Establishment of left-right asymmetry in the *Caenorhabditis elegans* embryo: a multistep process involving a series of inductive events

Harald Hutter and Ralf Schnabel*

Max-Planck-Institut für Biochemie, D-82152 Martinsried, FRG

*Author for correspondence

SUMMARY

Bilateral pairs of blastomeres derived from the founder cell AB, the anterior blastomere of the 2-cell stage, in the *Caenorhabditis elegans* embryo are initially equivalent in their developmental potential. Recently, we showed that an induction at the 12-cell stage by a blastomere called MS is necessary to establish the differences between left and right pairs of blastomeres in the anterior part of the embryo. Further analysis of the process of creating left-right asymmetry reveals that the induction at the 12-cell stage is only the first of a series of inductions establishing the left-right asymmetry of the embryo. We describe here two further inductions that create additional asymmetries in the posterior part of the embryo. One induction occurs at the 24-cell stage among AB descendants themselves. This induction is restricted to the left side of the embryo as a consequence of the fate changes induced by MS at the 12-cell stage. The second induction requires again blastomeres of the MS lineage and also occurs around the 24-cell stage. Together these inductions establish the fate differences observed in the development of left-right pairs of blastomeres in the embryo.

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

INTRODUCTION

Many animals exhibit an overall bilateral symmetry but nevertheless show left-right asymmetries often with invariant handedness in the number and positioning of internal organs. A few mutants affecting the handedness have been described: a maternal effect mutant affects the direction of coiling in snails (Boycott and Diver, 1923; Sturtevant, 1923) and two different mutants in the mouse result in a situs inversus in either half (Hummel and Chapman, 1959; Layton, 1976) or all (Yokoyama et al., 1993) of the mutant embryos. In all these cases, the left-right asymmetry is still present and only the handedness is reversed. To our knowledge only in *C. elegans* have mutants been found where the left-right asymmetry of the body plan i.e. cell fates is completely lost (Hutter and Schnabel, 1994).

The generation of left-right asymmetry can be divided into two separate steps. First an asymmetrical cue has to be created that afterwards is translated into differences in cell fates between left and right pairs of cells. In the *C. elegans* embryo, the first step corresponds to an asymmetry in the positioning of blastomeres at the 6-cell stage, when the left pair of AB descendants is positioned more anterior than the right pair during the third division round in the embryo (Deppe et al., 1978; Fig. 1A). Recently we demonstrated that the second step, the translation of the asymmetrical cue into differences in cell fates, is largely achieved by the induction of left-right asymmetries by the MS blastomere at the 12-cell stage. This induction breaks the equivalence of bilateral pairs of blastomeres (Wood, 1991) by inducing fate changes in the two ABa descendants that contact MS. This explains why the bilateral pairs of ABa descendants develop very differently on the left and right side (Sulston et al., 1983; Fig. 1C). A new symmetry leading to a bilaterally symmetrical larva is formed much later in embryogenesis. Cell-cell contacts are essential for the left-right induction to occur and confer the specificity of the induction (Hutter and Schnabel, 1994), because an experimental alteration of cell-cell contacts leads to changes in the pattern of induction by MS (Hutter and Schnabel, 1994; Moskowitz et al., 1994; Fig. 4 in Hutter and Schnabel, 1995). The maternal effect lethal gene *glp-1*, encoding a Notch-like cell-surface receptor (Yochem and Greenwald, 1989), is probably the receptor for this left-right induction (Hutter and Schnabel, 1994). The competence of the blastomeres to respond to the left-right induction is influenced by two earlier inductions acting along the anterior-posterior axis (Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994; Hutter and Schnabel, 1995).

Here we show that the establishment of left-right asymmetry in the AB-derived part of the embryo depends on a cascade of inductions. Together with the induction originating from MS at the 12-cell stage, two later inductions successively subdivide the left and right pairs of AB descendants into blastomeres with unique developmental potential and establish the left-right asymmetry of the AB lineage in the *C. elegans* embryo.

MATERIALS AND METHODS

Strains

The experiments were carried out with the *Caenorhabditis elegans* wild-type strain N2 Bristol cultivated under standard culture conditions (Brenner, 1974; Wood, 1988).

Microscopy, laser ablations, time-lapse recordings, lineage analysis and immunostainings

Microscopy, laser ablations, time-lapse recordings of laser ablated embryos and lineage analysis were carried out as described previously (Hutter and Schnabel, 1994). Briefly, embryos were prepared and mounted as described (Sulston et al., 1983). Embryos of the desired stage were selected and blastomeres were irradiated with the laser microbeam. Blastomeres of the 6-cell stage (ABal, ABar) were irradiated for 1-2 minutes with 20 laser pulses per second. Irradiation times for blastomeres at later stages become successively shorter due to the smaller size of the blastomeres and were about 20 seconds at the 24-cell stage. Ablated blastomeres usually did not divide any more during development or showed a few aberrant mitoses and cytokineses. Laser operations were carried out at 25°C. Time-lapse recordings were made using a multifocal plane time-lapse recording system, also called 4D-microscope (Hird and White, 1993). A series of 25 focal levels was recorded every 35 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. For immunostaining, the operated embryos were incubated either at 15°C overnight (15-17 hours) or at 25°C for 6-7 hours. Slides were then frozen on dry ice and the cover slip was removed with a razor blade. The embryos were then fixed in methanol for 5 minutes at −20°C, postfixed in acetone for 5 minutes at −20°C and then processed for immunostaining as described (Wood, 1988). The monoclonal antibody 3NB12 (Priess and Thomson, 1987) was used to stain pharyngeal muscle cells.

