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Cells Remain Competent to Respond to Mesoderm-Inducing Signals Present during Gastrulation in *Xenopus laevis*

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During gastrulation, the vertebrate embryo is patterned and shaped by complex signaling pathways and morphogenetic movements. One of the first regions defined during gastrulation is the prospective notochord, which exhibits specific cell behaviors that drive the extension of the embryonic axis. To examine the signals involved in notochord formation in *Xenopus laevis* and the competence of cells to respond to these signals, we performed cell transplantation experiments during gastrulation. Labeled cells from the prospective notochord, somitic mesoderm, ventrolateral mesoderm, neural ectoderm, and epidermis, between stages 9 (pregastrulation) and 12 (late gastrulation), were grafted into the prospective notochord region of the early gastrula. We show that cells from each region are competent to respond to notochord-inducing signals and differentiate into notochordal tissue. Cells from the prospective neural ectoderm are the most responsive to notochord-inducing signals, whereas cells from the ventrolateral and epidermal regions are the least responsive. We show that at the end of gastrulation, while transplanted cells lose their competence to form notochord, they remain competent to form somites. These results demonstrate that at the end of gastrulation cell fates are not restricted within germ layers. To determine whether notochord-inducing signals are present throughout gastrulation, grafts were made into progressively older host embryos. We found that regardless of the age of the host, grafted cells from each region give rise to notochordal tissue. This indicates that notochord-inducing signals are present throughout gastrulation and that these signals overlap with somite-inducing signals at the end of gastrulation. We conclude that it is the change of competence that restricts cells to specific tissues rather than the regulation of the inducing signals. © 2000 Academic Press

Key Words: **cell fate; embryo; induction; gastrulation; morphogenesis; notochord; pattern formation; competence; *Xenopus laevis.***

INTRODUCTION

At the onset of development cells are pluripotent and can give rise to many different cell types. As development progresses cells become biased and eventually restricted to a single cell type. The progressive bias of cells toward a specialized cell type has long been the subject of extensive research. Mangold (1923) first showed that at the onset of urodele gastrulation, fragments of ectoderm were still capable of adopting steps toward forming mesodermal and endodermal organs. Spemann (1938) concluded that the cells of the urodele gastrula remained labile at the onset of gastrulation. More recently, cell transplantation studies in mice (Lawson *et al.,* 1991), chicks (Garcia-Martinez and Schoenwolf, 1992), and zebrafish (Ho, 1992) have shown that cells remain pluripotent during the morphogenetic movements involved in gastrulation.

Unlike observations made in other vertebrate systems, studies of cell competency in *Xenopus* have suggested that cell fates become restricted by the onset of gastrulation. For example, single-cell transplantations to the blastocoel cavity of *Xenopus laevis* blastulae and gastrulae showed that vegetal pole cells become committed to the endoderm (Heasman *et al.,* 1984) and animal pole cells become committed to the ectoderm (Snape *et al.,* 1987) at the onset of gastrulation. Similarly, in tests of mesoderm-inducing capabilities of activin and bFGF, Green (1990) found that animal cap cells lose their competence to form notochord

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prior to gastrulation (stage 9). These studies suggest that cells are restricted to a specific germ layer at the onset of gastrulation.

Our observations of gastrulation and the formation of the notochord suggested that prevailing theories of cell commitment and competence during gastrulation warranted reexamination. First, notochord formation is driven by a progression of stereotyped cell behaviors known as mediolateral intercalation behavior (MIB) (Shih and Keller, 1992a,b). MIB begins anteriorly and laterally and progresses posteriorly and medially within the prospective notochord region. This progression of cell behaviors suggests that signals are present during gastrulation to organize the step-by-step formation of the notochord (Shih and Keller, 1992b). Second, nonnotochordal cells grafted to the notochord region at the onset of gastrulation adopt MIB in the same progression as the host notochordal cells. These results suggest that notochord-organizing signals instruct the progression of cell behaviors and that these signals can induce nonnotochordal cells to differentiate into notochord (Domingo and Keller, 1995). We found that cells grafted from both the prospective epidermal and the somitic regions remained competent to change their original cell fate to notochordal at the onset of gastrulation. These results led to three questions: (1) What cell types are responsive to notochord-inducing signals during gastrulation? (2) When do these cells lose their competence to respond to notochord-inducing signals? (3) How long do notochord-inducing signals persist?

To determine which cell types are responsive to notochord-inducing signals, we grafted cells from various regions to the prospective notochord region of *X. laevis* gastrulae where they could be exposed to the full complement of signals responsible for notochord formation. Cells were grafted from the prospective somitic mesoderm, ventrolateral mesoderm, neural ectoderm, and epidermis into the prospective notochord region of host embryos. Grafted cells were assayed for notochord and somite differentiation in tadpoles. We found that cells grafted from all these regions were capable of differentiating into notochordal and somitic tissues. To evaluate the time course of their competency, we performed heterochronic grafts in which we varied the age of the donor cells. We show that these cells are not committed to a particular tissue type prior to gastrulation and remain competent to respond to notochord-inducing signals during gastrulation. By the end of gastrulation most grafted cells lose their ability to differentiate into notochordal tissue; however, they remain competent to differentiate into somitic tissue. These results indicate that cells are not restricted to a specific germ layer by the end of gastrulation and that they remain pluripotent during *Xenopus* gastrulation. These observations are consistent with observations made in other vertebrate embryos (Lawson *et al.,* 1991; Garcia-Martinez and Schoenwolf, 1992; Ho, 1992).

To evaluate the temporal sequence of notochord-inducing signals, we performed heterochronic grafts in which we varied the age of the host embryos. We show that notochord-inducing signals are present throughout gastrulation and likely persist into neurulation. Likewise, we find that somite-inducing signals are also present throughout gastrulation and possibly during neurulation. We conclude that recruitment of cells into a specific morphogenetic field is a matter of competing signals that can be discriminated by changes in cell competency.

MATERIALS AND METHODS

Preparation of Donor Cells and Hosts

X. laevis eggs were obtained, fertilized, and dejellied by standard methods (Kay and Peng, 1991). Host embryos were transferred to 33% modified Barth's solution (MBS) and cultured at 16°C until needed. Donor embryos were transferred into full-strength MBS containing 5% Ficoll and microinjected at the one-cell stage with a 33 mg/ml dilution of either fluorescein-dextran amine or rhodamine-dextran amine dissolved in 0.2 N KCl (Gimlich and Braun, 1985). The injected embryos were transferred to petri dishes coated with 2% agar and filled with 33% MBS and cultured at 16°C until needed.

Embryos were staged according to Nieuwkoop and Faber (1967). The prospective regions of the gastrula were identified according to the gastrula fate map (Keller, 1975, 1976, 1991).

Grafting of Labeled Cells

Grafting experiments were performed from the pregastrula (stage 9) through the late gastrula stages (stage 12) (Fig. 1). The dorsal midline of stage 9 embryos was identified by a Nile blue mark (Kay and Peng, 1991) made at the two-cell stage, when the pigmentation pattern of the animal hemisphere is paler on the dorsal side than on the ventral side (Vincent *et al.,* 1986). Donor and host embryos were transferred to modified Danilchik's solution (Danilchick *et al.,* 1991; Sater *et al.,* 1994) and vitelline envelopes were removed with forceps. Using hairloops and eyebrow hair knives, a small clump of about 30 cells was dissected exclusively from the deep cell layers of a labeled donor embryo; cells in the superficial layer were not used. To place donor cells into the prospective notochord, which lies in the deep region, an eyebrow hair knife was used to make a small incision through the superficial endodermal layer. With the tip of an eyebrow hair knife, a clump of about 30 labeled cells was inserted into the prospective notochord. This procedure was repeated using cells dissected from the prospective epidermis (EP), neural ectoderm (NE), notochord (N), somitic mesoderm (SM), and ventrolateral mesoderm (VLM) at various stages of gastrulation (Fig. 1).

Experimental Design

Two types of experiments were performed. The first consisted of grafting progressively older gastrula cells (stages 9 through 12) into early gastrulae (stage 10) to reveal the competence of different regions of the gastrula to differentiate into notochord (Fig. 2A). The second consisted of grafting cells from early gastrulae (stage 10) to host embryos at progressively more advanced stages of gastrulation (stages –10 through 12) to determine whether notochord-inducing signals persist throughout gastrulation (Fig. 2B).

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We performed a third type of experiment to confirm the predicted fates of the donor cells (Fig. 2C). This technique consisted of dissecting small pieces of rhodamine-labeled tissue from the prospective somitic mesoderm, ventrolateral mesoderm, neural ectoderm, and epidermal regions of gastrulae (stage 10). Each piece of tissue was divided into 10 small clumps of about 30 cells. Each clump was grafted either to the same region it came from (homotopic graft) or to the prospective notochord region (heterotopic graft) of host gastrulae (Fig. 2C).

