What mechanisms drive cell migration and cell interactions in *Pleurodeles*?

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ABSTRACT **Embryogenesis implies a strict control of cell interactions and cell migration. The spatial and temporal regulation of morphogenetic movements occurring during gastrulation is directly dependent on the early cell interactions that take place in the blastula. The newt *Pleurodeles waltl* is a favorable model for the study of these early morphogenetic events. The combination of orthotopic grafting and fluorescent lineage tracers has led to precise early gastrula mesoderm fate maps. It is now clear that there are no sharp boundaries between germ layers at the onset of gastrulation but rather diffuse transition zones. The coordination of cell movements during gastrulation is closely related to the establishment of dorsoventral polarity. Ventralization by U.V. irradiation or dorsalization by lithium treatment modifies the capacity for autonomous migration on the fibronectin coated substratum of marginal zone cells accordingly. It is now firmly established that mesodermal cells need to adhere to a fibrillar extracellular matrix (ECM) to undergo migration during gastrulation. Extracellular fibrils contain laminin and fibronectin (FN). Interaction of cells with ECM involves receptors of the ß1 integrin family. A *Pleurodeles* homolog of the α_v integrin subunit has been recently identified. Protein α_v expression is restricted to the surface of mesodermal cells during gastrulation. Integrin-mediated interactions of cells with FN are essential for ECM assembly and mesodermal cell migration. Intracellular injection of antibodies to the cytoplasmic domain of ß1 into early cleavage embryos causes inhibition of FN fibril formation. Intrablastocoelic injections of several probes including antibodies to FN or integrin $\alpha_5ß_1$, competitive peptides to the major cell binding site of FN or the antiadhesive protein tenascin all block mesodermal cell migration. This results in a complete arrest of gastrulation indicating that mesodermal cell migration is a major driving force in urodele gastrulation. It is now possible to approach the role of fibroblast growth factor (FGF) during cell interactions taking place in urodele embryos. Four different FGF receptors (FGFR) have been cloned in *Pleurodeles*. Each of them has a unique mRNA expression pattern. FGFR-1, FGFR-3, and the variant of FGFR-2 containing the IIIb exon are maternally expressed and might be involved in mesodermal induction. During gastrulation, FGFR-3 and FGFR-4 have a restricted pattern of expression, whereas FGFR-1 mRNA is nearly uniformly distributed. Splicing variants FGFR-2IIIb and FGFR-2IIIc have exclusive expression patterns during neurulation. IIIb is expressed in epidermis and IIIc in neural tissue, suggesting a function in the differentiation of ectodermal derivatives.**

KEY WORDS: *Pleurodeles waltl, amphibian, embryogenesis, cell interactions, urodele cell migration*

Introduction

Cell interactions and cell migration are important for the ordered spatial and temporal pattern of cell differentiation that results in morphogenesis. During cleavage, mesoderm induction — which involves cell interactions between the presumptive endoderm and the adjacent ectoderm — leads to the formation of an equatorial zone, or marginal zone (MZ), of mesoderm (Nieuwkoop, 1969, 1977; Sudarwarti and Nieuwkoop, 1971). The most immediate consequence of mesoderm induction is the extensive morphogenetic movements that occur during gastrulation. These movements involve various region-specific cellular activities. Formation of bottle cells below the dorsal marginal zone (DMZ) leads to an invagination site: the blastopore. The blastopore first forms as a depression and rapidly grows into a small curved slit (Fig. 1). In the meantime, DMZ cells begin to migrate toward and then over the

Abbreviations used in this paper: DMZ, dorsal marginal zone; ECM, extracellular matrix; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; MZ, marginal zone.

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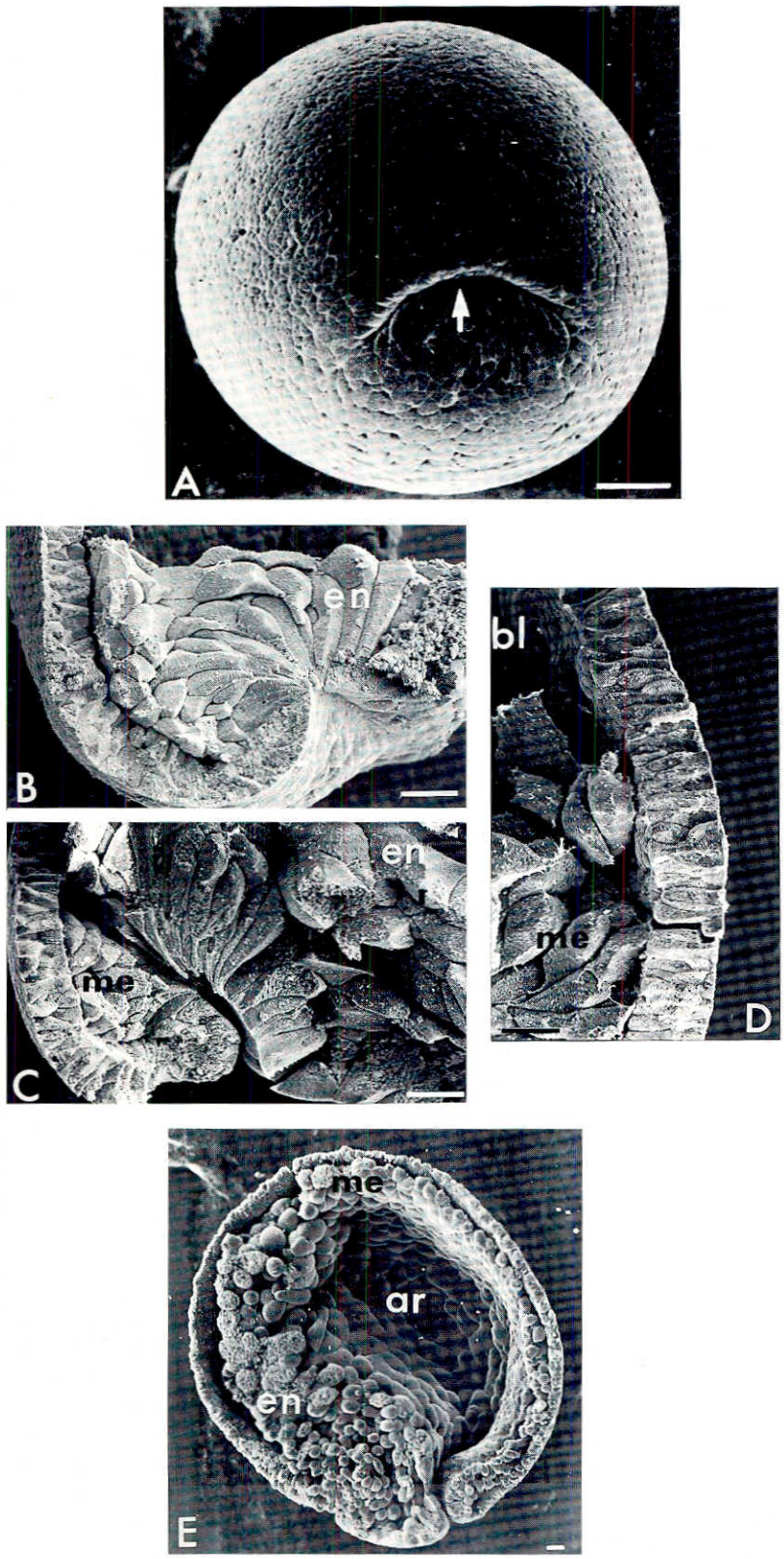

