-mediated liver regeneration. BMP9 suppressor effects were confirmed in vitro, as BMP9 decreases oval cell number and induces apoptosis. It is well known the critical pro-regenerative activity of the HGF/c-Met pathway in the liver. Using a model of OC lines harboring a genetically inactivated HGF receptor (c-Met) tyrosine kinase (Met-/- cells) and its control (Metflx/flx cells) we found that HGF/c-Met signaling inhibits BMP9 suppressor effects while potentiating BMP9-triggered Smad activation. We searched for mechanisms mediating the BMP9/HGF crosstalk and we found that ALK1 expression, a TGF-b type I receptor, is up-regulated in response to BMP9 and HGF. Furthermore, ALK1 knockdown abolishes both the HGF-mediated amplification of BMP9-triggered Smad signaling and HGF protective effects. In conclusion, BMP9 emerges as a novel regulator of OC population exerting suppressor effects. We also provide novel evidence of an interesting signaling crosstalk between BMP9 and HGF/c-Met pathways in OC regulation during CLD.

**53 Potentiation by melatonin of gemcitabine effect on mutant FLT3-ITD acute myeloid leukemia cells**

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Acute myeloid leukemia (AML) is a clonal disorder of the myeloid line of blood cells characterized by accumulation of immature blast cells in the bone marrow and peripheral blood that interferes with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age. It is a heterogeneous disease at both cytogenetic and molecular levels. Many of these molecular abnormalities, like FMS-like receptor tyrosine kinase Internal Tandem Duplication (Fit3-ITD), are currently used as biomarkers of poor prognostic and clinical outcome. The therapy of AML has largely remained unchanged from the standard cytarabine plus anthracycline. Although, it should be noted the current chemotherapy treatment presents a high toxicity with known side effects on normal dividing cells and a poor disease free survival due to development of tumor cell resistance to the compounds. Therefore, development of new therapeutic strategies to nullify tumor chemoresistance is necessary. Melatonin is an indolamine without relevant side effects that has been recently shown to present proapoptotic properties in hematological malignancies. In the present work we study the possible synergistic effect of gemcitabine in combination with melatonin in MOLM-13 (Fit3-ITD) and HL60 (wild type) leukemia cells. We have found that combination presents a synergistic cytotoxic effect in mutant MOLM-13 cells which is not appreciated in wild type HL-60 cells. Taken together, our results open the doors for a new promising approach to the treatment of AML, decreasing drug doses and so its toxicity.
54 Identification of conserved MEL-28/ELYS domains with essential roles in nuclear assembly and chromosome segregation

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Nucleoporins are the constituents of nuclear pore complexes (NPCs) and are essential regulators of nucleocytoplasmic transport, gene expression and genome stability. The nucleoporin MEL-28/ELYS plays a critical role in post-mitotic NPC reassembly through recruitment of the NUP107-160 subcomplex and is required for correct segregation of mitotic chromosomes. MEL-28 has a dynamic behavior: it localizes to nuclear pore complexes and chromatin during interphase and shuttles to kinetochores during cell division. However, it is unknown how MEL-28 localization and activity is regulated. Here we present a systematic functional and structural analysis of MEL-28 in C. elegans early development and human ELYS in cultured cells. We have identified functional domains responsible for NPC and kinetochore localization, chromatin binding, mitotic spindle matrix association and chromosome segregation. Surprisingly, we found that perturbations to MEL-28’s conserved AT-hook domain do not affect MEL-28 localization although they disrupt MEL-28 function and delay cell cycle progression in a DNA damage checkpoint-dependent manner. Our analyses also uncover a novel meiotic role of MEL-28. Together, these results show that MEL-28 has conserved structural domains that are essential for its fundamental roles in NPC assembly and chromosome segregation. To understand the function of MEL-28 chromatin binding we have used DamID to identify the chromatin regions with which MEL-28 associates. Interestingly, MEL-28 is enriched at transcribed genes and correlates positively with active histone marks, suggesting that it may be involved in regulation of gene expression. We compared the MEL-28 chromatin profile with the profile of another nucleoporin, NPP-22, which is permanently anchored to the nuclear pore complex. Surprisingly, we found that the chromatin association profile of NPP-22 was more similar to the profile of the nuclear lamina protein LMN-1 than to MEL-28’s profile, suggesting that individual NPPs interact with specific chromatin domains. Finally, GO-term analysis reveals that MEL-28-associated genes are related to larval and reproductive development. This suggests that MEL-28 has postembryonic functions that have not yet been studied.

SIGNALLING IN DEVELOPMENT & DISEASE

55 A connection between male abdominal segment elimination and the genitalia rotation

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Hox genes determine the development of different structures along the anteroposterior axis in bilaterians. The Hox gene Abdominal-B (Abd-B) of Drosophila melanogaster determines, during pupal development two processes; i) the suppression of the posterior abdominal segments, the larval A8 (in both sexes) and the imaginal A7 (only in males), and ii) the rotation of the male genitalia. Cells of the male A7 undergo extrusion under the control of Abd-B and the sexual determination pathway, requiring also the activity of genes like extramacrochetae (emc) and the down-regulation of the EGFR and Wnt pathways. As to male genitalia rotation, Abd-B regulates, in the male genital disc, myoID expression, a key molecule in determining circumrotation. Therefore, Abd-B controls both segment elimination and the genitalia rotation. Moreover, both processes overlap in time and may have arisen in a coordinated manner in the evolution of the Diptera. We have found that reducing emc expression or activating the EGFR pathway allows a small male A7 to develop, but also prevents full genitalia rotation. Since emc codes for a HLH protein that acts by forming inactive heterodimers with other HLH proteins, we are searching for Emc partners involved in such events. We also are investigating how the interaction of three different cell types (polytene larval cells, histoblasts and genital disc cells) allows or determines genitalia rotation. Our preliminary data suggest a coordination of mechanisms to shape the posterior region of the fly under Abd-B control.
The activation and regulation of differentiation programs in stem cells is a fundamental process during animal development and regeneration, required for proper tissue and organ formation and maintenance. Freshwater planarians are an excellent model to study the behavior of stem cells in vivo as they possess a population of pluripotent stem cells called neoblasts. Neoblasts are a heterogeneous population containing many different lineage-specific progenitors, identified by the expression of particular transcription factors. However, little is known about how these lineage-committed neoblasts differentiate into mature cell types. The EGFR signaling pathway has been shown to play an important role during key steps of cell differentiation and organogenesis in all studied model systems. Previous studies have showed that Smed-egfr-1 is required for pharynx and eye pigment cells regeneration and maintenance. Here we show that egfr-1, which is expressed in the digestive system, is additionally essential for correct gut regeneration and homeostasis, as Smed-egfr-1(RNAi) animals fail to regenerate new gut branches and dramatically lose the pre-existing ones during homeostasis. Moreover, their gut has a very reduced lumen, aberrant tissue organization, and significantly less gastrodermal cells. Importantly, the loss of gut cells in Smed-egfr-1(RNAi) animals is not due to an increase in apoptotic levels in the gastrodermis, suggesting that the gut-associated phenotype is likely caused by defects in gut cell differentiation. This is further corroborated by the reduced number of newly differentiated gut EdU+ cells after silencing Smed-egfr-1. Remarkably, double labeling indicates that Smed-egfr-1 is co-expressed with the gut progenitor markers gata4/5/6 and hnf4 in the mesenchyme around the gut. After silencing Smed-egfr-1 the number of hnf4- and gata4/5/6-positive cells increases in the mesenchyme. Overall, our results indicate that the defects observed in the regeneration and maintenance of the gut could be caused by the failure of those progenitors to differentiate into mature gut cells. Therefore, the EGFR pathway would have a key role regulating the differentiation of gut cells from their specialized progenitors. This work reports for the first time the role of the EGFR signaling pathway in the differentiation of planarian neoblasts.

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. By studying Meis1 and Meis2 conditional knock-out we also propose that Meis genes are determinant for limb antero-posterior (A-P) patterning from the early pre-patterning to Shh induction. Meis1Het; Meis2 KO limbs showed lost of posterior digits and reduction or lost of the posterior element of the zeugopod. In these mutants Shh expression is very reduced or lost. Meis1;2 KO do not develop limbs. At E10.5 these limbs did not show Shh expression. Moreover, the A-P pre-patterning was disturbed. In a wild type situation HOXD9 induces Hand2 posteriorly, which restricts GLI3 to the anterior region. The first A-P domains of the limb are then established. Meis1;2 KO forelimb (FL) showed reduced Hoxd9 in the flank, leading to the subsequent failure in Hand2 induction and extension of GlI3 through the whole A-P axis. Hand2 misexpression results also in the absence of Shh. In the hindlimb (HL) however, Hand2 is expressed through the whole A-P axis and GlI3 is not expressed. Therefore, in the HL the absence of MEIS would be, somehow, interfering with the restriction of HAND2 to the posterior region. Therefore, Meis genes play a role in limb A-P patterning and may act through different mechanisms in FL and HL.
58 **Tbx5 genes in early zebrafish development**

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Tbx5 is expressed in the developing heart, eyes and anterior appendages. Mutations in human TBX5 cause Holt-Oram syndrome, a condition characterized by heart and upper limb malformations. Tbx5-knockout mouse embryos have severely impaired forelimb and heart morphogenesis from the earliest stages of their development. However, zebrafish embryos with compromised tbx5 function show a complete absence of pectoral fins, while heart development is disturbed at significantly later developmental stages and eye development remains to be thoroughly analyzed. We identified a novel tbx5 gene in zebrafish-tbx5b- that is coexpressed with its parologue, tbx5a, in the developing eye and heart and hypothesized that functional redundancy could be occurring in these organs in embryos with impaired tbx5a function. We have investigated the consequences of tbx5a and/or tbx5b downregulation in zebrafish to reveal that tbx5 genes have essential roles in the establishment of cardiac laterality, dorsoventral retina axis organization and pectoral fin development. Our data show that distinct relationships between tbx5 paralogues are required in a tissue-specific manner to ensure the proper morphogenesis of the three organs in which they are expressed. Furthermore, we uncover a novel role for tbx5 genes in the establishment of correct heart asymmetry in zebrafish embryos and analyze the consequences of tbx5a/b morphants in left-right asymmetry establishment during development.

