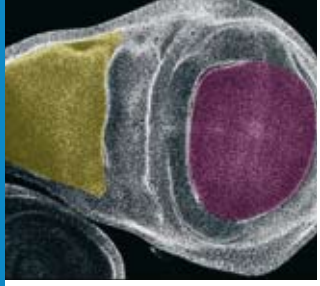

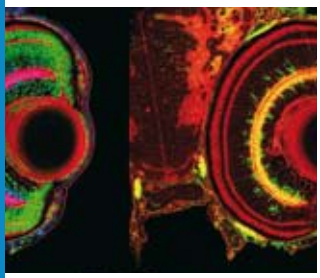

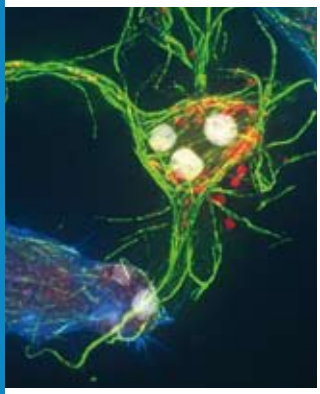

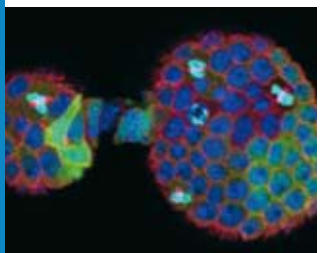

Joint Meeting of the British & Spanish Developmental Biology Societies

24th-27th September 2008
Seville, SPAIN

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Seville, 24-27 September 2008

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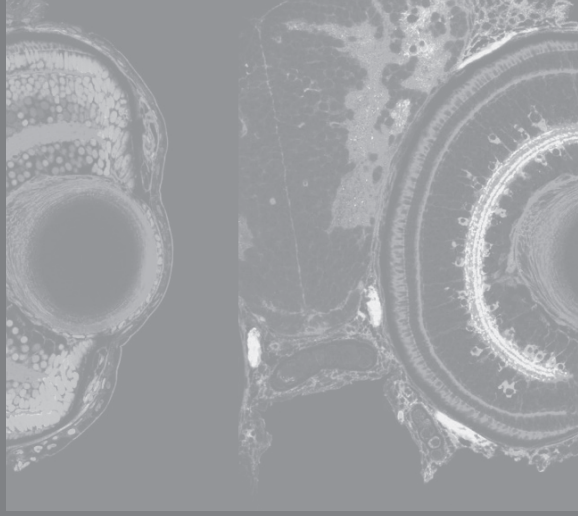

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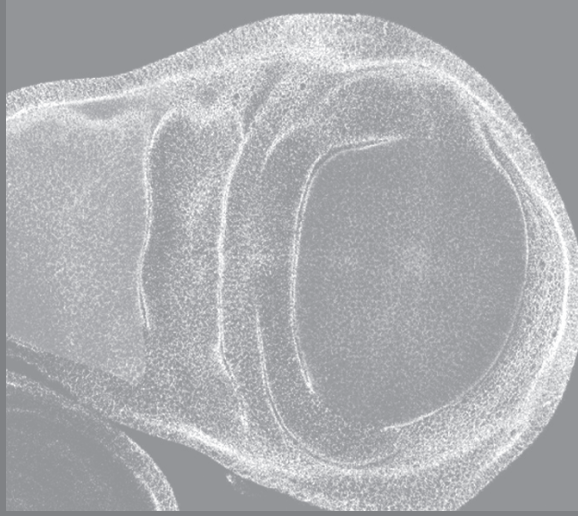

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SOCIAL EVENTS

WELCOME COCKTAIL
Wednesday 24th September – 20:00h
Hotel Silken Al-Andalus ****
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CONFERENCE DINNER (with "Rociero Show")
Friday 26th September – 20:30h (Buses leaving from Hotel at 20:00h)
Real Venta de Antequera
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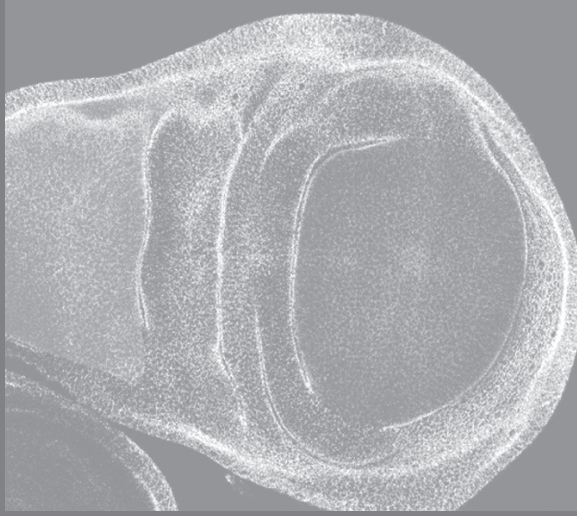


SCIENTIFIC PROGRAM

Scientific Program

Joint Meeting of the British and Spanish Developmental Biology Societies
Seville 24-27 September 2008

Wednesday 24th

16:00-18:00 . **Registration**
19:00 . . . . . Opening Lecture:
Angela Nieto (Inst. de Neurociencias, CSIC/Univ Miguel Hernández Alicante, Spain)
The Snail gene family in embryonic development and adult tissue homeostasis
20:00 . . . . . Welcome Cocktail.

Thursday 25th

9:00 . . . . . **Stem cells sesssion**
Emili Saló (Univ. Barcelona, Spain)
BMP and Wnt pathways control and maintain axial polarity during continuous planarian morphogenesis
Elaine Dzierzak (Erasmus Medical Center, Holland) AstraZeneca Lecture.
Blood stem cells and their niches in the mouse embryo

15 min. **Short talks**
Miguel Manzanares, (CNIC, Madrid, Spain)
Differential requirement for miRNAs in embryonic versus extra-embryonic stem cell populations of the mouse embryo
María José Sánchez (CABD, Seville, Spain)
Hemato-vascular contribution potential of foetal liver cells

Coffee break

11:00 . . . . . **Model systems for human pathologies session**
Cayetano González (IRB, Barcelona, Spain)
New asymmetries in stem cell development and malignant transformation in Drosophila
Alberto Pascual Bravo (Hospital Universitario Virgen del Rocío, Seville, Spain)
The role of GDNF in adult catecholaminergic neuron survival

15 min. **Short talks**
Ramón Muñoz-Chapuli (Univ. of Málaga, Spain)
Cardiac injury induces myocardial marker expression in epicardially-derived cells from cultured mouse embryonic hearts
James W. Bloor (Univ. of Kent, UK)
Modelling integrin-associated cardiomyopathy in the Drosophila larval heart

Lunch

14:30 . . . . . **Functional genomics and evolution session** (Genoma España Session)
Thomas Becker (SARS Centre, Norway)
Gene regulation in megabase domains around developmental control genes
Michalis Averof (IMBB, Greece).
New model systems for comparative developmental studies in arthropods

15 min. **Short talks**
Juan Pablo Couso, (Univ. of Sussex, UK)
Ancestral Notch segmentation in arthropods- Did the segmentation clock trigger the Cambrian explosion?
Lenardo Beccari (Instituto Cajal, Madrid, Spain)
SIX3 expression in the forebrain is directly regulated by SOX2 and PAX6

Coffee break

16:30 . . . . . **Systems biology session** (Genoma España Session)
Ben Lehner (CRG, Barcelona, Spain)
Genetics as a complex system
Sarah A. Teichmann (Medical Research Council, Cambridge, UK)
The evolutionary dynamics of constraints on genes in the human lineage
Jussi Taipale (University of Helsinki, Finland) EMBO YIP Lecture
Transcriptional control of growth

15 min. **Short talks**
Pedro Coutinho, (HGU, Edinburgh, UK)
Deciphering transcriptional networks: The PAX6 paradigm.

Poster session
18:00-19:00 . Odd numbered poster presentations
19:00-20:00 . Even numbered poster presentations

19.30-20.00 **Concurrent talk with poster session:**
David J. Fogarty, (Int. J. Dev. Biol. Managing Editor)
On the conception, gestation and parturition of a scientific manuscript: two protocols for writing better papers

Dinner

Friday 26th

9:00 . . . . . **Cell proliferation and apoptosis session**
Ginés Morata (CBM-SO, CSIC/Univ. Autónoma Madrid, Spain)
Cell competition and tumour progression in Drosophila imaginal discs
Nicolas Tapon (Cancer Research UK, London, UK) EMBO YIP Lecture
Control of Drosophila Src activity in epithelial cell maintenance and proliferation

15 min. **Short talks**
Marian Mellén, (CIB, Madrid, Spain)
The autophagic machinery is necessary for removal of cell corpses from developing retinal neuroepithelium
Ana V. Sánchez- Sánchez (CIPF, Valencia, Spain)
Canonical WNT signalling regulates cell cycle progression of retinal progenitors in medaka

Coffee break

11:00 . . . . . **Organogenesis and morphogenesis session**
Magdalena Zernicka-Goetz (Gurdon Inst., Univ. Cambridge, UK)
Switches between pluripotency and differentiation: formation of the three cell types of the mouse blastocyst
Andrew Fleming (Univ. of Sheffield, UK)
Control of organ size and shape: the role of the epidermis in leaf morphogenesis

15 min. **Short talks**
Florencia Cavodeassi (UCL, London, UK)
Cytoskeletal reorganisation at the edge of the eye field is required for early stages of eye morphogenesis
Noelia Pinal Seoane (LMCB, Cell Biology Unit, MRC, UCL, London, UK)
Moesin acts downstream of Rhodopsin1 in regulating morphogenesis of the Drosophila photoreceptor apical membrane

Lunch

14:30 . . . . . **The polarised cell in development session**
W. James Nelson (Univ. of Stanford, California, USA) Leica Lecture
Regulation of Cell-Cell Adhesion
Damian Brunner (EMBL, Germany)
Changes in Cell Architecture during Drosophila Dorsal Closure

15 min. **Short talks**
Thomas Widmann (MPI-CBG, Dresden, Germany)
Dpp signalling promotes apical-basal elongation through opposing Rho1
Anne Pacquelet (ETH, Zurich, Switzerland)
Regulation of polarity and asymmetric division: PAR-6 levels are regulated by NOS-3 in a CUL2 dependent manner in C. elegans

Coffee break

16:30 . . . . . **Migrating cell and folding tissues session**
Enrique Martín-Blanco (IBMB, CSIC, Barcelona, Spain)
Control of proliferation and invasion during epithelial morphogenesis in Drosophila
Carl Philipp Heisenberg (Max Planck Institute Dresden, Germany)
Adhesive and tensile forces control gastrulation movements in zebrafish

15 min. **Short talks**
Nicole Gorfinkiel, (Univ. of Cambridge, UK)
Mechanical constraints pattern cellular behaviour during dorsal closure in Drosophila
Patricia Ybot González (UCL, London, UK)
Neural plate morphogenesis during mouse neurulation is regulated by antagonism of BMP signalling

Poster session
18:00-19:00 . Odd numbered poster presentations
19:00-20:00 . Even numbered poster presentations

20:00 . . . . . **Gala dinner**

Saturday 27th

9:00 . . . . . **Cell communication session**
Marcos González Gaitán (Univ. of Geneva, Switzerland)
Endosomes and cell division: symmetric and asymmetric
Thierry Lepage (Nice, France)
Dorsal ventral axis formation in the sea urchin embryo

15 min. **Short talks**
Fernando Casares (CABD, Seville, Spain)
SoxF participates in a novel feedback loop in the WNT/WG pathway to regulate tissue growth in Drosophila
Anja Hagemann, (Gurdon Institute, Univ. of Cambridge, UK)
TGFß morphogen movement and signalling in live tissue

Coffee break

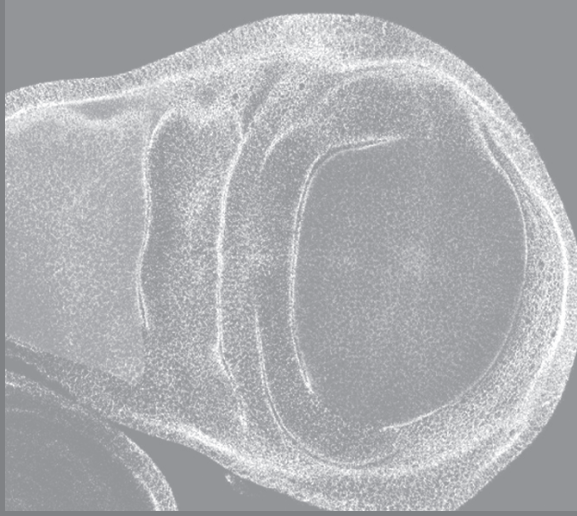
11:00 . . . . . **Architecture of the nervous system session**
Michael Bate (University of Cambridge,UK)
The embryonic origins of coordinated movement in Drosophila
Oscar Marín (Inst. de Neurociencias, CSIC/U. Miguel Hernández, Spain)
Mechanisms of cell migration in the developing cerebral cortex

15 min. **Short talks**
John Chilton, (IBCS, Peninsula Medical School, UK)
Neuronal migration pathways and axonal morphology are controlled by the actin-binding protein Drebin
Marianne Malartre, (CABD, Seville, Spain)
The oncogene and guanine exchange factor vav controls axon guidance during Drosophila development

12:30 . . . . . **The EMBO Lecture**
Christine Holt (University of Cambridge, UK)
RNA-based mechanism of directional steering in growth cones

13:30 . . . . . **Meeting of SEBD**

14:00 . . . . . **Farewell Cocktail**

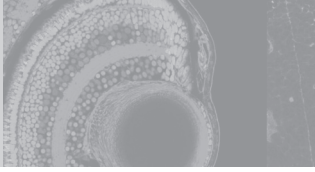


CONFERENCES SHORT TALKS

THE SNAIL GENE FAMILY IN EMBRYONIC DEVELOPMENT AND ADULT TISSUE HOMEOSTASIS

M. Angela Nieto
Instituto de Neurociencias CSIC-UMH. Alicante, Spain

For the last 15 years we have been working on the functional analysis and the evolution of the Snail gene family of zinc-finger transcription factors. The peculiarities of this gene family and its fundamental role in processes that imply profound cell movements have guided us through a fascinating journey. Not only Snail genes are crucial for the formation of many tissues during embryonic development but also their pathological activation in the adult leads to several prominent pathologies. As such, Snail aberrant activation in tumour cells leads to the acquisition of invasive and metastatic properties, while its activation in the adult kidney leads to renal fibrosis. Both are related at the cellular level, since they involve the Snail-mediated induction of the epithelial-to mesenchymal transition (EMT). Effective cell migration is incompatible with high proliferation, and survival is instrumental for both normal embryonic and malignant tumour cells to reach their final destination and form organs or metastasis, respectively. Interestingly, Snail factors not only to regulate epithelial cell adhesion and shape but also attenuate cell proliferation and induce resistance to cell death, increasing the efficiency of cell migration and colonization. Although known to function in epithelial cells, we have recently found that Snail can also act in non-epithelial cells, such as in chondrocytes, where it is unable to induce EMT but still controls cel division. By regulating chondrocyte proliferation, Snail controls the longitudinal growth of the long bones and its deregulated expression leads to achondroplasia, the most common form of dwarfism in humans. In summary, Snail factors play multiple roles in embryonic development through the regulation of different cellular properties including adhesion, proliferation and survival, which can operate simultaneously or separately depending on the cell context. I will discuss our efforts to understand the spectrum of Snail capabilities in different contexts and the rationale behind its integration into developmental programs and the aetiology of adult pathologies.



BMP AND WNT PATHWAYS CONTROL AND MAINTAIN AXIAL POLARITY DURING CONTINUOUS PLANARIAN MORPHOGENESIS

E. Saló[1], M. Iglesias[1], M.D. Molina[1], J.L. Gomez-Skarmeta[2], F. Cebrià[1], T. Adell[1]
[1] Dep. Genetics and Institute of Biomedicine. Univ. Barcelona. Spain
[2] CABD, University Pablo de Olavide-CSIC, Sevilla, Spain.

Freshwater planarians (phylum Platyhelmintes) have the remarkable ability to regenerate a whole animal from a small piece of their bodies. Little is known about the molecular mechanisms responsible for axial re-establishment or maintenance and cell fate determination during regeneration and homeostasis. Such planarian morphogenetic plasticity offers us an ideal model to elucidate this issue. During the last years we studied in planarian to basic signalling pathways, BMP and Wnt. BMP pathway has been shown to play an important role in the establishment of the dorso-ventral axis during development in both vertebrate and invertebrate organisms. We have functionally characterized several homologues of the BMP pathway in the planarian Schmidtea mediterranea. RNA interference (RNAi) knockdowns of Smed-BMP or Smed-Smad1 lead to a partial ventralization of the dorsal side of the animal, which in most cases results in the duplication of the nervous system. These defects are observed not only during regeneration but also in intact non-regenerating animals, suggesting that BMP pathway is a key element in both regeneration and maintenance of the dorso-ventral pattern (Dev. Biol. 311, 79-94). The Wnt/β-catenin signalling pathway is an evolutionary conserved mechanism to confer polarity to the embryo: it specifies the main axis in cnidarians and echinoderms, and the antero-posterior (A-P) axis in most bilaterians. In classical models for regeneration studies, as fish and amphibians, the Wnt/β-catenin signalling pathway is required for regenerative outgrowth but has no reported function in axis establishment. Here we show that β-catenin is required for A-P axis re-establishment and maintenance during regeneration and homeostasis in planarians. Loss of function of planarian β-catenin (Smed-βcat1) in regenerating and in intact planarians abolishes the A-P axis, inducing their gradual transformation to radial-like fully-cephalized animals ('medusa-like' planarians). Furthermore, the impressive plasticity of planarians provided the chance to report the unique example of complete and specific abolishment of one body axis, whereas its loss of function in other models leaded to too complex and severe defects. Strikingly the inhibition of only one gene results in a radical transformation of the body symmetry, from bilateral to radial-like. To our knowledge, 'medusa-like' phenotype in planarians is a unique example of a radial-like symmetry standing on its D-V axis (Development: 135, 1215-1221).

POSITIONAL INFORMATION IN THE DEVELOPMENT OF ADULT HEMATOPOIETIC STEM CELLS

Elaine Dzierzak, Tomomasa Yokomizo, Marian Peeters, Karine Bollerot, Francesco Cerisoli, and Catherine Robin
Dept. Cell Biology and Genetics, Erasmus U. Medical Centre, Rotterdam

The aorta-gonad-mesonephros (AGM) region generates the first hematopoietic stem cell (HSC) at midgestation in the mouse embryo and the study of the microenvironment surrounding this highly specialized embryonic region should provide insight into the cells and the molecules that affect the induction of HSCs. As compared to other embryonic hematopoietic tissues (yolk sac and placenta), the AGM region has a well-defined morphologic structure consisting of the medially positioned dorsal aorta and laterally positioned urogenital ridges. Tissues located dorsally to the AGM include the neural tube and notochord. Somites are positioned dorso-laterally, and endodermal tissues such as the gut are positioned ventrally. The temporal appearance of the first HSCs in the midgestation AGM is coincident with appearance of hematopoietic clusters closely associated with the ventral endothelium of the aorta. These clusters and endothelium express Ly-6A GFP and Runx1 and have been shown to possess adult hematopoietic repopulating activity. In contrast, clusters found on the dorsal aortic wall in the mouse midgestation aorta do not contain HSCs (Taoudi et al 2007). Our whole tissue imaging data have mapped and quantitated aortic clusters in normal and hematopoietic transcription factor deficient mouse embryos. To examine what molecules and signalling pathways induce the development of hematopoietic clusters and whether the induction of HSCs in the ventral aorta is dependent upon a specialized ventral microenvironment, we have performed explant cultures with tissues dorsal and ventral to the AGM. These tissues differentially affect the growth of AGM HSCs. Hedgehog and BMP4 have been identified as two of the factors that are involved in the enhancement of AGM HSC induction/growth. These data suggesting that all hematopoietic clusters in the embryonic vasculature are not alike, will be discussed.

DIFFERENTIAL REQUIREMENT FOR MIRNAS IN EMBRYONIC VERSUS EXTRA-EMBRYONIC STEM CELL POPULATIONS OF THE MOUSE EMBRYO

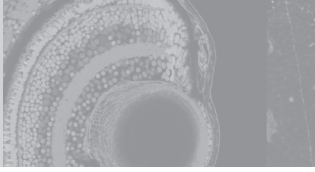
Manzanares, Miguel / Pernaute B / Spruce T / Di Gregorio A / Manzanares M / Rodríguez T
Fundacion CNIC, Cardiovascular Developmental Biology

The two first cell fate decisions in the mammalian embryo generate three distinct stem cell populations, the embryonic epiblast and the extra-embryonic trophoblast and primitive endoderm. It is not known if the same gene regulation mechanisms are utilised in embryonic and extra-embryonic stem cell populations to control gene expression. Micro RNAs (miRNAs) have emerged over the last decade as critical post-transcriptional regulators of gene expression in both development and disease. We have analysed their function in embryonic versus extra-embryonic stem cell lines by studying a null mutation in Dicer, a protein essential for the processing of mature miRNAs. We find that both in vivo and in vitro miRNAs have very different roles in embryonic and extra-embryonic stem cells. In the pluripotent epiblast miRNAs are required to inhibit apoptosis but play little role in the initiation of patterning of the three germ layers. In contrast to this, we find that in the multi-potent extra-embryonic trophoblast stem cells and primitive endoderm stem cells, miRNAs are essential to inhibit differentiation. Furthermore we observe critical differences in the repertoire of miRNAs expressed in these embryonic versus extra-embryonic stem cell populations. Together, these data argue that fundamental differences exist regarding how stem cells maintain their developmental potential in embryonic and extra-embryonic tissues.

HEMATO-VASCULAR CONTRIBUTION POTENTIAL OF FOETAL LIVER CELLS

Sanchez, María José / García A.M. / Quintero C. / Roldan E. / Cañete A.
CABD, Seville, Spain

Foetal and adult haematopoietic progenitor cells have differential phenotypical and functional properties. Most comparative studies have been focused on haematopoietic contribution capabilities but less is known about vascular potential of foetal hematopoietic cells, a critical aspect considering that vascular endothelium can be derived from adult bone marrow cells. Here we performed repopulation assays with E12 foetal liver cells transferred into the blood stream of busulfan-treated newborn recipient mice. High-level haematopoietic chimeras were selected and vascular contribution analyzed in kidney, liver and heart of recipient mice. For vascular/haematopoietic cell lineage tracing we used the Stem Cell Leukaemia gene (SCL) enhancer (SCL3'Enh-PLAP), active in endothelium and blood progenitors cells. The data showed that foetal liver chimeras presented a distinct pattern of SCL3'EnhPLAP+ donor cells distributed into extended vascular-like patches, not present in bone marrow chimeras. Multi-colour confocal analysis demonstrated that the SCL3'EnhPLAP+ patches are composed of CD31+CD45- endothelial cells and to a less extent of CD45+ haematopoietic cells. Also there is a population of PLAP+CD45-CD31- associated mostly to the kidney. Further data will be presented on the characterization of FL-derived SCL3'EnhPLAP+ cell homed into the vascular niches. Supported by the Spanish MEC Grant SAF64679 and SAF03448/ Junta de Andalucia PAI-CVI 295 supporting grant



NEW ASYMMETRIES IN STEM CELL DEVELOPMENT AND MALIGNANT TRANSFORMATION IN DROSOPHILA.

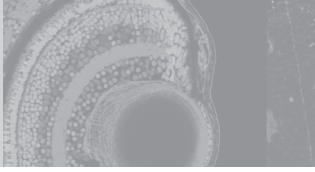
Cayetano González.
IRB-Barcelona. Spain.

In dividing Drosophila larval neural precursors -the stem cells that originate the fly's CNS-cell fate determinants are unequally segregated between the two resulting daughter cells, thus priming one of them to differentiate and contribute tissue mass, while the other retains stem-cell identity and can go through further rounds of asymmetric mitosis. Loss-of-function of any of several genes that control the asymmetric division of these stem cells results in the growth of malignant neuroblastomas. We are carrying out a number of genetic screens to identify new elements of the molecular machinery that prevents tumour formation in these cells. I will present and discuss some of the results that we are obtaining, particularly those regarding the hypothetic role of abnormal centrosome function and genome instability in tumourigenesis in this model system.

THE ROLE OF GDNF IN ADULT CATECHOLAMINERGIC NEURON SURVIVAL

Alberto Pascual Bravo
Hospital Universitario Virgen del Rocío, Seville, Spain

GDNF is a potent neurotrophic factor that protects catecholaminergic neurons from toxic damage and induces fiber outgrowth. However, the actual role of endogenous GDNF in the normal adult brain is unknown, despite GDNF-based therapies are considered promising for neurodegenerative disorders. We have generated a conditional GDNF-null mouse to suppress GDNF expression in adulthood, hence avoiding the developmental compensatory modifications masking its true physiologic action. After GDNF ablation animals showed a progressive hypokinesia and a selective decrease of brain tyrosine hydroxylase (TH) mRNA, accompanied of pronounced catecholaminergic cell death, affecting most notoriously the locus coeruleus (LC), which practically disappears, the substantia nigra (SN) and the ventral tegmental area (VTA). These data unequivocally demonstrate that GDNF is indispensable for adult catecholaminergic neuron survival, and also show that in physiologic conditions down-regulation of a single trophic factor can produce massive neuronal death.



CARDIAC INJURY INDUCES MYOCARDIAL MARKER EXPRESSION IN EPICARDIALLY-DERIVED CELLS FROM CULTURED MOUSE EMBRYONIC HEARTS

Muñoz-Chapuli, Ramon / Guadix JA / González Rosa JM / Perez-Pomares JM
University of Málaga, Department of Animal Biology

Epicardial progenitor (proepicardial) cells contribute to coronary blood vessel development. The avian proepicardium also has the potential to differentiate into myocardium in vitro but not in vivo. Moreover, embryonic Tbx-18-positive cells, supposedly derived from the proepicardium, contribute to adult myocardium in mice. However, no myocardial differentiation from epicardial or epicardially-derived cells (EPDCs) occurs neither in avians nor in mouse embryos after E11.5. This suggests the existence of some kind of “molecular lock” preventing myocardial differentiation in EPDC, although the epicardium seems to play an active role in the regeneration of the fish heart3. We have tested whether EPDCs can express myocardial markers after ventricular injury. Mice with either beta-galactosidase or GFP expression under control of the Wilms’ tumor gene promoter (only expressed by the epicardium and EPDCs in the heart) were used to test EPDCs differentiation. E12.5 and E14.5 hearts were sectioned or cryocauterized, and cultured (24 h). E12.5 hearts showed a blastema-like clump of cells co-expressing reporter genes and cardiac troponin, tropomyosin and sarcomeric myosin. A weaker response was found in E14.5 embryos. These results suggest that the myocardial potential of the epicardial lineage can be recovered after an extensive damage, opening the opportunity to identify the factor(s) that induce this differentiation.

MODELLING INTEGRIN-ASSOCIATED CARDIOMYOPATHY IN THE DROSOPHILA LARVAL HEART

Bloor, James / Ma L / Bradu A / Podoleanu A / Bloor JW
University of Kent

Integrins are associated with a number of cardiac diseases in both human patients and vertebrate models, including cardiac hypertrophy, ischemic cardiomyopathy and dilated cardiomyopathy. Here we exploit the Drosophila larval heart as a system in which to explore integrin cardiac function. The larval heart is composed of two parts, an anterior tubular aorta and a posterior cardiac region that is thicker, contractile, and which contains valves that allow directional flow of hemolymph through the organ. To explore the role of integrins and integrin-related genes in cardiac function we combine heart-specific RNAi expression with a novel label-less imaging technology called Optical Coherence Tomography (OCT) that provides direct imaging of the heart in wild type and knockdown larvae. To date we have examined the role of the PS2 integrin in larval cardiac function. RNAi constructs targetted to myospheroid or inflated (corresponding to betaPS and alphaPS2 integrin subunits) cause a similar dilated heart defect associated with a reduction in cardiac output. Moreover we have developed our OCT system to produce a “stethoscope” that can monitor both heart rate and the speed at which the heart wall contracts. Both are reduced in integrin knockdown hearts. We are currently extending this work by 1) examining the cell biology underlying the dilated heart defect and 2) determining which downstream signalling pathways are involved.

SYSTEMATIC HUMAN/ZEBRAFISH COMPARATIVE IDENTIFICATION OF CIS-REGULATORY ACTIVITY AROUND VERTEBRATE DEVELOPMENTAL TRANSCRIPTION FACTOR GENES

Thomas S Becker [1], Pavla Navratilova, David Fredman, Thomas A. Hawkins , Katherine Turner, Boris Lenhard
[1] Sars Centre for Marine Molecular Biology, Univ. of Bergen, Norway

Pan-vertebrate developmental cis-regulatory elements are discernible as highly conserved noncoding elements (HCNEs) and are often dispersed over large areas around the pleiotropic genes whose expression they control. On the loci of two developmental transcription factor genes, SOX3 and PAX,6 we demonstrate that HCNEs conserved between human and zebrafish can be systematically and reliably tested for their regulatory function in multiple stable transgenes in zebrafish, and their genomic reach estimated with confidence using synteny conservation and HCNE density along these loci. HCNEs of both human and zebrafish function as specific developmental enhancers in zebrafish. We show that human HCNEs result in expression patterns in zebrafish equivalent to those in mouse, establishing zebrafish as a suitable model for large-scale testing of human developmental enhancers. Orthologous human and zebrafish enhancers underwent functional evolution within their sequence and often directed related but non-identical expression patterns. Despite an evolutionary distance of 450 million years, one pax6 HCNE drove expression in identical areas when comparing zebrafish vs. human HCNEs. HCNEs from the same area often drive overlapping patterns, suggesting that multiple regulatory inputs are required to achieve robust and precise complex expression patterns exhibited by developmental genes.

ESTABLISHING A CRUSTACEAN MODEL FOR COMPARATIVE DEVELOPMENTAL STUDIES: TRANSGENESIS, GENE TRAPPING AND INDUCIBLE MIS-EXPRESSION OF HX GENES IN PARHYALE HAWAIENSIS

Michalis Averof, Anastasios Pavlopoulos, Zacharias Kontarakis, Alexandros Kiupakis, Vassilis Douris, Nikos Konstantinidis
IMBB, Greece

Genetic approaches available in a small number of model organisms have been crucial for elucidating fundamental mechanisms that control development in all animals. We need to extend these approaches to new species, to explore the changes that make different animals look and behave different. The crustacean Parhyale hawaiensis is emerging as an attractive model for comparative developmental studies; lineage tracing, experimental embryology, gene knockdown and transgenesis approaches are already established in this species. I will present our efforts to develop tools for genetic analysis in Parhyale, focusing on a case study, the mis-expression of the Hox gene Ultrabithorax (Ubx).

ANCESTRAL NOTCH SEGMENTATION IN ARTHROPODS DID THE SEGMENTATION CLOCK TRIGGER THE CAMBRIAN EXPLOSION?

Couso, Juan Pablo / Pueyo J.I. / Lanfear R.
University of Sussex, School of Life Sciences

Data on the molecular and genetic basis of animal development, and on genome sequences have been challenging our established assumptions about animal evolution for the last decade. The evolution of segmentation is a prime concern, in particular whether segmentation and metameric bodies have arisen just once or several times in evolution. Segmentation and metamerism are striking developmental and body organisations that exist, in varying degrees, in many complex animals but the traditional view holds that this is the result of convergent evolution. However, I will present data from basal insects supporting the control of their segmentation by Notch signalling, very much like vertebrates. These data beg us to take another look at whether similarities in developmental and genetic segmentation mechanisms in current animals are the product of a common inheritance (homology) or convergent evolution (analogy). I will review paleontological and developmental information in support of the hypothesis that a metameric body plan is not only a likely ancestral character of bilaterian animals, but also a possible trigger for the Cambrian explosion in body morphology and complexity. This hypothesis is supported by the phylogenetic distribution and prevalence of metameric phyla in the Cambrian, and the similarity of the genomes and segmentation mechanisms across current bilaterian phyla.

SIX3 EXPRESSION IN THE FOREBRAIN IS DIRECTLY REGULATED BY SOX2 AND PAX6

Beccari, Leonardo / Cisneros E / Conte I / Bovolenta P
Instituto Cajal, CSIC - Dept. Neurobiología del Desarrollo

Vertebrate forebrain derivatives are specified at early stages of gastrulation in the anterior neural plate by the overlapping expression of several transcription factors including Six3/6, two members of the Six/sine oculis family of homeobox containing transcription factors. Due to genome duplication, in the teleost medaka fish (Oryzia latipes) there are two copies of the Six3 gene: olSix3.1 and olSix3.2. Sequence comparison and expression analysis demonstrated that the latter is most closely related to the mammalian Six3 gene. To begin to understand the molecular network of vertebrate forebrain specification, we took advantage of the particular compact genome of the medaka fish and identified a cluster of highly conserved non-coding sequences surrounding the olSix3.2 gene. By transgenesis analysis we demonstrated that these sequences have enhancer, silencer and silencer blocker activities, which are differentially combined to control the entire distribution of olSix3.2 (Conte and Bovolenta, Genome Biol. 2007;8(7):R137). Here, we will present an in silico analysis, which predicts the presence of several putative binding sites for known transcription factors. On the basis of expression analysis, luciferase reporter and chromatin-protein interaction assays, we will also show that Sox2 and Pax6 are direct regulators of the early and late expression of olSix.2 in the forebrain. In vivo validation of these results is ongoing.

THE TISSUE-SPECIFICITY AND DOSAGE-SENSITIVITY OF INTERACTION NETWORKS

Ben Lehner
EMBL-CRG Systems Biology Unit, CRG, Barcelona

We use both experimental and computational approaches to understand the global and quantitative behaviour of genetic systems. Here I will discuss two problems that we have been addressing recently. The first is to understand the tissue-specificity of protein interaction networks. The second is to understand the mechanisms responsible for phenotypic change in response to the overexpression of a gene, and to understand why biological systems function robustly to the overexpression of many, but not all genes. I will present our conclusions from this work.

GENOME-WIDE TRANSCRIPTION FACTOR REPERTOIRES

Sarah A. Teichmann
MRC Laboratory of Molecular Biology, Cambridge, UK.

Regulation of gene expression involves a complex combination of several mechanisms. A key component of the system is regulation by DNA-binding transcription factors. This class of proteins recognizes specific DNA sequences and either activates or inhibits transcription initiation of target genes. Many cellular processes, including development and differentiation, are controlled in this way. Therefore, detailed knowledge of transcription factors and their expression patterns is important for understanding the biology of multi-cellularity.

For most genomes, the repertoire of transcription factors is only partially known. Hitherto transcription factor annotation has been largely based on genome annotation pipelines that use pairwise sequence comparisons, which detect only those factors similar to known genes, or on functional classification schemes that amalgamate many types of proteins into the category of 'transcription factor'. To fill this void, we have developed a novel transcription factor identification method, providing genome-wide transcription factor predictions for organisms from across the tree of life, available at www.transcriptionfactor.org. For the model organism D. melanogaster, we have expanded our automatic annotation pipeline to include extensive manual curation in the FlyTF database www.flytf.org.

We have investigated the evolution of transcription factors in both prokaryotes and eukaryotes. A general trend emerges across all different groups of organisms, showing that transcription factors, as well as other classes of regulatory molecules evolve more quickly than genes in other functional categories, such as enzymes for instance. This suggests that transcription factors are 'evolvable' in the sense that duplications and losses of transcription factors are tolerated more easily than for core functional classes. The changes in transcription factor repertoires are likely to play a large part in evolution of development and complexity. At the same time, there is a large range of conservation amongst transcription factors, with some conserved across all animals for instance, and others being primate-specific.

By integrating annotated transcription factors with expression data, we have started to gain insight into the dynamics of transcription factor expression under different cellular conditions in a unicellular organism (Luscombe et al,. 2004) and in different developmental stages and tissues in a multi-cellular organism. Both analyses reveal the importance of combinatorial action of transcription factors to determine the state of a cell, and the role of ubiquitous transcription factor hubs.

TRANSCRIPTIONAL NETWORKS CONTROLLING CELL GROWTH

Jussi Taipale
University of Helsinki and National Public Health Institute, Finland.

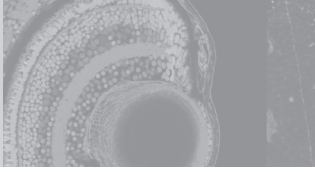
Organ specific growth control remains one of the major questions in developmental biology that has not been resolved; it is not understood what determines organ size and shape (reviewed in Lecuit and Le Goff, Nature 450:189, 2007). It is also not clear why tumors arising in different tissues harbor different oncogenic mutations (Taipale and Beachy, Nature 411:349, 2001). Much of what we know about physiological mechanisms controlling cellular growth in mammals has been revealed by human cancer genetics. These studies have revealed that a large number of genes can contribute to aberrant cell growth; there are more than 300 genes that have been linked to cancer, and mutations found in cancer are often cell type specific. For example, PTCH mutations are common in medulloblastoma, APC in colon cancer, and TMPRSS2-ERG in prostate cancer, suggesting that different pathways in different cell lineages are coupled to the cell cycle machinery. Our hypothesis is that the problems of organ-specific growth control and specificity of oncogenes to particular tumors represent two sides of the same coin; that is, mutations in tumors are tissue specific, because tumors arise by the most economical mutagenic route, aberrantly activating the organ-specific growth mechanisms.

