

# Fates and migratory routes of primitive streak cells in the chick embryo

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## SUMMARY

We have used carbocyanine dyes to fate map the primitive streak in the early chick embryo, from stages 3<sup>+</sup> (mid-primitive streak) to 9 (8 somites). We show that presumptive notochord, foregut and medial somite do not originate solely from Hensen's node, but also from the anterior primitive streak. At early stages (4<sup>-</sup> and 4), there is no correlation between specific anteroposterior levels of the primitive streak and the final position of their descendants in the notochord. We describe in detail the contribution of specific levels of the primitive streak to the medial and lateral halves of the somites.

To understand how the descendants of labelled cells reach their destinations in different tissues, we have

followed the movement of labelled cells during their emigration from the primitive streak in living embryos, and find that cells destined to different structures follow defined pathways of movement, even if they arise from similar positions in the streak. Somite and notochord precursors migrate anteriorly within the streak and pass through different portions of the node; this provides an explanation for the segregation of notochord and somite territories in the node.

Key words: primitive streak, fate map, notochord, somite, gastrulation, cell movements, chick embryo

## INTRODUCTION

In higher vertebrate embryos (birds and mammals), the homologue of the amphibian Spemann's organizer is located at the tip of the primitive streak, in a structure known as Hensen's node (Waddington 1932; Hara 1978). This is substantiated by comparison of the amphibian dorsal lip of the blastopore with Hensen's node in terms of the fates of their cells, the expression of several genes such as *gooseoid*, *HNF-3 $\beta$*  and *Sonic hedgehog* (see Levin et al., 1995) and their ability to induce an ectopic embryonic axis upon transplantation. The finding (e.g. Grabowski 1956) that the embryo can regulate for extirpation of such an important region is therefore surprising.

One possible explanation is that regions of the primitive streak just posterior to the node contains some precursors with the same fates as cells contained in the node. However, although the fate of the primitive streak has been studied extensively (Peebles 1898, Gräper 1929; Wetzel 1929, 1936; Kopsch 1934; Pasteels 1937, 1943; Spratt 1942a,b, 1946, 1947, 1952, 1955, 1957; Spratt and Codon 1947; Bellairs 1953a,b; Spratt and Haas 1962a, 1965; Nicolet 1965, 1967, 1970, 1971; Rosenquist 1966, 1970a-f, 1971a-d, 1972, 1982, 1983; Orts-Llorca and Collado 1968; Stalsberg and DeHaan 1969; Schoenwolf and Sheard 1990; Schoenwolf et al., 1992; García-Martínez and Schoenwolf 1993; García-Martínez et al., 1993; Inagaki et al., 1993), these previous studies have all used different methods to define the site of marking and therefore it is difficult to compare them. In addition, most of these studies

are incomplete in that they either concentrate on a single stage or do not cover the entire length of the primitive streak.

Here we present detailed fate maps of the primitive streak of the chick embryo constructed between mid-primitive streak and early somite stages, using carbocyanine dyes. We find that tissue types normally considered as derivatives of Hensen's node, such as notochord and the medial parts of the somites, have some progenitors situated posterior to the node, in the primitive streak. In addition, we have followed the migration of cells labelled in the anterior primitive streak up to the time that they reach their destination in different tissues. We find that cells destined to different structures follow defined pathways of movement, which appear to correlate more closely with the tissue to which they will contribute than to their position in the streak at the time of labelling.

## MATERIALS AND METHODS

### Embryo techniques and fate mapping experiments

Fertile hens' eggs (Rhode Island Red  $\times$  Light Sussex or White Leghorn) were incubated at 38°C for 12-30 hours to give embryos at stages 3<sup>+</sup>-9. Embryos were explanted ventral side uppermost in modified New culture (New 1955; Stern and Ireland 1981). For fate mapping studies, embryos were staged according to Hamburger and Hamilton (1951) and labelled with DiI (Molecular Probes). Methods for this have been described previously (Selleck and Stern 1991; Ruiz i Altaba et al., 1993); briefly, a 0.25% stock of DiI in absolute alcohol was diluted 1:10 in 0.3 M sucrose at 45°C and this injected by air

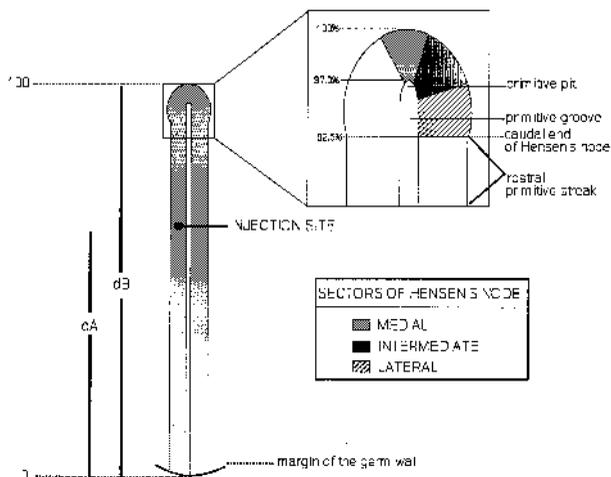
pressure through a micropipette made by pulling a 50  $\mu$ l capillary (Sigma) in a vertical electrode puller. The position of the labelling site was determined (see below) and the embryos cultured at 38°C in a humid chamber for up to 30 hours, when they had reached stages 9-13. The fate of the labelled cells was assessed both in whole mounts under fluorescence illumination and after photooxidation of the dye and histological processing (see below).

### Definition of the injection site

Immediately after injection, each embryo was staged and the length of the primitive streak measured (Fig. 1). We plotted the position of the injection site both in terms of distance (in mm) from the tip of Hensen's node and as a percentage of the length of the primitive streak. When constructing fate maps from these sets of data and comparing the two approaches, we found no significant difference between the two methods in terms of the positions of the boundaries between different prospective regions. Furthermore, there are large variations in the length of the primitive streak for any one stage. For these reasons, we chose to use the relative method to present the data.

### Observation of migratory pathways

Immediately after labelling (see above), embryos were photographed (see below). They were then placed in a humid chamber at 38°C. At intervals from one to a few hours, embryos were removed from the incubator and rapidly staged and photographed. The removal of the embryos from the incubator, the repeated changes in temperature and the exposure to fluorescent light did not appear to affect development substantially; the average time required for the addition of new somites was  $104.8 \pm 17$  minutes (data from 27 embryos), which is similar to that in New (1955) culture (in control embryos, the average time of somite formation was  $94.7 \pm 14$  minutes;  $n=13$ ) and in ovo (Menkes and Sandor 1969; Primmitt et al., 1989; Birgbauer et al., 1995).



**Fig. 1.** Definition of the injection site. The dye was applied either in the midline, lateral or intermediate region of the primitive streak, on the left or right side of the embryo. The length of the primitive streak from the anterior tip of Hensen's node to the edge of the posterior germ wall (dB) was fitted into a 100 unit scale using an eyepiece graticule in a dissecting microscope with a zoom objective. The position of the injection site (dA) was then calculated as a percentage of this total length ( $dA/dB \times 100$ ). After this, the size of the graticule scale was calibrated against a ruler to obtain a scale in mm. The subdivision of Hensen's node into sectors is shown in relation to the position of the primitive pit and to the caudal border of Hensen's node (normalized for a stage 4 node and adapted from Selleck and Stern 1992a,b).