59 **Canoe loss synergistically interacts with scribble mutants to promote tumor growth via ras activation**

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Over the past years an intriguing connection between asymmetric cell division (ACD), stem cells and cancer biology has emerged. Stem cells divide asymmetrically to give rise to one daughter cell that inherits the self-renewal potential of the mother cell and another daughter cell that is committed to differentiate. We previously showed that the PDZ protein Canoe (Cno) regulates ACD in embryonic neuroblasts (NBs), the neural stem cells of Drosophila CNS. In this work, we wondered whether the loss of cno in asymmetrically dividing NBs would lead to tumor-like overproliferation. We show that Cno is expressed in NBs and Intermediate Progenitors of Drosophila larval brain type II NB lineages (NBII). cno NBII mutant clones display ACD defects, reduced NB size and abnormal morphology and cellular composition. Despite these failures, cno mutant clones do not overgrow. Additionally, we have found that NBII mutant clones of scribble (scrib), a well-known tumor suppressor gene, do not overgrow either. Conversely, most scrib mutant clones die. However, cno, scrib double mutant clones exhibit a tumor-like overgrowth through both Ras activation and a severe disruption of ACD. Moreover, cno also synergistically interacts with scrib to promote overgrowth in epithelia by activating the Ras-MAPK pathway. While the scrib functionally related genes discs large and lethal (2) giant larvae do not show such a synergism with cno, they contribute to repress Ras-MAPK signaling in imaginal discs. Hence, our work uncovers novel cooperative interactions between all these tumor suppressors to ensure a tight regulation of the Ras signaling pathway.
60 Epigenotoxic effects of bisphenol A impair heart development
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Bisphenol A (BPA) is an intermediate in the production of epoxy resins. Its range of toxicity results from their multiplies effects being those involving the cardiovascular system one of the main health concerns since high levels of this toxic in urine are linked to cardiovascular diseases. BPA is an endocrine disruptor which potentially interferes with estrogen, androgen and thyroid hormone receptors, all of them involved in cardiomyogenesis. In addition, epigenotoxic effects of BPA have also been reported, providing a possible explanation for the transgenerational inheritance of heart defects. The aim of our work was to analyze the changes in the epigenetic profile promoted by BPA in germinal cells (PGCs) and cardiac precursors using zebrafish. Embryo were exposed to BPA (100, 2000 and 4000 µg/L) during 24 hours after fertilization. To evaluate embryo phenotype a histological analysis was performed. Global DNA methylation (5mC) and histone acetylation (H3AcK9) were analyzed by whole mount immunofluorescence in PGCs and cardiac precursors at different stages of development using vasa and cmlc2 as lineage markers. Moreover, expression of genes crucial for differentiation of cardiac progenitors (nkx2.5, gata5), heart tube formation (has2, hand2) and looping and ballooning (bmp4, tnt2a) was assessed. Results showed a significant increase in the rate of cardiac edema and heart failures of exposed embryos, an altered expression of gata5 and bmp4 as well as significant changes in the epigenetic profile in early embryos. The observed decrease of 5mC could be linked to an altered expression of dnmt3, whose mutants display a similar phenotype.

61 Uncovering novel therapeutic targets for p63-related hereditary malformations
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P63 transcription factor plays an important role during epidermis and neural crest derivatives formation. Mutations affecting its coding sequence or its target genes lead to the development of malformations mainly characterized by cleft palate, ectodermal dysplasia and limb malformation. To date, the search of genetic events underlying these malformations has been mainly focused on exonic regions. However, impairment on the transcriptional regulation of genes related to these phenotypes might cause similar diseases. The major problem of the studies focused on transcription factors binding sites is the difficulty to associate a certain regulatory region with its target gene. In general, this association is solely established having into account the proximity between these elements, which might lead to wrong assignments and waste of effort and resources in deeply studying the incorrect genes. To overcome this problem, we designed an approach based in binding sites and chromatin 3D interactions, which will allow us to properly associate transcriptional regulatory elements with their target genes. First, we performed a ChIP-seq in zebrafish to find p63 binding sites, and then 4C-seq experiments centered in some of these putative regulatory regions to uncover their regulatory landscape. The integration of these data will provide us a list of genes laying in those regions, which could potentially belong to the p63 genetic network and be involved in p63-related diseases.
62 Patterning-Hox interaction during organogenesis: regulation of the Drosophila Six3 gene Optix during wing and haltere development
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During development, cells integrate information concerning their position and identity. This information includes a HOX code that defines position along the head-to-tail body axis, and morphogens, which convey positional information at smaller scales. The result is the expression of specific sets of genes at defined locations within an organ. Especially interesting is the regulation of transcription factors, as these encode gene expression regulators themselves. Here we investigate the expression and function of the optix gene, which encodes a Six3-type transcription factor. In addition to its role during the development of visual structures, we find that optix is expressed in the developing wing and haltere, two serially homologous organs whose difference depends on the haltere-specific expression of the HOX gene Ubx. In wing and haltere discs optix is expressed with similar, but not identical patterns. Attenuating optix function generates also distinct phenotypes: while in the wing the area anterior to the vein2 is severely reduced, the loss of optix causes the ectopic development of bristles in the haltere with no effect on its size. To understand how the domains of optix are differentially positioned within developing wings and halteres we analyzed optix expression relative to the signaling domain of Dpp, a major morphogen, and when Dpp signaling is reduced or abrogated. In all, these experiments indicate that both in the wing and haltere, Dpp signaling acts as a repressor of optix expression, even though this repression may be exerted through different, organ-specific mechanisms.

63 Dechipering the regulatory transcriptional network controlling Drosophila wing disc regeneration
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Most organisms possess some ability to repair and regenerate damaged tissues, however while some can generate whole body parts or entire limbs others can only superficially seal wounds or restore small patches of tissue. Successful regeneration processes demand a hierarchical and well-controlled balance between proliferation, differentiation and metabolic functions, which are mostly orchestrated by signaling molecules and transcriptional regulation. Although similar gene networks participate in development and regeneration, there are differences in the intensity of the signals or the levels of transcription. The ultimate goal of our research group is to understand how transcription is regulated during development and regeneration using Drosophila wing imaginal discs, epithelia that develop adult structures and that is able to regenerate upon an injury or cell death. We have used a genetic approach to study regeneration which consists in genetic activation of apoptosis, followed by RNA-Seq and ATAC-Seq analyses at different times after induction of damage. Then we have correlated expression data with chromatin accessibility and we have applied motif discovery tools to search for transcription factors that could bind to the identified regulatory regions. We have identified genes that are induced or repressed upon cell- death induction and we have characterized several putative enhancer regions that may mediate the regulation of genes following damage.
Segment identity changes during imaginal disc regeneration
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One major question in regenerative biology is how determined cells can acquire pluripotency to reconstruct missing parts of the organism. Drosophila imaginal discs are a good model to study this process. Drosophila segments are specified early in the development by Hox genes. It has been reported that cells from the anterior compartment of the wing disc can transgress the A/P boundary, change fate and contribute to the regeneration of the posterior compartment, but it is unknown if Hox gene expression also changes during regenerative processes. To address this question we have studied regeneration in segments or compartments with a Hox expression different from that of adjacent cells. We have inflicted massive cell death in: a) the anterior and posterior compartments of bithorax and postbithorax haltere discs, in which the Hox gene Ultrabithorax is expressed just in one compartment, or b) the analia primordium (A10) of the genital disc, which expresses caudal and is adjacent to the A9 of the same disc, which expresses the Hox gene Abdominal-B. By lineage tracing we have followed the descendants of the damaged cells and analyzed Hox gene expression after regeneration. We have observed in the mutant haltere discs that cells may cross the boundary and gain or lose Ultrabithorax expression; similarly, cells from the A9 segment (expressing Abdominal-B) can cross to the A10 segment and gain caudal expression. Our results suggest that cells from one segment that contribute to the regeneration can acquire the Hox address of damaged cells in an adjacent segment.

Translating a static signaling source into a dynamic patterning process: the role of Hh signaling in the differentiation of Drosophila ocellus
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The function of sensory organs relies on the precise patterning of their cellular components. This patterning is controlled by secreted signaling molecules, called morphogens. Molecules of the Hedgehog (Hh) family are key conserved morphogens with a prominent role in the patterning of neural and sensory structures. In order to understand in detail how Hh carries out this function, we have resorted to the Drosophila ocelli. Each of the three ocelli is a simple eye, formed by a retina capped with a large lens. The specification and size of the ocellar retina is controlled by a static Hh source that generates a signaling gradient. Further, we have found that differentiation of retinal photoreceptors (PRs) proceeds as a wave, moving away from the Hh source. So, how does a static Hh source direct a differentiation wave? Our results suggest that the wave can be split mechanistically in two independent but linked waves. The first one is a proneural wave (that can be monitored by Sens expression) that is controlled by the Hh gradient. The second one transforms the proneural cells into PRs, and is driven by EGF. We find evidence that this second wave is further regulated by a counter-gradient, likely of Wnt signaling. In order to understand the dynamics of these complex interactions we are simultaneously developing a mathematical model. Since the Hh, EGF and Wnt signaling pathways are evolutionarily conserved and broadly used during development, the model we are developing may reveal general properties of these pathways and their integration.
66 Mosaic levels of Notch activity are necessary for regulated endothelial cell proliferation during vascular development

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Blood vessel formation through angiogenesis requires the temporal and spatial coordination of sprouting, proliferation and maturation of endothelial cells to generate a functional vascular network. During this process VEGF and Notch signaling pathways have been shown to control endothelial cell proliferation and organize the migration of new sprouts with the generation of more cells to form the vessel wall. However the cellular and molecular mechanisms that regulate endothelial cell proliferation are still poorly defined. We show the pharmacological inhibition of endothelial Notch signaling during angiogenesis causes a speeding of cell cycle and disorganized endothelial cell expansion. This in turn induces early cell cycle exit and endothelial cell exhaustion. Cell mosaic experiments indicated that this exhaustion leads to endothelial cell with reduced Notch activity being outcompeted by normal endothelial cells. In turn cells with higher Notch activity are also outcompeted due to blockade of cell cycle transitions. Inducible single-cell mosaics also showed that reduced VEGF signaling is required for endothelial cells clonal expansion after Notch inhibition. Additional data suggest that the speeding of proliferation might be driven by changes in the metabolism of molecules involved in cell cycle progression. These results suggest that Notch activity, through a lateral inhibition mechanism, restricts VEGF-independent endothelial cell cycle progression during angiogenesis. In the highly mitogenic environment of organogenesis this could be a mechanism not only to coordinate different cellular processes like migration and proliferation, but also as a safeguard for the detrimental effects that excessive proliferation could generate in differentiated endothelial cells.

