SEBD 1st SEBD-SFBD joint Meeting

Development, Stem cells and Evolution

Toulouse, November 7-10, 2009

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General Informations

1st SEBD-SFBD joint Meeting
Development, Stem cells and Evolution

Toulouse, 7-10 Novembre , 2009
Toulouse, November 7-10, 2009

Meeting Organizers in Toulouse

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Aknowledgements

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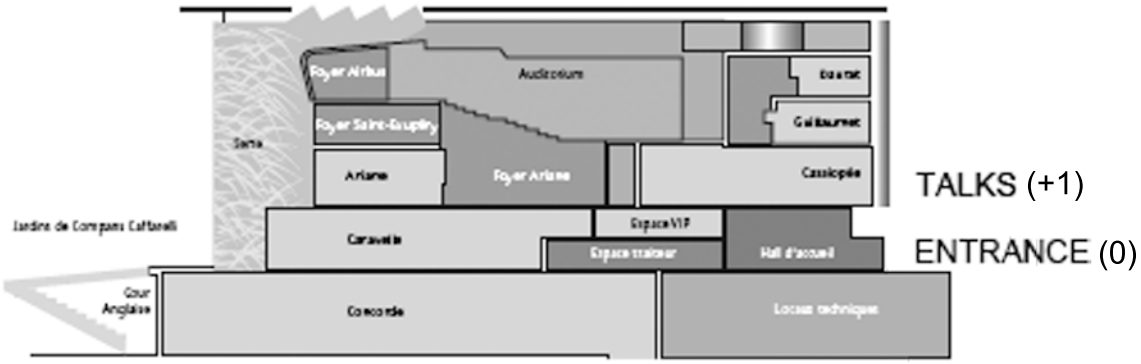
Stem Cell technologies

GENERAL INFORMATIONS

Oral presentartions will take place in the Cassiopée hall (Level 1).

Posters and exhibitions are located in the Ariane hall (Level 1).

Lunch will be served in Caravelle 2 (Level 0).
(See Map next page)



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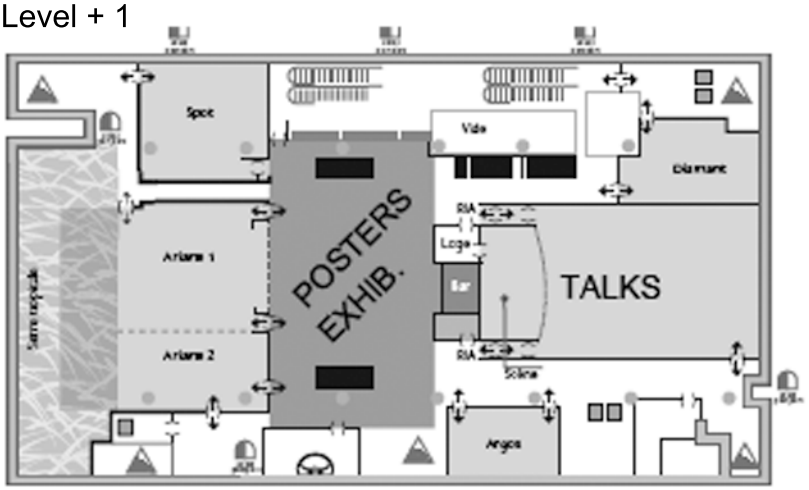

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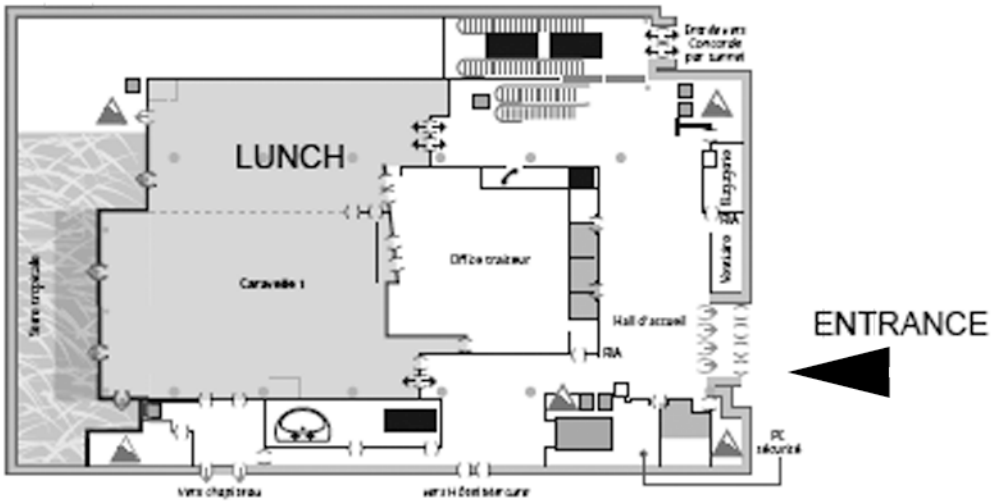

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Program

1er Colloque SEBD-SFBD
1st SEBD-SFBD joint Meeting

Développement, Cellules Souches et Evolution
Development, Stem cells and Evolution

Toulouse, 7-10 Novembre , 2009
Toulouse, November 7-10, 2009

Programme
Program

Samedi / Saturday 7

16h00-21h30 **Inscription/Registration ; informal discussions**

19h00-21h30 **Buffet.**

Dimanche / Sunday 8th

8h50 Opening Remarks

Session 1 : Régénération tissulaire et Modèles Evo/Devo
Tissue regeneration and Evo/Devo models

Sponsored by International Journal of Developmental Biology

Chairperson : Juan Arechaga

9h00-10h30

Brigitte Galliot " *Cell death, compensatory growth and regeneration in evolution ".*

Evelyn Houliston " *Establishment of embryo polarity in the cnidarian Clytia hemisphaerica ".*

Arp Schnittger " *Endoreplication controls cell fate maintenance ".*

10h30-11h00 **Pause Café / Coffee break**

11h00-12h30

Emilo Salo " *Planarian regeneration and axial polarity : The BMP and Wnt pathways ".*

Vincent Laudet " *Retinoic acid and teeth Evo/Devo in teleost fishes ".*

***Jenifer Croce** " *Dynamics of the Delta/Notch Pathway on Endomesoderm Segregation in the Sea Urchin Embryo".*

***Alexandre Alié** " *Evolutionary origin of stem/germ cells: insights from the ctenophore Pleurobrachia ".*

12h30-14h00 **Déjeuner / Lunch**

14h00-15h30 ***Session Poster / Poster / Sponsor Exhibition***

Session 2 : Métabolisme et Croissance Tissulaire
Metabolism and Tissue Growth

Chairperson : Michèle Crozatier

15h30-17h00

Alex Gould " *Food for thought : nutritional regulation of CNS growth in Drosophila ".*

Pierre Leopold " *The humoral control of growth in Drosophila ".*

***P. Bardet** " *Signalling pathways leading to the activation of apoptosis in the cell-polarity mutant crumbs in Drosophila " .*

***Alexandre Djiane** " *Pyd, the Drosophila ZO-1 homolog, binds to Nedd4 E3-Ubiquitin Ligases and controls Notch signaling and epithelial growth " .*

17h00-17h30 **Pause Café / Coffee break**

17h30-18h45

François Schweisguth " *Notch ligand activity is modulated by glycosphingolipid membrane composition in Drosophila".*

Miguel Angel Blasquez " Control of vessel maturation during plant vascular development ".

***François Agnès** " *Double paracrine signaling through the JAK-STAT pathway activates Hid-mediated induction of apoptosis of ovarian supernumerary polar cells in Drosophila ".*

19h00-20h30 **Dîner/ Diner**

20h30-22h30 ***Session Poster / Poster session*** *(impair/odd-numbered)*

Lundi / Monday 9th

Session 3 : Modelage Tissulaire / Tissue patterning

Chairperson : Elisabeth Dupin

9h-10h30

Marie-Hélène Verlhac "*Control of spindle positioning during asymmetric divisions of mouse oocytes*".
***D. Mesnard** " *Deciphering proprotein convertase activity around gastrulation* ".
***Guillaume Luxardi** " *Understanding the fate/morphogenesis interface : The Nodal pathway induces mesendoderm and activate gastrulation effectors* ".
Pilar Cubas "*Evolution of branching patterns in angiosperm*".

10h30-11h00 **Pause Café / Coffee break**

11h00-12h30

Charlie Scutt " *The evolution of carpel development* ".
Fernando Casares "*Genetic architecture of the Drosophila head and the control of eye development*".
François Payre " *Patterning the denticle field of Drosophila embryos : novel insights into an old problem* ".

12h30-14h00 **Déjeuner / Lunch**
13h00-14h00 **Assemblée generale de la SEBD / SEBD general assembly**

14h00-15h00 **Conférence Pléinière / Keynote Lecture**

Andreas Trumpp "*Dormancy in Stem Cells* " .
Sponsored by The Company of Biologists, Cambridge, UK

Session 4 : Hématopoièse et Myogenèse / Hematopoiesis and Myogenesis

Chairperson : Lucas Waltzer

15h15-16h30

Ana Cumano " *Identification of the immediate progenitors of hematopoietic stem cells* " .
***Matthias Kieslinger** " *Expression of Ebf2 in Osteoblastic Cells Regulates Homeostasis of Hematopoietic Stem Cells* ".
Margaret Buckingham "*Pax gene regulation of skeletal muscle stem cells* ".

16h30-17h15 **Pause Café / Coffee break**

17h15-18h45

R. Manoz-Chapuli "*Cardiovascular development: An evolutionary approach*".
***R. Sambasivan** "*Genetic analysis of distinct classes of skeletal muscle stem cells* ".
***Jonathan Enriquez** "*Control of Muscle Diversity by Hox Proteins in the Drosophila embryo* ".
***Fabienne Lescroart** "*Branchiomeric Head Muscles and Anterior Second Heart Field derivatives share a common progenitor* ".
***E. Velasco** "*Pitx2 and Pitx3 modulate cell proliferation vs differentiation in myoblasts* ".

19h00-20h30 **Dîner / Diner**

20h30-22h30 ***Session Poster / Poster session*** *(pair/even-numbered)*

Mardi / Tuesday 10th

Session 5 : Neurogenèse / Neurogenesis

Chairperson : Morgan Locker

9h00-10h30

Alice Davy *" Ephrin reverse signaling in neural progenitors ".*

***D. Sapede** *" Hedgehog (Hh) signalling governs the development of sensory epithelium and its associated innervation in the zebrafish inner ear " .*

*** Myriam Roussigné** *" Understanding the function of Fgf signaling in collective cell migration during the establishment of left / right asymmetry in the brain " .*

***Steven Zuryn** *" In vivo Epithelial-to-Neuron Reprogramming in C. elegans ".*

***C. Borday** " Interactions between canonical Wnt pathway and Hedgehog signalling in retinal stem/precursor cells ".

10h30-11h15 **Pause Café / Coffee break**

11h15-12h30

Ricardo Pardal *" Peripheral nervous system stem cells sustain adult neurogenesis ".*

Cathy Soula *" Temporal modulation of Shh signalling and gliogenesis ".*

***Samuel Tozer** *" A dynamic gradient of BMP signalling controls neuronal subtype identity in the dorsal neural tube ".*

12h30-14h00 **Déjeuner / Lunch**

13h00-14h00 **Assemblée générale de la SFBD / SFBD general assembly**

14h00-15h30

Hitoyoshi Yasuo *" Patterning of the ascidian neural plate via sequential and combinatorial inputs from Nodal, Delta/Notch and FGF signalling pathways ".*

Hernan Lopez-Schier *" A two-step mechanism underlies the recovery of tissue architecture in the regenerating zebrafish lateral line ".*

Laure Bally-Cuif *" Notch signaling in adult neural stem cell maintenance and recruitment ".*

15h30-16h30 Remise des prix des meilleurs posters/ best poster prizes

Sponsored by The Company of Biologists, Cambridge, UK

16h30 Départ/ Departure

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Oral presentations

Cell death, compensatory growth and regeneration in evolution

Brigitte GALLIOT, Luiza GHILA and Simona CHERA

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Keywords: apoptosis, Wnt pathway, Hydra, stem cells

Hydra polyps cut at any level along the body column will heal and invariably regenerate from the lower half a full head with mouth and tentacles (head-regeneration), and from the upper half a basal disk (foot-regeneration). These two types of regeneration are dramatically different at both cellular and molecular levels. We recently showed that after mid-gastric section, the head-regenerating half, but not the foot-regenerating one, immediately initiates a complex cellular remodeling involving apoptosis, engulfment and cell proliferation of distinct cell subpopulations, leading to the complete regeneration of the missing structure in less than three days (Chera et al., Dev Cell, 2009). The apoptotic cells transiently overproduce Wnt3 and activate the canonical Wnt pathway in interstitial progenitors, an event that promotes their synchronous proliferation. In various bilaterian contexts cell death also promotes compensatory proliferation and regeneration and we will discuss the arguments for a possible evolutionarily-conserved mechanism supporting epimorphic regeneration.

Notes

Establishment of embryo polarity in the cnidarian *Clytia hemisphaerica*

Houliston, E., Amiel, A., Chevalier, S., Chang, P., Fourrage C. and Momose, T.

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keywords : cnidarian, polarity, oocyte, Wnt, mRNA localisation

Cnidarians are positioned phylogenetically as a sister group to the Bilateria, and so can offer an informative evolutionary perspective on basic questions of developmental biology. We have been using a new cnidarian model species, *Clytia hemisphaerica*, to dissect the cellular and molecular basis of embryonic polarity development. Unexpectedly, we found that *Clytia* eggs, despite their lack of visible polarity, contain maternal determinant mRNAs with three distinct distributions along the animal-vegetal axis: Fz1 mRNA exhibits a declining animal-vegetal gradient in the cytoplasm; Wnt3 mRNA is localized at the animal cortex and Fz3 at the vegetal cortex. These RNAs direct development of the oral-aboral axis of the embryo by activating the canonical Wnt pathway on the future oral side of the embryo. Thus, as is typical in bilaterian species, establishment of the *Clytia* body plan involves early Wnt pathway activation, and can be traced back to polarisation events occurring in the oocyte prior to fertilisation. More recently, in a small-scale screen, we have identified several more maternal localised RNAs showing these distribution patterns, along with other RNAs associated with a germ plasm-like region at the animal pole of the egg. Another distinct pattern for maternal RNA localisation is shown by Pix, whose RNA and protein appear to associate with mitochondria.
To address the origins of RNA localisation in *Clytia*, we traced the localisation patterns of Fz1, Fz3 and Wnt3 mRNAs through oogenesis and oocyte maturation. Fz1 RNA acquires its polarised cytoplasmic distribution during the latter phase of vitellogenesis by a microtubule-dependent mechanism, whereas the cortical RNAs CheFz3 and CheWnt3 successively adopt their polarized cortical localisations during meiosis completion. The vegetal localisation of CheFz3 RNA requires both microtubules and an intact gonad structure, while the animal localisation of CheWnt3 RNA is microtubule independent and oocyte autonomous. Thus in *Clytia* three temporally and mechanistically distinct RNA localisation pathways contribute to the establishment oocyte polarity and thus to directing body plan development.

Notes

Endoreplication controls cell fate maintenance

A. Schnittger[1,2,3], J. Bramsiepe [1], K. Wester[4], C. Weinl[2], F. Roodbarkelari[2], M. Huelskamp[4], J. Larkin[5]

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Pattern formation, cell fate, endoreduplication, trichome, Arabidopsis

Cell fate specification is typically thought to precede and determine cell cycle regulation during differentiation. Using Arabidopsis trichomes as a model system we provide here a striking example of how endoreplication, a special cell cycle variant that is associated with cell differentiation but also frequently occurs in malignant cells, controls cell fate. For our study we have analyzed trichomes on cell cycle mutant plants and plants overexpressing cell cycle inhibitors under a trichome-specific promoter. Strikingly, a reduction of endoreplication resulted in reduced trichome numbers and caused trichomes to loose their identity. Live observations of young Arabidopsis leaves revealed that dedifferentiating trichomes re-entered mitosis and were re-integrated into the epidermal pavement cell layer acquiring the typical characteristics of the surrounding epidermal cells. Conversely, when we promoted endoreplication in glabrous patterning mutants, trichome fate could be restored. This revealed that endoreplication is an important determinant of cell identity leading to a new model of cell fate control and tissue integrity during development and disease.

Notes

Planarian regeneration and axial polarity: The BMP and Wnt pathways

E. Saló[1], M.D. Molina[1], M. Iglesias[1], Ignacio Maeso[1], A. Neto[2], M. Almuedo[1], A. Aboobaker [3], J.L. Gomez-Skarmeta[2], Kerstin Bartscherer[4], F. Cebrià[1] & T. Adell[1]

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3 Institute of Genetics, University of Nottingham, Nottingham, England
4 German Cancer Research Center, Div. of Signaling and Functional Genomics, and University of Heidelberg/Faculty of Medicine Mannheim, Dept. of Cell and Molecular Biology, 69120 Heidelberg, Germany.

Key words: Planarian, regeneration, BMP, wnt

Planarians can regenerate a whole animal from a tiny fragment of their body, and have become an important model for stem cell and patterning studies. To study the re-establishment and maintenance of dorsal–ventral (D–V) and anterior–posterior (A–P) polarity during planarian regeneration and homeostasis, we characterized evolutionary conserved BMP and Wnt signalling pathways.

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. Recently we identified homologs of the BMP signaling pathway inhibitors. We reported a large number of noggin genes, two canonical noggin genes and eight noggin-like genes, which are characterized by the presence of an insertion of 50-60 aminoacids inside the noggin domain. Their pattern of expression and function during the establishment of the D/V axis in *Xenopus* and planarian will be discussed.

The Wnt/β-catenin signalling pathway 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. Recent studies have characterized several elements of the Wnt/β-catenin signalling pathway in planarians, demonstrating the functional conservation of this pathway in cell-fate determination and A-P axial polarity establishment in these animals. Two β-catenins have been reported from the planarian *Schmidtea mediterranea*. The silencing of one of them, *Smed-βcatenin1*, leads to an extreme phenotype: 'radial-like hypercephalized' planarians, showing large circular cephalic ganglia together with several ectopic eyes all around the planarian body. While gain of Wnt signalling by interfering with elements of the degradation complex as Axins, produces the reciprocal phenotype "anterior tails" a tail appears in regenerating planarians instead of a head. Such phenotype was deeply analyzed with neural markers and a brain rudiment differentiate in the anterior wounds. The systematic RNAi silencing of each *S. mediterranea* 8 wnt genes shows that *Smed-wntP-1* and *Smed-wnt11-2* inhibition originates 'Two-headed' and 'Tailles' planarians, demonstrating that, at least, these 2 wnts signal through Smed-βcatenin1 and would be the morphogens which pattern planarian A/P axis. Despite not expressed posteriorly, the remaining *S. mediterranea* wnts also show a very specific area of expression: *Smed-wntA* is specifically expressed in the posterior part of the cephalic ganglia and *Smed-wnt5* is expressed in the most external region of the CNS. Furthermore, inhibition of *Smed-wntA* by RNAi induces the expansion of the brain posteriorly, and inhibition of *Smed-wnt5* induces the deflection and lateral expansion of the cephalic ganglia. Finally, the interference of the planarian Wnt secretion by disrupting Smed-*evi/wls* function, a transmembrane protein specifically required for the secretion of wnt ligands, produces the phenotypes described in all planarian wnt genes. All that results suggest that not only the A/P axis but the whole planarian body could be patterned through the integrated morphogenetic activity of several wnts.

Notes

Retinoic acid and teeth Evo/Devo in teleost fishes

V. Laudet[1], Y. Gibert[1], L. Bernard[1], M. Debiais-Thibaud[2], F. Bourrat[3], J.S. Joly[3], K. Pottin[4], A. Meyer[5], S. Retaux[4], W. R. Jackman[6] and G. Begemann[5]

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Keywords: Retinoic acid; Tooth; Zebrafish, Pharynx, Evo/Devo

One of the goals of evolutionary developmental biology is to link specific adaptations to changes in developmental pathways. The dentition of cypriniform fishes, which in contrast to many other fish species contains pharyngeal teeth but lacks oral teeth, provides a suitable model to study the development of feeding adaptations. Here, we have examined the involvement of retinoic acid (RA) in tooth development and show that RA is specifically required to induce the pharyngeal tooth developmental program in zebrafish. Perturbation of RA signaling at this stage abolished tooth induction without affecting the development of tooth-associated ceratobranchial bones. We show that this inductive event is dependent on RA synthesis from *aldh1a2* in the ventral posterior pharynx. FGF signaling has been shown to be critical for tooth induction in zebrafish and its loss has been associated with oral tooth loss in cypriniform fishes. Pharmacological treatments targeting the RA and FGF pathways revealed that both pathways act independently during tooth induction. In contrast, we find that in Mexican tetra and medaka, species that also posses oral teeth, both oral and pharyngeal teeth are induced independently of RA. Our analyses suggest an evolutionary scenario in which the gene network controlling tooth development obtained RA-dependency in the lineage leading to the Cypriniformes. The loss of pharyngeal teeth in this group was cancelled out through a shift in *aldh1a2* expression, while oral teeth might have been lost ultimately due to deficient RA signaling in the oral cavity.

Notes

Food for thought: nutritional regulation of CNS growth in *Drosophila*

Louise Cheng, Andrew Bailey, and **Alex P. Gould**

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Keywords: neural stem cells, growth, nutrition, insulin.

Moderate nutrient deprivation during development often results in undersized yet viable adults. However, not all organs scale down proportionately with body size. For example, dietary restriction during human pregnancy can result in small-for-gestational-age newborns with relatively large brains, a process known as brain sparing (Gruenwald 1963). We find that a similar phenomenon occurs when developing *Drosophila* larvae are starved during the late phase of growth. In this case, a near-normal sized CNS is contained within a half-sized larval body. For most larval cell types, growth and division are dependent upon extrinsic signals that are high under fed conditions, such as Insulin and amino acids. Surprisingly, neural stem-cell like progenitors (neuroblasts) are highly atypical in that they can divide in the absence of Insulin Receptor or the amino-acid sensing TOR kinase. Instead, clonal analysis indicates that neuroblast divisions are dependent upon an atypical PI3-kinase pathway that is constitutively active under both fed and starved conditions. Together, these results begin to provide a molecular mechanism for brain sparing.

Gruenwald, P. (1963). *Biol Neonat* 5, 215-65.

Notes

The humoral control of growth in Drosophila

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In metazoans, tissue growth relies on the availability of nutrients - stored internally or obtained from the environment - and on the activation of insulin/IGF signaling (IIS). In *Drosophila*, growth is mediated by several insulin-like peptides (Dilps) that act through a canonical IIS pathway. During the larval period, when animals feed, Dilps produced by the brain couple nutrient uptake with systemic growth. We recently found that during maturation/metamorphosis, when feeding has stopped, a Dilp produced by the fat body (Dilp6) is specifically required to relay the growth signal. Remarkably, DILP6 expression is also induced upon starvation. The expression of *DILP6* during development is controlled by the steroid hormone ecdysone, linking the control of growth with the developmental clock. In addition, both developmental and environmental expression of *DILP6* require activity of the *Drosophila* FoxO transcription factor, therefore defining a feedback regulation on IIS. This study reveals a specific class of insulin-like peptides induced upon metabolic stress that promote growth in condition of nutritional deprivation or following developmentally-induced cessation of feeding.

