

Commitment of mesoderm cells in Hensen's node of the chick embryo to notochord and somite

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Summary

Hensen's node in the chick embryo contains prospective notochord cells in a V-shaped midline region of the mesoderm, and prospective medial half somite cells in the lateral portions of the node mesoderm, whilst lateral somite cells are derived from the rostral part of the primitive streak (Selleck and Stern, *Development* 112, 615-626, 1991). In the present study, we have investigated the commitment of these mesoderm cells to their fates by grafting between these regions of the node and primitive streak. By challenging the cells in this way, we have attempted to discover whether prospective notochord and somite cells can change their fates.

We find that the mesoderm cells in the midline V-shaped portion of Hensen's node, when grafted into a different region, continue to integrate into the notochord, or form a separate notochord-like structure. By contrast, the prospective somitic cells do not appear to be committed to a somitic fate.

Key words: Hensen's node, somites, notochord, mesoderm, cell commitment, DiI, organiser.

Introduction

During embryonic development, cells become committed to their fates gradually, through a hierarchy of developmental decisions until they finally differentiate - that is, they acquire the morphological and biochemical characteristics of a particular cell type (see Slack, 1991). Little is known about the decisions made by cells early in the development of higher vertebrate embryos.

In addition to the ability of its cells to differentiate autonomously into a number of cell types (Hunt, 1931; Willier and Rawles, 1931; Viswanath and Mulherkar, 1972; Leikola, 1975, 1978; Veini and Hara, 1975), Hensen's node is considered to be the 'organiser' of the amniote embryo because it can induce the formation of an extra axis when grafted into a host embryo (Waddington, 1932, 1933; Waddington and Schmidt, 1933; Vakaet, 1965; Galla, 1971; McCallion and Shinde, 1973; Dias and Schoenwolf, 1990). Furthermore, when the node is grafted into the anterior margin of the developing limb bud, it can induce supernumerary digits (Hornbruch and Wolpert, 1986; Stocker and Carlson, 1990). These remarkable properties are not shared with any other region of the blastoderm.

Recently, the carbocyanine dye DiI and single-cell-labelling experiments have been used to produce a

detailed fate map of Hensen's node in the early chick embryo (Selleck and Stern, 1991). This study revealed that the node is spatially organised: at stage 4, there is a V-shaped region in the anterior midline which contains prospective notochord cells, and posterolateral regions whose deep portions contribute to the medial halves of the somites and whose dorsal portions contribute to the notochord. A region between the medial and lateral sectors contains cells whose progeny contribute to both notochord and somites. Since single cells in this region can populate more than one structure, these cells cannot be committed to either fate at this stage.

In order to investigate cell commitment, it is necessary to challenge the fates of cells by placing them in novel environments to see whether they behave according to their original position, or whether they now change their fates. In the present study, various microsurgical experiments were performed to test the state of commitment of the notochord and somite precursor cells in the medial and lateral parts of the node and in the rostral portion of the primitive streak, which contains cells that contribute to the lateral half of each somite. We find that the prospective notochord cells in the midline V-region of Hensen's node appear to be committed to become notochord, but prospective somite cells are not committed.

Materials and methods

Intranodal and primitive streak grafting of Hensen's node sectors

Fertile hens' eggs were incubated for 16 to 20 h to give embryos at definitive streak stage (stage 4; Hamburger and Hamilton, 1951). Host embryos were explanted by the technique of New (1955), modified as in Stern and Ireland (1981). Mounted A1 insect pins were used for all operations. A ring of outer *area opaca* was removed to prevent expansion from tearing open the graft site (Bellairs, 1963; Stern and Bellairs, 1984). The preparation was placed into a 30 mm plastic dish over a shallow pool of thin egg albumen and the culture placed into a humidified chamber at 38°C.

To ensure that the extirpation of the expansion margin itself did not lead to abnormal development, 12 control embryos at stage 4 were operated in this way and cultured for 12–18 h. Of the 12 embryos, 10 survived to stage 8–10. They appeared largely normal, although most were slightly stunted compared to unoperated embryos and often the rostral end of the neural tube was enlarged mediolaterally. All embryos had somites, although in 5 cases these were not well separated from each other, and were closely packed. Therefore, removal of an annulus of *area opaca* does not greatly perturb normal axis formation, in agreement with the findings of Stern and Bellairs (1984) who reported reduced rates of regression of Hensen's node and *area pellucida* elongation following this operation, but otherwise normal somite morphogenesis.

Donor embryos were explanted into phosphate buffered saline (PBS) and pinned out in dishes coated with Sylgard (Dow Corning, BDH). In most cases, the region to be grafted was labelled with the carbocyanine dye, DiI (see below). Different regions of the node and primitive streak were

dissected and the graft transferred to the host embryo where it was placed into the appropriate site.

The experiments and controls for the intranodal grafts are illustrated in Figs 1 and 2. In 'lateral-into-medial' grafting experiments (Fig. 1), the V-shaped midline sector of the node was replaced with a piece of lateral node. In control embryos ('medial-into-medial'), a V-segment from a donor embryo was grafted homotopically into a host. In 'medial-into-lateral' grafts (Fig. 2), the lateral portion of the node was replaced with a midline V-shaped portion from a donor, either unilaterally or bilaterally. Control embryos ('lateral-into-lateral') were grafted homotopically with lateral pieces of Hensen's node.

Primitive streak grafting experiments are illustrated in Fig. 3. In a series of 'medial-into-streak' experiments, midline V-shaped portions of the node were grafted into the rostral primitive streak. In another series ('lateral-into-streak'), the lateral sectors of the node were grafted into the rostral primitive streak. In yet another series ('streak-into-medial'), pieces of rostral primitive streak were grafted into the midline region of Hensen's node of a host. Controls for this set of experiments ('streak-into-streak') involved homotopic grafting of a portion of rostral primitive streak.

Operated embryos were grown for between 18 and 24 h, after which they were fixed in buffered 4% formal saline (pH 7.0).

Grafting of node segments into the segmental plate

Hens' eggs were incubated for 35–48 h to give host embryos with 10–19 somites. These were operated *in ovo*, as described previously (Stern and Keynes, 1987). A small hole was made in the vitelline membrane over the segmental plate region of the embryo. Using a needle, the ectoderm above a region of

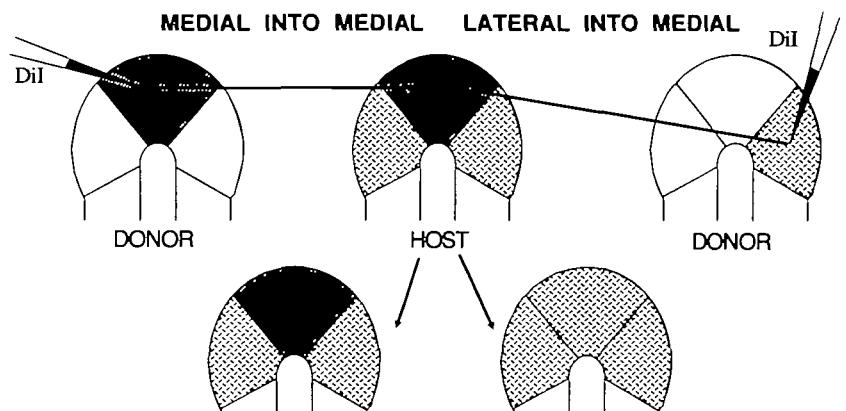


Fig. 1. Procedure for intranodal grafting experiments. The medial sector of Hensen's node is replaced with either a medial portion (controls) or lateral portion (experiments) from a donor embryo.

