Development 128, 3623-3634 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV3473



# The organizer of the mouse gastrula is composed of a dynamic population of progenitor cells for the axial mesoderm

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*Accepted 18 June 2001*

## SUMMARY

**An organizer population has been identified in the anterior end of the primitive streak of the mid-streak stage embryo, by the expression of *Hnf3β*, *Gsc$^{lacZ}$* and *Chrd*, and the ability of these cells to induce a second neural axis in the host embryo. This cell population can therefore be regarded as the mid-gastrula organizer and, together with the early-gastrula organizer and the node, constitute the organizer of the mouse embryo at successive stages of development. The profile of genetic activity and the tissue contribution by cells in the organizer change during gastrulation, suggesting that the organizer may be populated by a succession of cell populations with different fates. Fine mapping of the epiblast in the posterior region of the early-streak stage embryo reveals that although the early-gastrula organizer contains cells that give rise to the axial mesoderm, the bulk of the progenitors of the head process and the notochord are localized outside the early gastrula organizer. In the mid-gastrula organizer, early gastrula organizer derived cells that are fated for the prechordal mesoderm are joined by the progenitors of the head process that are recruited from the epiblast previously anterior to the early gastrula organizer. Cells that are fated for the head process move anteriorly from the mid-gastrula organizer in a tight column along the midline of the embryo. Other mid-gastrula organizer cells join the expanding mesodermal layer and colonize the cranial and heart mesoderm. Progenitors of the trunk notochord that are localized in the anterior primitive streak of the mid-streak stage embryo are later incorporated into the node. The gastrula organizer is therefore composed of a constantly changing population of cells that are allocated to different parts of the axial mesoderm.**

Key words: Gastrula organizer, Node, Axial mesoderm, Cell movement, Cell fate, Mouse embryo

## INTRODUCTION

The pioneering experiments of Spemann and Mangold in 1924 first demonstrated that cells located in the dorsal blastopore lip of the amphibian embryo have the ability to induce a secondary axis when grafted ectopically (Spemann and Mangold, 1924). This cell population was named the Spemann's organizer (Harland and Gerhart, 1997). A structure with axis-inducing abilities was also found in the chick (Wetzel, 1925; Waddington, 1933) and named Hensen's node after its original discoverer (Hensen, 1876). Later, in mammals, an equivalent organizer was found first in the rabbit and (Waddington, 1936; Viebahn, 2001) later in the mouse (Blum, 1992; Beddington, 1994). Similar to the amphibian, this population of cells in mouse has the ability to induce a secondary axis when grafted ectopically. Cells found within the organizer in all species show similar tissue fates and are the primary source of the axial mesoderm, which comprises the head process (prechordal plate and prechordal mesoderm) and the notochord (chordamesoderm) (Harland and Gerhart, 1997; Camus and Tam, 1999; De Robertis et al., 2000; Stern, 2001; Viebahn, 2001). In vertebrate embryos, segmental and dorsoventral patterning of the central nervous system is subject to the inductive activity of the axial mesoderm (Jessell, 2000; Wurst and Bally-Cuif, 2001; Lemaire et al., 1997; Camus et al., 2000).

In the early-streak (ES) stage mouse embryo, cells that are fated for the axial mesoderm are localized to a region of the posterior epiblast within 50-100 μm of the embryonic-extraembryonic junction of the gastrula (Lawson et al., 1991, Tam et al., 1997a). At the late-streak (LS) stage, the progenitors of the notochord are found in the node located at the anterior end of the primitive streak (Sulik et al., 1994; Beddington, 1994). Consistent with the properties of the organizer of fish, frog and bird embryos, cells at these two sites of the mouse gastrula can induce a new neural axis when they are transplanted as an intact tissue fragment to an ectopic site in the late-gastrulation embryo, and they self-differentiate to form

the axial mesoderm of the new body axis (Beddington, 1994; Tam et al., 1997a). These two cell populations are thus regarded as the organizer of the gastrula embryo: the early-gastrula organizer (EGO) and the node (or Hensen's node see Viebahn, 2001) which may be the equivalent of the late-gastrula organizer in other vertebrate embryos (Camus and Tam, 1999). Cells of the organizer at these two stages of gastrulation display different developmental fates regarding their relative contribution to different anteroposterior segments of the axial mesoderm, to other types of mesoderm and to the gut endoderm (Beddington, 1994; Sulik et al., 1994; Tam et al., 1997a). Furthermore, cells in the EGO and the node express different combinations of organizer-specific genes (Camus and Tam, 1999; Davidson and Tam, 2000). These findings suggest that, although the EGO and the node possess similar organizing activity, there may be significant difference in the composition or the lineage potency of the cell population at different phases of gastrulation.

In the mouse, very little is known about the ontogeny and cellular composition of the organizer, and the morphogenetic movement of the organizer derivatives in the gastrulating embryo. The development of the organizer and the allocation of cells to the axial mesoderm are more extensively studied in the chick embryo, which, like the mouse, gastrulates and forms germ layers by the ingression of cells at the primitive streak. In the early chick gastrula, precursor cells of Hensen's node reside first in the region of the Koller sickle in the posterior blastoderm. During gastrulation, these precursors, which express the *GSC* gene and are morphologically distinct from the overlying blastoderm and the underlying hypoblast (Izpisua-Belmonte et al., 1993), are displaced anteriorly with the advancing primitive streak (Lawson and Schoenwolf, 2001). In the mouse, cells of the EGO, which is localized in the posterior epiblast but away from the primitive streak, also express *Gsc* activity (Filosa et al., 1997). At the early-streak stage (HH stage 2-3), the tissues in the anterior primitive streak of the chick embryo can only induce the formation of a new primitive streak when transplanted to ectopic sites and do not induce any neural tissue or a complete body axis (Lemaire et al., 1997). By contrast, the mouse EGO can induce an incomplete axis, but lacks anterior characteristics (Tam et al., 1997a). In the chick, after the meeting of the advancing *GSC*-expressing cell population and the epiblast cells in the central part of the blastoderm, both cell populations are incorporated into the nascent Hensen's node. The combined cell population then acquires the ability to induce the formation of a neural axis complete with anterior and posterior characteristics (Storey et al., 1992; Lemaire et al., 1997, Izpisua-Belmonte et al., 1993). Whether the equivalent organizer of the mouse embryo at a comparable stage of gastrulation will induce an axis has not been tested. After the chick primitive streak has attained its full length, *GSC*-expressing cells that are destined for the prechordal plate and the anterior head process leave Hensen's node and extend anteriorly in a tight column along the midline of the embryo. This movement of the axial mesoderm differs from that of the paraxial and lateral mesoderm, which first migrate laterally out of the primitive streak and then anteriorly towards the cranial end of the embryo (Psychoyos and Stern, 1996). The departure of the *GSC*-expressing cells seems to coincide with the loss of the ability of Hensen's node to induce a full neural axis (Storey et al., 1992). The change in inductive potential of Hensen's node and its predecessor parallels the gain of *GSC* activity, as well as the arrival of the incoming *GSC*-expressing population from the Koller sickle to the node and the departure of the head process mesoderm (Izpisua-Belmonte et al., 1993). Similarly, the absence of *Gsc* activity from the node (Belo et al., 1998) is associated with the inability of the node to induce a complete neural axis (Beddington, 1994). It is not known, however, whether this may be correlated with the passage of the head process mesoderm. At the end of gastrulation in the chick, the primitive streak regresses and the node moves posteriorly and concomitantly lays down the notochord in its wake (Catala et al., 1996). Although the precursors of the axial mesoderm are derived mainly from the organizer in the chick recent evidence suggests that cells located outside of the organizer regions can contribute to the axial mesoderm, these cells first migrate into the organizer region and then anteriorly to form the midline (Joubin and Stern, 1999). It is not known if this occurs in the mouse.

Our work addresses the following questions, which have been raised by our current knowledge of the mouse gastrula organizer: (1) Is a population of organizer cells with comparable axis inductive property, genetic activity and cell fates present in the embryo between the early and late stage of gastrulation? (2) Does the mouse organizer always contain the full complement of progenitors of the entire axial mesoderm? (3) Which migratory route is taken by the cells that are destined for the axial mesoderm?

## MATERIALS AND METHODS

### Transgenic mouse strains

*HMG-lacZ;EGFP* (B5X) mice were used to produce embryos from which transgenically tagged cells were obtained for the fate mapping and cell tracking experiments. These mice are derived from crosses of the H253 mice that express the *HMG-CoA-nls-lacZ* transgene (TgN (HMG-*lacZ*) 253Tan; Tam and Tan, 1992) and the B5/EGFP mice that express the *β-actin-CMV-EGFP* transgene (TgN(GFPU)5Nagy, Hadjantonakis et al., 1998). The compound transgenic mice express both transgenes widely in the embryonic and extra-embryonic tissues.

