Instituto Juan March
de Estudios e Investigaciones

153 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

The Dynamics of Morphogenesis: Regulation of Cell and Tissue Movements in Development

Organized by

C. D. Stern and M. A. Nieto

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Introduction

Claudio D. Stern and M. Angela Nieto

The cellular events that direct embryonic development have broadly been subdivided into three kinds of processes: fate allocation, pattern formation and morphogenesis. *Fates* are allocated to cells through a combination of their lineage history (through cytoplasmic and nuclear determinants of fate) and cell interactions ("inductions"). *Pattern formation* is the set of processes that shape a more or less uniform field of cells by assigning different fates to cells according to their position with respect to their neighbours. *Morphogenesis* is also a set of processes that generate form, but here the main driving forces are cell movements and tissue reorganizations rather than fate allocation. To illustrate the difference: an example of pattern formation is the generation of the periodic mosaic of ommatidia in the compound eye of Drosophila; an example of a morphogenetic process is gastrulation, where cells arrange themselves into three layers which will then behave differently.

In the last few decades considerable effort has been spent in understanding how cells are assigned different fates and how pattern formation occurs in systems like the Drosophila eye and the vertebrate limb. Morphogenetic processes, on the other hand, have received comparatively little attention. Genetic and molecular analyses have concentrated on elucidating *pathways*, but very little is known about *how these pathways are integrated in space and time*. Interest is now turning to try to understand the processes that orchestrate these dynamics of cell behaviour. This applies not only to embryonic development, but also to the adult and to pathological processes such as cancer, where invasive behaviour can be considered a form of morphogenesis that profoundly influences the morbidity of the disease.

This meeting aimed to explore the current status of this field, at a time when many laboratories are starting to acknowledge that no solid understanding of development or pathology can be gained until our view can also includes an understanding of how gene expression and cell behaviour are integrated in space and time.

Some of the questions are:

- We already understand something about the *mechanics* of cell movement, but what signalling cues control the directed migration of cells?
- Is there directed migration, or just assortments of permissive, repulsive and attractive sites? How general a mechanism is chemotaxis?
- To date, several protein families have been implicated in signalling outside the nervous system (including FGFs, Wnts and HGF/SF), but different signalling proteins have been shown to guide axon navigation in the CNS and PNS (including netrins, semaphorins, collapsins, NOGO, Ephrins/Eph receptors, etc.). Is this a true difference? If so, how/why did different mechanisms evolve to regulate what seems like the same process in different parts of the same organisms?
- As cells migrate, they respond to local inductive/repressive cues from their neighbours which change their gene expression profiles and consequently alter their behaviour. How are the many signals they encounter integrated? For example, as prospective mesoderm cells approach the organizer region of the vertebrate embryo, they start to express organizer-specific genes, and then turn them off as they leave the region.

When do they receive the signals that activate/repress the expression of specific genes, and how much of the timing of this is controlled by combinations of regulatory genetic elements, and how much by the precise positioning and physical range of the inductive/repressive signals?

- How does the signalling machinery result in directional movement? Can cells really sense concentration differences between one of their sides and the other?
- Do other cellular asymmetries (such as asymmetric cell division, epithelial polarity) involve similar directional cues, which direct the migration of intracellular organelles?
- How many of the signalling systems involved in development are also implicated in wound healing and cancer invasion/metastasis? Why do embryos heal their wounds without scars, while adults always scar? Why are some tumours invasive and some not?

Of course, a two-and-a-half-day meeting could not possibly answer all of these important questions. However, by focusing on the major current "model" processes (gastrulation, neural crest cell migration, germ cell migration, tubulogenesis and branching morphogenesis, epidermal wound healing and cancer), and "model organisms" (Drosophila, C. elegans, Sea Urchin, zebrafish, Xenopus, chick and mouse), we can obtain an inkling of the great diversity of mechanisms that exist to coordinate complex cell behaviours in time and space. Perhaps one critical conclusion that was achieved during the meeting was that biology itself is extremely diverse and that the whole concept of "model" processes and systems can lead to oversimplifications and to false generalisations. The principles of evolution and development will be best understood by exploiting the advantages of each system, and by opportunities such as this one, to exchange views and experiences between those that have been thinking very deeply about their favourite organism and biological event.

The organisers,
May 2003

Session 1: Morphogenesis and embryo patterning
Chair: Claudio D. Stern

Imaging cell and tissue migrations in the developing embryo

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The explosion of progress in the fields of cell biology, biochemistry, and molecular biology has offered unprecedented knowledge of the components involved in embryonic development. The dramatic progress of these reductionistic approaches poses the challenge of integrating this knowledge into an understanding of the underlying mechanics. The classic publications in the field of experimental embryology illustrate the power of describing cell behavior (cf. lineages, movements) and perturbing the embryo to test hypotheses of the underlying mechanisms. Advanced imaging techniques offer an important stepping stone between these disparate approaches, permitting questions about cellular and molecular events to be posed in the most relevant setting of the intact embryo.

There are two major limitations of any approach based on fluorescence in living embryos. First, the image degrades dramatically as the microscope is focused below the surface layers of the embryo. This results from increased optical aberrations in the objective lenses and increased light scattering as the light passes through a significant pathlength of tissue. Second, the fluorochromes are bleached by irradiation with the exciting light and the by-products of this bleaching can be toxic. Two-photon laser scanning microscopy (TPLSM) offers a means to minimize these concerns. TPLSM uses an intense red or infra-red ultra-fast laser to excite fluorochromes in the UV or visible wavelength range through the concurrent absorption of two photons. Because the statistics of two photons being absorbed depends on the square of the intensity and the intensity drops by the square of distance from the focal plane, the technique selectively excites fluorochromes at the focal plane. Light scattering and optical aberrations decrease the probability of the excitation, but decrease the resolution in the image much less than in other light based microscopies. Based on its performance to date, two-photon microscopy may offer the best means for detecting fluorescent labels in intact tissues. Multispectral approaches, in which the entire spectrum is taken of the emitted light from each pixel, offers a potential solution, as the spectral data can be decomposed into its component parts by simple mathematics. Fluorochromes as similar as GFP and fluorescein can be separated unambiguously, and even small amounts of FRET can be detected by our approach (now available as the Zeiss LSM-510 Meta).

In systems in which light-based imaging is problematic, we are employing microscopic MRI. In MRI, radio frequency energy is used to excite the protons of the water, which generates no toxic by-products. Spatial resolution is created by imposing gradient magnetic fields on the specimen, thereby making it possible to encode the signals from individual volume elements (voxels) by their resonant frequency and phase. By increasing the magnitudes of the

static and gradient magnetic fields, and by improving the electronics, it has become possible to increase the resolution of MRI from the 1mm voxels of a clinical instrument to ~10μm. This approach has the promise of making imaging analyses possible in the systems with limited access to the embryos (e.g. mouse) or in which light scattering renders deep structures invisible (e.g. frog).

Here, MRI microscopy will be used to follow the fates and motions of the amphibian Spemann organizer, originally defined by its ability to induce and organize a secondary body axis when grafted to the future ventral side of an embryo. The Spemann organizer plays a central role in directing a subregion of the surface ectoderm to adopt a neural fate. Recent molecular and experimental embryology studies have defined a set of molecular events involved in the establishment and the function of the Spemann organizer and have shown that the lessons learned from the amphibian Spemann organizer have important parallels in a variety of species. A secondary body axis forms when the "organizer" region of the early embryo is grafted to an ectopic site in species ranging from fish to chicken and mouse. Similarly, many of the molecular correlates of organizer function in the frog have been found in a variety of species. As a result, many findings in other species are reported in the context of the amphibian organizer. Despite the frog serving as the standard by which results in other species are cast, significant open questions remain as to the nature of neural induction in the amphibian. Two opposed pathways of neural induction remain viable: vertical signaling models, in which the mesoderm and endoderm (mesendoderm) involute around the blastopore lip and then induce the overlying ectoderm; planar signaling models argue that the critical inductive interactions take place between mesendoderm and ectoderm in the plane of the embryonic surface before the involution motions of gastrulation. Microscopic magnetic resonance imaging allows us to follow cell movements and contacts in the Spemann organizer region before and during gastrulation. Key events such a vegetal rotation and epiboly bring surface ectoderm and meesendodderm into contact long before the outward signs of gastrulation. A surprising resolution of the debate between vertical and planar signaling models is the finding that the axial mesoderm is internally localized and in vertical contact with much of the future neurectoderm throughout early development. These observations are consistent with the direction of signaling proposed by vertical models and the early timing of the signaling proposed by planar models.