dorsal lip of the blastopore in a movement known as involution. Once over the blastoporal lip, DMZ cells representing head mesoderm, somitic mesoderm and the chordamesoderm, actively migrate across the blastocoel roof. Simultaneously, cells of the chordamesoderm become rearranged from a short broad group into a long, narrow, anteroposterior group. This movement has been called convergent extension (Keller, 1984). As DMZ cells involute, their vacated space on the surface of the embryo is occupied by spreading ectodermal cells. This movement of ectodermal cells is known as epiboly and is closely coordinated with convergent extension (Keller, 1985). By the end of gastrulation the ectoderm has completely surrounded the embryo. During gastrulation, a new cavity, called the archenteron, grows from the blastopore as the invagination deepens and expands toward the animal pole inside the embryo. By the end of gastrulation, the endoderm is inside the embryo and the blastocoel is nearly entirely obliterated. During this complex series of morphogenetic movements, the blastopore grows from a curved slit into a crescent, and then a circle. Once gastrulation has been completed the blastopore closes.

What is the fate of mesodermal cells in the *Pleurodeles waltl* gastrula?

Fate maps of the early gastrula in urodele embryos have been extensively studied by Vogt (1929) and Pasteels (1942) using the application of vital dyes to the surface of the embryo. Unfortunately, their methods were not refined enough to give detailed information concerning the mixing of cells and the relative contributions of deep and superficial cells in areas corresponding to the mesoderm in the early gastrula. Fate mapping in the anuran *Xenopus laevis* revealed that mesodermal cells are derived from deep blastomeres, with superficial ones forming exclusively ectoderm and endoderm (Keller, 1975, 1976; Løvtrup, 1975; Landström and Løvtrup, 1979). Smith and Malacinski confirmed Keller's earlier observations in the anuran *Xenopus laevis* and showed that mesodermal cells arise from deep cells in the DMZ. In contrast, in urodele gastrulae such as *Ambystoma mexicanum*, studies with cell surface labeling and lineage tracers have shown that superficial cells of the DMZ contribute to mesodermal derivatives (Smith and Malacinski, 1983).

Recently, Delarue *et al.* (1992) have obtained more detailed information about the fates of early gastrula mesodermal cells in the urodele *Pleurodeles waltl*. They used orthotopic grafts of small fragments of tissue where all cells were labeled with a fluorescent lineage tracer, and superficial cells were iodinated with Bolton-Hunter reagent (Katz *et al.*, 1982). Double-labeled explants were grafted in unlabeled host embryos.

Fig. 1. Scanning electron micrographs of *Pleurodeles waltl* embryos during gastrulation. (A) *External view. Early gastrula stage (stage 8b). Formation of the blastopore as a depression in the dorsal region of the embryo (arrow). Scale bar, 200 μm.* **(B-D)** *Sagittal sections during gastrulation. Formation of bottle cells below the dorsal marginal zone* **(B)**. *During gastrulation, mesodermal cells involute* **(C)** *and migrate under the ectodermal cell layer* **(D)**. *Bar, 15 μm.* **(E)** *Sagittal section at the end of gastrulation. The primary body plan of the embryo is established; a new cavity called the archenteron emerges. Bar, 50 μm. ar, archenteron; bl, blastocoel; en, endodermal cells; me, mesodermal cells.*

This method allowed Delarue *et al.* (1992) to construct a fate map. It indicates that the germ layer boundaries should not be drawn as sharp lines, but rather as diffuse transition zones with considerable overlap (Fig. 2). In the early gastrula five different mesodermal regions are arranged on a neat dorsal-to-ventral polarity axis starting with the most dorsal and ending with the most ventral and including: notochord, somites, pronephros, lateral plate mesoderm and blood islands. Mesodermal cells nearest the blastopore end up in the anterior portion at the tailbud stage. Conversely, mesodermal cells farthest from the blastopore end up in the posterior portion. As in *Xenopus laevis* gastrulation, where prospective anterior mesoderm involutes first followed by more posterior mesoderm (Keller, 1976), the anteroposterior regionalization of *Pleurodeles waltl* mesoderm may be related to the position of prospective mesodermal cells relative to the dorsal lip of the blastopore at the early gastrula stage.

