INTRODUCTION

The developmental fate of cells in the Zebrafish, *Xenopus*, chicken and mouse embryo during germ layer formation has been mapped by tracing cell lineages during gastrulation (Kimmel et al., 1990; Stainer et al., 1993; Keller, 1975, 1976; Vakaet, 1984; Schoenwolf and Sheard, 1990; Tam, 1989; Lawson et al., 1991; Lawson and Pedersen, 1992). From these studies, fate maps that broadly describe the morphogenetic movements and the multitude of differentiation pathways normally taken by the cells have been constructed. The most significant finding of these fate-mapping studies is that the mouse embryo is strikingly similar to other vertebrate embryos at gastrulation regarding the organisation of the basic body plan (Lawson et al., 1991). Despite this, it is not known whether the mouse embryo also shares similar morphogenetic and molecular mechanisms for germ layer induction. Results of this study provide compelling evidence that the precursor population of the neural tube is contained in the distal cap epiblast of the early-primitive-streak-stage embryo. Furthermore, the regionalisation of cell fate within this small population suggest that a preliminary cranio-caudal patterning may have occurred in the neural primordium before neurulation.

SUMMARY

Neuroectodermal fate of epiblast cells in the distal region of the mouse egg cylinder: implication for body plan organization during early embryogenesis

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Key word: epiblast, distal cap, egg cylinder, gastrulation, neuroectoderm, mouse embryo

INTRODUCTION

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In the early-streak stage (H&H stage 3-4) of chick embryos, the neural precursors are localised to a site in the epiblast anterolateral to the Hensen’s node (Nicolet, 1971; Vakaet, 1984). The entire neural tube seems to be derived from a small epiblast population of about 1500 cells in the early embryo. There is no morphological definition of the prospective population destined for different axial levels of the neural tube. Results of this study provide compelling evidence that the precursor population of the neural tube is contained in the distal cap epiblast of the early-primitive-streak-stage embryo. Furthermore, the regionalisation of cell fate within this small population suggest that a preliminary cranio-caudal patterning may have occurred in the neural primordium before neurulation.

Key word: epiblast, distal cap, egg cylinder, gastrulation, neuroectoderm, mouse embryo
dorsal blastopore lip in the animal hemisphere (Keller, 1975). At the late gastrula stage, the neuroectoderm expands and encompasses over 4/5 of the animal cap ectoderm and begins to display anterior-posterior difference in cell fate (Nieuwkoop et al., 1985) and the expression of region-specific genes (Ruiz i Altaba, 1994).

In the mouse, fate-mapping studies reveal that the epiblast (embryonic ectoderm) of the 6.5-day embryo at early gastrulation contains not only the precursor cells of the ectodermal derivatives but also of the endoderm and mesoderm (Lawson et al., 1991; Lawson and Pedersen, 1992; Lawson and Hage 1994; Tam and Beddington, 1992). The proximal part of the epiblast is populated by precursors of the extraembryonic mesoderm, the amnion ectoderm and the primordial germ cells. The lateral and posterior epiblast have a predominantly mesodermal fate and contribute cells to the heart, lateral and paraxial mesoderm. Ectodermal precursors are mostly localised to the anterior and distal regions of the egg cylinder that makes up about 45% of the total epiblast population (Snow, 1977). We do not know whether there is any delineation within this population, of the neuroectoderm from other ectodermal derivatives. However, by the late-primitive-streak-stage at 7.5 days, over 80% of cells in the embryonic ectoderm are destined to form the neural tube, and specific populations of embryonic ectodermal cells can be mapped to different segments of the neural tube (Tam, 1989). Such expansion of the neural precursor population may be the result of cell proliferation (Snow, 1977; Poelmann, 1980) and the morphogenetic movement of cells during gastrulation (Tam et al., 1993). Specifically, there may be an epibolic expansion, of the neuroectodermal and surface ectodermal cells, into the space vacated by the non-ectodermal cells that depart via ingestion at the primitive streak. To resolve this possibility, it is critical to find out whether there is indeed a separate pool of neuroectodermal precursors in the epiblast at early gastrulation. If so, it is of interest to locate precisely their position in the epiblast in relation to other cell lineages such as the surface ectoderm and the embryonic mesoderm. To trace the morphogenetic movement of the neuroectodermal precursors during germ layer formation, it is necessary to find out if there is any pattern of cell fate in this population that may bear upon their disposition first to the neural plate blueprint at late gastrulation, and then to their final destination in the fully formed neural axis.