67 Live analysis of Drosophila female germline stem cells reveals a role for ECM-regulator Timp in stem cell division

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Stem cell activity must be strictly regulated to ensure a proper balance between proliferation and differentiation. This regulation is possible because stem cells reside in specific and restricted microenvironments called niches. The extra cellular matrix (ECM) is an essential component of these niches, where it provides structural support and facilitates proper signalling. For this reason, ECM remodelling in niches ought to be tightly regulated. Matrix metalloproteinases (MMPs), well-known ECM proteases, have been implicated in ECM metabolism in several tissues. MMP activity is generally controlled by Timp (tissue inhibitor of metalloproteinases) proteins. Studying the function of the only Drosophila timp gene in the female germline stem cell (GSC) niche, our laboratory has shown that timp mutant ovaries contain lower amounts of the structural ECM component Collagen IV and possess softer ECM than controls. Moreover, mutant ovaries display an aberrant organisation of the niche and show inefficient gamete production, phenotypes that could be linked to the increased MMP activity typical of timp ovaries. Interestingly, the reduction in gamete production is not due to GSC loss or to increased cell death. Instead, our in vivo analyses support the hypothesis that the reduced generation of germline cysts (and thus of female gametes) is a consequence of mutant GSCs taking longer to divide. In order to demonstrate a link between a prolonged cell cycle in timp mutant GSCs and tissue stiffness, we are studying in detail the cell cycle of control and timp mutant GSCs, with a focus on centrosome/centriole behaviour, spectrosome dynamics and mitosis.
**68 miR-106 regulates muscle repair through control of muscle stem cell fate**

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Several recent evidences indicate that miRNAs are previously unrecognized regulators of muscle progenitor cell proliferation, fate specification, and differentiation during embryogenesis and modulating quiescence and cell proliferation of skeletal muscle satellite stem cells during adult myogenesis. Recent data reported by our laboratory demonstrated that miR-106b displays a tissue-specific expression pattern on satellite cells. Moreover, in vitro analysis in freshly isolated satellite cell cultures showed that miR-106b modulates cell proliferation by targeting cyclin D1 and cyclin D2 3'UTRs. More importantly, miR-106b also target the Myf5 3’UTR and down-regulation of miR106b in freshly isolated satellite cells leads to increase the Myf5+ cell population suggesting that miR-106b can play important role in the acquisition of a myogenic cell fate. Here, we present data demonstrating that miR-106b expression is down-regulated during the course of muscle regeneration in mice, at the same time that myogenic factor up-regulation occurs (day 3 after muscle injury). Importantly, miR-106b expression is increased in a mouse model for DMD (DMD/mdx mice). Intramuscular injection of antimiR-106b in DMD/mdx mice leads to improve muscle regeneration with a significant functional recovery. To further investigate miR-106b functions during adult myogenesis, we are currently performing RNAseq analysis on antimiR-106b-injected muscles. These findings support the notion that miR-106b plays a key role during the process of muscle regeneration and may open new therapeutic perspectives by identifying new molecular tools to improve the regenerative capacity in muscular dystrophies.

**69 Tolerance to defective zygotic repair of paternal DNA damage in external fertilizers**

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Sperm DNA integrity is crucial to proceed with development, most of the de novo mutations and chromosome aberrations are paternally transmitted. DNA injuries accumulated during spermatogenesis depend on the maternal repair machinery and the activation of DNA damage response (DDR) in the zygote. Fertilization with DNA damaged sperm (DDS) promotes in mammals a highly effective DDR, mis-repairing inducing early embryo loss, implantation failures and occasional abnormal outcomes at birth. We have analyzed the combined effects of DDS and zygotic repair in fish: external fertilizers, very prolific and with weak sperm selection mechanisms. DDS was promoted by UV irradiation (400 µw/cm² 10min). Egg batches from females spawning at different temperatures were fertilized. DNA damage and egg repairing capacity were analyzed. Fertility, abortion and malformation rates, expression of genes involved in DNA repair and apoptotic activity were assessed during embryo development. Eggs maturing at higher temperatures showed an altered repairing profile. Upon fertilization with DDS the embryos from modified eggs showed at epibolia an enhanced repairing activity (overexpression of ogg1, ung, lig3 and rad1), at organogenesis a lower apoptotic activity and higher abortion rates, rendering at hatching higher malformation rates. These results support our previous studies on the inhibition of zygotic repair, pointing to a DDR no as restrictive with development in fish as is in mammals, allowing the progression to advanced phases of development and boosting the survival of individuals tolerant to residual damage, prone to accumulate mutations. This scenario suggests a reproductive strategy enhancing genetic drift instead of strict control.
**70 High-throughput analysis of proximo-distal transcriptional profiles in limb development**

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Limbs develop from limb buds composed of a core of limb progenitor cells covered by the surface ectoderm. Cell tracking experiments in chick showed that the limb progenitors located at the distal tip of the limb bud progressively restrict their developmental potential in the proximo-distal axis. The mechanisms and models by which the limb progenitors acquire progressively more distal fates are subject to intense investigation and debate. To shed light into this issue we have generated expression profiles for each of the progressive differentiation step by RNA-seq. In parallel we have also generated the temporal transcriptome of the distal ectoderm including the Apical ectodermal ridge (the AER). We seek to unravel expression pattern specific of each of the progressive stages of the limb progenitors and of the AER trying to identify essential descriptors of their biological state (morphogenetic potential). Of 46,480 Ensembl-annotated genes, 3,416 were differentially expressed between ectoderm and mesoderm. Intriguingly, we observed that the number of genes changing between stages is similar between ectoderm and mesoderm, with the bulk of changes occurring from E9.5 to E10.5. Expression pattern validation was confirmed by in situ hybridization and previously published data. Gene Ontology analysis revealed, in general terms, a downregulation of transcription factors and an upregulation of extracellular molecules with age. We have identified a specific expression of HoxC genes in the limb ectoderm, which is currently on evaluation.

**71 Analysis of the mechanism underlying the function of Sp6 and Sp8 in the limb ectoderm**

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We have recently shown that the transcription factors Sp6 and Sp8 are conjointly necessary for limb development as no limbs form in their absence or substantial reduction. The study of the allelic series of double Sp6;Sp8 mutant also revealed that mice with only a functional copy of Sp8 exhibit the Split-hand/split-foot malformation. Based on the molecular study of mutant limbs, we have postulated that Sp6 and Sp8 are necessary in the limb ectoderm, downstream of Wnt/β-catenin, to activate Fgf8 and downstream of Bmp signaling, possibly cooperating with Smads for dorso-ventral patterning. Here we have used co-immunoprecipitation (CoIP) and bimolecular fluorescence complementation (BiFC) to explore these suspected protein-protein interactions. To this end, Sp6, Sp8 and Smads were tagged with Myc or Flag epitopes to their N-terminal end and with YFP full length or YFP moieties to their C-terminal end. By CoIP and BiFC we show that Sp6 and Sp8 can homo and heterodimerize and that they can also interact with Smads. Finally, by generating different truncated versions of Sp8 we resolve the protein regions responsible of these interaction. Our study underscores the mechanisms by which Sp6/8 mediate Wnt/β-catenin and Bmp signaling in the limb ectoderm.
Understanding Cdon-Hh interaction in zebrafish eye formation
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Patterning of the vertebrate optic vesicle into proximal/optic stalk and distal/neural retina involves midline-derived Hh signalling, which promotes stalk specification. In the absence of Hh signalling, the stalk is not specified, forming a single cyclopic eye. However, work from our laboratory has shown that the cell adhesion molecule Cdon presents a complementary expression pattern to the canonical Hh receptor Ptc and acts as a negative Hh signalling regulator during the formation of the eye in zebrafish. At the neural retina/optic stalk border Cdon binds Hh, serving as a decoy receptor to protect the neural retina from Hh activity, likely preventing its diffusion and limiting its long-range signalling. How Cdon controls Hh dispersion shaping the gradient responsible for the correct P-D patterning of the eye remains unknown. To address this question we have generated cdon mutant lines using the CRISPR/Cas9 technology. We targeted the ATG region (exon 2) of the gene in order to generate a truncated protein (knockout) and the domains need it for Shh (exon 14) or Ptc (exon 8) binding (knockdown), respectively. To investigate how loss of cdon affects the patterning of the eye we are currently analyzing the phenotype of the mutants through a combination of morphological studies and the expression pattern of optic vesicle markers by WISH. In addition, we are generating diverse tools to visualize the interaction of Hh/Cdon in vivo.

Breaking the Atoh1 autoregulatory loop in neurosensory precursors: the repression of Atoh1 by Neurog1 during inner ear development
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The functional unit of the inner ear consists of hair cells (HC), supporting cells (SC) and neurons. In amniotes, a multipotent pool of cells generates first neurons and, later on, HCs and SCs. Atoh1 is a bHLH protein crucial for HC development. It is induced early in development, but expressed only after neuronal differentiation, resulting in a delay in HC formation. Atoh1 activates its own transcription through a classA E-box located in a 3’Atoh1-enhancer composed of two regions, A and B. Atoh1 function is counteracted by Neurog1 another bHLH transcription factor related to neuronal commitment. The aim of this work is to understand the molecular mechanisms underlying Atoh1 repression during early inner ear development. Reporter assays on chick and P19 cells showed that enhancer B of the 3’Atoh1-enhancer is the main region involved in Atoh1 auto-activation. Unlike the whole 3’Atoh1-enhancer, enhancer B activity is not restricted to the neurosensory domain, showing also higher activation, while enhancer A did not show any activity. This parallels the accessibility of the enhancer in the mouse developing otocyst, only enhancer B being accessible in ATAC-Seq analysis. Neurog1 is able to prevent Atoh1 auto-activation at both the 3’Atoh1-enhancer and enhancer B, as well as HC differentiation. Neurog1 requires the flanking regions of the class A Ebox of enhancer B. Surprisingly, Neurog1 does not require its DNA-binding domain to repress Atoh1, but the Helix1, which is essential for heterodimerization. However, Neurog1 does not seem to sequester any Atoh1 co-factor essential for its function, but instead it promotes Atoh1 degradation by the proteosome. Funding: The work has been supported by MINECO and La MaratóTV3, Spain
74 The Intermediate Mesoderm, a new player in the establishment of organ laterality?
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Bilateral symmetry is a common feature of vertebrates. However, this external symmetry hides a surprise: internal organs such as heart, gut, liver and pancreas are asymmetrically organized with respect to the left-right (LR) axis. The establishment of LR asymmetries is a crucial initial step in organogenesis and involves a sequence of molecular and morphogenetic events. Work from our lab showed that the cell adhesion molecule N-cadherin plays a role in LR patterning in the chicken embryo. While searching for cell adhesion regulators of laterality in zebrafish, we came across Cadherin11 (Cdh11). Unpublished data from our lab shows that cdh11 knockdown, using morpholino technology, leads to organ laterality defects. The surprise came when we were unable to find cdh11 transcripts in known tissues related to LR patterning (i.e. LRO, notochord, LPM). Instead we, and others, detected cdh11 mRNA in the IM in the time period when the laterality signal is being relayed to the LPM (12-14 hpf) and also in the PN during LPM migration stages (24-30 hpf). This suggests a role for these tissues in organ laterality.

