IX Meeting
of the Spanish Society for Developmental Biology
November 12-14, 2012 Granada
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Programme
Monday November 12th

14:00-16:45 – Meeting Registration

16:45-17:00 – Welcome and Opening Remarks
   Angela Nieto (SEBD President), Mike Levine (SDB President) and Antonio Jacinto (SPBD president)

17:00-18:00 – Opening Lecture:
   Eric Olson (Southwestern Medical School, University of Texas, Dallas, USA)
   The Molecular Circuitry of Heart Development, Disease and Regeneration
   Sponsored by SBD

Session 1 – Developmental Genomics
   Chairs: Paola Bovolenta / Anton Moorman

18:00-18:30 – Invited speaker: José Luis Gómez-Skarmeta (CABD, CSIC-UPO, Sevilla)
   Genomic architecture, gene regulation and human diseases.

18:30-19:00 – Invited speaker: Anne C. Ferguson-Smith (University of Cambridge, UK)
   Genomic imprinting and the regulation of adult neurogenesis

19:00-19:30 – Talks chosen from abstracts
   19:00-19:15 - Melisa Gómez-Velázquez (CNIC, ISCIII, Madrid)
      Genomic architecture in heart development: exploring the role of CTCF.
   19:15-19:30 - Srividya Tamirisia (IBMB, CSIC, Barcelona)
      Functional analysis of genes involved in wound healing: A novel role of TCP-1 subunits in wound healing.

19:30-21:00 – Poster Session I (& wine)

Tuesday November 13th

Session 2 – Tissue Patterning and Differentiation
   Chairs: Antonio Jacinto/ Diego Franco

09:00-09:30 – Invited speaker: Anton Moorman (AMC, Amsterdam, Netherlands)
   Development of the cardiac building plan

09:30-10:00 – Invited speaker: Mar Ruiz (CBMSO, CSIC-UAM, Madrid)
   Drosophila nephrocytes: A suitable model to study podocyte function.

10:00-10:45 – Talks chosen from abstracts
   10:00-10:15 Javier López-Ríos (University of Basel, Switzerland)
      Loss of anterior-posterior limb polarity underlies digit reduction in cows
   10:15-10:30 Julián Cardozo (CBMSO, CSIC-UAM, Madrid)
      The Shh binding protein Cdon is required for vertebrate optic vesicle patterning and morphogenesis.
   10:30-10:45 Emili Saló (University of Barcelona)
      Neoblasts and jnk, two fundamental elements in the planarian exquisite regeneration capacity.

10:45-11:15 – Coffee break
Session 3 – Cell adhesion and Migration  
Chairs: James Castelli-Gair Hombría/Mike Levine

11:15-11:45 – Invited speaker: Antonio Jacinto (Institute of Molecular Medicine, Lisboa, Portugal)  
Actomyosin cable formation during wound healing involves highly dynamic cytoskeletal changes and calcium signalling. Sponsored by SPBD

11:45-12:15 – Invited speaker: Oscar Marin (Institute for Neurosciences, CSIC-UMH, Alicante)  
Understanding the mechanisms that build the surface of the cerebral cortex

12:15-12:45 – Talks chosen from abstract

12:15-12:30 Óscar Ocaña (Institute for Neurosciences, CSIC-UMH, Alicante)  
Prrx1, a novel class of EMT inducer in embryos and cancer cells.

12:30-12:45 Dolores Martín-Bermudo (CABD-CSIC, UPO, Sevilla)  
The GEF VAV regulates collective cell migration downstream of guidance receptors by locally activating RAC at the leading edge.

12:45-13:15 – Light Sheet Microscopy from Carl Zeiss - Dr. Jens Rietdorf, Zeiss Microscopy Labs, Munich  
Sponsored by Zeiss Microscopy S.L.

13:15-14:45 – Lunch

Session 4 – Modelling & Systems Biology  
Chairs: Margaret Buckingham/ Miguel Manzanares

14:45-15:15 – Invited speaker: Mike Levine (University of California, Berkeley, USA)  
Transcriptional Precision in the Drosophila Embryo  
Sponsored by SBD

15:15-15:45 – Invited speaker: James Sharpe (EMBL/CRG, Barcelona)  
Engineering synthetic development: the 7 ways to make a stripe

15:45-16:15 – Talks chosen from abstracts

15:45-16:00 Karl Wotton (EMBL/CRG, Barcelona)  
Generating anterior-posterior polarity in cyclorrhaphan flies: A story of missing maternal factors.

16:00-16:15 Tristan Rodríguez (Imperial College, London UK)  
MicroRNA regulation of naïve and primed pluripotent states.

16:15-16:45 – Coffee break

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Workshop on imaging (Sponsored by Leica)
Chairs: Jose Luis Gómez-Skarmeta/ Leonor Saude

16:45-17:05 – Timo Zimmerman (EMBL/CRG, Barcelona)
Super-resolution light microscopy using Stimulated Emission Depletion (STED) and Ground State Depletion (GSD) methods.

17:05-17:25 – Juan Ramón Martinez-Morales (CABD, CSIC-UPO, Sevilla)
Numb/Numbl-Opo antagonism controls retinal epithelium morphogenesis by regulating integrin endocytosis.

17:25-17:45 – Invited speaker: Elisa Marti (IBMB, CSIC, Barcelona)
To divide or differentiate: Growth control in the neural tube.

17:45-18:05 – Leica Microsystems

18:05-20:00 – Poster Session II

18:05 – SEBD/SPBD Board of Directors Meetings

Wednesday November 14th

Session 5 – Stem Cells
Chairs: Acaimo Gonzalez/Claudio Stern

09:00-09:30 – ISDB-MOD lecture: Margaret Buckingham (Pasteur Institute, Paris, France)
Regulation of Entry into the myogenic programme in stem cells in the embryo and in quiescent and activated satellite cells in the adult.

09:30-10:00 – Invited speaker: Isabel Fariñas (University of Valencia)
Developmental molecules for adult stem cells in mammalian neurogenic niches

10:00-10:45 – Talks chosen from abstracts

10:00-10:15 Cristina Clavería (CNIC, ISCIII, Madrid)
Cell competition in the mammalian epiblast selects cells with higher Myc levels.

10:15-10:30 Andreu Casali (IRB, Barcelona)
Conserved mechanisms of colorectal cancer tumorigenesis in the Drosophila adult midgut highlight a role of Dpp pathway in tumor suppression.

10:30-10:45 José Belo (University of Algarve, Portugal)
Integrative studies on the role of Ccbe1 in cardiogenesis: from the embryo to ES cell derived cardiac tissue.

10:45-11:15 – Coffee break

Session 6 – Evolution and Development
Chairs: Jordi García-Fernández/ Ramón Muñoz-Chapuli

11:15-11:45 – Invited speakers: Patricia Beldade (Gulbenkian Institute of Science, Oeiras, Portugal)
Evo-Devo: the genetic and environmental components of variation and diversification in phenotype. 
Sponsored by SPBD

11:45-12:15 – Invited Speaker: Pilar Cubas (CNB, CSIC, Madrid)
The evolution of BRANCHED1 genes and branching patterns of Solanaceas.
12:15-12:45 – Talks chosen from abstracts

12:15-12:30 James Cast.-G. Hombría (CABD, CSIC-UPO, Sevilla)
Evidence for the common origin of trachea and endocrine organs from a segmentally repeated ectodermal precursor.

12:30-12:45 Yacine Graba (IBDML, CNRS/AMU Marseille, France)
A C-TER plastic extension of the homeodomain provides a novel mode of HOX-PBC interaction.

12:45-14:15 – Lunch

Session 7 – SEBD-SPBD-SDB Postdoctoral Symposium
Chairs: Virginio García-Martínez/ Marian Ros

14:15-16:15 – Postdoctoral speakers

Selected by SEBD

14:15-14:35 Andrés Garelli (Institute for Neurosciences, CSIC-UMH, Alicante)
DILP8 synchronizes developmental timing with growth.

14:35-14:55 Manuel Irimia (University of Toronto, USA)
Muscleblind-like proteins regulate embryonic stem cell-specific alternative splicing and reprogramming.

Selected by SPBD

15:55-15:15 Claudia Gaspar (Institute of Molecular Medicine Lisbon, Portugal)
Understanding cell fate decisions in the embryonic neural retina.

15:15-15:35 José M. Inacio (University of Algarve, Portugal)
The dynamic right-to-left localization of Cerl2 regulates and terminates Nodal activity in the mouse node.

Selected by SDB

15:35-15:55 Elizabeth Rideout (University of Calgary, Canada)
Drosophila Maf1 Controls Body Size and Developmental Timing By Modulating tRNAiMet Synthesis and Systemic Insulin Signaling.

15:55-16:15 Sunjin Lee (Yale University, New Haven, USA)
Forward genetics identifies Edf1 as a novel regulator of epidermal development and stem cell quiescence.

16:15-16:45 – Coffee break

16:45-17:45 – EMBO lecture: Claudio Stern (University College London, UK)
From cells to embryo: the magic of gastrulation

18:00 – SEBD/SPBD General Assembly

19:00-20:30 – Visit to Granada

20:30-23:00 – Dinner
Principal Lectures
The Molecular Circuitry of Heart Development, Disease and Regeneration

Main author
Author: Eric Olson

Affiliations
1.- UT Southwestern Medical Center

We seek to define the gene regulatory networks that govern cardiovascular development and disease. Recently, we discovered that the hearts of neonatal mice can fully regenerate after partial surgical resection, but this capacity is lost early in life. We are currently exploring the molecular underpinnings of the neonatal regenerative response of the heart, with the long-term goal of discovering combinations of genes and drugs that promote cardiac repair and regeneration. We are also optimizing strategies for reprogramming of cardiac fibroblasts toward a cardiomyocyte cell fate as a means of replacing heart muscle following myocardial infarction. We have shown that four transcription factors can cooperatively reprogram fibroblasts into beating cardiac-like myocytes in vitro. Forced expression of these factors in dividing non-cardiomyocytes in mice reprograms these cells into functional cardiac-like myocytes, improves cardiac function and reduces adverse ventricular remodeling following myocardial infarction. The signaling pathways and transcriptional networks that control cardiac development and disease are intertwined with a collection of microRNAs (miRNAs) that act as negative regulators of gene expression. We have identified miRNAs associated with diverse cardiovascular disorders, including cardiac hypertrophy, heart failure, myocardial infarction, and angiogenesis. Gain- and loss-of-function studies in mice have revealed striking functions for these miRNAs in numerous processes, such as the control of muscle regeneration, myosin expression, fibrosis, myocyte survival, and metabolism. Identification of miRNA targets has uncovered new mechanisms and regulators of cardiovascular development and disease. Disease-inducing miRNAs can be persistently silenced in vivo through systemic delivery of miRNA inhibitors, allowing for therapeutic modulation of disease mechanisms. Opportunities for manipulating miRNA biology in the settings of cardiovascular disease and regeneration will be discussed.
Invited Speakers
Session 1 – Developmental Genomics
The generation of distinctive cell types that form different tissues and organs requires precise, temporal and spatial control of gene expression. This depends on specific *cis*-regulatory elements distributed in the non-coding DNA surrounding their target genes that became active or inactive at particular developmental stages. On the top of this, the 3D structure of the chromatin plays an essential role in facilitating the access of such *cis*-regulatory elements to particular promoters. In this seminar, I will discuss the importance of the chromatin architecture and the dynamic of *cis*-regulatory elements during development and its implication in human diseases and genome evolution.
Session 2 – Tissue Patterning and Differentiation
Development of the cardiac building plan

Author: Antoon FM Moorman

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The vertebrate heart has undergone remarkable structural and functional evolutionary changes. One important event was the independent evolution in mammals and birds of high blood pressures, high heart rates and fully divided hearts from a reptilian ancestor with low pressure, low heart rate and an undivided ventricle. The high heart rates of mammals and birds are only possible because their hearts are endowed with a well-developed cardiac conduction system that ensures an appropriate delay between atrial and ventricular contractions as well as a fast activation of the ventricle. How did a similar conduction system evolve independently in mammals and birds? Because there is no anatomical indication of a conduction system in extant reptiles and other lower vertebrates, this question has bothered comparative biologists for the past century. To address this issue we studied transcription of genes central to the development of the mammalian and avian cardiac conduction system in lizards (Anolis carolinensis and A. sagrei), frogs (Xenopus laevis) and fish (Danio rerio); Nppa, Bmp2, Tbx2, Tbx3, Gja5. The transcription domains in the formed hearts of these ectotherms were found to be comparable in form to those of embryonic mammalian and avian hearts. We then optically mapped the spread of the activating action potentials over the adult cardiac ventricles with the voltage-sensitive fluorescent dye di-4-ANEPPS. The patterns of activation in fish (Danio rerio), frogs and lizards were found to resemble those of embryonic mammals and birds before the formation of the compact walls, the interventricular septum and maturation of the specialized conduction system. We conclude that a slow-conducting atrioventricular canal and fast-conducting trabecular ventricular wall represent the building blocks of the atrioventricular conduction system in vertebrates. Only mammals and birds acquire a dramatically thickened compact myocardium to take over pumping, allowing the trabecules to differentiate into the His-Purkinje system making their uniquely high heart rates possible.
Kidney podocytes and Drosophila nephrocytes have developed a highly specialized cellular junction, the slit/filtration diaphragm, which functions as a size selective barrier during the processes of blood and haemolymph filtration. There are striking similarities at the ultra-structural, molecular and functional level between both structures. Furthermore, in vertebrates it has been shown that post-translational modifications of the major components of the slit diaphragm, nephrin and NEPH1, regulate diaphragm behaviour by modifying the activity of several signalling pathways. Here I present evidence that some regulatory mechanisms affecting filtration diaphragm constituents are also conserved in Drosophila. We find that phosphorylation of Dumbfounded (Duf), the orthologue of NEPH1, is important for the maintenance of the nephrocyte filtration diaphragm. Moreover, the identification of the Src kinase involved in Duf phosphorylation and the study of Src loss-of-function and gain-of-function phenotypes indicate that the activity of these kinases has to be tightly regulated in order to maintain the integrity of the nephrocyte's diaphragms. I will discuss the significance of these findings together with additional data that reveal the suitability of nephrocytes as a novel model to study podocyte biology and podocyte-associated diseases.
Session 3 – Cell Adhesion and Migration
Actomyosin Cable Formation During Wound Healing Involves Highly Dynamic Cytoskeletal Changes And Calcium Signalling

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Epithelial wound healing relies on tissue movements and cell shape changes. Our work shows that immediately after wounding there is a dramatic cytoskeleton remodelling consisting in a pulse of actomyosin filaments that assemble around the wound edge and flows towards the margin of the wound. We show that this actomyosin flow is regulated by Dia and Rok and that it elicits a wave of apical cell constriction that culminates in the formation of the leading edge actomyosin cable, a structure that is essential for wound closure. Calcium signalling plays an important role in this process as its intracellular concentration increases dramatically immediately after wounding and downregulation of TRPM, a stress activated calcium channel, also impairs the actomyosin flow. Lowering the activity of Gelsolin, a known calcium activated actin filament severing protein also impairs the wound response, suggesting that the remodelling of the cytoskeleton starts by cleavage of the existing actin filament network.
Session 4 – Modelling & Systems Biology
Transcriptional Precision in the Drosophila Embryo

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We are interested in mechanisms of transcriptional precision, namely, how are complex patterns of gene expression reproducibly deployed in the different embryos of a population? Whole-genome assays have identified two potential mechanisms of precision, “shadow” enhancers and paused RNA polymerase (paused Pol II). I will present evidence that the snail shadow enhancer helps ensure a normal pattern of activation within the presumptive mesoderm of embryos grown at elevated temperatures. Paused Pol II appears to foster synchronous activation of gene expression within the different cells of a tissue. Replacing the paused snail promoter with a nonpaused promoter results in stochastic activation of snail expression and a curious bistable mutant phenotype: 20% of the embryos exhibit normal invagination of the mesoderm during gastrulation, while 80% fail to gastrulate and exhibit the sna- mutant phenotype. I will attempt to explain the basis for this gastrulation bistability.
One classic paradigm in developmental biology is Wolpert's French-flag model of stripe formation: at high, middle or low concentrations of a morphogen, a “blue”, “white” or “red” gene stripe is activated, respectively. A complete understanding of morphogen gradient systems will require going beyond a case-by-case analysis of real morphogen interpretation mechanisms by mapping out the exhaustive “design space” of gene regulatory networks. Our collaborators recently generated the first computational atlas of design space for stripe-forming gene networks (J. Cotterell and J. Sharpe, *Mol Syst Biol* 6, 425, 2010). All possible topologies of networks consisting of three genes were simulated with the condition that one of the genes was activated by the morphogen. Using 30,000 randomly chosen parameter sets for each topology, the desired output was a single stripe of gene expression. 471 gene networks were able to produce a stripe in a noise-robust fashion. We are now applying a variety of systems and synthetic biology approaches to explore these predicted networks using multicellular experimental systems, combined with computational modelling.
Workshop on imaging (Sponsored by Leica)
Super-resolution light microscopy using Stimulated Emission Depletion (STED) and Ground State Depletion (GSD) methods

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In the last years, several light microscopy methods have managed to provide image details of microscopy samples below the diffraction limit (super-resolution microscopy). Most of these techniques use different forms of switchable behaviours of fluorophores to either either shrink the detection volume of a scanned beam (STED) or to improve the localization accuracy by pinpointing sparse signals with high precision.

We have studied different fluorophores for use with a STED microscope with an orange depletion laser to understand their potential as dyes for super-resolution. Compared to the spectral range available for conventional confocal microscopy, the choice of fluorophores is constrained to a more narrow spectral window that is limited by the shortest possible excitation (in our case 458 nm) and the very intense depletion laser light at 592 nm. Additionally, the chosen fluorophores have to be susceptible to stimulated emission at the wavelength of the depletion laser, while ideally not being directly excitable by it, neither in the ground nor in the excited state.

Even within the given limitations many dyes and fluorescent protein combinations can be used for multicolor STED imaging. We have evaluated several commercial green standard dyes (Alexa 488, Oregon Green 488, Atto 488) and spectral variants (BD 500, Atto425, Oregon Green 514) that have already been tested for continuous wave (CW) STED in addition to several new candidates in terms of their depletion efficiency, their photostability under depletion conditions and their excited state lifetimes. The characterization of the properties of these dyes provides valuable information for the comparison of the performance of fluorophores for superresolution imaging (“STEDable dyes”) and serves as a guideline for fluorophore selection for different STED applications like multicolor or in vivo CW STED imaging. Also, a fuller understanding of the individual fluorophores’ properties allows specific recommendations for the optimization of STED imaging conditions.

STED works on the principle of efficient depletion of the excited state of fluorophores. Of the localization based super-resolution approaches, Ground State Depletion (GSD) also works with a depletion approach, pushing fluorophores away from the ground state into transient dark states by strong illumination and then pinpointing single molecules as they return from the dark state at different times. This behavior also depends strongly on the fluorophore properties and the environmental conditions in the sample and we have studied the behavior of several dyes to determine improved imaging conditions.
Numb/Numbl-Opo antagonism controls retinal epithelium morphogenesis by regulating integrin endocytosis

Thematic area: Stem Cells

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Summary: Polarized trafficking of adhesion receptors plays a pivotal role in controlling cellular behavior during morphogenesis. Particularly, clathrin-dependent endocytosis of integrins has long been acknowledged as essential for cell migration. However, little is known on how oriented integrin trafficking contributes to the morphogenesis of epithelial tissues. In our laboratory we studied how the transmembrane protein Opo, previously described for its essential role during optic cup folding, plays a fundamental role in this process. Through interaction with the PTB domain of the clathrin adaptors Numb/Numbl via an integrin-like NPxF motif, Opo antagonizes Numb/Numbl function and acts as a negative regulator of integrin endocytosis. Accordingly, numb/numbl gain-of-function experiments in teleost embryos mimic the retinal malformations observed in opo mutants. Taking advantage of the polarized architecture of the vertebrate retina, we studied the folding of the optic cup as a model system for epithelial basal constriction. We propose that the developmental regulators Numb/Numbl and Opo are essential components of the endocytic machinery directing the basal constriction that shapes the vertebrate retina epithelium.
The early embryonic vertebrate spinal cord (the neural tube, NT) is an excellent model to study basic morphogenetic events since it is composed of proliferating neural stem cell precursors and functionally differentiating neurons. We study the role of extracellular signals and the genetic networks that control cell numbers and cell identity during the embryonic development of the NT, focusing our attention in the activity and the integration of three main signalling pathways: Sonic hedgehog, the Wnts and the Bone Morphogenetic proteins, in the acquisition of different cell identities along the dorsal-ventral axis. These same factors are re-used to control growth of the neural tube, and we recently demonstrated that the Sonic hedgehog activity is required upstream of Wnt signalling to control proliferation of neural stem cells. Whether these growth factors equally regulate the proliferative (P-P) or the neuron-generating divisions (either P-N, or N-N) is currently being investigated. To that end, we have generated molecular tools to unequivocally identify the three different types of divisions in the developing chick NT and to in vivo follow their behaviour.
Session 5 – Stem Cells
Regulation Of Entry Into The Myogenic Programme In Stem Cells In The Embryo And In Quiescent And Activated Satellite Cells In The Adult

Thematic area: Stem Cells

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Pax transcription factors play important roles in the regulation of organ and tissue development (1). In the case of skeletal muscle, Pax3, together with its orthologue Pax7, when it is expressed, controls the entry of cells into the myogenic programme in the embryo (2). Once a myogenic determination factor, such as Myf5, is expressed, cells differentiate to form muscle fibres. In the adult, satellite cells, present under the basal lamina of fibres, are the progenitor cells responsible for regeneration (3). These cells are marked by the expression of Pax7 and, in many muscles, also Pax3. However it was shown recently that these genes are not essential for the formation of new fibres (4). Unlike the Pax-positive muscle progenitor cells of the embryo, most satellite cells already transcribe the myogenic determination gene, Myf5, however without activation of the myogenic programme. We show that microRNA-31 regulation of Myf5mRNA, through specific sites in the 3' UTR, together with sequestration of this microRNA with Myf5mRNA in ribonucleoprotein particles, characterises the quiescent satellite cell. On activation, this post-transcriptional repression is released and the cell rapidly enters the myogenic programme (5). We therefore propose a model in which post-transcriptional mechanisms hold quiescent stem cells poised to enter a tissue specific differentiation programme.


Developmental molecules for adult stem cells in mammalian neurogenic niches

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A characteristic feature of all stem cell populations in long-lived metazoans is their capacity to balance self-renewal with differentiation during lifespan. Capacity for self-renewal endows stem cell populations with regenerative potential but also underlies susceptibility to neoplastic transformation and, therefore, knowing the molecular regulation of this property is essential to both stem cell and cancer research. One approach to the understanding of self-renewal is to analyze the mechanisms that regulate the maintenance of normal stem cells in their natural environment. The adult brain subependymal zone (SEZ) is a very active neurogenic niche in which a relatively quiescent population of radial glia/astrocyte-like GFAP+ neural stem cells (NSC) continually produce new neurons and oligodendrocytes, via a population of rapidly-diving transit-amplifying progenitor cells. Although some intrinsic determinants are known to regulate stem cell division, the observation that stem cells can respond to excessive cellular demand in pathological situations or after traumatic injury suggests that they have ways to increase their number in response to external signals. Within the specialized microenvironments in which stem cells reside, vascular elements appear to play an important role in the regulation of stem cell self-renewal vs. commitment, both under normal and pathological conditions but the signalling pathways involved are still under investigation. We will present our most recent data on intrinsic and extrinsic regulators of NSC maintenance, activation, and homing. In particular, we will comment on new results on the effects of neurotrophic factors, such as neurotrophin-3, and cell-adhesion molecules like N-cadherin.
Session 6 – Evolution and Development
Evo-Devo: the genetic and environmental components of variation and diversification in phenotype

Thematic area: Evolution and Development

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The diversity of shapes and colors in living organisms results from the reciprocal interactions between developmental and evolutionary processes. This diversity is the product of natural selection acting on phenotypic variants produced by development, and it has many compelling examples in insects. Despite being universal, until recently variation was seen more as a nuisance in experimental biology, where research typically focused on single (often inbred) laboratory strains of a handful of model organisms kept in constant (often very unnatural) laboratory environments. This situation is changing with the expansion of new disciplines that focus specifically on the genetic basis of intra-specific variation and inter-species diversity, and on the role of the external environment in organismal development. My research uses butterfly wing patterns to explore the mechanisms involved in producing variation and diversity. I will discuss the contribution of genetics and environment to adaptive variation in this trait.
**Invited Speakers**

**Thematic area:** Evolution and Development

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The evolution of BRANCHED1 genes and branching patterns of Solanaceae

In angiosperms, shoot branching greatly determines overall plant architecture and affects fundamental aspects of plant life. Branching patterns are established by genetic pathways conserved widely across angiosperms. However, despite this general conservation, a great diversity of branching patterns is found in angiosperms. In *Arabidopsis thaliana* (Brassicaceae, Rosidae) *BRANCHED1 (BRC1)* plays a central role in this process, acting locally to arrest axillary bud growth¹. This gene encoding a TCP transcription factor², is a putative target gene for selection during the evolution of new branching patterns. To investigate the relevance of the molecular evolution of *BRC1* genes during the evolution of branching patterns, we have isolated and analyzed the function of *BRC1*-like genes in two Solanaceae species with branching patterns divergent from those of Arabidopsis, tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*). We have found that a duplication of the *BRC1* gene has taken place in these species and we are analysing their function³. Our current view of the molecular evolution and functional divergence of these two gene paralogs will be presented.

Talks choosen from abstracts
Session 1 – Developmental Genomics
Genomic architecture in heart development: exploring the role of CTCF

abstract ID: 79

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The central question in developmental biology is to understand how a single cell becomes a complex multicellular organism. For this to occur, gene expression must be highly and tightly controlled in time and space. The information regarding the genes that need to be expressed at a certain time and place is coded in regulatory elements that sit throughout the genome. The distribution and location of these elements are going to define on which genes they can act. This is one of the reasons why the genome needs to be exquisitely organized in three dimensions. CTCF, an 11 zinc finger protein, has been recently associated at multiple levels in this process, as it can act as an insulator factor, separating adjacent genomic regulatory blocks, a looping factor that brings together proximal and distal gene regulatory elements, and an enhancer-promoting factor. We are interested in understanding how genome architecture is involved in early development. Here, we focus our attention in heart development and in order to decipher the role that CTCF plays here, we are specifically deleting the gene in cardiac tissue by using a conditional *Ctcf* allele and tissue-specific Cre drivers. When doing so, the embryos die at stage E13. As a first approach to understand the underlying defects we are analyzing by *in situ* hybridization at E9.5 and E11.5 the expression pattern of genes that could be deregulated by the loss of genomic structure due to lack of CTCF. More specifically, we are studying genes that are organized in tandem on the genome, that show divergent expression pattern in the developing heart, and that are separated by stable CTCF binding sites. Genes that show these features include transcription factors of the *Irx* and *Tbx* families. Preliminary data of this analysis will be presented.
Epithelia act as barriers protecting the body from the outside environment. Animals ranging from insects to mammals respond to epithelia wounding by triggering a complex process including clotting and re-epithelization. Although multiple studies have been carried out to understand the process of healing, it is still puzzling why some wounds fail to heal. No major functional genomic analyses have been done in a genetically amenable organism. Drosophila represents a good in vivo model to study this process.

Drosophila imaginal disc are composed of two layers of epithelia, a columnar (CE) and a squamous epithelium (PE). When imaginal discs are cut, they heal and regenerate in the abdomen of adult flies or in culture. CE and PE cells near the wound emit filopodia, proliferate and migrate towards the gap, zipping up by a contractile actin cable. This process is dependent on the JNK cascade on those cells engaged in healing. To identify those genes and pathways involved in healing in imaginal discs, we performed a genome wide gene expression analysis. JNK activity positive (healing) cells were compared to sisters and GO tools were used to single out potential regulators.

Relevant genes showing significant upregulation in healing were functionally assayed by RNAi in two processes, the fusion of imaginal discs, which resembles healing and also depends on JNK and healing itself. More than 300 RNAi’s were tested for disc fusion and those showing closure defects (failing on migration/polarity) were tested for repair. A short list showed robust phenotypes in both assays. We then concentrated in potential regulators of the actin cytoskeleton. They include Act42A itself, Scarface, Capping Protein beta and two subunits of the chaperonin TCP-1 complex. These last two highlight a novel mechanism of actin dynamics regulation by protein folding. Further a functional characterisation of this chaperone complex has been done to study the novel role of this complex in actin synthesis, folding and wound healing. Their detailed analysis and potential interactions will be presented.
Session 2 – Tissue Patterning and Differentiation
Loss of anterior-posterior limb polarity underlies digit reduction in cows

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Introduction

Bovine limbs exemplify the skeletal diversification and digit reduction that occurred in the artiodactyl clade. We have used mouse and bovine embryos to identify and study the functional relevance of the molecular alterations in the spatio-temporal expression of key regulators of vertebrate limb bud development.

Results

This analysis revealed that anterior-posterior (AP) asymmetry, signaling centers and feedback loops are established alike in mouse and bovine limb buds. However, the initial AP asymmetry was lost during outgrowth as evidenced by the rather apolar expression of several markers including Hoxd13 and Grem1 during progression of bovine limb bud development. Genetic analysis in the mouse has shown that up-regulation of Ptch1 expression by the mesenchymal cells responding to SHH is crucial to normal limb bud development. In contrast to the mouse, Ptch1 expression is not upregulated in the posterior-distal mesenchyme in bovine limb buds in spite of normal Shh expression. As Gli1 expression is upregulated but expressed more uniformly in bovine limb buds, this pointed to a rather specific alteration of SHH signal transduction that affects mesenchymal Ptch1 expression. It is possible that the cis-regulatory region(s) that control the up-regulation of Ptch1 in response to SHH signal transduction in the limb bud mesenchyme diverged during evolutionary diversification of bovine and other artiodactyls. This hypothesis was assessed by deep-sequencing of the Ptch1 locus from different artiodactyl species (bovine, sheep, goat, pig). Indeed, several artiodactyl-specific variations in candidate cis-regulatory regions were identified, while no significant alterations were detected in cis-regulatory regions that control Ptch1 expression in structures other than the limb bud mesenchyme. The functional relevance of this Ptch1-specific loss of mesenchymal responsiveness to SHH signaling was evidenced by analyzing mouse embryos lacking Ptch1 specifically in the limb bud mesenchyme. Molecular analysis revealed striking similarities in the expression of several key genes in bovine and mouse Ptch1 mutant limb buds.

Conclusions

The failure to sense the SHH morphogenetic signal by up-regulating Ptch1 in the limb bud mesenchyme and associated loss of anterior-posterior polarity is a likely key alteration that underlies the loss of digits and identities in the artiodactyl clade.
The Shh binding protein Cdon is required for vertebrate optic vesicle patterning and morphogenesis

abstract ID: 8

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Hedgehog (Hh) family members activate an evolutionarily conserved signal transduction pathway crucial for embryonic development and adult tissue homeostasis. Pathway activation is initiated by binding of the ligand to the seven-pass transmembrane protein Patched (Ptc) but recent studies have demonstrated that other membrane-associated proteins, including Cdon (cell-adhesion-molecule-related/down-regulated by oncogenes), can bind Hh proteins and cooperate in Hh mediated signaling. Consistent with its proposed function as a Hh co-receptor, genetic inactivation of Cdon in mice causes holoprocencephaly (HPE), a human congenital anomaly defined by forebrain midline defects that is often due to mutations in genes of the Hh pathway and hence to diminished signaling activation. HPE is also frequently associated to multiple eye defects but whether Cdon is relevant to vertebrate eye development is still poorly explored.

To address this issue, we investigated Cdon distribution during zebrafish and chick early eye formation and interfered with its expression using injections (zebrafish) or electroporations (chick) of specific antisense oligonucleotides (morpholinos, MO). The resulting phenotypes were analyzed using immunohistochemistry or in situ hybridization with tissue and cell specific markers.

In both chick and zebrafish embryos, Cdon is expressed in the early axial mesoderm and then in the presumptive neural retina and dorsal forebrain, among other regions. Previous studies have demonstrated that loss of Hh function causes a cyclopic phenotype with reduction of the optic stalks. In contrast, knockdown of Cdon activity caused a marked expansion of the expression of optic stalk markers, such as Pax2 and Fgf8 and interfered with optic fissure closure (a defect known as coloboma). These defects were also associated with an abnormal nasal temporal patterning of the optic cup. Because alterations of Fgf signaling have been associated to an abnormal nasal-temporal patterning of the optic vesicles, we are currently attempting to rescue the Cdon morphant phenotype with pharmacological blockade of both Hh and Fgf pathways.

Recent studies in Drosophila have suggested that Cdon homologs (Ihog/Boi), besides acting as Hh coreceptors, can also limit Hh diffusion thereby interfering with the expression of Hh target genes. This raises the possibility that Cdon may act in a similar way during optic vesicle patterning. To address this possibility, we have designed splice-site specific MO, which efficiently remove Shh or Ptc interacting domains in the Cdon protein. Notably, abrogation of Cdon-Ptc interaction seems to have no effect whereas lack of Cdon–Shh binding causes coloboma and Pax2 up-regulation.
Thus, our data point to an important role of Cdon in eye formation. They also suggest that, as in *Drosophila*, Cdon might be needed to limit Shh signaling, which, in turn, regulates Fgf8 expression.

Acknowledgements

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References


4- Yan D *et al*. Development. 2006.
Neoblasts And Jnk, Two Fundamental Elements In The Planarian Exquisite Regeneration Capacity

abstract ID: 129

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NEOBLASTS AND JNK, TWO FUNDAMENTAL ELEMENTS IN THE PLANARIAN EXQUISITE REGENERATION CAPACITY.

Introduction
Regeneration of missing tissues requires precise mechanisms to detect the missing part and respond appropriately, with a tight coordination between stem cell proliferation, differentiation, and cell death. The mechanisms that allow the integration of these cellular processes in order to regenerate a well-proportioned organism are still poorly understood. To address this question, we use freshwater planarians, since they can regenerate anew, any missing part of their body and undergo extensive re-patterning and remodeling, to give rise a complete proportioned animal. This amazing regenerative ability relies on the presence of a population of pluripotent adult stem cells (Neoblasts), which, in response to amputation, enter proliferation in a temporally coordinated manner and differentiate in all cell types of the adult planarian.

Materials and methods
In order to better characterize Neoblast biology, a comparative RNA seq (DGE) was performed between Neoblasts and differentiated cells isolated by fluorescent activated cell sorting (FACS).

Results.
The mapping of the 30,000 unique tags obtained from the neoblast population, over the transcriptome of *S. mediterranea* has allowed us to select a number of transcription factors and oncogenic proteins over-expressed in neoblasts. RNAi experiments demonstrate that around half of them are specifically required for neoblast maintenance. Some examples are transcription factors homologous to Serum Response Factor, Forkhead, Prep class homeobox and basic helix-loop-helix proteins. We also found that c-Jun-N-terminal kinase (JNK), a mitogen-activated protein kinase family member, responsible for the transduction of multiple stressing stimuli, was enriched in the Neoblast population. JNK controls essential cellular events, as cell death and survival, differentiation and proliferation.
Here we show that JNK is activated in the wounds and controls the expression of early wound response genes. Although, JNK is highly expressed in Neoblasts, its role is not to maintain their viability, but to act as a temporal control in their dynamics of proliferation by attenuating G2/M transition. In addition, planarian JNK also shows a strong pro-apoptotic role. Thus, in JNK silenced animals, neoblasts can leave the cycle and start making fate decisions, but the unbalance between cell proliferation and cell death rates yields impaired terminal differentiation and prevents the regeneration of a well-proportioned animal.

Conclusions

RNAseq of planarian stem cells versus differentiated cells allowed the isolation of several transcription factors and oncogenic proteins fundamental for proper planarian regeneration. Among them, the functional characterization of JNK support its crucial role in coordinating an essential issue of regeneration: how signaling from a wound is translated at the single cell level and allows them to respond consequently to produce a functional and proportionate new whole organism.
Session 3 – Cell Adhesion and Migration
**Prrx1, A Novel Class Of Emt Inducer In Embryos And Cancer Cells**

**abstract ID:** 108

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**Prrx1, A NOVEL CLASS OF EMT INDUCER IN EMBRYOS AND CANCER CELLS**

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The epithelial to mesenchymal transition (EMT) is a process that converts adherent and polarized epithelial cells into mesenchymal cell that can move individually. While EMT is crucial for the progress of embryonic development, the abnormal reactivation of some developmental programs could induce a pathological EMT in the adult and is related with the acquisition of the invasive properties in epithelial tumours (Nieto, 2011). The main triggers of EMT belong to several families of transcription factors, including Snail1, Zeb and Twist.

We performed expression screening for transcription factors associated with the early mesoderm, a relevant tissue in terms of the EMT in search of new EMT inducers. We found that the paired-related homeobox transcription factor Prrx1 is expressed in a subset of lateral plate mesodermal (lpm) cells that did not express either Snail1 or Snail2, the main EMT inducers expressed during early mesoderm development. Interestingly, ectopic expression of Prrx1, both in chick embryos and in epithelial and cancer cells, results in morphological and behavioural changes compatible with the activation of a full EMT.

*Prrx1* gain of function experiments in zebrafish embryos induces a dramatic invasion phenotype, whereby lpm cells became invasive and they violated the embryonic boundaries to enter the extraembryonic tissues. By contrast, *prrx1* loss of function prevents the migration of the mesodermal cells, failing to colonize their normal territory.

The analysis of a panel of human tumor cell lines indicates that the expression of *Prrx1* correlates with that of *Twist1* in metastatic and highly invasive lines and that both factors cooperate conferring invasive properties to cancer cells.

As *Snail1*, *Prrx1* is induced by members of the TGFβ family in embryos and cancer cells in a Snail-independent manner, pointing to the existence of two parallel and probably non-redundant EMT pathways. Furthermore, it differs from other EMT inducers in that it uncouples EMT from the acquisition of stem cell properties (see also abstract from Corcoles et al.).

Together, our data show that Prrx1 is a new, Snail independent, EMT inducer in embryos and cancer cells.

The Gef Vav Regulates Collective Cell Migration Downstream Of Guidance Receptors By Locally Activating Rac At The Leading Edge

abstract ID: 31

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Cell migration is essential in the development and maintenance of multicellular organisms. Guidance by spatial cues is essential for the migrating cells to reach the correct target tissue. Thus, during wound healing and immune responses cells are guided to site of injury and tumor cells may be guided to target tissues. One of our aims is to understand how guidance signals control cell migration. For this purpose, we use the migration of the border cells (BCs) of the Drosophila ovary as a model system. BCs are a group of 6-8 anterior specialized follicle cells that at stage 9 of oogenesis delaminate and initiate their migration between the germline toward the oocyte (1). This directed cell migration is controlled by the activity of two receptor tyrosine kinases, PVR and EGFR, which are activated in BCs in response to their ligands produced by the oocyte (2). Although there is some evidence suggesting that Rac and its activator Mbc can act as mediators of these guidance signals, the molecular mechanisms by which the activity of these two receptors control Rac are still poorly understood. Recently, we have identified Vav, a guanine nucleotide exchange factor (GEF) for Rac, as putative downstream target of Pvr activation. Loss of Vav function impairs BC migration. Two hybrid and co-IP experiments show that Vav interacts physically with Pvr. Furthermore, we find that stimulation of Pvr in S2 cells induces Vav activation. Finally, we show by FRET analysis that Rac activity is substantially reduced in the absence of Vav and that ectopic Vav activation leads to a non-polarized distribution of Rac activity. Taken altogether, we propose a model in which Vav acts as a signal transducer that couples signalling downstream of guidance receptors to Rac activation during directed cell migration.


Session 4 – Modelling & Systems Biology
Generating Anterior-Posterior Polarity In Cyclorrhaphan Flies: A Story Of Missing Maternal Factors

abstract ID: 5

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In dipterans (flies, midges and mosquitoes), segment determination occurs very early in embryogenesis through a hierarchy of genes activated by maternal factors. These include the anterior determinant *bicoid (bcd)*, found only in Cyclorrhapha or "higher" flies, and also *caudal (cad)*, *hunchback (hb)*, and *nanos (nos)*. Knock-down or mutation of any one of these genes in *Drosophila* results in the loss of segments, and in some cases duplication of anterior or posterior structures at the opposite pole of the egg. However, in none of these single knock-downs or mutants is global polarity lost. In contrast, knock-down of both maternal *bcd* and *hb* results in symmetrical embryos (mirror-abdomen or bicaudal phenotypes) in which global anterior-posterior polarity is lost along with expression of the central gap gene *Krüppel (Kr)*. Similar bicaudal phenotypes can be generated in two other fly species, the hover fly *Episyrphus balteatus* and the scuttle fly *Megaselia abdita*, by knocking down only maternal *bcd*. In *Episyrphus*, this has been explained by a lack of maternal *hb*, a supposed activator of *Kr*. However, in *Megaselia* this factor is present (though maternal *cad* is not) so another explanation must be found. To investigate this we have generated gene knock-downs for each of the maternal factors in *Megaselia* and analysed the resulting gene expression patterns against our wild-type *Megaselia* expression data sets. We identify loss of *Kr* expression via expansion of the *knirps (kni)* domain as the likely cause of the loss of polarity. Furthermore, we identify differences in *cad* and gap gene expression that account for the more anterior plane of symmetry observed in *Megaselia* bicaudal phenotypes. These differences suggest that a number of regulatory changes in the gap gene network have occurred since the divergence of these dipteran lineages. We are testing these inferred changes by performing more knock-down experiments, combined with data-driven modeling of the gap gene system in this species. Finally, we revisit the question of *Kr* activation in *Drosophila* and suggest an evolutionary scenario for the development of anterior-posterior polarity in dipterans.
MicroRNA regulation of naïve and primed pluripotent states

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Two distinct phases of pluripotency have been proposed in the early mammalian embryo, a naïve state found in the epiblast of the 3.5 days post coitum (dpc) mouse embryo and in embryonic stem cells (ESCs) and a primed state, found in the epiblast of the 5.5-6.5dpc embryo and in epiblast stem cells (EpiSCs). These two states of pluripotency are thought to be regulated by different mechanisms as different combinations of growth factors are required for their maintenance both in vivo and in vitro. MicroRNAs (miRNAs) are small non-coding RNAs that repress gene expression post-transcriptionally. In ESC miRNAs are required for proper proliferation and for exit from the naïve pluripotent state, however little is known about their roles in the primed phase of pluripotency. We have found that in contrast to the naïve pluripotent state, miRNAs regulate cell survival of primed pluripotent cells, but are not required for the initiation of differentiation, either in vitro or in vivo. Profiling in embryos from 5.5dpc to 8.5dpc has identified four miRNA families that account for over 75% of the total miRNA content at these stages. These miRNAs show dynamic expression during the initial phases of epiblast differentiation and are likely to be responsible for the defects observed in embryos and EpiSCs lacking miRNAs. This work provides insight into how miRNAs regulate the different pluripotent states and contributes to the understanding of the regulatory networks involved in stem cell homeostasis, pluripotency and differentiation during early embryo development.
Session 5 — Stem Cells
Cell competition in the mammalian epiblast selects cells with higher Myc levels

abstract ID: 43

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The epiblast is the mammalian embryonic tissue that contains the pluripotent stem cells that generate the whole embryo. We have established a method for inducing functional genetic mosaics in the mouse. Using this system we found that induction of a mosaic imbalance in c-Myc expression provokes the expansion of cells with higher c-Myc levels through the apoptotic elimination of cells with lower levels, without disrupting development. In contrast, a homogeneous shift in c-Myc levels did not affect epiblast cell viability, indicating that the observed competition results from comparison of relative c-Myc levels between epiblast cells. During normal development we found that c-Myc levels are intrinsically heterogeneous among epiblast cells, and that endogenous cell competition refines the epiblast cell population through the elimination of cells with low relative c-Myc levels. These results show that natural cell competition in the early mammalian embryo contributes to the selection of the epiblast stem-cell pool.