Analysis of cell-cell contacts

Cell-cell contacts were scored using the time-lapse recordings of either normal or ablated embryos. Most of the cell-cell contacts can be scored reliably in this way (Hutter and Schnabel, 1995).

RESULTS

The development of the left-right asymmetry in the ABpla/ABpra lineage depends on an induction from ABalap

The ablation of the MS blastomere at the 8-cell stage affects the development of the left-right asymmetry in the complete AB lineage (Hutter and Schnabel, 1994). In the ABa lineage, major alterations corresponding to fate changes occur in two blastomeres of the 12-cell stage. In contrast, only minor changes occur in the ABp lineage, since this part of the embryo develops mainly symmetrically (Fig. 1C). More specifically, after ablation of MS at the 8-cell stage, the right ABpraaa lineage is transformed into the corresponding left ABplaaa lineage and the left ABplpappa lineage develops like the right ABprpappa lineage. The induction of fate changes by MS in the ABa lineage depends on cell-cell contacts (Hutter and Schnabel, 1994; Moskowitz et al., 1994; Fig. 4 in Hutter and Schnabel, 1995). Since, in contrast to the ABa descendants, the contacts between MS and the ABp descendants do not correlate with the observed effects of the ablation of MS, we argued earlier that the effect of MS on the development of left versus

right fates in ABp descendants might be an indirect rather than a direct effect (Hutter and Schnabel, 1994). To investigate whether blastomeres other than MS are involved in the specification of the asymmetry of the ABp lineage, we ablated various ABa descendants and scored the development of the left-right asymmetry of the ABp lineage. The AB lineage developed normally after the ablation of ABar (see legend to Fig. 2). However, after the ablation of ABal, the asymmetric part of the left ABpla lineage was affected. In the ablated embryos, the hypodermal cells of the left ABplaaa lineage no
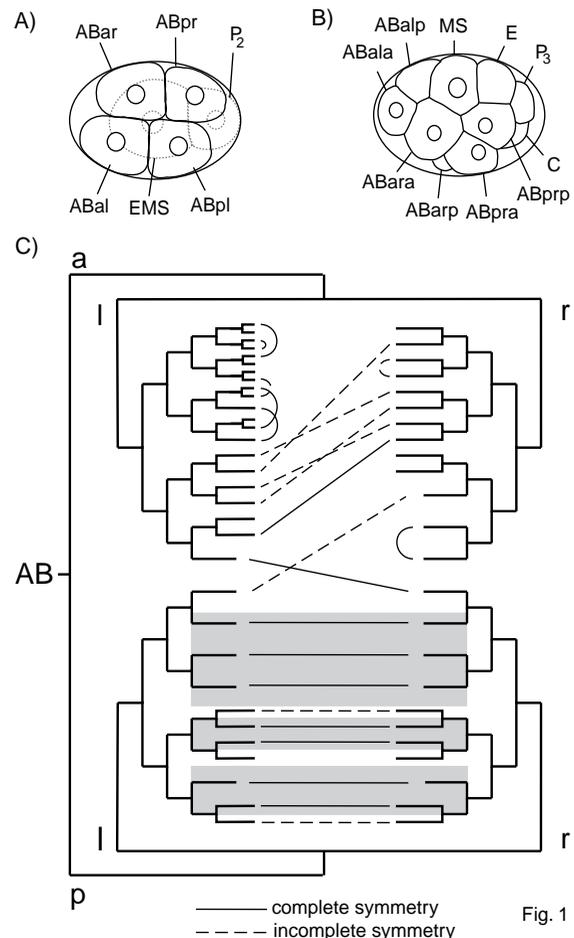

Fig. 1. Left-right asymmetry in the AB lineage of the *C. elegans* embryo. (A) Drawing of a 6-cell stage embryo, dorsal view, anterior to the left. This is the first stage where a left-right asymmetry is visible in the positioning of the AB descendants. (B) Drawing of a 12-cell stage embryo, ventral right view. The asymmetry of the 6-cell stage embryo now results in cell-cell contacts that are side-specific. For example, the right blastomere ABara touches MS, whereas its bilateral homolog ABala does not touch MS. At this stage, the primary induction of left-right asymmetry occurs (Hutter and Schnabel, 1994). (C) Symmetry relationship within the AB lineage adapted from Sulston (1983). The parts of the lineage that give rise to identical structures on the left and right side of the animal are connected by lines. Note that the ABa-derived part of the lineage is completely asymmetrical whereas the ABp-derived part is mainly symmetrical. The parts of the lineage that are not connected give rise either to unique structures like the excretory cell or are parts of the animal that exhibit a 3-fold symmetry like the pharynx (Albertson and Thomson, 1976) or a 6-fold symmetry like parts of the nervous system (Sulston et al., 1983).

longer developed the characteristic morphology of hypodermal cells, a clear nucleus containing a prominent nucleolus, but instead divided once more (Fig. 2A) and developed features of neuronal cells. The left ABplaaa lineage therefore developed like the corresponding right ABpraaa lineage, which normally produces neuronal cells (Fig. 2A). Hypodermal cells derived from the ABplaap lineage, the symmetric part of the ABpla lineage, always developed normally (Fig. 2A). This lineage transformation is the opposite of that observed after the ablation of MS, which we already mentioned above. After ablation of MS, neuronal lineages from the right ABpraaa lineage develop like hypodermal cells as their bilateral homologs of the left ABplaaa lineage do (Hutter and Schnabel,