In all cases, grafted embryos were transferred to agar-coated dishes containing 33% MBS. At tailbud stages (25–28), embryos were fixed in MEMFA (0.1 M Mops, pH 7.4, 2 mM EGTA, 1 mM $MgSO_4$, 3.7% formaldehyde) for 2 h at room temperature, washed in 100% methanol, and transferred to benzyl benzoate/benzyl alcohol (2:1) for viewing under a Nikon fluorescence microscope. The position and morphology of labeled cells with respect to the intact embryo were visualized. Some embryos were embedded in Paraplast and serially sectioned (10 μm) using a rotary microtome. Sections were mounted on microscope slides, dewaxed with xylenes, rehydrated through an ethanol series, and mounted in Aqua/Polymount (Polysciences).

Scoring and Analysis

Each embryo was analyzed to learn the fate of the labeled cells. Embryos between stages 22 and 30 were examined for the presence of labeled cells in the notochord. In addition, labeled cells were found in other regions, particularly in the somites. Labeled cells that had a "stack of coins" array, which appeared elongated parallel to one another in the mediolateral direction, contained vacuoles, and were located within the notochord were scored as giving rise to notochord. These morphological characteristics are *exclusive* to notochord cells (Keller *et al.*, 1985). Labeled cells that adopted the characteristic anteroposterior orientation and morphology of myotome cells were scored as giving rise to somitic tissue (Hamilton, 1969). At the tailbud stages, these morphological characteristics are unique to each tissue type and not easily confused (see Results, below). If cells were found in both the notochord and the somites, the graft was counted in both the notochord and the somite categories. If a single labeled cell adopted the morphological criteria for a notochord and/or somite cell, then the embryo was scored as a positive data point. However, the majority of positive embryos had numerous labeled cells in the notochord and/or somites (see heterotopic graft, Table 2).

RESULTS

Identification of Regions during Gastrulation

Stages 9 through 12 which encompass most of gastrulation were examined in detail. The Keller vital dye fate maps (1975, 1976, 1991) and morphological landmarks were used to identify the relevant tissues (Fig. 1). Pregastrula embryos (stage 9) are characterized by the equatorial position of the involuting marginal zone (IMZ). At this stage, the blastocoel expands and epiboly displaces the IMZ from the equator to a subequatorial position by the onset of gastrulation, stage 10- (Keller, 1980). At stage 10-, the blastoporal pigment line forms dorsally as the prospective bottle cells constrict their apices and the prechordal mesoderm begins to involute. The prospective notochord is located in the deep region just above the pigment line. As the pigment line forms laterally and ventrally (stage 11), the leading edge mesoderm involutes and migrates across the blastocoel roof. The prospective SM and VLM begin to involute as well. By stage 12, most of the IMZ has involuted and the blastopore is nearly closed (for details see Keller, 1991).

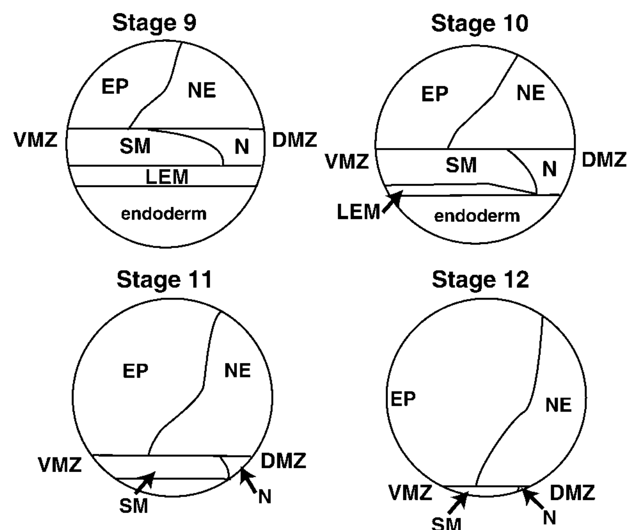

FIG. 1. Fate maps of the interior show the prospective tissues for the pregastrula (stage 9), early gastrula (stage 10), mid gastrula (stage 11), and late gastrula (stage 12). These fate maps and nomenclature are based on Keller (1975, 1976, 1991). The prospective areas include EP, epidermis; NE, neural ectoderm; N, notochord; SM, somitic mesoderm; VLM, ventrolateral mesoderm; DMZ dorsal marginal zone; and LEM, leading edge mesoderm.

Nonnotochordal Cells Are Competent to Respond to Notochord-Inducing Signals throughout Gastrulation

Cells were grafted from labeled embryos at stages 9, 10, 11, and 12 to the notochord region of host embryos at stage 10 (Fig. 2A). To confirm the original fate of the donor cells a combination of homotopic and heterotopic grafts was performed at stage 10 (Fig. 2C). For all grafts the fate of the labeled cells was examined at tailbud stages. Embryos were scored according to whether the grafted cells gave rise to notochordal tissues and/or somites. We will describe the results according to the origin of the transplanted cells.

Notochord grafts. To control for our effectiveness in grafting cells into the prospective notochord region, we grafted cells from the prospective N region of a labeled embryo to the notochord region of an unlabeled host at the onset of gastrulation (stage 10). Grafts into 72 embryos yielded labeled cells in 69% of the host notochords (Fig. 3A). Sections of some of these embryos confirmed that the labeled cells had differentiated into notochord (Fig. 3B). The donor cells intercalated between the host cells, adopted the

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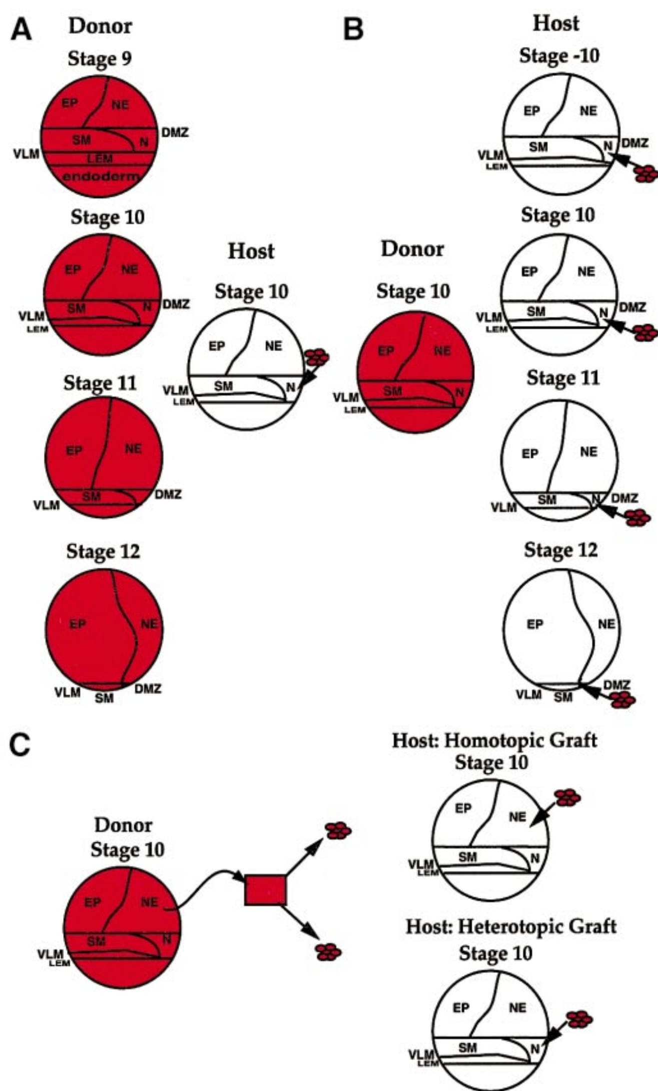

FIG. 2. Experimental design. (A) Embryos were injected with either fluorescein or rhodamine dextran at the single cell stage. At stages 9 through 12, approximately 30 cells were grafted from the deep cell layers of the N, SM, VLM, NE, and EP to the prospective notochord (N) region of unlabeled, stage 10 embryos. (B) Approximately 30 cells were grafted from fluorescently labeled embryos at stage 10 to the prospective notochord region of embryos at stages 10 through 12. (C) A piece of tissue was dissected from the SM, VLM, NE, and EP regions of rhodamine-labeled gastrulae (stage 10). Small clumps of about 30 cells were then grafted either to the same region they came from (homotopic graft) or to the prospective notochord region (heterotopic graft) of host gastrulae. Five homotopic and five heterotopic grafts were performed using the same piece of donor tissue. In all cases, the grafted embryos developed to tailbud (stages 22 to 28) and the fate of the grafted cells was determined.

characteristic stack of coins array along the length of the notochord and formed vacuoles (Keller, 1985). These morphological characteristics are *unique* to notochord cells; no other cell types form vacuoles at tailbud stages. We repeated this experiment using donor cells from stages 9, 11, and 12. Cells grafted from stage 9 embryos differentiated into notochord cells in 57% of the embryos. Using stage 11 and 12 donors resulted in 90 and 75% of hosts containing labeled cells in the notochord, respectively (Table 1). In addition, differentiation into somitic tissue increased from 30% using donors at stage 10 to 42% using stage 12 donors.

