Induction of notochord cell intercalation behavior and differentiation by progressive signals in the gastrula of \textit{Xenopus laevis}

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SUMMARY

We show that notochord-inducing signals are present during \textit{Xenopus laevis} gastrulation and that they are important for both inducing and organizing cell behavior and differentiation in the notochord. Previous work showed that convergent extension of prospective notochordal and somitic mesoderm occurs by mediolateral cell intercalation to produce a longer, narrower tissue. Mediolateral cell intercalation is driven by bipolar, mediolaterally directed protrusive activity that elongates cells and then pulls them between one another along the mediolateral axis. This cell behavior, and subsequent notochordal cell differentiation, begins anteriorly and spreads posteriorly along the notochordal-somitic boundary, and from this lateral boundary progresses mediolaterally towards the center of the notochord field. To examine whether these progressions of cell behaviors and differentiation are induced and organized during gastrulation, we grafted labeled cells from the prospective notochordal, somitic and epidermal regions of the gastrula into the notochordal region and monitored their behavior by low light, fluorescence videomicroscopy. Prospective notochordal, epidermal and somitic cells expressed mediolateral cell intercalation behavior in an anterior-to-posterior and lateral-to-medial order established by the host notochord. Behavioral changes were induced first and most dramatically among cells grafted next to the notochordal-somitic boundary, particularly those in direct contact with the boundary, suggesting that the boundary may provide signals that both induce and organize notochordal cell behaviors. By physically impeding normal convergent extension movements, notochordal cell behaviors and differentiation were restricted to the anteriormost notochordal region and to the lateral notochordal-somitic boundary. These results show that mediolateral cell intercalation behavior and notochordal differentiation can be induced in the gastrula stage, among cells not normally expressing these characteristics, and that these characteristics are induced progressively, most likely by signals emanating from the notochordal-somitic boundary. In addition, they show that morphogenetic movements during gastrulation are necessary for complete notochord formation and that the prospective notochord region is not determined by the onset of gastrulation.

Key words: gastrulation, mesoderm, \textit{Xenopus}, motility, induction, notochord, morphogenesis

INTRODUCTION

Here, we address whether the progressive expression of cell behaviors driving convergent extension of the dorsal mesoderm during \textit{Xenopus} gastrulation (Keller et al., 1992a) reflects the activity of mesodermal-inducing or organizing signals during gastrulation. Convergent extension movements involve a stereotyped sequence of cell behaviors that are highly patterned in space and time. These behaviors occur in two phases. First, at the beginning of gastrulation, between stages 10 and 11, a single round of cell division is accompanied by radial intercalation between several layers of deep mesodermal cells to extend the notochordal and somitic regions along the anteroposterior axis (Wilson and Keller, 1991). Second, beginning at stage 10.5, mediolateral intercalation occurs between cells within each layer to form a narrower and longer array (Keller and Tibbetts, 1989). Mediolateral cell intercalation is the result of a stereotyped pattern of motility displayed by deep mesodermal cells, called mediolateral intercalation behavior (MIB). MIB occurs as the protrusive activity is restricted to the medial and lateral ends of the cells. As a result, cells exert traction on adjacent cells causing them to elongate and align parallel to one another, and to intercalate along the mediolateral axis, thus producing mediolateral cell intercalation (Shih and Keller, 1992a). The onset of MIB, as assayed by cell elongation and alignment, is progressive across the dorsal mesoderm, beginning anteriorly and laterally and progressing posteriorly and mediolaterally in both the prospective notochordal and somitic regions (Shih and Keller, 1992b). Therefore, the medial-posterior regions of both the somitic and notochordal territories are the last to express these cell behaviors, and the last to differentiate into their respective tissues.