The relative contributions made by deep and superficial cells to organs at the tailbud stage have been studied by the use of double-labeled explants. Observations showed that superficial cells make a significant contribution to many different mesodermal organs. Such a result is in agreement with the Smith and Malacinski (1983) demonstration that urodele gastrulae have presumptive mesodermal cells both in the deep and superficial portions of the marginal zone. Furthermore, there is a decreasing contribution to mesodermal derivatives from the superficial portions of presumptive mesodermal regions along the dorso-ventral axis. The most dorsal regions contribute the largest number of superficial cells to mesodermal derivatives. The superficial cell population in the dorsal marginal zone makes an important contribution to mesodermal structures, especially in the notochord. The simplest interpretation of these results is that there is extensive mixing of superficial and deep cells among cells that involute first but much less mixing among cells that involute later from the lateral and ventral blastopore lips. It is clear that there is a decreasing gradient of cell intermixing along the dorsal-to-ventral axis in the *Pleurodeles waltl* gastrula.

What features characterize initiation and temporal-spatial regulation of mesodermal cell migration in the gastrula?

We have examined the arrangement of DMZ cells in the *Pleurodeles waltl* gastrula using a cell lineage tracer, microsurgery experiments and scanning electron microscopic studies (Shi *et al.*, 1987). As gastrulation occurs the DMZ is a bilayered structure approximately 120 μm thick. It contains polygonal cells extending terminal filopodia. In rare cases a cell extends through the two layers. Later, when the blastopore becomes crescent shaped, the DMZ decreases in thickness as cells intercalate to form a single layer. Just at the level of the blastopore, two or three layers of involuting cells are found. Simultaneously, involuted mesodermal cells migrate ahead towards the animal pole. They adhere to the inner surface of the blastocoel roof, extending numerous lamellipodia or filopodia.

Shi *et al.* (1989, 1990) have shown that DMZ explants deposited on fibronectin-coated-substratum comprise a useful *in vitro* model to study mesodermal cell behavior. Mesodermal cells spread and migrate as a cohesive cell sheet mimicking movements that occur *in vivo* in gastrulating embryos. Using this *in vitro* assay in *Pleurodeles waltl*, they found that the capacity of mesodermal cells to undergo autonomous migration is acquired as early as the 32-cell stage for the DMZ, and between the 64 and 128 cell stages for the lateral and ventral marginal zones. They also showed that the initiation of DMZ cell migration is probably due to a programmed developmental "clock" because in such an *in vitro* assay dorsal cells from the DMZ of different developmental stages begin to migrate at the same time as in control embryos.

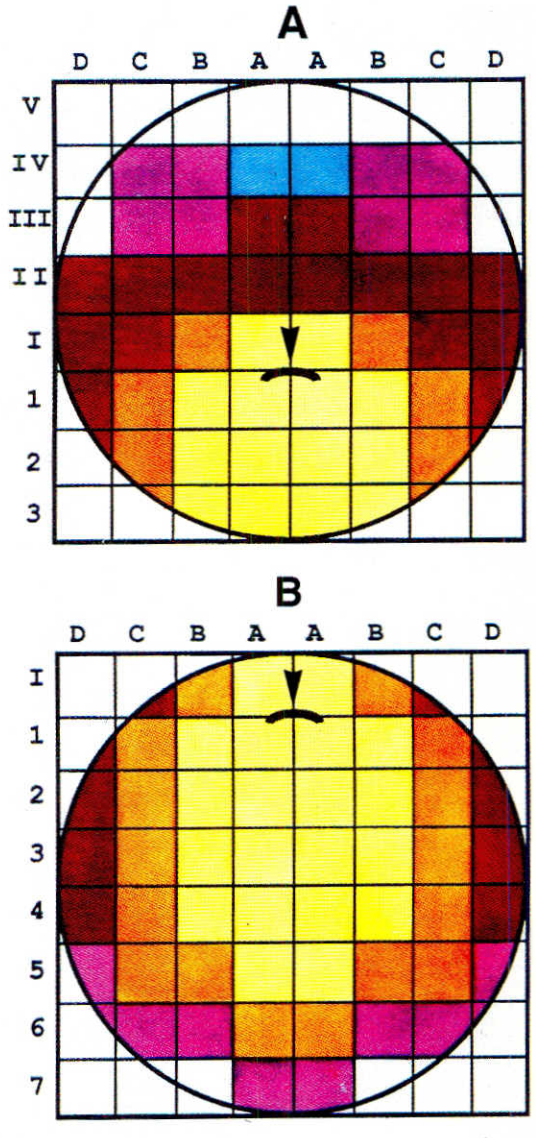

Fig. 2. Summary diagram illustrating the fate map for presumptive mesodermal regions in the *Pleurodeles waltl* early gastrula according to the results obtained with RLDx-labeled grafts of the circumblastoporal regions. *Red, regions forming only mesodermal derivatives. Yellow, regions forming only endoderm. Blue, regions forming only neuroectoderm. Purple: regions forming mesoderm and ectoderm. The blastopore is indicated by the arrow.* **(A)** *Dorsal marginal zone.* **(B)** *Vegetal region.*