1994; Fig. 2A). No other defects in the development of the AB-derived part of the embryo were found in the lineage analysis of ABal-ablated embryos (see legend to Fig. 2). This is corroborated in immunostainings with a monoclonal antibody recognizing a total of 21 pharyngeal muscle cells, 2 derived from the ABal lineage, 5 derived from the ABar lineage and 14 derived from the MS lineage. In ABal-ablated embryos, we counted 19 ± 2 ($\pm$s.d.; $n=15$) pharyngeal muscle cells stained (Fig. 3B), which corresponds to the contribution of the ABar and MS lineages. When ABal and the two daughters of MS were ablated, we found 4 ± 1 ($\pm$s.d.; $n=6$) pharyngeal muscle cells (Fig. 3C). This suggests that pharyngeal muscle cells derived from ABar develop normally after the ablation of

Fig. 2. Development of the asymmetry in the ABpla/ABpra lineage after the ablation of other blastomeres in the embryo. (A) The figure shows part of the ABp lineage arranged to show left-right symmetries and asymmetries adapted from Sulston et al. (1983). Dashed lines indicate the cells whose fate was traced. Outer columns show the fates of these cells in normal development. The other columns show the fates of the cells in embryos, where certain blastomeres were ablated. Data for the column 'P2 ablated' were taken from Hutter and Schnabel (1995) and data for the column 'MS ablated' were taken from Hutter and Schnabel (1994). The arrows along the midline indicate the direction of the fate transformations, left to right or vice versa. The fates shown in the figure are always a consensus of several embryos. Five out of six embryos where ABal was ablated developed as shown in column 'ABal-ablated' with the aberration in the ABplaaa lineage, only one of them showed the normal pattern indicated in the outer column. In three of these embryos, the following additional parts of the AB lineage were analysed: ABaraaaapp (1 descendant), ABarpaap (4), ABarpapp (4), ABarppap (4), ABarppppp (4), ABplaaaa (4), ABplappa (4), ABplppaaap (1), ABplppppapp (1), ABprappa (4), ABprppaaap (1), ABprpppapp (1); together with the 20 shown in the figure a total of 53 lineages. The only aberration that we found in these lineages were additional mitoses of the two hypodermal cells of the ABplaaaap lineage. This extends the defects in the ABpla lineage to the whole asymmetric part of the ABpla lineage. All other lineages developed normally. In three embryos, we ablated MS at the 8-cell stage and ABara at the 12-cell stage. A total of 60 lineages was followed, one of them could not be scored, all others developed as indicated by the consensus. In addition, we ablated ABar in three embryos and analysed the following lineages: ABalaapapa (1), ABalaappaa (1), ABalapapaa (1), ABalappaaa (1), ABalppaaaa (1), ABalppaapa (1), ABplaaap (4), ABplaapp (4), ABplappa (4), ABplpappa (2), ABplppaaap (1), ABplppppapp (1), ABpraaap (4), ABpraapp (4), ABprappa (4), ABprppaaap (1), ABprppppapp (1), a total of 108 lineages. Eleven of these could not be scored and six of them were found to be aberrant with respect to the normal fate. Since this aberration is within the range of lineage errors in this kind of analysis we conclude that the AB lineage develops normally after the ablation of ABar. (B-E) Schematic representation of the development of the asymmetry in the ABpla/ABpra lineage after various ablations. (B) After ablation of ABal or ABala (see Fig. 4A), ABpla develops like ABpra due to the prevention of an interaction between these lineages. (C) After the ablation of MS at the 8-cell stage, the ABala-fate is duplicated and also executed by the ABara blastomere on the right side of the embryo. Therefore an interaction between the ABara and ABpra lineages is probably responsible for the transformation of ABpra into ABpla. (D) ABpra develops normally when both MS and ABara are ablated, which provides evidence for the proposed interaction between the ABara and ABpra lineages. (E) When P2 is ablated at the 4-cell stage, the ABala fate is also duplicated and, in this case, executed by ABarp (Hutter and Schnabel, 1995). In an analogous way, the observed transformation of ABpra into ABpla could here be due to an interaction between the ABarp and ABpra lineages.
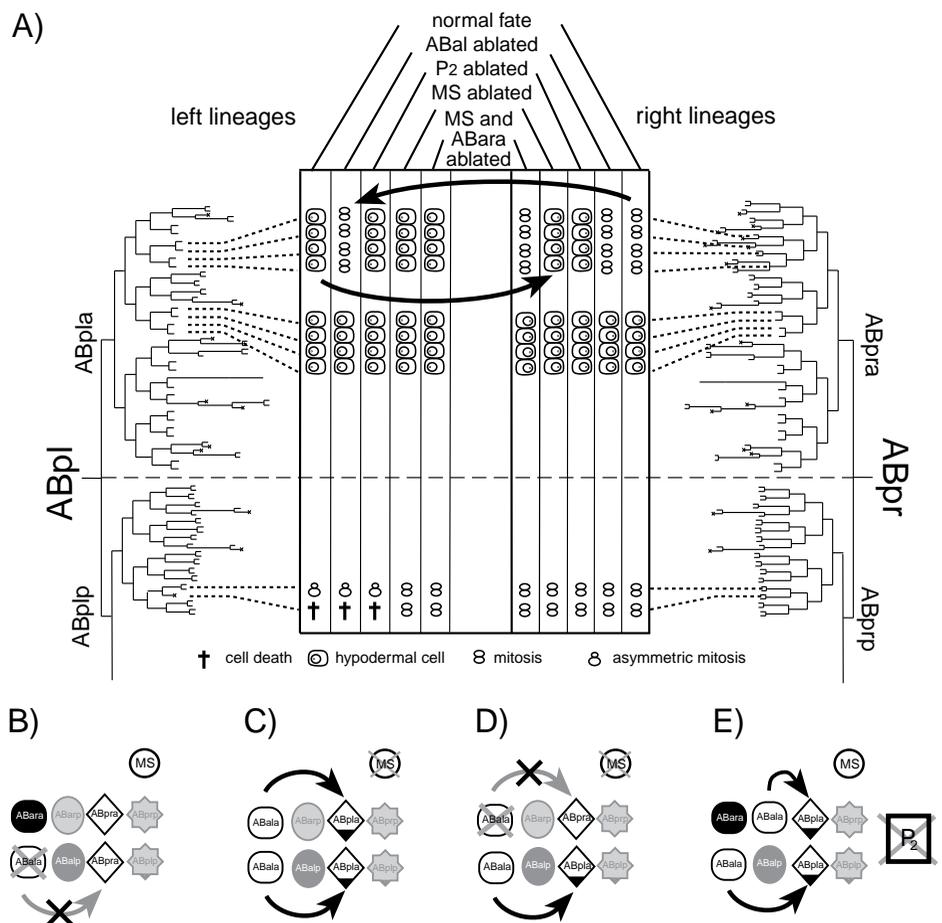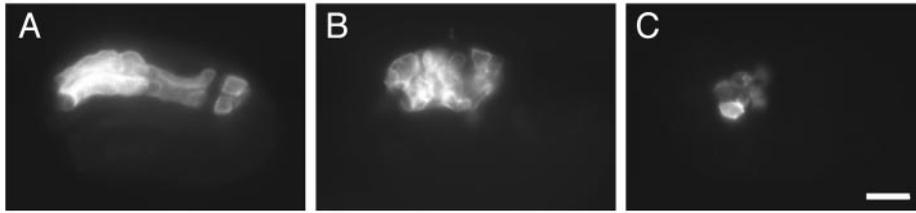
Fig. 3. Development of pharyngeal muscle cells after the ablation of the ABal blastomere. The figure shows embryos stained with the antibody 3NB12, which recognizes 21 pharyngeal muscle cells, 14 derived from the MS lineage, 5 derived from the ABar lineage and 2 derived from the ABal lineage (Priess and Thomson, 1987). (A) Normal embryo. (B) Embryo in which ABal was ablated at the 6-cell stage. We counted 19±2 (±s. d., n=15) pharyngeal muscle cells, an indication that the MS- and ABar-derived pharyngeal muscle cells develop normally in these embryos. (C) Embryo, in which ABal, MSa and MSp were ablated. 5 pharyngeal muscle cells should be left from the ABar lineage; we counted 4±1 (±s. d., n=6). Thus the ABar-derived pharyngeal muscle cells develop normally after the ablation of ABal. Bar, 10 µm.

