glp-1 and inductions establishing embryonic axes in *C. elegans*

Harald Hutter and Ralf Schnabel*
Max-Planck-Institut für Biochemie, D-82152 Martinsried, FRG

*Author for correspondence

SUMMARY

Two successive inductions specify blastomere identities, that is complex cell lineages and not specific tissues, in a major part of the early *C. elegans* embryo. The first induction acts along the anterior-posterior axis of the embryo and the second along the left-right axis. During the first induction a specific lineage program is induced in the posterior of the two AB blastomeres present in the four cell embryo. During the second induction, almost all of the left-right differences of the embryo are specified by interactions between a single signalling blastomere, MS, and the AB blastomeres that surround it. In both cases the inductions break the equivalence of pairs of blastomeres. The inductions correlate with the cell-cell contacts to the inducing blastomeres. The stereotype cleavage pattern of the early embryo results in invariant cell-cell contacts that guarantee the specificity of the inductions. Both inductions are affected in embryos mutant for *glp-1* suggesting that in both cases *glp-1* is involved in the reception of the signal.

Key words: left-right asymmetry, pattern formation, cell lineage, micromanipulation, *C. elegans*

INTRODUCTION

Nematodes like *Caenorhabditis elegans* display a stereotype cell lineage during embryogenesis. This was taken earlier as an indication of a strictly cell autonomous 'determinate' development (zur Strassen, 1959). Recent work on *C. elegans* has shown that the nematode also depends on cell-cell interactions to assign certain cell fates (Priess et al., 1987; Priess and Thomson, 1987; Schierenberg, 1987; Schnabel, 1991; Bowerman et al., 1992b; Goldstein, 1992). The focus of these experiments concerned only the specification of tissues and not the general organization of the body plan. However, two experiments in which changes in the relative positions of certain blastomeres resulted in viable embryos (Priess and Thomson, 1987; Wood, 1991) also indicated that cell-cell interactions can specify cell fates on a larger scale. Schnabel (1991) showed that the development of large parts of the embryo is affected by removing either of two signalling centres in the early embryo.

Here we present evidence that a major part of the embryo, that derived from the first founder cell AB (Fig. 1), is specified by a hierarchy of inductions acting on groups of equivalent cells. The initial anterior-posterior polarity of the *C. elegans* embryo (Schierenberg, 1988) gives rise to blastomeres that differ from one another intrinsically. Certain blastomeres endowed with signalling activity create large-scale differences in the body pattern by initiating specific lineage programs in neighboring blastomeres. The topographic left-right asymmetry created by a stereotype cleavage pattern in the early embryo is transferred into the specification of blastomere identity by an induction correlated with specific cell-cell contacts.

The early cleavages of a *C. elegans* embryo are asymmetric divisions that produce a set of somatic founder cells (see Fig. 1A; Deppe et al., 1978; Sulston et al., 1983). The first cleavage results in a large anterior blastomere AB, the first founder cell, and a smaller posterior one called P1. P1 continues to divide asymmetrically to produce the remaining founder cells. In contrast AB divides symmetrically first along the anterior-posterior axis to generate the daughters ABa and ABp (Fig. 1B). Priess and Thomson (1987) showed that ABa and ABp are initially equivalent in their developmental potentials. The next division of the two AB descendants occurs along the left-right axis of the embryo (Fig. 1C). This division always places the left daughters slightly more anterior to the right ones leading to the first visible asymmetry along the left-right axis. As in the preceding division, the left and right daughters are initially equivalent in their developmental potentials with respect to 'left' or 'right' fates and therefore require interactions with other blastomers to develop differently (Wood, 1991). The third division of the AB descendants occurs again along the anterior-posterior axis (Fig. 1D).

The initial asymmetry in the position of the AB descendants with respect to the left-right axis results in a completely asymmetric embryo. In the 8-cell embryo the MS blastomere is located on the ventral-right and the C blastomere on the dorsalsegmental side. Therefore MS or C could be the source of a signal breaking the equivalence of the left-right pairs of AB descendants (Schnabel, 1991). The MS blastomere is the daughter of EMS (see Fig. 1A). It was shown earlier that EMS or its descendants participate in an inductive event, since the removal or ablation of EMS causes, among other defects, a failure in the development of the anterior pharynx muscle cells which are derived from the anterior blastomere ABa (Priess and Thomson, 1987; Schnabel, 1991).
MATERIALS AND METHODS

Strains
The experiments were carried out with the *C. elegans* wild-type strain N2 Bristol and two strains of glp-1, glp-1(e2072)/eT1 III; him-3(e1147) IV; +/eT1 V kindly provided by J. R. Priess and glp-1(e2144ts)III cultivated under standard culture conditions (Brenner, 1974; Wood, 1988).