Notes

Notch ligand activity is modulated by glycosphingolipid membrane composition in *Drosophila*

François Schweisguth[1,3], Sophie Hamel[1] and Jacques Fantini[2]

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keywords: Notch, E3 ubiquitin ligase, endocytosis, Glycosphingolipids, Drosophila

Cell-cell signaling mediated by Notch receptors regulates a wide range of developmental processes and perturbations of Notch signaling activity underlie various human diseases. Following interaction of Notch with its extracellular ligands, intramembrane proteolytic cleavage of Notch results in the release of the intracellular domain from the membrane and transcriptional activation of Notch target genes. Activation of Notch is thus irreversible and a plethora of post-translational regulatory mechanisms control this irreversible step. One key mechanism involves the ubiquitination-dependent endocytosis of the Notch ligands. Genetic and biochemical analyses have shown that endocytosis of the transmembrane ligands Delta (Dl) and Serrate (Ser) is required for the proper activation of Notch receptors. In *Drosophila*, the E3 ubiquitin ligase Mindbomb1 (Mib1) regulates the ubiquitination of Delta and Serrate and thereby promotes both ligand endocytosis and Notch receptor activation during wing development.

To gain novel insights into the role and regulation of Notch ligand trafficking, we performed a genetic modifier screen for gain-of-function suppressors of a dominant-negative form of Mib1. We identified the *α1,4-N-acetylgalactosaminyltransferase1* (*α4GT1*) gene as a gain-of-function suppressor of Mib1 inhibition. Expression of *α4GT1* suppressed the signaling defects of Dl and Ser resulting from the inhibition of *mib1* activity. Genetic and biochemical evidence indicated that *α4GT1* plays a regulatory but non-essential function in Notch signaling and that rescue of *mib1* inhibition requires the synthesis of a specific glycosphingolipid (GSL), N5, produced by α4GT1. We further identified a conserved GSL binding motif in Dl and Ser, raising the possibility that direct GSL-protein interaction may underlie this activity of α4GT1 in Notch signaling. We will discuss how specific GSLs might act to modulate the signaling activity of Notch ligands.

Notes

Control of vessel maturation during plant vascular development

M.A. Blázquez[1], E.G. Minguet[1], F. Vera-Sirera[1], L. Muñiz[2], E. Pesquet[2], J. Carbonell[1], H Tuominen[2]

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Keywords: cell death, xylem, polyamines

Xylem differentiation is initiated from the stem cells that constitute the procambium and vascular cambium. This process involves extensive changes in gene expression, as well as cell wall thickening based on the patterned deposition of lignin and cellulose, that will allow water and solute transport through the plant. Xylem differentiation invariably culminates with cell death. The program that executes the construction of mature xylem cells must therefore be coordinated with their eventual death, to ensure proper development. The importance of this coordination has been highlighted by the analysis of mutants such as *acaulis5* (*acl5*) in Arabidopsis. This mutant was initially identified for its reduced size, and this growth defect has been later shown to be linked to defective vascular development. Specifically, xylem cells of the *acl5* mutant never reach the final maturation state, indicated by careful analysis of the presence of different vascular cell types. To test if this problem was due to premature death of the cells undergoing xylem differentiation, we constructed transgenic plants expressing diphteria toxin A under the control of the *ACL5* promoter. These plants mimicked the phenotype of *acl5* mutants,with respect to shoot growth and xylem defects, suggesting that ACL5 participates in a safeguard mechanism that maintains differentiating cells alive until the whole process is finished. To identify possible genetic targets of ACL5 action in the control of cell death during xylem differentiation, we carried out an EMS-mutagenesis screen looking for extragenic suppressors of the *acl5* mutation. We found more than 40 independent dominant mutants that restored wild-type plant size. Positional cloning of a few of these suppressors has led to the conclusion that most of the mutations are located in the 5'-UTR of three genes, named *AJAX*, that encode bHLH transcription factors. Molecular analysis of these genes has allowed us to propose a role for ACL5 in translational control of *AJAX* genes, and suggest the involvement of the corresponding bHLH transcription factors in the control of the expression of genes involved in xylem maturation, for instance those encoding nucleases and proteases involved in cell death.

Notes

Control of spindle positioning during asymmetric divisions of mouse oocytes

M.-H. Verlhac[1], J. Azoury[1], Karen W. Lee[1], V. Georget[2], P. Rassinier[1], B. Leader[3]

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Keywords: asymmetric divisions, F-actin, Formin 2, oocyte, mouse

Female meiosis in higher organisms consists of highly asymmetric divisions, which retain most maternal stores in the oocyte for embryo development. Asymmetric partitioning of the cytoplasm results from the 'off-center' positioning of the spindle, which, in mouse oocytes, depends mainly on actin filaments. This is a unique situation compared to most systems where spindle positioning requires interactions between astral microtubules and cortical actin filaments. Formin 2, a straight actin filament nucleator, is required for first meiotic spindle migration to the cortex and cytokinesis in mouse oocytes. Although the requirement for actin filaments in the control of spindle positioning is well established in this model, no one has been able to detect them in the cytoplasm. Through the expression of an F-actin specific probe and live confocal microscopy, we show the presence of a cytoplasmic actin meshwork, organized by Formin 2, controlling spindle migration. These filaments organize into a spindle-like structure of F-actin in late meiosis I, that is connected to the cortex. At anaphase, global reorganization of this meshwork allows polar body extrusion. In addition, using actin-YFP, our FRAP analysis confirms the presence of a highly dynamic cytoplasmic actin meshwork, tightly regulated in time and space.

Notes

Evolution of branching patterns in angiosperms.

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One of the central questions in Biology is how morphological diversity arises from the evolution of genomes. In flowering plants, genetic studies indicate that axillary bud development is controlled by conserved genetic pathways evolved before the radiation of flowering plants. However, despite the general conservation of genes and pathways controlling lateral shoot development, a wide diversity of branching patterns, timing of AM initiation, branch outgrowth, response to environmental cues and degrees of apical dominance are found. This suggests that the modulation of this process has diverged in different clades. Inside the bud, a TCP transcription factor 1, tb1/BRC1 is responsible for the suppression of bud outgrowth in monocots and dicots 2-9.

Our working hypothesis is that the evolution of BRC1-like genes must have played a key role in the evolution of branching patterns. We are analysing the evolution and function of BRC1-like genes in the Solanaceae family (subclass Asteridae). We have isolated BRC1-like genes in several Solanaceae species, analyzed in detail their expression patterns and generated loss of function lines for each gene in tomato and potato. Taking into acccount all this information we propose a model for the evolution of BRC1-like genes in Solanaceae.

Notes

Evolution of carpel development

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Keywords: carpel, flower, angiosperm, evolution, Surface Plasmon Resonance

We are beginning to unravel the molecular events surrounding the origin of the carpel, which is the female reproductive organ and precursor of the fruit in the angiosperms, or flowering plants. The carpel is the principal defining feature of the angiosperms, and probably made an important contribution to the evolutionary success of this group, which arose from an unknown ancestor in the Lower Cretaceous Period and rapidly diversified to form some 300 000 species alive today. Our approach begins with the network of genes that controls carpel development in the model angiosperm, *Arabidopsis thaliana*. To identify molecular changes that may have been important for early carpel evolution, we then study orthologous network components in gymnosperms and ANA grade angiosperms, whose lineages diverged from that of *Arabidopsis* before and after, respectively, the origin of the flowering plants. One important practical problem for this approach concerns the current lack of methods for the *in vivo* analysis of gene function in the non-model species of interest to our project. However, many of the genes we are studying encode transcription factors, whose functions at the biochemical level may be open to investigation using *in vitro* approaches. To this end, we have compiled lists of the direct target genes of several carpel development transcription factors from *Arabidopsis thaliana,* and furthermore identified the orthologues of these transcription factors, and of their direct target genes, from ANA grade angiosperms and gymnosperms. We are currently beginning to study conservation, between different plant lineages, of transcription factor-target gene interactions using a novel application we have developed of the Surface Plasmon Resonance technique (Moyroud *et al,* 2009). This technique enables us to measure transcription factor binding to entire gene promoters taken from any organism. The results of our molecular studies should provide insight into the immediate causes of morphological novelty during early angiosperm evolution. However, to provide a complete explanation for the sudden rise and rapid diversification of the flowering plants, environmental, geographical and biological factors pertaining in the Lower Cretaceous Period might also have to be taken into account. It is therefore probable that an interdisciplinary approach, integrating both molecular and ecological data, will be needed to provide full insight into the origin of the flowering plants- an event famously described by Charles Darwin as an "abominable mystery"!

Reference
Moyroud E, Reymond M, Hamès C, Parcy F, Scutt CP (2009). The analysis of entire gene promoters by Surface Plasmon Resonance. The Plant Journal 59: 851-858.

Notes

Genetic architecture of the *Drosophila* head and the control of eye development

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Most structures of the *Drosophila* head –eyes, antennae, palps, ocelli and head capsule- derive from a pair of eye-antennal imaginal discs (AEID). This disc is formed by cells coming from at least six embryonic head segments [1]. However, despite of this multisegmental origin, cells within the EAID remain multipotent until relatively late in development, so that the progeny of a single EAID cell can develop into parts of organs as diverse as the eye and the antenna [2]. Therefore, it is striking that until recently the study of the development of eyes or antennae had been carried out as if these organs were developing in isolation. This has changed with the realization that signaling molecules such as Wg (Wnt-1), Hh and Dpp (BMP2/4), which control growth, specification and patterning of the eye, are mostly produced by prospective head capsule cells [3]. In this talk, I will describe our efforts to determine the genetic architecture of the EAID using lineage-tracing experiments, with special attention to the specification of the prospective head, and discuss them as an integral component of the gene regulatory network that controls eye development. As part of this network, the tight control of the proliferation of eye progenitor cells impacts directly on the final eye size, and indirectly on its patterning. I will present evidence that the progenitor-specific transcription factor homothorax (hth) and the signaling molecule Dpp are engaged in a regulatory module which couples cell cycle with the progression from uncommitted proliferating progenitor cells into quiescent retinal precursors, and that this operation is key in the control of organ size.

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Notes

Patterning the denticle field of *Drosophila* embryos: novel insights into an old problem

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The external morphology of insect species is characterized by epidermal cell extensions, called denticles or trichomes, arranged in stereotyped arrays. Numerous studies using the trichome pattern as a readout have lead us to an exquisite understanding of the gene regulatory networks that progressively pattern embryonic tissues during *Drosophila* development. However, how these early regulatory cascades are translated, within individual epidermal cells, to program and realize the remodelling of their shape for trichome formation has remained elusive. We identified a transcription factor, Shavenbaby (Svb), which plays a key role in determining which cells form trichomes [1], and have shown that Svb directly controls the transcription of a wide range of effectors of cell shape remodelling [2,3]. Analyses of the mechanisms underlying evolution of the trichome pattern have also shown that, in all case studied so far, this is due to modifications of *svb* expression between species [4,5]. We will present recent advances towards understanding how the transcription of *svb* is controlled by upstream regulatory cascades. Moreover, it has been recently discovered that a novel class of small peptides is required for trichome formation [6]. We provide evidence that these peptides indeed regulate the activity of the Svb protein, thereby eventually orchestrating changes in the shape of epidermal cells.

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2- Chanut *et al*, **Plos Biology** 4(9):e290
3- Fernandes *et al*, submitted
4- Sucena *et al*, **Nature** 424(6951):935-8
5- Mc Gregor *et al*, **Nature** 448(7153):587-90
6- Kondo *et al*, **Nat Cell Biol** 9(6):660-5

Notes

Dormancy in normal and malignant stem cells

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Adult stem are required to maintain highly regenerative tissues such as the skin, the intestinal epithelium and the hematopoietic system. Mouse hematopoietic stem cells (HSCs) are the most well characterized somatic stem cell to date, and serve as a model for understanding other adult stem cells present in the mammalian body. Using two types of label-retaining assays we have identified a long-term dormant population within the most immature HSCs (Lin-Sca1+cKit+CD150+CD48-CD34-). Computational modeling suggests that dormant HSCs (d-HSCs) divide about every145 days, or 5 times per lifetime. d-HSCs harbor the vast majority of multi-lineage long-term self-renewal activity. While they form a silent reservoir of the most potent HSCs during homeostasis, they are efficiently activated to self-renew in response to bone marrow injury or G-CSF stimulation. After re-establishment of homeostasis activated HSCs return to dormancy, suggesting that HSCs are not stochastically entering the cell cycle, but reversibly switch from dormancy to self-renewal under conditions of hematopoietic stress[1,2]. One of the reasons cancer stem cells are thought to escape anti-proliferative chemotherapy is their relative dormancy[3]. We now have shown that treatment of mice with Interferon-alpha family leads to the activation and proliferation of dormant HSCs in vivo, which sensitizes them to chemotherapy drugs. HSCs lacking either the interferon-a/b receptor, STAT1 or Sca-1 are insensitive to IFNa stimulation, demonstrating that STAT1 and Sca-1 mediate IFNa induced HSC proliferation[4]. The implications of these results for the design of strategies to target dormant CML stem cells not targetable by imatinib alone will be discussed.

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Notes

Identification of the immediate progenitors of hematopoietic stem cells.

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Blood cells are constantly produced in the bone marrow (BM) of adult mammals. This constant turnover ultimately depends on the hematopoietic stem cells (HSCs). Definitive HSC are first found in the dorsal aorta of the Aorta Gonads Mesonephros (AGM) after the stage of E10.5, during mammalian embryonic development and sequentially colonize the fetal liver, the spleen, and finally the BM. It has been described that there is a close association between the first adult-type HSCs generated in the lumen of the dorsal aorta (DA) and the endothelial cells. We used Flk1-GFP knock-in mice to show high expression of GFP in the DA endothelium at a time where no hematopoietic cells are detected (E8.5). Between E9.5 and E10 cells so far indistinguishable from endothelial cells generate functional multipotent hematopoietic progenitors. In E10.5 AGM high expression of GFP is found in multipotent hematopoietic progenitors while in E11.5 embryos multipotent progenitors belong to GFP^{low} population. We show that de novo generation of HSC is limited in time to the E9.5-10.5 in the dorsal aorta.

The developmental steps that give rise to HSC are unclear. By using a long-term reconstituting assay into $Rag2\gamma c^{-/-}$ and $Rag^{-/-}$ mice, we identified a new population of HSC called immature HSC that are first detected in the DA in E9. Importantly, when cultured in vitro, immature HSC evolve into a population of functional HSC capable to reconstitute $Rag2^{-/-}$ mice. Until now it was accepted that HSC generated in the AGM colonize the fetal liver around E11 where they expand. We show that immature HSC precede definitive HSC in the fetal liver. A 4-day organ culture of fetal liver isolated between from E9.5 resulted in the expansion and maturation of the hematopoietic progenitors into an adult phenotype and the capacity to reconstitute NK+ mice. This capacity is accompanied by the up-regulation of MHC class I expression and the down regulation of Rae1, a NKG2D ligand. We show that the presence of mature HSC in fetal liver is partially due to their production in situ from the immature HSC present in the fetal liver of E9.5.

Notes

Regulation of muscle stem cell fate by *Pax* genes

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Pax3 and Pax7 mark myogenic progenitor cells and play a critical role in regulating their entry into the myogenic programme. We had shown that in the *Pax3/7*$^{-/-}$ double mutant the myogenic determination genes, *Myf5* and *MyoD* are not activated, leading to a major deficit in skeletal muscle. Pax3 directly activates *Myf5*, thus promoting a myogenic cell fate. However, it is essential to maintain a balance between differentiation and renewal of the progenitor cell population. We show that this can be achieved by Pax3 modulation of FGF signaling via *Sprouty1* and *Fgfr4*, which is a direct Pax3 target. Other Pax3 targets will be discussed, including *Dmrt2* and *Foxc2*, both expressed in the dermomyotome, the part of the somite from which skeletal muscle cells derive. *Foxc2* is negatively regulated by Pax3 and in turn feeds back negatively on *Pax3/7* expression. This negative feedback loop is implicated in cell fate decisions of the multipotent Pax3/7 positive stem cells of the dermomyotome. These cells can form derm, brown fat endothelial and smooth muscle cells of blood vessels as well as skeletal muscle. Taking the latter tissues as an example, we show genetically that up-regulation of Foxc2 promotes endothelial and smooth muscle cell fates whereas Pax3/7 promote myogenesis. This is also demonstrated by manipulation of these factors in somite explants. Signaling from adjacent tissues, such as the dorsal ectoderm, affects the equilibrium between *Pax3/7*:*Foxc2* and the choice of cell fate of the multipotent cells expressing these genes in the dermomyotome.

Keywords: Cell fate choices, myogenesis, vaculargenesis, somites, Pax3 and Foxc2.

Notes

Cardiovascular Development: An evolutionary approach

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Keywords: Cardiac development, evolution, endothelium, epicardium

The vertebrate circulatory system is unique in two aspects: 1) It is constituted by a network of vessels internally lined by endothelium. 2) The heart is constituted of myocardial cells surrounded by connective tissue derived from non-muscular embryonic layers (epicardium and endocardium) and endowed with complex and specialized valvular, coronary vessel and conduction systems. In contrast, hemal systems of invertebrates always lack of endothelium, and their hearts, although sharing a common genetic program underlying cardiac specification and differentiation, are much simpler and they contain less components than the vertebrate heart.

We will analyse in our speech the origin of the evolutionary novelties of the vertebrate circulatory system and how this origin can be tracked along cardiovascular development. We will pay special attention to new hypotheses on the evolutionary origin of the endothelium and the epicardium from ancestral blood cells and pronephric progenitors, respectively. We propose that endothelial cells originated from a type of specialized blood cells, called amoebocytes. The transition between amoebocytes and endothelium involved the acquisition of an epithelial phenotype. Angiogenic growth of vessels can thus be regarded as a reacquisition of the invasive ability of amoebocytes. On the other hand we can summarize the main evolutionary novelties of the vertebrate heart as follows: 1) acquisition of an inner lining by an endothelium with ability to transform into valvuloseptal mesenchyme; 2) acquisition of an outer lining derived from an ancestral pronephric glomerular primordium with a high vasculogenic potential, supplying coronary vessel progenitors to the heart; 3) a neural crest cell population which reach the heart from the pharyngeal region and 4) incorporation of new myocardial progenitors at both ends of the primitive cardiac tube from a so-called “secondary heart field”. The complex interactions between all these elements did originate an exceptionally powerful blood pump which was essential to allow vertebrates to reach their characteristic large size and activity.

Notes

Ephrin signaling in the nervous system

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Keywords: ephrins, neural progenitors, miRNAs, cortical development

Ephrins and Eph receptors are cell surface proteins involved in cell-cell communication regulating cell and tissue morphogenesis during embryonic development. In the nervous system this family of proteins was first identified for its role in axon pathfinding, however, more recently, it has been implicated in controlling the switch between maintenance and differentiation of neural progenitors. The molecular basis for this latter function is still unknown. To address this issue, we study ephrin-B1 which is the only member of the ephrinB family whose expression is restricted to neural progenitors and turned off as these cells differentiate. I will discuss our recent work characterizing the function of ephrin-B1 in the mammalian cortex and will present evidence that the pro-neuronal miRNA, miR-124 is both a target and a regulator of ephrin-B1 in neural progenitors.

Notes

Peripheral nervous system stem cells sustain adult neurogenesis

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Keywords: carotid body / peripheral neurogenesis / adult neural stem cells

The carotid body (CB), the main peripheral chemoreceptor in mammals, is a neural crest-derived organ whose major physiological role is to detect oxygen tension in the arterial blood. The CB parenchyma is organized in clusters of sensory (glomus) cells innervated by numerous afferent nerve fibers, which in response to acute hypoxemia activate the brainstem respiratory centers to evoke hyperventilation. In situations of chronic hypoxia (as experienced by high altitude residents or by patients with chronic obstructive lung disease), the CB parenchyma grows in size, thus allowing adaptation of the organisms to a maintained low oxygen tension. We have recently shown that this classic adaptive response of the CB to chronic hypoxia depends on the activation of a population of neural progenitors able to proliferate and differentiate into new neuronal cells (Pardal et al., Cell. Vol. 131 (2007); pp 364-377). CB stem cells change their phenotype from quiescence to proliferation, and back to quiescence, in response to hypoxia-induced niche signaling. In addition to this hypoxia-dependent adaptive role in the adult organ, CB stem cells acquire a definitive glia-like phenotype postnatally, and contribute to the final maturation of the juvenile organ. Abrogation of postnatal neurogenesis by disrupting mitochondrial function in the glia-like progenitors impedes the CB from correct development and maturation. Therefore, the CB is a neurogenic center in the adult and represents a clear example of how postnatal neurogenesis ensures correct maturation and the acquisition of adaptive physiological functions of specialized regions in the nervous system.

Notes

Temporal modulation of Shh signalling and gliogenesis: Sulfatase 1, a new player in Shh-mediated induction of oligodendroglial fate.

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Keywords: neural progenitors, oligodendrocyte specification, Shh signaling, Sulfatase 1

In the embryonic ventral spinal cord, the emergence of oligodendrocyte precursors (OLPs) is a relatively late event that depends on prolonged Sonic Hedgehog (Shh) signaling and is initiated precisely when ventral neural progenitors stop producing neurons. We have shown that an experimental early increase in the concentration of Shh is sufficient to induce premature specification of OLPs at the expense of neuronal genesis, indicating that the relative doses of Shh received by ventral neural progenitors determine whether they become neurons or glial cells. Accordingly, we observed that Shh accumulates at the apical surface of neural progenitors just prior to OLP specification, indicating that these cells are subjected to a higher concentration of the morphogen when they switch to an oligodendroglial fate. We recently evidenced that Sulfatase 1 (Sulf1), a secreted enzyme acting as a regulator of the sulfation state of HSPGs, is expressed in ventral neural progenitors just prior to OLP specification. We further showed that experimental overexpression of Sulf1 in chicken neural tube leads to apical concentration of Shh on neural progenitors, concomitantly to activation of the Shh pathway, arguing in favour of Sulf1 being a positive regulator of Shh signaling responsible for the ventral neuroglial switch. We are currently investigating this question by analysing the consequences of Sulf1 loss of function on OLP specification using various vertebrate animal models.

Notes

Patterning of the ascidian neural plate via sequential and combinatorial inputs from Nodal, Delta/Notch and FGF signalling pathways

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Keywords : neural plate, patterning, ascidian, motoneurons

The ascidian neural plate has a grid-like organisation, with six rows and eight columns of aligned cells, generated by a series of stereotypical cell divisions. We have defined unique molecular signatures for each of the eight cells in the posterior-most two rows of the neural plate - rows I and II. Using a combination of morpholino gene knockdown, dominant-negative forms and pharmacological inhibitors, we tested the role of three signalling pathways in defining these distinct cell identities. Nodal signalling at the 64-cell stage was found to be required to define two different neural plate domains - medial and lateral - with Nodal inducing lateral and repressing medial identities. Delta2, an early transcriptional target of Nodal signals, was found to then subdivide each of the lateral and medial domains to generate four columns. Finally, a separate signalling system along the anteroposterior axis, involving restricted ERK1/2 activation, was found to promote row I fates and repress row II fates. Our results reveal how the sequential integration of three signalling pathways - Nodal, Delta2/Notch and FGF/MEK/ERK - defines eight different sub-domains that characterise the ascidian caudal neural plate. Most remarkably, the distinct fates of the eight neural precursors are each determined by a unique combination of inputs from these three signalling pathways.

Notes

Mechanisms of regeneration of tissue architcture in a sensory organ

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Keywords: hair cells, regeneration, zebrafish, lateral line, tissue architecture

Mechanosensory hair cells show substantial similarities in their development and physiology across species. However, while their loss is irreversible in mammals, other vertebrates can recover hair cells after a damage of their sensory epithelia. In the lateral-line organs of aquatic vertebrates, for example, regeneration follows a choreographed set of steps, but the mechanisms that coordinate the spatial and temporal production of hair cells and the extent of their regeneration are not understood. Here we used quantitative three-dimensional live imaging to identify resident hair-cell progenitors as unipotent transient amplifying cells. Our results demonstrate the existence of resident bona fide hair-cell progenitors, and provide a comprehensive picture of the spatiotemporal control of hair-cell regeneration. We also define a framework for a detailed molecular interrogation of the regenerative process in vivo in an intact mechanosensory organ.