An important observation, also made by Shi *et al.* (1989, 1990), is that perturbation of the dorsoventral polarity affects the migration of mesodermal cells. The U.V. irradiation of fertilized amphibian eggs interferes with the determination of axial structures (Malacinski *et al.*, 1975, 1977; Scharf and Gerhart, 1980, 1983; Youn and Malacinski, 1981). In embryos that develop from U.V. irradiated

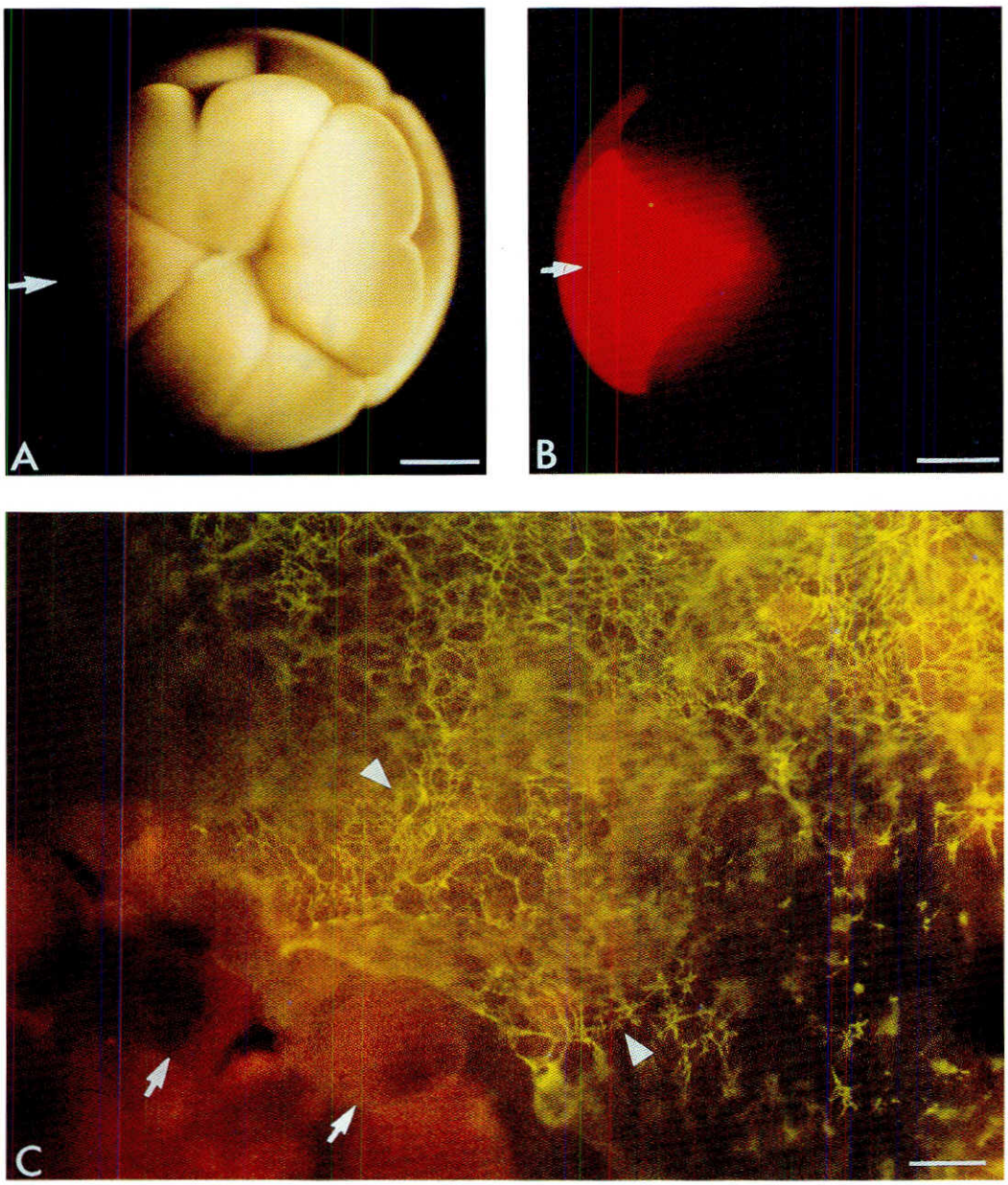

Fig. 3. Inhibition of fibronectin-fibril formation in selected blastomeres by Fab′ to cytoplasmic domain of integrin $ß_1$. *Monovalent antibodies against the cytoplasmic domain of integrin $ß_1$ subunit were injected into one blastomere at the 16-cell stage accompanied by rhodamine lysinated dextran. The embryos were cultured at 18°C. At the desired stage, the blastocoel roof was dissected and fixed. Detection of fibronectin was performed by indirect immunofluorescence.* **(A)** *Animal pole view of a living embryo (16-cell stage). Arrow shows the injected blastomeres.* **(B)** *Animal pole view under rhodamine illumination. The injected blastomere (arrow) and its progeny could easily be determined. Bars, 250 µm.* **(C)** *Whole-mount of an injected embryo. Late blastula stage (stage 7). Fibronectin labeling visualized with fluorescein-specific optics shows absence of fibronectin on the surface of rhodamine-labeled cells (arrows). Fibronectin is present in fibrils on the surface of unlabeled cells (arrowheads). Bar, 5 µm.*

fertilized eggs, the entire marginal zone is similar to the ventral marginal zone of unirradiated control embryos. In *Pleurodeles waltl*, U.V. irradiation is associated with a complete inhibition or a pronounced decrease in DMZ cell migration at either the 32- or the 64-cell stage. In later stages, there is an obvious synchrony in the migration capacity of mesodermal cells everywhere in the margins of explants. The results from scanning electron microscopic analysis and *in vitro* migration assays indicate that in such U.V. irradiated embryos the entire marginal zone behaves like the ventral marginal zone of normal embryos. Conversely, lithium treatment applied at the 32-cell stage dorsalizes the marginal zone due to interferences with the inositol triphosphate diacyl-glycerol second-messenger pathway. In *Pleurodeles waltl* again, the comparison of the migratory behavior of DMZ explants from lithium-treated or control embryos showed that all marginal cells acquired the same autonomous capacity for migration as dorsal marginal cells under the action of lithium. These observations support the idea that the capacity of mesodermal cells to migrate is related to the establishment of dorsoventral polarity.