For assessing the differentiation of the transplanted cells, mice expressing a *lacZ* reporter gene that has been integrated into the *Gsc* locus ($Gsc^{lacZ}$; Rivera-Perez et al., 1999) were used. The $Gsc^{lacZ}$ mice were crossed to the B5/EGFP mice to produce compound transgenic mice that widely express the EGFP transgene in the embryonic tissues but express only $Gsc^{lacZ}$ activity when the cells differentiate into the prechordal mesoderm and the foregut endoderm of embryos at the early-somite stage.

### Embryo collection, micro-manipulation and culture

Pregnant mice were dissected at 6.5, 7.0 and 7.5 days post coitus to collect embryos at the ES, mid-streak (MS) and LS-to-early bud (EB) stages, respectively (Downs and Davies, 1993). Isolated fragments of tissues from the organizer were dissected from embryos that provided cells for fate mapping and cell tracking experiment. For the ES and MS stage embryos, the tissue fragments were further dissected to remove any adherent visceral endoderm. For the EB stage embryo, the node was isolated by trimming away the neighboring primitive streak and germ layer tissues. The tissue fragment was then broken up into clumps of 5-20 cells for transplantation.

Host ARC/s strain embryos that matched the developmental stage of the donor embryo were used as recipients of cell transplantation. They were dissected free of the decidual tissues and parietal yolk sac, and kept in culture until manipulation. Cell clumps from donor

embryos were grafted to orthotopic sites in the host embryo using a Leitz micromanipulator under a Wild M3Z dissecting microscope (Kinder et al., 2000). After grafting, embryos were photographed (see below) and incubated for 1 hour in the chamber of NUNC culture slides to allow the incorporation of the graft in either RS medium (for ES and MS stage embryos) or DR75 medium (for LS and EB stage embryos, Sturm and Tam, 1993). ES and MS stage embryos were then cultured individually in eight-well NUNC slides in RS (100% rat serum) or DRH (25% DMEM, 50% rat serum, 25% human cord serum) medium under 5% $CO_2$ in air. LS and EB stage embryos were cultured individually in roller bottles in DR75 medium in 5% $CO_2$, 5% $O_2$ and 90% $CO_2$ (Sturm and Tam, 1993).

### Combined cell grafting and dye labeling of MS stage embryo

To track the movement of specific cell populations in the MS embryo, cells were tagged either by the EGFP transgene or the carbocyanine dye (DiI: 1,1-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, Molecular Probes). Transgenic cells were isolated from the mid-gastrula organizer (MGO) of the B5X strain and grafted orthotopically to the MGO region of the host embryo. After culturing the host embryo for 1 hour to allow the grafted cells to be incorporated into the host tissues, the mesoderm adjacent to the MGO was labelled by microinjecting a small volume (0.25-0.5 μl) of a 0.05% dye solution. The embryos were photographed immediately after labeling to record the position of the EGFP-expressing cells and the dye-labeled mesoderm (see below). The host embryos were cultured for 36 hours to the early-somite stage, fixed in 4% paraformaldehyde overnight and examined for the distribution of the fluorescent cells using fluorescence microscopy (Quinlan et al., 2000).

### Analysis of gene expression

#### In situ hybridization

Whole-mount mRNA in situ hybridization for the genes *Otx2*, *Sox2*, *Cer1*, *Chrd* and *Hnf3β* was performed to determine the location of the organizer at different gastrulation stages and during axis induction experiments. The protocol used for in situ hybridization was as described by Wilkinson and Nieto (Wilkinson and Nieto , 1993) with the following modifications: the Ampliscribe kit (Epicentre Technologies) was used in conjunction with Dig-11-UTP (Roche) to synthesize RNA probes, SDS was used instead of CHAPS in the hybridization solution and post-hybridization washes. Only 5× SSC was used in the hybridization solution, whereas post hybridization washes carried out at 70°C and excluded formamide. No RNA digestion was performed after hybridization.

### Immunohistochemistry for *HNF3β*

Immunostaining was carried out on whole ES stage embryos using a rabbit anti-HNF3β antibody at a dilution of 1:2000 (Davies et al., 1991). Staining of the tissues was visualized by reaction with the streptavidin-biotin reagent (ABC kit, Vector Laboratory) and diaminobenzidine.

### Detection of *lacZ* transgene activity

The expression of the *lacZ* transgene was assessed by histochemical reaction with X-gal reagent. ES to early-somite stage embryos containing either $Gsc^{lacZ}$ or *HMG-lacZ*-expressing cells were fixed for 5 minutes in 4% paraformaldehyde (PFA), followed by staining overnight at 37°C in X-gal staining solution. Stained embryos were re-fixed in 4% PFA, photographed and processed for wax embedding. Serial 8 μm sections were counterstained with nuclear fast red. The localization and the number of X-gal-stained cells in the host tissues were scored.

### Fluorescence microscopy of EGFP-expressing cells and DiI-labeled cells

Experimental embryos that contained EGFP-expressing or DiI-labeled cells were examined by fluorescence microscopy (Quinlan et al., 2000). Immediately after cell grafting and at 6 hour intervals until the embryo developed to the early neural plate stage (after 12, 24 and 30 hours of in vitro development for EB, MS and ES embryos, respectively), the embryo was taken out of the culture vessel and placed in a phosphate-buffered (PB1) medium for fluorescence imaging. The EGFP fluorescence was recorded using a SPOT 2 digital camera (SciTech, Melbourne, VIC) attached to a Lieca MZFLIII fluorescence microscope with a GFPIII filter set (adsorption at 495 nm). For each imaging session, a color bright field image and a monochrome high-resolution fluorescent image were taken consecutively. For dual EGFP and DiI detection, a third image was captured using a Rhodamine filter (absorption at 574 nm) after the bright field and the green fluorescence image. The pictures were superimposed to compose an image showing the location of the EGFP-expressing cells in the early neural plate embryo, using the image manipulation software that operates the digital camera and Photoshop 5.5 (Adobe). After photography, the embryos were then returned to the culture vessel until the next time point for imaging. After the last imaging session at the early neural plate stage, the embryos were further cultured until they reached the early somite stage. They were then harvested and fixed in 4% PFA for histological analysis of the distribution of the graft-derived *HMG-lacZ* transgenic cells in the embryonic tissues. Combined EGFP and DiI fluorescent specimens were examined by fluorescence microscopy at 36 hours and using an Olympus Fluoview FV300 confocal microscope using the Ar+HeNe lasers, and GFP and Rhodamine filters. Confocal images of the same specimen were collated using the Fluoview application software to assemble composite pictures showing the dual fluorescence.

## RESULTS

### An organizer cell population is present throughout gastrulation

Cell populations that display organizer activity have previously been identified in the ES and LS-to-EB stage embryos as the early-gastrula organizer (EGO) and the node, respectively, based on cell fate and axis induction activity (Sulik et al., 1994; Beddington, 1994; Tam et al., 1997a; Tam and Steiner, 1999). These two populations also express genes, such as *Hnf3β* (*Foxa2* – Mouse Genome Informatics) that are associated with the organizer of other vertebrate embryos (Camus and Tam, 1999; Davidson and Tam, 2000). To determine the location of the organizer at the intermediate stage of gastrulation, the expression of *Hnf3β* (which marks the EGO and the node at the beginning and the end of gastrulation respectively), $Gsc^{lacZ}$ (*Gsc* is expressed early in the organizer) and *Chrd* (which is expressed in the node and the head process mesoderm) were examined and compared with that of *Cer1* (which marks the endoderm adjacent to the EGO and later the definitive endoderm) and *Otx2* (the anterior shift of its expression domain reflects the progression of gastrulation) in stage-matched specimens at different stages of gastrulation. In the ES embryo, an evident overlap in the expression domain of *Hnf3β*, $Gsc^{lacZ}$ and *Cer1* is seen in the posterior germ layer tissues at the location of the EGO (Fig. 1A,C,D, ES). $Gsc^{lacZ}$ is co-expressed with *Hnf3β* in the epiblast distal to the newly formed primitive streak (Filosa et al., 1997) and with *Cer1* in the endoderm (data not shown). Expression of *Hnf3β*, however, is not restricted to the organizer, and both the transcript and the protein are found in the anterior visceral endoderm (Fig. 1A, ES). In the MS embryo, *Hnf3β* and *Chrd* expression overlap in the region at the anterior end of the elongating primitive streak (Fig. 1A,B,