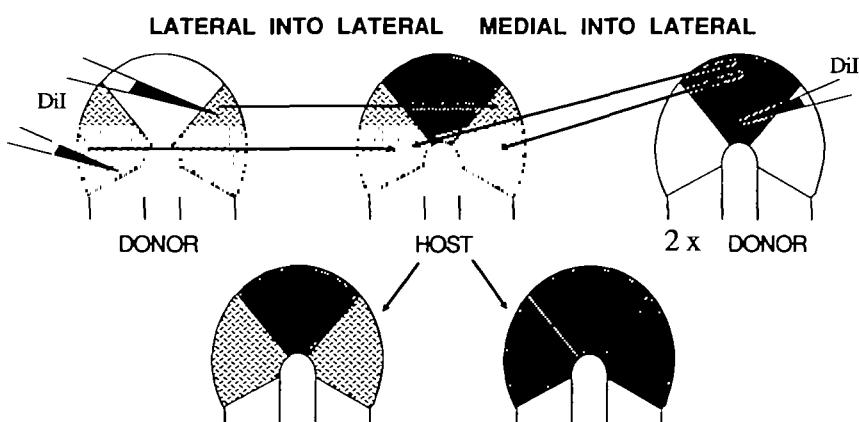


Fig. 2. Procedure for intranodal grafting experiments. The lateral sector of Hensen's node is replaced with either a lateral portion (controls) or medial portion (experiments) from a donor embryo.

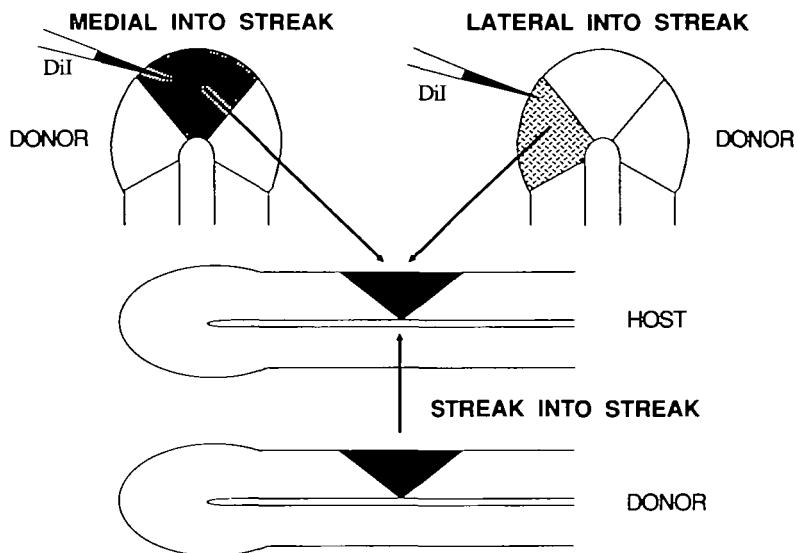


Fig. 3. Primitive streak grafting experiments. The top part of the diagram illustrates the experiments in which portions of the node are grafted into the rostral primitive streak. In controls (lower part of the Fig.), a portion of primitive streak is grafted homotypically into a host embryo.

segmental plate was reflected and a length of plate incised or evacuated to make room for the graft.

Using the same technique as for intranodal grafting experiments, sectors of Hensen's node, some of which had been labelled with DiI, were dissected from donor embryos and implanted into the evacuated region of the host segmental plate.

After the operation, the egg was sealed and a few drops of antibiotic/antimycotic (Sigma) added. The eggs were then incubated for a further 24 h at 38°C in a humid environment. After this time, the embryos were explanted into PBS, pinned out in Sylgard dishes and fixed in buffered formol saline.

DiI labelling

The method used has been described previously (Stern, 1990; Selleck and Stern, 1991). Briefly, microelectrodes were made using 50 µl Yankee Disposable Micropet capillary tubes (Clay Adams) or Clarke borosilicate electrode glass (1.5 mm outer diameter, with glass fibre), pulled with an Ealing vertical microelectrode puller. The electrodes were then filled with DiI (1,1'-dioctadecyl-3,3',3'-tetramethyl indocarbocyanine perchlorate; Molecular Probes): DiI was first dissolved at 0.5% in absolute ethanol and this diluted 1:9 with 0.3 M sucrose in distilled water at 40°C (see Serbedzija et al. 1990). By applying gentle air pressure, a bolus of dye was applied to the node region. DiI, a lipophilic carbocyanine dye, inserts into the membranes of the cells lying adjacent to the injection site (see Honig and Hume, 1989).

After incubation, the embryos were explanted and fixed in 0.25% glutaraldehyde in buffered 4% formol saline (pH 7.0), and examined in the whole mount (see below). Since DiI-labelled embryos cannot be sectioned directly, embryos had to be processed by photo-oxidation of 3,3'-diaminobenzidine (DAB) (Maranto, 1982; Buhl and Lübke, 1989; Stern, 1990; Selleck and Stern, 1991). Embryos were removed from the fixative and rinsed twice in 0.1 M Tris (pH 7.4), each for 1 h. They were then placed in 500 µg ml⁻¹ DAB in Tris. The specimens were then illuminated at the excitation wavelength (547 nm) until no fluorescence remained visible. After this, the embryos were rinsed three times in Tris, dehydrated and embedded in Paraplast. Embryos were then sectioned at 10 µm, dewaxed, hydrated and mounted in Hydromount (BioRad) and examined.

Immunostaining with the Not1 antibody

Whole-mount staining

A few embryos from the intranodal, primitive streak and segmental plate grafting experiments were stained with the antibody Not1 (a kind gift of Dr Jane Dodd, Columbia University), specific for notochord in chick embryos (Yamada et al., 1991).

Following incubation, embryos were explanted into PBS, fixed in 4% buffered formol saline (pH 7.0) for 1 h and rinsed in PBS. Endogenous peroxidase activity was blocked with 0.25% hydrogen peroxide for 2–3 h. The specimens were then washed in PBS and PBT (PBS containing 0.2% bovine serum albumin [BSA], 1% Triton X-100 and 0.01% thimerosal) and then finally with PBT containing 5% heat-inactivated goat serum. Not1 supernatant was added 1:1 and the embryos incubated overnight at 4°C. Specimens were washed thoroughly in PBT and PBT with goat serum, and then placed in a 1:200 dilution of peroxidase-conjugated goat anti-mouse IgG (Jackson) overnight at 4°C. Following several washes in PBS and 0.1 M Tris (pH 7.4), peroxidase activity was revealed by placing embryos into 1 mg ml⁻¹ DAB (3,3'-diaminobenzidine tetrahydrochloride [Aldrich]) in Tris with 0.001% H₂O₂. Embryos were then dehydrated up an alcohol series, cleared in xylene and mounted in DePeX (BDH).

Staining of frozen sections

Specimens were embedded in 7.5% gelatin (Sigma, '300 Bloom') in 15% sucrose in PBS, prior to cryostat sectioning at 10 µm. Sections were de-gelatinised and washed in PBS, and then incubated with Not1 supernatant (1:1 in PBS containing 0.2% Triton X-100) for 2 h at room temperature. Following several washes in PBS containing 1% heat-inactivated goat serum, the sections were incubated in a 1:100 dilution of peroxidase-conjugated goat anti-mouse IgG (Jackson) for 3 h. Following thorough washing, peroxidase activity was revealed by 0.5 mg ml⁻¹ DAB in Tris containing 0.0003% H₂O₂. Sections were washed thoroughly in water and mounted in Hydromount.