Microscopy, laser ablations and immunostaining
For microscopy we used Zeiss Axioplan microscopes equipped with Nomarski optics. A VSL-337ND dye laser (wavelength 450 nm; Laser Science Inc.) attached to it was used for the laser ablation of blastomeres. Embryos were prepared by cutting gravid adult hermaphrodites and transferring eggs to multi-well slides coated with polylysine (Schnabel, 1991). Eggs of the desired stage were selected and blastomeres were irradiated with the laser beam. EMS blastomeres were

Fig. 1. Early development of *C. elegans*. (A) Early lineage diagram showing the timing of cell divisions up to the 12-cell embryo (adapted from Sulston et al., 1983). The vertical axis indicates time of development at 25°C. The stem cell-like P blastomeres produce in unequal divisions the somatic founder cells AB, MS, E, C, D and the germ line precursor P4. The main tissues produced by the founder cells are shown under the lineage diagram. The progeny of the founder cells are named according to their position after cleavage (e.g. ABal is the left daughter of the anterior daughter of AB). (B-D) The first three cleavages of the AB blastomere. (B) The first cleavage is along the anterior-posterior axis and produces the daughters ABa and ABp. (C) The second cleavage is along the left-right axis. (D) The third cleavage is again along the anterior-posterior axis. Bold print in C and D indicates the blastomeres that have cell-cell contact to MS. The dashed line of ABpra indicates that the contact of this blastomere to MS varies from embryo to embryo. (E-G) Nomarski micrographs of an upper (E), medial (F) and lower focal plane (G) of the embryo. (H-J) Drawings corresponding to the Nomarski micrographs. The topographical relations of all 8 AB descendants can be deduced from the micrographs and drawings. Blastomeres touching MS are printed in bold. The contacts were analysed in recordings of embryos and, with one exception, were invariant in all 20 analysed embryos. A contact between ABpra and MS exists only in long but not in short eggs. Cell contacts in 12-cell stage embryos have been analysed previously (Schnabel, 1991). This analysis was based on three embryos directly observed under the microscope. In one of those embryos ABprp and ABplp had no contact to MS and were therefore scored as variable contacts. This embryo appears to be unusual because these contacts invariably existed in all 20 embryos analysed here.
irradiated for 3-5 minutes and MS blastomeres for 1-3 minutes with 20 laser pulses per second. During irradiation the blastomeres started to flatten and developed patches of clear cytoplasm in the interior and at the surface of the cell. The nucleus often broke down and increased motion of yolk granules was visible in the ablated blastomeres. Ablated blastomeres usually did not divide at all or divided very late in development, at most twice. Laser operations were carried out at 25°C. For immunostainings with the restrictive temperature of 25 °C 12 hours before embryos were prepared. For immunostainings with glp-1 embryos large amounts of embryos were collected as described earlier (Goh and Bogaert, 1991) and stained. glp-1(e2072) embryos were identified in MH27/NE2-1B4 double stainings by the absence of the anterior pharynx.

Time lapse recordings and lineage analysis

Time lapse recordings of embryos with ablated blastomeres were carried out using a 4-D microscope (Hird and White, 1993). Embryos were mounted as described by Sulston (1983). A series of 25 focal levels was recorded every 30 seconds for 5-6 hours at 25°C. This allows a three-dimensional reconstruction of the embryo at any recorded time point. For lineage analysis the recording was replayed in a time-lapse mode and the cell of interest was followed on a monitor through its divisions (see Results and Fig. 3).

Micromanipulation of embryos and temperature shift experiments with glp-1(e2144ts)

In the experiment where we pushed ABa towards P1, one-cell embryos were mounted on coverslips coated with polylysine. To prevent squashing of the embryos the coverslip was mounted between two coverslips fixed to a microscope slide. The P1 blastomere was irradiated with the laser microbeam for 1 minute, 2-4 minutes after division of P0. The coverslip was then brought to a Zeiss inverted microscope equipped with two micromanipulators, 9-10 minutes after division of P0 (21°C) we applied pressure to the anterior end of the egg with a microneedle. This constraint leads to a symmetrical positioning of the AB daughters with respect to the anterior-posterior axis after the division of AB. The needle was removed 10 minutes after division of AB and the coverslip with the embryo was placed on an agar pad for the recording of further development. After the microneedle was removed, one of the two AB descendants slipped into a more anterior position than the other within the next minutes. Following the nomenclature of Sulston et al. (1983) we named this blastomere ABa (Fig. 7E-F). P1 divided in one embryo in the middle, in another at the end of the 4-AB cell stage. In the third embryo P1 divided at the 8-AB cell stage. In the second and third embryo the P1 blastomere divided abnormally symmetrically. Three control embryos were only irradiated for 1 minute and then recorded without any further manipulation. We analysed 48 lineages in each embryo. The fate of the four descendants of the lineages ABalaapp, ABalaapa, ABalapp, ABplappa, ABplppaa, ABplpppa, ABprappa, and ABprppaa were found to be normal. Therefore the identity of ABa and ABp is not affected in these embryos. However, the lineages of ABalppapp and ABalpppp indicated a transformation of the ABalp blastomere to execute the ABapp fate. This suggests that the brief irradiation of P1 already disturbs the left-right asymmetry normally induced by its granddaughter MS.

For the removal of the P1 blastomere, eggs were mounted on coverslips as described above. Excess water was removed and 50 µl of culture medium added. The standard minimal culture medium RPMI (GIBCO BRL) diluted with double distilled water to 80% of the original concentration was used as the culture medium. For one embryo this medium was supplemented with 30% of embryonic growth medium (kindly supplied by Lois Edgar). On the inverted microscope the egg was punctured at the posterior end with a sharp microneedle, 8-10 minutes after division of P0 (20°C). Pressure was applied then with a blunt microneedle from the anterior end to squeeze the P1 blastomere out of the egg. This was achieved within a minute. The coverslip was then inverted onto a multi-well slide for the recording of further development. AB blastomeres were removed using the same technique.