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Mesenchymal migration and cell fate regulation in the sea urchin embryo

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Mesenchymal ingression temporally precedes invagination of the archenteron in the sea urchin embryo. Primary mesenchyme cells undergo an epithelial to mesenchymal transition as they invade the blastocoel. The immediate trigger for PMC ingression is not known but is downstream of several identified transcription factors. In the process of ingression the cells endocytose their epithelial membranes and the adhesive proteins contained therein. At the same time the PMCs insert new membranes containing new adhesion molecules onto the surface. This allows for a rapid deployment of the mesenchymal adhesive phenotype. Pre-PMCs prepare for this rapid transition for many hours: a gene regulatory network begins to specify micromeres at fourth cleavage. By 8^{th}-10^{th} cleavage the specified micromeres begin to store proteins in vesicles for recruitment at ingression. In a distinct specification sequence secondary mesenchyme cells begin their specification sequence, also at 4^{th} cleavage, and are separated from general endomesoderm by Notch signaling by 7^{th}-9^{th} cleavage. SMCs both ingress and lead the invagination of the archenteron. The trigger for initiation of archenteron invagination appears to be through activation of Rho kinase. The invagination of endoderm into the archenteron requires a wave of *brachyury* expression. That expression is necessary for many cytoskeletal and motility molecules involved in convergent extension morphogenesis of the gut. The wave of *brachyury* expression is controlled, at least in part, by a *gatae* activator, and a *foxA* repressor.

Morphogenetic movements that establish the anterior-posterior axis after implantation respect the bilateral symmetry of the embryo rather than the uterine axis

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Although the anterior-posterior axis of the mouse embryo becomes explicit morphologically at embryonic day (E) 6.5, the first molecular signs of the anterior-posterior polarity after implantation are the asymmetric expression of several genes along the proximo-distal axis of the egg cylinder (for review see Robertson and Beddington, 1999). Lineage tracing has shown that this proximo-distal polarity translates back to the axis of bilateral symmetry of the blastocyst, which in turn corresponds to the polarity of the fertilised egg (Weber et al., 1999; for review see Zernicka-Goetz, 2002). It appears that asymmetric cell movements between the blastocyst and gastrula stages are key to development of this polarity (Thomas and Beddington, 1996, Weber et al, 1999). The extent to which these movements reflect differential growth of the egg cylinder, changes in its shape or cell migration is unknown. Also unknown is the relationship of these movements to the organisation of the post-implantation embryo with reference to the axes of the uterus. To gain insight into the transformations of the post-implantation embryo, we have analysed the dynamics of its morphogenetic changes from implantation until the initiation of gastrulation with particular reference to the expression pattern of Cer-l-GFP, a marker of the future anterior (Belo et al, 1997). At implantation the bilateral symmetry of the embryo is aligned with the long axis of the uterus. This relationship is lost between E5.5-E6.25 but then re-gained in a perpendicular orientation at the time of gastrulation. The asymmetric distal to anterior movement of the visceral endoderm cells that is initiated at E5.5 relates to the embryo's axes of symmetry and not to the axes of the uterus. Unexpectedly this movement occurs predominantly along the short and not the long axis of the embryo. Subsequent alignment of the embryo's anterior-posterior axis with the axis of the uterus occurs through limiting the expression domain of anterior markers. We suggest this is facilitated by rotation of the egg cylinder within extra-embryonic tissues or changes in shape of the egg cylinder itself. An understanding of these processes has to be accommodated within this new perception of anatomical dynamics during implantation.

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The cell motility, biomechanics, and the self-deforming "skeleton" of the amphibian gastrula

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Involution of the mesoderm, blastopore closure, and axis elongation during gastrulation and neurulation of *Xenopus* are due largely to convergence and extension of the axial and paraxial mesoderm and posterior neural tissue. During these movements, cells actively intercalate between one another along the mediolateral axis to form a narrower, longer array, but at the same time, they collectively form a stiff beam and exert a pushing force. New evidence concerning polarized protrusive activity and extracellular matrix supports a cell-traction/cell-substrate mechanism that allows self-deforming tissues such as these to actively rearrange their cells but at the same time form a self-supporting, force-generating morphogenic machine that can also serve as a dynamic "skeletons" of the embryo. Understanding how self-deforming, force-producing tissues function is important for understanding a number of morphogenic movements. Other work shows that in addition to cell intercalation, urodele amphibian embryos use an epithelial mesenchymal transition, expressed in the form of a bilateral "primitive streak", to generate the forces produced by cell intercalation alone in *Xenopus*. These results argue that biomechanical strategy is the key parameter in evolution of gastrulation mechanisms.

FGF signalling and the control of gastrulation movements in Xenopus

Jeremy M. Sivak, Stephen L. Nutt and Enrique Amaya

Signal transduction through the FGF receptor is essential for the specification of the vertebrate body plan. Blocking the FGF pathway in early Xenopus embryos inhibits mesoderm formation and results in truncation of the anterior-posterior axis. We have previously shown that Xenopus sprouty2 is an intracellular antagonist of FGF-dependent calcium signalling. In addition we have shown that Xsprouty2 inhibits convergent extension movements and have suggested that there are at least two distinct FGF-dependent signal transduction pathways: a Sprouty insensitive Ras/MAPK pathway required for the transcription of most mesodermal genes, and a Sprouty sensitive pathway required for co-ordination of cellular morphogenesis. We have recently identified additional antagonists of FGF signalling, including Xsprouty1, Xspred1, Xspred2 and XSef and are currently studying their role during mesoderm formation and morphogenesis.

Unexpectedly, preliminary results from mis-expression and morpholino experiments suggest that the functions of these proteins diverge during Xenopus gastrulation. These differences support a model in which the various FGF signalling antagonists may cooperate to modulate cell behaviours and mesoderm formation. Our eventual aim is to understand how FGF signalling is controlled in space and time resulting in the co-ordination of mesoderm formation and cell movements during gastrulation.

Session 2: Integrating cell behaviours
Chair: Scott E. Fraser

Regulation of cell movements during zebrafish gastrulation and tail formation

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During the vertebrate gastrulation movements of convergence and extension (C&E) the entire embryo and most organ rudiments narrow along the mediolateral axis while extending their anterior-posterior dimension (Solnica-Krezel and Cooper, 2002). In zebrafish C&E movements are driven by directed cell migration and intercalation of mediolaterally polarized cells and require non-canonical Wnt signaling and are regulated by ventral to dorsal gradient of Bmp activity (Myers et al., 2002a; Myers et al., 2002b). We have previously identified a glypican Knypek (Topczewski et al., 2001), membrane protein Trilobite/Strabismus (Jessen et al., 2002) and Rho kinase alpha (Rok2) (Marlow et al., 2002) as components of this pathway.

In other systems, Rho kinase impacts cell behaviors and cytoskeleton by phosphorylating Myosin regulatory light chain (Mrlc). We have cloned two zebrafish *mrcl* genes and showed that their transcripts and encoded proteins are present ubiquitously during early zebrafish embryogenesis. Overexpression of a phosphomimetic, constitutively active form of Mrlca disrupts C&E, phenocopying non-canonical Wnt signaling mutants. Epistatic analyses place Mrlc downstream of Wnt 11 in regulation of C&E. Impaired dorsal convergence movements in embryos overexpressing are associated with defective cell polarity and protrusive activity.

Tail morphogenesis entails both continuation of CE movements, as well as unique movements like subduction. We tested functional interactions between components of the non-canonical Wnt signaling pathway and a transcription factor Notail (Brachyury). Mutations in the knypek gene, encoding a glypican, and pipetail encoding Wnt5, impair CE movements resulting in shortened trunk and tail. *notail* mutants exhibit defective notochord and tail truncations. *kny;ntl* and *ppt;ntl* double mutants show similar, synergistic phenotypes characterized by truncation of posterior trunk and tail, but relatively normal head. Gene expression studies reveal that this synergistic phenotype is not due to defective specification of caudal tissues. Furthermore, neither decreased cell proliferation nor excess cell death can account for body shortening of double mutants. Tracing movements of cell populations in vivo revealed that defects in several types of cell movements contribute to posterior body shortening in double mutants including: extension, convergence and subduction. Hence, the non-canonical Wnt signaling cooperates with Notail to regulate morphogenetic movements that shape trunk and tail in vertebrate embryos. Our work initiates the genetic dissection of posterior body morphogenesis and links genes to specific tail-forming movements. Moreover, we provide genetic evidence for the notion that posterior body development entails a continuation of mechanisms that operate during gastrulation together with mechanisms unique to posterior body.

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Control of cortical reorganization during planar polarization in *Drosophila*

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A critical step in the organization of epithelial tissues is planar polarization, that is, the development of intracellular polarity along an axis within the plane of the epithelium. The coordinate beating of cilia in the oviduct and the alignment of steriocilia bundles in sensory hair cells are two striking examples of planar polarization. The alignment of hairs and bristles in the insect cuticle is one of the best-studied examples of the process. How is this polarity generated and coordinated with the overall shape of the tissue? Over the last twenty years, genetic analysis as identified a class of 6 "tissue polarity" mutations that disrupt the planar polarity of the cuticle secreting epithelial cells and produce disordered arrays of hairs and bristles [Gubb, 1993 #28]. In the last three years, analysis of the proteins encoded by these genes has shown that they organize proximal-distal cortical polarity [Adler, 2001 #359]. The distribution of these proteins is initially uniform around the junctional region of each cell. Approximately 10 hours before hairs form, they become asymmetrically distributed and segregate into proximal and distal domains with different compositions. The process is highly cooperative and polarization fails if any one of the proteins is missing. The proximal-distal cortical domains have the striking ability to propagate their polarity from cell to cell [Usui, 1999 #318; Feiguin, 2001 #403; Tree, 2002 #404]. Our lab is interested in the cell biological mechanisms underlying this fascinating process. Does it involve polarized delivery of membrane proteins? Polarized endocytosis or recycling? Polarized cytoskeletal linkage?