Somitic mesoderm grafts. Grafts of labeled cells from the prospective SM region of stage 9 donors differentiated into notochord tissue in 69% of cases. Grafting cells from stage 10 embryos yielded labeled cells in the notochord in 51% of the cases and in the somitic tissue in 32% of cases (Table 1). Labeled cells in the notochord region adopted notochord-specific characteristics of the stack of coins array (Fig. 4A) and vacuoles (Fig. 4B). Cells grafted from stage 11 embryos differentiated into notochordal tissue in 44% of the cases and into somitic tissue in 35% of cases (Table 1). Grafted cells from progressively older donor embryos differentiated into somitic tissue in greater proportions (arrowhead, Fig. 4C), while still giving rise to notochord tissue (arrow, Fig. 4C). By stage 12, at the end of gastrulation, cells

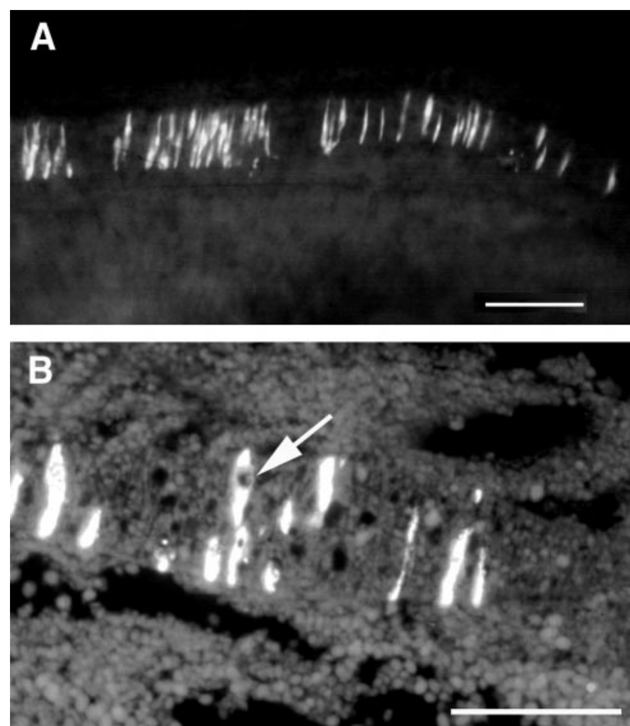

FIG. 3. Distribution of labeled cells grafted from the prospective notochord. Cells were grafted from a stage 10 donor into a stage 10 host. (A) A cleared whole-mount embryo (stage 27) with labeled cells scattered throughout the notochord. (B) A longitudinal section of a grafted embryo, showing that the grafted cells differentiated into notochordal tissue as indicated by the bipolar, elongated cell shapes and the formation of vacuoles (arrow). Scale bars, 100 μm.

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TABLE 1
Results from Cells Grafted to the Prospective Notochord of Stage 10 Embryos

Donor stage	Sample size	Notochord	Somite	Donor stage	Sample size	Notochord	Somite
9				10			
N	23	13 (57%)	7 (30%)	N	72	49 (69%)	4 (6%)
SM	51	35 (69%)	14 (28%)	SM	37	19 (51%)	12 (32%)
VLM	51	36 (71%)	12 (24%)	VLM	19	7 (37%)	9 (47%)
NE	47	43 (92%)	10 (21%)	NE	47	31 (65%)	7 (15%)
EP	45	29 (64%)	5 (11%)	EP	84	37 (44%)	9 (11%)
11				12			
N	51	46 (90%)	17 (33%)	N	12	9 (75%)	5 (42%)
SM	85	37 (44%)	30 (35%)	SM	11	0 (0%)	6 (55%)
VLM	58	11 (19%)	37 (64%)	VLM	31	3 (10%)	22 (71%)
NE	39	15 (39%)	8 (21%)	NE	34	3 (9%)	11 (32%)
EP	42	4 (10%)	1 (2%)	EP	10	0 (0%)	0 (0%)

from the prospective somitic region were unable to differentiate into notochordal tissue (Table 1). However, in 55% of these cases grafted cells differentiated into somitic tissue, suggesting that the grafted cells have become biased toward a somitic fate by stage 12.

To confirm that the transplanted cells come from a region that is fated to become somitic, a combination of homotopic and heterotopic grafts was performed using labeled cells from a single piece of tissue dissected from the prospective somitic mesoderm (Fig. 2C). Small clumps of cells were grafted to either the somitic mesoderm (homotopic graft) or the notochord region (heterotopic graft) of host gastrulae. In grafts to the prospective somitic region, labeled cells primarily differentiated into somitic tissue (SM homotopic graft, Table 2). Figure 4E demonstrates the distinct myotomal morphology adopted by the grafted cells. In all cases except individual IV, labeled cells were also found dorsal and/or ventral to the notochord; these numbers were included in the category "Total number of cells" (SM homotopic graft, Table 2). In one case (individual V), 30% of labeled cells were found in the notochord. Although labeled cells were found in several regions, the majority of the grafted cells differentiated into somitic tissue. When cells were grafted from the same donor piece of tissue to the notochord region of host gastrulae they differentiated into notochord tissue (SM heterotopic graft, Table 2) at a frequency similar to that described in Table 1. These results confirm that although the donor cells are fated to give rise to somitic tissue when grafted to the notochord region, they are competent to change their program and give rise to notochordal tissue.

Ventrolateral mesoderm grafts. Cells grafted from the VLM showed behavior similar to that of cells grafted from the somitic mesoderm. Prior to gastrulation (stage 9), cells grafted from the VLM to 51 hosts yielded labeled cells in the notochord in 71% of cases. As gastrulation proceeded the percentage of cases showing differentiation into notochord decreased to 37% using cells from stage 10 donors and to 19% using stage 11 donors (Table 1). In these embryos labeled cells were found in the notochord (see arrow, Figs. 5A and 5B) and in the matrix-filled region that separates the notochord from the adjacent somites (see arrowhead, Figs. 5A and 5B) as well as in the somites. When cells were grafted from stage 12 embryos, cells differentiated into notochordal tissue in only 10% of the cases (Table 1). However, the majority of the grafted embryos (71%) contained labeled cells within the somites.

To confirm the origins of our donor cells, labeled cells were grafted from the VLM to the VLM of host gastrulae. Cells from the VLM are thought to give rise to ventral blood islands and posterior somites (Lane and Smith, 1999). In all five cases labeled cells were found scattered around the posterior and ventral region of the tailbud embryo (VLM homotopic graft, Table 2). Figure 5D shows the typical scattered-like pattern of labeled cells in these grafts, which likely represents blood islands. In one case (individual I), labeled cells were also present in the somites and in another case (individual IV), labeled cells were present in the ventral epithelium. Together these results show that donor cells from the VLM give rise to ventral cell types as predicted by the Keller (1991) fate map. To confirm that cells from the same piece of VLM tissue can differentiate into notochordal tissue, we grafted a subset of cells from the same donor tissue to the notochord region of host gastrulae. In two of the four cases we found that labeled cells differentiated into notochordal tissue (VLM heterotopic graft, Table 2). The frequency observed in this experiment is similar to that reported in Table 1. Thus, although cells from the VLM are not programmed to give rise to notochordal tissue when transplanted to the prospective notochord, a subset of cells is capable of changing its fate and differentiating into notochord cells.

Neural ectoderm grafts. Cells grafted from the NE differentiated into notochord cells throughout gastrulation

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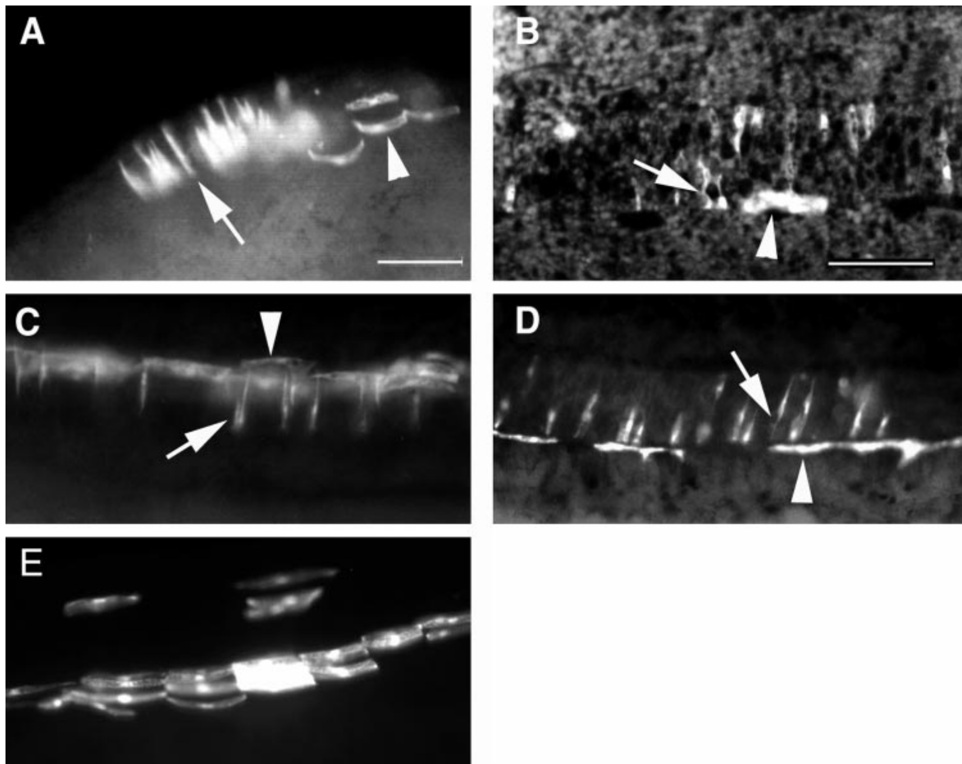