Examination of the embryos

All embryos were examined as whole mounts and photographed with Kodak TMAX 100 film. In addition, DiI-

labelled embryos were viewed with an Olympus Vanox-T microscope with epifluorescence optics (rhodamine filter set) and photographed with Kodak TMAX 400 or Fuji 1600P film. Dil-labelled embryos that had been photo-oxidised and sectioned were examined using Nomarski optics and photographed with Kodak TMAX 100 or Technical Pan film.

Results

A total of 234 experiments were performed, of which 184 survived a 24 h incubation period.

Intranodal grafts

In this set of experiments, a total of 135 operations were performed, of which 95 survived (70%). The results are summarised in Table 1.

Lateral-into-medial grafts

Lateral-into-medial grafts (Fig. 1) were performed to investigate whether all cells of the lateral sector (including presumptive somitic cells) contribute to notochord when placed into a region that contains cells destined only for notochord. Graft-derived cells were found in the notochord in all cases (Fig. 4A,B), and in somite in only 13% of specimens. Since the lateral portion of the node contains both presumptive somitic cells and notochord cells (Selleck and Stern, 1991), it is important to note that no labelled cells were found in mesodermal tissues other than notochord except in this 13% of specimens. By contrast, unilateral homotopic control grafts ('lateral-into-lateral', Fig. 2) contributed to somite in 67% of cases (Fig. 6D-F). In all of these, the graft also contributed to the notochord.

Following photo-oxidation, labelled cells in experimental grafts were also found in the floor plate of the neural tube and in the endoderm. In one of the surviving embryos that had failed to form somites, labelled cells were restricted to the notochord and arranged in a periodic fashion along its length.

To investigate the phenotype of graft-derived cells, a few embryos were stained with the notochord-specific antibody, Not1, after photographing the labelled cells in the whole mount. Graft-derived cells became incorporated into the notochord and, in one case, into the head process and head mesenchyme. All labelled cells lying in the trunk notochord were found to be Not1 positive. In the embryo with labelled cells in the head,

the graft-derived cells in the head mesenchyme did not stain with the antibody (Fig. 5).

Medial-into-lateral grafts

Medial-into-lateral grafts were performed to investigate whether prospective notochord cells in the medial sector of Hensen's node can populate tissues other than notochord (Fig. 2). Medial sectors were grafted either unilaterally or bilaterally. After bilateral grafts, somites formed in only 27% of embryos. Labelled cells were found in the notochord and floor plate of the neural tube in all specimens, and in one case (1/9) also in the paraxial mesoderm. In one of the unlabelled embryos, a supernumerary notochord was seen.

Somites formed in 80% of embryos receiving a unilateral graft. Labelled cells were confined to the notochord in all but one case, in which somites were also labelled. One embryo showed a periodicity in the arrangement of labelled cells along the length of the notochord, with a period of about two somite-lengths (Fig. 6A-C).

Homotopic control experiments ('medial-into-medial') were also performed. The surviving embryos appeared normal and had formed somites. In all cases, labelled cells were found in the notochord, and in the somites in 2/7 cases (Fig. 4C,D).

Primitive streak grafts (Fig. 3)

A total of 42 embryos were operated in this series, of which 36 survived (85%). The results are summarised in Table 2 and Figs 5F, 7-8. Of the 4 control embryos ('streak-into-streak'), 3 survived and had normal morphology. Labelled cells were found only in the somites and segmental plate (Fig. 8A-C).

Medial-into-streak grafts

These experiments, in which midline portions of the node were grafted into the rostral part of the primitive streak, were designed to address the question of whether prospective notochord cells can change their fate when grafted outside the node into a region where no cells contribute to notochord. The embryos that survived (15/18) had a morphology comparable with that of controls. Dil-labelled, graft-derived cells populated the notochord in 12 embryos. The notochord occasionally looked thicker, and graft-derived cells (in 6 cases) extended along the notochord for most of its length in one half of the notochord only (Fig. 7A-D).

Table 1. Summary of intranodal grafting experiments. Contribution of a graft to a mesodermal structure was scored when 20 or more labelled cells could be found within it

Type	Specimen numbers			% with somites	Labelled cells in			
	Expt.	Surv.	Scored		Notochord	(%)	Somite	(%)
Lat→Med:	45	29	20	75	8/8	(100)	1/8	(13)
Med→Med:	14	11	11	100	7/7	(100)	2/7	(29)
Med→Lat: Bi	24	14	11	27	9/9	(100)	1/9	(11)
Med→Lat: Uni	30	23	20	80	8/8	(100)	1/8	(13)
Lat→Lat: Bi	11	9	5	80	3/3	(100)	1/3	(33)
Lat→Lat: Uni	11	9	9	100	6/6	(100)	4/6	(67)
Total	135	95	76					