### Examination of embryos, fixation and histology

Embryos were explanted into phosphate-buffered saline (PBS; pH 7.4) in Sylgard (Dow Corning)-coated dishes and fixed in PBS containing 0.25% glutaraldehyde and 4% formaldehyde. They were then examined as whole mounts and photographed as double exposures using epifluorescence and bright-field optics on Ektachrome or Fuji 1600 ASA film. Slides were then scanned into a Dell Pentium P-90 computer using a Kodak RFS 2035 Professional Plus film scanner. The illustrations in this paper were made using Adobe Photoshop (Adobe Systems, Inc) and printed on a Tektronix II SDX dye sublimation printer.

When photographing labelled embryos as whole mounts, some light scattering occurs from cells in other layers of the embryo, particularly at the time of labelling, when the intensity of emission is great. It is therefore important to note that some of the injections illustrated, particularly for time = 0 hours, appear larger than they are in reality.

A total of 36 embryos labelled with DiI were processed histologically to confirm the location of the labelled cells. For this, DiI was photoconverted by exposure to the excitation wavelength in the presence of 3,3'-diaminobenzidine (DAB; Aldrich) in 0.1 M Tris (pH 7.4) as described previously (Ruiz i Altaba et al., 1993), the embryos embedded in Paraplast and sectioned at 10  $\mu$ m. Sections were photographed on Kodak T-MAX 100 film, and the negatives obtained were scanned and printed as described above.

## RESULTS

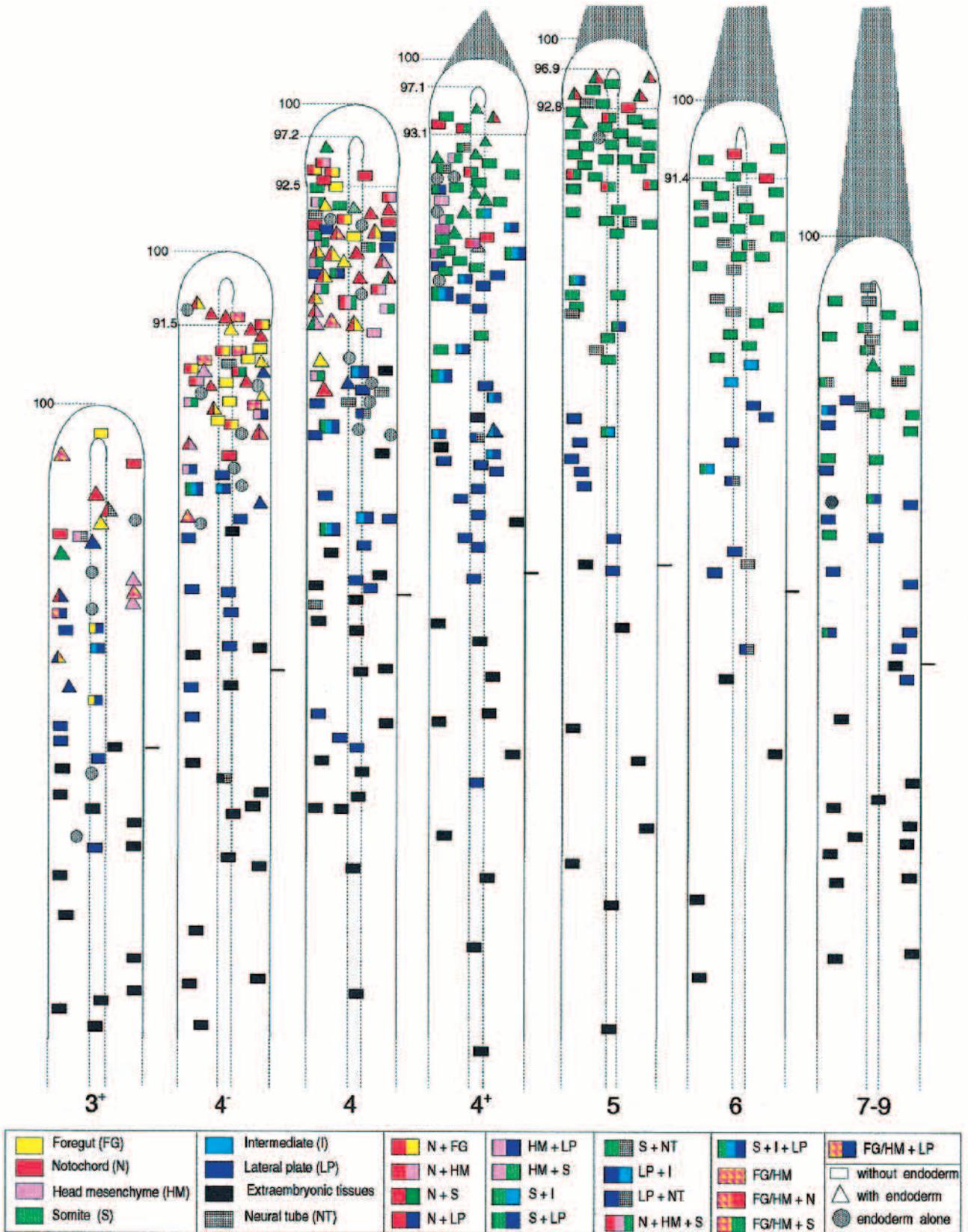
689 embryos were labelled, of which 448 were used for analysis. The remaining embryos were not considered because they either had not developed normally or had died before the desired stage. The results obtained are summarised in Fig. 2 and specific examples shown in Fig. 3.

At all stages, the area 0 (posterior end) to 75% of the length of the primitive streak contributes mainly to lateral plate mesoderm and extraembryonic tissues. By contrast, from stages 3<sup>+</sup> to 4, the most anterior quarter of the primitive streak contributes largely to axial tissue types, such as notochord, foregut, head mesenchyme and somites. From stage 4<sup>+</sup>, this region of the streak contributes mainly to somitic mesoderm. The following sections are organized according to the tissue type to which labelled cells contributed.

### Chordamesoderm, prechordal plate, foregut and head mesenchyme

Our results show that cells contributing to notochord are localized in the anterior end of the primitive streak (from 75-92% of the length of the streak), as well as in Hensen's node (see Selleck and Stern, 1991). Between stages 4 and 4<sup>+</sup>, there is a reduction in the number of notochord progenitors in the streak: within the region 75-92%, 18/39 (46%) injections at stage 4 produced labelled cells in the notochord, as opposed to only 3/22 (14%) of injections performed at stage 4<sup>+</sup> in this same region of the anterior primitive streak.

The contribution of the primitive streak to various levels of the notochord was also recorded (Fig. 4). Up to stage 4, some locations contribute cells to the entire length of the notochord (some including the prechordal region), while others tend to generate progeny restricted to short lengths of the notochord. From stages 4<sup>+</sup> to 6, the prospective notochord cells remaining in the streak contribute only to levels caudal to the first somite. After stage 6, no cells labelled in the primitive streak contribute to the notochord.