FIG. 4. Distribution of labeled cells grafted from the somitic mesoderm. (A) Cells were grafted from a stage 10 donor into a stage 10 host. A cleared whole-mount embryo shows that the grafted cells differentiated into notochord (arrow) and somitic (arrowhead) tissues. (B) A longitudinal section of a grafted embryo from the same experiment, showing that the grafted cells differentiated into notochordal tissue as shown by the presence of vacuoles (arrow). A couple of labeled cells are also found within the notochordal–somitic boundary (arrowhead). (C) Cells were grafted from a stage 11 donor to a stage 10 host. A cleared whole-mount embryo shows that the labeled cells have differentiated into notochordal (arrow) and somitic (arrowhead) tissues in about equal proportions. (D) Cells were grafted from a stage 10 donor to a stage 11 host. A cleared whole-mount embryo shows labeled cells that have differentiated into notochordal tissue (arrow) while other labeled cells are positioned ventrally to the notochord, within the notochordal–somitic boundary (arrowhead). (E) Cells were grafted from a stage 10 donor to the somitic region of a stage 10 host. A cleared whole-mount embryo shows that labeled cells have differentiated into somitic tissue. Scale bars, 100 μm.

(Table 1). Cells grafted from the NE at stage 9 to the prospective notochord region differentiated into notochord cells in 43 (91%) of the 47 grafted embryos. This frequency declined to 65% using stage 10 donors to 39% of cases using stage 11 donors and to 9% of cases with stage 12 donors. Cells grafted to the notochord region adopted distinct notochord characteristics such as vacuolation (arrow, Figs. 6A and 6B). Grafted cells from stage 12 donors were found more frequently in the floor plate of the neural tube and in the matrix-filled boundary between the notochord and the somites (Figs. 6C and 6D). Cells grafted from stage 10 embryos were found in the somitic tissue in 15% of cases. This frequency increased to 32% of cases using cells grafted from stage 12 donors.

To confirm the fate of the donor cells, we performed a homotopic graft in which cells were grafted from the prospective NE of a labeled donor gastrula to the same cite of unlabeled host gastrulae (Fig. 2C). Our results show that in every case, the donor cells differentiated into neural tissue (NE homotopic graft, Table 2). In fact, labeled cells were never found in any mesodermal tissue. Figure 6E shows labeled cells localized to the neural tube and some of the neurons formed long axonal projections (see arrow). As expected, when cells were grafted from the same donor piece of NE tissue to the prospective notochord region of host gastrulae, a proportion of the grafted cells differentiated into notochordal tissue in all five embryos (NE heterotopic graft, Table 2). These results clearly demonstrate that although the donor NE cells are fated to differentiate into neural tissue, when grafted to the prospective notochord region they can change their fate and differentiate into notochordal tissue.

Epidermal grafts. Cells grafted from the prospective EP region at stage 9 differentiate into notochord cells as frequently as cells from the prospective notochord region (Table 1). This frequency declines more rapidly for EP cells

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TABLE 2
Cell Fates from Stage 10 Homotopic and Heterotopic Grafts

Donor tissue	Individual (No. of cells)	Homotopic graft[a] No. of differentiated cells		Individual (No. of cells)	Heterotopic graft (N) No. of differentiated cells	
		*SM	N		SM	N
SM	I (89)	31 (35%)	0 (0%)	VI (45)	0 (0%)	43 (96%)
	II (34)	32 (94%)	0 (0%)	VII (39)	8 (21%)	26 (67%)
	III (129)	61 (47%)	0 (0%)	VIII (34)	15 (44%)	17 (50%)
	IV (29)	29 (100%)	0 (0%)	IX (34)	3 (9%)	29 (85%)
	V (43)	27 (63%)	13 (30%)	X (57)	2 (4%)	23 (40%)
		*VLM	N		VLM	N
VLM	I (28)	18 (65%)	0 (0%)	VI (23)	22 (96%)	0 (0%)
	II (10)	10 (100%)	0 (0%)	VII (16)	6 (38%)	0 (0%)
	III (6)	6 (100%)	0 (0%)	VIII (83)	69 (83%)	8 (10%)
	IV (120)	37 (31%)	0 (0%)	IX (31)	18 (58%)	10 (32%)
	V (21)	21 (100%)	0 (0%)	X (0)		
		*NE	N		NE	N
NE	I (53)	53 (100%)	0 (0%)	VI (51)	0 (0%)	2 (4%)
	II (74)	74 (100%)	0 (0%)	VII (95)	23 (24%)	59 (62%)
	III (28)	28 (100%)	0 (0%)	VIII (37)	0 (0%)	18 (49%)
	IV (46)	46 (100%)	0 (0%)	IX (62)	4 (6%)	18 (29%)
	V (79)	79 (100%)	0 (0%)	X (87)	6 (7%)	81 (93%)
		*EP	N		EP	N
EP	I (49)	49 (100%)	0 (0%)	VI (44)	0 (0%)	0 (0%)
	II (36)	36 (100%)	0 (0%)	VII (51)	0 (0%)	23 (45%)
	III (84)	84 (100%)	0 (0%)	VIII (19)	0 (0%)	0 (0%)
	IV (99)	99 (100%)	0 (0%)	IX (25)	0 (0%)	0 (0%)
	V (67)	57 (100%)	0 (0%)	X (26)	0 (0%)	9 (35%)

Note. Small pieces of tissue were dissected from fluorescently labeled gastrulae. Each piece was subdivided into 10 small clumps of cells, which were then transplanted either to the same location they came from (homotopic) or to the notochord region (heterotopic) of host gastrulae. At the tadpole stage, the fates and the total number of the transplanted cells were determined. The total number of cells includes labeled cells found in categories not shown (see Results for descriptions of various cell fates).

[a],* Indicates the site of the homotopic graft.

than for cells grafted from other regions of the gastrula. EP cells grafted from stage 10 were found in the notochord region in 44% of cases (Table 1). Labeled cells in the notochord adopted the stack of coins array and vacuolation characteristic of notochordal tissue (arrow, Figs. 7A and 7B). Using stage 11 donor cells the frequency of labeled cells in the notochord declined to 10% of the cases. In the majority of cases grafted cells from stage 11 donors were found lying ventrolateral to the notochord either in the hypochord or within a matrix-filled region that surrounds the notochord (Figs. 7C and 7D). EP cells grafted from embryos at late gastrula (stage 12) did not differentiate into notochordal tissue (Table 1). Unlike cells from the other regions, EP cells grafted from embryos between the stages of 10 and 12 were infrequently found within the somites, but instead were found to lie in the matrix-filled region between the notochord and the somites.