Since these mechanisms are basic to all cells, it is likely that mutating the genes that control them would cause early lethality or even cell lethality. To find such genes, we performed an EP overexpression screen. One interesting player we identified in this screen was *widerborst* [Hannus, 2002 #390]. Widerborst activity is absolutely required for cortical polarization; in its absence, the cortical domain proteins accumulate uniformly around the cell at a high level. Interestingly, Widerborst does not localize to the cortex itself. Instead, it is found on the distal side of a planar microtubule web that lies at the level of apical junctions. Although these microtubules do not appear to have any structural polarity, the localization of Widerborst suggests that they might be functionally polarized. Widerborst is a B' regulatory subunit of Protein Phosphatase 2A and presumably acts by targeting the catalytic subunit of the enzyme to a particular substrate on the distal microtubule web. We are performing a two-hybrid screen to find the target(s) dephophorylated by Widerborst.

What role might the planar microtubule web play in cortical polarization? Microtubules often act as tracks for the movement of intracellular membrane compartments. With this in mind, we are investigating the role of secretion, endocytosis and recycling in polarizing the distribution of cortical proteins. Our data indicate that endocytosis is essential for this process.

Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/EphrinB

Eduard Batlle & Hans Clevers

Cell renewal, lineage commitment and cell differentiation in the mammalian intestinal epithelium occur throughout postnatal life. These processes are intimately coupled to cell migration in a spatially organized manner. In the small intestine, the progeny of stem cells migrate in precise patterns. Absorptive, enteroendocrine, and goblet cells migrate toward the villus while Paneth cells occupy the bottom of the crypts. We have found that in the intestinal epithelium beta-catenin and TCF couple proliferation and differentiation to the sorting of cell populations by inversely controlling the expression of the EphB2/EphB3 receptors and their ligand ephrin-B1 along the crypt-villus axis. In EphB2/EphB3 null mice, the proliferative and differentiated populations intermingle. In adult EphB3-/- mice, Paneth cells do not follow their downward migratory path, but scatter along crypt and villus. We provide evidence that the proliferation/differentiation switch and the sorting of cell populations represent two independent outputs on the Wnt signaling pathway in the intestinal epithelium. Moreover, we show that during the first stages of intestinal tumorigenesis, Eph and ephrin expressing cells are further compartmentalized suggesting an additional outcome of the genetic program driven by beta-catenin/TCF in colorectal cancer.

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Genetic and physiological control of branching morphogenesis

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Many organs including the mammalian lung, vascular system, and kidney consist of branching networks of tubes. The branching pattern and the size and shape of the branches are critical for their transport functions, but the mechanisms controlling these properties are not well understood (1). Genetic and genomic approaches are being used to elucidate the developmental and physiological programs that govern branching morphogenesis of the Drosophila tracheal (respiratory) system, a ramifying network of ~10,000 epithelial tubes that delivers oxygen to the tissues (2). These studies have begun to reveal the cellular and molecular processes that induce sprouting and guide outgrowth of tracheal branches and dictate tube size and shape.

An FGF ligand guides the migrations of the tracheal epithelium as it grows out and assembles into primary branches (3). Cells at the ends of the primary branches are induced to express secondary and terminal genes, and go on to form secondary and terminal branches. Terminal branch outgrowth is controlled by the same FGF ligand, but this later expression of the ligand is not hard-wired but instead regulated by oxygen (4). We have identified the oxygen response pathway that is activated in oxygen-starved cells and induces expression of the FGF ligand to attract new terminal branches. This pathway also stimulates a remarkable cellular response called "hypoxipodia" formation which rearranges existing branches to improve cellular oxygen supply (5).

We have also begun to identify and characterize genes required in tracheal cells to promote outgrowth of new terminal branches in response to the FGF ligand. One of these genes encodes an oxygen-regulated transcription factor which is broadly expressed and translocates to the nucleus in response to low oxygen. Another is a downstream target gene induced by FGF signaling. It encodes a novel Ig domain protein that promotes terminal branch outgrowth and at high levels causes tortuousity of the branches. We will also present a progress report on a saturation genetic screen that has identified over a hundred new terminal branching mutants. These provide an overview of the genetic steps required to extend a terminal branch and create a tubular structure.

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How does the embryo coordinate cell movements and cell fate? Integrating different functions of FGF signalling

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During gastrulation the early embryo establishes three initial cell layers (ectoderm, mesoderm and endoderm) by coordinating multiple signalling events with complex cell movements. Shortly afterwards, the ectoderm is subdivided into neural and non-neural (mainly epidermis) sub-regions. A large body of work over the last decade has revealed that these complex patterning processes appear to be controlled by just a handful of signalling factors, and that the molecular pathways are conserved throughout the Animal kingdom. Among the most important signals are the Fibroblast Growth Factors (FGFs), which are required for mesoderm formation, as chemorepellents for the exit of mesoderm cells from the primitive streak, for neural induction and for caudalisation of the neural plate. These processes occur in the ectoderm, very close to each other in time and space – how do the receiving cells decide on the appropriate response?

Previous studies have revealed that FGF signalling is required, but not sufficient for neural induction (Streit et al., 2000; Streit et al., 1998; Wilson and Edlund, 2001). FGF8 induces the expression of the early pre-neural genes *Sox3* and *ERNI* within 2 hours, but unless cells are exposed to other (still unknown) signals, this expression is lost and cells revert to an epidermal fate. We have also found that 5 hours' exposure to either organiser (Hensen's node) signals or to FGF8 are required to sensitise cells to BMP antagonists, which then stabilise the expression of *Sox3*. It therefore became important to define the differences between cells that have or have not been exposed to an organiser for 5 hours.

To answer this, we conducted a differential screen to identify genes whose expression is regulated after 5 hours' exposure to the organiser. We will report the isolation and functions of one of these genes, which we have named *Churchill* (*ChCh*). It encodes a novel C4-type zinc finger that acts as a transcriptional activator, yet it represses the induction of mesodermal markers (*Brachyury* and *Tbx6L*) by FGF, suggesting that at least one of its targets is a transcriptional repressor. We identify one such target, *Smad-interacting-protein-1* (*Sip1*) and show that *ChCh* is required for normal expression of *Sip1* as well as for neural plate development. ChCh also sensitises cells to neural inducing signals from the organiser and regulates the cell movements of mesoderm formation. Together with the expression patterns of these components, these results suggest that ChCh functions as an important switch between gastrulation (mesoderm/endoderm formation) and neurulation. It also appears to act

as a gate separating two different functions of FGF signalling: in mesoderm formation and in neural induction. Finally, it provides a simple explanation for why cells exposed to FGF for 5 hours become sensitive to BMP signalling.

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Session 3: Directing cell migration
Chair: Ruth Lehmann

The eversion of *Drosophila* imaginal discs requires a JNK-regulated change in the adhesive and motile properties of peripodial cells

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The imaginal discs of *Drosophila* are epithelial sac-like invaginations that will develop the external exoskeleton of the thorax, head and genitalia of the adult fly. During the larval period, the imaginal discs hang from stalks linked to the larval epidermis into the body cavity. Later on, during the first hours of metamorphosis, they evert and fuse. Imaginal discs consist of two kinds of epithelial cells: cubic pseudostratified cells, forming the imaginal disc proper, and squamous or rather squamous peripodial cells, which constitute their stalk and peripodial membrane. Despite the fact that the process of eversion of imaginal discs represents one of the major morphogenetic events during metamorphosis, it has only been roughly described. By serial sectioning and in vivo time-lapse analysis, we have found that at early stages of eversion, the peripodial epithelium of all discs adhere to the external larval layer. At this point, peripodial and larval cells undergo a pseudo epithelial-mesenchimal transition, detaching from each other and, as a consequence, leading to the breaking of epithelium integrity in several points, which soon coalesce into a single hole. This gap widens through continuous loss of adhesion between peripodial cells, resulting in intercalation of new cells at the edge of the initial hole. Finally, the whole peripodial epithelium has rearrenged into a stripe of cells that proximally surrounds the already everted disc. This stripe of peripodial cells conforms the leading front of the discs in their movement over intervening larval cells to achieve the discs closure. Once the sealing is complete, these cells differentiate as normal epidermal cells. We have found that the whole eversion process depends of proper levels of JNK activity in peripodial cells, since mutations in both *hemipterous* (JNK-kinase) and *puckered* (JNK-phosphatase) affect across-layers adhesion and cell motility.

Regulation of cell-cell adhesion in wound edge epithelium by protein kinase C alpha

David Garrod and Mohamed Berika

Epidermal keratinocytes are bound tightly together by intercellular junctions, the desmosomes. Wounding promotes migration of cells at the wound edge to re-epithelialise the wound. To do this they partially down-regulate desmosomes so that they can move more easily. We present new data showing that desmosomal adhesion is modulated in epidermal wounds by a mechanism involving protein kinase C alpha signalling.