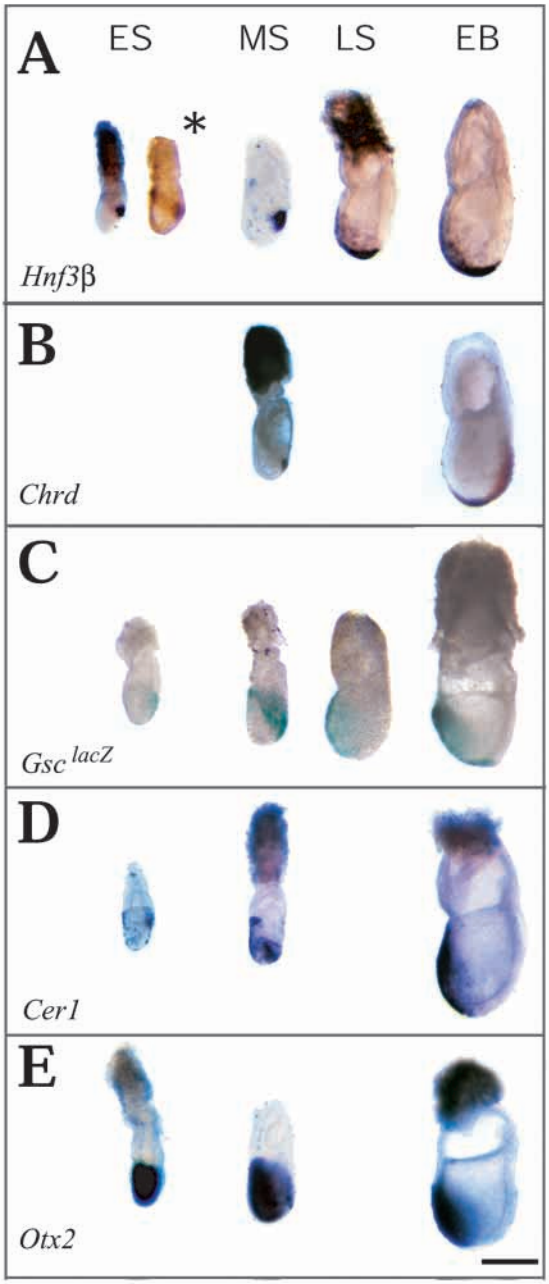


**Fig. 1.** The expression pattern of (A) *Hnf3β*, (B) *Chrd*, (C) *Gsc*$^{lacZ}$, (D) *Cer1* and (E) *Otx2* in the ES, MS, LS and the EB stage embryos. Gene expression was revealed by whole-mount mRNA in situ hybridization (A,B,D,E); by immunostaining (A, asterisk); and by X-gal staining for β-galactosidase activity (C). In (B), ES stage embryo, which displays no *Chrd* activity, is not shown and combined in situ hybridization of *T* and *Chrd* are for EB stage embryo. Scale bar: 300 μm.

MS), while *Cer1* is expressed in the adjacent endoderm (Fig. 1D, MS). *Gsc*$^{lacZ}$ is expressed in the anterior region of the primitive streak and widely in the newly formed mesoderm (Fig. 1C, MS). By the LS stage, the expression domain of *Hnf3β* is shifted distally, in concert with extension of the primitive streak (Fig. 1A, LS). Finally, by the EB stage, *Hnf3β* and *Chrd* are co-expressed in the node that abuts the anterior end of the primitive streak (Fig. 1A,B, EB). *Hnf3β* expression, however, is also evident in the mesoderm and endoderm in the prospective cranial region of the embryo (Fig. 1A, LS, EB). Its expression overlaps that of *Chrd* and *Gsc*$^{lacZ}$ in the axial mesoderm (Fig. 1B,C, EB) and of *Cer1* and *Gsc*$^{lacZ}$ in the prospective foregut gut endoderm (Fig. 1C,D, EB). During gastrulation, the expression domain of the *Otx2* gene recedes from the posterior germ layer tissues as the primitive streak extends anteriorly (Fig. 1E). In the midline of the MS and EB embryos, the posterior limit of *Otx2* expression borders on the position of the *Chrd*-expressing cells at the anterior end of the primitive streak (Fig. 1B,E).

The analysis of gene expression in the gastrula strongly suggests that cells in the anterior region of the primitive streak of the MS embryo might contain cells with organizer properties. The axis inducing activity of these cells were tested in an axis induction assay by grafting cells from the anterior region of the primitive streak of a B5/EGFP MS embryo to the lateral regions of LS host embryos (Fig. 2A). After 24 hours of in vitro development, graft-derived (EGFP-expressing) cells formed an elongated structure parallel to the host axis (Fig. 2B,C). The host ectoderm adjacent to the graft formed a thickened epithelium and expressed the neural specific *Sox2* gene (Fig. 2B) as well as the forebrain- and midbrain-specific gene *Otx2* (Fig. 2C). This ability to organize a neural axis suggests that the *Hnf3β* and *Chrd*-expressing cells in the anterior tip of the primitive streak of the MS embryo may function as an organizer (which we have named the mid-gastrula organizer, MGO) that may have full axis-inducing ability not found in either the EGO or the node (Beddington, 1994; Tam et al., 1997a; Tam and Steiner, 1999).

## Cell fates reflect the progressive change in the cellular composition of the gastrula organizer

### The overall pattern of tissue colonization by graft-derived cells

The developmental fates of cells in the gastrula organizer were investigated by transplantation experiments to test if different types of progenitor cells are present at successive stages of development. Cells were isolated from the gastrula organizer of the donor embryos and transplanted to the organizer of host embryos of matching developmental stage. The distribution of graft-derived cells, which are marked by the expression of the *HMG-lacZ* transgene, was examined after the host embryo has developed to the early-somite stage (Table 1).

After transplantation, descendants of EGO cells were found in the prechordal mesoderm that merges with the roof of the foregut and the foregut endoderm as well as the notochord at all levels of the anteroposterior axis (Fig. 3). There is a minor contribution to the neuroepithelium in the midline of the brain and the floor plate of the neural tube. EGO cells also contributed to other tissues such as the cranial mesenchyme, the heart and the somites. MGO-derived cells displayed a similar pattern of tissue distribution. However, more MGO than EGO descendants were found in the prechordal mesoderm and foregut, in the neural epithelium in the midline of the forebrain and hindbrain, and in the floor plate of the trunk neural tube; fewer were found in the cranial mesenchyme and the somites (Table 1). Contribution of the EGO and MGO cells to the foregut endoderm correlates well with the onset of *Cer1* activity in the endoderm immediately adjacent to the organizer (Fig. 1D). In contrast to EGO and MGO cells, node-derived

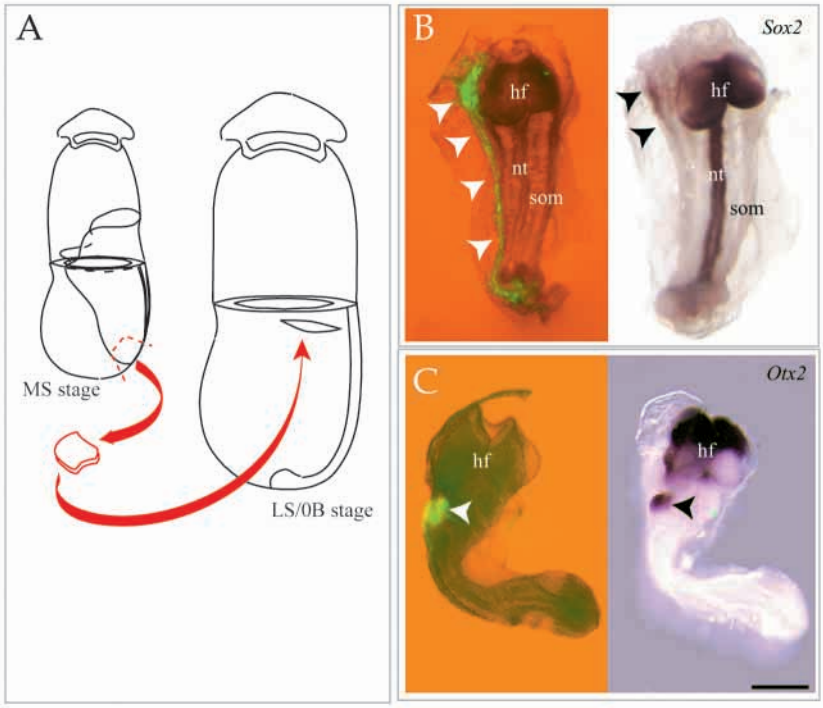


**Fig. 2.** Axis induction by the MGO. (A) The scheme of tissue transplantation: a tissue fragment dissected from the anterior end of the primitive streak (Region V, VI; Fig. 5C) of mid-streak (MS) stage embryo is transplanted to the lateral region of the late-streak/no bud (LS/0B) stage host embryo. (B) After 24 hours of in vitro development, the host embryo contains an elongated structure (white arrowheads) of EGFP-expressing graft-derived tissues on its left flank. *Sox2* activity, which is expressed in the neural tube of the host embryo, is also expressed in the ectoderm overlying the graft-derived tissue (black arrowheads, one out of two embryos containing grafts showed *Sox2* induction). (C) EGFP-expressing graft tissue (white arrowhead) located at hindbrain level has induced *Otx2* expression (black arrowhead, six of seven embryos containing grafts showed *Otx2* induction) which is normally expressed in the fore- and midbrain regions. hf, head folds; nt, neural tube; som, somites. Scale bar: 200 μm.

cells were absent from most of the cranial tissues, and are localized mainly to the notochord and the floor plate posterior to the hindbrain (Fig. 3). The node-derived cells also colonized the anterior region of the primitive streak and the somites.