**Fig. 2.** Summary of the results obtained. Each coloured block symbolizes the results of a single injection of dye in one embryo, in terms of the fates of the descendants of the labelled cells. Different colours (shown in the key) represent different tissue types. The combinations of colours within a single block represent combinations of tissues. The size of the symbols used in this diagram is slightly smaller than the injections made. The short horizontal line by each streak shows the 50% position.

From stage 3<sup>+</sup> to 4, the region of primitive streak that gives rise to notochord (73-92%) also contributes to prospective head mesenchyme (Fig. 3C,D) and foregut (Fig. 3A,B). After stage 4<sup>+</sup>, the primitive streak no longer contributed to the foregut, although it still contains precursors for head mesenchyme (Fig. 5). In 12 out of the 72 (17%) injections that gave rise to labelled cells in either foregut or head mesenchyme, it was not possible to determine whether the labelled progeny was located in the foregut, the head mesenchyme or both. In 14/31 cases, labelled cells were found in the dorsal foregut and in the prechordal region.

#### Migration routes of notochord and foregut precursor cells

We investigated the routes by which precursor cells that contribute to the notochord and foregut reach their destinations. Fig. 6 shows two examples (out of 19 embryos of this type analyzed) of the results obtained (summarised in Fig. 7, along with con-

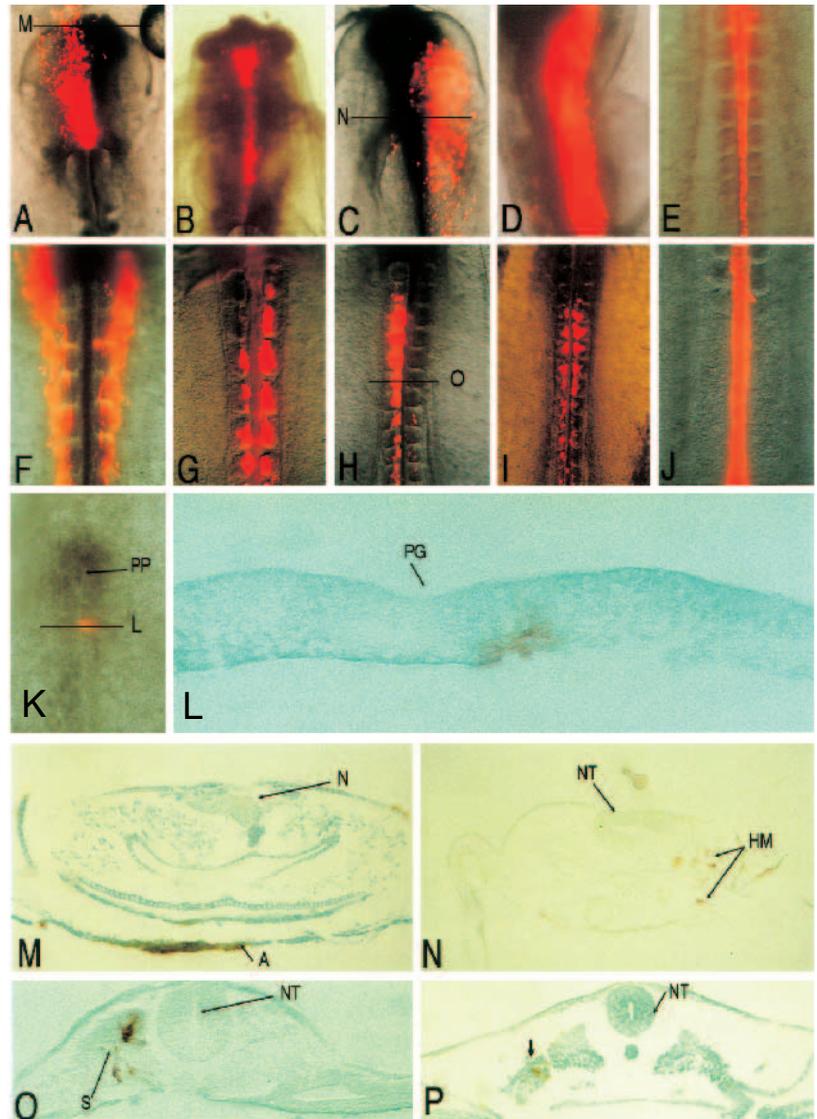
tributions to other tissue types). From analysis of embryos labelled in the primitive streak at stage 4, we find that the earliest descendants to leave the streak do so via a lateral route that does not include Hensen's node (Figs 6A-B, 7), but the stream of later emerging cells gradually shifts to the anterior-median sector of Hensen's node before leaving the streak (Fig. 7).

At all stages following the injection, some labelled descendants of the marked cells remain at or close to the injection site, in the primitive streak, while other descendants leave this region (e.g. Fig. 6A,B at 18 hours). Thus, there is a resident population of cells in the anterior primitive streak that persists even during node regression (arrows in Fig. 6).

#### Somite mesoderm

The contribution of the primitive streak to the somites is summarised in Fig. 8. The labelled cells can occupy different

**Fig. 3.** Examples of the results obtained. (A) Stage 9 embryo that had been marked in the midline of the streak at 96% at stage 3<sup>+</sup>; the amnion and the ventral midline of the foregut are labelled. (B) Stage 11 embryo that had been labelled at 92% at stage 4: the dorsal midline of the foregut and the region of the prechordal plate are labelled. (C) Stage 9 embryo injected at stage 3<sup>+</sup> at position 73%; the head mesenchyme and endoderm are labeled unilaterally. (D) Stage 11 embryo that had been labelled at position 87% at stage 4. Fluorescent cells are found in the head mesenchyme (in the same embryo, the most anterior somites were also labelled). (E) Stage 11 embryo that had been labelled at position 93% at stage 4<sup>-</sup>; the midline of the endoderm and a fragment of notochord extending from the level of somite 7 to the regressing node (out of the frame) are labelled; the fluorescent cells in the posterior part of the notochord appear as small groups. (F) Stage 11 embryo marked at position 71% at stage 4<sup>-</sup>; the intermediate mesoderm and blood vessels are labelled; cells in the aortae underlying the somitic mesoderm have a characteristic elongated shape. (G) Embryo that had been labelled at stage 4<sup>+</sup> at position 93%. Progeny are found in the medial portions of the somites. (H) Stage 11 embryo that had been marked at position 89% at stage 5; progeny are seen in the medial and more lateral regions of somites 3-5 and in the medial portions of somites 6-15. (I) After injection of dye into position 94% at stage 6, labelled progeny were found in the medial and more lateral regions of somites 5-10; more posteriorly, other structures were found to contain fluorescent cells. (J) Stage 12<sup>-</sup> embryo that had been injected at position 94% at stage 8<sup>+</sup>; the neural tube is labelled. (K) Control embryo in which an injection of dye was placed at position 73% at stage 4<sup>-</sup>. PP, primitive pit. (L) Transverse section through the embryo shown in K, at the level indicated by the transverse line, showing cells containing photooxidised DiI. PG, primitive groove. (M) Transverse section through the embryo in A at the level indicated. Labelled cells are found in the amnion (A). N, tip of the neural tube. (N) Transverse section through an embryo showing labelling similar to that in C at the level indicated, with photoconverted cells in the head mesenchyme (HM). NT, neural tube. (O) Transverse section through the embryo in H, at the level indicated, with labelled cells in the somite (S). NT, neural tube. (P) Transverse section through the embryo in F, at the level of the rostral end of the segmental plate. Labelled cells (arrow) are seen in the intermediate mesoderm and in the somatopleure. NT, neural tube.