75 Retinoic acid regulates chamber specification through miR-133 during early heart looping formation
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We have previously demonstrated the ability of retinoic acid to regulate the expression of atrial specific markers AMHC-1 and Tbx5 during early cardiac chamber specification. However, the molecular mechanisms responsible for this process still remain unclear. At present, microRNAs represent a novel layer of complexity in the regulatory networks controlling gene expression during cardiovascular development. The aim of this work is to study the intrinsic mechanism involved in this signaling pathway, since miR-133 has demonstrated to be involved in other earliest cardiac developmental processes. Our model is focused on developing chick at gastrula stages by in vitro electroporation of miR-133, a microRNA which is has been shown expressed at the level of linear cardiac tube. Our results show the miR-133 expression at the level of the primitive heart tube. Moreover, or work reveals that overexpression of miR-133 suppresses AMHC-1 and Tbx5 expression. These data support that miR-133, a putative microRNA that targets RARB 3’UTR, regulates the early cardiac chamber specification via retinoic acid pathway. This work has been partially financed with grants to research groups CTS005 (to VGM) from the Junta de Extremadura, with FEDER co-financing, and CVI-6556 (to DF) from the Junta de Andalucía Regional Council, and PB01/2016 FIFRA Foundation Grant.

76 Role of miR-130 in Bmp2 pathway signalling during cardiac atrial differentiation
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It is known that Bmp2 plays a relevant role in specific cardiac gene expression during early cardiac tubular heart formation to regulate the cardiac chamber specification. However, the molecular mechanisms responsible for this process still remain unclear. At present, microRNAs represent a novel layer of complexity in the regulatory networks controlling gene expression during cardiovascular development. In this work we analyze the role of miR130 as an intrinsic mechanism involved in early heart looping formation. Our model is focused in developing chick at gastrula stages by means of gain- and loss-of-function experiments on primitive streak precardiac cells by in vitro electroporation of both Bmp2 and miR-130, and anti-miR-130, respectively. Embryos were subjected to whole mount in situ hybridization. Our results reveal that both Bmp2 and miR-130 suppress the expression of Tbx5 and AMHC-1, while anti-miR130 electroporation increases the areas corresponding to these specific atrial genes here analyzed. Our data support that miR-130 constitutes a necessary linkage in the control of atrial specific markers, Tbx5 and AMHC-1, induced by Bmp2 activity. This work has been partially financed with grants to research groups CTS005 (to VGM) from the Junta de Extremadura, with FEDER co-financing, and CVI-6556 (to DF) from the Junta de Andalucía Regional Council, and PB01/2016 FIFRA Foundation Grant.
Alternative splicing control over naïve and primed pluripotency throughout mammalian embryo development

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¹CRG

During mammalian development, the embryo goes through two sequential pluripotency states before lineage commitment. These two cell identities have been defined by their contrasting ability to initiate differentiation. While naïve pluripotent stem cells found in the pre-implantation embryo, are not yet competent to differentiate, primed pluripotent stem cells, found later in the post-implantation embryo have the ability to initiate this process. Despite the efforts made to characterise these two cell identities through gene expression, protein and epigenetic profiling, the molecular program that defines them remains incompletely understood. Recently, alternative splicing was found to be tightly regulated in stem cells and crucial for promoting a pluripotent cell identity, however its role during the transition between the naïve and primed pluripotency states, both in vitro and in vivo, remains largely unexplored. We have performed ultra-deep coverage RNA sequencing on pre- and post-implantation embryos and embryo derived stem cells, and identified over 650 exons that are differentially spliced in the naïve and primed pluripotency states. In order to uncover the functional impact of these isoform switches in the establishment of pluripotency, we are presently performing targeted exon deletions in mouse embryonic stem cells using the CRISPR/Cas9 gene editing system. This, together with the identification of master regulators of alternative splicing responsible for the isoform switches seen in the two pluripotent states, will provide new insights into the regulatory networks governing pluripotency maintenance and the gain of differentiation potential.

Elucidating novel functions for the EGF receptor pathway during liver regeneration and hepatocarcinogenesis

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The aim of this study was to study the specific role of Epidermal Growth Factor receptor (EGFR) during liver regeneration (LR) and hepatocarcinogenesis. A novel transgenic mouse model expressing a hepatocyte-specific truncated form of the human EGFR, which lacks its catalytic activity, was generated. EGFR livers displayed lower and delayed proliferation during LR after partial hepatectomy (PH), correlating with overactivation of the TGF-β pathway. Interestingly, lipid synthesis was severely inhibited after PH, revealing a new function for EGFR kinase activity. In spite of this, EGFR livers fully regenerate by overactivating compensatory signals, such as c-Met. EGFR catalytic activity was also critical in early pre-neoplastic stages of the liver, EGFR mice showing delayed appearance of diethyl-nitrosamine-induced tumors. This effect correlated with decreased inflammation, the driving force of hepatocarcinogenesis, indicating that EGFR pathway in hepatocytes would be involved in the regulation of the inflammatory environment under pre-neoplastic situations. To study the role of EGFR pathway in liver tumor cell migratory/invasive phenotype, we used different hepatocellular carcinoma (HCC) cell lines where the EGFR expression has been attenuated with shRNA. Surprisingly, knock-down of EGFR per se changed the phenotype, cells showing decreased cell-to-cell and cell-to-matrix adhesion, although only a slight increase in migration. However, silencing of EGFR increased the migratory response to TGF-β in an ameboid-like mode. In conclusion, our studies provide key mechanistic insights into how the EGFR pathway regulates liver pathophysiology.
Hippo signaling controls cell death, cell cycle and differentiation in planarians

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Growth control is an open basic question in developmental biology. The Hippo signaling emerges as an essential pathway in the organ size control for its unique ability to simultaneously regulate cell proliferation and apoptosis, and thus to enable a balanced stem cell versus post-mitotic cell compartment. Although the core of the signaling pathway is well understood, the downstream transcriptional regulation and the main biological processes regulated remain still obscure since they are highly context-dependent. We have studied the function of the Hippo elements in Planarians, which stem cell based plasticity provides us an ideal scenario to approach the role of the pathway in the different cell compartments. Our results show that Hippo silencing in Planarians does not lead to an increase of organ size but to the formation of overgrowths that are not the result of an increase in cell number but rather to a problem in cell differentiation. Cellular analysis demonstrates that in Planarians Hippo regulates the mitotic division in stem cells and the apoptosis and maintenance of cell differentiation in post-mitotic cells.

WNT5-ROR and Slit-ROBOc signals generate a mutually dependent system to position the cns along the medio-lateral axis in planarians

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The acquisition of bilateral symmetry was a key evolutionary step, allowing the development of a centralized nervous system (CNS). However, the developmental signals that position bilateral symmetric structures in relation to the midline are still poorly understood. Planarian plasticity demands continuous positional information to maintain body proportions and axial information during regeneration and homeostasis. This ability offers us an ideal context to study the signals required to position the CNS in relation to the midline. Here we demonstrate that Wnt5 and Slit are axon repulsive cues in planarians which exert their function from opposite domains of the CNS. We found that the receptors ROR2 and ROBO-c, respectively, mediate their effect on axonal growth. Interestingly, ROR2 and ROBO-c are expressed in neurons but also in muscular cells that express slit and wnt5, respectively. Since muscular cells exert the positional control in planarians, we hypothesized that WNT5-ROR and SLIT-ROBO-c signals could conform a self-regulated system to define their expression boundaries in addition to guide the axonal path, allowing the self-maintenance of the medio-lateral positional information. We are currently modelling the biological data to deepen into the proposed model.
Molecular mechanisms involved in naso-temporal patterning of the retina in zebrafish

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The earliest known determinants of retinal nasotemporal identity are the transcriptional regulators Foxg1, which is expressed in the prospective nasal optic vesicle, and Foxd1, which is expressed in the prospective temporal optic vesicle. Our previous work has shown that, in zebrafish, the evaginating optic vesicles become partitioned into prospective nasal and temporal domains by the opposing actions of Fgfs and Shh. Fgf signals from the dorsal forebrain and olfactory primodia are required to specify nasal identity in the dorsal, prospective nasal, optic vesicle, while Hh signalling from the ventral forebrain is required for specification of temporal identity in the ventral optic vesicle and is sufficient to induce temporal character when activated in the prospective nasal retina. This work also showed that in absence of Fgf activity, foxd1 expression is established irrespective of levels of Hh signalling, indicating that the role of Shh in promoting foxd1 expression is only required in the presence of Fgf activity, and suggesting that other signals contribute to the establishment of nasal and temporal domains. Here, we will present our recent results, exploring the putative role of the secreted signals Bmps and Nodal in the establishment of the naso-temporal subdivision in the retina. We will also put forward a model with which we attempt to explain how the acquisition of nasal and temporal fates by the eye field cells is coordinated with their dynamic reorganisation during the evagination of the optic vesicles.

RUNX1c regulates hematopoietic differentiation of human embryonic stem cells through activation of pro-inflammatory signaling

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hESCs are a unique model to study early human development. Runx1 is a master hematopoietic transcription factor essential for definitive HSC emergence. Runx1-deficient mice die during early embryogenesis due to the inability to establish definitive hematopoiesis. Here we analyzed the role of RUNX1 in human embryonic hematopoiesis. RUNX1a, b and c were expressed in CD45+ hematopoietic cells; however, RUNX1c was the only one expressed in hemato-endothelial progenitors (HEPs). Constitutive expression of RUNX1c in hESCs enhanced the appearance of HEPs and promoted subsequent differentiation into blood cells. Conversely, specific deletion of RUNX1c virtually abrogated the hematopoietic potential of HEPs, indicating that RUNX1c is a master regulator of human hematopoietic development. Gene expression profiling of HEPs revealed a RUNX1c-induced pro-inflammatory molecular signature, supporting previous studies demonstrating pro-inflammatory signaling as a regulator of hematopoietic stem cell emergence. Collectively, RUNX1c orchestrates hematopoietic specification of hESCs, likely in cooperation with pro-inflammatory signaling.

ROS-induced signaling is required for Drosophila imaginal disc regeneration

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The nature of the signals involved in epithelial regeneration is an issue that has captivated scientists since centuries. This can be studied in Drosophila imaginal discs, which are epithelial sacs that are able to regenerate after damage. Upon apoptotic stimuli, epithelial cells fill the gaps left by dead cells by activating proliferation. This has led to the proposal that dying cells signal to surrounding living cells to restore homeostasis. Whether Reactive Oxygen Species (ROS) emerge from dead cells and what is the genetic response triggered by ROS is pivotal to understand Drosophila imaginal disc regeneration. In this work, we genetically induced cell death, monitored the production of ROS and analyzed the signals required for repair. We found that cell death generates a burst of ROS that propagate to the nearby surviving cells. Propagated ROS activate p38 and induce tolerable levels of JNK, and ultimately the expression of the cytokines Unpaired (Upd). The JAK/STAT signaling activated by Upd is essential to trigger regeneration. Our findings demonstrate that this ROS/JNK/p38/Upd stress responsive module restores tissue homeostasis. This module is not only activated after cell death induction but also after physical damage and reveals one of the earliest responses for imaginal disc regeneration.
84 Functional relationship between Snail1 and Prrx1 EMT transcription factors in embryonic development
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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. Prrx1 was recently identified as a novel EMT-TF in our lab (Ocaña et al. 2012). We have observed a complementarity between the expression patterns of Prrx1 and the classical EMT-TF Snail1 during embryonic development. The described phenotype of single mutants indicate that although they differ, they affect similar cell populations, including mesoderm and neural crest derivatives. In my project, I am generating mouse models in which the expression of both transcription factors is compromised with the aim of better understanding putative genetic interactions and/or cooperation during embryonic development, which can also shed new light into the interpretation of the phenotypes observed in pathological contexts. We have carried out a preliminary analysis of the phenotype of the single and double Prrx1/Snail1 heterozygous embryos. A copy of both transcription factors is sufficient to allow neural crest cell migration, but the loss of one copy of Prrx1 causes defects in the tectum posteriorus, and this defects are enhanced in the double heterozygotes.