Recently we reported that the desmosomes in cultured confluent epithelial cell sheets are resistant to disruption by extracellular calcium chelation: they are calcium independent. When such a cell sheet is wounded, desmosomes of wound edge cells become calcium dependent within 1 hour. This effect is propagated to cells deep within the cell sheet. Treatment of the cells with protein kinase C (PKC) activators switches desmosomes rapidly from calcium independence to calcium dependence, and PKC inhibitors do the reverse. Wounding causes translocation of PKC alpha to the cell periphery were it is localised to desmosomes, and antisense depletion of PKC alpha from calcium dependent cells promotes calcium dependence.

We now show that entirely similar changes occur in desmosomes at the wound edge in mouse epidermis. PKC alpha becomes localised to the desmosomes, seemingly penetrating the dense plaques. Desmosomes become calcium dependent and lose their characteristic midline. The effect is propagated to at least 50 cells from the edge. We propose that PKC activation primes desmosomes for internalisation by cells, and that this mechanism down-regulates adhesion. These results have important implications for epithelial cells movement in embryonic development and cancer metastasis.

Early germline development in Drosophila

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In many organisms primordial germ cells (PGCs) form in a specialized germ plasm. In Drosophila PGCs form by budding at nuclear cycle 10 while the somatic cells form by polarized cellularization at nuclear cycle 14 in the early embryo (1). We have identified a membrane-associated protein, Slow as molasses as well as other maternal effect mutations whose function is only required for polarized cell growth but not for germ cell formation (2). This suggests that somatic and germ cells already differ by their modes of cell formation. The first molecular manifestation of germ cell specification is the lack of transcriptional activity in PGCs (3, 4). We have identified transcripts expressed in PGCs and have used these to study transcriptional repression during PGC development. Our analysis suggests that relief of transcriptional repression in PGC is mediated by a series of steps. A non-protein coding RNA is needed for the initial repression of transcription by possibly interfering with the activity of RNA polymerase II. Subsequently, members of the Brahma chromatin-remodeling complex also affect early germ line transcriptional quiescence, while the translational repressor protein Nanos plays a role during later stages.

While the PGCs originate at the posterior pole of the embryo, the cells, which will contribute the somatic portion of the gonad, derive from the mesoderm. To reach the gonadal mesoderm PGCs navigate through and along different embryonic tissues. Here, these two cell populations coalesce to form the embryonic gonad, which during the larval and pupal stages differentiates into the ovary and testis. At stage 10 of embryogenesis, germ cells move through the posterior midgut, they then move on the midgut towards the extending germ band. The germ cells then transfer from the gut to the mesoderm. In the mesoderm PGCs associate with three clusters of gonadal mesoderm cells on each side of the embryos. Arrangement of germ cells into two lateral lines and coalescence into the embryonic gonads, is regulated by the reorganization and presumably changing properties of the gonadal mesoderm (5).

In a series of genetic screens we have shown that attractive and repellant signals guide germ cells (6). The repellant signal depends on the activity of the enzyme phosphatidic acid phosphohydrolase, encoded by two redundantly acting genes *wunen* (*wun*) and *wunen-2* (*wun-2*) (7-9). An independently acting, attractive signal is produced by the gonadal mesoderm. We showed that *hmgcr*, the gene encoding the Drosophila homolog of 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMG-CoAR), is required for PGCs to leave the midgut and to associate with the gonadal mesoderm precursors (10). Within the germ cells,

G-protein coupled receptor signaling medicates directional response to migratory cues. Final coalescence of the gonad requires the activity of the transmembrane protein FOI and E-cadherin (11).

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Cell migration and morphogenesis of the Drosophila tracheal system

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Cell migration is a widespread phenomenon in many biological processes, both in development and in the adult organism. In particular, cells move in an ordered pattern, following defined paths of migration, probably by recognition of distinct cues and substrates. Thus, the establishment of specific interactions between cells and their substrates is a crucial step in migration, a process ultimately determined by molecules expressed at their surface.

The tracheal system of Drosophila is an especially appropriate model for the study of the genetic control of morphogenesis and, in particular, for the study if the mechanisms that guide cells to migrate in specific directions. The larval tracheal system of Drosophila is a complex tubular network that conducts oxygen from the exterior to the internal tissues. It arises from the tracheal placodes, clusters of ectodermal cells that appear at each side of ten embryonic segments. The cells of each cluster invaginate and migrate in different and stereotyped directions to form each of the primary tracheal branches (for review, see Hogan and Yingling 1998; Metzger and Krasnow 1999). The general conclusion from many studies is that the direction of migration of the tracheal cells relies on a set of positional cues provided by nearby cells. On the one hand, branchless (bnl), a gene encoding an FGF homologue, is expressed around the developing tracheal system in clusters of cells at each position in which a new branch will form and grow. Activation of the Breathless (Btl) receptor in the tracheal cells by Bnl is thought to stimulate and guide tracheal migration toward these positions (Sutherland et al. 1996). On the other hand, Dpp, EGF, and Wnt signalling have a role in the choice between the alternative directions of migration (Llimargas and Casanova 1997; Vincent et al. 1997; Wappner et al. 1997; Chihara and Hayashi 2000; Llimargas 2000). However, it is not known how the signals from Dpp, EGF, and Wnt specify a particular migratory path or what cell surface proteins are used by the tracheal cells to interact with their specific substrates.

To understand the mechanisms that are responsible for the distinct migratory pathways of the cells of the different branches we have first analysed how the tracheal cells migrate in the context of the whole organism. We have found that the alternative migratory pathways of the tracheal cells are associated with distinct subsets of mesodermal cells (Franch-Marro and Casanova 2000). We have then addressed which are the cell surface proteins involved in the recognition of these migratory pathway. In particular, we have found that migration of the cells of the visceral branches, the ramifications of the tracheal tree that transport oxygen to the gut, is mediated by the restricted expression of the PS1 integrin in theirs cells, which is matched by the complementary expression of the PS2 in the cells of their

migratory substrate. Moreover, we have found that the signalling pathways that mediate the choice between alternative paths of migration regulate the appropriate expression of the alfa PS1 subunit in the subset of the tracheal cells of the visceral branches (Boube et al., 2001). These results support a model in which signalling by transduction pathways specifies the particular migratory pathways of tracheal cells by regulating a precise array of adhesion proteins such as integrins at their surface. We think that this can be a very general mechanism to regulate integrin expression in distinct subpopulations of cells within a wider field and to confer them different substrate recognition properties. At present, we are focusing our study on the identification of new genes, specially coding for additional cell surface proteins involved in tracheal cell migration and morphogenesis of the tracheal system.

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Genetic control of cell migration in Drosophila

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Border cell migration in the Drosophila ovary has emerged as a useful genetic model for studying the conversion of stationary epithelial cells to invasive migratory cells in vivo. Three signaling pathways have been defined, which control different aspects of the migration (reviewed in Montell, 2003). A steroid hormone signal regulates when the cells become migratory and may regulate adhesion dynamics. Signaling through the JAK/STAT pathway defines which epithelial cells become migratory. Finally a growth factor signal contributes to guiding the cells to their destination. Each of these types of signaling pathways is known to be upregulated in a variety of human cancers, suggesting that the same signaling pathways that are known for promoting cell proliferation and survival may also contribute to metastasis by regulating cell motility and invasiveness. To test this hypothesis we have studied the effects of inhibiting JAK/STAT signaling on the motility of human ovarian cancer cell lines.

The small GTPase Rac is also required for border cells to migrate, and we have conducted a genetic screen in vivo for genes that, when over-expressed, suppress the migration defects due to dominant-negative Rac. Over-expression of wild-type Rac, actin, profilin, or a Rac exchange factor suppresses the migration defect. In addition we identified a new mediator of Rac-dependent actin polymerization in this screen.

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Session 4: Cell movements and the developing nervous system
Chair: M. Angela Nieto

Molecular analysis of neural crest formation

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Neural crest cells arise within the ectoderm during neurulation and give rise to most of the peripheral nervous system. Following neural tube closure, they come to lie within the dorsal neural tube from which they emerge and subsequently migrate extensively to numerous and characteristic sites. There, they differentiate into neurons and glia of the peripheral nervous system, cartilage and bone of the face, melanocytes and various other cell types. Fate mapping experiments have demonstrated that the neural crest arises at the juncture between presumptive epidermis and neural plate. However, injection of lineage tracer into individual cells reveals that single neural fold cells are not committed to a neural crest fate; rather these cells can form all ectodermal derivatives (epidermis, neural tube, neural crest).

Inductive interactions between the neural and non-neural ectoderm can generate neural crest cells, suggesting that signals travel through the epidermis to generate neural crest cells prior to neural tube closure. Induction of the neural crest appears to be a multiphasic process and involves a combination of an early Wnt signal, likely mediated by Wnt6, together with later functions for BMP signaling pathways. We show that Wnt is both necessary and sufficient for this stage of neural crest induction. Recent experiments have focused on the initiation of neural crest induction in the gastrulating embryo. Our results suggest that cells in the gastrula are already conditionally specified to form neural crest cells. Finally, we have been taking a genomics approach to identify the array of molecules expressed as a result of neural crest induction and will discuss the results of these screens.