portions of the mediolateral extent of the somites. We have classified the patterns of labelling observed into three classes:

(a) In **'medial type'** patterns (black symbols in Fig. 8), embryos contained labelled cells in a medial portion of the somites (note that the labelled region can encompass more or less than half of the somite); however, somites with **only** lateral labelling are not observed in this type of pattern, but some somites might be labelled throughout.

(b) **'Lateral type'** patterns (white symbols in Fig. 8) are the converse of those described above: somites labelled only in a medial portion are not observed.

(c) Finally, **'whole somite'** patterns (hatched squares in Fig. 8) consist of somites that are labelled throughout their width; these embryos do not contain somites in which a restricted part of their mediolateral extent is labelled.

'Medial type' patterns arise from the region 80-95% of the primitive streak, 'lateral type' patterns from the region 65-80% and 'whole somite type' patterns from the region 76-85%. As found with notochord progenitors, labelling a given site at a more advanced stage contributes only to more posterior somites (Fig. 9).

#### Migration pathways of somite precursor cells

We followed the pathways taken by prospective somitic cells arising in the primitive streak to their destinations in the somites ( $n=12$ ). When somite progenitors are labelled at approximately the 90% position, cells emerge anterolaterally, passing through the lateral part of the node. Subsequently, these cells move anteriorly as the primitive streak shortens and become confined to the medial aspect of the paraxial mesoderm (Figs 7, 10A). Somite precursors situated more posteriorly (around the 85% position) in the streak emerge laterally and the stream remains separate from the edges of the node (Fig. 7; in Fig. 10, compare A and B). These cells also become confined to the medial aspect of the paraxial mesoderm. As injections are made more posteriorly, the pathways taken by labelled cells become progressively more lateral and further from the node, as found by Rosenquist (1966) and Schoenwolf (1992) (Fig. 7).

As found for notochord and head mesenchyme precursors, the emerging cells that contribute to somites form a stream from the injection site, with some labelled cells always remaining in the area of the regressing node (Fig. 10A,B).

#### Neural tube, heart and intermediate and lateral plate mesoderm

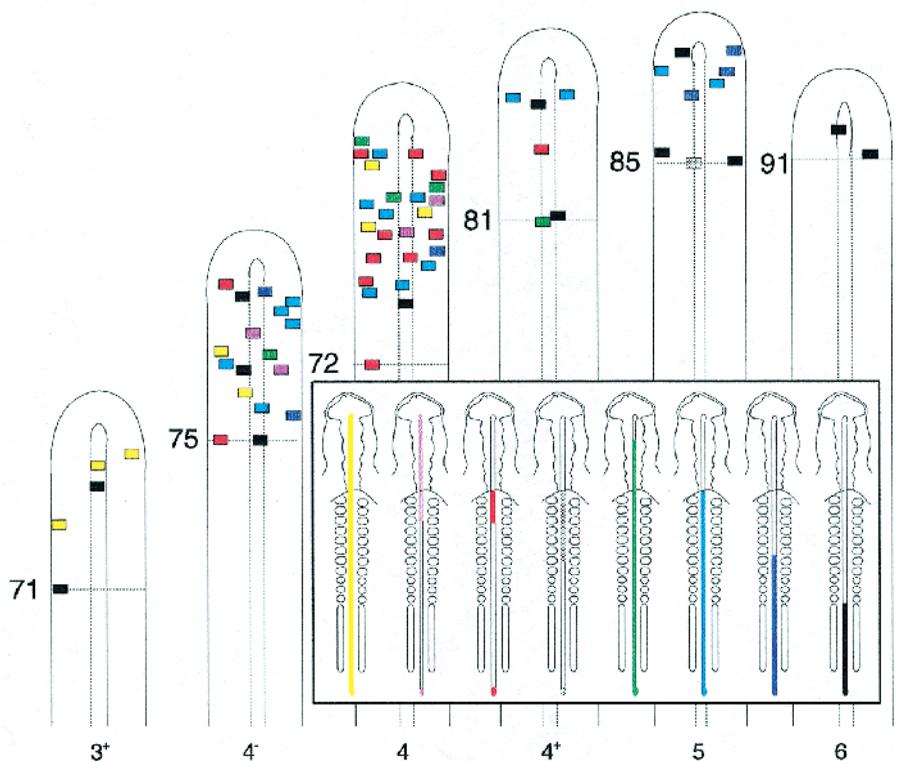
At all stages studied, the region 40-92% of the primitive streak contributes cells to the neural tube (Figs 2, 3J). All areas contributed to the medial hinge

point ('notoplate'), as well as to dorsal and/or lateral walls of the neural tube. No correlation was found between stage or position in the streak and the pattern of labelling observed in the neural tube.

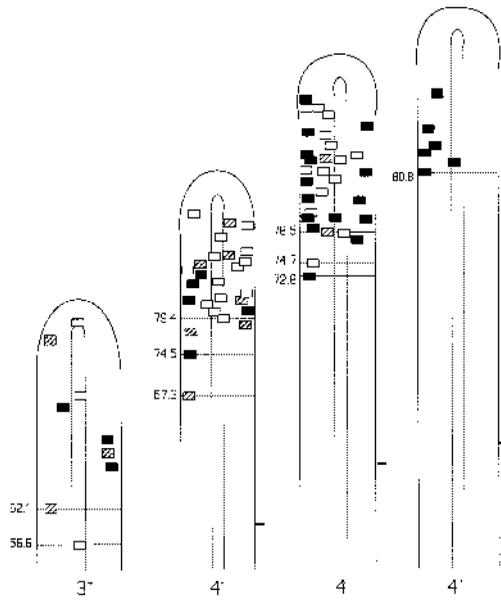
Sites that contribute to intermediate mesoderm are concentrated in a zone at 60-80% of the length of the primitive streak, overlapping with regions that contribute to the lateral parts of the somite and lateral plate mesoderm (Figs 2, 3F,P). Most of the length of the primitive streak (0-75%) contributes to the lateral plate and blood vessels (Figs 3F, 11) at all stages studied. The contribution of the primitive streak to the heart ceases at stage 4<sup>+</sup> (Fig. 11).