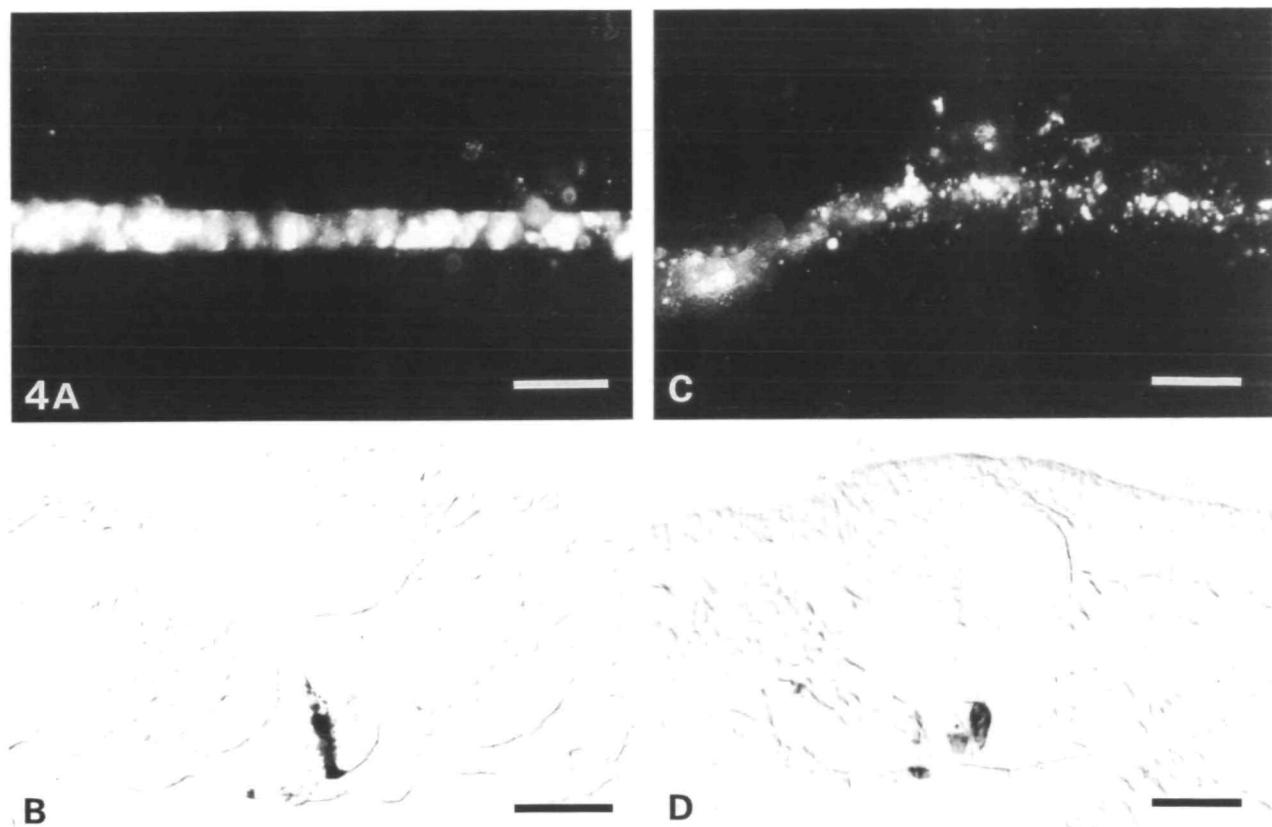


Fig. 4. Grafts placed into the medial sector of Hensen's node always contribute to notochord. **A** and **B** show the labelled descendants of a graft of lateral node. In control experiments where medial sector is grafted homotypically (**C**, **D**), the cells continue to contribute to notochord. A few labelled cells may also be seen in the endoderm, illustrating that the graft has integrated completely into the host Hensen's node. (**A**, **C**) Epifluorescence, showing DiI; (**B**, **D**) transverse sections through the same embryos, after photo-oxidation. Scale bars: 100 µm (**A**, **C**), 50 µm (**B**, **D**).

Table 2. Summary of primitive streak grafting experiments

Type	Specimen numbers		Labelled cells in			
	Expt.	Surv.	Notochord	(%)	Somite	(%)
Med→Streak	18	15	12/15	(80)	2/15	(13)
Lat→Streak	10	9	7/7	(100)	7/7	(100)
Streak→Med	10	9	8/8	(100)	2/8	(25)
Streak→Streak	4	3	0/3	(0)	3/3	(100)
Total	42	36				

All cells within the enlarged notochord stained with the Not1 antibody (Fig. 5F). In addition to the mesoderm, labelled cells frequently contributed to the endoderm and to the floor plate of the neural tube.

In one specimen, the notochord was split at its rostral end, caudal to the foregut; between the two rods of notochord lay a row of unlabelled somites. In two cases, labelled cells were found in the somites of the host embryo. In one case, the labelled cells were scattered throughout the embryo.

Lateral-into-streak grafts

A total of 10 experiments were performed and 9 of the

embryos survived. In 2 cases, labelled cells could not be found. In the remaining 7 embryos, cells were found to populate both the notochord and the rostral somites (Fig. 7E-G) and, in one case, the segmental plate. In one specimen, the labelled notochord lay external to the host notochord and fused with it posteriorly. In another specimen, an extra row of about five somites was also seen at the branch point of a split, labelled notochord.

Streak-into-medial grafts

These experiments were designed to investigate

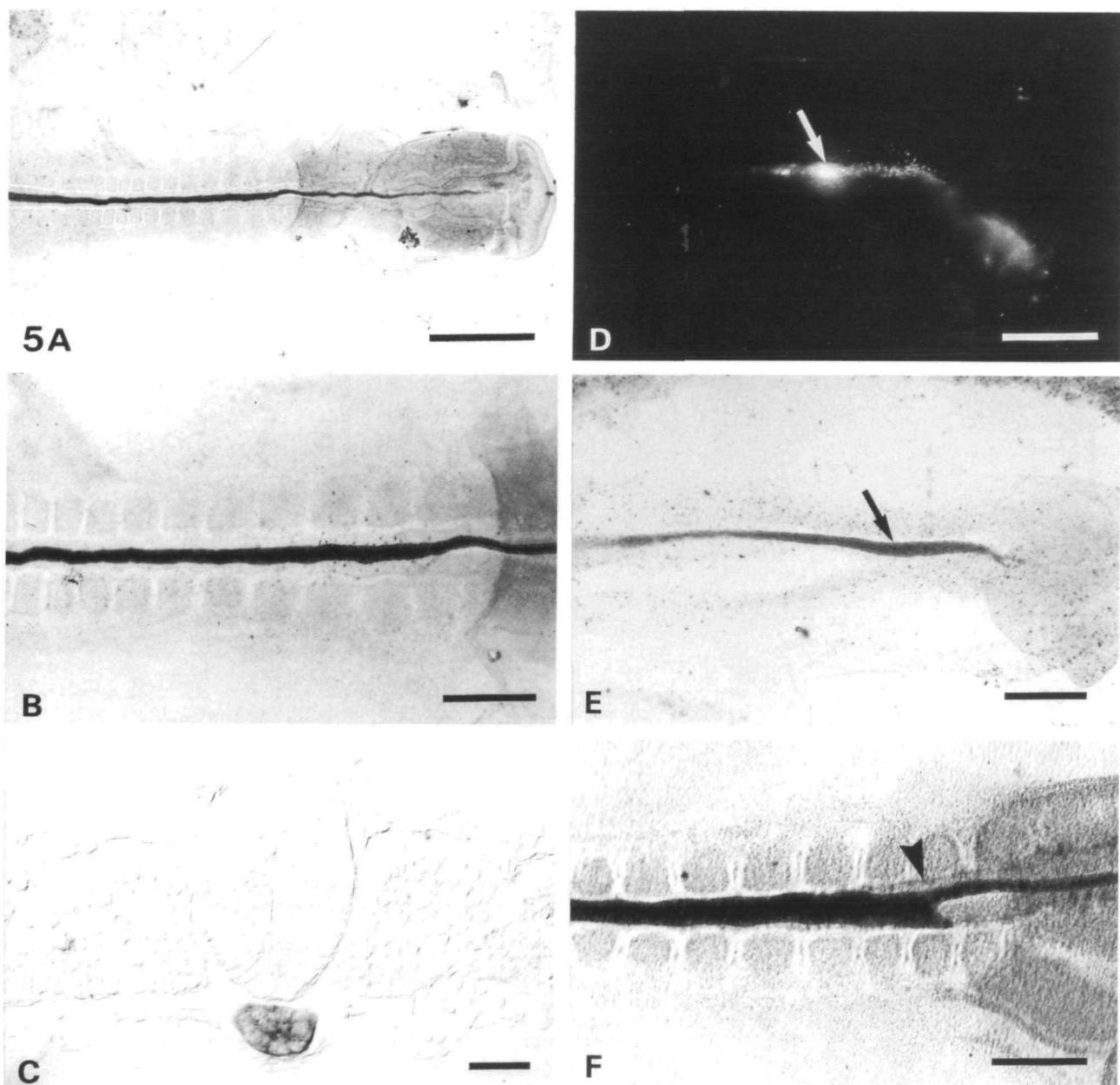


Fig. 5. Monoclonal antibody Not1 recognises notochord and head process. A-C show the notochord labelled in immunostained embryos in the whole mount (A, B) and in section (C). When a lateral sector of Hensen's node is grafted into the medial portion of a host node, labelled progeny are found in the notochord and head process (D). Following immunocytochemistry with Not1, the notochord, but not the head process, is stained (E). The arrows in D and E point to the same position in the embryo. (D) Epifluorescence, showing Dil-labelled cells. (E) The same embryo, stained with Not1 by immunoperoxidase. When a medial sector of Hensen's node is grafted into the rostral primitive streak of a host embryo, the graft-derived cells stain with Not1 and incorporate into the host notochord. (F) In this specimen, stained with the Not1 antibody, the notochord is greatly enlarged caudal to the point indicated by the arrow, due to the additional contribution of graft-derived cells. (A, B, D-F) Rostral lies to the right. Scale bars: 500 µm (A, D, E), 200 µm (B, F), 50 µm (C).

whether prospective somite cells of the rostral primitive streak can change their fate and become notochord. Labelled rostral primitive streak was grafted into the ∇ -portion of the node in 10 embryos, of which 9 survived. Labelled cells were found in the notochord and head process (Fig. 8D-F). In one case, the Dil fluorescence

was too faint to allow accurate localisation of the labelled cells. In two cases, labelled cells were also found in the head mesenchyme. In one case, label was found in the caudal notochord and in the somites, and the head process had failed to form. Graft derived cells in the notochord stained with Not1.