E6.5 and E9.5 control mosaic embryos
Conserved mechanisms of colorectal cancer tumorigenesis in the Drosophila adult midgut highlight a role of Dpp pathway in tumor suppression

abstract ID: 63

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Conserved mechanisms of colorectal cancer tumorigenesis in the Drosophila adult midgut highlight a role of Dpp pathway in tumor suppression

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Colorectal cancers (CRC) usually arise as benign lesions which progress to CRC due to the accumulation of genetic alterations in oncogenes and tumor suppressor genes. Whereas the series of genetic events leading to CRC have been well established, the precise functions that these alterations play in tumor progression and how they disrupt intestinal homeostasis remain poorly characterized. As mammalian and Drosophila’s intestines share many similarities, we have explored the alterations induced in the Drosophila adult midgut by the combined activation of the Wnt signaling pathway with gain of function of Ras signaling, the two main drivers of the onset of human CRC. Our results show that compound Apc-Ras clones but not clones bearing the individual mutations expand as aggressive intestinal tumor-like outgrowths. These lesions reproduce many of the human CRC hallmarks such as increased proliferation, blockade of cell differentiation and cell polarity, disrupted organ architecture and expression of tumoral markers. Moreover, we have identified a Ras-driven tumor suppression mechanism, and show that the down-regulation of the Dpp/TGFb pathway activity, a feature also present in human CRCs, is essential for tumor growth. Finally, our results demonstrate that these flies suffer a progressive deterioration in intestinal homeostasis, providing a simple readout that could be used in screens for tumor modifiers or therapeutic compounds. Taken together, our results illustrate the conservation of the mechanisms of CRC tumorigenesis in Drosophila, providing an excellent model system to unravel the events that, upon mutation in Apc and Ras, lead to CRC initiation and further progression, and highlight a role of the Dpp pathway as a tumor suppressor.
Integrative studies on the role of Ccbe1 in cardiogenesis: from the embryo to ES cell derived cardiac tissue.

abstract ID: 174

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A central challenge for the future perspective of cardiac regenerative medicine is the generation of large numbers of patient-specific cardiac myocytes.

Ccbe1 encodes a secreted molecule that was firstly identified using an Affymetrix GeneChip differential screen for chick heart precursor cells expressed genes (Bento et al., 2011). In mouse and chick, Ccbe1 is expressed in major cardiac progenitor lineages that contribute to distinct heart structures during heart organogenesis (Facucho-Oliveira et al., 2011). Moreover, analysis of gain and loss of function performed in both mouse and chick embryos showed abnormal cardiac morphogenesis and aberrant chamber formation further elucidating the role of Ccbe1 for cardiac development. Similarly, in mouse and human ES cells, increased levels of Ccbe1 expression were detected after cardiac lineage commitment demonstrating well-coordinated expression of various early and late cardiac specific markers and Ccbe1. Knock-Down in mouse and human ES cells demonstrated the requirement of Ccbe1 for proper cardiogenesis. Modulation of mCcbe1 activity in differentiating mES cells using media supplemented with mCcbe1 recombinant protein has demonstrated a remarkable inductive potential of mCcbe1 to enhance cardiogenesis.

Taken together, this data strongly suggest that Ccbe1 has the ability to direct the expression of cardiac inducers and to control cardiac progenitor expansion in vitro and in vivo, allowing the generation of non-genetically manipulated cardiac cells from a renewable cell source for regenerative cardiovascular medicine.
Session 6 – Evolution and Development
Evidence For The Common Origin Of Trachea And Endocrine Organs From A Segmentally Repeated Ectodermal Precursor

abstract ID: 18

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Segmented organisms present serially repeated homologous organs along the body. Homologous organs may become specialized in some segments to perform specific functions. The divergence of homologous structures normally occurs during their development when the genes required for their morphogenesis are differentially regulated by segment specific transcription factors as exemplified by the Hox proteins.

The tracheal tree of Drosophila is a segmentally repeated epithelial organ. It develops from the invagination of ten ectodermal placodes present from the second thoracic to the eighth abdominal segments. These placodes express two main tracheal regulators: the tracheal (trh) and the ventral veinless (vvl) genes. The segmental vvl expression is not restricted to the tracheal placodes but is present at homologous positions in other segments with placodes that also invaginate. We show that the placode on the maxillary segment is the primordium of the corpora allata, and the one on the labium the primordium of the prothoracic gland, which are two of the most important endocrine organs in the fly. We observed that Vvl is required for the formation of these endocrine organs, and that the activation of vvl in each placode is under the regulation of different Hox proteins: Deformed is required for vvl expression in the corpora allata; Sex combs reduced in the prothoracic gland; and the central Hox genes for vvl expression in the tracheal placodes. We also observe that these endocrine organs transform into trachea when besides Vvl they express Trh.

The development of trachea and endocrine organs initiates with the invagination of the polarized epithelial cells. The morphological divergence occurs shortly after invagination, when activation of Snail in the endocrine primordia induces an epithelial to mesenchymal transition. This is followed by the coalescence of the corpora allata and prothoracic gland primordia that migrate dorsally fusing, first, to the corpora cardiaca and, later, to the contralateral primordium in what becomes the ring gland. Our data are important because: (1) they complete the genetic and developmental description of the ring gland and (2) indicate that the respiratory tracheal organs and two main endocrine glands arose through a process of divergent evolution from an ectodermal repeated structure that lead to extremely different morphological and functional organs.
A C-Ter Plastic Extension Of The Homeodomain Provides A Novel Mode Of Hox-Pbc Interaction

abstract ID: 24

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Protein function is encoded within protein sequence and protein domains, which often serve for protein-protein interactions. Hox proteins are homeodomain (HD) containing, evolutionary conserved, transcription factors, which gain functional specificity through interaction with PBC class proteins. This interaction relies on a short motif preceding the HD, termed the hexapeptide (HX), which is instructive in positioning the HD N-terminal arm into the DNA minor groove.

We have shown that HX-independent modes of Hox-PBC interaction exist and have identified a short peptide involved C-terminal to the HD (1-5). Structural analyses highlight an orchestrating role for a structurally-plastic C-terminal extension of the HD, which provides a topologically constrained contact with the PBC class protein. This contact has a potential for subtle positioning of the HD recognition helix within the DNA major groove, ultimately defining base specific DNA recognition.

This novel mode of Hox-PBC interaction define a complementary mechanism for defining HD/DNA interaction, which together with the HX-mediated interaction accounts for both minor and major groove DNA contacts. The conservation of similarly structurally organized C-terminal sequences in other HD-containing proteins further suggests that the mechanism uncovered may broadly contribute to specify the DNA binding properties of HD containing proteins in general.

REFERENCES

5- Hudry B. et al., PLoS Biology, In press
Session 7 – SEBD-SPBD-SDB Postdoctoral Symposium
DILP8 synchronizes developmental timing with growth

abstract ID: 145

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Developing animals adjust their growth programs and/or their maturation or metamorphosis to compensate for growth disturbances -such as injury or tumor- and ensure normal adult size. Such plasticity entails tissue and organ communication to preserve their proportions and symmetry. We have identified dilp8, a gene encoding a *Drosophila* insulin-like peptide that is autonomously activated in imaginal discs to communicate abnormal growth and postpone maturation. DILP8 delays metamorphosis by inhibiting ecdysone biosynthesis, slowing growth in the undamaged imaginal discs, and generating normal-sized animals. Loss of dilp8 yields asymmetric individuals with an unusually large variation in size and a more varied time of maturation. Thus, DILP8 is a fundamental element of the machinery governing the plasticity that ensures developmental stability and robustness.
Muscleblind-like proteins regulate embryonic stem cell-specific alternative splicing and reprogramming

abstract ID: 147

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Previous investigations of the core gene regulatory circuitry that controls embryonic stem cell (ESC) pluripotency have largely focused on the roles of transcription, chromatin and non-coding RNA regulators. Alternative splicing (AS) represents a widely acting mode of gene regulation, yet its role in the regulation of ESC pluripotency and differentiation is poorly understood. Here we characterized ESC-specific exon networks in mouse and human by analyzing RNA-Seq datasets from several ESCs and a wide variety of differentiated tissues and cell lines. These networks are highly conserved, with nearly half of the alternative exons showing similar ESC-specific regulation. The level of conservation of AS regulation is similar to that for gene expression; however, the genes modulated by each of the two mechanisms are largely non-overlapping, suggesting complementary roles for each regulatory layer. Genes with ESC-specific exons are enriched in cytoskeleton, kinase activity and cell adhesion gene ontology categories. In addition, we provide evidence that many of these exons may have important roles in modulating protein-protein interactions. Finally, by analyzing gene expression patterns of splicing factors, we identified Muscleblind (Mbnl) as a key conserved negative regulator of human and mouse ESC-specific AS. Using functional knockdown experiments followed by RNA-Seq analyses and CLIP-seq data, we show that MBNL proteins regulate approximately half of ESC-specific AS events. Consistent with a central role for MBNL proteins in the core pluripotency circuitry, their knockdown promotes the expression of key pluripotency genes and formation of SSEA1-positive colonies early on during the reprogramming of somatic cells to induced pluripotent stem cells. These results demonstrate the existence of a conserved and tightly regulated ESC-specific exon network in mammalian species, which likely plays numerous important roles in ESC biology.
Understanding cell fate decisions in the embryonic neural retina

abstract ID: 164

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The adult neural retina consists of seven different cell types: retinal ganglion cells (RGC), amacrine cells (AC), horizontal cells (HC), photoreceptors (PR), bipolar cells (BP) and Mueller glia cells (MG), born between embryonic day E11.0 and postnatal day P11. All cell types derive from a pool of multipotent retinal progenitor cells (RPCs) and are born in a conserved chronological sequence. Our aim is to unravel the molecular mechanisms underlying the generation of neuronal diversity in the developing neural retina. The Notch pathway has been associated with cell fate decisions during development, controlling neurogenesis and creating diversity. In the retina, two Notch ligands (Dll1 and Dll4) are expressed in overlapping patterns, with Dll4 being expressed in isolated cells throughout the retina, while Dll1-expressing cells are more abundant and appear in clusters. Neither ligand is expressed in the ciliary margin zone.

To address the function of the two Notch ligands, Dll1 and Dll4, we have been carrying out loss of function studies by conditional deletion of Dll4 and/or Dll1 in the mouse retina during embryogenesis, using a retina specific Cre-driver Chx10:Cre. Our results show that Dll1 and Dll4 exhibit non redundant roles in retinal neurogenesis. Dll1 is mainly involved in the control of RGC generation while Dll4 is necessary for the control of PR, AC and HC fates.

We are also addressing which and how proneural bHLH proteins (Math5, NeuroD, Ptf1a, Ngn2, Olig2, bHLHb5) interact to prime multipotent retinal progenitors (RPCs) into the different cell fates. We have found that different combinations of proneural bHLH genes are expressed not only in RPCs but also in differentiating neurons, overlapping with Dll4 expression.

Our data supports a model in which the simultaneous expression of proneural genes in differentiating retinal neurons is central to their multipotent character, with Dll/Notch signaling acting to generate the observed spatio-temporal pattern of neuronal specification in the retina. Dll1 acts upstream of Dll4 to inhibit RGC fate and control the pool of progenitors while Dll4 acts upon a subset of RPCs to promote the binary fate cell fate decision between PR/AC or HC fates.
The determination of left-right body asymmetry in mouse embryos depends on the interplay of molecules in a highly sensitive structure, the node. These interactions generate an asymmetric signal that is transferred preferentially towards the left side of the embryo. Cerl2 is a secreted 20-kDa protein belonging to the family of TGF-b antagonists, Cerberus/DAN, whose gene transcripts can be detected in the perinodal region at the early headfold (EHF) stage of mouse embryo development. Moreover, Cerl2 knockout mice show a wide range of laterality defects including randomization of Nodal expression in the LPM.

Here, in order to access the contribution of Cerl2 in LR asymmetry mechanism, we show that the localization of this protein does not correlate to its expression pattern. Instead, Cerl2 displays a nodal flow-dependent dynamic behavior that controls the activity of Nodal in the node, and the transmission of the laterality information to the left lateral plate mesoderm. Our results indicate that Cerl2 initially localizes and prevents the activation of Nodal genetic circuitry on the right side of the embryo, and later its right-to-left translocation shuts down Nodal activity in the node. The consequent prolonged Nodal signaling in the node by the absence of Cerl2 affects Nodal expression, and its consequent prolonged activity in the node keeps longer its expression in the LPM.
How growth and size are controlled during animal development is an important question in biology. Several families of conserved cell-cell signaling pathways regulate organ size by controlling cell growth, proliferation and survival. In addition, environmental factors such as nutrients, oxygen and temperature influence tissue and organismal growth during development. The conserved target-of-rapamycin (TOR) kinase is perhaps the best-understood nutrient-dependent regulator of cell metabolism and growth in animals. The key effectors underlying this growth are, however, unclear. Here we show that Maf1, a repressor of RNA Polymerase III-dependent tRNA transcription, is an important mediator of nutrient-dependent growth in Drosophila. We find nutrients promote tRNA synthesis during larval development by inhibiting Maf1. Genetic inhibition of Maf1 accelerates development and increases body size. These phenotypes are due to a non cell-autonomous effect of Maf1 inhibition in the larval fat body, the main larval endocrine organ. Inhibiting Maf1 in the fat body increases growth by promoting the expression of brain-derived insulin-like peptides and consequently enhanced systemic insulin signaling. Remarkably, the effects of Maf1 inhibition were reproduced in flies carrying one extra copy of the initiator methionine tRNA, tRNA_{Met}. These findings suggest the stimulation of tRNA_{Met} synthesis via inhibition of dMaf1 is limiting for nutrition-dependent growth during development.
Forward genetics identifies Edf1 as a novel regulator of epidermal development and stem cell quiescence

Sunjin Lee-Wölfel, Yong Kong and Scott D. Weatherbee

The outermost layer of the skin, the epidermis, plays a key role in animal survival by acting as a barrier to prevent infection and desiccation. Stem cells in the interfollicular epidermis (IFE) undergo a series of cell fate choices during the differentiation program to form a stratified epidermis. The appropriate balance between proliferation and differentiation is crucial for epidermis function, and alterations in this process can cause human diseases, such as psoriasis and skin cancer. However, the factors that regulate cell fate choices of stem cells in the IFE are not well understood. To identify new mediators involved in these processes, we performed a forward genetics screen in mice and identified a novel regulator of skin development, the Epidermal differentiation factor 1 (Edf1) gene. Mice carrying a homozygous mutation in Edf1 develop a hyperproliferative, poorly differentiated epidermis. We have shown that Edf1 function is essential to curb stem cell proliferation and for normal differentiation of their progeny. We further demonstrate that Edf1 and the cell cycle regulator Stratifin (Sfn; 14-3-3s) act together to regulate keratinocyte differentiation and epidermal barrier formation. The transcription factor p63 is a master regulator of epidermal development and strongly expressed in the stem cell compartment. Edf1 mutants, however, exhibit increased levels of p63 throughout the IFE and reduction of p63 dosage in Edf1 mutants rescues many aspects of the phenotype, indicating that Edf1 modulates p63 levels. Together, our findings identify Edf1 as a novel regulator of epidermal stem cell proliferation and differentiation that regulates p63 expression and acts with Sfn to balance these processes.
Posters
Session 1 – Developmental Genomics
The interplay of alternative splicing with protein expression levels of hypertrophy-associated factors during postnatal remodelling of the heart

abstract ID: 3

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Introduction: Expression of two genes of the fetal cardiac program, ankrd1 (encoding cardiac ankyrin repeat domain 1 protein, a transcriptional co-factor involved in cardiac gene expression and stress response) and nppb (encoding B-type natriuretic peptide, a cardiac hormone involved in cardiac myocyte growth and survival), is re-activated during postnatal hypertrophy remodelling, as well as in failing myocardium. Previously, we identified spliced variants of ankrd1 (generated through intron retention) and nppb (generated through exon skipping) which all are expressed in neonatal pig and human myocardium. Objective: The aim of the present study was to evaluate the roles (if any) of spliced ankrd1 and nppb variants in regulation of protein expression levels of these factors in postnatal heart remodelling. Results and Discussion: The intron-retaining ankrd1 transcripts, identified in the pig and human heart, are functionally intact, efficiently translated into protein in Cos-7 cells and exported to the cytoplasm in cardiomyocytes in situ. In the piglet heart, both the intronless and intron-retaining ankrd1 mRNAs are co-expressed in a chamber-dependent manner and co-upregulated in the piglet model of diastolic heart failure (DHF). In these settings, the higher level of intron-retaining ankrd1 transcripts was always associated with a higher ANKRD1 protein content in myocardium. In vivo forced expression of intronless ankrd1 resulted in up-regulation of endogenous intron-retaining ankrd1 variants (but not of the endogenous regular transcript), which, in turn, was associated with enrichment of endogenous ANKRD1 protein products in transfected myocardium thus suggesting a positive feedback loop in posttranscriptional downstream events of ankrd1 expression. The expression of an alternatively spliced variant of pig NPPB, resulting from exon 2 skipping (designated as delta-E2-NPPB), is downregulated in both ventricles shortly after birth, but it is markedly re-activated in myocardium at DHF. Our study provided evidence that delta-E2-NPPB can post-transcriptionally repress both NPPB protein production and secretion in cell-based assays, thereby revealing a novel negative feedback mechanism that might influence the production of normally spliced NPPB in cardiomyocytes in vivo. Inhibition of NPPB protein accumulation-secretion by delta-E2-NPPB did not affect the levels of normally spliced NPPB mRNA suggesting that decreased translational yield of NPPB could either be due to the decreased translatability of the normally spliced nppb message or to the diminished stability of the encoded polypeptide. In addition, exon-skipped nppb transcripts were detected in normal and failing human myocardium, suggesting the existence of similar mechanisms by which levels of normally spliced NPPB might be regulated in patients. Conclusions: In normal and failing neonatal myocardium, both ankrd1 and nppb transcripts are alternatively spliced in a manner which is stress-sensitive, suggesting that balance between spliced variants of these factors plays a role in postnatal heart development and adult heart function.
Global analysis of opo cis-regulatory landscape reveals an early role of chx10 in optic cup morphogenesis

abstract ID: 15

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Global analysis of opo cis-regulatory landscape reveals an early role of chx10 in optic cup morphogenesis.

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Introduction:

Ojoplano (opo) is a vertebrate-specific gene associated in humans with genetic diseases such as hereditary craniofacial malformations and schizophrenia.(1)(2)

This gene encodes a developmentally regulated transmembrane protein, which is essential for the morphogenesis of the eyes and other tissues including the fins, heart and neural crest.(1) Opo is localized in a 2Mb gene desert flanked by insulator sequences, between the genes SLC35B and TFAp2a. This region, synthetic in all vertebrates, locates at the distal region of the chromosome 6p in humans. This locus represents only 2% of the chromosome 6, however it includes 23% of the all conserved cis-regulatory regions in this chromosome.(3)(4)

Methods:

Using bioinformatics analyses we observe that the majority of these putative enhancing sequences are also targets of histone H3K4 methylation and H3K27 acetylation, thus suggesting they may act as enhancers. We perform transgenesis assays in zebrafish for 28 putative regulatory regions and screened for enhancer activity using ZED construct. (5)

Results/Conclusions:

A total of 9 stable transgenic lines showing tissue specific enhancer activity were identified. All the enhancers were highly conserved and the majority shows both H3K4me1 and H3K27ac marks. The sum of their expression patterns, recapitulate opo expression.

In order to explore the functionality of the enhancers, we performed a bioinformatics analysis to search for transcription factor binding sites in the enhancer’s sequences. We observe that H6:1013 opo enhancer has two chx10 motifs and share a common eye expression pattern in specific developmental stages with this transcription factor. Moreover, opo expression levels increase in chx10 overexpressing embryos, at 24hpf. It is known that chx10 plays an essential role in neural
retina specification (6). Its expression at early stages in optic cup, and its binding to the identified opo eye enhancer suggest a contribution of chx10 during retina folding.

We hypothesize that chx10 is a regulator of opo expression, essential for eye development. We aim to confirm physical interactions between H6:1013 enhancer and chx10 by ChIP-qPCR; check that the enhancer activity of H6:1013 depends on chx10 motifs, by deletions in H6:1013 sequence, and use splicing morpholinos to explore the role of chx10 in optic cup morphogenesis.

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CABD – Fish room facility

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References:

Temporal lack of DNA methylation-mediated repression is a universal feature of vertebrate development

abstract ID: 25

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Introduction:

DNA methylation is a major epigenetic modification of vertebrate genomes usually associated with transcriptional repression. Sites of DNA methylation can be bound by methyl-CpG binding proteins that recruit complexes with histone deacetylase (HDAC) activity and promote formation of silent chromatin on targeted loci. Yet, it was recently shown that DNA methylation is uncoupled from transcriptional repression during pluripotency stages in the *Xenopus* embryo. Our work aims at elucidating the function and the cause of this phenomenon in vertebrate organisms.

Methods: MethylCap-seq, Next Generation Sequencing, RNA-seq, quantitative proteomics, DNA affinity precipitation, transgenesis

Findings:

By using global DNA methylation profiling (MethylCap-seq) combined with transgenic approaches we demonstrate that this temporary lapse in epigenetic regulation is conserved across the vertebrate subphylum and that many active genes exhibit strong promoter-proximal methylation. Surprisingly, the majority of the known components involved in DNA methylation-mediated repression pathways are robustly expressed during pluripotency stages suggesting that this differential interpretation of the DNA methylation signal can be explained by stage-specific recruitment of chromatin components. We have therefore set up a strategy to identify DNA methylation “readers” at different stages of early zebrafish development. Essentially, methylated DNA probes are immobilized on magnetic beads and incubated with zebrafish nuclear extracts. The beads are then thoroughly washed and the eluates subjected to quantitative mass spectrometry. This will result in the precise quantification of differences in DNA methylation-specific binding between subsequent developmental stages and provide more insight into the cause of differential DNA methylation interpretation during early development.

Conclusions:

In the present study we demonstrate that DNA methylation marks promoters of active genes some of which are poised for transcriptional repression. Furthermore, we show that this temporal uncoupling of DNA methylation and transcriptional repression is conserved in zebrafish and chick embryos. To identify stage-specific DNA methylation binding proteins we are currently using affinity precipitation assays combined with quantitative mass spectrometry.
Identification and characterisation of new nuclear envelope proteins

abstract ID: 64

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Identification and characterisation of new nuclear envelope proteins.

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The nuclear envelope (NE) constitutes a physical barrier between the nucleoplasm and the cytoplasm in eukaryotic cells. The NE is composed by inner and outer nuclear membranes, each enriched for numerous transmembrane and peripheral proteins. Within the NE nuclear pore complexes (NPC), which are composed of multiple copies of around 30 nucleoporins, are responsible for transport in and out of the nucleus. In addition, the NE plays a critical role in organisation of nuclear architecture by providing an anchoring surface for chromatin. Although the list of NE components has expanded considerably during recent years, it is conceivably that numerous, important NE proteins remain to be identified.

To identify novel NE proteins we are performing a genome-wide RNAi screen for genes that show synthetic lethality with mutations in genes encoding either the nucleoporin Nup50/NPP-16 or the NE transmembrane protein LEM-2. Our screen consists in three steps: (1) genome-wide analysis in liquid cultures in 96-well plates; (2) verification on single NGM plates; (3) second verification step in triplicates. We have completed the first step and will present the outcome of the verification steps at the meeting.

In addition to a detailed analysis of top candidate genes from the screen, our future objective is to study NPP-16 and LEM-2, which are both relatively uncharacterised. Combined with the results of the RNAi screen we expect to describe their role in NE structure and function.

Key words: nuclear envelope, nucleoporins, RNAi screening
PROTEIN KINASE VRK-1 AND ITS ROLE IN CELL PROLIFERATION AND DIFFERENTIATION

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Phosphorylation of proteins is an important regulatory mechanism that controls numerous biological processes. Vaccinia-Related Kinase 1 (VRK1) is a conserved protein kinase that is related to cell proliferation and survival. In mammals, loss of VRK1 leads to sterility and may cause neurological disorders. VRK1 is known to phosphorylate chromatin proteins histone H2A and BAF and transcription factors p53, c-Jun and ATF2. In Caenorhabditis elegans VRK-1 plays critical roles in development of the vulva and uterus, as well as in germ cell proliferation.

In order to characterize the dynamics of VRK1 we have generated several VRK1 single-copy transgenic C. elegans strains and human cell lines. We report here that, like previously described in C. elegans embryos, human VRK1 is nuclear during interphase and is associated with condensed chromosomes in mitosis. Moreover, we have identified a short C-terminal domain of both, C. elegans and human VRK1, which localizes properly to the nucleus and, for human VRK1, is able to bind chromatin during cell divisions. Fluorescence Recovery After Photobleaching (FRAP) analysis suggests transient association of VRK1 with chromatin. Identical kinetics were observed in interphase and mitosis, suggesting VRK1 may interact with the same chromatin protein(-s) throughout the cell cycle.

Our new C. elegans transgenic strains show expression of VRK-1 not only in previously reported cells (neurons, hypodermal cells and vulva precursor cells), but also in the anchor cell (AC), that plays an essential role in vulval development. During C. elegans vulval development the AC fuses with uterine cells to form the utse syncytium. Defects in utse formation cause in adults a protruding vulva phenotype. Using tissue-specific knockdown and rescue strategies, we show that the AC fails to fuse in vrk-1 mutants, most likely due to the loss of VRK-1 from uterine tissue, which is characterized by defects in proliferation and differentiation.
Retinoic Acid (RA), a lipophilic molecule and a metabolite of Vitamin-A (all-trans-Retinol), is a well known morphogen that affects gene transcription and modulates a wide variety of biological processes in vertebrates. The targets of all-trans-Retinoic Acid include a multitude of structural genes, oncogenes, transcription factors and cytokines. In this work we use a unique collection of zebrafish reporter lines generated in our lab to detect tissue specific down stream targets of RA. Here we present preliminary results suggesting that mycb, an oncogene whose misregulation has been related to different types of cancers, is repressed specifically in the branchial arches by RA, situation that leads to a decrease in cell proliferation. By this work we demonstrate the suitability of this collection of zebrafish reporter lines to identify responsive genes to a specific effector. In addition, to better understand the cis-regulation of mycb expression, in particular RA-dependent, we are analyzing the genomic landscape of this gene using Circularized Chromosome Conformation Capture (4C). This way we will identify genome wide DNA sequences that interact with the promoter of mycb which might correspond to elements of the regulatory landscape of this gene. These sequences will be tested for enhancer and silencer activity using transgenesis assays. Here we will present the latest results of this screen.
Regulatory landscape of the vertebrate six2/six3 locus

abstract ID: 69

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ABSTRACT

Six3 and Six2 are transcription factors from the SIX family of homeoproteins. The Six genes play important roles during development controlling patterning, cell proliferation and migration. Across bilaterian evolution, Six3 and Six2 genes have been maintained in very close proximity in the genome despite displaying largely different expression patterns. This suggests that these genes are exposed to different regulatory environments. Using zebrafish as a model, we are trying to precisely define the regulatory landscape for each gene and determine how and why synteny is maintained and differential expression patterns is generated. For this goal, we have used our zebrafish histone epigenomic data to select and test putative enhancers for both genes. We also use circular chromatin conformation capture (4C) derived technologies to elucidate the global chromatin architecture of each promoter. In addition, BAC recombineering will allow us to test the influence of regulatory elements and potential insulators on gene expression and chromatin architecture of the locus.
Animal size is remarkably constant within species although the mechanisms that control this are still unclear. In insects, size-dependent events control the timing of larval molts and establish when the larva is of sufficient size to enter metamorphosis. In Drosophila most of the body growth occurs at the larval stages, during which animals increase in mass approximately 200-fold. Communication between the different organs and tissues is important on the overall larval growth rate and body size, especially in response to the availability of dietary nutrients. The interplay between the insulin and the steroid hormone ecdysone pathways influences this process. Ecdysone is the major steroid hormone in all holometabolous insects responsible for driving the metamorphosis of larval tissues into adult structures. During metamorphosis, ecdysone is essential for upregulating the genes required to control apoptosis and differentiation. In addition, ecdysone directs cell growth and division in many tissues throughout the larval to pupal transition. We are interested in the effect of ecdysone on the growth of larval organs. In larvae without ecdysone, imaginal discs do not reach the proper size, which can be reached when the hormone is exogenously administrated. We are studying how the hormone influences organ size and which are the factors involved.
Stat92e activates transcription through counter-repression

abstract ID: 80

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The JAK/STAT signalling pathway is highly conserved among vertebrates and invertebrates where it is not only required for key developmental processes, but also for homeostasis, with misregulation of the pathway associated with the development of several types of cancer. For this reason, identification of STAT downstream targets has been of the utmost importance. Despite this, the actual molecular mechanism of STAT in the activation of its targets remains unclear.

Drosophila, with a simple JAK/STAT pathway compared to vertebrates, provides a good model to address how STAT is able to regulate gene expression. Although several STAT-activated enhancers have been previously described, little is known about the function of STAT in the activation of these cis-regulatory elements. Previous work showed that, in Drosophila, JAK/STAT is required for posterior spiracle morphogenesis, where it directs the transcription of specific target genes. One of these targets is the cell polarity gene crumbs (crb) that is up-regulated in the posterior spiracle primordia through the direct activation by JAK/STAT of an enhancer localized in an intronic region. The activation of this enhancer exclusively in the posterior spiracles and not in other cells where the pathway is active, provides a model to study gene specific activation by STAT. We have dissected the enhancer and analyzed it genetically and biochemically. We will present evidence suggesting that despite STAT being required for the activity of the spiracle enhancer in its normal genomic context, STAT does not act as a direct transcriptional activator. Instead, we show that other factors provide the spatial specific activation of the crb enhancer with STAT, rather than directly activating the enhancer, being required to counteract the activity of a repressor. To our knowledge, this is the first time that such a function has been proposed for STAT proteins.
Effects of fertilization with DNA fragmented sperm on gene expression of trout larvae

abstract ID: 91

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EFFECTS OF FERTILIZATION WITH DNA FRAGMENTED SPERM ON GENE EXPRESSION OF TROUT LARVAE

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Recent studies have changed our perception about the sperm chromatin role on fertilization and beyond, reporting their contribution to the regulation of gene expression during embryo development. Fish, very prolific and with weak sperm selection mechanisms, facilitates designing of experimental approaches to correlate gamete quality and reproductive outcome. In a previous report we referred that trout larvae obtained with DNA cryodamaged sperm over-expressed 5 from 8 genes related to growth and development as well as the telomerase reverse transcriptase gene (Tert), producing larvae with longer telomeres (Pérez-Cerezales et al 2011). These data are compatible with alterations in the regulation of embryo gene expression. In the present study we promoted DNA fragmentation using a suboptimal procedure of sperm cryopreservation. Eggs were fertilized with fresh (control) and frozen (DNA damaged) sperm. Some embryo batches were treated with 3-aminobenzamide (3AB), an inhibitor of the poly (ADP-ribose) polymerase (enzyme of the base excision repair –BER- pathway), during the first cleavage. The abortions rate was analyzed during embryo development and mRNA was extracted after hatching using the TRIZOL® reagent kit. Transcriptome was analyzed using cDNA microarrays. Results demonstrated that spermatozoa with 10% of DNA fragmented produces viable larvae and that the BER pathway is able to repair at least 10% DNA fragmentation at early cleavage. Blocking of the base excision repair pathway promotes changes in the expression of 7.280 genes, whereas little differences were observed between transcriptomes of larvae from control and DNA damaged spermatozoa. The analysis of these affected genes could help us to understand the actual contribution of sperm chromatin to embryo development.

Keywords: DNA-cryodamaged; gene expression; sperm chromatin
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REFERENCE

Epigenetic control of drosophila imaginal discs regeneration

abstract ID: 99

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Introduction
Regeneration is the ability of an organism to rebuild a body part that has been damaged or amputated and can be studied at the molecular level. Key issues that are common to regenerative events may include: determination of the regenerative capacity, proliferation, dedifferentiation, or targeting of regenerative signals (Poss, 2010). Regeneration induces considerable changes at the transcription level and dynamic changes in the chromatin are needed to establish or reset gene expression programs of cells involved in the process. The switch of transcription state from off to on will be more feasible at flexible chromatin than at inactive chromatin, modulated by the change of balance of PcG and TrxG activities during regeneration. In this study we are trying to understand the role of epigenetic mechanisms of cellular reprogramming during tissue regeneration using Drosophila imaginal discs, which are the larval primordial of adult cuticular structures.

Methods
We induce regeneration in wing imaginal discs by either manual fragmentation, implantation and culture into the abdomen of an adult female or by using a cell death inducible system, based on Gal4/Gal80 and UAS-rpr constructs, to ablate a particular region of the mutant disc.

Results
In previous expression profile analyses of wing discs at different time points of regeneration (after fragmentation and implantation into adult females), we identified members of the JNK and Notch signalling pathways and chromatin regulators as differentially regulated (Blanco et al. 2010). We are now performing a screening for loss of function mutants of chromatin modifiers to gain insight into which genes are involved in the resetting of the cells epigenetic program. We observe a delay in regeneration in some of these mutants and, depending on the time point of cell death induction, we see wing patterning defects, suggesting these genes are important for the survival of wing disc cells and their proper differentiation.

Conclusions
By analyzing different time points we expect to unravel the temporal requirement of chromatin modifiers during regeneration. Understanding how plasticity and identity are controlled during regeneration may help to improve, on the long run, reprogramming strategies.

References
Advances in understanding tissue regenerative capacity and mechanisms in animals.
Poss KD.


Gene expression following induction of regeneration in Drosophila wing imaginal discs. Expression profile of regenerating wing discs.

Blanco E, Ruiz-Romero M, Beltran S, Bosch M, Punset A, Serras F, Corominas M.

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Understanding the evolution of hypothalamic compartments based on gene expression patterns. Insights from chondrichthyanas

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UNDERSTANDING THE EVOLUTION OF HYPOTHALAMIC COMPARTMENTS BASED ON GENE EXPRESSION PATTERNS: INSIGHTS FROM CHONDRICHTHYANS.

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Introduction

During vertebrate brain development, key genes—which mostly code for transcription factors and signaling molecules—, tend to be expressed in longitudinal and transverse domains. In the prosencephalon, transcription factors of the Dlx, Nkx, T-box and Pax gene families seem to be involved in the development of the hypothalamus playing a role in the establishment of different histogenetic compartments and their boundaries. Signaling molecules as sonic hedgehog (Shh) seem also to have a key role in the regionalization of the hypothalamus. The expression pattern of these genes in tetrapods has led to the identification of several compartments in the vertebrate prosencephalon including the preoptic area and hypothalamus.

Methods

We used in situ hybridization and immunohistochemistry to analyze the spatiotemporal expression pattern of key transcription factors (among others; Dlx2, Dlx5, Nkx2.1, Tbr-1, Pax6, Otp, Emx2) and signaling molecules (Shh) within the hypothalamus of developing embryos of the lesser spotted dogfish Scyliorhinus canicula, a small shark currently used as developmental "model organism" among chondrichthyanas (cartilaginous fishes).
Results

As in other vertebrates, these markers lead to the identification of histogenetic compartments in the developing hypothalamus of these basal vertebrates, representatives of the gnathostome ancestral condition. The preoptic (Shh+, Nkx2.1+), alar hypothalamus (Otp+, Dlx2+) and basal hypothalamus (Nkx2.1+ and, at certain extension, Shh+) compartments were identified. In the alar hypothalamus, the expression of Dlx2 allowed the identification of a ventral (suprachiasmatic) compartment and a dorsal (supraoptoparaventricular) compartment (Otp+, Nkx2.1-, Shh-), where the preoptic magnocellular nucleus differentiates. In the basal hypothalamus, the overlapping of Shh and Nkx2.1 expressions may correspond to the compartment that will form the tuberal hypothalamus while in the mamillary compartment there was not Shh expression. Sub-compartments and hypothalamic boundaries were also identified with the help of additional markers. Although there is a great conservation in the identity of these main compartments among vertebrates, differences can also be noted both in the preoptic area and hypothalamus. Sharks possess a preoptic compartment that seems to be typical of gnathostomes, as it is absent in lampreys. On the other hand, the mamillary hypothalamus of sharks seems to be more related to that of the agnathans due to lack of Shh.

Conclusions

The chondrichthyan model seems to be an intermediate form in terms of brain genetic specification between agnathan and osteichthyan lineages. Comparisons of the expression pattern of particular genes among different vertebrates increase our knowledge on brain evolution and developmental programs.

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The role of rex1/zfp42 in preimplantation development pertains regulation of endogenous retroviral elements

abstract ID: 105

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Rex1 is a nuclear Zinc Finger protein. In line with its association to more pluripotent subpopulations of embryonic stem cells (ES) cells and iPS cells, Rex1 is widely used as a marker for pluripotency. The equivalent of ES cells appear during preimplantation development in the Inner Cell Mass of the preimplantation mouse embryo. We study mouse preimplantation development to understand selfrenewal and pluripotency in embryonic stem cells. Although Rex1 expression is an excellent marker for ES cells, we show that REX1 is expressed in both ICM and trophectoderm of the blastocyst. In contrast to the absence of preimplantation phenotypes in loss-of-function models, we show that Rex1 gain-of function negatively interferes with both blastocyst development, and selfrenewal of mouse ES cells.

To identify the transcriptional circuit regulated by Rex1, we have carried out genome-wide chromatin association studies in mouse ES cells using chromatin immunoprecipitation of endogenous protein coupled to high-throughput sequencing (ChIP-Seq). Analysis of the genomic loci identified indicates that REX1 associates with (1) the non-coding RNA Tsix, which regulates X inactivation in ES cells; (2) binding sites that center on or adjacent to endogenous retroviral-like elements (RE). Surprisingly genes associated with the elements identified are not necessarily highly expressed in mouse ES cells, although Rex-1 depletion does alter expression levels. They represent a set of genes whose expression is highly regulated during preimplantation development. Endogenous retroviral-like elements (ERV) are Retro-transposable Elements (RE), which cover about 8% of the mammalian genome. Rex1 depletion in mES caused increased expression of several ERV elements, and REX1 strongly associates in ChIP assays with ERV elements, especially MuERV-L.

Using gain-and-loss of function approaches, we have identified successive waves of gene regulation by Rex1 during preimplantation development in two cell stage embryos (Zscan), 8 cell stage embryos (muERV-L elements) and in the blastocyst. We conclude that gene expression by Rex-1 is mainly related to regulation of retroviral elements and genes controlled in cis by degenerate REs. Expression patterns of the genes regulated by Rex1 in the blastocyst stage highlight a potential role for REX1 in priming genes expressed during preimplantation development for posterior activity in placenta.

References:


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Search for STAT coregulators responsible for tissue specific transcriptional activation in Drosophila

abstract ID: 127

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The JAK/STAT signalling pathway is required for many different functions during development and homeostasis. Despite great interest on this pathway, it is not clear how the STAT transcription factor proteins can achieve cell specific activation of different targets in different cell types. This issue is complicated in vertebrates where seven different STAT proteins exist but is more tractable in Drosophila where there is a single STAT protein.

Several directly regulated STAT target genes have been identified in Drosophila. While some of them are activated in every single cell where the pathway is active, as exemplified by the SOCS36E gene and the x10STAT-reporter gene taken from it; others are only activated in a subset of tissues where the pathway is active.

To understand what gives cell specificity to the STAT transcription factor we have started analyzing two enhancers controlling the expression of the ventral veinless (vvl) gene. Although both are directly activated by STAT, one is expressed in the trachea primordia (vvl1+2) while the other is expressed in the hindgut (vvlds1.5risk) (Sotillos, 2010).

To try to identify STAT cofactors, we analyzed in detail the vvl1+2 tracheal enhancer by using three complementary approaches:

1- Systematic mutagenesis,

2- Bioinformatics approach

3- Yeast-one-hybrid assays.

1- First, to delimitate the main part of the enhancer we subdivided the 680 bp vvl1+2 into three smaller 200bp fragments. While the two external elements (vvlMiniS1 and vvlMiniS3) do not drive trachea expression by themselves, the central part (vvlMiniS2) drives tracheal expression although has some derepression between the tracheal pits. To find the missing repressor elements we have extended vvlMiniS2 by adding small neighbouring cis regulatory sequences to get a minimum enhancer with the complete information. In parallel, the vvlMiniS2 enhancer was systematically mutated every ten base pairs to identify elements that cause changes in its expression pattern.

2- To identify putative binding sites by bioinformatics we used two methods: identification using known matrixes of transcription factors or identification "de novo" by comparing the sequence of the vvl1+2 enhancer with other STAT-controlled enhancers with similar expression pattern described in Sotillos et al, 2010
3- We used a Yeast one hybrid assay using a cDNA library of 0-24h Drosophila embryos to uncover transcription factors that could bind to the enhancer.