To test this hypothesis, we have developed computational and experimental methods to identify direct target genes of oncogenic transcription factors, and used high-throughput RNAi screening to identify genes required for cell cycle progression. Combining these two sets of data allows identification of specific regulatory elements which drive growth in particular tissues and tumor types. Preliminary evidence suggests that Hedgehog (Hh) and Wnt signals appear to be directly coupled to expression of N-myc and c-Myc genes, but only in tissues and cell-types that display a proliferative response to these factors.

DECIPHERING TRANSCRIPTION NETWORKS: THE PAX6 PARADIGM.

Coutinho, Pedro / Kleinjan DA / Van Heyningen V
HGU-MRC, Medical and Developmental Genetics

The pax6 gene has been defined as a selector gene, responsible for the determination of multiple cell fates in different developing tissues: the eye, central nervous system and pancreas. This multi-tasking is achieved by complex spatiotemporal and quantitative regulation of pax6 expression at the transcriptional level. Pax6 transcripts encode proteins that are highly conserved across evolution. Furthermore, Pax6 cis-regulatory sequences are also well conserved across vertebrates. Using comparative genomics, we are investigating transcriptional regulation by pax6. We have developed a bioinformatic procedure to identify genes that are transcriptionally regulated by specific transcription factors (TFs). This procedure makes use of combined data and tools, such as bibliographic knowledge of transcription factor binding site (TFBS), genome sequences and probabilistic models to predict evolutionarily conserved sequences (ECRs) and the target genes under direct regulation. We have identified more than one hundred vertebrate genes that are putative pax6 targets and some have been validated experimentally in zebrafish, using methodologies such as morpholino induced knock downs, in situ hybridization and ChIP. This set of loci is highly enriched for genes that encode proteins with DNA-binding or transcription factor activity, reinforcing the idea of Pax6 as a major transcriptional modulator.



CELL COMPETITION AND TUMOUR PROGRESSION IN THE IMAGINAL DISCS OF DROSOPHILA

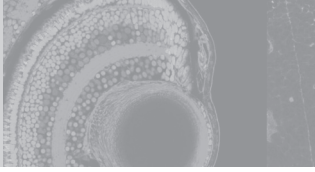
Ginés Morata
Centro de Biología Molecular Severo Ochoa, CSIC-UAM
Universidad Autónoma de Madrid, Spain.

Cell competition was reported over 30 years ago in the imaginal discs of Drosophila to describe the elimination of slow-dividing but otherwise viable cells when surrounded by more rapidly proliferating cells. Later work showed that the elimination of out-competed cells is achieved by apoptosis mediated by the activity of the JNK pathway. We are testing the role of cell competition during the process of tumour progression in the imaginal discs of Drosophila. Larvae homozygous for mutations at the tumour suppressor genes lethal giant larvae (lgl), scribble (scribb) or disc large (dlg) develop extensive neoplastic tumours in the central nervous system and the imaginal discs. However, clones of cells mutant for these genes do not form a tumour if surrounded by non-mutant cells. We are studying the behaviour of lgl mutant clones in a normal wing disc and find that they are eliminated by a process akin to cell competition: they enter apoptosis mediated by the JNK pathway and the interactions leading to the disappearance of the mutant cells take place at the border of the clones, where mutant and non-mutant cells are in close proximity. We have also found that when mutant lgl cells contain an additional factor conferring a high proliferation rate, they are transformed into “supercompetitors” cells, which are able to eliminate surrounding non-tumour cells and give rise to invasive neoplastic tumours that colonise the entire disc. Based on these observations we propose that cell competition is a key phenomenon during tumour progression.

CONTROL OF DROSOPHILA SRC ACTIVITY IN EPITHELIAL CELL MAINTENANCE AND PROLIFERATION.

Nicolas Tapon
Cancer Research UK, London Research Institute

Src-family kinases (SFKs) control a variety of biological processes, from cell proliferation and differentiation to cytoskeletal rearrangements. Abnormal activation of SFKs has been implicated in a wide variety of cancers and is associated with metastatic behavior. SFKs are maintained in an inactive state by inhibitory phosphorylation of their C-terminus by Carboxy-terminal Src kinase (Csk). We have identified Drosophila Ankyrin-repeat, SH3-domain and Proline-rich-region containing Protein (dASPP) as a new regulator of Drosophila Csk (dCsk) activity. dASPP is the homolog of the mammalian ASPP proteins, which are known to bind to and stimulate the pro-apoptotic function of p53. We have shown that dASPP is a positive regulator of dCsk. Firstly, dASPP loss-of-function causes overgrowth and strongly enhances the specific phenotypes of dCsk mutants in wing epithelial cells. Secondly, dASPP interacts physically with dCsk to potentiate the inhibitory phosphorylation of dSrc. Our results suggest a new role for dASPP in maintaining epithelial integrity through dCsk regulation. We have also identified Boa as a direct dASPP interactor. boa mutants have a similar overgrowth phenotype to dASPP mutants and Boa is required for dASPP localization in the apical domain of imaginal disc cells. We are currently dissecting the role of Boa and dASPP in dCsk activation and epithelial integrity.



THE AUTOPHAGIC MACHINERY IS NECESSARY FOR REMOVAL OF CELL CORPSES FROM DEVELOPING RETINAL NEUROEPITHELIUM

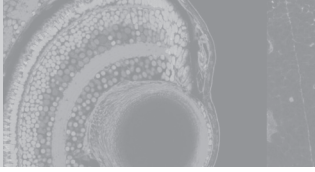
Mellén, Marian / De La Rosa EJ / Boya P
CIB-CSIC, Molecular and Cellular Physiophatology

Disruption of autophagic machinery interferes with embryonic development in several species, although the underlying cellular processes affected remain unclear. Here, we investigate the role of autophagy during the early stages of chick retina development, when the retinal neuroepithelium proliferates and starts to generate the first neurons, the retinal ganglion cells. These two developmental processes are accompanied by programmed cell death. Upon treatment with the autophagic inhibitor 3-methyladenine retinas accumulated numerous TUNEL-positive cells that correlated with a lack of the “eat-me” signal phosphatidyl-serine. In consequence, neighbouring cells did not engulf apoptotic bodies and they persisted as individual cell corpses, a phenotype that was also observed after blockade of phagocytosis with phospho-L-serine. Supplying the retinas with methyl-pyruvate, a cell permeable substrate for ATP production, restored ATP levels and the presentation of phosphatidyl-serine at the cell surface. Hence, engulfment and lysosomal degradation of apoptotic bodies was also re-established. These data point to a novel role for the autophagic machinery during the development of the central nervous system.

CANONICAL WNT SIGNALING REGULATES CELL CYCLE PROGRESSION OF RETINAL PROGENITORS IN MEDAKA.

Sánchez Sánchez, Ana Virginia / Leal Tassias, A / Mullor Sanjosé, JL
Dep. of Veterinary Basic Sciences, Royal Veterinary College

A functionally mature eye requires that there is a precise coordination of retinal progenitor cell proliferation and differentiation after cell cycle exit. One of the signaling pathways that plays important roles during eye development is the Wnt pathway. However, little is know about how this signaling pathway works during retinal differentiation, although many components of the pathway are expressed at various stages in the developing vertebrate eye. In addition, recent studies show that Wnt pathway takes part in regulating cell proliferation, differentiation and polarity in the eye (1). To determine the exact role of Wnt pathway during vertebrate eye formation, we have used different pharmacological modulators and two medaka transgenic lines that contain a heat-shock (HS) inducible promoter with either the Wnt8 gene (HS-Wnt8) or a dominant-negative form of this gene (HS-dnWnt8). The HS promoter allowed us to modulate canonical Wnt signaling with precise temporal control. In our study we provide evidence that canonical Wnt pathway may have different roles on proliferation and differentiation depending on the developmental stage of the retinal cell precursors. Our results suggest that Wnt signaling acts on cell cycle regulation of retinal progenitors during the early stages of proliferation and differentiation. After the first wave of differentiation, Wnt signaling regulates only differentiation, but not proliferation. 1. de Iongh RU, Abud HE, Hime GR. 2006. Front Biosci.



SWITCHES BETWEEN PLURIPOTENCY AND DIFFERENTIATION: FORMATION OF THE THREE CELL TYPES OF THE MOUSE BLASTOCYST

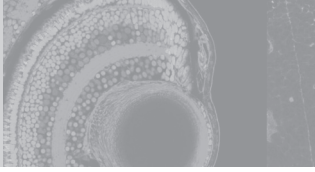
Magdalena Zernicka-Goetz
Gurdon Inst., Univ. Cambridge, UK

We are interested in the molecular mechanisms of the switches that direct cells from pluripotency to differentiation in the developing mammalian embryo. The first cell fate decision serves to set apart pluripotent inner cell mass (ICM) cells from outside cells that differentiate into the extra-embryonic trophectoderm. There have been two alternative hypotheses to explain how this cell fate decision might be achieved: one stressed the importance of cell position ("inside-outside") and the other cell polarisation and the asymmetric divisions that make cells different even before they reach different, inside versus outside, positions. We found that these hypotheses are not mutually exclusive and show how both cell position and polarisation together regulate the expression of cell fate-determining genes. The commitment of outside cells to trophectoderm is mediated at the blastocyst stage by Cdx2. We find that Cdx2 can also act earlier to influence cell polarisation and the pattern of symmetric versus asymmetric divisions that allocate cells to either trophectoderm or ICM. This early expression of Cdx2 commences at the 8-cell-stage and is heterogeneous between blastomeres. Natural, or experimentally induced, high levels of Cdx2 leads to a higher frequency of symmetric divisions and, consequently, allocation of more cells to trophectoderm. Conversely, reduced Cdx2 expression leads to an increase in asymmetric divisions and contribution to the ICM. We find that the level of Cdx2 expression affects the extent of cell polarisation, but cell polarity in turn leads Cdx2 transcripts to become asymmetrically distributed in blastomeres. Thus the asymmetric divisions that generate inside and outside cells are truly asymmetric in terms of differentially distributing fate instructions between daughters. Such a feedback loop between cell polarisation and gene expression ensures the switch from pluripotency to differentiation is robust. Finally we find that the heterogeneity of Cdx2 expression between blastomeres does not arise at random, but depends on cell history and specifically upon how the contents of the zygote becomes partitioned between the cells. Underlying this are differential levels of specific epigenetic modifications between 4-cell blastomeres instrumental in assigning different developmental potential to these cells: those with higher levels of methylated arginines in histone H3 being most pluripotent. Together our results indicate that the history of cells affects their later interactive capacity. Such bias in development would maximise chances of developmental success while retaining developmental plasticity.

ORGAN MORPHOGENESIS: A COMBINED COMPUTATIONAL AND MOLECULAR ANALYSIS IN ARABIDOPSIS

Andrew Fleming
Univ. Sheffield, UK

Morphogenesis in the plant leaf involves the co-ordination of cell division and growth, yet the mechanism underpinning this co-ordination remains unknown. Understanding this mechanism would provide the link from the identified transcriptional blueprints that define organ size and shape to the final output that is morphology. Using a combination of imaging and computational techniques, we have generated models linking parameters of cell division and growth with specific facets of organ morphogenesis. These models are now being tested using an array of molecular tools. Our results highlight the importance of specific cell types as developmental boundaries (in our case the leaf perimeter) and provide an insight into a novel potential mechanism by which growth and polarity could be co-ordinated in a growing multicellular system.



CYTOSKELETAL REORGANISATION AT THE EDGE OF THE EYE FIELD IS REQUIRED FOR EARLY STAGES OF EYE MORPHOGENESIS

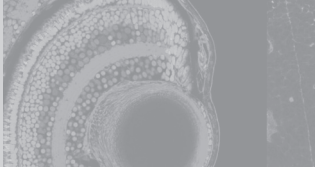
Cavodeassi, Florencia / Ivanovitch K / Wilson SW
Anatomy and Developmental Biology UCL, London, UK

The vertebrate optic vesicles are formed as evaginations of the forebrain, but prior to this, the group of cells that will give rise to the eyes exist as a single bilateral domain called the eye field. Soon after the specification of the eye field, extensive morphogenetic movements split this domain in two, one on each side of the midline. Each of these domains will give rise to one optic vesicle. Optic vesicle evagination is a very dynamic process involving a combination of processes, from passive movements induced by the morphogenesis of the surrounding tissues to coordinated migration by cells within the eye field. However, the molecular mechanisms underlying these processes are still unclear. Here we make use of the advantages of the zebrafish model system to analyse cell behaviour and organisation during early stages of eye morphogenesis. We will show that at the onset of optic vesicle evagination, the cells at the edge of the eye field organise an actomyosin cable that seems to be essential for their coordinated evagination. We will discuss the significance of this requirement and our ideas on the signals that might be involved in regulating actomyosin activity during this process.

MOESIN ACTS DOWNSTREAM OF RHODOPSIN1 IN REGULATING MORPHOGENESIS OF THE DROSOPHILA PHOTORECEPTOR APICAL MEMBRANE.

Pinal Seoane, Noelia / Pichaud FP
LMCB, UCL, London, UK

G-protein coupled receptors (GPCRs), such as the sensory visual pigment rhodopsin1 (Rh1) form a large conserved family of transmembrane receptors. In the fly eye, Rh1 is expressed in the outer photoreceptors (R1 to R6). Rh1 is involved in phototransduction in these cells, but is also required in late pupation for the morphogenesis and maintenance of the apical organelle, the rhabdomere, that consists of a stack of microvilli. In addition, the Rho-GTPases Rac and Cdc42 have been shown to act downstream of Rh1 in apical organelle morphogenesis (Chang and Ready, Science 2000). However, besides the Rho-GTPases, the nature of the pathway involved in this process is not clear. Here we present evidence indicating that rh1 function during apical organelle morphogenesis is largely conserved through evolution and is independent of Gαq. Importantly, we show that rh1 function relies on the activity of the ERM protein Moesin, a factor involved in linking the F-actin cytoskeleton to the plasma membrane. Our work highlights a novel aspect of sensory pigment function that is independent of the phototransduction cascade and is required for sensory neuron morphogenesis.



CYTOMECHANICS OF CELL-CELL ADHESION

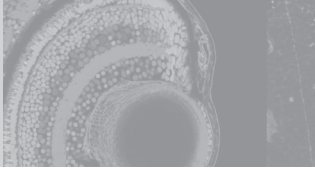
W. James Nelson
Stanford University, Stanford CA, USA

Cell-cell recognition and adhesion is mediated by the cadherin family of Ca++-dependent cell adhesion proteins. Cadherins bind to a number of cytoplasmic proteins including members of the catenin family, b-catenin and a-catenin. While it had been shown that cadherin, b-catenin and a-catenin form a complex, and that a-catenin is an actin bundling protein, the evidence that the cadherin/catenin complex bound to the actin cytoskeleton was weak and indirect. Direct studies testing this possibility with purified proteins revealed, however, that the cadherin/catenin complex does not bind actin filaments in vitro (Cell, 123: 889; ibid 123: 903, 2005); a potential molecular explanation of the role of a-catenin was revealed by the finding that a-catenin exists as a monomer which binds β-catenin, and a dimer that does not bind β-catenin but binds more strongly to actin filaments and can inhibit Arp2/3-mediated actin polymerization. Based on these in vitro studies, it was proposed that a-catenin locally regulates actin organization and dynamics in the cytoplasm and thereby membrane dynamics to enable maintenance of weak initial cell-cell contacts formed by cadherins. I will describe recent results examining the regulation of actin cytoskeleton during cell-cell adhesion, and the role of the Rho family of small GTPases in the spatial control of actin dynamics. I will also describe recent studies that test the role of a-catenin by changing the equilibrium of pools of a-catenin bound to cadherins and in the cytoplasm in epithelial cells. Our results show that a-catenin plays an unexpected role in cell migration by regulating actin and plasma membrane dynamics that provide novel insight into the roles of a-catenin in cell-cell adhesion.

CHANGES IN CELL ARCHITECTURE DURING DROSOPHILA DORSAL CLOSURE

Damian Brunner, Jerome Solon, Aynur Kaya, Julien Colombelli
Cell Biology and Biophysics Unit, EMBL Heidelberg

We investigate the mechanisms that control cell shape and cell shape changes using two model organisms, the fruit fly Drosophila melanogaster and the single cellular fission yeast Schizosaccharomyces pombe. We are in particular interested in the role of the actin and microtubule cytoskeleton. In Drosophila we focus on dorsal closure (DC), a wound healing related process where a gap in the epithelium at the dorsal side of the embryo is closed. DC begins with the convergence of two lateral, epidermal cell sheets and ends with the final zippering where epidermal cells with identical positional identity recognize each other and fuse. The initial convergence step involves shape changes of the amnioserosa cells, which cover the dorsal opening. They are thought to exert a pulling force by gradually constricting their apical surface. Closure is critically dependent also on the formation of a contractile supra-cellular ribbon of actin that surrounds the opening like a purse-string. The epidermal cells that later are directly involved in zippering also modify their shape. Like all epidermal cells they elongate during the process and in addition they produce cellular protrusions at their dorsal apical surface, which interact with the corresponding target cell. We have shown that the formation and correct functioning of these cellular protrusions requires microtubules, which transiently re-arrange to form bundles that align parallel to the long cell axis at the apical side of the cell. Whilst studying the DC microtubule cytoskeleton our attention was drawn to an intriguing pulsing behavior of the amnioserosa cells, which is superimposed on their gradual apical constriction. We have launched an investigation, combining real-time fluorescence imaging with automated, quantitative image analysis and computer simulations. Results will be presented and discussed that describe these fluctuating cell shape changes and that provide evidence for a specific force generation role during DC.



DPP SIGNALING PROMOTES APICAL-BASAL CELL ELONGATION THROUGH OPPOSING RHO1

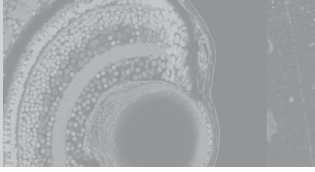
Widmann, Thomas / Dahmann C
MPI-CBG, Dresden, Germany

Morphogenesis is largely driven by changes in the shape of individual cells. However, how cell shape is regulated in developing animals is not well understood. Here we show that the onset of TGF-b/Dpp signaling activity correlates with the apical-basal elongation of cells in developing Drosophila melanogaster wing imaginal disc epithelia. Dpp signaling is necessary for maintaining this elongated columnar cell shape and overactivation of the Dpp signaling pathway results in precocious cell elongation. Moreover, we find that the small GTPase Rho1 and non-muscle myosin II promote cell shortening. Finally, we demonstrate that decreased Rho1 activity rescues the shortening of Dpp signaling compromised cells. Our results identify novel cell-autonomous roles for Dpp signaling and Rho1 in the regulation of epithelial apical-basal cell length and show that Dpp signaling functions by antagonizing Rho1.

REGULATION OF POLARITY AND ASYMMETRIC DIVISION: PAR-6 LEVELS ARE REGULATED BY NOS-3 IN A CUL-2 DEPENDENT MANNER IN C. ELEGANS

Pacquelet, Anne / Zanin E / Ashiono C / Gotta M
Institute of Biochemistry, ETH

The conserved PAR proteins have an essential function in establishing polarity in many cell types, in particular in asymmetrically dividing cells. In C.elegans, the PAR proteins allow the asymmetric division of the one-cell embryo where they define an anterior and a posterior cortical domain: PAR-3, PAR-6 and PKC-3 localize at the anterior cortex, while PAR-1 and PAR-2 are restricted to the posterior cortex. Regulators of the anterior PAR proteins can be identified by their ability to suppress the polarity defects of par-2 mutant embryos. We found that a nos-3 loss of function mutant suppresses the phenotypes of par-2 mutants by regulating PAR-6 protein levels. Interestingly, this suppression requires the activity of the genes fem-1/2/3 and of the cullin cul-2. FEM-1 is a substrate specific adaptor for a CUL-2-based ubiquitin ligase (CBCFEM-1); FEM-2 (a phosphatase) and FEM-3 (a novel protein) interact with CBCFEM-1 and increase its activity. We found that CUL-2 is required for the regulation of PAR-6 levels and that PAR-6 physically interacts with FEM-1. Our data strongly suggest that PAR-6 levels are regulated by the CBCFEM-1 ubiquitin ligase thereby uncovering a novel role for the FEM proteins and cullin-dependent degradation in regulating PAR proteins and polarity processes.



CONTROL OF PROLIFERATION AND INVASION DURING EPITHELIAL MORPHOGENESIS IN DROSOPHILA

Enrique Martín-Blanco, Nikolay Ninov, Cristina Manjón
IBMB, CSIC, Barcelona, Spain

The adult abdominal epidermis of Drosophila is formed by cells descending from histoblasts, which are founder imaginal cells specified during embryonic stages. During metamorphosis, stem-like histoblasts suffer a series of sequential transitions from a non-proliferative to a growing, and finally to an invasive epithelium replacing pre-existant larval epidermal cells (LECs). We have been studying the mechanisms regulating histoblasts cell divisions and characterizing the signaling events controlling the cellular changes associated with histoblast invasiveness. By a combination of clonal genetic interference and live imaging analysis, we have identified the transcription of String (Cdc25) in response to ecdysone as the trigger element stimulating histoblasts exit from their quiescent G2 state. Further, we characterized the signaling pathways instructing histoblasts to dynamically change speed as they divide. We found that, initially, histoblasts undergo a series of growth- and G1-less fast divisions employing pre-stored G1/S regulators (Cyclin E). Afterward, histoblasts proceed into a second stage of slow divisions dependent on Epidermal Growth Factor Receptor (EGFR) and Insulin Receptor/PI3K signaling. Altogether these results uncover an internal logic modulating the cell cycle where growth and proliferation become sequentially uncoupled and coupled. On the other hand, at the onset of histoblast nests expansion, cells at the periphery become motile and move by planar intercalation between adjacent LECs. This spatial and temporally-controlled change in behavior provide an elegant model to analyze and study the mechanism that trigger and maintain epithelia motility and invasion. We found that this process is controlled by the TGFb homologue Decapentaplegic (Dpp). Dpp secreted from LECs leads to the graded activation of the pathway and, across the nests, confers differential cellular plasticity and motility to leaders. Invasiveness of leading histoblasts controlled by Dpp associates with changes in actin dynamics, reduction of cell-cell adhesion and downregulation of cell substrate adhesion. The identification of the key regulators and the dissection of the mechanistic backbone underlying histoblasts morphogenesis makes this a relevant model for other processes of similar nature in stem cell, cancer or developmental biology.

THE ROLE OF CELL ADHESION AND CORTEX TENSION FOR GERM LAYER ORGANIZATION DURING ZEBRAFISH GASTRULATION

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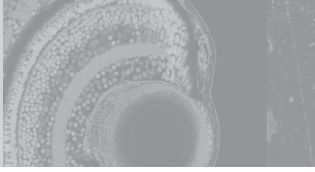
Tissue morphogenesis during embryonic development is brought about by mechanical forces which are generated by the specific biophysical properties of its constituent cells. It has also been suggested that embryonic tissues behave like immiscible liquids with a given surface tension and that differences in surface tension between tissues determine their spatial configuration during embryogenesis. To understand how single cell biophysical properties regulate tissue surface tension and how tissue surface tension controls tissue organization in development, we are studying the specific function of germ layer progenitor cell adhesion and cell cortex tension in determining germ layer organization during zebrafish gastrulation. We found that the combinatorial activity of progenitor cell adhesion and cortex tension determines germ layer tissue surface tension and that differences in germ layer tissue surface tension influence germ layer organization during gastrulation. We will discuss these findings in the light of different hypotheses explaining how single cell biophysical properties determine tissue surface tension in development.



MECHANICAL CONSTRAINTS PATTERN CELLULAR BEHAVIOUR DURING DORSAL CLOSURE IN DROSOPHILA

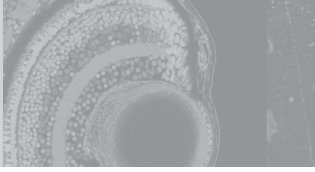
Gorfinkiel, Nicole / Blanchard GB / Adams RJ / Martinez Arias A
University of Cambridge, Department of Genetics

Half way through embryonic development, the epidermis of Drosophila exhibits a gap at the dorsal side covered by a different epithelium, the amnioserosa (AS). Dorsal closure (DC) is the process whereby interactions between these two epithelia establish epidermal continuity. It is a powerful model system for epithelial sheet movements and wound healing. Genetic and biomechanical analysis of DC have revealed that the AS is required for DC by generating a contractile force that contributes to the process. However, we still don't know how individual cell behaviours are coordinated and transformed into macroscopic forces and movements. To analyze this, we performed a kinematic analysis of AS contraction during DC using a novel method to quantify strain rates in local domains of cells. Our analysis reveals that the asymmetric contraction of the AS results from the apical constriction of cells in the medio-lateral orientation at a rate that accelerates with time. Changes in cell shape are not homogenous across the AS but exhibit differential spatial and temporal patterns, reflecting the influence of an asymmetric environment. The application of this anlysis to myospheroid mutants reveals that ßPS integrin also has a role in transmitting a patterning signal from the epidermis to the AS. Altogether, our analysis suggests that the patterned contraction of the AS is the result of an autonomous program of cell contraction on which mechanical constraints are acting.

NEURAL PLATE MORPHOGENESIS DURING MOUSE NEURULATION IS REGULATED BY ANTAGONISM OF BMP SIGNALLING

Ybot Gonzalez, Patricia / Gaston Massuet C / Girdler G / Greene ND / Copp A J
Institute of Child Health

Dorsolateral bending of the neural plate, an undifferentiated pseudostratified epithelium, is essential for neural tube closure in the mouse spinal region. If dorsolateral bending fails, spina bifida results. In the present study, we investigated the molecular signals that regulate formation of dorsolateral hinge points (DLHPs). We show that Bmp2 expression correlates with upper spinal neurulation where DLHPs are absent, Bmp2 null embryos exhibit premature, exaggerated DLHPs, and local release of Bmp2 inhibits neural fold bending. Therefore, Bmp signalling is necessary and sufficient to inhibit DLHPs. In contrast, the Bmp antagonist noggin is expressed dorsally in neural folds containing DLHPs, noggin null embryos show markedly reduced dorsolateral bending, and local release of noggin stimulates bending. Hence, Bmp antagonism is both necessary and sufficient to induce dorsolateral bending. Local release of Shh suppresses dorsal noggin expression, explaining the absence of DLHPs at high spinal levels where notochordal expression of Shh is strong. Our findings reveal a molecular mechanism underlying regulation of DLHP formation during mouse spinal neural tube closure, based on antagonism of Bmp signalling.



ENDOSOMES AND CELL DIVISION: SYMMETRIC AND ASYMMETRIC

Marcos González Gaitán
Univ. of Geneva, Switzerland

Recent studies have uncovered a key role of endocytosis during Notch signalling after the asymmetric division of the fly sensory organ precursor cells (SOP): directional signalling is mediated by differential endocytosis of the ligand Delta and the Notch effector Sanpodo in one of the SOP daughters, the pIIb. We discovered a novel mechanism of directional signalling based on the trafficking of Delta/Notch molecules already internalized in the SOP and targeted subsequently to the pIIa daughter. We could show that, in the SOP, internalized Delta and Notch traffic to an endosome characterized by the localization of the endosomal protein Sara. During SOP division, Sara endosomes and Notch/Delta therein move first to the central spindle and then to the pIIa cell in a process mediated by the Par-complex. Subsequently, in pIIa (but not pIIb), Notch appears cleaved in Sara endosomes in a process that requires gamma-secretase and Delta internalization implying that the intracellular Notch tail has been released to mediate transcription of Notch target genes. We thus uncovered two phases during biased Notch signalling: i) in the mother, receptor molecules internalized into Sara endosomes are targeted to the pIIa, thereby increasing the level of Notch signaling in pIIa and decreasing it in pIIb and, ii) subsequently, biased signaling is also mediated by endocytic trafficking leading to Sanpodo downregulation and Delta recycling/ activation in the pIIb daughter cell.

DORSAL VENTRAL AXIS FORMATION IN THE SEA URCHIN EMBRYO

Thierry Lepage, François Lapraz, Ryan Range, Magali Quirin, Véronique Duboc, Eric Röttinger, Alexandra Saudemont, Lydia Besnardeau
CNRS and Université Pierre et Marie Curie, Villefranche-sur-Mer, France

The organizer of the vertebrate gastrula directs patterning of the embryo along the dorsal ventral axis but the evolutionary origin of this important signaling centre remains unclear. Echinoderms constitute a sister group of the chordates and their basal phylogenetic position among deuterostomes makes them an interesting group to study the evolution of developmental mechanisms. Our laboratory is interested in understanding how the dorsal-ventral axis of the sea urchin embryo is established. The TGF-β family member Nodal, which is expressed very early in the presumptive ventral ectoderm, plays a crucial role in this process. Overexpression of Nodal is sufficient to specify most ectodermal cells with a ventral fate. Intriguingly, although Nodal is produced ventrally, its activity is required for specification of both the ventral and the dorsal territories. Furthermore, rescue experiments revealed that Nodal expressing cells have a long range organizing activity and are capable of restoring dorsal-ventral polarity over the whole embryo. These experiments suggest that the ventral ectoderm of the sea urchin embryo is a signalling centre that directs patterning along the whole dorsal-ventral axis. There are additional striking similarities between this ventral organizer and the dorsal organizer of vertebrates. First, formation of this ventral organizer, like that of the Spemann organizer, critically requires the activity of TCF/beta catenin as well as that of Univin, the orthologue of Vg1. Second, the sea urchin embryo expresses many dorsal ventral patterning genes (for example nodal, goosecoid, foxA) in a pattern reminiscent of those of their vertebrates orthologues but in an inverted orientation (ventral in the sea urchin and dorsal in vertebrates). Finally, in the sea urchin as in vertebrates, ectopic activation of the Nodal signaling pathway results in duplications of the dorsal ventral axis. Taken together, these findings further reinforce the view that the sea urchin ventral ectoderm is an organizing center that shares several features with the Spemann organizer in vertebrates.

SOXF PARTICIPATES IN A NOVEL FEEDBACK LOOP IN THE WNT/WG PATHWAY TO REGULATE TISSUE GROWTH IN DROSOPHILA.

Casares, Fernando / Dichtel-Danjoy MI / Caldeira
CABD, CSIC, UPO, Seville, Spain

Normal organ growth requires both the restricted expression of mitogenic signals and the regulated activity of their signaling pathways. Wnt molecules act as mitogens in several developmental contexts, and the aberrant activity of their pathway is associated to a number of cancer types. Therefore, the production of Wnts and the activity of their signaling pathway must be tightly regulated. We have investigated this regulation in the Drosophila hinge, a tissue that depends on the fly Wnt-1 ortholog, wingless (wg), for its growth. We identified the HMG transcription factor SoxF as a candidate regulator of the wg pathway: SoxF is expressed specifically in the hinge and its homolog, Sox17, has been shown to interact with the Wnt/beta-catenin in vertebrates. Here we show that SoxF is part of a novel negative feedback loop in the wg pathway: SoxF expression is activated by Wg and, in turn, SoxF represses wg transcription and attenuates its signaling. In the absence of SoxF, wg expression spreads through the hinge and causes its overgrowth. Finally we have observed that SoxF is expressed earlier than wg during hinge formation and that its expression is restrained by the transcription factor Rn, which in turn allows the onset of wg expression in the hinge. The feedback mechanism we describe tightly controls the proliferative activity of the Wnt pathway to regulate hinge growth and might be relevant to human disease, as human SoxF genes are implicated in colon carcinoma.

TGFβ MORPHOGEN MOVEMENT AND SIGNALLING IN LIVE TISSUE

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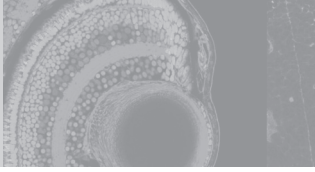
Wellcome Trust/ CRUK Gurdon Institute, Developmental Biology

During embryonic development, morphogens act in a gradient as positional cues for cell fate specification and tissue patterning. In early frog development, TGFβ family members like nodal related proteins are essential for mesoderm induction and patterning. One important aspect of morphogen function, the method of progression, is extensively discussed in the literature for a variety of model systems and molecules. Activin, a member of the TGFβ family, has all features of a mesoderm patterning morphogen. We used a fluorescently labelled form of the protein to visualize the ligand, together with Bimolecular Fluorescent Complementation (BiFC) to study Smad complex dynamics responsible for TGFβ signalling. The combination of both imaging techniques allows us to observe morphogen behaviour and direct response in living cells. Our experiments suggest that in contrast to several other morphogens, the passage of Activin ligand through neighbouring cells can be excluded. Activin appears to travel exclusively through the extracellular space while the number of cognate receptors presented on cell surfaces is crucial for the range of progression and signalling. Inhibition of endocytosis successfully blocks cellular uptake of Activin, but does not interfere with either signalling capacity or range.



THE EMBRYONIC ORIGINS OF COORDINATED MOVEMENT IN DROSOPHILA

Michael Bate
University of Cambridge, UK

An important issue for our understanding of the development of circuits that underlie behaviour is to show how these circuits first become functional and to investigate the role of this early function in adjusting and tuning network properties. We use an imaging technique to reveal the earliest patterns of movement in the Drosophila embryo and experimental methods that allow us to manipulate early activity in the motor network. The results of this work will be presented here. We find that the earliest outputs of the central pattern generating circuits are apparently unpatterned, but quite quickly evolve into coordinated sequences that resemble those of normal larval movement. The significance of these findings will be discussed together with additional data that link early activity to the differentiation of a pattern of synaptic contacts on the dendritic arbors of identified motorneurons.

NOVEL FUNCTIONS OF ROBO RECEPTORS IN NEURAL DEVELOPMENT

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Instituto de Neurociencias, CSIC & Universidad Miguel Hernández, Spain

Robo1 and Robo2, receptors for Slit proteins, are well known for their roles in regulating neuronal migration, branching and axon guidance during the development of the nervous system, both in vertebrates and invertebrates. In this context, expression of Robo receptors has been typically associated to postmitotic neurons. We have found that most progenitor epithelia in the developing forebrain and spinal cord express Robo receptors at relatively early stages of development. Loss of both Robo1 and Robo2 function leads to slightly smaller brains at the time of birth. Consistent with this, Robo1/2 double mutants have a minor but significant decrease in the number of precursor divisions in different regions of the forebrain and spinal cord at mid-gestation, whereas no major changes in cell death exist during these embryonic stages. Detailed analysis of the developing cerebral cortex in Robo1/2 double mutants also revealed an alteration in the relative proportions of primary and secondary progenitors, suggesting that Robo receptors may modulate cell division dynamics in the developing brain. These findings provide evidence that Robo signaling influences progenitor proliferation, and that this evolutionarily ancient mechanism for guiding postmitotic neuronal processes has been co-opted to regulate the neuronal composition of the developing brain.

NEURONAL MIGRATION PATHWAYS AND AXONAL MORPHOLOGY ARE CONTROLLED BY THE ACTIN-BINDING PROTEIN DREBRIN

Chilton, John / Dun X. / Allen J.
Peninsula Medical School, Institute of Biomedical and Clinical Science

The neuronal leading edge can direct local somal movement or extend huge distances as the axonal growth cone. Such morphological changes require exquisite cytoskeletal control. The actin-binding protein Drebrin localises to leading edges, associates with both actin and microtubule networks and potently induces filopodia in neuronal and non-neuronal cells. Two, alternatively-spliced, isoforms occur during embryonic development; the shorter (E1) isoform is initially present in post-mitotic neurons, a switch to E2 occurs a few days later. We have found that the duration and relative levels of their expression vary widely within the developing nervous system, suggesting complementary but distinct roles in processes including migration and axonogenesis. We have focussed on the role of Drebrin in the translocation and axonal growth of tangentially migrating neurons. C-terminally truncated Drebrin - which enhances its ability to induce filopodia - dramatically disrupts the tangential migration of oculomotor neurons. However, in precerebellar neurons the same construct produces axons of normal length but with multiple growth cones. Conversely, N-terminally truncated Drebrin - which blocks filopodia formation - results in axons which grow but with stalled, club-shaped growth cones. Our in vivo results will be extended by live-cell imaging and biochemical data to suggest how Drebrin isoforms interact with the cytoskeleton to produce these differing phenotypes.