Local tissue interactions across the dorsal midline underlie morphogenesis of asymmetric forebrain nuclei

Miguel L. Concha, Claire Russell, Jennifer C. Regan, Marcel Tawk, Jon Clarke and Stephen W. Wilson

Animals show behavioural, cognitive and neuroanatomical asymmetries but the mechanisms that establish these asymmetries are not well understood. The dorsal diencephalon (epithalamus) is one of the most evolutionarily conserved sites of asymmetry in the vertebrate brain (Concha and Wilson, 2001). In this region, the habenular nuclei are usually asymmetric and the photoreceptive parapineal, when present, is frequently positioned or projects axons asymmetrically. Our previous studies in collaboration with Alex Schier's group showed that the Nodal signalling pathway is necessary for specifying laterality in the epithalamus, but is not essential for the development of asymmetry per se (Concha et al. 2000). More recently we have been analysing the morphogenetic events and tissue interactions that regulate the development of asymmetric nuclei in the epithalamic region of the dorsal forebrain. We show that the unilateral parapineal organ has a bilateral origin and that some parapineal precursors migrate across the midline to form this left-sided nucleus. The parapineal subsequently innervates the left habenular nucleus, which itself derives from ventral epithalamic cells directly adjacent to the parapineal precursors. We show that ablation of cells in the left ventral epithalamus of wild type embryos can lead to reversal of epithalamic laterality and that such ablations can impose the direction of CNS asymmetry in embryos in which laterality is normally randomised. These data lead us to propose that laterality is determined by a competitive interaction between the left and the right epithalamus. We are currently testing the notion that the role of Nodal signalling is to bias the outcome of this competition.

Our collaborators on this and related projects include Becky Burdine, Samuel Sidi, Darren Gilmour, Enrique Amaya, David Kimelman, Lauro Sumoy, Teresa Nicolson, Stefan Gründer, Miranda Gomperts and Alex Schier.

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Axon migration at the midline of the *Drosophila* central nervous system

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Neurons within the central nervous system (CNS) extend axons that migrate towards, near to or away from the midline. The specialised cells that lie at the midline produce a range of signals with a key role in regulating the guidance decisions of these neurons. Those axons that do migrate towards the midline also switch their behaviour so that they do not remain at the midline but extend across to the contralateral side. These axons rarely recross but ignore previously attractive midline cues and extend within a longitudinal tract or exit the CNS. The changes in axonal behaviour are produced in part by the modification of cell surface receptor protein activity. The full extent of the mechanisms used and how they switch the response of axons is not yet fully understood. It is clear that there is tight spatial and temporal regulation of the surface expression of guidance receptors as well as the activation of novel molecular interactions that modify axonal responses to midline signals as the axons cross the midline cells. Commissureless (Comm) is one of the few molecules known to have an important role to regulate the levels of a receptor protein necessary for axon guidance at the midline. Comm is able to down-regulate levels of the Roundabout (Robo) protein that acts as a receptor for the midline derived axon outgrowth inhibitor Slit. Down-regulation of Robo is necessary for axons to cross the midline.

Comm is a novel transmembrane molecule that is expressed on midline cells and is seen to accumulate on commissural axons. Comm protein is required in the embryo for normal axon guidance. We have discovered that Comm is required and expressed both in commissural neurons and in midline cells. Comm localisation within these cells is dynamic where it is found within intracellular vesicles and at the cell surface. Comm can bind Robo and is able to sequester Robo within the intracellular vesicles within commissural neurons prior to crossing the midline. This allows the commissural axons to extend toward the midline cells that express the outgrowth inhibitory Robo ligand Slit. The intracellular portion of Comm is essential for its function since a truncated form of the protein lacking this region is inactive. Using a yeast two-hybrid screen we identified a *Drosophila* protein homologous to the known vertebrate protein Nedd4 which specifically binds the intracellular portion of Comm. Nedd4 is a modular protein containing three or four WW domains, a C2 Ca^{2+} /lipid binding domain and an E3 ubiquitin ligase domain. We have identified the amino acid sequence within Comm that binds to two of the Nedd4 WW domains. We have identified that Nedd4 catalyses the ubiquitination of Comm, is necessary for Comm localisation to the

vesicles and for Comm to sequester Robo away from the cell surface. We have further identified that an interaction between Nedd4 and Comm is also necessary for overexpressed Comm to downregulate Robo in the embryo.

The Comm protein expressed by the commissural axons accumulates at the midline and is thus prevented from acting on Robo once the axons have crossed the midline. Specific domains within the extracellular and transmembrane regions of Comm appear to be necessary for this targeting of the Comm protein. Comm can also bind itself through its extracellular domains and a possible trans-interaction between Comm at the midline and on the commissural axons may confine high Comm activity to the commissures.

Molecular mechanisms of cell migration in the telencephalon

Oscar Marín

Neurons are most frequently born at a distance from the place where they finally become integrated in a specific neuronal circuit, so that they have to migrate to reach their final destination. The process of cell migration requires young neurons to perfectly synchronize multiple actions, including the timing for initiation and cessation of the movement as well as the appropriate responses to multiple guidance cues encountered through their trajectory.

The telencephalon is undoubtedly one of the most intricate regions of the mammalian brain, and its extraordinary degree of organization reflects the complexity of the migratory movements required to generate it. Two general modes of migration are distinguished in the telencephalon: radial migration, which established the general cytoarchitectonical framework of the different telencephalic subdivisions, and tangential migration, which increases the cellular complexity of its circuits by allowing the dispersion of multiple neuronal types. This later type of migration appears to be governed by mechanisms similar to those that control the guidance of growing axons, i.e. contact guidance (permissive and non-permissive substrates for migration) and diffusible gradients (attractive and repulsive cues).

The movement of interneurons from the basal telencephalon to the embryonic cortex is one of the most prominent examples of tangential migration in the telencephalon. Despite being a highly directional process, the molecular mechanisms controlling this migration are still poorly understood. Here, I will review the cellular and molecular mechanisms underlying the migration of interneurons to the embryonic cortex and discuss how emerging concepts in neuronal migration are reshaping our understanding of brain development in normal and pathological situations.

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Hoxa2 overexpression in rhombomere 1 reveals rhombic lip autonomous patterning of tangential migration

Mark Eddison, Leah Toole, Esther Bell, Andrew Lumsden and Richard J. T. Wingate

The rhombic lip of rhombomere 1 gives rise to a unique population of tangentially migrating precursor cells, which condense as the external granule cell layer of the cerebellum. As the most rostral hindbrain segment, rhombomere 1 is defined by an absence of Hox gene expression. To assess the role of segmental identity in specifying rhombic lip development, we have used both infection by RCASBP(B)Hoxa2 virus and electroporation of an RCASBP(B)Hoxa2 construct to drive ectopic Hoxa2 expression in chick. Overexpression of Hoxa2 within r1 results in the downregulation of granule cell markers (Pax6, ErbB4), while the expression of Phox2a, which normally characterises the trochlear nucleus and locus coeruleus in ventral rhombomere 1, is upregulated throughout the cerebellum. To follow the fate of migratory cells, Hoxa2 misexpression was targeted to rhombic lip precursors by combining electroporation with the microsurgical construction of quail-chick chimaeras. This reveals that the loss of granule cell markers in cerebellum expressing ectopic Hoxa2 can be attributed to a respecification of the migration path of cerebellar rhombic lip derivatives to a single ventrolateral, extra-cerebellar target. A complementary grafting strategy confirms that specification of migration path is autonomous to the rhombic lip.

Establishment of cell polarity in the chick

Cristina Afonso, Domingos Henrique

We are particularly interested in understanding the mechanisms of polarized membrane growth during neural tube morphogenesis. While much emphasis has been given to the contribution of the actin cytoskeleton per se, the role of protein and membrane trafficking in remodeling the cell surface and establishing polarized structures within the neuroepithelial cell has received little attention.

We have isolated several chick homologues of the C. elegans par-3 and par-6 genes, which encode PDZ proteins, and studied their expression during neural development. Using antibodies against one of the PAR-3 proteins and one of the PAR-6 proteins, we have recently found that dividing neuroepithelial cells in the chick embryo assemble a basally-located molecular complex during mitosis, including the PAR3 and PAR6 proteins, the NUMB protein and some proteins known to be involved in vesicular trafficking.

Given the known role of these molecules, our current hypothesis is that the PAR3/PAR6 proteins nucleate the building of a macromolecular complex, which includes probably other proteins like the GTPases CDC42 and Rac. This complex would orchestrate various steps during creation of neuroepithelial polarity, like the reorganization of the cytoskeleton and directed trafficking of proteins and membranes that leads to the polarized growth of neuroepithelial cells, after exit of mitosis.

To test this hypothesis, we are following the localization of PAR3 and PAR6-GFP fusion proteins in vivo, using time-lapse recording in chick neural tube slices. We also use other markers to study the organization and dynamics of organelles, as well as the intracellular transport of integral membrane proteins in these live slices of chick neural tube. We also try to interfere with the normal function of the PAR3/PAR6/... basal complex, by overexpressing different forms of its constituent proteins in single neuroepithelial cells, using electroporation of embryonic neural tube. We are following subsequent events inside the cell, dynamically, by timelapse microscopy of cultured neural tube slices, and evaluate the effects on neuronal division, migration and differentiation.