We are now testing the expression of the vvl enhancers in mutants for proteins either detected in the yeast one hybrid screen or in the bioinformatics approach to confirm the functionality of the predicted sites.
Function of lines and the odd-skipped gene family in the regulation of abd-b protein activity

abstract ID: 137

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Abd-B hox protein promotes the formation of the posterior spiracles in the dorsal A8 of Drosophila larvae through the activation of a genetic cascade where the first activated genes are sal, ct, ems and upd. Sal is expressed in the cells that will form the external part of spiracle (stigmatophore), while cut, ems and upd are expressed in cells that will give rise to the internal part (spiracular chamber). In the past, it was proposed that Lin protein function as co-factor of Abd-B since in lin mutants Abd-B is normally expressed but it is unable to activate its downstream targets and consequently these larvae lack of posterior spiracles. Here, we propose that Lin regulates the Abd-B activity through the Drm/Lin/Bowl regulatory cassette already described in many tissues. It is known that Lin induces the degradation of Bowl while Drm allows the accumulation of Bowl in the nucleus by translocating Lin towards the cytoplasm. Bowl and Drm are proteins from the Odd-skipped family. It has been described that Odd and Sob, other proteins from this family, also block the degradation of Bowl having redundant function with Drm. We have demonstrated that Bowl is able to repress the Abd-B activity in A8. Moreover, Lin prevents the repression of Abd-B by the degradation of Bowl while Drm allows the accumulation of Bowl in the nucleus by translocating Lin towards the cytoplasm. Since Bowl is not expressed in A8 and all of these observations has been made in overexpression conditions we decided to study the effect of Bowl over the Abd-B activity in a tissue where both proteins naturally coexist. We have observed that Bowl and Abd-B coexist in two groups of cells of the genital discs. Moreover, some of the Bowl positive cells express Dll, which is repressed in the rest of the genital disc by Abd-B. Then, this is the perfect context to analyse the functional interaction between Bowl and Abd-B, and this is one of the main goal of our study at present.

We have also observed that mutants with a deficiency containing drm, odd and sob genes (Df(2L)drmP2) show defects in the stigmatophore of posterior spiracles. Since single mutants of drm, odd and sob have no phenotype, it seems that all of these genes have another redundant function. Moreover, this role seems to be Bowl independent because bowl mutants do not show any phenotype in A8. Since Df(2L)drmP2 larvae have flat spiracles which resemble those of sal mutants we have analysed the expression of sal in A8 of Df(2L)drmP2 larvae. Expression is normal at stage 11 while at stage 14 is affected in the cells of the anterior compartment suggesting that odd-skipped genes are not required for early expression of sal in A8, which is regulated by Abd-B, but they are required for the maintenance of the late expression in the anterior cells of the spiracle primordium. The second objective of our study is to better understand the regulation of sal gene by the odd-skipped genes.
Characterization of the mouse Snai1 locus

abstract ID: 152

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The Snai1 gene is a member of the Snail/Scratch family of Zn finger transcription factors. The members of this family have crucial roles in metazoan development and disease. The Snail members regulate cell-cell adhesion and trigger epithelial-to-mesenchymal transition (EMT). With the exception of the nervous system and epidermis, almost every cell in a metazoan has undergone at least one round of EMT. Expression of the Snail proteins is selectively prevented in the adult stage. The only Snail expression that can be detected in adults corresponds to responses to insult or oncogenic processes. Therefore, the expression of the Snail genes needs to be under a tight control during development and in particular in the adult. We have started the characterization of the mouse Snai1 locus in order to find the control elements that can explain this sophisticated expression control.

Using whole genome analysis and inter-species comparisons we have searched for the boundary elements of the mouse Snai1 gene. Using zebrafish as experimental animal model we have identified regions with insulator/boundary activity that have allowed us to identify the limits of the mouse gene.

We have performed whole gene analysis for active and inactive epigenetic marks. The use of different cell line models, combined with the use of material obtained from selected tissues with differential regulation of the Snai1 gene, has allowed us to obtain a picture of the relevant epigenetic marks deposited in the Snai1 gene.

Taking advantage of transient mouse and zebrafish transgenesis we have been able to test the function of some selected small regions of the Snai1 gene that might contribute to our understanding of the Snai1 gene regulation.
Study of the interaction of environmental and genetic factors, during mouse embryogenesis

abstract ID: 167

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Abstract:
Introduction: Embryopathies observed as a consequence of maternal diabetes, have been highly investigated in experimental and clinical studies. It has been reported that the hyperglycemic state is able to downregulate the expression of non canonical Wnt-PCP pathway’s components (1), like Dishevelled-associated activator of morphogenesis 1 (Daam1). Daam1 is a member of the formin protein family and it is essential for the actin polymerization and cytoskeleton reorganization (2). Daam1 is highly expressed in some organs during mice development, including the eye and the heart. Daam1 knock-down embryos exhibit ocular and heart malformations (3), very similar to ocular and cardiac malformations found in embryos from diabetic mice mothers (4).

Methods: Daam1 expression was analyzed by in-situ hybridation and X-gal staining in mouse embryos between stages E9.5 and E12.5. The diabetes was induced in C57 females mice by streptozotozin injections. Diabetic females were crossed with Daam1gt/+ males and, at stage E11.5, embryos were dissected, genotyped and analyzed by eosin and hematoxilin staining techniques.

Results: During development Daam1 mRNA was localized in the eye in the optic cup, in the optic stalk and in the heart was found in the ventricular and auricular myocardium. This expression pattern was corroborated by X-gal staining. Daam1gtgt and Daam1g/+ embryos presented ocular defects, anophthalmia or microphthalmia, and heart malformations, double outflow right ventricle and interventricular septal defects. Embryos from diabetic mothers developed the same ocular and cardiac defects than in Daam1 mutants. The study of the interaction of these two factors, Daam1 mutation and maternal diabetes, revealed an increase in the number and severity of specific embryopathies.

Conclusion: The inhibition of the non-canonical Wnt-PCP pathway by maternal diabetes seems to the responsible for the ocular and cardiac defects observed in the offspring of diabetic mothers.

Acknowledgements: We would like to thanks Deborah Henderson, Juan Ramón Martinez Morales, Rocío Durán, and Dr. Domingo Acosta. This project was supported by Instituto de
Salud Carlos III project CP08/00111 and PS09/00050 and la Consejería de Salud de la Junta de Andalucía project PI-0438-2010.

Non-canonical wnt-pcp pathway in mouse spinal neurulation

abstract ID: 171

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NON-CANONICAL WNT-PCP PATHWAY IN MOUSE SPINAL NEURULATION

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Introduction

Neural tube closure is a complex developmental process that takes place early during embryogenesis and is a key step in neurulation. In humans, neural tube defects (NTD) constitute the second most frequent cause of congenital abnormalities affecting one in a thousand pregnancies. NTD give rise to syndromes including spina bifida and other lethal defects. In neurulation, the neural plate undergoes shaping by “convergent extension” that involves the rearrangement of polarized cells within the plane of the neural plate. This is a necessary process for the initiation of neural tube closure and it is regulated to a great extent by the non-canonical Wnt-PCP (planar cell polarity) signaling pathway, resulting in a reorganisation of the cytoskeleton of the neural ectoderm. Mouse embryos homozygous for Wnt-PCP gene mutations exhibit craniorachischisis, making difficult to study the role of those genes during the later stages of the neural tube closure. Heterozygous embryos has offered instead better perspectives into the molecular study of NTD. Disheveled-associated activator of morphogenesis 1 (Daam1) and Vangl2 has been described to be important in establishing planar cell polarity and thus functions downstream of the Wnt-PCP pathway.

Methods

*In situ* hybridation technique was used to analysed the expression of Daam1 and Vangl2 in the spinal neural tube closure (posterior neuropore) at different stages of neurulation. Cryosections of the area of interest were immunostained with phalloidin and anti-myosin IIB antibody. Heterozygous “gene trap” Daam1 mice (BayGenomics) were crossed with the Looptail mice (mutant of Vangl2) to produce double heterozygous Daam1+/−; Lp−/−. Yolk sacs were used to genotype the obtained offspring.

Results and Conclusions

Our data shows a clear overlapping of Daam1 and Vangl2 mRNA expression in the midline of the posterior neural tube as development progresses. Cytoskeletal immunostaining
demonstrated a clear cell polarity in the area of interest. Moreover, the double heterozygous Lp/+; Daam<sup>−/−</sup> have a higher incidence of developing spina bifida.

In conclusion, our results suggest that in addition to be crucial for the initiation of the neural tube closure, the non canonical Wnt-PCP signalling pathway, has a role during the closure of the spinal neural tube. Double heterozygous Lp/+;Daam<sup>−/−</sup> mice represent the first spina bifida model due to alterations in the Wnt-PCP pathway. Elucidating the molecular mechanisms underlying NT closure, specially at the posterior spinal site, will lead to a better comprehension of the embryo neural tube development, and will mean the first steps in the identification of the mutations responsible of human spina bifida.

**Acknowledgements:** This project was supported by Instituto de Salud Carlos III project CP08/00111 and PS09/00050, la Consejería de Salud de la Junta de Andalucia project PI-0438-2010 and FPU 2009 Programme from the Spanish Ministry of Innovation and science (MICINN).
Functional analysis of Rex1 during preimplantation development

abstract ID: 178

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Functional analysis of Rex1 during preimplantation development.

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Rex1/Zfp42 is a nuclear protein unique to placental mammals and widely used as an embryonic stem cell marker. Although Rex1 expression is associated with enhanced pluripotency, loss-of-function models recently described do not exhibit major phenotypes, and both preimplantation development and embryonic stem cell derivation appear normal in the absence of Rex-1.

Our analysis of RT-PCR expression and immunostaining of the protein shows that REX1 is present at all stages during mouse preimplantation development, with a mixed and dynamic pattern of nuclear, perinuclear and cytoplasmic localization. Chromatin association seemed to be altered in 8-cell embryos and in the blastocyst we found Rex-1 localized almost exclusively in the nucleus.

We assessed a functional role for Rex1 in vivo using gain-and-loss of function approaches. Embryos with attenuated levels of Rex1 (siRNA injection of zygotes), did not exhibit defects in preimplantation development in vitro. In contrast, overexpression of Rex1 interfered with cleavage divisions and proper blastocyst development, although we failed to detect significant alterations in the expression of lineage markers (Nanog, Cdx2) or preimplantation markers (Stella, Oct4). In accordance with the presence of Rex-1 throughout preimplantation development, we demonstrate changes in gene expression caused by Rex1 loss or gain-of-function at different time points: Zscan4 in 2-cell embryos, muERV-L and in 8-cell embryos. Separately, we show that Rex-1 controls expression of a group of genes regulated through cis-acting degenerated ERV-derived elements during blastocyst development. Combined our results suggest that Rex-1 plays a role as a regulator of gene expression during preimplantation development . We have also detected the presence of REX1 in specific cell types in 11.5E
mouse placenta. Using ChIP assays we show REX1 binding to specific retrotransposon elements, with a different specificity compared to ES.

Transcription of RE (retroviral elements) in mammals is silenced in differentiated cells/tissues by DNA methylation. Coinciding with lower levels of DNA methylation, the expression of ERVs is increased in the germ line, preimplantation embryos and in the placenta. Our results suggest that Rex1 participate in the regulation RE and degenerate retroviral elements during these stages.
Characterization of the cdkn2b gene during zebrafish development

abstract ID: 182

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Cyclin-dependent kinase inhibitor 2B (CDKN2B), is known for its role in cell aging, senescence, proliferation, apoptosis and function as a tumor suppressor. In humans, CDKN2B is in a region where a new risk locus associated with intracranial aneurysms was identified.

In order to overcome the complexity of human genetic etiology, we have studied an ortholog (cdkn2b) of this gene in the zebrafish, a very attractive model system to study vertebrates. Since there is no information about this gene in zebrafish, we started by characterizing the gene in wild-type fish and then did knock-down experiments by injecting a morpholino in fli1a:EGFP zebrafish, a transgenic that has the blood vessels marked with GFP.

The gene is transcribed in all tissues of the fish, but has higher expression in the brain. At the protein level, there is higher expression on the epidermis, especially in the tail. When injecting a morpholino against cdkn2b, an abnormally curved tail is formed, and the caudal vein of the fish is affected.

This is the first report to show the expression of cdkn2b in Zebrafish and its potential importance of it in the correct formation of the tail and the vessels of the fish.
Session 2 – Tissue Patterning and Differentiation
Conditional inactivation of Gata4 and Gata6 leads to pancreatic agenesis

abstract ID: 4

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Introduction: Spontaneous heterozygous mutations in the human GATA6 gene have been linked to neonatal diabetes associated to pancreas agenesis or severe pancreas hypoplasia. In the mouse, GATA4 and GATA6, have been shown to be part of the transcriptional network controlling the formation of endoderm-derived organs, including the pancreas.

Methods: To investigate the contribution of GATA4 and GATA6 to pancreas formation, we have conditionally inactivated these transcription factors in the embryonic pancreatic precursors, marked by Pdx1 expression domain.

Conclusions: Single inactivation of either Gata4 or Gata6 does not have a major impact in pancreas morphogenesis. Remarkably, inactivation of both Gata4 and Gata6 leads to pancreatic agenesis. Gata4/Gata6 compound mutant mice die shortly after birth and display severe hyperglycemia. Morphological defects in Gata4/Gata6 mutant pancreata become apparent as early as embryonic stage 13.5, where the epithelium fails to expand as a result of defects in cell proliferation and differentiation. Expression of multipotent pancreatic progenitors, including Pdx1+ progenitors, is also reduced in the Gata4/Gata6 mutant pancreatic epithelium. We identified two conserved GATA sites in the Pdx1 promoter region that are required for promoter activity in transgenic embryos. Altogether, our data indicate that GATA4 and GATA6 play redundant roles in pancreas morphogenesis and the activity of these transcription factors are crucial for the proper development of the pancreas, likely via activation of Pdx1.

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Median pre-placodal precursors uniquely contribute to the primordium of the adenohypophysis

abstract ID: 6

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Vertebrate sensory placodes arise from a continuous “pre-placodal” domain located at the interface between the anterior neural plate and the prospective ectoderm. Their specification occurs with a well-defined spatial organization, in which the adenohypophysis (AH), olfactory (O) and lens (L) placodes occupy the most rostral position with a medial to lateral order. Previous studies have proposed that cells in the pre-placodal domain share a common ground state, which is then progressively restricted, generating all placodal derivatives. Different cell signaling pathways play a mayor role in this restriction: indeed FGF8 contributes to the specification of the olfactory placode whereas SHH promotes the adenohypophysis fate. Tracing studies in chick embryos have also shown that adjacent pre-placodal progenitors contribute to both O and L placodes, suggesting that placode formation involves lateral migration and/or intercalation. We have recently shown that cells at the median neural border do not undergo medial to lateral intercalation raising the question whether median pre-placodal cells, thought to originate the AH placode, behave in a similar manner.

Using fate map analysis of the rostral pre-placodal domain in gastrulating chick embryos, we confirmed the restricted median location of AH precursors and further demonstrated that this position is already fixed at stage HH5. Notably, from HH6 to HH12, AH-placode progenitors remained restricted to the midline and were never observed intermingling with laterally located O/L placodes. In embryos in which median pre-placodal precursors were ablated at HH4-5, the AH rudiment was largely absent, whereas O/L placodes were apparently normal.

Taken together, these observations support that median pre-placodal precursors behave differently from those positioned in more lateral regions and uniquely contribute to the primordium of the AH. Current studies will test whether these characteristics are molecularly or positionally imprinted and how Shh and Fgf signaling contribute to AH placode behaviour.

Acknowledgments

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References


Wnt/b-catenin signaling during odontogenesis is essential for cell differentiation, structure and expression of signaling factors

abstract ID: 19

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Many signalling pathways and molecules are involved in odontogenesis. The aim of our work is to evaluate the importance of Wnt (Wingless MMTV Integration site) pathway in each stage of dental development.

In order to specifically determine the role of an activation of the Wnt pathway from the morphogenesis stage of tooth development, we used 6-bromoindirubin-3'-oxime (BIO), a specific inhibitor of glycogen synthase kinase 3 (GSK-3). First molars of 14.5 day-old mouse embryos (E14.5) were cultured for 6 or 12 days in the presence or absence of BIO 20µM. Samples were processed for histology, in situ hybridization and scanning electron microscopy.

The 6 days BIO-treated molars showed a delay on development as well as deficiencies in the formation of the inner dental epithelium. To assess the reversibility of this effect, we cultured E14.5 teeth during 12 days, the first 6 days in the presence of BIO and 6 additional days with drug-free medium. These cultured molars showed apparently normal development and dental histology was similar to that observed in controls. Both odontoblast differentiation and dental matrix secretion appeared to have recovered. These results indicate that the action of BIO for 6 days on E14.5 teeth in vitro is reversible. To test whether the delay on development was also present at the adult stage of the tooth, E14.5 first molars were treated with BIO or MetBIO for 6 days, and then explants were transplanted into the testes of recipient mice for 21 days. When comparing control molars with treated teeth, remarkable differences in terms of morphology and mineralization were observed. Finally, in order to evaluate the effect of BIO on Lef1, Msx1 and Bmp4 target genes of the Wnt/b-catenin canonical pathway we performed in situ hybridization on E14.5 first molars. These samples were cultured with 20mM of BIO for 48h and a clear increase in the expression of target genes was observed in the dental mesenchyme when compared to control cultures. To know whether upregulation of Lef1, Msx1 and Bmp4 expression was retained in explants after cease of BIO application, we performed a new set of experiments on E14.5 first molars. We detected that Lef1 expression was rescued after 24h of cease of BIO, but upregulation of Msx1 and Bmp4 expression was maintained. After 48h of cease of BIO the basal expression of Msx1 and Bmp4 was achieved. These results suggest that the target genes of the pathway return to their regular expression when the activity of Gsk3β is restored (Aurrekoetxea et al., 2012).

In conclusion, we report that stimulation of the Wnt/b-catenin pathway at a morphogenesis stage of development is sufficient to produce significant changes in embryonic and adult tooth development.
Cells derived from the coelomic epithelium contribute to multiple gastrointestinal tissues in mouse embryos

abstract ID: 20

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Gut mesodermal tissues originate from the splanchnopleural mesenchyme. However, the embryonic gastrointestinal coelomic epithelium also gives rise to mesenchymal cells, whose significance and fate are still little known. We have investigated the contribution of coelomic epithelium-derived cells to the intestinal development. We have used the transgenic mouse model Wt1-Cre/Rosa26R-eYFP, where the lineage of the cells expressing the Wilms' tumor suppressor gene expresses the yellow fluorescent protein (YFP). In the intestine, Wt1 is specifically expressed in the coelomic epithelium and in the cells that delaminate from it. YFP was colocalized with a number of differentiation markers through confocal microscopy and flow cytometry of dissociated intestines.

YFP+ cells were very abundant throughout the intestine during midgestation, declining in neonates. YFP+ cells were also transiently observed within the mucosa, being apparently released into the intestinal lumen. YFP was detected in cells contributing to intestinal vascularization (endothelium, pericytes and smooth muscle), visceral musculature (circular, longitudinal and submucosal) as well as in Cajal and Cajal-like interstitial cells, where YFP colocalized with c-Kit and CD34, respectively. YFP+ mesenchymal cells expressed FGF9, a critical growth factor for intestinal development, as well as PDGFRα, mainly within developing villi. Thus, a cell population derived from the coelomic epithelium incorporates to the gut mesenchyme and contribute to a variety of intestinal tissues, probably playing also a signaling role in development. Our results also support the origin of interstitial cells of Cajal and visceral circular muscle from a common progenitor expressing anoctamin-1 and SMCα-actin. Coelomic-derived cells contributed to the differentiation of at least a part of the interstitial cells of Cajal.
Cell lineage analysis and localisation of the sinoatrial node precursor cells within the posterior secondary heart field in the mouse

Abstract ID: 22

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CELL LINEAGE ANALYSIS AND LOCALISATION OF THE SINOATRIAL NODE PRECURSOR CELLS WITHIN THE POSTERIOR SECONDARY HEART FIELD IN THE MOUSE

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Keywords: heart development

Introduction

The early heart forms from two mesodermal cell populations, the First and Second Heart Fields (F&SHF). The FHF forms the cardiac crescent (E7.5 in mouse). The SHF lies dorsally and medially, in pharyngeal mesoderm, and is defined by islet1 expression. The left ventricle (LV) derives exclusively from FHF whereas the distal outflow tract (OFT) is SHF derived. The proximal OFT and RV are predominantly SHF derived, and the atria are of mixed F&SHF origin. A subportion of the SHF expresses Fgf10, as shown by the 1V-24 lacZ reporter, which labels the anterior HF (AHF). The AHF is fated to contribute to the OFT/RV but not the inflow/atria.

Using dye-labeling injections at the posterior portion of the SHF (pSHF), which is characterized by Islet1+/1v-24- expression, we have demonstrated that IF region arise from pSHF (see Galli et al, 2008) and also atrioventricular canal (AVC) and OFT are pSHF-derived cardiac regions (Domínguez et al, 2012; submitted).

Lineage studies using islet1-cre and islet1-mer-cre-mer mice crossed with the ROSA26R have described that sinoatrial node (SAN) and a central region of the atrioventricular node (AVN) are derived from islet1 precursor cells (SHF) (Moretti et al, 2006; Sun et al, 2007), whereas it is assume that ventricular conduction system (His-Purkinje system) is a non-SHF derived. In this regard, an accurate lineage analysis of SHF-islet1 cells contribution to the distinct components of the cardiac conduction system (CCS) is missing. Moreover, the localization of precursor cells forming the SAN and AVN central regions from the SHF is unknown.

Base on our previous data, we are interesting now to determine whether SAN and AVN islet1+ precursor cells are placed at the pSHF and, moreover, whether islet1+ progenitor cells participate in His-Purkinje system development during cardiogenesis.

Methods
We are labelling small groups of cells of 4-6 somites mouse embryos, throughout the pSHF using fluorescent dyes. After that, we culture the embryos for 48 hours and then we perform immunohistochemistry in whole mount against HCN4, a well-known SAN molecular marker.

**Results**

Preliminary results evidence that, in most cases, right and left pSHF derived cells populate the right and left atria of the developing heart (HCN4-), respectively. However, some dye-labelled cells were placed at the right atria-sinus venous junction, where HCN4 is expressed and define the putative SAN precursor.

**Conclusions**

These results would suggest that islet1+ progenitors cells localized at the pSHF participate in the developing of both, the working and conduction system myocardium, and open new ways to explore the putative SHF origin of the distinct cardiac conduction system elements.
AbdB and Dsx control the growth of A9 primordium during Drosophila genital disc development through the modulation of dpp and Hippo pathways.

abstract ID: 23

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The terminal part of the adult fly develops from the genital disc, which is formed by the fusion of three different embryonic primordia, A8, A9 and A10. In females, most of the structures of genitalia are derived from the A8, while the A9 forms the small accessory glands called parovaria. Thus, the A8 grows more than the A9 during larval stages. It was previously shown that the Double-sex Female protein (DsxF) is controlling female A9 growth by repressing decapentaplegic (dpp) expression. We assumed that the Hox gene Abdominal-B (Abd-B) may also be required for this repression, since Abd-B is the Hox gene expressed in the genital disc and needed to form the genitalia. There are two Abd-B proteins, Abd-BM and Abd-BR, the latter specifically expressed in the female A9. The Hox gene cofactors Homothorax (Hth) and Extradenticle (Exd) are also expressed, at high levels, in this primordium. Our experiments show that Abd-B, Hth and Exd, together with the DsxF isoform, are required to repress dpp in this segment of the genital disc. We have characterized a 610 bp long DNA fragment from the dpp region that reproduces the endogenous dpp expression (and lack of it in the A9) and that is highly conserved in the twelve Drosophila sequenced genomes. We are studying how repression of dpp by these factors is sufficient to control growth in the A9 primordium. We show that the Hippo pathway is down-regulated in the female A9 and we provide genetic evidence that this is due to the repression of Dpp. In mutants that derepress dpp, growth ensues as a result of the possible interaction of PMad and Yorkie, the effector of the Hippo pathway.
Soxd genes interaction with wnt signaling pathway in the control of cell proliferation and patterning formation

abstract ID: 27

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During the development of the nervous system, a vast number of different neurons and glial cells are generated from a small population of self-renewing progenitor cells. At the same time that neural progenitors exit the cell cycle and differentiate, distinct neuronal subtypes emerge from progenitor cells in a highly controlled spatial order, partitioning the dorso-ventral axis of the neural tube into discrete regions. The correct coordination of these events requires tight spatio-temporal control. Sox genes could be at the core of that control as they play crucial roles during neural development. We have previously determined that Sox5 controls cell cycle exit of neural progenitors through the inhibition of the mitogenic Wnt signaling pathway (Martínez-Morales et al., EMBO reports 2010). More recently, using gain- and loss-of function approaches in chicken embryos, we have determined that Sox5 controls cell fate specification of dorsal neural progenitors in the spinal cord inducing the transcription of the Wnt pathway negative regulator Axin2. Using a combination of EMSA, luciferase reporter transcriptional analysis and ChIP assays we have established that Sox5 cooperates with βcatenin in the transcriptional activation of Axin2, through direct binding to Axin2 enhancer regions. Thus, Sox5 restricts proliferation and prevents the extent of dorsal identity, both imposed by Wnt signaling in the central nervous system.
Rca1-dependent regulation of intracellular lumen formation

abstract ID: 30

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The tracheal system of *Drosophila melanogaster*, a model for tubulogenesis and angiogenesis, is a network of interconnected epithelial tubes that targets the tissues throughout the body to supply them with oxygen. The main branches of the *Drosophila* tracheal system have an extracellular lumen because their cells fold to form a tube with an apical lumen. In contrast, terminal cells of the tracheal system, specialized tip cells in some of the main branches, form fine unicellular tubes as they reach their target tissues. They do so by elongating their cytoplasm and forming an intracellular lumen. Recent work has shown that this intracellular lumen formation depends on a mechanism based on asymmetric actin accumulation and microtubule network organization. However, how intracellular tube formation is regulated by both intracellular and extracellular cues remains an open question.

From an EMS mutagenesis screen we isolated mutant line G012 that displays embryonic terminal cell luminal bifurcations. These bifurcations result from one terminal cell extending two or more intracellular lumina, in contrast with embryonic wild-type (wt) tracheal terminal cells that only display one lumen per cytoplasm. We mapped and sequenced the mutation responsible for this phenotype. Allele G012 has a mutation in rca1 (*regulator of cyclin A*), encoding an F-box containing protein that has been characterized as a regulator of APC/C (*anaphase-promoting complex/cyclosome*) activity during the cell cycle. APC/C was initially identified on the basis of its role in cell cycle control, but post-mitotic APC/C function is also required during later stages of development, such as in dendrite morphogenesis and axonal growth. We were able to separate mitotic and post-mitotic functions of Rca1 in terminal cell development and will discuss how, in post-mitotic tracheal terminal cells, rca1 functions to regulate intracellular luminal branching decisions.
Integrins are required for proper cell cycle progression and differentiation

abstract ID: 32

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Coordinating differentiation with exit from the cell cycle is critical for proper organogenesis, yet how this is achieved remains largely unknown. The development of the follicular epithelium of the *Drosophila* ovary represents an ideal system to study the mechanisms controlling the transition from cell cycle exit to differentiation. The ovary of the adult *Drosophila* female is composed of various tubular structures called ovarioles that contains a line of egg Chambers at different developmental stages. Each egg chamber begins as a 16-cell germline cyst surrounded by a monolayer of somatic follicle cells (FCs) precursors. During the early stages (up to stage 6), FCs undergo a mitotic division program giving rise to approximately 1000 FCs, which will form a monolayer known as the follicular epithelium. After stage 6, FCs differentiate and switch from normal mitotic cycle to undergo three rounds of endoreplication. Later in oogenesis, four different loci synchronously initiate a gene amplification event (Reviewed by 2). Two main pathways that have been described to control the proliferation-to-differentiation switch are the Notch pathway and the Hippo pathway 3-5. By clonal and FACS analysis, we show that integrins are required for proper proliferation-to-differentiation switch. Interestingly, although integrin mutant cells exit mitosis they remain in an undifferentiated state and do not enter endocycle. In addition, integrin mutant follicle cells do not initiate the amplification event. At present we are investigating the molecular mechanisms by which integrins regulate the cell cycle exit to differentiation switch. Our results suggest that integrin mediated signalling controls this transition by modulating the activity of the Notch pathway and the Hippo pathway. As a consequence, key cell cycle regulator proteins, such as Cyclin B and Dacapo, are deregulated causing the differentiation phenotype.

Identification of target genes of n-myc during inner ear development

abstract ID: 34

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Introduction.

The N-myc gene displays a complex expression pattern during inner ear development spanning from the otic placode to the adult organ. This pattern of expression suggests important roles of N-myc during otic vesicle patterning at early stages and cell specification and differentiation during later development. To address the function of N-myc in the mouse inner ear we have generated conditional mouse mutants for this gene.

Methods.

Transgenic mice

Mouse lines: N-myc\textsuperscript{lox/lox}, ROSA 26 Cre-Reporter and CrePax2 and Foxg1Cre lines.

Microarray analysis.

RNA was isolated from otic vesicles, or dissected cochleas at day 15. cDNAs were hybridized with Mouse Gene 1.0 ST arrays from Affymetrix.

RNA in situ hybridization.

RNA in situ analysis was performed in whole mount on embryos, inner ears and cochleas, using riboprobes for LFng, Pax2, Bmp4, Fgf10, Otx1, Otx2 and NeuroD. For detection of N-myc mRNA, a probe encoding the complete cDNA was used (MG207382; Origene).

Results.

Morphogenesis of the inner ear in N-myc mouse mutants is severely disturbed and mutant cochleas are characterized by an increased number of cells exiting the cell cycle that express the cyclin-dependent kinase inhibitor p27\textsuperscript{Kip1}. Analysis of different molecular markers in N-myc mutant ears reveals the development of a rudimentary sensory epithelium (Organ of Corti). The Kölliker’s organ, a transient structure neighbouring the Organ of Corti and a potential source of ectopic sensory hair cells, is absent or reduced in the mutant ears. In situ analysis confirms that Fgf10 and Lfng which are expressed in the presumptive Kölliker’s organ are downregulated in the apical aspect of the cochlear duct in N-myc mutants. However, Bmp4 expression which marks the Outer Sulcus that borders the Organ of Corti on the opposite side is unaffected.

Additionally, RNA in situ analysis on mutant ears carried out between embryonic day 13.5 to 14.5 revealed misregulation of dorsal markers of the cochlear duct such as Otx genes. Otx genes may control the development of dorsal structures such as the Reissner’s membrane. Analysis at early stages of inner ear development, confirmed that Nmyc controls expression of Otx1 and Otx2 that are present on the lateral aspect of the otic vesicle and highlights the relevance of Nmyc for otic vesicle patterning.
To identify further potential target genes for N-myc in the cochlea we are using microarray-based screening of wild type versus mutant tissue.

Conclusions

N-myc contributes to inner ear morphogenesis throughout development. Sensory and non-sensory areas are affected in mutant ears. In non-sensory areas, Otx1 expression in the lateral aspect of the otic vesicle and later on in the dorsal aspect of the cochlear duct is downregulated in N-myc mutants and Otx2 severely misregulated. At later stages of development, mutant cochleas are shortened and show rudimentary sensory epithelia. Downregulation of Fgf10 and LFng confirms the requirement of N-myc during formation of the Kölliker’s organ. The phenotypes observed in mutants are mirrored by the complex Nmyc expression pattern observed during inner ear development.
Role of apoptosis and activation of myosin ii in tarsal joint development of drosophila leg

abstract ID: 36

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The specific elimination of cells by programmed cell death is a fundamental mechanism in many morphogenetic processes that require changes in cell number to shape tissues. One of such processes is the morphogenesis of Drosophila tarsal joints, where apoptosis is needed to sculpt a fold that precedes joint formation. In order to study how fold formation and apoptosis are shaping the presumptive joint, our results have been obtained both in fixed leg disc as in time-lapse movies that allow seeing gene expression and morphogenesis in vivo. In our work we have found that, after the initial activation of cell death, both GTPase Rho1 and its activating factor RhoGEF2 are expressed at high levels at the position where the fold that precedes the joint develops. The activation of Rho1 leads to the monophosphorylation of myosin II, and these two proteins are active during and after the apoptotic cell delamination and extrusion at the basal region of the epithelium. In the final part of the process that maintains the fold, it seems that myosin II is in its diphosphorylated state. The two processes described, apoptosis and myosin II activation, are closely related during fold formation: on one hand, we show that the activation of myosin II and Rho1 are necessary for apoptosis to occur in specific domains. On the other hand, we have seen that if apoptosis is prevented myosin distribution and expression levels are altered. The interaction between the two mechanisms, therefore, is crucial for the correct development of the joint. Our work describes a gene network that coordinates mechanical properties required to eliminate apoptotic cells, directing the morphological changes needed for the development of tarsal joints.
In the embryonic development, the rostral neural tube is segmented in three main vesicles. The most caudal, the rhombencephalon or hindbrain is posteriorly subdivided in rhombomeres, seven main rhombomeres and four crypto-rhombomeres, these last segments do not display physical constrictions. All of them are specify by the particular combination of expression patterns of the Hox genes. The rhombomere 1 displays special features. It is rostrally limited by the isthmus, a well-known secondary organizer needed for the specification and differentiation of the midbrain and hindbrain, and obviously by rhombomere 2 caudally. It is a unique segment, as it does not express any Hox gene and also due to its dimension, it doubles the normal size of any other rhombomere. Its dorsal aspect contributes mainly to the generation of the cerebellar hemispheres and its ventral part gives rise to several different populations such as the Rafe formation, the interpeduncular nucleus, the tegmental nuclei, reticular formation, etc. In the transition area we can find the Locus coeruleus and the lateral lemniscus nucleus among others. Some of these populations occupy its final location by radial migration but in the case of the rhombencephalon many of them travel tangentially before reaching their terminal position. The aim of our work is subdivided in two objectives. The first objective is to describe the generation of the neuronal populations of the rhombomere 1. The second objective is to analyze the role of the morphogene Sonic Hedgehog in this process and its possible role in the guidance of the tangentially migrating early neurons.

The results obtained, when we altered the function of Shh, have revealed that the populations generated close to the floor plate, natural source of Shh, are completely absent. The Rafe formation and the prodromal part of the interpeduncular nucleus are not detected. But the basal neuronal nuclei of alar origin (tangentially migrated ventrally) are still present but partially disorganized. The rostral interpeduncular nucleus and the ventral tegmental nucleus are detected but the caudal interpeduncular nucleus is disoriented and it is not located in its final location. A plausible hypothesis for the maintenance of the ventral migration in the absence of Shh is the preservation of the expression of the signaling molecule netrin1 in the rhombomere 1 floor plate.

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The absence of Shh produces severe abnormalities during cerebellar development

Abstract ID: 47

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The absence of Shh produces severe abnormalities during cerebellar development

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During embryonic development, several molecules known as morphogens direct the specification and differentiation of the neuronal populations of the central nervous system. One of these morphogens is Sonic hedgehog (Shh); it is expressed in the axial mesoderm (notochord and prechordal plate) and in the floor plate of the neural tube. The genetic pathway induced by Shh controls the generation of all the ventral neuronal populations of the central nervous system. It also plays another role in specific parts of the brain. In the cerebellum, Shh is expressed in the Purkinje cells during its development. Its function in this structure is to control the proliferation and migration of the granule cells precursors from the rhombic lip. The aim of our work is to deeply analyze the development of a Shh deprived cerebellum in order to identify other possible functions of this molecule. We generated a conditional mutant in which we generate a non-functional Shh protein in the territory of Engrailed 1 expression (midbrain and rostral hindbrain). Therefore, the Purkinje cells generated in this conditional cerebellum are not able to produce a functional Shh protein. We obtained a low proportion of postnatal conditional mutant that displayed a clear cerebellar ataxia with a severe motor impairment. The macroscopic morphological analysis showed an important reduction in cerebellar size. The histological analysis revealed a great disorganization in the cerebellar layers. The outer molecular and the inner granular layers were absent or severely disorganized. Surprisingly, the phenotype displayed a clear antero-posterior gradient, being less severe in the caudal part of cerebellum. The number of Purkinje seems to be maintained but they were not able to form the classical monolayer. The deep nuclei appears to be unaffected by the lack of Shh. We detected an unexpected ectopic mass of cerebellar cells in the inferior colliculus. These were mainly Purkinje cells. We detected the ectopic location of these cells from postnatal day 0 onwards. This ectopia was located at both sides of the midline, being the cerebellar vermis completely unaffected. In previous publications, it has been described that alterations in granular cells signaling produce a similar phenotype. This population generates a boundary that contains the cerebellar neurons in their territory. The working hypothesis for this process is the signaling network of netrin1 and its receptors Unc5c and Dcc. Therefore, in our conditional mutant, the ectopic location of the Purkinje cells could be due to the complete absence of the granular cells in the rostral cerebellum.

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Control of Pattern, Size and Shape of the Fly’s Ocelli by hedgehog, Wnt and optix/Six3.

abstract ID: 50

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During development, extracellular signaling molecules interact with intracellular gene networks to control the specification, pattern and size of organs. One such signaling molecule is Hedgehog (Hh). Hh is known to act as a morphogen, instructing different fates depending on the distance to its source. However, how Hh, signaling across a cell field, impacts organ-specific transcriptional networks is still poorly understood. Here have investigated this issue during the development of the ocellar complex, a Drosophila sensory structure located at the top of dorsal head. Formation of the ocellar complex requires the definition of three domains: the prospective anterior and posterior ocelli (or simple eyes) plus the intervening interocellar cuticle –that is, an O-I-O pattern. hh is transcribed in a single domain, that corresponds to the prospective “I”. To generate the “O” fate, Hh activates eya and so/Six2 expression at long range. However, Hh also induces the transcriptional repressor engrailed (en) at shorter range, which feeds back negatively on the Hh pathway and attenuates it. This attenuation generates a region where eya/so are no longer expressed and which thereby becomes the “I” fate. This complex feedback demands a mechanism to maintain en expression in a Hh-independent manner, which we propose to require Dl/Notch signaling. This hh-driven gene network would generate a symmetrical O-I-O pattern. However, the expression profiles of anterior and posterior O domains differ. This asymmetry suggests different genetic controls acting on the anterior and posterior ocelli, which might be related to the different scaling of the anterior and posterior ocelli show in fly species with different body sizes. Interestingly, the Six3 gene Optix is expressed in the anterior region of the ocellar complex and its downregulation reduces specifically the size of the anterior ocellus. Moreover, in addition to Hh signaling, the Drosophila Wnt1, wingless (wg), is expressed surrounding the prospective ocellar region. Previous works have suggested that Wg promotes dorsal head capsule fates. However, wg mutant adult flies not only show absence or reduced dorsal head capsule, but also have larger and abnormally shaped ocelli. We will further describe research aimed at understanding the interplay between Hh and Wg signaling, and Optix/Six3 in the control of the pattern and size of the ocellar region.
Regulation of quasi-stable cell states by the Brahma complex subunit Bap60 during eye development

abstract ID: 52

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INTRODUCTION: Drosophila eye development is a widely used model to investigate cell specification mechanisms. During development, eye primordium cells undergo a series of successive steps: from undifferentiated progenitors, through determined precursors to differentiating retinal cell subtypes. The progenitor cell state is maintained by a combination of transcription factors that include Pax6 eyeless (ey), the zinc-finger tsh and the Meis1 homologue, hth. To characterize the progenitor-to-precursor transition, we need to identify which genes lie downstream of these transcription factors. RESULTS: After carrying out a screen to functionally validate a group of potential Ey targets, we identified the Brahma associated protein 60kDa (Bap60), a component of Brahma chromatin remodeling complexes, as a gene required for correct eye development. Moreover, in our recent RNAseq experiments to characterize progenitor and precursor cells, Bap60 expression is higher in progenitors and therefore, it is a potential Hth target. We found that Bap60 is necessary for both cell survival and proliferation in the early phases of development and that Bap60 is required downstream of Eyeless for ectopic eye development. At the molecular level, Bap60 appears to control the expression of key retinal genes, such aseya and hth, although the mechanism involved may differ between the two. The control mechanism seems to be transcriptional for hth, but in the case of eya it may involve protein stability. CONCLUSIONS: The progenitor-to-precursor transition requires the consistent and simultaneous repression of hth and upregulation of eya, to ensure that cells are unambiguously specified. Our results suggest that Bap60-containing complexes are part of the mechanism that ensures this binary fate decision. In the absence of Bap60, the expression of two critical genes, hth and eya, is not properly balanced and this deregulation might be affecting more generally other members of the retinal determination gene network. Mechanistically, complexes containing Bap60 might be involved not only in transcriptional regulation through chromatin remodeling, but also in the control of protein stability.
Contribution of gap junction communication during early morphogenetic activity of fgf8 arising from the mouse isthmic organizer

abstract ID: 54

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During early brain development, correct patterning of both mesencephalon and rhombencephalon depend on a precise cocktail of transcription factor and secreted diffusible molecules. The latter belong to those molecules with morphogenetic activity. These morphogens arise from signalling centres at distinct brain locations. Between midbrain and hindbrain vesicles is located the isthmic organizer (IsO), at the isthmic constriction. The key secreted signalling molecule abutting from the IsO is FGF8, a member of the Fibroblast Growth Factor (FGF) family. The specification of these brain structures requires different concentrations of FGF signal activity, which can be quantified by the detection of phosphorylated forms of Extracellular Rich Kinase molecules 1 and 2 (ERK1/2). Gap Junction Communication (GJC) in many developmental systems appears to be organized in a developmentally significant manner suggesting an important role in development. We are investigating the contribution of GJC (connexin 43) during morphogenetic planar instructions of FGF8 signal activity (through ERK1/2) activation during midbrain and hindbrain patterning.
Identification of a new gene si-vegf2 of the vascular endothelial growth factor family, its temporal and spatial expression during sea urchin development

abstract ID: 55

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Growth factors regulate basic cellular processes, including cell proliferation, differentiation, migration and apoptosis, and play a vital role in embryonic development of many animals. Vascular endothelial growth factors (VEGFs) are widely known as proteins involved in guide cell migration and cell differentiation during development in both vertebrates and invertebrates. The aim of our study is to investigate the role of VEGFs during early development of sea urchin, using as a model object the sea urchin *Strongylocentrotus intermedius*. Three different Vegf genes have been predicted in the genome of a closely related sea urchin *S. purpuratus*: Sp-Vegf1, Sp-Vegf2 and Sp-Vegf3. The expression of a Sp-Vegf3 homolog and its function have been previously described (Duloquin et al., 2007). In this work we demonstrated a high similarity of the PDGF/VEGF superfamily domain between predicted Si-Vegf2 gene in *S. purpuratus* and a new member of the Vegf family in *S. intermedius*, called Sp-Vegf2. Using RT-PCR, we examined the time course of Si-Vegf2 expression during sea urchin development and found the Si-Vegf2 transcripts beginning from the zygote until the 4-arm pluteus stage. RT-PCR findings were supported by in situ hybridization results: the Si-Vegf2 transcripts were uniformly distributed along the animal-vegetal axis in cleavage stage embryos, but after the late gastrula stage its distribution became bilaterally symmetrical; the Si-Vegf2 transcripts were detected in a few primary mesenchymal cells. Taking into account these data, we suggest that the Si-Vegf2 can play an essential role in skeleton formation. In summary, we have characterized the expression of a new member of the VEGF family in sea urchin, Si-Vegf2, to build the foundation for future functional and regulatory studies (This work was partly performed at the “CHROMAS” center, St. Petersburg State University, Russia, and supported by the RFBR (grant no. 12-04-00363a), the Far Eastern Branch of the Russian Academy of Sciences (grant no. 12-I-06-015), the Program at the Far Eastern Federal University (grant no. 11 G34.31.0010 and 12-III-B-06-031) and OPTEC company grant for junior researchers).
Analysis of genes functionally related to scabrous in the Drosophila eye

abstract ID: 58

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Introduction: Epithelial planar cell polarity (PCP) in the Drosophila eye is generated when immature ommatidial preclusters acquire opposite chirality in the dorsal and ventral halves of the eye imaginal disc and subsequently rotate 90° towards the equator, an imaginary dorsoventral midline. In consequence, mutations in genes involved in PCP establishment can cause defects in R3/R4 photoreceptors specification (reflected by chirality changes and symmetric ommatidia) and/or in the degree/direction of ommatidial rotation. The scabrous (sca) gene, encoding a fibrinogen-related secreted protein, was initially described to be involved in R8 photoreceptor differentiation and in the correct spacing of ommatidial clusters in Drosophila eye imaginal discs, and subsequently was shown to be required for the ommatidial rotation process. We have recently found that sca overexpression in the eye produces a high percentage of symmetric (achiral) ommatidia, suggesting a potential role for this gene in R3/R4 specification. To identify genes functionally related to sca during PCP establishment we performed a genetic screen using the sev>sca line (which overexpress sca in the eye) and a collection of iRNA lines. We identified 88 candidate genes to be functionally related to sca during this process.