THE ONCOGENE AND GUANINE EXCHANGE FACTOR VAV CONTROLS AXON GUIDANCE DURING DROSOPHILA DEVELOPMENT

Malartre, Marianne / Martin Bermudo, MD
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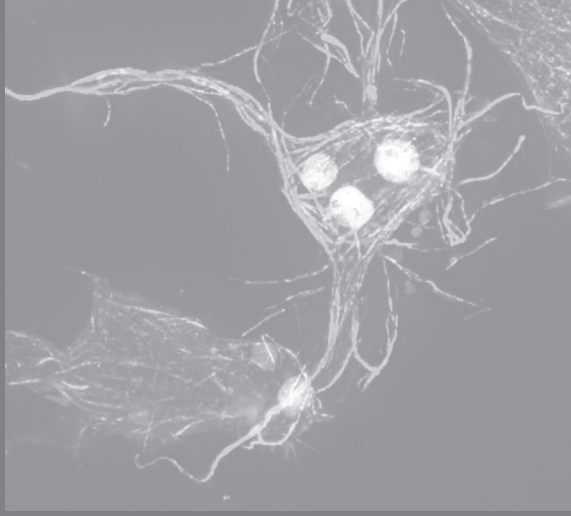
The regulation of the actin cytoskeleton is a crucial event controlling many biological processes such as cell division, migration and axon guidance. This is mainly achieved by guanine exchange factors (GEFs), such as the Vav proteins, that trigger the activation of the Rho GTPases. Despite their intense characterization in mammalian immune response, there is little information outside of this field regarding the role of the vav genes during development. Here we demonstrate for the first time that vav plays an important role in axon growth and guidance in a developing organism. First we observed that the unique Drosophila vav homolog is ubiquitously expressed during development with higher levels in the ventral midline. Then, we use vav null mutant alleles that we have generated to show that vav is required to prevent some axons from crossing the embryonic midline. We also found that vav activates the pak signalling pathway to regulate photoreceptor axon targeting to the optic lobe in larvae. Finally, adult vav mutant escapers present locomotion problems. Interestingly, these vav mutant flies display axon growth defects in the ellipsoid body, the part of the brain controlling locomotion. As these axons are generated during metamorphosis, we can conclude that vav is required for proper axon growth and guidance from embryo to pupae. Altogether, these results suggest that vav participates in a general rather than specific mechanism to control axon pathfinding during development.

RNA-BASED MECHANISM OF DIRECTIONAL STEERING IN GROWTH CONES

Christine Holt
Dept. of Physiology, Development and Neuroscience, Anatomy Building, University of Cambridge, UK.

Axons from the eye accurately pioneer the route to their distant synaptic targets in the midbrain. A major goal of our research is to understand the cellular and molecular mechanisms that underlie the precision of this long-range navigation. Guidance molecules, such as netrin and semaphorin, act as 'signposts' that attract or repel axonal growth cones at various points along the pathway. Recently it has become clear that the directional steering responses of growth cones to some guidance cues depends on local protein synthesis and degradation. Our work has focused on the following key questions: What proteins are synthesised in response to guidance cue stimulation? How does local translation contribute to directional steering? Our findings relating to these questions will be presented.



POSTERS

Ref: 1

MYOCARDIN REGULATES THE REACTIVATION OF THE FETAL CARDIAC GENE PROGRAM DURING POSTNATAL DEVELOPMENT AND AT HEART FAILURE

Mikhailov, Alexander; Torrado M., Centeno A. / López E. / Mikhailov A.T.
University La Coruña. Institute of Health Sciences

Reactivation of the fetal cardiac gene program is a characteristic feature of hypertrophied and failing hearts that correlates with impaired cardiac function. However, the molecular mechanisms governing the reversible expression of fetal cardiac genes in postnatal myocardium are not yet precisely delineated. We have demonstrated that myocardin (myocd), a positive modifier of serum response factor activities in cardiac muscle cells, is up-regulated at both physiological hypertrophy and heart failure (HF). Here we show that in-vivo forced expression of myocd up-regulates expression of a set of target genes in ventricular myocardium of early neonatal piglets. Moreover, neonatal piglets transfected with myocd-expression vectors exhibit a short-time impaired systolic function. We demonstrate that genes, encoding fetal myosin light chain 3f and smooth muscle actin, are significantly induced in myocd-transfected ventricular myocardium and can be, therefore, responsible for cardiac dysfunction. In neonatal piglets, myocardial myocd-transfection followed by induction of HF-phenotype resulted in a significant lowering of survival rates of experimented animals. Our results indicate myocd to be a key factor in molecular pathways regulating re-expression of fetal cardiac genes in the early postnatal heart and suggest that cessation of myocd overexpression at HF might represent a safety feature from a therapeutic point of view.

Ref: 2

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF NEUROTRANSMITTERS AND GROWTH FACTORS RECEPTORS DURING PLANARIAN REGENERATION

Cebrià, Francesc, Barberán S. / Cebriá F.
University of Barcelona. Department of Genetics, Faculty of Biology

The high regenerative capabilities of freshwater planarians are based upon a population of totipotent stem cells, known as neoblasts. Over the years several studies have suggested an important role of the nervous system in planarian regeneration. However, the exact nature of this neural influence remains to be characterized at the molecular level. As a first step, we have recently characterized distinct neuronal populations in the model planarian Schmidtea mediterranea based on the immunolocalization of several neuroactives molecules such as GYRFamide, neuropeptide F, serotonin and allatostatin. Also, planarian homologues of a high variety of growth factors, neurotransmitters and neuropeptides receptors have been isolated (e.g. serotonin, dopamine, octopamine, somatostatin, FMRFamide, neuropeptide F, allatostatin and opioid growth factors receptors). The undergoing determination of their expression patterns by in situ hybridizations together with RNAi-based functional analyses should provide insights into the role of these molecules during planarian regeneration.

Ref: 3

THE X. TROPICALIS MUTATION KALEIDOSCOPE TRUNCATES THE COPPER TRANSPORTER ATP7A AND MODELS MENKES DISEASE

Vendrell, Elisenda; Vendrell E / Zimmerman L
NIMR. Developmental Biology

The chemically-induced X. tropicalis mutation kaleidoscope (kal), results in strikingly variegated retinal pigmented epithelium, and is among the first mutations in X. tropicalis to be genetically mapped. Kal lens and retinal neuroepithial layers are properly organized, but gaps are observed in the RPE layer. Mutants also show reduction in melanocyte number as well as specific cartilage defects. We used a meiotic mapping approach to define a genetic interval containing the kal mutation between two simple sequence repeat polymorphisms in Scaffold 10, 0.5 Mb apart on X. tropicalis Linkage Group 5. This interval includes the ATP7a copper transporter, defects in which cause Menkes disease. Analysis of ATP7a revealed a base change at the exon 20 splice acceptor, resulting in mis-spliced mRNA deleting a domain responsible for subcellular localization of ATP7a to the trans-golgi network. Kaleidoscope demonstrates the utility of X. tropicalis genetics in developing models for human disease.

Ref: 5

DISSECTION OF DROSOPHILA STAT TO FIND NEW FUNCTIONAL DOMAINS RELATED TO ITS POLARIZED SIGNALLING.

Sotillos Martín, Sol; Castelli-Gair Hombría, J
CABD/CSIC/UPO. Gene regulation and morphogenesis

The JAK/STAT signalling pathway is involved in processes ranging from immune response to organogenesis. We recently showed that JAK/STAT signalling in the Drosophila ectoderm is strongly influenced by the cell's polarity. In contrast to the conventional vertebrate signalling model based on cell culture experiments where the receptor does not localise to any particular domain and the inactive STAT is in the cytoplasm, we showed that the receptor, JAK kinase and STAT protein localize apically in the ectoderm. STAT's apical localization is dependent on the polarity protein PAR-3 and this apical localization is required for efficient signalling. To find out what domains of STAT are required for the apical localization and function, we are dissecting the protein and analyzing the function of the different domains in vivo. Using immunoprecipitation assays we are also analyzing what domains bind directly to PAR-3. We are also studying Xenopus STAT proteins to find out if polarized apical localization is a general feature also occuring in vertebrates.

Ref: 7

REGULATION OF THE VENTRAL VEINLESS (VVL) GENE OF DROSOPHILA

Espinosa Vázquez, José Manuel; Sotillos Martín, S. / Espinosa Vázquez, J.M. / Castelli-Gair Hombría, J.
CABD CSIC-UPO

The ventral veinless (vvl) gene encodes a POU-domain transcription factor required for the development of several organs including the trachea, the midline glia, the chordotonal sensory organs and the wing. Vvl is expressed during development in a complex pattern and it is a possible direct target of STAT and the STAT competitor KEN/BCL-6. To prove this we are searching the direct binding sites of STAT and KEN in the vvl cis regulatory region that will help us understanding the regulatory interactions between these proteins. The vvl mRNA lacks introns and is located in a "gene desert" region separated 28kb from the closest up-stream gene and by 120kb from the next down-stream gene. We will present the results of our ongoing work that to this date has resulted in the localisation of two embryonic epithelial enhancers, three independent tracheal enhancers, an oenocyte enhancer, and at least two imaginal wing disc enhancers. Including the previously reported autoregulatory enhancer, this work describes over 30kb vvl cis-regulatory regions. We present data showing that an early (st10) vvl tracheal enhancer is regulated by JAK/STAT signalling pathway, underscoring the important role of this pathway for trachea specification.

Ref: 9

A PLATFORM FOR GENE EXPRESSION: APPLICATIONS IN DEVELOPMENTAL BIOLOGY

Jiménez Lozano, Natalia; Segura Mora, J. / Macías González, J.R. / Carazo García, J.M.
Centro Nacional de Biotecnología. Biocomputing Unit.

This work presents a platform that integrates gene expression data from mouse with spatial-temporal anatomic data by means of an intuitive visualiser: http://bioweb.cnb.uam.es/VisualGenomics/visualgenomics.html. The main questions that can be solved by means of this platform are: what are the genes that are expressed in a given mouse anatomical component, over a development stage (as defined by its Theiler Stage)?, in which anatomical components and TS, a given gene is expressed?. An application of this integration effort is the study of the gene expression profile during the development of a gene known to be involved in a pathological process. We have taken as reference EMAP ontology (for TS1-TS27) and the MA ontology (TS 28) of mouse anatomical terms as the pillar that holds up expression data from EMAGE and GXD gene expression databases. This system is evolving continuously by means of the inclusion of resources centred in specific parts of the mouse anatomy or even in other organisms as rat or human. Moreover, a new version of the visualiser containing a virtual mouse model in which the user could select a specific anatomical part simply by clicking on it will be available soon.

Ref: 11

EXPERIMENTAL ANALYSIS OF A GENE REGULATORY NETWORK UNDERLYING ZEBRAFISH MELANOCYTE DEVELOPMENT

Kelsh, Robert N.; Greenhill E. / Kelsh R.N.
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The genetic study of mouse, human, and, more recently, zebrafish melanocytes has identified many genes required for their specification, survival and differentiation. Mitf encodes a transcription factor that plays a central role in melanocyte development, acting as a master regulator of this fate. Work in mouse and zebrafish has made clear that melanocyte specification from the neural crest depends upon transcriptional regulation of Mitf, and shows that Sox10 and Wnt signaling are both critical factors underlying melanocyte specification. However, a possible role for Sox10 at later stages in melanocyte development is controversial. We begin by proposing a simple gene regulatory network mediating melanocyte development, taking as an analogy one proposed for sympathetic neuron development. We test multiple predictions of this model, including those regarding the timing of sox10 expression and the effects of ectopic sox10 expression on mitfa and melanocyte differentiation genes. Whilst many of these predictions have been largely upheld, other results have been unexpected, and suggest key refinements to the initial gene regulatory network model. This work represents an initial attempt to formally model the developmental genetics of zebrafish melanocyte that will be the stimulus for further characterization of the precise roles of Sox10, Mitfa and other factors.

Ref: 12

WNT SIGNALLING REGULATES TRANSCRIPTION FACTOR NETWORKS IN VERTEBRATE HEART DEVELOPMENT

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The heart is the first organ to become functional in the vertebrate embryo; however the mechanisms regulating its formation are not yet fully understood. One important step in this process is the decision of mesodermal cells to assume a cardiac fate and subsequently differentiate into heart muscle. This is provoked by the interaction of signalling cascades and extra-cellular cues including the Wnt family of secreted signalling proteins. In this study we investigated the hierarchy of effects that β-Catenin and GATA family members overexpression have on the formation of Xenopus hearts. This was achieved using a combination of experiments utilizing inducible, constitutively active mRNA constructs and a Wnt signalling agonist BIO. The effects of these manipulations were assessed by analysing gene expression of cardiac markers by RNA In-Situ Hybridization and by Quantitative PCR. Further experiments have also been carried out to ascertain whether this regulation is cell autonomous or not using a lineage marker to map the migration of the β-Catenin-GR throughout the embryo. Results show that overexpression of β-Catenin leads to down regulation of heart markers, whereas GATA overexpression leads to an increase in marker expression. Overexpression of both β-Catenin and GATA results in a return to normal levels of expression in these markers suggesting that GATA activity is downstream of β-Catenin and is a relevant target of Wnt/β-Catenin in heart development.

Ref: 14

THE EXPRESSION OF A CXCR4 GENE IN XENOPUS EMBRYO

Sáez, Francisco J. / Alonso E / Madrid Jf / Valbuena G / Sáez Fj
Universidad del País Vasco. Dpto. de Biología Celular e Histología.

Our aim was to identify a homologue of zebrafish cxcr4b in Xenopus, which could be involved in primordial germ cell (PGC) guidance migration. After a BLAST analysis, the clone gi 27519681, homologous to the zebrafish gene z-cxcr4b, was identified, inserted into pCMV-SPORT6 plasmid and cloned in Escherichia coli. The expression of x-cxcr4b was analyzed by RT-PCR in embryos. X-cxcr4b was weakly expressed maternally but expression was increased after the mid-blastula transition (MTB), declining significantly when PGCs migration is complete at stage 45. After isolation of presumptive PGCs, RT-PCR showed strong maternal expression at stage 8, which decreased by stage 10 post-MTB and was not detected at stage 14. Whole mount in situ hybridization in Xenopus embryos using x-cxcr4b mRNA showed that this gene is expressed in haematopoietic and neural tissues, thus the expression of this gene should be linked to important processes during embryonic development of these systems. Expression of x-cxcr4b was never coincident with that of Xpat mRNA, which labels PGCs restricted to the posterior endoderm, although sometimes weak staining could be seen within the anterior endoderm. It could be concluded that maternal x-cxcr4b is specifically downregulated within PGCs at pre-migratory stages while it is expressed in other organs.

Ref: 18

ELECTROPORATING CHICK EMBRYO DORSAL AORTA ENDOTHELIUM TO ASCERTAIN THE ROLE OF THE HOMEOBOX GENE MEIS1 IN DEFINITIVE HEMATOPOIESIS

Rossello Castillo, Catalina Ana; Azcoitia Borghi, V. / Torres Sánchez, M.
Fundación CNIC-Instituto Carlos III. Dept. of Cardiovascular Developmental Biology

Meis genes (Myeloid Ecotropic viral Insertion Site) are homeodomain transcription factors from the TALE (Three-Aminoacid Loop Extension) family. Meis1-deficient mice display defects in the birth and establishment of the first definitive hematopoietic stem cells (HSCs) in the aorta-gonads mesonephros (AGM) region of the mouse embryo. While Meis1 overexpression is linked to the induction of leukaemias, in close association with other TALE family genes (Pbx) as well as with other homeobox genes (Hox). We present here data on the expression of these Meis1 partners in the AGM of 10.5 dpc mouse embryos, showing that no Hox gene is expressed in the hemogenic potential area, while HSCs clusters do express Pbx1b protein. Since also Hox-independent functions have been described for Meis proteins, new candidate Meis1 partners could take part in HSCs birth. To address this question, we set up a system for the electroporation into the dorsal aorta endothelium of HH16-18 chicken embryos and overexpressed the main transcription factors described for HSCs (SCL/Tal1, Lmo2, GATA2, cMyb, RunX1, PU.1) alone or joint with Meis1 and Pbx1. Such analysis will yield the “cocktail” of genes that an endothelial cell needs to activate when becoming a HSC. Finally, we compared by microarray technology the expression profiles of wild type and Meis1-mutant AGM region of 10.5 dpc mouse embryos in the aim of finding Meis1 targets in HSCs generation. KEYWORDS: Homeobox, TALE family, AGM, hematopoiesis, HSCs.

Ref: 19

ROLE OF THE HOMEOBOX GENE MEIS1 IN DEFINITIVE HEMATOPOIESIS

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Meis genes (Myeloid Ecotropic viral Insertion Site) are homeodomain transcription factors from the TALE (Three-Aminoacid Loop Extension) family. Meis1-deficient mice display defects in the birth and establishment of the first definitive hematopoietic stem cells (HSCs) in the aorta-gonads mesonephros (AGM) region of the mouse embryo. While Meis1 overexpression is linked to the induction of leukaemias, in close association with other TALE family genes (Pbx) as well as with other homeobox genes (Hox). We present here data on the expression of these Meis1 partners in the AGM of 10.5 dpc mouse embryos, showing that no Hox gene is expressed in the hemogenic potential area, while HSCs clusters do express Pbx1b protein. Since also Hox-independent functions have been described for Meis proteins, new candidate Meis1 partners could take part in HSCs birth. To address this question, we set up a system for the electroporation into the dorsal aorta endothelium of HH16-18 chicken embryos and overexpressed the main transcription factors described for HSCs (SCL/Tal1, Lmo2, GATA2, cMyb, RunX1, PU.1) alone or joint with Meis1 and Pbx1. Such analysis will yield the “cocktail” of genes that an endothelial cell needs to activate when becoming a HSC. Finally, we compared by microarray technology the expression profiles of wild type and Meis1-mutant AGM region of 10.5 dpc mouse embryos in the aim of finding Meis1 targets in HSCs generation. KEYWORDS: Homeobox, TALE family, AGM, hematopoiesis, HSCs

Ref: 20

DALLY AND DALLY-LIKE ARE TWO NOVEL GENE TARGETS OF DACHSOUS AND FAT DURING IMAGINAL DISC DEVELOPMENT.

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CSIC. Centro de Biologia Molecular

Dachsous (Ds) and Fat (Ft) are two protocadherins of Drosophila that participate during imaginal disc development in several processes as cell proliferation, proximo-distal patterning and planar cell polarity (PCP) among others. Some of the ds and ft phenotypes resemble those caused by changes in the signalling of Wnt, TGF-b and Hh suggesting that Ds and Ft might be regulating these pathways. We show that dally and dally-like genes are regulated by Ds and Ft and this transcriptional control is mediated by the Hippo pathway. We propose a mechanism by which the control of growth and patterning through Hippo pathway is achieved by modulating the expression of the glypicans Dally and Dally-like that control simultaneously the activity of different signalling pathways.

Ref: 21

A NOVEL MORPHOGENETIC FUNCTION FOR THE ANTERIOR VISCERAL ENDODERM IN RESTRICTING EPITHELIAL-TO-MESENCHYMAL TRANSITION TO THE PRIMITIVE STREAK

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Antero-posterior (A-P) axis specification is crucial to determine the body plan of the embryo. Before gastrulation, the mouse embryo is a uniform structure formed by two adjacent epithelia: the visceral endoderm (VE) and the epiblast, which will give rise to the embryo proper. The anterior VE (AVE) has a key role in the orientation of the A-P axis and in proper patterning of the underlying epiblast. This is achieved by the secretion of inhibitors of the Wnt and TGFβ signaling which normally induce posterior cell fates and primitive streak formation. Our results show that AVE cells express the transmembrane protein FLRT3 that in other contexts enhances signaling by FGFR and regulates E-cadherin internalization. FLRT3-/- embryos die at midgestation displaying dramatic malformations in anterior structures. This effect is not due to patterning problems but to the rupture of the basement membrane (BM) underlying the AVE. As a consequence, epiblast cells loose polarity, leave the epithelium and acquire mesodermal fate. Hence, the AVE has a dual role, as signaling/patterning center counteracts posterior fate and as a morphogenetic regulator of the BM restricts epithelial-to-mesenchymal transition to the posterior primitive streak region of the mouse embryo.

Ref: 22

DEVELOPMENT OF CRANIAL FORAMINA IN THE CHICK EMBRYO

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Cranial foramina are tiny holes that allow the entry and exit of blood vessels and nerves through the skull. Malformations of cranial foramina in the embryo and also closure in adulthood can lead to blindness, deafness and facial paralysis and also raised intracranial pressure, which can be fatal. The cellular and molecular mechanisms of cranial foramina development have never been investigated. We have examined the appearance of cranial foramina from day4 to day20 of development in the chick embryo, using alcian blue and alizarin red stained whole skeletal preparations and histological stained sections. This analysis has shown that overt cranial foramina appear at day6 once cartilage has begun to differentiate. Furthermore they reduce in size as development proceeds. Immunohistochemical analysis of cranial foramina in the chick embryo has revealed that all cranial foramina contain a nerve and blood vessel. Additionally we have identified a unique type of blood vessel which isn't immunoreactive to smooth muscle actin, appearing in some cranial foramina. We suggest these blood vessels are part of a glomus body contained within the cranial foramina. These blood vessels can be seen from day4 of development and may proove useful in identifying regions where cranial foramina will form before morphologically overt foramina are seen. The role of nerves in the development of cranial foramina in the chick embryo is being investigated by removing developing cranial nerves before axon extension. We are focusing on ablation of hypoglossal nerves and bilateral enucleations (which result in loss of the optic nerves). The appearance of these hypoglossal and optic foramina are being examined in whole skeletal preparations and tissue sections. We are also examining the expression of early cartilage marker genes such as Sox9 and aggrecan in the mesenchyme around blood vessels, nerves and glomus bodies before morphologically distinct foramina are visible. These investigations will aid our understanding of whether cranial foramina can be identified as discrete regions where cartilage differentiation is inhibited from the time blood vessels and nerves invade head mesenchyme, or whether this process occurs subsequent to this.

Ref: 23

NON-AUTONOMOUS CONTROL OF LEADING VERSUS TRAILING CELL MIGRATION AND CELL FATE IN DROSOPHILA TRACHEA BY SEQUOIA, A REPRESSOR OF FGF EXPRESSION

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IRB Barcelona (IBMB-CSIC) - Cell and Developmental Biology Programme

Coordination and integration of cell changes during development enables organs to adapt their final function, shape and size to the proper performance of the full organism. Cells can respond to different signals by adopting different fates and/or changes in their properties and developmental programmes. The migratory ability and behaviour of each cell depends on extracellular signals and the sensing of its surroundings. The Drosophila tracheal system is a model to address this as its many features, in particular the migration of the tracheal cells, rely on a set of positional cues provided by their neighbours. At the ventral side of the embryo, a single terminal cell forms at the tip of each ganglionic branch (GB) which migrates towards the embryonic CNS. Here we report that the Sequoia (Seq) transcription factor, which is expressed in the nervous system, is responsible to restrict terminal fate to a single cell per GB. We show that in the GB of seq mutants some otherwise trailing cells do not follow the leading cell and also act as leading cells. Seq acts as a repressor of Bnl and that ectopic expression of bnl in the ventral midline of the CNS mimics the seq phenotype. In agreement, we detect genetic interactions between seq and bnl and pointed (pnt) mutants. These data indicate that the extent of Bnl signal determines how many cells in the GB will adopt a terminal fate and controls how many cells adopt a leading migratory behaviour instead of acting as trailing cells.

Ref: 26

GATA4 AS A NODE OF TRANSCRIPTIONAL NETWORK IN PANCREAS SPECIFICATION

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CABIMER

It is widely acknowledged that information gathered from the field of developmental biology has been crucial for the design of in vitro differentiation strategies, including the generation of insulin-producing cells (of endodermal origin) from embryonic stem cells. The zinc finger transcription factor GATA4 has been shown to play important roles in endoderm development. As a first step to understand how endoderm formation is controlled and how the cells within the endoderm differentiate and adopt a pancreatic fate, we propose to study the transcriptional regulation of Gata4 in this specific cell lineage. A search for conserved non-coding sequences of the Gata4 locus revealed two highly conserved regions that direct expression to the early endoderm and pancreas, respectively, in transgenic mice. We show that Gata4 is activated in the early endoderm by Fox factors and in the pancreatic endoderm by Hox factors, via the two identified enhancers. Based in our data and others we propose a model in which GATA4 serves as a node of the transcriptional network for pancreas specification.

Ref: 28

ROLE OF VRK-1 IN C. ELEGANS VULVA AND UTERUS DEVELOPMENT

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CABD - UPO - CSIC

Our work focuses on the single Caenorhabditis elegans homolog of the mammalian Vaccinia Related Kinases, named VRK-1. We have previously described an important role of VRK-1 in post-mitotic nuclear envelope formation during early embryogenesis# and are now characterising the function of VRK-1 in postembryonic development. Homozygous vrk-1(ok1181) mutants show a fully penetrant protruding vulva phenotype and do not form a uterus lumen nor the connection between uterus and vulva (utse). Cell proliferation and specification occur normal in the vulva but are strongly impaired in uterine cells. A specialised gonadal cell termed the anchor cell invades through the basement membranes separating the epithelial vulva cells and the somatic gonad during larval stage L3 in wild-type animals. In contrast, anchor cell invasion in vrk-1(ok1181) mutants is strongly delayed. Expression of a VRK-1-GFP fusion protein from the vrk-1 promoter restores proper anchor cell invasion and rescues the uterus and utse defects. Interestingly, VRK-1-GFP is expressed in all vulva precursor cells and reaches highest levels in the central P6.p and its descendants. Genetic studies show that vrk-1 is required for expression of EGL-17/FGF of the LET-60/Ras signalling pathway in the developing vulva. We are currently working to place vrk-1 precisely in the pathways essential for vulva and uterus formation. #Klerkx EPF and Gorjanacz M et al., EMBO J 2007, 26:132-43.

Ref: 29

IN VIVO ANALYSIS OF MORPHOGENESIS IN CULTURED IMAGINAL DISCS: A NEW ROLE FOR MYOSIN II

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MRC-LMB. Cell Biology

The morphogenesis of any organ is an extremely complex and dynamic process. Initially simple monolayer epithelia develop into multiple different organs, each with a different shape closely related to its function. We have used the eversion of the Drosophila wing imaginal disc during metamorphosis as a model to understand the morphogenetic mechanisms that sculpt tissues. We have combined a novel technique of ex vivo culture and imaging of the discs with the powerful genetic tools of Drosophila. In our movies we use fluorescent reporters to see in detail the different steps of eversion, and at the same time analyse the function of relevant genes in the process. This method has led to the identification of a novel function of myosin II unrelated to its previously described roles in apical constriction and intercalation/ rotation of cells. Myosin II accumulates in two stripes of cells in the peripodial membrane that act in unison as cables to direct folding of the proper disc during eversion. This represents a non-autonomous movement of an epithelium (the proper disc) driven by another (the peripodial membrane). Our detailed dissection of the eversion process demonstrates the enormous potential of in vivo imaging of Drosophila epithelia for the analysis of developmental processes.

Ref: 33

EMBRYONIC STEM CELL TARGETING AND CHIMERA PRODUCTION FACILITY: GENERATION OF A NOVEL GENETIC ABLATION MOUSE MODEL

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UCL-Institute of Child Health. Neural Development Unit

We have established a core facility at the UCL Institute of Child Health that provides a customised ES cell targeting service. We perform targeting and random integration experiments in ES cells as well as generating chimeric mice and embryos by blastocyst injection. In the last three years, we have generated more than 13 new mouse models and carried out more than 28 experiments using ES cells. One important model we have generated is the ROSA26-eGFP-DTA mouse line, by introducing an eGFP-DTA (enhanced Green Fluorescent Protein - Diphtheria Toxin fragment A) cassette into the ROSA26 locus by homologous recombination in ES cells. This mouse expresses eGFP ubiquitously, but DTA expression is prevented by the presence of eGFP, a Neo cassette and a strong transcriptional stop sequence. Mice carrying this construct are normal and fertile indicating absence of DTA expression. However, upon Cre-mediated excision of the floxed region, DTA expression is activated resulting in the specific ablation of Cre-expressing cells. As an example of this approach, we have ablated Nkx2.5 and Wnt1 expressing cells by using the Nkx2.5:Cre and Wnt1:Cre mouse lines, respectively. We observed loss of the precise tissues in which Nkx2.5 and Wnt1 are expressed. Apart from being a valuable general GFP reporter, the ROSA26-GFP-DTA mouse line should provide a useful resource for genetic ablation of specific groups of cells.

Ref: 34

THE DROSOPHILA PDZ PROTEIN CANOE: A NOVEL PLAYER DURING ASYMMETRIC CELL DIVISION

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Instituto de Neurociencias de Alicante. Developmental Neurobiology

Asymmetric cell division is a conserved mechanism to generate cell diversity during development and a key process in stem cell biology. Neuroblasts (NBs), the progenitors of the Drosophila central nervous system, undergo asymmetric divisions. In a stem cell-like fashion, polarized NBs divide to give rise to an apical NB, which keeps the stem cell-like properties, and a basal ganglion mother cell, committed to differentiate. We have analyzed the function of the PDZ protein Canoe (Cno) during asymmetric NBs division. We found that Cno colocalizes with the PDZ protein Bazooka/Par3 apically in metaphase NBs. cno loss-of-function mutants showed an altered distribution of the cell-fate determinants Numb, Prospero and Brat, as well as randomized orientation of the mitotic spindle in metaphase NBs. The unequal size of NBs daughter cells was also affected in cno mutants. Indeed, neuronal lineages were compromised in cno mutants. Epistatic interactions between Cno and other apical proteins placed Cno downstream of Inscuteable-Partner of Inscuteable (Pins)-Gαi and upstream of the NuMA-related protein Mushroom body defect. Furthermore, co-immunoprecipitation assays showed that Cno is forming a complex with Pins in vivo. Hence, our data unveil a function of Cno as a new player during asymmetric NB division.

Ref: 36

FUNCTIONAL ANALYSIS OF THE RHOGAP CV-C PROTEIN DOMAINS

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The crossveinless-c (cv-c) gene encodes a RhoGAP required for the actin reorganisation during morphogenesis. Cv-c and its human homologs (Deleted in liver cancer 1 and 2 [DLC]) enhance the weak constitutive GTPase activity of Rho leading to its inactivation. Cv-c is expressed in the mesoderm and in the ectoderm, and its mutation leads to abnormalities including defects in midgut constriction, head involution, salivary glands, trachea and posterior spiracle invagination, dorsal closure and Malpighian tubule formation. Analysis of Cv-cGFP fusion proteins showed that Cv-c localises to the basolateral membrane of ectoderm epithelial cells in an opposing localisation to that of the apical RhoGEF activators. As this observation suggests that both Cv-c enzymatic activity and its subcellular localisation are fundamental for its function we have started analysing how the different protein domains contribute for this RhoGAP protein function and localisation. Cv-c/DLC has four conserved domains: two protein-protein interaction SAM domains, a Rho GTPase interacting GAP domain and a putative lipid binding START domain. We have analysed the effects caused on embryogenesis of ectopic expression of Cv-c or of protein variants lacking different domains. We have also analysed the requirement of the different protein domains for Cv-c's subcellular localisation. We will present these results as well as others analysing the requirement of Cv-c membrane localisation for the protein's function.

Ref: 37

EXPRESSION OF GENES INVOLVED IN ORBITAL CARTILAGE DEVELOPMENT

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Royal Veterinary College. VBS

Orbital cartilage encircles the eye and is essential for bony eye socket formation. It is derived from cranial neural crest cells (NCC), cells that migrate from the dorsal neural tube in vertebrate embryos. NCCs are pluripotent generating a number of cell types including neurons, glia, and melanocytes. Uniquely in the developing head, NCCs also make skeletal derivatives that form the majority of the craniofacial skeleton. Differentiation of NCCs into cartilage in the head requires inductive interactions between NCCs and the local environment. The nature of these interactions is largely unknown. We hypothesise that formation of the eye socket requires interactions between the eye and the NC during early development. This is supported by evidence in animals and humans where lack of eyes (anophthalmia) or formation of small eyes (microphthalmia) result in craniofacial abnormalities, in particular lack of a socket and misalignment of jaws. We are interested in identifying the molecules involved in this interaction in the chick embryo. We have examined gene expression patterns around the developing eye, including those involved in cartilage induction and differentiation. Aggrecan, encircling the eye in the region of presumptive scleral cartilage, follows Sox9 expression. We also demonstrate that expression of these genes is altered following eye removal in early development, suggesting a role for the eye in scleral cartilage induction. Funded by the Wellcome Trust

Ref: 38

POLYCOMB-DEPENDENT DROSOPHILA ULTRABITHORAX HOX GENE SILENCING INDUCED BY HIGH ULTRABITHORAX LEVELS

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The Ultrabithorax gene of Drosophila specifies the third thoracic and first abdominal segments. Ultrabithorax expression is controlled by several mechanisms, including negative regulation by its own product. We have discovered that if Ultrabithorax expression levels are inappropriately elevated, overriding the autoregulatory control, a permanent repression of Ultrabithorax is established. This continuous repression becomes independent of the presence of exogenous Ultrabithorax and leads to the paradoxical result that an excess of Ultrabithorax leads to a phenotype of Ultrabithorax loss. The mechanism of permanent repression depends on Polycomb-group genes and also requires the UbdA motif of the Ultrabithorax protein. Absence of endogenous Ultrabithorax transcription when Ultrabithorax levels are highly elevated probably allows the formation of active Polycomb complexes on a Polycomb response element located in the Ubx major intron. This leads to the permanent repression of Ultrabithorax transcription. Similar results are obtained with the gene engrailed, showing that this mechanism of permanent repression may be a general one for genes with negative autoregulation when levels of expresion are transitorily elevated.

Ref: 39

THE HOMEOBOX GENE SIX3 IS REQUIRED FOR NORMAL PITUITARY DEVELOPMENT

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The pituitary gland is a master regulator of homeostasis in vertebrates and controls vital physiological functions such as metabolism, growth, fertility and stress response. The homeobox genes Hesx1 and Six3 are expressed in the developing anterior pituitary. Mutational analyses in mice and humans have uncovered a fundamental role for Hesx1 in pituitary formation, but the functional relevance of Six3 is unknown. We present genetic evidence demonstrating, for the first time, a novel role for Six3 in pituitary gland development. Six3+/-;Hesx1Cre/+ double heterozygous mice exhibit dwarfism with abnormal thyroid and gonad development and die at the 5th-6th week of age. They show enlarged and bifurcated pituitary glands as a consequence of an increased proliferation of periluminal progenitors, possibly due to an elevation of the Wnt/b-catenin signalling. We report for the first time an involvement for Six3 in pituitary gland formation.

Ref: 40

SNAIL1 TRANSCRIPTION FACTOR IN BONE DEVELOPMENT AND HOMEOSTASIS

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The Snail gene family is fundamental during embryonic development in processes that imply cell movements including mesoderm and neural crest formation. However, they must be maintained silent in the adult as its pathological activation leads to several prominent pathologies, its aberrant activation in tumours leads to the acquisition of invasive and metastatic properties while its activation in the adult kidney leads to renal fibrosis. Both involve the Snail-mediated induction of the EMT. In addition, Snail factors attenuate cell proliferation and induce resistance to cell death, necessary for normal embryonic and malignant tumour cells to form organs or metastasis, respectively. Interestingly, Snail also functions in non-epithelial cells, such as chondrocytes, where it is unable to induce EMT but still controls proliferation. Indeed, its deregulated expression in the developing bone leads to achondroplasia in transgenic mice. Achondroplasias are associated with activating mutations in FGFR3. Snail1 is the transcriptional effector of FGFR3 signaling as the inhibition of Snail1 abolishes its signaling even through the pathological activating FGFR3 forms. Snail1 expression is very tightly regulated in the bone and after having shown its importance during fetal bone development we wondered whether its aberrant activation in the adult had any impact on bone homeostasis. Our preliminary data indicate that indeed, Snail1 activation disrupts mineralization in the adult bone.

Ref: 42

TYROSINE HYDROXYLASE INVOLVEMENT IN CARDIAC DIFFERENTIATION

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Tyrosine hydroxylase (TH) catalyzes the conversion of L-tyrosine to L-DOPA, the rate limiting step in the biosynthesis of catecholamines. In postnatal organisms, the catecholamines act as hormones and neurotransmitters. However, the role of TH during embryonic development is still unknown. In the chick embryo, we detected TH mRNA by RT-qPCR as early as at gastrulation stage (st. 5). At later stages, TH mRNA and protein were predominantly found in the cardiac tube. In addition, L-DOPA was already detected by HPLC at st. 8. To address the role of TH in cardiac development we performed pharmacological and genetic approaches. We implanted heparin-acrylic beads coated with L-DOPA or dopamine laterally to the heart forming region in st. 5 embryos. L-DOPA and dopamine induced the ectopic expression of the cardiac markers AMHC, VMHC and Tbx5. Conversely, beads coated with an inhibitor of DOPA production (3-iodo-tyrosine) or dopamine biosynthesis (meta-hydroxybenzylhydrazine) inhibited the expression of AMHC and VMHC. We overexpressed TH by means of a bicistronic vector coding for TH and GFP. The plasmidic vector was injected in the primitive streak and electroporated. Embryos overexpressing TH showed a large increase in AMHC and VMHC heart expression, as well as expanded expression domains. Work funded by Grants BFU2007-66350 and BFU2007-61055 of the Spanish Government and PRI07A005 of the Junta de Extremadura.

Ref: 43

MULTIPLE ROLES FOR NOTCH SIGNALLING PATHWAY DURING LEFT-RIGHT DEVELOPMENT

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It is known that Notch signalling plays an important role in left-right determination in vertebrates. We show that in zebrafish not only it is likely involved in transcription of left sided genes but it is also crucial for the correct distribution and growth of the nodal cilia. So, for the first time we report that Notch signalling is involved in proper cilia growth or cilia maintenance of the ciliated cells lining the fish node called Kupffer's vesicle. Our results imply that notch signalling pathway affects the left-right process in at least two different events that occur in overlapping time windows.