Notes

Notch signaling in adult neural stem cell maintenance and recruitment

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Keywords: zebrafish, adult neurogenesis, Notch, quiescence, neural stem cells

An important limit to the generation of neurons during adulthood is the balance between neural stem cell (NSC) quiescence and recruitment. We are using the germinal zone of the zebrafish adult telencephalon to examine this issue and determine how the frequency of NSC divisions is regulated. We identified in this domain three progenitor states: quiescent radial glial cells (state I), dividing radial glial cells (state II) and cycling neuroblasts committed towards differentiation (state III). A detailed analysis of molecular markers shows a clear parallel between these states and the progenitors described in the adult mammalian brain. Using this model, we show that progenitors transit back and forth between the quiescent and dividing states according to varying levels of Notch-activity: Notch induction drives progenitors into quiescence, while blocking Notch massively re-initiates NSC division, and subsequent commitment towards becoming neurons. Notch activation appears predominantly to be imposed by newly recruited progenitors on their neighbours, suggesting that a self-limiting mechanism of neurogenesis control takes place in adult germinal zones. These results identify for the first time a lateral inhibition-like mechanism in the context of adult neurogenesis, and suggest that the equilibrium between quiescence and neurogenesis in the adult brain is controlled by fluctuations of Notch activity, thereby regulating the number of adult-born neurons.

Notes

Double paracrine signaling through the JAK-STAT pathway activates Hid-mediated induction of apoptosis of ovarian supernumerary polar cells in *Drosophila*

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Keywords: Polar cell, apoptosis, Hid, JAK/STAT, Drosophila

Programmed cell death by apoptosis is widely used during development to shape organs and control cell number by precise elimination of individual cells. Although execution of cell death programs by apoptosis have been characterized, how cells communicate between each other to initiate these programs is still poorly understood and the number of physiological models is limited. In the Drosophila ovary, polar cells (PC) undergo a highly-regulated cell death program whereby a small excess of PC that is produced (1 to 4 cells) at early stages of oogenesis is eliminated by apoptosis such that by midoogenesis all follicles contain exactly 2 PC at each extremity. We have shown that PC apoptosis requires a Hid-Diap1-Dronc-Drice cascade and that hid is positively regulated in the PC destined to die, while DIAP1 is specifically downregulated in these cells in a hid-dependent manner. Unpaired1 (Upd1), one of the three known ligands of the Drosophila JAK/STAT pathway is expressed in PC at all stages of oogenesis. PC-specific RNAi-mediated inactivation of upd1 leads to prolonged survival of supernumerary PC as well as a strong inhibition of JAK/STAT reporter activity in PC and neighboring terminal follicle cells. Importantly, specific RNAi-mediated inactivation of dome, encoding the receptor for Upd1, in terminal follicle cells, but not in PC, also inhibits PC apoptosis. Moreover, clones of stat92E mutant terminal follicle cells adjacent to PC are found associated with groups of supernumerary PC in advanced stages of oogenesis. These results indicate that JAK-STAT signal transduction in terminal follicle cells is necessary for polar cell apoptosis. Finally, we show that RNAi-mediated inhibition of upd1 function within PC abolishes Hid expression in cells destined to die and alters down-regulation of DIAP1 expression in these cells. Altogether, our data provide the first evidence for a pro-apoptotic role of the JAK/STAT pathway during development in Drosophila. They also support a model whereby double paracrine signaling through the JAK/STAT pathway is required to eliminate supernumerary PC. According to this model terminal follicle cells are the source of a STAT-regulated relay signal promoting specific hid transcription in PC destined to die. Given the highly conserved structure and function of the JAK/STAT pathway during evolution and its known implication in cancers, it would be of great interest to test if this pathway also fulfills a proapoptotic role during mammalian development.

Notes

Evolutionary origin of stem/germ cells: insights from the ctenophore *Pleurobrachia pileus*.

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key words : evo-devo, stem cells, ctenophores, vasa, piwi

The reconstruction of an ancestral “molecular fingerprint” of stemness would be a milestone towards understand the origin of animal stem cells. However, this goal is still far for being achieved, as even among the classically studied bilaterian models (e.g. vertebrates and fly), it remains unclear if there is a common molecular signature of stemness. We focalized on a set of RNA regulators (*Piwi, Vasa, Bruno, PL10*) with widely conserved functions in germ stem cells across bilaterians. Previous studies have pointed to a role for this germ line cassette in somatic stem cells as well, at least in some bilaterians (e.g. planarians) and in the hydrozoan cnidarians. To determine if this gene set was indeed ancestrally associated with all kinds of stem cells (rather than only with the germ line) we investigated their expression in embryos and adults of the ctenophore *Pleurobrachia pileus*. Ctenophores are a phylum of marine non-bilaterian animals which a recent phylogenomic analysis positioned as the sister-group of cnidarians. As such, they represent an essential piece of the puzzle for reconstructing the evolutionary history of stem cells. We found that all investigated genes were expressed in male and female germ cells in the ctenophore gonads, confirming their ancient involvement in the germ line. Furthermore, the complete gene set was co-expressed in various populations of somatic stem cells (previously characterized by morphological studies) that continuously provide new cells for regenerating the tentacles tissues. We further used these genes as molecular markers for identifying additional, previously unknown, populations of stem cells in various parts of the adult body, highlighting the so far underestimated complexity of the ctenophore body plan. Finally, we will present preliminary data showing that these markers also allow for tracing the origin of stem/germ cells during embryonic development. That *Piwi, Vasa, Bruno, PL10* are co-expressed in the germ line as well as in various types of somatic stem cells (including neural and muscle stem cells) in ctenophore strongly suggests that they were important components of RNA regulation in stem cells of the last ancestors of Eumetazoa (Ctenophora + Cnidaria + Bilateria) and most likely Bilateria, while they became secondarily confined to the germ line in some bilaterian lineages (e.g. vertebrates).

Notes

Signalling pathways leading to the activation of apoptosis in the cell-polarity mutant *crumbs* in *Drosophila*.

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Keywords: Apoptosis, cell polarity, cell signalling

A precise control of cell death and survival is crucial for a correct tissue patterning during development. We sought to uncover the signalling pathways involved in the control of cell death/survival choices during *Drosophila* development. The Crumbs protein is essential for cell polarity maintenance in the embryonic epithelia. Thus, many epithelial cells in the *crumbs* mutant fail to establish an apical domain and undergo apoptosis. We took advantage of this mutant background with ectopic apoptosis to unravel the regulation of apoptosis in response to loss of epithelial integrity. We first described the precise spatiotemporal pattern of apoptosis activation. We show that some cells specifically survive in the *crumbs* mutant, concomitantly with the maintenance of their apico-basal polarity. Cell death in the rest of epidermis mainly depends on the transcriptional activation of pro-apoptotic gene *reaper*. Based on these premises, we have explored the potential signals leading to this transcriptional activation. One key signalling pathway appears to be essential to trigger cell death in the *crumbs* mutant. We also have preliminary results suggesting that the Hippo pathway contributes to this activation of cell death. Altogether, we are building a new model to explain how loss of Crumbs and mispolarisation of epithelial cells lead to apoptosis activation in epithelial cells. This represents an original model to further understand the link between epithelium integrity maintenance and cell survival.

Notes

Interactions between canonical Wnt pathway and Hedgehog signalling in retinal stem/precursor cells

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Keywords: Canonical Wnt pathway, Hedgehog pathway, Retina, Stem cell, Maintenance

Neural stem cells represent a promising tool to treat a wide range of neurological disorders. Comprehensive analysis of their properties is also of utmost importance in cancerology due to their high similarities with some types of tumour cells. However, therapeutic exploitation of these cells primarily requires achievements in fundamental research. In this context, our work focuses on neural stem cells in *Xenopus* retina. The amphibian retina contains a population of neural stem cells in a defined niche, localized at the margin of the retina, allowing continuous tissue growth throughout the animal's life, as well as regeneration following retinal damage. This system has proved to be very powerful in our previous studies highlighting the role of Wnt and Hedgehog signalling pathways in the control of retinal stem/precursor cell proliferation. We now aim at unravelling the interactions established by these two pathways to sustain stem cell behavior. We first characterized the cellular source of Wnt/Hedgehog morphogens in retinal stem cell niche. We next undertook an *in vivo* functional analysis *(i)* by studying Wnt signalling activity (target gene *CyclinD1* expression, transgenic reporter line) following Hedgehog pathway pharmacological interference *(ii)* by exploring components and target genes expression of the Hedgehog signalling upon pharmacological or genetical perturbation of the Wnt pathway and *(iii)* by investigating retinal cell proliferation and determination phenotypes following simultaneous inhibition or activation of the two pathways. Altogether, our data suggest that Wnt and Hedgehog morphogens form opposite gradients within retinal stem cell niche and that these signalling pathways antagonize with each other to control retinal stem/precursor cell proliferation and multipotency. Although our retinal gradient model is reminiscent to the neural tube patterning model, it is the first time that such an antagonistic interaction between Wnt and Hedgehog signalling is proposed in the context of neural stem cell proliferation.

Notes

Dynamics of the Delta/Notch Pathway on Endomesoderm Segregation in the Sea Urchin Embryo.

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Keywords: embryogenesis, sea urchin, cell fate decision, endomesoderm, Notch pathway

In many triploblastic animals, most of the endoderm and mesoderm arise from a common progenitor, the endomesoderm (Rodaway and Patient, 2001). In sea urchin, the endomesoderm cells emerge at 6th cleavage, in the vegetal half of the embryo, and the endoderm and mesoderm lineages segregate only several cleavages later (Logan and McClay, 1999; Ruffins and Ettensohn, 1996). This event involves primarily the Delta/Notch signaling pathway and two early transcription factors, Gcm and FoxA, expressed in mesoderm and endoderm, respectively (Oliveri et al., 2006; Ransick and Davidson, 2006; Sherwood and McClay, 1999). By looking in detail at the expression patterns and the regulatory relationships of three molecules over the course of sea urchin embryogenesis, we found first that endomesoderm segregation occurs in this embryo at hatching, assuming that definitive mesoderm and endoderm paths begin when cells lose expression of either Gcm or FoxA respectively. Furthermore, experiments that will be presented here will point out that in addition to its initial role in activating Gcm expression in the mesoderm precursor cells (Ransick and Davidson, 2006), the Delta/Notch signal is required for a continuous period of about two cell cycles (or about 2.5 hours) before Gcm expression can continue on its own, independently of Delta, and therefore before the cells have truly adopted the mesoderm path. Thus, our work provides new insights into the timing mechanisms and the molecular dynamics of endomesoderm segregation during sea urchin embryogenesis and into the mode of action of the Delta/Notch pathway in mediating mesoderm fate.

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Notes

Pyd, the Drosophila ZO-1 homolog, binds to Nedd4 E3-Ubiquitin Ligases and controls Notch signaling and epithelial growth.

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ZO-1, Adherens Junctions, Ubiquitin Ligase, Notch, Growth

The polarization of epithelial cells is established and maintained by the asymmetric segregation of evolutionary conserved protein complexes. In vertebrates, the ZO proteins (ZO-1, 2, 3) are MAGUK scaffolds localized at the level of the tight junctions. The role of the vertebrate ZOs in epithelial polarity maintenance has been controversial, even though a role in tight junction integrity is well documented. Vertebrate ZO proteins have also been implicated in directed exocytosis and in growth control through the cytoplasmic sequestering of transcription factors. In order to get insights in the role of ZO proteins, we have generated null mutations in the single *Drosophila* ZO homolog, *polychaetoid* (*pyd*). In the fly epithelia, Pyd is localized at the adherens junctions. *pyd* null mutants are viable, suggesting a non essential role for Pyd in cell polarization. However, *pyd* mutants display slightly overgrown epithelial structures, revealing a role for ZOs in growth control. Finally, *pyd* mutants have extra external sensory organs, a process tightly regulated by Notch signaling. In *pyd* mutant clones the Notch receptor accumulates at the membrane and the Notch pathway is impaired. Pyd binds to the E3-Ubiquitin Ligases Su(dx) and Nedd4, which have been implicated in the regulation of Notch receptor trafficking and downregulation of the pathway. *In vivo* genetic interactions and cell culture experiments suggest that Pyd acts antagonistically to Su(dx) and therefore suggest a model whereby the apically localized ZO proteins control cell signaling by regulating endocytosis.

Notes

Multi-step Control of Muscle Diversity by Hox Proteins in the *Drosophila* Embryo.

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Key words: Hox proteins, cis-regulatory modules, Collier/EBF, Nautilus/MyoD, myogenesis.

Hox proteins are evolutionarily conserved transcription factors which play essential functions in controlling morphogenetic diversity within the animal kingdom. The segment-specific pattern of *Drosophila* skeletal muscles offers an ideal model to study the role of Hox proteins in controlling cell diversification in a context-specific manner. Each muscle is seeded by a founder cell and the properties specific to each muscle reflect the expression by each founder cell of a specific combination of 'identity' transcription factors. Using as a paradigm the dorsal DA3 muscle lineage, we show here that Hox proteins play a decisive role in establishing the *Drosophila* muscle pattern by regulating the expression of muscle identity transcription factors such as Nautilus and Collier (Col). Founder cells arise from asymmetric division of progenitor cells specified at fixed positions within the somatic mesoderm. High-resolution analysis of the activity of two independent *col* cis-regulatory modules, using a newly engineered intron-containing reporter to detect primary transcripts, demonstrates that the progenitor stage is the key step when segment-specific information carried by Hox proteins is superimposed on positional information generated by the segmentation and dorso-ventral patterning processes. The segment specific regulation of *col* transcription by the Hox proteins Antennapedia and Ultrabithorax is mediated by distinct cisregulatory modules. Our data further show that Hox activity subsequently controls the segment-specific number of myoblasts which is allocated to the DA3 muscle. These findings show that Hox proteins both regulate and contribute to the combinatorial code of transcription factors specifying muscle identity and provide a new framework for dissecting the fundamental, multi-step role of Hox proteins in controlling cell diversification and pattern formation in a context-specific manner. Submitted for publication

Notes

Expression of Ebf2 in Osteoblastic Cells Regulates Homeostasis of Hematopoietic Stem Cells

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Haematopoiesis is dependent on the interaction of haematopoietic progenitors with specialized microenvironments. Osteoblastic cells have been implicated in the regulation of the haematopoietic stem cell niche. Ebf2, a member of the Ebf family of transcription factors, is expressed in immature osteoblastic cells, and deletion of *Ebf2* leads to an age-dependent, postnatal decrease in haematopoietic cell numbers. While all lineages are affected due to changes in the environment, the reduction is most pronounced in the lymphoid compartment. The frequency of haematopoietic stem cells as well as common lymphoid progenitor cells is reduced in the bone marrow of Ebf2-deficient mice. Interestingly, bone sections show that Ebf2-expressing osteoblastic cells are in cell-to-cell contact with immature haematopoietic cells. Comparison of osteoblastic cells from *Ebf2+/-* and *Ebf2-/-* mice by DNA chip reveals the deregulation of genes that have already been implicated in the maintenance of HSC as well as genes novel in this process. Finally, we have made advances in characterizing some of these novel genes and their role in the support of hematopoietic stem cells. Taken together, the data suggest a role for Ebf2 in the regulation of immature haematopoietic cells via its expression in immature osteoblastic cells and its modulatory function on secreted and transmembrane proteins in these cells.

Notes

Branchiomeric Head Muscles and Anterior Second Heart Field Derivatives share a common progenitor

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Keywords: clonal analysis, branchiomeric muscles, anterior second heart field

Branchiomeric head muscles are derived from the unsegmented paraxial mesoderm while trunk muscles are derived from the somites (Noden et al., 2006) and are governed by a different regulatory gene program (Mootoosamy and Dieitrich, 2002). It has been of particular interest that some markers involved in head myogenesis, such as *Tbx1, Pitx2, Isl1, Nkx2.5, Tcf21* and *Msc,* are also critical for the development of the anterior second heart field derivatives (right ventricle and arterial pole myocardium) (reviewed in Grifone and Kelly, 2007). Moreover cell-labeling experiments in chick have shown that myocardial cells of the arterial pole and branchiomeric head muscles arise from the same region of the cranial paraxial mesoderm (Tirosh-Finkel et al. 2006). Genetic lineage analyses with *Mef2c-cre* or *Isl1-cre* lines have also shown that heart myocardium and branchiomeric muscles are derived both from *Isl1*+ and *Mef2c*+ progenitor cells (reviewed in Tzahor 2009). We have performed a retrospective clonal analysis at E14.5 using α *-cardiac actinnlaacZ1.1/nlaacZ1.1* mice. We have first found that branchiomeric head muscles are derived from two distinct subpopulations of progenitor cells. We have also found a clonal relationship between the 1st category of branchial arch muscles and the myocardium of the right ventricle and a clonal relationship between the 2nd category of branchial arch muscles and the myocardium of the arterial pole. Moreover left head muscles are clonally related to the myocardium surrounding the pulmonary artery while right head muscles are related to the myocardium surrounding the aorta. This is the first clonal evidence in mice that branchiomeric head muscles share a common progenitor with anterior second heart field derivatives.

Notes

Understanding the fate/morphogenesis interface : The Nodal pathway induces mesendoderm and activate gastrulation effectors

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Nodal, Gastrulation, Planar cell polarity, Cell adhesion

Little is known about how the transition from fate specification to morphogenesis is operated in developing embryos. The first example of such transitions is the gastrulation process, whereby mesendoderm progenitors must internalise, migrate and intercalate to give rise to a three-layered polarised embryo. In vertebrates, mesendoderm is formed in response to Nodal signalling. Here, we demonstrate that Nodal also controls gastrulation movements independently of its role in mesendoderm induction. Using time-dependent inhibition, and taking advantage of the sub-functionalisation among Nodal ligands in Xenopus, we have been able to uncouple these two functions. Following an early phase where Xnr5 and Xnr6 activate the mesendoderm program, Xnr1 and Xnr2 together control a second genetic program, made up of known as well as novel movement effector genes. Interestingly, this program does not include the non-canonical Wnt/PCP pathway, which controls tissue and cell polarity. Nodal activity is important for cell intercalation and convergence-extension in the chordal mesoderm, as well as for head mesoderm migration. One of the main cellular property controlled by Nodal appears to be adhesion. This work reveals that the same signalling pathway coordinates fate adoption, and the capacity to undergo morphogenesis of embryonic cells.

Notes

Deciphering proprotein convertase activity around gastrulation

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Keywords: gastrulation, Nodal, proproteins convertases, biosensor.

Axis and germ layer formation in vertebrates depends on instructive interactions between the epiblast and surrounding extraembryonic tissues and are orchestrated by the secreted proprotein convertases (PC) Furin and PACE4. Genetic evidence in the mouse suggested that Furin and PACE4 are provided by the extraembryonic ectoderm (ExE) to activate the Nodal precursor in adjacent epiblast during gastrulation, but soluble forms of these proteases, their distribution and activity have never been directly observed in vivo. In addition, we hypothesized that Nodal signalling may be stimulated already after implantation by an early wave of transient Furin expression in the visceral endoderm (Mesnard et al., 2006), possibly to achieve maximal Nodal signal duration (BenHaim et al., 2006). To specifically visualize ExE-derived Furin, and to monitor its effect on Nodal signalling, we expressed a Furin-GFP transgene in the ExE of wild-type and Furin-/-;Pace4-/- double mutant embryos. Then to directly monitor PC activity in the epiblast we developed a transgenic line for a PC specific biosensor and analysed its response to different PC genetic backgrounds. Together these approaches allowed us to substantially progress in understanding the mechanism by which PC may control the fate of pluripotent epiblast cells at gastrulation. Of importance, this data strongly suggests that paracrine PC activity may be of relevance in vivo. In addition, our PC biosensor showed to be a powerful tool to decipher the implication and potential redundancy of different PC within a specific tissue, and promises critical advances in understanding the wide implication of PC in vivo.

Notes

Understanding the function of Fgf signaling in collective cell migration during the establishment of left/right asymmetry in the brain.

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Keywords: Left right brain asymmetry, Fgf signalling, cell migration, zebrafish

Brain lateralisation is a widespread feature among vertebrates that is thought to improve cognitive performance and underlie lateralised behaviour. Functional lateralisation of the brain is likely a consequence of differences in brain structure and circuitry between the left and right hemispheres but the mechanisms by which these left right (LR) neuroanatomical asymmetries develop with a consistent laterality is still poorly understood. The zebrafish has emerged as a leading model for understanding how LR anatomical asymmetries develop and how this impacts on cognitive function and lateralized behaviour. In the zebrafish epithalamus, a small group of cells, called the parapineal organ, migrates from the dorsal midline to the left side and is required for subsequent elaboration of epithalamic asymmetries. Recently we described that Fgf8 is required for parapineal migration as the parapineal fails to migrate in *ace/fgf8* mutant embryos and parapineal migration can be rescued in *fgf8* mutants by locally supplied exogenous Fgf8 (Regan J. et al, 2009). Parapineal cells express Fgf Receptor 4 (FgfR4) suggesting that they are able to respond directly to Fgf signals. To better understand the molecular and cellular mechanism by which Fgf8 promotes parapineal migration, we have analysed the spatial and temporal expression of an Fgf reporter transgene. Interestingly, we observe that Fgf signaling is activated in only a few cells on the left side of the parapineal just prior its migration. Results from live imaging analysis reveal that a high level of Fgf signalling correlates with active migratory behaviour suggesting that the Fgf pathway needs to be activated in only few parapineal cells (that might define the parapineal leading cells) to promote migration of the whole nucleus. Finally, we have preliminary data that suggest a role for the Notch pathway in restricting the activation of Fgf signalling to few parapineal cells.

Regan, J. C., Concha, M. L.*, Roussigne, M.*, Russell, C. and Wilson, S. W. *(2009). An Fgf8- dependent bistable cell migratory event establishes CNS asymmetry. Neuron* ***61****, 27-34. * These authors contributed equally.*

Notes

Genetic analysis of distinct classes of skeletal muscle stem cells

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The genetic interactions among the regulatory factors that govern skeletal myogenesis in the body has been well studied. However, the hierarchical relationships within the regulatory network in head muscle development remained unresolved. We have dissected the distinct regulatory cascades that govern muscle progenitor cell fate in two different muscle groups in the head – extraocular muscles (EOMs) that govern eye movements and first pharyngeal arch derived jaw muscles. We show that EOMs have an obligate requirement for the bHLH muscle regulatory factors (MRFs) *Myf5* or *Mrf4* for initiating myogenic fate. Moreover, *Myf5* expressing cells are critical for EOM development. In contrast, *Mrf4* is dispensible for initiating jaw muscle progenitor fate. Notably, almost all first arch derived jaw muscles are absent in *Tbx1:Myf5* double mutants indicating that the T-box transcription factor *Tbx1* acts synergistically with *Myf5* for jaw muscle development. This is reminescent of complementarity between the paired-box transcription factor *Pax3* and *Myf5* for myogenesis in the body. We also show that *Myod*, a key Mrf determination gene, acts epistatically to the initiating cascades in the head as in the body muscle progenitors. Interestingly, these diverse muscle progenitors maintain their respective embryonic regulatory signatures in the adult. However, heterotopic transplantations of adult extraocular satellite cells to the limb show that these intrinsic signatures are not sufficient to ensure the unique muscle phenotypes, since the expected differentiated phenotype is not manifested. These findings identify unique skeletal muscle founder stem cell populations during development. These genetic relationships may provide insights into myopathies which often affect only subsets of skeletal muscles.