Methods: The interactions previously found in the screen were validated by analyzing the effect of loss-of-function (LOF) alleles of these candidate genes on the percentage of symmetric ommatidia in sev>sca eyes. In addition, we analyzed adult retina sections of iRNA lines or LOF alleles of the candidate genes and Sca localization by immunostaining of third instard larval eye imaginal discs in these mutants.

Results and conclusions: We found that LOF alleles of genes already known to be implicated in PCP establishment, regulation of cell adhesion/extracellular matrix composition, microtubule formation and Notch signalling, significantly modified the sev>sca phenotype thus supporting a functional relation between these genes and sca during R3/R4 specification. Moreover, the phenotypic analysis of iRNA lines or LOF alleles of the validated genes in the eye showed that some of them also caused ommatidial chirality defects. The Sca protein is synthesized by the morphogenetic furrow and is transported through vesicles to ommatidial row 6-8. Interestingly, we observed that LOF of one of the validated genes, the cytoskeleton-related gene Dhc64C, modified the distribution pattern of Sca during ommatidial development, thus suggesting a potential mechanism by which Dhc64C could be functionally related to sca. These results provide additional data about the mechanisms that govern PCP establishment in the Drosophila eye and reveal new genes potentially involved in R3/R4 specification.

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Coordination in the reproductive system of drosophila melanogaster during pupal stages: tissues and genes involved

abstract ID: 59

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During pupal development the genital disc, placed at the back of the animal, evaginates and contact with the gonads, located in the fifth abdominal segment. Our main interest is to elucidate how this contact and fusion is produced to give rise to a fertile adult. Previous studies have shown that genital disc and gonad may develop functionally correctly in the absence of each other, although some morphological changes might occur. We are confirming and extending these results, and show that in the absence of gonads the oviducts move towards the direction where the gonads should be normally located and are later on folded back and attached to the seminal receptacle. Similarly, lack of genital disc does not prevent development of the gonads, although some morphological abnormalities are observed: female gonads are smaller than in the wildtype, similar to gonads in earlier stages of development, and testes do not elongate and are not coiled, again as it is observed in younger pupae. Our results suggest that coiling of the testes may be related to muscle migration. Accordingly, when we prevent muscle development and division in the vas deferens and around the area where genital disc and gonads contact, we obtain a wide range of phenotypes, depending on the time of inhibition, that seem to correlate with the extent of muscle migration. The role of different genes in modulating these morphogenetic movements is being analyzed.
Novel roles of sonic hedgehog in spinal cord morphogenesis

abstract ID: 60

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Introduction

During spinal cord development, proliferative neural progenitors arrayed along the dorsal-ventral axis differentiate into postmitotic neurons with distinct functions and morphologies.

Sonic hedgehog (Shh), one of the key inductive signals involved in spinal cord patterning, is secreted by the notochord and floor plate, generating a gradient of Hh signalling activity that will define distinct progenitor domains, thus assigning distinct fates along the dorsal-ventral axis of the spinal cord.

The best-studied function of the Hh signalling pathway in the nervous system is its role as a morphogen. However, in the last years have been uncovered the diversity of developmental processes in which Shh is involved with their specific actions changing with both space and time, such as its mitogenic role in controlling neural progenitor proliferation and axonal pathfinding.

During the zebrafish neurulation several events take place simultaneously in a short time window: morphogenesis, patterning and proliferation. Despite the well-established roles of Hh signalling in patterning and proliferation, it is unclear how these events are orchestrated with the early morphogenic events of the spinal cord. Through the analyses of Hh loss- and gain-of-function phenotypes we analyzed the effects of Hh signalling in neural keel morphogenesis.

Methods

We took advantage of the transparent zebrafish embryo to perform early manipulation of the Hh pathway through mRNA co-injection with fluorescent reporters at one cell stage. For single cell analysis we performed mosaic labelling in 32 to 64 cells stage embryos. In situ hybridization, immunostaining, and live confocal imaging followed the embryo phenotype

Results

We show that changes in Hh levels at early developmental stages affect neural keel morphogenesis. Preliminary results suggest a role for Hh-activity in the convergence of neural progenitors to the midline, thus affecting neurulation and giving rise to phenotypes reminiscent to that of Vangl2 and N-Cadherin mutants.

In a transcriptome screening performed in our laboratory, we found that activation or repression of Shh signalling highlighted the regulation on several polarity and adhesion proteins, thus suggesting a novel role for Shh in the development and/or maintenance of cell polarity and adhesion properties of neuroepithelial cells. To investigate the mechanisms underlying these observed morphogenic defects we are examining neural progenitor polarity establishment, adhesion properties and proliferation in the neural keel of Hh loss- and gain-of-function embryos.

Conclusion

Results suggest a novel role played by Shh signalling in spinal cord morphogenesis in the zebrafish embryo.
Sox21 is required for the posterior lateral line patterning by regulating FGF signalling

abstract ID: 67

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The posterior lateral line (PLL) is a mecanosensory organ present in fishes and amphibians. During development, this structure is formed from a group of cells that migrate from a region near the otic vesicle to the tip of the tail, leaving neuromasts in its trail. Patterning in the PLL primordium occurs mainly by the antagonistic activity of two morphogens, Wnt and FGF. Two morphogens play a crucial role in PLL development. Wnt is required to maintain the proliferative state of cells and the counteracting FGF triggers the first steps of differentiation and epithelialisation (1). Currently upstream regulators of these two pathways remain unknown. Several genes of the Sox family are also expressed in the PLL primordium, among them sox21a, a gene required to the transition of progenitors to differentiated neurons in the vertebrate central nervous system (2), although its function in the PLL development is not yet known. Recently it has been shown that this gene in central nervous system trigger proliferated cells into differentiated state. Recently, we started to study the role that sox21a may play during PLL development. Here we present a functional assay of sox21a in the zebrafish PLL development. To that aim we performed knockdown experiments using morpholinos and in vivo reporters as readouts. In these assays we observed a delay in PLL migration and impairment of epitheliarization. In addition, in this same sox21a morphant condition, we observed a down regulation of specific molecular markers downstream of FGF, suggesting that sox21a is an upstream regulator of this signalling. These phenotypes are similar to loss of function of FGF. To that end, we postulate that sox21a is an upstream regulator of FGF signal in PLL. In total, we identify sox21a as new important player in the patterning of the PLL by controlling FGF signalling.


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The specification of the skeletal muscle lineage in craniofacial development is dependent on the activity of MYF5 and MYOD, two members of the myogenic regulatory factor family. In the absence of MYF5 or MYOD there is not an overt muscle phenotype while in the double Myf5/MyoD knockout branchiomeric myogenic precursors fail to be specified and skeletal muscle is not formed. The transcriptional regulation of Myf5 is controlled by a multitude of regulatory elements acting at different times and anatomical locations, with at least five operating in the branchial arches. In contrast, only two enhancers have been implicated in the regulation of MyoD. We have recently shown that the transcriptional activity of one of these enhancer elements (ECR-1), which drives Myf5 expression in the branchial arches in isolation from 9.5 dpc, is dependent on two highly conserved E-boxes. In addition, we have shown that the correct levels of expression of Myf5 and MyoD are the result of the activation by MUSCULIN and TCF21 through direct binding to specific enhancers and that in the absence of Msc the timing of activation of Myf5 and MyoD is not affected but the expression levels are significantly reduced. We have now undertaken a comprehensive analysis of the different Myf5 branchial arch enhancers and we show that their functions in isolation are not equivalent to their functions in the context of the locus. By generating several BAC constructs carrying different deletion combinations of these enhancers we are starting to understand their functional relationship within the locus and show that a series of cis-interactions between the enhancers takes place in order to generate the full expression pattern. In addition, we are studying the interactions between genes involved in branchiomatic development (Tbx1, Pitx2, Msc, Tcf21, Myf5 and MyoD) and the different Myf5 and MyoD enhancers in order to understand the complex regulatory mechanisms behind the pattern formation during development.
Ecori as a space-controllable x-ray alternative for d. Melanogaster

abstract ID: 72

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Ablation of a certain domain at some point of development is a very valuable procedure in developmental biology studies. X-ray irradiation, meanwhile, is the time-honored way to produce DNA damage – and subsequent apoptosis – randomly over the whole organism, and so it has been very useful for research in development and regeneration over the years.

For some studies of regeneration during development it would be of great utility to have the ability to produce some DNA damage analogous to that of X-rays – double-strand breaks – in a space-controlled manner, so that the full cellular response to genetic damage is activated but only in certain cells. In order to achieve this, we put EcoRI – a very well studied and robust type-II restriction endonuclease that has the key property of entering the nucleus without modification – under the control of the UAS/Gal4 Drosophila system.

UAS-EcoRI flies are fully viable when Gal4 is not present and show extensive apoptosis in the appropriate domain when some Gal4 driver is used. If activated in random clones, UAS-EcoRI reasonably mimics the effect of X-rays with the added benefit that cells that are suffering DNA damage can be marked. Taking advantage of the temporal control provided by Gal80TS, we have used UAS-EcoRI to efficiently kill certain domains of the developing wing disc at a selected timepoint in a way that affected cells can activate the full physiological response to DNA damage. Altogether, we propose UAS-EcoRI as an effective addition to the Drosophila death-inducing toolset, one specially indicated when an irradiation-like death is desired in a space-controlled manner.
USE OF THE TH-GFP MICE TO CHARACTERIZE A TRANSIENT CATECHOLAMINERGIC CELL POPULATION OF THE CRANEAL GANGLIA

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Sensory neurons innervate visceral tissues and sensory organs where they are activated by sensory inputs and send projections into the Central Nervous System. The cell bodies of the sensory neurons are located in ganglia outside of the neural tube and are derived either from neural crest cells or from embryonic placodes. The cranial ectodermic placodes are transient thickenings of the head embryonic ectoderm in which neuronal cells are born and, after a process that involved delamination and internal migration, coalescent to form a ganglion. The majority of the sensory neurons of the cranial sensory ganglia originate from the embryonic placodes whereas those of the trunk sensory ganglia are derived of the neural crest. The epibranchial placodes comprise the geniculate, petrosal, and nodose placodes associated in sequence with the first, second and third branchial clefts; they give rise to the viscerosensory neurons of the VII (facial), IX (glossopharyngeal) and X (vagal) cranial nerves, respectively.

Tyrosine hydroxylase (TH) is the first and rate-limiting enzyme in catecholamine biosynthesis. Although it has been reported that TH is transiently expressed in the cranial sensory ganglia during embryogenesis, nothing is known about the role of this transient expression in sensory neurons whose final phenotype is other than catecholaminergic. The aim of our study is to characterize initially the expression and, later on, function of TH in the developing cranial sensory ganglia of the mouse embryo. We therefore have analyzed the transient TH positive cell population in the TH-GFP transgenic embryo, in which the expression of the green fluorescent protein (GFP) is under the control of the Th promoter. At embryonic day (E) 10.5 we found GFP-positive cells associated to the geniculate, petrosal, and nodose ganglia. This expression pattern is in accordance with that of the endogenous Th gene, as detected by whole mount In situ hybridization. Thus, the TH-GFP transgenic mice are a valid model to characterize the transient catecholaminergic cell population. Confocal microscopy analysis of TH-GFP embryos immunostained for Islet1, a marker of neuronal differentiation, revealed that all GFP positive cells co-expressed Islet1 although only a proportion of the former ones are GFP positive. These results indicate that a subpopulation of the cranial ganglia neurons transiently expressed TH. To get insight on the relevance of TH expression, we have analyzed the Th-null mouse. Preliminary results have shown a decrease in the number of islet expressing cells in the nodose ganglion indicating that catecholamines may play a role in the generation or survival of sensory neurons. In addition, we have analyzed the expression of Sox-10, a marker of the neuroglial subpopulation of the migrating neural crest cells that follows migratory pathways overlapping with those of the placodal sensory neurons. It appears that there is a decrease in Sox10 expression in the cranial ganglia of Th null embryos compared to heterozygous or wild-type mice, suggesting that TH may also be necessary for the development of neural crest precursors migrating along this pathway.
FGF modulates the response to Shh at the onset of ventral patterning in the spinal cord

abstract ID: 75

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Development of the nervous system involves the control of proliferation, cell cycle exit and cell fate assignment which is dependent on the position of neural progenitors and neurons at their time of birth. In the spinal cord these processes are coordinated with caudal extension of the embryonic axis. The spinal cord primordium is located in the vicinity of the node and regresses with the primitive streak leaving on its wake and progressively spinal cord cells. Once they are far from the influence of caudal-most signals, cells are able to exit the cell cycle and acquire dorso-ventral specific characteristics such as the activation of important transcription factors that govern the acquisition of neuronal subtype fates. The transition from an immature to a more mature spinal cord state is governed by FGF signaling. Cells located in the primordium region produce FGF8 that impairs cell cycle exit and as they leave this region and stop production of FGF they have the ability to differentiate as neurons. In addition, downregulation of FGF is required for proper acquisition of ventral pattern as well as for specification of neural crest cells.

The ventral spinal cord gives rise to motor neurons and different types of inter-neurons in a spatially and temporally ordered way initiated by the restricted expression of specific combination of transcription factors that confer identity such as FoxA2, Nkx2.2, Nkx6.1, Olig2. Expression of these genes depends on Shh signaling which is activated in a ventral to dorsal gradient as spinal cord precursor cells are exposed to Shh emanating first from the notochord and later from the floor-plate.

Downregulation of FGF8 coincides with the activation of ventral gene expression and we have previously shown that FGF can repress the expression of ventral genes suggesting a role of this signaling pathway in the control of ventral patterning.

Here, we explore the requirement of FGF signaling for proper ventral patterning and the mechanism responsible for FGF mediated interference with the Shh pathway. We identify Ptc2 as a crucial component of this interaction both in chick and mouse models.
The homeostatic mechanisms during the growing of wing imaginal discs

abstract ID: 76

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During the development of a given organ or tissue, growth and patterning are controlled in a coordinated manner in order to give rise to a well-formed structure with a particular size, shape and pattern.

We use an RNAi-based approach to block cell cycle in dorsal and posterior compartments of Drosophila imaginal discs. Compartments in which cell division is prevented exhibit reduced size and are underdeveloped, as suggested by the expression pattern of the wingless gene.

We observe a high proliferation rate close to the border between the normal and the blocked compartment. This suggests a misregulation of the signalling pathways involved in growth control and some sort of regulatory interactions between developing compartments.

Conversely, blocking the growth of wing domains not defined by lineage does not produce a significant change the size of the disc and of even the affected domain. We find that the lack of growth of the blocked domain is compensated by recruiting cells from close regions, which ensure the maintainance of the size and pattern. These results suggest the existence of an overall mechanism of tissue homeostasis that can cope with differential growth rates.
Wnt signalling and epicardial development

abstract ID: 81

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Proepicardial cells, which constitute a part of the pericardiac mesoderm located at the venous pole of the heart (cardiac inflow), give rise to the epicardium and their mesenchymal derivatives (EPDC). Proepicardial/epicardial cells and EPDC contribute to the formation of both coronary blood vessels and cardiac interstitial cells, indirectly modulating ventricular myocardium growth and inflow myocardial differentiation. In accordance to the multipotent properties of proepicardial cells, the differentiation of this tissue is crucial to the formation of the venous pole of the heart. Our results in avian (chick) and mammalian (BAT-Gal reporter line) embryos indicate that active Wnt canonical signalling is involved in the control of cardiac inflow patterning by modulating myocardial differentiation from epicardial progenitors (proepicardial cells). Activation or inhibition of Wnt signalling affects proepicardial cell differentiation and proliferation in proepicardial cells by interacting with other signalling pathways active in the region. This study identifies Wnts as key signals in the developmental coordination of cardiac muscle differentiation at the venous pole of the heart.
Tissue homeostasis in the wing disc of drosophila

abstract ID: 82

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Living organisms have developed response mechanisms to restore potential injuries or tissue damages that may occur during development or adult life. The term regeneration refers to the ability of some organisms to rebuild damaged or missing portions of their bodies almost completely after injury, and this capacity to regenerate is different among animals. Drosophila imaginal discs, the precursors of adult cuticular structures, are capable to regenerate.

To study tissue regeneration in Drosophila, we have used a genetic method (Smith-Bolton et al, 2009) to induce tissue ablation and then recovery of specific areas of the wing imaginal discs. Our experiments reveal a very powerful homeostatic mechanism that is able to repair injured domains contemporaneously with the damaging process. In addition, we have investigated the molecular signals underlying this repair mechanism. We have examined the activity of Wg and Dpp signalling pathways, known to be key regulators for the growth of the wing disc, and found that they do not play a specific role in the repair process. Moreover, we have tested the role of the Jun-Kinase pathway during the wing disc repair.
Post-translational regulation of lipophorin receptors

abstract ID: 83

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Mammalian Low Density Lipoprotein Receptor (LDLR) proteins are single pass transmembrane proteins involved in multiple processes, most notably lipid metabolism. Drosophila has two genes with homology to the human LDLR known as lipophorin receptor 1 and 2 (lpr1, lpr2) that are essential for neutral lipid uptake by several cell types. Each gene has two alternative promoters (P and D) that give rise to multiple isoforms (P- and D-isoforms) having strikingly different functions and distributions. These isoforms are subjected to differential post-translational regulation when expressed in imaginal discs affecting their subcellular distribution and activity. We have mapped the protein domains required for this post-translational regulation to the signal peptide characteristic of P-isoforms. We are currently studying how the signal peptide affects protein stability and function. In mammals, the E3-ubiquitin ligase IDOL regulates the LDLR post-translationally promoting its degradation. Our data indicates that a Drosophila IDOL distant homolog, known as defense repressor 1, is also critical for the regulation of the Lipophorin Receptors. Our work reveals the functional relevance of isoform-specific post-translational regulation of LDLR proteins.
The hox gene Ultrabithorax regulates growth of the metanotum during pupal development

abstract ID: 85

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The development of the wing and haltere of Drosophila melanogaster constitutes one of the more deeply studied models for Hox gene function. Both appendages are homologous structures present in the second thoracic (T2) and third thoracic (T3) segments, respectively, but show different size and shape due to the activity of the Hox gene Ultrabithorax (Ubx), only present in the T3 cells.

The proximal region of the wing and haltere imaginal discs (the larval structures which develop into the adult appendages) also give rise to the mesonotum (T2) and metanotum (T3), respectively, which altogether constitute the notum (dorsal region of the thorax) of the adult fly. The mesonotum is approximately 3 times bigger than the metanotum at the third larval stage. However, in the adult, the difference in size between the two segments is significantly increased: the mesonotum constitutes nearly all of the adult notum while the metanotum consists on a small stripe of cuticle between the halteres.

We focused our study on how Ubx controls the different growth rate of the mesonotum and metanotum during pupal development. We have found that the metanotum forms a double layered structure along with some posterior T2 cells, previously named mesothoracic phragma, located at the boundary between the thorax and the abdomen, which penetrates in the thoracic cavity. Surprisingly, we found that although the metanotum is smaller than mesonotum, its cells are more than twice bigger in the adult fly, but that cell proliferation seems to be specifically blocked in metanotum cells.

We propose that Ubx prevents cell division in the metanotum during pupal development and that this accounts for the different size of the T2 and T3 in the adult.
miR-23a/27a/24-2 TRANSCRIPTIONAL REGULATION IS DIFFERENTLY MODULATED IN CARDIAC AND SKELETAL MUSCLE CELLS.

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MiRNAs are small non-coding RNAs that regulate stability and translation of mRNA targets. Today, we know that these small RNAs are involved in almost every biological process, including early development, lineage commitment, growth and differentiation, cell death, and metabolic control. Misregulation of a variety of miRNAs has been recently related with cardiac and skeletal muscle illness. To date, there are experimental data demonstrating that miR23a is involved in hypertrophy regulation response in cardiac muscle, also playing a role in muscle response to atrophic stimuli. On the other hand, miR27a is capable to increase cardiac β-MHC gene expression in ventricular myocytes and promotes myoblast proliferation. Both miRs belong to the intergenic miR-23a/27a/24-2 cluster. However the transcriptional regulation of miR-23a/27a/24-2 cluster and how their expression is altered in these pathological states remains unclear. The aim of this work is to shed light in the transcriptional regulation of miR-23a/27a/24-2 cluster. Our results demonstrate that conserved sequences up and downstream miR-23a~27a~24-2 genomic loci are implicated in its transcriptional control. We show that transcription factors with important roles in skeletal and cardiac myogenesis and homeostasis are able to modulate miR 23a~27a~24-2 cluster promoter activity, either repressing, like Myogenin or Myod1, or activating, like Myf6, Mef2c and Srf. Finally, we demonstrate a strong modulation of miR-23a~27a~24-2 promoter activity mediated by angiotensin II and norepinephrine in cardiac but not in skeletal myocytes. Taking together, our results provide new insights into the transcriptional control of the miR-23a~27a~24-2 cluster in cardiac and skeletal muscle.
Single-enhancer knockout to study the contribution of the dorsal dermomyotome to adult musculature

abstract ID: 89

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The determination and specification of skeletal muscle in vertebrates is orchestrated by the activation of a cascade of the myogenic regulatory factors (MRFs) that ultimately leads to the formation of the different muscles in the adult body. Myf5 is the first of these MRFs to be expressed in the embryo and, at least in the trunk, initiates and co-ordinates the myogenic cascade.

The different knockout alleles generated indicate that Myf5 is essential for the determination of the skeletal muscle phenotype; in its absence myogenic progenitors fail to be specified at the correct developmental stage. Only after the activation of MyoD, another member of the family, is the phenotype rescued and myogenesis progresses in a normal way, giving rise to adult individuals without an overt muscle phenotype. In double Myf5/MyoD KO animals this rescue does not take place and animals lack all adult and embryonic skeletal muscles.

We and others have extensively analysed the transcriptional regulation of the Myf5 gene during development and show that there are more than 25 regulatory elements responsible for the generation of the complex expression pattern of the gene. Most of these regulatory elements are able to drive expression at specific developmental times and anatomical locations. The Early Epaxial Enhancer (EEE) operates in the dorsomedial lip of the dermomyotome and is one of the best characterised enhancers in the locus.

Although the contribution of the different regulatory elements to the expression pattern is well defined we still lack an understanding on the contribution of the different subpopulations of muscle progenitor cells to adult musculature. Furthermore, there is still no connection between the spatiotemporal activation of Myf5 and its function within the particular set of myogenic precursors in which it is active.

In order to address these questions we have generated a single-enhancer knockout in which the EEE has been targeted. For this we used the cre-lox technology and flanked the EEE by loxP sites. As we needed to incorporate a selectable marker for the targeting of ES-cells, and we know that the promoters from selectable markers can interfere with transcriptional regulation, it was flanked by FRT sites and included within the loxP-flanked interval. By crossing this allele to the β-actin-Flp deleter strain the selectable marker is removed leaving an intact EEE allele (flanked by loxP sites) while crossing into the β-actin-cre deleter strain generates the enhancer-specific knockout allele.

Preliminary data indicates that homozygous Myf5\textsuperscript{EEE-EEE-} animals do not have an overt skeletal muscle phenotype, indicating that, as in the full Myf5-KO alleles, the phenotype is being rescued by MyoD and that differences in the onset of myogenesis between different progenitors do not have a phenotypical effect. We are performing an in-depth analysis by immunohistochemistry and in situ hybridisation to determine if in this allele myogenesis is delayed in the progenitors originating from the dorsal dermomyotome.
Finally, we have undertaken a comprehensive analysis of the new KO allele by comparative RNAseq of wild type and Myf5$^{EEE/-EEE}$ embryos, which should give us a clear picture of the downstream targets of Myf5 in the dorsal dermomyotome.
Ck2alpha is essential for heart proliferation

abstract ID: 90

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Introduction: Mice deficient for CK2alpha, a highly conserved serine-threonine kinase, die around embryonic day (E)11 showing heart malformations that may be the cause of the lethality. Since defective cardiogenesis leads to congenital heart disease, we studied the mechanism utilized by CK2alpha to control cardiogenesis.

Methods: We used three or more pairs of somite-matched wild-type (WT) and CK2alpha/-embryos for the following analyses. We used histological and morphometric analysis to study morphological defects; immunofluorescence and immunoblot to assess protein expression; RT-qPCR and in situ hybridization for transcript expression; and FACS for cell cycle studies.

Results: CK2alpha-/ mice showed severely defective hearts at E9.5; this late phenotype was due to the presence of maternal CK2alpha protein until E8.5. Characterization of the cardiac malformations in CK2alpha mutants showed shortened and dysmorphic outflow tracts (OFT) and hypoplastic right ventricles (RV) at E9.5 compared to WT embryos. In addition, at E9.5 and E10.5, CK2alpha deficient hearts showed poorly developed ventricular trabeculations. At E9.5 and E10.5, differentiation happened normally in CK2alpha deficient hearts as they expressed pan-cardiac gene and protein markers. To understand these morphological defects, we analyzed the levels and expression pattern of Isl1, and the mitotic and apoptotic indexes in the heart tube. At E9.5, the number of Isl1+ progenitor cells in the OFT, and the levels of Isl1 transcript and protein were indistinguishable from WT. Apoptosis was not affected in CK2alpha deficient hearts. The mitotic index was decreased throughout the heart tube in CK2alpha mutants compared to WT embryos at E9.5 and E10.5. A mitotic defect was not apparent at E8.5, when maternal CK2alpha protein was still present. The observed decrease in proliferation did not correlate with cell cycle arrest, but with reduced expression of cardiac proliferative markers such as n-myc and proliferative marker gene expression.

Conclusion: CK2alpha is necessary for proper heart tube morphogenesis, at least in part, through regulating cell proliferation and proliferative marker gene expression in the heart tube. These data suggest that the mechanism underlying the OFT and RV defects is different from progenitor integration defects.

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Role of lgr family members in AER renewal during limb bud development

abstract ID: 92

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During embryonic development, a signaling centre termed the Apical Ectodermal Ridge (AER) controls limb bud outgrowth along its proximal-distal axis. The AER consists on a thickening of ectodermal cells at the distal tip of the limb. This transient embryonic structure is essential both for patterning and limb outgrowth, and it is a conserved feature among vertebrates. The structure of the AER is maintained through limb development until last phalanx is formed when it disappears by apoptosis. Although AER induction and maintenance are orchestrated by complex interactions between the FGF, WNT/β-catenin and BMP signaling pathways, little is known about the molecules involved in the maintenance of the proliferation versus apoptosis of the cells that configure the AER. In this work we present evidences of the involvement of two more molecules in this process, lgr5 and lgr6, which are known as adult stem cells markers. In here, we describe the expression pattern of lgr5 and lgr6 during limb bud development using the chicken embryo as a model and show that their expression patterns in the limb bud are consistent with the areas of cell proliferation within the AER. Moreover, we performed lgr5 gain-of-function experiments by in ovo electroporation and studied the relationship of lgr5 and lgr6 with different signaling pathways known to be involved in the AER induction and maintenance. The phenotypes obtained point to the involvement of lgr5 in the maintenance of a proliferative niche of proliferation in the AER. We also present here evidences showing that lgr6 is controlled by WNT signaling. Our results support a model in which lgr5 and lgr6 control the activation and maintenance of a niche of undifferentiated cells that are in continuous proliferation at the base of AER. This niche can be responsible for the renewal of AER until it disappears by massive programmed cell death.
Rewiring the fly retinal determination gene network through the analysis of string/cdc25 regulation

abstract ID: 93

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Most organs are formed by the timed recruitment of precursor cells from a pool of proliferative organ progenitors. This recruitment is tightly coupled to the control of the cell cycle, as progenitors exit the cell cycle prior to their terminal differentiation. The coupling between cell cycle control and the progenitor-to-precursor transition is particularly clear during the development of the Drosophila eye. During the third instar stage, eye progenitor cells undergo a transient amplification phase, known as first mitotic wave (FMW), prior to their synchronous entry in G1 and proneural genes expression upregulation. The FMW is therefore a hallmark for the transition into the precursor state and is characterised by the transcriptional burst of string/cdc25 (stg), the universal mitotic trigger. Study of stg transcriptional regulation can thus be used as a proxy to uncover the factors that govern the progenitor-to-precursor transition, during eye development. Our previous results showed that upregulation of stg expression at the FMW is under the control of the transcription factor Homothorax (Hth), the Drosophila homologue of the Meis family of proto-oncogenes. We have recently identified the FMW stg-enhancer region. We are using this enhancer to address the molecular mechanisms underlying this enhancer’s activity and to identify other key regulators of the progenitor to precursor cell state transition. Combined genetic and molecular analysis reveals that the fly Pax6 homologues Eyless (Ey) and Twin of Eyless (Toy) are required redundantly for stg-FMW transcription. Eyes absent (Eya) and Sine oculis (So), two other central components of the retinal determination network also play a role in the regulation of stg expression. However, how these factors interact and how the progenitor to precursor transition is regulated is still unclear. I will present our latest results on the genetic and molecular regulation of stg at the FMW and our current view on the network that regulates the progenitor-to-precursor transition in the developing eye.
Cabut, the Drosophila ortholog of vertebrate TGF-b-inducible early-response gene (TIEG) proteins, is functionally related to ecdysone signaling during dorsal closure

abstract ID: 96

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Introduction: The steroid hormone 20-hydroxyecdysone (20E) triggers the major developmental transitions in the Drosophila life cycle and provides a model system for defining the developmental and molecular mechanisms of steroid signaling. 20E acts via a heterodimer of two nuclear receptors, the ecdysone receptor (EcR) and Ultraspiracle, to directly regulate target gene transcription. A high titer 20E pulse occurs midway through embryogenesis and is required for epithelial movements occurring during germband retraction, head involution and dorsal closure (DC). DC is a morphogenetic process that occurs at the end of embryogenesis, in which embryos exhibit a large dorsal hole that closes by migration of the lateral epithelial sheets and subsequent fusion at the dorsal midline. This process is primarily regulated by the JNK and Wnt/Wg pathways, but the role of ecdysone signaling during DC is unknown. Cabut (Cbt), the Drosophila ortholog of vertebrate TIEG proteins, is a transcription factor involved in this process that regulates epithelial migration, actomyosin cable formation and dpp expression downstream of JNK signaling. Interestingly, previous analyses using larval organ culture in combination with microarray technology revealed that cbt is a 20E-primary response gene dependent on EcR during metamorphosis. Besides, different assays have led to identify new JNK target genes during Drosophila DC that encode proteins involved in ecdysone response. Since preliminary analyses of microarray results indicated that cbt regulates the expression of the 20E-inducible genes ImpE1 and ImpL1 during DC, we have analyzed the involvement of Cbt in ecdysone signaling during this process.

Methods: Gene expression levels in different genetic backgrounds and experimental conditions have been performed by RT-PCR. Lethality assays, cuticle preparations and immunostainings of ImpE1 and ImpL1 mutant embryos were also performed. Lethality rescue assays of mutant embryos were also carried out by transgenes overexpression using the UAS/GAL4 system.

Results and conclusions: Results obtained by qRT-PCR analyses confirmed that Cbt regulates ImpE1 and ImpL1 expression during DC. Consistently, cuticle preparations of ImpE1 and ImpL1 mutant embryos revealed that they show DC and head involution defects. To determine their role during these processes, immunohistochemical analyses of ImpE1 and ImpL1 mutants with anti-FasIII and phaloidin were performed. We observed defects in elongation and polarization of the dorsal-most epidermal cells as well as in actomyosin cable assembly at the leading edge in such embryos, suggesting a role of Imp-genes in the lateral epidermis and in cytoskeleton regulation during DC. We also found that cbt embryonic lethality is rescued by overexpression of different EcR isoforms in the epidermis, thus demonstrating that they regulate cbt expression in this tissue during DC. Consistently, we found that cbt mutant escapers are able to develop until L2 stage and present molting defects similar to those observed when 20E signaling is compromised. Taken together, these findings reveal that cbt expression is regulated by ecdysone signaling during Drosophila mid-embryogenesis and that it is a key player in 20E response at this developmental stage.

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Dissecting the pancreas genetic networks using a novel Enhancer Disruption screen in zebrafish

abstract ID: 97

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Cell therapy for diabetes requires a deep knowledge of the molecular pathways underlying normal and disturbed pancreas development. To that end, we are performing a genetic screen in zebrafish using a new tool developed in our laboratory, the Enhancer Disruption (ED) vector. ED is an enhancer trap vector with strong mutagenic capacity that can disconnect cis-regulatory elements of genes. So far we were able to isolate 45 insertions that show expression in the pancreas. Further genetic analysis from the isolated mutations combined with morpholinos studies of the targeted genes will help us dissect the function of new and unknown pancreas expressed genes during the development of this organ.
Negative feed back fgf-bmp is controlled

abstract ID: 101

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Introduction.

At early gastrula stages of the chick embryo, cells with cardiogenic potential arise from a localized region of the primitive streak, just caudal to Hensen’s node. These groups of cells migrate in an anterior-lateral direction to form the bilateral cardiogenic mesoderm, between the ectoderm and the endoderm, in the most anterior and lateral part of the embryo, constituting the primary cardiac field. At this moment, the precardiac mesoderm is surrounded by the adjacent endoderm, which comes from the more cranial part of the primitive streak, and which plays a crucial role during cardiac specification with the involvement of several molecular factors. Therefore, previous evidences suggest that Nkx2.5 expression is induced by Bmp2, emanating from the adjacent endoderm, which is also regulated by Fgf8.

Methods.

To further analyze Bmp2-Fgf8 interaction during cardiac specification, we have analyzed the role of microRNA-130, which is expressed in the endoderm adjacent to precardiac mesoderm, in early cardiogenesis. For this purpose, we have electroporated Bmp2, Fgf8 and miR130 in precardiac cells as they pass the primitive streak. Our study has been complemented by experiments of ectopic administration of Bmp2, Noggin, Fgf8 and SU5402 (an inhibitor of Fgfr1).

Results.

We observed that Bmp2 induces the specific cardiac markers cNkx-2.5 and Gata4, and suppresses the endodermal endogenous expression of Fgf8. Moreover, Fgf8 suppresses Bmp2, cNkx-2.5 and Gata4 expression, while SU5402 administration suppresses the endogenous Fgf8 expression. Overexpression of miR130, a microRNA that binds to specific sequences in the 3’UTR of target gene Erk1/2 (Mapk1), induces the expression of Bmp2, cNkx-2.5 and Gata4, and suppresses the expression of Fgf8. The electroporation of antipremiR130 shows the opposite results.

Conclusions.

All our data supports the establishment of a negative feed-back between Bmp2 and Fgf8 through miR130, constituting a crucial element involved in the precise patterning and early development of the heart.

Acknowledgements. Funding: Spanish Ministry of Science and Innovation [BFU2007-66350], and the Junta de Extremadura with FEDER co-funding [CTS005].
Scratch2 prevents cell cycle re-entry by repressing mir-25 in postmitotic primary neurons

abstract ID: 106

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ABSTRACT

The Scratch family is integrated into the highly conserved Snail Superfamily of zinc finger transcription factors (Barrallo-Gimeno and Nieto, 2009). Snail factors have multiple functions during embryonic development in processes that involve cellular movements, and they are reactivated in adult pathologies such as tumor progression by inducing EMT, the Transition of Epithelial cells into Mesenchymal cells with invasive and migratory properties (Nieto, 2011). By contrast, Scratch genes do not seem to be involved in the EMT process since their expression appears restricted to the central nervous system in different model organisms.

In addition to EMT induction, several Snail family members have been shown to promote cell survival and to stop proliferation both during normal development and cancer cells (Nieto, 2011). We have recently shown that scratch2 gene in zebrafish is involved in neuronal survival (Rodríguez-Aznar and Nieto, 2011) and wondered whether cell cycle control could be another ancestral function associated with the family.

During nervous system development the regulation of cell cycle, differentiation and survival is tightly interlinked. Newly generated neurons must keep cell cycle components under strict control, as cell cycle re-entry leads to neuronal degeneration and death. Despite their relevance, the mechanisms controlling this process remain largely unexplored. We have found that scratch2 regulates the cell cycle of spinal neurons in the developing zebrafish embryo, since scratch2 knockdown induces postmitotic neurons to re-enter mitosis. Scratch2 prevents neurons from reverting into a proliferative state by increasing the expression of the cycle inhibitor p57 through the downregulation of miR-25. Thus, Scratch2 appears to safeguard the homeostasis of postmitotic primary neurons.

References


Keywords: neuronal cell cycle re-entry /p57 /Snail genes
From precursor muscle cells to fast fiber-type: a defined fiber-type specific mouse model

abstract ID: 112

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Introduction

Early events of fast fiber-type specification had not been well characterized. Fiber-type specification begins during early embryogenesis, whereby skeletal muscle myotubes switch isoform muscle protein-type to establish fast, slow and mixed fiber muscle groups with distinct functions. In the mouse, skeletal muscle fibers are formed during the second half of embryogenesis (E10.5-E16.5). Myogenic precursor cells undergo two overlapping waves of differentiation that form the primary and secondary myotubes, respectively. Primary and secondary myotubes can be distinguished by their morphology, location and by the skeletal myosin subtypes they produce. A new level of complexity is produced in the adult muscle, since each muscle is composed of thousands of fibers, each of which is a syncytium of hundreds of cells stretching from one tendon to another. The absence of experimental and genetic tools to manipulate and track precursor muscle cells, fiber-type conversions and muscle dynamics in vivo have impeded to better understand the process of myogenesis in development, aging and in response to external or pathological stimuli. We have developed an excellent tool for rapid tracking of precursor muscle cells from somites to limbs or to body wall and to examine directly primary and secondary myogenesis in whole mount embryos by non-invasive fluorescent imaging technology.

Methods

In the laboratory, we have created transgenic mouse lines, carrying the FURE/FIRE enhancer elements from the mouse $fTnI$ gene upstream of the green fluorescence reporter gene, to drive the expression of the fluorescent markers to precursor muscle cells and fast fibers (Guerrero et al, 2010). Homozygous offspring have been produced. This will enable the use of non-invasive fluorescence imaging technology in live anesthetized embryos (figure 1) and animals to track precursor muscle cells and assess the changes in fast-fibers within a muscle in the same animal in response to different stimuli.

Results and Discussion

Here, we present fiber-type characterization of the embryo and mouse transgenic lines. Our studies revealed that GFP expression correlates very well with the endogenous $fTnI$ expression. Type I fibers (slow) do not express GFP. GFP-expressing fibers are all fast-twitch fibers, i.e. type Ila, IIX and IIB. There are mixed fibers, those expressing more than one MHC antibody. A gradation in GFP fluorescence intensity is observed in muscle positive sections. Type IIX fibers express higher GFP quantities. We also will present hindlimb muscles and diaphragm maturation in P1, P7 & P14 mice. A gradation of GFP fluorescence intensity can be observed at these stages. This gradation mirrors that of adulthood. Fast myosin starts to appear while slow myosin expression decreased over time. This switch from slow MHC to fast MHC occurred earlier in diaphragm (P7) than in the rest of skeletal muscles (P14).

Conclusion

The FUFI-EGFP transgenic mice are an excellent tool for rapid tracking of precursor muscle cells and fast-twitch fibers.
Rrole of glial cell line-derived neurotrophic factor in embryonic pancreas colonization by neural precursors

abstract ID: 113

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Introduction: The mammalian pancreas is richly innervated by both the sympathetic and parasympathetic nervous system that control exocrine and endocrine secretion. The intrinsic innervation of the pancreas is derived from neural crest-derived enteric nervous system precursors during embryonic development. Recent studies have suggested that neural crest cells might provide signals that regulate pancreatic embryonic formation. We have characterized the colonization of pancreas by neural crest cells using in vivo and ex vivo approaches in mice. We have identified a key player in the neural colonization of the pancreas, the neurotrophic factor Glial Cell Line-Derived Neurotrophic Factor (Gdnf).

Material and methods: Gdnf expression was studied by the use of a lacZ-based transgenic mouse throughout embryonic development and adulthood. Cre/lox recombination was used to conditionally eliminate GDNF in mouse pancreatic cells. The pancreata of the mutant mice was analyzed by immunochemistry and molecular biology techniques.