Migrating mesodermal cells adhere to a fibrillar extracellular matrix in the gastrula

During gastrulation, migrating mesodermal cells adhere to an anastomosed network of extracellular fibrils on the basal surface of the blastocoel roof. These fibrils were first discovered by Nakatsuji *et al.* (1982) and Boucaut and Darribère (1983) in gastrulae of *Ambystoma maculatum* and of *Pleurodeles waltl*, respectively. The fibrils are sparse prior to gastrulation, but during gastrulation a dense meshwork of fine fibrils appears lining the basal surface of epithelial cells that make up the roof of the blastocoel. The organization and arrangement of these fibrils have been well

documented both by light and electron microscopic studies in urodeles (Nakatsuji and Johnson, 1983a,b; Darribère *et al.*, 1985).

In the course of gastrulation, migrating pioneer mesodermal cells extend lamellipodia and filopodia at the leading margin, whereas the trailing edge remains round or associated with an elongated process. They move as a stream of loosely packed cells. Examination of scanning electron micrographs at high magnification revealed that filopodia are firmly adherent to and aligned with extracellular fibrils. Furthermore, the presence of the statistically significant alignment of such fibrils along the blastopore-animal pole axis (Johnson *et al.*, 1992; Nakatsuji *et al.*, 1982), indicates an interesting possibility for an actual role *in vivo* for guidance by an aligned fibrillar network (Nakatsuji, 1984).

The notion that extracellular fibrils covering the inner surface of the blastocoel roof provide a substratum for mesodermal cells to adhere to and migrate along has been illustrated directly by grafting animal pole explants. Boucaut *et al.* (1984a) have shown that when the ectodermal layer of the blastocoel roof was inverted 180° so that the surface facing the external medium now faced into the blastocoel, mesodermal cells do not migrate because extracellular fibrils were not available. Another interesting observation is that cells from DMZ labeled explants grafted in the animal pole region migrate on the inner surface of the ectodermal layer (Shi *et al.*, 1987). Such migration of labeled cells was not found in control embryos bearing labeled animal pole explants.

To test the hypothesis that the extracellular fibrils guide mesodermal cells, Nakatsuji and Johnson (1984) transferred these extracellular fibrils onto a coverslip surface. A rectangular piece of the ectodermal layer was dissected from the dorsal part of early gastrulae of *Ambystoma maculatum* and explanted onto a plastic coverslip with the inner surface facing down. After incubation to allow transfer of the fibrillar extracellular matrix, the explant was mechanically removed from the coverslip. Dissociated mesodermal cells isolated from DMZ were then seeded on the conditioned surface. Cell movement was recorded by time-lapse cinemicrography, followed by fixation for scanning electron microscopy. By developing a computer program to analyze alignment of cell trails and extracellular fibrils, Nakatsuji and Johnson (1983a) were able to establish that isolated mesodermal cells move preferentially towards the blastopore-animal pole axis of the conditioned substrate.

Shi *et al.* (1989) showed that DMZ explants of *Pleurodeles waltl* early gastrulae had outgrowths turning towards the animal pole region of substrata that had been conditioned by a fragment of the blastocoel roof extending from the dorsal lip of the blastopore to the animal pole. These observations have two important implications. First, they suggest that there is some polarized information in the extracellular fibrils deposited on the conditioned medium. Second, dorsal mesodermal cells are able to respond to the guidance of the fibrillar network in spite of the discrepancy between the direction of migration and their original polarity.

What is the molecular composition of the fibrillar extracellular matrix in the gastrula?

In early *Pleurodeles waltl* embryos fibronectin (FN) is a major component of the extracellular fibrils lining the entire internal surface of the blastocoel roof. Maternally derived FN mRNAs are activated and translated during the blastula stage (Darribère *et al.*, 1984). FN synthesis occurs prior to gastrulation, and FN containing fibrils are first detectable at the early blastula stage. In *Pleurodeles waltl* blastulae FN becomes localized to the inner surface of the ectodermal layer despite the fact that the protein is apparently synthesized by cells in both the animal and vegetal parts of the embryo. These observations are supported by immunoprecipitation, *in situ* hybridization, and RNAase protection results.