Session 5: Cell movements, development and disease
Chair: Marianne Bronner-Fraser

The migratory phenotype of Snail-expressing cells

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The Snail family of zinc-finger transcription factors is involved in processes that imply profound cell movements both during embryonic development and tumour progression. We and others have previously shown that they are crucial for the formation of the mesoderm and the neural crest and for the acquisition of migratory and invasive properties in epithelial tumours through the triggering of the epithelial-mesenchymal transition (EMT) (reviewed in 1).

The function of Snail in the triggering of EMT is in part mediated by the direct repression of E-cadherin transcription (2,3). However, the EMT implies a dramatic phenotypic change concomitant not only with the loss of epithelial markers but also with the gain of mesenchymal markers and changes in cell shape. Since Snail is able to induce a full EMT in epithelial cells, it must have additional targets. Indeed, there are other direct targets already identified such as the epithelial Mucin-1 (4) or components of the tight junctions such as claudin and occludin (5). Through the analysis of Snail transfected cells and overexpression experiments in embryos we found that in addition to cell adhesion molecules, Snail is upstream of molecules involved in cytoeskeletal changes (6). We are currently further characterizing the phenotype of the Snail-expressing cells and have found that this transcription factor is also involved in cell cycle control and cell survival. I will discuss these results in the frame of the properties that both embryonic and tumour cells share when they become migratory and invasive.

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Parallels between morphogenesis and wound healing in embryos

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Embryos heal wounds very rapidly and efficiently and without leaving a scar. Studying how they do this can tell us much about the natural morphogenetic movements of embryogenesis as well as suggesting ways in which we might make adult tissues repair more efficiently. Using live confocal imaging of transgenic Drosophila embryos expressing gfp-actin in epithelial tissues we have revealed the key actin machineries that drive the paradigm morphogenetic process of dorsal closure which appears to bear striking analogy with re-epithelialisation of a vertebrate skin wound. Using embryos expressing mutant forms of the various small GTPases, we have tested the function of each of these actin-based elements - the actin cable and dynamic filopodia and lamellipodia – in both dorsal closure and the repair of laser-generated wound holes in the fly embryo. Our experiments in embryonic chicks and mice and in the neonatal PU.1 null mouse, which is genetically macrophageless, suggest that an inflammatory response is not essential for healing and may indeed be causal of fibrosis in post-embryonic animals. Consequently, we have used a microarray approach with this mouse in order to identify a portfolio of candidate inflammation/fibrosis genes. Finally, by taking advantage of the translucency of the zebrafish larval tail we have begun to make DIC movies of the inflammatory response and to dissect the genetics of this process by screening for mutants that fail to recruit leukocytes to the wound site and by morpholino knockdown of candidate "inflammation" genes. Our hope is that these basic cell and molecular studies in genetically tractable organisms will supply us with the clues we need to design the new repair and regeneration medicines of the future.

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Genes that control cell migration during mouse embryo

Carmen Birchmeier

During development, cell migration is an important process and it is frequently observed that cells are born at one position and subsequently move to their final locations. In development, migration events are tightly controlled. Cells are released at defined stages and positions, and they move along defined routes to their particular target sites. Molecules that control such migration events have received much attention. Of particular interest is the molecular nature of signals responsible for the release of the cells, for the maintenance of cellular motility, or for directed migration and target finding. I will summarize here data from my laboratory that contributed to an understanding of the molecular control of cell migration in the embryo. Over the years, we analysed genes that control migration of two developing cell types, neural crest cells and muscle progenitor cells. Both cell types are released form epithelial structures, and in order to detach and become motile they first undergo an epithelial-mesenchymal conversion. Mechanistically, the release of the cells and their subsequent motility resembles the process that is observed late during the progression of malignant carcinomas. There, cells detach from the primary tumor by epithelial-mesenchymal conversion and migrate in an uncontrolled manner to form metastases at sites distant of the primary tumor. Because of the mechanistic similarities, it is not so astonishing that genes implicated in tumor progression play also important roles in cell migration events during development. In particular, work from my laboratory demonstrated that tyrosine kinase receptors implicated in tumorigenesis, like the c-ErbB or c-Met tyrosine kinase receptors, turn out to control decisive steps in migration of embryonic cells.

POSTERS

Hyaluronan Synthase 2 is required for dorsal migration of lateral mesodermal cells during zebrafish gastrulation

Jeroen Bakkers, Carina Kramer, Joris Pothof, Nicolette Quaedvlieg, Herman Spaink and Matthias Hammerschmidt

The large polysaccharide Hyaluronan (HA) and its synthesizing enzymes (Has) have been implicated in regulating the migratory potential of metastatic cancer cells. Here, we analyze the roles of zebrafish Has2 in normal development. Antisense morpholino oligonucleotide (MO)-mediated knockdown of zebrafish Has2 leads to severe migratory defects during gastrulation, somite morphogenesis, and germ cell development. During gastrulation, ventrolateral cells of *has2* morphant embryos fail to develop lamellipodia and to migrate dorsally, resulting in a blockage of dorsal convergence, while extension of the dorsal axis is normal. The effect is cell autonomous, suggesting that HA acts as an autocrine or local signal. Upon ectopic expression in axial cells, *has2* causes the formation of supernumerary lamellipodia and a blockage of mediolateral cell-cell intercalations and axis extension. Epistasis analyses indicate that these effects of Has2 are mediated by activating the Rho GTPase Rac1. Together, the data suggest that Has2 and Rac1 are necessary and sufficient components of a linear pathway inducing the migration of lateral cells, while blocking extension movements. This provides first genetic evidence that convergence and extension are separate morphogenetic movements of gastrulation.

Analysis of the genetic program of epithelial cell plasticity

Geert Berx, Joke Comijn, Bram De Craene, Kristin Strumane, Frans Van Roy

Epithelial-mesenchymal transitions (EMTs) are a manifestation of epithelial cell plasticity during morphogenesis, wound healing, and tumour progression. Loss of the cell cell adhesion molecule E-cadherin seems to be heavily involved in EMT appointing E-cadherin as one of the key caretakers of the epithelial phenotype. In cancer the amount of E-cadherin is decreased through mutations, chromosomal loss or active repression of the E-cadherin promoter. Different transcription factors like SIP1 (smad interacting protein 1), Snail and Slug repress E-cadherin transcription in vitro and in vivo by binding to E-box sequences of the E cadherin promoter. The transcription factor SIP1 belongs to the dEF-1/Zfh-1 family of two-handed zinc finger/homeodomain proteins, which can efficiently repress cell-cell adhesion and induce invasion. Conditional expression in an epithelial cell modelsystems of SIP1 modulates epithelial plasticity by repressing E-cadherin and inducing EMT. Transfer of E-cadherin in an E-cadherin negative model cellsystem showing high SIP1 expression resulted in restoration of cell-cell adhesion and epithelial differentiation. We performed a comparative transcriptome screen from these model cellsystems using cDNA arrays of 17.268 cDNAs to delineate signaling pathways and transcriptional events that determine epithelial cell plasticity controlled by E-cadherin, and SIP1. Analysis of the identified pathways and genes will advance the understanding of the molecular mechanisms that underlie tumor invasiveness and metastasis.

Requirement of Sfrp1 in eye anlage specification in medaka embryos

Pilar Esteve, Javier Lopez-Rios, Josana Rodriguez- Sanchez and Paola Bovolenta

It is currently believed that the levels of Wnt activity may specify posterior to anterior fates in the neural plate and that the most rostral fates will develop only in a Wnt-signalling free zone. In line with this idea Hourt et al. (2002, Neuron 35 ; 255-265) have demostrated that in zebrafish, telecephalic specification require the activity of a Wnt inhibitor of the Secreted Frizzled Related family (SFRP), known as tlc. We had previously isolated in chick (Esteve et al, 2001; Esteve et al, 2003) and medaka fish a different member of this family, Sfrp1. In both species, Sfrp1 is strongly expressed in the anterior neural plate including the eye anlage. To test whether this molecule could also contribute to the specification of anterior neural territories, we have attempted to interfere with the expression of this gene by injecting specific morpholinos into medaka fish (Oryzias latipes) embryos at the two cell stage. Morphological and molecular analysis, using anterior or retinal specific markers (Rx, Six3, Emx1, Pax6, BF1), indicates that morpholino treated-embryos have a severely reduced eye field. On the other hand, over-expression of Sfrp1 in medaka embryos leads to an expansion of the anterior neural fates, particularly of the eye anlage, as judged by molecular analysis. This "anteriorization" of the embryos is associated either to A-P axis truncation or axis duplication. Though over-expression experiment might be complicated by a general interference with Wnts activities, affecting both the canonical and the PCP pathway and we believe that altogether our result point to a specific role of Sfrp1 in eye anlage specification.

Association between the cell cycle and neural crest delamination through specific regulation of G1/S transition

Tal Burstyn-Cohen and Chaya Kalcheim

The neural crest (NC) is a group of transient progenitors which rapidly disperses in the embryo and differentiates into a rich variety of derivatives (Le Douarin and Kalcheim, 1999). Successful migration is essential for cells to reach their homing sites where differentiation occurs. To engage in migration, epithelial pre-migratory cells must convert into mesenchyme. Epithelio-mesenchymal transitions are, therefore, an essential prerequisit for the development of many embryonic tissues and organs. When converting into mesenchyme, they become loosely associated or fully individualize, acquire motile properties and invade initially the extracellular matrix surrounding the dorsolateral neural tube (Le Douarin and Kalcheim, 1999, Kalcheim, 2000, Nieto, 2001). The identity of signals triggering NC cell delamination remained elusive until recently. Sela-Donenfeld and Kalcheim (1999) have reported that a gradient of BMP4 activity created along the dorsal neural tube triggers emigration of NC progenitors.