#### Tissue combinations

Comparison of the fate maps shown in Figs 2 and 8 shows that 50% (33/66) of injections that contributed to the notochord also produced labelled cells in the medial part of the somitic mesoderm ( $n=13$ ), in the foregut ( $n=15$ ) or in the head mesenchyme ( $n=5$ ). Comparison between Figs 2 and 8 also shows that, in those injections that give rise to intermediate mesoderm and/or lateral plate in addition to somites ( $n=15$ ), the somites tend to display 'lateral type' patterns (see above). By contrast, when injections give rise to head mesenchyme or notochord in addition to somites ( $n=21$ ), the latter display 'medial type' patterns.



**Fig. 4.** Progenitors of the notochord. Diagrams summarising the contribution of different regions of primitive streak and lateral Hensen's node to different rostrocaudal portions of the notochord. Each coloured block represents one injection, some of whose progeny was found to have contributed to the notochord. The horizontal dashed lines represent the posterior limit of the region contributing to notochord at each stage.



**Fig. 5.** Progenitors of the head mesenchyme and foregut. Each open square represents one injection that contributed labelled cells to the foregut. Black squares represent injections contributing to the head mesenchyme. Hatched squares correspond to injections that contributed to both of the above structures, or where it was impossible to distinguish between them.

## DISCUSSION

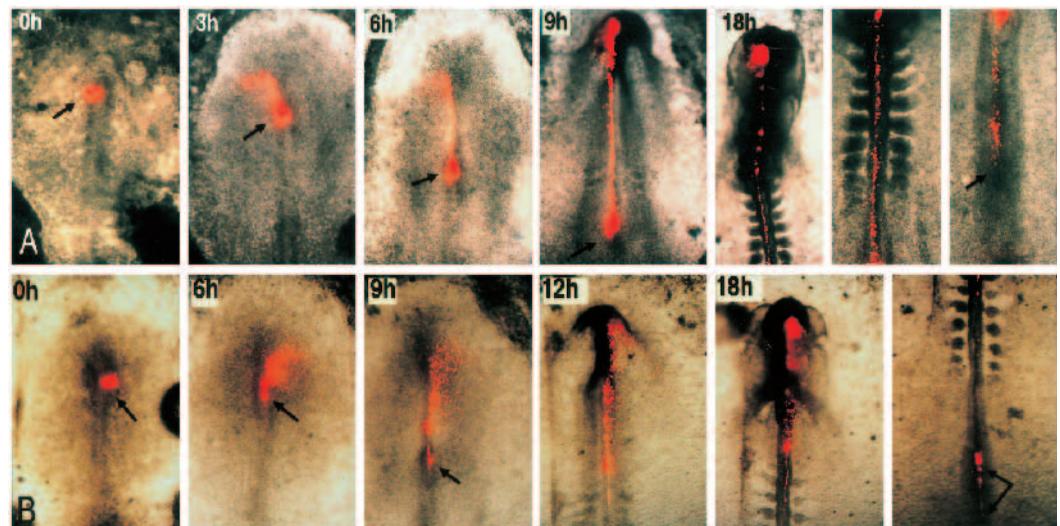
The fate maps presented in this study confirm and extend those of previous authors, produced by a variety of methods (Peebles 1898; Gräper 1929; Wetzel 1929, 1936; Kopsch 1934; Hunt 1937; Pasteels 1937, 1943; Spratt 1942a,b, 1946, 1947, 1952, 1955, 1957; Spratt and Codon 1947; Bellairs 1953a,b; Spratt and Haas 1962a,b, 1965; Nicolet 1965, 1967, 1970, 1971; Rosenquist 1966, 1970a-f, 1971a-d, 1972, 1982, 1983; Rosenquist and DeHaan 1966; Orts-Llorca and Collado 1968;

Stalsberg and DeHaan 1969; Schoenwolf et al., 1992; Schoenwolf and Sheard 1990; García-Martínez and Schoenwolf 1993; García-Martínez et al., 1993; Inagaki et al., 1993). In general, they reveal that the primitive streak is organized such that anterior regions give rise to more dorsal/axial descendants than posterior regions, which contribute mainly to lateral tissue types. In agreement with previous authors, we find that this pattern changes with time. The precursors of some cell types (such as endoderm) are present in the streak for only a relatively short time, while the progenitors of other types (such as notochord and somite) remain in the streak throughout its development. Our results give additional information about the subdivision of somites into lateral and medial portions, as well as on the pathways taken by the descendants of primitive streak cells as they join different tissues of the embryo.

### Somites and their subdivision into medial and lateral parts

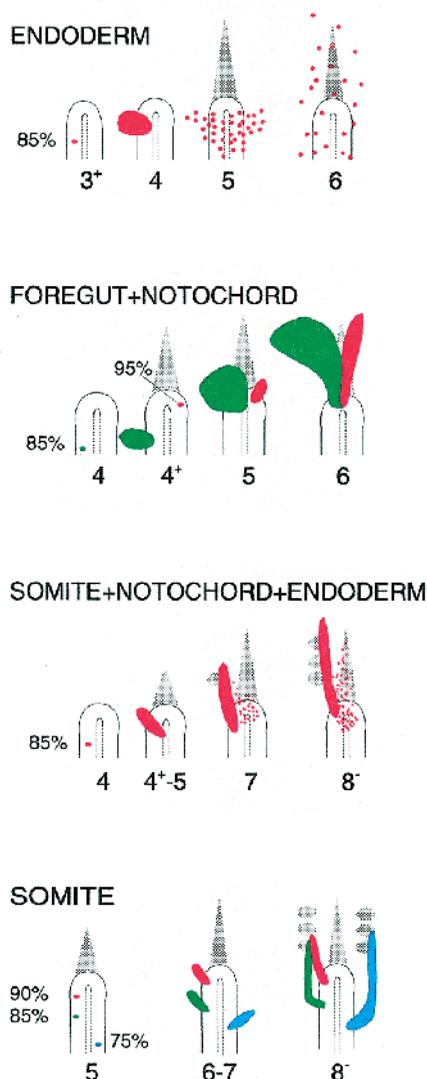
Selleck and Stern (1991) reported that the progenitors of somites are segregated into those that contribute to the medial half, situated in Hensen's node, and those that give rise to the lateral halves, situated more posteriorly. This was correlated with the finding by Ordahl and Le Douarin (1992) that the two halves of the dermomyotome have different fates in terms of the muscles to which they will give rise. In contrast, Schoenwolf et al. (1992) reported that there is no such segregation into medial and lateral somite precursors in the primitive streak at stage 4<sup>+</sup>. It was therefore important to resolve this apparent discrepancy, applying the dye as described by Selleck and Stern (1991) but extending the analysis to more posterior regions of the streak. We find that there is indeed a segregation into medial and lateral precursors, but there is considerably more overlap than suggested in Selleck and Stern's (1991) study of the node. The node itself, and particularly at stage 4, is the only region in which cells contribute **solely** to the medial part of the somites ('medial type' patterns) (Fig. 3G). The region contributing to lateral portions of the somites is much larger and extends from position 57% to 91% of the streak at this stage

**Fig. 6.** Movement of cells contributing to notochord and foregut. (A) Embryo labelled at position 93% at stage 4, photographed at intervals during incubation. Initially, cells emerge anteriorly and laterally. As incubation proceeds (6 hours and beyond), the paths of newly emerging cells become more medial. Note that at all stages, a group of labelled cells remains at the level of the regressing node. Three views are shown for the 18 hour time point (stage 9<sup>+</sup>), covering the entire length of the axis. (B) Embryo in which DiI was injected at position 85% at stage 4<sup>-</sup>, showing ipsilateral movement of labelled cells to the foregut, prechordal plate and axial endoderm. The pattern of movements seen is similar to that shown in A for notochord progenitors, except that the cells that emerge initially (6 hours) are more dispersed. Again, a group of labelled cells remains at the level of the regressing node throughout the period studied. At the 18 hour time point, two views are shown, to cover the whole axis.