Results: Gdnf expression was detected at embryonic day 10.5 (E10.5), soon after the pancreas evaginates for the foregut, and E12.5 along with progenitor markers such as Pdx-1 but not in differentiated glucagon producing cells. At E14.5, after the secondary transition, expression persisted in Pdx-1-expressing progenitor cells in the ducts. From E15.5 on, Gdnf was expressed only in differentiating ducts albeit at much lower levels, and after birth became almost undetectable. Gdnf was detected exclusively within pancreatic epithelial cells, with no expression in mesenchyme throughout the embryonic development. This is in sharp contrast to other regions of the gut where Gdnf is expressed only in mesenchyme. Gdnf was specifically inactivated in developing pancreatic epithelium using the Pdx-1-Cre transgenic mouse line. No differences in pancreas morphology were observed between Gdnf knockout mice and control mice, demonstrating the GDNF function is dispensable for pancreas formation. However, a profound loss of neural cells was found in the pancreata of Gdnf knockout mice. Innervation of other regions of the gut remained unaffected. Our analysis of early stages of embryonic pancreas formation and ex vivo experiments indicate that migration of neural progenitor cells is compromised in pancreas of Pdx-1-Cre Gdnf mutant mice.

Conclusion: Gdnf is expressed in the pancreatic progenitor compartment during early embryonic development becoming restricted to ducts at later stages. Our results show that GDNF is dispensable for pancreas formation. Our results demonstrate that pancreatic GDNF acts as a neurotrophic factor for gut-resident neural crest derived cells. The pancreas-specific Gdnf mutant mouse might be a useful tool to study the role of innervation in pancreatic embryonic formation and to analyze neural-pancreas interactions in adult endocrine and exocrine pancreas.

Funding: Grants from the Spanish Ministry of Science and Innovation (SAF2008-02469), Andalusian Ministry of Health (PI-0250/2008) and Andalusian Ministry of Science and Innovation (P08-CVI-3727)
Dampening the signals transduced through hedgehog via microrna mir-7 facilitates notch-induced tumorigenesis

abstract ID: 114

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Introduction
A fundamental question in biology and in the study of cancer is what instructs cells to stop growing when the proper size is attained to commence terminal differentiation. One strategy is the establishment of spatially confined domains called organizers along the dorsal-ventral (DV) and anterior-posterior (AP) axes.

The compound eye of Drosophila melanogaster is a powerful in vivo experimental system to study this issue because growth depends on asymmetric activation of Notch receptor along the DV boundary and retinal differentiation depends on the AP organizer Hedgehog (Hh) [1].

Methods
qRT-PCR, immunofluorescence staining, luciferase reporter assay and enhanced GFP sensors.

Results
The direct overexpression of mir-7 together with Nocth ligand Delta, under an eye promoter provoked tumour growth.

We tested candidate target genes of mir-7 looking for eye tumor growth, using RNAi lines in conjunction with Delta overexpression and we found Interference of Hedgehog (Ihog) as an important target of mir-7 which has been recently identified, together with Brother of Ihog (Boi) as Hh co-receptors in Drosophila [2].

As miRNAs negatively regulate gene expression by mRNA degradation with its binding to sequences in the UTRs, in vitro we found less activity of a luciferase reporter containing the full-length ihog 3’ UTR compared with one with a point mutation in the ihog 3’UTR and in vivo we found repression of an ihog 3’UTR eGFP sensor in wing discs overexpressing mir-7.

The endogenous ihog mRNA was inhibited by miR-7 in vivo as heat shock induction of mature mir-7 overexpression, assayed by qRT-PCR.

Although boi does not appear to be a target of miR-7, there is a functional overlap in the roles of Ihog and Boi. Consequently, we verified the status of boi transcription in relation to eye disc growth and we found that we can expand or reduce the region of expression of boi by the ubiquitous expression of eyegone (a Notch’s effector in eye growth) and fringe (that causes defective Notch receptor activation) due to a widening or the thinning of the DV organizer, respectively [3].
Conclusion

We found that tumourigenesis was provoked by oncogenic cooperation between the microRNA miR-7 and the Notch pathway converging on the silencing of hedgehog signalling. In mechanistic terms, miR-7 silenced the ihog Hedgehog receptor, while Notch repressed expression of the brother of ihog (boi) receptor. These findings reveal an unanticipated cooperative antagonism between two pathways extensively used in cancer.

References


Acknowledgements

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Dynamic extracellular proton fluxes during adult vertebrate regeneration

abstract ID: 116

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Endogenous electric currents are known to be important for vertebrate organ regeneration. Nevertheless, many questions remain: (i) what is the ion nature of these currents? (ii) how is cellular ion dynamics during regeneration? (iii) which are the molecular signalling pathways that transduce electric cues into cellular responses? To gain new insights into these questions, we are using zebrafish caudal fins as an adult regeneration model in vertebrates. Our approach couples specific extracellular ion flux measurements, using a non-invasive Scanning Ion-Specific Electrode Technique (SIET), with transcriptional profiling and genetic functional analysis.

Our data points to a role for protons (H+) during the regeneration process. Ion-specific flux measurements suggest that H+ effluxes are triggered and maintained during crucial stages of the regeneration. A microarray analysis revealed the V-ATPase as a putative mediator of such H+ fluxes, and we confirmed its specific expression in the regenerating tissue (blastema) at both mRNA and protein levels. Morpholino and pharmacological knockdown of V-ATPase expression or activity respectively, decreased the regeneration rate.

To further investigate this relationship between proton flux, likely mediated by the V-ATPase and regeneration kinetics, we took advantage of the fact that in the same fin there can be different regeneration kinetics, depending on the plane of amputation at the proximal-distal axis: the more proximal the amputation (removal of a bigger section of the fin), the higher the regeneration rate. Our data revealed that after proximal amputation, proton efflux starts earlier, and has a higher magnitude than those at the distal stumps. V-ATPase expression follows the same pattern.

Overall, our results suggest tightly-regulated ion-driven phenomena as part of the mechanism of regulation of adult tissue regeneration kinetics.
The Notch ligand Jagged 1 is essential for cardiac valve and chamber development

abstract ID: 117

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The Notch ligand Jagged 1 is essential for cardiac valve and chamber development

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Notch is an evolutionarily conserved signaling pathway that regulates cell-fate specification, differentiation, and patterning. The extracellular region of Notch can interact with membrane-bound ligands of the Delta and Jagged families. At early stages of development the heart is a tube with two layers, an outer myocardial layer and an inner endocardial endothelium. The role of Notch signaling in the endocardium is to promote EMT and the formation of endocardial cushions, the primitive cardiac valves. Notch is also required for ventricular chamber development. In the developing heart, Jag1 is expressed in chamber myocardium and endocardium but its specific role in these tissues is still not clear. We have used different murine loss of function models to reveal the role of Jag1 during heart development. We crossed Jag1
t with endocardial Cre lines to study the role that Jag1 has in the endocardium and with myocardial Cre lines to analyze its role in myocardium. Our results indicate that deletion of Jag1 in the endocardium impairs valve maturation, causes ventricular septum defects and a thinning of the compact myocardium. Deletion of Jag1 in the myocardium affects chamber maturation leading to a thinner compact zone wall and the formation of larger trabecules invading the light of the ventricles. These data indicate that the deletion of Jag1 affects valve cushions fusion and morphogenesis but also plays a crucial role in ventricle patterning and maturation. Interestingly, the Notch1 receptor is active in the endocardium, suggesting that a myocardium-endocardium Notch ligand-receptor signaling interplay is crucial for the development of these cardiac structures and we are currently investigating the underlying molecular mechanisms.
The extended ArmcX cluster and the origin of the complex eutherian brain

abstract ID: 118

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Three eutherian-specific gene subfamilies known as ARMCX (or GASP) and BEX/WEX (or BEX/TCEAL) map to the q22 region of the X chromosome. The BEX/WEX and the ARMCX genes share no evident similarities at the protein level, but they all share a common exonic structure, as well as an intriguing motif in their 5' UTR. The whole cluster probably originated through retroposition of a single copy ancestor gene (Armcl0) followed by quick expansion and subsequent tandem duplication. After this, rapid sequence divergence gave rise to the three families. The homology in the 5' UTR has been preserved through several events of gene conversion within and between the three families, up to the creation of chimaeric genes. Furthermore, events of gene conversion comprising the coding region have also occurred between genes of the same family. These frequent events of gene conversion may have been important to homogenize the regulation of these genes both transcriptionally (preserving the sequence motifs inside the promoters to which transcription factors bind) and post-transcriptionally (concerning mRNA processing).

Excitingly, the sudden appearance of this cluster in the origin of eutherian mammals, consisting of more than 20 genes expressed preferentially in the brain, has been proposed to be linked to the increased brain complexity found in this group, namely the development of an expanded and complex. In addition, ARMCX cluster genes have been shown recently (López-Domenech et al., Nature Communications, 2012) to play a role in regulating mitochondrial dynamics and transport in neurons, which are key processes for nervous system activity. Here, we show the step-by-step events that generated the cluster in the early evolution of eutherian mammals, gaining further insights into the regulation of the cluster. We also show data on the expression of the clustered and the pre-duplicative genes during the embryonic development of amphioxus, zebrafish and mouse, and discuss their co-option to get a complex brain.
Cell competition in cardiogenesis: mechanisms for tissue optimization

INTRODUCTION:

Cell competition is a well established mechanism for tissue homeostasis in *Drosophila*, where it has been shown to promote the expansion of the fitter cells at the expense of suboptimal but otherwise viable cells. Recent evidence suggests that this mechanism could be playing a major role in metazoan development, tissue homeostasis and organ regeneration, performing so in a phenotypically silent manner. In *Drosophila*, Myc has been shown to be a key factor in triggering cell competition through its ability to control many aspects of the cell's anabolic machinery.

METHODS:

In order to understand the occurrence and relevance of cell competition triggered by Myc, we have generated a novel system of inducible genetic mosaics that allows us to manipulate Myc levels and apoptotic patterns. This system consists in a knock-in insertion in the Rosa26 locus which carries two pair of lox sites. Upon Cre mediated recombination, two cell populations arise, allowing us to overexpress c-Myc in a mosaic fashion.

RESULTS:

To address whether cell competition remains an active process during cardiogenesis we have overexpressed Myc in a mosaic fashion both in cardiac progenitors and in differentiated cardiomyocytes. During heart formation, cardiomyocytes are as well able to respond to heterogeneity in cell anabolic activity and eliminate the suboptimal ones by apoptosis. We have seen an important enrichment in Myc overexpressing versus WT cardiomyocytes at birth, which does not take place when apoptosis is blocked in WT cardiomyocytes. Moreover, this phenomenon takes place in a dose-dependent fashion: a direct relationship is found between myc levels and final contribution to the heart.

CONCLUSIONS:

Thus, differentiating cardiomyocytes are able to eliminate less fit cells through cell competition and this feature is not exclusive of pluripotent cells. We are currently exploring how heart progenitors respond to Myc heterogeneity and also the ability of cell competition to induce the displacement of less active postmitotic cardiomyocyte populations in homeostatic and injured adult hearts.
Looking for new partners in crime for the tumorigenic activity of pipsqueak

abstract ID: 122

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The BTB-containing transcription factor Pipsqueak induces invasive tumors when co-expressed with the Notch ligand Delta in *Drosophila melanogaster*. Previously, we have found that mutations in either the BTB protein-protein interaction domain or the sequence-specific DNA-binding domain of Pipsqueak disrupt its oncogenic activity, implying that both protein-protein interactions and DNA-binding activity of Pipsqueak contribute to tumorigenesis. Here, we have identified proteins that interact with Pipsqueak using yeast two hybrid experiments and we are investigating their potential role in Pipsqueak-mediated oncogenesis. Several of the Pipsqueak interacting factors have known roles in sumoylation, therefore we are currently investigating if Pipsqueak itself is sumoylated *in vivo* and if this modification influences its oncogenic activity.
Motile cilia need to be coordinated and ciliary beat frequency (CBF) is characteristic of different types of cilia depending on their physiological function. In zebrafish, monociliated cells arise in the tailbud at the end of gastrulation in a transient spherical organ called Kupffer's vesicle (KV). Using zebrafish as a model, our group has been studying cilia length regulation and motility in wild-type (wt) and aei<sup>−/−</sup> mutant embryos. These mutants carry a premature stop codon in the deltaD gene. Recently, our group showed that Notch signaling was directly involved in the control of cilia length in the KV cells given that the aei<sup>−/−</sup> mutant present shorter cilia in KV cells. The goal of this project is the characterization of the CBF and beat patterns of aei<sup>−/−</sup> KV cilia vs. wt cilia. We did spectral analysis of individual cilia associated with high-speed digital videomicroscopy. By decomposing and comparing the obtained frequencies with Fourier Transform we have identified significant differences in KV cilia motility pattern between the wt and the aei<sup>−/−</sup> mutants. So far, we show that not only are the cilia shorter in the KV of aei<sup>−/−</sup> mutants but also their motility pattern is different resulting in an overall defective fluid flow.
Joubert Syndrome is a ciliopathy that can be caused by a mutation in Arl13b protein. This syndrome is characterized by problems in embryonic development, especially at the neurological level. Arl13b is a protein that belongs to the small GTPase family, but presents the double size of a normal GTPase, because it has a different C-terminus with a coiled-coil domain and proline rich region. It is known that Arl13b localizes to the cilium and recent data showed that Arl13b is in the ciliary membrane. However the molecular function of Arl13b is unknown. This work is based on a functional study of this protein where we used zebrafish as a vertebrate model organism to study the embryonic development in a situation of loss or gain of function of Arl13b. In both situations we observed cardiac edema and abnormal body curvature. This work shows that in an over-expression situation cilia length is increased in the Kupffer’s vesicle, leading to randomization of the left-right gene expression cascade. This study contributes to a better understanding of Arl13b protein function in an organism since its molecular mechanism is not yet known, and provides new information on the localization of this protein in motile cilia.
Defining the requirements for efficient gene expression during early embryonic development.

abstract ID: 134

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Transcriptional elongation has been reported to occur at a rate of proximately 1-2 kilobases per minute. Paradoxically, Drosophila transcriptional activation of the quiescent zygotic genome after egg fertilization (early zygotic transcription) occurs during the extremely fast syncytial nuclear divisions, when the interphases can be as short as 5 minutes. We and others have hypothesized that syncytial nuclear divisions impose a significant constraint in early zygotic expression. Supporting this hypothesis, early zygotic genes are frequently small and intronless.

We isolated a mutant (which we named fandango) that is specifically defective for pre-mRNA splicing of early zygotic but not maternal pre-mRNAs. Analysis of this mutant suggests that these differences are due to distinct developmental contexts, and not due to the transcripts sequence per se.

Our work suggests that Fandango is required for RNApolII phosphorylation and the efficient recruitment to the elongating RNApolII complex of distinct proteins important for early zygotic expression. We hypothesize that Fandango function becomes particularly rate limiting in developmental conditions when gene expression needs to be particularly efficient.

The main aim of this proposal is to define the function of Fandango in transcriptional elongation and pre-mRNA splicing, and better understand the constraints impose by rapidly dividing cells in gene expression.
Trkb-dependent cdc2 inhibition prevents g2/m transition in developing tetraploid neurons

abstract ID: 138

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1) Introduction

In the developing chick retina, a proportion of nascent chick retinal ganglion cells (RGCs) reenter the cell cycle in response to the interaction of NGF with the common neurotrophin receptor p75 (p75NTR). These neurons remain in a G2-like state, thus becoming tetraploid, but in the absence of BDNF they undergo mitosis followed by apoptosis (Morillo et al, 2010). In this work, we focus on the mechanism used by BDNF through its specific receptor TrkB to block the G2/M transition in RGCs tetraploid cells.

2) Methods

Chicken embryos were staged according Hamburger and Hamilton (1951). E6 chick embryos were used in this study. Cell cultures were made as described previously (Frade and Rodríguez-Tebar, 2000). Retinal fragments were electroporated with a plasmid containing Cdc2 and its activator Cyclin B1. The fragments were dissociated and cultured with different combinations of NGF and BDNF. Cdc2 activity was analyzed as an estimation of the mitotic index. Immunostaining was performed as described previously (Morillo et al, 2010). Immunoprecipitation was performed as described previously (Kranenburg et al., 1995). Western blot and ELISA analysis were performed according to the protocols described in Santos et al. (2012).

3) Results

We have observed that TrkB, the BDNF neurotrophic receptor, is expressed by a subpopulation of differentiating RGCs susceptible to reactivate the cell cycle. When used at concentrations specific for the TrkB receptor, BDNF reduces the expression of cdc2 and inhibits the activity of both endogenous cdc2 and exogenously-expressed cdc2/cyclin B1 in TrkB-positive, differentiating retinal neurons in vitro. BDNF seems therefore to post-translationally modify cdc2, as it has been shown to occur with the cdc2 family member cdk5 (Cheung et al., 2007). We show that this inhibition depends on the phosphorylation of cdc2 at Tyr15, and that this modification is specific since BDNF cannot prevent the activity of a constitutively active form of cdc2 (Tyr15Phe) when expressed in differentiating retinal neurons.

4) Conclusions

The inhibition of cdc2 activity by BDNF results from its phosphorylation in Tyr15. This, along with the capacity of BDNF to reduce the expression of both cdc2 (this study) and cyclin B1 (Frade, 2000), likely contributes to the maintenance of tetraploid RGCs in a G2-like state.

5) Acknowledgements

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6) References

Cheung et al. (2007) Plos Biol 5: 865-877


Hamburguer and Hamilton (1951) J Morphol 88:49-92


Drosophila melanogaster rhogef cg43658 is required for proper dorsal vessel formation

abstract ID: 142

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Rho GTPases are key regulators of cytoskeletal dynamics in a wide variety of morphogenetic events, such as cell migration, axonal guidance, vesicle trafficking, cytokinesis and endocytosis. Additionally, they control many other biological functions, such as cell-cycle progression, contraction, gene expression, contraction, cell-cell and cell-matrix adhesion and cell polarity [1]. Recent data have indicated a role of Rho GTPases in Drosophila melanogaster heart formation, also called dorsal vessel. Mutations in genes controlling heart development and abnormalities in any step of heart development frequently cause fatal cardiac malformations, the most common type of birth defect in humans [2]. Comparative studies between vertebrates and Drosophila highlight a significant conservation in the embryology and molecular regulation of heart development. Hereby, Drosophila melanogaster represents a powerful model for genetically dissecting this complex developmental process [3]. Rho GTPases are activated by the RhoGEFs, which promote the exchange of GDP to GTP, and are inactivated by the RhoGAPs, which promote the hydrolysis of GTP to GDP. During embryonic development, RhoGEFs and RhoGAPs are predicted as expressed in restricted patterns in order to regulate locally the activities of their target RhoGTPases [4]. Our aim is to characterize the expression of all 23 RhoGEFs in Drosophila embryos in order to identify RhoGEFs specifically expressed in the dorsal vessel.

For this, we used embryo Whole Mount In Situ Hybridization to analyze the RhoGEFs expressed in all embryonic developmental stages. We identified a previously uncharacterized RhoGEF (CG43658) as expressed in the dorsal vessel during embryonic stages 13-17.

To confirm that CG43658 is specifically expressed in the dorsal vessel, we used Fluorescent In Situ Hybridization followed by an immunostaining against the epithelia marker engrailed or the muscle marker Mef2. We then generated a homozygous viable mutant of CG43658 using the FLP-FRT recombination technique. Finally, we obtained a mutant reporter line (by combining our CG43658 mutant with either Tup-GFP or Toll-GFP) that allowed us to characterize in vivo dorsal vessel formation defects during stage 16 of embryonic development. We found that deletion of CG43658 causes a misalignment in the aorta cardioblasts between segments T4-T5 of stage 16 embryos.

This is the first time that CG43658 has been described to function during dorsal vessel formation in Drosophila. The homolog in human, RhoGEF10, is known to also be expressed in the heart and is a susceptibility gene for atherothrombotic stroke. These resemblances between human and Drosophila once again show that the fly can be a powerful model system to study human-related diseases.

The embryo molecular clock in temporal control of hox gene expression

abstract ID: 143

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Introduction: Embryo development proceeds under strict spatial and temporal control. Positional information along the vertebrate body is specified by differential Hox expression. An embryonic molecular clock (EC) was first evidenced by cyclic hairy1 expression underlying somite formation periodicity and is known to operate in multiple other systems. A link between temporal collinear activation of Hox gene expression and the EC has been proposed, but definitive evidence is required to connect these processes.

Methods: We have performed a meticulous characterization of HoxB cluster gene expression activation in chick embryos from late blastula to 6-somite stages using a tailored RT-qPCR array. Misexpression of EC genes was performed by in vivo electroporation and the HoxB gene expression obtained was compared with the molecular signatures previously obtained in control embryos.

Results: This large gene expression study confirmed temporal collinearity of HoxB expression initiation in early embryos and unveiled stage-specific HoxB gene expression signatures. EC genes present dynamic expression at early gastrulation stages, when Hox activation takes place. We found that development was clearly delayed upon misexpression of EC genes, accompanied by a corresponding delay in HoxB gene expression signature. We will present a model, whereby EC oscillations may temporally control Hox gene expression initiation through cyclic protein complex formation with a transcription factor known to mediate activation of Hox expression.

Conclusion: Our data strongly suggest that the EC is regulating temporal collinearity of Hox gene expression initiation, thus coupling temporal and positional information in the early embryo.

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Wnt signaling shapes planarians

abstract ID: 146

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Introduction

Planarians are a classical model for regeneration studies due to their striking plasticity. They can regenerate a whole animal from any piece of their body and show a continuous remodeling. This capacity relies on the pluripotency of their adult stem cells, which, according to recent results, is accompanied by the continuous activation of major developmental signaling pathways. The evolutionary conserved Wnt family of secreted proteins exerts a critical control of the intercellular communication. Despite its broad range of functions, the ßcatenin-dependent Wnt signaling is a conserved mechanism to specify endomesoderm and pattern the antero-posterior axis during embryogenesis across metazoans. The ßcatenin-independent Wnt signaling includes unrelated branches, involved in the regulation of different processes such as planar cell polarization (PCP), cell division orientation, ciliogenesis or neural circuitry assembly.

Methods

RNAi silencing of the Wnt pathway elements was performed to study their function in the planarian species Schmidtea mediterranea and Schmidtea polychroa. A specific antibody against bcatenin1 was raised to analyze its nuclear localization (activation).

Results

In adult planarians, both during regeneration and homeostasis, silencing of bcatenin1 produces a striking fully anteriorization, demonstrating that the ßcatenin-dependent signal is a conserved mechanism to pattern the antero-posterior axis in adult planarians. Out of the nine Wnts which integrate the Schmidtea Wnt family, four of them are expressed in the posterior part of the animal, being a Wnt1 homolog the main bcatenin1 activator.

Our analysis of bcatenin1 protein expression throughout the planarian life cycle (embryogenesis, sexual maturation and regeneration) supports 1) its ancestral role in endomesoderm specification, as it is specifically nuclearized in the blastomeres of the transient embryonic pharynx; 2) its function in antero-posterior patterning and cell differentiation also during embryogenesis, as a burst of ßcatenin1 nuclearization occurs in the transition from the radial ‘larvae’ to the bilateral adult; 3) its nature of master regulatory gene essential for general patterning, since in late embryos, juveniles and adults, ßcatenin1 is activated in the anterior and posterior tips, in the dorso-ventral border and in every organ; and 4) although during regeneration, ßcatenin1 is nuclearized in both anterior and posterior blastemas, its kinetics is different, spatially and temporarily coinciding with Wnt1 expression. Interestingly, Wnt1 silenced animals, which regenerate a head instead of a tail, specifically down-regulate bcatenin1 in the posterior blastema.

We have already reported the functional conservation of two different ßcatenin-independent Wnt branches: a Wnt-independent one, integrated by Dishevelled, Van-Gogh and Diversin, representing the first PCP network reported in lophotrocozoans, and required for apical positioning of the cilia basal bodies of planarian epithelial cells; and a Wnt5-dependent one, which controls the...
proper medio-lateral positioning of their central nervous system. Here we show that a Ror-family receptor tyrosine kinase (Ror) is the responsible for receiving the Wnt5 signal in planarians. We are currently exploring whether Wnt5/Ror signaling governs neuronal migration or axonal projections.

**Conclusions**

Planarians βcatenin-dependent and -independent Wnt signals are functionally conserved both during embryogenesis and during post-embryonic development, highlighting the close relationship between adult plasticity and maintenance of embryonic signals.

During embryogenesis, βcatenin is first nuclearized in the embryonic pharynx (the first endodermal structure) (arrows in A); later on it is nuclearized in the central nervous system, in the ventral nerve cords (B) and in the main organs. The βcatenin-independent Wnt5/Ror-dependent signal is required for correct medio-lateral positioning of the cephalic ganglia in adult planarians (arrows in C indicate ectopic expanded brain in wnt5 RNAi animals).
HOXB GENE EXPRESSION ACTIVATION IN DEVELOPING CHICK EMBRYOS

abstract ID: 151

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Introduction: Positional information along the vertebrate embryo body axis, reflected on the identity of trunk segments, is specified by differential Hox gene expression during early development (1). Hox genes are organized in the genome along clusters such that the anterior limit of their expression in the embryo is collinear with their chromosomal position, defined as spatial collinearity. Hox genes also present temporal collinearity, as the onset of gene expression occurs in a temporal sequence that respects the ordering of the genes in the cluster. Aiming at a better understanding of temporal control of the acquisition of positional information during embryo development, we have studied HoxB cluster gene activation in the chick embryo.

Methods: We have characterized the temporal progression of HoxB gene activation in the early chick embryo, by evaluating gene expression patterns of HoxB cluster genes HoxB1, HoxB2, HoxB3, HoxB5, HoxB8 and HoxB9, from late blastula to 6-somite stage by in situ hybridization and subsequent cross section histology analysis.

Results: Temporal collinearity of HoxB cluster gene expression activation was observed. Anterior HoxB cluster genes are expressed earlier in development, than the posterior-most genes. HoxB gene expression initially appears at the posterior primitive-streak level and progressively extends along the primitive streak and surrounding epiblast, resulting in a radial gradient of expression. This is not the case for HoxB5, which is mostly expressed in the extra-embryonic tissue.

Conclusions: HoxB cluster gene expression activation presents Temporal Collinearity in the early chick embryo. Spatial collinear expression was also observed in the primitive streak and in neural tube tissue, with few exceptions. The expression patterns of HoxB2 and HoxB5 in early chicken embryos are described for the first time. The results obtained with this study intend to contribute towards a better understanding of temporal control of the acquisition of positional information during embryo development.

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The epithermal growth factor receptor 1 and its role during the regeneration and homeostasis in the planarian Schmidtea mediterranea

abstract ID: 153

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Introduction: The amazing regenerative abilities shown by freshwater planarians are due to the presence of a population of pluripotent stem cells called neoblasts. Several studies have been addressed to determine the molecular mechanisms that control those cells during both regeneration and homeostasis. Thus, it has been hypothesized that neoblasts could express different receptors specific for growth factors, which would control their proliferation and differentiation. This premise together with the fact that some studies have suggested that the epidermal growth factor (EGF) could act as a mitogenic agent for the planarian stem cells, have made the EGFR signaling pathway a good candidate to be regulating planarian regeneration. In our laboratory, we have identified so far three putative planarian EGFR homologues and my work is focused on one of them, the Smed-egfr-1.

Methods: Smed-egfr-1 was silenced using RNAi technique. The phenotypes were analyzed using in situ hybridizations and immunochemistry with several markers in both, whole animals and histological sections. In order to identify putative downstream targets of Smed-egfr-1 we constructed and sequenced several libraries for Digital Gene Expression (DGE) analyses. We have compared the transcriptomic profiles of control and Smed-egfr-1-knockdown animals after 2 and 4 weeks of RNAi treatment.

Results: Smed-egfr-1 is mainly expressed in the gut, mesenchyme and pharynx and its silencing results in a variety of phenotypes at different levels of cellular and tissular organization. First of all, Smed-egfr-1 RNAi animals display reduced or absent eye pigment cells compared to controls. Moreover those animals fail to differentiate a normal pharynx and instead they show aberrant pharynxes which are smaller, more rounded and with a reduced lumen. Another tissue that seems to be affected after the RNAi of Smed-egfr-1 is the gut. In RNAi animals the gut branches are less ramified and appear to have a reduced lumen with an abnormal gastrodermis. Finally the most intriguing consequence of the silencing of Smed-egfr-1 is a general hyperproliferation all throughout the animal which is associated with the development of dorsal outgrowths within a variety of cell types and tissues differentiate. To gain further insights into the role of Smed-egfr-1 and to find candidate downstream genes, we have performed DGE analyses after its silencing. Preliminary studies of the results obtained show that many matrix metalloproteinases as well as several extracellular matrix components such as collagens are miss-regulated in Smed-egfr-1 RNAi animals. This could be in agreement with the remodeling that the treated animals are suffering as a consequence of the formation of the dorsal outgrowths and the lost of their normal gut and pharynx architecture. Moreover, various neoblast markers appear up-regulated which is consistent with the hiperproliferation observed. At present, we are analyzing in further details some of those candidate genes and their connection with Smed-egfr-1 and the defects displayed after its silencing.

Conclusions: Our results demonstrate that Smed-egfr-1 is necessary not only for the proper differentiation of specific cell types, such as the eye pigment cells but also for the correct regeneration and homeostasis of relatively complex organs as for instance the pharynx and the gut. Moreover the hiperproliferation observed after its silencing suggests that it might be playing a role as well in controlling neoblast proliferation.
En-light-enig the embryo segmentation clock

abstract ID: 157

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Timing is essential for proper embryonic development. It has been shown the presence of periodic gene expression during embryogenesis, with a 90min period in somitogenesis and 6h in the limb, in the chick. These oscillations give the notion of time, unveiling the existence of an Embryonic Clock. Several cell and tissue circadian rhythms have been described after birth, in many organisms. Still, the importance of external cues such as light/dark cycles remains greatly neglected concerning embryogenesis.

Here we demonstrate that several circadian clock genes, such as clock, bmal1, cry1, per2, cki are also expressed very early in chick development. We know that the Circadian Clock genes are characterized for being influenced by light entrainment. Routinely in the lab the embryos are grown in constant darkness and development proceeds normally. This could suggest that light has no capacity to influence embryo development. However, it is known that the Circadian Clock is preserved in dark conditions, being significantly affected only in constant light. In order to determine to what extent different light conditions affect the Embryonic Clock, we have evaluated these same parameters in embryonic tissues directly exposed to light using our chick explant culture system: the control explant will be grown in constant darkness and the experimental half will be submitted to constant light. We have already performed several experiments with different incubation periods obtaining very promising results: constant light appears to affect the expression of Embryonic Clock genes hairy1 and hairy2.

Taken together, these results show that light affects 1/3 of the cultured explants, regarding Embryonic Clock genes. We know that light affect the Circadian Clock via CREB/MAPK and that FGF also induces ERK/MAPK oscillatory activity accompanied by cyclic expression of Embryonic Clock genes. Could mimic the role of Fgf8 in the embryo?
Secreted Frizzled Related Proteins (SFRPs) compose a family of soluble molecules structurally related to Frizzled receptors. Several studies have demonstrated that these proteins, besides acting as Wnt ligand antagonists, have several additional Wnt-related and unrelated functions. Among others, our laboratory has recently demonstrated that in the central neural retina, Sfrp proteins bind and inhibit the Adam10 metalloproteinase, which is the α-secretase responsible for cleavage of the Notch receptor and thereby of subsequent signalling activation. In contrast, in the retinal periphery, Sfrp proteins positively modulate Wnt/β-catenin signalling, favouring Wnt ligand diffusion. We will show that Sfrp1 has a similar dual role during the development of the telencephalon, where it is strongly expressed. In Sfrp1−/− mice, the brain is reduced in size, presents small lateral ventricles, abnormal layering of the cortex with agenesis of the posterior corpus callosum and expansion of the retro-splenial cortex at the expenses of the hippocampal region. The abnormal thickening of the cortical neural epithelium is associated with a transient up-regulation of Notch signalling at early embryonic stages that, similarly to what happens in the retina, causes an increase in early neural progenitor proliferation and differentiation of early-born, lower layer projection neurons. Furthermore, proteolytical processing of other Adam10 substrates is also altered, supporting that in the cortex Sfrp1 normally modulate this α-secretase. Previous studies have shown that hippocampal development depends on the activation of canonical Wnt signalling. In Sfrp1−/− embryos, the hippocampal primordium is reduced, expresses very low levels of Wnt canonical target genes, such as Left1 and Axin2, and does not show detectable active β-catenin, strongly supporting that Sfrp1 acts as a positive modulator of Wnt/β-catenin cascade, as observed in the retinal periphery.

Together, these data demonstrates that Sfrp1 has a key function in telencephalic development, modulating both Notch and Wnt signalling with different mechanisms, and establish an interesting parallel between the events that control retinal and telencephalic development.

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Redundant and specific roles of the zebrafish tbx5 genes

abstract ID: 162

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Mutations in the T-box transcription factor \(TBX5\) cause Holt-Oram syndrome, an autosomal dominant human “heart-hand” condition characterised by upper limb and heart malformations. In zebrafish and although \(tbx5\) is conspicuously expressed in the developing heart, eye and pectoral fin precursor cells from their earliest stages of determination, embryos with compromised \(tbx5\) function show a complete absence of pectoral fins, whereas heart and eye development are not so highly disturbed. We identified a new \(tbx5\) gene in zebrafish that we call \(tbx5b\). This duplicate gene is present in all teleost genomes whose sequence is available, suggesting it resulted from the teleost-specific genome duplication event that took place during fish evolution. Detailed analysis of \(tbx5a\) and \(tbx5b\) expression patterns showed that \(tbx5b\) has lost the characteristic forelimb/pectoral fin expression of Tbx5 genes but has retained the eye and heart expression, overlapping with that of its parologue, now referred to as \(tbx5a\)\(^1\). Our hypothesis is that functional redundancy of \(tbx5a\) and \(tbx5b\) in the eye and heart would therefore explain the mild phenotypes observed during development of these organs in fish embryos with compromised \(tbx5a\) function.

To address this, we are currently using morpholinos in combination with loss of function alleles in the background of early heart, pectoral fins and eye precursor reporter lines to study the cellular phenotypes of \(tbx5a\), \(tbx5b\) as well as double \(tbx5a/tbx5b\) compromised embryos. Our most recent results illuminating those redundant versus gene specific functions will be presented.

\(^1\)Albalat et al., 2010. GEP 10:24-30
Sp6 and sp8 transcription factors mediate wnt/b-cat dependent function in the limb ectoderm

abstract ID: 163

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The apical ectodermal ridge (AER) is a specialized thickened epithelium located at the distal rim of the developing limb, along the dorso-ventral (DV) boundary. Through the synthesis of several members of the Fibroblast Growth Factor (FGFs) family, it controls limb proximo-distal development. The induction of the AER is a complex process that relies on intricate interactions amongst Fgf, Wnt and Bmp signalling pathways operating within the ectoderm and between the ectoderm and mesoderm of the early limb bud. Furthermore, the induction of the AER is linked to the establishment of DV patterning.

Interestingly, two members of the Specificity Protein family of transcription factors, Sp6 and Sp8, also known as Epiprofin and Buttonhead respectively, have been shown to function downstream of Wnt signalling and upstream of Fgf8 in the limb ectoderm. Sp6 and Sp8 have been individually disrupted. Disruption of Sp6 prevents AER maturation and results in syndactyly with partial dorsolization of the digital tips whereas disruption of Sp8 results in limb truncation due to the premature regression of the AER.

To unravel the possible redundancy between Sp6 and Sp8, we have generated double Sp6;Sp8 null mutants. We have also generated Sp6null;Sp8 conditional mutants using the Ap2-Cre and the Msx2-Cre lines. Our results show that double Sp6;Sp8 mutants are amelie although an initial budding with no detectable expression of Fgf8 occurs. The phenotype of mutants bearing a single functional copy of Sp6 (Sp6"+/Sp8"+) is indistinguishable from that of double mutants whereas the presence of a single functional allele of Sp8 (Sp6"-;Sp8"+) results in a Split Hand Foot Malformation (SHFM) phenotype. We will present the molecular characterization of these limbs and discuss the role of Sp6 and Sp8 in limb development.
Stat proteins expression during lung development in nitrofen-induced congenital diaphragmatic hernia rat model

Abstract ID: 169

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Introduction
The signal transducers and activators of transcription (STAT) are best characterized as downstream mediators of cytokine signaling. Along with cytokines, STATs have been implicated in the signal transduction of other major instructive pathways of fetal lung development including growth factors such as FGF, VEGF, PDGF. Further evidence has implicated STAT proteins in the pathogenesis of allergic airway diseases but also in lung inflammation and repair. Although improved understanding of normal and abnormal lung development may unveil new therapeutic targets to rescue impaired lung growth, common to a spectrum of human developmental diseases, such as congenital diaphragmatic hernia (CDH) STATs involvement in normal and abnormal fetal lung development remains largely underexplored.

Methods
Western Blot and Immunohistochemistry were performed to evaluate the expression pattern of STAT protein family (STAT1-6) during normal lung development. CDH was induced by maternal administration of a single dose of nitrofen on day 9.5 of gestation (term, 21.5 days). Cesarean section was performed and fetuses were harvested on days 15.5 through 21.5. Compared characterization of gestational expression levels of STAT protein family (STAT1-6) in normal rat lung and in CDH lung, between days 15.5 and 21.5, was performed by western blot analysis.

Results
STAT protein family members are constitutively expressed in pulmonary tissues during fetal lung development. STAT1, STAT4 and STAT5 display increased pulmonary expression at the latest gestational stage, day 21.5, in both normal and CDH lungs. Whereas STAT3 and STAT6 display increased pulmonary expression in CDH cases significantly different from control lungs from day 17.5 onwards.

Conclusions
The present study provides evidence of the presence of STAT protein family in fetal lung development. Furthermore our data point towards a disease-induced STAT3 and STAT6 overexpression, which is suggestive of a role for STATs in the poorly understood pathogenetic events in CDH.
An eye-targeted double-rna screen identifies a functional interplay between drosophila dpp signalling and viriato

abstract ID: 170

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Introduction: The correct patterning of body structures, like the fly eye or any other organ, is regulated by cell-cell signalling pathways that orchestrate the timing and spatial organization of cellular proliferation and differentiation. In Drosophila, the Dpp (BMP2-4 class of the TGF-beta family of cytokines) signalling pathway plays multiple essential developmental roles. During eye development, Dpp plays a dual role: early on it is required for the proliferation and survival of undifferentiated cells, but later on it has a role in the initiation and progression of retinogenesis. At a cellular level, the nucleolus plays a major role in this control as it is been characterized as a regulatory compartment involved in important cellular processes as ribosome biogenesis, cell-cycle control, apoptosis and cellular stress response. Previous studies in our lab identified Drosophila Viriato (Vito) as a nucleolar-localised protein that is necessary for cell proliferation and survival and thus, required for proper tissue growth development (Marinho et al. 2011).

Methods: We performed a targeted double-RNAi screen using the driver eyelessGal4 to identify pathways working with Vito in the promotion of tissue growth and differentiation in the developing eye. As a read-out we used the L3 eye-antennal imaginal disc photoreceptor differentiation as well as the adult fly retina size.

Results: We have identified a strong genetic interaction between vito and members of the Dpp signalling pathway (including TGF-beta receptor type I and II, thickveins and put and the co-SMAD medea). Interestingly, this interaction is not restricted to growth control but also it suggests a role of Vito mediating Dpp induced differentiation. Moreover, we found that nucleolus size is dynamic modulated during differentiation: before differentiation occurs in the eye disc (2nd instar larvae) all the cells present a prominent nucleolus, however as soon as the differentiation wave starts, the undifferentiated cells closer to the photoreceptors present a reduction in the nucleolus size. This dynamic behaviour of the nucleolus might be important for the photoreceptor differentiation as when we reduced the levels of other nucleolar protein Nopp140 in a sensitized background, we identified a strong effect on retinal differentiation.

Conclusions: Putting together, our data suggest that Vito besides being important for growth control and nucleolar function may have an important role in differentiation. This may reflect a novel role for nucleolar-based events in the modulation of signalling pathways regulating photoreceptor differentiation.

References:

Signalling gradients, embryonic clock and limb structure formation

abstract ID: 173

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Development of all embryo structures requires precise spatial-temporal orchestration of cell proliferation and differentiation to correctly shape each structure at the right time and place. During embryo development, a Hairy-Enhancer-of-split (HES) family gene, *hairy2* was found to present cyclic expression in the distal limb chondrogenic progenitor cells and proposed to be providing temporal and proximal-distal (PD) positional value (Pascoal et al., 2007). This tissue is maintained in an undifferentiated proliferative state by Fibroblast Growth Factors (FGFs) produced by the distal apical ectodermal ridge (AER), which also dictates PD outgrowth and patterning. Identification of this limb clock supports the existence of an intrinsic oscillator in the distal limb, previously predicted by the Progress-Zone (PZ)-model for limb PD patterning (Summerbell et al., 1973). Presently, a Two-Signal (TS)-model, based on opposing proximal-to-distal Retinoic Acid (RA) and distal-to-proximal AER-FGF signalling gradients is accepted to account for limb PD-patterning (Tabin & Wolpert, 2007). This model does not contemplate the requirement of a time counting mechanism for PD-patterning, however this issue remains controversial (Towers et al., 2012).

By manipulating AER-FGF and RA signalling pathways through meticulous tissue ablation and/or bead implantation experiments, we recently described their crucial role for the clock gene *hairy2* expression in the distal limb (Sheeba et al., 2012a, b). We further showed that Erk/MAPK, Akt/PI3K and Gli3 activity underlie this regulation. In addition, the signalling molecules governing limb anterior-posterior (AP) patterning, Sonic Hedgehog (SHH) secreted by the zone of polarizing activity (ZPA), is also involved in *hairy2* expression regulation through Gli3 activity modulation (Sheeba et al., 2012a). AER-FGF functions as a short-term, short-range instructive signal; ZPA-SHH acts as a long-term, long-range permissive signal and RA is capable of performing both instructive and permissive roles on *hairy2* expression, although in a transient manner.

While the requirement of temporal information for limb patterning is challenged by the TS-model, our findings have clearly associated the confronting gradients of RA and FGF signalling with *hairy2* expression and revealed that the limb clock is tightly monitored by the PD-patterning molecules FGF/RA and the AP patterning morphogen SHH. Considering the influence of the limb patterning gradients on the limb clock, we propose a model for integrated PD and AP limb patterning, combining time with signalling gradients.


High levels of gli3r do not prevent the development of recombinant limbs made with anterior mesoderm

abstract ID: 176

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A recombinant limb (RL) can be described as a limb bud-like structure formed by the experimental assembly of limb bud mesoderm inside a limb bud ectodermal jacket. This procedure permits a huge grade of manipulation of the limb components before the recombination. When grafted to a host embryo, the RL develops into a normal limb or into a limb-like structure depending on the origin/manipulation inflicted to the limb components. The use of RLs has notably contributed to the understanding of the mechanisms controlling patterning in the limb bud including recent molecular studies on proximo-distal specification.