Here we show that NC cells emigrate in the S-phase of the cell cycle. Moreover, we establish that this feature is part of the mechanism of cell delamination as treatment of explanted neural primordia with the G1-S transition inhibitors olomoucine, AG555 or mimosine prevented initial delamination of NC cells, that could be rescued upon removal of the inhibitors. In contrast, similar treatments with aphidicolin or VM-26, which inhibit the cycle at S and G2 phases, respectively, had no effect. To further examine the significance of G1-S transition on cell delamination in the living embryo, we electroporated hemi-neural tubes with several constructs leading to a cell cycle arrest at the G1 phase, resulting in an almost total prevention of NC delamination in the treated side.

Taken together, we demonstrate for the first time that the transition between G1 to S is necessary for the epithelial-to-mesenchymal conversion of premigratory NC. Molecular events occurring during the progression of G1-S transition may therefore be translated into downstream signals that generate cell movement and/or be required for the cells to respond to the environmental factors that trigger delamination.

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New insights into the initiation of gastrulation in the mouse embryo

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In the mouse embryo, gastrulation starts at 6.5 days of development with the appearance of the primitive streak at the posterior pole of the embryo. Fate mapping studies and gene expression analysis have hinted at the existence of a complex cellular choreography between the time of implantation and the initiation of gastrulation. However, current data offer only a fragmented view of these processes and we still lack a full understanding of their dynamics.

Using histology, expression studies, optical coherence tomography and cell-lineage, we documented the cellular and molecular events that precede the initiation of gastrulation at 6.5 dpc. Our marker studies show that the antero-posterior (AP) axis of the embryo at 6.0 dpc, shortly before gastrulation, is perpendicular to the direction which would have been inferred from earlier studies. However, through a mechanism which can involve asymmetrical regulation of gene expression and associated changes in the behaviour of a subset of epiblast cells, its orientation, given by the formation of the primitive streak (PS) at 6.5 dpc, can change rapidly and ends up being particular to each embryo. Our results therefore suggest additional steps in the establishment of the AP axis in the mouse embryo. We are currently investigating whether early positional clues in the preimplantation embryo have a predictive value regarding the position at which the primitive streak forms at later stages.

Dynamics of cell rearrangement in early zebrafish gastrulation

Miguel L. Concha, Benjamin Feldman, Daisy Faruque, Derek L. Stemple, Stephen W. Wilson, and Richard J. Adams

Gastrulation defines the process by which the three germ layers, ectoderm, mesoderm and endoderm are formed. We have been studying the cellular mechanisms that underlie this crucial event by using in vivo time-lapse imaging and cell movement analyses. In zebrafish, gastrulation involves extensive cell internalisation along the blastoderm margin to form the prospective mesendoderm, a process that is intimately co-ordinated with the vegetally directed epibolic movements of marginal cells, enveloping layer (EVL), and yolk syncytial layer (YSL). As gastrulation begins, marginal cells accumulate into a sheet that moves coherently vegetal-ward in close association with epiboly of the EVL and YSL. Strikingly, this movement of the blastoderm persists and a velocity gradient develops along the animal-vegetal axis of this tissue -- the fastest moving cells being those closest to the margin -- even when epiboly of the YSL itself pauses.

This strongly suggests that the generating force for internalisation resides at the margin. During this time the YSL changes shape and marginal blastomeres within two rows of the margin start to internalise. Although internalisation occurs concurrently in neighbouring marginal cells the patterns of cell rearrangement, motility and shape changes indicate that cells are moving autonomously giving the impression of active ingression. Hence, the process of cell internalisation in zebrafish represents a model in which individual cell ingressions are constrained to the narrow edge of a cell sheet. Examination of mutant and morphant embryos reveals a key role of Nodal signalling in regulating the dynamics of internalisation. Enhancement of Nodal signalling by depletion of lefty1/2 proteins induces an extended period of active internalisation that leads to an excessive accumulation of hypoblast cells, seen as an exaggerated thickening of the germ ring and shield. This phenotype contrasts with the complete failure in cell internalisation and absence of germ ring and shield in embryos lacking Nodal signalling.

Notch lateral activation promotes epithelial-mesenchymal transitions during heart development and neoplasic transformation

Luika A. Timmerman, Joaquin Grego, Esther Bertrán, José María Pérez-Pomares, Juan Diez, Sergi Aranda, Sergio Palomo, Angel Raya, Frank McCormick, Juan Carlos Izpisúa-Belmonte & José Luis de la Pompa

The Notch pathway regulates cell-fate choices in embryonic and adult tissues via mechanisms termed lateral inhibition and lateral activation, the latter being poorly understood in vertebrates. Disruption of Notch has severe developmental consequences and ectopic expression of its intracellular domain (Notch IC) is oncogenic in mammals. Here we show that Notch ligands and receptors are co-expressed at high levels in cells of the embryonic endocardium, including cells which overly the heart valve primordia (endocardial cushions). Ablation of the Notch1 receptor or the effector RBPJK curtails expression of Notch ligands, receptors and the target gene HRT1, demonstrating the existence of a positive feed-back loop consistent with lateral activation. Notch-expressing endocardial cells undergo a TGFb-mediated endothelial-mesenchymal transition (EMT), to cellularize developing cardiac valves. Loss of Notch activity attenuates expression of TGFb2 and its receptors, prevents local expression of the snail transcriptional repressor, and stabilizes expression of the endothelial cellular adhesion molecule VE-cadherin, resulting in concomitant loss of endocardial EMT. Conversely, transient ectopice expression of Notch1IC in zebrafish embryos results in abnormally enlarged and hypercellular cardiac cushions. Overexpression of Notch1IC in endothelial cells in vitro also induces severe attenuation of VE-cadherin expression and loss of contact inhibition. These transformed cells undergo an apparent EMT, as evidenced by independent cell migration on plastic, through collagen, and in xenografts. We conclude that Notch plays an unexpected, novel role in the promotion of EMT in both developmental and tumor formation settings, in part via regulation of the cellular adhesion system.

Guidance of border cell migration by the Drosophila EGFR and PDGF/VEGF receptor orthologs

Peter Duchek and Pernille Rorth

Border cells are a group of about 8 specialized follicle cells that perform a stereotypic migration during Drosophila oogenesis. This developmentally controlled cell migration serves as an in vivo model system that can be genetically modified, allowing both gain-of-function as well as loss-of-function analyses. It was not known what guides border cell migration, but the graded expression of guidance cues might serve this purpose. We reasoned that uniform overexpression of such guidance factors should destroy the gradient and lead to inefficient migration. In a genetic screen we overexpressed random endogenous genes in the tissue through which the border cells migrate. We found that uniform activation of the EGFR or the newly identified PDGF/VEGF receptor ortholog PVR inhibits border cell migration, as is expected for guidance receptors. The EGFR ligand Gurken as well as the PDGF/VEGF-like protein PVF1 are expressed in the oocyte, which is the target for border cell migration. Although border cells mutant for either EGFR or PVR are still able to migrate properly in the majority of cases, co-expression of dominant negative forms of both EGFR and PVR completely inhibits border cell migration, indicating a requirement in vivo and a redundancy of both RTKs for the guidance function. Surprisingly, we found that this guidance function is independent of the PI3K, PLCgamma, and ERK pathways.

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Regulation of patterning and morphology in the zebrafish embryonic forebrain

Luca Caneparo, Isabelle Foucher, Karima Kissa and Corinne Houart

In the past decade, tremendous progress has been made in understanding the molecular and cellular events leading to the establishment of the body plan in vertebrate. The most amazing finding was the great conservation in the genetic pathways used for any given process. The making of a limb, an eye or a brain is depending upon very similar cascades of molecular events from fish to human. However, the morphology of each of those structures differs drastically amongst vertebrates. We chose the zebrafish (danio rerio) as a model organism to study the early events defining the, size and shape of the forebrain compartments (telencephalon, eye field and diencephalons). Our study let us to identify signalling events, inside the neural plate, responsible for the early regionalisation of the forebrain territory (Houart et al, 1998; Houart et al. 2002). In the course of our study, we were surprised to observe that modifications in patterning events have often a very weak impact on morphogenesis. Reciprocally, changes in morphology can be found without altering the patterning of the brain. We will present a set of data showing striking cases were morphology and patterning seem to be controlled independently and will discuss the possibility of such an uncoupling as a general mechanism for evolution of shapes.