In this study, we have mapped the location of the neuroectodermal precursors in the epiblast of 6.5-day early primitive-streak-stage embryos. Specifically, we have analysed cell fate in the distal (cap) region of the epiblast by tracking cells marked in situ with carbocyanine dyes, or by monitoring the differentiation of lacZ-expressing epiblast cells that have been grafted to the distal cap region of the egg cylinder.

**MATERIALS AND METHODS**

**Experimental strategy**

The developmental fate of distal cap epiblast cells of the 6.5-day early-primitive-streak-stage embryo was analysed by monitoring the distribution of labelled cells in the neural tube during in vitro development. Labelling of small groups of epiblast cells was achieved by delivering pico-litre quantities of carbocyanine dyes by microinjection to defined sites in the distal epiblast. Their location in the embryonic ectoderm and neural tube of the experimental embryo was examined by fluorescence microscopy and confocal imaging after 24 and 48 hours of in vitro development. The normal fate of cells at specific regions of the epiblast was also studied by analysing the pattern of tissue colonisation by cells that have been grafted to the distal cap epiblast. The grafted cells were taken from embryos of a transgenic mouse line that expresses an X-linked lacZ transgene ubiquitously throughout development. The transgene-expressing cells in the experimental chimaeras were identified by the histochemical detection of β-galactosidase using the X-gal substrate.

**Isolation and culture of early-primitive-streak-stage embryos**

Early-primitive-streak-stage mouse embryos of ARCS strain were obtained from pregnant mice at 6.5 days post coitum (noon on the day the vaginal plug was 0.5 day post coitum). The egg cylinder was dissected from the decidua in PB1 media and the Reichert’s membrane was removed. Embryos were staged according to Downs and Davies (1993) and cultured in 4-well chamber slides (NUNC), in groups of 8-10 per well, at 37°C under 5% CO2 in air. The culture medium was made up of 25% Dulbecco’s modified essential medium (DMEM) and 75% human cord serum (HCS), or 25% DMEM, 50% rat serum and 25% HCS (Sturm and Tam, 1993).

**Defining the injection sites**

The distal cap region of the epiblast was demarcated by a transverse plane passing through the distal limit of the proamniotic cavity and by the arc of an approximately 30° sector centred on the plane at the level of the amniotic folds (Fig. 1A). The distance along the diameter of the cap was 50-65 µm. Approximately 125-150 cells (25% of total epiblast population, Snow, 1977) were contained in the distal cap. Epiblast cells along the embryonic midline at (A) the anterior, (B) the tip and (C) the posterior regions of the distal cap and two additional sites (D,E) immediately outside the distal cap and about 2/3 the way down the anterior and posterior sides of the epiblast were labelled by microinjection. Grafting of transgenic cells was made only to Sites A, B and C. To ensure that injections and grafts were always made to the embryonic midline, egg cylinders were held in an orientation so that the sagittal plane was always in focus (Fig. 1B).

**In situ cell labelling by microinjection**

Cells were labelled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indo-carbocyanine percholate (DiI) and 3,3′-dioctadecyloxycarbocyanine percholate (DiO, Molecular Probes). Stock solutions (at 0.5% w/v) were prepared in 100% ethanol. The stock solution was diluted with 0.3 M sucrose at 1:5 (DiI) and 1:2 (DiO) for injection (Serbedzija et al., 1985) and the expression of region-specific genes (Ruiz et al., 1992). Embryos for labelling were placed in a hanging drop of PB1 medium in a Leitz micromanipulation chamber under paraffin oil. The embryo was held, using a polished thick-walled glass micropipette, by gentle aspiration of the endoderm next to the epiblast site to be labelled (Fig. 1B). The injection pipette (i.d. 2 µm) containing the dye solution was inserted into the egg cylinder through the extraembryonic tissue and the proamniotic cavity and punctured the luminal side of the epiblast opposite the holding pipette (Fig. 1B). About 1-3 pl of dye was injected into the epiblast. The injection pipette only contacts the epiblast at the injection site. Inadvertent labelling of the extraembryonic tissue did not affect the results because it makes no contribution to the embryo proper.

The embryos were fixed in 4% paraformaldehyde in air. The culture was examined using a confocal laser scan microscope.
Neural fate of distal epiblast

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Optical sections to visualise the labelled cells were always taken at specific depth of the flat-mounted specimens: between +30 to +80 µm for 24-hour embryos and from 0 to +50 µm for 48-hour embryos. Labelled cells found in these optical sections were most likely to be within the ectodermal layer. Labelled cells ... were summarised using a Canvas 3.0 program on the Macintosh computer.