Ref: 44

INTEGRINS AND FOLLICULAR EPITHELIUM MORPHOGENESIS

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The ovary of the adult Drosophila female is composed of various tubular structures called ovarioles within which eggs are formed. Each ovariole contains a line of egg chambers at different stages of development, from stage 1 (most immature) to stage 14 (most mature). Each egg contains two classes of cells, germline cells and somatic cells. During the early stages of oogenesis, the germline (15 nurse cells plus 1 oocyte) is enveloped by somatic cells that will eventually form a monolayer known as the follicular epithelium. As oogenesis progresses, the follicular epithelium differentiate, a process that involves changes in gene expression and cellular rearrangements, which are essential for correct egg formation. Here, we demonstrate that integrins, the main cell-ECM adhesion receptors, regulate different aspects of the follicular epithelium morphogenesis. Using clonal analysis, we show that integrins control the proper differentiation of the follicular epithelium, as integrin mutant cells remain in a precursor state. Our analyses also show that loss of integrin function results in an increase in the number of polar cells, suggesting that integrins regulate polar cell fate. This results in a concomitant rise in the number of follicle cells with the ability to migrate, the so-called border cells. Finally, preliminary data reveal possible interactions between integrins and the main pathways controlling follicle cell maturation, such as Notch, EGFR and the Hippo pathways.

Ref: 45

NEUROPILIN 1 AND 2 COOPERATIVELY GUIDE THE MIGRATION OF SYMPATHETIC NEURAL CREST CELLS

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Neuropilin (NRP) receptors and their class 3 semaphorin (SEMA3) ligands are well known for their contribution to axon guidance and neuronal migration in the developing vertebrate nervous system. In contrast, their role in guiding the neural crest cell precursors of the peripheral nervous system is not well understood. On the one hand, SEMA3F/NRP2 signalling guides the segmental migration of trunk neural crest cells, but mouse mutants lacking SEMA3F or NRP2 do not have obvious defects in the segmental organisation of the dorsal root ganglia or the assembly of the sympathetic nervous system. On the other hand, SEMA3A/NRP1 signalling was thought to be dispensable for trunk neural crest migration in the mouse. We now show that loss of SEMA3A or NRP1 caused sympathetic neural crest cells to stray into ectopic territories, where they differentiated into neuronal progenitors, often in the vicinity of blood vessels that do not normally receive sympathetic innervation. Strikingly, mutants lacking semaphorin signalling through both NRP1 and NRP2 were more severely affected than mutants lacking NRP1 alone. This observation suggests that the predominant role of SEMA3F/NRP2 signalling during sympathetic neural crest migration is to provide a backup pathway for SEMA3A/NRP1. We conclude that neuropilins pattern the sympathetic nervous system by guiding the migration of its neural crest cell precursors.

Ref: 46

THE ROLE OF HOX GENES IN INITIATION OF LIMB OUTGROWTH

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Tbx4 and Tbx5 are expressed in prospective hind- and forelimb territories, respectively, in vertebrates. We showed that despite their limb-type specific expression pattern, these genes do not participate in the acquisition of limb-type specific morphologies, but play equivalent roles in the initiation of hind- and forelimb growth. We suggested that distinct combinations of Hox proteins expressed in rostral vs caudal domains of the LPM are involved in the limb-type restricted expression of Tbx4 and Tbx5 and in the determination of limb-type specific morphologies. To determine whether Hox genes expressed in the LPM control the limb-type restricted expression of Tbx genes, we have isolated the minimal regulatory element required to drive the earliest forelimb-restricted expression of the mouse Tbx5 gene. We find that a 363bp region located in the second intron of the gene recapitulates the forelimb expression of the gene when linked to a reporter. It contains six predicted Hox binding sites required for the regulatory properties of this region. Using co-electroporation studies and site-directed mutagenesis of transgenic constructs, we show that Hox proteins indeed regulate the forelimb-restricted expression of Tbx5. We further demonstrate that Hox proteins bind directly to these putative Hox binding sites in vitro. These data confirm that an axial Hox code regulates the forelimb-restricted expression of Tbx5 to control the axial position at which forelimb outgrowth is initiated

Ref: 49

SPROUTY GENES CONTROL SHH-MEDIATED GRANULE CELL EXPANSION IN THE POSTNATAL CEREBELLUM

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Previous studies have demonstrated a key role for Fgf8 in development of the cerebellum from dorsal rhombomere 1 in the embryo. However, many of the developmental processes that shape cerebellar morphogenesis occur during the first two weeks after birth. All four sprouty genes (Spry1-4), which encode feedback antagonists of Fgf signalling, are expressed throughout cerebellar development with high levels of expression in neuronal precursors in the postnatal cerebellum. Conditional gene deletion studies indicate that Spry1, Spry2 and Spry4 function redundantly during development of the cerebellum. Mid-hindbrain-specific Spry1;2 and Spry1;2;4 knockout animals have smaller cerebella with abnormal foliation patterns. When Spry1 and Spry2 are deleted specifically from granule cell precursors after birth, these mutants also exhibit smaller cerebella indicating that sprouty genes function in granule cell precursors in the postnatal cerebellum. We show that the expression of Fgf target genes, Pea3 and Erm are increased, and SHH target genes, Gli1 and Ptch1 are decreased in the sprouty mutant cerebella. Our results suggest that sprouty genes function as Fgf inhibitors and that the absence of the sprouty genes results in a decrease in SHH-induced proliferation of granule cell precursors in the postnatal cerebellum.

Ref: 50

FGF EXPRESSION IN THE DEVELOPING CEREBELLUM SUGGESTS ROLES IN CEREBELLAR MORPHOGENESIS AND DEVELOPMENT

Yaguchi, Yuichiro / Yu T / Ahmed M / Mason I / Basson M.A
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Earlier studies have defined important roles for FGF8 signalling during development of the midbrain and cerebellum. The major classes of cerebellar neurons are only born and differentiate after embryonic day (E) 12.5, when expression of Fgf8 has been reported to cease. A key process required for normal cerebellar development is the extensive proliferation of granule cell precursors in the external granule cell layer during the early postnatal period. After a period of proliferation, the cells exit the cell cycle, start differentiating and migrate inwards to form the internal granule cell layer, where final maturation takes place. This process is not completed until approximately postnatal day (P) 21. To investigate whether Fgf signaling is required during these later stages of cerebellar morphogenesis, we determined the expression of all Fgf genes at key stages of cerebellar development after E12.5. Several Fgf genes are expressed in cell type- and region-specific patterns, suggesting multiple functions during the development of different cell types and specialised regions within the cerebellum. By analyzing the effects of modulating FGF signaling on cerebellar development in vivo, we provide evidence suggesting that FGF signaling regulates the expansion of granule cell precursors during the early postnatal period by antagonizing SHH signalling.

Ref: 51

ANTERO-POSTERIOR AXIS DETERMINATION IN THE EARLY CHICK EMBRYO: ROLE OF GATA2 AS INHIBITOR OF AXIS FORMATION

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Although polarity (head-tail axis) of the chick embryo is specified by the time of egg-laying, this is not yet irreversibly fixed: when a blastula-stage embryo (about 20,000 cells) is cut in half both halves can develop an embryonic axis spontaneously. In the normal embryo, inhibitory mechanisms prevent formation of multiple axes (Bertocchini and Stern, 2002; Bertocchini et al., 2004). Although several known genes are expressed posteriorly at these stages, only one, the transcription factor Gata2, is stronger anteriorly. Here, we investigate its role in specification of embryonic polarity. At very early stages, Gata2 and Vg1, a member of the TGFβ family of signalling molecules, are expressed in complementary domains. When the anterior half is isolated, Gata2 is upregulated along its whole circumference. Knock-down of Gata2 with a morpholino causes ectopic axis formation or displacement of the axis. This suggests that Gata2 is involved in inhibiting axis formation anteriorly.We are currently investigating the epistatic relation between Gata2 and Vg1. Bertocchini, F., Skromne, I., Wolpert, L. and Stern, C. D. (2004). Determination of embryonic polarity in a regulative system: evidence for endogenous inhibitors acting sequentially during primitive streak formation in the chick embryo. Development 131, 3381-90. Bertocchini, F. and Stern, C. D. (2002). The hypoblast of the chick embryo positions the primitive streak by antagonizing nodal signalling. Developmental Cell 3, 735-44.

Ref: 52

SOX3 IS A DIRECT REPRESSOR OF SNAIL AND BOTH REGULATE EMT AT GASTRULATION

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Snail genes are key factors controlling epithelial plasticity and EMT during embryonic development and in the adult. At gastrulation stages, the primitive streak (PS) forms at the posterior end of the embryo from which mesendodermal precursors delaminate upon undergoing EMT. Epithelial and neural markers are concomitantly repressed at the PS. Loss of function experiments have shown that Snail1 in the mouse and Snail2 in the chicken are crucial for the EMT process at the PS. Through gain of function experiments and analysis of cell movements with time-lapse confocal microscopy we have observed that: (1) Snail2 overexpression is able to induce an ectopic EMT in territories that would otherwise give rises to epidermal or neural cells. (2) Snail2 overexpression represses the neural marker Sox3 and the epithelial marker B-cadherin in the presumptive neural and ectodermal territories respectively. (3) Conversely, Sox3 overexpression in the PS area represses Snail2 expression and blocks EMT while expression of a dominant-negative form of Snail2 expands Sox3 expression up to the embryonic midline also blocking EMT. (4) SOX3 and SNAIL2 bound and repressed respectively Snail2 and Sox3 promoters, suggesting a direct and reciprocal negative transcriptional regulation. Altogether these data confirm the antagonistic function of Snail and SoxB genes in the patterning of the amniotes gastrula, Snail allowing mesendoderm formation while SoxB acts to maintain the epithelial character of the epiblast.

Ref: 53

A COMPARATIVE ANALYSIS OF GENE NETWORKS INVOLVED IN EMBRYONIC PLURIPOTENCY AND BLASTOCYST LINEAGE SPECIFICATION

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Morphological evolution proceeds by changing developmental programmes, what results in the appearance of novel structures and cell populations. The early stages of mammalian development are remarkably different from other vertebrates, and can be considered in that sense as an evolutionary innovation. The first decision to occur in the mouse embryo is the specification of the embryonic versus the extraembryonic lineages, giving rise to the inner cell mass on one hand, and the trophoblast on the other, a mammalian-specific character. This lineage decision is controlled by a limited set of transcription factors that include Oct4, Cdx2, Nanog and Eomes. Taking into account these facts, we are trying to identify key regulatory events leading to the stem cell phenotype present in the mammalian embryo and to understand how the distinguishing feature of mammals has arisen. Taking an evolutionary approach, we are comparing the expression of these genes, as well as those involved in the cross-talk between embryonic and extraembryonic regions of the mouse, between mouse and chicken early embryos, trying to unravel the regulatory networks involved in these early lineage determination events.

Ref: 57

THE RETINAL DETERMINATION GENE DACHSHUND CONTROLS THE DYNAMICS OF CELL SHAPE CHANGES AND CELL CYCLE EXIT DURING THE DIFFERENTIATION OF THE DROSOPHILA EYE.

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The Drosophila dachshund (dac) gene, the founder member of the DACH subfamily of nuclear proteins, forms part of the retinal determination (RD) pathway. In Drosophila, previous work indicated that dac is required for the initiation of retinal differentiation. Once it has started, lack of Dac does not preclude retinal differentiation, but the resulting retina is severely impaired. Since recent work has implicated the vertebrate Dac homologues, Dach1 and Dach2, in the control of cell proliferation and migration, we decided to investigate further the function of Dac during Drosophila eye development to better understand the biological processes controlled by this conserved gene family. Our work uncovers at least two novel roles for dac during retinal differentiation: first, it is required for the propagation of retinal differentiation, as dac-mutant cells show abnormal cell shape dynamics. Second, dac is necessary to prevent cell-cycle re-entry after the second mitotic wave, which normally marks the region where the last retinal percursors become postmitotic. This latter role of dac seems to be conserved, since different studies with mammalian normal and cancer cells demonstrate that the RD genes directly interact with cell-cycle control determinants.

Ref: 59

LOW-DENSITY LIPOPROTEINS FROM EMBRYONIC CEREBROSPINAL FLUID ARE REQUIRED FOR NEURAL DIFFERENTIATION

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Embryonic cerebrospinal fluid (E-CSF) is involved in cell survival, proliferation, and differentiation of the neuroepithelial cells. We have identified a complex pattern of proteins in chick and rat E-CSF, including apolipoproteins. Apolipoproteins play a critical role in the function of lipoproteins by interacting with receptors to deliver the lipid cargo to target cells. We characterized the chick E-CSF lipoprotein profile and analyzed the role of its lipoprotein fractions in neurogenesis. The lipoprotein pattern of E-CSF differed significantly from that of adult plasma, with a major proportion of apoB-containing lipoproteins. Further, supplementation of lipoprotein-depleted fraction with E-CSF VLDL and LDL resulted in 25% and 60%, respectively, of the neurogenesis induced by the whole E-CSF in neuroepithelium explants, whereas HDL caused the lowest induction. We investigated the potential role of E-CSF LDL in vivo by analyzing neural differentiation in the neuroepithelium of wild-type (WT) and LDL receptor-knockout (LDLR KO) mouse embryos. E-CSF lipids were mainly associated with LDL in both WT and LDLR KO, and the latter exhibited a substantial increase in LDL lipids compared with WT. Externally, LDLR KO embryos were normal but they exhibited up to 26% reduction in the number of neural differentiating cells in comparison with WT mice, although this finding was not statistically significant. These data suggest that E-CSF LDL plays a critical role during early neurogenesis.

Ref: 60

A BLOOD-CSF BARRIER FUNCTION CONTROLS EMBRYONIC CSF PROTEIN COMPOSITION AND HOMEOSTASIS DURING EARLY CNS DEVELOPMENT

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Universitat de Barcelona. Departament de Genetica

In vertebrates, early brain development takes place at the expanded anterior end of the neural tube, which is filled with embryonic cerebrospinal fluid (E-CSF), a protein-rich fluid which plays cruc ial roles in CNS development, promoting neuroepithelial stem cells survival, proliferation and neurogenesis. Two important questions are how E-CSF is manufactured and how its homeostasis controlled. We injected a number of proteins into the outflow of the heart and into the cephalic cavities in chick embryos, and examined their transport rate and route between these two embryo compartments. Our results indicate that a functional blood-CSF barrier dynamically controls E-CSF protein composition and homeostasis at the beginning of primary neurogenesis, before the formation of functional choroid plexuses, through transcellular routes, in a specific area of the brain stem lateral to the ventral midline, in particular blood vessels close to the ventral mesencephalic and prosencephalic neuroectoderm. Moreover, several water channel proteins (AQP1 and AQP4) as well as other barrier markers, such as GLUT1, Caveolin1, Kir4.1, alkalin phosphatase and γ-glutamyl transpeptidase, are also present in the same blood vessels, indicating the presence of a transient blood-CSF barrier controlling E-CSF composition from the beginning of brain embryogenesis, and thus contributing to CNS development.
References: Current Proteomics 4 (2007): 89-106; Developmental Biology, in press (2008).

Ref: 61

UNDERSTANDING THE MECHANISMS INVOLVED IN THE FORMATION AND MORPHOGENESIS OF A POLARIZED EPITHELIUM DURING DROSOPHILA EARLY EMBRYONIC DEVELOPMENT

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Instituto Gulbenkian de Ciencia. Early FlyDevelopment

Proper epithelium integrity is essential throughout animal development. We are interested in studying epithelium formation and morphogenesis. For this purpose, we took advantage of a maternal screen previously done in the laboratory of Dr. Ruth Lehmann (NYU-Medical Center, USA) where we isolated Drosophila mutants with defects in the formation of embryonic cuticle; which is a good marker for apical polarization. Initially, we isolated 6 complementation groups and 28 singleton lines. Since three of the genes isolated in our screen are known to be involved in cytoskeleton regulation (scraps) and cell-cell adhesion/polarity (DRhoGEF2, DaPKC) we are confident that our screen was successful. In order to further explore our mutant collection we decided to better analyze our putative singletons. We used two experimental approaches: 1) an adult eye clonal analysis screen, and 2) a more detailed complementation analysis of our singletons. We found that 8 singletons have defects in the development of the adult eye. Within those, we found two new complementation groups and another allele of the scraps gene. Additionally, we also identified a new complementation group without obvious defects in adult eye. Here, we present data concerning the characterization of these three newly isolated complementation groups.

Ref: 62

ROLE OF RETINOIC ACID IN PROXIMO-DISTAL LIMB PATTERNING

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Specification of fate in the PD axis is one of the questions remaining unanswered about vertebrate limb development. Based on grafting experiments and on the manipulation of the retinoic acid (RA) metabolism and signaling, we hypothesize that the amount of intracellular RA is a key parameter. Exogenously-added RA (free to diffuse) can proximalize the affinity and fate of a PZ graft to a certain extent, however, this proximalization is not seen when the PZ is grafted to an endogenous RA-producing site in the embryo. We hypothesize that endogenous RA diffusion may not contribute to RA levels in limb bud cells. In this scenario the earliest limb bud cells would contain an initial RA load derived from previous synthesis in the flank but will not receive further RA contributions. RA levels would then decrease progressively due to proliferation-driven dilution and active degradation by Cyp26 enzymes. This would provide a molecular framework underlying the progressive generation of PD fates

Ref: 63

CHEMOKINES IN VERTEBRATE LIMB DEVELOPMENT

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Chemokines are a family of small, basic, structurally related molecules that regulate migration of cells in the developing and adult organism through their binding to seven transmembrane-Gprotein-coupled receptors. Classically, they have been associated with leukocyte trafficking in host defense mechanisms but, in the recent years, they have become essential modulators in the development, homeostasis, and function of the Immune System. Furthermore, with the advance in the analysis of knock-out models, some chemokines and their receptors have turned out to be key molecules in the development of non-hematopoietic cell lineages; One example is CXCR4 and its ligand SDF1 which can regulate the migration of muscle progenitors cells that form the hypaxial muscle (Vasyutina et al. 2005). In our laboratory, we have performed a microarray experiment in which we compare proximal vs. distal genes in the mouse forelimb bud at different developmental stages and the results show that several chemokines and chemokine receptors have a dynamic expression in this part of the embryo. In this work we focus our attention on BRAK/CXCL14, a CXC chemokine that functions as a potent monocyte chemoattractant (Kurth et al. 2001) and has been considered to be and inhibitor of angiogenesis "in vitro" and "in vivo" (Shellenberger TD et al.2004). Using mouse and chicken embryo as a model we describe CXCL14 expression pattern and speculate about its possible role on some limb bud populations like tendon precursors or endothelium. These preliminary findings, along with other works in this field, support the idea of considering chemokines as general modulators involved in different developmental processes.

Ref: 64

ANALYSIS OF MOUSE KREISLER MUTANTS REVEALS NEW ROLES OF HINDBRAIN-DERIVED FGFS IN OTIC NEUROGENESIS

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Tissues surrounding the otic primordium, and more particularly the adjacent segmented hindbrain, have been implicated in specifying structures along AP and DV axes of the inner ear. We characterized the generation and axial specification of the otic neurogenic domain, and we investigated the effects of the mutation of kreisler/MafB -a gene transiently expressed in the rhombomeres 5 and 6 of the developing hindbrain- in early otic patterning and cell specification. We show that kr/kr embryos display an expansion of the otic neurogenic domain, due to defects in otic patterning. Although many reports have pointed to the role of FGF3 in otic regionalization, we provided evidence that FGF3 is not sufficient to govern this process. Neither Krox20 nor Fgf3 mutant embryos, which display a downregulation or absence of Fgf3 in r5 and r6, display ectopic neuroblasts in the otic primordium. However, double mutants Fgf3 -/- Fgf10-/- show a very similar phenotype to kr/kr embryos: they present ectopic neuroblasts along the AP and DV otic axes. Partial rescue of the kr/kr phenotype is obtained when Fgf3 or Fgf10 are ectopically expressed in the hindbrain of kr/kr mutants. These results highlight an example of how transcription factors from the hindbrain influence neurogenesis in the peripheral nervous system via FGF signaling

Ref: 65

HOMOTHORAX COUPLES THE TRANSITION FROM MULTIPOTENT CELLS TO PROGENITORS WITH CELL CYCLE REGULATION DURING DROSOPHILA EYE DEVELOPMENT

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During Drosophila eye development, multipotent cells enter a progenitor state before their terminal differentiation. Multipotent-to-progenitor transition is coupled with a synchronic amplification phase, known as “First Mitotic wave”. After the FMW, cells pause their cycle in G1 and become atonal-expressing retinal progenitors. Retinal founder cells, or R8s, are specified among these progenitors. Then, each R8 nucleates the formation of one ommatidium, or unit eye, by successive recruitment of adjacent cells. Thus, the first mitotic wave determines the overall size of the final eye by providing the pool of raw progenitors among which the R8s will be singled out. The TALE-homeodomain transcription factor homothorax (hth) has been shown to be necessary to keep the multipotent, proliferative state in the eye primordium, though the mechanisms through which hth operates are still unknown. Here we investigate how hth and its control regulate this transition. We show that hth imposes an extended G2 phase in multipotent cells through transcriptional down-regulation of string/cdc25. Repression of hth by TGF-β and hh signals relieves this control, allowing entry into mitosis. Entry into mitosis is boosted by a burst of cdc25/string transcription, induced by RDGs and allowed by the absence of hth. We propose a model in which hth is a central node in the integration of patterning signals and cell cycle regulators that control the FMW and the acquisition of a G1-arrested progenitor fate.

Ref: 66

THE ROLE OF PS1 INTEGRIN IN CVM MIGRATION

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CABD-UPO

In our lab we investigate the role of cell surface proteins such as integrins in cell migration during Drosophila embryonic development. Integrins are a family of heterodimeric transmembrane receptors composed of an a and a b subunit. The extracelullar domain of both subunits contributes to the binding site for extracellular ligands while the intracellular domains interact with different cytoplasmic proteins. We have focused in studing the role of integrins during the migration of the caudal visceral mesoderm (CVM) cells. These cells will form part of the visceral musculature of the larval digestive tube. We have found that PS1 integrin is required in CVM cells, whereas PS2 integrin is required in the visceral mesoderm for proper CVM migration. Our data reveal that normal CVM migration requires alpha PS1 subunit extracellular domain to occur while removal of PS1 subunit cytoplasmic domain results in an important delay. These results suggest that PS1 is the main integrin involved in cell migration and its specificity resides in the extracellular domain.

Ref: 68

CHICKEN PRETECTAL MOLECULAR MAP AS INTERMEDIATE ANALYTICAL STEP BETWEEN FATE AND SPECIFICATION MAPS.

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Nervous system development progresses through several specification steps that generate specific structures of the final adult form. Fate maps clarify relations between early parts of the brain and specific derivatives arisen from them, but do not resolve the molecular basis that underpins the progresive specification process. Causal explanations of pattern formation during nervous system development, which would tend to generate specification maps require in principle a complete knowledge about all gene expression patterns present at every stage analyzed. This kind of information can be understood as a “molecular map” and it would provide the conceptual framework previous to perform functional studies aiming to decode gene network activities that represent real specification events. In that sense we present here an ample molecular map of the chicken pretectal region around stages HH23-25. Our large-scale mapping of genes includes more than 25 new gene expression patterns (and 15 published in Ferran el al. 2007), centred mainly on transcription factors, because these are the main actors involved in the stabilizing and changing gene network activities. These molecular data identify the specific radial localization (ventricular, mantle or superficial strata) for every expressión pattern in each pretectal subdomain. The results agree with our published model of pretectal region molecular regionalization and suggests relevant gene network interactions to be tested in the future.

Ref: 69

DUAL REQUIREMENT OF IROQUOIS GENES DURING XENOPUS KIDNEY DEVELOPMENT

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The Iroquois (Irx) genes encode homeoproteins evolutionary conserved. We report that Xenopus genes Irx1, Irx2 and Irx3 have dynamic patterns of expression during Xenopus pronephros development. They are initially expressed during mid neurulation in broad domains extending most of the prospective pronephric teritory. This onset of expression occurs after the kidney anlage is specified but before pronephric organogenesis occurs. Later, during nephron segmentation, they become restricted to the intermediate tubule region of the proximo-distal axis. Loss- and gain-of-functions studies using specific morpholinos and inducible wild type and dominant negative constructs, clearly reveal that Irx1 and Irx3 genes have a dual requirement during pronephros development. They are initially required during neurula stage to maintain the specification and define the size of the pronephric territory and they are later required for the proper formation of the intermediate tubule. We also show that both Irx genes are activated in the pronephros by the retinoic acid signalling.

Ref: 71

COLLECTIVE MIGRATION OF NEURAL CREST CELLS IS CONTROLLED BY PLANAR CELL POLARITY (PCP) SIGNALLING.

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Cell migration is a central process in the development and maintenance of multicellular organisms, and it is also critical in pathological situations such as metastasis of cancer cells. Here, we use the Neural Crest (NC) cell as a model to study cell migration in vivo. The NC is an embryonic stem cell population that develops dorsally to the neural tube from where it migrates to almost all the regions of the embryo. By using zebrafish and Xenopus embryos expressing fluorescent proteins in the NC we have been able to perform time-lapse microscopy of migrating NC. Our observations show for the first time that NC migrates in vivo as a compact group of cells showing coherent directional migration. However this directional migration is lost in isolated NC cells. This collective migration is similar to the migration of mesodermal and cancer cells. We show that NC collective migration is dependent on the regulation of cell-cell contacts and on the localized production of cell protrusions. Furthermore, we show that this cell behaviour is controlled by the non-canonical Wnt PCP signalling and the proteoglycan Syndecan4. Thus, in addition of this novel mechanism of NC migration we ascribe a new function for the PCP signalling.

Ref: 72

STRUCTURE FUNCTION DISSECTION OF THE FRIZZLED GENE IN DROSOPHILA MELANOGASTER

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The Frizzled (Fz) family of receptors is known to act in multiple pathways throughout development. The best characterised of these is the canonical Wnt pathway. Some, though not all, Fz receptors are also known to function in a second pathway, the planar polarity (or planar cell polarity, PCP) pathway. In Drosophila melanogaster, Fz, the founder member of the family, has been shown to function in both the Wnt and planar polarity pathways. A second member of the family, Dfz2, functions in the Wnt pathway, but is unable to transduce planar polarity signals. A number of studies have used reverse genetics to analyse the structural requirements for coupling either to the Wnt or planar polarity polarity pathway (Boutros et al 2000; Rulifson et al 2000; Strapps & Tomlinson 2001), but have not led to consistent findings. To gain a better understanding of the coupling of Fz receptors to the Wnt and planar polarity pathways, we generated a library of chemically induced random point mutations in a fz transgene, which are deficient for planar polarity function. From this library we have identified a number of mutations in which the function of the planar polarity pathway is impaired, but the canonical pathway remains unaffected. We studied these mutants in more detail, and we found that stable recruitment of Dsh to the apical membrane is not essential for canonical function.

Ref: 73

DOES CELL COMPETITION FUNCTION IN MAMMALS?

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Cell competition, a process discovered in Drosophila melanogaster, occurs when cells with different metabolic activity are in contact with each other: the cells with higher metabolic activity compete with the disadvantaged neighbouring cells and win them over by inducing their apoptosis. It is thought that cell competition could be involved in the pre-tumour stages of cancer as inferred from the competitive behaviour of clones of cells over-expressing the Drosophila homologue of c-Myc in a wild-type wing imaginal disc. This competitive cell-cell interaction has also been shown to take place naturally in the adult Drosophila germline stem cells niche, possibly as a means to regulate stemness. Due to the increasing significance of the cell competition phenomenon in development and disease, it has become necessary to confirm the role of this process in mammals. To address this issue, we employ two main tools: mosaic mice created by inducing the expression of c-MycER in marked clones of cells and mice KO for a novel gene, the Drosophila homologue of which was found to be over-expressed during cell competition.

Ref: 74

OSA, A MEMBER OF THE CHROMATIN REMODELLING COMPLEX BRAHMA, IS REQUIRED FOR GENE EXPRESSION IN RESPONSE TO EGFR SIGNALLING

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Gene expression is regulated in part by protein complexes containing ATP-dependent chromatin remodelling factors of the SWI/SNF family. In Drosophila there is only one SWI/SNF protein, named Brahma, which forms the catalytic subunit of two complexes composed of different proteins. The protein Osa defines the BAP complex. We have analysed the functional requirements of Osa during Drosophila wing development, and found that osa is needed for imaginal cells growth and survival, and for the correct patterning of sensory organs and veins. Genetic interactions between osa alleles and mutations affecting the activity of the EGFR pathway suggest that one aspect of Osa is intimately related to the response to EGFR activity. Thus, loss of osa and EGFR signalling result in similar wing vein phenotypes, and Osa is required for the expression of several nuclear targets of EGFR signalling, such as Delta, rhomboid and argos. We suggest that Osa facilitates the transcriptional responses to EGFR signalling in the wing, making the regulatory regions of EGFR target genes available for both activators and repressors. The function of Osa is also shared by other members of the BAP complex, such as Snf1, Bap55, Mor and Brm, indicating that chromatin remodelling is a key component of transcriptional regulation in response to EGFR signalling.

Ref: 75

SPROUTY GENES AND TBX1 GENETICALLY INTERACT DURING PHARYNGEAL ARCH DEVELOPMENT

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Velocardiofacial or DiGeorge syndrome is associated with a 1.5-3MB microdeletion on chromosome 22q11. The Tbx1 gene, which lies within this critical region, has been implicated in the aetiology of this syndrome. Tbx1 is a T-box transcription factor and previous studies have suggested that Fgf8 expression is regulated by Tbx1. Both Tbx1-/- and Fgf8 hypomorphic embryos have defects in the formation of the caudal pharyngeal arches, phenocopying many of the defects characteristic of this syndrome such as thymus and parathyroid hypoplasia and aortic arch anomalies. These data suggest that aberrations in FGF signaling may be associated with these phenotypes. The Sprouty genes encode feedback antagonists of FGF signalling and we found that Spry1-/-;Spry2-/- embryos also have defects in many of the same organs. Interestingly, although neither Spry1-/-;Spry2-/- nor Tbx1+/- embryos have clear defects in aortic arch arteries, Spry1-/-;Spry2-/-;Tbx1+/- embryos exhibit a high incidence of these defects. No Spry1-/-;Spry2+/-, Spry1+/-;Spry2-/- or Tbx1+/- embryos examined have thymus defects, whilst combining these mutations results in an increased incidence of thymus hypoplasia and ectopia. Our results suggest that the Sprouty genes can act as genetic modifiers of 22q11 deletion syndromes and potential mechanisms to account for our observations will be discussed.

Ref: 76

PLANAR CELL POLARITY: IS FOUR-JOINTED AN ECTOKINASE?

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Epithelial cells are often coordinately polarised in the plane of the sheet; a feature known as planar cell polarity (PCP). There are, at least, two separate genetic systems responsible: The "Dachsous system" and the "Starry night system". The "Dachsous system" involves three genes, two encoding large cadherin molecules, Dachsous (Ds) and Fat (Ft) and a Golgi resident protein Four-jointed (Fj) of unknown molecular function. Fj was discovered in / Drosophila / long ago and more recently it has been implicated in PCP where it probably acts by modulating Ds and/or Ft. However little is known about its real molecular function. Here I present evidence that Fj may be a rare extracellular kinase: the sequence shows a putative kinase domain in the extracellular (Golgi-lumenal) part of the molecule, Fj binds to its likely substrates Ds and Ft, and mutations in those amino acids essential for kinase activity block function when assayed in flies.

Ref: 77

THE 11-AMINOACID LONG TARSAL-LESS PEPTIDES TRIGGER A CELL SIGNAL IN DROSOPHILA LEG DEVELOPMENT

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We have characterised the Drosophila gene tarsal-less (tal). tal is non-canonical in two aspects: firstly, it is polycistronic as a single tal mRNA contains 4 short Open Reading Frames, and secondly, these 4 sORFs encode for four related peptides of 11, 11, 12 and 32 aminoacids respectively. A single 11aa peptide fulfills tal function. tal may represent a whole new class of eukaryotic genes, as tal homologues have been found in other insects and arthropods. tal is required for the development of the tarsal region. During embryogenesis tal is necessary for proper development of ectodermal tissues undergoing morphological changes such as invaginated organs (mouthparts, trachea, hindgut) and for denticle differentiation. tal controls denticle differentiation through the regulation of the cytoskeleton independently of the denticle patterning cascade. Interestingly, tal denticle role is non-autonomous suggesting that Tal peptides may act as a cell signal or trigger a signalling mechanism. However, we do not know if the non-autonomy is a general feature of tal function, nor which genes relate to the non-autonomous effect, as no target genes have been found. We show that cell signalling is a general feature of tal function. The tal-dependent signal has a range of 2-4 cells in the legs. tal regulates the transcription of tarsal genes in a specific manner. These regulatory genetic interactions explain how distal leg patterning proceeds and the requirement for tal in this process.

Ref: 78

SEARCHING FOR NEW MOLECULAR “PIECES” OF CELL COMPETITION

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The cell competition phenomenon has been studied for a long time, without any reporter gene that could define the diseases in which cell competition could be involved. So far, we have identified some novel genes that could be the missing tools needed to distinguish what cell competition is and what it is not. One of these newly discovered genes belongs to the zinc finger transcription factor protein family. This particular feature coverts it into a very interesting target of the cell competition study, as a key to discovering other specific genes and their possible regulation. Moreover, some recent results give us data about the possible role of this new protein in the control of the mechanism that allows a wing imaginal disc to have normal surface after an aggressive cell death previously caused by cell competition. This new zinc finger protein, Caronte, could become the key of Pandora´s box that the cell competition community has long been waiting for.

Ref: 79

THE POTASSIUM CHANNEL ERG1 AS A NEW PLAYER IN THE REGULATION OF VERTEBRATE LIMB DEVELOPMENT

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To understand the role of ion dynamics during vertebrate limb development, we are studying an ERG potassium channel, which is well characterized as a key molecular component of cardiac repolarization, although other important functions have recently been suggested. In situ hybridization in chick embryos showed that erg1 is expressed at the prospective forelimb mesoderm simultaneously with tbx5, suggesting a role in limb initiation. Later, erg1 transcripts are detected in the progress zone, necrotic zones, and myogenic precursors. It is also expressed during digit patterning, at the interdigit and in the phalanxes, surrounding the epiphysis. Functional studies were done by RNAi downregulation of erg1 expression and selective blockade of ERG1 activity by antagonists. When inhibition is performed in the presumptive limb field of stage 11-12 HH embryos, resulting limbs were either truncated or smaller than control limbs, suggesting a role for this channel in cell proliferation, as also shown for cancer cells. When this is done in the interdigit of 5 day-old embryos, the autopods remained with interdigital membranes, which, together with the fact that erg is not expressed at the interdigit of duck embryos, reinforces the idea of a role also in apoptosis. Altogether, our results show that ERG1 is a new molecular player in limb development. Work supported by FCT POCTI/BCI/47972/2002, POCI/SAU-MMO/63284/2004, SFRH/BPD/29957/2006 and FP6 EU Network “Cells into Organs”

Ref: 80

EARLY EMBRYONIC PROGRAMMING OF NEURONAL LEFT/RIGHT ASYMMETRY IN C. ELEGANS

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In C. elegans, the bilaterally symmetric gustatory neuron pair ASEL/ASER displays directional asymmetric expression of several cell fate markers and senses and discriminates distinct inputs. How L/R asymmetric functional features are superimposed onto an essentially bilaterally symmetric nervous system is poorly understood. We have previously shown that the specification of ASE asymmetry is regulated by a cell-autonomous bi-stable double-negative feedback loop involving both microRNAs and transcription factors. However, it is currently unclear what biases the outcome of this bistable system.Using a combination of genetics and laser ablations to manipulate early embryonic lineages we demonstrate that the adult laterality of the ASE neurons is specified embryonically at the 4-cell stage and is dependent on the asymmetric lineage origins of ASEL and ASER. To understand the molecular nature of the link between this early embryonic symmetry-breaking event and the bi-stable feedback loop we are currently performing a genome-wide RNAi screen to uncover new players involved in the specification of ASE cell fate and we will present our latest findings. To date we have already identified a number of factors that are required for ASE specification including hlh-14/aceate-scute. We find that hlh-14 is required for the specification of both ASEL and ASER. Moreover, it is asymmetrically expressed in the embryonic ASE lineages suggesting it may be involved in establishing ASE asymmetry.

Ref: 81

INCIPIENT ADENOHYPOPHYSIS BOUNDARIES IN EARLY CHICK EMBRYOS

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Classical views distinguish that the adenohypophysis has its origin in Rathke's pouch, a deep diverticulum which extends upward from stomodaeum up to adhere to the floor of the diencephalon (Rathke, 1838; Romanoff 1960). Based on morphological and experimental studies in chick embryos, several groups proposed that the prospective adenohypophysis is derived from rostral median neuroectoderm(ANR). In contrast, some neural plate fate-map studies in the chick led to the idea that the adenohypophysis anlage is located in median extraneural domains close to the ANR at stages HH4 and HH8. A third model suggests that the prospective adenohypophysis originates through the interaction of neural and oral ectodermal tissues. In regard to resolve this issue about the primary adenohypophysial anlage, we carried out a new fate map using either injection of DiI/DiO or homotopic grafting of CFSE-fluorescently-labeled donor tissue in early chick blastoderms. The embryos were processed by in situ hibridization for neural and non-neural gene markers and inmunodetection of the CFSE-labeled cells derived from the grafts. Our results conclusively show that the prospective adenohypophysis is located in a median rostral extraneural domain, close to the prospective anterior neural ridge. The prospective adenohypophysis develops after this primordium adheres secondarily to a prospective basal plate domain of the neural tube, in contrast to previous belief that it contacts the floor plate.