Notes

Hedgehog (Hh) signalling governs the development of sensory epithelium and its associated innervation in the zebrafish inner ear

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Keywords: Hh signalling, neurosensory elements, otic neurons, hair cells, inner ear

The inner ear is responsible for the perception of motion and sound in vertebrates. Its functional unit, the sensory patch, contains mechanosensory hair cells innervated by sensory neurons of the vestibular and acoustic ganglia that project to the corresponding nuclei in the brainstem. How hair cells develop at specific positions, and how otic neurons are sorted to specifically innervate each endorgan and to convey the extracted information to the hindbrain is not completely understood yet. In this work, we integrated several of these different aspects and study how, when and where the formation of first-order neurons and their target hair cells takes place. We study the generation of macular sensory patches and investigate the role of Hh signalling in the production of their neurosensory elements. Using zebrafish transgenic lines to visualize the dynamics of hair cell and neuron production, we show that the development of the anterior and posterior maculae is asynchronic, suggesting they are independently regulated. Tracing experiments demonstrate the statoacoustic ganglion is topologically organized in two different neuronal subpopulations, which are spatially segregated and innervate specifically each macula. Functional experiments identify the Hh pathway as crucial in coordinating the production of hair cells in the posterior macula, and the formation of its specific innervation. Finally, gene expression analyses suggest that Hh influences the balance between different SAG neuronal subpopulations. These results lead to a model in which Hh orients functionally the development of inner ear towards an auditory fate in all vertebrate species.

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Notes

A DYNAMIC GRADIENT OF BMP SIGNALLING CONTROLS NEURONAL SUBTYPE IDENTITY IN THE DORSAL NEURAL TUBE

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key words: spinal cord, BMP, morphogen, dorsal interneurons, stem zone

In the spinal cord, BMPs secreted by the roof plate, have been proposed to act as morphogens to specify the pattern of generation of dorsal interneurons dI1-3. However the way in which BMPs perform this function remains unclear. Here we show *in vitro* that the progenitors of dI1 and dI2 are both induced by sustained exposure to a range of BMP4 concentrations. However, dI2 progenitors are induced before dI1. By removing the ligand at different time points, we show that dI2 progenitors are maximally induced after a transient exposure to BMP while dI1 induction required prolonged signalling. Increased exposure times resulted in higher levels of signalling activity. Thus, we could define distinct levels of BMP activity that exclusively induce dI2 and dI1 neurons. Furthermore, by blocking the BMP pathway *in vivo* at progressively later stages, more dorsal interneurons were specified, suggesting that dI3, dI2 and dI1 are sequentially induced. Finally, we analysed the distribution of BMP activity in the progenitor zone of the spinal cord. We observed the establishment of a dorso-ventral gradient over time. Together these data suggest that dI1-3 interneurons are progressively specified by a growing gradient of BMP activity in the “stem zone” of the spinal cord.

Notes

Pitx2 and *Pitx3* modulate cell proliferation *vs* differentiation in myoblasts

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Keywords: *Pitx2*, *Pitx3*, *Pax3*, myogenesis

Pitx2 is a paired-related homeobox gene that has been shown to play a central role during development. *Pitx2* expression has been detected in many tissues during development, including myotomes as well as putative migrating myoblasts. Its expression is also maintained in *Pax3* positive cells that have completed migration at the proximal limb bud. The related *Pitx3* gene is required for ocular development and for the development and maintenance of a subset of midbrain dopaminergic neurons. *Pitx3* is also expressed in differentiating muscle cells concomitant with the onset of myoblast differentation and its expression is maintained in all skeletal muscles while *Pitx2* expression decreases thereafter. Interestingly *Pitx3* mutant mice display normal muscle development and maintain *Pitx2* expression in all skeletal muscles suggesting that *Pitx2* and *Pitx3* may have partly redundant roles. We have previously documented that overexpression *Pitx2*c –isoform in undifferentiated myoblasts (Sol8 myogenic cell line) resulted in upregulation of cell cycle genes (c-myc, *cyclinD1* and D2) 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* differentiation during skeletal myogenesis. By using *Pitx2c* transient transfections we have demonstrate that the *Pitx2*c effects in myoblasts are dose-dependent. Therefore, we have determined at which doses of transfection *Pitx2*c began to induce changes in cell phenotype, inhibiting myocyte differentiation and myotube formation. Interestingly, we found that transient transfections with high *Pitx3* doses also results in modulation of proliferation *vs* differentiation in myoblasts. Additionally, we have currently analyzing whether microRNA-27 (recently reported as regulator of Pax3) can mediate the putative *Pitx2c*-dependent changes in *Pax3* expression. Initial data could support the hypothesis of a role of microRNA-27 mediating *Pitx2-Pax3* interactions in myoblasts.

Notes

In vivo Epithelial-to-Neuron Reprogramming in *C. elegans*

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Keywords: reprogramming; *C. elegans*, stem cell cell identity, regenerative medicine

Processes that underlie the ability of a cell to be reprogrammed and change its identity remain elusive. Understanding the molecular events that mediate such cell plasticity is of interest, not only from a developmental standpoint, but also because of the implications in cancer and regenerative medicines. Various examples of cellular reprogramming have been described, including the direct conversion of pancreatic exocrine cells into beta cells or the switch in drosophila photoreceptor identity. We have established *C. elegans* as a powerful model to study cell plasticity and have characterised an epithelial-toneuron reprogramming that occurs during the development of the worm. We use this unique *in vivo* system to understand the mechanisms underlying the reprogramming of a cell. We have explored factors pertaining to competence, lineage and local environment. We found that this event does not depend on fusion with a neighbouring cell; and that competence to be reprogrammed is restricted and requires 2 transcription factors and the LIN-12/Notch signalling. To identify further genetic cascades important for cellular reprogramming, we have isolated and characterised mutants defective in this event from an EMS screen. Interestingly, we find that there are mutants that halt epithelial-neuron reprogramming at different intermediate stages. Our data suggest that reprogramming is multifaceted and proceeds through transient cellular steps, rather than through concommitant loss and gain of the initial and final identities, even in absence of cell division. These intermediary steps probably do not represent reversion to a more blastic state. We are currently in the process of identifying more mutants through deep sequencing, which we expect will help us further piece together the details of cellular reprogramming *in vivo*.

Notes

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Short talks

Double paracrine signaling through the JAK-STAT pathway activates Hid-mediated induction of apoptosis of ovarian supernumerary polar cells in *Drosophila*

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Keywords: Polar cell, apoptosis, Hid, JAK/STAT, Drosophila

Programmed cell death by apoptosis is widely used during development to shape organs and control cell number by precise elimination of individual cells. Although execution of cell death programs by apoptosis have been characterized, how cells communicate between each other to initiate these programs is still poorly understood and the number of physiological models is limited. In the Drosophila ovary, polar cells (PC) undergo a highly-regulated cell death program whereby a small excess of PC that is produced (1 to 4 cells) at early stages of oogenesis is eliminated by apoptosis such that by midoogenesis all follicles contain exactly 2 PC at each extremity. We have shown that PC apoptosis requires a Hid-Diap1-Dronc-Drice cascade and that hid is positively regulated in the PC destined to die, while DIAP1 is specifically downregulated in these cells in a hid-dependent manner. Unpaired1 (Upd1), one of the three known ligands of the Drosophila JAK/STAT pathway is expressed in PC at all stages of oogenesis. PC-specific RNAi-mediated inactivation of upd1 leads to prolonged survival of supernumerary PC as well as a strong inhibition of JAK/STAT reporter activity in PC and neighboring terminal follicle cells. Importantly, specific RNAi-mediated inactivation of dome, encoding the receptor for Upd1, in terminal follicle cells, but not in PC, also inhibits PC apoptosis. Moreover, clones of stat92E mutant terminal follicle cells adjacent to PC are found associated with groups of supernumerary PC in advanced stages of oogenesis. These results indicate that JAK-STAT signal transduction in terminal follicle cells is necessary for polar cell apoptosis. Finally, we show that RNAi-mediated inhibition of upd1 function within PC abolishes Hid expression in cells destined to die and alters down-regulation of DIAP1 expression in these cells. Altogether, our data provide the first evidence for a pro-apoptotic role of the JAK/STAT pathway during development in Drosophila. They also support a model whereby double paracrine signaling through the JAK/STAT pathway is required to eliminate supernumerary PC. According to this model terminal follicle cells are the source of a STAT-regulated relay signal promoting specific hid transcription in PC destined to die. Given the highly conserved structure and function of the JAK/STAT pathway during evolution and its known implication in cancers, it would be of great interest to test if this pathway also fulfills a proapoptotic role during mammalian development.

Notes

Evolutionary origin of stem/germ cells: insights from the ctenophore *Pleurobrachia pileus*.

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key words : evo-devo, stem cells, ctenophores, vasa, piwi

The reconstruction of an ancestral “molecular fingerprint” of stemness would be a milestone towards understand the origin of animal stem cells. However, this goal is still far for being achieved, as even among the classically studied bilaterian models (e.g. vertebrates and fly), it remains unclear if there is a common molecular signature of stemness. We focalized on a set of RNA regulators (*Piwi, Vasa, Bruno, PL10*) with widely conserved functions in germ stem cells across bilaterians. Previous studies have pointed to a role for this germ line cassette in somatic stem cells as well, at least in some bilaterians (e.g. planarians) and in the hydrozoan cnidarians. To determine if this gene set was indeed ancestrally associated with all kinds of stem cells (rather than only with the germ line) we investigated their expression in embryos and adults of the ctenophore *Pleurobrachia pileus*. Ctenophores are a phylum of marine non-bilaterian animals which a recent phylogenomic analysis positioned as the sister-group of cnidarians. As such, they represent an essential piece of the puzzle for reconstructing the evolutionary history of stem cells. We found that all investigated genes were expressed in male and female germ cells in the ctenophore gonads, confirming their ancient involvement in the germ line. Furthermore, the complete gene set was co-expressed in various populations of somatic stem cells (previously characterized by morphological studies) that continuously provide new cells for regenerating the tentacles tissues. We further used these genes as molecular markers for identifying additional, previously unknown, populations of stem cells in various parts of the adult body, highlighting the so far underestimated complexity of the ctenophore body plan. Finally, we will present preliminary data showing that these markers also allow for tracing the origin of stem/germ cells during embryonic development. That *Piwi, Vasa, Bruno, PL10* are co-expressed in the germ line as well as in various types of somatic stem cells (including neural and muscle stem cells) in ctenophore strongly suggests that they were important components of RNA regulation in stem cells of the last ancestors of Eumetazoa (Ctenophora + Cnidaria + Bilateria) and most likely Bilateria, while they became secondarily confined to the germ line in some bilaterian lineages (e.g. vertebrates).

Notes

Signalling pathways leading to the activation of apoptosis in the cell-polarity mutant *crumbs* in *Drosophila*.

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Keywords: Apoptosis, cell polarity, cell signalling

A precise control of cell death and survival is crucial for a correct tissue patterning during development. We sought to uncover the signalling pathways involved in the control of cell death/survival choices during *Drosophila* development. The Crumbs protein is essential for cell polarity maintenance in the embryonic epithelia. Thus, many epithelial cells in the *crumbs* mutant fail to establish an apical domain and undergo apoptosis. We took advantage of this mutant background with ectopic apoptosis to unravel the regulation of apoptosis in response to loss of epithelial integrity. We first described the precise spatiotemporal pattern of apoptosis activation. We show that some cells specifically survive in the *crumbs* mutant, concomitantly with the maintenance of their apico-basal polarity. Cell death in the rest of epidermis mainly depends on the transcriptional activation of pro-apoptotic gene *reaper*. Based on these premises, we have explored the potential signals leading to this transcriptional activation. One key signalling pathway appears to be essential to trigger cell death in the *crumbs* mutant. We also have preliminary results suggesting that the Hippo pathway contributes to this activation of cell death. Altogether, we are building a new model to explain how loss of Crumbs and mispolarisation of epithelial cells lead to apoptosis activation in epithelial cells. This represents an original model to further understand the link between epithelium integrity maintenance and cell survival.

Notes

Interactions between canonical Wnt pathway and Hedgehog signalling in retinal stem/precursor cells

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Keywords: Canonical Wnt pathway, Hedgehog pathway, Retina, Stem cell, Maintenance

Neural stem cells represent a promising tool to treat a wide range of neurological disorders. Comprehensive analysis of their properties is also of utmost importance in cancerology due to their high similarities with some types of tumour cells. However, therapeutic exploitation of these cells primarily requires achievements in fundamental research. In this context, our work focuses on neural stem cells in *Xenopus* retina. The amphibian retina contains a population of neural stem cells in a defined niche, localized at the margin of the retina, allowing continuous tissue growth throughout the animal's life, as well as regeneration following retinal damage. This system has proved to be very powerful in our previous studies highlighting the role of Wnt and Hedgehog signalling pathways in the control of retinal stem/precursor cell proliferation. We now aim at unravelling the interactions established by these two pathways to sustain stem cell behavior. We first characterized the cellular source of Wnt/Hedgehog morphogens in retinal stem cell niche. We next undertook an *in vivo* functional analysis *(i)* by studying Wnt signalling activity (target gene *CyclinD1* expression, transgenic reporter line) following Hedgehog pathway pharmacological interference *(ii)* by exploring components and target genes expression of the Hedgehog signalling upon pharmacological or genetical perturbation of the Wnt pathway and *(iii)* by investigating retinal cell proliferation and determination phenotypes following simultaneous inhibition or activation of the two pathways. Altogether, our data suggest that Wnt and Hedgehog morphogens form opposite gradients within retinal stem cell niche and that these signalling pathways antagonize with each other to control retinal stem/precursor cell proliferation and multipotency. Although our retinal gradient model is reminiscent to the neural tube patterning model, it is the first time that such an antagonistic interaction between Wnt and Hedgehog signalling is proposed in the context of neural stem cell proliferation.

Notes

Dynamics of the Delta/Notch Pathway on Endomesoderm Segregation in the Sea Urchin Embryo.

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Keywords: embryogenesis, sea urchin, cell fate decision, endomesoderm, Notch pathway

In many triploblastic animals, most of the endoderm and mesoderm arise from a common progenitor, the endomesoderm (Rodaway and Patient, 2001). In sea urchin, the endomesoderm cells emerge at 6th cleavage, in the vegetal half of the embryo, and the endoderm and mesoderm lineages segregate only several cleavages later (Logan and McClay, 1999; Ruffins and Ettensohn, 1996). This event involves primarily the Delta/Notch signaling pathway and two early transcription factors, Gcm and FoxA, expressed in mesoderm and endoderm, respectively (Oliveri et al., 2006; Ransick and Davidson, 2006; Sherwood and McClay, 1999). By looking in detail at the expression patterns and the regulatory relationships of three molecules over the course of sea urchin embryogenesis, we found first that endomesoderm segregation occurs in this embryo at hatching, assuming that definitive mesoderm and endoderm paths begin when cells lose expression of either Gcm or FoxA respectively. Furthermore, experiments that will be presented here will point out that in addition to its initial role in activating Gcm expression in the mesoderm precursor cells (Ransick and Davidson, 2006), the Delta/Notch signal is required for a continuous period of about two cell cycles (or about 2.5 hours) before Gcm expression can continue on its own, independently of Delta, and therefore before the cells have truly adopted the mesoderm path. Thus, our work provides new insights into the timing mechanisms and the molecular dynamics of endomesoderm segregation during sea urchin embryogenesis and into the mode of action of the Delta/Notch pathway in mediating mesoderm fate.

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Notes

Pyd, the Drosophila ZO-1 homolog, binds to Nedd4 E3-Ubiquitin Ligases and controls Notch signaling and epithelial growth.

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ZO-1, Adherens Junctions, Ubiquitin Ligase, Notch, Growth

The polarization of epithelial cells is established and maintained by the asymmetric segregation of evolutionary conserved protein complexes. In vertebrates, the ZO proteins (ZO-1, 2, 3) are MAGUK scaffolds localized at the level of the tight junctions. The role of the vertebrate ZOs in epithelial polarity maintenance has been controversial, even though a role in tight junction integrity is well documented. Vertebrate ZO proteins have also been implicated in directed exocytosis and in growth control through the cytoplasmic sequestering of transcription factors. In order to get insights in the role of ZO proteins, we have generated null mutations in the single *Drosophila* ZO homolog, *polychaetoid* (*pyd*). In the fly epithelia, Pyd is localized at the adherens junctions. *pyd* null mutants are viable, suggesting a non essential role for Pyd in cell polarization. However, *pyd* mutants display slightly overgrown epithelial structures, revealing a role for ZOs in growth control. Finally, *pyd* mutants have extra external sensory organs, a process tightly regulated by Notch signaling. In *pyd* mutant clones the Notch receptor accumulates at the membrane and the Notch pathway is impaired. Pyd binds to the E3-Ubiquitin Ligases Su(dx) and Nedd4, which have been implicated in the regulation of Notch receptor trafficking and downregulation of the pathway. *In vivo* genetic interactions and cell culture experiments suggest that Pyd acts antagonistically to Su(dx) and therefore suggest a model whereby the apically localized ZO proteins control cell signaling by regulating endocytosis.

Notes

Multi-step Control of Muscle Diversity by Hox Proteins in the *Drosophila* Embryo.

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Key words: Hox proteins, cis-regulatory modules, Collier/EBF, Nautilus/MyoD, myogenesis.

Hox proteins are evolutionarily conserved transcription factors which play essential functions in controlling morphogenetic diversity within the animal kingdom. The segment-specific pattern of *Drosophila* skeletal muscles offers an ideal model to study the role of Hox proteins in controlling cell diversification in a context-specific manner. Each muscle is seeded by a founder cell and the properties specific to each muscle reflect the expression by each founder cell of a specific combination of 'identity' transcription factors. Using as a paradigm the dorsal DA3 muscle lineage, we show here that Hox proteins play a decisive role in establishing the *Drosophila* muscle pattern by regulating the expression of muscle identity transcription factors such as Nautilus and Collier (Col). Founder cells arise from asymmetric division of progenitor cells specified at fixed positions within the somatic mesoderm. High-resolution analysis of the activity of two independent *col* cis-regulatory modules, using a newly engineered intron-containing reporter to detect primary transcripts, demonstrates that the progenitor stage is the key step when segment-specific information carried by Hox proteins is superimposed on positional information generated by the segmentation and dorso-ventral patterning processes. The segment specific regulation of *col* transcription by the Hox proteins Antennapedia and Ultrabithorax is mediated by distinct cisregulatory modules. Our data further show that Hox activity subsequently controls the segment-specific number of myoblasts which is allocated to the DA3 muscle. These findings show that Hox proteins both regulate and contribute to the combinatorial code of transcription factors specifying muscle identity and provide a new framework for dissecting the fundamental, multi-step role of Hox proteins in controlling cell diversification and pattern formation in a context-specific manner. Submitted for publication

Notes

Expression of Ebf2 in Osteoblastic Cells Regulates Homeostasis of Hematopoietic Stem Cells

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Haematopoiesis is dependent on the interaction of haematopoietic progenitors with specialized microenvironments. Osteoblastic cells have been implicated in the regulation of the haematopoietic stem cell niche. Ebf2, a member of the Ebf family of transcription factors, is expressed in immature osteoblastic cells, and deletion of *Ebf2* leads to an age-dependent, postnatal decrease in haematopoietic cell numbers. While all lineages are affected due to changes in the environment, the reduction is most pronounced in the lymphoid compartment. The frequency of haematopoietic stem cells as well as common lymphoid progenitor cells is reduced in the bone marrow of Ebf2-deficient mice. Interestingly, bone sections show that Ebf2-expressing osteoblastic cells are in cell-to-cell contact with immature haematopoietic cells. Comparison of osteoblastic cells from *Ebf2+/-* and *Ebf2-/-* mice by DNA chip reveals the deregulation of genes that have already been implicated in the maintenance of HSC as well as genes novel in this process. Finally, we have made advances in characterizing some of these novel genes and their role in the support of hematopoietic stem cells. Taken together, the data suggest a role for Ebf2 in the regulation of immature haematopoietic cells via its expression in immature osteoblastic cells and its modulatory function on secreted and transmembrane proteins in these cells.

Notes

Branchiomeric Head Muscles and Anterior Second Heart Field Derivatives share a common progenitor

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Keywords: clonal analysis, branchiomeric muscles, anterior second heart field

Branchiomeric head muscles are derived from the unsegmented paraxial mesoderm while trunk muscles are derived from the somites (Noden et al., 2006) and are governed by a different regulatory gene program (Mootoosamy and Dieitrich, 2002). It has been of particular interest that some markers involved in head myogenesis, such as *Tbx1, Pitx2, Isl1, Nkx2.5, Tcf21* and *Msc,* are also critical for the development of the anterior second heart field derivatives (right ventricle and arterial pole myocardium) (reviewed in Grifone and Kelly, 2007). Moreover cell-labeling experiments in chick have shown that myocardial cells of the arterial pole and branchiomeric head muscles arise from the same region of the cranial paraxial mesoderm (Tirosh-Finkel et al. 2006). Genetic lineage analyses with *Mef2c-cre* or *Isl1-cre* lines have also shown that heart myocardium and branchiomeric muscles are derived both from *Isl1*+ and *Mef2c*+ progenitor cells (reviewed in Tzahor 2009). We have performed a retrospective clonal analysis at E14.5 using α *-cardiac actinnlaacZ1.1/nlaacZ1.1* mice. We have first found that branchiomeric head muscles are derived from two distinct subpopulations of progenitor cells. We have also found a clonal relationship between the 1st category of branchial arch muscles and the myocardium of the right ventricle and a clonal relationship between the 2nd category of branchial arch muscles and the myocardium of the arterial pole. Moreover left head muscles are clonally related to the myocardium surrounding the pulmonary artery while right head muscles are related to the myocardium surrounding the aorta. This is the first clonal evidence in mice that branchiomeric head muscles share a common progenitor with anterior second heart field derivatives.

Notes

Understanding the fate/morphogenesis interface : The Nodal pathway induces mesendoderm and activate gastrulation effectors

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Nodal, Gastrulation, Planar cell polarity, Cell adhesion

Little is known about how the transition from fate specification to morphogenesis is operated in developing embryos. The first example of such transitions is the gastrulation process, whereby mesendoderm progenitors must internalise, migrate and intercalate to give rise to a three-layered polarised embryo. In vertebrates, mesendoderm is formed in response to Nodal signalling. Here, we demonstrate that Nodal also controls gastrulation movements independently of its role in mesendoderm induction. Using time-dependent inhibition, and taking advantage of the sub-functionalisation among Nodal ligands in Xenopus, we have been able to uncouple these two functions. Following an early phase where Xnr5 and Xnr6 activate the mesendoderm program, Xnr1 and Xnr2 together control a second genetic program, made up of known as well as novel movement effector genes. Interestingly, this program does not include the non-canonical Wnt/PCP pathway, which controls tissue and cell polarity. Nodal activity is important for cell intercalation and convergence-extension in the chordal mesoderm, as well as for head mesoderm migration. One of the main cellular property controlled by Nodal appears to be adhesion. This work reveals that the same signalling pathway coordinates fate adoption, and the capacity to undergo morphogenesis of embryonic cells.

Notes

Deciphering proprotein convertase activity around gastrulation

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Keywords: gastrulation, Nodal, proproteins convertases, biosensor.

Axis and germ layer formation in vertebrates depends on instructive interactions between the epiblast and surrounding extraembryonic tissues and are orchestrated by the secreted proprotein convertases (PC) Furin and PACE4. Genetic evidence in the mouse suggested that Furin and PACE4 are provided by the extraembryonic ectoderm (ExE) to activate the Nodal precursor in adjacent epiblast during gastrulation, but soluble forms of these proteases, their distribution and activity have never been directly observed in vivo. In addition, we hypothesized that Nodal signalling may be stimulated already after implantation by an early wave of transient Furin expression in the visceral endoderm (Mesnard et al., 2006), possibly to achieve maximal Nodal signal duration (BenHaim et al., 2006). To specifically visualize ExE-derived Furin, and to monitor its effect on Nodal signalling, we expressed a Furin-GFP transgene in the ExE of wild-type and Furin-/-;Pace4-/- double mutant embryos. Then to directly monitor PC activity in the epiblast we developed a transgenic line for a PC specific biosensor and analysed its response to different PC genetic backgrounds. Together these approaches allowed us to substantially progress in understanding the mechanism by which PC may control the fate of pluripotent epiblast cells at gastrulation. Of importance, this data strongly suggests that paracrine PC activity may be of relevance in vivo. In addition, our PC biosensor showed to be a powerful tool to decipher the implication and potential redundancy of different PC within a specific tissue, and promises critical advances in understanding the wide implication of PC in vivo.