The highly organized pattern of MIB could be established
at the blastula stage, when mesoderm is thought to be induced and patterned, or it could reflect organizing events at the gastrula stage. Previous work on mesoderm induction and patterning has focused on the blastula stage, when signals emanating from the endoderm appear to induce the animal cap ectoderm to form mesoderm (Nieuwkoop, 1969a; Sudarwati and Nieuwkoop, 1971; Nakamura and Kishiyawa, 1971) and, in the process, also determine its dorsoventral polarity (Nieuwkoop, 1969b). In attempts to identify these signals, several peptide growth factors, including FGF (Kimelman and Kirschner, 1987; Slack et al., 1987), members of the TGFβ family, including activin (Smith, 1987), bone morphogenetic protein 4 (BMP4) (Jones et al., 1991; Dale et al., 1992) and Vg1 (Thomsen and Melton, 1993), have been characterized and shown to have mesoderm-inducing or patterning activity when assayed on isolated animal caps (Symes and Smith, 1987) or dissociated animal caps (Green et al., 1992) or by expression in the embryo. Injection of RNAs coding for mutated receptors of FGF (Amaya et al., 1991) and activin (Hemmati-Brivanlou and Melton, 1992) block mesoderm induction in different ways, suggesting that each receptor-ligand system has a specialized role in induction and patterning of the mesoderm.

Although these and other experiments show that mesoderm is induced and patterned to some extent in the blastula stage, the expression pattern of MIB suggests that these cell behaviors are instructed during gastrulation (Shih and Keller, 1992b). If signals are organizing notochord and somitic formation during gastrulation, then cells competent to respond to these signals should adopt MIB and differentiate in the same lateral-to-medial and anterior-to-posterior progressions when grafted into the notochordal or somitic field of the early gastrula. We tested this hypothesis by grafting cells from various regions of the gastrula to the prospective notochord region and recording the progression of MIB with time-lapse videomicroscopy. We show that the prospective notochord region instructed cells grafted from the prospective notochordal, somitic and epidermal regions to express MIB and differentiate into notochord cells in the same anterior-to-posterior, ventral-to-dorsal progressions as the host notochord cells, regardless of their source or the geometry of their placement in the host prospective notochord. We also show that if convergent extension movements are physically retarded, MIB and notochord differentiation are limited to cells in the anterior notochord and cells in contact with the notochordal-somatic boundary. These results suggest that the signals involved in inducing and organizing the notochord emanate from the anterior notochord region and the notochordal-somatic boundary, and that these signals are likely to be of limited range and thus require convergent cell movements to bring the lateral source of the signal closer to the responding, medial cells.

**MATERIALS AND METHODS**

**Preparation and dissection of embryos**

*Xenopus laevis* eggs were obtained, fertilized, dejellied and stored in Modified Barth’s Solution at 16°C (Winklbauer, 1986) by standard methods (Kay and Peng, 1991). Staging was done according to Nieuwkoop and Faber (1967). Embryos were transferred at gastrula stages to modified Danilchik’s solution (DFA) (Keller et al., 1985; Sater et al., 1994). Vitelline envelopes were removed with forceps and open-faced explants were made from early to late gastrulae (stages 10-11.5) as described previously (Shih and Keller, 1992a). These explants consisted of an involuting marginal zone (IMZ), composed of an epithelial layer of prospective endoderm and several layers of deep cells of prospective mesoderm, and a noninvoluting marginal zone (NIMZ), composed of prospective neural ectoderm in both epithelial and deep layers. Explants were cultured at room temperature or 16°C with the deep cell layer facing down on a coverslip glued with high vacuum grease across a 20 mm hole in a plastic Petri dish. Each explant was restrained from above with a small piece of coverslip glass supported with high vacuum grease.

**Grafting of labeled cells**

Dejelled embryos were placed in 5% Ficoll in MBS and injected by air pressure with 15 nl of either 33 mg/ml of fluorescein-dextran amine (FDA) or rhodamine-dextran amine (RDA) (Molecular Probes) in 0.2 N KCl (Gimlich and Braun, 1985) and grown in 33% MBS in dishes coated with 2% agar. Early gastrulae were transferred to DFA and their vitelline envelopes were removed. Clumps of about 40 cells were removed from specific regions of labeled embryos with an eyebrow hair, teased apart and gently wedged, in random orientation, into the prospective notochord region of an unlabeled, open-faced explant (Fig. 1). Grafted explants were grown to stage 25, fixed in MEMFA (0.1 M MOPS, pH 7.4, 2 mM EGTA, 1 mM MgSO₄, 3.7% formaldehyde), washed in 100% methanol for 1 hour, and transferred to benzyl benzoate/benzyl alcohol (Kay and Peng, 1991) for viewing on a Nikon fluorescence microscope.