ABal. However, one of us (Schnabel, 1991) reported that the AB-derived part of the pharynx is missing after the ablation of ABal. It is not clear to us whether the difference in the number of pharyngeal muscle cells after the ablation of ABal is due to differences in the ablation techniques used or due to improved staining procedures.

The effect of the ablation of ABal on the development of the ABplaaa lineage appears to reflect a direct interaction between the ABal and the ABplaaa lineages. In order to identify the signalling blastomere and to determine the time course of the

interaction between the ABal and the ABplaaa lineages, we ablated various descendants of the ABal blastomere and scored the development of the ABplaaa lineage. During the search for the signalling blastomere, it turned out that only the ablation of ABal descendants that have contact to the corresponding precursors of the affected ABplaaa blastomere results in an aberrant development of the ABplaaa lineage (Figs 4A, 5A). For instance, the ABplaaa lineage developed normally after ablation of ABalaa, a blastomere that has no contact to ABplaa, the precursor of the ABplaaa lineage (Fig. 4A). In contrast, an

Fig. 4. Time course of the inductions establishing the left-right asymmetry in the ABp lineage. The horizontal axis shows the time of development in minutes after the division of the zygote at 25°C. The bars on top show the division times of the AB and MS lineages. On the vertical axis, the fraction of embryos is indicated, in which the analysed lineage (ABplaaa, ABpraaa or ABplpappa) developed normally despite the ablation. The name of the ablated blastomere(s) and the number of analysed embryos are indicated at each time point. (A) Development of the ABplaaa lineage after the ablation of various ABal descendants at different stages during development. In order to judge the development of the ABplaaa lineage, the fates shown in Fig. 2A were analysed. All embryos, whose development was scored abnormal developed as expected as described in column 'ABal ablated' in Fig. 2A. The other embryos developed normally, as indicated in column 'normal fate' in Fig. 2A. The blastomeres ABal, ABala and ABalap have cell-cell contact to the precursors of the ABplaaa lineage, whereas ABalp and ABalaa lack such contacts. (*) ABal was ablated early in the 4-AB-cell stage in four of the six embryos and late in the 4-AB-cell stage in the remaining two embryos. (**) ABalp was ablated early in the 8-AB-cell stage in two of the three embryos and late in the 8-AB-cell stage in the remaining one. ABalx is the abbreviation for ABala and ABalp, ABalax is the abbreviation for ABalaa and ABalap. In experiments where ABalaa was ablated early in the 16-AB cell stage its sister ABalap divided with a delay of 9-20 minutes. In the reverse experiment, ablation of ABalap at the same time, ABalaa also divided with a delay of 10-20 minutes. Since the ablation of ABalaa, which led to a delay in the division of the ABalap blastomere, had no effect on the induction of the left-right asymmetry of the ABpla/ABpra lineage, we think it is reasonable to assume that the effect visible after the ablation of ABalap is due to the damage of ABalap itself and not due to the minor damage of ABalaa. (B) Development of the ABpraaa and ABplpappa lineages after ablation of various MS descendants at different stages during development. Circles indicate the development of the ABplpappa lineage, squares the development of the ABpraaa lineage, which was scored in the same embryos in which the development of the ABplpappa lineage was scored. In order to score the development of these lineages, the fates indicated in Fig. 2A were scored and judged as normal or aberrant in a way analogous to that described for A. The blastomeres MS, MSa, MSp, MSap and MSapp all have contact to precursors of the ABplpappa lineage. MSx is the abbreviation for MSa and MSp, MSapx is the abbreviation for MSapa and MSapp. The time course for the ABpraaa lineage reflects the time course for the primary induction of left-right asymmetry by the MS blastomere at the 12-cell stage. The development of the ABplpappa lineage in contrast becomes independent from the MS lineage only at the 51-cell stage (32 AB descendants).