Transplantation of transgene-expressing cells

Cells used for transplantation were isolated from 6.5-day embryos produced by mating H253 transgenic mice that express an X-linked lacZ transgene ubiquitously during embryogenesis (Tam and Tan, 1992). The egg cylinder was cut transversely using fine glass needles to isolate the distal cap fragment. The endoderm layer was removed after a 5-minute incubation in 0.5% trypsin, 2.5% pancreatin in Ca<sup>2+</sup>/Mg<sup>2+</sup>-free PBS (Sturm and Tam, 1993). The apical epiblast was further dissected into clumps of 10-15 cells for transplantation to 6.5-day host ARC/s strain embryos. Because of the difficulty in keeping track of the orientation of the specimen is questionable.

RESULTS

Embryos developed normally in culture

Early-primitive-streak-stage embryos were cultured for 24 or 48 hours after injection with carbocyanine dyes (Table 1). After 24 hours, 67-86% of embryos developed to the early-allantoic-bud stage showing normal formation of the three germ layers, a recognisable embryonic axis marked by the head process, the primitive streak and the allantois. The embryos were morphologically similar to the 7.5-day late-primitive-streak-stage embryos in utero (Fig. 2B), except that the cultured embryos developed a more fully expanded visceral yolk sac and amniotic cavity. After 48 hours in culture, 71-78% of the injected embryos had developed a complete neural axis with cephalic neural folds, fused cervical neural tube and open posterior neuropore (Fig. 6A). The embryos also formed a beating heart and established good vitelline circulation. The paraxial mesoderm also differentiated into cranial mesenchyme, 5 pairs of somites (Table 2, range: 3-10 pairs) and unsegmented presomitic mesoderm. Development of the 6.5-day embryos in vitro is therefore comparable to that in vivo at least up to the 8.5-day early-somite-stage.

Fate-mapping by carbocyanine dye labelling

Microinjection to specific sites

Eleven 6.5-day embryos (Fig. 2A) were examined by confocal microscopy 1 hour after injection of the carbocyanine dye into Thomas) using a vertical electrode puller (David Kopf) and fabricated using a microforge (Narishige). The embryos were held with a polished holding pipette made from thick-walled capillaries (Leica) and the donor cells were grafted by microinjection to Sites A, B and C in the distal cap (Beddington, 1987). Embryos that have developed for 48 hours in culture were fixed for 3-5 minutes with 4% paraformaldehyde and then stained with X-gal solution overnight at 37°C to detect the β-galactosidase activity in lacZ-expressing cells (Beddington and Lawson, 1990). Embryos containing labelled cells were photographed using a Wild M340 photoautomat system fitted to a Wild M3Z dissecting microscope and then were processed for wax histology.

Table 1. Embryos labelled by microinjection of carbocyanine dye and cultured in vitro for 24 to 48 hours

<table>
<thead>
<tr>
<th>Site</th>
<th>Time in culture (hours)</th>
<th>No. injected</th>
<th>No. developed normally* ( % injected)</th>
<th>No. fully analysed by confocal microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>24</td>
<td>–</td>
<td>104</td>
<td>80 (77%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>48</td>
<td>112</td>
<td>87 (78%)</td>
</tr>
<tr>
<td>B</td>
<td>24</td>
<td>57</td>
<td>57</td>
<td>49 (86%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>48</td>
<td>63</td>
<td>44 (70%)</td>
</tr>
<tr>
<td>C</td>
<td>24</td>
<td>58</td>
<td>168</td>
<td>113 (67%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>48</td>
<td>58</td>
<td>41 (71%)</td>
</tr>
</tbody>
</table>