For the temperature shift experiments with glp-1(e2144ts) two-cell embryos raised at 25°C were mounted for recording. They were kept at 25°C under the microscope until they reached the 8-cell stage. The slide was then put into a 15°C incubator. During the next minutes the microscope was also cooled to 15°C. The slide was brought back to the microscope and the recording was started. The temperature was kept at 15°C until the end of recording. To control the temperature of the microscope slide during all of our recordings, water of the desired temperature was circulated through a modified stage and through a copper tube bent around the objective.

RESULTS

MS induces AB descendants around the 12-cell stage

To determine when the induction of pharyngeal muscle occurs, we inactivated the EMS blastomere or its daughter MS by laser ablation at various stages in development and tested for the presence of the anterior pharynx by staining the embryos with a monoclonal antibody that recognizes a subset of pharyngeal muscle cells (Fig. 2A-C). The pharyngeal muscle cells are absent or strongly reduced when EMS or MS is ablated in the 4 to 8-cell embryo (2 to 4 AB descendants present; Fig. 2A,B). They are, however, present when MS is ablated in the 12-cell embryo (8 AB descendants; Fig. 2A,C). This indicates that MS induces pharyngeal development very late in the 8-cell embryo (4 AB descendants) or very early in the 12-cell embryo.

MS induces the left-right asymmetry of the AB lineage

A more complete account, on a cellular level, of the alterations caused by these ablations was achieved by tracing the descendants of the early blastomeres to their final fate in time lapse recordings of manipulated embryos. This allows a comparison of the lineage in manipulated embryos with that in normal embryos (Deppe et al., 1978; Sulston et al., 1983; Schnabel, 1991). The resolution of the recordings is sufficient to follow cells through 9 divisions. Most of the hypodermal cells start differentiating after 8 divisions, at a time when early cell deaths occur. These cells can be identified by their characteristic morphology, position and behaviour during development. Dead cells have a ‘lentil-like’ appearance. Hypodermal cells form rows at the surface of the embryo and have large ‘fried-egg’ shaped nuclei (Fig. 3). In embryos where MS was ablated in the 8-cell embryo (4 AB descendants), not only the pharynx but also other parts of the AB lineage were affected (Fig. 4). The observed lineage alterations indicate the abolition of left-right asymmetry in the AB lineage. Half of the asymmetric lineages were transformed into either their left or right counterparts.

When fate transformations occur, the name of the blastomere (see legend to Fig. 1) is no longer identical to its fate, which now may be the fate normally executed by a different blastomere. To distinguish the names and the fates of blastomeres we will refer for example to the blastomere ABa by...
using the term ‘ABara blastomere’ and to its fate by using the term ‘ABara-fate’. The major transformations after the ablation of the blastomere MS occur in the ABa lineage. The ABara blastomere executes the ABala-fate and the ABalp blastomere adopts the ABarp-fate. Major parts of the ABp lineage are symmetrical and are not affected by the ablation of MS. Only the minor asymmetrical parts of ABp are affected. In the future therefore we will refer to this induction as left-right induction.

The anterior pharynx is derived from parts of the asymmetrical AB lineage and is deleted as a result of the lineage transformations. This extends the observation of the loss of pharyngeal muscles detected by immunostaining to a complete failure to develop an anterior pharynx.

Additional hypodermal cells are produced in the manipulated embryos, since the blastomere ABalp, which normally does not produce hypodermis, now executes the ABarp-fate, which includes the production of hypodermal cells. A specialised subset of the hypodermal cells, the seam cells, can be used to assess whether the terminal fates scored in the lineage analysis indeed reflect discrete lineage transformations. Wild-type embryos contain 20 seam cells, which are stained by a specific antibody (NE2-1B4) late in development. One would expect the production of a maximum of 14 additional seam cells, if the observed lineage transformations are complete (Sulston et al., 1983). When MS was ablated in the 8-cell embryo (4 AB descendants), we counted 30±2 (±s.d., n=11; highest count 35) stained cells. However, when MS was ablated in the 12-cell embryo (8 AB descendants), only the normal equivalent of 20±1 (±s.d., n=10) seam cells was stained (Fig. 2D,E). This observation indicates that the transformations are complete up to the terminal fate of the cells.

To understand the observed lineage transformations, we examined the arrangement of cells in the early embryo. We analysed the cell-cell contacts between MS and the AB descendants in recordings of 20 embryos (Fig. 1). In the 8-cell embryo (4 AB descendants) all AB descendants contact MS. One cell division later in the 12-cell embryo, only a subset of the 8 AB descendants still contact MS (Fig. 1E-J). These contacts differ on the two sides of the embryo. Of the four granddaughters of ABa, ABalp and ABara both contact MS and are transformed by ablation of MS, whereas their sisters ABarp and ABala never contact MS and are never affected. We therefore propose that differential cell-cell contacts confer specificity to the induction by MS.