**Low light fluorescence time-lapse recording**

Time-lapse video recordings of grafted, labeled cells were made using a Hamamatsu C2400-08 SIT camera, a Nikon zoom ocular and a Diaphot fluorescence microscope. Contrast adjustment and frame averaging (16 to 64) were done with an IMAGE I (Universal Imaging Media, PA) image processor. Images were saved on a Panasonic TQ-2028F optical memory disc recorder. The explants were illuminated only during collection of video frames at intervals of 30 seconds to several minutes, using a Uniblitz shutter (Vincent Associates, PA), controlled by the image processor.

**Immunocytochemistry and in situ hybridization**

Embryos and explants were fixed in MEMFA for 1 to 2 hours at room temperature and stored in methanol at –20°C. Whole-mount immunocytochemistry was done with the notochord-specific monoclonal antibody Tor 70 at a 1:500 dilution (Kushner, 1984; Bolce et al., 1992) and the neural-specific monoclonal antibody N-CAM (4d) at a 1:1000 dilution (Watanabe et al., 1986). Both were visualized with an HRP-conjugated secondary antibody by the method of Hemmati-Brivanlou and Harland (1989). Digoxigenin-labeled antisense probe to endoB, a notochord marker (LaFlamme and Dawid, 1990), was used for wholemount in situ hybridization according to Harland (1991). Afterwards, the explants were refixed in MEMFA, stained with the somite-specific antibody 12-101 (Kinter and Brockes, 1984), and visualized with an HRP-conjugated secondary antibody. Explants were transferred to 100% methanol for 1 hour and cleared in benzyl benzoate/benzyl alcohol (Kay and Peng, 1991) for viewing on a Nikon Diaphot.

**RESULTS**

**Notochordal cells grafted to random positions within the prospective notochord region adopt cell behaviors synchronously with the host notochord**

To test whether the progressive expression of cell behaviors and cell type differentiation in the organizer is due to a
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signaling process active during gastrulation, we grafted labeled prospective notochord cells from an early gastrula, in random order, into the prospective notochord region of another unlabeled early gastrula explant. If the hypothesis is correct, the grafted cells should express the characteristic cell behaviors at the appropriate time with respect to their new position within the host notochord. Alternatively, if these behaviors were pre-programmed at the onset of gastrulation, the grafted cells should express them in a chaotic pattern that reflects our deliberate disorganization of their spatial relationships. Since the notochord is not yet defined morphologically in the early gastrula, we made the grafts to the deep region immediately animal to the dorsal blastoporal lip, which is the prospective notochord (Keller, 1976) (Fig. 1). We then recorded the behaviors of the labeled cells by low-light video microscopy (Fig. 2).

Five time-lapse video recordings showed that the grafted, prospective notochord cells adopted the progression of cell behaviors consistent with their new position. A video recording shows the formation of the anterior region of the notochordal-somatic boundary and an anterior cell at or near the boundary that adopted MIB (see arrow, Fig. 2B,B'). As the boundary progressed posteriorly (see arrow), more posterior cells in contact with the boundary adopted MIB (Fig. 2C,C'). Eventually, an anterior-medial cell adopted MIB (see arrow, Fig. 2D,D'). As the most posterior-lateral cell adopted MIB (see arrow), middle-lateral cells associated with the boundary formed protrusions in the medial direction that contacted middle-medial cells (see arrowhead, Fig. 2E,E'). Finally, MIB progressed posteriorly among medially positioned cells (see arrowheads, Fig. 2D,D'-F,F'). Both the host cell behaviors and the position of the opposite notochordal-somatic boundary, which is located about one cell diameter away from the medially labeled cells, were observed by epi-illumination (data not shown). The progression of cell behaviors among the host notochordal cells is the same as the grafted cells and similar to those observed previously by Shih and Keller (1992b). The anteroposterior progression of MIB along the notochordal-somatic boundary is relatively fast, whereas the medial progression from the lateral boundaries towards the center of the notochord region is slow and occurs after the behaviors are well established along the boundary. The grafted notochord cells expressed MIB according to the temporal pattern of the host regardless of the geometry in which the labeled cells were introduced. In the explant, these behaviors occurred during 14.5 hours whereas, in the embryo, they are most likely to occur in less than 10 hours. This discrepancy in time may be due to the retardation of convergence and extension from the coverslip glass which keeps the explant flat and allows better viewing of the cell behaviors.