Notes

Understanding the function of Fgf signaling in collective cell migration during the establishment of left/right asymmetry in the brain.

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Keywords: Left right brain asymmetry, Fgf signalling, cell migration, zebrafish

Brain lateralisation is a widespread feature among vertebrates that is thought to improve cognitive performance and underlie lateralised behaviour. Functional lateralisation of the brain is likely a consequence of differences in brain structure and circuitry between the left and right hemispheres but the mechanisms by which these left right (LR) neuroanatomical asymmetries develop with a consistent laterality is still poorly understood. The zebrafish has emerged as a leading model for understanding how LR anatomical asymmetries develop and how this impacts on cognitive function and lateralized behaviour. In the zebrafish epithalamus, a small group of cells, called the parapineal organ, migrates from the dorsal midline to the left side and is required for subsequent elaboration of epithalamic asymmetries. Recently we described that Fgf8 is required for parapineal migration as the parapineal fails to migrate in *ace/fgf8* mutant embryos and parapineal migration can be rescued in *fgf8* mutants by locally supplied exogenous Fgf8 (Regan J. et al, 2009). Parapineal cells express Fgf Receptor 4 (FgfR4) suggesting that they are able to respond directly to Fgf signals. To better understand the molecular and cellular mechanism by which Fgf8 promotes parapineal migration, we have analysed the spatial and temporal expression of an Fgf reporter transgene. Interestingly, we observe that Fgf signaling is activated in only a few cells on the left side of the parapineal just prior its migration. Results from live imaging analysis reveal that a high level of Fgf signalling correlates with active migratory behaviour suggesting that the Fgf pathway needs to be activated in only few parapineal cells (that might define the parapineal leading cells) to promote migration of the whole nucleus. Finally, we have preliminary data that suggest a role for the Notch pathway in restricting the activation of Fgf signalling to few parapineal cells.

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Notes

Genetic analysis of distinct classes of skeletal muscle stem cells

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The genetic interactions among the regulatory factors that govern skeletal myogenesis in the body has been well studied. However, the hierarchical relationships within the regulatory network in head muscle development remained unresolved. We have dissected the distinct regulatory cascades that govern muscle progenitor cell fate in two different muscle groups in the head – extraocular muscles (EOMs) that govern eye movements and first pharyngeal arch derived jaw muscles. We show that EOMs have an obligate requirement for the bHLH muscle regulatory factors (MRFs) *Myf5* or *Mrf4* for initiating myogenic fate. Moreover, *Myf5* expressing cells are critical for EOM development. In contrast, *Mrf4* is dispensible for initiating jaw muscle progenitor fate. Notably, almost all first arch derived jaw muscles are absent in *Tbx1:Myf5* double mutants indicating that the T-box transcription factor *Tbx1* acts synergistically with *Myf5* for jaw muscle development. This is reminescent of complementarity between the paired-box transcription factor *Pax3* and *Myf5* for myogenesis in the body. We also show that *Myod*, a key Mrf determination gene, acts epistatically to the initiating cascades in the head as in the body muscle progenitors. Interestingly, these diverse muscle progenitors maintain their respective embryonic regulatory signatures in the adult. However, heterotopic transplantations of adult extraocular satellite cells to the limb show that these intrinsic signatures are not sufficient to ensure the unique muscle phenotypes, since the expected differentiated phenotype is not manifested. These findings identify unique skeletal muscle founder stem cell populations during development. These genetic relationships may provide insights into myopathies which often affect only subsets of skeletal muscles.

Notes

Hedgehog (Hh) signalling governs the development of sensory epithelium and its associated innervation in the zebrafish inner ear

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Keywords: Hh signalling, neurosensory elements, otic neurons, hair cells, inner ear

The inner ear is responsible for the perception of motion and sound in vertebrates. Its functional unit, the sensory patch, contains mechanosensory hair cells innervated by sensory neurons of the vestibular and acoustic ganglia that project to the corresponding nuclei in the brainstem. How hair cells develop at specific positions, and how otic neurons are sorted to specifically innervate each endorgan and to convey the extracted information to the hindbrain is not completely understood yet. In this work, we integrated several of these different aspects and study how, when and where the formation of first-order neurons and their target hair cells takes place. We study the generation of macular sensory patches and investigate the role of Hh signalling in the production of their neurosensory elements. Using zebrafish transgenic lines to visualize the dynamics of hair cell and neuron production, we show that the development of the anterior and posterior maculae is asynchronic, suggesting they are independently regulated. Tracing experiments demonstrate the statoacoustic ganglion is topologically organized in two different neuronal subpopulations, which are spatially segregated and innervate specifically each macula. Functional experiments identify the Hh pathway as crucial in coordinating the production of hair cells in the posterior macula, and the formation of its specific innervation. Finally, gene expression analyses suggest that Hh influences the balance between different SAG neuronal subpopulations. These results lead to a model in which Hh orients functionally the development of inner ear towards an auditory fate in all vertebrate species.

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Notes

A DYNAMIC GRADIENT OF BMP SIGNALLING CONTROLS NEURONAL SUBTYPE IDENTITY IN THE DORSAL NEURAL TUBE

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key words: spinal cord, BMP, morphogen, dorsal interneurons, stem zone

In the spinal cord, BMPs secreted by the roof plate, have been proposed to act as morphogens to specify the pattern of generation of dorsal interneurons dI1-3. However the way in which BMPs perform this function remains unclear. Here we show *in vitro* that the progenitors of dI1 and dI2 are both induced by sustained exposure to a range of BMP4 concentrations. However, dI2 progenitors are induced before dI1. By removing the ligand at different time points, we show that dI2 progenitors are maximally induced after a transient exposure to BMP while dI1 induction required prolonged signalling. Increased exposure times resulted in higher levels of signalling activity. Thus, we could define distinct levels of BMP activity that exclusively induce dI2 and dI1 neurons. Furthermore, by blocking the BMP pathway *in vivo* at progressively later stages, more dorsal interneurons were specified, suggesting that dI3, dI2 and dI1 are sequentially induced. Finally, we analysed the distribution of BMP activity in the progenitor zone of the spinal cord. We observed the establishment of a dorso-ventral gradient over time. Together these data suggest that dI1-3 interneurons are progressively specified by a growing gradient of BMP activity in the “stem zone” of the spinal cord.

Notes

Pitx2 and *Pitx3* modulate cell proliferation *vs* differentiation in myoblasts

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Keywords: *Pitx2*, *Pitx3*, *Pax3*, myogenesis

Pitx2 is a paired-related homeobox gene that has been shown to play a central role during development. *Pitx2* expression has been detected in many tissues during development, including myotomes as well as putative migrating myoblasts. Its expression is also maintained in *Pax3* positive cells that have completed migration at the proximal limb bud. The related *Pitx3* gene is required for ocular development and for the development and maintenance of a subset of midbrain dopaminergic neurons. *Pitx3* is also expressed in differentiating muscle cells concomitant with the onset of myoblast differentation and its expression is maintained in all skeletal muscles while *Pitx2* expression decreases thereafter. Interestingly *Pitx3* mutant mice display normal muscle development and maintain *Pitx2* expression in all skeletal muscles suggesting that *Pitx2* and *Pitx3* may have partly redundant roles. We have previously documented that overexpression *Pitx2*c –isoform in undifferentiated myoblasts (Sol8 myogenic cell line) resulted in upregulation of cell cycle genes (c-myc, *cyclinD1* and D2) 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* differentiation during skeletal myogenesis. By using *Pitx2c* transient transfections we have demonstrate that the *Pitx2*c effects in myoblasts are dose-dependent. Therefore, we have determined at which doses of transfection *Pitx2*c began to induce changes in cell phenotype, inhibiting myocyte differentiation and myotube formation. Interestingly, we found that transient transfections with high *Pitx3* doses also results in modulation of proliferation *vs* differentiation in myoblasts. Additionally, we have currently analyzing whether microRNA-27 (recently reported as regulator of Pax3) can mediate the putative *Pitx2c*-dependent changes in *Pax3* expression. Initial data could support the hypothesis of a role of microRNA-27 mediating *Pitx2-Pax3* interactions in myoblasts.

Notes

In vivo Epithelial-to-Neuron Reprogramming in *C. elegans*

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Keywords: reprogramming; *C. elegans*, stem cell cell identity, regenerative medicine

Processes that underlie the ability of a cell to be reprogrammed and change its identity remain elusive. Understanding the molecular events that mediate such cell plasticity is of interest, not only from a developmental standpoint, but also because of the implications in cancer and regenerative medicines. Various examples of cellular reprogramming have been described, including the direct conversion of pancreatic exocrine cells into beta cells or the switch in drosophila photoreceptor identity. We have established *C. elegans* as a powerful model to study cell plasticity and have characterised an epithelial-toneuron reprogramming that occurs during the development of the worm. We use this unique *in vivo* system to understand the mechanisms underlying the reprogramming of a cell. We have explored factors pertaining to competence, lineage and local environment. We found that this event does not depend on fusion with a neighbouring cell; and that competence to be reprogrammed is restricted and requires 2 transcription factors and the LIN-12/Notch signalling. To identify further genetic cascades important for cellular reprogramming, we have isolated and characterised mutants defective in this event from an EMS screen. Interestingly, we find that there are mutants that halt epithelial-neuron reprogramming at different intermediate stages. Our data suggest that reprogramming is multifaceted and proceeds through transient cellular steps, rather than through concommitant loss and gain of the initial and final identities, even in absence of cell division. These intermediary steps probably do not represent reversion to a more blastic state. We are currently in the process of identifying more mutants through deep sequencing, which we expect will help us further piece together the details of cellular reprogramming *in vivo*.

Notes

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Posters

FGF signalling and glial development in the chick spinal cord

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During central nervous development system, various classes of neurons are generated along the entire dorso ventral axis of the spinal cord then glial progenitors are generated from discrete regions of the neuroepithelium. In vivo, Shh and BMPs, secreted respectively in the floor plate and the roof plate of the neural tube have been involved in the neurogenesis. However, if FGF signalling has long been shown to control glial development in vitro, the role of this morphogen is still controversial in vivo.

We have used the embryonic chick spinal cord to study the role of FGF signaling regarding the mechanisms controlling glial development. In previous work, we have shown that *sulfatase1* is expressed in the ventral spinal cord where it is a new oligodendrocyte lineage marker. Sulfatases are secreted enzymes removing sulphate moieties from heparin sulphate proteoglyans and have been shown to affect activity of various signalling pathway including Wnt, FGF and Shh.

In this work, we show that the FGF receptors expression is regionalised in the ventral neuroepithelium and that FGF signalling pathway is activated when glial cells are generated around E6 in the chick spinal cord. In the chick spinal cord. We are currently analysing the relationship between sulfatases expression in the ventral spinal cord, the activity of FGF pathway and gliogenesis.

Control of telencephalic development and Foxg1 expression by the cephalic neural crest: role of Smad1 molecule

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Key words: Neural crest, Forebrain, Avian chimeras, RNA interference, *Bmp* pathway The neural crest (NC) is a transient structure of the vertebrate embryo, which plays a crutial role in head development: NC cells generate most of the skeletal tissue encasing the developing forebrain and provides the prosencephalon with functional vasculature and meninges. In additional, recent findings show that, early in development, NC cells control morphogenetic activities of brain organizers and stimulate the growth of prosencephalic alar plate. Our project is to understand how NC cells receive distinct morphogenetic cues. Here, we have focused our interest on *Smad1* molecule: *Smad1* is early expressed in cephalic NC cells and can integrated multiple signaling pathways. We have tested the effects of *Smad1* silencing in cephalic NC cells by RNA interference. We show that *Smad1* loss-of-function downregulates *Fgf8* in the anterior neural ridge and *Shh* in the floor plate, but promotes the upregulation of the *Bmp4*. In absence of *Smad1*, gene expression at the dorsal midline is severely perturbed and *Foxg1* activity in the telencephalon is completely abolished. As a consequence, the telencephalic hemispheres fail to develop. Our results support the idea that transient Smad1 expression in cephalic NC cells is required for forebrain development.

Identification of new factors involved in the specification of satellite cells.

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KEYWORDS: Pax3, satellite cells, single fiber, microarray, muscle stem cells

Around birth, fetal muscle progenitor cells adopt a satellite cell position, becoming embedded within the basal lamina in close contact to the muscle fibers. Importantly, in addition to this morphological change, the emerging satellite cells enter quiescence, a molecular state poorly characterized *in vivo*. During post-natal growth or in response to injury or disruption of the basal lamina, a subset of the satellite cells become activated, proliferate and either fuse to form multinucleated myotubes or re-establish a residual pool of quiescent satellite cells that have the capability of supporting additional rounds of growth/regeneration.

We are interested in identifying new molecular pathways involved in the progression from a proliferating population to a quiescent post-natal progenitor cell population. Pax3 is a paired-homeobox transcription factor expressed in muscle progenitor cells throughout development, including post-natal satellite cells. The team leader has previously generated a *Pax3*$^{GFP/+}$ allele (Relaix et al., 2005), which provides direct and efficient access to the Pax3-expressing muscle progenitor cells through FACS-sorting.

We have performed expression profiling of *Pax3*$^{GFP/+}$ cells during development and in early post-natal muscle progenitor cells and identified factors specifically induced during specification of muscle satellite cells. Results and validation of the screen as well as functional analysis of novel candidate genes will be presented.

Relaix, F., Rocancourt, D., Mansouri, A. and Buckingham, M. (2005). A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. **Nature**, *435, 948-953*.

Cognitive deficits and aberrant cortical lamination stem from an early disorganization of the cortex in an EphrinB1 mutant mouse model.

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Keywords: ephrins, cortical development, cognitive function

The Ephrin-Bs, ligands for EphB receptor tyrosine kinases are an evolutionarily conserved family of proteins. Though primarily known for their roles in early developmental processes, recent works have shown the importance for two members, ephrinB2 and -B3 in the adult CNS; largely as regulators of synaptic plasticity. To date little is known on the role of ephrinB1 in adult CNS. Mutations in the ephrinB1 gene results in a human disorder termed craniofrontonasal syndrome, in which few studies have reported learning deficits. To determine the role of ephrinB1 in the adult CNS, and whether it participates in higher cognitive functions, we performed a series of cognitive and morphological analysis using an ephrinB1 knockout mouse model. Our findings show that the ephrinB1 mutant mice performed poorly in the object recognition test and, by histological analysis we show evidence for disorganization of cortical laminae. Interestingly, we found no evidence for ephrinB1 expression in the adult cortex. These findings prompted us to investigate ephrinB1 expression and function during early cortical development, since it is highly expressed in neural progenitor cells in the developing mammalian cerebral cortex. We found that in embryonic corticogenesis ephrinB1 mutant embryos displayed an overall disorganization of the cortex including a disorganization of the radial glial scaffold and a disruption in the integrity of the pial basement membrane (BM). Therefore, our findings indicate that ephrinB1 influences the organization of the developing cortex thus impacting on cortical laminar patterning, which is required for learning and memory tasks. Altogether, our findings suggest that early cortical organization is necessary for cognitive function.

Defects in odontoblast and ameloblast differentiation by alteration of Shh and Wnt pathways

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Keywords: odontoblast, ameloblast, differentiation, Wnt, Shh.

Odontogenesis, beginning in the mouse at embryonic day eleven (E11), encompasses epithelial-mesenchymal interactions. Conditional inactivation of Shh causes profound alterations in dental growth and morphogenesis, as well as a failure in amelogenesis. In addition, overactivation of the canonic Wnt pathway in the dental epithelium, through conditional overexpression of β-catenin, produces a phenotype whereby multiple extra teeth are formed. Shh and Wnt pathways could act in coordination to regulate multiple aspects of tooth development. The aim of this work was to study the importance of Shh and Wnt pathways during the dental morphodifferentiation stage of odontoblasts and ameloblasts.

For this purpose, we developed an organotypic culture system of mouse molars at E17.5, prior to the differentiation of odontoblasts and ameloblasts. Molars were cultured with the presence or absence or Lithium Chloride (LiCl) or Cyclopamine. LiCl is an inhibitor of GSK-3, and activates Wnt signalling. Cyclopamine acts as a primary inhibitor of the Hedgehog signal-transduction pathway, by direct binding to the heptahelical bundle of Smoothened.

After 6 days in culture, the enamel organ of E17.5 molars in the presence of Cyclopamine or LiCl showed anomalous deep invaginations towards the underlying mesenchyme, which resulted in the formation of very pronounced dental ridges. Aditionally, the cervical loops of the treated samples showed poorly developed edges compared to controls, which is indicative of possible malformations at the tooth root.

Cyclopamine treatment caused an appreciable reduction in the numbers of differentiated odontoblasts compared to the controls. LiCl seemed drastically affect the odontoblast polarization process, because only few patches of these cells differentiate from mesenchyme, secreting a tiny layer of predentin. In addition, the internal enamel epithelium appeared disorganized and completely undifferentiated. These results were also confirmed by using nestin and amelogenin, an odontoblast and ameloblast markers, respectively.

These results indicated a clear blockade of the differentiation process of mesenchymal cells to odontoblasts and epithelial cells to ameloblasts. Overactivation of Shh and Wnt pathways could therefore induce profound alterations in the conformation and structure of hard tissues such as dentin and enamel.

Sumoylation modulates Spalt activity during wing development

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Keywords: Spalt, wing, Sumo, Smt3, transcriptional regulation

The Spalt family of zinc finger transcription factors is conserved throughout evolution and is involved in fundamental processes during development, as limb and nervous system formation. The function of these proteins might be conditioned by their posttranslational modifications. We analyzed the modification of Spalt (Sal) and Spalt related (Salr) in *Drosophila melanogaster* by the small ubiquitin-like modifier Smt3 (Sumo) and the functional consequences of this modification. We identified by sumoylation assays *in vitro* one functional Sumo binding motif on Sal and two on Salr. The presence of Smt3 modifies the sub-cellular localization of Sal and Salr in cultured cells. Smt3-Sal or Smt3-Salr fusion proteins can mimic these changes on the localization. We demonstrated that Sal and Salr act as transcriptional repressors in cultured cells and that Smt3 modulates their activity. In addition, Sal and Salr interact genetically with Sumo *in vivo* during wing imaginal development. Furthermore, the capacity of these proteins to promote vein formation and regulate downstream genes depends on their sumoylation status.

The Hox gene *Deformed* (*Dfd/Hoxb4-d4*) modulates cell adhesion and segregation within the drosophila eye-antennal disc.

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Keywords: Cell adhesion, Hox, morphogenesis, Drosophila, organogenesis.

Most of the adult Drosophila head derives from the composite eye-antennal imaginal disc and can be thus considered as a representative example of the development of multiple organs from a composite rudiment. The antennal disc gives rise to two distinct appendages: the antenna (Ant) and maxillary palp (Mx), both olfactory organs. We found that the Mx territory is detectable within the antennal disc by expression of the Hox gene *Deformed* (*Dfd/ Hoxb4-d4)* while Ant cells express the transcription factor Cut. This exclusive Cut-Dfd expression pattern seen as early as mid-L2 appears concomitant with establishment of a clonal restriction between Mx and antennal fields and is maintained throughout the head development. We analysed the segregation of these two cells populations (Dfd-expressing for Mx cells and Cut expressing for Ant cells) within the antennal disc. By loss of function experiments, we identified an antagonism between Cut and Dfd which may be involved in forming the Mx/Ant boundary. Furthermore, we found that differential expression of Dfd modulates cell adhesion properties probably involved in the sorting and the physical segregation of the two cell populations giving rise to the Mx and Ant primordia. Accordingly, Dfd appears to act acts at the Ant-Mx boundary as a local organizer, required for Mx differentiation. This novel function of a Hox gene in cell affinity and in separation of cell populations sheds a new light on Hox function in organ morphogenesis.

Establishment of anteroposterior patterning of the cardiac field.

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Congenital heart disease (CHD) is the most common class of birth defect. Abnormalities are thought to originate during early cardiogenesis. Therefore, lucidation of genetic pathways operating in heart development is important. Establishment of anteroposterior (AP) polarity in the vertebrate embryonic heart tube is crucial for proper morphogenesis of the mature heart. However, the molecular details of this process are poorly understood. Studies using various animal models have implicated retinoic acid (RA) in the communication of AP polarity to the heart. Transgenic analysis, Cre-mediated cell tracing analysis and retrospective clonal lineage analysis have changed our view on the origin of cardiac progenitor cells in the mouse embryo: the linear heart tube, derived from the first heart field (FHF), provides a scaffold to which cells from the second heart field (SHF) add at both arterial and venous poles to build the future cardiac chambers. Based on this new model, we have recently reinvestigated the phenotype of embryos lacking *Raldh2* (required for RA biosynthesis) and shown that RA is required to restrict the SHF posteriorly [1]. Moreover, we have shown that the contribution of the SHF to both arterial (anterior) and venous (posterior) poles was perturbed under RA deficiency, leading to disorganization of the heart tube. To clarify RA contribution, we have analysed the expression of several Hox genes, known as RA targets, within the lateral mesoderm. Our data show that *Hoxa1* and *Hoxb1* are expressed within the Raldh2 domain, adjacent to and overlapping with cardiac progenitor cells respectively. Using lineage tracing of *Hoxa1- and Hoxb1*-expressing progenitors, we demonstrate a common origin of both poles of the heart. Moreover, *Hoxa1*- and *Hoxb1*-Cre lineage analysis shows a difference in the contribution of these populations along the proximal and distal OFT myocardium. Together, these data suggest a role for *Hox* genes during establishment of cardiac AP fates.

[1] Ryckebusch et al. 2008. PNAS, 105, 2913-2918

Amphioxus reveals the evolution of FGF signalling in chordates

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Keywords: Evo-Devo, amphioxus, FGF

Fibroblast Growth Factors (FGF) and their receptors are well known for having major implications in cell signalling controlling embryonic development in all metazoans. In vertebrates, 22 genes coding for FGFs and 4 genes coding for their receptors are known whereas only three genes coding for FGFs and two genes coding for their receptors were described in the model system *Drosophila*. In *Ciona intestinalis*, which belongs to urochordates that are the sister group of vertebrates, only 6 FGF genes can be found in the genome. Did the diversification of the FGF family in vertebrates participate to the acquisition of vertebrates' specific morphological features? How FGF signalling implication during development evolved at the transition between invertebrates to vertebrates? In *Ciona* the control of early developmental events is not inductive as it is in vertebrates and comparison of early developmental processes between the two groups do not give information on how they evolved during the invertebrate to vertebrate transition. The cephalochordate amphioxus is placed at the base of the deuterostomes and represents the best model to answer our questions. We have characterized in *Branchiostoma lanceolatum* 8 genes coding for FGFs as well as a unique gene coding for their receptor. The expression during embryonic development of these genes reveals putative conserved and non-conserved function of FGF signalling in chordates, and treatments with inhibitor of the FGF receptor during embryonic development shows that mesoderm induction seems to be controlled in amphioxus as in vertebrate by the FGF pathway.