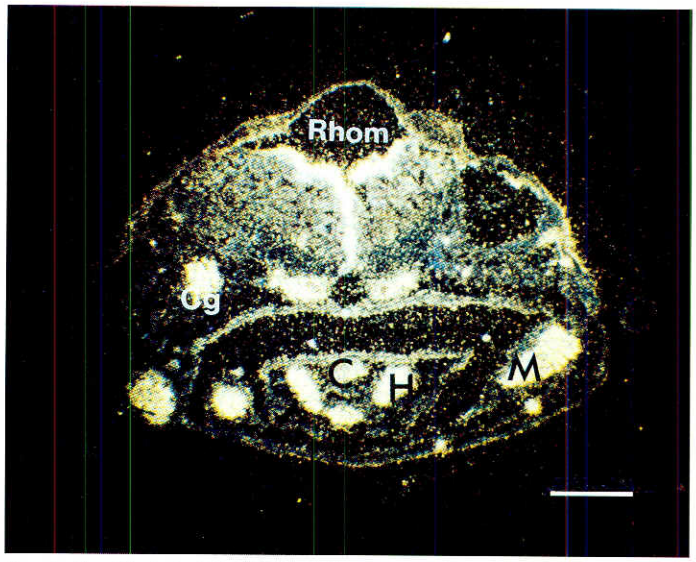

Fig. 4. *In situ* hybridization of the expression of an FGF receptor (PFR3) mRNA at the prelarval stage (stage 38). *Cross-section at the level of rhombencephalon. Strong labeling is noticed in the proliferative ventricular zone of the rhombencephalon (Rhom) and in the mesenchymal condensation sites of Meckel's cartilage (M), hyoid (H) and copula (C). The cranial ganglion (cg) is also highly labeled. Bar, 40 μm.*

The FN protein is secreted into the extracellular medium as a disulfide-bonded dimer. Each monomeric form consists of three types of amino acid repeats referred to as types I, II, and III. Type I and II repeats contain residue involved in intra-chain disulfide bonds. Type III repeats contain conserved aromatic residues. The arrangement of the three types of repeat defines a series of functional domains with binding properties to other extracellular components or to the cell surface (for a review, see Hynes, 1990; Potts and Campbell, 1994). Protein isoforms of FN are generated by alternative splicing of a single gene in mammals, avians and *Xenopus* (Schwartzbauer *et al.*, 1983, 1987; Kornblihtt *et al.*, 1984; Tamkun *et al.*, 1984; Norton and Hynes, 1987; DeSimone *et al.*, 1992). Three different regions of alternative splicing have been identified. Two alternatively spliced type III repeats designated as EIIIA and EIIIB are located in the central region of the molecule. Both EIIIA and EIIIB are encoded by two different exons that can be either entirely included or excluded from the FN transcript. The third region where alternative splicing occurs is the so-called V-region or type III connecting segment (IIICS) of FN.

cDNA clones encoding the carboxy terminal half of *Pleurodeles waltl* FNs have recently been isolated and the sequence determined (Clavilier *et al.*, 1993). The cDNA clones comprise all three alternatively spliced regions designated EIIIA, EIIIB and V-region, respectively. As expected, RNAase protection experiments show that the three spliced regions are continuously included in all FN transcripts of early embryonic stages, whereas from the tailbud stage onward the V-region was partially excluded.

In urodele gastrulae, antibodies directed against mouse laminin stain the fibrillar extracellular matrix on the blastocoel roof (Nakatsuji

et al., 1985; Darribère *et al.*, 1986). Immunoprecipitation experiments show that laminin is synthesized in *Pleurodeles waltl* oocytes, during cleavage and throughout early development. There is evidence that at least until the late blastula stage, laminin is the translation product of stored maternal mRNA. Finally, comparison of laminin and FN synthesis suggests that both extracellular glycoproteins have a common pattern of synthesis in *Pleurodeles waltl* early embryos (Riou *et al.*, 1987). By using a *Xenopus* cDNA homologous to the mouse laminin ß1 chain and antibodies raised against the corresponding protein, Fey and Hausen (1990) have analyzed the expression and distribution of laminin in *Xenopus* early embryos. They provide evidence for the presence of laminin transcripts at the midgastrula stage and for the appearance of the protein in extracellular matrix at the early neurula stage. These observations suggest that there may be a significant difference in the composition of the fibrillar extracellular network on the basal surface of the blastocoel roof in urodeles and *Xenopus*.

Identification of cellular receptors for the fibrillar extracellular matrix

Attention has been focused on the ß1 family of integrins, which includes several well characterized receptors for FN and laminin. These receptors consist of heterodimeric complexes made up of noncovalently associated α and ß subunits. Various αß combinations differ in their ligand-binding specificities. For example integrins $\alpha_5\beta_1$ and $\alpha_V\beta_1$ are monospecific for FN. Conversely, $\alpha_2\beta_1$ receptor complex binds different ligands, such as collagens, laminin, and FN.

A cDNA encoding the integrin β_1 subunit from *Pleurodeles waltl* has been isolated (D.L. Shi and D.W. DeSimone, unpublished). Recent studies indicate that β_1 transcripts are present in the oocyte and then steadily expressed in all regions of the early embryo. Immunoprecipitation experiments using a polyclonal antibody against the extracellular domain of β_1 showed that both the immature and mature forms of the protein are expressed in early stages of development. Labeling experiments using iodination showed that the mature form of β_1 was first incorporated into the plasma membrane at the blastula stage (T. Darribère. and D. Alfandari, unpublished).

More recently, PCR amplification experiments have yielded two cDNAs encoding the integrin α_V subunit from *Pleurodeles waltl*. The embryonic expression of α_V transcripts has been determined by RNAase protection analyses. α_V mRNAs are expressed in oocytes and in all regions of the embryo from the gastrula to the tail-bud stages. Prior to the blastula stage there is no integrin α_V subunit detectable on the cell surface. At the blastula stage, however, immunostaining for α_V becomes apparent on the surface of cells from the marginal zone. By the early gastrula stage, a striking increase in amount of α_V staining is observed on the surface of mesodermal cells (Alfandari *et al.*, 1995).

What features characterize fibronectin matrix assembly in the blastula?