## EGO and MGO cells but not node cells contribute to the anterior mesoderm and endoderm

The significant colonization of the prechordal mesoderm and foregut endoderm by the EGO and MGO cells but not by the cells of the node suggests that the progenitors for these tissues are present in the organizer only at the earlier stage of gastrulation. In the intact normal embryo, the mesoderm and endoderm of the head process specifically expressed the *Gsc* gene (as revealed by the activity of the $Gsc^{lacZ}$ transgene; Fig. 4A). To test whether graft-derived cells that colonize the prechordal mesoderm and foregut endoderm were correctly specified, donor EGO and MGO cells that co-express the $Gsc^{lacZ}$ and the EGFP transgenes were tested for differentiation in the host tissues. Transgenic cells that were transplanted orthotopically to the EGO of the ES embryo produced descendants that are distributed widely in the axial tissues of the host embryo at the early-somite stage (Fig. 4B). However, the $Gsc^{lacZ}$ activity was observed only in EGO descendants that colonized the prechordal mesoderm and the foregut (Fig. 4C-E). Specific expression of *Gsc* activity in the prechordal mesoderm and foregut endoderm was also observed after orthotopic transplantation of $Gsc^{lacZ}$ and EGFP transgenic MGO cells (Fig. 4F-H). By contrast, descendants of node cells did not colonize the anterior mesoderm or foregut endoderm and those found outside the anterior mesoderm could not activate the $Gsc^{lacZ}$ transgene (data not shown). Cells grafted to the central region of the node contributed principally to the notochord and the floor plate (data not shown), while cells grafted to regions posterior to the node and in the primitive streak contribute mainly to the paraxial mesoderm and only make a minor contribution to axial mesoderm (Kinder et al., 1999). These findings demonstrate that progenitors of the prechordal

**Table 1. Tissue contribution of the EGO, MGO and node to both mesoderm and endoderm lineages at the early somite stage**

| | | | Tissue contribution (% of total graft derived population) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tissue | Number of embryos | Total graft-derived cells | Brain* | Cranial mesenchyme | Heart | AME | Notochord | Floor plate | Trunk neural tube | Somites | Lateral plate mesoderm | Primitive streak | Gut‡ |
| EGO | 41 | 2294 | 6.9 | 14.7 | 3.9 | 32.2 | 15.6 | 6.9 | 1.4 | 14.4 | 1.4 | 3.7 | 0 |
| MGO | 39 | 4118 | 12.2 | 4.7 | 6.5 | 45.3 | 13.4 | 15.2 | 0.6 | 2.9 | 0.2 | 3.1 | 1.6 |
| Node | 22 | 1333 | 0 | 0 | 0 | 4.9 | 24.6 | 31.4 | 1.9 | 11.5 | 0 | 25.8 | 0 |

Graft-derived cells (from regions I-III, V-VII and the node) were detected by X-gal staining, and the percentage contribution of graft cells to each lineage is shown above.

Percentages have been rounded to one decimal place.

*Midline (ventral) neuroepithelium in the fore- and midbrain.

‡Other gut endoderm.

AME, anterior mesendoderm (includes prechordal mesoderm and foregut endoderm).

mesoderm and the foregut are present in the EGO and MGO, but are absent from the node of the gastrulating embryo. Whether the node cells may still have the potential to differentiate into prechordal mesoderm cells when they are transplanted heterotopically to the EGO or MGO has yet to be tested.

## Recruitment of cells to the gastrula organizer

Analysis of the developmental fates of the cells of the gastrula organizer reveals that there is a progressive shift from the predominant contribution to the anterior mesoderm and endoderm, to an increasing contribution to the midline (ventral) neuroectoderm of the brain and the notochord, and finally to almost exclusive contribution to the floor plate and the notochord of the trunk. These findings strongly suggest that the there is a significant change in the cellular composition of the organizer during gastrulation, either owing to the acquisition of novel fate or the recruitment of new tissue progenitors to the organizer. To test if the organizer contains progenitors of different tissue lineages, finer mapping of the cell fates of the EGO and MGO was performed.

In the ES embryo, *Hnf3β* and *Gsc*$^{lacZ}$ expression demarcates the EGO, which occupies a 50 μm domain in the mid region of the posterior epiblast between the junction of the epiblast with the extra-embryonic tissues and the distal tip of the cup-shaped embryo (Fig. 5A, regions III and IV). Donor posterior epiblast (encompassing all four regions) cells that co-express the *HMG-lacZ* and EGFP transgenes were grafted to the regions I, II, III or IV (Fig. 5A) in the posterior epiblast. The precise position of the graft was determined immediately after grafting by visual inspection of the distance of the EGFP-expressing cells to the junction of the epiblast and the extra-embryonic tissues. When the host embryos were examined after 48 hours of in vitro development (Fig. 5B), 31% of the embryos with cells transplanted to region III contained graft-derived cells in the anterior and trunk axial mesoderm (Fig. 5E). In embryos with cells

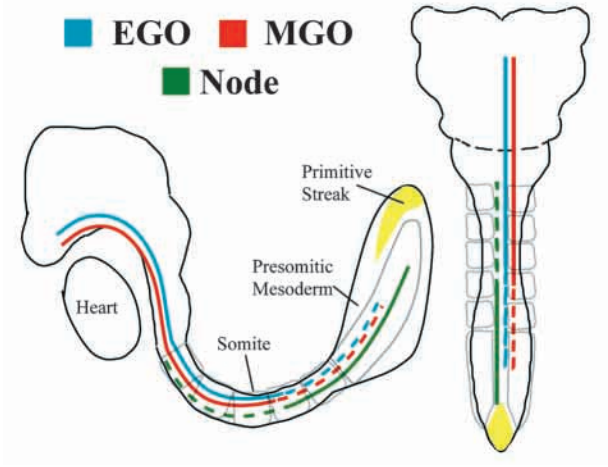


**Fig. 3.** The contribution of the organizer of the ES (EGO), MS (MGO) and the LS (node) stage embryo to the axial mesoderm and the neuroectoderm (ventral tissue of the forebrain and the floor plate of the neural tube) of the early-somite stage embryo. Both the EGO and the MGO contribute to the axial mesoderm and the neuroectoderm from the most anterior region to the level of the presomitic mesoderm. The node contributes to the notochord and floor plate spanning from the second to third somite to the anterior primitive streak (PS). Unbroken line, major contribution; broken line, lesser contribution. The early-somite stage embryo is shown from lateral (left) and the dorsal (right) views.