In 87% of 101 grafts, the grafted notochord cells differentiated as notochord (Fig. 3), as determined by location within the notochord field, bipolar cell shape and vacuolation. In 12% of grafts, labeled cells were either in the ectoderm due to misplacement during the grafting procedure or scattered throughout the explant displaying signs of damage that included blebbing and lysis, probably due to injection of excessive dextran. Although we did not quantify this fact, our impression from examining 101 explants is that grafts of several small clumps rather than one large clump, increased the likelihood of inducing notochord differentiation among the grafted cells, perhaps by increasing the area of contact between the graft and the host. This contact behavior was also observed between grafted cells (Fig. 2E,F) suggesting that MIB may begin by directly contacting cells already expressing MIB.

**Acquisition of notochord cell behaviors and fate by cells grafted from the prospective epidermal and somitic regions**

Despite our efforts to scatter cells randomly in the above experiment, we may have actually grafted them into positions congruent with their original positions in the donor and their subsequent differentiation may have matched that of the host notochord. Although remote, this possibility can be eliminated if MIB is expressed progressively by grafts of non-notochordal cells, which would never have expressed these behaviors on their own.
Cells from the prospective epidermal region were grafted to the prospective notochord region at the early gastrula stage (Fig. 3). In 46% of 87 grafts, the labeled cells differentiated into notochord. In 54% of cases under the category of ‘Neither’, cells were found either in the ectoderm, in the boundary region between the notochord and somites, or scattered throughout the explant. Since the labeled cells in the boundary did not show a somitic or a notochordal phenotype, we examined whether these cells were subjected to neural inducing rather than notochord-inducing signals. We found that the grafted epidermal cells located in both the ectoderm and next to the notochord expressed N-CAM (Watanabe et al., 1986) (data not shown). Furthermore, we observed that grafts excluded from the notochord also remained in clumps, whereas those that differentiated into notochord, dispersed among the host notochordal cells.

Six low-light video recordings showed that labeled, prospective epidermal cells differentiated into notochord cells by adopting MIB in the same progressive pattern characteristic of the host notochord. A video recording shows labeled prospective epidermal cells scattered in the prospective notochord region (Fig. 4A,A'). As the notochordal-somatic boundary progressed along the anteroposterior axis (see arrow, Fig. 4B,B'-E,E'), cells located anteriorly extended filopodial protrusions in the medial direction, thus becoming elongated and aligned parallel to one another (see arrowhead, Fig. 4C,C'-H,H'). This behavior progressed to more posterior cells and eventually progressed medially from its lateral origin to span the entire notochordal region (Fig. 4E,E'-H,H'). The grafted cells moved towards the notochordal midline as they adopted MIB. Finally, the grafted cells differentiated into notochord cells and formed vacuoles in the same anterior-to-posterior progression displayed by the preceding cell behaviors (see dark spots at the end of the arrows, Fig. 4H,H').

Likewise, cells were grafted from the prospective somitic region to the prospective notochord region at the early gastrula stages (Fig. 3). In 39% of 33 grafts, labeled cells differentiated into notochordal tissue, in 12% of grafts, labeled cells were found in both somitic and notochordal tissues and, in 33% of cases, labeled cells differentiated into somitic tissue. There are two likely reasons why cells grafted from the prospective somitic region differentiated into notochordal and somitic tissues with nearly equal frequencies. Although we attempted to graft them into the prospective notochord region, some may have been grafted close to the host somitic tissue and thus may have been more likely to respond to signals emanating from this territory. Alternatively, the grafted cells may be heterogenous in their ability to respond to notochord-inducing signals. Since somitic tissue also differentiates in a lateral-to-medial progression (Shih and Keller, 1992b), lateral somitic tissue may be more committed to a somitic fate and thus less responsive to notochord-inducing signals than medial cells, at a particular time.