85 Regenerative response of different territories of the Drosophila wing imaginal disc
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Regeneration is a classical theme in Developmental Biology. In this work, we have investigated the response to ablation of specific regions of the notum and of the wing and found that the notum regions show very limited regenerative response. This is in contrast with the appendage regions, which possess a high regenerative potential. We have also studied the response of the notum cells to massive cell death in the entire wing primordium and of that of the wing cells to massive apoptosis of the notum precursors. All together, our results indicate that even though they are parts of the same imaginal disc, the notum and the wing become developmentally isolated from each other and acquire different regenerative potential.

86 Diversity of fate outcomes in cell pairs under lateral inhibition
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Notch/Delta signalling is responsible for cell fate determination in a variety of systems. Typically, Notch activates Delta in the neighbouring cell, where Delta represses Notch. This lateral inhibition mechanism then leads to symmetry breaking of signalling states between neighbouring cells, commonly resulting in salt-and-pepper fate patterns in fields of cells. Here we consider the case of signalling between isolated cell pairs, and find that the bifurcation properties of a standard mathematical model of lateral inhibition can lead to stable symmetric signalling states. We apply this model to the adult intestinal stem cell (ISC) of Drosophila, whose fate is stochastic but dependent on the Notch/Delta pathway. Our experiments show a correlation between the signalling state of pairs of cells and the contact area between them. Using our model we interpret this behaviour in terms of the response of the system to population variability in signalling thresholds. Our results suggest that the dynamics of Notch/Delta signalling can contribute to explain stochasticity in stem cell fate decisions, and that the standard model for lateral inhibition can account for a wider range of developmental outcomes than previously considered.
Deciphering the molecular function of the novel nuclear protein Trnp1 in brain development

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Evolution of the mammalian brain encompassed an increase in size of the cerebral cortex (CCTX) including tangential and radial expansion. We have identified Trnp1 as a regulator of CCTX expansion in both of these dimensions (Stahl et al., 2013). Trnp1 overexpression promotes tangential expansion by increasing neural stem cell (NSC) self-renewal while knockdown promotes radial expansion leading to folding of the otherwise smooth murine CCTX. Remarkably, Trnp1 expression exhibits regional differences in the human developing CCTX anticipating radial or tangential expansion. Trnp1 is a novel nuclear protein without any known motif or conserved domain. Predictions of Trnp1 secondary structure revealed intrinsically disordered regions (IDRs) at the N- and C-terminus of the protein and an alpha-helix domain with the capacity to form coiled coil (CC) structures in the central region. Using different mutant proteins we now demonstrate that the first conserved 16 aa are important to promote NSC self-renewal, the CC is important for the nuclear localization, and the IDRs are important for Trnp1 oligomerization. We further determined the Trnp1 interactome by MS revealing several groups of interactors involved in functions that IDR containing proteins have been implicated in, such as regulation of transcription, splicing and the organization of chromatin. Taken together, our data highlight a novel nuclear complex with crucial roles in brain development and evolution.

Dissecting the role of Yap/Taz-TEAD activity in a pool of hindbrain progenitors

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During embryogenesis, the posterior-most vesicle of the brain (hindbrain) is transiently metamerized into segments named rhombomeres. This process of compartmentalization is followed by the induction of a specialized population of cells at the interface between two segments, called rhombomere boundary cell population (rBCP). The rBCP is a non-neurogenic population relevant to the building up of hindbrain architecture since i) it is a signalling centre that contributes to hindbrain patterning [Terriente et al, 2012], and ii) it acts as a cell mesh that avoids cell intermingling between adjacent rhombomeres [Calzolari et al, 2014], keeping them as lineage-restriction compartments. Little is known about the properties of this cell population. In this work we aim to understand the behaviour of rBCP in terms of cell proliferation and cell fate. First, by temporal analysis of specific rBCP gene expression we showed that this cell population is kept as a transient two-cell layer with a neutral growth at rhombomeric interfaces. Second, cell cycle behavior studies suggested that the rBCP actively proliferates, although harbors a small pool of cells which are arrested in G1. In addition, previous and ongoing work suggests that boundary cells endow a specific cytoarchitecture in terms of i) cell shape [Gutzman et al, 2010], ii) cytoskeletal organization, and iii) tissue tension [Calzolari et al, 2014]. To investigate the potential impact of tissue architecture on cell fate and behaviour we analysed the state of Hippo pathway in hindbrain boundaries. Preliminary results show that a subpopulation of hindbrain boundary Sox2+ cells display Yap/Taz-TEAD activity, and loss-of-function studies suggest that this activity depends on cytoskeleton stability. Current work is focused on unveiling the role of Yap/Taz-TEAD activity in hindbrain boundaries, investigating the contribution of both effectors in the system and sorting out the involvement of the pathway in progenitor maintenance and cell fate.
Role of cell dynamics in neuronal specification of the zebrafish inner ear

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Neural patterning is established by secreted morphogens that regulate positional information mechanisms. The signals that influence these patterns have been extensively studied. Recently, cell movements were also implicated in the refining or progression of these patterns. Specifically, the role of cell behavior in neural specification is still unknown. Here we use the establishment of a neurogenic domain in the zebrafish inner ear as a model to evaluate contributions of cell dynamics to neuronal specification. Until now, otic specification was conceived to occur in a static tissue. Single cell quantitative 4D imaging allows us to analyze how otic primordium morphogenesis is coordinated with expression of the proneural gene neurog1. We identify a group of migrating cells that express neurog1 outside the organ and ingress into the primordium, becoming the first otic neuronal progenitors. After ingression, other cells of the primordium express neurog1 and this pool is expanded by apical symmetric divisions. Laser ablation of the ingressing cells revealed that they also play an instructive role by promoting the specification of the resident cells of the domain. Finally, tracking of photoconverted nuclei indicate that FGF signaling regulates ingestion of these cells. We propose a novel view for otic neurogenesis integrating cell dynamics whereby FGF-dependent ingestion of pioneer cells instruct local neuronal specification.

The role of the acetyltransferase CBP in neural development

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Rubinstein-Taybi syndrome (RSTS) is a genetic neurodevelopmental disorder characterized by mental impairment of variable severity and a wide spectrum of congenital abnormalities, that is caused by hemizygous mutations in the genes encoding the KAT3 family of transcriptional co-activator CREB binding protein (CBP) and the E1A binding protein P300 (p300). Both factors have intrinsic lysine acetyltransferase (KAT) activity and are critically involved in the transcriptional and epigenetic regulation of gene expression. Consistent with this central role, CBP and p300 knockout (KO) mice exhibit early embryonic death and neuronal tube closure defects. However, the precise function of these proteins during development of the central nervous system has not been clearly stated. In this study we assess whether CBP is necessary for the maintenance and/or differentiation of neural progenitors and postmitotic neurons, since this could provide an explanation for the neurological alterations associated with RSTS. Conditional removal of CBP in differentiated retinal ganglion cells demonstrates that this protein is not essential to maintain a differentiated neuronal stage in vivo. In contrast, ablation of CBP from retinal pluripotent cells reveals that CBP is required for neural differentiation. These data support the hypothesis that abnormalities observed in RTS patients may be the consequence of the lack of CBP activity during neural differentiation processes. Ongoing genomic screens may further clarify the specific role of KAT3 proteins during neuronal differentiation and circuit formation.

91 The ciliary margin zone of the mammalian retina generates retinal ganglion cells
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The retina of lower vertebrates grows continuously by integrating new neurons generated from progenitors in the ciliary margin zone (CMZ). Whether the mammalian CMZ provides the neural retina with retinal cells is controversial. By live imaging of embryonic retina expressing eGFP in the CMZ we show here that CMZ cells move laterally to the neural retina where differentiated retinal ganglion cells (RGCs) reside. Cyclin D2, a cell cycle regulator, is enriched in ventral CMZ and we used Cyclin D2-/- mice as a tool to perturb this region and investigate whether the CMZ is a source of retinal cells. Neurogenesis was diminished in the CMZ of Cyclin D2 mutants leading to a reduction in the number of peripheral RGCs. In line with these findings we also observed that albino retinas, which have fewer ipsilaterally projecting RGCs, have fewer Cyclin D2+ cells. Together, these results implicate the mammalian CMZ as a neurogenic site that produces subsets of RGCs, whose proper generation depends on Cyclin D2 activity.

92 The role of the synaptic protein Sv2b in embryonic development of the cerebral cortex
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The Outer Subventricular Zone (OSVZ) is a unique germinal layer crucial for the evolutionary expansion of the mammalian cerebral cortex, promoting gyrencephaly. By using ferret as animal model of folded brains, we have recently identified a critical period during early embryonic development when apical Radial Glia Cells (aRGCs) in the ventricular zone (VZ) undergo self-consuming divisions to produce massive amounts of basal Radial Glia Cells (bRGC). These early-formed bRGCs are the founder progenitor cells of the OSVZ, and blockade of bRGC production during this critical period profoundly impairs the formation of the OSVZ. The gene encoding for Synaptic Vesicle Glycoprotein 2B (Sv2b) is differently expressed in the VZ before, during and after the critical period for OSVZ formation, and hence it is an attractive candidate to regulate this process. Importantly, Sv2b is expressed in the germinal layers of the developing ferret cortex but not in mouse, with a small and smooth cortex without bRGCs nor OSVZ. We have overexpressed Sv2b in progenitor cells of the embryonic mouse cortex by in utero electroporation at E14, and found that in the short-term (E17) it disrupted the distribution of electroporated cells. Sv2b overexpression disassembled the laminar organization of the VZ, induced the delamination of aRGCs to basal positions and altered their proliferation. At mid-term (P5), we observed the formation of a fold-like bulge in the electroporation site, with normal lamination of cortical neurons. We propose that this gene may play important roles in the developmental formation of bRGCs and the OSVZ, and hence in the expansion and folding of the mammalian cortex.
93 **Looking for mechanisms to increase the neural potential of neuro-mesodermal progenitors**  
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During vertebrate embryonic development, both the spinal cord and the somitic mesoderm derive from a multipotent progenitor population called neuro-mesodermal progenitors (NMPs). The knowledge of the molecular identity of these progenitors as well as the conditions that may mediate their expansion, are fundamental goals to develop regeneration strategies, mainly in the case of spinal lesions. To identify the molecular fingerprint of these progenitors, we established a genetic strategy to isolate NMPs from the tail bud of developing mouse embryos and we are currently obtaining the transcriptomic profile of these progenitors using RNA sequencing. Furthermore, to address the possibility of favoring the neural fate from NMPs, we used the CRIPSR/Cas9 technology to inactivate the Tbx6 gene (specific of paraxial mesoderm) in mouse embryonic stem cells (mESCs). Gene expression analysis showed that Tbx6-negative cells exhibited an upregulation of neural progenitor markers. Moreover, when these cells were induced to differentiate through the mesodermal route, we observed that expression of neural markers was further promoted together with a downregulation of mesoderm markers. Our preliminary data thus shows that the absence of Tbx6 could indeed promote differentiation of NMPs in a neurogenic pathway without inducing mesoderm fates, suggesting the potential of this strategy for regeneration therapies of the spinal cord.