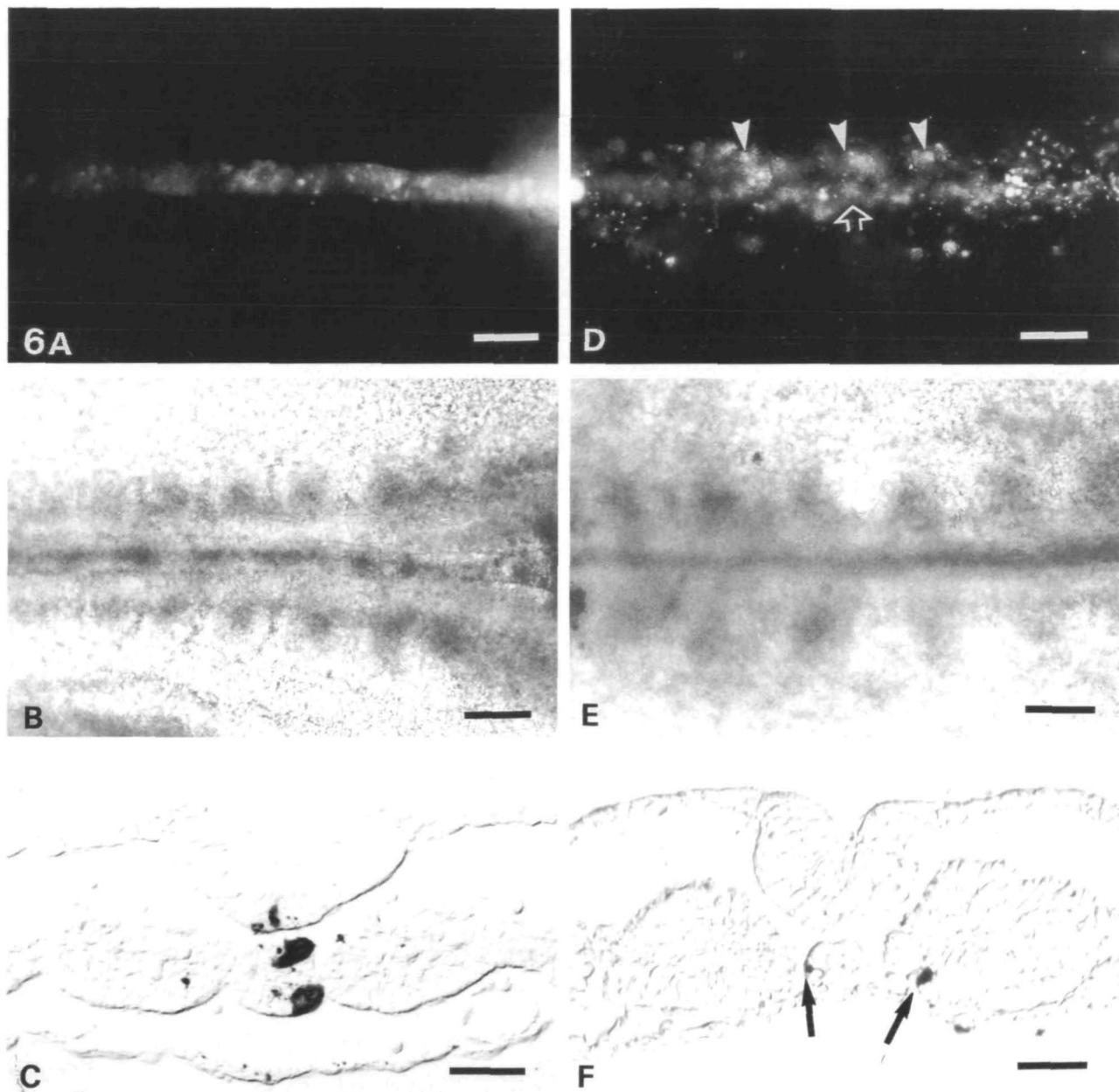


Fig. 6. When medial V-portions of Hensen's node are grafted into the lateral portion of the node, the cells of the graft continue to populate the notochord. (A, B) DiI-labelled cells viewed by epifluorescence optics (A) and bright-field (B) (rostral to the right) and in sections (C). The cells seem to be arranged periodically along the length of the notochord, with the intensity of labelling decreasing in more caudal groups of cells. In control experiments (D, E, F), lateral node cells grafted homotypically populate both the notochord and the somites. In D, the open arrow indicates labelling in the notochord, whilst the solid triangles indicate labelling in the medial parts of three consecutive somites. (A,D) Epifluorescence, showing DiI; (B,E) bright-field images of same embryos; (C, F) transverse sections of same embryos, after photo-oxidation. Scale bars: 100 µm (A, B), 80 µm (D, E), 50 µm (C, F).

Segmental plate grafts

To test whether the mechanisms that can cause changes in fate operate at later stages of development, or in more developmentally mature tissues, grafts of sectors of Hensen's node were placed into the segmental plate, a region where the somitic mesoderm becomes more mature as it progresses from posterior to anterior. In some cases, the ectoderm of the grafts was removed,

since some cells present in this layer, in both medial and lateral sectors, are destined for notochord. A total of 57 grafting experiments were performed; 53 embryos survived.

Grafts of midline V-pieces into segmental plate
Of the 28 operated embryos, 26 survived. Grafts were