Control grafts of prospective epidermal cells to the prospective epidermal region of host gastrulae were also performed (Fig. 2C). In all five cases labeled cells exclusively differentiated as epidermal tissue (EP homotopic graft, Table 2). Labeled cells were found in the epidermis of all five grafted embryos and showed distinct epidermal-like morphology (Fig. 7E). Cells grafted from the same donor tissue into the prospective notochord region of host gastrulae gave rise to notochord cells in two of the five grafted embryos (EP heterotopic graft, Table 2). In all cases labeled cells were also found scattered dorsal and ventral to the notochord, and in three of the five grafted embryos, labeled cells were in the somites (these cells are included under the

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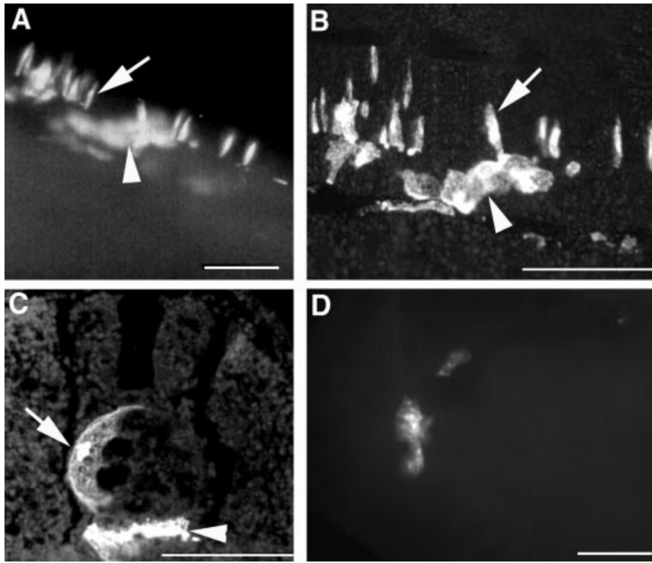

FIG. 5. Distribution of labeled cells grafted from the ventrolateral mesoderm. (A) Cells were grafted from a stage 10 donor to a stage 10 host. A cleared whole-mount embryo shows labeled cells both in the notochord (arrow) and ventral to the notochord (arrowhead). (B) This embryo was longitudinally sectioned and the labeled cells were found in the notochord showing the typical bipolar elongated cell shape (arrow). Cells excluded from the notochord did not show the bipolar cell shape (arrowhead). (C) Cells were grafted from a stage 10 donor to a stage 11 host. A transverse section shows the labeled cells within the notochord (arrow) and also a labeled cell ventral to the notochord (arrowhead). (D) Cells were grafted from a stage 10 donor to the VLM region of a stage 10 host. A cleared whole-mount embryo shows groups of labeled cells that are likely blood islands.

category "Individual (No. of cells)"). These experimental results show that cells grafted from the prospective epidermal region will give rise to epidermis; however, if grafted to the prospective notochord region, a certain population of cells can change its fate and differentiate as notochordal tissue.

Nonnotochordal Cells Lose Their Ability to Differentiate into Notochordal Tissue by the End of Gastrulation

Pregastrula cells grafted from the SM, VLM, NE, and EP were all capable of differentiating into notochordal tissue at the same frequency as cells that originated within the notochord. However, this pluripotency diminished at a relatively constant rate during gastrulation such that by the end of gastrulation, the grafted cells rarely populate the notochord (Table 1). As expected, cells grafted from the notochord region of late gastrulae continued to differentiate into notochordal tissue. Interestingly, as the grafted cells progressively lost their ability to differentiate into notochordal tissue, they differentiated into somitic tissue at increasingly higher frequencies with the exception of cells grafted from the EP (Fig. 8B). Of particular note is the fact that cells grafted from the NE at the end of gastrulation remain competent to give rise to somitic tissue. This result reveals that cell fates, in the case of the NE, are not even restricted to a specific germ layer by the end of gastrulation.

Notochord-Inducing Signals Are Present throughout Gastrulation

We now turn to the question of how long do notochord-inducing signals persist. To answer this question, we grafted cells from stage 10 embryos to the prospective notochord region of embryos from stages 10– to 12 (Fig. 2B). Stage 10– hosts were used as our starting point rather than stage 9 embryos because bottle cells have just begun to form and they are a more reliable indicator of notochord position than Nile blue marks made at the two-cell stage. The grafted embryos were assayed at tailbud stages for labeled

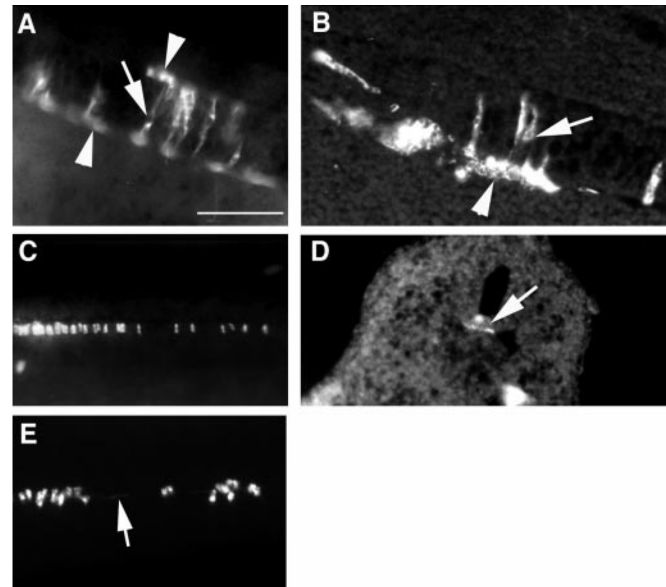

FIG. 6. Distribution of labeled cells grafted from the neural ectoderm. (A) Cells were grafted from a stage 10 donor to a stage 10 host. A cleared whole-mount embryo shows labeled cells in the notochord with vacuoles clearly visible (arrow). In addition, labeled cells are present both dorsal and ventral to the notochord (arrowheads). (B) A sectioned embryo from this experiment shows labeled cells that are elongated and vacuolated in the notochord (arrow). Ventral to the notochord are labeled cells that are more rounded and lack vacuoles (arrowhead). (C) Cells were grafted from a stage 12 donor to a stage 10 host. A cleared whole-mount embryo shows labeled cells in the floor plate of the neural tube. (D) A transverse section shows that the labeled cells are located to the floor plate of the neural tube (arrow). (E) Cells were grafted from a stage 10 donor to the neural ectoderm of a stage 10 host. A cleared whole-mount embryo shows labeled cells in the neural tube. The arrow points at the axonal projections extending from the labeled cells in the neural tube.

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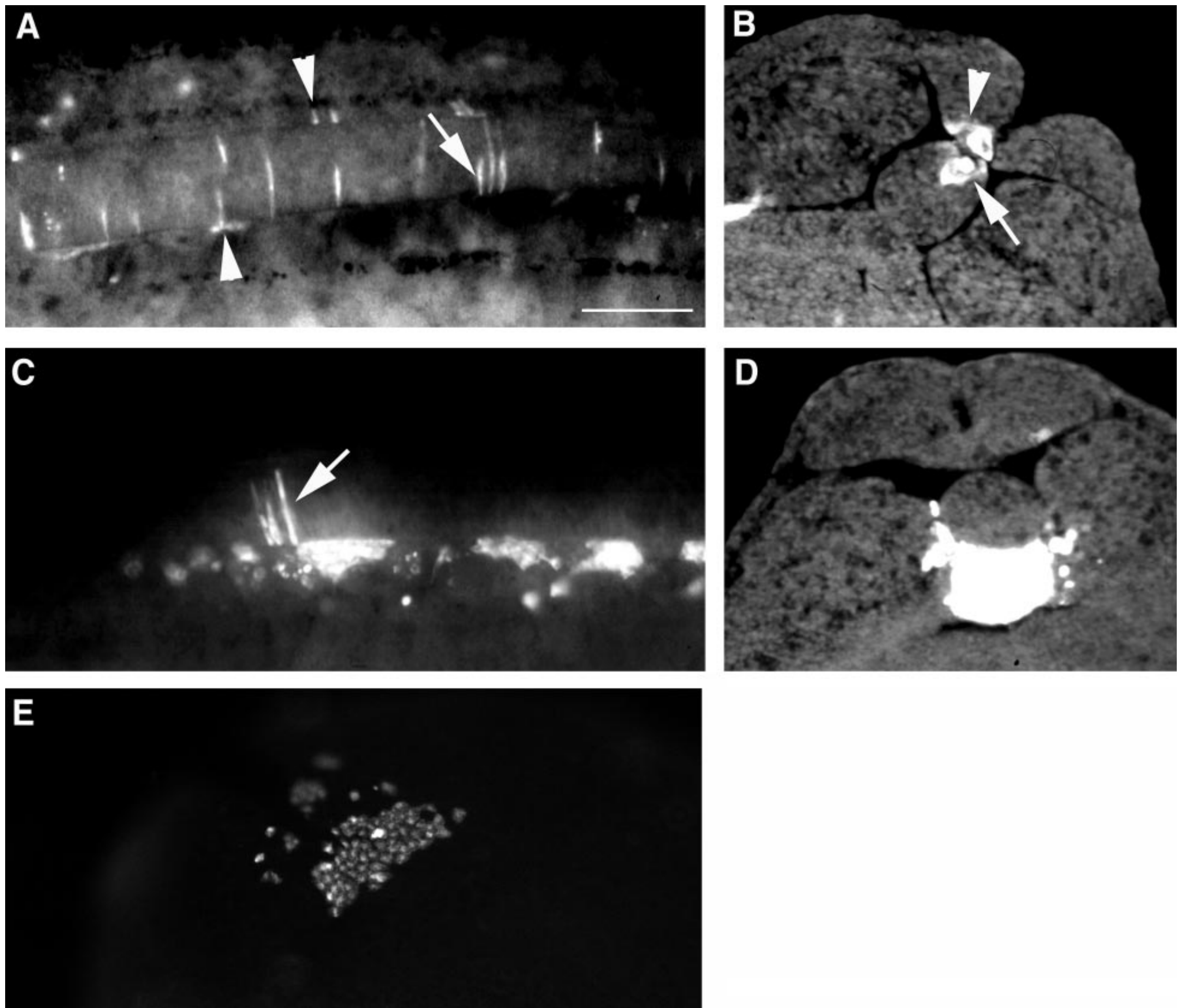