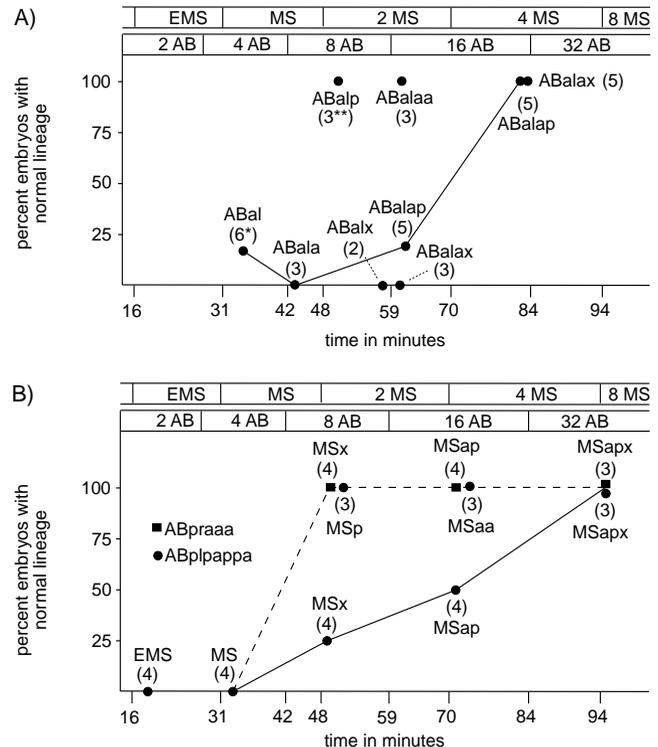
early ablation of ABalap, which has contact to ABplaa, resulted in abnormal development of the ABplaaa lineage in most of the cases (Fig. 4A, see also legend to Fig. 4). When ABalap is ablated later, shortly before its division, the ABplaaa lineage always developed normally, which indicates that the interaction is already completed at that time. The time course shown in Fig. 4A indicates that the induction occurs around the 24-cell stage (16-AB cell stage) and that ABalap and ABplaa are the interacting blastomeres.

After the ablation of MS, a transformation of the right ABpraaa lineage into the left ABplaaa lineage is observed (Hutter and Schnabel, 1994), i.e. the opposite transformation of that observed after ablation of ABala. One major effect of the ablation of MS is the duplication of the ABala-fate which is not only executed by the left ABala blastomere but also by the ABara blastomere on the right side of the embryo (Hutter and Schnabel, 1994). The observed loss of ABpraaa-fate therefore probably is a consequence of an ectopic induction of ABplaaa-fate by ABarap, which now develops like its left homolog ABalap (Fig. 2C). This can be tested by ablation of ABara and MS in the same embryo, which should restore the normal development of ABpraaa since this ablation should remove the ectopic signalling activity from the ABara lineage. After such ablations, we indeed found that both ABplaaa and ABpraaa executed their normal lineage patterns (Fig. 2A,D), which confirms that the effect of the MS ablation on ABpraaa is mediated by the ABara lineage. Such an ectopic induction of the ABplaaa lineage on the right side of the embryo might require a contact between ABarap and ABpraa, the inducing and induced blastomeres in this case. These blastomeres indeed have cell-cell contacts in normal as well as in MS-ablated embryos (Fig. 5C).

The development of the left-right asymmetry in the ABplp/ABprp lineage is induced by MSap

The asymmetry between the ABplp and the ABprp lineages was never affected in either ABar- or ABal-ablated embryos (Fig. 2). The establishment of this asymmetry therefore does not depend on the fate changes induced by MS in the ABa lineage but may directly depend on the MS lineage. Because we observed earlier that the asymmetries in ABplp were still affected when the MS blastomere was ablated after the induction of the major anterior left-right asymmetries that occurs at the 12-cell stage (Hutter and Schnabel, 1994), we now tested whether the specification of the posterior asymmetries occurs later in development. The asymmetry between ABplp and ABprp was still affected when the daughters of MS were ablated immediately after the division

of MS (Fig. 4B). In such embryos, a characteristic cell death derived from ABplpappap on the left side does not occur. The cell instead continues to divide like the corresponding right ABprpappap cell. Furthermore, the asymmetric division of the left ABplpappaa cell, which normally gives rise to the large excretory cell, is symmetrical instead indicating that the excretory cell is not properly formed (Fig. 2, compare rows 'normal fate' and 'MS ablated'). This indicates that not MS itself, but descendants of MS are responsible for the generation of the asymmetry between ABplp and ABprp. The time course shown in Fig. 4B reveals that this interaction occurs one cleavage after the primary induction of left-right asymmetry around the 24-cell stage (16-AB cell stage) and therefore that ABplpa and most likely MSap are the interacting blastomeres. Inducing and induced blastomeres again have cell-cell contact (Fig. 5B).