The glp-1 mutation e2072 affects the left-right asymmetry of the AB lineage

The phenotype of MS-ablated embryos, lack of anterior pharynx and additional hypodermal cells, is reminiscent of the
Establishment of left-right asymmetry

phenotype of embryos from mothers mutant for the maternal effect lethal gene glp-1 (Priess et al., 1987). glp-1 codes for a protein homologous to Notch, a cell surface receptor from Drosophila (Yochem and Greenwald, 1989) and is involved in cell-cell interactions not only in embryogenesis but also in germ cell development (Austin and Kimble, 1987). In order to test the possibility that glp-1 is involved in the reception of the inductive signal from MS we analysed the AB lineage of embryos from mothers mutant for a non-conditional allele of glp-1(e2072) (for convenience from now on these embryos will be referred to as mutant glp-1 embryos). We observed fate transformations in the AB lineage identical to the MS-ablated embryos (Fig. 4). The blastomere ABara executed the ABala-fate, the blastomere ABalp adopted the ABarp-fate. This explains both the absence of the anterior pharynx and the additional hypodermal cells found earlier in glp-1 embryos (Priess et al., 1987). We also observed the additional seam cells expected from the lineage transformation in these embryos (see legend to Fig. 5). Immunostainings with the mAb NE2-1B4 showed additional seam cells inserted into the normal rows of these cells (Fig. 5A,B). We counted 31±2 (± s.d., n=21; maximal count 34) seam cells in the mutant glp-1(e2072) embryos.

In the posterior AB lineage of mutant glp-1 embryos an interesting difference to MS-ablated embryos was observed. The cell death in ABplpappoccurred normally in glp-1(e2072) embryos but was absent in MS-ablated embryos (Fig. 4). In glp-1(e2072) embryos the sister cell ABplpapp divided asymmetrically as in normal development to create the large excretory cell. In MS-ablated embryos, however, this cell

Fig. 3. The fates of individual cells as it was analysed in the recording of the development of MS-ablated embryos. All times are times of development after the division of P0 at 25°C. Anterior is to the left. A,C,E show the outcome when MS was ablated in the 8-cell embryo (4 AB descendants) to prevent the left-right induction. Fate transformations occur. B,D,F show the subsequent development when MS was ablated in the 12-cell embryo (8 AB descendants) after the left-right induction occurred. The cells develop into their normal fates. The thick arrow in A and C points to the MS blastomere, which has not divided any more during development. (A) Embryo in which MS was ablated at the 8-cell stage (4 AB descendants) after 4 hours and 36 minutes of development. The thin arrow points to the cell ABaraapp, which has started to undergo programmed cell death a few minutes previously. In normal development this cell does not die but divides once more. The arrowheads point to ABarapaapp and ABalapaapp, which will develop into hypodermal cells instead of dividing further as in normal development. (C) The same embryo after 5 hours and 45 minutes of development. ABarapaapp and ABalapaapp (small arrows) are now members of a row of cells at the surface of the embryo. The cells have the characteristic morphology of hypodermal cells, part of which is the prominent nucleolus that is clearly visible in the posterior of the two marked cells. During normal development both cells divide once more and contribute to the pharynx. (B,D) An embryo in which the MS blastomere was ablated at the 12-cell stage (8 AB descendants). Despite the ablation the cells now execute their normal fate. (B) The embryo after 4 hours and 40 minutes of development. The cell ABalapaapp has divided normally instead of dying (compare with A). The small arrows point to the daughter cells immediately after division. (D) The same embryo after 4 hours and 25 minutes of development. ABarapaapp divided normally instead of differentiating into a hypodermal cell (compare with C). The small arrows point to the daughter cells immediately after division. ABarapaapp also divided normally in this embryo (not shown). (E,F) Schematic drawings of the lineages of the cells shown in (A-D). Arrows indicate abbreviated lineage parts. (E) Lineage of the embryo where MS was ablated in the 8-cell embryo (A,C). ABara follows a lineage pattern that is different from the wild-type pattern (compare with F) and is executed normally by its right partner ABara. The lineage of ABara is also shown for comparison. (F) Lineage in embryos where MS was ablated in the 12-cell embryo (B,D). Here ABara executes its normal lineage pattern. Bar, 10 μm.
cell death
hypodermal cell
not dividing any more
tracing not possible
or result ambigous

left lineages
right lineages

fate in wild type
MS ablated

fate in wild type
MS ablated

left lineages
right lineages
Fig. 4. The left-right asymmetry is abolished in embryos with an ablated MS blastomere and in embryos from mutant glp-1(e2072) mothers. The figure shows the complete AB lineage adapted from Sulston et al. (1983) arranged to show left-right symmetries and asymmetries. The symmetric parts of the lineage are shaded. Pharyngeal lineages are enclosed in boxes. Dashed lines indicate the cells whose fate was traced. Outer columns show the fates of these cells in normal development. Middle columns show the fates of cells in e2072 embryos. Inner columns show the fates of cells in embryos where MS was ablated in the 8-cell embryo (4 AB descendants). Arrows along the midline indicate the direction of fate transformations, left to right or vice versa. In normal development the position of a blastomere in the lineage also corresponds to its fate. In those cases where blastomeres acquire new fates after a transformation these fates are indicated in bold print beside the normal identity of the blastomeres. The MS-ablated embryos show a complete breakdown of left-right asymmetry caused by fate transformations of corresponding left-right blastomeres. In glp-1(e2072) almost identical transformations occur. The only difference is in the ABplpappa lineage, which is normal in glp-1(e2072) but aberrant in MS-ablated embryos. In two embryos where MS was ablated in the 12-cell embryo (8 AB descendants) no deviation from wild-type fate was found, with the exception of the cell death in ABplpappap, which did not occur. This could reflect a requirement for MS or descendants later in development in order to induce the left-right asymmetry here. However, we cannot exclude the possibility that this blastomere was damaged during ablation, since divided symmetrically indicating the absence of the excretory cell. Whereas ablation of the MS blastomere affects the left-right asymmetry of the entire AB lineage, a small part of the ABp lineage is still asymmetrical in mutant glp-1(e2072) embryos.