(Fig. 8). Since cells that contribute **some** progeny to the medial portions of somites extend posteriorly as far as 73% at stage 4, there is considerable, but not complete, overlap between prospective medial and lateral cells, as suggested by Schoenwolf et al. (1992).

Our findings contrast with the report of Kopsch (1934), of a direct correlation between 'one segment of the primitive streak and one somite in the embryo' (cited from Romanoff, 1960). While it is generally true that rostral somites originate from rostral levels of the primitive streak, as reported by Rosenquist (1966) and Schoenwolf et al. (1992), our results reveal that the correlation only applies to the rostral **limit** of labelling; we often see the whole length of the somitic mesoderm with labelled cells (Fig. 9). This is consistent with our finding of labelled cells remaining at the site of injection during subsequent development (see below).



**Fig. 7.** Summary of initial migration routes taken by labelled cells as they contribute to different structures. The colours are arbitrary and the shapes of the regions represent typical results of single injections rather than the change of shape of whole prospective regions. Although in many cases it was observed that a single injection contributes labelled cells to both sides of the embryo, only one side is shown in most of the diagrams, for clarity.

Our study of cell migration reveals a mechanism by which the segregation into medial and lateral portions of the somite occurs. Medial precursors migrate very close to (or through) Hensen's node to reach the medial part of the paraxial mesoderm, whereas precursors for the lateral halves migrate more laterally, to reach the lateral half of the paraxial mesoderm (Fig. 7). An important question remaining to be addressed is whether this distribution is the result of the position of precursors in the primitive streak, or a reflection of the state of commitment of the cells.

### Multipotent cells, or mixtures of committed cells?

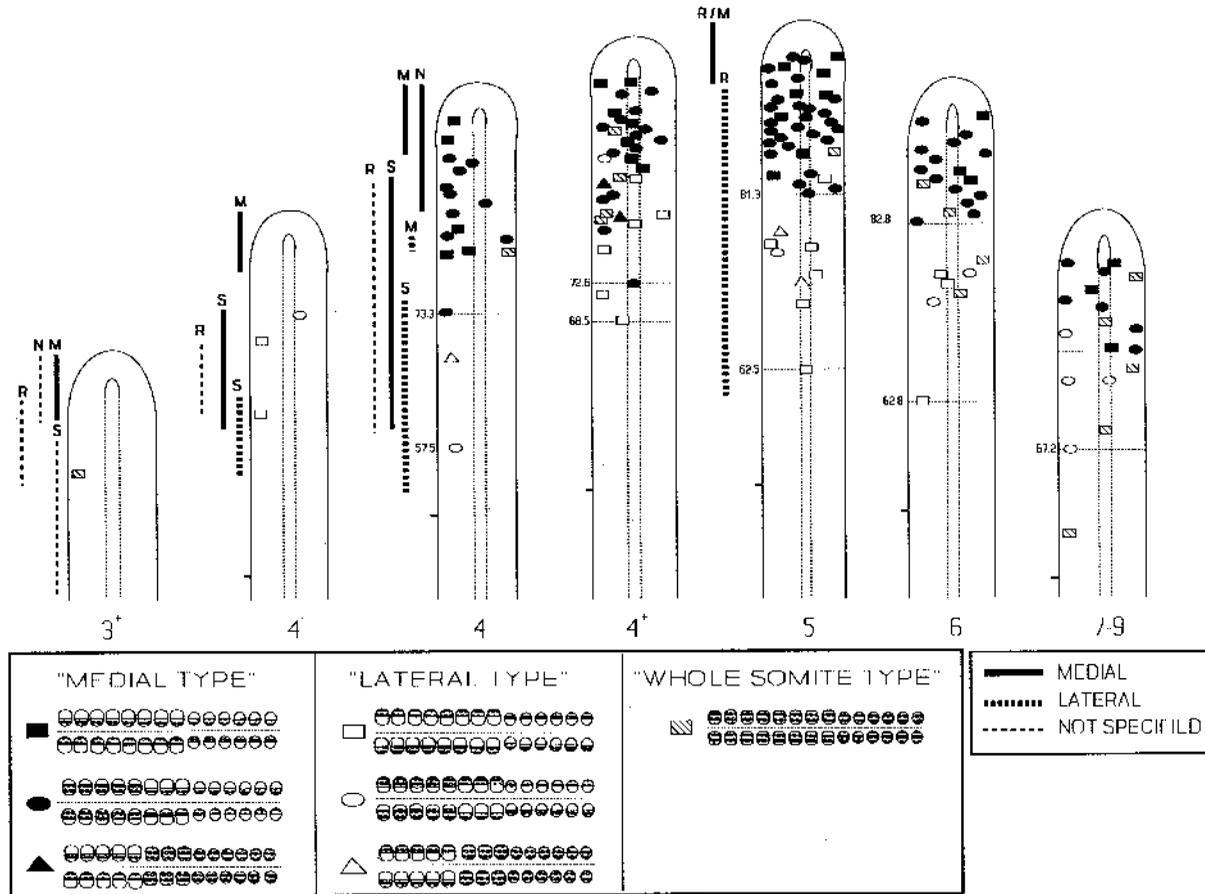
Despite the general arrangement of prospective cell types in the primitive streak, with more dorsal/axial precursors occupying more anterior positions, there is considerable overlap between them. Many injection sites contributed cells to different tissues, and this is particularly so at early stages of development and in anterior portions of the streak. Thus, the anterior part of the early primitive streak contains a condensed population of precursors for most of the dorsal/axial tissues. Given the high density of progenitors for different dorsal/axial organs in the anterior streak, does the multitude of fates seen after a single injection of dye reflect the existence of multipotent cells, or is it the result of a complex mixture of cells each committed to a particular fate? Since our labelling method marks many cells, it is impossible to answer this question for all cell types from the present data. However, the results obtained highlight the anterior quarter of the primitive streak as the most interesting region for future studies by single cell lineage analysis.

The method used to apply DiI also makes it difficult to decide the location of the labelled cells in terms of superficial and deep layers. This may be particularly important in resolving the question of multipotentiality, since previous single cell lineage analysis has shown that, in Hensen's node, it is cells in the upper (epiblast) portion that are most multipotent (Selleck and Stern 1991). This situation is also likely to extend to the primitive streak.