Ref: 82

DROSOPHILA LIPOPHORIN RECEPTORS MEDIATE CELLULAR UPTAKE OF LIPIDS AND ARE REQUIRED FOR FEMALE FERTILITY.

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We have identified two partially redundant Drosophila proteins, Lipophorin Receptors 1 and 2, that belong to the Low Density Lipoprotein Receptor family and are essential for efficient uptake and accumulation of neutral lipids by several cell types including oocytes, oenocytes and cells of the imaginal discs. The Lipophorin Receptors mediate the endocytosis of lipophorins, lipoprotein particles that carry neutral lipids and distribute them through the body via hemolyph. Even though other receptors or mechanisms can also mediate lipophorin endocytosis, only the Lipophorin Receptors efficiently promote lipid uptake by most cell types. However, they are not required for the storage of lipids in the fat body. Flies that are mutant for the lipophorin receptors are viable but female-sterile.

Ref: 83

SMT3 IS NECESSARY FOR THE METAMORPHOSIS OF DROSOPHILA MELANOGASTER

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We have studied in vivo the role of the ubiquitin-like protein Smt3 (Sumo) during Drosophila development. We generated transgenic flies carrying the transgene UAS-smt3i to reduce smt3 mRNA levels in specific groups of cells. Low smt3 in the prothoracic gland prevents metamorphosis. RNAi knockdown larvae stop their development in their last larval stage and remain alive for up to a month. Their prothoracic glands have fewer, but larger cells than normal. They also have lower ecdysteroid titre than WT. After dietary administration of exogenous ecdysone these larvae form pupal cases, but do not proceed further in development and die. We observed that, in larvae with lower levels of smt3 the subcellular localization and expression levels of factors involved in the regulation of ecdysteroids synthesis are altered. Interestingly, their prothoracic gland cells present reduced intracellular channels and a reduced content in lipid droplets and cholesterol, which could contribute to the deficit in steroidogenesis

Ref: 84

SUBDIVISIONS OF THE AVIAN DORSAL VENTRICULAR RIDGE AND THE NOVEL CAUDO-DORSO-LATERAL CORTICOID AREA STUDIED BY MEANS DACH2 AND FOXP1 EXPRESSION PATTERNS IN THE DEVELOPING TELENCEPHALON.

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Universidad de Murcia. Anatomía Humana y Psicobiología

The telencephalon is initially divided in pallial and subpallial parts. As development advances, the pallium becomes subdivided at least in four domains, called ventral, lateral, medial and dorsal pallia (Puelles et al., 2000). The ventral and the lateral pallial domains (in a different terminology: nidopallium and mesopallium) jointly build the dorsal ventricular ridge (DVR; classical hipopallium). Using the Dach2 gene (a transcription cofactor) as a ventropallial marker (Szele et al., 2002) and the transcription factor FoxP1 (Teramitsu et al., 2004) as lateropallial marker, and following backwards development from advanced stages (HH44) to earlier ones (HH28), we resolved some controversial boundaries and recognized the so-called caudo-dorso-lateral corticoid area (CDL) as a molecularly distinct part of pallium, in agreement with the postulate presented by Puelles et al. (2007). Supported by NIH grant 1-R01-MH070370-01A2, CIBERER-U736 (FIS-Spanish Ministry of Health), and MEC (Spanish Ministry of Education) grant BFU2005-09378-C02-01 to LP. (J.E.S. is a predoctoral NIH fellow and J.L.F. is postdoctoral CIBERER fellow, all in the LP lab).

Ref: 86

STUDY OF THE NATURE OF THE DIOXIN TOXICITY USING DROSOPHILA AS A MODEL ORGANISM

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The aryl hydrocarbon receptor (Ahr) is a key component in the adaptive response to dioxins, and an intermediary of the toxic effects of 2,3,7,8-tetradichlorodibenzo-p-dioxin (TCDD). Ahr is also important in development. spineless (ss), the orthologue of Ahr in Drosophila, is a transcription factor involved in leg and antenna patterning. The Ss and Ahr proteins show high sequence conservation in the bHLH and PAS domains. In this work, we have tested the effects of expression of Ahr in Drosophila and compared them with those caused by misexpression of ss, demonstrated the ability of Ahr to rescue the ss mutant phenotype, studied the effects of Ahr on the subcellular localization of Tgo (binding partner of Ss), and described the nature of the dioxin toxicity in transgenic lines. We also have identified two Zinc-finger transcription factor of the C2H2 Kruppel-type functionally related to ss in limb patterning and eye development.

Ref: 87

ANALYSIS OF CEREBELLAR CHANGES IN SHAPE AND SIZE ALONG DEVELOPMENT BY USING GEOMETRIC MORPHOMETRICS COORDINATES

Blanco, María José / Peña-Melián A. / Bastir* M.

The cerebellar anlage is subject to a multifactorial process, with intrinsic and extrinsic factors, to acquire a complex adult morphological structure. The spectacular cortical development provokes dramatic changes in shape and size of the initial structure, that course with the formation of folia and fissures. The study of these transformations from a global point of view is the goal of the present study. For this approach, midsagittal sections of chick embryonic cerebella from stages HH36 to HH44 were used to obtain sets of landmarks analyzed by geometric morphometrics. This tool exploits not only the morphology per se, but the spatial relationships of anatomical structures in a quantitative and visual way. Preliminary results indicate that changes in the cerebellum are mainly observed at the transition between stages HH37-HH38, HH39-HH40 and HH40-HH41. The observed changes are summarized: 1)- the cavum ventricularis is progressively reduced to be converted into a cleft; 2)- centrifugal expansion of the folia, specially from HH40 onwards; 3)- fixation of the base of the fissures to the cerebellum core during the centrifugal expansion of the folia.

Ref: 88

PROPERTIES OF NEUROEPITHELIAL CELLS DURING NEURULATION IN NORMAL AND PAX3 MUTANT MICE

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During primary neurulation, the flat neural plate is transformed into a closed neural tube throughout a series of morphogenetic changes driven by a combination of intrinsic and extrinsic forces. Neuroepithelial cell properties like adhesion, polarity, movements and changes in cell shape act as intrinsic forces that determine morphogenesis of the neural tube. In particular, the formation of dorsolateral bendings in the neuroepithelium (dorsolateral hinge-points, DLHP) are crucial events for the neural tube to close in the cranial region. It has been suggested that the transcription factor Pax3 regulates cellular properties involved in the formation of DLHP, since null mutant embryos show flapping neural folds and failure of the neural tube closure in the cranial region. In this study, we analyze the expression pattern of Pax3 in relation to the formation of DLHP. The possible regulation of the cellular properties involved in the bending process is studied in the Sploth embryos (Pax3 mutants) showing exencephaly. The role of neural crest cells, as a dynamic population that affects the architecture of the neuroepithelium during neurulation in the mouse, was also investigated.

Ref: 90

IDENTIFICATION AND VALIDATION OF IGF-I TARGET GENES IN MOUSE LUNG PRENATAL MATURATION

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Insulin-like Growth Factor-I (IGF-I) is a key factor during lung development. IGF-I deficiency in the neonatal mouse causes respiratory failure due to severe lung hypoplasia, collapsed alveolar spaces and altered expression of pulmonary markers. Prenatal lungs of IGF-I-deficient embryos displayed altered alveolar epithelium and capillary remodelling. To identify new genes targeted by IGF-I in lung maturation we analyzed RNA differential expression in Igf-I -/- lungs at embryonic stage (E) 18.5 using Affymetrix arrays. Downregulated genes in Igf-I -/- lungs corresponded to vascular development, morphogenesis and cellular growth biological functions and to MAP-kinase, Wnt and cell-adhesion molecular pathways. In accordance, ECM was found des-organized and alveolar capillaries abnormally dilated. It was striking to find up-regulation of immunity and cell defense related genes that were corroborated with the increased presence of granulocytes and expression of CD3 antigen, revealing an “inflammatory phenotype” in the Igf-I-/- lungs. Among the lung regulatory genes with validated expression at the protein level stand out the transcription factors Nfib (up-regulated) and KLf2 and Egr-1 (both down-regulated). Addition of IGF-I to E16,5 Igf-I -/- lung explants in culture induced alveolar morphogenesis and increased the expression of Nfib, Klf2 and Cyr61 proteins. Our results demonstrate the functional implication of IGF-I in lung maturation by regulating specific target genes.

Ref: 92

ROLE OF PITX2C DURING MYOGENESIS

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Pitx2 is a member of the bicoid family of homeodomain transcription factors that plays a relevant role in morphogenesis. Pitx2 expression is maintained in Pax3 positive cells that have completed migration at the proximal limb bud. We have previously documented that overexpression Pitx2c -isoform in undifferentiated myoblasts resulted in upregulation of cell cycle genes while it arrests differentiation into mature myotubes by upregulating Pax3 and downregulating myogenic transcription factors such as MyoD and myogenin. These observations indicate that c-isoform of Pitx2 plays a pivotal role modulating proliferation vs differentiationin myoblasts. We report herein that the Pitx2c effects in this cell line are dose-dependent. Therefore, we have determined at which doses of transfection Pitx2c began to inhibit myocyte differentiation and myoyube formation, coinciding with cell cycle genes (Cyclin D1 and Cyclin D2) as well as Pax3 upregulation, whereas myogenic regulatory factors (MyoD, Myogenin) become down-regulated at low doses of Pitx2c-transfection before the onset of changes in the phenotype. These data suggests that regulation of genes involved in the maintenance of proliferative stages in myoblast and genes involved in the onset of differentiation (MyoD and Myogenin) require different Pitx2c doses. Interestingly, we found Pax7 down-regulation after low doses of Pitx2c-transfection coinciding with MyoD and Myogenin down-regulation suggesting that Pitx2 could play a role modulating Pax3/7 function in adult satellite cells. These findings may have future applications in the regeneration of skeletal muscle using myoblast theraphy.

Ref: 93

THE NESTHOCKER MUTATION REVEALS NOVEL REQUIREMENTS OF AMINO-SUGAR SYNTHESIS FOR FGF-SIGNALING IN DROSOPHILA

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Mesoderm migration in the Drosophila gastrula is dependent on FGF signalling. In a genetic screen for maternal effect genes, we identified nesthocker (nst), a mutation that phenocopies the FGF-receptor heartless (htl) mesoderm migration defect. htl-dependent-MAPK activation at the leading edge of migrating mesodermal cells is absent in nstmat,zyg embryos indicating that Nst is essential for Htl-signalling. Genetic epistasis experiments revealed that Nst function is required downstream of the Htl receptor. We cloned the nst gene and found that it is allelic to CG10627 encoding GlcNAc (N-acetyl phosphoglucosamine) mutase. The nst16923 mutant allele encodes a catalytically inactive enzyme. Furthermore, a genomic construct of CG10627 rescued nstmat,zyg mutant embryos to viability. The level of UDP-N-acetyl hexosamines (UDP-HexNAc), a pool of downstream metabolites of GlcNAc mutase is reduced in nstmat,zyg embryos. The potential pathways that might be affected by reduced UDP-HexNAc levels are N-/O-glycosylation, O-GlcNAcylation, GPI-anchor formation and proteoglycan biosynthesis. In nst mutants, other heparansulfate proteoglycan -dependant signalling pathways and GPI-anchor formation are not compromised. We are currently investigating which other pathway is most sensitive to reduced UDP-HexNAc levels towards understanding the glyco-biochemical requirements for FGF signalling during Drosophila mesoderm migration.

Ref: 94

DEADEASY: AUTOMATIC CELL COUNTING IN VIVO IN DROSOPHILA

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Knowledge of cell number in vivo is relevant for understanding development and diseases, such as cancer and neurodegeneration. However, counting cells in vivo has been either unfeasible or carried out manually. Here, we have developed DeadEasy software for the automatic quantification in vivo in Drosophila of apoptotic and mitotic cells, neuronal and glial nuclei. DeadEasy software employs image filtering and mathematical morphology techniques. It counts cell number from a confocal stack of images, by analysing cells in 2D and 3D throughout the whole stack. Segmentation techniques are applied to each image of a stack avoiding corrections to intensity attenuation. 2D techniques are employed while grey-scales images are processed and 3D techniques are only used after binarisation, gaining speed in the process. Quantification is automatic, accurate, objective and very fast, enabling reliable comparisons of multiple specimens of diverse genotypes. We show here the application of DeadEasy for counting apoptotic and mitotic cells and glia in Drosophila embryos and larvae, in different genetic backgrounds. DeadEasy enables an unprecendent quantitative analysis of growth during development providing new insights into the genetics underlying cell number control. DeadEasy programmes have been developed as freely available ImageJ plug-ins.

Ref: 96

IDENTIFICATION OF NEW MECHANISMS REGULATING THE EXPRESION OF THE RETINAL DETERMINATION GENE EYES ABSENT.

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Eyes absent (Eya) is one of the transcription factors involved in Drosophila eye specification and development. Eya expression is positively regulated by Decapentaplegic (Dpp) and Hedgehog (Hh), and negatively regulated by Wingless (Wg) signaling pathways. When expressed ectopically, Eya protein can induce ectopic eyes. However, this ability is heavily dependent on the context. To investigate the basis of this behaviour, we used different drivers to express Eya in Drosophila imaginal discs, using target genes and ectopic eye formation as readouts for Eya activity. Regardless of the driver used, Eya protein and Eya-induced targets were detected only at specific areas of the imaginal discs where endogenous Dpp signalling is high. In addition, Eya protein levels are higher and the frequency of ectopic eyes is increased if the Dpp signaling is activated concomitantly to Eya expression, while Eya protein is almost undetectable and its effects negligible when Wg signaling is activated in Eya expressing cells. Therefore, there must be a mechanism repressed by Dpp signalling and induced by Wg signaling which governs both Eya protein stability and activity. Preliminary results seem to rule out proteasome mediated Eya degradation, suggesting that Eya expression might be regulated through the degradation or translational repression of eya's mRNA. We will provide data on different experiments designed to address this question.

Ref: 97

DROSOPHILA NEUROTROPHINS REVEAL A COMMON MECHANISM FOR NERVOUS SYSTEM FORMATION

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It has long been thought that neurotrophic factors are missing from Drosophila. Neurotrophins (NTs) are the main vertebrate signalling molecules that link nervous system structure and function: they regulate neuronal survival, targeting, synaptic plasticity, memory and cognition. Impaired NT function underlies human psychiatric disorders. The presumed lack of NTs in fruit-flies had three relevant consequences: 1) it precluded in vivo genetic and cellular analyses of NT signalling in Drosophila. 2) Presumed mechanistic differences between Drosophila and vertebrates created a void in studies of neuronal function in fruit-flies. 3) It suggested that invertebrate nervous systems differ from those of vertebrates, being "hard-wired". Accordingly, NTs are a vertebrate innovation that enabled the emergence of brain complexity during evolution. There is evidence for the existence of neurotrophic factors in Drosophila, but to date, none had been found. Here we show the identification of the first NT in flies, Drosophila Neurotrophin (DNT1), structurally related to all NTs and highly conserved in insects. DNT1 maintains neuronal survival in a target dependent fashion and enables axonal targeting. We show that there is a further fly NT, DNT2, and that Spz also has neurotrophic function. This work provides a missing link between aspects of neuronal function in flies and vertebrates, and it opens the opportunity to use Drosophila as an in vivo context to study NT function and signalling.

Ref: 98

DIFFERENTIAL REGULATION OF SISTER CHROMATID COHESION IN FEMALE DROSOPHILA GERM LINE STEM CELLS.

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During mitosis different types of cells can have differential requirements for the correct segregation of chromosomes. We isolated two new alleles of the separation anxiety gene (san). san was previously described in Drosophila and in humans to be required for centromeric sister chromatid cohesion (Hou et al., 2007; Williams et al., 2003). Our work confirms and expands the observation that san is required in vivo for normal mitosis of different types of somatic cells. In addition, our work now suggests that san is also important for the correct condensation of chromosomes, implying a more general function of this acetyltransferase. Surprisingly, during oogenesis we cannot detect any obvious mitotic phenotypes in germ line stem cells (GSCs) mutant for san. We hypothesize that female GSCs have differential requirements for sister chromatid cohesion.

Ref: 102

USING ENHANCERS TO STUDY GENE INTERACTIONS DURING EARLY INNER EAR DEVELOPMENT

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The inner ear is one of the major sensory organs of the head and is responsible for balance and sound detection in vertebrates. The otic placode generates the different cell types required for sensory function. The first developmental event in the otic placode is the specification of a neural competent domain. We have been characterizing the transcription factors and signalling pathways that regulate neurosensory development, such as patterning genes as Sox3, Tbx1, Lmx1b, FGF and Notch signalling. Gain and loss of functional experiments are starting to elucidate the genetic interactions between these transcription factors and pathways. Our current model points towards a pivotal role of Sox3 in otic neural commitment as overexpression of Sox3 leaded to ectopic neuronal precursors. The restriction of Sox3 activity to the anterior otic region was regulated by local FGF signaling. In particular, our data suggests that a local FGF8-FGF10 cascade drives ectodermal cells into otic neural fate independently of the early process of otic induction. By using quantitative data from real-time PCR, a computational model of otic neural specification is being built. In order to further expand the knowledge on the gene regulatory network working during this process, we have begun the search and analysis of specific HCNR/enhancers of neurosensory locus (Sox3,Lmx1b,FGF8,FGF10,Tbx1). Our aim is to elucidate a common signature of composite TFBS that may co-direct this developmental process.

Ref: 103

THE ROLE OF MULTICELLULAR ROSETTES DURING ANTERIOR VISCERAL ENDODERM MIGRATION IN THE MOUSE EMBRYO

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In the mouse embryo, anteroposterior patterning is specified 5.5 days after fertilization by a specialized extra-embryonic epithelial tissue called anterior visceral endoderm (AVE). The AVE is essential for the proper orientation of the anterior-posterior axis of the embryo. The AVE migrates from the distal tip of the egg cylinder to the future anterior where it instructs the adjacent epiblast (from which the foetus derives) to acquire anterior pattern. Embryos mutant for Cripto or Otx2 fail to effect this movement, leading to a mispatterned epiblast. Recently, Blankenship et al. showed that germband extension in the Drosophila embryo is mediated by multicellular structures called rosettes. Rosettes are groupings of five or more cells meeting at a point, and are a distinct intermediary in convergent extension movements, that require planar polarity within the epithelium in order to be organized properly. We show that rosettes exist in the visceral endoderm of living and fixed E5.5 mouse embryos and increase in number during AVE migration. We also show mutant embryos with impaired AVE migration show a statistically significant decrease in the number of rosettes as compared to wild-type embryos. These findings point to an involvement of planar polarity and convergent extension in AVE migration.

Ref: 104

APOPTOSIS REQUIREMENT DURING THE EVERSION OF THE DROSOPHILA DISCS.

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Ecdysone is the main hormone that drives the metamorphosis of Drosophila. The raise in ecdysone titter produces the eversion of the discs as well as the death of many larval tissues. We have developed an ex vivo culture system that allows us to image and follow the eversion of the disc. This high-resolution technique has shown the necessity of ecdysone-drive apoptosis during different moments in the eversion. The opening of the stalk and the disintegration of the peripodial membrane required the apoptosis. Apoptosis if not essential seems to be quite important for the right eversion of the disc. We have analyzed genetically this requirements as well as the relation between Myosin and cell death during this process.

Ref: 105

SOCS36E SPECIFICALLY INTERFERES WITH SEVENLESS SIGNALING DURING DROSOPHILA EYE DEVELOPMENT

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During the development of multicellular organisms the fate of individual cells is specified with great precision and reproducibility. Although classical genetic approaches led to the identification of many of the signaling pathways contributing to cell fate specification, they have provided little insight into the mechanisms that ensure robustness and reproducibility. We have used the specification of the R7 photoreceptor cells controlled by the Sevenless (Sev) pathway to screen for modulators of pathway activity and to uncover the mechanisms underlying the robustness of cell fate decisions. Here we provide genetic evidence that the Drosophila SOCS36E acts as an attenuator of Sev signaling. Overexpression of Socs36E strongly suppresses the specification of extra R7 photoreceptor cells in response to constitutive activation of Sev, and loss of Socs36E function suppresses the loss of R7 cells when Sev activity is impaired. In a wild-type background, however, loss and gain of Socs36E function exhibits little effect on R7 specification. We show that Socs36E expression is suppressed by high Sev pathway activity. Genetic evidence indicates that the presence of SOCS36E in cells with low Sev activity competes with the SH2 adaptor Drk for binding to the activated receptor and thus prevents precocious activation of the Ras/MAPK cascade. Therefore, Socs36E constitutes a novel partially redundant feedback mechanism that contributes to the robustness of R7 specification.

Ref: 107

WOUND HEALING: THE TRANSCRIPTOME

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Efficient wound healing, including clotting and subsequent re-epithelisation, is essential for animals ranging from insects to mammals to recover from epithelial injury. Genes involved in wound healing are conserved through phylogeny and therefore, Drosophila may be a useful in vivo model system to identify genes necessary during this process. To define the transcriptome programme, identify pathways and genes involved in wound healing in Drosophila imaginal discs, we have carried out genome-wide expression profiling on imaginal disc cells actively involved in healing (JNK-activity positive) versus their non-engaged siblings. Data processing based on GO terms, comparison with published genome-wide data and clustering analysis have allowed us to identify a set of potential wound healing specific genes and to define specific co-expressed gene families participating in the healing process. Besides identifying new genes, we have functionally tested many of their gene products by genetic interference and overexpression in two different processes, the fusion of imaginal discs during metamorphosis and wound healing in cultured discs. This non-saturated analysis has identified a number of genes whose change in expression levels and functionality is significant to understand the factors that influence morphogenetic plasticity and tissue repair.

Ref: 108

ORIENTED CELL DIVISION DURING ZEBRAFISH GASTRULATION

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Oriented cell division is thought to play an important role in tissue morphogenesis throughout development. During zebrafish gastrulation, ectoderm progenitors divide along the anterior-posterior (AP) axis thereby contributing to the AP-extension of the embryonic body axis. Wnt/planar cell polarity (Wnt/PCP) signaling has been shown to be required for this AP cell division orientation. However, the mechanisms by which Wnt/PCP functions in this process, and whether other signaling pathways are also involved, are not yet fully understood. To systematically analyze which signaling pathways control cell division orientation during gastrulation, we monitored and quantified cell divisions in embryos mutant for the Wnt-, BMP- and Nodal- signaling pathways. Consistent with previous observations, we found that ectoderm cell division orientation is defective in Wnt/PCP mutant embryos. Furthermore, we observed that embryos with ectopic BMP signaling show aberrant orientation of ectoderm cell divisions similar to Wnt/PCP mutants. We are currently analyzing how the Wnt/PCP and BMP signaling pathways interact to orient cell divisions during gastrulation.

Ref: 109

ROLE OF ABC TRANSPORTERS IN THE EXPORT OF A LIPID MODIFIED GERM CELL ATTRACTANT

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In most organisms, germ cells migrating to the gonad through different embryonic tissues are oriented by repellent and attractive cues expressed along their path. In Drosophila the last step of migration is guided by the enzyme Hmgcr (HMGCo-A reductase) which controls a rate-limiting step for the production of geranyl moieties. In S. cerevisiae, the small peptide a-factor is produced from a precursor, prenylated and then exported by an ABC transporter to guide the fusion of mating yeast during sexual reproduction. We had postulated, therefore, that Hmgcr might be required for the prenylation of a small, secreted peptide which attracts germ cells to the somatic gonadal precursors (SGPs). Amazingly, we found by genetic analysis that the same peptide export and prenylation pathway utilized in yeast is also required in the mesoderm in Drosophila for germ cell association with the SGPs. We extended these results by developing a new transwell migration assay which demonstrated that germ cells move towards a secreted attractant and by using RNAi that the production of this attractant is dependent on hmgcr and ABC transporters. Our results support the idea that sexual reproduction from yeast to Drosophila requires a prenylated guidance peptide for proper germ cell function.

Ref: 110

MAINTENANCE OF MAPK SIGNALING REGULATES INCISOR DEVELOPMENT

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The MEK/ERK cascade is related to mechanisms that control cell cycling and cell survival. However, the roles of mitogen-activated protein kinase cascades in incisor remain to be determined. In this study, we intensively investigated epithelial patterning and tooth growth via MAPK signaling in the mouse incisor. In addition, we used anisomycin, a protein synthesis inhibitor that activates MAP kinases (MAPKs), and U0126, a MEK inhibitor which blocks ERK1/2 phosphorylation, to examine the role of MAPKs in incisor development of mice. Also we performed immunohistochemistry and in vitro culture. We found pERK and pMEK localization in developing incisor tooth germs and failure of Anisomycin and U0162 to produce incisor development. In addition, immunohistochemistry staining showed that anisomycin stimulated the phosphorylation of ERK1/2 and that the phosphorylations were blocked by the U0126. In order to check up-stream and down-stream of MAPK, we performed reverse transcriptase-PCR analysis with RNA isolated from tissue treated with Anisomycin and U0126 and cultured in vitro. These findings suggest that MAPK signaling plays an important role in incisor development and morphogenesis.

Ref: 111

BMP4 SIGNALING REGULATES HERS FORMATION DURING TOOTH ROOT DEVELOPMENT

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Hertwig's epithelial root sheath performs an important function in the formation of the tooth root, yet the developmental mechanisms which control HERS growth and differentiation remain to be thoroughly elucidated. Bone morphogenetic protein 4, which is secreted by mesenchymal cells, acts on the dental epithelium as a regulator of cell differentiation during crown formation. In this study, in an effort to determine whether BMP4 specifically regulates the development of HERS in the dental epithelium, localizations of BMP4, BMP receptor-IB and -II were assessed during molar root formation in the mouse. HERS cells were shown to express the BMP receptors -IB and -II. BMP4 positive cells were detected in the dental papillae around HERS, suggesting that BMP4 participates in HERS formation. Besides, BMP4-, NOGGIN- and PBS- beads were implanted into the pulp cavity under culture condition, and the length of HERS was evaluated with regard to the proliferating cells. After 48 hours, the HERS elongation was significantly shorter in the BMP4-treated group. In addition, proliferative cell nuclear antigens were detectable only in the NOGGIN and PBS-treated groups. These findings demonstrate that mesenchymally-expressed BMP4 regulates HERS development by preventing elongation and maintaining cell proliferation. BMP4 may, therefore, prove useful in a variety of tissue engineering applications, as a root formation regulatory agent.

Ref: 112

DYNAMIC EXTRACELLULAR ION FLUXES ARE INVOLVED IN FIN REGENERATION IN ZEBRAFISH

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The specific ion nature of the electric currents that accompany wound healing and tissue regeneration remains unknown as well as the role of cellular ion dynamics during the regeneration process and the molecular signalling pathway that transduces electric cues into cellular responses. We use zebrafish caudal fin regeneration as a model to unveil the specific ionic composition of the currents associated with wound healing and regeneration. We have developed a non-invasive Ion-Specific Scanning Microprobe setup to measure specific extracellular ion fluxes in live adult fish at several time points during fin regeneration. This technique measures ionic concentration differences between one point closer to and one farther away from the tissue (30-50 μm); these values are then converted into fluxes (pmol.cm-2.sec-1) giving an output on weather a specific ion in entering (influx) or leaving (efflux) the tissue. Our data suggests a role for K+ only during wound closure. H+ efflux is triggered during this process and maintained throughout regeneration. Other ions such as Ca2+, Na+ and Cl- are also under study. We are presently dissecting the functional role of H+ dynamics using both genetic and pharmacological approaches coupled with advanced pH imaging using genetically-encoded pH probes. This work is supported by the European Network of Excellence "Cells into Organs" and grants from FCT. ACC and JRL are supported by FCT fellowships; JFM acknowledges IEFP fellowship.

Ref: 113

HOXD REGULATION AND THE EVOLUTIONARY TRANSITION FROM FISH FINS TO TETRAPOD LIMBS.

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The fossil record suggests that fish fins suffered sequential transformations, giving rise to limbs close to the origin of tetrapods. However, the molecular mechanisms that underlie this evolutionary transition remain largely unclear. HoxD genes were proposed to be involved in the origin of digits, the main innovation of the tetrapod limb. In this group of organisms, these genes are re-expressed in the presumptive region of the digits due to the activation of a second wave of transcription in the 5' end of the HoxD cluster. This new wave of expression may have been caused by the acquisition of new regulatory modules ("cis" evolution hypothesis) and/or to the recruitment of new transcription factors to pre-existent 5' HoxD gene enhancers ("trans" evolution hypothesis). To test these hypotheses, we are currently testing the enhancer activity of highly conserved non-coding regions upstream of the HoxD cluster that may have caused the activation of this second wave of 5' HoxD gene transcription during evolution. The implications of our results for the fin to limb transition will be discussed.

Ref: 115

A FIBRONECTIN MATRIX IS ESSENTIAL TO REGULATE THE CELL REARRANGEMENTS DRIVING SOMITE FORMATION

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Tremendous advances have been made to comprehend the temporal and spatial regulation of somitogenesis. However, the morphogenic events that drive somite formation remain elusive. Until recently two distinct models persisted, one advocating the role of an ectoderm-derived factor, and another proposing fibronectin as crucial in morphological somite formation. We recently provided new evidence that support and fuse the two models (Rifes et al., Development 134: 3155, 2007) We showed that the enzymatic treatments used to remove the ectoderm, also remove fibronectin. The extensive fibrillar fibronectin matrix surrounding the presomitic mesoderm is assembled in cooperation with the overlying ectoderm, which serves as the main producer of Fn1 transcripts. Furthermore, when we inhibit fibronectin matrix assembly, even in the presence of ectoderm and all surrounding tissues, somite formation is impaired. In this study, we show that the inhibition of fibronectin matrix assembly does not alter the molecular identity of the paraxial mesoderm, since Paraxis and Tbx6 are correctly expressed. Rather, the inhibition of fibronectin fibrillogenesis leads to deficient cell arrangements, in that cells are less elongated and do not display the typical N-cadherin enrichment. Thus, we conclude that a fibronectin matrix provides cues that regulate the cell shape changes driving somite formation.

Ref: 116

REGULATION OF CELL POLARITY IN CONVERGENT EXTENSION

Panousopoulou, Eleni
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Deuterostome primary body axis elongation is driven by a narrowing and lengthening of the chordamesoderm during gastrulation. In Xenopus laevis, this convergent extension, is achieved by trunk cells that initially protrude lamellipodia in random directions before they become elongated and extend lamellae in the mediolateral direction. Traction on mediolaterally adjacent cells drives intercalation, converging towards the dorsal midline of the embryo. Planar cell polarity (PCP) non-canonical Wnt pathway proteins are required for this convergent extension and we have undertaken a detailed study of in vivo intracellular localization of one such protein, Dishevelled, as well as another polarity protein, with a known role in epithelial apicobasal polarity, PAR-1. We find that both polarity proteins are enriched at mediolateral protrusions of intercalating cells. Before convergent extension commences, the mesoderm is an epithelial-like bilayer surrounded by fibronectin and disruption of this structure impairs correct orientation of intercalation. This suggests that apicobasal polarization is also critical. We will therefore report the results of a study looking in detail at the localization of apicobasal polarity proteins, including PAR-1, at these stages.

Ref: 117

NEW INSIGHTS IN THE PROCESS OF VERTEBRATE SEGMENTATION

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Somitogenesis is the first overt sign of segmentation in the vertebrate embryo. Somites give rise to the vertebral column, the skeletal musculature and dermis. Somites are made sequentially during axis elongation, from two rods of presomitic mesoderm (PSM) at the caudal end of the embryo. Cells at the rostral end of each PSM bud off with a remarkable periodicity as an epithelial sphere of cells to form the new somite. In chick and mouse a somite pair forms every 90 and 120 minutes, respectively. It is widely accepted this process is regulated by the segmentation clock that drives periodic expression of a number of clock genes in the PSM. Expression of these clock genes appears as a wave of transcription that sweeps across the PSM caudorostrally in a cyclical fashion with a periodicity that matches somite formation. The majority of clock genes identified so far belong to the Notch pathway. Studies over the last ten years investigating the function of clock genes have implied an essential role for Notch, Wnt and recently FGF in the mechanism of the clock. We will present our recent data providing new insights into the segmentation clock using Lfng and Hes7 null mouse embryos.

Ref: 129

IN VIVO GENETIC STUDY OF THE DEVELOPMENT OF THE DROSOPHILA EMBRYONIC MYOTENDINOUS JUNCTION

Estrada, Beatriz
Cabd-Univ.Pablo Olavide. Desarrollo invertebrados

The molecular mechanisms underlying muscle guidance and formation of myotendinous junctions are poorly understood both in vertebrates and Drosophila. I am using the development of the embryonic Drosophila myotendinous junction as an in vivo model system to study these mechanisms. I am developing an in vivo high resolution microscopic assay to visualize myotendinous junction development in real time both in wild type and in different genetic backgrounds. One of these backgrounds being mutant muscles for the gene perdido, (perd), which we recently identified to be essential for the formation of proper muscle projections and stable attachments to the epidermal tendon cells. Perd encodes a conserved single-pass transmembrane cell adhesion protein that contains laminin globular domains and a small intracellular domain with a C-terminal PDZ-binding consensus sequence. We proposed that Perd regulates projection of myotube processes and subsequent differentiation of the myotendinous junction by priming formation of a protein complex through its interaction with two proteins. Intracellularly, with the Glutamate receptor interacting protein (Grip), and extracellularly, by transient engagement with the tendon cell-expressed laminin binding αPS1-βPS integrin. I am currently investigating the interaction between perd and mew to test this hypothesis.

Ref: 130

SPROUTY GENE FUNCTION IS REQUIRED FOR DEVELOPMENT OF THE SENSORY CRANIAL NERVES

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The twelve cranial nerves are the sensory and motor nerves of the head, heart and gut. The sensory cranial nerves are derived from the late migrating neural crest cells and regions of ectodermal thickenings called placodes, whereas the motor cranial nerves originate in the neural tube. Fibroblast Growth Factors (Fgfs) have been implicated in olfactory, epibranchial and otic placode development, as well as neural crest migration. The Sprouty (Spry) gene family encodes feedback antagonists of Fgf signalling. At E8.75 Spry1, 2 and 4 are expressed transiently in the region of the developing epibranchial placodes. Embryos lacking both Spry1 and Spry2 exhibit abnormal morphology in the proximal and/or distal regions of the cranial nerves III, V, IX and X. Sensory cranial ganglia in neural crest-specific conditional Spry1;2 double knockout (Wnt1cre;Spry1flox/-;2flox/-) were normal, suggesting that the sprouty genes are required for normal placodal development. This hypothesis is strengthened by the observation that the expression of early neuronal markers is altered in some placodes at E9.5. Interestingly, although the formation of certain placodes has been shown to be dependent on endodermal signals, no effect on endodermal expression of Bmp7 was observed at E9.5.

Ref: 132

RNA TURNOVER AND ITS ROLE IN DEVELOPMENTAL PROCESSES IN DROSOPHILA.

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The exoribonuclease pacman (Xrn1) is highly conserved in all eukaryotes and has been shown to be involved in many cellular processes including RNA interference and regulation of gene expression via micro-RNAs. Pacman and its homologues have recently been shown to be located in cytoplasmic particles, termed P-bodies, where localised translational repression and degradation can take place. We have constructed a number of mutations in pacman by P-element excision and characterised the resulting phenotypes. The most striking phenotype observed is a defect in thorax formation such that the two halves of the thorax on the dorsal side do not join together correctly. This epithelial cell M.V.movement is very similar to that seen in dorsal closure in Drosophila, ventral enclosure in C. elegans and wound healing in humans. We have also tested whether pacman is involved in the wound healing process in Drosophila. For all or our pacman mutants tested, the survival rate was lower after wounding, even though the rates of clotting were identical to controls. These results suggest that pacman/xrn-1 targets a specific subset of mRNAs that encode proteins involved in cell shape change or adhesion. Our analysis of the possible targets of the pacman ribonuclease will be presented.

Ref: 133

THE PHYLOGENY OF THE SNAIL GENE SUPERFAMILY

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The Snail gene superfamily encodes transcription factors of the zinc finger class, containing a SNAG domain at the N-terminal end. This superfamily is clearly separated from other SNAG-containing zinc finger transcription factors, and can be divided into two families: Snail and Scratch. Snail genes play crucial roles in the induction of morphogenetic movements, such as the formation of mesoderm or the neural crest, while Scratch genes are exclusively expressed in the nervous system. We have searched for members of this superfamily in public databases and data-mined the genomes representative of the major metazoan clades, from Cnidaria to Vertebrates, and isolated more than 20 new members making a total of 150 genes identified to date. Using different phylogenetic methods, we have reconstructed its evolutionary history and found that: (i) there is one Snail and one Scratch family member in Cnidaria and therefore the proposed duplication that originated the two families must have occurred in the metazoan ancestor; (ii) the founding member of the superfamily, Drosophila snail, is the result of a genus-specific tandem duplication, as all other insect genomes analyzed have a single family member more similar to escargot than to snail, and closer to vertebrate Snail genes than to Drosophila snail itself; (iii) the snail-like retrogene found in the human genome is primate-specific, and located within an area rich in repetitive elements that may justify its retrotransposition.