However, certain aspects of this powerful system are not fully understood. For example, the anterior mesoderm that is unable to survive if isolated from the posterior mesoderm (Shh producing cells), shows a high morphogenetic potential and makes digits when it is dissociated-reaggregated in the RLs situation (aRLs). Our aim is to clarify the reason for these two highly contrasted behaviors of the anterior mesoderm.

With this purpose we have embarked on a complete characterization of the RL model, in particular of the aRLs. First, we analyzed the state of Shh signalling in these recombinants. Our results showed that Shh expression and that of its targets Gli1 and Ptc, was undetectable. Accordingly, the expression of Gli3 occurred all across the anterior-posterior axis of the aRL. However, the aRLs express genes typical of the posterior mesoderm, such as the 5'Hoxd genes, what could explain their morphogenetic capacity and suggests a possible failure in the processing of Gli3 to its repressor short form (Gli3R) or in its function.

Since the primary cilium is essential for Hh signalling and also for processing and signalling of Gli proteins, we analysed whether the procedure to perform the aRLs could damage the cilium. However, our confocal and electron microscopy study ruled out this possibility as the primary cilium appeared normal in all the phases of aRL development analyzed. Accordingly, we showed by immunoblot analysis that the majority of the Gli3 protein was processed to Gli3R. Furthermore, both immunoblot and immunofluorescence assays revealed that in fact this Gli3R translocates to the nucleus.

Thus, our results show that the aRLs develop with high levels of Gli3R that however do not prevent the expression of posterior genes, neither impact their morphogenetic capacity. These results will be discussed in the context of the current understanding of anterior-posterior patterning of the limb bud.
Involvement of XAdtk1 and XAdtk2, members of a novel tyrosine kinase family in PCP signaling.

abstract ID: 177

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During vertebrate early development, Planar Cell Polarity signaling plays important roles in the development of a wide variety of organs and tissues. Namely, controls tissue polarity and cell movement that occurs during gastrulation and neural tube closure. However, the mechanisms controlling these events are poorly known.

Here we show that both XAdtk1 and XAdtk2, two members of a novel family of serine/threonine/tyrosine kinases are involved in neural tube closure and neural crest migration. During gastrulation both these genes are expressed in the organizer region more precisely in the anterior dorsal endoderm, however later they are expressed in different tissues. Loss-of-function of either XAdtk1 and XAdtk2 display severe defects in neural tube closure and neural crest migration. Curiously, both XAdtk1 and XAdtk2 are induced by both Wnt signaling pathways. However they could only regulate the PCP signaling pathway since both XAdtk1 and XAdtk2 recruit Disheveled to the plasma membrane through the DEP domain. All together these results show that XAdtk1/2 are unique intracellular molecules that converge the signals from both Wnt canonical and non-canonical signaling pathways, into the activation of the Wnt non-canonical pathway leading to cell migration and movement.
Cell contribution to blastema formation and plasticity during zebrafish (danio rerio) caudal fin regeneration

Abstract ID: 181

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Mammals repair some tissues such as blood and liver, but most organs fail to regenerate. In contrast, zebrafish regenerates complex organs and is a popular metazoan in regeneration research [1]. Fin regeneration is an epimorphic process dependent on the formation of a specialized structure, the blastema, mesenchymal-like cells localized between the stump and wound epidermis, that grows and differentiates to restore the lost parts [2].

A key research question is whether blastema originates through tissue dedifferentiation or from a pool of undifferentiated, resident progenitor. A second related question is whether cells are lineage committed during regeneration.

Recent studies suggested that differentiated cells de-differentiate and contribute to blastema formation but remain lineage committed [3-5]. These cells undergo partial dedifferentiation but cell lineage is restricted, contributing solely to new bone related cells. Nevertheless, osteoblast ablation experiments [6] suggest distinct pool of osteogenic progenitor cells, such as fibroblasts. The question remains about the true source and cell commitment during regeneration.

Here we present the strategy that we will use in an attempt to elucidate these fundamental questions.

We will use transgenic zebrafish lines marking osteoblast and/or fibroblast at different differentiation stages: Osx, Runx2b and Oc2 for osteoblasts and Ctgf and Op for fibroblasts. These fluorescent reporters will allow cell fate tracing and contribute to establish the requirement different cell types for regeneration. With this information, directed cell conditional ablation will be induced in osteoblasts and fibroblasts in order to determine whether specific cells contribute to caudal fin regeneration. We will also attempt cell reprograming by inducing expression of genes potentially osteogenic in non osteogenic cells. Finally, cell plasticity between different lineages will be assessed using a recently developed in vitro blastema culture. By isolating the fluorescently marked cells from the transgenic lines (osteogenic cells), we will determine their differentiation fate, and attempt to trigger differentiation, by culture conditions manipulation, of osteogenic cells into a distinct lineage or non-osteogenic cells into osteoblasts.

These data should provide valuable insights on mechanisms of cell differentiation and regeneration, particularly in relation to bone.

Acknowledgements

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References


A role of the zebrafish c-myc in adult caudal fin regeneration

abstract ID: 184

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Preservation of tissue morphology and function is a fundamental competence of multicellular organisms. Some vertebrates such as the urodele amphibians and the teleost fish have an extraordinary capacity to regenerate countless of its organs upon amputation through a process defined as epimorphic regeneration. Thanks to its accessibility, its fast and robust regeneration and its simple architecture, the zebrafish caudal fin is a very powerful model for regenerative studies. After caudal fin amputation, a regenerative event is initiated and in two weeks this structure is completely reformed with exactly the equivalent amount of lost tissue, the correct final size and functionality. A common feature of all organisms that are able to efficiently regenerate amputated appendages is the ability to make and shape a blastema. Although the active cell proliferation of the blastema is required for the progression of regeneration, little is known about the origin and fate of the blastema cells in the fish fin. Recent studies demonstrated that in zebrafish, such as in urodele amphibians, the blastema cells originate from a process of dedifferentiation of adult differentiated cells. Nevertheless, in these studies is a definitive marker of dedifferentiation is still missing. Thus, a big challenge now is to understand how dedifferentiation events during regeneration are being modulated and by which signalling pathways. Dedifferentiation also occurs in the induction of pluripotent stem cells when a set of transcription factors (Oct4, Sox2, Klf4 and c-Myc) is over expressed in mature cell types and since these factors are upregulated in regenerating newt limb and lens tissues we put forward that they may be involved in fin regeneration. We analyzed the expression levels of c-myc in regenerating and non-regeneration tissues and our preliminary results indicate an up-regulation of myc activity within the blastema. Hence in this work we will focus our efforts in trying to unravel a possible role of c-Myc in caudal fin regeneration.
Mechanisms involved in bristle formation in the abdomen of D. melanogaster

abstract ID: 187

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The adult epidermis of Drosophila is formed by cells that descend from histoblasts, stem-like founder cells that are specified during embryogenesis and organized as small nests. From them, different adult cell types arise as the epidermis, the sensory precursors, the oenocytes and the tendon cells.

In imaginal discs, sensory precursors get patterned and selected through lateral inhibition, differentiating in distinct types of adult bristles. Their topographical and temporal patterning is autonomously determined by the activity of the proneural genes of the achaete-scute complex (AS-C).

*achaete (ac)* and *scute (sc)* proneural genes form functional heterodimers with the ubiquitously expressed bHLH protein Daughterless (Da), and bind E-box sequences in target enhancers to activate transcription. One of these targets is Senseless (Sens) that encodes a zinc finger transcription factor. Loss of Sens results in loss of sensory organs. In the abdomen has been suggested that Asense (Ase), another proneural gene, takes over the proneural functions together with Sc. However, we observed that, while Sens is expressed very early during the histoblast nest expansion, Ase is expressed later, indicating that Ase does not act as a proneural protein in the abdomen. Since in the anterior wing margin (AWM) Sens and Da take over the proneural functions, the same could be happening in the abdomen.

In imaginal discs, prepatter genes established the landscape where proneural genes will express in clusters. This is eventually refined to single out individual precursors by lateral inhibition mediated by Notch. Interestingly, sequential time points depicting the expression of E(Spl)ma-GFP reporter (a read out of Notch activity) in dorsal nests also show a wave of expression originating from the posterior edge of the anterior nest.

In histoblasts this process has not been explored before. When and where are sensory precursors born? Which mechanisms lead to their determination? How does the bristle pattern get established? Our data suggests that in the dorsal abdomen, proneural and neurogenic activities proceed almost simultaneously and initiate at 14-15h APF immediately after the transient amplification stage is completed. Both activities spread anteriorly as a front wave from the posterior edge of the anterior compartment and we are characterizing in detail this process.

These findings lead to several important questions to be answered: Which are the mechanisms that activate the expression of Sens and Notch? How Sens works? Does it affect sensory organ precursors specification, differentiation or survival? How and when lateral inhibition is implemented during sensory organs specification in the abdomen?

Preliminary results of this work will be presented.
Study of the role of ccbe1 during early cardiogenesis

abstract ID: 188

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Genetic evidence has implicated several genes as being critical for heart development. However, the inducers of these genes as well as their targets and pathways they are involved with, remain largely unknown.

Therefore, a greater understanding of the molecular control of heart development including the characterization and functional analysis of novel genes involved in cardiogenesis has major implications for treating congenital and adult heart diseases. With this in mind, our laboratory focused on the identification and study of novel genes expressed and involved in the correct development of the vertebrate heart/hemangioblast precursor cell (HPC) lineages. A differential screening using Affymetrix GeneChip Chick system was performed, leading to the identification of several new genes expressed in the haematopoiesis, angiogenesis or cardiogenesis precursor lineages. Among these novel genes Ccbe1, a secreted protein, containing a collagen and calcium binding EGF-like domains was found to be upregulated by 7.8 fold.

Expression analysis indicated that ccbe1 is expressed since very early in cardiac mesoderm precursor cells at HH4 and later in the secondary heart field (SHF).

Ccbe1 contains calcium binding EGF domains that has been shown to be involved in the control of proliferation. Since ccbe1 is expressed in the SHF precursors and cell derivatives, we hypothesise that ccbe1 signalling pathway might be required for appropriate SHF development. Indeed, knockdown of ccbe1 using morpholino antisense oligonucleotides resulted in aberrant heart formation, in which the fusion of the heart fields was incomplete or failed to fuse. The potential role of ccbe1 in the heart morphogenesis is currently being studied and the results will be discussed.

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Uncovering cellular mechanisms of spinal cord regeneration in zebrafish

abstract ID: 189

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Introduction: The vertebrate spinal cord has a key role in the integration of sensory inputs and in the coordination of motor output. Spinal cord damage has severe effects in mammals, due to its limited capacity of cellular regeneration and functional recovery (1). In contrast to mammals, the lesioned spinal cord of adult zebrafish is able to regenerate axons and reestablish the connections to the appropriate targets, thus recovering motor function. Moreover, the zebrafish spinal cord is able to trigger the formation of new motor neurons in response to trauma (2). Our work aims to address the cellular and molecular mechanisms that allow the de novo formation of glia and neurons and ultimately contribute to the recovery of lesions in the adult zebrafish spinal cord.

Methods: To investigate the regenerative events of the zebrafish spinal cord we established a spinal cord crush injury protocol. To assess the degree and progression of regeneration we examined the swimming behaviour of the fish at different times. In parallel, we monitored the cellular events underlying this regenerative process using molecular and histological analyses. Additionally, we are generating zebrafish transgenic lines to genetically label specific subsets of spinal cord cells, using the Cre-loxP system.

Results: In this study we characterized the structure of the ependymal region, which is thought to contain a population of adult neural stem cells. We show that the central canal of the zebrafish spinal cord has a similar organization to the mouse spinal cord, with biciliated ependymal cells (3). Likewise, ependymal cells express stem cell markers and show increased proliferation in response to injury. Moreover, ependymal cells show an altered ciliary profile upon trauma, consistent with the upregulation of the cilia marker Foxj1a. To assess the role of ependymal cells in the repair of the injured spinal cord, we are developing a lineage-tracing procedure, which will allow us to permanently label populations of ependymal cells and their progeny. Using this approach we will establish which spinal cord cell types are able to originate new oligodendrocytes and neurons and contribute to the recovery of spinal cord function.

Conclusions: This work provides initial indication of the morphological similarities between zebrafish and mouse spinal cords. In addition, this work provides a base for the future identification of adult neural stem cells in zebrafish and for the understanding of the signals that promote their proliferation and plasticity. This knowledge could help identify the factors that restrict the progression of regeneration in the mammalian spinal cord.

References:


Asymmetric n-cadherin expression is essential to cease the leftward cell movements around hensen's node

abstract ID: 190

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Introduction: The vertebrate body appears bilaterally symmetric on the outside, however on the inside it exhibits a stereotypical left-right asymmetry distribution of its internal organs. A conserved symmetry breaking mechanism in vertebrates is the leftward fluid flow generated by motile cilia in the embryonic node. However, the chicken embryo is an exception, since molecular asymmetries are detected before the appearance of motile cilia in the Hensen’s node. Indeed, it was recently shown that the asymmetric expression of shh and fgf8 in the Hensen's node is the result of a transient leftward movement of cells from the right to the left side of the node. How are these cell movements ceased once the asymmetry is established is still unknown.

Methods: The adhesion molecule N-cadherin seems to be a good candidate to stop the leftward cell movements since it is asymmetrically expressed on the right side of the node and inhibition of its function leads to heart misposition in the chicken embryo. We used loss- and gain-of function experiments combined with cell tracking in vivo imaging analysis to test this possibility.

Results: We were able to show that, when N-cadherin’s function is blocked, the leftward cell movements lasted for longer and when N-cadherin was asymmetrically expressed at earlier stages the leftward cell movements were ceased prematurely. We also found that when N-cadherin function is compromised, asymmetric expression of fgf8, wnt3a and nodal around the node is lost. Consequently, this incorrect information from the node is mistranslated to the left lateral plate mesoderm resulting in the abnormal expression of the asymmetric genes like snail and caronte and will ultimately lead into heart misposition.

Conclusions: We conclude that asymmetric levels of N-cadherin expression are crucial to maintain asymmetric gene expression in the node previously established by the leftward cell movements simply by allowing these movements to occur during a transient period of time.

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Notch signalling is required for proper formation of muscle fibers and cartilage in zebrafish pectoral fin

abstract ID: 191

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INTRODUCTION: The development of many structures and organs starts with the formation of a primordium at specific embryonic locations in response to combinatorial positional signals. This is the case of the appendages. The homogeneous limb/fin mesenchyme precursor cells protrude from the trunk of the embryo to form a small bud covered by a layer of ectoderm and will give rise to precise arrangements of differentiated cells such as cartilage/bone and muscle (Capdevila and Izpisua Belmonte, 2001; Mercader, 2007). In the tetrapod limb, Notch signalling seems to be required for a variety of functions like AER function, muscle and chondrogenic differentiation. Nevertheless, the function of the Notch pathway during pectoral fin development is still unknown.

METHODS: To investigate the role of Notch in pectoral fin development we have performed several techniques as in situ hybridization and immunostaining using several antibodies, we also take advantage of the morpholino-technique, drugs (DAPT) and mutants to block the function of Notch signalling at different levels of the pathway.

RESULTS/CONCLUSIONS: Here we demonstrate that all core elements necessary for a functional Notch pathway are expressed during pectoral fin development, particularly in or near prospective fin muscle territories. When Notch signalling pathway is disrupted, overall smaller pectoral fins with a clear disorganized endoskeletal disc and a misshapen fin fold are observed. When immunostaining was performed using phalloidin and DAPI, to label F-actin/muscle fibres and nuclei, respectively, we could observe that muscle fibres are wavy, disconnected and with gaps between them and that the cartilaginous cells are misshaped and present high levels of actin at its periphery. In addition, Desmin and Vinculin protein levels appear down-regulated in Notch-disrupted pectoral fins suggesting that an impairment of mechanical forces produced by unstable muscle fibres could result in the phenotype observed. Moreover, depletion in Pax7-positive cells is observed in Notch-disrupted pectoral fins. Thus, we can speculate that the lack of integrity of the muscle fibres observed results from an inefficient muscle regeneration due to depletion of progenitor cells through premature uncontrolled differentiation.

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Keratocan, lumican and decorin as targets of lmx1b regulation in the limb, kidney and brain

abstract ID: 192

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Lmx1b is a homeodomain transcription factor that regulates limb dorsalization and kidney development. In humans, Lmx1b haploinsufficiency causes a condition known as Nail-Patella syndrome (NPS). Individuals with NPS typically have under-developed nails, absent patellae, and impaired kidney function. In Lmx1b knockout (KO) mice, the absence of Lmx1b function disrupts limb dorsalization and mice have nearly symmetrical ventral-ventral limbs. In addition, Lmx1b KO mice have impaired glomerular filtration abating urine production and deficient cerebellums. Currently, the downstream targets of Lmx1b responsible for limb dorsalization are unknown, and the Lmx1b regulated molecules involved in glomerular and cerebellar development are incompletely characterized.

To identify genes targeted by Lmx1b, we used three complementary approaches. First, we compared gene arrays from normal and Lmx1b KO mice during limb dorsalization (e11.5-13.5). Differentially expressed genes were confirmed by real-time PCR and whole mount in situ hybridization. Second, we searched for conserved noncoding regions (CNRs) that contained putative Lmx1b binding sites (TAATTA) in the loci of the genes identified as potential targets by gene arrays. To identify CNRs, we used the National Institutes of Health VISTA Enhancer Browser and compared the human sequence to divergent species (Dog, Mouse, Opossum, and Chicken). Sequences that were conserved across all 5 species with greater than 70% identity were evaluated for Lmx1b binding sites. CNRs with putative Lmx1b binding sites were cloned into a reporter construct, electroporated into chick embryos and evaluated for activity during limb, kidney and cerebellar development. Third, we used chromatin immunoprecipitation (ChIP) technology to confirm Lmx1b binding to putative CNRs associated with genes differentially expressed.

Our gene arrays identified 18 confirmed targets that were differentially expressed during all three stages of limb dorsalization examined. Keratocan (Kera) showed the greatest difference (10 fold) in expression between normal and Lmx1b KO mice. Interestingly, Lumican (Lum) and Decorin (Dec), two closely-associated proteoglycans, were also regulated by Lmx1b. In situ hybridization localized these three genes to dorsal presumptive tendon mesoderm. The expression of Kera, Lum and Dec was also found in the kidney and cerebellum, other sites regulated by Lmx1b. These three genes are clustered together within a 140 kb genetic locus. Upstream of the Keratocan, Lumican and Decorin (KLD) locus is a 900 kb gene desert. We identified 11 CNRs within the KLD locus and associated gene desert; two of these CNRs (CNR3 and CNR10) also contained conserved Lmx1b binding sites. CNR3 exhibited enhancer activity in the developing cerebellum. Furthermore, ChIP for Lmx1b in human embryonic kidney cells (HEK 293) isolated the CNR3 regulatory element. Collectively, these findings suggest that the KLD proteoglycans play a role in Lmx1b regulated patterning and differentiation.
Session 3 – Cell Adhesion and Migration
In vivo analysis of cephalic neural crest cell behaviour in ojoplano

abstract ID: 12

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During embryogenesis, genetically programmed morphogenetic events occur through modifications in cellular architecture. These modifications may trigger behaviours such as the contraction of epithelial layers or cell migration events. In vertebrates, a key morphogenetic event takes place when neural crest cells delaminate from the dorsal neural tube and experiment a massive migration to finally contribute to a variety of tissues throughout the body. Our laboratory recently described the ojoplano (opo) mutant, named after its eye phenotype, which is caused by optic cup folding defects (Martínez-Morales et al., 2009). Interestingly, opo also presents a neural crest phenotype, with many derivatives starkly reduced in number, albeit correctly differentiated. To understand what causes this phenotype, we are currently using an in vivo time-lapse imaging approach to study cell polarization and early migration in the neural crest, and specifically the role of opo during these events.
Src kinases mediate apical determinant Bazooka/PAR3 interaction with STAT92E and increase signalling efficiency in Drosophila ectodermal cells

abstract ID: 13

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Intercellular communication depends on the correct organization of the signal transduction complexes. In many signalling pathways the mechanisms controlling the overall cell polarity also localize components of these pathways to different domains of the plasma membrane. In the Drosophila ectoderm, the JAK/STAT pathway components are highly polarized with apical localization of the receptor, the associated kinase and the STAT92E protein itself. STAT92E’s apical localization is independent of the receptor complex and is due to its direct association to the apical determining protein Bazooka (Baz). Here we find that Baz-STAT92E interaction depends on the presence of the Drosophila Src kinases. In the absence of Src, STAT92E cannot bind to Baz in cells or in whole embryos, and this correlates with an impairment of JAK/STAT signalling function. We believe that the requirement of Src proteins for STAT92E apical localization is mediated through Baz, as we can co precipitate Src with Baz but not with STAT92E. We also show that the pool of proteins binding to Baz is formed by inactive STAT92E dimers. This is the first time that a functional link between cell polarity, the JAK/STAT signalling pathway and the Src kinases has been established in a whole organism.
Study of the establishment of epithelial polarity: search for new proteins that interact with apkc

abstract ID: 16

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A key issue in developmental biology is the relationship between cell polarity and signal transduction pathways. Most eukaryotic cells are polarized with an asymmetric distribution of molecules and organelles resulting in different functional regions required for cell physiology. The control of this polarity in space and time is essential to coordinate changes in cell morphology with proliferation and morphogenetic movements required for the development of the organism. This control is carried out by signalling pathways, which in many cases are regulated by the subcellular localization of their components. In fact, there is a close relationship between polarity and the control of cell proliferation, since many receptors of intercellular communication pathways that regulate proliferation are located and activated in specific domains of the plasma membrane. Therefore, the understanding of the signalling pathways-cell polarity relationship is crucial for the knowledge of how signals are integrated to induce morphogenesis but also how are modified in aberrant processes as those occurring in cancer.

Despite the great diversity of polarized cell types, the machinery required for this process is evolutionarily conserved. Among this machinery there are three protein complexes: Par-3/aPKC/Par-6, Crumbs/PATJ/PALS1 and Lethal giant larvae/Disc large/Scribble, which participate in most of the biological processes involving ectodermal cell polarity establishment or maintenance. Of all these determinants of polarity only the atypical protein kinase C (aPKC) has an enzymatic activity. aPKC is involved in all polarity processes and is crucial in these events by interacting, depending on the process, with different regulators and modifying different substrates. In addition, aPKC is an oncogene and it is known that this protein is overexpressed in many forms of cancer. To understand how cell polarity is established, maintained and modified and also how this polarity can regulate signalling processes we have focused on to find out new proteins that interact with aPKC. To this end, we are looking, biochemically and genetically, for new proteins that interact and can be modulated by aPKC.

1D. St Johnston and B. Sanson, Current opinion in cell biology 23 (5), 540 (2011).


Regulation of valve development by microRNAs

Valve development is a multistep process involving the activation of the cardiac endothelium, an epithelial-mesenchymal transition (EMT) and the progressive lining and differentiation of distinct mesenchymal cell types into the fibrosa and spongiosa layers. Several pathways such as Notch/delta, Tgf-beta and/or Vegf signalling have been involved in crucial steps of valvulogenesis. We have previously demonstrated that discrete changes on microRNAs expression occur during ventricular chamber development. Several of these microRNAs were predicted to target Bmp- and Tgf-beta signalling, which are crucial steps during valvulogenesis. We have therefore analyzed the expression profile of candidate microRNAs in the atrial, ventricular and atrioventricular canal regions at four different developmental stages, HH13, HH19, HH27 and HH32. qRT-PCR analyses of microRNAs demonstrated a highly dynamic and distinct expression profile within the atrial, ventricular and atrioventricular canal regions of the developing chicken heart. Several microRNAs, such as miR-23b displayed increased expression at early but not late AVC developmental stages as compared to the atrial and ventricular chambers, whereas others such as miR-130a display decreased expression levels. Furthermore, we have implemented an in vitro EMT model to study microRNA function. Preliminary results demonstrated that over-expression of miR-23b or miR-199 significantly blocks EMT in a similar fashion as high glucose administration controls. Analyses of the distinct signalling pathways involved in valvulogenesis are currently been scrutinized and will be presented providing thus insights into the discrete role of microRNAs in valve development.
Forces shaping a Hox morphogenetic gene-network

abstract ID: 37

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The crossveinless- c (cv-c) gene of Drosophila encodes a RhoGAP protein required for actin reorganization. Cv-c inactivates Rho1 by enhancing its low intrinsic GTPase activity. cv-c is a common downstream target of the selector genes that control the organogenesis of the salivary gland, the trachea and the posterior spiracles. Mutation of cv-c causes morphogenetic defects in these tissues.

We have analyzed the effect of inducing Cv-c in ectodermal cells that normally do not express it. These cells change their shape and increase their motility. Interestingly Rho1 inactivation due to Cv-c expression in naive epithelial cells has collateral effects on the maintenance of epithelial polarity and adhesion with a strong downregulation of E-Cadherin and the apical determinants Crb and aPKC.

These defects do not occur in tissues expressing Cv-c endogenously suggesting that the epithelial cells where cv-c is normally expressed possess compensatory mechanisms allowing transient Rho1 downregulation without losing cell polarity or adhesion. We show that the selector proteins that activate cv-c simultaneously activate the transcription of molecules that counteract the loss of polarity caused by transient Rho1 downregulation. We propose that the cooption of a morphogenetic gene to a gene network creates a strong selective pressure leading the same gene network to recruit compensatory regulators for deleterious collateral defects.
Basement membrane as a regulator of “global tissue rotation” during drosophila melanogaster oogenesis

abstract ID: 38

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Elongation of Drosophila eggs depends on a particular type of morphogenetic movement during oogenesis termed “global tissue rotation”. It consists in the rotation of the egg chambers (developing eggs) along their long axes. Similarly to the traditional migration of an epithelial sheet, interactions between the epithelial cells of the egg chamber and the basement membrane (BM) are essential for the rotation movement. We have used live imaging to clarify the role that the BM-cell interactions play in the regulation of egg chamber rotation. We have found that mutant egg chambers with low levels of Laminins, an essential component of all the BMs, undergo premature rotation. We also show that the rotation axis depends of the position of the polar cells, pairs of specialized cells located at both ends of the egg chambers. Finally, we have characterized the formation of highly dynamic filopodia-like protrusions in the basal side of the epithelial follicle cells that seem to control the direction of rotation. We propose that BM composition regulates the timing of rotation by supporting the formation of basal filopodia-like projections in follicle cells.
Integrins are required for effective migration of embryonic haemocytes in drosophila

abstract ID: 49

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Cell migration plays a key role in a wide variety of biological phenomena. During embryogenesis, many cells travel substantial distances to reach their final destinations. A example of these migration are the haemocytes, the haemocytes star migration from head mesoderm at approximately two hours after gastrulation. After mesoderm invagination, once inside the embryo, haemocytes migrate to populate the whole embryo following several stereotypical paths (Tepass, 1994). During the complex and stereotype migration described above, haemocytes migrate along several tissues, which are surrounded by components of the extracellular matrix (ECM). Among the adhesion receptors found to be involved in the migration of different cell types, integrins constitute a major family of receptors promoting cell migration over the ECM.

In these study, by using confocal microscopy and live imaging, we have investigated the function of βPS integrins during macrophage migration. We demonstrate that the phases of extensive migration require βPS function predominantly in the macrophages themselves and to a much lesser extent within the substrate they migrate on. βPS-deficient macrophages constantly form and retract protrusions without efficiently moving forward, suggesting a βPS function for the adhesion of protrusions to the substrate. We present evidence for a cooperation of αβS1 and αβS3 with βPS in mediating the migration of macrophages. In addition we show that the maturation of macrophages and the phagocytosis of apoptotic cell corpses by macrophages occur independently of any βPS function. Finally, we have identified laminins as the major ECM components required for haemocytes migration.
Lmo4 is an essential cofactor for the snail2 mediated emt in neuroblastoma and neural crest cells

abstract ID: 56

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Introduction

The epithelial-to-mesenchymal transition (EMT) is the process by which cells switch from a non-motile to a migratory and invasive behavior. This process is not only relevant in morphogenesis, but is fundamental in cancer metastasis. Migration of neural crest cells from the neural tube, fulfill a paradigmatic EMT and blockade of neural crest development causes neuroblastoma tumour formation. Here we study the contribution of LMO4 in neural crest cell development, delamination and migration and also its role in cancer by studying neuroblastoma cell lines.

Methods

By means of a genome-wide expression screen we found the LMO4 to be expressed in the chick embryo neural crest. Taking advantage of in ovo electroporation methodology we did in vivo gain-and loss-of-function experiments in chick embryos. Also, migratory and invasive capacity was studied by in vitro assays with neuroblastoma cell lines.

Results

In vivo and in vitro experiments demonstrated the requirement of LMO4 for delamination of neural crest cells, as well as for the invasive capacity of neuroblastoma cells. Also in search for the mechanism of LMO4 activity, we found that LMO4 is a required cofactor for the Snail-mediated transcriptional repression of cadherin expression.

Conclusions

LMO4 plays a crucial role for both EMT in the neural crest and in neuroblastoma cell lines.

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Delamination of neural crest cells requires inhibition of wnt canonical activity by dapper2

abstract ID: 61

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INTRODUCTION: Wnt signalling is reiteratively used at multiple steps of neural crest development. Acquisition of the neural crest identity requires Wnt canonical activity, and the same canonical activity is again reused for neural crest lineage restriction. On the other hand, acquisition of the migratory behaviour depends on the non-canonical Wnt signalling. However, in this study we show that activation of the Wnt canonical pathway inhibits delamination of the neural crest from the dorsal neural tube, and therefore the requirement for a timely and spatially controlled inhibition of Wnt activity to allow delamination of neural crest cell.

Thus we set to identify the local and transient inhibitors of Wnt canonical pathway needed for neural crest cell delamination and epithelial-to-mesenchymal transition.

METHODS: By means of electroporation in the chick neural tube, we set for a genome wide search of genes differentially expressed in neural crest cells, and we identified the Wnt-inhibitor Dapper2.

RESULTS: We show that Dapper2 is a potent inhibitor of the Wnt canonical pathway specifically expressed in premigratory and early migratory neural crest cells in the chick and Xenopus embryos. Taking advantage of the TOP-Flash reporter, we show that Dapper2 strongly inhibits the Wnt canonical activity upstream of TCF. By in vivo gain- and loss-of-function experiments in chick and Xenopus embryos we show that Dapper2 is both necessary and sufficient for neural crest delamination from the dorsal neural tube, without regulating the motion properties of neural crest cells. Moreover, Dapper2 is also sufficient to rescue the delamination of neural crest cells blocked by Wnt canonical activation. In search for the mechanisms regulating this activity we show that Dapper2 interacts with and translocate βCatening to abnormal ring shape-PML nuclear bodies, thus preventing βCatening function as a transcriptional co-activator.

CONCLUSIONS: We propose a two-step model for neural crest development in which Wnt activity is necessary for the specification of neural crest progenitors (by regulating the expression of neural crest genes), but subsequent local down-regulation of the Wnt-pathway is required at the time of neural crest delamination. Our results pose Dapper2 as a candidate to play this role probably by sequestering βCatening into specific nuclear bodies.
The proteoglycan Perdido and the alphaPS2betaPS integrin cooperate to mediate muscle adhesion to the tendon extracellular matrix in the Drosophila embryo.

abstract ID: 95

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The development of functional muscles requires multiple cellular events, such as cell migration, recognition and adhesion to tendon cells. We are studying the genetic and molecular mechanisms underlying the development of the muscle-tendon junction in Drosophila as a model to better understand these cellular processes. The transmembrane proteoglycan Perdido (Perd), orthologue of the mammalian cell surface receptor Condrotin Sulfate Glycoprotein (CSGP)/NG2, is required for muscle-tendon attachment. Nevertheless, the exact role of this protein during these cellular interactions is still unknown. We have observed that Perd interacts with Integrins at the genetic and biochemical level suggesting they are cooperating in muscle-tendon junction. In particular, we have found that Perd enhances ability of the αPS2βPS integrin to promote cell adhesion. Moreover, this adhesion is mediated by their binding to the tendon extracellular matrix protein Thrombospondin, which is also essential for muscle-tendon junction. Thus, we propose that Perd and the αPS2βPS integrin are engaged in a protein complex at the muscle membrane that promotes muscle-tendon adhesion through the tendons extracellular matrix.
The Role of proteglycan Versican in neural crest migration

abstract ID: 104

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The neural crest (NC) is a population of highly migratory embryonic cells that colonize diverse regions throughout the body and give rise to a wide variety of cell types. In order for NC cells to effectively navigate, they must be presented with the appropriate environmental cues and must have an underlying ECM substrate of the appropriate density and molecular composition. The ECM is a complex, highly-ordered lattice composed of fibrous proteins, such as fibronectin and laminins, and proteoglycans. Among the proteoglycans are the family of chondroitin sulphate proteoglycans, including Aggrecan, Brevican, Neurocan and Versican.

Versican is present along neural crest migratory routes, yet our understanding of its role in NC migration is complicated by contradictory findings. Studies in mice have shown that Versican acts as a non-permissive substrate, effectively creating a barrier to NC migration. Yet others have shown a different role: experiments in chick suggest that Versican might attract NC cells directly, thereby functioning as a haptotactic factor.

To better understand Versican’s role in mediating NC migration, we have examined its function using the Xenopus laevis embryo. We found that Xversican is expressed between migrating NC streams, consistent with a role in demarcating NC migratory paths. Interestingly, we found that the Xversican V0-1 and V3 isoforms are up-regulated at a time consistent with a role in NC migration in our system.

In embryos lacking Versican, NC cells failed to migrate. Similarly, in an explant invasion assay, NC cells failed to invade Versican-deficient tissue, indicating that NC cells require the presence of Versican in the surrounding tissue for proper migration. Paradoxically, in vitro culture experiments showed that neural crest cells avoided areas containing Versican, suggesting that Versican acts as a negative cue. To resolve these seemingly-contradictory observations, we performed a stripe assay. NC explants were cultured on either fibronectin alone or fibronectin corridors bordered by Versican-containing regions. Strikingly, we found that as opposed to fibronectin alone, where NC would disperse radially, explants bordered by Versican-containing regions displayed clear directional collective migration.

Our results support the notion that Versican acts as a non-permissive substrate and suggest that Versican is required for coordinated directional migration of NC cells by establishing exclusionary boundaries. We are currently employing mathematical modelling to validate the requirement of restrictive cues in funnelling NC cells into streams and driving coordinated directional migration.
Fat3 conveys retinal ganglion cell signals regulating amacrine cell migration

abstract ID: 111

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Introduction: In the vertebrate retina, neurons are organized by function into discrete layers that communicate via precisely ordered synaptic connections. The laminar development and synaptic organization of the retina requires neuronal migration into the appropriate nuclear layers followed by dendritic extension into the appropriate plexiform layer(s). Previous results from our laboratory have established a key role for the atypical cadherin fat3 in these processes; ensuring the unipolar morphology of amacrine cells (ACs) and establishing the proper distribution of ACs between the inner nuclear layer (INL) and ganglion cell layer (GCL). Fat3 is expressed by both ACs and retinal ganglion cells (RGCs) and likely functions by regulating developmental interactions occurring between these cell types.

In the absence of fat3, laminar development of the retina is altered with two additional plexiform layers resulting from changes in the distribution of ACs and formation of ectopic AC dendrites. When fat3 is conditionally removed from ACs using ptf1a-Cre, the migration phenotype does not occur and these mutant ACs are faithfully retained in the INL. In contrast mutant ACs continue to form ectopic dendrites demonstrating that the two developmental events, migration and dendrite formation, are dependent upon Fat3 function in RGCs and ACs respectively. Specifically these results predict a non-autonomous function for fat3-expressing RGCs in creating a “boundary” that prevents ACs from migrating across the inner plexiform layer (IPL) and laminating inappropriately in the ganglion cell layer.

Methods: The hypothesis that fat3-expressing RGCs control AC migration was tested by generating ganglion cell-specific CKOs. The brn3b/Pou4f2 transcription factor is expressed in 70–80% of RGCs. We have crossed brn3b-Cre mice (V. Bennett, Duke, unpublished) with our fat3floxed line to produce a CKO in which fat3 is severely reduced, if not eliminated, from the RGCs. The effect of Fat3 signaling between RGCs and ACs was determined by measuring the distribution of BHLHB5-positive ACs between the INL and GCL. This method has been previously used in our lab to demonstrate the AC migration phenotype in fat3∆TM KOs.

Results and Conclusions: The brn3b-Cre transgenic mouse efficiently promotes Cre-mediated recombination in the majority of RGCs and some neurons in the INL. When fat3 is conditionally removed from RGCs we observe an increase in the number of BHLHB5-positive ACs that fail to stop migrating at the INL:IPL boundary and become trapped in the IPL or GCL. These findings are similar to the aberrant AC migration phenotype that occurs in fat3 KOs. Altogether our results support a model in which Fat3 regulates AC development by acting autonomously in ACs to determine dendrite number and non-autonomously in RGCs as part of an RGC:AC signal to control AC movements. We describe a broader role for Fat signaling, originally characterized for its role in planar cell polarity (PCP), and tissue growth and patterning, that has likely been adapted for new functions in vertebrates. This unexpected commitment from a classical PCP signaling molecule in the control of cell migration and dendrite number is both novel and significant.
Aggregation factor and serotonin are colocalized in larval mucous cells and are the main stimuli of Bougainvillia superciliaris (Hydrozoa) metamorphosis.

abstract ID: 126

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Introduction:
Coelenterates are basal multicellular animals with possess nervous system, sensory structures, myoepithelial cells, digestive epithelium, massive structures of extracellular matrix responsible for the tissue structures integration. Bougainvillia superciliaris is a typical Hydrozoa species with methagenesis in life cycle. Therefore it is an appropriate model organism in developmental biology.

B. superciliaris metamorphosis can process in two different ways with larval settling followed by polyp metamorphosis, or by zygosis consisting of planulas aggregation leading to the complicated heterogeneous colony formation.

Larvae ectodermic mucous cells release specific secreta significantly stimulating B. superciliaris metamorphosis. We named it aggregation factor because it forms structures of extracellular matrix responsible for the individual association during larval settling. Another stimulus of metamorphosis is the serotonin release. In 2007, G.Zega discovered that, some ectodermic cells being partly nonneural secrete serotonin.

The purpose of this work was discovering the serotonin role and aggregation factor in B. superciliaris metamorphosis.

Methods:
Adult medusae were collected at Novik Creek at Russkiy Island (Peter the Great bay, Sea of Japan), kept in the aquariums and fed with nauplii Arthemia salina. Free-swimming planulas were collected and cultivated in the climate chamber in Petri dishes filled with filtered sea water. Serotonin was added to the water to the final concentration 1 µM.

Embryos and larvae were fixed in 4% formaldehyde and paraffin embedded. Sections were stained using Mouri method for glycan-positive structures. The total preparations of larvae were immunostained with following primary antibodies: anti-β III-tubulin to identify neural structures; anti-serotonin to discover serotonin-positive structures. Antibodies for the aggregation factor were raised in mice and used to discover secretory structures.

The results:
Embryos inside the maternal organism still have some yolk. Both serotonin positive and mucous cells are absent. While the yolk disappears the mucous cells with the aggregation factor secreta emerge. After the mucous cells are formed and completely filled with the secreta, the nervous system’s singular cells appear and nervous system forms.
At the stage of swimming planula, the complicated neural network consists of cells concentrated at the front pole and adjoined directly to the serotonin-positive cells.

Before metamorphosis, planula begins to settle down. At this moment the mucous cells are extra rich with the aggregation factor secreta where the serotonin-positive structures are also colocalized, so that mucous cells are the cells producing serotonin.

*B. superciliaris* larvae simultaneously release aggregation factor and serotonin from the cell deposits when serotonin reaches the cultivation medium.

So, when one planula secretes serotonin, the other planulae become stimulated to settle and to metamorphose, thus the complicated colony can form due to the synchronization.

Conclusions:

- *B. superciliaris* mucous cells formation, primary serotonin secreta and aggregation factor accumulation happen at the embryonal stage.
- Colocalizational analysis shows that planula mucous cells also produce serotonin.
- Aggregation factor and serotonin release stimulates active metamorphosis accompanied by hydroid larvae aggregation.
The role of pH in cilia length regulation

abstract ID: 131

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Using the zebrafish mutant for the deltaD gene (after eight or aei⁻/⁻), our group has showed that Notch signaling was involved in the control of cilia length in the cells of the fish laterality organ, the Kupffer’s vesicle (KV). Further research based on a tissue specific microarray screening allowed the discovery of several genes with differential expression in KV cells from aei⁻/⁻ mutants compared to WT embryos.

One of these genes, rabconnectin3 or rc3 was found to be severely downregulated. Homologs of this gene have recently been associated with Notch signaling in Drosophila and mammalian cells through the regulation of the V-ATPase activity. Furthermore, the activity of this pump has also been associated with the ciliogenesis in the KV.

Using a Morpholino against rc3, we caused a decrease in the cilia length of the KV. We propose that such decrease in the cilia size present in the KV of the aei⁻/⁻ mutants and the rc3 morphants is caused by the deregulation of the VATPase pump as shown by the lack of acidic vesicles in these embryos.
Tight Junctions: is there a role in morphogenesis?

abstract ID: 139

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Introduction: Tight Junctions (TJs) are multiprotein complexes located on the apical surface of differentiated epithelia where they regulate tissue permeability and form a barrier between internal organs and the external environment. However, the role of TJs during morphogenetic processes is largely unknown. Our main interest is to gain insight into the role of these structures in dynamic processes such as embryonic development, where epithelial integrity must be maintained while at the same time the tissue is being remodelled.

In the developing zebrafish embryo, during the epiboly process, TJs are enriched at the boundary between the enveloping layer (EVL) and the yolk syncitial layer (YSL), suggesting a role in the maintenance of tissue integrity in a migrating sheet of cells (1).

Methods: We have undertaken a morpholino-based knockdown approach (2) of TJ components in order to characterize its function during development and wound healing using immunofluorescence, confocal and two-photon live imaging.

Results: Our results show that downregulation of the Tight Junction Protein-3 (Tjp-3/ZO-3) causes an epiboly delay due to impaired adhesion between the EVL and YSL. Moreover, the knockdown of this single component seems to impair the assembly of the whole TJ complex.