DISCUSSION

Many animals show an overall bilateral symmetry with left-right asymmetries in the number and positioning of internal organs that usually arise with invariant handedness during embryonic development. It is a long-standing question how left-right asymmetries are specified in initially symmetrical embryos. The *C. elegans* embryo is also initially left-right sym-
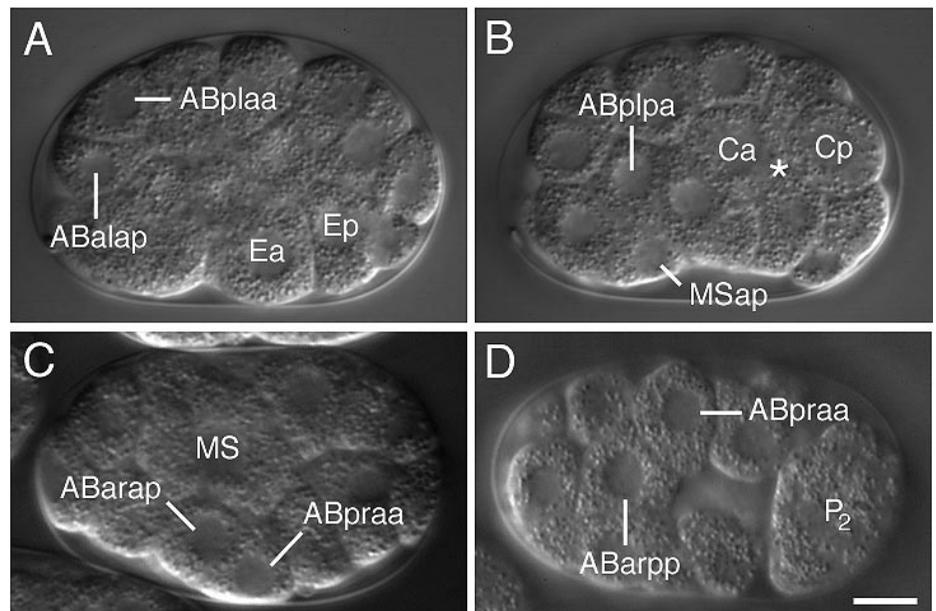
Fig. 5. Cell-cell contacts relevant for the induction of left-right asymmetry in the ABp lineage. The figure shows Nomarski light micrographs of embryos at the 26-cell-stage. Anterior is to the left. Bar 10 μm. (A,B) Normal embryo, left-dorsal view. (A) Medial focal plane. The two AB descendants ABalap and ABplaa touch each other. The position of the two gut precursors Ea and Ep is also indicated. (B) Upper focal plane. ABplpa and MSap have cell-cell contact. The positions of the Ca and Cp blastomeres are also indicated. The star (*) indicates the position of the ABprpa blastomere that is on the other side of the embryo visible only in the bottom focal plane. Descendants of the ABplp and MS lineages are also in contact one cleavage before and after this stage (not shown). (C) Embryo, in which MS was ablated at the 8-cell stage, ventral view. ABarap and ABpraa have cell-cell contact. This contact also exists in normal embryos. The position of the ablated MS blastomere is indicated. (D) Embryo, in which P_2 was ablated at the 4-cell stage. ABarpp and ABpraa are in contact. The large undivided P_2 blastomere is indicated.

metrical. An asymmetry along the left-right axis in the positioning of blastomeres becomes visible in the 6-cell-stage embryo, where the left pair of AB descendants is placed more anterior than the right pair during the third cleavage round (Fig. 1A). Despite the asymmetrical positioning, the bilateral pairs of blastomeres are not intrinsically different from the beginning but depend on interactions to acquire their different developmental potential (Wood, 1991). We showed that the MS lineage is the initial source responsible for all left-right asymmetries in the AB lineage of the embryo. The major asymmetries in the embryo in the ABa lineage are induced in the 12-cell-stage embryo (Hutter and Schnabel, 1994). Here we describe two further inductions occurring later in development creating further asymmetries in the ABp lineage. Together with the primary induction by MS, they form a cascade of inductive events successively subdividing bilateral pairs of blastomeres into cells with unique left or right developmental potential.

The primary induction of left-right asymmetry by MS at the 12-cell stage restricts a later induction to one side of the embryo

In contrast to the ABa lineage, which develops completely asymmetrically, the ABp lineage is mainly symmetrical with only a few asymmetrical parts (Fig. 1C). The establishment of all the asymmetry of the AB lineage depends on an intact MS blastomere. Unlike the contacts between MS and ABa descendants, the contacts between MS and the ABp descendants do not correlate with the changes observed after the ablation of MS (Hutter and Schnabel, 1994). Whereas ABpra has contact to MS only in about half of the analysed embryos, it is always affected by the ablation of MS, and whereas both ABplp and ABprp have contact to MS at the 12-cell stage only the ABplp lineage, but not the ABprp lineage, is affected by the ablation of MS (Hutter and Schnabel, 1994). Since cell-cell contacts are essential for the induction of fate changes by MS in the ABa descendants (Hutter and Schnabel, 1994; Hutter and Schnabel, 1995), we proposed that the changes observed in the ABp lineage after ablation of MS are secondary effects possibly due to the fate changes observed in the ABa lineage. The ablation experiments reported here indeed reveal an interaction between the ABa descendants and ABp descendants around the 24-cell stage (Fig. 4A). The ABalap blastomere appears to be the source for the signal. The interaction is required for the proper development of hypodermal lineages derived from the asymmetric part of ABpla (Fig. 2A) and therefore is responsible for the establishment of the asymmetry between the ABpla and ABpra lineages. As in the initial left-right induction by MS at the 12-cell stage, the induction between the AB descendants appears to depend on cell-cell contacts between inducing and induced blastomeres. Analysis of cell-cell contacts, however, shows that there is not only a contact between the inducing ABalap blastomere and the induced ABplaa blastomere on the left side of the embryo (Fig. 5A) but also between the bilateral homologs ABarap and ABpraa on the right side (Fig. 5C). The restriction of the induction to the left side therefore does not depend on the differences in cell-cell contacts between the left and right sides of the embryo as in the specification of left-right asymmetry in the ABa lineage but depends on the different fates executed by the left and right blastomeres ABalap and ABarap, respectively. This difference in cell fate in turn depends on the primary left-right induction by MS. In