Ref: 134

FUNCTIONAL ANALYSIS OF SOX5 DURING THE DEVELOPMENT OF THE VERTEBRATE SPINAL CORD

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Instituto Cajal. Neurobiologia Celular, Molecular y del Desarrollo

The Sox family of transcription factors participates in a variety of processes during central (CNS) and peripheral (PNS) nervous system development. Amongst other functions, they are involved in neuroectodermal competence, self-renewal and pluripotency of neural stem-cells, macroglial cells differentiation and in the specification and maintenance of neural crest cells (Wegner and Stolt, 2005). We have established that Sox5, one of the SoxD group members, is involved in the specification and migration of cephalic neural crest cells (Perez-Alcala et al., 2004) and possibly in the specification of the cells of glial lineage in the cranial ganglia (Morales et al., 2007). However, the role of Sox5 during spinal cord neuronal development has not been explored. Results from our laboratory have shown that Sox5 is expressed in discrete progenitor domains of the developing chick neural tube and Sox5 expression is switch off as differentiation progresses. In gain of function experiments, Sox5 maintained expression cause cell cycle withdrawal, but the postmitotic cells fail to complete the full program of differentiation and enter apoptosis. Conversely, lack of Sox5 function keep cells proliferating through elevated CyclinD1 levels and cause a decrease in neuronal differentiation. The integration of Sox5 function in the signalling pathways involved in keeping the proliferation/ differentiation balance in the neural tube will be discussed.

Ref: 135

IDENTIFICATION OF POU3F4 REGULATORY ELEMENTS AND THEIR DIRECT RELATIONSHIP WITH DEAFNESS TYPE 3 (DFN3)

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During early inner ear development several complex cell-cell interactions take place. These interactions are controlled by inductive or inhibitory regulatory signals from surrounding tissues that are interpreted by different transcription factors. One of these factors is Pou3f4, which is expressed and required during inner ear development. Different molecular and genetic studies have shown that both coding and regulatory mutations at the Pou3f4 loci are associated with a X-linked nonsyndromic deafness type 3, DFN3. In our work, we aimed to identify cis-regulatory elements that control Pou3f4 expression in otic vesicle. For that purpose, we have use transgenic experiments in Xenopus laevis and Danio rerio (zebrafish) to analyze the enhancer activity of highly conserved non-coding regions located within 1 Mb upstream of the Pou3f4 coding region. We have found several regulatory sequences in this genomic interval that promote expression of a reporter gene (GFP) in the developing inner ear. Although this enhancer screen was done with Xenopus genomic DNA, we have demonstrated that the human homologous sequences promote expression in the same domains when tested in Xenopus or zebrafish transgenic assays. The implications of our results will be discussed in this report.

Ref: 136

TSH AND THE EXD/HTH COMPLEX BLOCK THE ACTIVITY OF ABDB TO FORM POSTERIOR SPIRACLES

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The Drosophila Hox transcription factor AbdB induces the formation of the posterior spiracles in the eighth abdominal segment (A8) through the activation of its targets. There are two different Abd-B isoforms, the morphogenetic (m) and the regulatory (r) isoform. AbdBm is preferentially expressed in A8 where it is responsible for posterior spiracles formation. In contrast, AbdBr is expressed in two segments lacking posterior spiracles, the A9 and A10 segments, where it represses the expression of AbdBm. Although these observations suggest that Abd-Br lacks morphogenetic capacity, various authors have proven otherwise. We have reanalysed both AbdB isoforms to find out what is responsible for their differential function. We have found evidence that Tsh and the Exd/Hth complex are capable to compete with the activity of both AbdB isoforms. We find that as AbdBm is able to repress the transcription of tsh, exd and hth, it can prevent the repression exerted by them. AbdBr is less efficient in repressing Tsh, Exd and Hth expression thus explaining the different morphogenetic capacity of both proteins. Although we do not know the molecular mechanism by which Tsh and the Exd/Hth complex block AbdB transcriptional activity. It seems that this repression does not require the action of more anterior Hox proteins. We believe that Tsh and the Exd/Hth complex block the cis regulatory regions of AbdB target genes interfering with AbdB transcriptional activity.

Ref: 138

FUNCTIONAL CHARACTERIZATION OF FEZ (FOREBRAIN EMBRYONIC ZINC-FINGER) IN DROSOPHILA MELANOGASTER.

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CABD

Developmental genetic studies suggest that the embryonic vertebrate brain consists in a forebrain/ midbrain, a hindbrain and an intervening midbrain/hindbrain boundary region. Fez is a zinc-finger gene originally isolated as a forebrain- and OSN-specific gene in Xenopus. Identification of a homolog (Fez-like), and of orthologs in zebrafish, mouse and human, revealed that there are two highly related genes, Fez and Fez-like (Fezl). In Drosophila the embryonic brain also has a tripartite ground plan which consists of protocerebrum, deutocerebrum and tritocerebrum. The protocerebrum forms the anterior brain and the visual system, as the forebrain does in vertebrates. We found a single fez homolog in Drosophila which is expressed in the protocerebral neurectoderm during embryonic stages and in the optic lobes of third instar larvae. The fact that there is only one fez homolog gene in Drosophila makes this organism a good model to understand the function of this gene family. To this purpose we will analyze fez expression in mutants for genes such as otd (Otx), so(Six3/6) and tll (Tlx) which contribute to the eye/optic lobe fate and are expressed in the protocerebral neurectoderm, like fez during early embryonic stages, in Drosophila. We will test different RNAi lines for fez and we will perform overexpression studies of fez in order to analyze a possible function in anterior brain development. OSN: Olfactory sensory neuron; otd: orthodenticle; tll: tailless; so: sine oculis.

Ref: 139

THE ROLE OF FIBROBLAST GROWTH FACTOR (FGF) SIGNALLING IN THYMUS AND PARATHYROID ORGANOGENESIS

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The thymus and parathyroid glands develop from the 3rd pharyngeal pouch endoderm in the mouse embryo. Previous research has shown that Fgf8 is necessary for the formation of the 3rd pouch by E9.5 and that signalling through FGFR2(IIIb) is required for proliferation of the thymic primordium at E12.5. It is not known whether Fgf signalling plays a role between E10.5 and E12.5, stages when subdivision of the 3rd pouch into prospective thymus and parathyroid domains becomes apparent. Sprouty genes encode intracellular feedback antagonists of Fgf signalling. Simultaneous deletion of both Spry1 and Spry2 results in thymus and parathyroid hypoplasia, and thymic migration defects. Even though the 3rd pouch forms in these mutants, pouch morphogenesis is clearly abnormal. Gene expression analyses indicate that Fgf signalling is involved in patterning of the 3rd pouch endoderm into thymus and parathyroid domains before E11.5. Since Spry1 and Spry2 are expressed in all tissues of the 3rd pharyngeal arch at E10.5, we are making use of conditional gene knockout approaches to address the function of these genes. We show that neural crest-specific sprouty gene knockouts have parathyroid hypoplasia suggesting that the regulation of Fgf signalling in the neural crest is essential for normal parathyroid development.

Ref: 140

THE PLANARIAN BMP PATHWAY: STUDYING THE FUNCTIONAL CONSERVATION OF THE NOGGIN GENES

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Freshwater planarians constitute an excellent model in which to study axis re-specification during regeneration. Remarkably, planarians are able to regenerate a whole animal from a small piece of their bodies. Recently, we have shown that RNAi knockdowns of BMP and Smad1 homologues on intact and regenerating planarians lead to the disappearance of dorsal markers and the ectopic expression of ventral markers on the dorsal side of the treated animals. In addition, and in most cases, a duplicated central nervous system differentiates dorsally. These defects suggest that the BMP signalling pathway has been conserved in planarians and that it plays a key role in the regeneration and maintenance of the dorsoventral (DV) axis. In order to expand our knowledge on the function of the BMP pathway in planarian DV axis specification, we have started to identify and characterize putative antagonist elements of this pathway. So far, no chordin homologue has been found in the planarian genome yet. However, we have found several noggin-like genes that are expressed in different regions of the animal. With the aim of determining if planarian noggin-like genes have a conserved role in DV patterning we have performed RNAi on planarians as well as functional assays using Xenopus embryos.

Ref: 141

FOLIC ACID DEFICIENCY ALTERS THE PALATAL MEDIAL EPITHELIUM AND MESENCHYME IN C57 MICE

Pérez Miguelsanz, María Juliana; Maldonado E, López Y, Maestro C, Barrio MC, Del Río A, Murillo J, Martínez-Sanz E, Casado I, Paradas I, Martínez-Álvarez C*, Pérez-Miguelsanz J*.

A deficit of dietary folic acid (FA) leads to cleft palate (CP). We studied the effects of a FA deficiency in the palate of embryos from mouse females under a FA deficient diet. C57 mouse females received either a control (2 mg FA/kg diet) or FA deficient (0 mg FA/kg diet -experimental group-) diets for 2-16 weeks. Embryonic day (E) 13.5 palatal shelves (n=39) were cultured for 18 or 36 hours. E17 phoetuses (n=205) were examined to check the presence of CP. Both cultures and heads were embedded in paraffin, sectioned and haematoxilin and eosin stained. TUNEL assay and measurement of palatal shelf adhesion/fusion were performed in cultures. Palates from embryos progeny of 2 week experimental females showed a significant reduction of MEE cell death, palatal shelf adhesion and fusion. Alteration of palatal ossification was observed in E17 phoetuses from 4 week experimental females onwards. Partial or complete CP was observed in 4% of the whole experimental group phoetuses, starting in the progeny from 2 week FA deficient diet mouse females. Therefore, FA deficiency alters both the palatal medial edge epithelium and mesenchyme. Work supported by grants FIS (PI06/0184) and UCM-CM to the Complutense Research Group 920202, 2006.

Ref: 142

FOLIC ACID SOFTENS THE SEVERITY OF THE CLEFT PALATE OF TGF-B3 NULL MICE

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Tgf-b3 null mice (Tgf-b3 -/-) have cleft palate (CP). Supplements of folic acid (FA) reduce the risk of this malformation. We investigated whether FA added either to Tgf-b3 +/- dams or Tgf-b3 -/- palatal shelf cultures benefits the CP presented by the null mice. The diet of Tgf-b3 +/- females was 2 mg FA/kg diet (control) or supplemented with 40 mg FA/kg diet for 2 to 16 weeks. Pregnant females were sacrificed at embryonic day (E) 17, and the palate of the null embryos studied. E13.5 Tgf-b3 -/- palatal shelves were apposed and cultured for 18 or 36 hours in the absence (control) or presence of a media supplemented FA. TUNEL assay and measurement of palatal shelf adhesion/fusion were performed. The Tgf-b3 -/- progeny of FA supplemented dams had 17.24% presence of a milder form of CP, only observed in 3.4% of the controls. No differences between groups were observed in the culture study. Therefore, dietary FA supplement to Tgf-b3 +/- females softens the severity of the cleft palate presented by Tgf-b3 -/- embryos, through mechanisms other than MEE cell death, adhesion and fusion. Work supported by grants FIS (PI06/0184) and UCM-CM to the Complutense Research Group 920202, 2006.

Ref: 143

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF GLUCOSE TRANSPORTER GLUT1 IN DEVELOPING CHICK BRAIN

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GLUT1 is known to transport glucose through the endothelial cells of rodent cerebral capillaries exhibiting blood brain barrier properties and is also present in ependymal cells of the choroid plexus, in astrocytes and in hypothalamic tanycytes. In the developing rodent brain, high expression of GLUT1 was reported in the neuroectoderm of early embryonic stages and was related to the presence of tight (strand) junctions. Conversely in the pineal gland, GLUT1 was present through the development but not thereafter. In the chicken brain, quantitative studies showed high GLUT1 protein and mRNA at early and late embryonic stages. We describe here the immunocytochemical localization of GLUT1 in the chick developing brain from E3 to P45. The most intense staining occurred in blood vessels penetrating from the perineural vascular plexus in the ventral neural tube at E5 and increasingly in the remaining zones of the brain. The retina and lens were positive from E3 on, and the pineal gland showed positive reaction from E6 until P45. Choroid plexus, at E21, showed a strong reaction in the basolateral membrane but, at postnatal stages, only a weak immunoreaction in the apical pole of the choroidal ependymal cells was visible. The neuroepithelium showed only a weak, if any, immunoreactivity in discrete zones at different stages. Our results showed important differences with respect to mammals. Grants: Junta de Andalucia (P07-CVI-03079), MICINN(BFU2006-11754), Ring PBCT-Word Bank ACT-02.

Ref: 144

THE HOMEOBOX GENE HESX1 ACTS AS A NEGATIVE REGULATOR OF WNT SIGNALLING

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Hesx1 is a conserved transcriptional repressor required for normal anterior forebrain (AFB) and pituitary development in vertebrates, including Xenopus, mice and humans. Through genetic fate mapping and marker analysis, we have recently demonstrated that the absence of HESX1 leads to a posterior cell fate transformation of the AFB. Our data suggest that HESX1 functions to antagonize the activation of Wnt/beta-catenin signalling within the AFB, in a cell autonomous manner. However, the precise molecular mechanism by which HESX1 modulates this pathway is not known. Aiming to uncover HESX1 molecular function, we have generated a Hesx1-Egfp mouse line and performed GeneChip profiling studies from FACS purified precursors at 8.0dpc. Together with conditional gain and loss of beta-catenin function studies in the anterior forebrain, the results confirm the role of HESX1 as a Wnt/beta-catenin signalling modulator and have revealed novel putative HESX1 targets.

Ref: 146

A PRIMITIVE HEART-KIDNEY CONNECTION CAN ACCOUNT FOR THE EVOLUTIONARY ORIGIN OF THE VERTEBRATE EPICARDIUM

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The epicardium is the outermost layer of the vertebrate heart. The embryonic epicardium and its derivatives contribute to the formation of coronary vasculature and modulate the proliferation of ventricular myocardium. The embryonic epicardium arises from an extracardiac, paired progenitor called the proepicardium, a proliferation of coelomic cells at the posterior cardiac limit. Proepicardial cells spread over the heart giving rise to the epicardium. Invertebrate hearts lack of epicardium. However, no hypothesis has been hitherto proposed about the origin of this tissue in vertebrates. We have studied the epicardial development in the lamprey (Petromyzon marinus) a representative of the most primitive living lineage of vertebrates, the agnathans. The lamprey epicardium develops by cell migration from a paired proliferation of coelomic cells in the dorsal part of the coelomic cavity, between the pronephros and the gut. Later, these outgrowths differentiate into a well-known structure, the pronephric external glomerulus (PEG) which is composed of capillary networks, mesangial cells and podocytes. This observation suggests that the primordia of the most anterior pair of PEG in agnathans were conserved in gnathostomes as the proepicardial outgrowths. This new hypothesis accounts for the striking (pro)epicardial expression of Wt1, Tbx18 and Pod1, transcription factors involved in genitourinary development.

Ref: 148

THE MATRICELLULAR GLYCOPROTEIN OSTEONECTIN IS REQUIRED FOR SKELETAL DEVELOPMENT AND HEMATOPOIETIC MATURATION.

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Sparc (Osteonectin), a matricellular glycoprotein expressed by many differentiated cells, is a major non-collagenous constituent of vertebrate bones. Recent studies indicate that Sparc expression appears early in development, although its function and regulation during embryogenesis are largely unknown. We cloned zebrafish sparc and investigated its role during development, using a morpholino antisense oligonucleotide-based knockdown approach. Knockdown of Sparc function resulted in specific skeletal, vestibular and haematopoietic defects that are highlighted by changes in gene expression, morphology and behavior. We rescued the knockdown phenotypes by co-injecting sparc mRNA, providing evidence that the knockdown phenotype is due specifically to impairment of Sparc function. We conclude that the matricellular protein osteoenectin is required for bone formation, vestibular development and normal differentiation of erythoid and myeloid lineage cells.

Ref: 149

PATTERNING EFFECTS OF MORPHOGENS AND HOMEOBOX TRANSCRIPTION FACTORS EXPRESSED AT THE PRETECTAL ROOF PLATE

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University of Murcia. Human Anatomy and Psicobiology

The pretectal region (alar and roof plate of prosomere 1) has three main anteriorposterior domains and several dorsoventral (dv) subdomains (Ferran et.al. 2007). These pattern subdivisions are thought to be organized via still vaguely understood signals acting differentially upon the AP and DV dimensions of the local genetic network. The signals affecting the DV pretectal subdomains probably involve molecules spreading from the local roof and floor plates. The aim of the present work was the analyze the expression pattern of members of Wnt and Bmp families and transcription factors known to be related with the respective signalling pathways in the pretectal region between HH16 and HH25. We found that genes coding for some but not all Bmp- and Wnt-family morphogens are expressed at the roof plate of p1, and transcripts of some related transcription factors (e.g., Msx1 or Zic3) were localized not only at the roof plate but also extended to the most dorsal pretectal subdomains. These results increase fundamental knowledge about the molecular specification of the pretectal region at the caudal part of the diencephalon, and underpin ulterior studies of the patterning mechanisms associated to signaling effects spreading from the roof plate.

Ref: 151

MORPHOGENETIC PLASTICITY AND TOLERANCE TO HYPOXIA IN THE EARLY CHICK EMBRYO

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The chick embryo (Gallus gallus) has a propensity for regulating its overall rate of development. Temperature, p[O2] and p[CO2] have been shown to affect rates of morphogenesis and/or growth of the embryo, and time of hatch. The mechanisms by which any of these factors may interact with embryonic development are largely unknown. We hypothesised that the chick embryo would be most able to regulate its developmental rate via morphogenetic mechanisms and therefore early in development. We chose hypoxia as our incubation variable because of the well-characterised HIF pathways. Fertile hens' eggs were incubated in 10% O2 for any one of the first 4 days of development before being returned to normoxia (n=52), or in normoxia alone (n=11), and embryos scored for size, weight and developmental stage at Day 4. All 50/52 live embryos were of a normal Day 4 stage, but reduced size and weight (mean+-SD hypoxic 37.5+-9.7mg; control 54.4+-17.9mg) (t-test p<0.001). 32 additional eggs received the same hypoxic treatments but were then incubated normoxically to Day 10. Their embryos were also morphologically normal but smaller and lighter (mean+-SD heads 1.05+-0.08g; trunks 0.98+-0.11g) than 9 normoxic controls (mean+-SD heads 1.15+-0.05g; trunks 1.13+-0.16g) (t-test heads p<0.001; trunks p<0.001). We conclude that the chick embryo can regulate its overall rate of development via early morphogenetic mechanisms and can tolerate substantial acute hypoxia during the first 4 days of incubation.

Ref: 152

CHANGES IN EMBRYONIC MYOCARDIUM COHESIVITY DEPEND ON RETINOIC ACID-DEPENDENT EPICARDIAL SECRETED SIGNALS

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Before HH18 (3 incubation days) the chick embryonic myocardium is a simple thin muscular wall constituted of two or three cell layers. Interaction between cardiomyocytes and the neighbouring non-muscular epicardial cells gives rise to the ventricular compact layer, wich will proliferate and mature into the working myocardium of late embryonic, postnatal and adult hearts. It has been proposed that the epicardium provides instructive and proliferative signals to the adjacent myocardium that are dependent on the autocrine activation of retinoid signalling in the epicardium. Effects of the epicardial secretome on the proliferation and maturation of the myocardium in vivo are difficult to evaluate. We have thus used a volume-independent in vitro technique, tissue surface tensiometry, to test whether the signals provided by the epicardium account for the differentiation and compaction of the underlying myocardium. Our results show that RA-treated epicardial cells, but not control epicardial cells, produce a secretome able to increase the surface tension of myocardial cell aggregates. These results can be explained using the Differential Adhesion Hypothesis, which predicts that when two tissues meet and fuse, the one with the higher surface tension will become enveloped by the tissue of lower surface tension.

Ref: 153

FGF SIGNALLING REGULATES A SUBSET OF PLURIPOTENT GENES IN MOUSE ES CELLS

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Fibroblastic growth factors (FGFs) are central to many major developmental processes and together with the downstream activation of the Ras-Erk signalling cascade are critical stimuli for proliferation and differentiation in many cell types. FGF4 has an essential role in early embryo development. We have previously shown that Fgf4-/- ES cells have a severe defect in neural differentiation, as well as defects in differentiation into other lineages, a defect rescued by the addition of recombinant FGF4 ligand to the culture medium. Thus, the FGF pathway is required at a very early of ES cell differentiation. In order to better understand the mechanism by which FGF4 affects early lineage commitment, we have attempted to identify which pluripotency-associated genes are regulated by FGF4 signalling by using Fgf4-/- ES cells stimulated with recombinant FGF4 for various times. To complement the above studies, two wild-type ES cell lines were treated with PD173074, an FGFR inhibitor, for different times, and the expression pattern of a number of these genes was analysed by qPCR. Initial observations have shown that some pluripotent genes are regulated by FGF signalling. Nanog mRNA and protein were down-regulated by FGF signalling, Oct4 was unaffected, and two pluripotent markers were consistently and acutely up-regulated by FGF signalling. These observations provide genes to investigate the mechanism by which ES cells initially enter into lineage commitment.

Ref: 154

INCREASED CILIAL BEAT FREQUENCY IMPEDES LEFT RIGHT DETERMINATION.

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Medical Research Council. Mammalian Genetics Unit

Externally we are mirror symmetrical between left and right, yet internally we show left-right (L-R) asymmetry in organ positioning and patterning. Whilst much is understood about the establishment of asymmetry, gaps clearly exist in our understanding of this process. In mammals symmetry is believed to be broken when mono-cilia, in the embryonic node, beat so as to cause a leftward flow of liquid. This “nodal flow” somehow directs left sided gene expression, in turn resulting in the correct establishment of organ asymmetries. Previously we performed a phenotype driven genetic screen in mice. This identified the bull-whip mutant, which shows defects in L-R patterning. A significant proportion of mutants demonstrated situs inversus totalis; other phenotypes include partial, left or right pulmonary isomerism. On examination of the embryonic node, an unusually fast cilial beat pattern is observed, suggesting that that increasing the speed of nodal cilia rotation affects L-R patterning. This is the first mutation that has been demonstrated to increase cilial beat frequency at the node. Work to characterise the mutant is ongoing.

Ref: 155

PADA, A NEW PROTEIN INVOLVED IN CELL DIFFERENTIATION IN DICTYOSTELIUM DISCOIDEUM

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The social amoeba D. discoideum is an excellent model system to study basic processes of cell differentiation and development. We have identified a new gene involved in cell differentiation in D. discoideum. The padA- mutant displays a pleiotropic phenotype, grows poorly on axenic medium and cell differentiation and terminal development are impaired. Spore formation is also defective and only 20% of them achieve maturation. PadA bears structural similarity to the Neurospora crassa negative transcriptional regulator, NmrA, which is involved in nitrogen metabolite repression. Ammonium levels play a critical role during D. discoideum development. Like NmrA, PadA function depends on the integrity of dinucleotide-binding and GATA transcription factor-interacting domains. Our results show that proper cell differentiation of two prestalk cell subpopulations, pstA and pstAB, is impaired in the mutant and this severely hinders terminal differentiation. We also demonstrate that the presence of ammonium and other weak bases interferes with padA- development and two ammonium transporters appear to be derepressed in the mutant. We propose that PadA belongs to a new family of NAD(P)+-binding proteins that link metabolic changes to gene expression and is required for cell differentiation

Ref: 157

CELL SHAPE CHANGES INDUCED BY MECHANICAL FORCES DURING DROSOPHILA GERM-BAND EXTENSION

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Convergence and extension is a morphogenetic movement commonly seen during the development of embryos and organs. During Drosophila gastrulation, the trunk of the embryo (the germ-band) elongates in the antero-posterior (A/P) axis and narrows in the dorso-ventral axis, thus providing a genetically accessible model to study convergence and extension movements. Cell intercalation is known to contribute to this process, but how much this cell behaviour accounts for tissue deformation at a given time and location is unknown. Here we apply a novel method to continuously measure the contribution of cell intercalation and cell shape changes to tissue deformation. We find that contrarily to what was previously thought, cell shape changes contribute to germ-band extension (GBE) in wild-type embryos. In mutants such as kruppel- in which A/P patterning is defective, directed cell intercalation is reduced, but this is fully compensated in the first half of GBE by an increase in the contribution of cell shape changes. By contrast, the contribution of cell shape changes is decreased in mutants such as twist- which are defective in mesoderm invagination. We will provide evidence that mesoderm invagination produces mechanical forces that contribute to GBE by orienting cell shape changes in the direction of extension. Our quantitative analysis of cell behaviours during GBE provides a start point to model how tissues and cells respond to mechanical forces in vivo.

Ref: 158

THE FUNCTION OF SPCS IN THE DEVELOPMENT OF DROSOPHILA

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Bone Morphogenetic proteins (BMPs) are synthesized as inactive pre-cursors that are cleaved by Subtilisin like pro-protein convertases (SPCs) to yield the active signaling molecule. In vertebrates, SPC -1 has been implicated in processing of BMP-4, however in vivo studies suggest that several different SPCs may be able to cleave the pro-protein. To better address this issue, we have studied the Drosophila SPC family members Furin1 (Fur1), Furin2 (Fur2) and amontillado (amon). We have generated mutations in Fur1 and Fur2 and phenotypic analysis show that both are larval lethal, and thus not functionally redundant. In addition, hypomorphic Fur1 mutants show a wing phenotype: they have small, dusky wings and their tricomes, especially on the veins, produce a cluster of small hairs surrounding the base, unlike the well-defined singular hairs produced by wild-type flies. Clonal analysis reveals that Fur1 act cell - autonomously in this aspect of wing morphogenesis. Fur2 and amon on the other hand do not appear to be involved in wing development. Interestingly, Fur1 and Fur2 mutants either singly or in combination, do not show phenotypes reflecting a role in BMP signaling either in the embryo or larva. These results suggest that cleavage by SPCs may not be an essential factor that is required for Drosophila BMP function in vivo.

Ref: 159

ANALYSIS OF THE ROLES OF DBRWD3 DURING DROSOPHILA DEVELOPMENT

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University of Sheffield. Biomedical Sciences

We have previously identified the dBRWD3 locus as a positive regulator of the Drosophila JAK/STAT signal transduction pathway in a genome wide RNAi screen undertaken in Drosophila tissue culture cells. We have also demonstrated genetic interactions between dBRWD3 and JAK/STAT signalling in vivo. In addition, analysis by another group (who subsequently termed this locus ramshackle), suggests that dBRWD3 interacts with Hedgehog (Hh) pathway signalling. Here we present our recent analysis of dBRWD3 and its developmental interactions with both the Hh and JAK/STAT pathways and examine the significance of these results in the light of a potential role for human BRDW3 in human chronic lymphocytic leukaemia.

Ref: 160

THE PLANAR POLARITY PATHWAY AND TRACHEA FORMATION IN THE DROSOPHILA EMBRYO

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Signalling through the Frizzled-dependent planar polarity pathway is a conserved mechanism that polarises cells in a plane perpendicular to the apical-basal axis. Interactions between the core planar polarity proteins lead to their asymmetric distribution, resulting in polarised activity within a cell. Epithelial cells in many organisms exhibit planar polarity. But roles for the planar polarity pathway during Drosophila embryonic development have not been extensively characterised. The formation of trachea in the Drosophila embryo involves the transformation of an epithelial monolayer sheet into a tubular network. This process requires cell migration and intercalation leading to the formation of a network of interconnected tubes. Cells in tracheal branches are initially organized into pairs connected via intercellular adherens junctions (AJ's), which maintain cell-to-cell contact. To ascertain whether the intercalation of these paired cells requires the planar polarity pathway, we studied the process in Drosophila embryos mutant for core planar polarity genes. Data suggests that endogenous Frizzled protein localises to the cell junctions of the tracheal branches. Null mutants of planar polarity pathway components result in incomplete formation of autocellular AJ's between some of the cells. This may be a result of cells being unable to polarise and therefore pair and align correctly prior to the alteration of cell-to-cell junctions from intercellular to autocellular AJ's.

Ref: 161

SFRP1 AND SFRP2 ARE REQUIRED FOR PROPER PATTERNING OF THE EYE AND NEURAL RETINAL DIFFERENTIATION

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Secreted Frizzled Related Proteins (Sfrps) compose a family of soluble factors involved in the control of embryonic development and homeostasis of adult tissues. Although Sfrps act as antagonists of Wnt signalling in many contexts, there is evidence supporting alternative functions, including binding to Frizzled receptors or interference with BMP signalling. We have previously shown that interference with Sfrp1 expression causes alteration in the patterning of the eye field and defects in retinal neurogenesis that cannot be explained by canonical Wnt inhibition. In the attempt to further elucidate this issue, we have analysed the eye phenotype of Sfrp1/Sfrp2 double null embryos. Although optic vesicle development initiates normally in the mutants, strong alterations of the proximal eye structures are observed from optic cup stages: the lens are enlarged, the cornea is absent and the ciliary bodies are displaced due to conversion of the ciliary margin into neural retina-like tissue. This eye phenotype is reminiscent of that observed after inactivation of Wnt/βcatenin signalling, suggesting that Sfrp1 and Sfrp2 may act as positive modulators of the canonical Wnt cascade. Notably, a prominent increased in retinal differentiation as well as alterations of vasculature formation are also observed, raising the possibility that in the retina Sfrps may act independently of Wnt canonical signalling because this pathway is largely inactive during retinal neurogenesis.

Ref: 162

THE 5'- 3' EXORIBONUCLEASE PACMAN HAS MULTIPLE ROLES IN DROSOPHILA DEVELOPMENT.

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It is becoming increasingly clear that transcript degradation via ribonucleases is an important step in the control of developmental processes. pacman (pcm) is the Drosophila homologue of yeast XRN1 and is the only known cytoplasmic 5' - 3' exoribonuclease in eukaryotes. To determine the effects of this exoribonuclease in development we have constructed a number of mutants and transgenics in pacman and characterised the resulting phenotypes We have previously generated a small allelic series of pcm mutants via imprecise excision of a P-element. We have recently generated a larger series of pcm alleles using the same techniques. Some of these are lethal lines (including at least one null allele). We are currently characterising these lines at the molecular and phenotypic level. We also have a number of UAS-pcm (cDNA) constructs, both wild-type and nuclease-dead versions. These constructs are being used to investigate the effects of ectopic/over-expression of wildtype and nuclease dead PCM. To date, we have investigated the phenotypic consequences of perturbing pcm expression during Drosophila development. These include defects in processes such as dorsal closure in embryonic development, thoracic closure and wing morphogenesis in pupal development and effects on wound healing in adult flies. We have shown that pcm interacts with the JNK pathway via the phosphatase puckered. We will present our recent findings on the mechanisms of action of PCM in development.

Ref: 163

REGENERATION IN SITU

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One of the classic problems in biology is to understand the genetic and molecular mechanisms by which the process of regeneration is regulated during development. Drosophila imaginal discs are a suitable model system for studying this process. A standard approach to study regeneration in Drosophila has been by performing “in vivo” culture of the regenerating structures. One of the problem of this method is that is impossible to reproduce the physiological condition that occur during normal development. We have developed a new method to remove a part of the wing imaginal disc inside the larva. Using this method, it is not necessary to transplant the disc afterwards. Thus, we can study the process of regeneration in its normal developmental context. Further, the effect of the wound in the final adult structures can be assessed.

Ref: 166

IDENTIFICATION OF A NEW TARGET GENE OF THE JNK SIGNALLING PATHWAY REQUIRED FOR EMBRYONIC DORSAL CLOSURE IN DROSOPHILA

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Dorsal closure during Drosophila embryogenesis provides a valuable model for epithelial morphogenesis and wound healing. It is the last major morphogenetic movement of Drosophila embryogenesis and is the process whereby a gaping dorsal epithelial hole is covered by lateral epithelia from both sides of the embryo, which meet and fuse at the dorsal midline. This process involves extremely coordinated morphological changes of both the epidermal and the amnioserosal cells, and is driven by coordinated assembly and contraction of the actomyosin cytoskeleton in restricted populations of epithelial cells. There are clear parallels between the events of dorsal closure and morphogenetic episodes that occur in higher vertebrates, such as neural tube closure. In particular, it is likely that there will be significant conservation of mechanism for the events that finally knit together two epithelial faces during any morphogenetic movement. We focused on the study of a novel gene, target of JNK signalling which is required for the process of dorsal closure in Drosophila.

Ref: 167

CELL-AUTONOMOUS AND NON-AUTONOMOUS SHH SIGNALLING MEDIATES THE GROWTH AND GUIDANCE OF MOUSE RETINA GANGLION CELL AXONS

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Retinal ganglion cells (RGCs), which transmit visual information from the retina to the brain, are cells that secrete and receive Sonic hedgehog (Shh), a signalling protein with important function in vertebrate embryo development. In chick and fishes midline derived-Shh has an important role in restricting the growth of RGC axons to the contralateral side of the brain. In mice visual projections are different from those of fishes and birds and two RGC populations ensure binocular vision by projecting either ipsilaterally or contralaterally at the chiasm midline. Here we have asked if these two populations respond differently to Shh and if RGC-derived Shh contributes to the growth of the axons toward the midline. We show that ispilateral and contralateral projecting RGCs respond differently to Shh in vitro and that this distinct behaviour correlates with a differential expression of Shh signalling components in the retina. We will also show that in vivo interference with Shh signalling causes abnormal growth and navigation of contralaterally projecting axons in the initial portion of the pathway as well as alterations in the distribution of retinal axons at the optic chiasm, clearly indicating that both RGC- and midline-derived Shh participates in RGC axons' guidance. Collectively these data underscore the importance of Shh in funnelling and sorting of visual fibres at the midline and highlight a novel cell autonomous mechanism by which Shh can influence growth cone behaviour.

Ref: 168

TETRAPLOIDIZATION OF A SUBPOPULATION OF RETINAL GANGLION CELLS INDUCED BY NERVE GROWTH FACTOR

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A subset of neurons in the vertebrate nervous system contains double the normal amount of DNA in their nuclei. Most of these neurons are large, with long axons and extensive dendritic trees, and they have been shown to represent subpopulations of projecting neurons such as retinal ganglion cells (RGCs). However, how vertebrate neurons with a 4C DNA content in their nuclei arise is completely unknown. We show that endogenous nerve growth factor (NGF) induces DNA replication in a subpopulation of migrating RGCs that express both the neurotrophin receptor p75 (p75NTR) and the E2F1 transcription factor, but that lack the retinoblastoma protein. Although some migrating RGCs that attempt cell cycle re-entry undergo apoptosis, a substantial proportion of these cells remain alive in the ganglion cell layer with double the nuclear DNA content. We conclude that NGF and p75NTR are critical for the specification of tetraploid neurons in the chick retina.

Ref: 169

ROLE OF ADHESION IN MESENDODERM PROGENITOR MIGRATION DURING ZEBRAFISH GASTRULATION

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Gastrulation is the first major morphogenetic process in vertebrate development and leads to the formation of the three germ layers: ectoderm, mesoderm and endoderm. Morphological changes shaping the germ layers are proposed to be governed by adhesive and mechanical properties of the constituent progenitor cells. In a previous study we measured adhesion and cortex tension of the three germ layer progenitor cell types using atomic force microscopy (AFM). By comparing these data with results of in vivo and in vitro cell sorting experiments we found that differentially regulated acto-myosin-dependent cell cortex tension constitutes a key factor directing progenitor cell sorting. To evaluate the contribution of cell adhesion to progenitor cell movement in vivo, we designed a transplantation assay that allows us to analyze and quantify adhesion-dependent mesendoderm progenitor cell migration and morphology. The aim of this approach is to determine the degree of cell adhesion required for optimal mesendoderm progenitor cell migration during gastrulation.

Ref: 170

ARE FURIN CLEAVAGE SITES REQUIRED FOR BMP FUNCTION IN DROSOPHILA?

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Bone Morphogenic Proteins (BMPs) are expressed as pre-proteins consisting of an N-terminal pro-domain and the C-terminal ligand-domain. In vitro studies in vertebrates have suggested that Bmp-4 has to be sequentially cleaved at two Furin cleavage sites (RXXR) for secretion of the active ligand domain. In contrast, vertebrate Bmp-5, -6, -7 and -8 contain only one Furin cleavage site between the pro- and the ligand-domain, suggesting that these BMPs are cleaved by a different mechanism. The Drosophila Bmp-5/6/7/8 orthologs Glass bottom boat (Gbb) and Screw (Scw) contain a main Furin cleavage site between the pro- and the ligand-domain, followed by a cryptic site. In addition Gbb contains two and Scw four more cleavage sites within their pro-domains. We tested the requirement for each individual site by site directed mutagenesis in a genomic rescue construct and assayed their ability to rescue gbb or scw mutants, respectively. We found that none of the sites in the pro-domain nor the cryptic sites are essential for function. Curiously, while the main site in Scw appears to be essential, the main site in Gbb is not. This result raises the question of whether Furin cleavage is essential for BMP function in vivo or merely provides a mechanism of fine tuning of BMP signalling. To further address the requirement of Furin cleavage in BMP signalling we are currently testing if the three potential cleavage sites in the Bmp-2/-4 ortholog Decapentaplegic are essential for its function.

Ref: 171

FUNCTIONAL DIVERGENCE OF A RECENTLY DUPLICATED BMP ORTHOLOG IN DROSOPHILA

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Drosophila has three BMP-related signalling molecules: the Bmp-2/4 ortholog Decapentaplegic (Dpp), and the Bmp-5/6/7/8 orthologs Glass bottom boat (Gbb) and Screw (Scw), that modulate the activity of the main morphogen Dpp. Sequence comparison and gene structure analysis from different Dipteran species reveals that gbb has been duplicated several times independently and progressively lost introns in the more derived species. The data suggests that a recent duplication of gbb within the lineage leading to higher Diptera resulted in the emergence of scw which thereafter rapidly diverged from the ancestral gbb sequence. However, Scw has retained 6 blocks of conserved ancestral Gbb sequence within its pro-domain. Chimeras of the Gbb pro-domain fused to the ligand domains of human Bmp-5, Bmp-6 and Bmp-7 will rescue gbb mutants, whereas the ligand domains of the human BMPs are not able to rescue scw mutants. Rescue assays with Gbb-Scw chimeras show that both genes are not able to rescue mutations in the other gene suggesting that they are functionally distinct. This functional difference is not due to differences in gene expression but is based on alterations within the protein coding sequence.