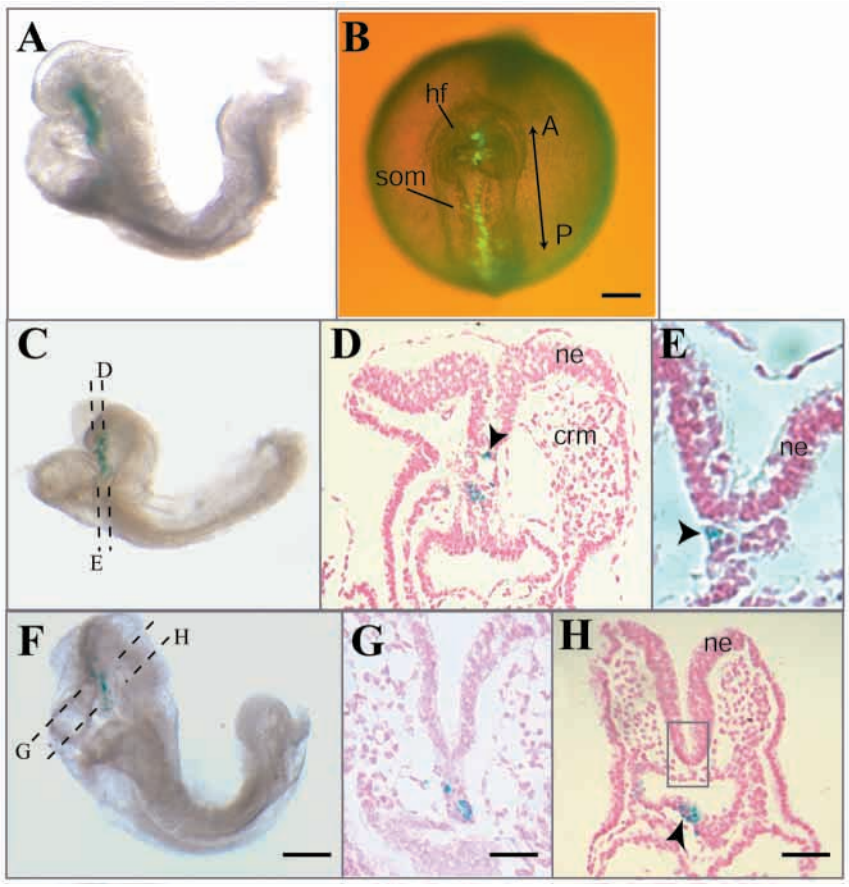


**Fig. 4.** Contribution of the EGO and MGO to the anterior mesoderm and foregut endoderm. (A) *Gsc*$^{lacZ}$-expressing tissue is found in the prechordal mesoderm and the foregut of the early-somite stage embryo. (B) EGFP-expressing cells derived from the transplanted EGO cells are distributed throughout the anteroposterior axis of the early somite stage host embryo, but only those that are localized (C-E) in the prechordal mesoderm (D), the foregut endoderm and the head processes (E) express the *Gsc*$^{lacZ}$ transgene (arrowheads). (F) MGO-derived cells that are found in the cranial tissues of the early-somite stage host embryo also express *Gsc*$^{lacZ}$ transgene when they are localized in the prechordal mesoderm (G) and the foregut (H, arrowhead), but not in the notochord of the hindbrain (box). Broken lines in C,F indicate the planes of section shown in D,E,G,H. Abbreviations: A-P, anterior-posterior axis; crm, cranial mesenchyme; hf, head-fold; som, somite; ne, neuroectoderm. Scale bars: 200 μm in A,B,C,F; 50 μm in D,H; 20 μm in E,G.

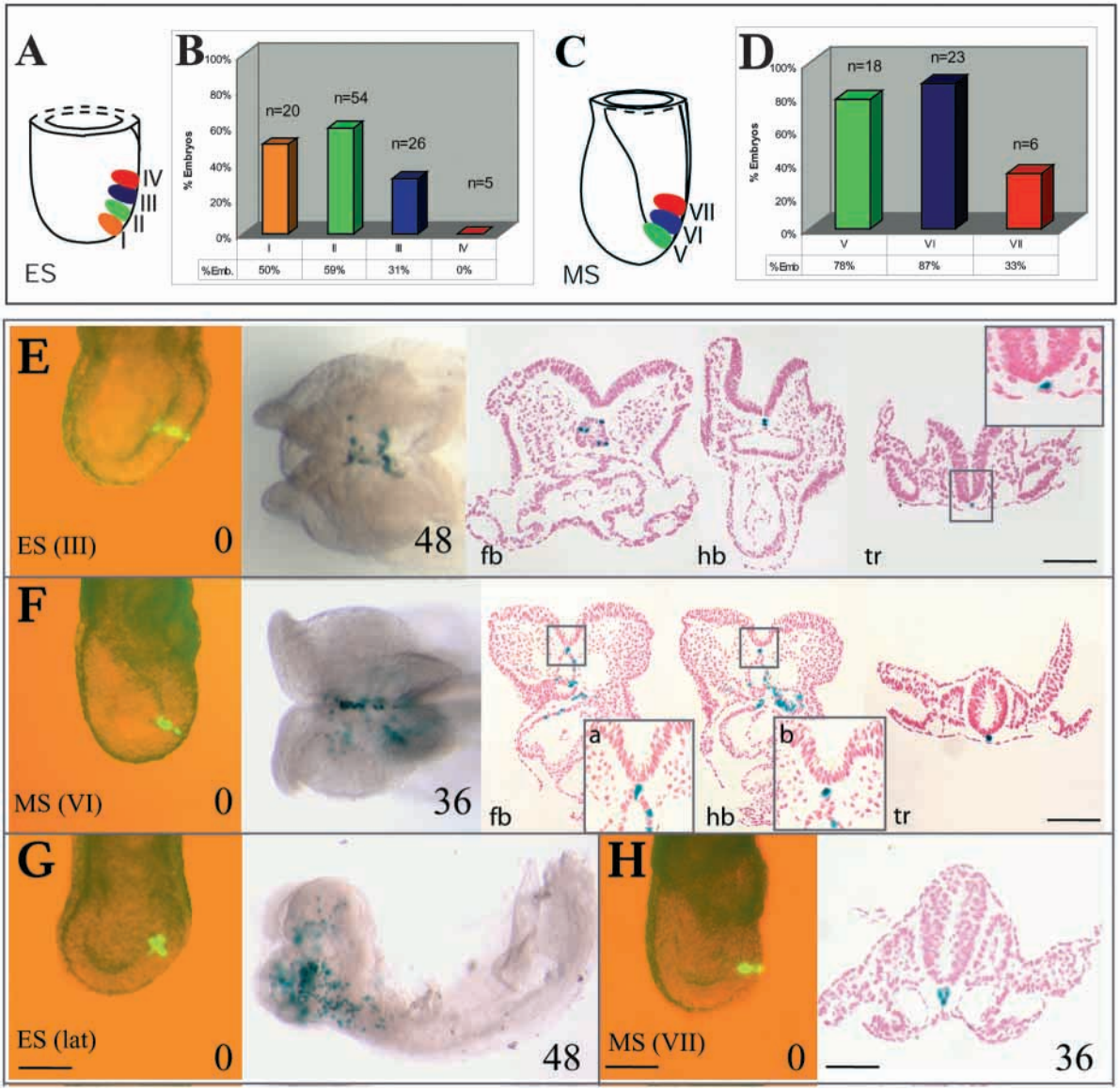


**Fig. 5.** Fine mapping of the developmental fate of cells within and adjacent to the EGO and MGO. (A) Regions I-IV in the posterior midline epiblast of the ES embryo, where cells are tested for contribution to axial mesoderm. Regions I to IV are located anterior to the recently formed primitive streak, with region III and IV located in the domain of the EGO delineated by *Hnf3β* and *Gsc*$^{lacZ}$ activity (Fig. 1). (B) The contribution to axial mesoderm by cells in Regions I to IV, expressed as the percentage of embryos showing graft-derived cells in this tissue. (C) Regions V-VII in the posterior midline epiblast of the MS embryo, where cells are tested for contribution to axial mesoderm. Regions V and VI are located in the anterior end of the elongating primitive streak within the domain of the MGO, which is defined by the expression of *Hnf3β, Chrd* and *Gsc*$^{lacZ}$. Region VII is located in the anterior segment of the primitive streak. (D) The contribution to axial mesoderm by cells in Regions V to VII, expressed as the percentage of embryos showing graft-derived cells in this tissue. (E) Cells that were transplanted to Region III of the early-streak embryo (t=0) colonized the axial tissues of the host embryo after 36 hours of culture (*t*=36). Histological sections at the level of the forebrain (fb),hindbrain (hb) and trunk (tr) of an embryo showing graft-derived cells in the prechordal mesoderm and the notochord (inset). (F) Cells that were transplanted to Region VI of the MS embryo (*t*=0) colonized the axial tissues of the host embryo after 36 hours of culture (*t*=36). Histological sections at the level of the forebrain (fb), hindbrain (hb) and trunk (tr) of an embryo showing graft-derived cells in the prechordal mesoderm (inset a), the notochord of the midbrain (inset b) and the trunk. (G) Cells transplanted to epiblast lateral to Region III of the ES embryo (*t*=0) colonized the cranial mesenchyme and the heart of the host embryo after 48 hours of culture. (H) Cells transplanted to Region VII of the MS embryo (*t*=0) colonized the notochord in the trunk of the host embryo after 36 hours of culture (*t*=36). EGFP-expressing cells show green fluorescence and *lacZ*-expressing cells are stained blue by X-gal reagent. Scale bars: in E, 50 μm; in G (*t*=0) and H (*t*=0),100 μm; in F, 50 μm; in H (*t*=36), 50 μm.