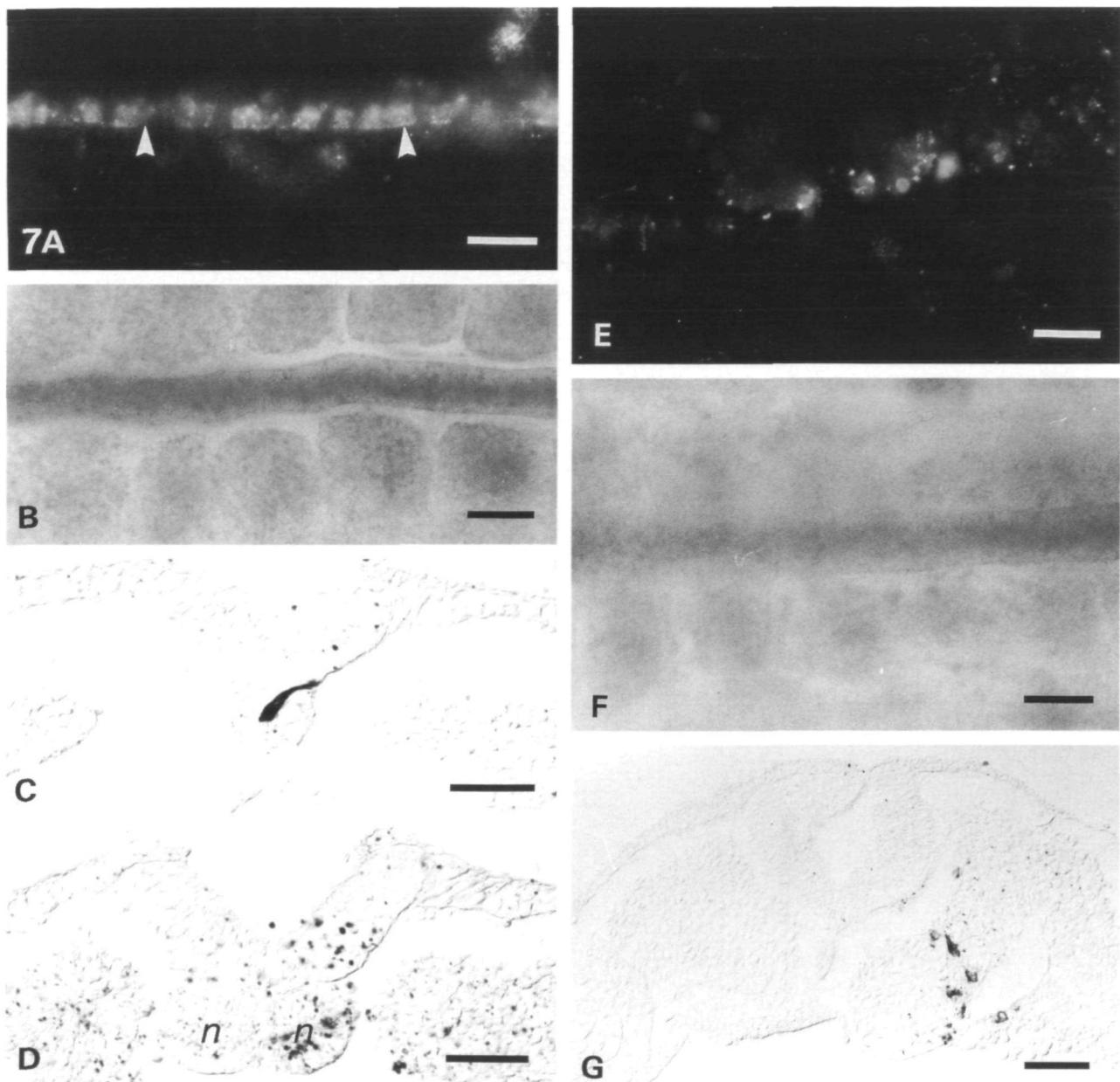


Fig. 7. Grafts of Hensen's node sectors into the rostral primitive streak. (A-C) Medial portions of the node grafted into the streak populate the notochord - some on one side only. The arrowheads (A) indicate labelled cells, restricted to one side of the notochord. In one case (D), a double notochord was formed, and only one of the notochords contains labelled cells. (E-G) Lateral portions of the node, when grafted into rostral primitive streak, contribute to both notochord and somites of the host embryo. (A, E) Epifluorescence, showing Dil; (B, F) bright-field views of same embryos; (C, D, G) transverse sections of same embryos after photo-oxidation of Dil. Scale bars: 80 μm (A, B, E, F), 50 μm (C, D), 30 μm (G).

placed at different rostrocaudal positions in the segmental plate.

With ectoderm ($n=14$). Labelled cells were found associated with both somite and notochord. A similar finding was made when the graft was placed half-way along the segmental plate. The graft tended to remain coherent, with only a few cells spreading away from the graft, mainly towards the midline. In several cases a rod-like structure with a central lumen had developed,

which projected dorsally beneath the ectoderm (Fig. 9A,B), which stained with Not1 (Fig. 9D).

Without ectoderm ($n=12$). In 2 specimens, no labelled cells could be found. When the graft was placed at the caudal end of the segmental plate ($n=2$), a mass of labelled cells was found adjacent to the somites and many cells seemed to align rostrocaudally in the midline (Fig. 9C). In one case, a rod-like structure developed, as described above. As the graft was placed more

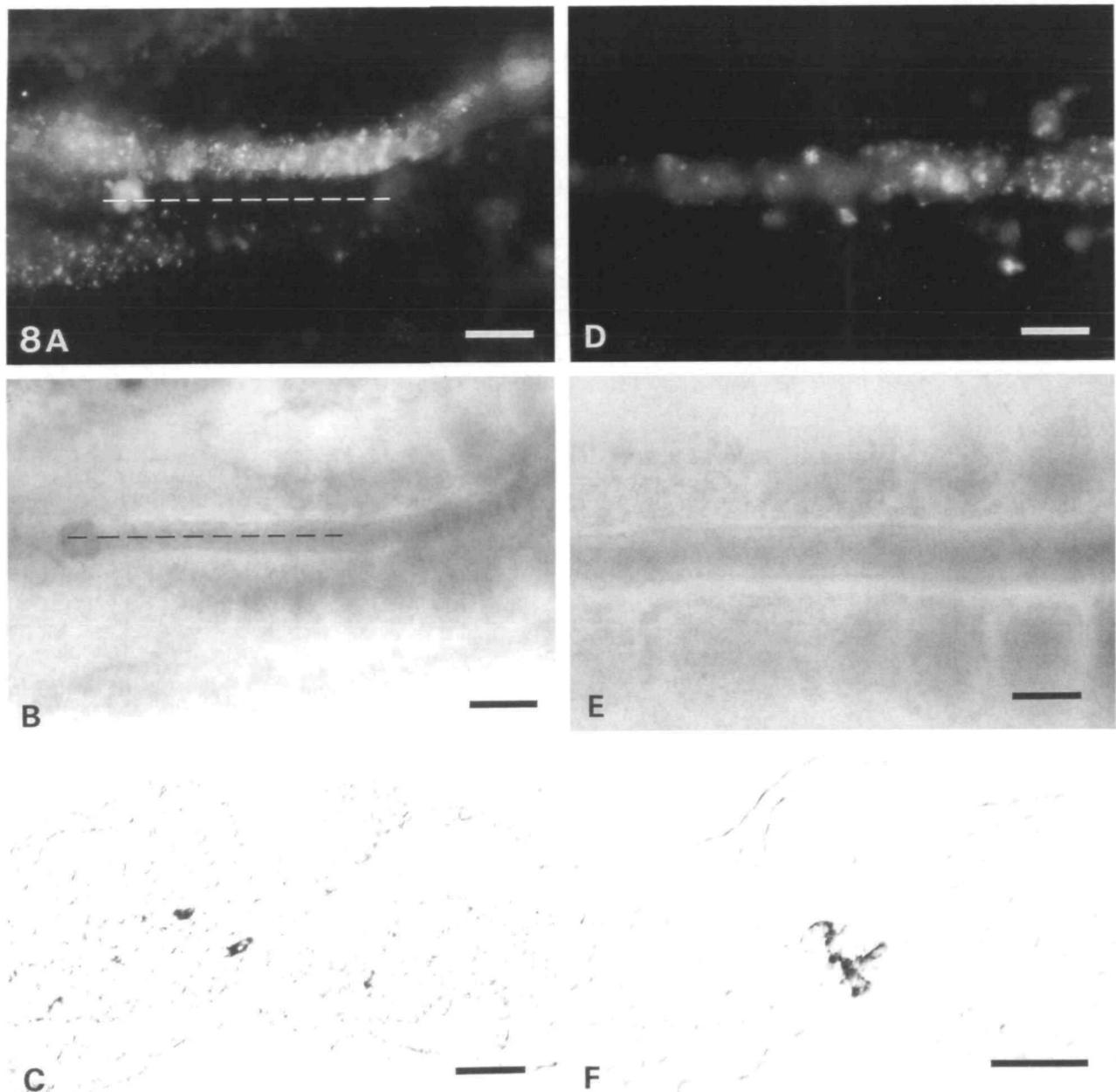


Fig. 8. (A-C) Grafts placed homotopically into the rostral primitive streak contribute only to paraxial mesoderm. The dotted lines (A, B) indicate the position of the notochord in this embryo. (D-F) Grafts of rostral primitive streak into the medial V-sector of the node contribute to the notochord. (A, D) Epifluorescence, showing Dil labelled cells; (B, E) bright-field images of same embryos; (C, F) transverse sections of same embryos after photo-oxidation. Scale bars: 100 μm (A, B, E), 50 μm (C, F).

rostrally in the segmental plate, fewer cells could be found adjacent to the notochord.