Ref: 172

ROLES OF THE DROSOPHILA G-PROTEIN COUPLED RECEPTOR KINASE GPRK2 AND THE B-ARRESTIN KURTZ IN SMOOTHENED SIGNALING

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Signalling by Smoothened (Smo) plays fundamental roles during Drosophila development and is deregulated in a variety of human cancers. Smo is a transmembrane protein with seven transmembrane domains, and shows several characteristics of G-protein coupled receptors (GPCR). We find that the protein Gprk2, a Drosophila member of the GPCR regulatory proteins termed G protein-coupled receptor kinases (GRKs), plays a positive role in the Hh signal transduction pathway whereas the protein Kurtz, the b-arrestin Drosophila homologue, plays a negative role in this signalling pathway. Thus, lowering Gprk2 levels in the wing disc or augmenting Kurtz levels causes a phenotype reminiscent to loss of Hh function and prevents the expression of Hh-targets in the corresponding wing discs. The reduction in Smo signalling is correlated by Smo accumulation in the cell membrane when Gprk2 is reduced or by Smo elimination of the cell surface when Kurtz is augmented. We propose that the phosphorylation step mediated by Gprk2 leads to Smo internalization to a signaling compartment. The b-arrestin Kurtz might internalize Smo to a degradation compartment in an independent manner of Gprk2.

Ref: 173

A ROLE OF THE CELL ADHESION MOLECULE F3/CONTACTIN IN THE PROLIFERATION AND DIFFERENTIATION OF CEREBELLAR GRANULE CELLS

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In the postnatal mouse cerebellum, precursors of granule cell neurons (GCPs) proliferate and then begin to differentiate and migrate, first tangentially, then radially until they settle in the internal granule cell layer as mature neurons. During this process, L1-like cell adhesion molecules such as F3/contactin (F3) and TAG-1 are expressed in a highly ordered manner. TAG-1 is expressed first in the proliferating and tangentially migrating GCPs of the EGL while F3 is expressed later on post-mitotic cells. In transgenic mice where F3 is expressed prematurely, a transient reduction in the relative size of the cerebellum occurs due to a decrease in the number of GCPs, which was in turn due to a reduction in their proliferation. In order to elucidate the role of F3 in this process we have investigated the effects of purified soluble F3 protein (F3/fc) on primary GCPs in culture. Addition of purified F3/fc protein to GC cultures in the presence of Shh, a major mitogenic factor of GCPs, resulted in a decrease in the Shh-induced proliferation of GCPs. We are currently investigating whether F3 is simply inhibiting GCP proliferation or also promoting differentiation as well as the possible signalling pathways mediating the effect of F3.

Ref: 176

NEUROD-MEDIATED ACTIVATION OF SIX6 CONTROLS RETINA PROLIFERATION AND DIFFERENTIATION.

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Timely generation of appropriate numbers of distinct neural cell types from competent progenitors is fundamental for the generation of a functional retina. In vertebrates Six6, a transcription factor of the Six/sine oculis family, is initially expressed in multipotent retina progenitors and then becomes restricted to differentiated retinal ganglion and amacrine cells as well to the proliferative ciliary marginal zone. How Six6 expression in the retina is controlled and what are its precise functions is still unclear. To begin to address this issue we have used bioinformatic searches and transgenic approaches in medaka fish, identifying a highly conserved regulatory module in the Six6 locus responsible for gene expression in differentiating and adult retina. Luciferase and super-shift assays together with gain-of-function studies indicated that NeuroD, a bHLH transcription factor, is one of the factors that directly bind to this module regulating Six6 activity in the retina. Notably, NeuroD over-expression in medaka fish embryos promotes unorganized retina cell proliferation and increases the expression of amacrine cell markers associated with a decrease of photoreceptor specific gene expression. Because these effects are prevented by the injection of morpholinos designed against olSix6, we propose that NeuroD-mediated regulation of Six6 in the retina controls the appropriate generation of progenitor cells pushing them toward an amacrine phenotype.

Ref: 177

POSTNATAL EFFECTS OF HYDROXYUREA ON THE NEUROGENESIS AND SETTLING PATTERNS OF CEREBELLAR GRANULE CELLS.

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Effects of hydroxyurea (HU), a DNA synthesis inhibitor, were evaluated during postnatal development of rat cerebellum. Firstly, one injection of HU (1 mg/g) was administered to 4-day-old rats, to study granule-cell (GC) precursors in the external granular layer (EGL). Secondly, a group of animals received two consecutive doses when 10 and 11 days old, followed by the 5-bromo-2'-desoxyuridine (BrdU) administration at 15 days old, in order to assess the GCs settling patterns into the internal granular layer (IGL). Different parameters were evaluated at short and long survival times. Moreover, an immunocytochemistry for BrdU, incorporated into GC precursors, was used to examine regional differences in the distribution of GCs along the anterior and central lobe. In the first experiment, a reduction in EGL thickness was observed, accompanied by cell death images and low presence of mitotic cells. In the second experiment, decreases in vermal and IGL areas were found in rats sacrificed at day 30. Furthermore, a maximum percentage of BrdU+-GCs in IGL was observed in the central lobe of both control and treated rats. Thus, although reductions in the number of tagged cells were detected, this settling pattern was not disturbed by HU treatment.

Ref: 178

EFFECTS OF PRENATAL HYDROXYUREA-TREATMENT ON THE NEUROGENESIS OF PURKINJE CELLS

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Hydroxyurea (HU), a ribonucleotide reductase inhibitor, blocks DNA synthesis. To determine whether this drug disturbs the pattern of Purkinje cell (PC) generation in the cerebellum, pregnant rats were treated with 350 mg/kg b.w. of HU at embryonic days (E) 13 and 14, when PCs are being generated. Furthermore, 5-bromo-2´desoxyuridine (BrdU) was injected (20 mg/kg b.w.) on two successive days in an overlapping series, in accordance with the following time-windows: E13-14, E14-15 ..., and E17-18. The offspring were examined on postnatal day (P) 28. Immunoperoxidase staining used to detect BrdU-labelled PCs showed that neurogenesis, at vermis, extended in both experimental groups from E13 to E17 with a production peak located on E15. However, proportions of BrdU-positive PCs at E16-17 were significantly higher in HU-treated than in control groups. PC neurogenesis at cerebellar hemispheres covered a period of time ranging from E12 to E16. In control rats, the generation peak was found on E14, while E15 represented the maximum point in the treated group. Although HU treatment modified the PC developmental timetables, no loss of these macroneurons was observed in the studied regions, which suggests that a compensatory mechanism keeps the number of PCs constant in adult animals.

Ref: 179

DROSOPHILA LRP1 MODULATES BMP SIGNALING

Parra Peralbo, Esmeralda / Culí J
RGYM

The Low Density Lipoprotein Receptors (LDLRs) are members of an ancient and evolutionarily conserved family of proteins implicated in endocytosis of multiple ligands as well as in cell signaling. The Drosophila genome contains seven LDLRs. Here we present results about the LDL-receptor-related protein 1 (LRP1). In mammals, LRP1 acts as a scavenger receptor having a broad ligand-binding specificity. Its ligands include apolipoproteins, proteases, protease inhibitors, lipases and other functionally diverse macromolecules. Additionally, LRP1 modulates signaling pathways such as PDGF in vascular smooth muscle cells. We have generated null lrp1 mutations in Drosophila, which are homozygous viable and display extra veins in the wing. To identify the molecular basis of this phenotype, we tested if lrp1 mutations genetically interact with the signaling pathways known to be involved in wing vein formation. We detected an interaction solely with the integrin subunits myospheroid and multiple edematous wings. Integrins play an important role in vein formation by regulating Bmp signaling, which is essential for vein differentiation. It was shown that integrins regulate the diffusion of Short gastrulation (Sog), a Bmp antagonist. Accordingly, we detected a strong interaction between lrp1 mutations and Sog overexpression in the wing. Based on our results on Drosophila vein differentiation, we suggest a novel molecular model linking LRP1 activity and BMP signaling.

Ref: 180

ANALYSIS OF THE POSSIBLE EXISTENCE OF AN EARLY LINEAGE RESTRICTION IN THE MOUSE OPTIC VESICLE BY CELL TRACING STUDIES

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Optic vesicles are the first visible eye structures in vertebrates. Genetic studies have suggested that specification of the optic vesicle neuroepithelium into optic stalk, neural retina and retinal pigment epithelium (RPE) may occur during folding of the vesicles into the optic cup. Whether there is an earlier lineage restriction in the cells that compose the vesicle is however unexplored. Here we have begun to address this question by exploiting a recently established and validated system (Arques et al., 2007, Development, 134, 3713-3722) that enables the induction of LacZ-positive somatic clones in the mouse embryo at the developmental time point of interest and at the desired frequency. Clones were induced in E6.5-E9.5 pregnant mice and their topological distribution was analysed in the optic cups of E12.5-E14.5 embryos. This analysis suggests the establishment of a very early lineage restriction between progenitors that occupy the central neural retina and those of the central RPE. In the periphery of the retina (ciliary marginal zone, CMZ) instead, this restriction does not seem to exist. Notably, there also seems to be a lineage restriction boundary between the CMZ and the remaining of the neural retina since intermingling between positive cells of one or the other region does not occur. Additional analysis and specific marker studies are ongoing to confirm these results.

Ref: 181

THE ROLE OF JNK IN ZEBRAFISH EARLY MORPHOGENESIS

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The zebrafish embryo is an ideal model system to study the molecular and cellular mechanisms implied in the expansion and fusion of epithelial sheets. At epiboly, the coordinated movement of the diferent cell layers suggests specific mechanism directing both cell polarity and cell migration. The interactions between the enveloping layer (EVL), the deep cells layer (DCs) and the yolk syncitial layer (YSL) could be directed by many signalling mechanisms.We propose JNK signalling pathway as a potential candidate to regulate the processes of epiboly and convergence and extension in the zebrafish embryo. Knock-down of zebrafish JNK leads to an epiboly phenotype, in which migration of the majority of deep cells is affected in the morphant embryos, while epibolic expansion of the superficial EVL and the YSL nuclei seems to progress at a normal rate. This suggests that JNK controls cell adhesion between the different layers. We monitor changes in cell behaviour by confocal videomicroscopy and in fixed sections with markers for both cell adhesion and cytoeskeleton. Embryos that overcome the DCs epiboly phenotype show a convergence and extension phenotype with a shorter and broader body. They also have a curled body and display circling movements. We propose that JNK could be controlling cell shape changes and motility underlying mophogenetic movements in early zebrafish embryogenesis.

Ref: 182

CONTROL OF NEUROEPITHELIAL CELL CYCLE PROGRESSION REQUIRES INTEGRATION OF WNT AND SONIC HEDGEHOG ACTIVITIES

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The Wnt canonical pathway and Hedgehog signalling have been linked to cell proliferation in a variety of systems, however interaction of these pathways to control cell cycle progression have not been studied. In the developing vertebrate nervous system, although Shh and Wnt ligands are expressed at the opposite ventral and dorsal signalling centres, reports demonstrate that proliferation of neural progenitors require both activities throughout the dorsoventral axis. Here we demonstrate the integration of both pathways to control the length of G1 phase, and the absolute requirement of an upstream Hedgehog activity for the Wnt-mediated regulation of the key cell cycle activator CyclinD1 expression and for G1 progression. Although Wnt canonical activity appeared restricted to the control of G1 phase, Hedgehog activity additionally regulates the length of G2 phase through the regulation of late cell cycle activators such as CyclinA2 and CyclinB2/3. These findings support a key role for Hedgehog in growth control, as a regulator of G1 and G2 phases of cell cycle and importantly as an upstream regulator of the canonical Wnt activity.

Ref: 183

GENE EXPRESSION ANALYSIS IN HUMAN FOREBRAIN DEVELOPMENT, USING THE ELECTRONIC ATLAS OF THE DEVELOPING HUMAN BRAIN.

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The developing human brain is a complex structure and developmental processes are still poorly understood. During embryonic development, many genes are active simultaneously and gene expression patterns change over time. Currently, the Electronic Atlas of the Developing Human Brain (EADHB) is under construction. This atlas is based on three dimensional models of human embryos, obtained by optical projection tomography (OPT). Spatio - temporal activity of genes can be mapped on the anatomical structures and expression of genes can be visualized simultaneously, providing insight in the spatial relationships of gene expression patterns and developing anatomical structures. The EADHB is available as an online reference for researchers world wide. Recently a number of genes involved in brain development has already been studied and mapped on anatomical structures. Analysis of gene expression patterns over time may provide insight in origin of structures, since gene activity can be studied before any morphological change is visible. In this study, we analyse expression patterns of genes that play a role in brain development and have shown to be markers for anatomical structures. The genes are involved in brain malformations and disorders such as holoprosencephaly (Zic2, Six3), microcephaly (Arx) and Dandy-Walker malformation (Zic1). Additionally, we will analyse expression patterns of genes which are known to be expressed differently in human forebrain compared to mouse.

Ref: 184

FATE MAP OF ROSTRAL AND CAUDAL PARANEURAL ECTODERM AREAS AT STAGE HH4 OF CHICK EMBRYOS

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Cranial placodes are regions of thickened cephalic ectoderm that form in characteristic positions in the head of vertebrate embryos. They contribute to the paired sense organs (nose, eyes, ears and lateral line) and to neurons of cranial sensory ganglia. In anteroposterior order, the cranial placodes include the hypophyseal, olfactory, lens, trigeminal, otic ,lateral line, epibranchial, and presumably also the recently discovered hypobranchial placodes. It has been suggested the existence of a “pre-placodal region” impliying that all placodal precursors arise from a continuous domain of the embryonic ectoderm. Many of the placodal fate maps have been performed after neurula stages when precursors for different placodes are confined to unique regions. In this sense, we have been make a placodal fate map at late gastrula/early neurula stage (HH3d/4), using the limits of the neural plate previously established in our laboratory. We have fated the proximal non-neural ectoderm surrounds the neural plate primordium, it share with it some molecular determinants and forms what we call rostral and caudal paraneural ectoderm areas (RPN and CPN). Fate mapping of the placodes was performed using homospecific fluorescently labelled, homotopic grafts in New-cultured chick embryos. The labelled cells derived from the graft were detected immunocytochemically in wholemounts at stages HH10-12, when precursors of different placodes are easily distinguished, and cross-sections were later obtained. The positions of the graft-derived cells were analyzed to establish the placodes boundary at stages 3d/4. This work was supported by MEC Grants BFU2006-15330-C02-02 to LRG and BFU2005-09378-C02-01 and CIBERER Institute to LP.

Ref: 185

THE ROLE FOR THE EXORIBONUCLEASE PACMAN IN DROSOPHILA SPERMATOGENESIS

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University of Sussex. Brighton and Sussex Medical School

The Drosophila gene pacman is highly homologous to the major yeast exoribonuclease XRN1. Pacman/xrn-1 has been shown to be involved in cytoplasmic degradation of mRNA, nonsense-mediated decay, RNA interference and degradation of micro-RNAs. We have examined the function and localization of the exoribonuclease Pacman in Drosophila testis. The advantage of examining testis cells is that the differentiation pathway from stem cells to mature sperm is well understood and that many of the cells are large. Adult male flies carrying mutations in pacman have small, abnormally shaped testes, produce fewer offspring and show a higher rate of decline in fertility. Using confocal microscopy we have shown that Pacman is normally expressed in cytoplasmic particles at the tip of the testes where the initial mitosis takes place. In mature spermatocytes pacman protein appears to be located in cup-shaped structures, that surround particles containing the translational repressor Fragile-X mental retardation protein (dFMR1). In humans and Drosophila, mutations FMR1 also affect male fertility. The cytoplasmic particles containing Pacman are likely to be similar to yeast and human “P-bodies”. When male flies are subjected to heat shock the numbers and sizes of these putative “P-bodies” increase in a similar way to that observed in yeast and human cells. Our experiments indicate that Pacman co-localises with the decapping protein Dcp1 and the helicase Me31B.

Ref: 186

FUNCTIONAL STUDY OF FLATWORM STEM CELLS BY RNA INTERFERENCE

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University of Innbruck. Institute of Zoology

The flatworm Macrostomum lignano possesses a remarkable stem cell system, which has been analyzed extensively on the cytological and ultrastructural level. These findings and recent studies with molecular markers have led to a broad understanding about morphology, cell cycle, neoblast distribution, migration and the stem cell role during development and regeneration. However, despite the knowledge about the expression of stem cell and germ line genes in Macrostomum, little is known about their function or molecular regulation. We have produced a subtractive cDNA library enriched for stem cell and germ line genes to use as a source for candidate genes, which are then tested by RNA interference. The library has been produced from irradiated animals lacking proliferating cells and 3072 clones of differentially expressed genes have been sequenced. The goal is to analyze gene knock-downs and molecular regulation of pluripotent/totipotent flatworm stem cells. RNA interference of three stem cell and germ line genes show interesting phenotypes and were analyzed by morphology, BrdU stainings, in situ hybridization and immunocytochemistry. Our analysis will help to better understand stem cells in flatworms and also higher organisms, including human. Supported by LFU-Nachwuchsforschungsförderung to D.P., FWO to K.D.M. and FWF project 18099 to P.L.

Ref: 187

SPATIAL EXPRESSION PATTERNS OF MIRNAS IN ADULT AND REGENERATING PLANARIANS

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University of Nottingham. Developmental Genetics and Gene Control

miRNAs are an abundant class of non-protein-coding small RNAs whose specific functions in animals remain to be elucidated. There is emerging evidence suggesting that miRNAs may be crucial for stem cell maintenance, cell fate determination and differentiation. We take advantage of planarian flatworms as model organism. These animals posses a large population of pluripotent somatic stem cells that continuously participate in the normal cell turnover of the animal and in regeneration. We have begun to investigate if miRNAs play a major regulatory role during regeneration and in maintaining the neoblast cell population. Here we examine the differential spatial expression pattern of miRNAs in adult and regenerating planarians by in situ hybridization to nascent miRNA transcripts. Our results represent the first Lophotrocozoan animal where miRNAs expression patterns have been determined. We have characterised the expression pattern of 42 miRNAs in adult planarians and in regenerants. Together these data suggest an important role for miRNAs in stem cell regulation and in neural cell plasticity in planarians.

Ref: 188

IDENTIFICATION AND CHARACTERIZATION OF TWO MOUSE MUTANTS WITH DEVELOPMENTAL DEFECTS

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University of Manchester. Life sciences

Congenital abnormalities can be modelled in the mouse using genetic approaches such as phenotype driven mutagenesis screens. These screens can elucidate the function of genes based on the phenotypes recovered. A region-specific mutagenesis screen directed at mouse chromosome 11 allowed the isolation of the l11Jus27 and embryonic hydrocephalus (EHC) mouse mutants. The l11Jus27 mutant has defective cardiovascular development causing lethality at mid-gestation. Morphological analysis revealed that pericardial effusion and reversal of left/right asymmetry are prevalent in mutants. Whole mount in-situ hybridization indicates that mutant cardiac muscle development is impaired. Meiotic mapping has refined the l11Jus27 candidate interval to 5Mb. The EHC mutant exhibits neural tube and cardiac defects leading to lethality at late gestation. EHC mutants develop non-communicating hydrocephalus caused by blockage of the spinal canal. Histological analysis revealed that mutants have enlarged cardiomyocytes that fail to undergo proper cytokinesis resulting in binucleation. Additionally defects in cell adhesion are observed in mutant epicardium. Mapping data and western blot analysis indicates that the EHC phenotype is due to a mutation in the nonmuscle Myosin II-B gene. Mutants such as l11Jus27 and EHC can identify genes involved in embryonic development that may contribute to congenital birth defects in humans.

Ref: 189

HEMANGIOBLAST DIFFERENTIATION: PATHWAYS INTERACTING WITH THE NOTCH/DELTA SIGNAL.

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University of California, Los Angeles. Molecular, Cellular and Developmental Biology

The discovery of a hemangioblast precursor in Drosophila (Mandal, 2004), has led us to investigate the mechanisms involved in its differentiation. The two theories that are the most probable for the distinction of vascular cells from blood cells from a single precursor include asymmetric cell division or the presence of an asymmetrically distributed signal. Our data supports the latter of the two theories. Notch signaling has been found to be the main factor deciding hemangioblast cell fate and studies have shown a localization of Delta expression in cells flanking the blood precursors. Currently, we are studying different signaling pathways that may play a role in spatially restricting the expression of Delta and thereby effecting hemangioblast cell fate. Detailed genetic analysis will be presented regarding the combinatorial signaling cascades that give rise to the hemangioblast in Drosophila.

Ref: 192

CELL FATE AND COMMITMENT OF THE EARLY CARDIAC PRECURSORS

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Faculty of Medicine. Univ. Extremadura. Human Anatomy and Embryology

We describe a detailed study of the precardiac cells located at the level of the primitive streak, combining double transplantations, microinjections, and immunocytochemistry. Most cells of the more rostral segments of the primitive streak were found to contribute cells to the endodermal layer, adjacent to precardiac mesodermal cells of the heart forming region whose provenance was in the immediately more caudal segments of the primitive streak. We established a close spatio-temporal relationship between the two cells layers and the expression of their specific cardiac markers. We analyzed the ability of precardiac cells to differentiate when they are transplanted to ectopic locations or are subjected to the influence of the organizer. We propose that the precardiac cells of the primitive streak form two groups with different significance. One, regulated by mediation of the organizer, is located in the more rostral region of the primitive streak. It consists of the prospective cells of the endoderm layer, with a hierarchic pattern of expression of different genes characterized by its capacity for induction and regulation of a second group of cells, which is located in the more caudal segments, and is fated to form the precardiac mesoderm, whose differentiation would be characterized by the expression of various specific genes. This work was supported by grants BFU2007-66350/BFI from the Spanish MEC and PRI07A005, from the Junta de Extremadura.

Ref: 193

THE ROLE OF WNT SIGNALLING IN ENDODERMAL ORGANOGENESIS IN ZEBRAFISH

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NIMR, London. Developmental Biology

Several signalling pathways have been implicated in early liver development but only a recent study in zebrafish has isolated a gene specifically required for liver specification - prometheus/wnt2bb. prt/wnt2bb is bilaterally expressed in the lateral plate mesoderm (LPM), adjacent to the forming liver. Consistently, mosaic analysis reveals an essential involvement of the LPM in liver formation. Although liver specification initially fails to occur in prt/wnt2bb mutant embryos, a small number of endodermal cells start expressing liver specific genes at later stages, suggesting that additional factors may act in parallel in liver specification and compensate for its absence. Work carried out predominantly in chick has revealed a complex pattern of defined Wnt gene expression domains along the digestive system. Wnt ligands closely related to prt/wnt2bb that are expressed in a temporal and spatial appropriate window represent good candidates to act in conjunction with Prt/Wnt2bb in liver formation. Detailed expression analyses, followed by loss-of-function studies are performed to identify relevant Wnt ligands and to determine their role in endodermal organogenesis. Results of these studies will be reported during this meeting.

Ref: 194

FUNCTION AND REGULATION OF ODD-SKIPPED RELATED GENES IN KIDNEY DEVELOPMENT

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Odd-skipped genes encode evolutionary conserved zinc finger transcription factors (Odd, Drm, Bowl and Sob in Drosophila and Osr 1 and 2 in vertebrates). In Xenopus and zebrafish, Osr1 and Osr2 are necessary and sufficient for the development of renal structures (pronephros) (Tena et al, 2006). The aim of our study is to unravel how Osr genes are regulated during the pronephros specification using Xenopus and zebrafish as models. To study how the regulation of Osr transcription occurs we pretend to identify and characterize regulatory elements responsible for activation of Ors transcription during renal development. To further study the molecular mechanisms of the activity of Osr genes in renal development we are investigating the possible crosstalk with the retinoic acid signalling pathway, that is known to be required for the specification of pronephric cell fate (Cartry et al, 2006; Wingert et al, 2007).

Ref: 195

TIMETABLE OF SENSORY SPECIFICATION IN THE DEVELOPING CHICK INNER EAR.

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The inner ear is a complex three-dimensional sensorial structure with auditory and vestibular functions. It originates from transient embryonic ectodermal placodes, the otic placodes, which invaginate to form the otocysts. These ovoid-shaped vesicles give rise to neurosensory and non-sensory elements of the adult membranous labyrinths and acoustic-vestibular neurons. A key aim of developmental studies is to understand the molecular and cellular mechanisms involved in epithelial patterning and cell differentiation relative to the specification of sensory patches. A hypothesis based on descriptive evidence suggests that the acquisition of discrete sensory patches during evolution could be generated by the subdivision of an early pan-sensory domain. In order to gain insight into understand this developmental event, we carried out a detailed analysis of the spatial and temporal expression patterns of Fgf genes and different markers of hair cell differentiation with in situ hybridization on serial cryostat sections from otic vesicles up to 8 embryonic days. Being markers of sensory patches, the Bmp4 expression patterns and otic innervation patterns were also considered. Taken together, the results allowed us to determine a timetable of sensory specification in the developing chick inner ear which strongly supports the proposed hypothesis. *E-mail address: mhidalgo@unex.es. This work was supported by Grants BFU2006-15330-C02-02 to L.R-G.

Ref: 196

MIDLINE1 AND THE DEVELOPMENT OF THE CRANIAL PERIPHERAL NERVOUS SYSTEM

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Genetic abnormalities involving the skull and facial region account for around 1/3 of birth defects. Opitz BBB/G syndrome is one such disorder that gives rise to craniofacial malformations, as well as midline defects that cause gastrointestinal anomalies. Patients with X-linked Opitz BBB/G syndrome have loss of function mutations in the gene Midline 1 (Mid1). Using a chick model of cranial development, we demonstrate the expression pattern of the chick orthologue of Mid1 and report a novel function for cMid1 in the formation of the cranial ganglia. In order to study the role of cMid1, we ectopically expressed cMid1 in subpopulations of neural crest cells (NCC's) from rhombomere 4 (r4), normally devoid of the protein. We find that ectopic targeting of cMid1 to r4 NCCs transiently induces the formation of a larger facial ganglion. Subsequent work to down regulate endogenous cMid1 activity in rhombomere 2 demonstrates a reduction in the size of the developing trigeminal ganglia. Current work aimed at elucidating the mechanisms underlying these observations will be presented.

Ref: 197

FUNCTIONAL ANALYSIS OF CG13625 DURING DROSOPHILA WING DEVELOPMENT

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We are studying the requirements and molecular process affected by the Drosophila gene CG13625, which encodes a conserved but uncharacterised 647 amino acids protein. CG13625 has a N-terminal Proline-rich domain and a C-terminal domain of 50 amino acids very similar to S. cerevisae BUD13. BUD13 belongs to the pre-mRNA retention and splicing (RES) complex, which is required for efficient splicing in vitro and in vivo, and also to prevent pre-mRNA leakage from the nucleus. Previous data have identified CG13625 as a gene involved in protein secretion and Golgi organization, and Protein-protein interactions screens using a yeast two-hybrid approach uncovered an association of CG13625 with several cell-cycle regulators, including, CyclinG, CDC2kinase, Dacapo and CyclinD. We generated a polyclonal antibody against CG13625, and found that the protein is present in the cell nucleus of all imaginal cells. The expression of a specific RNA interference construct against CG13625 results in a strong reduction of CG13625 protein levels in the wing disc, and results in wings with reduced size, loss of the wing margin and missing longitudinal veins. Genetic interactions between CG13625 and members of several signalling pathways suggest that a key component of CG13625 activity is related to normal BMP4/Dpp and EGFR signalling.

Ref: 198

EFFECT OF EXPOSURE TO EXTERNAL ENVIRONMENT ON CELL DEATH IN MOUSE PREIMPLANTATIONAL EMBRYOS.

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Dpto. Anatomía, Biología Celular y Zoología. UEX

Some studies have showed that apoptosis can be induced in preimplantational mice embryos by external factors during embryo culture. In the present study we have addressed the incidence on cell death of harmful changes in the protocol used for embryo culture as different media and variations in manipulation and temperature. We have analyzed the percentage of apoptosis using the TUNEL/DAPI technique in mouse embryos of three days (morula stage, Ed3) and of four days (blastocyst stage, Ed4). We have found that embryos developed in vivo do not show cell death. Apoptosis increases when the embryos are cultured in vitro, being the Ed4 embryos more sensitive than Ed3. The pre-culture of the embryos in the oviduct prior to the collection diminishes the apoptotic effects of the in vitro culture. Also, when a slight decrease of temperature is induced in the culture for a short period there is a reduction in cell death. In sum, Ed4 are more sensitive than Ed3 to handling, whereas a short incubation in the oviduct prior to the collection of embryos counteract the harmful effects of the handling. These results could be used to modify current protocols in IVF procedures in order to its improvement.

Ref: 199

FUNCTIONAL ANALYSIS OF MIB2 DURING ADULT MYOGENESIS

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CSIC. CBMSO

Our laboratory aim is to study how myogenesis is regulated and the role that myoblast diversification plays in this process. To this end we are interested in genes that control distinct aspects of myoblast differentiation programmes. Recently, we characterized Mib2 as founder-specific modular protein with putative E3 ubiquitin ligase activity. Mib2 performs through different structural domains multiple functions in Drosophila myogenesis. During embryogenesis, Mib2 is restricted to founder myoblasts and it is necessary for completion of myoblast fusion and for the maintenance of sarcomeric structure. The regulation of the fusion process requires the E3-RING domains of Mib2, however, these motifs appear dispensable for muscle integrity. In adult muscles, Mib2 accumulates at the sarcomeric Z bands and plays a structural role in muscle stability. We will present the data obtained from the analysis of the contribution of the different functional domains of Mib2 to adult myogenesis.

Ref: 201

THE MEDICAL RESEARCH COUNCIL AT HARWELL: PROVIDING THE RESEARCH COMMUNITY WITH TOOLS AND SERVICES FOR MOUSE FUNCTIONAL GENOMICS

Teboul, Lydia
MRC - MLC

The Mouse Genetics Unit and the Mary Lyon Centre are two Medical Research Council units that are based at Harwell, Oxfordshire, UK. The MRC at Harwell offers a range of tools and services to the scientific community for the creation, maintenance, study and archiving of mouse models for human diseases: ∑ A state of the art specific pathogen free mouse holding and breeding facility. ∑ The Frozen Sperm and Embryo Archive which act as the UK node for European Mouse Mutant Archive consortium. ∑ Mutagenesis services for the production of chemically induced mutations in the mouse genome and the creation of transgenic mice by pronuclear injection and gene targeting ∑ Advanced phenotyping platforms including metabolic, sensory and behavioural testing. The MRC at Harwell is a member of both the EUCOMM and EUMODIC consortia. We present in further details some of the tools and services available at the MRC Harwell.

Ref: 202

REGULATION OF APICO-BASAL POLARITY DURING DROSOPHILA WING DISC DEVELOPMENT

Learte, Anabel / Sotillos S / Caminero E / Campuzano S
Centro Biología Molecular (CSIC-UAM). CBMSO Madrid

Growth and pattern formation in the imaginal wing disc of Drosophila is mainly controlled by intercellular signalling pathways operating among the polarized epithelial cells of the disc. We aim at understanding the molecular mechanisms that trigger the spatial segregation and the functional antagonism of members of the polarity complexes (Par, Crumbs and the basolateral complex) in the wing disc epithelium and their relationship with intercellular signalling events. Analysis of the subcellular distribution of the different polarity determinants in the wing disc shows that PATJ colocalizes with DaPKC, whereas Crb, in contrast with its embryonic colocalization with the par complex localizes subapically to DaPKC. Clones of DaPKC null cells are extruded from the epithelium and accumulate high levels of actin and show reduced levels of Crumbs as well as accumulation of Echinoid. None of these phenotypes are found in crumbs mutant clones that show neither extrusion of the epithelium nor accumulation of actin. Constitutive activation of DaPKC causes tumorous overgrowth and delocalization of polarity determinants similar to tumour suppressor gene inactivation phenotypes. Our results further suggest that intracellular trafficking might be compromised in the DaPKC mutant cells.

Ref: 203

DELETION ANALYSIS OF THE IROQUOIS COMPLEX GENES

González-Pérez, Esther / Barrios N / Letizia A / Campuzano S
CBMSO (CSIC-UAM). Developmental Biology

The Iroquois complex (Iro-C) of Drosophila contains three genes, araucan (ara), caupolican (caup) and mirror (mirr) that share cis-regulatory elements which drive their expression in partially overlapping patterns. Ara and Caup proteins are very similar; they share almost identical expression patterns and are involved in the same processes, whereas Mirr is more divergent both in sequence and functions. These genes are thought to act redundantly in several developmental contexts such as notum specification and bristle patterning. To address this question we have generated a set of novel deletions (using the Exelixis collection of P-element and piggyBac insertions) that independently eliminate their activity without affecting shared regulatory sequences. The penetrance and expressivity of the loss-of-bristle phenotype is increased whenever dose of any of the Iro-C proteins is reduced, indicating that the three genes act redundantly to specify development of the notum and patterning of the nervous system. In some genetic combinations of Iro-C mutants, the rostral membrane of the ventral head is disrupted, the maxillary palps are often absent or deformed, and the eyes are dorsally enlarged. We are examining the relation of these phenotypes with the loss of Iro-C from the peripodial membrane of the eye disc.

Ref: 208

ROLE OF DRIL2 IN LIMB AER DEVELOPMENT

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CNIC. Biología del Desarrollo Cardiovascular

The Apical Ectodermal Ridge (AER) is an important signalling centre crucial for vertebrate limb development. This specialized epithelium is located at the dorso-ventral boundary in the distal-most edge of the limb bud and is essential for limb outgrowth. Although the molecular mechanisms involved in its initial induction and dorso-ventral positioning are well established, less is known about the cellular mechanisms leading to its compaction at the tip of the bud from an initially broader domain and maturation to acquire its special histological features. We have identified a new gene, Dril2, a member of the ARID family of transcription factors, which is expressed in the AER. Functional experiments in chicken embryos support an important role for this gene in AER formation: gain of function by overexpressing the wt gene or loss of function by expressing a dominant-negative version or morpholino oligonucleotides lead to alterations in AER morphology. This is confirmed by the AER phenotype observed in KO mouse embryos, where the AER is shorter in the A/P axis but wider in the D/V axis. Our results suggest that Dril2 is not involved in either Wnt signalling leading to Fgf8 expression or the dorso-ventral pathway. Rather, AER maturation seems to be affected. We are analysing in which cellular process Dril2 may be acting.

Ref: 209

FUNCTIONAL ANALYSES OF THE CANONICAL WNT SIGNALLING COMPONENTS IN SCHMIDTE A MEDITERRANEA

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Planarians (phylum Platyhelminthes), are renowned for their regenerative capacities and extensive tissue turnover as part of their normal homeostasis facilitated by the presence of neoblasts, pluripotent adult stem cells. Since TH Morgan's classical work one of the major unanswered mysteries was how the anterior-posterior (A-P) axis is properly re-established and maintained during regeneration and homeostasis. In order to unravel the molecular mechanisms underlying A-P axis determination, we are characterizing the role of the Wnt pathway in the planarian Schmidtea mediterranea. Recently, we have demonstrated that β-catenin (Smed-βcat1), probably as the effector molecule of the of the canonical Wnt pathway, is required for the re-establishment and maintenance of the A-P axis in planarians. RNAi knockdowns of Smed-βcat1 result in an anteriorized phenotype: planarians regenerate heads instead of tails. In order to characterize the function of other elements of the canonical Wnt pathway we have identified their homologues in S. mediterranea. The silencing of some of these genes, particularly using rigorous RNAi methodology, results in planarians regenerating with A-P axis defects. Altogether these results indicate that the canonical Wnt pathway controls planarian A-P axis during regeneration and homeostasis and that previously published work using RNAi by feeding was not efficient at removing functional levels of transcript.

Ref: 210

HAEMATOPOIETIC STEM CELL EMERGENCE AND DIFFERENTIATION IN ZEBRAFISH TIF1GAMMA MUTANTS

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During vertebrate development, primitive haematopoiesis is followed by a definitive wave that includes the stem cells (HSCs) that need to last the adult for its entire life. In zebrafish, primitive red blood cells and the HSCs arise from the posterior lateral mesoderm which migrates to and coalesces at the midline, forming the dorsal aorta and the intermediate cell mass (ICM). Definitive HSCs arise in the ventral wall of the dorsal aorta (DA), immediately juxtaposed to the ICM. Despite this close spatial relationship between the primitive blood and the HSC lineages, it has been possible to distinguish them genetically. One example is the transcriptional intermediate factor, TIF1gamma (or Trim33), which is a nuclear RING-domain ubiquitin ligase that modulates the TGFbeta pathway by interacting with Smad proteins. The zebrafish mutant, moonshine (mon), harbours a mutation in the Smad-interacting domain of TIF1gamma which induces a complete loss of primitive, but not definitive, blood cells. Here we will report on the further exploration of the mon mutant phenotype to gain insight into the emergence, self-renewal and controls over differentiation of the HSC population in zebrafish.