*About 45% of these embryos were found not to contain any labelled cells. 13% of embryos were analysed completely by confocal microscopy. The remaining embryos were only analysed by fluorescent microscopy and most of them displayed patterns of distribution of labelled cells similar to those shown in Figs 3 and 5. These embryos were not suitable for confocal analysis because of inappropriate level of labelling, or when the orientation of the specimen is questionable.
the distal cap epiblast. In all cases, a discrete patch (10-15 µm in diameter which corresponded to about 8-12 cells) of the epiblast was labelled. The injected dye did not spread indiscriminately to other regions of the epiblast nor to the adjacent endoderm (Fig. 2, Sites A, B and C). The entry site of the injection pipette in the extraembryonic parts of the egg cylinder was often also labelled by dye adhering to the outer surface of the pipette (data not shown). Table 1 summarises the number of embryos that have been injected with carbocyanine dye. About 55% of the embryos harvested at the end of the experiment were found to contain labelled cells when examined by fluorescence microscopy. 7-10 well-labelled and properly
mounted embryos of each group were selected for a complete analysis by confocal microscopy. Data of confocal analyses were summarised in Figs 3 and 5, which show only the neuroectodermal contribution by labelled cells. The distribution of labelled cells in the other embryos, which were examined by fluorescent microscopy without detailed confocal imaging, was generally similar to the selected embryos. Some weakly labelled embryos that contained few labelled cells showed only a subset of the overall pattern of distribution.

Distribution of labelled cells in embryos cultured for 24 hours

Site A

Labelled cells derived from the anterior region of the distal cap epiblast were found in anterior proximal regions of the embryonic ectoderm of the late-primitive-stripe-stage embryo (Fig. 2C). The data from 7 embryos were summarised in Fig. 3 (Site A), which shows that the labelled cells were localised to the regions occupied by the prospective forebrain and midbrain neuroectoderm. There is variation between embryos in the distribution of the labelled cells. In 3 embryos, cells were found to spread widely over the whole anterior proximal ectoderm, but in 4 other embryos, labelled cells remained close together as a small patch in the anterior ectoderm. No labelled cells were found in the distal, lateral or the posterior regions of the embryonic ectoderm (Fig. 3 Site A).

Site B

Labelled cells were localised mostly to the anterior, lateral and distal regions of the embryonic ectoderm (Figs 2D, 3 Site B). Cells from site B were distributed more extensively than those from site A and spread over wider areas along the craniocaudal axis. Similar to cells from site A, cells from site B colonised the anterior proximal ectoderm that is destined to form the forebrain and midbrain. In addition, site B cells were also found in the distal and lateral ectoderm (domain of prospective hindbrain). A minor contribution was also found in the posterior ectoderm next to the primitive streak. A closer examination of the data from 10 fully analysed embryos have identified some differences among individual embryos. In 2 embryos, the distribution of labelled cells was remarkably similar to that of site A. In 2 other embryos, cells from site B were restricted mostly to the distal ectoderm and, lastly, in the other 6 embryos, labelled cells were found in the anterior proximal and lateral regions of the ectoderm.

Site C

Cells labelled in this site contributed significantly to the distal and posterior regions of the embryonic ectoderm (Fig. 3 Site C), corresponding to the prospective hindbrain and spinal cord. In 6 out of 9 embryos, labelled cells were found in the posterior proximal regions of the ectoderm towards the caudal parts of the neural axis (Fig. 2E). In the other embryos, labelled cells were found in a similar distribution to that found in some site B embryos.

Distribution of labelled cells in embryos culture for 48 hours

Site A

Labelled cells were mostly found in the neuroectoderm of the forebrain and the midbrain (Fig. 4A). In the hindbrain, labelled cells were invariably found in regions anterior to the preotic sulcus. Fluorescent cells spread evenly, but usually unilaterally, in the neural plate and colonised more than one brain part (Fig. 5 Site A). Some labelled cells were also found in the adjacent surface ectoderm.

Site B

The distribution of the labelled cells from Site B in the forebrain, midbrain and upper hindbrain was very similar to those from Site A. A major difference was the more caudal distribution of Site B cells to the lower hindbrain and the spinal cord at the somite level and the posterior neuropore (Figs 4B, 5 Site B). In addition, 8 other embryos contained labelled cells only in the spinal cord and the cranial aspect of the posterior neuropore (Fig. 5 Site B, stippled areas).