FIG. 7. The distribution of labeled cells grafted from the prospective epidermis. (A) Cells were grafted from a stage 10 donor to a stage 10 host. A cleared whole-mount embryo shows labeled cells within the notochord showing the stack of coins array (arrow). Some labeled cells are also present ventral to the notochord and dorsal to the notochord (arrowheads). (B) Transverse section through an embryo from this experiment shows labeled cells both in the notochord (arrow) and in the floor plate of the neural tube (arrowhead). (C) Cells were grafted from a stage 11 donor to a stage 10 host. A cleared whole-mount embryo shows a few labeled cells present in the notochord (arrow) and several cells ventral to the notochord. (D) A transverse section shows labeled cells positioned ventral to the notochord within the matrix-filled region surrounding the notochord and in the hypochord. (E) Cells were grafted from a stage 10 donor to the prospective epidermal region of a stage 10 host. A cleared whole-mount embryo shows labeled cells in the epidermis.

cells that differentiated into notochord tissue and/or somites.

Notochord grafts. Cells grafted from the prospective notochord region differentiated into notochord tissue at high frequencies regardless of the age of the host. Using stage 10– hosts, grafted cells differentiated into notochordal tissue in 64% of cases (Table 3). This frequency increased to 80% of cases using stage 11 hosts and decreased to 69% of cases using stage 12 hosts. The frequency in which grafted cells differentiated into somites increased over time from 0% of cases using stage 10– hosts to 47% of cases using stage 12 hosts (Table 3).

Somitic mesoderm grafts. Cells grafted from the SM to the prospective notochord region at all stages of gastrulation were able to differentiate into notochordal tissue to varying degrees. When grafted to the notochord region of stage 10– hosts, SM cells differentiated into notochordal tissue in 33% of cases, half the frequency observed in

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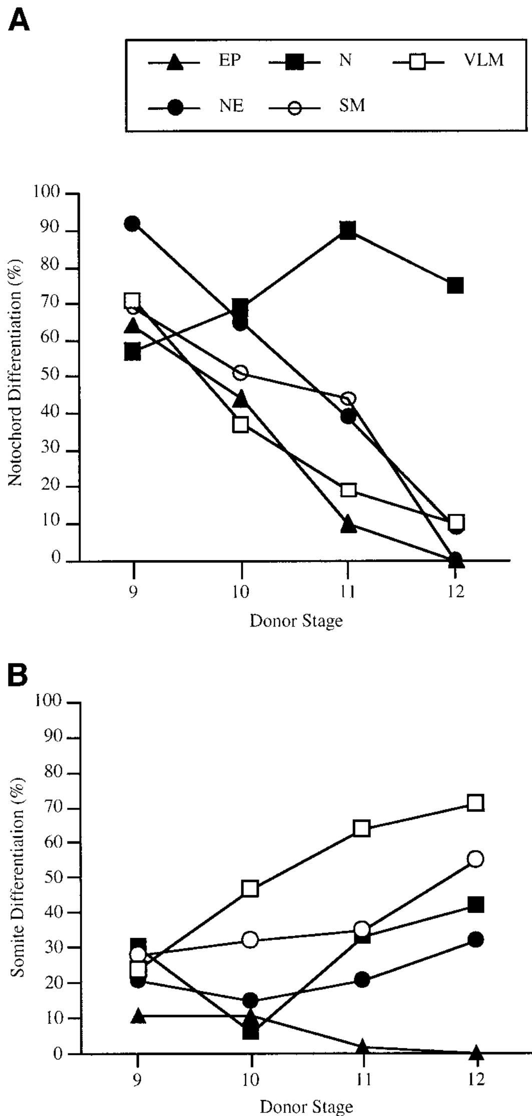

FIG. 8. Stage-dependent competence to form notochord and somites. (A) A line graph shows the percentage of cases in which grafted cells gave rise to notochord cells. The *y* axis represents the percentage of cases in which grafted cells differentiated into notochord cells. The *x* axis represents the age of the donor embryos. (B) A line graph shows the percentage of cases in which grafted cells gave rise to somitic cells. The *y* axis represents the percentage of cases in which grafted cells differentiated into somitic cells. The *x* axis represents the age of the donor embryos. EP, epidermis; NE, neural ectoderm; N, notochord; SM, somitic mesoderm; VLM, ventrolateral mesoderm.

comparable notochord cell grafts (Table 3). This frequency increased to 51% of cases using stage 10 hosts and to 68% of cases in stage 11 hosts. Labeled cells in the notochord clearly expressed notochord-specific characteristics such as vacuolation (arrow, Fig. 4D). Grafts to stage 12 hosts gave rise to notochordal cells in 21% of cases. On the other hand, the frequency at which the grafted cells differentiated into somitic tissue continued to rise from 22% of cases in grafts using stage 10– hosts to 90% of cases using stage 12 hosts (Table 3). In addition to finding more labeled cells in the somitic region as the stage of the host increased, we also found more labeled cells in the matrix region surrounding the notochord (arrowhead, Fig. 4D).

Ventrolateral mesoderm grafts. Similar to cells grafted from the SM, but to a lesser extent, cells grafted from the VLM can differentiate into notochordal tissue regardless of the age of the host (Table 3). Grafts to stage 10– hosts were found within the notochord in only 11% of cases. This frequency rose to 37% of cases using stage 10 hosts and declined to 25 and 21% of cases using hosts at stage 11 and 12, respectively. In stage 11 hosts, labeled cells were present in the notochord (arrow, Fig. 5C) as well as in the matrix region, ventral to the notochord (arrowhead, Fig. 5C). As observed among cells grafted from the SM, cells grafted from the VLM differentiate into somitic tissue at similar rates depending on the age of the host. For example, in stage 10– hosts, cells grafted from the VLM differentiated into somitic tissue in 38% of cases and this increased to 86% of cases in stage 12 hosts (Table 3).

Neural ectoderm grafts. Of all the nonnotochordal regions examined in these experiments, cells grafted from this region have the greatest capacity to differentiate into notochordal tissue (Table 3). In grafts performed on stages 10– and 10 hosts, labeled cells differentiated into notochordal tissue in 67 and 65% of cases, respectively, essentially the same frequency as cells grafted from the prospective notochord region. This trend is also observed with stage 12 hosts in which grafted cells differentiated into notochordal tissue in 75% of cases; a similar frequency is found in cells grafted from the prospective notochord (Table 3). Another commonality between cells grafted from the NE and the prospective notochord is the frequency with which they differentiate into somitic tissue. Cells grafted into stage 10– hosts do not differentiate into somitic tissue. However, when NE cells are grafted to stage 12 hosts, they give rise to somitic tissue in 52% of cases (Table 3).

Epidermal grafts. Similarly, cells grafted from the prospective EP region differentiated into notochordal tissue regardless of the age of the host. EP grafts to stage 10- hosts differentiated into notochord cells in 75% of cases. This frequency decreased to 44% of cases with grafts to stage 10 hosts. EP grafts to stage 11 and 12 hosts gave rise to notochord tissue at similar rates of 22 and 28% of cases, respectively (Table 3). Interestingly, grafts to stage 12 hosts were found in the somitic tissue in 58% of cases, a significant increase over the 14% of cases with grafts to stage 11 hosts (Table 3).

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TABLE 3
Results from Cells Grafted from Stage 10 Embryos to the Notochord Region of Progressively Older Embryos

Host stage	Sample size	Notochord	Somite	Host stage	Sample size	Notochord	Somite
−10				10			
N	11	7 (64%)	0 (0%)	N	72	49 (69%)	4 (6%)
SM	9	3 (33%)	2 (22%)	SM	37	19 (51%)	12 (32%)
VLM	8	1 (11%)	3 (38%)	VLM	19	7 (37%)	9 (47%)
NE	9	6 (67%)	0 (0%)	NE	47	31 (65%)	7 (15%)
EP	8	6 (75%)	4 (50%)	EP	84	37 (44%)	9 (11%)
11				12			
N	15	12 (80%)	2 (13%)	N	32	22 (69%)	15 (47%)
SM	31	21 (68%)	13 (42%)	SM	19	4 (21%)	17 (90%)
VLM	52	13 (25%)	9 (17%)	VLM	28	6 (21%)	24 (86%)
NE	35	18 (51%)	8 (23%)	NE	44	33 (75%)	23 (52%)
EP	59	13 (22%)	8 (14%)	EP	50	14 (28%)	29 (58%)

Notochord-Inducing Signals Persist throughout Gastrulation

Cells grafted from the prospective EP, NE, SM, and VLM were able to differentiate into notochord cells to varying degrees regardless of the age of the host embryo. Cells grafted into stage 12 hosts differentiated into notochordal tissues in at least 21% of cases with cells grafted from the SM and VLM and at most in 75% of cases with cells grafted from the NE (Fig. 9A). Cells grafted from the NE were just as likely to differentiate into notochordal tissue as cells grafted from the notochord. These results indicate that notochord-inducing signals are present at the end of gastrulation and most likely persist into neurulation. Interestingly, as gastrulation progresses grafted cells are more likely to differentiate into somitic tissue (Fig. 9B). Cells grafted into the prospective notochord region of stage 12 hosts differentiated into somitic tissue in at least 47 to 52% of cases with cells grafted from the notochord and NE, respectively, and at most in 86 to 90% of cases with cells grafted from the VLM and SM, respectively. These results indicate that signals involved in somite differentiation are also active at the end of gastrulation.