Boucaut and Darribère (1983) and Darribère *et al.* (1990) have studied the time course and pattern of FN fibril formation in *Pleurodeles waltl*. Immunofluorescent staining for FN was done in whole-mounted specimens of the blastocoel roof. At first, when the blastocoel begins to form in 8-cell embryos there is no fluorescence for FN on the surface of blastomeres. The formation of FN fibrils begins in early blastulae where FN fibrils are first situated on the inner surface of small ectodermal cells. In midblastulae, fibrils appear on all cells of the blastocoel roof. They are located all around the cell periphery and also across adjacent boundaries. Later, FN fibrils elongate from these peripheral sites toward the central supranuclear region. In late blastulae, they are now well developed over all cells forming the roof of the blastocoel. Finally a complex anastomosing FN fibrillar meshwork covers the entire surface of the blastocoel roof in early gastrulae.

How do cell interactions with FN control the assembly of FN into fibrils on the inner surface of the ectodermal layer? FN is a multifunctional molecule consisting of a series of specific binding-sites that mediate its function (Akiyama *et al.*, 1990; Hynes, 1990). A domain located at the aminoterminus interacts with heparin and also contains a site of binding to fibrin. A collagen binding site has been identified in a 40 kDa region near the amino terminus, and the 110 kDa central cell-binding domain is adjacent to it. That cell-binding region of the molecule is of crucial importance since it mediates cell attachment via interactions between the RGDS recognition sequence and the integrin receptor $\alpha_5\beta_1$ (Yamada and Kennedy, 1984). The two final domains contain a second binding-site for heparin and fibrin, respectively.

FN fibrillogenesis is a process that requires integrins, homophilic interactions, cross linking and association with other extracellular molecules such as collagens and proteoglycans. The factors governing FN assembly into fibrils have been investigated in *Pleurodeles waltl* by Darribère *et al.* (1990, 1992). When they microinjected exogenous bovine or human FN into the blastocoel of living embryos, they found that exogenous FN is assembled in the same spatio-temporal pattern as observed of endogenous FN. Competition experiments performed with exogenously added proteolytic fragments of human FN and perturbation with domain-specific monoclonal antibodies demonstrate that at least three FN sites are essential for assembly of FN fibrils in the blastula of *Pleurodeles waltl*. Two sites are located in the central cell-binding domain: the first is the RGDS sequence of the 11th type III homology, and the second is a synergistic site in the 10th type III repeat. A third site that spans the ninth type I and first type III homology sequences is also likely involved in FN-FN interactions.

Such observations indicate that the binding of FN to integrins is essential for the proper assembly of FN fibrils. Indeed, intracellular injection of antibodies to the cytoplasmic domain of integrin β_1 subunit into uncleaved embryos or into defined blastomeres produces a reversible inhibition of FN fibril formation and causes delay in development (Fig. 3). These results also suggest that gastrulation requires normal assembly of FN fibrillar extracellular matrix.

A maternal-effect mutation disturbs fibronectin matrix assembly in the *Pleurodeles waltl* embryo

A delay in gastrulation has been described in progeny from homozygous mutant females *(ac/ac)* in *Pleurodeles waltl* (Beetschen and Fernandez, 1979). The phenotype of *ac/ac* mutant gastrulae is strikingly similar to the appearance of control embryos injected with probes that perturb interactions between cells and FN (see below). In studying these *ac/ac* progeny, Darribère *et al.* (1991) have found interesting results with respect to the consequence of the defect of FN assembly on the morphogenetic movements of gastrulation. First, they provide evidence that mutant embryos are able to synthesize FN and both α_5 and β_1 integrin subunits.

Second, the integrin receptor $\alpha_5\beta_1$ is expressed on the inner surface of ectodermal cells in a similar pattern in both mutant and normal embryos. Third, as shown by microinjection experiments, *ac/ac* progeny show a conspicuous defect in the assembly of either endogenous FN or exogenous FN into a fibrillar matrix. Although the precise target of this maternal effect mutation is unclear, the morphological similarity between *ac/ac* progeny and probed embryos once again suggests that a normal FN matrix assembly is required to support gastrulation in *Pleurodeles waltl*.

Probes which disrupt cell matrix interaction inhibit mesodermal cell migration and subsequently block gastrulation

Boucaut *et al.* (1984a) showed that injection of the Fab' fragment of anti-FN IgG into the blastocoel of late blastulae or early gastrulae disrupts mesodermal cell migration and the entire morphogenetic cell movements of gastrulation. Arrested embryos have a characteristic morphology. The blastopore formed a circular constriction in the equatorial region which divided the embryo into two hemispheres. The animal hemisphere became extensively folded with convolutions and deep furrows. The endodermal mass remained on the outer surface of the vegetal hemisphere. Scanning electron microscopic examination of injected embryos reveals a large blastocoel that was not collapsed. Migrating mesodermal cells formed a ring-like collection in the marginal zone, but they failed to migrate across the inner surface of the blastocoel roof, presumably because they were unable to gain an appropriate foothold there. When injected into the blastocoel of late blastulae or early gastrulae, RGDS-containing peptides also disrupt mesodermal cell migration in a dose-dependant manner (Boucaut *et al.*, 1984b). Inhibited embryos were strikingly similar to inhibited embryos injected with Fab' anti-FN. Embryos injected with control FN peptide from the collagen-binding region, ACTH, BSA, preimmune Fab' and Fab' anti-FN preabsorbed with FN all develop normally. When Darribère *et al.* (1988) injected Fab' fragments of anti $\alpha_5\beta_1$ integrin complex IgG into living early gastrulae, gastrulation was also completely inhibited. They interpreted these results to mean that antibodies blocked the integrin receptor $\alpha_5\beta_1$ function, thus once again preventing interactions between migrating mesodermal cells and FN fibrils.