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Modeling neurulation: computer simulations of genetic networks, cell interactions, mechanics and motion

Michel Kerszberg

I introduce ctrl-Dev, an experimental computer system adapted to the description and simulation of genetical, molecular and biomechanical processes in cells and groups of cells, i.e. tissues. The system is one of the first to integrate within a single framework cell motility and genetic interactions. I shall present a study using ctrl-Dev as applied to the early events in neurogenesis (neurulation). The genetic networks responsible for neurulation in Insects and Vertebrates (Xenopus, and chick to a lesser extent) exhibit remarkable homologies. Yet the morphogenesis is very different: the Insects have a (ventral) neural cord consisting of discrete ganglia arising from delaminating neuroblasts; Vertebrates have a (dorsal) neural tube. Here I use ctrl-Dev to test the workability of an hypothesis which might account for these diverse morphologies (though not for the dorso/ventral inversion itself). The genetic networks included in the simulations are (using the Vertebrate "language"): the neural induction system (messages such as chordin and Sonic hedgehog secreted by the notochord, and BMP protein); "memory" networks for differentiation of neural plate (e.g. Nkx2, Pax3) and of neurons [AS-C, E(spl)]; and a cytoskeletal network responsible for apical/basal differentiation and cell motions. I demonstrate how a simple switch in upstream control of this last network can explain alternatively the formation of a neural tube or of ganglia, thus proposing a testable mechanism for their evolutionary divergence. A film of tube formation will be shown.The program is now being applied to Insect segmentation and to a simulation of cell movements during gastrulation and primitive streak formation.

Defining the molecular determinants of epimorphin/syntaxin-2-mediated mammary epithelial morphogenesis

D. C. Radisky, S. Bennett, Y. Hirai, and M. J. Bissell

The fully differentiated mammary gland is highly optimized for secretion of milk into the lumen of a highly branched ductal epithelial structure, and tubulogenesis is an essential component of the development and ramification of the mammary ductal system. We have identified a particular role in this process for epimorphin/syntaxin-2 (EPM), a mesenchymal protein that directs tubular morphogenesis in mammary gland development. EPM is a transmembrane protein localized to the cell surface that has been shown to exist in both intracellular and extracellular topological orientations. When presented to the extracellular surface of mammary epithelial cells, EPM acts as a morphogen and produces distinct morphogenic outcomes depending upon the mode of presentation. When presented in a polar, basal fashion, EPM produces branching morphogenesis, whereas presented in an apolar fashion, EPM leads to luminal cyst morphogenesis. We have used a three-dimensional (3D) collagen gels to define EPM function, and have found that the activity of EPM is contained within an N-terminal three-helix bundle. We have used the well-studied and highly-homologous syntaxin-1A (which shows no activity as a mammary morphogen) as a basis for homology modeling of EPM, to predict the active site of EPM action. We performed site-specific mutagenesis of syntaxin-1A to alter the six residues that are different in EPM at this predicted active site, and we found that this hybrid product was functional; in doing so, we created an active morphogen from an inactive template. This information can be used to more completely define the role of EPM in mammary morphogenesis.

Prickle1 regulates cell movements during gastrulation and neuronal migration in zebrafish

Filipa Carreira-Barbosa, Miguel L. Concha, Masaki Takeuchi, Naoto Ueno, Stephen W. Wilson and Masazumi Tada

During vertebrate gastrulation, mesodermal and ectodermal cells undergo convergent extension, a process characterised by prominent cellular rearrangements in which polarised cells intercalate along the medio-lateral axis leading to elongation of the antero-posterior axis. Recently, it has become evident that a non-canonical Wnt/Frizzled (Fz)/Dishevelled (Dsh) signalling pathway, related to the planar cell polarity (PCP) pathway in flies, regulates convergent extension during vertebrate gastrulation. Here we isolate and functionally characterise a zebrafish homologue of *Drosophila prickle (pk)*, a gene implicated in the regulation of PCP. Zebrafish *pk1* is expressed maternally and in moving mesodermal precursors. Abrogation of Pk1 function by morpholino oligonucleotides leads to defective convergent extension movements, enhances the *silberblick (slb)/wnt11* and *pipetail/wnt5* phenotypes and suppresses the ability of Wnt11 to rescue the *slb* phenotype. Gain-of-function of Pk1 also inhibits convergent extension movements and enhances the *slb* phenotype, most likely due to the ability of Pk1 to block the Fz7-dependent membrane localisation of Dsh by down-regulating levels of Dsh protein. Furthermore, we show that *pk1* genetically interacts with *trilobite (tri)/strabismus* to mediate the caudally directed migration of cranial motor neurons as well as convergent extension. These results suggest that during zebrafish gastrulation, Pk1 acts in part through interaction with the non-canonical Wnt11/Wnt5 pathway to regulate convergent extension cell movements, but is unlikely to simply be a linear component of this pathway. In addition, Pk1 interacts with Tri to mediate posterior migration of branchiomotor neurons, probably independent of the non-canonical Wnt pathway.

Transcriptional regulation of Cerberus during embryonic development

Ana Teresa Tavares, Ana Cristina Borges, Jose Antonio Belo

In the post genome-sequencing era, one of the main challenges will be to identify the sequence information that specifies when and where a given gene is expressed, that is, its cis-regulatory program. These programs are essential in the control of developmental processes, and changes in their sequence and organisation are one of the major causes of animal evolution (Davidson, 2001). Cerberus-like genes code for cystine-knot secreted proteins and exhibit both common and specific expression patterns during Xenopus, chick and mouse embryonic development (Bouwmeester et al., 1996; Rodriguez-Esteban et al., 1999; Belo et al., 1997). In order to study the regulation of chick Cerberus, we have cloned and analysed portions of its cis-regulatory regions. In brief, 5'-genomic sequences were subcloned in EGFP expression vectors and introduced into early chick embryos by electroporation (in New culture). Deletion and site-directed mutagenesis analyses led to the identification of distinct candidate regulatory regions that direct expression in the anterior mesendoderm and in the left side mesoderm. Since the stability of the EGFP RNA and protein is higher than that of Cerberus RNA we were able to trace the fates of the Cerberus-expressing cells. Interestingly, our observations indicate that the anterior mesendodermal cells that express Cerberus constitute a population of precursors precursor cells common to the foregut endoderm, heart and anterior blood islands. Additionally, the ongoing cross-species analysis will help to understand the evolutionary divergence of Cerberus-like gene regulation. Preliminary observations of chick Cerberus-EGFP transgenic mice suggest that the upstream regulators of chick Cerberus expression are also present in the mouse anterior mesendoderm and left side mesoderm.

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TGFβ-dependent regulation of trophoblast invasion during placental implantation

Patrick Fafet, Carolina Segura-Morales, Jean Marie Blanchard, Thierry Maudelonde and Marie-Luce Vignais

The placenta is the first organ to form during mammalian embryogenesis. This organ is vital as it provides the interface between fetal and maternal tissues and enables nutrient exchange between the mother and the fetus. Within the placenta, the trophoblast lineage constitutes the most important cell type, with the syncytiotrophoblast and the extravillous cytotrophoblast playing important structural and functional roles for the exchanges between the maternal blood system and the fetus vasculature. Implantation of the placenta requires migration of the trophoblast cells within the maternal endometrium in an invasion process which is controlled both in time and space.

We are studying the molecular mechanisms of trophoblast invasion in humans and their regulation by the TGFβ signaling pathway. We have designed a culture system based on the coculture of human primary endometrial fibroblasts and of trophoblastic villi obtained from elective abortions. Using placenta from early pregancies (3 to 5 weeks), we can reconstitute the invasion process which occurs in utero with both active syncytiotrophoblast and extravillous cytotrophoblast. These cocultures are analyzed by phase contrast microscopy in time lapse experiments, under controlled CO2 and at 37°C, for periods of 24 to 48 hours. We observe that trophoblast cells invade the endometrial fibroblast layer and form lacuna.

This invasion process is reversed by TGFβ. We are currently investigating the role of metalloproteases in this invasion and its regulation using immunofluorescence and RT-PCR on cells isolated by laser microdissection. These studies are carried out in parallel in primary human dermal and endometrial fibroblasts.

Functional interaction of p120catenin with the Rho family of small GTPases during early Xenopus morphogenesis

Malgorzata Ciesiolka, Mieke Delvaeye, Frans Van Roy and Kris Vleminckx

We are studying early morphogenetic processes in the early Xenopus embryo.

We are especially interested in the functional regulation of cadherin mediated cell-cell adhesion during cellular migration and tissue rearrangements. A good candidate regulator of cadherin functionality is p120ctn, a protein that is situated at the interplay between the cadherin adhesion complex and the cytoskeleton.We investigated the expression of Xp120ctn during early embryogenesis. In order to understand the function of Xp120ctn in early development we either overexpressed Xp120ctn or mutants of E-cadherin that can not bind Xp120ctn. In both cases the head structures were affected as reflected by malformations of the eyes and the craniofacial skeleton. Interestingly, as has also been documented in vitro, we observed that overexpressed Xp120ctn modulates the activity of endogenous RhoA. Moreover, Xp120ctn overexpression was phenocopied by dominant-negative RhoA and the morphological defects obtained by Xp120ctn overexpression could be fully rescued by coinjection of wild type or constitutive active RhoA. In addition, coinjection of XE-cadherin with Xp120ctn could rescue the embryos and relieved the RhoA inhibition, possibly by sequestering the excess of cytosolic p120ctn. These results indicate that Xp120ctn is critically involved in regulating morphogenesis in the early Xenopus embryo both through action on the cytoskeleton and by regulating the activity of the cadherins.

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