Regulation of Nodal during the establishment of left-right asymmetry in the sea urchin Embryo

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Key-words : Nodal, left-right asymmetry, Sea urchin

Most animals display characteristic left right handedness in the positioning of internal organs. However, the mechanisms responsible for breaking the initial symmetry during early development are not well understood. In vertebrates, different processes have been implicated in the establishment of left-right asymmetry. One of implied mechanism is an oriented movement of a specific population of cilia localized in the node region of the mouse embryo. This ciliary movement would generate an asymmetric flow towards the left side driving expression of the TGFβ *nodal* on the left side. Asymmetrical *nodal* expression is a conserved feature in all deuterostomes and plays key roles in the molecular pathway responsible for left-right specification. More recently, others signaling pathways have been implicated in localization of *nodal* expression including the Delta/Notch and the TGFβ GDF1. Delta/Notch pathway is required for the initial expression of *nodal* in the node region. GDF1, which is thought to heterodimerize with Nodal and allow long range Nodal signaling on the left side. Establishment of left-right asymmetry is also a key feature of sea urchin development. At pluteus stage, an imaginal disk called the rudiment forms on the left side of the larva. We showed previously that left-right positioning of the rudiment is regulated by asymmetrical *nodal* expression on the right side *(Duboc et al. 2005)*. Our current research aims at understanding molecular mechanisms acting upstream of *nodal* expression. For the moment, we did not find evidence that cilia are involved establishment of left-right asymmetry in sea urchin. On the other hand, our current data show an involvement of Delta/Notch signaling to localize *nodal* expression on the right side. However, its involvement seems to be indirect. Univin (GDF1) seems to be a good candidate to be an intermediary between Delta/Notch signaling and *nodal* expression. First, it is expressed more strongly on the right side. Second, *nodal* expression is dependent of Univin and third, expression of *univin* is dependent to Delta/Notch. Interestingly, we found that inhibition of ERK signaling randomizes *nodal* expression. We are presently investigating the relationship between these actors and are trying to understand how these signaling pathways act to direct asymmetrical nodal expression.

Lack of Maternal Heat Shock Factor 1 provokes multiple cellular and developmental defects, altered redox homeostasis and reduced survival in mammalian oocytes

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Oocytes, heat shock factor, oxidative stress, fertilization, survival

Heat Shock Factor (HSF1) is a maternal effect gene required in oocytes to regulate the expression of heat shock protein 90alpha (Hsp90alpha) and mediate meiosis progression. It was also previously noted that *Hsf1*$^{-/-}$ females were totally infertile due to the early developmental arrest of their embryos. To better understand this phenotype, we examined intracellular morphology of mutant oocytes, zygotes and 2-cell embryos, followed by organelle ultrastructure analysis, determination of biochemical parameters of homeostasis and survival. MetaphaseII-arrest, cortical granule exocytosis were impaired in *Hsf1*$^{-/-}$ oocytes and zygotes, and this was followed by embryo degeneration before the 2-cellstage. Pre-ovulatory *Hsf1*$^{-/-}$ oocytes revealed ultrastructural abnormal and strongly oxidized mitochondria associated with elevated oxidative load, due to reactive oxygen species (ROS). Finally, in most mutant oocytes and embryos the apoptosis effector caspase, caspase-3 was activated, reflecting the pro-apoptotic tendency of *Hsf1*$^{-/-}$ oocytes. Reduction in Hsp levels (Hsp25, Hsp70.1, Hsp90alpha, Hsp105) is likely implied in this phenomenon. In conclusion, our study using a genetic model which was known to alter the maintenance of redox homeostasis demonstrates that early post-ovulation events are particularly sensible to such an insult which definitely abrogates the developmental competence of affected oocytes

Functional links between Mediator complex subunits and GAT A transcription factors during Drosophila development.

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Key words: Mediator, transcriptional regulation, Med1, CDK8 module, GATA

Mediator (MED), a conserved ~30 subunit modular complex, plays a pivotal role by bridging sequence-specific transcription factors (STF) to the PolII transcriptional machinery. We are using Drosophila as a model to analyze the functional specificity of MED subunits *in vivo*. We previously showed that the four MED subunits of the regulatory CDK8 module (Med12, Med13 Cdk8 and CycC) share some functions but also have distinct roles in developmental gene regulation. Here, we analyzed the functional relationships between the CDK8 module subunits and the core Med1 subunit, whose mammalian counterpart is a direct interactor of GATA-type STFs and nuclear hormone receptors. Our recent generation of *Med1* null mutations and the availability of dsRNAs transgenic lines showed that like the Cdk8 module subunits, Med1 is required for normal development but not for cell viability. Loss of function phenotypes indicate that *Med1* is required for leg, wing and thorax development, as *Med12-13*, suggesting a functional link between Med1 and Med12 as previously shown in *C. elegans*. Nevertheless, the absence of eye phenotypes indicates that Med1 does not share all Med12-13 functions. Given that thoracic closure depends on the GATA transcription factor Pnr, we analyze at which level(s) the Med1 Med12 and Med13 subunits functionally interact. In parallel, we study the role of MED subunits during embryonic haematopoiesis, a process depending on Serpent, another GATA transcription factor.

A role for NeuroD1 in terminal neuronal differentiation during postnatal olfactory bulb neurogenesis

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Keywords: postnatal neurogenesis, electroporation in vivo, transcription factor, stem cell

In postnatal and adult mammals, the periventricular region (PVR) lining the lateral wall of the lateral ventricle contains stem cells that generate transit amplifying precursors that, in turn, give rise to neuroblasts. These cells migrate along a specific pathway, the rostral migratory stream (RMS) to reach the olfactory bulb (OB), where they differentiate into GABAergic and dopaminergic interneurons. We showed previously that migratory neuroblasts undergo exclusively glial differentiation when transplanted into non-neurogenic regions in brain repair paradigms. Therefore, we aimed at the identification of factors inducing irreversible neuronal differentiation. For this purpose, we isolated defined precursor and neuron populations from the olfactory neurogenic system by microdissection and magnetic cell sorting (MACS®) and investigated their gene expression profile using microarray technology. We found that the bHLH transcription factor NeuroD1 is absent from the neuroblast but strongly induced in the mature neurons. To investigate the function of this gene we performed gain and loss of function approaches based on in vivo electroporation of the postnatal forebrain. We show that overexpression of NeuroD1 alone is sufficient to induce immediate morphological neuronal differentiation and the expression of neuronal markers such as NeuN and MAP2 in the PVR. In contrast, we found that shRNA induced knock down of NeuroD1 prevents neuronal differentiation in the olfactory bulb. Altogether, these finding allow show that the NeuroD1 is both necessary and sufficient to induce terminal neuronal differentiation. This finding might provide a new tool for cell manipulation in therapeutic approaches to neurodegenerative disease implicating loss of neuronal transmission like, for example, Parkinson's disease.

Functional characterization of eye regeneration genes in the flatworm *Schmidtea mediterranea*

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Keywords: planaria, eye regeneration.

The flatworm *Schmidtea mediterranea* can regenerate a complete organism out of a tiny piece of its body. Further it is able to undergo dramatic changes in size without changing its functionality. This process includes the reconstitution of the eye organ. The regeneration of these simple eye structures consisting of just two cell types, the pigment cells and light sensing photoreceptor cells, occurs within just one week after amputation. Several eye genes have been characterized previously. The genes *Sine Oculis* (1) and *Eye Absen*t (2) produces a non-eye phenotypes after RNA interference but *Pax6A* and *Pax6B* (3) RNAi do not interfere in eye regeneration. The gene network of eye regeneration is still unclear. Molecular biology tools such as in situ hybridization, immunostaining, RNA interference and a genome draft, are feasible for this organism. Here we will present the actual state of art on flatworm eye regeneration and new genes that are currently under investigation.

(1) Pineda et al 2000, (2) Mannini et al 2004, (3) (Pineda et al 2002)

Role of β-catenin and PTEN in the development of the melanocyte lineage

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Keywords: melanoblast, β-catenin, PTEN, development, hyperpigmentation

Melanoblasts are derived from neural crest cells (NCC) and generate melanocytes, the pigment-producing cells of the skin. In the murine trunk region, melanoblasts are determined at around E9. Then they proliferate and migrate dorso-laterally to the somites invading the dermis, then the epidermis and the hair bulb. Unfortunatelly, melanocytes may undergo transformation and give rise to melanoma, a highly invasive and lethal skin cancer. β-catenin and PTEN are two proteins implicated in cell proliferation. They are expressed in murine melanoblasts and were found to be mutated in melanoma. Moreover, PTEN regulates β-catenin activity. To understand the role of β-catenin and PTEN during the establishment of the melanocyte lineage, we analyzed the coat color of mice expressing either an activated form of β-catenin (Δex3βcat) or lacking PTEN in melanoblasts. Such mice were produced thanks to the Cre/LoxP system. Mice expressing specifically the Cre recombinase in melanoblasts (Tyr::Cre mice) from E10.5 were previously produced (1). The Δex3βcat and PTEN mutant mice present a hyperpigmented phenotype suggesting an essential role of these two proteins in the development of melanocytes. We are currently investigating these phenotypes, which appear be due to a hyperproliferation of melanoblasts. In order to get insights on the function of β-catenin and PTEN during the development of the melanocyte lineage, we decided to perform a transcriptomic analysis of wild-type and mutant melanoblasts at different embryonic stages. Melanoblasts are dispersed in the skin and their number is very limited. We designed a method to isolate them during development. In this respect, we genetically labeled melanoblasts using Z/EG mice (2) producing conditionnally the fluorescent reporter EGFP. We isolated them by FACS, extracted RNA of good quality (RIN>8) and are perfoming the transcriptomic analysis.

1. V. Delmas et al., Genesis **36** (2003) p73.
2. A. Novak et al., Genesis **28** (2000) p147.
3. SC was supported by a MESR fellowship. This work was supported by La Ligue Nationale Contre le Cancer (équipe labellisée), INCa, Cancéropôle IdF. We thank Z. Maciorowski and A. Viguier for technical

Larval haematopoiesis and the cellular immune response to wasp parasitisation

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Larval hematopoiesis takes place in the lymph gland (LG). The LG is composed of the "Posterior Signalling Center" (PSC) which express Collier, a medullary zone (MZ) containing progenitors and a cortical zone (CZ) composed of differentiated hemocytes: plasmatocytes (macrophages) and crystal cells (melanisation). In normal conditions, only plasmatocytes and crystal cells differentiate. A third type of hemocytes, the lamellocytes devoted to encapsulation of foreign bodies too large to be phagocytised differentiate only under specific immune conditions such as wasp infestation. We established that the PSC plays a key role in controlling the maintenance of a pool of multipotent progenitors in the LG. The role of the PSC is reminiscent of the hematopoietic "niche" of vertebrates, a micro-environment required for survival and self-renewing of Hematopoietic Stem Cells (HSC). The maintenance of a pool of multipotent progenitors which is a prerequisite for lamellocyte differentiation in response to wasp parasitisation requires to maintain JAK/STAT signalling on in progenitor cells. We show that CG14225/latran, which encodes a short cytokine receptor, is required for efficiently switching off JAK/STAT signalling in prohemocytes and allowing massive differentiation of lamellocytes following wasp parasitization. Latran antagonises the function of Domeless, the Drosophila type I cytokine-related receptor in a dose-dependent manner, via the formation of inactive heterodimers. The specific role of *latran* in controlling a dedicated cellular immune response via the repression of JAK/STAT signalling raises the question of whether short, non signalling receptors could also control specific aspects of vertebrate immunity.

Key words: JAK/STAT, hematopoiesis, Drosophila, lymph gland, lamellocyte.

Analysis of Sulfatase1 function in Shh-dependant oligodendrocyte specification in the ventral spinal cord.

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Keywords: HSPG, sulf, Shh, oligodendrocyte

Neurons and glial cells (astrocytes and oligodendrocytes) composing the adult central nervous system, arise from neural progenitors during embryonic development. The spinal cord is a simple model to study the emergence of the diverse neural populations. In the ventral region, the Sonic Hedgehog (Shh) morphogenetic gradient, originating from notochord and floor plate, establishes the formation of distinct neural domains generating different neuronal subpopulations. Subsequently to neuronal production, Shh induces the specification of oligodendrocyte precursors (OPC) in the ventral spinal cord, raising the question of the molecular mechanisms underlying this temporal change of neural progenitor response to the same signal. The sulfatase Sulf1 is a potential player involved in these events. Indeed, its expression starts after the main waves of neuronal production in the floor plate and later expands into the ventral neural progenitors just prior to OPC specification in this domain. This spatio-temporal pattern of expression is strongly conserved among vertebrates, including chick and zebrafish. Sulf1 encodes for an enzyme hydrolysing sulphate groups at specific position from Heparan Sulfate Proteoglycans (HSPG) at the cell surface. This modification of HSPG sulfation pattern modulates their affinity for extracellular ligands and therefore their activity in regulating signalling pathways. Studies from Drosophila and Vertebrates have shown that transport and activity of ligands from Hedgehog family depends on HSPG. Gain of function studies in chick indicate that Sulf1 regulates Shh extracellular distribution and signalling. We tested *sulf1* function in zebrafish by morpholino loss of function experiments. In *sulf1* morphants, expression of Shh target genes is downregulated in the ventral neural tube. Our preliminary results indicate that production of ventral neurons is not impaired whereas OPC are absent in *sulf1* morphants. These findings suggest that Sulf1 is a positive regulator of Shh activity required for OPC specification in the ventral spinal cord. We propose that Sulf1 triggers a temporal modulation of Shh activity in the ventral progenitors that induce their switch from producing neuron to oligodendrocyte in the vertebrate spinal cord.

Antero-posterior axis specification by the Wnt/βcatenin pathway in the hemichordate *Saccoglossus kowalevskii*.

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To understand the emergence of the chordate body plan, the hemichordates appear to constitute a very valuable out-group. Despite vast morphological differences with vertebrates, developmental genetic analysis in the hemichordate *Saccoglossus kowalevskii* have revealed strikingly similar patterning mechanisms controlling antero-posterior or dorso-ventral axis. Here, we describe two specific functions of the Wnt/βcatenin pathway in antero-posterior axis establishment. Early during development, this pathway is essential to specify the endomesodermal fate in the vegetal cells. This function appears to be deeply conserved since very comparable situations have been described in tunicates, echinoderms, protostomes and even cnidarians. We further show the vegetal endomesoderm precursors organise ectodermal patterning along the antero-posterior axis by secreting posteriorising molecules. Accordingly, in the absence of endomesoderm, the ectoderm adopts the anterior-most fate of the embryo. Later during embryogenesis, expression of the Wnt ligands and their secreted inhibitors defines staggered antero-posterior domains. Experimental manipulation of the Wnt pathway shows that its activation is incompatible with anterior fate and actively blocking its activity is necessary to define the anterior portion of the embryo. This later function is comparable to what is seen in vertebrates head formation. Part of the molecular control of head patterning thus predates emergence of the vertebrates and is consequently not coupled to the vertebrate specific morphological innovations. Moreover, it suggests that at least part of this patterning machinery was lost in both amphioxus and tunicates.

In vitro reprogramming of human melanoma cells by the post-implanted mouse embryo microenvironment

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Embryonic stem cells and malignant tumor cells share several functional characteristics, such as the undifferentiated phenotype and the plasticity of differentiation. This is especially evident in the so-called *"developmental tumors",* like teratocarcinoma and neuroblastoma. Thus, an increasing number of today studies support the idea of some common regulating mechanisms in both embryonic and neoplastic cells. The current "*cancer stem cell theory"*, which postulates that the origin of cancer lies in tumor stem cell reservoirs which give rise to a *caricature* of normal tissue renewal (Aréchaga,1993), is one of the fruits of this way of thinking. In this regard, it has been communicated the reversion of the cancer phenotype of human melanoma cells by the zebrafish (Lee *et al.*, 2005) and chick (Kasemeier-Kulesa *et al.*, 2008) embryos which are useful models for these kinds of studies, since all developmental stages can be followed *in vitro*. However, the use of a mammalian model, such as the mouse embryo, could provide conditions and cues closer to those found in the human biological microenvironments. The aim of our work was to test the possible regulation of human melanoma cells by post-implanted mouse embryos. For this purpose we used an embryo culture system, which allows the maintenance of post-implanted mouse embryos for a few days *in vitro*, using New's method (Aréchaga, 1997). A375 human melanoma cell line, expressing green fluorescent protein (GFP), were attached to developing visceral endoderm of 7.5 dpc mouse embryos and cultured *in vitro* for three days. The position changes of transplanted human melanoma cells were monitorized by confocal microscopy. Our results show that melanoma cells were internalized and migrated inside the embryo body in a way reminiscent of neural crest cells, which give rise to melanoblasts. The absence of localized tumor growth, after 72 hours of *in vitro* embryo culture, suggests that malignant phenotype inhibiting factors are active at the gastrulating stage, as was shown previously during later embryonic development (Gerschenson *et al*, 1986). Further research is needed to elucidate the final fate of melanoma cells in relationship with the initial place of the transplants and the involved signaling pathways of tumor growth regulation. However, the present results illustrate on the biological reprogramming of human melanoma cells by embryo microenvironments and, thus, represents a possible starting point for the future development of more physiological anticancer therapies.

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Heterogeneity of radial glial progenitor cells and involvement in brain repair of adult zebrafish

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Unlike that of mammals, the brain of adult teleost fish presents a very intense neurogenic activity linked to the persitence of neural progenitors: the radial glial cells (RGC). Interestingly, in fish, an important proportion of these cells strongly express aromatase B (AroB), an enzyme converting androgens into estrogens and produced by the cyp19a1b gene. In this study, we first tried to further characterize the AroB$^+$ RGC by looking at other molecular markers in order to see if these progenitor cells express a particular combination of markers. We investigated the expression of Brain Lipid Binding Protein (BLBP), a member of the fatty acid binding protein family, and CXCR4, one of the receptors to the chemokine CXCL12 (SDF1). Taking advantage of a transgenic zebrafish line cyp19a1b-GFP, we show that most RGC co-express AroB and BLBP. A statistical analysis shows that most dividing RGC (PCNA$^+$) co-express AroB and BLBP. In addition, we evidence that CXCR4 is also expressed in a subset of RGC. We next studied the potential involvement of RGC and AroB in brain repair after mechanical injury of the telencephalon of adult zebrafish. A 300% increase of the proliferative activity is observed around the lesion only in the ipsilateral hemisphere. Moreover, preliminary results demonstrate that most of the dividing cells (PCNA$^+$) correspond to RGC expressing AroB and/or BLBP. Interestingly, these RGC extend long cytoplasmic processes towards the lesion, suggesting a role in both renewal of neural cells and migration of newborn cells after injury. Altogether, these data contribute to a better characterization of RGC in fishes and suggest that RGC do not constitute an homogeneous population. These data indicate that RGC are implicated in brain repair and could also suggest a role of AroB expression and thus estrogens in mechanisms sustaining the proliferative activity of RGC in the brain of fish.

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Keywords: Aromatase; Brain repair; Lesion; Neurogenesis; Radial glial cells

Role of Sprouty3 in the regulation of BDNF/TrkB signalling during *Xenopus* neuronal development

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Keywords: *Xenopus*, motorneuron, BDNF, signalling, Sprouty

The elaboration of axon branches during development is controlled by the interplay between environmental cues, which provide the branching signals and the intracellular components that elicit the response of the neuron to these signals. The majority of studies have focused on extracellular factors that affect axon branching, while very little is known about the intracellular players that mediate this process. Signalling by the ligand BDNF (Brain Derived Neurotrophic Factor) and its receptor TrkB controls many aspects of neuronal differentiation, survival and morphology. Here, we show that Sprouty3 is an intracellular modulator of BDNF-TrkB signalling regulating specifically the ability of BDNF to induce neurites growth along the axon shaft of motor neurons.

Sprouty family members are well-characterised intracellular regulators of receptor tyrosine kinase signalling in general and FGF in particular. There are four family members in vertebrates and most studies have focused on the role of Sprouty1, 2 and 4 while nothing is known about Sprouty3. *Sprouty3* is expressed exclusively in motor and sensory neurons in zebrafish, *Xenopus* and mouse embryos indicating a conserved role during neuronal development. To gain insight about Sprouty3 function, we have performed morpholino-mediated knockdown experiments of Sprouty3 expression in *Xenopus* embryos. We observed a significant increase of branching of spinal motor axonal tracts *in vivo* in Sprouty3 morphants compared to control embryos. We have also showed that *sprouty3* expression is dependant on signalling by BDNF/TrkB suggesting that Sprouty3 may participate in a regulatory feedback loop. Sprouty3 overexpression in mouse cortical neurons reduces axon branching, showing that Sprouty3 regulates axon branching in different types of neurons. Furthermore, real time imaging of *Xenopus* spinal cord neurons in culture reveals that Sprouty3 knockdown increases the number of filopodia along the axon shaft, a process known to be BDNF-dependant.

At the molecular level, Sprouty3 inhibits strongly Ca^{2+} release induced by BDNF, has only a modest effect on the activation of MAPK and no effect on the activation of Akt. Therefore, Sprouty3 specifically regulates a subset of the intracellular pathways activated by BDNF/TrkB. Taken together, these data show that Sprouty3 limits specifically BDNF-mediated axon branching by inhibiting the Ca^{2+} pathway while not inhibiting other cellular responses downstream of BDNF in motor neurons during embryonic development.

Neural crest cells include multipotent neural-osteogenic progenitors

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Neural crest, stem cell, osteoblast, quail embryo, cell culture

A diversity of vertebrate cell types originates from neural crest cells (NCC), such as melanocytes, PNS neurons and glial cells, and endocrine cells. In the cranial region, the NCC also produce chondrocytes, osteocytes, adipocytes and smooth muscle cells; in the trunk, these mesenchymal cells arise from mesoderm. How skeletogenic progenitors are segregated in NCC and whether they are multipotent is still an open question. Here we have characterized osteogenic progenitors in cranial NCC cultures and investigated whether trunk NCC have osteogenic capacity in vitro.

We show that mesencephalic NCC from 6-8 somite-stage quail embryos differentiate in vitro into Runx2-expressing osteoblasts of two distinct types: i) endochondral-like osteoblasts located in perichondrium, which respond to Sonic Hedgehog (Shh) by enhanced proliferation and differentiation, and ii) dermal-like osteoblasts that condense without association with chondrocytes. In single cell cultures, 90% of clonogenic cranial NCC give rise to osteoblasts in multilineage clones. We disclose a novel highly multipotent NCC that yields glia, neurons, melanocytes, myofibroblasts, chondrocytes and osteoblasts, and is lying upstream of all the other NC precursors identified so far. We also describe that trunk NCC have the capacity to give rise to osteoblasts in culture, albeit with lower frequency than cephalic NCC. Preliminary data indicate that a significant subset of trunk NCC clonally produce both osteoblasts and PNS neural cells. Altogether these results argue that neural and osteogenic lineages are not segregated in the early NCC.

Control of the cell cycle during neurogenesis: role of the CDC25B phosphatise

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During the development of the nervous system, neural progenitors give rise to multiple types of neurons and glia in a stereotyped sequence involving coordination of proliferation, specification and differentiation. In this context, we are studying the control of progenitor cell divisions during neurogenesis using the developing Vertebrate spinal cord as a model. This process has to be tightly regulated to generate the correct number of cells required for the mature spinal cord. Here we investigated the role of core positive cell cycle regulators, the CDC25 phosphatases. We found that only CDC25A is expressed in self-renewing neural progenitors, suggesting that it is sufficient to drive proliferative divisions. CDC25B expression is initiated concomitantly with the onset of neuronal differentiation and progresses in correlation with the wave of neurogenesis. To determine the relevance of that correlation, we used a micro-RNA based vector to knock-down CDC25B. Down-regulating CDC25B leads to an increase in the pool of cycling neural progenitors but to a decrease in neuron production revealing that CDC25B plays a key role in the fate decision of a neural progenitor to become a neuron. We previously showed that CDC25B expression is initiated by the morphogen Shh (Bénazéraf et al., Dev Biol, 2006), our present findings thus reveal a novel mechanism for promoting neuronal differentiation from specified neural progenitors.