Site C

Labelled cells from Site C were found mostly in the lower

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Table 2. Regionalisation of cell fate in the distal cap epiblast of 6.5-day early primitive streak stage embryo, tested by orthotopic grafting of cells expressing the lacZ reporter gene

<table>
<thead>
<tr>
<th>Sites</th>
<th>No of embryos grafted</th>
<th>Somite no. after 48 hours</th>
<th>No. analysed</th>
<th>AM</th>
<th>SE</th>
<th>FB</th>
<th>MB</th>
<th>HB</th>
<th>SP</th>
<th>PPore</th>
<th>HT</th>
<th>CrM</th>
<th>LSM</th>
<th>NCD</th>
<th>YSM</th>
<th>PS</th>
<th>GN</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35</td>
<td>5.13±0.45 (15)</td>
<td>17</td>
<td>3</td>
<td>5</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>33</td>
<td>4.70±0.45 (10)</td>
<td>14</td>
<td>1</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>5</td>
<td>3</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>28</td>
<td>4.75±0.41 (28)</td>
<td>15</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AM, amnion; SE, surface ectoderm; FB, forebrain; MB, midbrain; HB, hindbrain; SP, spinal cord; PP, posterior neuropore; HT, heart; Cr, cranial mesenchyme; LSM, lateral and somitic mesoderm; NCD, notochord; YSM, yolk sac mesoderm; PS, primitive streak and/or allantois; GN, gut endoderm.
hindbrain, the spinal cord and the posterior neuropore (Fig. 4C). Labelled cells were distributed evenly and often bilaterally in the neural tube and in the neuroepithelium of the posterior neuropore. Some labelled cells were also found in the surface ectoderm and the allantois (Fig. 5 Site C), especially in embryos with the labelled cells localised to the most lateral or caudal regions of the neuroepithelium (eg. Fig. 4F,G and results of double labelling experiment). In some cases, labelled cells were found in the deeper tissues of the trunk and the posterior regions. Although their precise tissue localisation cannot be ascertained by confocal imaging, these labelled cells were likely to be cells in the paraxial and lateral mesoderm (data not shown).

Double labelling experiments

The divergent displacement of cells initially localised within the distal cap epiblast to different segments of the neural tube was further tested by comparing the distribution of labelled cells in embryos that have been simultaneously labelled at Sites A and C with DiO and DiI, respectively (Fig. 4E,F,G). Such double labelling experiments were difficult. Proper adjustment of the quantity of dye injected was required so that both labels will persist equally well and produce comparable fluorescence signals after 48 hours of culture. Often labelled embryos were not analysable because one of the labels has diminished to below the level of detection or there was an obvious difference in the signal intensity that may bias the observation. Of the 18 embryos successfully analysed, 11 displayed DiO-labelled Site A cells localised to the neuroectoderm of the cephalic neural tube (Fig. 4E) and DiI-labelled Site C cells to the spinal cord (Fig. 4F), posterior neuropore (Fig. 4G), posterior surface ectoderm and allantois (data not shown). In the remaining 7 embryos, DiO-labelled Site A cells were confined to the fore- and midbrain but the DiI-labelled site C cells were distributed not only to the caudal parts of the neural axis but were also found in the hindbrain as rostral as the preotic sulcus.

Mapping cell fate outside the distal cap

8 embryos were labelled at site D and labelled cells were found to contribute to the anterior headfold (2 embryos), hindbrain (1 embryo), extraembryonic (amniotic) and surface ectoderm (5 embryos). 7 embryos were labelled at site E and labelled cells colonised the allantois (3 embryos Fig. 4D), the neuroepithelium of the posterior neuropore immediately anterior to the allantois (3 embryos) and the posterior neuropore and adjacent amnion (1 embryo). Labelling of cells at these sites therefore resulted in different patterns of tissue colonisation compared to labelling in the distal cap.

Blanket labelling of the entire epiblast of 6.5-day early-streak-stage embryos was also performed by injecting the carbocyanine dye into the proamniotic cavity. From these experiments, 42 out of 67 embryos analysed after 24 hour of in vitro development had labelled cells in all three germ layers. In some cases, the presence of labelled cells in the mesoderm was difficult to ascertain because of the distortion of the embryo after flat mounting.

The wider distribution of labelled cells when epiblast outside the cap or the entire epiblast was labelled, strongly suggests that the dye placed in the distal cap epiblast has not spread indiscriminately to other non-cap cells. Cells that are found in the neural tube of the early-somite-stage embryos are therefore mostly derived from descendants of the distal cap epiblast.