94 **Molecular mechanisms regulating wiring specificity**  
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A fundamental requirement in the assembly of neural circuits is that neurons establish synaptic connections with their appropriate partners. In many systems, this involves extension into a particular synaptic layer, and selection of the appropriate partner among all the cells in the layer. Virtually nothing is known about the mechanisms governing the establishment of specific connections in any system. Our hypothesis is that the molecular differences that exist between neuronal subtypes, with similar developmental origin and function, contribute to their distinct connectivity. We study the differential layer selection of the closely related Drosophila R7 and R8 photoreceptors. Each eye contains 750 R7 and 750 R8 cells, and the entire population of each subtype proceeds synchronously to their respective final synaptic layer during pupal development. Taking advantage of such precise coordination, we have profiled the R7 and R8 transcriptomes right before this final extension. Our bioinformatics analysis has identified differentially expressed genes between the R7 and the R8. We have focused on 229 R8 enriched genes and performed an RNAi screen. Out of 186 genes analyzed we have identified 44 candidate genes showing layer selection defects. We are currently confirming our findings using mutant alleles. We expect that characterization of these genes contributes to the understanding of the molecular mechanisms regulating R7 and R8 differential layer selection.
The role of glial ionic homeostasis in glia-neuron communication

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The importance of glia-neuron communication during development and adulthood is becoming evident. Deregulation of this interaction can result in leukodystrophy, that is vacuolization and edema of the brain due to disruption of the myelin sheath. The CLCN2 chloride channel has been related to some types of leukodystrophy. Analysis of CLCN2 mutant mice suggests that leukodystrophy is a result of impaired ion glial homeostasis early in development. However, the consequences of this ionic impairment on glial function, and the secondary effects on neurons are still unknown. We turned to Drosophila since the intimate relationship between glia and photoreceptors early in development of the visual system allows studying the role of this channel in glia-neuron interactions. We have detected expression of the CLCN2 Drosophila homolog gene ClC-a in glial cells in the developing brain. Mutant ClC-a animals show defects in photoreceptor axon guidance during development and RNAi depletion of ClC-a transcripts exclusively in glia phenocopies the guidance defects. The glia-photoreceptor interaction in the early development of the visual system is mediated by Slit/Robo signaling. Slit secretion from glial cells is necessary for the correct guidance of photoreceptors. ClC-a mutant glia is morphologically wild type and transcribes slit. Together with the genetic interaction observed between ClC-a and slit, these results suggest that ClC-a mediated glial homeostasis regulates Slit secretion. Our hypothesis is that impairment of ionic homeostasis in glia might be causing defects in secretion of molecules important in glia-neuron communication events.

Role of non-canonical Wnt pathway during cellular organization and differentiation in spinal neural plate development

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Neural tube closure is a complex developmental process that takes place early during embryogenesis and is a key step in neurulation. It has become evident that some posterior neural tissue is generated independently of the mechanism that induces the anterior neural plate. This spinal neural tissue derived from a bipotent neuromesodermal progenitors located in the caudal lateral epiblast. Neurulation is regulated to a great extent by the non-canonical Wnt signalling pathway, which in turns regulates actin cytoskeleton responsible for cell shape during development, directs polarized cellular orientation within the plane and directional cell migration. There is an increasing evidence for the non-canonical Wnt pathway to be involved in the final step in neurulation, the closure of the posterior neuropore. VanGL+/lp embryos presented disruption of the apical accumulation of actin microfilaments, an abnormal cell morphology and alteration in the expression of genes related with the neuromesodermal progenitors in the posterior embryo, alterations that are directly implicated in the failure of spinal neural tube closure.
Neuroblastoma cancer stem cells modulation by Nxph1/alpha-Nrxn system

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Cancer research is orienting toward the design of novel therapeutic strategies that directly target cancer stem cells, the crucial effectors of tumor malignancy. Developing such strategies is particularly relevant in the case of highly aggressive cancers such as neuroblastoma, a paediatric cancer deriving from the developing peripheral nervous system and which accounts for 10-15% of cancer-related childhood deaths.

So far, the existence of neuroblastoma cancer stem cells (NB-CSCs) has been hypothesized, yet not firmly established. It is assumed that NB-CSCs might share many similarities with their normal stem cells counterparts, the neural crest cells (NCCs), which represent a transient population of pluripotent stem cells that rise to the peripheral nervous system. Therefore, we decided to compare the NCCs transcriptomic signature with the transcriptomic profiles established for clinically relevant groups of NB patients. Among the candidates highlighted by our double screening method, we retrieved Neurexophilin 1: an extracellular glycoprotein known to bind alpha-Neurexins.

To determine whether Neurexophilin1/alpha-Neurexins system is effectively related to NB-CSCs, we analyzed their expression in a panel of established human NB cell lines in conditions of stem cell enrichment. The marked induction of alpha-Neurexin1/2 mRNA levels in the stem cell-enriched fraction (as assessed by both the side population and sphere-forming assays) suggest that they might represent NB-CSCs markers, a notion we are currently trying to confirm.

In parallel, we initiated a series of loss- and gain-of-functions experiments aimed at modulating Neurexophilin1/alpha-Neurexins functions in vitro to further investigate whether this pathway might control NB-CSCs amount or behaviour, and thereby NB malignancy.

Developmental mechanisms underlying chick secondary neurulation

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Development of the posterior neural tube in human embryos is a complex process that involves both primary and secondary neurulation (SN). Because normal development is not well understood, pathogenesis of neural tube defects remains elusive, even though these are high incidence birth defects (approximately 1 in 1000 births). Here we propose to take advantage of the chick embryo to build a model of human secondary neurulation as the corresponding anatomical region extends up to the lumbar level, closely resembling human development. Chick SN initiates when neuromesodermal progenitors aggregate in the tissue dorsal midline and form a densely packed medullary cord. The cells eventually become elongated and undergo mesenchymal-to-epithelial transition (MET). We have analyzed markers for apical and basal epithelial polarity and showed that cells located dorsally and peripherally are the first to undergo MET and, subsequently, the epithelialisation propagates ventrally. The centrally located cells do not exhibit localized apical markers and they remain mesenchymal till the very end of the process. Lumen opening finally starts by the formation of small cavities of varied size and shape at the boundaries of these two cell populations that later coalesce, from dorsal to ventral, into a single central lumen. Central cells are cleared from the lumen but apoptosis is not involved, as caspase-3 positive cells were never associated to the multiple foci of lumen openings. This suggests that cavitation does not play a major role in SN and that central cells intercalate in the lateral walls of the developing neural tube. We have also investigated the role of mitosis in secondary lumen growth and showed that mitotic rounding cells contribute to lumen expansion by pulling the luminal surface.
A mouse model of DYRK1A-related intellectual disability syndrome shows altered gliogenesis and defects in myelination

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Individuals with de novo mutations in DYRK1A present microcephaly and intellectual disability (ID), in most cases accompanied with astrogliosis and hypomyelination. Human DYRK1A is located in chromosome 21 and its triplication contributes to the neurological alterations associated to Down syndrome (DS). DYRK1A encodes a protein kinase with a conserved function across evolution in the developing brain where it regulates neurogenesis and neuronal survival and differentiation. There is evidence that the overexpression of DYRK1A contributes to the astrogliosis associated to DS. However, the role of DYRK1A in gliogenesis or glial cell function has not been studied. Here we show that adult haploinsufficient Dyrk1a+/- mice phenocopy the ID-related DYRK1A syndrome exhibiting an increased number of astrocytes in the telencephalon and altered myelination of the corpus callosum, alterations that arise early in development. Neural progenitors isolated from Dyrk1a+/- embryos show an altered capacity to differentiate into astrocytes and oligodendrocytes. In vivo, an excess of cortical astrocytes in the Dyrk1a+/- model is evident one week after birth. In contrast, at perinatal stages, there is a deficit of oligodendrocyte precursors in the corpus callosum, which is due to a reduced embryonic oligodendrogenesis. After the second postnatal week the population of oligodendrocytes reaches normal numbers in Dyrk1a+/- mice. However, they show a defect in myelination that persists in the adult. Our results suggest a new role of DYRK1A in glial cell development that could contribute to the ID and other neurological problems in patients carrying heterozygous mutations in the DYRK1A gene.