MS-ablated embryos, the right ABara blastomere adopts an ABala fate (Hutter and Schnabel, 1994, Fig. 2C). Consequently, the left ABplaa-fate is also induced on the right side leading to a transformation of the right ABpraaa blastomere (Fig. 2A,C,D). One result of the primary induction of left-right asymmetry in the ABa lineage by MS therefore is the restriction of a secondary induction to one side of the embryo, which then establishes additional asymmetries.

The transformation of the right ABpraaa lineage into the left ABplaaa lineage is also observed after the ablation of P$_2$, indicating a requirement for P$_2$ at the 4-cell stage for the establishment of this asymmetry (Hutter and Schnabel, 1995). This observation was puzzling because only the ABp blastomere itself is present at the 4-cell stage, which appears to be completely symmetrical. The fact that the execution of the ABala-fate on the right side of the embryo results in the transformation of the right ABpraaa lineage into the left ABplaaa lineage, however, provides a simple explanation for the transformation of the ABpraaa lineage in P$_2$-ablated embryos. One effect of the ablation of P$_2$ is a duplication of the ABala-fate, which in this case is also executed by the ABarp blastomere on the right side (Fig. 2E) due to the breakdown of the anterior-posterior difference between ABara and ABarp blastomeres after the ablation of P$_2$ (Hutter and Schnabel, 1995). Since there is a contact between ABarpp (developing like ABalap) and ABpraa in P$_2$-ablated embryos (Fig. 5D), the loss of ABpraaa-fate again appears to be a consequence of an ectopic induction of ABplaaa-fate in this case by ABarpp. It is interesting to note that in this case the ABplaaa-fate is induced on the right side not by the bilateral homolog of the cell that normally induces the ABplaaa-fate on the left side but by a completely unrelated cell that accidentally also has the appropriate cell-cell contact.

Blastomeres of the MS lineage are required twice to induce asymmetries in the AB lineage

Asymmetries in the ABplp/ABprp-derived part of the AB lineage also depend on an intact MS blastomere. The ablation of the ABa-descendants that are affected by the MS ablation did not interfere with the development of this part of the asymmetry indicating that the establishment of this asymmetry directly depends on the MS lineage. Further ablation of MS descendants showed that the induction of the asymmetries in the ABplp/ABprp-derived part of the AB lineage follows a different time course than the induction of the other asymmetries that depend on MS (Fig. 4B). The induction does not occur at the 12-cell stage but around the 24-cell stage (16-AB-cell stage). ABplpa and most likely MSap are the interacting blastomeres. As in the induction described above there is not only a contact between the interacting blastomeres on the left side but also between the corresponding blastomeres on the right side, MSpp and ABprpa, respectively. It appears that again the specificity of the induction could be due to differences in the signalling properties of corresponding left and right blastomeres, MSap and MSpp respectively. This suggests that MSap and MSpp already have different properties at that stage.

Only part of the asymmetries in the ABplp/ABprp lineage can be scored by a lineage analysis. One cell death that is characteristic for the left ABplp lineage and the asymmetric division of ABplpappa on the left side giving rise to the large excretory cell can be scored this way (Fig. 2A). Other asym-

metric lineages derived from the ABplp/ABprp part of the lineage contribute to structures in the rectum like the anal depressor muscle, the sphincter muscle and the pair of valve cells linking the intestine to the rectum. The asymmetric part of the ABplp/ABprp lineage does not correspond to a single lineage block as the asymmetry in the ABpla/ABpra lineage does (Fig. 1C). It is not clear whether the induction of asymmetry in the ABplp/ABprp lineage depends on still more inductions or whether all of them are induced during the induction described here.