The glp-1 mutation e2144ts also affects the anterior-posterior axis

The temperature sensitive allele of glp-1, e2144ts, shows extensive morphogenetic defects in addition to defects in pharynx development. Lineage analysis of mutant e2144ts embryos revealed the same left-right transformations in the ABa lineage as in e2072. In addition the ABp blastomere ABplp lies just beneath MS at the time of ablation. This blastomere divided with a delay of several minutes after ablation in these embryos. In addition to the cells shown in the figure we examined the division of the sister of ABplpappap, the cell death mentioned above. This cell normally divides highly asymmetrically to give rise to the large excretory cell. This division always was symmetrical instead of asymmetrical in all MS-ablated embryos where ABplpappap did not die, indicating the absence of a normal excretory cell. This division was normal in the other embryos. No AB descendant was damaged when MS was ablated in the 8-cell embryo (4 AB descendants). A total of 76 fates, mainly hypodermal cells and cell deaths in the nervous system, were analysed in each embryo. The figure shows a consensus of several embryos. Lineages where less than half of the embryos showed the same fate are marked with a question mark. Three e2072 embryos were analysed completely and the aberrant lineages of two more were checked. A total of 282 fates were analysed, 23 thereof could not be scored and 4 were aberrant with respect to the consensus fate. The fates in 2 lineages were ambiguous and had no consensus, indicated by a question mark in the figure. Four embryos where MS was ablated in the 8-cell embryo (4 AB descendants) were analysed and showed identical alterations with the exception of one embryo where the lineages from ABalpa and ABraaa behaved normally. In the four embryos a total of 304 fates were analysed, 20 thereof could not be scored and in addition to the exception described above 3 were aberrant with respect to the consensus fate. The fates in 3 lineages were ambiguous and had no consensus, indicated by a question mark.

![Image](57x161 to 558x295)

Fig. 5. The differentiation of seam cells in wild-type and glp-1 embryos. The figure shows embryos stained with a combination of the monoclonal antibodies MH27 and NE2-1B4. This combination allows one to determine the number of seam cells (see Fig. 2). (A) Wild-type embryo. Part of one seam cell row is visible. This embryo has the normal number of 20 seam cells arranged in two lateral rows. (B) A glp-1(e2072) embryo with a symmetric AB lineage. Seam cell lineages are duplicated by the left-right transformations, in particular by the duplication of the ABarp-fate (Fig. 4), and additional seam cells are inserted into the row (arrows). We counted 29 seam cells in this embryo. (C) A glp-1(e2144ts) embryo where the ABp blastomere executed an ABa-fate. Here the seam cells no longer form rows, but usually two clusters, a sign of the severe morphogenetic defect. The ABarp-fate is not only duplicated as in e2072 embryos but quadruplicated since it is also executed twice by the ABp blastomere (Fig. 6A). We counted 40 seam cells in this embryo.
Fig. 6

A) 

B) 

C) 

\[ \text{ABp-fate} \]

\[ \text{ABala-fate} \]

\[ \text{ABalp-fate} \]

\[ \text{ABala-fate} \]

\[ \text{ABp-fate} \]

\[ \text{cell death} \]

\[ \text{hypodermal cell} \]

\[ \text{mitosis} \]

\[ \text{non-hypodermal cell, not dividing any more} \]

\[ \text{tracing not possible or result ambiguous} \]
The removal of P₁ affects the complete AB lineage

In order to test the role of the P lineage in specifying ABp-fate, we removed P₁ in the middle of the two cell stage with a microneedle and recorded the development of the isolated AB blastomeres (Fig. 7G-I). They divided at a normal rate but with aberrant directions of cleavages. The first division occurred along the anterior-posterior and the second along the left-right axis as in normal development. The left-right division lacked its characteristic asymmetry, which normally places the left daughters anterior to the right ones, and was symmetric instead. From the third division onward the orientation of cleavages was completely aberrant. It was therefore impossible to perform a lineage analysis in these embryos. This type of analysis depends on the correct orientations of cell divisions because they are the basis for determining the identities of blastomeres during development.