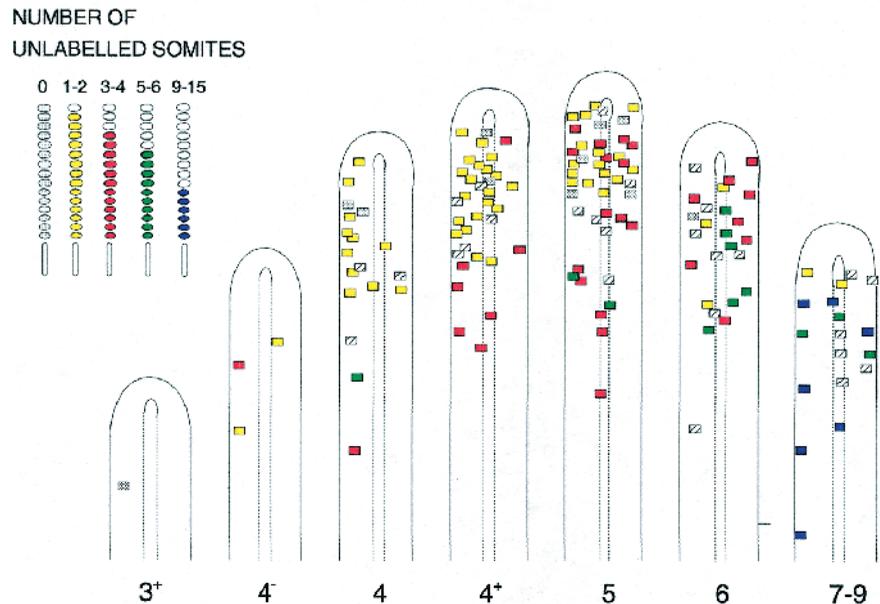
An interesting finding from the present study is that cells destined for different structures follow defined pathways of movement, which appear to correlate more closely with the tissue to which they will contribute than to their position in the streak at the time of labelling. Thus, although notochord and somitic precursors share some of the same locations in the streak, the former migrate anteriorly via the node, while some of the latter emerge from the lateral parts of the streak close to the injection site (Fig. 7). This suggests that cells destined for notochord and those destined for somite mesoderm are already specified in the streak. However, a recent study (Stern et al., 1995) has shown that, even at primitive streak stages, epiblast and primitive streak cells retain their ability to respond to activin as a mesoderm-inducing factor, consistent with there being uncommitted cells within the primitive streak.

### Apparent commitment of regions of the node

Selleck and Stern (1992a), in a series of transplantation and exchange experiments between different node fragments, found that the median-anterior quadrant of the node (which contains mainly notochord precursors) would give rise to ectopic notochord, but not somites, when used to replace the lateral quadrants. By contrast, when a lateral quadrant was used to

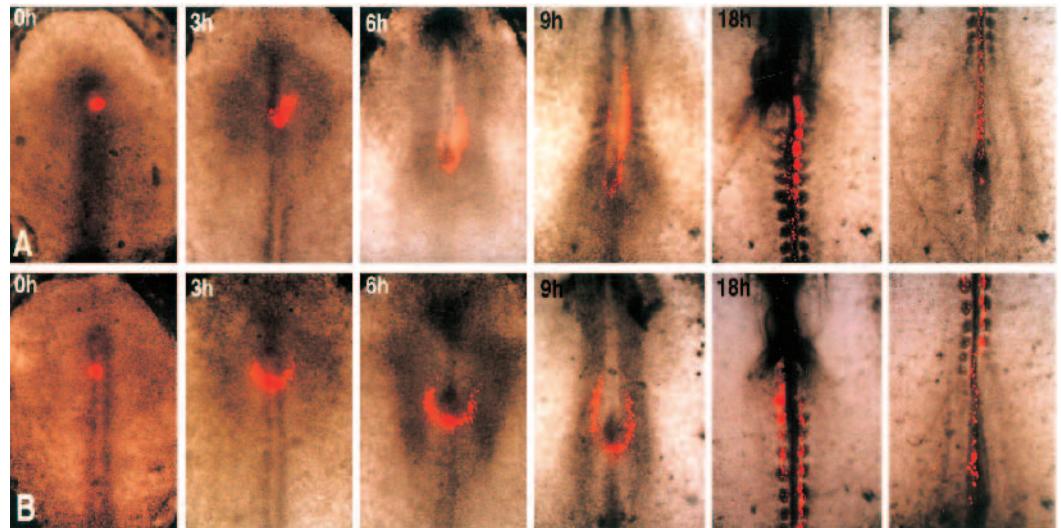


**Fig. 8.** Progenitors of the somitic mesoderm. The patterns of labelling obtained were classified into three major categories: ‘medial type’ patterns (black symbols) are those seen in embryos containing labelled cells in a medial portion of the somites (the labelled region can encompass more or less than half of the somite) but which did not contain any somites with labelling confined to the lateral side. ‘Lateral type’ patterns (white symbols) are the converse of those described above: somites labelled only in a medial portion are never observed. Finally, ‘whole somite type’ patterns (hatched squares) consist of somites that are labelled throughout their width; these embryos do not contain somites in which a restricted part of their mediolateral extent is labelled. Next to each diagram, the vertical lines indicate the results obtained by previous authors: R, Rosenquist 1966; N, Nicolet 1967; M, Selleck and Stern 1991; S, Schoenwolf et al., 1992.



**Fig. 9.** Contribution of the primitive streak to different rostrocaudal levels of the somitic mesoderm. Each colour represents a labelling pattern according to the most rostral somite that contained labelled cells, as shown in the inset. Hatched squares correspond to embryos that were not analysed in this way.

**Fig. 10.** Movement of cells contributing to somites and other structures. (A) DiI was injected at position 90% at stage 4. Labelled progeny are found in the medial halves of the somites, head mesenchyme, the midline of the endoderm and the notochord. Cells emerge laterally very close to the node and begin to overlap with the node between 3 and 6 hours after labelling. Cells are confined to the medial aspect of the forming paraxial mesoderm as early as stage 7 (6 hours). Two views of the 18 hour time point are shown. (B) DiI injected embryo at position 83% at stage 4, showing bilateral movement of cells to the medial halves of the somites and to the underlying blood vessels. Note that cells emerge laterally from the site of labelling without passing through the node. At later stages, the pathways of migration become progressively more medial. Even for this more caudal injection, cells remain at the level of the regressing node throughout the incubation period. Two views of the 18 hour time point are shown.