Fate-mapping of the distal cap epiblast by cell transplantation

Table 2 summarises the pattern of tissue colonisation displayed by the transplanted distal cap epiblast cells in the host embryos after 48 hours of in vitro development. Embryos
Fig. 4. (A) Confocal image of labelled cells from Site A in the head fold of the early-somite-stage embryo at 48 hours of culture. Labelled cells are found in the neural plate of the prospective forebrain (fb), midbrain (mb), ng, neural groove. (B) Confocal image of mid-portion of the early-somite-stage embryo at 48 hours of culture showing the distribution of labelled cells from Site B in the lower hindbrain (hb) and the spinal cord (sp). (C) Confocal image showing the localisation of labelled cells from Site C in the neuroepithelium of the posterior neuropore (pn), in the primitive streak (ps) and the caudal surface ectoderm (se) of the early-somite-stage embryo at 48 hours of culture. (D) Confocal image of the caudal region of the early-somite-stage embryo at 48 hours of culture, showing the presence of labelled cells derived from Site E in the allantois (al). (E-G) Results of double labelled experiment. The 6.5-day embryo was labelled at Site A with DiO and at Site C with Dil and examined at the early-somite-stage after 48 hours of culture. (E) The localisation of DiO-labelled cells from Site A in the forebrain (fb) and midbrain (mb). (F,G) The Dil-labelled Site C cells in the same embryo that are found in the spinal cord (sp) and the posterior neuropore (pn). Labelled cells outside the neural tube are likely to be in the surface ectoderm (arrowheads) because they are located in the same optical plane as the labelled cells in the spinal cord and posterior neuropore. sm, somite. Specific regions of the embryo that are illustrated in A-G are marked by boxes on the line drawing of an early-somite-stage embryo. Labelled cells show a false colour (white in A-E and blue green in F,G) as a result of computer imaging and some examples are highlighted by small arrows. Open arrows point cranially. Bar, 50 µm.
developed to the early-somite stage with an average of 5 pairs of somites and a well-developed neural tube showing early delineation of the primitive brain parts and the spinal cord. Cells grafted to site A of the distal cap often colonised the fore- and midbrain of the host embryo (Fig. 6A,E). Graft-derived cells were also found more caudally in the spinal cord and the posterior neuropore in 2 embryos and the craniofacial surface ectoderm over the pericardium in 3 embryos. Cells derived from those transplanted to Site B were distributed to the rostral parts of the neural tube (Fig. 6B,F) but, in 5 embryos, cells were distributed widely to the whole length of the neural axis. In some embryos, labelled cells were also found in the cephalic surface ectoderm and yolk sac mesoderm (Table 2). Cells in Site C were distributed more caudally in the neural axis and were found in the caudal hindbrain, the neural tube at the level of the somites (Fig. 6C,H) and the neuroepithelium of the posterior neuropore (Fig. 6D,G). In contrast to the other two sites, cells from Site C also contributed to non-ectodermal tissues such as the endocardial heart tube (Fig. 6C), the cranial mesenchyme, the somitic and lateral mesoderm, notochord (Fig. 6G), yolk sac mesoderm and the endoderm of foregut and hindgut (Table 2). The cell grafting experiment therefore produced results similar to that of the dye labelling study for the three sites. Between 20 and 70 X-gal stained cells were found in the experimental embryos. The graft-derived cells usually interspersed with host cells, but occasionally labelled cells existed as clumps in the neural tube, the heart and the allantois.

**DISCUSSION**

**Distal epiblast displays a predominantly neuroectodermal fate**

Results of this fate-mapping experiments have provided com-
pelling evidence that almost the entire precursor population of the neural tube is contained in the distal cap region of the epiblast of the early-primitive-streak-stage embryo. Clonal descendants of a population of 125-150 cells could colonise all parts of the neural tube of the 8.5-day early-somite-stage embryo. There are minor contributions to the embryonic (paraxial and lateral) and extraembryonic (allantoic and yolk sac) mesoderm especially from the epiblast cells localised at the posterior side of the distal cap. Epiblast in the most anterior region of the distal cap also contributed to non-neural ectoderm cells of the amnion and craniofacial surface ectoderm. Similar results were also obtained by tracking the differentiation of epiblast cells transplanted orthotopically to the distal cap regions. Results of our study therefore extend those obtained by clonal analysis of single cells (Lawson et al., 1991), that the apical epiblast cells not only give rise mostly to ectodermal tissues but display a predominantly neuroectodermal fate. The present study, however, only tested the fate of distal epiblast cells in the embryonic midline. It is therefore not known whether cells located on the lateral aspect of the distal cap also have a similar neuroectodermal fate. It was found in this study that the progeny of epiblast cells from Sites B and C can spread to more lateral regions of the embryonic ectoderm of the late-primitive-streak-stage embryo and by the time of neurulation cells from all 3 sites colonise the full mediolateral span of the neural plate. This finding would suggest that the midline cells do make a significant contribution to the whole neural plate and certainly would have supplemented those derived from the lateral regions of the distal cap epiblast. Anterior epiblast cells outside the distal cap (eg. Site D) also colonise the neuroectoderm but the majority of the descendants are found in non-neural tissue lineages such as the surface ectoderm and amniotic ectoderm. Those initially residing posterior to the distal cap (eg. Site E) mostly contribute to the posterior surface ectoderm and extraembryonic mesoderm. Results of our labelling studies have therefore provided a finer delineation of the domain occupied by the neuroectodermal precursors. Descendants from this population are distributed to the regions of the embryonic ectoderm of the late-primitive-streak-stage embryo that are occupied by prospective segments of the neural tube. The apical epiblast-derived neuroectodermal cells are likely to have mixed extensively with non-neural cells originating in other parts of the epiblast (Lawson et al., 1991; Lawson and Pedersen, 1992). The embryonic ectoderm at 7.5 days has been shown to contain both neural and non-neural precursors (Tam, 1989). Results of the present study suggest that the neural precursors are those that are derived from the 6.5-day apical epiblast and are by 7.5-day interspersed with the non-neural precursors.