The terminal phenotypes of the P₁ deleted embryos were similar to those described earlier (Laufer et al., 1980; Priess and Thomson, 1987). A detailed analysis of the terminal phenotypes showed that the development of both ABa and ABp between the anterior and posterior daughters produced by the third cleavage of AB descendants. Therefore only two different fates of the 8-AB cell stage are executed: The ABala- and the ABarp-fate (Fig. 6A). These two fates are repeated along the anterior-posterior axis and executed on the left as well as on the right side of the embryo. Thus the phenotype of e2144ts can be explained as a combination of two fate transformations. The first removes the difference between the ABa and ABp blastomeres along the anterior-posterior axis, the second the differences along the left-right axis.
was severely disturbed and no obvious transformation occurred. In contrast to our other manipulation experiments the cellular differentiation of the embryos showed considerable variation. For instance the number of early cell deaths was 5, 15 and 17. One of the cell deaths even occurred one cleavage round earlier than normal. In normal development 13 early cell deaths occur in the AB lineage and 20 are expected when ABp is transformed into ABa as in mutant glp-1(e2144ts) embryos. Cells with the characteristic morphology of hypodermal cells could not be found by following lineages and by inspection of terminal embryos. This is not due to the manipulation itself, because ‘isolated’ P1 blastomeres (we removed most of the AB blastomere from the two-cell embryo) differentiated into cells of all major tissues derived from this lineage, i.e. intestine, muscle and hypodermal cells (not shown). We therefore conclude that the presence of an intact P1 blastomere is essential for the anterior-posterior induction. The experiments are consistent with the notion that the P1 lineage is the source of the signal specifying ABp fate. We cannot exclude entirely, however, that the eggshell also plays a role in specifying the AB lineage.

**Left-right induction correlates with cell-cell contacts**

In order to test the idea that the cell-cell contacts of MS in the 12-cell embryo (8 AB descendants) are essential for the left-right induction, it is necessary to establish atypical contacts.
Fig. 8. Summary: Two inductions establish the anterior-posterior and left-right axes of the C. elegans embryo. The figure summarises schematically our experiments and observations (A-B) and shows a model of the two inductions (C). Different shapes and patterns represent the different fates, i.e. identities of blastomeres that are indicated within the schematic representations of the blastomeres. (A) An induction along the anterior-posterior axis induces the ABp-fate. In the absence of the anterior-posterior induction in glp-1(e2144ts) embryos (Fig. 6A) the blastomere ABp executes the ABa-fate. Establishing a close contact between ABa and P1 (Fig. 7D-F) induces ABp-fate ectopically in ABa (Fig. 6B). (B) A left-right induction alters the fate of equivalent blastomeres along the left-right axis and thus establishes the left-right asymmetry of the AB lineage. In the absence of the induction, in embryos where MS was ablated or in glp-1 embryos (Fig. 4), the left-right asymmetry breaks down. It is likely that only ABara and ABalp are directly affected. In temperature shifted glp-1(e2144ts) embryos the ABp blastomere adopts an ABa fate since the anterior-posterior induction is prevented at the restrictive temperature. After the down shift, the left-right induction occurs at the permissive temperature (Fig. 6C). Left-right fate changes are induced ectopically in those ABp descendants that have cell contact to MS (cells with contact to MS are marked with an asterisk). (C) Model of the two inductions. First the anterior-posterior induction specifies the ABp-fate. P2 is most probably the source of the signal and glp-1 is involved in the reception of the signal on the ABp blastomere. The MS blastomere is the source of the signal for the left-right induction. Here only the ABa descendants, ABara and ABalp, are directly affected. glp-1 is again the receptor.
between MS and AB descendants competent to respond to the MS signal. In embryos where the ABp blastomere adopts an ABa-fate the transformed ABp descendants should be competent to respond to the MS signal. If cell-cell contacts are indeed essential for the left-right induction to occur, all ABp descendants contacting MS in the 12-cell embryo (8 AB descendants; Fig. 1) should be induced during the left-right induction. In temperature shift experiments of glp-1 embryos the morphogenetic defect occurs before the pharynx defect (Priess et al. 1987; C. C. Mello and J. R. Priess, personal communication; H. H. and R. S., unpublished data). According to our analysis, the morphogenetic defect corresponds to the anterior-posterior induction and the pharynx defect to the left-right induction. Since both inductions are affected by the temperature sensitive allele e2144ts, it is possible to prevent the anterior-posterior induction but to allow the left-right induction by appropriate temperature shift. We incubated glp-1(e2144ts) embryos up to the 8-cell embryo (4 AB descendants) at the non-permissive temperature to prevent the anterior-posterior induction and then down-shifted the embryos to allow left-right induction to occur (for a full account of this experiment see legend to Fig. 6). As predicted, the AB descendants in the 12-cell embryo (8 AB descendants), which had contact to MS, altered their fate (Fig. 6C) when compared to e2144ts embryos incubated only at the non-permissive temperature (Fig. 6A). As expected from the induction of left-right asymmetry in the AB lineage, two different fate changes were induced depending on whether the affected cell was an anterior or posterior daughter of the third cleavage round. The blastomeres ABa and ABp adopted the ABa-fate, whereas the blastomeres ABalp, ABprp and ABpla executed the ABplp-fate (Figs 6C, 8B). AB descendants without contact to MS were not affected. We therefore conclude that the cell contacts in the 12-cell embryo (8 AB descendants) are indeed essential for the left-right induction.

DISCUSSION

A detailed analysis of the phenotype of embryos from mothers mutant in the gene glp-1 and a series of manipulation experiments show that two successive inductions specify the fates of descendants of the AB blastomere.