DISCUSSION

Evaluation of Cell Competence during Gastrulation

Meaningful evaluation of cell competence requires that the cells under examination be exposed to the normal tissue-inducing signals. Previous experiments examining the competence of cells during the blastula and gastrula stages suggested that the germ layers of *X. laevis* are determined by the onset of gastrulation (Heasman *et al.,* 1984; Snape *et al.,* 1987). The approach of these papers was to transplant single cells to the blastocoel cavity where grafted cells were likely exposed to an undefined combination of signals, ranging from the advancing pharyngeal endoderm to the leading edge of the mesoderm. In this context, negative results could lead to concluding the lack of cell competence, whereas, in fact, the cells may not have been exposed to the proper inducing signals and therefore their competence was not tested.

Other approaches in the study of cell competency have been to expose tissues to various concentrations of specific proteins. This can be done either by soaking tissue in media containing specific growth factors (Green *et al.,* 1990) or by overexpressing the RNA encoding the protein of interest. Using a hormone-inducible *Brachyury* construct, Tada *et al.* (1997) showed that animal caps remained responsive to *Brachyury* at gastrula stages as assayed by cardiac actin gene expression. However, since *Brachyury* alone cannot induce notochord tissue (Cunliffe and Smith, 1992), they were not able to assay the competence of animal caps to form notochord tissue. Although these approaches may hint at the potential effect that a single molecule or combination of molecules has on cell competency, they can not mimic the complexity of signals and the geometric context of the embryo's environment. Furthermore, these approaches assay competency by the activation of specific genes rather than by determining the final fate of the cell in the context of the embryo. By analyzing cell fate in the context of the embryo more rigorous measurements such as unique morphological characteristics (i.e., vacuolation) and position with respect to various tissues and organs can be used.

Measurements of cell competency are context dependent. The competence of the gastrula cells has not changed between our experiments and previous ones; the context in which the assay is performed has changed. In our approach, we evaluate cell competence in a more realistic way. Cells have been grafted into the environ-

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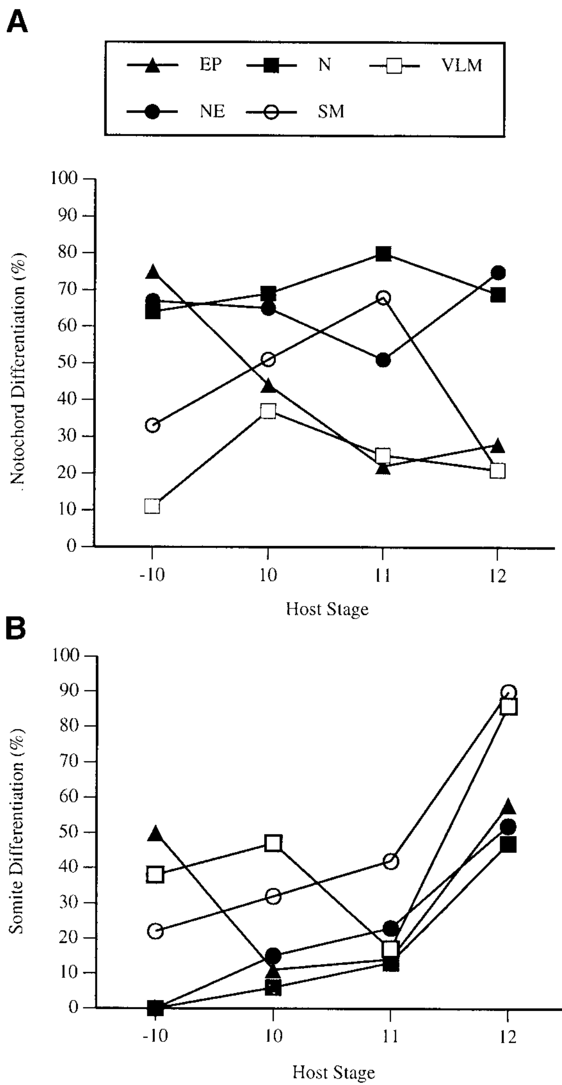

FIG. 9. Notochord-inducing signals persist through gastrula stages and overlap with somite-inducing signals throughout gastrulation. (A) A line graph shows the percentage of cases in which grafted cells gave rise to notochord cells. The *y* axis represents the percentage of cases in which grafted cells differentiated into notochord cells. The *x* axis represents the age of the host embryos. (B) A line graph shows the percentage of cases in which grafted cells gave rise to somitic cells. The *y* axis represents the age of the host embryos. EP, epidermis; NE, neural ectoderm; N, notochord; SM, somitic mesoderm; VLM, ventrolateral mesoderm.

ment in which a notochord is being made. Although the signals are unknown, presumably they are the relevant ones that are actually functioning to build a notochord. In this context, we show that cells throughout the gastrula remain competent to form notochord tissue until the end of gastrulation and somitic tissue into neurulation.

Gastrula Cells Remain Competent to Form Notochord Tissue through Most of Gastrulation

In this paper we grafted cells to a defined region, the prospective notochord, where we previously showed that notochord-organizing and -inducing signals are present at the onset of gastrulation (Domingo and Keller, 1995). We proposed that if a cell is transplanted into the proper context, and thus exposed to the full array of signals responsible for a particular cell fate, it would be more likely to respond by changing its fate. To examine cell competence we designed our experiments to include the following features: (1) the grafts consist of a small group of cells in order to avoid a "community effect" (Gurdon *et al.,* 1993) and to increase the likelihood that all or most of the cells are exposed to the inducing signals, (2) the grafts are placed directly within the putative signaling region, (3) the grafted cells are placed in an environment in which they can appropriately respond to the signals, and (4) heterochronic grafts are use to determine the time course of the potency of the inducing signal.

We grafted labeled cells from various regions of the gastrula into the prospective notochord region to specifically examine the competence of cells to respond to notochord-inducing signals present within the prospective notochord region during gastrulation. We performed a combination of homotopic and heterotopic grafts to show that cells are not restricted to a specific germ layer or cell fate during gastrulation. Homotopic grafts were first used to confirm the prospective fates of our donor cells. We show that in every homotopic graft, labeled cells differentiated into tissues as predicted by the Keller fate maps (1975, 1976, 1991). For example, cells grafted from the prospective epidermal region differentiated into epidermis when grafted to a similar site in a host embryo. These results confirmed that we were able to accurately predict the fate of cells at the gastrula stage based on the Keller fate maps (1975, 1976, 1991). Moreover, in all homotopic grafts except one (SM, Table 2), labeled cells never adopted notochord characteristics and were never present in the notochord region. Next, we performed heterotopic grafts in which cells were grafted from the same donor piece of tissue used in the homotopic grafts, but in this case were grafted to the prospective notochord region of host gastrulae. Results from the heterotopic grafts showed that cells grafted from the prospective SM, VLM, NE, and EP regions differentiate into notochordal tissue to varying degrees (Table 2). To determine whether these cells remain competent to differentiate into notochordal tissue throughout gastrulation, we performed additional grafting experiments in which we used progressively older donor tissue (Table 1). Our results show that cells throughout the gastrula remain competent to respond to notochord-inducing signals from midgastrulation (stage 11) until the end of gastrulation (stage 12) when they lose their competence to differentiate into notochord tissue. However, cells from the NE, VLM, and SM remain competent to differentiate into somitic tissue at the end of gastrulation (Fig. 8).

Live video imaging of cells has revealed that notochord

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differentiation is an active process (Domingo and Keller, 1995). Incompetent cells that are grafted into the notochord region will not adopt mediolateral intercalation behavior (Shih and Keller, 1996a). The inability to participate in the local cell behaviors will physically force incompetent cells to be excluded from the developing notochord. These incompetent cells are usually found within the matrix-filled boundary region between the notochord and the somites. This observation rules out the possibility that grafted cells are trapped within the developing notochord. Thus, all labeled cells that are found within the notochord have adopted MIB and have actively participated in the process of notochord cell differentiation as indicated by the presence of vacuoles, a unique characteristic found only in differentiated notochord cells.