Historical analysis of the pattern of tissue colonisation and differentiation of lacZ-expressing cells has provided additional information on the developmental fate of distal epiblast. Cells from Sites A and B display a predominantly neuroectodermal fate. Some Site A cells however will colonise cranial surface ectoderm and amnion, probably because of the lability of cell fate similarly to that shown by clonal analysis (Lawson et al., 1991). Site B cells are almost exclusively found in the neural tube. We have observed that, in carbocyanine-labelled embryos, some labelled cells from Site C were found in the non-neural tube tissues. The cell transplantation study has shown that, apart from the posterior neural tube, Site C cells also frequently contribute to the heart mesoderm, the paraxial mesoderm, the allantoic mesoderm and the notochord. Most interestingly, graft-derived cells from Site C were found in nearly all craniocaudal levels of mesoderm and the notochord and in the neural tube caudal to the upper hindbrain. By contrast, the segmental distribution of cells from Sites A and B are more restricted. It seems therefore, some cells grafted to Site C may have adopted a new fate by their incorporation into a stem cell population normally residing close to Site C. This stem cell population then acts as a constant source of new cells for the formation of tissues of embryonic axis (Tam, 1984; Tam and Beddington, 1987; Tam and Tan, 1992).

Analysis of the mitotic activity in the epiblast of mouse egg cylinders has indicated that cell proliferation of the distal cap epiblast may be sufficient to account for the increase in cell population in the ectoderm layer and other germ layers (Snow, 1977, but see Poelmann [1980] who argues that the distal epiblast is not proliferating any faster than the rest of the epiblast). It is therefore possible that as a result of cell proliferation alone, the whole neuroectodermal population is derived entirely from the distal epiblast, a speculation that had been made based on phenotypic anomaly of the $^{*}{/8}$ mutant embryos (Snow and Bennett, 1978). Furthermore, the apparent expansion of the neuroectodermal population from the distal cap to encompass a major part of embryonic ectoderm has lent support for a role of epibolic tissue movement reputed to be instrumental to the formation of the definitive ectoderm (Tam et al., 1993).

Regionalisation of cell fate within the distal epiblast

The most important finding of this fate-mapping study is the discovery that cells in different regions of the distal epiblast are biased in their colonisation of different craniocaudal divisions of the neural tube. Differently labelled cells separated from each other by a distance of 50-65 µm in the distal cap are displaced in divergent directions during neurulation and contribute to the whole length of the embryonic axis. When the distribution of the distal epiblast cells was examined at the late-primitive-streak stage, they were found to localise in the appropriate prospective neural segments in accordance to their original anterior-posterior positions in the distal cap. This anterior-posterior regionalisation is later translated into a proper segmental address when morphogenesis takes place in the neural plate at the early-somite-stage. Even allowing for intermingling of cells from different sites and the differential tissue proliferation among brain parts (Morris-Kay et al., 1994), these findings suggest that a basic craniocaudal pattern of the neural tube may have already been established during the allocation of epiblast cells to the neuroectodermal lineage. Regionalisation of cell fate, however, does not imply fate determination. A more stringent test by heterotopic transplantation experiments will be performed to resolve whether an early pattern really exists in the distal epiblast. It is also important to know how such cell fate pattern is specified.