One induction specifies the anterior-posterior axis in the AB derived part of the embryo

The first division of the founder cell AB along the anterior-posterior axis (Fig. 1B) produces two daughter cells, ABa and ABp, with initially identical developmental potential (Priess and Thomson, 1987). In embryos from mothers mutant for glp-1(e2144ts) ABp is transformed into ABa (Figs 6A, 8A). Since glp-1 is thought to be a receptor for cell-cell interactions (Austin and Kimble, 1987; Priess et al., 1987; Yochem and Greenwald, 1989; Kimble et al., 1992), it is likely that an induction specifies the ABp fate. The ABp fate indeed depends on an induction because establishing a close contact between ABa and P1 results in a duplication of the ABp fate by the ABa blastomere (Figs 6B, 8A). Removal of P1 from two-cell embryos causes completely aberrant behaviour of the remaining AB blastomere. The phenotype of these P1 deleted embryos showed considerable variation and no obvious fate transformations occurred as observed in all our other experiments. This effect could either be due to the altered topography of the remaining AB descendants in the embryo or it could reflect a direct requirement of P1.

A second induction establishes the asymmetry along the left-right axis

The second division of the AB descendants occurs along the left-right axis (Fig. 1C) and produces equivalent daughter cells (Wood, 1991). The laser ablation experiments presented here show that the induction of left-right asymmetry in the AB lineage occurs around the 12-cell stage of the embryo (8 AB descendants) and that the MS blastomere is the source of the inductive signal. Ablation of MS before this stage leads to a complete loss of asymmetry in the AB lineage (Figs 4, 8B) because of fate transformations along the left-right axis. Since the main part of the ABp lineage is symmetrical, the major transformations occur in ABa. These transformations correlate with cell contacts to the inducing blastomere MS in the 12-cell embryo (8 AB descendants; Fig. 1E-J).

The contacts of the blastomeres of the ABp lineage do not correlate with the induced asymmetries (Figs 1, 4). Although a part of the lineage of ABpra is affected by the irradiation of MS this blastomere does not always touch MS. Both ABplp and ABprp always touch MS, but only parts of the lineage of ABplp are affected. The asymmetries in ABp are small consisting only of lineage parts corresponding to cells of the 32-AB cell stage and later stages (Fig. 4). In mutant glp-1(e2072) embryos the same left-right lineage transformations as after ablation of MS are observed in ABa, and in most of the embryos also in the anterior part of ABp. A small part of the ABp lineage (ABplpappa), however, remained asymmetric in all glp-1(e2072) embryos (Fig. 4). This can be explained by assuming that the generation of asymmetry within the AB lineage is a multi-step process. In a first step, MS induces different fates only in ABa descendants. This step is glp-1 dependent. Further steps are necessary to create the minor asymmetries in ABp descendants. Here glp-1 function may not be essential, but they still depend on MS as a source of asymmetry. The simplest explanation for the observed alterations in the ABp lineage of glp-1(e2072) embryos is to assume that the left-right asymmetries in ABp also depend on glp-1 function but are very differently dosage dependent. e2072 is thought to be a partial loss-of-function mutation (Kodoyianni et al., 1992), which would support this idea. Another possibility is that lin-12, a gene homologous to glp-1, may be also involved in this step in a redundant way (Lambie and Kimble, 1991). Analysis of lin-12 glp-1 double mutants (Lambie and Kimble, 1991) revealed defects in the asymmetric parts of ABp, e.g. a loss of the excretory cell and a duplication of the excretory pore cell. This phenotype can be interpreted as a loss of left-right asymmetry in ABp as it is observed in MS-ablated embryos. lin-12 might substitute for glp-1 function here.

glp-1 is involved in both inductions

The phenotypes of glp-1 mutant embryos suggest that glp-1 is involved in both inductions. In the allele e2072 only the left-right induction is affected whereas in the allele e2144ts the anterior-posterior induction is also disturbed. glp-1 protein is found in the membrane of ABa and ABp in the 4-cell embryo.
(2 AB descendants) and on all AB descendants up to the 28-cell embryo (16 AB descendants; Kimble et al., 1992). The expression pattern is consistent with the notion that glp-1 is involved in the reception of the signals inducing both anterior-posterior polarity and left-right asymmetry.

Both blastomeres ABa and ABp are competent to respond to the anterior-posterior induction (Figs 6B, 8A). In contrast, only descendants of ABa but not those of ABp are competent to respond to the left-right induction. In the temperature shift experiment carried out with glp-1(e2144ts) embryos the anterior-posterior induction was prevented and the ABp blastomere adopted an ABa-fate. When the left-right induction then occurred at the permissive temperature, not only ABa descendants but also ABp descendants altered their fate (Figs 6C, 8B). One consequence of the anterior-posterior induction in normal development therefore is to restrict the competence for the left-right induction to the ABa descendants.

A third step in the specification of the AB lineage is independent of the two inductions described

The third division of the AB descendants to the 8-AB cell stage occurs along the anterior-posterior axis (Fig. 1D). The anterior daughter cells of this division are different from their posterior sisters. This difference is independent of the inductions described above. This can be seen in glp-1(e2144ts) embryos where the AB descendants execute two different fates, the ABala-fate and the ABarp-fate, alternately along the anterior-posterior axis (Fig. 6A). It is also evident from the different responses to the left-right induction. After induction AB descendants adopt one of two possible fates: either an ABara- or an ABarp-fate (Figs 6C, 8B). This difference is specified by another induction from the P1 lineage (Schnabel, 1991; H. H. and R. S., unpublished data).