Four low-light video recordings of labeled cells grafted from the prospective somitic region to the notochordal region adopted MIB in anterior-to-posterior and lateral-to-medial pro-

Fig. 2. Video frames from a recording of labeled, stage 10 prospective notochord cells grafted to the prospective notochord region at gastrulation (stage 10-10.5). Below each frame a computer generated contour map outlines the labeled cells and a pen drawing outlines areas of interest. Anterior (a), posterior (p), lateral (l), medial (m). Scale bar: 50 μm.
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Changes in shape of grafted cells is not passive

The elongated shapes of the grafted cells are not passive responses to compressive forces within the explant, but reflect active formation and retraction of protrusions. To demonstrate this point further, we made heterochronic grafts in which either the host tissue was less capable of inducing MIB and notochord differentiation or in which the donor tissue could no longer respond to the signals (Domingo, 1995). Invariably, cells that did not express MIB were excluded from the notochord. For example, stage 10.5 prospective somitic cells grafted next to the notochordal-somatic boundary of a stage 11.5 explant, were excluded from the notochord within several hours (see arrowhead, Fig. 7A,A,B,B) to take residence in the host somitic region where some labeled cells differentiated into somitic tissue (see arrowhead, Fig. 7C,C). Other labeled cells that were excluded from the notochord, lost their competence to differentiate into somitic tissue by the time they reached the host somitic region. None of the grafted cells adopted the bipolar protrusive activity characteristic of the host notochord cells.

Likewise, stage 11.5 prospective epidermal cells, which have lost their competence to respond to a notochord-inducing signal, were transplanted to the prospective notochord region of a stage 10.5 explant. The grafted cells did not adopt MIB and were eventually excluded from the notochord (Fig. 7D,D') to the somitic region or to the ectodermal region of the explant as observed by epi-illuminating the explant. Thus, we were unsuccessful in passively mimicking the elongated cell shape, characteristic of MIB, by trapping uncooperative cells within the notochord.

Morphogenetic movements are required for notochord formation

If inducing and organizing signals originate at and propagate from the lateral notochordal-somatic boundaries towards the center of the notochord field, we should be able to visualize their range by preventing convergence of the boundaries. We addressed this contention by physically retarding convergence by flattening explants between two coverslips as described previously (Shih and Keller, 1992a) and thus preventing the boundaries from approaching one another. Notochord differentiation was assayed by the expression of a notochord-specific proteoglycan recognized by the antibody, Tor 70 (Kushner, 1984; Bolce, et al., 1992). In explants showing considerable convergence, Tor 70 expression occurred across the entire notochord field (Fig. 8A), whereas in explants showing limited convergence, expression was restricted to the anterior notochord region and to the notochordal-somatic boundaries (see arrows, Fig. 8B). Both MIB, assayed by cell elongation and alignment, and Tor 70 expression were absent from the center of the prospective notochord region (see arrowhead, Fig. 8B). Since both MIB and Tor 70 expression are coincident, this suggests that both may be dependent on a common upstream signal that originates at the notochordal-somatic boundaries but has a limited range such that cells in the center of the notochord...
field never receive it. In explants with reduced convergence, cells in the medial region form a third notochordal-somitic boundary located across the prospective posterior notochord. In this case, cells in the posterior-medial region of the explant do differentiate as notochord, forming a tri-radiate notochord with somitic mesoderm on all three sides (Fig. 8C).

**DISCUSSION**

**Notochord-inducing and organizing signals are present during gastrulation**

The anterior-to-posterior and lateral-to-medial progressions of MIB (Shih and Keller, 1992b; Keller et al., 1992a) raised the possibility that the prospective notochord region is patterned during gastrulation. Previous studies have shown that the dorsal mesoderm is established, to a large extent, in the blastula through an instructive interaction between the dorsal, vegetal blastomeres and the blastomeres immediately animal to them (Nieuwkoop, 1969a,b; Sudarwati and Nieuwkoop, 1971; Nakamura and Kishiyawa, 1971; Gimlich and Gerhart, 1984; Gimlich, 1985). Thus, at the blastula stage the precursors of the dorsal mesodermal cells could be instructed to eventually express MIB in the gastrula in the progressive patterns observed. However, the progeny of the three dorsal animal blastomeres at the 32-cell stage are mixed

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**Fig. 4.** Video frames from a recording of labeled, stage 10 prospective epidermal cells grafted to the prospective notochord region at gastrulation (stage 10-10.5). Below each frame a computer generated contour map outlines the labeled cells and a pen drawing outlines areas of interest. Anterior (a), posterior (p), lateral (l), medial (m). Scale bar: 50 μm.
extensively by the mid gastrula stage (Bauer et al., 1994; Vodicka and Gerhart, 1995), making it unlikely that the cells could still retain any detailed spatial pattern of progressive behavior imposed on them many stages earlier. Although general properties of the mesoderm may be induced in the blastula stage, the detailed patterns of motility and differentiation must be determined by signals operating during gastrulation.