An efficient approach to isolate STAT regulated enhancers uncovers STAT92E fundamental role in *Drosophila* tracheal development

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ABSTRACT:

The *ventral veinless* (*vvl*) and *trachealess* (*trh*) genes are determinants of the *Drosophila* trachea. Early in development both genes are independently activated in the tracheal primordia by signals that are ill defined. Mutants blocking JAK/STAT signalling at any level do not form a tracheal tree suggesting that STAT92E may be an upstream transcriptional activator of the early trachea determinants. To test this hypothesis we have searched for STAT92E responsive enhancers activating the expression of *vvl* and *trh* in the tracheal primordia. We show that STAT92E regulated enhancers can be rapidly and efficiently isolated by focusing the analysis in genomic regions with clusters of putative STAT binding sites were at least two of them are phylogenetically conserved. Detailed analysis of a *vvl* early tracheal enhancer shows that non-conserved sites collaborate with conserved sites for enhancer activation. We find that STAT92E regulated enhancers can be located as far 60kb from the promoters. Our results indicate that *vvl* and *trh* are independently activated by STAT92E which is the most important transcription factor required for trachea specification.

Key words: STAT gene-regulation, Trachea specification, ventral veinless, trachealess, enhancer localization.

Role of Heparan sulfate proteoglycans sulfation state modulation on Hedgehog signalling pathway in drosophila.

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Hedgehog, sulfotransferase, extracellular matrix, heparan sulfate proteoglycan, drosophila

Heparan sulfate proteoglycans (HSPGs) are major components of the extracellular matrix and play a key role in regulating multiple signalling pathways during metazoan development. HSPGs are macromolecules that are composed of a core protein to which heparan sulfate (HS) glycosaminoglycans chains are attached. These chains are initially synthesised as linear polysaccharides composed of disaccharide repeating units and subjected to marked structural modification by sulfation. After secretion, HSPGs can be further modified by extracellular enzymes. Recent work from our group has shown that DSulf1, the only drosophila extracellular 6-Oendosulfatase encoding gene, is involved in modulation of Hedgehog (Hh) morphogen signalling during drosophila wing development (Wojcinski et al., submitted). This enzyme modifies the sulfation pattern of HS at the cell surface, by catalysing specifically removal of 6-O sulfate from HS chains. This study shows for the first time an involvement of HSPGs sulfation state in regulating Hh signalling pathway. Moreover, HSPGs are characterised by a “sulfation code” depending on the activity of several intracellular sulfotransferases. These enzymes, by their specific activities, catalyse the addition of sulfates in differents positions (2-N-, 2-O, 3-O- and 6-O) on disaccharide units forming the HS chains. This raises the question of the importance of specific sulfation patterns for regulating morphogens signalling pathways. Due to the few representatives in drosophila of all these modifying enzymes, we will use the development of the wing as a model to analyse the potential role of each modification in the modulation of Hh signalling. We will present preliminary data showing the expression pattern of each enzyme in this tissue. We will next analyse the function of these enzymes by using already existing mutants for the 2-O- sulfotransferases (*hs2st* and *pipe*) and the 6-Osulfotransferase (*hs6st*), or generating mutants for the 3-O- sulfotransferases (*hs3st-A* and *hs3st-B*). In these mutants we will analyse the expression pattern of the Hh target genes and the distribution of Hh morphogen.

FUNCTIONAL ANALYSIS OF A NOVEL FAMILY OF ERM PROTEIN PARTNERS DURING CELL DIVISION

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Cell division involves a stereotyped sequence of changes in cell morphology, regulated by localized acto-myosin contractions of the cell cortex. Whilst the microtubule spindle is well known to influence location of the cleavage furrow, how cell shape transformations are coordinated with spindle reorganization throughout mitosis remains largely elusive. ERM (Ezrin, Radixin, Moesin) proteins, which are deregulated in several cancers, are renowned to link cortical actin to membrane, upon signal-mediated activation. We found that localized activation of dMoesin, the unique drosophila ERM, is required both for cell shape changes and mitotic spindle positioning during cell division. To better understand dMoe regulation, we carried out an *in vivo* genetic screen to identify functional partners of dMoe. Among genetic interactors, *GIM* (Genetic Interactor of dMoesin) is a pioneer gene which defines a novel evolutionarily conserved protein family. We therefore decided to explore a putative function of GIM during cell division. We show that GIM specifically co-localizes with activated dMoe, at the cortex in pro/metaphase and at the cleavage furrow in ana/telophase. Interestingly, GIM also localizes at centrosomes and midbody. Similarly to dMoe inactivation, depletion of *GIM* in S2 cells leads to severe defects in mitotic cell shape, with large cytoplasmic bulges that deform the cortex, and spindle misorientation. Phylogenetic analysis allowed identification of 4 *GIM* orthologs in mammals. We characterized human *GIM*-like genes and analyzed the distribution of one out the 4 hGim proteins. During cell division, we show that it localizes both at the cleavage furrow and midbody. Taken together, our results show that the GIM family of ERM partners plays an important role in the control of cell division in *Drosophila*. Its functional conservation in humans may be relevant to understand regulation of ERM proteins during cell division, in normal and pathological situations.

Planarian stem cells transcriptomes

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keywords: stem cells, planaria, transcriptome, massive parallel sequencing

In multicellular organisms, stem cells are able to recapitulate a complete differenciation program (multipotent or pluripotent cells) or to achieve an entire developmental process (totipotent cells). Understanding the molecular and physiological characteristics at the root of cell stemness is of paramount importance for the understanding of metazoan biology and cellular pathologies such as cancer, and for much expected-from therapeutic fields such as regenerative medicine. The planarian flatworm is a particularly well suited model to study cell stemness since this animal possesses a large number of totipotent stem cells called neoblasts. Planaria are regeneration champions due to the existence of neoblasts: an entire animal can develop from almost any small piece of tissue in record time. The genome sequence of one reference species, Schmidtea mediterranea, has been completed. With the advent of ever more powerful high-throughput digital gene expression approaches and accompanying bioinformatics methods, it is now possible to obtain and analyze very comprehensive transcriptomes and proteomes. To repertoriate most if not all of the genes expressed in the planaria neoblasts, we have obtained transcriptomes of animals with and without stem cells (lost by irradiation) using the SAGE and massive parallel sequencing methods (Solexa). 373 500 different gene tags have been listed (10,5 millions total) which could encompass, theoritically, the vast majority (if not all ?) of the mRNAs expressed in the animal. A primary data sorting revealed that 545 gene tags were significantly under-represented in irradiated versus untreated animals, whereas 2028 tags were over-represented. Under-expressed tags potentially represent genes specifically expressed in neoblasts, whereas over-expressed tags likely pinpoint genes induced in response to the irradiation stress. A large collection of transcripts differentially expressed in planaria with and without stem cells has thus been obtained. Data validation and gene identification are underway, using high through-put qPCR techniques and gene knock –outs (RNAi). This work should lead to the description of genetic networks active in the planarian stem cells, and contribute to reveal global stem cell features conserved across the species.

Laser Microdissection of sensory organ precursor cells of *Drosophila* microchaetes

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Keywords: Cell fate determination, Microarray, Laser microdissection, SOP, Precursor cells

In *Drosophila*, each external sensory organ originates from the division of a unique precursor cell (the sensory organ precursor cell or SOP). Each SOP is specified from a cluster of equivalent cells, called a proneural cluster, all of them competent to become SOP. Although, it is well known how SOP cells are selected from proneural clusters, little is known about the downstream genes that are regulated during SOP fate specification.

In order to better understand the mechanism involved in the specification of these precursor cells, we combined laser microdissection, to isolate SOP cells, with transcriptome analysis, to study their RNA profile. Using this procedure, we found that genes that exhibit a 2-fold or greater expression in SOPs versus epithelial cells were mainly associated with Gene Ontology (GO) terms related with cell fate determination and sensory organ specification. Furthermore, we found that several genes such as *pebbled/hindsight, scabrous, miranda, senseless*, or *cut*, known to be expressed in SOP cells by independent procedures, are particularly detected in laser microdissected SOP cells rather than in epithelial cells.

These results confirm the feasibility and the specificity of our laser microdissection based procedure. We anticipate that this analysis will give new insight into the selection and specification of neural precursor cells.

Cdc42 protein regulates cytokinesis in mouse oocytes and embryos

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Keywords: oocytes, embryos, meiosis, mitosis, cytokinesis

During meiosis, mouse oocytes undergo two subsequent divisions with unequal cytokinesis, which lead to the formation of two polar bodies and large haploid eggs. The polarity of mouse oocytes is then reflected in the position of the meiotic spindle near the cortex of the cell.

Many evidences have shown that a small GTPase from the Rho family, Cdc42 (Cell division cycle 42), takes part in the regulation of cell division, both in mitosis and meiosis. In mitosis. Cdc42 may participate in the proper attachment of microtubules (MTs) to the kinetochores (Yasuda et al., 2004) and also in the organization of actin filaments during contractile ring assembly (Dutartre et al., 1996).

During meiosis, Cdc42 may be involved in regulation of asymmetric divisions of vertebrate oocytes (Bielak-Zmijewska et al, 2008; Na and Zernicka-Goetz, 2006). In mouse oocytes, it is thought that Cdc42 is involved in the migration of the meiotic spindle (Na and Zernicka-Goetz, 2006) We have re-investigated these observations. We show here that the inhibition of the binding activity of Cdc42 does not disturb the migration of the meiotic spindle but blocks the extrusion of the first polar body. This effect is due to the disorganization of actin filaments and improper localization of its effector protein, IQGAP1 (Bielak-Zmijewska et al, 2008). We have also shown for the first time that in mice there might be a difference in the mechanism by which Cdc42 regulates meiosis of oocytes and the first mitosis of one-cell embryos.

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Molecular basis of Pax6 repression by Neurogenin 2 in spinal cord neuronal precursors

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Neurogenesis process involves the action of transcriptional programs, cooperating to produce a multitude of functionally specialized cells that are derived from a common pool of neural precursors. Proneural genes, encoding bHLH transcription factors play a key role in this process as they are necessary and sufficient to confer a neuronal identity to a neural progenitor. In addition, many others transcription factors act at different levels to control cell cycle exit, specification or differentiation of neuronal precursors. Among them, Pax6 has a pleiotropic effect, being required in the spinal cord for motoneurons specification, neuronal commitment and maintenance of an immature pool of neuronal precursors[1]. We recently showed that Pax6 prevent premature differentiation of neuronal precursors by counteracting Neurogenin 2 proneural activity. Hence, Pax6 extinction is a gate for neuronal differentiation and we found that its down regulation involves negative feed back by Neurogenin 2. We started to study the molecular mechanisms of this repression. The results obtained so far show that in this context, Neurogenin2 functions as a transcriptional activator and that an intermediate gene is required to trigger Pax6 repression. In order to identify which gene is involved, we performed a transcriptomic approach to uncover early response targets of Ngn2 in the young neural tube. Our different results will be presented.

Keywords : Ngn2, Pax6, regulation, transcriptome

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Characterization of a novel gene involved in epithelia morphogenesis in *Drosophila melanogaster* ovarian follicle

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Keywords: Epithelia morphogenesis, adhesion, stretch cells, ovarian follicle, *Drosophila melanogaster*

Epithelial morphogenesis occuring during development of multicellular organisms is a key step that allows the formation of every organs and tissues. To do so, epithelial cells must be able (to adopt various identities and) to adapt their adhesive properties, in order to maintain epithelia integrity during morphogenesis. Molecular and cellular mechanisms that control differentiation and change in adhesiveness of epithelial cells are not well known yet. Drosophila ovarian follicle is used as an experimental model to study morphogenetic processes occuring in epithelial somatic cells during oogenesis, such as stretching or apical constriction. These mechanisms are allowed by an accurate control of adhesion remodeling. A new gene, *CG9932*, has been identified by enhancer-trap as a gene required for morphogenesis of epithelial cells in the Drosophila ovarian follicle during oogenesis. Indeed, RNAi experiments against *CG9932* have shown that this gene is required for the control of dynamic, pattern and degree of adhesion remodeling during stretching of epithelial follicle cells.

Notchless regulates adult hematopoietic stem cell homeostasis

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Keywords : hematopoietic stem cell, niche, WD40 protein, conditional mutagenesis.

The Hematopoietic Stem Cell (HSC) is the best-characterized adult somatic stem cell so far. Crosstalks between the HSC and bone marrow microenvironment ensure a proper balance between self-renewal and differentiation during homeostasis. Several factors regulating HSC function and/or niche activity have been identified during the last decade thanks to mouse models. Using in vivo conditional mutagenesis, we showed that *Notchless (Nle)*, encoding a widely expressed nuclear WD40 protein that plays a critical role in the maintenance of embryonic pluripotent cells, also regulates the pool of adult HSCs. Indeed, acute ubiquitous inactivation of *Nle* in the adult using the *RosaCre-ERT2* mice provoked a severe disturbance of hematopoietic tissues and the death of the mice within 10 days after induction. In the bone marrow, we observed a rapid and drastic exhaustion of the Lin-Sca1+c-kit+ population, which includes stem cells and multipotent progenitors. Depletion of stem cell and progenitor pools was not due to increased apoptosis indicating that *Nle* is not directly acting on survival of these cells. Preliminary data suggest that following the deletion of *Nle*, HSCs enter cell cycle, indicating that *Nle* is essential for maintaining HSCs quiescence. Reciprocal bone marrow transplantation between wild-type and conditional mutant mice revealed both an intrinsic and a systemic effect of *Nle* inactivation on the maintenance of HSCs and progenitors. Altogether, these data identify *Nle* as an important regulator of adult HSCs homeostasis.

A new model for neural induction: FGF-activated calcium channels control neural gene expression in Xenopus.

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Although it has been shown that neural induction would be a “by default” process resulting from the inhibition of BMP signalling, increasing evidences are rather in favour of an instructive mechanism involving in addition the activation of calcium[1] and FGF signalling pathways.
Previously, our studies with Xenopus embryos showed that an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), via dihydropyridine-sensitive Ca^{2+} channels (DHP-sensitive Ca^{2+} channels) is necessary and sufficient to direct the ectodermal cells toward a neural fate, and that Ca^{2+} directly controls the expression of neural genes.
The important question is to determine the missing link between BMP signalling inhibition and DHP-sensitive Ca^{2+} channels gating.
Here we show that activation of FGF receptor can control the opening of the DHP-sensitive Ca^{2+} channels during neural induction. Using isolated ectoderm tissue, we demonstrated that FGF-4 depolarises the membrane of ectodermal cells and induces an increase in $[Ca^{2+}]_i$. This Ca^{2+} increase can be blocked by SU5402, an FGF receptor inhibitor, and by DHP-sensitive Ca^{2+} channel antagonists. These inhibitors also block the induction of neural genes[2].
We present a possible gating mechanism for the activation of DHP-sensitive Ca^{2+} channels via the FGF signalling pathway, which involves arachidonic acid and TRPC1 channel activation and we propose a new model of neural induction to modulate the concept of the ‘by default’ mechanism.

Keywords: neurogenesis; calcium signalling; calcium channels; Xenopus

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Role of HSF1 and HSF2 (Heat Shock Factor) during oogenesis in mice

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HSF1 and HSF2 belong to the HSF family that regulates stress inducible synthesis of heat shock proteins (HSPs). Although only one HSF exists in yeast or drosophila, there are five members in mammals, HSF1-5. HSF1 and HSF2 are expressed ubiquitously and remain the most studied factors. HSF4 is expressed predominantly in brain and lens where it is required for normal development. HSF3 and HSF5 have been recently identified and their functions remain unknown. Mouse knockout experiments have shown HSF1 is a maternal factor required for female fertility which severely alters oocytes meiosis(*1, 2*). In contrast, Hsf2-/- females are fertile and can produce offspring(*3*). In most tissues and cells lines, HSF1 and HSF2 are coexpressed and, recently accumulating data have shown these factors are able to interact and co-regulate some target genes(*4, 5*). However, in oocytes, we found HSF1 is 100-fold more expressed than HSF2 suggesting that these factors might control a distinct transcriptome in oocytes compared to somatic cells. These data prompted us to perform genome wide transcriptomic analysis using wildtype, Hsf1-/- and Hsf2-/- oocytes. We used 200 oocytes per genotype (4-5ng of RNA) to generate cDNA and hybridize on NimbleGen microarray (42600 genes). This approach allowed us to find 650 genes regulated by HSF1, 296 genes regulated by HSF2 and 51 genes regulated by both factors. Among all these genes, global analysis revealed enrichment in various processes like chromatin remodelling, MAPK pathway and cell cycle regulation. For further analysis, we selected candidates implicated in meiosis (Msh4, Syce1, SA-2) which can explain Hsf1-/- phenotype. All these data will help us to better understand role played by HSF1 and HSF2 in this particular germ cell, oocyte.

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Implication of XHairy1/2 transcription factors in embryonic retinal stem cell maintenance

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Keywords: Retinal stem cells, Hairy, Proliferation, Cell cycle, RPE

The recent identification of various sources of stem cells in the mammalian retina has raised the possibility that cell-based therapies might be efficient strategies to treat a wide range of incurable eye diseases. The successful therapeutic exploitation of these cells primarily requires to unravel intrinsic and extrinsic molecular cues that control their proliferation and cell lineage determination. *In vivo* investigations on retinal stem cells are however presently limited by the lack of reliable markers. The ciliary marginal zone (CMZ) of fish and amphibian offers an exceptional model for retinal stem cell marker identification, as stem cells are confined in an identified area, located at its most peripheral edge. We recently identified two novel markers, *XHairy1* and *XHairy2* (orthologs of human Hes1 and Hes4, respectively), which are specifically expressed in the stem cell-containing region of the Xenopus retina. Following an early expression in the optic field, their expression domain progressively get restricted to the presumptive retinal pigmented epithelium (RPE), then to the developing CMZ and finally to the stem cell niche at post-embryonic stages. Such a dynamic expression profile suggests that XHairy1 and XHairy2 might be involved in the segregation and maintenance of a cell subpopulation dedicated to generate adult retinal stem cells. In line with this, *XHairy1/2* overexpression affects precursor proliferation by slowing down cell cycle speed and preventing cell cycle exit. In addition, gain of function experiments suggest that XHairy1/2 may inhibit differentiation of retinal and RPE precursor cells. As a whole, we propose that XHairy1 and XHairy2 maintain the stemness of a cell subpopulation by keeping it in an undifferentiated and slowly proliferative state until adulthood.

BMP and Notch signalling control ciliogenesis in the frog epidermis

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Mucociliary epithelia are essential for homeostasis of many organs and consist of few cell types including mucus-secreting goblet cells and ciliated cells. Here, we present the ciliated epidermis of *Xenopus* embryos as a simple and accessible model for *in vivo* molecular studies of mucociliary epithelial development. It has long been known that epidermal induction depends on BMP signaling, but the role of this pathway on subsequent epidermis cell type specification has never been investigated. In this study, we addressed this question. To modulate BMP activity in the developing epidermis, we used multiple approaches including treatment with dorsomorphin, a reversible selective chemical inhibitor of Alk1/2/3 receptors, blastocoelic injection of recombinant BMP4 or its antagonist Noggin, and targeted injection of mRNAs encoding constitutively active, or dominant negative BMP receptors. In all cases, we found that BMP activity promotes goblet cell specification and repress ciliated cell formation. Interestingly, these effects are identical to the ones caused by modulation of the Notch signalling pathway, a known regulator of ciliogenesis in frog epidermis. We thus investigated the epistatic relationships between BMP and Notch signals. The results suggest intricate connections between the two signalling systems, rather than a simple hierarchical organisation. Our strategy to try and unravel these connections will be presented. Altogether, our data reveal a novel biological function of BMP signalling, that may eventually be relevant to biomedical research as frog epidermis is akin to human airway epithelia, and the role of Notch in controlling the goblet/ciliated cell balance is conserved.

Role of ephrinB1 in the development of the neuromuscular system

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Keywords : axon guidance, dorsal root ganglion, mouse

The neuromuscular system, composed of motor neurons, sensory neurons and muscles allows coordination of limb movements. Motoneurons are born in the ventral spinal cord and extend their axons towards their target muscles in a stereotypical fashion. At the same time, the sensory neurons, located in dorsal root ganglion (DRG), send axons in order to innervate the skin and axial muscles. These pathfinding processes are highly regulated by a number of guidance molecules, including Eph receptors and ephrins.
The aim of this study is to analyze the role of ephrinB1 in the development of the neuromuscular system. We showed that ephrinB1 is expressed in the limb bud mesenchyme and sensory neurons but not in motor neurons during the development of the sensory-motor circuit. Moreover, we have identified a motor and sensory axon branching defect in efnb1 deficient embryos. To ask whether ephrinB1 acts autonomously or non-autonomously in guiding growing axons, we established DRG explants cultures. Our preliminary results indicate that ephrinB1 non-autonomously regulates sensory axon extension, probably by activating Eph receptors at the growth cone.
Altogether our results suggest that ephrinB1 could be an important player in the development of the neuromuscular system.

Three clustered Ngn1 binding sites act in a cooperative manner for specific *deltaA* transcriptional regulation.

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Proneural genes which are necessary to the formation of neurons code for bHLH transcription factors. The two families of proneural genes: *achaete-scute* and *atonal* are conserved from *Drosophila* to vertebrates. These two families have divergent activities in the formation of neurons both in *Drosophila* and mouse. The transgenic lines, allowing the misexpression of *neurogenin1* (*ngn1*) or *achaete-scute-like1a* (*ascl1a*) after heat-shock induction, show that proneural genes of the *achaete-scute* and *atonal* family also have divergent activity in zebrafish. Moreover, rescue experiment of the Mauthner neuron in *ngn1-/-* and *spg-/-* mutants embryo, show that Ngn1 and Ascl1a differ in their abilities to save mutants. Proneural genes are transcription factor, making it likely that divergent activity between *atonal* and *achaete-scute* family comes from transcriptional regulation of different targets genes. In an attempt to understand these divergences, we have begun a study of *deltaA* gene regulation, a potential target of Ngn1 and Ascl1a in zebrafish. Both gain and loss of function experiment suggest that *deltaA* is a target of Ngn1 and Ascl1a. The functional analysis of the *deltaA* promoter led us to identify a fragment of 470pb which is necessary for a regulation by Ngn1. In this fragment, there is a cluster of three potential Ngn1 binding sites (E-Box) which appear to act in a cooperative manner to allow DNA binding and regulation of *deltaA* by Ngn1. This study provides a novel mechanism for the regulation of target genes by proneural genes of the neurogenin family and leads us to gain further insight in the molecular basis explaining divergences of activity between proneural genes of the *achaete-scute* and *atonal* families.

Keywords : Proneural genes, Zebrafish, Transcriptional regulation, Neurogenesis.

Tshz3 deficiency causes functional renal tract obstruction by impeding ureteric smooth muscle differentiation

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ureter, smooth muscle, differentiation, Teashirt 3, Sox9, mouse

The ureter plays a pivotal role in the urinary system. After filling the renal pelvis with urine, the upper portion of the ureter undergoes peristaltic contractions to propel urine down to the bladder. To ensure this essential function, proper differentiation of mesenchyme surrounding the urothelium into smooth muscle (SM) has to be achieved prior urine production starts at E15. Teashirt (Tshz) genes encode zinc finger transcription factors, which orchestrate embryonic development. Mouse ureteric smooth muscle cell precursors express Teashirt-3 (TSHZ3) and *Tshz3* null mutant mice have congenital hydronephrosis not associated with evident anatomical obstruction. Furthermore, in null mutant embryos, a failure of ureteric SM differentiation antedated the urinary tract dilatation and implicated TSHZ3 as model of 'functional' urinary tract obstruction (Caubit, Lye, Martin et al 2008). To identify new factors involved in renal tract development and to profile gene networks active in this process, we sought to identify protein partners of TSHZ3 with a yeast two-hybrid screen. Among the positive clones, SOX9 was identified as a protein binding partner. Finally, we observe that SOX9 is expressed in an overlapping expression pattern with TSHZ3 and that *Sox9* expression is maintained in *Tshz3* mutant ureter. The closely overlapping expression patterns of TSHZ3 and SOX9 suggest a role for SOX9 in ureter development. We are investigating of the relevance of TSHZ3 and SOX9 interaction during the ureter morphogenesis.