Trisomy of the down syndrome DYRK1a gene favors apical versus basal neurogenesis reducing the ratio of parvalbumin/somatostatin cortical interneurons

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One of the causes of Down syndrome intellectual disability is an altered connectivity of the cerebral cortex due to an imbalance between excitatory and inhibitory neurons. This imbalance arises in the embryo by an abnormal development of both types of neurons. We previously showed that trisomy of DYRK1A gene, which encodes a kinase involved in several nervous system functions, contributes to the reduced neurogenesis of excitatory cortical neurons in Down syndrome. To determine if DYRK1A trisomy is involved in the excess of inhibition observed in Down syndrome cerebral cortex, we have characterized the population of GABAergic interneurons in a transgenic mouse that carries an extra copy of the entire Dyrk1a mouse gene. Our results reveal an imbalance of the two main subtypes of GABAergic interneurons in the cerebral cortex of adult Dyrk1a trisomic mice: reduced number of parvalbumin interneurons, but increased number of somatostatin interneurons. A developmental characterization in the medial ganglionic eminence, the region where both neuronal subtypes are generated, shows that Dyrk1a trisomy enhances neuronal production at early developmental stages by increasing the proportion of somatostatin-generating apical progenitors at expenses of the parvalbumin-generating basal progenitors. This bias in the type of progenitor leads to an early exhaustion of progenitors and a reduction of neuronal production at later stages, which exacerbates the deficit of cortical parvalbumin interneurons. Our results provide evidences that DYRK1A regulates neurogenesis in the medial ganglionic eminence and that trisomy of DYRK1A may contribute to the altered number of cortical interneuron subtypes associated to Down syndrome.
101 ZEB1 promotes transcription of invasiveness genes in glioblastoma multiform cancer stem cells via a novel LEF1/TCF dependent mechanism

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Glioblastoma Multiform (GBM) harbors a population of Cancer Stem Cells (CSCs) thought to contribute to treatment resistance and relapse. Here we investigated the function of the zinc-finger factor ZEB1 in GBM CSCs, a classical inducer of Epithelial-to-Mesenchymal Transition (EMT) previously implicated in invasion, chemoresistance and tumorigenesis of GBM. Although a role for ZEB1 in repressing gene transcription is well established, little is known on the molecular basis for its activities in GBM. We combined genome wide location analysis with transcriptional profiling upon gene knock-down, in order to characterize the transcriptional program of ZEB1 in GBM CSCs. We found that Zeb1 binding associates with both activation and repression of gene expression, and that this dual role results from two distinct modes of recruitment to regulatory regions. Direct binding to its consensus binding sequences mediates target gene repression, while indirect recruitment via Lef1/Tcf factors occurs at promoters of genes activated by Zeb1. The latest include putative regulators of cell invasion/migration such as Prex1 or Nrp2. In vivo binding and transcriptional assays confirm Zeb1 strongly potentiates Lef1/Tcf-mediated activation of target gene promoters, and that this activity does not require active Wnt signaling. Our results demonstrate how Zeb1 can activate and repress simultaneously gene transcription, providing an important molecular frame for the activity of this important player in GBM.

102 Study of the role of Ebf-1 in medium spiny neurons specification and maturation

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Ebf-1/Olf-1 is a helix-loop-helix transcription factor previously described as being to be strongly expressed in the Lateral and Medial Ganglionic Eminences from early embryonic stages (E11) until postnatal stages. Its disruption affected the proper pattern of expression of genes involved in striatal neurogenesis. However, little is known about the mechanism of action of Ebf-1. In this work we further characterize Ebf-1 expression and its specific roles in striatum. We demonstrate that Ebf-1 is not only expressed by mature neurons but also by astrocytes. However, it does not co-localize with oligodendrocyte markers. It is expressed by Medium Spiny Neurons (MSNs) positive for Ctip2. Specifically, we show that it is not expressed by DRD2 positive MSNs, revealing specific functions for Ebf-1 in DRD1 neurons in agreement with previous studies. Interestingly, we also found some Ebf-1 positive cells in the cortex that might be interneurons due to Ebf-1 expression in the medial ganglionic eminence and its co-localization with some interneuron markers in vitro such as CHAT, Calretinin and PV. Analysis of Ebf-1 knockout mice shows a reduction in striatal size as early as E16.5 pointing out a possible implication in neuronal differentiation and/or maturation. In fact, when we overexpressed Ebf-1 in striatal proliferating progenitors we observed a decrease of Ki67+ cells. In addition, its overexpression in striatal cultures induces an increase of the calbindin+ neurons along with a reduction of the number of Nkx2.1+ cells. Overall, our findings indicate potential roles of Ebf-1 in cell cycle exit and cell specification and maturation during striatal development.
FLRT adhesion molecules regulate cerebral cortex folding by controlling neuron migration

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Folding of the cerebral cortex into valleys (sulci) and ridges (gyri) represents a fascinating evolutionary mechanism that impacts on neuronal networking and cognitive capacities of large mammals. While recent studies suggest that gyri develop in areas with an amplification of basal progenitors, the developmental mechanisms controlling sulci formation remain largely unknown. Previously, we have established that genetic ablation in mice of FLRT3, a member of the FLRT family of cell adhesion molecules leads to an altered distribution of pyramidal neurons during cortical development, forming a repeated pattern of clusters (Seiradake et al., Neuron, 2014). Here we report that FLRT1-/-;FLRT3lx/null;Nestin-cre double mutant mice show enhanced pyramidal neuron clustering and develop macroscopic cortical folds during embryogenesis (with 35% penetrance). This process appears to happen independently of cell proliferation, since the numbers of apical and basal progenitors are not altered in FLRT1/3 mutant cortex. Moreover, cortex-layered organization and radial glial morphology are normal in FLRT1/3 mutants. Instead, we find that neuron migration is altered: Analysis of cell morphologies after in utero electroporation of Cre recombinase into FLRT1-/-;FLRT3lx/null;Nestin-cre double mutant mice revealed a higher proportion of mutant neurons in upper versus lower cortical plate (CP), and a higher proportion of immature neurons in upper CP of mutant versus control embryos. These and further results on dissociated neuron cultures suggest a model in which the absence of FLRT1/3 reduces intercellular adhesion, accelerates immature neuron migration and promotes neuron clustering in the tangential axis, thereby leading to sulcus formation in the normally smooth mouse neocortex.

Generation of Vsx1/Vsx2 transcription factor double mutant in zebrafish using Crispr/cas9 system

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Visual system homeobox genes Vsx1 and Vsx2 are transcription factors that play an essential role in eye patterning and differentiation and have been linked to human diseases like microphthalmia/anophthalmia and coloboma. Taken advantage of the CRISPR/Cas9 system, we have generated several zebrafish lines with different deletions in the vsx1/vsx2 homeodomain, in order to obtain null mutations for phenotypic analysis. So far, we have generated 2 CRISPR lines for Vsx1 with deletions of 66bp and 245pb, both lacking most part of exon 3, which is the core DNA binding motif in vsx TFs. For vsx2 we generated 3 different alleles, two with small deletions in exon1 and exon 3 (8bp and 4bp, respectively) and one with a 73bp deletion in exon3. We found no clear developmental defects in vsx2E1∆8bp maternal zygotic homozygous mutant, vsx2E3∆4bp zygotic mutant and vsx1∆66bp zygotic mutant. Currently, we are analyzing if compensatory changes in vsx1/vsx2 gene expression can explain the lack of morphological phenotype observed. In addition, we are crossing both vsx1 and vsx2 alleles in order to generate the double mutant, which will be used as a tool to investigate the impact of vsx paralogs in the gene regulatory network involved in the specification of the neural retina domain.
RNA-binding protein MEX3A regulates adult neurogenesis
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The subependymal zone (SEZ) in the lateral ventricles and the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus are the two main neurogenic niches in the mammalian adult brain. Subependymal neural stem cells (NSCs) in adult mice continually generate neuroblasts and oligodendroblasts that migrate to the olfactory bulb and corpus callosum, respectively. Meanwhile, subgranular NSCs generate new neurons that integrate into the DG. NSCs in both niches are highly regulated by both extrinsic and intrinsic factors. Recently, RNA-binding proteins have emerged as interesting key regulators of many cellular processes such as cell division and differentiation, as they can quickly switch gene expression by stabilizing or promoting degradation of mRNAs. Here, we show that RNA-binding protein MEX3A is highly expressed in neuroblasts in vivo, both in the SEZ and the SGZ. Accordingly, it colocalizes with both doublecortin (DCX) and polysialylated-neural cell adhesion molecule (PSA-NCAM). Interestingly, Mx3a expression is also observed in NSCs and progenitors by flow cytometry analysis, although these populations show lower levels of expression. This suggests that MEX3A might be playing different roles in each population within the niche. Preliminary results show that Mx3a deficiency results in increased proliferation of PSA-NCAM+ subependymal neuroblasts. Although functional experiments are still in progress, it seems that MEX3A may be involved in controlling the cell cycle of neuroblasts while it might have other functions in NSCs.

MODELLING CELL BEHAVIOUR AND MORPHOGENESIS

Understanding the cues regulating morphogenesis of meristematic cells during lateral root formation in Arabidopsis thaliana
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The pericycle tissue gives rise to lateral root founder cells (LRFC) through a reprogramming process, and subsequently, distinctive cell fates are specified through asymmetrical divisions. Morphogenesis of lateral roots initiates with the asymmetric division of LRFC to generate small and large cells. These divisions require external inputs (auxin hormone) and are driven by intrinsic cues (such as polarity and nuclear migration). We hypothesize that self-organizing properties of founder cells are controlled by a regulatory network which incorporates external cues such as auxin.

Trough double Fluorescent Activated Cell Sorting we will be able to know the expression levels of genes in pericycle, lateral root founder cells and its daughters. To these end, we have already generate a range of plants carrying cells markers: a) pericycle cells capable of undergoing reprogramming will be isolated using the line carrying the markers J0121_pSKP2B0.5:ER::3xmcherry, b) pericycle cells undergoing reprogramming trough the line carrying DR5:D2eGFP::eGFP_pSKP2B0.5:NLS::3xmcherry, c) founder cells trough the marker line pWOX5:FP_pSKP2B0.5:NLS::3xmcherry, and the LRFC daughter cells will be isolated using d) the line carrying the markers pWOX5:FP_pSCR::ER::3xmcherry for the small daughter cell, and e) the markers pWOX5:FP_pHB53-2K::ER::3xmcherry for the large daughter cell.

This approach will define the regulatory program between crucial developmental states (pericycle, lateral root founder cells and its daughters) associated to root organ morphogenesis addressing how two distinct fates are specified from a single cell. We expect that our approach provides novel relationships between pluripotency and cell identity.
List of authors
Dominic, Ritler 53
Dooley, Steven 49
Downward, Julian 17
Duboule, Denis 61
Duran-Bello, Enya 14

E
Egea, Gustavo 49
Elefanty, Andrew 66
Escriva, Héctor 12
Escoll, Maribel 46
Escudero, Luis M 12, 30
Esgleas, Miriam 68
Espinosa, Luis 23
Espinosa Vázquez, José M 33
Estévez, Raúl 72

F
Fabio, Piano 53
Fabra, Àngels 51
Fabregat, Isabel 22, 49, 51, 52, 64
Fanlo, Lucia 73
Fargas, Laura 69
Fariña, Isabel 46
Fernandes, Sara F. 63
Fernandez, Anita G 53
Fernández, Eduardo 74
Fernández, Margarita 52, 64
Fernández, Covadonga 68
Fernández Díez, Cristina 45, 56, 60
Fernández Pineda, Alejandra 71
Fernández-de-Manuel, Laura 29
Fernández-Guerrero, Marc 61
Fernando, Joan 51
Ferran, Jose Luis 39
Ferrández-Roldán, Alfonso 14, 41
Ferreira, Ana 23
Ferrero-Galve, Susana 70
Flames, Núria 24
Forne, Ignasi 68
Francisco, García-Asencio 67
Franco, Diego 60, 63
Franco-Zorrilla, José Manuel 35