Our results strongly support this contention. Labeled cells, grafted from the prospective notochordal, somitic and epidermal regions of the gastrula into the notochordal region of explants adopted anterior-to-posterior and lateral-to-medial progressions of both MIB and notochord differentiation, regardless of their placement within the notochord field or their original prospective fate. In the case of prospective notochord cells, some prior instruction may have been received at the blastula stage, but these instructions were over-ridden and the specific spatial and temporal expression of MIB was acquired after the cells were grafted, during gastrulation. In the case of prospective epidermal and somitic cells, these non-notochordal cells were induced to become notochord during gastrulation, showing that the notochordal region can not only organize the motility of notochordal cells, but it can induce other tissue types to become notochord and express notochordal cell behaviors. Although somitic cells also undergo a form of MIB on their own accord (Shih and Keller, 1992b), when grafted to the notochordal region, they expressed MIB according to the temporal and spatial patterns of the notochord. Moreover, prospective epidermal cells, which never express MIB in their original environment, were induced to adopt MIB and notochord differentiation in the same progressive manner as the host notochord cells. Therefore, the grafted cells are responding to notochord-inducing and organizing signals present during gastrulation.

**Why organize mesodermal cell behavior in spatially progressive patterns?**

The spatial and temporal progressions of cell behaviors are necessary for driving involution and blastopore closure. Involution of the IMZ is driven by the posterior progression of convergence on the inside of the blastoporal lip, thus pulling the IMZ over the lip without additional forces (Keller and Jansa, 1992; Keller et al., 1992a; Shih and Keller, 1992b). In fact, if the mediolateral continuity of the converging cells is broken, then involution and blastopore closure will fail, the notochord will extend across the yolk plug and the somitic mesoderm will extend around the blastoporal lip (Schechtman, 1942; Keller, 1981, 1984). The anteroposterior progression of MIB appears to be important in rolling the marginal zone inside, since in its absence all the cells within the marginal zone would adopt MIB concurrently and the IMZ would form a constricting band around the yolky endoderm, thus squeezing the yolk rather than involuting itself. This phenotype may be similar to that observed in lithium-treated embryos where massive convergent movements occur concurrently throughout the marginal zone to produce a prominent radial proboscis that consists of notochordal tissue (Kao and Elinson, 1988). Furthermore, this tissue differentiates as a synchronous mass rather than in an axial progression and, consequently, lacks the normal extended notochord morphology (Cooke and Smith, 1988).

**How are patterns of cell behavior related to gene expression?**

By the onset of gastrulation, the organizer is a specialized region expressing a number of mRNAs in specific patterns, including *goosecoid* in the prechordal mesoderm (Cho et al., 1991), *noggin* in both the deep and epithelial layers of the organizer (Smith and Harland, 1992), Xnr 3 in the epithelial layer of the organizer (Smith et al., 1995), Xnot in the deep and epithelial layers of the prospective notochordal region (von Dassow et al., 1993), Xbra in all the preinvoluval axial mesoderm and in the postinvoluval, notochordal mesoderm (Smith et al., 1991), and X-lim1 in the organizer and prechordal mesoderm (Taira et al., 1992). All are thought to be important in the function of the organizer or the adjacent regions of the dorsal mesoderm, but their patterns of expression do not directly reflect the progressive pattern of...
cell behaviors described above (Domingo, 1995). Therefore, the expression of these molecules, which span all or part of the dorsal marginal zone, may act upstream of the immediate control mechanism responsible for the progression of cell behaviors.