Wt1 is required for mesenchymal cardiovascular progenitor cells formation through transcriptional regulation of Snail and E-cadherin.

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Embryonic development often requires the conversion of epithelial cells into mesenchymal ones. This event is mediated by a tightly controlled cellular phenomenon known as epithelial-to-mesenchymal transition (EMT). EMT disregulation can give rise to a variety of congenital defects or trigger cancer progression along postnatal life. During heart development, epicardial EMT generates cardiovascular progenitor cells that are able to differentiate into various cell types, including coronary smooth muscle and endothelial cells, perivascular and cardiac interstitial fibroblasts and, under certain conditions, also cardiomyocytes. Here we show that an epicardial-specific knockout of Wt1 leads to a reduction in this population of mesenchymal progenitor cells and their derivatives. We demonstrate that Wt1 is essential for EMT in cultured epicardial cells and embryoid bodies (EB), through direct transcriptional regulation of Snail and E-cadherin, two of the major EMT mediators. Some mesodermal lineages fail to form in Wt1 null EB but this effect can be rescued by the expression of Snail, underlining the importance of EMT in generating these differentiated cells. These results provide important information on the molecular mechanism regulating the appearance and function of progenitorlike epicardial derivatives, providing a further rationale to analyze some congenital heart diseases as well as to sustain future cell-based therapies to repair the damaged heart.

Implication of the Notch pathway in the regulation of myogenic cell fate in mouse embryos

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Keywords: Myogenesis – Notch - Pax3 – Progenitors - Cell fate

Vertebrate skeletal muscles derive from transitory mesodermal structures called somites. Within the dorsal part of the somite, the dermomyotome, there are progenitors that express the transcription factor Pax3. It is well established that the dermomyotome gives rise to the derm of the back and all skeletal muscles of the body [1]. More recently, it was shown that single Pax3 positive cells, in this structure, contribute to derm and skeletal muscle, and to endothelial cells and smooth muscle [2,3,4]. Moreover, a common progenitor also gives rise to brown fat cells and skeletal muscle [5].

Mechanisms that control such cell fate decisions are not well elucidated, but recent results suggest that the Notch pathway could be a potential candidate. The Notch pathway is an evolutionarily conserved system that generates cellular diversity during development. In the context of the dermomyotome, it promotes smooth muscle cell differentiation at the expense of skeletal muscle [4]. Furthermore the Notch pathway leads to the maintenance of the myogenic progenitor pool within differentiated skeletal muscle masses [6,7]. We are investigating the role of this pathway in murine myogenesis by using a mouse model in which a sequence coding the intracellular constitutively activated form of Notch (NIC) was inserted into *Pax3,* with an *nLacZ* reporter. *Pax3* $^{NIC\text{-}IresnlacZ/+}$ embryos present defects in appendicular muscle development and trunk myogenesis. Concerning limb myogenesis, the number of progenitors that migrate to the limbs is reduced, and the analysis of the balance between progenitors and differentiated muscle cells is perturbed. Ongoing work focuses on the cause of the reduction in migrating muscle progenitors and on whether activation of the Notch pathway in *Pax3* $^{NIC\text{-}IresnlacZ/+}$ embryos leads to changes in cell fate decisions of multipotent Pax3 positive progenitors.

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Role of *Xash1* transcription factor in GABAergic cells specification in *Xenopus* retina

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Mechanisms sustaining the specification of the different neuronal subclasses are largely unknown. My project aims at unraveling the molecular mechanisms controlling GABAergic neurons specification during retinogenesis in order to contribute to the elaboration of a retinal subtype determination model. Recently, *Ptf1a* has been implicated in GABAergic cell specification in the retina. To increase our knowledge on this process, I focussed my word on a potential regulator of *Ptf1a, Mash1*. I thus examined the function of the *Xenopus* homolog, *Xash1*, in GABAergic phenotype specification. I found that *Xash1* is a unique bHLH factor able to promote GABAergic neurons and is able to inhibit glutamatergic neurons genesis in ectopic conditions. My data also highlighted the role of *Xash1* in favouring a GABAergic destiny within the retina. I also discovered that *Xash1* is epistatic on *XNgnr-1*, a glutamatergic inducer. Surprisingly, I demonstrated that *Xash1* and *XNgnr-1* acts synergistically in directing the GABAergic phenotype. Next, I found that *Xash1* is also able to favour a dopaminergic destiny in the retina. Last, I demonstrated that *(i) Xash1* is able to promote *Ptf1a* expression, *(ii) Xash1* knock down leads to a decrease of *Ptf1a* expression in the retina and *(iii) Xash1* GABAergic inducing activity is inhibited upon loss of *Ptf1a* function. These three experiments strongly suggest that *Xash1* functions through the *Ptf1a* pathway to promote a GABAergic destiny.

Keywords : retina, determination, transmitter phenotypes, GABAergic neurons, bHLH transcription factor *Xash1/Mash1*.

Control of larval haematopoiesis: cell lineages of the lymph gland

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Keywords: Hematopoiesis, *Drosophila*, Lymph Gland Lineages

Larval hematopoiesis takes place in a specialised organ, the lymph gland. Two types of hemocytes differentiate in 3rd instar larvae in normal conditions: plasmatocytes (macrophages) and crystal cells (melanisation). A third type of hemocytes, the lamellocytes, are devoted to encapsulation of foreign bodies too large to be phagocytised and only differentiate under specific immune conditions such as wasp parasitism. The lymph gland is composed of a cortical zone (CZ) where hemocytes differentiate, a medullary zone (MZ) containing immature pro-hemocytes and a "Posterior Signalling Center" (PSC). We have previously shown that the PSC plays a key role in the maintenance of a pool of multipotent progenitors, which is a prerequisite for lamellocyte differentiation in response to wasp parasitism. The role of the PSC is reminiscent of the hematopoietic "niche" of vertebrates, a micro-environment required for survival and self-renewing of Hematopoietic Stem Cells (HSC). To better understand the communication between progenitors and their micro-environment, we performed lineage analyses. Our results show that distinct pools of progenitors are restricted to a plasmatocyte or crystal cell fate early during larval development while the lineage restriction between PSC cells and other LG cells is already established in embryos. A genome-wide analysis is in progress to identify new genes expressed in the medullary zone and/or the PSC and better characterise the mechanisms involved in the maintenance of pro-hemocytes and the segregation of the different hemocyte lineages.

Molecular and functional characterisation of the hematopoietic niche in *Drosophila melanogaster*

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Drosophila hemocytes (blood cells) originate from a specialised hematopoietic organ, the lymph gland (LG). Larval hematopoietic progenitors (prohemocytes) give rise to three types of circulating hemocytes: plasmatocytes (phagocytosis), crystal cells (melanisation) and lamellocytes. Lamellocytes, which are devoted to encapsulation of large foreign bodies only differentiate in response to specific immune threats such as parasitization by wasps. We showed that a small cluster of signaling cells, termed the PSC (Posterior Signaling Center), acts in a non cell autonomous manner to control the balance between multipotent prohemocytes and differentiating hemocytes and is necessary for the massive differentiation of lamellocytes that follows parasitization. The key role of the PSC in controlling blood cell homeostasis is reminiscent of micro-environmental stem-cell niches that provide support for hematopoiesis in vertebrates. In order to study mechanisms involved in cellular communications between PSC cells and pro-hemocytes in normal conditions and after a immune challenge. Identification and characterisation of some news candidate genes involved in Drosophila larval hematopoiesis will be presented.

CARDIAC PHENOTYPIC CHARACTERIZATION OF A DROSOPHILA MODEL OF MYOTONIC DYSTROPHY TYPE 1

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Keywords : Myotonic dystrophy type 1, drosophila, mbl, bru-3, CTG repeats

Myotonic dystrophy type 1 (DM1) is a dominant multisystemic disorder caused by a CTG expansion in the 3'untranslated region of the Dystrophy Myotonic Protein Kinase (*DMPK*) gene. In muscle cells, the mutated DMPK transcript is retained in nuclear foci where it sequesters and induces alterations in the levels of splicing factors : MBNL1 and CUGBP1. Thus, DM1 patients exhibit decreased MBNL1 levels and increased CUGBP1 levels.

To understand the mechanisms underlying DM1 and investigate the involvement of CUGBP1 and MBNL1 in this myopathy, Drosophila is used as an experimental model. We show that larvae overexpressing bru-3 (CUGBP1 ortholog) or with attenuated mbl expression (MBNL1 ortholog) in the somatic muscles, display muscle loss, sarcomere disorganization and in addition mbl loss-of-function induces muscle attachment defects. Furthermore, bru-3 overexpression in the heart significantly increases adult heart rate, decreases fractional shortening (heart pumping capacities) and reduces lifespan. These perturbation of cardiac function that mimic cardiac defects in DM1 patients may result from altered cardioblast differentiation observed in flies overexpressing bru-3.

Live imaging of Hox-induced neuroepithelial cell clusters

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During embryonic development, the vertebrate hindbrain becomes segmented into a series of rhombomeres. The roles of Hox homeodomain proteins in assigning AP character have been well described but their downstream functions in the segmental subdivision process itself remain unclear. We show that, in the mouse, there is a redundant requirement for either Hoxb4 or Hoxd4 in specifying the boundary between rhombomeres 6 and 7. We find that Hox4 proteins in the mouse and chick hindbrain regulate many downstream target genes implicated in cell adhesion/repulsion, including several Ephs, Ephrins and a member of the LRRTM family. Mosaic expression of Hoxb4 (or several other Hox proteins) in electroporated chick hindbrains leads to targeted neuroepithelial cells forming large clusters that are not observed with GFP-alone or with other controls. Using live imaging, we have begun to analyse which cell behaviours might be responsible for these clusters. Surprisingly, we do not observe widespread sorting out, in the classic “oil-and-water” sense, of Hoxb4-expressing and non-expressing cells. Neither do we see significantly altered cell proliferation/apoptosis, thus also ruling out cell-competition based mechanisms. We are currently testing the hypothesis that Hox proteins restrict cell dispersal in a clonal manner, increasing cell affinities within neural progenitor clones more strongly than between them.

The *ParaHox* gene *Gsx/Anthox2* regulates neurogenesis in developing *Nematostella vectensis*

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Keywords: cnidarians, *Gsx ParaHox* genes, neurogenesis, morpholinos, reporter genes

Background: Neurogenesis in bilaterians rely on a shared genetic circuitry that involves different classes of transcription factors including homeobox genes. Among those, the *Gsx/Ind* gene family is involved in dorso-ventral patterning of the neural tube, brain formation and neuronal identity. In cnidarians, the first phylum that acquired a nervous system, the *Gsx* homologs (*cnox2*, *Anthox2*) are highly conserved and expressed in the nervous system as in *Hydra*, *Clytia*, *Acropora*. Here we investigated the neurogenic function of *Anthox2* during *Nematostella* development.

Results: The phylogenetic analysis confirmed the higher conservation of the cnidarian ParaHox families than the Hox-like ones. Moreover the structural conservation in *Gsx* orthologs possibly reflects the conservation of an ancestral function. We found that *Anthox2* expression precedes onset of neurogenesis early during embryogenesis. Subsequently *Anthox2* is expressed in putative neuronal precursors and differentiated neurons, which are exclusively detected in tentacles after metamorphosis. *Anthox2* morpholino inhibition altered the formation of the nerve net and the survival of the planulae. We also performed reporter assays and found that 3 kb of upstream *Anthox2* sequences suffice to drive expression in apical neurons. These sequences contain putative regulatory elements also present in the *Hydra cnox2* upstream sequences, suggesting a conserved genetic regulation for *Gsx* between anthozoans and hydrozoans.

Significance: These data suggest an essential role for *Anthox2* in *Nematostella* neurogenesis, likely shared among *Gsx* cnidarian homologs Hence the high degree of conservation of the *ParaHox* genes from cnidarians to bilaterians could reflect the constraints driven by their essential function in cell type innovation as neurogenesis, an innovation that was maintained and complexified among eumetazoans.

A transcriptional co-repressor involved in growth and development in *Dictyostelium discoideum*

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Growth and development are neatly separated in the life cycle of the model organism *D. discoideum*. Development, including cell differentiation and morphogenesis, starts when food is no longer available and *Dictyostelium* initiates a specific transcription program. The switch between both states has to be precisely regulated and at the same time, energy must be provided all through development. Proteins like NmrA, an *Aspergillus* transcriptional repressor, or the human HSCARG can differentially bind dinucleotides (like NAD, NADH, FAD) and this binding modulates their function and interactions with other proteins (1). Thus, these proteins can signal fluctuations in the cell metabolic state and produce changes in the transcription or the physiology of the cell. PadA, a *D. discoideum* protein, has been proposed to belong to this class of proteins (2). Besides the described developmental defects at 27ºC, we show here that the *padA*$^{-}$ mutant exhibits both ineffective aggregation and cell adhesion, and a temporal blockage during the slug-culminant transition at normal Tª. The mutant also shows slow or no vegetative growth under stressing conditions, like high Tª or minimal medium, and low oxygen consumption, revealing a basic metabolic deficiency. We suggest that PadA may play a regulatory role both in cell metabolism and development

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A Neoblast-specific Functional Screening in *Schmidtea mediterranea*

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Keywords: stem cell, planaria, neoblast, proteomics.

Recent metagenomic projects have proved how far we are of a complete catalog of protein functions yet. Despite having a large number of sequences, current nucleotide and protein databases cannot assist us to find species-specific functional sequences or completely novel undescribed ones. Proteins being expressed under determined experimental conditions can be detected by different proteomic approaches, including mass spectrometry. Using this information to define their genomic locations and to detect novel functions can be challenging, specially when the underlying genome is partially assembled, or not at all.

We have integrated the flatworm *Schmidtea mediterranea* genomic shotgun-traces with mass-spectrometry peptide data, in order to provide sets of putative proteins, containing both known and novel sequences, that can be experimentally validated. The protein set of control and irradiated planarians was compared. Irradiation affects cells that are actively replicating its DNA. Thus, it depletes the animal from neoblasts which are the only proliferating cells in this organism. In fact, neoblasts are the planarian stem cells, responsible for cell turnover and regeneration. We aimed to find proteins being expressed differentially at an undifferentiated stage or under a DNA-damage repairing scenario. We discuss here the computational protocol, the results on different datasets of open reading frame sequences, as well as experimental results validating this approach.

Induction of orbital cartilage by the CNS

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Orbital cartilage encircles the eye giving strength and support to the neural retina. It is derived from cranial neural crest cells (NCC), which can generate a number of cell types including neurons, glia, and melanocytes. Uniquely in the head, NCCs also make cartilage and bone of the craniofacial skeleton. Differentiation of NCCs into cartilage 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 NCCs 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. Orbital cartilage is found in the majority of vertebrates but the ability to induce it has been lost to mammals. A comparison of chick and mouse will help to determine the evolutionary mechanisms underlying changes in the orbital skeleton.

We have examined the gene expression patterns of cartilage markers and the definitive cartilage stain Alcian Blue, to show the development of orbital cartilage in the chicken embryo. Using these methods we also demonstrate that orbital cartilage is initiated in the mouse, but fails to differentiate. We demonstrate that cartilage formation is prevented in chick following early eye removal and the neural ectoderm derived- retinal pigment epithelium induces ectopic cartilage to form in cranial NCCs in ovo. Thus the RPE is a critical tissue that co-ordinates growth and development of the eye with the skeletal structures that support it.

Ligand and receptor gene expression patterns indicate a role for Fgfs in the development of this tissue.

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Keywords- Cartilage, Neural Crest Cells, Visual System, Development

Function of Sulf proteins in Shh-dependant glial specification

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Sulfatase 1 (Sulf1) belongs to a family of recently identified extracellular endosulfatases. These enzymes, by their unique ability to catalyze removal of 6-O sulfate from specific regions within the heparan sulfate (HS) chains of heparan sulfate proteoglycans (HSPGs), contribute to modulate signalling pathways by inducing or inhibiting interactions of signaling molecules or their antagonists with their receptors. In a previous work, our lab has identified Sulf1 as an early and reliable marker of oligodendrocyte progenitors (OLPs) originating from ventral embryonic chicken spinal cord. These ventral OLPs are specified after the onset of neuronal production in response to a rise in Sonic Hedgehog (Shh) activity. Accumulation of the morphogen factor at the apical surface of ventral neural progenitors immediately prior to OLP specification has been proposed to be responsible for this temporal change in Shh signaling. The good correlation between modification of Shh distribution and initial expression of Sulf1 in ventral neural cells was supportive of a function of Sulf1 as a regulator of Shh signaling responsible for OLP induction. In favour of this hypothesis, overexpression of Sulf1 in the developing spinal cord is sufficient to promote Shh activity in neural progenitors. To investigate the function of Sulf1 in Shh signaling and OLP specification, we recently initiated loss of function experiments in chicken. Two approaches were developed, overexpression of a dominant-negative form of Sulf1 and RNA interference. An electroporation method was first developed to target ventral cells of the embryonic spinal cord at various developmental stages. Our preliminary data showing that down-regulation of Sulf1 impedes OLP production from ventral neural progenitors strongly support a crucial role of this enzyme in regulating the emergence of OLPs. In parallel, we asked whether Sulf1 may also be involved in OLP specification in mammals. Our recent data showed that the spatial and temporal pattern of expression of Sulf1 is conserved in mice. We are currently analyzing the phenotype of *sulf1* knockout mice using various OLP makers.

The role of ARID3b in heart development

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Keywords: ARID3b, cardiac development

ARID3b is a transcription factor from the highly conserved ARID family, whose members share a common DNA-binding domain. ARID3b null-mice die early in embryonic development and present a severe phenotype in many structures. However, its roles in development are not clear. Here, we try to address the importance of ARID3b in the developing heart. We studied the pattern of expression of this gene in the embryonic heart and found that it is expressed at early stages of development in the tubular heart and later in the outflow tract, right ventricle, atria and sinus venosus. Using ARID3b knock out mice, we analyzed the cardiac defects produced by the absence of the gene. The most dramatic phenotype is observed at the level of the outflow tract, which is shortened or even absent. By analyzing several molecular markers of the secondary heart field and of chamber differentiation, we observed a reduction in the expression of some of these genes (BMP4, FGF8, Islet-1) in mutant's heart. Our results provide some evidence of a role of ARID3b in the mechanisms regulating the contribution of cells from the secondary heart field to the heart.

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The transcriptional repressor prdm1/blimp1 is required within the second heart field for the morphogenesis of the distal outflow tract.

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Keywords: mouse, heart development, transcriptional repressor, conditional deletion, prdm1

The *prdm1* gene encodes the prdm1/blimp1 protein, which is characterized by PR/SET and zinc finger domains. Blimp1 acts as a transcriptional repressor by recruiting co-repressors leading to direct repression and also the indirect activation of hundreds of genes. The *prdm1* gene is dynamically expressed during development and, strikingly, has been shown to control specific differentiation programs in each of the different domains studied to date.

During heart formation, *prdm1* is transiently expressed in the second heart field (SHF), a region that will contribute to the formation of the outflow tract (OFT). This structure will eventually form the pulmonary trunk and the aortic arch. In this context, *prdm1* is also expressed in the adjacent endoderm where it is required to support the growth of the mesenchymal and neural crest derived cells of branchial arches 2 to 6 [1]. As a result, *prdm1*$^{-/-}$ embryos display heart septation defects, including persistence of the *truncus arteriosus* [2]. In order to analyse a cell autonomous function in the SHF, we carried out a conditional deletion of *prdm1* using the heart specific *Mesp1Cre* (early cardiac mesoderm) and *Mef2cCre* (SHF) lines. Deletion of *prdm1* in the heart mesoderm does not interfere with the development of the 2nd and 3rd branchial arches confirming that the arch defect observed in null embryos is non-cell autonomous. *Mesp1Cre* conditional mutants die at birth and display defects in structures of the distal outflow tract (OFT), derived from the SHF: uneven semilunar valves, misalignment of the great arteries and severe aortic arch defects (interruption of the aortic arch type II (IAA-B), high arches, absence of *ductus arteriosus*…). *Mef2cCre* conditional mutants are viable, but display a retro-oesophageal right subclavian artery, a defect also observed in the *Mesp1Cre* mutants. Examination of pharyngeal arch artery (PAA) remodelling demonstrates that the aortic arch phenotypes are linked with a defect in the formation of the 4th PAA. In *MesP1Cre* conditional mutant embryos, the OFT is shortened. The difference in phenotypic severity between the two *Cre* lines used in this study suggests that *prdm1* plays an essential early regulatory role in mesodermal progenitor cells of the SHF that contribute to morphogenesis of the arterial pole of the heart.

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An *in vivo* RNAi screen identifies new genes controlling Drosophila blood cells homeostasis.

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Drosophila blood cells (hemocytes) ensure the defence of the organism in particular by eliminating pathogens and abnormal cells. These hemocytes belong to three lineages: the plasmatocytes, the crystal cells, and the lamellocytes. Lamellocytes are normaly scarcely present but their differentiation is massively induced after an immune challenge such as the parasitisation of the larvae by wasp eggs around which they will form a melanotic capsule. Likewise, several zygotic mutations have been described to induce the production of melanotic capsules in the absence of infection. These mutations have been linked to misregulation in several processes including hemocyte differentiation, defect in self tissues recognition or tissue degeneration. Yet, little is known about the mechanisms controlling self *versus* non-self or alter-self recognition and how the subsequent immune cellular response is orchestrated.

To identify new genes implicated in blood cell homeostasis, we used a collection of UAS-dsRNA transgenic lines to specifically induce loss of functions in the hemocytes (or in the hemocytes and the fat body) and we looked for melanotic tumour formation. Screening 10% of the *Drosophila* genes, we recovered around 50 melanotic tumour suppressors genes. This approach pinpointed several new pathways participating in blood cell homeostasis. Notably, results suggest that larval blood cell homeostasis is controlled by an intricate network of communications between the different immune tissues. Interestingly, we identified a set of genes that act cell-autonomously and we demonstrated that embryonic-derived plasmatocytes transform into lamellocytes. All together, our results shed new light on the control of Drosophila blood cell lineage development and plasticity.

Keywords: haematopoiesis, cellular immunity, Drosophila.

Hedgehog morphogen gradient is shaped by Sulfatase-1 modified HSPGs during *Drosophila* wing development.

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Over the past decade, intensive biochemical and genetic studies have elucidated the central components of the Hedgehog (Hh) signalling pathway. However, several important issues remain to be resolved concerning the mechanisms by which the distribution and movement of Hh is regulated in morphogenetic fields. Heparan sulfate proteoglycans (HSPGs), major components of the extracellular matrix, have clearly been shown to play crucial roles in regulating Hh movement during development. HSPGs consist a core protein to which heparan sulphate (HS) glycosaminoglycan chains are linked. HS chains are characterized by a specific sulfation pattern defined during their biosynthesis that is further modified at the cell surface by extracellular endosulfatase. Our lab has recently shown that such an enzyme, called sulf1, is a modulator of Sonic Hedgehog (Shh) signalling in the developing chick neural tube suggesting that the sulfation state of HS chain may play a role in modulating Hh signalling. In order to investigate this question, we turned to *Drosophila melanogaster* and analyse the function of *sulf1* during wing development. As a first step, we showed that *sulf1* is indeed expressed in the wing imaginal disc and its restricted expression pattern corresponds to future wing vein domains in agreement with a function in modulating Hh signalling. Then, we showed that *sulf1* loss and/or gain-of-function experiments in the wing disc lead to a misregulation of various Hh target genes indicating that Sulf1 play a role in Hh signalling regulation. Moreover we have established a correlation between these Hh signalling defects and a modification of apical Hh distribution. Our results allow us to propose a model where Sulf1, by modulating sulfation pattern of HSPGs and extracellular distribution of Hh, is necessary to fine-tune Hh signalling during wing development.

Key words: Hedgehog, HSPG, sulfatase, morphogen, gradient

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