G
Gaitán-Peñas, Héctor 72
Galardi-Castilla, María 28
Galceran, Juan 18
Gallardo, Viviana 62
Galvez García, Héctor 62
Gandolf, Pablo 20
Garcia, Esther 46
García Morales, Diana 58
García-Álvaro, María 52
García-Asencio, Francisco 67
García-Escudero, Ramon 46
Garcia-Fernández, Jordi 38, 39, 55
Garcia-Lopez, Virgilio 63
Garcia-Martínez, Virgilio 63
Garcia-Ojalvo, Jordi 15, 67
Garess, Rafael 47
Gargi, Ricardo 46
Garrabou, Gloria 50
Gaspar, Claudia 24
Gavilán, Brenda 40
Gavilán, Maria P. 20
Germann, Philipp 11
Giannelli, Gianluigi 51
Giorgetti, Alessandra 23
Giraldez Orgaz, Fernando 62
Girela, José Luis 48
Gómez Lamarca, María Jesús 43
Gómez Míguez, David 58
Gómez-Cadenas, Aurelio 50
Gómez-Gálvez, Pedro 30
Gomez-Gómez, Soledad 73
Gomez-Saldiar, Georgina 53
Gómez-Skarmeta, Jose Luis 16, 40
González Díaz, Sergio 76
González García, Juvencio 42
González-Gobartt, Elena 73
González-Iglesias, Ainara 46
Gonzalez-Martinez, Rocío 69
González Reyes, Acaimo 43, 59

H
Hachimi, Mariam 35
Hagey, Daniel 25
Hassan, Bassem 29
Henrique, Domingos 24
Heredia, Luis 59
Hermoso, Ana 59
Hernández Bejarano, María 66
Hernández-Torres, Francisco 60
Herráez, M. Paz 45, 56, 60
Herrera, Blanca 52
Herrera, Eloisa 69, 70
Herrera, Sc 58
Herrera, Carlos 38
Hirata, Y. 15
Hoijman, Esteban 34, 69

I
Imhof, Axel 68
Irastorza, Ibai 40
Irimia, Manuel 36, 38, 39, 64

J
J. Cohn, Martin 37
J. Widmann, Thomas 43
Jacinto, Antonio 34
Jiménez-Carretero, Daniel 29
Juárez Uribe, Ra 58
<table>
<thead>
<tr>
<th>K</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Kalender, Zeynep</td>
<td>14</td>
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</tr>
<tr>
<td>Kedishvili, Natalia Y.</td>
<td>14</td>
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<td>Khalid, Sania</td>
<td>70</td>
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<tr>
<td>Kisiswa, Lilian</td>
<td>57</td>
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<td></td>
</tr>
<tr>
<td>Klein, Cecilia</td>
<td>57</td>
<td></td>
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</tr>
<tr>
<td>Koudelkova, Petra</td>
<td>51</td>
<td></td>
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</tr>
<tr>
<td>Kuwajima, Takaaki</td>
<td>70</td>
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<tr>
<td>L</td>
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<tr>
<td>Laine, Sara</td>
<td>47</td>
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<tr>
<td>Laranjeira, Cátia</td>
<td>25</td>
<td></td>
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<tr>
<td>Laruy, Briane</td>
<td>42</td>
<td></td>
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</tr>
<tr>
<td>Lavarino, Cinzia</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lazcanoiturburu, Nerea</td>
<td>52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Le Dréau, Gwenvaël</td>
<td>73</td>
<td></td>
<td></td>
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<tr>
<td>Leal, Francisca</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Letelier, Joaquín</td>
<td>16, 31, 62, 76</td>
<td></td>
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<tr>
<td>Link, Brian A.</td>
<td>31</td>
<td></td>
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<td>Lobo Pecellín, María</td>
<td>59</td>
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<td>López, María-Fernanda</td>
<td>50</td>
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<td>Lopez-Delgado, Alejandra Cristina</td>
<td>30</td>
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<td>Lopez-Escobar, B.</td>
<td>72</td>
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<tr>
<td>López-Giménez, Juan F.</td>
<td>61</td>
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<tr>
<td>López-Luque, Judit</td>
<td>64</td>
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<td></td>
</tr>
<tr>
<td>López-Mayorga, Macarena</td>
<td>40</td>
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<tr>
<td>Lopez-Sanchez, Carmen</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martin-Blanco, Carlos</td>
<td>14, 29</td>
<td></td>
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<tr>
<td>Martínez, Luis Miguel</td>
<td>38</td>
<td></td>
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<td>Martínez, Pedro</td>
<td>40</td>
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<td>Martínez Corral, Rosa</td>
<td>67</td>
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<td></td>
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<tr>
<td>Martínez Morales, Juan Ramón</td>
<td>16, 28, 31, 62, 76</td>
<td></td>
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<tr>
<td>Martínez-Palacín, Adoración</td>
<td>64</td>
<td></td>
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<tr>
<td>Martinho, Rui</td>
<td>17</td>
<td></td>
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<tr>
<td>Martins, Gabriel G.</td>
<td>34</td>
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<td>Mas, Paloma</td>
<td>15</td>
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<td>Mason, Carol</td>
<td>70</td>
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<td>Mateo, Juan L.</td>
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<td>Mayor, Roberto</td>
<td>11</td>
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<td>McCarthy, Alicia</td>
<td>17</td>
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<td>Menéndez, Pablo</td>
<td>23, 50, 60, 66</td>
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<td>Meister, Peter</td>
<td>53</td>
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<td>Mercader, Nadia</td>
<td>28</td>
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<td>Merino, Javier</td>
<td>59</td>
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<td>Meyer, Christoph</td>
<td>49</td>
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<tr>
<td>Michael, Mauro</td>
<td>53</td>
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<td>Miesfeld, Joel B.</td>
<td>31</td>
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<td>Mikhailov, Alexander</td>
<td>44</td>
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<tr>
<td>Mikulits, Wolfgang</td>
<td>51</td>
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<td>Milán, Marco</td>
<td>23</td>
<td></td>
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</tr>
<tr>
<td>Milinkowitch, Michel</td>
<td>10, 38</td>
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<tr>
<td>Minguillón, Carolina</td>
<td>18, 55</td>
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<tr>
<td>Molina Gil, Sara</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Momma, Stefan</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moncusí, Anna</td>
<td>14, 41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montoya, María</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morata, Ginés</td>
<td>58, 67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moreno-Cáceres, Joaquim</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moreno-Mármol, Tania</td>
<td>28, 32</td>
<td></td>
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</tr>
<tr>
<td>Moreno-Risueno, Miguel</td>
<td>16, 77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morey, Marta</td>
<td>71, 72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morgado, Ramiro</td>
<td>34</td>
<td></td>
<td></td>
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<td>Muhr, Jonas</td>
<td>25</td>
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<td>50</td>
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<td>18, 44</td>
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<td>18, 36, 46, 67</td>
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<td>Prud’homme, Benjamin</td>
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</tr>
</tbody>
</table>
Puelles, Luis 39
Puente-Moncada, Noelia 51, 52
Pujades, Cristina 16, 68

R
Rago, Luciano 36
Ramaekers, Arianne 29
Ramos, Emilio 64
Rangan, Prashanth 17
Raposo, Alexandre 25, 75
Real, Pedro J. 66
Recasens, Carles 23
Reillo, Isabel 37
Relvas, João B. 18
Retaux, Sylvie 16
Ricolo, Delia 21
Rios, Rosa 20
Risueño, María-Carmen 48, 50
Rives-Quinto, Noemí 55
Rodríguez, Carmen 51, 52
Rodríguez, Sonia 47
Rodríguez Fraticelli, Alejo 35
Rodríguez-Buey, María Luisa 35
Rodríguez-Outeiriño, Lara 60
Rodríguez-Sanz, Héctor 50
Rodríguez-Vaello, Victoria 64
Rojas, Anabel 45
Rojas, José M. 47
Rojas-Galván, Natalia S. 14
Rojo-Laguna, Jose-Ignacio 65
Romero-Moya, Damià 50
Roncero, César 52, 64
Ros, Marian 61
Rosmaninho, Pedro 75
Ross, M Elizabeth 70
Ruediger, Klein 76
Ruiz, Mar 49
Ruiz, Alfredo 39

S
Sáenz Hidalgo, Hilda Karina 42
Salgüero, Sergio 32
Saló, Emili 33, 65
Sánchez, Aránzazu 52, 64
Sanchez-Alcazar, J.a. 72
Sánchez-Aragón, Máximo 29
Sanchez-Corronero, Alvaro 16, 77
Sánchez-Gutiérrez, Daniel 12
Sánchez-Herrero, Ernesto 53, 58
Sánchez-Sánchez, Ana M* 51, 52
Sandonis, Africa 62
Santa Bárbara Ruiz, Paula 66
Santa-Cruz Mateos, Carmen 43
Santamaria, Estrella 48
Santos, Marilia 18
Santos Pereira, José María 56
Santos-Ocaña, Carlos 50
Sanz, Julian 52
Sanz-Ortega, Laura 47
Saperas, Patricia 52
Saúde, Leonor 63
Secombe, Julie 17
Segovia, Jose-Carlos 64
Sekaran, Thileepan 65
Serra, Roberto 47
Serrano, Teresa 64
Serras, Florencí 57, 66
Sessa, Alessandro 25
Seyit-Bremer, Gönül 76
Sharpe, James 11
Silva, Dalila 63
Smith, Richard 11
Solís, María-Teresa 48, 50
Sotillos Martín, Sol 21
Soukupova, Jitka 51

T
Taberner, Laura 30
Takahashi, N. 15
Tanaka, Elly 18
Tavares, Ana Teresa 34
Tavares, Ligia 18
Teixeira, Vera 25, 75
Ten, Jorge 48
Ten Dijke, Peter 52
Tena, Juan J. 40, 56, 62
Terriente, Javier 16, 68
Tesser-Lavigne, Marc 38
Testillano, Pilar S. 48, 50
Tobias, Ruff 76
Tokuko, Haraguchi 53
Tomaz, Diogo 25
Tornero, Alba Rocío 47
Torrado, Mario 44
Torres, Miguel 29, 30, 54
Tozlouglu, Melda 12
Tzika, Athanasia 38

U
Undurraga, Cristian 31

V
Valencia Expósito, Andrea 43
Vallejo, Daniel 60
Vasconcelos, Francisca 25
Vázquez-Marín, Javier 31
Vermot, Julien 28
Vicente-García, Cristina 39, 40
Vijayaraghavan, D. 72
Villa-Fombuena, Gema 30
Villalba Requena, Ana 38, 70, 76
Villamayor Coronado, Laura 45
Vizcaya, Elena 57
Voltes, Adria 16, 68

W
Wandosell, Francisco 46
Wang, Qing 70
Wittbrodt, Joachim 31
Wyatt, Chris D R 36

Y
Yasuhiro, Hirano 53
Yasushi, Hiraoka 53
Ybot-González, P. 72

Z
Zhu, Qi 72
Zunzunegui, Sandra 61
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