**Early and late signals in mesoderm induction and organization**

How are the signals organizing the progression of notochord cell behaviors in the gastrula related to mesoderm-inducing signals in the blastula stage? There is evidence from induction experiments with animal caps, and from the use of dominant negative receptor constructs, that mesoderm induction may involve activin (Smith, 1987; Symes and Smith, 1987; Hemmati-Brivanlou and Melton, 1992), FGF (Kimelman and Kirschner, 1987; Slack et al., 1987; Amaya et al., 1991), noggin (Smith et al., 1993) and Vg1 (Thomsen and Melton, 1993). Although these molecules and signaling systems are involved in the early induction of the organizer, some also appear to be involved in the later, progressive organization of cell behavior and terminal differentiation. For example, the expression of a mutated FGF receptor that has a truncated intracellular kinase domain that prevents signaling, blocks the induction of posterior axial mesoderm and impedes normal gastrulation movements (Amaya et al., 1991). Recently, XeFGF has been shown to be important during gastrulation for the maintenance of Xbra expression in the postinvolution notochord (Isaacs et al., 1994). In addition, dorsal blastopore lips treated at gastrula stages with suramin, an agent that interferes with the action of growth factors, do not form notochord (Grunz, 1993). These experiments suggest that notochord formation requires signals present during gastrulation. Thus, these growth factor signaling systems may function in organizing the progression of cell behaviors in the gastrula, as well as inducing the general pattern of mesoderm formation in the blastula.

**Sources, range and direction of the organizing signals**

The first expression of both MIB and cell differentiation occurs anteriorly, near the prechordal mesoderm, and laterally, along the notochordal-somatic boundaries, seen both here and previously (Shih and Keller, 1992b), suggesting that these regions are the sources of the signals. Furthermore, the propagation of these signals is directional, acting posteriorly, from the prechordal mesoderm and medially from the notochordal-somatic boundaries. If the notochordal-somatic boundary were a symmetrical source of notochord-organizing signals, then cells in the prospective somitic region would respond by making notochord, and they do not. However, cells grafted from the prospective somitic region can respond to notochord-organizing signals when placed medial to the boundaries.

These signals also appear to be of limited range. Mechanically limiting convergence restricts expression of MIB and Tor 70 to regions near the notochordal-somatic boundaries. Perhaps the cells in the posterior-medial region, which do not differentiate under these conditions, are unhealthy. However, in many explants, a third notochordal-somatic boundary forms and the cells of this region, which now lie adjacent to the new boundary, do form notochord.

Little is known about the possible signals in the notochordal-somatic boundary at the gastrula stages. The boundary region contains a B1 chain of laminin at stage 12.5 (late-gastrulation) (Fey and Hauser, 1990) which is some time after the boundary begins forming at stages 11-11.5 (mid-gastrulation). The boundary may also contain heparan sulfate proteoglycan (HSPG), which has been shown to concentrate and deliver
basic FGF to the high-affinity FGF receptor (Yayon et al., 1991). Disruption of this matrix molecule with heparitinase affects both mesoderm formation and gastrulation movements in *Xenopus* (Brickman and Gerhart, 1994; Itoh and Sokol, 1994).

Cell-cell contacts may also be important for propagating the signals. Cells adopting MIB are invariably next to ones that are already expressing MIB. Furthermore, increasing the area of contact between grafted and host cells by breaking the clumps of grafted cells into individual cells, increased the likelihood of inducing their differentiation into notochord. These results explain a previous study that showed that large dorsal grafts to early gastrulae retained autonomy for cell movements independent of the host environment (Cooke, 1972). These larger grafts maintain their microenvironment by exhibiting a community effect (Gurdon et al., 1993) and becoming less responsive to signals from host whereas smaller grafts lack a community effect and are more responsive to signals from the host.

**Signaling routes: neural and mesodermal induction**

If the prospective notochordal region of the gastrula can induce prospective...
epidermal cells to express notochord-specific cell behavior and to differentiate into notochord cells, why does it not induce the overlying prospective ectodermal cells to become mesodermal cells rather than neural cells? One possibility is that signals passing planarily within the notochord field are mesodermalizing while those passing vertically to the adjacent ectoderm are neuralizing. This is supported by our observation that individual cells and small groups of ectodermal cells were more likely to be embedded within the notochordal tissue and to differentiate as notochord, whereas large clumps, which were more likely to rest on the surface of the notochord, were also excluded from the notochord and formed neural tissue. Thus, we propose that the organizer delivers different signals in different directions. This is not a new idea; assimilative induction, which involves mesoderm-inducing mesoderm and requires planar interactions, whereas neural induction could involve both vertical and planar induction (Spemann, 1938; Keller et al., 1992b; Doniach et al., 1992). Since short-range signals are involved in induction of notochordal convergent extension, as discussed above, and in induction of neural convergent extension, as well (Keller et al., 1992b), we conclude that precise patterning, fine geometric features and timing of the induced morphogenetic behaviors in both the mesodermal and neural regions demand short-range signaling and response systems.

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