Ref: 211

HOMEOBOX GENES IN PLANARIA

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Planaria possess a great developmental plasticity. They are able to grow and degrow in response to changing nutrition and are able to regenerate from just small body fragments. The underlying genetic networks that enable this astounding plasticity are just beginning to be unravelled. Homeobox genes are a family of transcription factors highly conserved through evolution and are found in animal, plants and fungi. The defining character of this family is a protein domain called the homeodomain which is about 60 amino acids in length and binds to DNA in a sequence dependent manner. Homeobox genes regulate many embryonic developmental programs like axis formation, limb development, haematopoiesis and organ development. In an ongoing reverse genetic screen to identify planarian homologues of transcription factors important for anterior-posterior axis formation we have identified several homeodomain containing genes affecting the positioning of organs along the anterior-posterior axis as well as their shape and number. We will present our results and discuss the role of these homeobox-genes in patterning the planarian body and the possible conservation of their functions from flatworms to vertebrates.

Ref: 212

IDENTIFICATION AND GENETIC, AND MOLECULAR ANALYSIS OF NEW GENES REQUIRED FOR THE CONTROL OF CELL PROLIFERATION AND NEURAL DIFFERENTIATION

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The definition of the size and pattern of an organ largely depends on the control of cell proliferation and differentiation. In order to better understand how these processes are regulated, it is critical to identify as many genes as possible involved in their regulation. Most of the screenings carried out for searching mutation that affect cell proliferation have been focused in the identification of loss of function alleles. One of the problems of this approach is that the lack of function of genes required for proliferation, usually induce cell death. In addition, it has been previously reported that, at least in Drosophila, the functions of some of the genes required for the control of cell proliferation are redundant. Both problems, the redundancy and the cell lethality effect, can be avoid in an over expression screening. This type of screening it is aimed to identify genes by their over expression phenotype. We have used a Gal4 line (GMR-Gal4) to drive the expression in the eye of a collection of about 500 P-UAS elements (EP) inserted randomly throughout the genome. Our screening is aimed to identify genes that affect the pattern of cell proliferation and neuronal differentiation in the second mitotic wave (SMW) during eye development. We have identified several genes that specifically affect one of these processes or both at the same time.

Ref: 213

OLSFRP5 FUNCTION IS REQUIRED FOR PROPER NEUROGENESIS OF THE RETINA AND THE OPTIC TECTUM IN MEDAKA FISH EMBRYOS

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Secreted Frizzled Related Proteins (SFRPs) are multifunctional modulators of the Wnt and BMP signalling pathways. Consistent with a key contribution of these pathways to vertebrate embryonic development, we have previously shown that altered levels of Sfrp1 expression interferes with eye field specification and retina neurogenesis. To test whether other Sfrp family members may be involved in eye formation, we have searched for Sfrp1 homologues in the medaka fish (Oryzia latipes). This search led to the isolation of olSfrp5. We will show that, as Sfrp1, olSfrp5 is strongly expressed in the developing eye but in addition localises to the midbrain and the foregut primordia. Morpholino-based interference with olSfrp5 expression causes a specific phenotype characterised by microphthalmia, sometime associated with coloboma, and reduced size of the optic tectum. These eye defects correlate with a reduced expression of genes specifically expressed in the ventral optic cup. Furthermore, in both the retina and the optic tectum of olSfrp5 morphants, cell proliferation as well as apoptotic cell death is increased. In the retina, this unbalanced generation of progenitor cell leads to a specific reduction in the number of differentiated neurons, affecting retinal ganglion and photoreceptor cells mainly. Together these results suggest that olSfrp5 is involved in the proper establishment of the dorso-ventral polarity of the optic cup as well as in the control of retina neurogenesis.

Ref: 214

POLARIZATION IS TIGHTLY ASSOCIATED WITH CDX2 EXPRESSION IN THE PREIMPLANTATION EMBRYO

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The mechanisms underlying the specification of the first two cell types in the mouse, the trophectoderm (TE, which later forms the placenta) and the inner cell mass (ICM, which later forms the embryo and the yolk sac) have been studied for more than half a century but are not completely understood to this day. Recent work has identified a number of transcription factors important for the formation of these lineages. However, as they are expressed in the same cells at the same time early on, the means by which their expression becomes restricted to different cell types is unclear. The purpose of this study was to determine the relationship between the restriction of TE specific transcription factor expression and two morphological events: compaction and polarization. We found that E-cadherin mutants (that do not undergo compaction) have an increased proportion of polarized cells. This increase in polarized cells is associated with increased TE marker expression. Further, we found that polarization anticipates TE marker expression during TE regeneration in isolated ICMs. These results demonstrate a strong relationship between polarization and the expression of TE markers. To clarify the nature of this relationship, we are using chemical and genetic means to disrupt polarization.

Ref: 215

NANOG INHIBITS MESENDODERM FORMATION AND INCREASES THE EXPRESSION OF GENES INVOLVED IN PLURIPOTENCY

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Embryonic stem cells (ESC) are self-renewing, multipotent cells controlled by cytokines and key transcription factors including Oct4, Sox-2 & Nanog. In vitro studies using ESC have demonstrated the important function of Nanog in cell proliferation and differentiation. In vivo studies have described the spatio-temporal expression pattern of Nanog during early embryogenesis and the important role of Nanog in germ layer specification. However, little is known about the molecular mechanisms that Nanog employs to exert its action during development. To investigate Nanog function and its genetic interactions during development we have microinjected hNanog into Xenopus tropicalis embryos and have performed whole mount in situ hybridization and quantitative real time PCR. Our results demonstrate that ectopic Nanog expression causes gastrulation defects and disrupts the expression of genes associated with mesendoderm formation. In addition ectopic Nanog expression results in increased expression of genes known to be target genes of hNanog in human ESC and which are involved in the maintenance of pluripotency. This suggests that hNanog functions in Xenopus through similar molecular pathways as in human cells and that Xenopus embryos are appropriate animal models to further investigate the molecular interactions of hNanog during development. Future experiments will provide important insights into the fundamental molecular mechanisms of stemness and differentiation across both species.

Ref: 216

EMBRYONIC CEREBROSPINAL FLUID REGULATE NEUROGENESIS VIA RETINOIC ACID.

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In the last decade, we showed that Embryonic Cerebrospinal Fluid (E-CSF) play a key role in early brain development. E-CSF plays a role in the control of the neuroepithelial cell precursor behavior, specially the neuronal differentiation. We previously show that Retinol and Retinol Binding Protein (RBP) are presents in chick embryos E-CSF and both are the substrate and the carrier for Retinoldehydrogenases, enzymes which are involved in Retinoic Acid synthesis, a morphogen involved in neurogénesis. These enzymes are only presents in brain chick embryos in the Mesencephalon-Rombencephalic Istmus (M-R Io). Consequently, we try to show that E-CSF in collaboration with the M-R Io cells are involved in neuroepithelial cell neurogenesis via Retinoic Acid control. In vitro cultures of M-R Io cells of chick embryos were co-cultured with F9-1.8 cells (which develop X-gal dependent blue color in presence of Retinoic Acid) showing that RBP activity from E-CSF was essential for M-R Io cells synthesis of Retinoic Acid. Also we show that E-CSF have “per se” Retinoic Acid like activity. By means organotípic cultures of mesencephalic neuroepithelium plus M-R Io we show that the Retinoic Acid activity regulated by E-CSF are involved directly in neuroepithelial precursors neurogénesis.

Ref: 217

THE DROSOPHILA HOX GENE ABDB COLLABORATES WITH ITS PRIMARY TARGET TO DIRECTLY REMODEL THE APOPTOTIC ACTIVITY IN POSTERIOR SPIRACLES

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Hox genes specify segment identities along the anterior-posterior body axis. As transcription factors, HOX proteins achieve this via regulation of their downstream genes, one group of which, termed “realisators”, performs fundamental cellular behavior under the control of “selectors”, the HOX proteins. However, our current knowledge about realisator genes is very limited, and till now direct regulation by a Hox protein and functional relevance of this regulatory interaction in respect to segment morphology has only been demonstrated for a single realisator gene. Through the study of a newly predicted Abdominal-B (AbdB) dependent Hox Responsive Element (HRE) for the proapoptotic gene reaper (rpr), we describe here another case study of the realisator rpr under direct and collaborative regulation of AbdB and its primary (very likely, “direct”) target Cut (Ct), so as to precisely modify apoptosis for the correct formation of Filzkoeper. We propose that this “feed forward” regulation model could be largely used by AbdB in specifying the posterior spiracles, and could also hold true for all the Hox genes in a sense that they are master regulators and much of cellular behavior may be under the direct inspection of Hox genes themselves.

Ref: 218

SIGNALLING PATHWAYS INVOLVED IN THE COORDINATION OF SOMITOGENESIS AND THE ONSET OF NEURAL CREST CELLS MIGRATION

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The neural crest is a population of pluripotent cells that originates at the border of the dorsal lips of the neural plate. Upon an ephitelial-mesenchimal transition, neural crest cells (NCC) delaminate and migrate away from the neural tube. They migrate following pathways controlled by adjacent segmented paraxial mesoderm. Initial delamination of NCC is induced by signals from the dorsal part of somites, while signals from the unsegmented mesoderm inhibit crest migration. We have explored if signalling pathways (such as FGF, Retinoic Acid and Wnt) involved in the sequential control of somitogenesis and the onset of neural differentiation could be responsible for triggering neural crest migration. To address this question, we have first characterized the spatial and temporal order of expression of several NCCs markers (Snail2, FoxD3, Sox9, Sox5 y Sox10) in relation with paraxial mesoderm segmentation. By blocking FGF signalling in dorsal neural tube we have observed a precocious onset of Sox10 and Sox5 expression that provoked a premature migration of neural crest cells. On the contrary, maintaining FGF signalling by FGF8 implanted beads caused a delay on NCCs delamination. These data suggest that FGF signalling controls NCC delamination to coordinate spatially and temporally the NCC exit with somite formation.

Ref: 219

GENETIC TOOLS TO STUDY NEURONAL CIRCUIT FORMATION IN ZEBRAFISH AND XENOPUS

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The processes, by which neurons acquire specificity, form a given neural network and participate during different chemical and physical stimuli, are far from understood. The simple anatomy of Danio rerio and Xenopus make them ideal models for the study of neural formation and gene expression, as well as for physiological studies. We aim to develop a series of transgenic lines in Danio rerio and Xenopus, which will be used as genetic tools to study the neural circuit formation during development. We are focusing on different types of neurons located in the spinal cord, and will use reporter constructs carrying regulatory regions for specific neural genes to generate transgenic lines.

Ref: 220

SHH/GLI ACTIVITY REGULATES EXPRESSION OF NOTCH LIGANDS TO RESTRICTED DV DOMAINS OF THE DEVELOPING SPINAL CORD.

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Sonic hedgehog (Shh), signalling through the canonical Gli pathway, regulates the expression of transcription factors that demarcate unique progenitor domains in the developing central nervous system. To identify new genes regulated by Shh activity during spinal cord development, we compare the transcriptome of neural progenitors expressing constructs that dominantly confer or inhibit Shh activity in a cell autonomous manner. We focussed on components of the Notch pathway since a putative transcriptional interaction between the hedgehog and the Notch pathways in neural development has not been investigated. After activation of the Shh/Gli pathway, expression of the Notch-ligand Jagged2 appeared up-regulated while other components of the pathway appeared down-regulated. In situ hybridization revealed the ligands of the Notch pathway to be expressed in highly restricted domains throughout the DV axis of the developing spinal cord, and these domains of expression have been regulated by Shh/Gli activity. Jagged1 is expressed in two narrow stripes, highly complementary to the expression of Delta1. Expression of Jagged2 is however restricted to a ventral domain identified as pMN by the co-expression of Olig2. Here we have addressed the role of Jagged2 in the generation of spinal motor neurons and its possible interaction with Olig2.

Ref: 221

INTERACTION BETWEEN NOTCH AND BMP TO DIRECT TRUNK NEURAL CREST DEVELOPMENT

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IBMB CSIC. BMC

Neural crest cells are a transient migratory population of multipotent progenitor cells, generated along the rostro-caudal axis of the developing nervous system at the border between the neural plate and non-neural ectoderm. Their generation depends on the activity of secreted signals, notably BMPs and Wnts, and the integration of the Notch pathway at the boundary between the neural and the non-neural ectoderm. Activation of the BMP pathway, by misexpression of constitutively active BMP receptors, resulted in the induction of the full set of neural crest progenitors markers including Sox9, FoxD3 and Snail2 throughout the DV axis. However, these committed neural crest progenitors lack the capacity to undergo the required epithelial-to-mesenchymal transition and thus to complete the neural crest differentiation programme. In search for additional signals regulating neural crest differentiation we have focused on components of the Notch pathway. Expression analysis revealed several components of the pathway including the receptor Notch4, the ligands Delta1 and Jagged2 and the effector Hes1, to be expressed at the proper times and locations for regulating neural crest differentiation. Furthermore, activation of the BMP pathway results in the ventral expansion of Hes1 expression. Here we have addressed the contribution of specific components of the Notch pathway in the generation of neural crest cells and their interaction with the BMP activity.

Ref: 222

DROSOPHILA TES, HOMOLOG OF THE TUMOR SUPPRESSOR TESTIN, IS REQUIRED FOR GAMETOGENESIS

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We describe dTES, a homolog of the focal adhesion protein Testin, which has tumor suppressor properties in vertebrates. Testin is thought to be a flexible adaptor protein that contributes to focal adhesion assembly/disassembly. dTES is expressed in both somatic and germ cells of the developing testes/ovaries. A P-element insertion in the promoter of dTES results in pleiotropic effects, with high embryonic lethality and few adult escapers. The homozygous adults have defects in gametogenesis, with complete sterility in males and reduced fertility in females. Mutant males show a striking disruption in sperm individualisation. Egg chambers show a range of phenotypes, including ring canal detachment and weakening of the follicular epithelium. Antibody staining reveals dTES at cellular adhesions as well as putative “organelle-attachment sites” within cells. Attempts to create new TES alleles have been unsuccessful, and may explain the inability to isolate deficiencies in the dTES region. Rescue experiments suggest that dTES function may be very sensitive to gene dosage, since extra copies of dTES lead to distinct earlier defects in spermatogenesis. Interestingly, loss of a single copy of Testin predisposes mice to cancer in a similar manner than loss of both copies, suggesting that tumor suppression by Testin is also haplosensitive. Work in progress is examining roles for dTES in embryonic and imaginal development as well as tumor suppression in fly models of cancer.

Ref: 226

TGF-BETA AND BMP DIFFERENTIALLY CONTRIBUTE TO NEUROGENESIS IN THE SPINAL CORD

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During development of the central nervous system, selected transcription factors of the basic helix-loop-helix (bHLH) family appear to play multiple key roles in driving progenitor cells from a proliferative to a terminally differentiated state at the proper times and locations. To supply the spinal cord with the appropriate number of cells, neural progenitors must proliferate sufficiently before differentiating. Id proteins (inhibitory HLH factors lacking the basic DNA binding region) promote cell cycle progression by interacting with components of the cell cycle machinery and inhibit neurogenic bHLH activity by sequestering bHLH factors. Subsequently, the transition from proliferation to neurogenesis involves a coordinated increase in pro-neural bHLH activity and a decrease in Id activity. Furthermore, positional information in the developing spinal cord is acquired by a gradient of extracellular signals, including members of the TGFb superfamily, which set up a combinatorial code of homeodomain and bHLH transcription factors. Here we have analysed the contribution of TGFb and BMP pathways in neurogenesis. By performing in vivo gain- and loss-of-function experiments in the chick neural tube, we demonstrate that Smad3-mediated TGFb activity hinders progenitor features by repressing Ids expression thereby resulting in cell cycle exit and neurogenesis. On the contrary, Smad1/5-mediated BMP activity promotes progenitor identities by maintaining Ids expression.

Ref: 227

ADULT STEM CELLS, ENGINEERED GROWTH FACTORS AND NOVEL BIOMATERIALS FOR SKELETAL REPAIR AND REGENERATION

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Due to the limited regenerating ability of skeletal tissues, severe injuries to bones often leave life-lasting sequels. Tissue engineering offers a promising alternative to traditional orthopedic devices. Our group studies skeletal regeneration, with a focus on stem cell-based skeletal tissue engineering. We are working in three different lines: Adult stem cell differentiation: Our work in bone marrow progenitor cells yielded a methodology (Patent PCT/ES2005/000287) that combines a 3D collagen scaffold with home-engineered growth factors to isolate, propagate and differentiate cells into the chondrogenic and osteogenic pathways. We are now further characterizing this method. Engineered growth factors: BMPs have proven the most effective osteogenic growth factors, but their low affinity for collagen and short half-life hamper them for therapeutic use. We are engineering BMPs to carry a collagen binding domain (CBD). The engineered molecules should get trapped in the extracellular matrix and then be slowly released. We have engineered two human BMPs: rhBMP2-CBD and rhBMP6-CBD. Their stability and osteoinductive properties are currently being tested. Biomaterials: We are testing the performance of our chondro/osteoinduced cells cultured on scaffolding biomaterials. The results show that our cells can be successfully grown on hidroxiapatite. We are presently testing a novel ceramic biomaterial (patent P200702694).

Ref: 229

REQUIREMENT OF NOTOCHORD FOR TIMELY SOMITE SEGMENTATION

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Somites are vertebrate embryonic structures that periodically bud off from the rostral presomitic mesoderm (PSM), latter giving rise to the axial skeleton and the muscles of the body. PSM molecular segmentation and morphological somite formation are processes believed to be independent of axial structures. We have studied the role of the notochord in molecular and morphological somite segmentation. Chick embryo explants were cultured for different time periods with or without notochord. We show that for time periods over 4,5 hours a smaller number of somites is formed in the absence of notochord, results also confirmed by in ovo experiments. Moreover, expression of segmentation markers in the notochord-excised explants was abnormal. Correct somite number formation can be rescued in notochord-ablated explants by the presence of notochord or by the juxtaposition of SHH-producing cells. We conclude that the undetermined PSM tissue requires a signal from the notochord, likely Shh, to correctly segment into somites.

Ref: 231

LOSS OF FUNCTION ANALYSIS OF THE POTENTIAL WNT TARGET GENE CST IN CORTICAL DEVELOPMENT

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The regulation of brain development is the result of complex cell-restricted and temporal expression profiles directed by signaling networks like BMPs, FGFs and WNTs. Despite the well established role of WNT signalling in the CNS patterning, a better understanding of WNT gene function in cortical development requires the identification and characterization of the target genes which are regulated by WNT signaling. The zinc finger transcription factor Cst is the mouse homologue of the Drosophila melanogaster gene Castor, which participates in a genetic cascade controlling temporal aspects of neurogenesis. Cst is expressed in the cortical hem, a signalling centre that produces WNTs and controls the development of the hippocampus and the dorsal neocortex. In order to determine Cst function in the telencephalon we have generated a Cst knock-out mice by homologous recombination of an IRES lacZ cassette into the first coding exon of the Cst gene. Wnt2b expression, a cortical hem marker, appears normal in homozygous mutant embryos. However E14.5 Cst -/- mutant seems to show downregulation of Mash1 in the dentate gyrus primordium of the hippocampus. From this loss of function analysis, we also expect to gain insights into potential Cst functions in the generation and differentiation of Cajal-Retzius neurons.

Ref: 232

HISTAMINE MODULATES THE LEVELS OF NITRIC ACID IN THE ISOLATED EYESTALK OF CRAYFISH.

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In the present study the basal nitric oxide (NO) levels in the isolated perfused eyestalk were measured through the use of the Griees method in saline solution for crustaceans, which had an average of 3.84± 0.24μM NO/mg prot. The nitrate levels were also obtained before, during, and after the application of a pulse of light of 11720 lx to the eyestalk for 30s, which obtained an increase of 41% in nitrate levels evoked by the pulse of light previously mentioned. The basal nitrate levels increased 140% under the first exposure of the light, registering values of 9.34±1.28μM NO/mg prot, a level which is not reached under a second luminary stimuli, which increases to 8.26±1.51μM NO/mg prot, 115% in relation to the basal levels, and with a third stimuli the levels only reached 5.13±0.199μM NO/mg prot, which would be an increase of 34%. When the isolated eyestalk were perfused with a saline solution of more than 10 mM of histamine, a threefold increase in the synthesis of NO was observed, reaching a value of 9.034±0.524 μM NO/mg prot. Finally, when the histamine was applied for prolonged periods, an adaptive reaction to the luminous stimuli was not observed, which suggests that it could play a role in the adaptation of photoreceptors to luminous stimuli. The present study shows evidence the NO levels are modified depending on the luminous intensity, and this response adapts to repetitive stimuli. It is important to note that HA modifies the adaptation to the luminary stimuli.

Ref: 233

DROSOPHILA RIAM REGULATES ACTIN BUNDLES INDEPENDENTLY OF INTEGRINS DURING OOGENESIS

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The regulation of the affinity of integrin receptors to their ligands is essential for their physiological functions, e.g. during leukocyte extravasation or blood clotting. Recent data suggest that in vertebrates an integrin activation complex containing RIAM and Talin binds to and activates integrins. Furthermore, RIAM regulates the actin cytoskeleton, but it is unclear if this process depends on RIAM's function during integrin activation or vice versa, the activation of integrins depends on RIAM's effect on the actin cytoskeleton. We took a reverse genetics approach to analyse the function of the Drosophila homolog of RIAM and found that Drosophila RIAM is essential for an integrin-independent process during oogenesis: Homozygous mutant females lay "dumpless" eggs due to the lack of actin bundles in nurse cells during the phase of rapid cytoplasm transport into the oocyte. Here we present our current data suggesting a model in which RIAM organises the formation of these actin bundles via Enabled and Profilin. These data show that RIAM's function during the regulation of the actin cytoskeleton is independent of integrins and their activation, at least in the case of Drosophila nurse cells.

Ref: 234

EXCITATION BY GABA OF CHICK VESTIBULAR AFFERENTS DURING DEVELOPMENT

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We studied the action of gamma-aminobutyric acid (GABA) in the vestibular system of chicken during the early days of postnatal development (P5-P15-P30; n=30). We used an in vitro preparation of the inner ear and recorded the electrical activity of the vestibular afferents using the multiunit extracellular recording technique. The application of GABA and Muscimol (10-3M; n=30) at postnatal ages caused an increase in the basal discharge; this excitatory response to GABA was of lesser magnitude in the presence of bicuculline, a GABAergic antagonist (10-5M; n= 10). Baclofen (10-5M; n=10) increased the basal discharge of the semicircular canal nerve fibers in P15 chickens, this effect was diminished by CGP35348, a GABAB receptor antagonist. In another experimental section with embryonic (E17) and postnatal (P15) chickens, a mechanical stimulation was applied (0.2Hz) simultaneously to the bath perfusion of bicuculline (10-5M; n=14). There were no significant changes in the response of the vestibular afferents to its natural stimuli. These results provide evidence indicating that GABA plays a significant role in the sensory coding in the vestibular system, participating as a neuromodulator of the afferent discharge. Its release from the hair cells may activate ionotropic and metabotropic postsynaptic receptors. We do not exclude the fact that GABA together with glutamate might be involved in the elaboration of dynamic vestibular responses on the mature system.

Ref: 236

FISHING FOR ENDOTHELIAL AND HAEMATOPOIETIC GENES

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Genetics

Zebrafish is an excellent model for studying vertebrate embryonic development mainly because of their external development, the transparency of their embryos and the possibility of doing forward genetic screens to identify genes involved in developmental processes. Tol2 transposons that were first identified in the teleost Medaka have recently become a valuable tool for insertional mutagenesis and germline transgenesis in zebrafish. Gene-trap vectors are particularly useful. They encode a fluorescent reporter gene downstream of a splice acceptor. Integration of the gene trap into an active gene's intron will, if in the correct reading frame, allow expression of the fluorescent protein and thereby allow identification of genes expressed in tissues of interest. In addition, it may also provide the opportunity of generating a knock-down or even knock-out allele of the respective gene if it interferes sufficiently strongly with the normal splicing of the primary transcript. Here, we are using a tol2-based gene trap vector in the hope to identify genes involved in vascular and haematopoietic development. We will present preliminary results of our expression screen and show transgenic zebrafish that express green fluorescent protein in a variety of different tissues, including the pronephric ducts, the retina, the olfactory placode, neurons of the spinal cord as well as the vasculature.

Ref: 237

EXPRESSION OF THE ACTIVATED FORM OF NOTCH1 IN BONE MARROW HEMATOPOIETIC PROGENITORS IMPAIRS THEIR HEMATOPOIETIC REPOPULATION CAPACITY

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The Notch signalling pathway is involved in different aspects of hematopoiesis. Some of its function is mediated through the expression in the bone marrow niche (Calvi LM. et al., 2003 Nature). To analyze the role of Notch1 in hematopoiesis we have generated transgenic mice that express the activated form of Notch 1 (NIC1) under the regulatory elements of the SCL 3'Enh. This enhancer is active in hematopoietic progenitors, endothelial and osteoblastic cells in the bone marrow (Pimanda JE. et al., 2005, Mol Cell Biol; Silberstein L. et al., 2005, Stem Cells). Analysis of NIC-1 transgenic mice showed NIC1-transcrip expression in BM cells. These mice present a mild but consistent decreased number of B cells and an increase in CFU-C hematopoietic progenitor cells in accordance with previously reported results using viral promoters (Varnum-Finney B. et al., 2000, Nat. Med.). Other hematopoietic cell populations did not present significant differences from wild type. In contrast to this mild phenotypes observed in transgenic animals, a very strong effect is observed when bone marrow NIC-1 cells are confronted with wild type cells in transplant experiments. Competitive long term reconstitution assays using newborn-busulfan treated mice showed an acute decrease in hematopoietic repopulation capacity of the BM-NIC1 donor cells compared with wild type cells. This suggest that activation balance of Notch pathway in niche cells (endothelial and/or osteoblast) and the interactive hematopoietic progenitors is critical for proper hematopoietic repopulation. Further results will be shown on the NIC1+ hematopoietic populations generated in the chimeras and the implications of Notch activation on engraftment and differentiation. Supported by the Spanish Ministry of Education and Science Grant SAF64679 and SAF03448/ Junta de Andalucía PAI-CVI 295 supporting grant

Ref: 238

COMBINATORIAL WNT- AND NOTCH SIGNALLING PATTERNS THE ZEBRAFISH OPTIC TECTUM

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The optic tectum (OT) is one of the most important processing centers of sensory (mainly visual) information in teleost fish. During embryonic development it develops from the simple neuroepithelium of the mesencephalic alar plate into a complex, multilayered structure, containing at least eleven different cell types. The continuous post-embryonic growth of the OT shows that its germinative zone retains proliferating cells during the whole life of the fish, providing a good model to study neural stem cell maintenance and differentiation. Using zebrafish embryos we show that different Notch-Delta receptor-ligand pairs are expressed in distinct, often complementary areas during tectal development. These expression patterns most likely reflect differential functional requirements for Notch and Delta proteins, as functional knock-down of different notch genes results in characteristic defects during tectal neurogenesis. We also provide evidence for the existence of a Wnt-responsive cell population in the dorsal tectum. We hypothesize that these cell are in fact the neuronal stem cells of the progenitor zone, as the impairment of the canonical Wnt signalling pathway late in development causes severe defects in tectal neurogenesis.

Ref: 239

SELF-MODULATION OF NOTCH SIGNALING DURING OMMATIDIAL DEVELOPMENT VIA THE ROUGHENED EYE TRANSCRIPTIONAL REPRESSOR

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The Notch (N) signaling pathway is involved in a vast number of patterning processes in all metazoans. The regulation of the core N pathway is largely understood, but little is known about fine-tuning modulatory effects. We have studied the role of the Drosophila Krüppel-family Zn-finger transcription factor roughened eye (roe) in the context of N-signaling. We demonstrate that during eye patterning N signaling regulates the expression of roe. In turn, Roe negatively modulates the expression of target genes of N-signaling activation. In the absence of roe function, expression of N target genes is elevated and the resulting phenotypes during patterning of the retina are similar to those of N gain-of-function scenarios. Importantly, our data show that Roe binds regulatory DNA sequences of N target genes of the E(spl)-complex both in vitro and in vivo, independently of Su(H)-DNA interaction. We propose a model in which binding of Roe to DNA regulatory sequences independently of Su(H) allows for N-target gene-specific modulation, leading to different levels of expression caused by the same level of N pathway activity.

Ref: 240

THE MEDIATOR COMPLEX PROTEIN MED31 IS REQUIRED FOR MAMMALIAN DEVELOPMENT

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We have previously identified a mouse mutant, L11Jus15, from a balancer chromosome mutangenesis screen. This mutant exhibits growth defects, oedema, and late-gestation lethality. Meiotic mapping and candidate gene analysis revealed that this phenotype results from a mutation in the Mediator complex gene Med31. Mutant embryos have fewer proliferating cells than controls, especially in regions such as the forelimb buds that expand rapidly during development. Likewise, fibroblast lines created from mutant embryos have a severe proliferation defect. As the Mediator complex is a transcriptional coactivator, these results suggest that Med31 functions to promote the transcription of genes required for embryonic growth and cell proliferation.

Ref: 241

IDENTIFICATION OF LIGAND-SPECIFIC MECHANISMS OF JAK/STAT REGULATION

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JAK/STAT signaling is highly conserved in vertebrates and invertebrates and is important in a range of developmental processes including cell proliferation and hematopoietic differentiation. The canonical JAK/STAT pathway in Drosophila consists of 3 ligands Upd (Unpaired), Upd2 and Upd3, a receptor Domeless (Dome), a JAK kinase, Hopscotch (Hop), and a transcription factor, Stat92E. Although the core components of JAK/STAT signaling and their roles are known, comparatively little is understood of the regulators and mechanisms used to control the pathway. Throughout development the different Upd-like ligands have been shown to stimulate JAK/STAT signaling to result in varying outcomes and transcript profiling has revealed that stimulation of JAK/STAT by Upd and Upd2 results in the expression of partly overlapping but clearly distinct transcriptomes. This raises the question of how a pathway with only one receptor, kinase and transcription factor can result in such diverse effects? We therefore aim to identify the ligand specific mechanisms used to produce differential outcomes to signaling from Upd, Upd2 and Upd3. This includes characterization of Upd3 function in Drosophila, in vitro studies to determine the modes of secretion and signaling of each of the Upd ligands and RNAi screens to identify ligand specific modulators of JAK/STAT signaling.

Ref: 244

A DROSOPHILA MODEL FOR COENZYME Q10 DEFICIENCY AND MITOCHONDRIAL DISEASES

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We isolated a previously uncharacterised gene, qless, in a Drosophila mosaic screen for mutants with reduced CNS growth. qless is the fly orthologue of the human PDSS1 gene, encoding a trans-prenyl transferase required for synthesising the lipid side chains of Coenzyme-Q (CoQ). Mutations in PDSS1 lead to CoQ10 deficiency, oxidative phosphorylation disorders and mitochondrial disease. In the Drosophila CNS, we show that qless activity is required in a cell-autonomous manner for neural progenitor divisions and neuronal survival. In qless mutant cells, the mitochondrial membrane is disrupted, cytochrome c is altered, caspases are activated and cell death occurs. Surprisingly, all these aspects of the cell-autonomous CNS phenotype can be rescued by dietary supplementation with CoQs of varying side chain lengths. Thus qless provides a valuable genetic tool for dissecting the relationship between mitochondrial functions and programmed cell death which, although well documented in mammals, remain unclear in Drosophila. In addition, as primary CoQ10 deficiencies and other mitochondrial diseases are rare and difficult to study in humans, the fly qless mutant is also likely to provide a useful disease model.

Ref: 246

EXPRESSION OF H-RASV12 IN A ZEBRAFISH MODEL OF COSTELLO SYNDROME CAUSES CELLULAR SENESCENCE IN ADULT PROLIFERATING CELLS

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Fondazione Ifom

Constitutively active, “oncogenic” H-RAS can drive proliferation and transformation in human cancer, or be a potent inducer of cellular senescence. Moreover, aberrant activation of the Ras pathway due to germline mutations can cause severe developmental disorders. In this study we have generated transgenic zebrafish that constitutively express low levels, or can be induced to express high levels, of oncogenic H-RAS. We observed that fish carrying the integration of the transgene in their germline display several hallmarks of the Costello syndrome, a rare genetic disease due to activating mutations in the gene H-RAS, and can be used as model for the disease. In Costello-like fish low levels of oncogenic H-RAS expression are associated with reduced proliferation and increased senescence markers in adult progenitor cell compartments of the brain and heart, together with activated DNA damage responses. Overexpression of H-RAS through a heat-shock inducible promoter in larvae led to hyperproliferation, activation of the DNA damage response and tp53-dependent cell cycle arrest. Thus, oncogene-induced senescence of adult proliferating cells contributes to the development of Costello syndrome and provides an alternative pathway to transformation in the presence of widespread constitutively active H-RAS expression.

Ref: 248

TEMPORAL AND SPATIAL WINDOWS DELIMIT ACTIVATION OF THE OUTER RING OF WINGLESS IN THE DROSOPHILA WING

Perea Menéndez, Daniel; Terriente Félix, J / Jiménez Díaz-Benjumea, F

Extracellular signalling molecules play many roles in the development of higher organisms. They are used reiteratively in different tissues and stages, but the response of the receiving cells is controlled in a context dependent manner. The pattern of expression of the signalling molecule Wingless/WNT in Drosophila is extraordinarily complex. We have studied the mechanism that controls its expression and function in the outer ring of the Drosophila wing hinge. Our findings indicate that wingless expression is controlled by a dual mechanism: its initial activation requires the product of zinc finger homeodomain 2 and is repressed by the product of the gene complex elbow/no ocelli. This tight regulation restricts the activation of wingless temporally and spatially. Later in development, wingless expression is maintained by an autoregulatory loop that involves the product of homothorax. We have analyzed the phenotype of a wingless allelic combination that specifically removes the outer ring, and our results show that Wingless is required to promote local proliferation of the wing base cells. Thus, cell proliferation in the proximal-distal axis is controlled by the sequential activation of wingless in the inner ring and the outer ring at different stages of development.

Ref: 249

VIRIATO, A NOVEL GENE THAT REGULATES CELL SURVIVAL AND DIFFERENTIATION IN THE EARLY DROSOPHILA RETINA

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The Drosophila eye has contributed much to our knowledge of patterning and cell differentiation. We have identified the viriato (vito) gene in a 1-hybrid screen for transcription factors binding the wg eye-disc enhancer. Like wg, the vito gene is expressed in the anterior unspecified region of the retinal epithelium, and in the peripodial epithelium. Our analysis of vito shows that it plays an important role in regulating cell survival and patterning in the early Drosophila retina. Overexpressing vito in the eye causes apoptosis and transdetermination with the formation of tubular structures in the retina. Reducing vito function by RNAi in the developing eye, resultes in smaller eye fields, and high apoptotic rates are detected in the anterior proliferating region. In support of these observations, we show that vito genetically interacts with Lobe, a gene specifically required for cell survival in the ventral eye disc. Vito belongs to the Nol12 family suggested to be associated with nucleolar functions. We show that reducing Vito levels results in nucleolar decondensation, while overexpressing Vito causes a reverse phenotype, with the nucleolus becoming a very dense and compact structure. Fibrillarin, a methyltransferase involved in pre-rRNA processing, accumulates in nucleoli of vitoRNAi-treated cells when compared with WT cells, while the overexpression of Vito results in loss of fibrillarin at the nucleolus. We are currently investigating the molecular function of Vito.

Ref: 999

THE ROLE OF ABDOMINAL-B IN THE CONTROL OF GROWTH IN THE DROSOPHILA GENITALIA.

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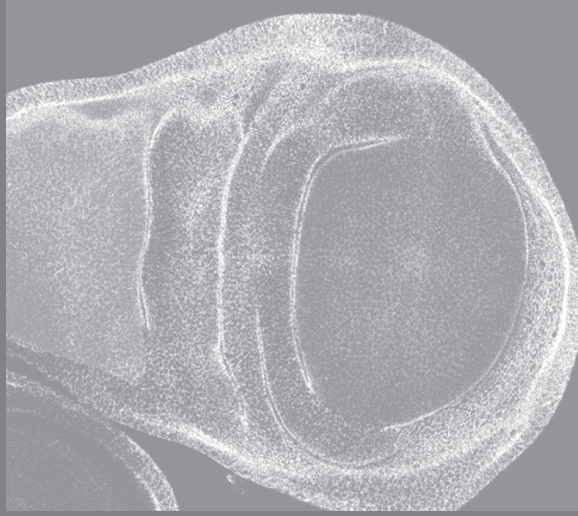
In Drosophila, female genitalia derive mainly from the A8 segment of the female genital disc and male genitalia from the A9 of the male one. The Abdominal-B (Abd-B) Hox gene is needed for the development of both female and male genitalia. There are two Abd-B proteins, Abd-B M and Abd-B R, with different distribution in the genital disc: Abd-B M is expressed in the A8, of both male and female genital discs, whereas Abd-B R is present in the A9 segment. In the female disc, the A9 segment is much smaller than the A8, and it has been shown that the gene double sex (dsx) represses growth in the former, probably by repressing decapentaplegic (dpp) expression. We have found that absence of Abd-B or homothorax (hth) in this segment also increases growth and activates dpp. dsx does not control hth or Abd-B expression, and Abd-B and hth does not regulate each other's expression, either. This suggests that dsx, hth and Abd-B may coordinately repress growth in this primordium of the genital disc.



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