transplanted to region IV, graft-derived cells were mostly found in the embryonic heart. These findings suggest that the EGO contains a heterogenous population of progenitor cells, some of which may differentiate into cranial or heart mesoderm as well as somitic mesoderm. Epiblast cells in regions I and II outside the EGO domain contributed significantly to the axial prechordal mesoderm and foregut endoderm, as would be expected of the organizer, suggesting that a significant

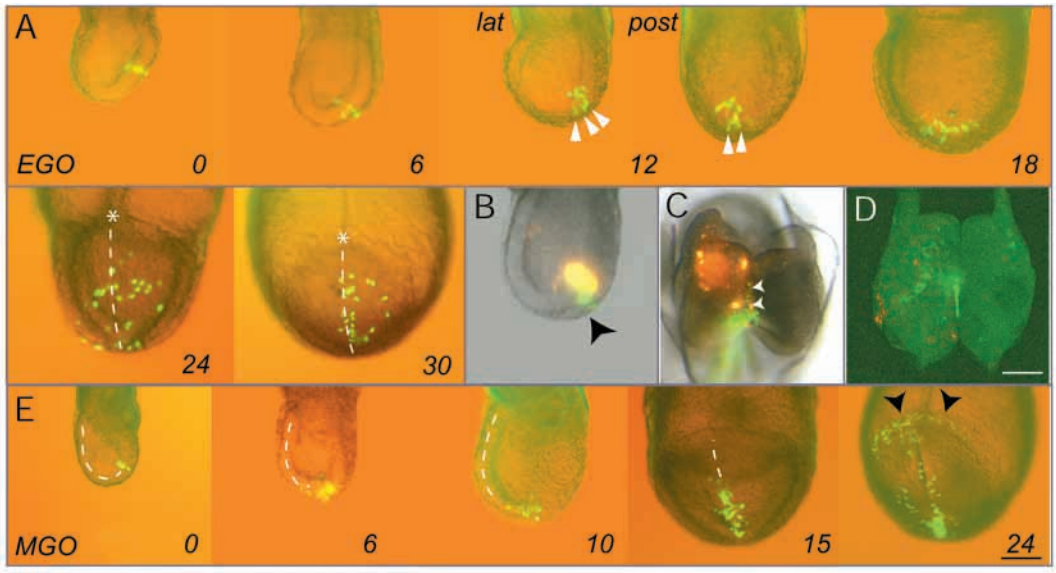


**Fig. 6.** Movement of cells derived from the EGO and the MGO. (A) Serial images taken at 6 hour intervals showing the localization of the EGFP-expressing cells derived from the EGO during 30 hours of in vitro development from the ES to the early neural plate stage. At 0-6 hours, the EGO-derived cells are displaced anteriorly to the site of the MGO, followed by the dispersal of the EGO-derived cells out of the MGO (lateral and posterior view at 12 hours) laterally to the mesodermal layer and along the midline of the embryo (arrowheads at 12 hours). The EGO-derived cells are spreading anteriorly in both the paraxial and axial mesoderm under the head folds (18-30 hours). Embryos are viewed from the left side at 0, 6, 12 and 18 hours, posterior side at 12 hours, and anterior side at 24 and 30 hours. (B) The EGO-derived cells in the mesodermal (yellow fluorescent DiI-labeled cells) and axial (green fluorescent EGFP-expressing cells, arrowhead) position in the MS stage embryo colonize, respectively, the paraxial (orange-red DiI-labelled cells) and axial mesoderm (green fluorescent cells, arrowheads in C) of the early-somite stage host embryo (C,D). (B) Lateral view, anterior towards the left; (C,D) dorsal view, anterior towards the top. (E) Serial images showing the localization of the EGFP-expressing MGO-derived cells during development from the MS to the early neural plate stage. The MGO-derived cells migrate anteriorly in a tight column along the midline at 6-15 hours. After 24 hours of culture, some MGO-derived cells are in the axial mesoderm as well as underneath the anterior of the neural plate. 0-10 hours: lateral view, anterior towards the left; 15-24 hours: anterior view. Broken lines mark the distance of the MGO cells from the anterior end of the body axis. Scale bars: in E, 150 μm in A,B,E; in D, 50 μm in C,D.

population of progenitors for organizer derivatives is present outside the EGO at early gastrulation and are yet to be incorporated into the gastrular organizer. The epiblast lateral to the EGO does not contain any precursors of organizer tissues. Their descendants were found in the heart and cranial mesenchyme and not in any axial mesoderm (Fig. 5G).

In the MS embryo, the MGO (delineated by *Hnf3β* and *Chrd* activity) is located in regions V and VI (Fig. 5C) at the junction of the anterior region of the primitive streak and the leading edge of the mesodermal layer. Most (over 70%) of the embryos with cells transplanted to the MGO (regions V and VI) showed colonization of the anterior axial mesoderm and foregut endoderm and the midline neuroepithelium of the neural tube by graft-derived cells (Fig. 5D,F). Cells grafted to the region (VII) posterior to the MGO contributed to axial tissues only in 33% of embryos (Fig. 5D). The descendants of these cells were found in the node after 18 hours of in vitro development (data not shown) and were later localized in the trunk notochord of the early-somite stage embryo (Fig. 5H histological section of host embryo after 36 hours of culture). This finding suggests that the precursors of the anterior axial mesoderm and foregut endoderm are localized in the MGO but that those of the node are still outside the MGO. Despite the fact that the donor cells for grafting are isolated from all the four and three regions in the early-streak and mid-streak embryo, respectively, no significant variation of cell fate is found among the specimens that received grafted cells at the same site. This suggests that cells in the posterior epiblast around the organizer region are not committed to a specific cell fate and can be allocated and differentiate into tissue types that are typical of the native cells at the site of transplantation.

## Morphogenetic movements of the cellular progeny of the gastrula organizer

Results of the cell fate analysis show that cells originating from the gastrula organizer that is localized in the posterior region of the embryo populate the axial mesoderm and endoderm in the most anterior part of the body axis. This prompts the question of what migratory pathways these cells may take to arrive at the axial destination during the morphogenesis of the germ layers at gastrulation. The recent application of EGFP transgenic mice to fate mapping (Kinder et al., 1999) allows an accurate migratory route to be determined in the same embryo for the whole duration of in vitro development.

### Two divergent paths of movement of the EGO cells

EGO cells that express both the *HMG-lacZ* and EGFP transgenes were transplanted to posterior epiblast (Fig. 6A, 0 hour) of the ES embryo. The host embryos were examined immediately after cell transplantation to ascertain the correct positioning of the cells. Incorrectly grafted embryos were excluded from this study. Altogether 41 embryos were examined and the position of the grafted cells was tracked by frequent observation by fluorescence digital imaging (Fig. 6). Cells grafted to the EGO region were displaced anteriorly as the primitive streak elongated (towards the tip of the cup-shaped embryo) in the first 6 hours but cells remained clustered. Twelve hours after grafting, cells began to disperse so that some cells were found in the adjacent mesoderm and some stayed in the region that corresponds to the MGO of the MS embryo. In the next 6 hours (18 hours after grafting), these two streams of EGO-derived cells moved in concert anteriorly along the midline and in the paraxial mesoderm. When the

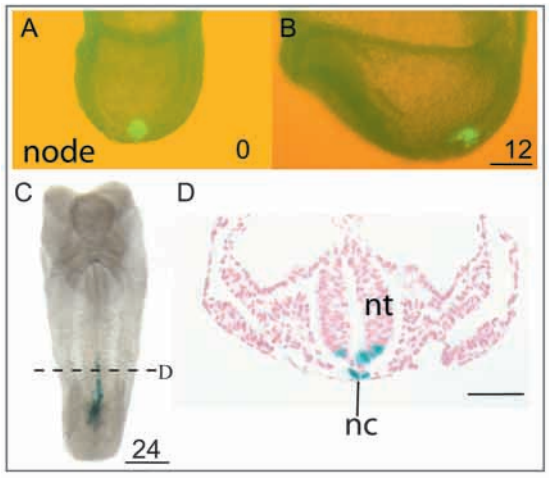


**Fig. 7.** Contribution of node-derived cells to the notochord. (A) EGFP-expressing cells transplanted to the node of the early-bud stage embryo (*t*=0 hour). (B) Posterior displacement of the grafted cells and the formation of a trail of notochordal as the node is drawn posteriorly as the primitive streak regresses (*t*=12 hours after grafting). (C) *lacZ*-expressing graft-derived cells in the notochord of the early-somite stage host embryo (24 hours after grafting). (D) A histological section of the embryo at the plane indicated by the broken line in C showing the colonization of the notochord (nc) and the ventral neural tube (nt) by the graft-derived cells. (A,B) Lateral view, anterior towards the left; (C) dorsal view, anteriorwards to the top. Scale bar in A, 150 μm in A,B and 50 μm in C,D.

embryos were examined at 24 and 30 hours after grafting, the EGO-derived cells were progressively displaced anteriorly and finally reached the most anterior point of the axis. Cells in the paraxial mesoderm moved at the same rate as the axial population, but were increasingly dispersed over a larger area in the cranial mesenchyme. By the early somite stage (48 hours after grafting), the EGO-derived cells (visualized by *lacZ* activity) were found in the axial mesoderm, foregut endoderm (of the head fold and the trunk) and the cranial mesenchyme (data not shown). However, we could not distinguish the relative contribution of the two streams of EGO-derived cells to the axial and non-axial tissues.