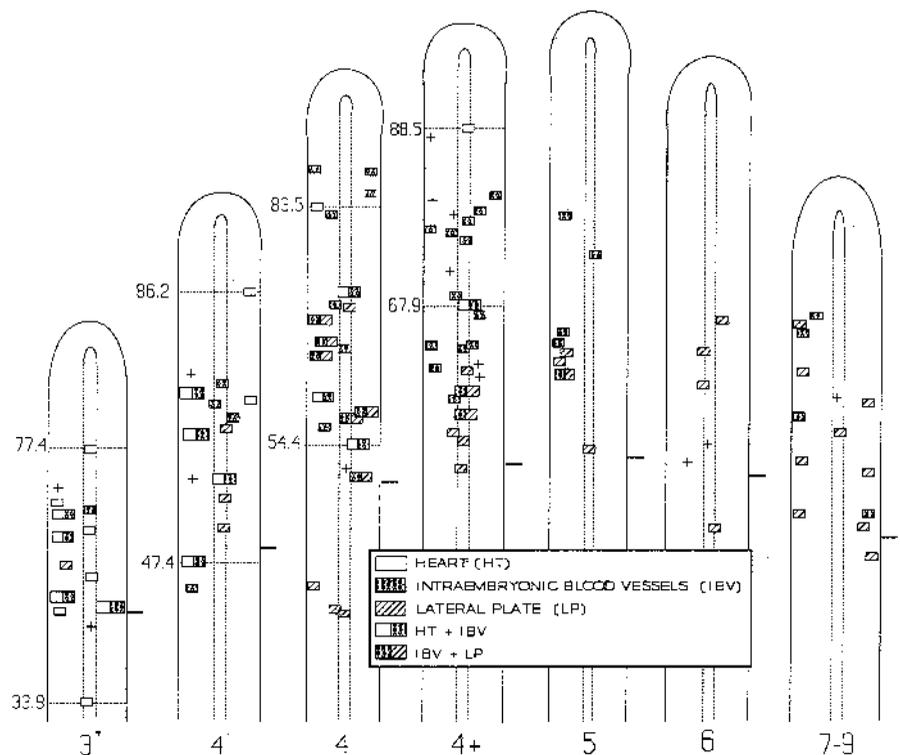
replace the median-anterior portion, notochord was also formed, and the grafted piece did not contribute to somites. This led to the suggestion that the median-anterior quadrant contains cells committed to a notochord fate, while the median-anterior quadrants are not yet specified.

The present results provide an explanation for these findings. The median-anterior quadrant of the node contains cells that show predominantly an anteriorly directed movement into the future head process/notochord. All cells destined to contribute to the somites have already emerged from the streak at more posterior levels (including the lateral quadrants of the node). Thus, while the lateral quadrants contain both somitic and notochord precursors, none of the former remain in the most anterior part of the node. Hence, rather than different states of commitment, the experiments of Selleck and Stern (1992a) appear to reflect the presence of different subpopulations of cells and different migratory pathways for the two regions. An interesting conclusion from this argument is that the median-anterior quadrant of the node might be considered as a posterior extension of the head process, rather than part of the node itself.

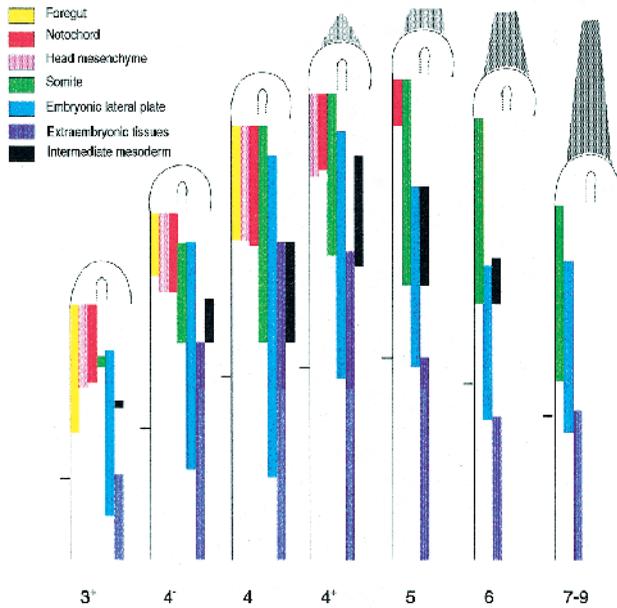
**Origin of some typical derivatives of Hensen’s node in the primitive streak**

As found by previous studies using different methods (Spratt 1955, 1957; Rosenquist 1966, 1970a-f, 1971a-d; Schoenwolf et al., 1992), we find that

tissues normally considered to derive from the node also have progenitors in more posterior regions. However, we now reveal that cell migration within the primitive streak leads to the movement of some of these precursors (mainly notochord,



**Fig. 11.** Progenitors of the lateral plate mesoderm. The conventions of the figure follow those in Fig. 2. Crosses represent cases where the labelled cells were found in the lateral plate, but which were not scored with respect to which specific structure in the later embryo contained labelled cells. The horizontal dashed lines correspond to the anterior and posterior limits of the region found to contribute to the heart at each stage.



**Fig. 12.** Summary fate maps of the primitive streak. The diagrams summarise the anteroposterior positions of the precursors to the main tissue types along the axis of the primitive streak. Although prospective cell types are shown side-by-side for clarity, there is no meaning to their mediolateral position in the diagram. The short horizontal line next to each streak corresponds to the 50% position. It is important to note that when a specific prospective region seems to appear or to increase in size between consecutive stages, this could be due to proliferation within the streak or to ingressions of cells from other regions of the embryo.

head mesenchyme and foregut) from the streak to the node before they exit. Notochord progenitors move in a direction that takes them through the anteromedial quadrant of the node, while others, such as progenitors for the medial somite, only pass through the lateral quadrants and do not invade the medial sector (Fig. 7). This finding has implications for studies of the ability of the embryo to regulate after ablation of Hensen's node (Grabowski 1956) or its replacement by posterior regions of the primitive streak (Abercrombie 1950; Abercrombie and Bellairs 1954). Clearly, these studies must now be repeated taking advantage of the present fate maps as well as of newly available molecular markers to identify specific cell types.

### Resident cells in Hensen's node

When the movements of descendants of cells labelled in a region destined to contribute to the notochord or somites were followed, it was found that labelled cells remaining in the primitive streak gradually moved to the node, where they remained at least up to stage 12 (Figs 6, 10), despite the substantial regression that takes place after stage 5. This does not support Spratt's (1955) conclusion that during streak regression the population of cells making up the streak is transient. While some cells clearly do exit the streak and undergo considerable migration within it, others remain in it for at least 24 hours, throughout regression. These findings are consistent with previous suggestions of the existence of stem cells in the node (Selleck and Stern 1992b; Beddington 1994).

### What is the primitive streak?

The primitive streak has variously been defined as a transient passageway for cells between epiblast and deeper layers (e.g. Balinsky 1975), as a blastemal centre for proliferation (e.g. Kölliker 1879; Balfour 1880; Peebles 1898; Novack 1902; Kopsch 1926, 1934; Wetzel 1929; 1936; Derrick 1937; Spratt and Haas 1960; 1961a,b; 1962a,b; Stern 1979), as well as by the prospective cell types contained within it (Nicolet 1971) and by the expression of molecular markers (Stern and Canning 1988). The present study (with fate maps summarised in Fig. 12) gives support to all of these views. On one hand, the population of cells it contains is transient in that some cells leave to contribute to different tissues while others presumably enter it as part of the process of gastrulation. On the other, there exists, particularly at the anterior end, a resident population of cells which accompanies regression and whose progeny leave the streak during this period. An interesting question remaining to be addressed concerns the control mechanisms that regulate gene expression for cells that enter, remain in or leave the primitive streak.

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