In the mouse embryo at early gastrulation, neural differentiation of the epiblast may be induced by an interaction with the mesendoderm that is normally associated with the neural plate ectoderm. It has also been shown that the competence for neural induction is restricted to the cells in the anterior epiblast of the early-primitive-streak-stage embryo (Ang and Rossant, 1993). In these experiments, the explanted anterior epiblast
includes both the distal cap epiblast and the presumptive non-
neural ectoderm, it would therefore be interesting to find out if the competence to response to mesendodermal induction is
restricted to the distal epiblast cells. If this is the case, then it
would suggest that the acquisition of a neuroectodermal fate
must precede the interaction with mesoderm, which does not
happen until later during gastrulation. An early signal of neural
specification might be involved. Studies in *Xenopus* have led
to the identification of an additional inductive process termed
planar induction. This differs from the more classical vertical
induction, which involves interaction of embryonic tissues that
are topographically bound together. Planar induction involves
a wave of inductive signals that spreads from a source through
the tissue mass, resulting in regional determination of cell fate
(Reviewed by Jacobson and Sater, 1988; Ruiz i Altaba, 1994).
In *Xenopus*, studies on the induction of region-specific marker
genes and the differentiation pattern of the neural tissue in the
exogastrulae suggest that planar induction by the organiser is
responsible for the anterior-posterior patterning of the neural
primordium (Ruiz i Altaba, 1992; Gilbert and Saxen, 1993).
Neural fate of distal epiblast

As fate mapping studies have localised the neural precursors of the *Xenopus* and the chick to a region immediately adjacent to the dorsal blastopore lip and Hensen’s node respectively, it is feasible that some type of planar induction evident from gene expression patterns is occurring. If an analogy could be drawn between the dorsal blastopore lip of the amphibian gastrula and the node of the avian and mouse gastrula (Lawson et al., 1991), then it is possible that the cranio-caudal regionalisation of the neural primordium may be subject to the inductive action of the node as early as the early-primitive-streak stage. In order to understand and to test the morphogenetic role of the node during neural plate specification, it is now necessary to map more precisely its precursor population in the early epiblast.

**Implication of a common mechanism for the organisation of early body pattern**

With the mapping of the neuroectoderm to the distal cap epiblast in the early-primitive-streak-stage embryo (this study) and the precursor for various mesodermal lineages to the lateral epiblast (Parameswaran and Tam, unpublished), a more detailed fate map for the epiblast can now be constructed (Fig. 7). Parallels between the gastrula fate maps of different vertebrates and the mouse have been drawn when a sufficiently detailed map for the mouse epiblast was first obtained by Lawson et al. (1991). A remarkably similar spatial organisation of precursors of various embryonic and extraembryonic tissues is evident. In our study, a comparison of the spatial organisation of tissue precursors in the mouse epiblast (presented in an inverted orientation without any topographical distortion, Fig. 7) with that in the early gastrula of *Xenopus* (Nieuwkoop and Sutasurya, 1979; Nieuwkoop et al., 1985; Jacobson and Sater, 1988) has revealed another perspective of the similar architecture of the basic body blueprint. We propose that the similarities between their body plans may be due to common patterning forces and processes. The inductive signals that establish the primary difference between animal pole and vegetal pole may be the same as those that cause, first, the delineation of extraembryonic versus embryonic lineages in the epiblast and, second, the specification of the mesoderm in lateral epiblast and the ectoderm in the most distal and anterior epiblast. This model also suggests that the source of inductive signals that specify the germ layer precursors may be found in the extraembryonic tissues (eg. the extraembryonic ectoderm and/or the primitive endoderm) of the egg cylinder. Many candidate molecules for germ layer induction have been proposed (Slack, 1994) but none have been shown unequivocally to have a critical function in the mouse. That the activin subunits are expressed in the mouse decidua (Albano et al., 1994) suggest that signals for embryonic induction may come from outside the epiblast. Evidence in *Xenopus* shows that...
there may be more than one molecule involved in the processes of neural and mesoderm induction (Green, 1994; Rao, 1994; Hemmati-Brivanlou, and Melton, 1994; Hemmati-Brivanlou et al., 1994). If this is also true for the mouse embryo, then single knockout experiments are not likely to be very informative. Verification of the role of molecules involved in the induction processes may require simultaneous nullification of several or all molecules involved in a particular inductive process so that a phenotypic change can be observed.

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REFERENCES


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