The fate of the two EGO-derived populations that follow divergent migratory paths was studied by an additional tracking experiment in which the two cell types were distinguished by different markers. EGFP-expressing epiblast cells were transplanted to the MGO of the MS embryo, followed by the labeling of the adjacent mesodermal cells by DiI (Fig. 6B). After 36 hours of in vitro development (*n*=4 embryos), EGFP-expressing cells were found mostly in the tissues at the midline of the embryo, while the DiI-labeled cells colonized mainly the

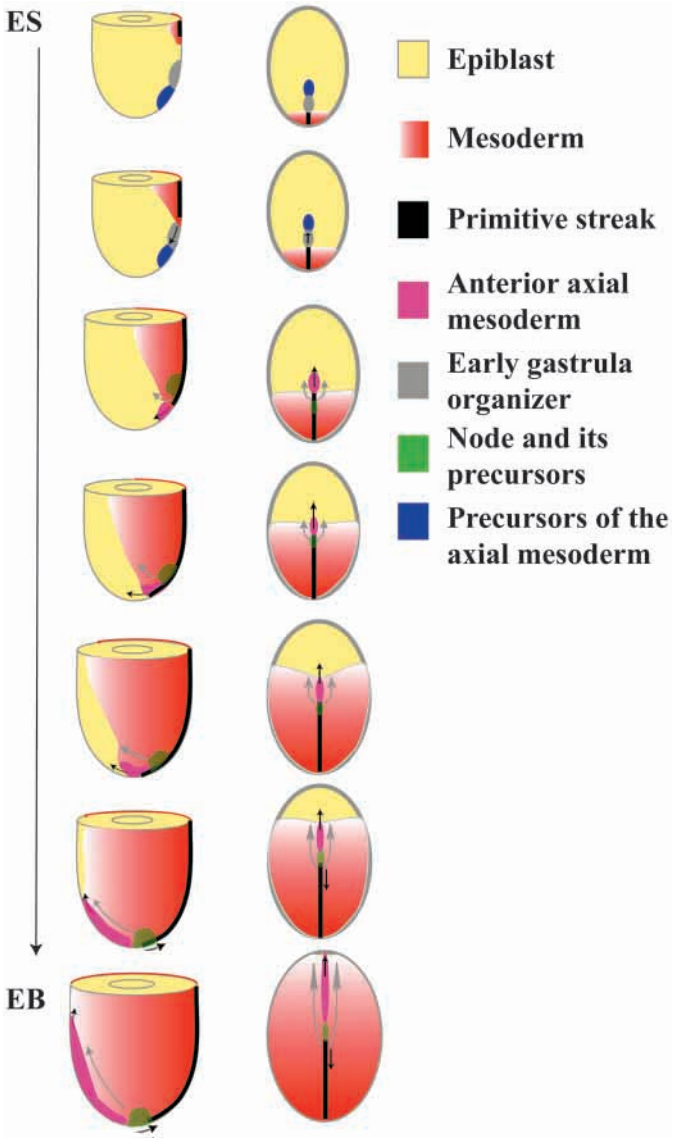


**Fig. 8.** The movement of cells derived from the gastrula organizer relative to the morphogenetic movement of the mesoderm during gastrulation from the ES (early-streak) to the EB (early-bud) stage. Left column: lateral view of the embryo with anterior towards the left; right column: flattened dorsal view, anterior towards the top. The early gastrula organizer (EGO, gray) cells are first found in the posterior epiblast at a distance of about 50 μm anterior to the newly formed primitive streak (black) of the ES embryo. These cells initially remain static in a tight cluster before the elongating primitive streak reaches them. Cells in the posterior region (Region IV, Fig. 5A) of the EGO are incorporated first into the advancing primitive streak and they ingress (grey arrows) to join the mesoderm (pink) lateral to the anterior end of the primitive streak. The cells in the anterior region of the EGO (the bulk of the progenitor of the prechordal mesoderm) stay in the midline and are carried anteriorly to meet the progenitors of the prechordal mesoderm and the head process in the epiblast of Regions I and II. These cells collectively constitute the mid-gastrula organizer (MGO). Cells leaving the MGO move in a tight column anteriorly along the midline of the embryo (black arrow, pointing anteriorly) and form the prechordal mesoderm and the head process. These progenitors of the axial mesoderm are moving in concert with the paraxial stream of EGO-derived cells in the mesoderm. The movement of these two streams of EGO-derived cells is part of the global movement of the mesoderm and the definitive (gut) endoderm towards the anterior region of the gastrulating embryo (Tam et al., 2001). At the MS stage, the bulk of the precursors of the trunk notochord is not found in the MGO and is localized in the primitive streak immediately posterior to the MGO. These cells are recruited into the node after the departure of the anterior axial mesoderm and they are laid down as the notochord as the node is displaced posteriorly (black arrow, pointing posteriorly) with the regression of the primitive streak.

cranial and heart mesenchyme with little contribution to the axial tissues (Fig. 6C,D). This result demonstrates that the EGO is composed of two types of progenitors: one population destined for the cranial and heart mesoderm, which transit through the MGO and migrate with the other cells in the mesodermal layer; and the other which resides transiently in the MGO and migrates anteriorly along the midline of the embryo to form the axial mesoderm and endoderm.

### Movement of the MGO cells is restricted to the midline

Donor MGO cells were transplanted to region V/VI (Fig. 5C) of the MGO of the MS host embryo (Fig. 6E, 0 hour). During the first 6 hours after grafting, the MGO cells were displaced further to the distal region of the cup-shaped embryo and began to spread anteriorly from the anterior end of the primitive streak. In the next 4-9 hours (10 to 15 hours after grafting), cells from the MGO advanced anteriorly along the midline of the embryo and deposited a trail of cells anterior to the primitive streak. Finally, by 24 hours after grafting, the MGO-derived cells had reached the most anterior part of the embryonic axis and covered the midline of the entire axis from the node to the anterior neural ridge of the head fold. For the duration of the tracking experiments, the movement of the MGO-derived cells was restricted to the midline of the embryo, and only spread bilaterally after they reached the anterior margin of the neural plate. By the early-somite stage (36 hours after grafting), the MGO-derived cells (marked by β-galactosidase activity) were found in the prechordal mesoderm, foregut, the notochord of the head and the trunk and the floor plate (data not shown).

### Axial distribution of the node-derived cells

EGFP-expressing node derived cells that were grafted orthotopically (Fig. 7A) remained clustered in the node in the first 12 hours after grafting (Fig. 7B). However, the position of the node appeared to have been displaced posteriorly in the body axis. By 24 hours after grafting, node-derived cells were found in the notochord (Fig. 7C,D) and floor plate of the most posterior embryo and the anterior primitive streak (Fig. 7C). This pattern of cellular distribution is consistent with the notion that the node regresses and leaves a trail of descendants in the midline as the embryonic axis extends posteriorly.

The profile of gene expression and cell fate analysis therefore reveals that (1) an organizer is present at successive stages of gastrulation; (2) the molecular properties of the organizer change during gastrulation, as does the cellular fate of organizer cells; and (3) the location of the organizer shifts in step with the elongation of the primitive streak.