The number of labeled cells found within the notochord varied from experiment to experiment. On average, our cell clumps contained about 30 cells. At the tailbud stage we counted an average of 50 cells, which suggests that a subset of labeled cells underwent a round of cell division. We observed that the number of grafted cells that differentiated into notochordal tissue in each embryo was proportional to the overall frequency observed in each experiment. In other words, when the frequency of cases was high each embryo contained a large number of labeled cells (20+ cells) in the notochord. The converse was also true. When the frequency of cases was low each embryo contained a smaller number of labeled cells (fewer than 5 cells) in the notochord. Thus, the overall rate in which cells differentiated into notochordal and/or somitic tissue reflects the number of labeled cells found in the respective tissues of each embryo.

The difference between our results and the previous studies is that in our experiments cells were grafted to the site where they were actively recruited into adopting MIB. The ability to participate in specific cell shape changes appears to play an important role in signaling cells to differentiate into notochordal tissue. A similar observation was made in a recent study of embryonic mesenchymal cells whereby Yang *et al.* (1999) showed that cell elongation is required for smooth muscle differentiation. In fact, changes in cell shape have been shown to activate specific genes (Rosette and Karin, 1995) as well as control cell growth and apoptosis (Chen *et al.,* 1997). By grafting cells to the notochord region where they could participate in the cell shape changes required for notochord differentiation, we have shown that the competency period can be extended significantly for cells within the ectoderm and mesoderm to the end of gastrulation.

Regional Differences in Changes of Competence to Form Notochord

Cells grafted from the NE were the most competent, of the nonnotochordal regions examined, to form notochordal tissue. This suggests that the prospective neural ectoderm is quite responsive to notochord-inducing signals. This may be due to the fact that cells from the NE, like cells from the notochord, undergo convergent extension movements to shape and pattern the neural plate (Elul *et al.,* 1997). Thus, the high frequency in which NE cells differentiate into notochordal tissue may relate to the fact that they are more capable of adopting MIB and integrating into the notochord than cells from any other nonnotochordal region. Interestingly, although prospective NE cells lose their ability to differentiate into notochordal tissue by the end of gastrulation, they remain competent to differentiate into somitic tissue. This suggests that the loss of competence among NE cells is tissue specific and not germ layer specific. Moreover, cells from the NE are not committed to the ectoderm by the end of gastrulation. Perhaps this pluripotency allows prospective NE cells to contribute to mesodermal tissues as the posterior axis extends. In support of this hypothesis, previous clonal analysis of A1 and B1 blastomeres revealed that extensive intermixing occurred among the progeny of these cells by the end of gastrulation (Vodicka and Gerhart, 1995). Thus, the final position of a cell at the end of gastrulation will define the fate rather than its ectodermal (A1) or mesodermal (B1) origin.

Prospective EP and VLM cells were the least likely to differentiate into notochordal tissue. This loss of competence could be due to a "ventralizing" signal present during gastrulation. A likely candidate for such a signal is the bone morphogenetic protein 4 (BMP-4), which is first detected at the onset of gastrulation (Dale *et al.,* 1992) and is expressed in the animal cap and ventral and lateral marginal zones during gastrulation (Fainsod *et al.,* 1994). BMP-4 protein has been shown to induce dissociated animal cap cells to differentiate into epidermal tissue (Wilson and Hemmati-Brivanlou, 1995). It is likely that prospective VLM and EP cells have been exposed to a BMP signal during gastrulation prior to transplantation into the notochord region and thus are well on their way to adopting a ventral mesodermal or epidermal cell fate.

Notochord-Inducing Signals Persist throughout Gastrulation and into Neurulation

The formation of the notochord involves a progression of cell behaviors that begins at gastrulation and continues into neurulation (Shih and Keller, 1992b). This progression of cell behaviors suggests that signals involved in notochord formation persist into neurulation. By grafting cells from the prospective SM, VLM, NE, and EP into the prospective notochord region of progressively older hosts, we show that notochord-inducing signals persist through gastrulation and into neurulation. Cells grafted from all these regions continued to differentiate into notochordal tissue even when grafted to host embryos at the end of gastrulation (Table 3). These results indicate that mesoderm induction continues into neurulation which extends mesoderm induction into much later stages than previously thought (Jones and Woodland, 1987). These signals may be involved in elongating the posterior axis at the end of gastrulation and during neurulation. Supporting this observation, Gerhart *et al.* (1989)

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showed that injection of suramin into gastrulae disrupted the binding of protein ligands to cell surface receptors such as FGF, which caused severe axis truncations. This experiment revealed that mesoderm-inducing signals as well as other signaling systems must be active during gastrulation for complete axis formation.

Somitic Mesodermal Organizing Signals Revealed in Late Gastrulation

In grafting cells to progressively older embryos, we observed a significant increase in the frequency in which grafted cells gave rise to somitic tissue. Cells grafted into stage 12 embryos showed a higher predisposition to give rise to somitic tissue than when grafted to stage 11 hosts, regardless of where the graft came from (Fig. 9). For example, cells grafted from either the prospective EP or VLM region to stage 11 hosts were most frequently found in the extracellular matrix surrounding the notochord. However, cells grafted from the EP and VLM to stage 12 hosts differentiated into somitic tissue in 58 and 86% of cases, respectively. One possibility is that as the prospective notochord field narrows (stage12), it produces a smaller target, increasing the likelihood that cells were grafted into the somitic region where they are exposed to somitic signals. However, this seems unlikely since cells grafted from the epidermal and ventral–lateral mesoderm regions differentiated into notochordal tissue at similar frequencies regardless of whether the hosts were at stage 11 or 12 (Table 3). Time-lapse videos of grafted nonnotochordal cells have shown that cells that do not participate in notochord cell behaviors are pushed out of the converging and extending notochord region and into the extracellular matrix between the notochord and the somites (Domingo and Keller, 1995). The key difference between stage 11 and stage 12 is that in grafts to stage 11 hosts the transplanted cells excluded from the notochord remain in the extracellular matrix, whereas in grafts to stage 12 host embryos these cells are found within the somites. A more likely possibility is that the grafts are accurately placed within the notochord region; however, depending on the presence of somite-inducing signals, the grafted cells will either stay in extracellular matrix or be recruited into forming somites. There are two possible explanations to address why cells grafted to stage 11 hosts are less likely to differentiate into somites than cells grafted to stage 12 hosts. One possibility is that somite-inducing signals become more prominent at the end of gastrulation. In this case, cells grafted to stage 11 hosts are less likely to encounter somite-inducing signals and, therefore, remain in the extracellular matrix. However, when cells are grafted to stage 12 hosts they are exposed to somite-inducing signals which have become more prevalent at this time. Supporting this observation, Kato and Gurdon (1993) found that signals present in the late gastrula were necessary for single presumptive somite cells transplanted to the blastocoel to differentiate into muscle cells. Another possibility is that somite-inducing signals are present throughout gastrulation, but they are not localized adjacent to the posterior notochord until the end of gastrulation. Thus, cells grafted at the onset of gastrulation are positioned more anteriorly and away from the somite-inducing signals. When cells are grafted to stage 12 hosts they will be positioned closer to the posterior somitic region where the somite-inducing signal is actively recruiting cells into forming somites. Thus, the timing and position of the grafted cells when they become excluded from the notochord may influence whether they will be exposed to somite-inducing signals and recruited into forming somites. We are currently grafting cells into different somitic regions to distinguish between these possibilities. Although we do not know the exact location of the somite-inducing signals, our results show that notochord-inducing signals and somite-inducing signals are both present throughout gastrulation.

Progressive Organization of Cell Behavior Is Necessary during Gastrulation

During gastrulation massive cell intercalation cause cells to be distributed along the entire anteroposterior axis (Keller and Tibbetts, 1989; Wilson *et al.,* 1989; Weliky *et al.,* 1991; Vodicka and Gerhart, 1995). At the onset of gastrulation the final position of a cell is not precisely predictable. Therefore, it seems unlikely that their participation in the process could be preprogrammed. Rather, it seems essential that cells be induced to participate in MIB progressively in an anterior to posterior order. For this to occur, the cells would have to remain responsive to a repertoire of signals during gastrulation.

In summary, our results show that the loss of competence of cells to respond to notochord-inducing signals restricts cells from differentiating into notochordal tissue at the end of gastrulation and that the notochord-inducing signal is not the limiting factor since it is present throughout gastrulation. In addition, these signals are temporally and spatially overlapping with somite-inducing signals. Thus, the picture emerging from this study is that cell recruitment into a specific morphogenic movement and tissue type is a matter of competing signals acting in fields of modulated competence. Moreover, changes in cell competence and strength of the inducing signals are a property of regional participation in morphogenesis, rather than a property of specific germ layers or tissue types.

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