Grafts of lateral node into segmental plate

17/19 survived. Grafts were placed in different rostro-caudal positions, with or without ectoderm attached.

With ectoderm (n=8). Compared to grafts of midline node, grafts of lateral node showed a greater tendency to form structures resembling epithelial somites, but which remained separate from the host somites. In all

cases, a rod-like structure had formed, similar to those generated after grafting the medial sector of the node. In one case where the graft had been placed at the very rostral end of the segmental plate, the cells came to lie lateral to the somites, in the mesonephros.

Without ectoderm (n=9). The rostrocaudal position of the graft did not seem to affect the outcome of the experiment. In 5/9 cases, cells of the graft arranged themselves into small epithelial spheres, out of register with the host somites (Fig. 9E,F). In three instances, cells had migrated away from the graft towards the

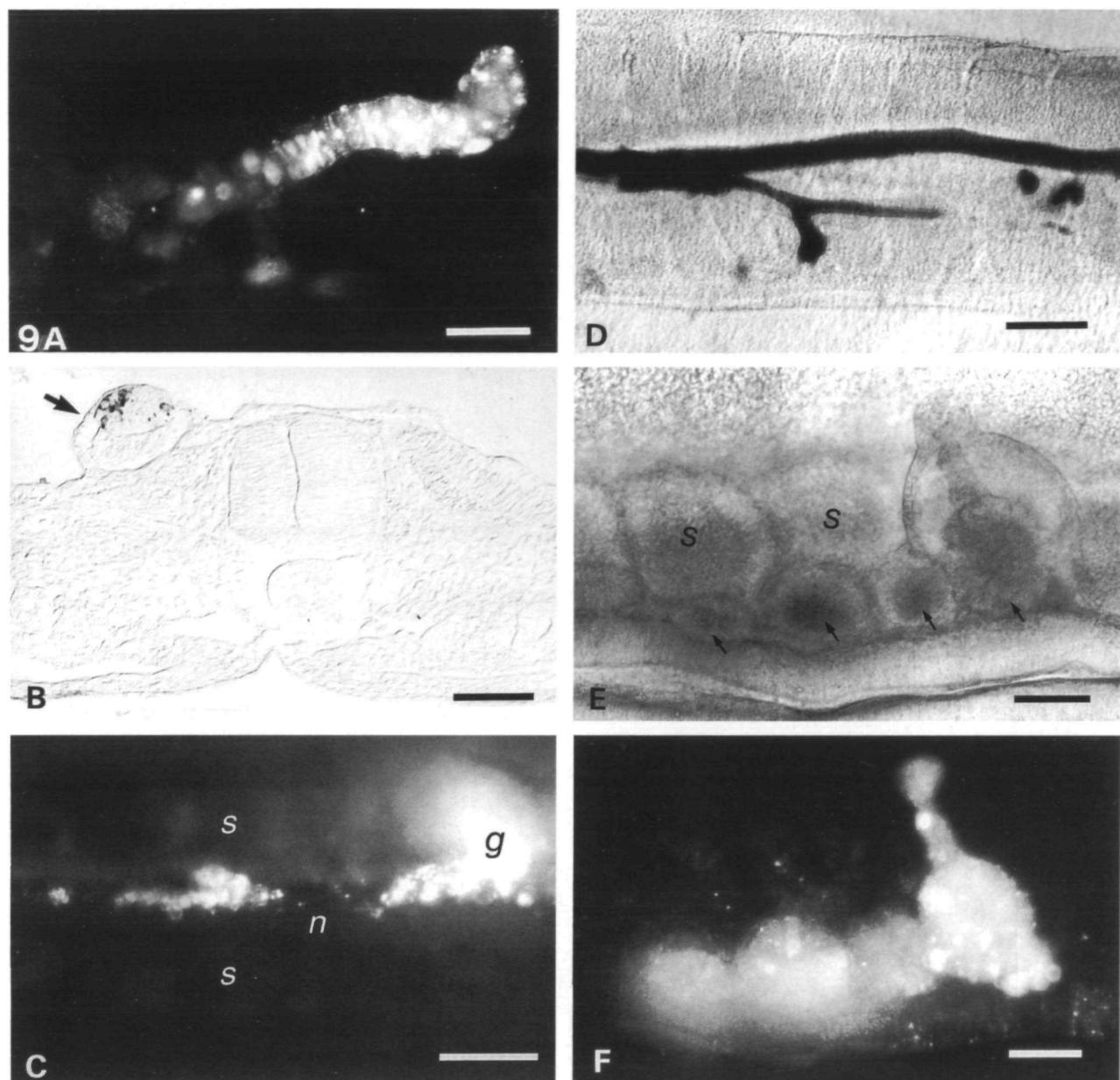


Fig. 9. Segmental plate grafting experiments. (A, B) Medial sector grafts of Hensen's node to the segmental plate produce notochord-like rods that project from the dorsal side of the embryo. (C) Some cells from medial sector grafts (without ectoderm) migrate towards the midline of the host embryo and align with it rostrocaudally. (D) Immunocytochemistry with the Not1 antibody reveals that graft-derived cells that form rod-like structures and some small groups of cells are recognised by the antibody. (E, F) Lateral sector grafts into the segmental plate give rise to small somite-like clusters (arrows). Note that these are out of phase with the host somites. (A, C, F) Epifluorescence showing labelled cells; (B) photo-oxidation of DiI in a similar embryo to that shown in A; (D) whole-mount immunoperoxidase with Not-1; (E) bright-field view of embryo in F. Scale bars: 100 μm (C, D) and 50 μm (A, B, E-F). g, graft; n, notochord; s, somite.

midline. None of the grafts lacking ectoderm gave rise to a projecting rod.

Grafts of segmental plate into segmental plate

Grafts ($n=10$) of rostral segmental plate (about three prospective somites long) placed into caudal host segmental plate did not remain as a coherent mass;

labelled cells were found in a number of consecutive host somites (maximum of four seen). In 4 cases, the cells appeared to arrange themselves around the periphery of the host somites. When the graft of rostral segmental plate was placed into the rostral part of the host segmental plate, the cells incorporated exclusively into the host somites.

Discussion

Dil-labelled portions of Hensen's node and rostral primitive streak were grafted into different positions within the embryo to address the question of whether cells in these regions are committed to their fates. We find that the presumptive notochord cells in the midline V-shaped portion of Hensen's node, if grafted into a new environment, continue to integrate into the notochord or form a notochord-like structure. The lateral portion of the node contains prospective medial somite and notochord cells, with the latter located more dorsally; the rostral primitive streak also contains cells destined to become somite (Selleck and Stern, 1991). The prospective somitic cells, unlike those in the presumptive notochord regions, do alter their fates when transplanted into a new site.

The commitment of notochord cells to their fates

The fates of cells in the midline V-portion of Hensen's node were challenged in three grafting experiments: to lateral regions of the node, into the rostral streak and into the segmental plate. Cells in the midline V-sector of the node continue to contribute to notochord after grafting into any of these areas.