Anterior-posterior inductions

Left-right inductions
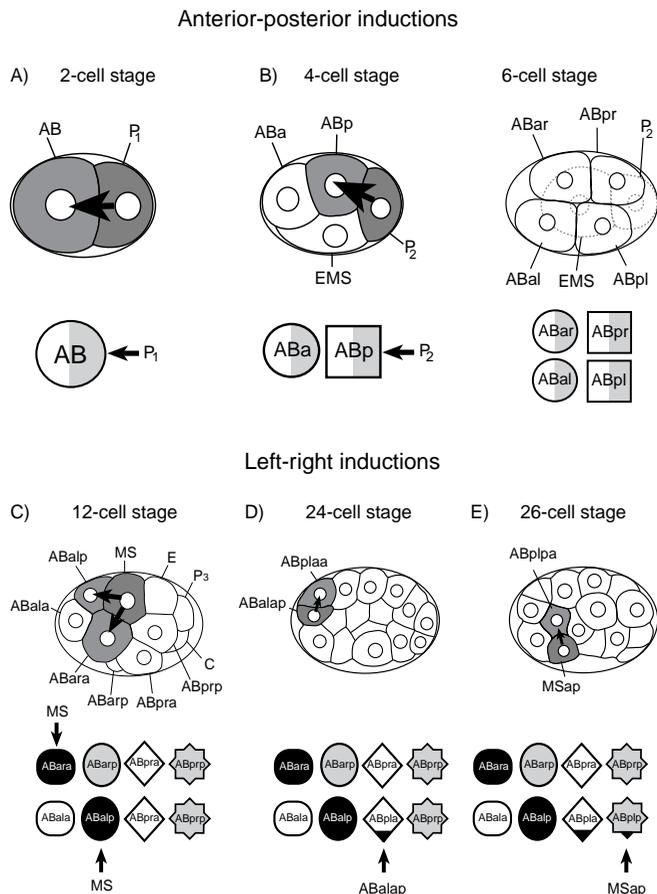
Fig. 6. Five inductions in the *C. elegans* embryo successively establish the 8 different fates of the AB-descendants present at the 12-cell stage. (A) At the 2-cell stage, the posterior P_1 blastomere polarises its anterior sister AB along the anterior-posterior axis (Hutter and Schnabel, 1995). (B) At the 4-cell stage, the P_2 blastomere induces the ABp-fate (Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994). (C) At the 12-cell stage, a primary induction by MS induces the left-right asymmetry of the ABa lineage (Hutter and Schnabel, 1994). Two ABa descendants, ABara and ABalp, are affected by this induction. It induces fate changes in these blastomeres and depends on cell-cell contacts between inducing and induced blastomeres which become side-specific at that stage. (D) At the 24-cell stage, ABalap induces the asymmetry of the ABpla/ABpra lineage by affecting the development of ABpla descendants. Cell-cell contacts again appear to be important. Since the contacts are symmetrical in this case (see Fig. 5), the asymmetry of this induction depends on the different fates adopted by the left ABalap and the right ABarap blastomere, which depend on the previous induction of ABara-fate by MS. (E) At about the same stage MSap, an MS descendant, is required for the establishment of the asymmetry in the ABplp/ABprp lineage.

Structures derived from the asymmetric part of the ABplp/ABprp lineage like the excretory cell, the sphincter muscle, the excretory pore and the valve cells are either duplicated or missing in mutants showing the so called Lag phenotype (Lambie and Kimble, 1991). This phenotype therefore can be interpreted as a breakdown of the asymmetry in the ABplp/ABprp lineage and is observed not only in mutants in the *lag-2* gene but also in the *glp-1/lin-12* double mutant (Lambie and Kimble, 1991). Interestingly, this part of the asymmetry within the ABp lineage is specified correctly in embryos mutant only for *glp-1* (Hutter and Schnabel, 1994), which suggests that *glp-1* and *lin-12* have overlapping functions in this process. Proteins encoded by these genes are proposed to be ligands (LAG-2; Henderson et al., 1994; Tax et al., 1994) or receptors (GLP-1 and LIN-12; Yochem et al., 1988; Yochem and Greenwald, 1989) in cell-cell interactions. It is therefore possible that the genes mentioned above are indeed the ligand (LAG-2) and receptors (GLP-1/LIN-12) for the induction of the asymmetry in the ABplp/ABprp lineage described here.

A series of inductions successively establishes the left-right asymmetry in the *C. elegans* embryo

Models for the establishment of handedness and left-right asymmetry that are derived from observations in mammals like the model proposed by Brown and Wolpert (1990) assume that the asymmetry along the left-right axis is reflected in a gradient along this axis that is somehow inevitably created during development. Our work on the establishment of asymmetry in the *C. elegans* embryo shows that a series of inductive events is required in this animal to break the equivalence of pairs of blastomeres along the left-right axis. The inductions depend on cell-cell contacts between the inducing and induced blastomeres rather than on the presence of a graded substance that acts at a distance. Cell-cell contacts on the left and right sides become different because left and right pairs of blastomeres are positioned asymmetrically at the 6-cell stage, the left pair always lying more anterior than the right one (Fig. 1A). The basis for the generation of the invariant handedness in this process is not known. The asymmetric positioning of blastomeres, however, creates the asymmetrical cue that is later translated into fate differences of bilateral pairs of blastomeres by inductions relying on side-specific cell contacts.

Together with previous work (Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994; Hutter and Schnabel, 1995), the inductions described here explain how the 8 AB descendants present at the 12-cell stage finally adopt their 8 different fates. In chronological order, the following sequence of 5 inductions takes place: at the 2-cell stage the posterior P_1 blastomere polarises its anterior sister AB in anterior-posterior direction (Fig. 6A; Hutter and Schnabel, 1995). This polarisation is transmitted through the following three divisions before it is used to specify the fates of the four posterior ABxxp blastomeres. At the 4-cell stage, P_2 induces the ABp-fate thus creating an additional difference along the anterior-posterior axis (Fig. 6B; Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994). A cascade of inductions then successively establishes differences in bilaterally symmetrical pairs of blastomeres. At the 12-cell stage, the MS blastomere induces the left-right asymmetry in the ABa-derived part of the embryo (Fig. 6C;

Hutter and Schnabel, 1994). One consequence of this induction is the restriction of a second induction among the AB descendants themselves to the left side of the embryo. This induction between the ABalap and the ABplaa blastomeres at the 24-cell stage establishes left-right differences in the ABpla/ABpra lineage (Fig. 6D, this work). Another induction originating from MSap, a descendant of the MS blastomere, further refines the left-right asymmetry in the ABplp/ABprp-derived part of the embryo (Fig. 6E, this work). In combination, these five inductions specify the 8 different fates of the AB descendants in the 12-cell stage embryo.

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