Conclusion: Taken together, our results suggest a novel role of Tight Junctions in Zebrafish development where ZO-3 protein is essential to maintain adhesion between two adjacent tissues: the EVL and the YSL. This shows for the first time that Tight Junctions mediate adhesion between tissues which has implications in epithelial morphogenesis and reveals that TJ functions are not restricted to tissue barrier and permeability of epithelia.

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References

Adhesion molecules in drosophila macrophage migration in vivo

Abstract ID: 141

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Cell migration is a key mechanism that occurs in many developmental and homeostatic processes, such as wound and tissue repair and immune surveillance. It is also critical in several clinically relevant pathologies such as chronic inflammatory diseases and cancer progression. Because of its biological and clinical importance, cell migration has been intensively studied in the past few decades using several in vitro methods. In this project we have developed an in vivo migration assay using Drosophila hemocytes as our model system. Hemocytes are immune cells analogous to vertebrate leukocytes in terms of features and functions. Drosophila hemocytes acquire varied morphological and adhesive properties depending on the developmental stage. During the larval stage, two sub-populations of hemocytes have been described, 1) the circulating hemocytes associated with the hemolymph patrolling the organism for invaders or damaged tissue, and 2) a sessile population located in dorsal periodic sub-epidermal patches. These sub-epidermal hemocytes maintain a round morphology and low migration profile during larval stages, and are unresponsive upon wounding of the larval epidermis in contrast to the hemocytes during the embryonic and late pupal stages. However, between 2 and 4 hours after pupa formation, the sessile sub-epidermal hemocytes acquire a spread morphology with filopodia and lamellipodia-like structures and initiate migration. The transition between the two distinct morphological and migratory behaviours makes these cells an ideal in vivo model system to systematically study the molecular mechanisms underlying cell adhesion, spreading and migration. Using RNAi against b-integrin (myospheroid) and some integrin-associated proteins, we have found that the migration was severely impaired. On the contrary, adhesion and spreading of the sessile hemocytes are independent of these molecules. We will continue to identify other components important for the integrin-mediated migration. This is the first report of integrins and integrin-associated proteins being involved in in vivo hemocyte migration in Drosophila.
Steroid hormone signalling synchronises macrophage activation with the onset of metamorphosis in drosophila

abstract ID: 154

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Steroid hormones are emerging as potent regulators of immunity and inflammation, underlying differences in immune competence and pathology at different life stages and between genders. In Drosophila, the steroid hormone ecdysone controls the major developmental transitions through the life cycle, including metamorphosis. In this study, we characterise the activation at pupariation of a pool of dormant macrophages, the so-called ‘sessile’ hemocytes, and show that these cells become highly migratory, change their morphology and acquire wound responsiveness. We demonstrate that this activation is synchronised with the onset of metamorphosis by ecdysone received directly by hemocytes triggering an EcR/Usp-tai-br signalling cascade. Hemocytes insensitive to ecdysone do not undergo these changes and present an impaired phagocytic activity. Individuals in which hemocytes are not activated are more susceptible to infection during metamorphosis, which we show to be a particularly vulnerable stage in development. We propose that sessile hemocytes act as ‘reinforcement troops’ ready to be activated at metamorphosis to protect the pupa and participate in pupal remodelling.
Septate junctions are essential during epithelial wound healing in drosophila

Abstract ID: 156

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Introduction: Epithelia are an organism’s first defense against the sinister environment. Simple epithelial tissues, such as those in a developing embryo, have the extraordinary capacity to resolve wounds in a rapid and efficient manner by means of a resealing mechanism that is conserved across species. This process involves dramatic cellular rearrangements and the assembly of a contractile supracellular cable formed by F-actin and myosin. The key cellular events and signaling pathways that regulate this process have now started to be identified, but many questions remain about how they are integrated and eventually lead to the closure of an epithelial hole (Razzell et al 2011). In order to identify novel genes essential for wound healing, our lab has performed a genetic screen based on laser wounding of Drosophila embryos (Campos et al 2009). One of the isolated mutations was in the gene Coiled, which encodes a protein required for septate junction (SJ) formation (Hijazi et al 2011; Nilton et al 2010). This prompted us to investigate the role of SJs in wound closure in Drosophila. SJs, which are analogous to vertebrate tight junctions, are crucial not only for the maintenance of the epithelial barrier function but also for regulating cell shape changes and adhesion during development (Banerjee et al 2006).

Methods: In order to determine the role of SJs during wound healing, we performed laser ablation of the ventral epidermis in the Drosophila embryo. We then followed the wound closure process in the developing embryo labeled with different live markers, using confocal time-lapse imaging.

Results and Conclusions: We found that SJs are essential for wound closure in the epidermis of Drosophila embryos. Furthermore, our preliminary data suggest that the localization of SJ components at the wound edge is very dynamic, indicating that the remodeling of these junctions during wound closure must be tightly regulated. We are now determining how the SJ complex is controlled during wound closure and how that affects the wound healing process.

References


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Grainyhead in wound healing

abstract ID: 168

Type: Poster

Thematic area: Cell Adhesion and Migration

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Epithelia are the first line of defense that an organism has against the surrounding environment. The ability to maintain a well-controlled barrier is fundamental for survival. Tight Junctions (TJ)/Septate Junctions (SJ) are important components of the permeability barrier and have been linked, in different organisms, to tissue repair. This suggests junctions are dynamically regulated during wound closure, although their detailed function in this process is still unknown.

The grainyhead (grh) gene family encodes an important group of transcription factors that regulate the development and repair of epidermal tissues. There is one grh gene in Drosophila, whereas in vertebrates there are up to four homologs (Grainy head-like genes – grhl). Their function seems to be conserved and they seem to be important in the formation and maintenance of TJ and adherens junctions. Their main functions are the control of cell polarity and the establishment of the permeability barrier.

Using laser-based tissue wounding, live imaging and morpholino/RNAi knock down, we are studying the role of grh and grhl genes during the formation and repair of invertebrate and vertebrate epithelia.

In Drosophila, we use the pupa notum epithelium to address the importance of grainyhead in wound closure and septate junction regulation. In zebrafish, we focus on two of the homolog vertebrate forms, grhl2b and grhl3, both expressed in the embryonic epithelium.

Our preliminary data indicate that grh is important for Drosophila thorax closure during pupariation, and for wound closure. In zebrafish, wound closure is delayed in grhl2b and grhl3 morphants during epiboly (first hours of development). Double morphants for these genes seem to have a stronger phenotype in wound closure, pointing to redundancy between them.

These findings point to a promising and novel role for grainyhead family genes in wound healing.
Planar Cell Polarity in Drosophila Abdomen: A Word to be Discovered.

abstract ID: 185

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Planar Cell Polarity (PCP) is a morphogenetic process that instructs cells to be coherently oriented within the plane of an epithelium. In Drosophila PCP is shown through oriented cytoskeletal protrusions (hairs and bristles) that normally point one way. Open questions in the planar polarity field are how PCP is determined over an entire field of cells, what are the molecular mechanisms needed to orient the cell in a coherent way and how gradients of polarizing cues can lead to a uniformly oriented growth. Our group aim to shed light on these fundamental aspects of planar cell polarity, looking at the dorsal abdominal epidermis from the very beginning of pupal development.

Indeed, little is known about establishment of planar polarization in the body wall of the fly.

We have first characterized the general properties of the abdominal epidermal cells (a.k.a histoblast cells), such as shape, packing and placement of hairs within each cell. We found that histoblast cells have a distinctive feature, as each cell exhibits elongated hexagonal shape that make it different from other epithelial cells (wing or thorax cells) in Drosophila. Moreover, we show that each abdominal cell give rise up to five posteriorly pointing hairs. As the Fat-Dachsous-Four-jointed system appears to be crucial for a proper planar polarization we first investigate how this set of genes are acting in early phases of abdominal development.

We show that four-jointed and dachsous genes are expressed in a mutual gradient fashion in each abdominal segmental compartment and that these gradients are early set up during pupal development.

We then reveal for the first time how the polarizing cue of four-jointed gradient is evolving over time during pupal development and how its slope correlate with the oriented growth of the tissue accordingly with the morphogenetic movements that lead to each abdominal segment specification.

Breaking the slope of the four-jointed and dachsous gradients generating mosaic patches that lack one of other gene we show dramatic changes in the normal shape, packing and placement of the hairs. These observations suggest that a group of cells lacking the proper polarizing cue cannot acquire a stable cell packing. Moreover, the mutant patch strongly influences neighboring cells.

We finally show that also a downstream protein of the Ft-Ds-Fj System, Dachs, shows an asymmetrical localization at the apical side of each histoblast cells. Furthermore, its localization nicely correlates with the oriented growth of the tissue during pupal development.
Apical contraction is a common cell shape change that drives morphogenesis in different contexts. During Dorsal Closure (DC) in Drosophila, amnioserosa (AS) cells apically contract and generate one of the major forces driving the closure of the epidermis. Apical contraction in these cells is pulsatile and driven by the activity of the actomyosin cytoskeleton, which localizes both at the medial apical region and at the level of cell-cell junctions. It is not known which is the structural organization of the actomyosin cytoskeleton in these cells neither how they generate the contractile force. In order to get some insights into the molecular mechanisms underlying apical cell contraction in AS cells, we have studied the localization and function of different Actin-binding proteins during DC using genetics, molecular biology and live imaging techniques. Our results suggest different roles for α-Actinin, Filamin, Wasp and Vinculin and suggest the existence of a tension-mediated mechanism involved in the reinforcement of cell-cell junctions.
Session 4 – Modelling & Systems Biology
Analysis of pulsed cellular behaviour during zebrafish optic cup morphogenesis

abstract ID: 14

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The development of the vertebrate eye is a complex process that involves the coordinated rearrangement of cells (Kwan et al 2012). Previous reports have shown that pulsatile contractile forces generated by the cellular acto-myosin network play an essential role during epithelial morphogenesis. This oscillatory behaviour has been mainly described during apical constriction in Drosophila epithelia (Solon et al. 2009, Martin et al 2009). To ascertain whether similar cellular behaviours may direct the folding of the vertebrate optic cup, we have generated the zebrafish eye-specific transgenic line VSX3::caaxGFP, which enables us to follow membrane dynamics in vivo by performing time-lapse confocal imaging. We are currently quantifying cell shape changes at the basal and apical surfaces of the optic cup neuroepithelium, to better understand how individual cell activity contributes to tissular morphogenetic changes. Furthermore, we would like to investigate whether there is any correlation between cell area oscillations and an accumulation of the actomyosin network during retinal folding. Finally, we aim to design functia interference will be examined both on cell shape changes as well as on the macroscopic shape of the optic cup.
Smad2 and Smad3 are both cooperative and antagonist in neurogenesis due to TGFβ pathway network architecture

abstract ID: 44

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Introduction - The cellular machinery is governed by interacting proteins, genes and metabolites that form complex and highly interconnected networks of interactions. This way, extracellular stimuli triggers pathways of biological events that regulate gene expression, protein activity, and ultimately, cell response. The transforming growth factor beta (TGFβ) pathway is one of the most conserved and prolific of these signaling cascades, involved in a wide variety of both adult and embryonic processes. Many studies have focused in comparing the roles of Smad2 and Smad3 following TGFβ activation. Smad2 and Smad3 often share a similar function, yet they appear as antagonist in other scenarios.

Results - We show in silico and in vivo that Smad2 and Smad3 cooperate and antagonize each other in the same cellular context, as a result of the particular wiring of the network of interactions of the TGFβ pathway. In particular, the interaction between the two transcription factors forming the hetero-trimeric complex Smad2-Smad3-Smad4 bias the signal towards antagonism or cooperation between the Smads, inducing positive or negative cooperation despite the fact that the two molecules target different regulatory sequences.

Methods - We first proceed by developing a simplified mathematical model of the TGFβ pathway to explore how the architecture of the pathway of interactions dictates the characteristics of the interplay between the R-Smads. Then, the model predictions are tested in vivo in the context of vertebrate neural development, where previous studies have shown that TGFβ pathway activation, and in particular Smad3, promotes cell-cycle exit and neurogenesis by inhibiting the expression of Id proteins while activating the expression of neurogenic factors. We proceed by characterizing the expression and function during neural development of Smad2, and compare it with Smad3. Next, we perform in ovo electroporation to induce overexpression and reduction of Smad2 and Smad3 and compare the effect on Smad2 and Smad3 specific regulatory sequences, as well as in neurogenesis. We finally incorporate the experimental observations to the mathematical model to observe that the experimental results fit with a scenario where the complex Smad2-Smad3-Smad4 differentially affects the regulatory sequences specific for Smad2 and Smad3, inducing simultaneously cooperation and antagonism, even in the same cellular context.

Conclusions - The interplay between the R-Smads dictates the dynamics of Smad2 and Smad3 targets after over-expression or deletion of the other partner. We hypothesize that this mechanism is responsible for the divergence between studies that focused on the interplay between Smad2 and Smad3 functions in other biological contexts. We hypothesize that this dual cooperation/antagonism scenario not only impacts neurogenesis, but also the other biological mechanisms regulated by the TGFβ pathway. A deeper understanding of how the two R-Smads interplay is key to understand the dynamics and function of the TGFβ pathway, to develop more efficient treatments against diseases that involve TGFβ pathway alterations, since disruption of the growth inhibitory function of TGF-β is considered a hallmark of cancer.
Theoretical model to study the role of introns in transcription

abstract ID: 51

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Introduction

Transcription is tightly regulated by a combination of elements such as promoter architecture, number of transcription factor binding sites, chromatin structure, amount of DNA-binding proteins and co-factors of the transcriptional complex [1].

In addition, intronic non-coding DNA is itself a core regulator of transcription since, apart from separating coding regions for genes to be processed appropriately, it also introduces time delays in the synthesis of mRNA [2]. It has been shown that these delay in transcription introduced by introns are crucial during development to ensure a proper temporal sequence in the expression of key genes regulators of developmental processes.

Since these precise timing is pivotal during development [3], we aim to understand the how introns induce these precise timing, and how this process interplays with the noisy nature of protein transcription.

Methods & Results

Previous studies measured the impact of intron length in the context of synthetic biology by implanting different intronic sequences in a oscillating genetic network [4]. These experiments revealed that introns did actually introduce an important delay that was reflected in the length of the oscillations.

We developed a mathematical model of the synthetic genetic network used in [4] to analyze the effect of the time constrains induced by introns of different length. We observe that the length of the intron induced longer delays, but it also amplifies transcriptional noise by inducing traffic jams of polymerases.

The existence of slow and fast transcription elongation rates eventually results in a stack of polymerases that piles up behind a slow polymerase, so we proposed that transcriptional noise, and its effect on intron dynamics, is caused by a “traffic jam” phenomenon of the polymerases transcribing the gen.

Conclusions

Our theoretical model is able to fit previous experimental data and also reveals hidden features of introns that introduce additional levels of regulation of transcription. Our studies can help us to understand important features of the role of intronic delays in developmental processes.
References

Theoretical models to understand GH and EPO ligand-receptor systems in developmental processes

abstract ID: 78

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INTRODUCTION

The growth hormone (GH) and the erythropoietin (EPO) are molecules that control several physiological processes during fetal, neonatal and adult life [2],[4],[5]. The interaction of these hormones with their receptors have the same characteristic scheme in an asymmetric and sequential 1:2 ligand-receptor configuration. In this scheme, the ligand presents two different affinities to its receptor, with one binding site of the ligand being 1000 times weaker than the other ([1],[3],[6]). This 1:2 scheme generates important self-regulatory properties that determine the dynamics and strength of the active complex signaling.

METHODS

We will use a mathematical approach to bring out the consequences of the differential binding process and its relevance in the regulation of the complex activation. A mathematical model will be designed to study the role of each of the two distinct affinity binding sites of the ligand towards each of the receptors in the complex. Those models will allow us to analyze the emergence of key properties of the system due to differential and sequential binding, such as adaptation, self-antagonism at high ligand concentrations and homodimer enhanced activation.

RESULTS AND CONCLUSIONS

Our mathematical approach help us to understand the dynamics and regulation of the interaction between EGF or EPO ligands and their receptors. We evidencie that the increase in the ligand local concentration increase due to the sequential binding is essential to form the active complex. Furthermore, we reveal the different roles of the two binding sites of the ligand during the formation of the active complex: while the weak binding site controls the signal strength and the amount of active signaling complexes, the strong binding site regulates the self-antagonist effect at high ligand concentrations and the optimal ligand concentration that induces maximum activity of the complex.

In summary, our mathematical model provides a tool to understand the whole mechanism and regulation of 1:2 ligand-receptor systems.

BIBLIOGRAPHY


Title of attached figure: 'Scheme for the 1:2 asymmetric sequential interaction model'
The reactivation of developmental programmes in organ degeneration

abstract ID: 107

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The epithelial-mesenchymal transition (EMT) occurs during embryonic development for the formation of many tissues and organs, but also occurs in the adult as a physiological response to injury and during the progression of cancer and other pathologies. The EMT was recruited during evolution to control epithelial plasticity, and therefore, the embryo holds the clues to the molecular and cellular mechanisms operating after its reactivation in the adult. The EMT is now established as an important step in the metastatic cascade of epithelial tumours and it is emerging as fundamental in organ fibrosis. The development of renal fibrosis is an excellent model to study the contribution of EMT to organ degenerative diseases and very importantly, it is the link between progressive loss of renal function and primary diseases such as glomerulonephritis, diabetes, toxic injury, congenital abnormalities, urinary tract obstruction and chronic rejection of transplanted kidneys.

We have previously shown that aberrant reactivation of Snail1, a well known EMT inducer is sufficient to provoke renal fibrosis and renal failure in adult transgenic mice, and that high Snail1 expression and evidence of EMT is found in the kidneys of patients with renal fibrosis. We have now asked whether Snail is not only sufficient but also required for the development of fibrosis, and furthermore, whether targeting Snail expression could be a valuable strategy to revert or ameliorate the disease. Our results show Snail inactivation is a promising target for the design of alternative ways to treat kidney fibrotic disease.

Understanding Gene Regulatory Network in Drosophila Eye Development: a New Perspective by Reverse Engineering

abstract ID: 130

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we are interested in the study of the gene regulatory network (GRN) that controls early eye development in Drosophila: from undifferentiated progenitors until committed progenitors. Several genes are known to be important in this process and traditional studies of loss/gain of function have led to the definition of a GRN. Although these kinds of studies have provided with fruitful results so far, they fail to establish certain relationships that lead to contradictory results when tested. On account of these deficiencies of the current model, we are taking a reverse engineering approach, with which we will analyze network behavior as a whole. As this procedure requires quantitative data at a single cell resolution, a 2D/3D ad hoc reconstruction software has been implemented in order to obtain the gene expression profiles (GEP) of several known members of the network. This is particularly challenging in the eye disc because it is a pseudostratified epithelium (i.e. the nuclei are located on different positions along the z axis). In addition, the tissue is pleated, and the degree of pleating varies from sample to sample. Our software copes well with both problems. In particular, the algorithm is capable of "stretching" the tissue, so that samples can be compared regardless of how pleated they are. Quantitative data of this sort can be used to derive GRN topologies with similar approaches as those used for the analysis of the early blastoderm embryo -basically the only other system where it has been used and with reasonable success. In addition, our data allow the study of noise which has been proposed to be essential to understand the effect of micro-RNAs in certain network topologies and network robustness under fluctuating conditions. Besides the fact that not all possible interactions with the known members have been tested yet, several other genes that have not been functionally defined show specific eye expression in the eye imaginal disc and so might contribute to the actual specification of cell fates in the eye.
Functional interaction between gaba and glutamate in the basal discharge of vestibular afferents during development

abstract ID: 98

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We studied the action of gamma-aminobutyric acid (GABA) in the vestibular periphery of chicken during the embryonic development (E15, E17, 19 and E21; n= 119). We used an in vitro preparation of the inner ear and record the electrical activity of vestibular afferents by means of a multunit extracellular technique. The application of GABA and muscimol (10^{-3}M; n=30) at embryonic ages caused an increase in the basal discharge. The excitatory effect of these GABAergic agonists was of smaller magnitude in presence of bicuculline (10^{-5}M) or picrotoxin (10^{-4}M), two GABAergic antagonists. At E17 the use of a Ringer solution with low in Ca^{2+} (0.22 mM) and high in Mg^{2+} (20 mM) produced a significant reduction in the response of afferent neurons to GABA (n=6) indicating both presynaptic and postsynaptic sites of action for GABA. The bath perfusion of DNQX 10^{-5}M, MCPG 10^{-5}M and 7-Cl-kyn 10^{-5}M (glutamatergic antagonists) diminished the excitatory action of muscimol (10^{-4}M; n=12) suggesting a functional interaction between GABAergic and Glutamatergic transmission. These data suggest that GABA functions as a neuromodulator of vestibular afferent activity. It could possibly activate GABA_A receptors or a mixed GABA_A-GABA_C receptor, functioning synergistically together with glutamate to provide the system with enough depolarization to permit the successful development of neurons and the consolidation of synaptic contacts necessary in the normal function of mature vestibular system.
Session 5 – Stem Cells
ROLE OF PITX2 ON SATELLITE CELLS AND SKELETAL MUSCLE REGENERATION

abstract ID: 7

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ROLE OF PITX2 ON SATELLITE CELLS AND SKELETAL MUSCLE REGENERATION

Estefanía Lozano-Velasco, Alejandra Contreras*, Daniel Vallejo; Diego Franco, Amelia Aránega


Introduction: Over the last decades we have gained insights about the molecular cues that govern the determination, formation and regeneration of the skeletal muscles. Regeneration of skeletal muscle mainly depends on adult muscle progenitors, named satellite cells. Some of these satellite cells are capable of both proliferation/self-renewal and differentiate along the skeletal muscle lineage, defining them as stem cells. Pitx2 is a paired-related homeobox gene that has been shown to play a central role during development. Pitx2 expression has been detected in many tissues during morphogenesis, including myotomes as well as putative migrating myoblasts. We have previously documented that c-isoform of Pitx2 plays a pivotal role modulating proliferation vs differentiation during myogenesis, balancing Pax3+/Pax7+ myogenic population in vivo, and regulating key myogenic transcription factors such as Pax3 by repressing miR-27.

Methods and Results: It has been previously shown that Pax3 and Pax7 play a role on the maintenance of satellite cells self-renewal in the adult skeletal muscle. Here we demonstrate that Pitx2c is expressed in a subset of satellite cells in both mouse and human adult limb muscles. Pitx2c overexpression in isolated mouse satellite cells leads to Pax3 up-regulation, Pax7 down-regulation and increase cell proliferation. Additionally, we have observed that Pitx2c is up-regulated in the mouse after muscle injury indicating a putative involvement of this transcription factor in muscle regeneration. Therefore, Pitx2c is widely increased after induced muscle injury in the mouse (cardiotoxin injection) while is mildly increased in Dmd/mdx transgenic mice (a mouse model of Duchenne muscular dystrophy). In this context, we have developed a strategy based on in vivo cell transplantation experiments to further investigate Pitx2c implications on regenerative myogenesis. Preliminary results shown that Pax3+ cells as well as cell proliferation rates are increased after cell transplantation of Pitx2c-overexpressing satellite cells into limb muscles of cardiotoxin injected and Dmd/mdx mice, indicating that Pitx2c seems to play a role in the process of satellite cells activation during muscle regeneration.

Conclusions: These results place Pitx2 as a new player on skeletal muscle satellite cell biology and will help us to identify unknown functions of Pitx2 during regenerative myogenesis.
Mechanisms of epicardium formation in the zebrafish

abstract ID: 29

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The epicardium is the outer cell layer enveloping the myocardium. It plays an important role as a progenitor cell source during development and adult homeostasis as well as during heart repair. The epicardium arises from the proepicardium (PE), a transient cluster of mesothelial cells located close to the cardiac inflow tract. While some of the genetic pathways involved in its specification have been described, little is known about how biomechanical forces influence its morphogenesis. The zebrafish poses an ideal model to study developmental processes such as epicardium formation in vivo. Here, we used an epicardial specific reporter lines and high-speed confocal microscopy to elucidate the mechanisms of epicardial attachment and the effect of heart beat on epicardial morphogenesis. Our results represent the first in vivo description of epicardium formation in a vertebrate animal model. We unexpectedly found that epicardial cells do not only derive from the PE, but also from defined areas of the pericardial wall located at the venous and arterial cardiac poles. PE cells are released into the pericardial cavity and adhere to discrete regions of the ventricle in an ordered pattern. Importantly, we found that heart beating controls not only PE formation but also adhesion of PE cells to the heart. We propose that biomechanical forces present in the pericardial cavity control the formation of the epicardium.
Regulation of the trophoblast fate in the blastocyst

abstract ID: 35

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To better understand how gene regulatory networks act in the mammalian blastocyst to define the first embryonic lineages, we are identifying cis-regulatory sequences that control the spatial and temporal expression of genes in the network. Our aim is to link regulatory sequences to early stochastic expression and late maintenance of central transcription factors in the network.

To do use, we are using a comparative genomic approach combined with transient transgenesis to find regulatory elements important in the transcriptional network responsible for lineage determination. Once these elements are characterized, their regulatory role is tested in vivo in mouse embryos by examining their ability to drive lineage-restricted expression of a reporter gene. We are also taking advantage of blastocyst derived stem cells –both trophoblast (TS) and embryonic (ES) stem cells– to find the specific signalling inputs and roles of the regulatory elements characterized in a tissue culture assay.

We have established several mouse lines in order to follow the dynamics of selected regulatory sequences in vivo and also as a tool to derive our own specific blastocyst derived stem cell populations.

We have studied the regulation of the key trophectoderm transcription factor Cdx2, finding that specific cis-regulatory elements are involved in different aspects of its expression in the trophectoderm of the blastocyst and in trophoblast-derived stem cells.
Study of the effect of cryopreservation on human MRNAS considered as potential fertility and pregnancy markers

abstract ID: 39

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Introduction:
Cryopreservation is a technique widely used for the conservation of spermatozoa in assisted reproductive technologies and it is known that this technique can produce changes in transcripts [1], as well as DNA damage [2] and epigenetic modifications [3]. The aim of this project is to analyze the effect of cryopreservation on two populations of spermatozoa mRNAs. The first set has been proposed as male fertility markers (BCL2-interacting killer, FSHβ polypeptide, protamine 1, protamine 2 and mesoderm specific transcript homolog (mouse)) [1][4][5] and the second as pregnancy success markers (activin A receptor type II like 1, adducin 1 alpha, androgen receptor, aryl hydrocarbon receptor nuclear translocator-and PAS domain protein 1 endothelial [1].

Methods:
Human semen samples were donated by six young men (24-28 years old). A preliminary analysis of the samples was carried out analyzing: sperm concentration, morphologic analysis and motility assessment. RNA extraction was done following Trizol Reagent (Invitrogen) protocol and a commercial kit, Cloned AMV First-Strand cDNA Synthesis Kit, was used for cDNA synthesis (Invitrogen). Designed primers were validated before qPCR, which was performed using SYBR Green (Applied Biosystems) in a StepOnePlus system (Applied Biosystems).

Results:
Results, comparing Ct values (fresh-cryopreserved), showed a significant decrease in the presence of transcripts of the following genes: FSHβ Polypeptide, protamine 1, protamine 2 and mesoderm specific transcript homolog (mouse) within group 1 and adducin 1 alpha and aryl hydrocarbon receptor nuclear translocator within group 2.

Conclusions:
These results demonstrated that cryopreservation, per se, can modify the presence of mRNAs with important roles in fertilization and in early embryonic development. These findings could make advisable to perform an evaluation of some relevant transcripts prior fertilization.

Acknowledgments: This work was supported by Fundación Ramón Areces and Ramón y Cajal program (RYC-2008-02339, MICINN, Spain). Authors would like to thank Cintia Miranda, and Dra. Martínez Guerra.

References:


An alternative cell source for genebanking in zebrafish: effect of cryopreservation procedures at genetic and epigenetic level

Abstract ID: 40

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Introduction

During last years many different transgenic and mutant lines have been generated in Zebrafish. All these lines are extremely valuable for science and usually, companies working with this model species use to cryopreserved sperm from them. These companies thaw the sperm and fertilize the eggs upon request. Surprisingly, DNA integrity is not evaluated after cryopreservation. It is well known that this technique can produce DNA damage via ROS (Reactive Oxygen Species), and damaging DNA in those valuable lines is a very undesirable effect. In this work we have cryopreserved zebrafish PGCs (Primordial Germ Cells), which we consider a better source for gene banking since both paternal and maternal genome can be preserved. Moreover, we have performed a deep study about the different possible effects that cryopreservation can cause, analyzing promoter methylation and quantifying DNA damage in several relevant genes as well as the presence of their transcripts.

Material and Methods

Promoter methylation was analyzed using the EpiTect Bisulfite Kit, DNA damage quantified by qPCR using the formula proposed by Rothfuss (1). The relative quantification of transcripts was done using qPCR by \( \text{delta delta Ct method} \). The following genes were analyzed: \text{cxcr4, vasa, pou5f1, sox3, dnd1 and sox2}.

Results and Discussion

The effect of cryopreservation seems to be mainly related to the alteration of different mRNAs in the PGCs. In one hand, a decrease of transcripts of certain gene was not always correlated with a hipermethylation in the minimal promoter of the gene. This could be explained considering that cryopreservation can not only affect transcripts by producing a decrease in transcription rates but also directly eliminating mRNAs, as was suggested in human spermatozoa (2), cells that lacks transcription activity. On the other hand, some mutations have been found in some of the studied genes, however, the cryopreservation protocol did not have a significant effect at this level in the most of genes.

Conclusions

The effects of cryopreservation can be diverse and should be analyzed in those zebrafish lines particularly valuable from a biotechnological point of view. Our protocol did not produce relevant alterations of DNA in the most of studied genes (neither DNA damage or modification of the methylation pattern in minimal promoters) but altered the presence of all the studied transcripts.

Acknowledgments
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References


Dna damage quantification in specific genes associated with fertilization and embryo development after human sperm cryopreservation

abstract ID: 41

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Introduction
Cryopreservation of human spermatozoa is a widely employed procedure for sperm storage before assisted reproductive technologies (ARTs) and for male fertility preservation before therapy for malignant diseases, vasectomy or other infertility treatments. However, this freezing/thawing process has been related to impair chromatin integrity, causing mutations and DNA fragmentation. The origins of these alterations are still under study but have been associated with an increase oxidative stress during cryopreservation [1]. There are many studies that have related DNA damage with an increased risk of pregnancy loss after IVF or ICSI, poor embryo quality, even with the health of the resulting offspring [2]. Therefore, the analysis of these damages is of vital importance for the successful of ARTs. There are many tests as comet assay, TUNEL or SCSA that analyze DNA fragmentation at global level. However, employing a qPCR assay for gene-specific damage study, we have observed in our laboratory that a total absence of fragmentation not always is associated with an absence on DNA damage. In the present work, we have employed for the first time this qPCR assay in human DNA sperm, to quantify the damages after cryopreservation in specific genes with roles in fertilization (prm1, prm2, peg1-mest, fsh-β).

Materials and Methods
Cryopreservation was done in 0.5 mL straws employing Sperm Freezing Medium as cryoprotectant (Irvine Scientific). The qPCR assay and lesions rate calculation has been carried out as Rothfuss et al. [3] described, with 3 ng of template DNA of each fresh, cryopreserved and hydrogen peroxide treated samples.

Results
Traditionally, the analysis of DNA damage in human spermatozoa is limited to a global fragmentation assessments that can be carried out by different techniques above mentioned. However, this work, point out the importance of using this novel technology for quantification of DNA damage produce by cryopreservation in specific genes, which play important roles in fertilization or early embryo development. Our group has used this technique for the first time for the evaluation of DNA damage caused by cryopreservation procedures. Within the four genes studied, we have observed a variable number of lesions showing different vulnerability to cryopreservation, as it was expected, depending on the genome region.

Conclusion
The quantification of DNA damage in relevant genes after cryopreservation provides valuable information that traditional techniques for DNA fragmentation assessment cannot detect. The use of this technique in combination with fragmentation studies would be strongly recommended.

Acknowledgments
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References


The canonical bmp activity promotes stemness in the vertebrate neural tube

abstract ID: 42

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Introduction: Bone Morphogenetic Proteins (BMPs) represent one of the main classes of multitasking secreted factors orchestrating Vertebrate development. In the context of central nervous system (CNS) development, their early contribution to patterning the neural tube has been known for a long time. Interestingly, our group recently demonstrated that the canonical (Smad-dependent) BMP activity is also required later, for the generation of discrete interneuron subgroups in the developing spinal cord of both mouse and chick embryos. Intriguingly, this requirement of BMP signaling is independent of its patterning activity, thus pointing to additional roles for this pathway during neural development.

Methods: To determine how the canonical BMP activity contributes to neurogenesis in the developing CNS, we took advantage of the chick in ovo electroporation methodology, and studied how modulating the canonical BMP activity affects the behavior of neural progenitors in vivo.

Results: FACS, transcriptional assays and immunohistochemical experiments demonstrated that the inhibition of Smad1/5 activity leads to a premature neuronal differentiation. The in vivo assessment of cell cycle length revealed that proliferating neural progenitors with decreased Smad1/5 activity harbor an S-phase shortening, a feature reported for neural progenitors committed to neuronal differentiation. Taking advantage of reporter constructs allowing the in vivo visualization of symmetric proliferative (P-P), asymmetric (P-N) and symmetric neurogenic (N-N) divisions, we show that inhibiting Smad1/5 activity increases the proportion of N-N divisions at the expense of P-P divisions, while the proportion of P-N divisions is maintained. Conversely, Smad1/5 hyper-activation results in an increased proportion of P-P divisions at the expenses of both P-N and N-N divisions.

Conclusion: These results demonstrate a novel requirement of the canonical BMP activity in the maintenance of neural stem cells during Vertebrate CNS development.
Mapping, characterization and identification of a new drosophila mutation that prevents germline cell proliferation

abstract ID: 45

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In our lab we have identified a mutation on the 3rd chromosome of *D. melanogaster* that is homozygous viable but sterile as the reproductive system of the adult flies (male and female) is agametic (absence of germ cells). Interestingly, because other somatic cell types can divide and proliferate normally when mutated, our mutation disrupts germline cells viability specifically. The agametic phenotype can be first observed at larval stages, where the mutant primordial germ cells die by apoptosis before the germline stem cell niche is formed. Induction of germline stem cells homozygous for the mutation in adult ovaries disrupts stem cell proliferation and provokes their degeneration. We have mapped the mutation to an intronic region within the *pointed* locus and we are currently trying to identify its molecular and genetic characteristics.
Function of perlecan in the drosophila ovary

abstract ID: 53

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The Drosophila female develops two ovaries, each composed of 16-20 egg-producing tubes called ovarioles. Eggs chambers are generated in the germarium, formed at the anterior end of each ovariole and home to two or three Germline Stem Cells (GSCs). Stem cells often reside in specialised cellular microenvironments or niches that offer stem cells structural support. In addition, signalling between support cells and stem cells is essential to regulate stem cell proliferation and to prevent their differentiation. The extracellular matrix plays a key role in controlling the homeostasis of stem cell niches, as it provides physical support and conveys extracellular signals. Perlecan, a heparan sulphate proteoglycan component of the extracellular matrix, has recently attracted much interest as it has been shown to act as a modulator of intercellular signals in development and morphogenesis. We will present our studies on the role of Perlecan in the maintenance of the Drosophila female germline stem cell niche and ovariole architecture. In addition, we will report the presence of different splicing isoforms of Perlecan in the basement membrane and in the interstitial matrix deposited in the GSC niche.
THE DYNAMIC DEPLOYMENT OF SONIC HEDGEHOG SIGNALING CONTROLS THE RENEWAL AND DIFFERENTIATION OF NEURAL PROGENITORS

abstract ID: 57

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INTRODUCTION
In the early developing nervous system, proliferative neural progenitors form a pseudostratified epithelium that undergo both proliferative (P-P) and neurogenic (P-N or N-N) divisions. Hence, they self-renew to maintain the progenitor pool or differentiate into neurons with distinct functions and morphologies. The intrinsic determinants regulating cell division and the resulting fate of the progeny are just beginning to be understood in vertebrates. However, very little is known about the role of extrinsic signals controlling the balance between these types of division.

METHODS
We use the chick spinal cord development as a model system to investigate how neural progenitor cells undergo the three types of division and maintain a normal tissue growth, and to study how this could be regulated by extrinsic signals.

RESULTS
Combining genetic manipulation and simulation tools, with specific reporters for each type of division, our data show that a normal balance between proliferative and neurogenic divisions requires a dynamic regulation of Sonic Hedgehog (Shh) signaling. Activation of Shh signaling maintains the competence of neural progenitors to undergo proliferative division. However as a cells switch from progenitor to differentiated states, Shh signaling needs to become attenuated.

CONCLUSION
Shh signaling is essential for the maintenance of neural stem cells.
THE DIFFERENTIAL EFFECT OF AN ACTIVATED FORM OF NOTCH1 IN HEMATOPOIETIC STEM CELLS IS RELATED TO THEIR FETAL VERSUS ADULT CHARACTERISTICS

abstract ID: 77

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THE DIFFERENTIAL EFFECT OF AN ACTIVATED FORM OF NOTCH1 IN HEMATOPOIETIC STEM CELLS IS RELATED TO THEIR FETAL VERSUS ADULT CHARACTERISTICS.

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Foetal hematopoietic stem cells (F-HSCs) are different from adult HSCs (A-HSCs) in terms of gene expression profile, surface markers, differentiation, and self-renewal capacity. F-HSCs maintain their own gene expression and functional profile up to three weeks post natal in mice and then they change and become A-HSC. Although mechanisms of this switch are not clear, it has been demonstrated that imposing the maintenance of a foetal HSC status during adult life results in leukaemia (He S, 2011). It is therefore of importance to understand how regulatory signals act differentially in foetal and adult HSCs.

The Notch signalling pathway plays a critical role in embryonic haematopoiesis. In particular, studies with Notch1 knockout mice have demonstrated that mutant embryos die at embryonic day 10.5 with defects in HSCs generation from hemogenic endothelium. However the effect of activation/inactivation of Notch signaling on F-HSCs is not clear. In adults, Notch activation induces self-renewal and blocks differentiation of HSCs. Studies on the deficiency of Notch signaling in A-HSC diverge from no effect to induction of mieloproliferative diseases (Iannis Aifantis et al, Nature 2011).

Our objective is to elucidate the effect of Notch activation during fetal, newborn and adult stages of HSC.

In this study, HSCs were obtained from transgenic mice for a mild-activated form of Notch1 (NIC) expressed under the regulatory elements of the stem cell leukaemia gene (SCL). The SCL3’Enh construct is active in HSCs (Silberstain, 2005). NIC-WT competitive hematopoietic engraftment assays were performed to determine NIC-HSC functionality at different developmental stages. Also the levels of Notch target genes (Hes1, GATA2 and Notch1), were assessed by quantitative RT-PCR.

Results showed that the activation of Notch signalling was observed in SCL3’EnhNIC adult bone marrow derived A-HSCs, concomitant with a decrease in engraftment potential (41±17%, animalsengrafted with NIC-HSCs versus 80±28% engrafted with WT-HSCs, p<0, 06). However, no increment of Notch-targeted genes was observed in NIC expressing F-HSCs and engraftment potential was no different from wild type (75±35% animals engrafted with NIC-HSCs versus 77±38% engrafted with WT-HSCs, p<0,9). Foetal HSC unresponsiveness to Notch activation was also a character observed in 3 weeks postnatal bone marrow. Interestingly, secondary transplantation of F-HSC derived from long-term chimeras showed impaired engraftment suggesting that the mechanisms that regulate Notch activation on HSCs depend on their foetal versus adult developmental stage.


Prrx1 FACTOR REGULATES EPITHELIAL PLASTICITY AND STEM CELL PROPERTIES

abstract ID: 109

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Prrx1 factor regulates epithelial plasticity and stem cell properties

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The epithelial to mesenchymal transition (EMT) is required in the embryo for the formation of tissues which cells originate far for their final destination. This programme endows cells with migratory and invasive properties and allows the transient dedifferentiation of embryonic epithelial cells. Interestingly, the reverse process, known as mesenchymal to epithelial transition (MET), is also essential in embryogenesis to allow the differentiation of tissues and organs once the embryonic migratory cells reach their final destination. The pathological activation of the EMT programme in the adult promotes tumour progression and organ fibrosis and recent findings indicate that the EMT can also confer stem cell properties. Similar to the situation in embryos, it has been suggested that a reversion of the EMT (MET) might also be necessary for metastatic colonization once malignant cells extravasate and find their niche in distant organs\(^1\).

The main inducers of the EMT are transcription factors of the Snail, Zeb and Twist families (EMT-TFs). Recently, we have identified Prrx1, as a novel EMT inducer both in embryos and in cancer cells (see also poster from Ocaña et al.). Importantly, the loss of Prrx1 in mesenchymal cancer cells induces a complete reversion to the epithelial phenotype as evidenced by the loss of mesenchymal and the gain of epithelial markers together with diminished invasive and migratory properties. Accordingly, it appears that the loss of Prrx1 is sufficient to revert the EMT.

A crucial difference between Prrx1 and other EMT-TFs is that Prrx1-induced-EMT does not concur with the described induction of stem cell-like properties concomitant with Snail-, Twist- or Zeb-mediated mesenchymal transitions. On the contrary, it is Prrx1 loss rather than gain in cancer cells what it is accompanied by the acquisition of stemness related capabilities, including anchorage-independent growth, mammosphere formation, increased cell proliferation and the conversion from a mostly double positive CD44/CD24 population to CD44high single-positive cells. Therefore, unlike the classical EMT transcription factors, Prrx1 uncouples EMT and stemness.

In conclusion, our data indicate that the loss of Prrx1 alone is sufficient and necessary to induce a MET accompanied by the acquisition of stem cell properties. These findings have important implications both in terms of the differentiation of embryonic stem cells, reprogramming and metastatic colonization.


Key words: Epithelial-mesenchymal transition, mesenchymal-epithelial transition, stemness.
Stem cells play important roles during embryonic development, but their involvement in adult processes is much more variable, and highly dependent upon the phylum studied. In flatworms, stem cells can regenerate the whole body, while in other organisms they are involved in self-renewal and tissue regeneration. They can be present in the germ line only, but also in the somatic lines. While some chordates can regenerate extensively as adults, the classical model being the salamander, others have much more limited regenerative ability. Recently, regeneration of axial structures—mainly tail—has also been extensively studied in the cephalochordate amphioxus (*Branchiostoma*)

Amphioxus is in a key evolutionary model system, as it has not suffered the genomic duplications characteristic of vertebrate evolution. Moreover, its embryonic development and morphology are apparently not as derived as those of vertebrates compared to those of the original early chordate. Following from the first description of stem cell populations in adult amphioxus (Somorjai et al. 2012), we aimed to study the evolution and expression in amphioxus of genes characteristically involved in stem cell maintenance and self-renewal,

Piwi proteins of the Argonaute family are present in some types of somatic stem or progenitor cells in a wide range of animal phyla. They are characterized by a PAZ domain and a PIWI domain, which are responsible for binding to the piwi-interacting RNAs (piRNAs) that are most abundantly expressed in the germ line. The Piwi-piRNA pathway mediates epigenetic mechanisms and post-transcriptional regulation, which may be responsible for its function in germ line specification, gametogenesis and stem cell maintenance, among other functions (Juliano et al. 2011).