When V-node was grafted into the lateral sector, labelled cells derived from the graft were always found in the notochord. Some control embryos that had been operated bilaterally failed to form somites and the labelled cells contributed to somitic mesoderm in only one third of cases. This suggests that the operation interfered with normal development. Control embryos operated unilaterally, on the other hand, developed more normally and there was a contribution of labelled cells to somite in the majority of cases. For this reason, experimental embryos operated unilaterally were more informative. The results show that when midline prospective notochord cells are grafted into the lateral sector, they still generate notochord and do not tend to contribute to somites, except in a few cases, which could be accounted for by contamination of the graft with more lateral cells.

Following a graft of midline node into the rostral streak, two-thirds of the embryos showed labelled cells in the host notochord. This is a rather surprising result, since the rostral streak does not contain prospective notochord cells (Rosenquist, 1966; Selleck and Stern, 1991). Two possibilities could account for the presence of cells in the host notochord and its enlargement. The first is that the grafted cells failed to migrate laterally away from the streak, and instead became incorporated into the node and/or notochord following regression of the primitive streak. The failure of the graft cells to migrate away from the streak is unlikely to be due to the trauma of the operation, because the control experiments reveal that when rostral primitive streak is grafted, cells migrate away from the midline as normal and are never found in the notochord. The second possibility is that the graft self-differentiated into a notochord which later merged with the host notochord. At present it is impossible to distinguish between these

two possibilities, and it is possible that both contribute to the results observed. The second hypothesis would explain the finding that in some cases only one half (either left or right) of the notochord was labelled.

After grafting V-node, stripped of its ectoderm, into the segmental plate, labelled cells were found to have migrated towards the midline, to take up positions adjacent to the notochord. This result suggests that presumptive notochord cells are capable of finding their appropriate position in the embryo, and is consistent with mechanisms such as chemotaxis or differential adhesion of notochordal cells. From electron microscope studies of chick (Bancroft and Bellairs, 1976) and time-lapse analysis of amphibian embryos (Wilson and Keller, 1991), it appears that there is, initially, a close relationship between prospective notochord and prospective somitic mesoderm. The existence of mechanisms that allow presumptive notochord and somite cells to sort out would ensure that the correct spatial relationships between these two cell types are maintained.

It appears, therefore, that the cells in the midline V-shaped region of the node are committed to a notochordal fate. This conclusion explains why a Hensen's node grafted into the *area opaca* of a host embryo can autonomously generate a well organised notochord (Waddington, 1933; Gallera, 1971; Nieuwkoop et al. 1985; Dias and Schoenwolf, 1990).

The commitment of somite cells to their fates

Our previous work revealed that somites are composed of cells from two separate regions of the definitive streak-stage embryo: the rostral part of the primitive streak and the lateral portions of Hensen's node (Selleck and Stern, 1991; see also Ordahl and Le Douarin, 1992). In addition to its contribution to the somites, the lateral portion of Hensen's node contains prospective notochord cells, which are located more dorsally. This accounts for the finding that injection of LRD into single cells in the mesoderm of the lateral sector gives rise to labelled cells only in the somite, while labelling the same region with Dil, which cannot be confined to the mesoderm, often marks notochord cells as well (Selleck and Stern, 1991). In this regard, it is interesting that in the present experiments the lateral node sectors in control grafts ('lateral-into-lateral') contribute to notochord in all cases but to paraxial mesoderm in fewer. It may be that the trauma of the operation and imperfect healing at the site of grafting may, in some cases, prevent the migration of presumptive somitic cells into the paraxial mesoderm.

We have tested the commitment of cells in both the lateral node sectors and the rostral primitive streak in several experiments.

(1) When lateral node was grafted into the midline V-sector, cells were found in the notochord in all cases and paraxial mesoderm in one case. When a portion of lateral node was replaced with a Dil-labelled lateral node, somites were labelled in two-thirds of the grafts; this experiment controls for the variation in size of tissue excised and for differences in the distribution of

somite cells in the node among the embryos. The results suggest that prospective somite cells are not committed to a somitic fate, as they can become incorporated into notochord. The possibility that the cells found within the notochord represent somitic cells that became trapped there can be ruled out because they express immunoreactivity with the notochord-specific antibody, Not1. In one specimen graft-derived cells in the head process and head mesenchyme did not stain with Not1. This could be taken to mean that these cells are the presumptive somitic cells, while the Not1-positive cells are derived from committed notochord precursors. This is unlikely, however, because this is the only embryo in the series that displayed this result. A more likely possibility is that the Not1-negative cells migrated out of Hensen's node before they could become committed to a notochord fate.

(2) Grafting the lateral portion of the node into the rostral primitive streak places prospective medial somite cells (with presumptive notochord cells dorsally in the associated epiblast; Selleck and Stern, 1991), into a presumptive lateral somite region. In all cases where the labelled cells could be located, cells contributed to notochord and somites. This result does not imply that the somite cells of the node are committed to their fates, but it does suggest that, like presumptive somite cells in the primitive streak, they can contribute to somite.

(3) Grafting lateral node into the segmental plate gave little information on the commitment of the somite cells to their fates. In one case, however, grafted cells were found in the mesonephros.

(4) When rostral primitive streak cells were grafted into the V-portion of the node, the cells contributed to paraxial mesoderm of the head or trunk in one-third of cases, a value similar to that obtained from embryos in which V-sectors were grafted homotopically. This suggests that rostral primitive streak grafts behave identically to medial sector grafts, and therefore that the former do not consist of committed cells.

All of the above results suggest that prospective somite cells are not committed to a somitic fate either in the node or in the streak at this stage. Studies by Stern et al. (1988) suggest that cells are not committed at the caudal end of the segmental plate, and others (Lance-Jones, 1989; Noden, 1989; Veini and Bellairs, 1991) find that even the cells of somites that have already segmented are not committed to form somitic derivatives. Veini and Bellairs (1991), for example, find that somites from stage 10-14 embryos grafted into younger hosts (stage 4-6) can contribute to pharyngeal endoderm, lateral plate and endothelium of the blood vessels. It therefore seems likely that the 'somatic state' is not defined as a separate, differentiated state during development. The remaining properties of somitic cells, such as their pathways of differentiation into muscle, dermis, skeletal elements and rostral and caudal halves (some of which decisions may occur earlier and some later than somite formation; see Stern and Keynes, 1986; Aoyama and Asamoto, 1988), appear to be designated independently of their condition as somitic.

It is worth pointing out that both regions containing presumptive somitic cells (the lateral sectors of the node and the rostral primitive streak immediately caudal to the primitive pit) are capable of forming independent, small somite-like structures when grafted into the segmental plate mesoderm of a host embryo (Fig. 9E,F). The finding that they do so independently of the periodicity of host segmentation suggests that these presumptive somite cells possess intrinsic information that determines the meristic pattern. Again, therefore, the ability to segment and the periodicity of such segmentation appears to be independent of the commitment of cells as somitic.

Conclusion

Our results indicate that whilst the presumptive notochord cells in the fate map of Hensen's node appear to be committed to a notochordal fate, the somitic cells in Hensen's node and primitive streak are still plastic.

If the medial portion of the node contains cells committed to a notochord fate, while prospective somite cells in the lateral segment are not committed, does this difference have any bearing on the neural inducing and regionalisation abilities of Hensen's node? Does the node play an important role in the control of segmentation? Experiments are in progress to address these questions.

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