Cell-cell contacts are important for the inductions

There are several possible ways to achieve the inductions described here. The signal could either be located in the eggshell or derived from a specific blastomere. It could either be released as a diffusible factor or remain fixed at the source. The ablation experiments show that the left-right induction clearly depends on the blastomere MS (Figs 2, 4). The temperature shift experiments with glp-1(e2144ts) embryos (Figs 6C, 8B) show that the fate changes induced by MS correlate with the cell-cell contacts between MS and the AB descendants in the 12-cell embryo (Fig. 1E-J). In our experiments one cannot discriminate whether direct cell-cell contact is sufficient or whether a short range diffusible signal exists. A long range diffusible signal, however, is improbable since the distance between the MS blastomere and the target blastomere is only a few micrometers.

The anterior-posterior induction also depends on the presence of a single blastomere. Pushing ABa posterior towards P1 is sufficient for an induction of ABp-fate in ABa (Figs 6B, 8A). The source of the signal therefore appears to be P1 or its descendants. Since P2, the posterior daughter of P1, is the only blastomere that has contact to ABp but not to ABa, it is possible that P2 is the source for the signal in normal development. Bowerman et al. (1992b) showed that ABp fails to produce two valve cells when P2 is ablated. This was taken as an indication that P2 is necessary to distinguish between the ABa and ABp fates.

The inductions affect blastomere identities and not tissues

Most of the previous studies on inductions early in the embryo have focused on tissues (Priess and Thomson, 1987; Schnabel, 1991; Bowerman et al., 1992b). Our results show that the inductions affecting the early AB descendants do not specify tissues but complex lineage programs leading to very different tissues. This occurs on different levels. P1 is able to induce the ABp-fate. MS induces the ABara- and ABarp-fate. The inductions act as switches in binary decisions taken by the blastomeres. The blastomeres that are competent for the inductions can therefore be described as an equivalence group (Kimble et al., 1979). After manipulation, AB descendants in very different positions in the embryo are able to adopt the same fate. In e2144ts the ABarp-fate is executed not only by ABarp itself but also by the blastomeres ABalp, ABppr and ABplp. The transformations are in most cases complete as far as we could judge by lineage analysis. It therefore seems unlikely that further major inductions are necessary for the execution of fates of AB descendants after the 12-cell embryo (8 AB descendants). For other blastomeres it has been suggested that blastomere identity is specified cell autonomously (Schnabel and Schnabel, 1990; Bowerman et al., 1992a; Mello et al., 1992) or by a combination of lineage dependent and inductive mechanisms (Goldstein, 1993). Thus two fundamentally different mechanisms to specify cell fate, cell autonomous determination and inductive events, may both operate on the same level, the identity of blastomeres.

Establishment of the left-right asymmetry

The problem of how left-right asymmetries and handedness are generated is poorly understood (Brown and Wolpert, 1990). Early C. elegans embryos show a left-right asymmetry with an invariant handedness, because during the first division along the left-right axis in the 4-cell embryo, the left daughters of the AB descendants are always located more anteriorly than their right counterparts. The basis for the invariance in the handedness of the embryo is not known. A series of stereotype cleavages under the physical constraint of the eggshell is responsible for the asymmetric arrangement of blastomeres in the 12-cell embryo. This arrangement of blastomeres results in only one member each of two bilateral homologs of blastomeres coming into contact with a single signalling blastomere. Inductions then initiate the complex lineage programs required to establish the left-right asymmetry of the embryo.

Modes of development

The modes of development of animals can be distinguished first on a descriptive level. Animals with so called ‘determinate’ development show an ‘embryonic lineage’, i.e. there is a clear correlation between descent and fate on the single cell level. By contrast in ‘indeterminate’ development no such correlation exists. It is usually implied that ‘determinate’ development reflects cell autonomous or ‘mosaic’ development while indeterminate development is often used synonymously with ‘regulative’ or non-autonomous development. Drosophila and amphibians are well studied paradigms for indeterminate development. Nematodes were considered to be a major paradigm for a determinate development. That determinate development only uses cell autonomous specification
was challenged by the demonstration of inductions during early development in *C. elegans* (Priess et al., 1987; Priess and Thomson, 1987; Schierenberg, 1987; Schnabel, 1991; Wood, 1991; Bowerman et al., 1992b; Goldstein, 1992) which showed that these two modes of development are not mutually exclusive. The results presented here show that the establishment of a large part of the body plan of *C. elegans* depends on a hierarchy of inductions. Thus it appears that the major difference between the determinate and indeterminate modes of development is the number of cells involved in embryogenesis. In addition to a small number of cells being involved, the orientations of the mitotic cleavage planes are fixed in determinate embryos so that the relationships between cells is invariant. This leads to reproducible patterns of cell interactions.

We thank Heinke Schnabel and Richard Feichtinger for helpful discussions and Thierry Bogaert, Thomas Bosch, Gerard Marriot, Christiane Weigner and in particular Charles N. David for critically reading the manuscript, and C. Mello and J. Priess for communicating unpublished results. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

**REFERENCES**


(Accepted 28 March 1994)