Tenascin is a non-collagenous glycoprotein of the extracellular matrix with a spatially and temporally restricted distribution. It was initially isolated by Chiquet and Fambrough (1984a,b). Studies in *Pleurodeles waltl* reveal that tenascin is expressed in the extracellular matrix in close association with neural crest cell migration, but is not normally present in ECM during gastrulation (Riou *et al.*, 1988). Tenascin has been reported to modify integrin mediated cell attachment to FN (Chiquet-Ehrismann *et al.*, 1988). Riou *et al.* (1990) have shown that tenascin has striking effects on mesodermal cell migration in *Pleurodeles waltl*. When tenascin was microinjected into the blastocoel of late blastulae, it co-localized with FN in the fibrillar extracellular matrix. Injected embryos exhibited a severe alteration of gastrulation, reminiscent of embryos injected with antibodies or synthetic peptides to disrupt mesodermal cell-FN interactions. Finally, when tenascin was preincubated with a monoclonal antibody masking the putative cell binding region, morphogenetic movements were not perturbed. Once again, a molecule which interferes with FN-integrin interaction specifically inhibits gastrulation.

Isolation and developmental expression of fibroblast growth factor receptors in early *Pleurodeles* embryos

Substantial progress has been made recently in the understanding of mechanisms of mesoderm formation in amphibian embryos, primarily due to the discovery that peptide growth factors belonging to the fibroblast growth factors (FGFs) and the transforming growth factor ß (TGFß) families are involved in mesoderm induction (for reviews see Kimelman *et al.*, 1992; Sive, 1993). The response of cells to FGFs is mediated by high-affinity receptors which belong to the tyrosine kinase superfamily. The characterization of FGF receptors (FGFRs) has progressed rapidly in the last few years. Four distinct FGFR genes classified into FGFR-1/Flg, FGFR-2/bek, FGFR-3 and FGFR-4 have been described in humans (Ruta *et al.*, 1988; Dionne *et al.*, 1990; Houssaint *et al.*, 1990; Keagan *et al.*, 1991; Partanen *et al.*, 1991). All these genes encode membrane-spanning proteins with three extracellular immunoglobulin-like domains and an intracellular tyrosine kinase domain.

Members of the FGF family are not only candidates as early endogenous inducers of mesoderm, but also have functions in the later development of the amphibian (Isaacs *et al.*, 1992; Tannahill *et al.*, 1992). A role for FGFs as endogenous inducing molecules is suggested by injection of RNAs encoding dominant negative mutations of FGFRs into early *Xenopus* and *Pleurodeles waltl* embryos (Amaya *et al.*, 1991; D.L. Shi, unpublished). In both cases mesoderm formation is defective.

To understand fully the roles played by FGFs in orchestrating inducing activities at early stages of embryogenesis, it is of course necessary to identify and characterize all the receptors for FGFs that are expressed in the embryo. cDNAs encoding *Pleurodeles waltl* FGFRs have recently been isolated and the sequences determined (Shi *et al.*, 1992, 1994a,b). Each of these FGFR *Pleurodeles waltl* genes has an unique expression pattern. The homologs of FGFR-1 and FGFR-3 are maternally expressed, and this expression persists during cleavage and from gastrula stage onwards. On the other hand, the homolog of FGFR-4 is only expressed after the blastula stage. In addition *in situ* hybridizations showed an overlapping but differential distribution pattern of FGFR-3 mRNA with respect to FGFR-1 and FGFR-4 mRNAs. At the early gastrula stage, FGFR-1 is nearly uniformly distributed, whereas FGFR-3 is expressed predominantly in the ectoderm, and FGFR-4 is mainly localized to the ectoderm and the ventral mesoderm. Later on at the late tail-bud stage, like FGFR-4, but different from FGFR-1, FGFR-3 is most abundantly expressed in the head region in defined sites of the brain (Fig. 4).

Shi *et al.* (1994b) have isolated five splice variants corresponding to the *Pleurodeles waltl* homolog of FGFR-2. These receptor variants differ in the second half of the third Ig-like loop in that they may include either a IIIb or a IIIc exon. This region was recently shown to confer ligand-binding specificity of the receptor (Johnson *et al.*, 1991; Werner *et al.*, 1992). FGFR-2 variants containing the IIIb exon are maternally expressed, whereas those containing the IIIc exon are zygotically activated. Results of RNase protection analysis clearly showed that the expression of IIIb and IIIc transcripts is mutually exclusive. At the early neurula stage, IIIb mRNA is mainly expressed in the epidermis, while IIIc mRNA is activated in the neural tissue. Interestingly, the zygotically expressed IIIc variant is activated by neural induction suggesting that members of the FGF family and their receptors may indeed be involved in the development of the nervous system. These observations provide

evidence that FGFRs are remarkably conserved from human to *Pleurodeles waltl*, and that they are developmentally and tissue specifically regulated. They imply that individual FGFRs may have different functions during early amphibian development. In addition, generation of multiple receptor variants with different ligand-binding specificities reflects the likely complexity of FGF signaling regulatory loops in the course of early embryogenesis.

Outlook

Although many different amphibian species have attracted developmental biologists since the beginning of experimental embryology, the newt *Pleurodeles waltl* is still a very exciting model for the study of early morphogenetic events. Because *Pleurodeles waltl* offers the possibility to combine microsurgical, cellular interactions, and molecular approaches, we are confident that this model system will provide other new insights into the mechanisms involved in the control of morphogenesis.

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