## DISCUSSION

### Changes in cell fate and molecular properties point to a dynamic cell population in the gastrula organizer.

Results of our fate-mapping experiments show that cells of the organizer contribute to different types of tissues as the embryo develops. There is a decreasing contribution to the anterior axial mesoderm and foregut endoderm, and the cranial paraxial mesenchyme and the ventral (midline) neuroectoderm of the brain, but an increasing presence of cellular progeny in the notochord, the floor plate of the spinal cord and the primitive streak (Table 1). With respect to the distribution of descendants of the organizer in the axial mesoderm, the EGO and MGO cells are found predominantly in the head process and notochord of the upper body, whereas the node cells are localized mainly to the notochord from the hindbrain to the rest of the body axis. Our analysis of genetic activity has revealed that the gastrula organizer does not express the same set of genes during gastrulation. In the early-streak embryo, where the axis-inducing activity can be localized to the EGO, cells in this region of the epiblast express *Hnf3β*, *Gsc* and *Lim1* (Filosa et al., 1997; Tsang et al., 2000; Kinder et al., 2001; this study). By the MS stage, the MGO expresses *Chrd* and *Noggin* in addition to *Hnf3β* and *G*sc (Belo et al., 1997; Belo et al., 1998; Bachiller et al., 2000). In the EB stage embryo, the node expresses an array of ten other genes besides *Hnf3β*, *Chrd* and *Noggin* (reviewed by Davidson and Tam, 2000). This coordinated change in cell fate and genetic activity is reminiscent of the loss of *GSC* activity from Hensen's node when the head process mesoderm is formed in the avian embryo (Lemaire et al., 1997); and the changes in the mesodermal fate are reminiscent of the loss of *GSC* activity in the *Xenopus* organizer (Lane and Keller, 1997; Zoltewicz and Gerhart, 1997). There is some indication that a regionalized pattern of genetic activity is present in the node and cells in the dorsal (epiblast) and ventral (endodermal) compartment of the node display neuroectodermal and notochordal fate respectively (reviewed by Camus and Tam, 1999). The changing profile of molecular activity and the cell fate strongly suggest that the mouse gastrula organizer contains precursor cells that display a progressive change in characteristics as defined by their ultimate fate.

### Different tissue progenitors are present in the organizer during gastrulation

Changes in the developmental fate of organizer cells may be brought about by changes in the lineage potency of a pool of pluripotent cells that act as a constant source of tissue progenitors during gastrulation, alternatively the organizer may progressively recruit multipotential progenitors from the epiblast outside the organizer region. Results of the lineage analysis show that the bulk of the descendants of the EGO and MGO are allocated to the derivatives of the organizer, and very few cells remain in the node. This suggests that primarily transient rather than resident cells populate the EGO and MGO. Some resident cells, however, are found in the node of the mouse embryo from late-gastrulation onwards (Beddington, 1994; Sulik et al., 1994, Bellomo et al., 1996; Tam et al., 1997a).

Within the EGO, as defined by the domain of *Hnf3β* and $Gsc^{lacZ}$ expression, progenitors of the head process mesoderm and the foregut endoderm are localized in the anterior region, and those of the cranial mesoderm and the heart are in the posterior region. The EGO therefore contains a heterogenous population of tissue progenitors, not all of which will give rise to axial mesoderm. A demonstration of the heterogeneity of lineage progenitors is provided by the finding that some single-cell EGO clones can contribute exclusively to axial mesoderm and not to multiple tissue types (A. Camus and P. P. L. T.. unpublished). A significant outcome of the cell fate analysis is

that the EGO cells only make a very minor contribution to the node and the notochord of the trunk. Fate mapping of the epiblast cells in the vicinity of the EGO reveals that additional progenitors of the head process are found in the epiblast located anterior to the EGO. The epiblast that is lateral and posterior to the EGO contains the progenitors of cranial mesoderm and the extra-embryonic mesoderm, respectively, but no axial mesoderm (this study; Kinder et al., 1999). No significant contribution to the node by the epiblast surrounding the EGO was observed. These findings suggest that the EGO and the adjacent epiblast contain mainly the precursors of the head process and that those of the node are either present as a minor component in and around the EGO or are localized elsewhere in the epiblast.

During early gastrulation, the primitive streak extends to reach the epiblast domain that seats the EGO. EGO cells are then incorporated into the primitive streak and are relocated anteriorly with the advancing primitive streak. Based on the relatively more anterior position of the MGO to the EGO, it is likely that the MGO would have incorporated both the EGO and the epiblast that is originally anterior to the EGO and contains the progenitors of the head process. An important implication of this result is that the head-inducing activity is indeed associated with the anterior mesendoderm. Such inducing activity is not detected while the precursor of the anterior mesendoderm is in the EGO, but only after it is incorporated, together with other precursors of the anterior mesendoderm into the MGO. Once these precursors have left the organizer region, the remaining cells no longer display anterior-inducing activity. This places the mouse gastrula organizer in line with the chick Hensen's node, regarding the acquisition and dispensation of head-organizing activity (Storey et al., 1992; Lemaire et al., 1997, Izpisua-Belmonte et al., 1993). However, like the EGO, the MGO makes only a minor contribution to the notochord. The epiblast lateral to the MGO contains precursors of the somite and lateral mesoderm but not any axial mesoderm. Progenitors of the notochord are found in the anterior segment of the primitive streak tissue immediately posterior to the MGO (this study; Kinder et al., 1999). By the early-bud stage, the bulk of the progenitors of the notochord are found in the node, but there may still be additional contribution to the notochord by the cells in the anterior region of the primitive streak (Kinder et al., 1999). Our findings therefore point directly to the possibility that the gastrula organizer is composed of a transitory rather than a permanent population of tissue precursors. These precursors are constantly recruited from outside the organizer to replenish the organizer population as cells are allocated to the various tissue lineages during gastrulation.

In the chick, a similar traffic of cells through the organizer has been described. Cells destined for the axial mesoderm are not all present in Hensen's node but some are found in the anterior part of the primitive streak and in epiblast lateral to Hensen's node (Psychoyos and Stern, 1996; Joubin and Stern, 1999; Lawson and Schoenwolf, 2001). Cell tracing studies show that cells from the lateral epiblast migrate through Hensen's node before they are allocated to the axial and paraxial mesoderm. These findings have led to the concept that Hensen's node does not define a population of cells with determined cell fates or molecular properties. Instead, the node marks a fixed spatial domain in the embryo to which cells are recruited and then acquire the organizing activity and/or lineage-specific characteristics (Joubin and Stern, 1999; Stern, 2001).

### Precursors of the axial mesoderm and other somatic tissues take different migratory routes but move in a concerted manner

The idea that the gastrula organizer is a site of active cellular trafficking has been examined by tracking the movement of the organizer cells during gastrulation. Our data demonstrates that as the EGO cells move anteriorly with the advancing primitive streak, the precursors for non-axial tissues (such as the heart and cranial mesoderm) leave the organizer and ingress through the primitive streak to the mesoderm. These cells then take a migratory route lateral to the body axis and move with the rest of the cells in the mesoderm to the anterior region of the embryo (Fig. 8). Those EGO cells that are destined for the axial tissues are displaced along the midline of the embryo until they merge with the other axial precursors that are originally localized anterior to the EGO (Regions I, II in Fig. 5A,B) to form the MGO (Fig. 8). Cells that are allocated to the head process move out of the MGO and advance in a tight column along the midline. The axial cells, however, are not moving in isolation but are in step with the adjacent paraxial mesoderm cells (Fig. 8). Previous analysis of the apical cap of the ES embryo has demonstrated that the precursors of the midline head neuroectoderm are located in the distal epiblast of the early gastrula adjacent to the EGO (Quinlan et al., 1995; Tam et al., 1997a; Tam et al., 1997b). This implies that the floor plate of the brain has to be displaced anteriorly and may co-migrate with the head process mesoderm derived from the EGO and MGO (Tam et al., 1997b). The gastrula organizer reaches its most anterior position in the embryo by late gastrulation and thereafter appears to be displaced posteriorly as the embryonic axis elongates (this study; Sulik et al., 1994). This apparent posterior movement is reminiscent of that in the chick where the primitive streak regresses towards the posterior embryo and the node lays down the notochord and floor plate in its wake (Catala et al., 1996). The remarkable similarity in the pattern of movement of the organizer and its derivatives in the avian and mouse embryo highlights a conserved morphogenetic process in the formation of the amniote body plan despite the differences in the fine details of the molecular and morphological characteristics of the organizer and the germ layers of the gastrulating embryo.

We thank E. M. De Robertis, R. Lovell-Badge and S.-L. Ang for the gift of riboprobes; David Loebel and Peter Rowe for reading the manuscript; Seong-Seng Tan for the use of transgenic mice; and Olympus Australia Ltd for the generous provision of the Fluoview FV300 confocal microscope and imaging facility. Our research is supported by the Human Frontier Science Program (project grant to R. R. B., S.-L. Ang, H. S. and P. P. L. T.), the National Health and Medical Research Council (NHMRC) of Australia, the Ramaciotti Foundation and Mr James Fairfax. P. P. L. T. is a NHMRC Senior Principal Research Fellow.

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