In order to gain some insight into the functional evolution of the Piwi family, we have isolated *piwi* genes in the European amphioxus *Branchiostoma lanceolatum*. Here, we present data that clarify their phylogenetic relationships, and characterize their expression during embryonic development. This study provides the basis for future studies of *piwi* function during stem cell evolution in chordates.
Pan-epicardial lineage tracing reveals that epicardium derived cells give rise to myofibroblasts and perivascular cells during zebrafish heart regeneration

abstract ID: 124

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Pan-epicardial lineage tracing reveals that epicardium derived cells give rise to myofibroblasts and perivascular cells during zebrafish heart regeneration

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Myocardial infarction (MI) leads to a severe loss of cardiomyocytes, which in mammals are replaced by scar tissue. Epicardial derived cells (EPDCs) have been reported to differentiate into cardiomyocytes during development, and proposed to have cardiomyogenic potential in the adult heart. However, mouse MI models reveal little if any contribution of EPDCs to myocardium. In contrast to adult mammals, teleosts possess a high myocardial regenerative capacity. To test if this advantage relates to the properties of their epicardium, we studied the fate of EPDCs in cryoinjured zebrafish hearts. To avoid the limitations of genetic labelling, which might trace only a subpopulation of EPDCs, we used cell transplantation to track all EPDCs during regeneration. EPDCs migrated to the injured myocardium, where they differentiated into myofibroblasts and perivascular fibroblasts. However, we did not detect any differentiation of EPDCs nor any other non-cardiomyocyte population into cardiomyocytes, even in a context of impaired cardiomyocyte proliferation. Our results support a model in which the epicardium promotes myocardial regeneration by forming a cellular scaffold, and suggests that it might induce cardiomyocyte proliferation and contribute to neoangiogenesis in a paracrine manner.
How important are CD41 positive cells for lymphangiogenesis?

abstract ID: 125

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Vascular development is essential for an embryo and understanding how endothelial cell behaviour is regulated has an impact on how we might treat many diseases. Cellular components of blood are excellent candidates as regulators of angiogenesis, and in particular zebrafish thrombocytes, mammalian embryonic megakaryocytes or CD41 positive cells may play an important role in the regulation of endothelial cell behaviour in vivo.

The basis of our working hypothesis is the vascular phenotype of Meis1 null mice, which dies between embryonic day 11.5 and 14.5 with internal hemorrhages and blood-filled lymphatics. Meis1 is essential for proper hematopoietic stem cell (HSC) development and differentiation of megakaryocytic lineage. The hematopoietic defect found in Meis1-/- animals is characterized by a decrease in HSC potential and is accompanied by the absence of megakaryocyte precursors and derived platelets. This phenotype resembles other hematopoietic mutants, however and in contrast to these other hematopoietic genes, Meis1 is never expressed in the vasculature, and such suggested a function for hematopoietic cells during angiogenesis. In fact, we found that platelets and the embryonic megakaryocytic lineage have an essential role in the separation of the blood and lymphatic vasculatures during embryonic angiogenesis (Carramolino et al 2010).

We are exploring the interaction between CD41 positive cells and the endothelium “in vivo” by making use of the live imaging potential of zebrafish and transgenic lines that label the vasculature and CD41 positive cells. Zebrafish hematopoiesis shares many similarities with mammalian blood development, however it does not have a specialized megakaryocytic lineage and instead has a functionally homologous cell type, the thrombocyte, which conserves the expression of CD41. In particular, we are focusing in the interaction between CD41 positive cells and venous sprouts, and if those correlate these with venous to lymphatic transitions, an event that might go wrong in the absence of these CD41 positive cells.

To further evaluate the requirement of CD41 cells for venous to lymphatic transitions we are progressing towards systems that permit the temporal and spatial control over the depletion of CD41 positive cells, either using the CRE-loxP or the Gal4:UAS binary approaches and from which we expect to present some preliminary data.
Hematopoietic cells marked by CD41 expression interact with migrating venous sprouts as the parachordial vessel and lymphatic primordium is formed.

Figure legend: Trunk vasculature of a transgenic 3 day-old zebrafish embryo. Two intersomitic vessels run vertically, the aorta and caudal vein run horizontally with the CHT (caudal hematopoietic tissue) developing between the aorta and caudal vein. A) A double transgenic /ag2ZeoGFP; kdriveCherry zebrafish embryo highlights the hematopoietic cell population of interest in green (CD41 positive cells that develop in the CHT) and the vascular tree in red under a confocal microscope. A maximum intensity projection of a z-stack that contains the left side of the embryo is shown, from the exterior of the embryo to the lumen of the aorta. B) A imaging software reconstructed view illustrating a venous sprout, in shades of grey (arrow), and a CD41 cell within that venous sprout, that will contribute to the parachordial vessel (PCV), which in turn is the source of lymphatic endothelial cells. These venous sprouts are fated to become lymphatic vessels 2 days later. aSV, arterial intersomitic vessel and vSVV, venous intersomitic vessel.
The role of Tissue Inhibitor of Metalloproteinases in maintaining stem cell niche morphology in the Drosophila ovary

abstract ID: 140

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Introduction:

The Drosophila ovary houses germline and somatic stem cell populations in a specialized structure known as the germarium. Maintenance of these stem cell populations depends on the local micro-environment or niche, comprising cell-cell signaling and physical interactions with surrounding stromal cells and the extracellular matrix (ECM). ECM components can be degraded by secreted Matrix Metalloproteases (MMPs). In this work we examine the role of Tissue inhibitor of metalloproteases (Timp), a secreted MMP inhibitor, in maintaining the specialized stem cell niche structures of the Drosophila ovary.

Methods:

Mutants homozygous for a Timp null mutation (1), or in combination with a deficiency uncovering the same genomic region, reach adulthood, allowing us to studying the requirement for Timp in adult tissues. Rescue and ectopic expression experiments were carried out using a UAST-Timp transgene (2) either alone or combined with different somatic GAL4 driver lines. Atomic force microscopy (AFM) was performed using a JPK nanowizard II AFM system at specific points of dissected live germaria.

Results:

Females lacking Timp lay very few eggs and exhibit abnormally small or disorganized ovaries. Ectopic expression of Timp in mutant females could rescue the ovary defects (1). Germaria from 1+ week old Timp mutant females were abnormally rounded and often exhibited dramatically altered stem cell niche domain organization. In addition, the number of new cysts produced in these mutant germaria is significantly reduced with respect to wild-type controls. Abnormally long interfollicular stalks were also often observed in Timp mutants. The ectopic expression of Timp within wild-type germarium, using the C587-GAL4 line (3), results in the formation of large elongated germaria containing multiple unseparated egg chambers. In these germaria, the presence of cells expressing stalk cell markers indicates a failure of stalk cell organization and/or migration rather than stalk cell specification. While alterations in the distribution of extracellular matrix components were not visible in Timp mutant germaria as judged by immunostaining and transmission electron microscopy (TEM), differences were detectable around interfollicular stalks. Significantly, analysis ex-vivo with Atomic Force Microscopy (AFM) revealed changes in the physically properties of the surface of mutant germaria.

Conclusions:

Our AFM results suggest the absence of Timp may result in a functional alteration to ECM organization that does not involve gross changes in its distribution or composition, but that grossly disrupts the ability of the ovarian niche to produce new developing egg chambers. Further studies...
will be required to determine the molecular basis for these changes. Together our results suggest that controlling the activity of ECM proteases is crucial for both maintaining adult stem cell niche organization and regulating the properties of their differentiated progeny in adult tissues.

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Modeling cancer stem cell in the adult drosophila midgut

abstract ID: 159

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Modeling cancer stem cell in the adult Drosophila Midgut

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Cancer stem cell model poses that a subpopulation of tumour cells with properties reminiscent to normal stem cells are responsible for cancer progression, recurrence and metastasis. Recently it has been suggested that cancer stem-like cells could be the cells of origin in some solid tumours, like colon cancers.

Adult Drosophila midgut contains Intestinal Stem Cell (ISC) able to replenish the tissue during normal homeostasis, injury and infection. ISCs express Delta, which signals to a daughter transient precursor cell, termed enteroblast, to generate an enterocyte or an enteroendocrine cell through differential activation of Notch signalling, thereby sustaining organ homeostasis.

We are exploiting this system to test cancer stem cell model(s) in vivo. To this end, we have constructed strains of Drosophila that allow us to trace the adult stem cells as well to mis(over)express combinations of oncogenes and/or tumour suppressors in the intestinal stem cell compartment or in their differentiated progeny. Although, it is generally accepted that disrupting stem cell asymmetric division may underlie tumorigenesis associated with normal stem cells, we found conserved molecular pathways capable of inducing in vivo invasive tumor behaviour when overexpressed in adult stem cells without blocking asymmetric cell division. These tumours exhibit remarkable heterogeneity and plasticity, resembling human solid tumours.
Adult drosophila midgut intestinal stem cells as a model to detect common mechanisms of cancer and aging

Abstract ID: 160

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The adult *Drosophila melanogaster* midgut harbors Intestinal Stem Cells (ISC) that are able to regenerate aging epithelial cells during normal tissue homeostasis, injury and infection. Adult stem cells are exposed to environmental stress and intracellular damages that go together with the process of aging. Longevity seems to be associated with increased renewal capacity via stem cells but also with increased cancer risk. In contrast, aging is associated with disturbance in stem cell number or function. This trade-off between longevity (aging) and cancer is further strengthened by findings that several solid cancer types harbor cancer stem cells that may derive from normal stem- or progenitor cells. Cancer stem cells are likely to contribute to therapy resistance and tumor relapse.

ISC in the *Drosophila* midgut respond to various signaling pathways like EGF, Hippo and Delta/Notch-signaling. A GS-screen aiming to identify new cooperators of Notch-signaling in tumorigenesis identified various genes that are not only responsible for tumorigenesis, but have also been involved in aging, e.g. Indy. Indy (I am not dead yet) mutant flies and mice exhibit an increased life span through caloric restriction and alterations in fat metabolism. Further analysis of the GS-screen data highlighted more genes being involved in cell metabolism and caloric restriction. This study employs the highly developed experimental model *Drosophila melanogaster* to test those genes in an adult intestinal stem cell model by overexpression and knockdown strategies.

Taken together we try to identify new pathways that connect Notch signaling and metabolism with tumorigenic functions and aging.
Rex1 and Yy2 have been generated by retrotransposition from Yy1. Their presence is exclusive to eutherian mammals which suggests a potential function in preimplantation and/or placental development. Henceforth, efforts to identify biological consequences of Rex1 loss-of-function have failed to uncover major phenotypes. We have started to consider compensation by Rex1 related ZincFinger proteins Yy1 and Yy2 as a cause for the weak phenotypes observed.

We have generated a rabbit polyclonal serum specific for YY2. We show the presence and localization of YY2 in embryonic stem cells (ES), trophectoderm stem cells (TS), and throughout mouse preimplantation development. Accordingly, we are studying a potential role for Yy2 in the regulation of preimplantation development. Loss-of-function assays of Yy2 using shRNAs and siRNAs in ES cells and embryos, respectively, have been performed. In both cases, we have successfully attenuated the levels of Yy2, and biological effects are under study. We have identified Rex1 as a regulator of retrotransposable elements (RE) during preimplantation development. In order to identify targets of regulation by Yy2, we have performed ChIP assays that demonstrate YY2 association to RE in both ES and TS cells. This is compatible with a role for Yy2 in regulation of RE during preimplantation development together with Rex1.

Recently, KDM1A/LSD1 has been discovered as a methylation-independent epigenetic regulator of ERV and ERV LTR-linked genes in ES cells. Moreover, LSD1-deficient ES cells present similarities to Rex1−/− cells, as both maintain selfrenewal but suffer deregulation of common targets upon differentiation. We show that Rex1 and YY2 can interact with LSD1 in overexpressing cells. Combined, we hypothesize that Rex1 and Yy2, in cooperation with Lsd1, play a role in control of transcription directed through RE and RE-derived elements during mouse preimplantation development.
Session 6 – Evolution and Development
Early morphohistogenesys of the thalamus in the amphibian anuran *Xenopus laevis*: a genoarchitectonic study

Abstract ID: 10

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Each structure that shapes the adult brain in vertebrates begins to differentiate from the earliest developmental moments by means of the activation of many regulatory genes that are interconnected by several gene networks. These networks will determine the conformation of each of the highly conserved histogenetic domains in vertebrates that gives rise to several more or less divergent derivatives. The study of the spatiotemporal expression patterns of different developmental genes within a given territory is very useful to identify each progenitor domain and morphogenetic domains that show a unique combinatory gene expression pattern that allows analyzing the corresponding derivatives of each progenitor domain as development proceed. Thus, recent genoarchitectonic studies used this approach for the analysis of several brain regions. The present work uses this powerful tool for the study of the early regionalization of the alar region of diencephalic prosomere p2, called thalamus. Among the genes studied, Sonic hedgehog (Shh) is a pivotal developmental morphogene that marks the basal plate and the zona limitans intrathalamica (Zli) and it is involved in the formation of the p2/p3 boundary and the molecular specification and regionalization of the diencephalon. Additionally, Gbx2 has been demonstrated to be involved in the development of the thalamus and in the formation of thalamic boundaries, in combination with the gene Xiro1. By means of single and double in situ hybridization combined with immunohistochemical techniques, we describe the patterns of expression for Shh in combination with Gbx2 and Xiro1, in the embryonic diencephalon of the amphibian *Xenopus laevis*. At early embryonic stages, Shh is expressed in the ventricular zone in the Zli. At the same time, Gbx2 is expressed in a thin band next to the alar-basal boundary, while Xiro1 is strongly expressed in the alar plate of p1 and p2. As development proceeds, Shh is gradually reduced in the Zli and is downregulated from the basal to alar regions. Simultaneously, Gbx2 is expanded in the mantle zone of p2, and Xiro1 keeps a strong expression in p1, at the p1-p2 boundary, and in the habenula.

Conclusions

Our results corroborate those found in other vertebrates, and support the early establishment of thalamic boundaries in *Xenopus* in a very reduced region initially that showed an alar expansion during development. Additionally, a ventrodorsal expansion and a rostrocaudal subdivision are supported by the synchronized complementary spatiotemporal expression patterns of the genes studied. This concurrence also supports a likely causal functional interaction between these genes.

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The evolutionary origin of the epicardium and the early cardiac vasculature is related to a primitive excretory system of vertebrates

abstract ID: 21

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The embryonic epicardium and its derived mesenchyme are critical to heart development, as the epicardial lineage contributes to the formation of the coronary vasculature and the cardiac interstitium, also modulating the proliferation of the ventricular myocardium. The embryonic epicardium arises from an extracardiac and originally paired progenitor tissue called the proepicardium, a proliferation of coelomic cells at the limit between the liver and the cardiac sinus venosus in all the vertebrates so far studied. However, proepicardium and epicardium have not been described in invertebrates, and the evolutionary origin of these structures in vertebrates is poorly known. We described the epicardial development in a representative of the most primitive vertebrate lineage, the lamprey *Petromyzon marinus* and we observed that the epicardium arises by cell migration from the primordia of a pair of pronephric external glomeruli that are fully functional in lamprey larvae. Hence, the proepicardium in gnathostomes might be regarded as an evolutionary derivative from pronephric external glomeruli that have lost their excretory role. Glomerular progenitor cells are highly vasculogenic and probably allowed for cardiac vascularization when the walls of the vertebrate heart increased their thickness. This hypothesis accounts for the striking similarities in gene expression between developing epicardium and kidneys. In fact, Wilms' tumor suppressor gene Wt1, Pod1/epicardin, Tbx18 and podoplanin are all involved in the development of both, epicardium and kidneys.

In order to test the hypothesis of a nephric origin of the epicardium and its progenitor tissue, the proepicardium, we are currently investigating if the ancient nephrogenic potentiality of the proepicardium can be still activated. We are checking the expression of glomerular and podocyte markers in cultured proepicardia of chick and mouse embryos as well as in proepicardia cocultured with embryonic kidneys. Our first results suggest that a number of podocyte-specific genes can be expressed in the proepicardia, thus supporting our hypothesis.

We think that the suggested ancestral association of the vertebrate heart with an excretory glomerulus is reminiscent of the heart-kidney complex of the hemichordates and the axial organ of echinoderms. This hypothesis can provide new approaches about the evolutionary origin of the vertebrate heart.
Comparative transcriptomics of early dipteran development

abstract ID: 26

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We are carrying out a comparative analysis of transcriptomic sequence data in early embryos of three emerging experimental dipteran model systems. *Clogmia albipunctata* belongs to a lineage of flies believed to have diverged early in the evolution of the dipters, while the lineage leading to *Megaselia abdita* branched intermediately, at the base of the cyclorrhaphans, and the lineage leading to *Episyrphus balteatus* diverged later in the cyclorrhaphans.

We have acquired and assembled transcriptomic sequences at early embryonic stages in *Clogmia albipunctata* and *Megaselia abdita*. We compare these sequences to those from *Episyrphus balteatus* as well as transcriptomic and genomic sequences from drosophilids and/or mosquitoes. These datasets form the basis of a new phylogenomic assessment of dipteran relationships. It places psychodid moth midges (such as *Clogmia*) within the brachyceran, rather than the culicomorph lineage, in contrast to another recent study (Wiegman et al., 2011). Furthermore, we have analysed patterns of gene duplication in our datasets. Finally, we have verified information present in our transcriptomes by manual curation, *in situ* hybridization, and verification of alternative splicing events among a subset of genes present in the data.
Dlk1 and dlk2 in notch signaling during salivary gland development

abstract ID: 48

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The morphogenesis of the salivary gland branches is dependent on epithelial-mesenchymal interactions and controlled by different growth factors and signaling molecules, expressed at specific times and locations.

The goal of our experiments was to investigate the role of Dlk1 and Dlk2 proteins on the Notch signaling pathway in salivary gland development.

First we studied the stage and cell distribution of Dlk1 and Dlk2 (Delta Like Proteins, transmembrane glycoproteins, which contain similar motifs to those present in the Delta/ Notch/ Serrate family signaling molecules) in submandibular salivary gland (SMG) development. We performed immunofluorescence against Dlk1 and Dlk2 on 13.5 to 19.5 day-old mouse embryo (E13.5-E19.5) SMGs sections and we compared cellular expression and distribution. The results revealed a clear difference in pattern expression of Dlk1 and Dlk2 in mouse embryo SMGs: Dlk1 was negative for the tubular and acinar epithelium but positive for the mesenchyme, while Dlk2 has an antagonistic expression pattern being positive for the epithelium and weakly expressed in the mesenchyme.

Our next step was to determine the mechanism of action of Dlk1 and Dlk2 on the Notch pathway in the salivary gland. To address this question, we conducted a luciferase assay in HSG salivary gland cell line. This experiment consists of measuring the transcriptional notch activity in cotransfected HSG cells with notch1 and dlk1 or dlk2 protein. The results clearly indicated that Dlk1 and Dlk2 significantly inhibited Notch1 receptor.

In order to know if Notch inhibition interferes in SMG development, we carried out organotypic cultures of E13.5 SMGs for 48 hours in the presence or absence of DAPT 20mM (N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester), an inhibitor of γ-secretase, which specifically disrupts Notch pathway. Histology of SMGs cultured in the presence of DAPT showed that acini were not completely developed and branching clefting were not always formed. This finding indicates that Notch pathway is crucial during first stages of salivary gland development.

In conclusion, Dlk proteins through Notch1 receptor seem to play an important role in SMGs development.

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Molecular basis of thermotolerance facilitates monitoring ecological and morphological changes under climate change

abstract ID: 62

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Understanding the molecular basis of thermal tolerance is key to predicting the range of species and evolutionary changes in response to climate change. Heat shock proteins (Hsps), heat induced chaperone molecules, have been widely used as markers to predict thermal tolerance and associated ecological and morphological changes. However, there has been little direct evidence that the expression level of these molecules predicts heritable levels of thermal tolerance.

Here I present that differences in thermal tolerance of embryo development in two sister species of sea squirts correlate with their geographic distributions. Using reciprocal hybridization I show that thermal tolerance is maternally-inherited. By transcriptome analysis we found expression of typical heat-induced genes like hsp60 and hsp83 do not predict maternal inheritance of thermal tolerance. In addition, expression of hsp70 was not positively correlated to the thermal tolerance. Instead I found the expression level of an endoplasmic reticulum chaperone, which do not increase the level of expression promptly therefore was neglected in study of thermal tolerance, is maternally inherited, and that its expression in embryos strongly predicts thermal tolerance. This molecular marker will facilitate tracing morphological evolutionary changes under climate change. I will also discuss developmental genes that accompany maternal inheritance of thermal tolerance.
Regeneration in acoels (Acoela; Acoelmorpha)

abstract ID: 68

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Regeneration in acoels (Acoela, Acoelmorpha)

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Background: Even though regeneration is a widespread phenomenon among animals the capacity to regenerate varies greatly among various taxa. A broad sampling of animal models should provide us with clear insights on the underlying principles. Interesting in this respect is the Acoela as they are a group with great regeneration potential. Now recognized as a different phylum, independent of the Platyhelminthes, they still seem to share a very similar stem-cell system. In order to provide more information on this understudied group of animals and lay a framework for future molecular analyses we have investigated the regeneration of the posterior part in the acoel Isodiametra pulchra (the head does not regenerate), using histology, electron microscopy, fluorophore-tagged-phalloidin labeling, and staining of S-phase and mitotic cells.

Results: Right after amputation the wound closes by contraction of local muscle fibres and the extension of the epidermis. Subsequently, neoblasts in the wounded area start to proliferate, forming an indefinite blastema. After two days of regeneration, the primordium of the male copulatory organ can be observed for the first time. A number of developing swallow's nest receptors can be detected at the terminal end of the body. During the third day the primordium of the female copulatory organ can be detected, anterior to the male copulatory organ primordium. The development of the copulatory organs continues progressively until day seven. The vagina, the bursal stalk and the female sphincter are the last structures to appear. Regeneration is complete after nine or ten days.

Conclusion: Isodiametra pulchra is capable to restore all the posterior structures within ten days of regeneration. The restoration order always starts with the male copulatory organ and it’s followed by the female copulatory apparatus, appearing first the bursal nozzle and later the female sphincter. This data suggests a mechanism of intercalary regeneration, as reflected by the restoration order (first the more distal, then the most proximal and subsequently the intermediate structures).
Non-stochastic assignment of brain asymmetry at the basis of the vertebrate lineage

abstract ID: 71

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Although roughly symmetrical, vertebrates exhibit marked asymmetries at the level of the visceral organs. In addition, in many vertebrate species major asymmetries are visible at the level of the epithalamus, a subdivision of the diencephalon composed of a paired nuclei set (the habenula) and a photoreceptive complex (the pineal organ). It has been proposed that the pineal complex originally consisted of paired photoreceptive organs that fuse at the midline and split into the pineal and parapineal organs. In actinopterygii, the left and right habenulae undergo a differential morphogenesis whereas this asymmetry is variable, faint or absent within sarcopterygii (aves, mammalia, reptilia, amphibia). Not surprisingly, a left-sided expression of the nodal/pitx2/lefty cassette has been detected in the presumptive diencephalon of both zebrafish and medaka but it turned out that nodal signalling is not controlling the habenalum asymmetry per se but the migration of the parapineal organ toward the left habenular nucleus. In contrast, experimental ablation of the parapineal organ abrogated the differential morphogenesis of the two habenulae. Since teleost is the only osteichthye group where asymmetric nodal expression has been reported, chondrichthye and agnatha models were necessary to know whether nodal co-option in the vertebrate epithalamus was set up only to direct laterality of the epithalamus or whether it is truly breaking its symmetry. We found clear molecular differences between the right and the left habenulae both in the lamprey, P. marinus, and in the catshark, S. canicula. Indeed, in both species, marked expression of a signalling effector was strongly detected in the right habenula compared to its left compound confirming that molecular asymmetries were already present in the vertebrate ancestor. As in zebrafish, the lamprey parapineal supplies the left habenal nucleus, which could account for the specific morphogenesis on this side but shark are devoid of parapineal organ. Furthermore, we demonstrated that they are not expressing FGF8, the secreted factor driving parapineal migration in ZF. As the catshark situation was not phenocopying the parapineal ablation in zebrafish, we wondered whether nodal pathway was already active in agnatha and at the basis of gnathostomes. We observed asymmetric expression of nodal genes in the diencephalon of lamprey and catshark embryos. The nodal/lefty module is considered to be an activator/inhibitor system for which the activator needs to activate its own expression. Consistent with this system we observed that abrogation of nodal signalling in catshark embryos inhibited pitx2 expression both in the diencephalon and in the lateral plate mesoderm but not in the branchial arches. Then, nodal pathway co-option in the brain is not an event occurred independently in the teleost lineage but a robust genetic pathway set up at the origin of vertebrate. In addition, our data suggest that Nodal activity could drive asymmetric morphogenesis of the epithalamus in vertebrate in the absence of any FGF8-directed cell movements.
An ancient process in a modern mollusc: development of the shell field in *Lymnaea stagnalis*

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Introduction

Molluscs constitute one of the most morphologically diverse and ancient phyla of the animal kingdom with an extensive fossil record dating back to the early Cambrian (543+ MYA). Although much of this morphological diversity can be attributed to the evolutionary plasticity of their external shell, surprisingly little is known about the cellular or molecular processes that underlie the development of this structure. Shell formation starts early in embryogenesis with the specification of a shell gland which then differentiates into a shell field, and finally into the adult shell-secreting organ. The initial differentiation of the shell gland can be observed by a thickening of the dorsal ectoderm which is in intimate contact with the underlying endoderm. Historically, this contact lead to the idea that shell gland specification is a contact-dependent process, representing a "specification by induction" mechanism. However, studies on precise cell movements and contacts during molluscan shell gland specification using modern high resolution techniques have not been performed.

Methods

We have employed a range of techniques to provide a detailed description of early shell gland and shell field development in the derived gastropod *Lymnaea stagnalis*. These include confocal laser scanning microscopy, electron microscopy and histochemical assays that detect endogenous peroxidase and alkaline phosphatase activity.

Results

Using these techniques we are able to detect most of the morphogenic events associated with shell gland development that have been described by previous workers, but we propose a different order of events. We detect ontogenetically earlier peroxidase and alkaline phosphatase activity than previously reported, and a pronounced initial bilateral invagination of the shell gland that has received little attention. Finally, our initial data is consistent with the concept of a contact-dependent endoderm-ectoderm induction event that specifies the shell field.

Conclusions

This work represents a platform from which we will conduct analyses aimed at the identification of the molecular regulators of shell gland specification. Given the evolutionary success of the phylum Mollusca, and the role that their shell has played in this success, understanding the evo-devo of the molluscan shell is likely to provide deep insight into the evolutionary events that supported the generation of much of today's molluscan diversity.
Effects of egf on the preimplantatory embryonic development

abstract ID: 94

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Introduction:

Epidermal growth factor (EGF) family and its receptors are primarily responsible for directing the process of embryo implantation. Therefore, the study of the EGF effects on preimplantation embryos has been a general objective in research on reproductive medicine. However, it is not clear whether the supplement of culture media in preimplantatory embryos with EGF induce an improvement in embryo implantation by means of better development to the blastocyst stage.

In order to clarify EGF role in early steps of development we have established the following objectives: 1º to determine the sensitivity to EGF during embryonic development by a EGF binding assay and 2º to analyze the changes in development produced by the presence of EGF in the culture media in which the embryos are growth from zygote to blastocyst.

Material and methods:

Mouse embryos were obtained by in vitro fertilization (IVF) accordingly to routine procedures or extracted directly from the uterus. To reveal embryo sensitivity to EGF, we used an EGF binding assay based on the union of a fluorochrome conjugated EGF probe to living embryos. To analyze the effects of EGF on development, we exposed preimplantatory embryos in different phases and intervals to recombinant EGF at desired concentrations. We estimate the effects of EGF on the cell proliferation by the number of cells of the embryos after reaching the blastocyst stage.

Results:

The period of sensitivity to EGF is opened at the time of fertilization, decreases gradually to the morula stage and is maximum at the blastocyst stage.

Embryos developed in utero have maximum values in sensitivity to EGF and in the number of cells at blastocyst stage.

The culture of IVF embryos with EGF during the later stages of preimplantational development represents a significant increase both in the sensitivity to growth factors and in the number of cells at the blastocyst stage. On the other hand, the presence of EGF in culture during the early stages of development (zygote to 2 cell stage) has not effect.

Discussion:

It has been confirmed that during later stages of preimplatatory development EGF has more affinity for epidermal growth factor. There is also a period of sensibility in the zygote stage that must be clarified. Besides, the supplementation of the media with EGF have positive effects both in increasing the number of cells and the affinity by EGF at the blastocyst stage of the embryos obtained by IVF.
Molecular basis of thermotolerance facilitates monitoring ecological and morphological changes under climate change

abstract ID: 110

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Understanding the molecular basis of thermal tolerance is key to predicting the range of species and evolutionary changes in response to climate change. Heat shock proteins (Hsps), heat induced chaperone molecules, have been widely used as markers to predict thermal tolerance and associated ecological and morphological changes. However, there has been little direct evidence that the expression level of these molecules predicts heritable levels of thermal tolerance.

Here I present that differences in thermal tolerance of embryo development in two sister species of sea squirts correlate with their geographic distributions. Using reciprocal hybridization I show that thermal tolerance is maternally-inherited. By transcriptome analysis I found expression of typical heat-induced genes like hsp60 and hsp83 do not predict maternal inheritance of thermal tolerance. In addition, expression of hsp70 was not positively correlated to the thermal tolerance. Instead I found the expression level of an endoplasmic reticulum chaperone, which do not increase the level of expression promptly and was neglected in study of thermal tolerance, is maternally inherited, and that its expression in embryos strongly predicts thermal tolerance. This molecular marker will facilitate tracing morphological evolutionary changes under climate change. I will also discuss developmental genes that accompany maternal inheritance of thermal tolerance.
The inner ear is a complex sensorial structure with hearing and balance functions. A key aim of developmental biology is to understand the molecular and cellular mechanisms involved in the patterning of the vertebrate inner ear. Fate specification of the developing otic epithelium seems to be governed by regulatory genes that provide positional identities to the parts of its complex three-dimensional structure. Despite the knowledge of axial patterning in the developing inner ear, little is known about the mechanisms implicated in the generation of the sensory and non-sensory elements. The vertebrate Iroquois (\textit{Irx}) genes, organized in two vertebrate clusters (cluster A: \textit{Irx1}, \textit{Irx2}, and \textit{Irx4}; cluster B: \textit{Irx3}, \textit{Irx5}, and \textit{Irx6}), play key roles in the early tissue specification of neuroectoderm, in particular regulating its late subdivision. In order to gain insight into the possible implication of \textit{Irx} genes in the inner ear development, we performed a detailed analysis of the expression patterns of cluster \textit{IrxA} in the chick inner ear at 5 embryonic days (stage 27) by \textit{in situ} hybridisation experiments on cryostat serial sections. \textit{Irx1}, \textit{Irx2}, and \textit{Irx4} genes were expressed in the lateral portion of the inner ear. \textit{Irx1}-expressing domain bordered all the cristae, which were devoid of \textit{Irx1} expression. The macula utriculi and macula sacculi showed very low or no \textit{Irx1} expression, whereas the distal basilar papilla, the macula lagena, and macula neglecta were \textit{Irx1} positive. Regarding the \textit{Irx2} gene, scattered \textit{Irx2}-positive cells were also observed in all the sensory patches. The acoustic-vestibular ganglion showed a clear \textit{Irx1/2} expression. These results suggest that IRX proteins could confer positional identities to coordinate inner ear morphogenesis and specification of sensory elements.

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Gabaergic interneurons cluster in the cerebral cortex according to lineage relationships

abstract ID: 144

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Functional networks in the cerebral cortex emerge during development through the interaction of two main classes of neurons, excitatory glutamatergic pyramidal cells and inhibitory gamma-aminobutyric containing (GABAergic) interneurons. The general principles underlying the allocation of pyramidal cells in the cerebral cortex are relatively well understood, but the mechanisms controlling the spatial organization of cortical interneurons remain to be elucidated. This is complicated by the fact that GABAergic interneurons are born remotely in the subpallium and undergo a long tangential migration before adopting their final position in the cerebral cortex. Here, we have investigated whether lineage relationships play a critical role in the final destination of specific classes of interneurons. To elucidate this question, we have developed a method to fate map clones of inhibitory interneurons in vivo by combining retroviral lineage-tracing technology with Cre/loxP system. This method allows the analysis of the final distribution of a relatively small number of interneurons originated in specific regions of the subpallium. We found that interneurons do not disperse randomly throughout the adult cortex. Instead, clonally-related interneurons have a tendency to cluster in relatively small regions of the cortex, independently of their origin within the subpallium. Our results suggest that lineage relationships strongly influence the final allocation of interneurons.
Evolutionary origin of the chordate hematopoiesis and cardiovascular system: insights from amphioxus

abstract ID: 150

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The ontogeny of the heart and the vascular system of vertebrates, mainly of mammals, has been widely studied, but little is known about the origin and evolution of vertebrate-specific cardiovascular novelties, and most of the literature in the field is limited to the heart only. Cephalochordates, a basal branch within the phylum Chordata, is very informative to assess the condition of the last common ancestor of chordates. Their circulatory system is nearly closed, and the hemal liquid fulfills concrete open spaces between basal membranes of the epithelium, or coeloms, covered by connective tissue. Despite the lack of endothelium, a vertebrate innovation, the presence of amebocyte-like cells inside the vascular lumen of the vessels, sometimes lining them, has been reported in amphioxus. This hemal cell type may well be the evolutionary precursor of the vertebrate endothelium (reviewed in Muñoz-Chápuli et al., 2005). Moreover, it has been recently reported that this kind of hemal cells can induce the opening of the vascular lumen of vessels of amphioxus during development (Kucera et al., 2009). To gain further insights into the origin and co-options of the gene networks involved in those cells and the amphioxus vascular system, here, we study the expression patterns of several key hematopoietic and cardiac markers involved not only in heart development but also in the formation of blood cells and vessels, in the European amphioxus B. lanceolatum. We will show data on those genes and mechanism and propose scenarios for the origin of the complex cardiovascular system of vertebrates.
Epidermal wound response and pigmentation pattern formation: developmental and evolutionary perspectives

abstract ID: 161

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The epidermis protects animals from the external environment. In case of damage, it is crucial for organisms to repair it rapidly and efficiently. The cellular and molecular mechanisms underlying this process have been studied in detail in several lab models. Studies have shown the evolutionary conservation of many aspects of wound response, but also key differences between species, and between developmental stages and tissues of the same organism. Wound response is typically tightly connected to pigmentation. An interesting example occurs in some Lepidopterans, the insect order of butterflies and moths, which develop organized pigmentation patterns around wound sites, resembling native color pattern elements. This system can provide novel insights into the mechanisms of wound-induced color patterning, including i) evolutionary considerations about the potential co-option of wound-response mechanisms to the formation of novel traits, such as butterfly wing patterns, and ii) analysis of wound response in post-growth, pre-adult tissues, a largely uncharted area. In my Ph.D. project I am exploring the mechanisms of wound-induced pattern formation by 1) assessing the contribution of immunity and tissue repair, two processes activated post-wounding, to the formation of wound-induced color patterns, and 2) characterizing the cellular (using standard markers) and molecular (following gene expression) processes during pattern-inducing epidermal healing.
The role of the environment in generating heritable variation

abstract ID: 165

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Heritable phenotypic variation is the raw material for evolution by natural selection. To fully understand evolutionary change we must study the processes that drive the formation of phenotypic variants. We aim to understand the role of the environment in producing adaptive genetic variation. We propose to study the mechanisms whereby the external environment impacts transposable element dynamics during oogenesis in *Drosophila melanogaster*. It is known that environmental stresses induce transposon jumping, and that the piRNA pathway inhibits transposition in the germline. We will test the hypothesis that environmental stresses influence the piRNA pathway, affecting germline transposition and creating novel heritable variants.
Tissue-specific splicing factors in deuterostomes: evolutionary remodelling of gene regulatory networks through alternative splicing

Abstract ID: 166

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Alternative splicing (AS) regulates gene expression from an immediately post-transcriptional level across many eukaryotic lineages, but its frequency and subtle regulation is higher in organisms with complex genomes. Regulatory networks have been identified through which splicing promotes dynamic remodeling of the transcriptome to control developmental processes. Here we study tissue-specific expression of alternative splicing regulatory genes in non-vertebrate deuterostome species. These results show the evolvability and conservation of tissue-specific expression of PTB, ESRP, QK, RBM24 and RbFox, among others. While certain factors seem to have different biological roles, some of them point to share homologous expression and hypothetically partial gene regulated networks. We also have identified several AS events in amphioxus and we have studied its percentage of conservation with respect to vertebrates.

Our phylogenetic analysis proves the existence of four RbFox genes in the last common gnathostome ancestor, and subsequent convergent loss of the same paralogue in different lineages. We also analyze the evolution of RbFox gene structure in diverse deuterostome branches. Focusing on alternative promoter subfunctionalization, some vertebrate family members have revealed its relation with tissue-specific redundant expression loss. Moreover, conserved and coherent function of RbFox in the regulation of tropomyosin isoforms has been detected in deuterostomes by in silico identification and relative localization of particular intronic elements, acting as binding sites for RbFox proteins.
T-box genes and vertebrate eye development

abstract ID: 180

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Eye formation is a complex developmental process that requires the coordination of a series of morphogenetic events and the regulated expression of several genes. Among these, a number of T-box genes, which encode transcription factors that contain a T-domain involved in DNA binding and protein dimerisation, have been implicated in the earliest events of dorso-ventral specification of the developing retina in vertebrates. In particular, some members of the Tbx2 subfamily (namely Tbx2 and Tbx5) have been involved in the establishment of dorsal retina identity and as such, are frequently used as markers of dorsal retina characteristics. However, the transcription factors and/or signalling pathways that directly restrict Tbx2 subfamily expression in this dorsal domain remain elusive.

To gain more insights about the earliest steps of vertebrate dorsal retina specification, we aimed to identify the factors/signalling pathways required to restrict the expression of the Tbx2 subfamily genes in this domain. The teleost zebrafish is especially suitable for these studies due to the great advantage of following the expression of a fluorescent reporter gene in the transparent, ex utero developing embryo. To this end, we have established stable transgenic lines that drive GFP expression under the control of the dorsal retina-specific enhancers of tbx2a, tbx2b and tbx5a that we have previously identified in silico by phylogenetic footprinting. Sequence comparison of these three enhancers followed by in silico identification of putative transcription factors binding sites highlighted the presence of two main domains that may be involved in dorsal retina expression of these genes using a “dorsal-positive/ventral negative” mode of regulation. Briefly, transcriptional activators would activate Tbx2 genes transcription in the dorsal retina through the first conserved domain, whereas their expression would be silenced by ventral repressors acting through the second conserved domain.

We are currently using genetic and gene manipulation techniques as well as interfering with certain signalling pathways using specific chemical inhibitors to ascertain whether this “positive-negative” regulation is indeed taking place. Our analyses will shed some light into the understanding of the initiation and maintenance phases of dorso-ventral retina specification in vertebrates.
Epigenetic reprogramming during pollen development and embryogenesis: dynamics of DNA methylation patterns and MET1 expression

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INTRODUCTION

DNA methylation, accomplished by DNA methyltransferases, constitutes a prominent epigenetic modification of the chromatin fiber which is locked in a transcriptionally inactive conformation. Changes in DNA methylation accompany the reorganization of the nuclear architecture during plant cell differentiation and proliferation. Stress-induced plant cell reprogramming involves changes in global genome organization, being the epigenetic modifications key factors in the regulation of genome flexibility.

After a stress treatment, in vitro-cultured microspores are reprogrammed and change their gametophytic developmental pathway towards embryogenesis to form haploid embryos and plants, convenient tools in crop breeding and biotechnology, the process constituting a useful system of reprogramming in isolated cells, for applied and basic research. Gene expression driven by developmental and stress cues often depends on DNA methylation, however, global DNA methylation and genome-wide expression patterns relationship is still poorly understood.

In this work, the dynamics of global DNA methylation patterns in relation to nuclear architecture, and the expression of DNA methyltransferase MET1 have been analyzed during pollen development and pollen reprogramming to embryogenesis in Brassica napus L by a multidisciplinary approach.

METHODS

Quantification of global DNA methylation by high performance capillary electrophoresis and in situ localization of methylated DNA by confocal microscopy analysis of 5-methyl-cytidine (5mC) immunofluorescence were performed on cryoprocessed samples at different developmental stages of in vivo pollen development and in vitro pollen embryogenesis. The spatial and temporal expression patterns of the B. napus DNA methyltransferase BnMET1, one of the enzymes responsible of DNA methylation, were approached by RT-PCR and fluorescence in situ hybridization (FISH) and confocal analysis in the same developmental stages. For comparison, similar assays were also performed on zygotic embryos developed in vivo.

RESULTS AND CONCLUSIONS

Results showed an epigenetic reprogramming regulated by MET1 expression after microspore embryogenesis induction which involved a decrease of global DNA methylation and its nuclear redistribution with the change of developmental program and the activation of cell proliferation, while DNA methylation increases with pollen and embryo differentiation in a cell-type specific manner. BnMET1 expression was regulated during pollen...
reprogramming and embryogenesis, accompanying DNA methylation dynamics. *BnMET1* was up-regulated during pollen differentiation, while after pollen reprogramming, *BnMET1* expression kept similar to the vacuolated microspore and increased at late embryogenesis stages. Zygotic and pollen embryos presented analogous patterns of DNA methylation and *BnMET1* expression, providing new evidences of a similar epigenetic regulation of both embryogenic programs.

ACKNOWLEDGEMENTS

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REFERENCES


Solís MT, Rodríguez-Serrano M, Meijón M, Cañal MJ, Cifuentes A, Risueño MC and Testillano PS (2012) DNA methylation dynamics and *MET1* expression are involved in the stress-induced pollen reprogramming to embryogenesis. *Submitted*
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