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Specification of anterior-posterior differences within the AB lineage in the *C. elegans* embryo: a polarising induction

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SUMMARY

In a *C. elegans* embryo the third cleavages of descendants of the anterior blastomere AB of the 2-cell stage create pairs of blastomeres that develop differently. By laser ablation experiments we show that the fates of all the posterior daughters of this division depend on an induction occurring three cleavages before these blastomeres are born. The time of induction precludes a direct effect on cell fate. Alternatively, we suggest that the induction creates a heritable cell polarity which is propagated through several divisions. We suggest a model to demonstrate how a signal could be propagated through several rounds of cell division. An important implication of our observations is that this early induction acts to specify blastomere identity, not tissue type. A detailed lineage analysis revealed that altering the inductive signal alters complex lineage patterns as a whole. The induction described here, together with two inductions described previously can be used to illustrate how the anterior portion of the *C. elegans* embryo can be successively subdivided into blastomeres with unique developmental potential.

Key words: anterior-posterior axis, pattern formation, cell lineage, micromanipulation, *C. elegans*, blastomere

INTRODUCTION

Fate changes during early development are thought to arise either through communication with neighbouring cells (induction) or through the unequal distribution of cellular material during cell divisions. A variety of early cell-cell interactions affecting most parts of the *C. elegans* embryo have been postulated and described (Priess and Thompson, 1987; Priess et al., 1987; Schierenberg, 1987; Schnabel, 1991, 1994; Wood, 1991; Bowerman et al., 1992; Goldstein, 1992, 1993; Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994). There is also some evidence for cell autonomous specification for the development of certain tissues (Strome and Wood, 1982, 1983; Schierenberg, 1988; Laufer et al., 1980; Cowan and McIntosh, 1985; Edgar and McGhee, 1986; Gendreau et al., 1994). However, recently it was shown that most of the blastomere fates are nevertheless subject to cell-cell interactions (Schnabel, 1994; R.S. unpublished data).

Most of the inductions described so far (op. cit.) affect the development of the AB blastomere, the anterior cell born in the first cleavage of the embryo (Fig. 1A). This work is concerned with the specification of the anterior-posterior differences of the 8 AB-derived blastomeres present at the 12-cell stage of the embryo. These blastomeres have distinct identities (Fig. 1B), reflected by complex and unique lineage patterns, which lead to the polyclonal formation of several tissues, including the hypodermis, the nervous system and the anterior part of the pharynx (Sulston et al., 1983). These 8 identities could be specified parsimoniously by a series of three binary decisions, two along the anterior-posterior axis and one along the left-right axis, each time creating a difference between pairs of equivalent cells. It was shown earlier that two of these decisions, one along the anterior-posterior axis (Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994) and the other along the left-right axis (Hutter and Schnabel, 1994) are binary and depend on inductions from non-AB cells contacting the induced cells. The remaining decision specifies the differences between anterior and posterior sisters created by the cleavages of the four AB descendants of the 8-cell stage. This difference could be specified cell autonomously or it could depend on yet another induction. We describe here a new induction affecting the development of the four posterior blastomeres of this third division of the AB blastomere. These posterior blastomeres develop like their anterior sisters when the induction is blocked by the ablation of the signalling blastomere P_1. The induction induces the complex lineage programs normally executed by these blastomeres and therefore serves to establish the correct identity of the blastomeres. Analysis of the timing of the induction revealed a unique feature of the induction. It occurs three cleavages before the affected blastomeres are born. The induction therefore does not induce cell fates directly but polarises the great-grandmothers of the blastomeres along the anterior-posterior axis. The polarity is then inherited through several cell divisions before being used to make cells different. This example of cell fate specification combines the two

basically different modes of induction and unequal distribution of developmental potential in a novel form. This induction, together with earlier work (Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994), allows us to propose a model for the specification of the identities of all AB blastomeres up to the 12-cell stage.

MATERIALS AND METHODS

Strains

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

Microscopy, laser ablations, time lapse recordings and lineage analysis

Microscopy, laser ablations, time lapse recordings of laser-ablated embryos and lineage analysis were carried out as described before (Hutter and Schnabel, 1994). Briefly, embryos were prepared and mounted as described by Sulston (1983). Eggs of the desired stage were selected and blastomeres were irradiated with the laser microbeam. P_1 blastomeres were irradiated for 3-5 minutes, blastomeres of the 4-cell stage, ABa, ABp EMS, or P_2, for 1-3 minutes and blastomeres of later stages for 1-1.5 minutes with 20 laser pulses per second. Differences in ablation time are due to differences in the size of the ablated blastomeres and differences in the strength of the laser varying somewhat between experiments. Ablated blastomeres often did not divide any more during development or showed few aberrant mitoses and cytokineses. Laser operations were carried out at 25°C. Time lapse recordings were made using a time-lapse multi-focal-plane recording system, the 4-D microscope (Hird and White, 1993). A series of 25 focal levels was recorded every 35 seconds for 6-7 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.

Analysis of cell-cell contacts

We compared cell-cell contacts in normal and laser-ablated embryos in order to judge whether the effects seen in laser-ablated embryos are due to the ablation itself or are secondary effects resulting from a disturbed topology of the embryo. Contacts were scored at the 12-cell stage, the first stage where the blastomeres that are affected by the ablation are present, by viewing the embryos in 4-D recordings. Most of the cell-cell contacts can be scored reliably in this way. Only rarely was it difficult to judge the contact of two cells at opposite ends of the embryo, e.g. between ABara and ABplp in the embryo shown in Fig. 3.

RESULTS

Ablation of the P_1 blastomere disturbs the correct specification of AB descendants

The division of the four descendants of the AB blastomere occurs along the anterior-posterior axis (Fig. 1B) and produces four pairs of blastomeres where all the anterior blastomeres develop differently from their posterior sisters. To test whether this difference is specified cell-autonomously or by inductions we laser-ablated the P_1 blastomere, the posterior sister of AB (see Fig. 1B) in 2-cell stage embryos. This operation should eliminate cellular sources for inductions outside the AB lineage itself. A detailed lineage analysis revealed two types of fate changes in the manipulated embryos. As expected from our previous work (Hutter and Schnabel, 1994) all fate differences along the left-right axis disappear (Fig. 2, row P_1 ablated). This is due to the missing left-right induction which depends on an intact MS blastomere, the granddaughter of the ablated P_1 blastomere. In addition anterior-posterior fate differences between the anterior and posterior sisters created in the third division of the AB lineage are often eliminated in manipulated embryos (Fig. 1C). The four posterior blastomeres ABxxp, i.e. ABalp, ABarp, ABplp, and ABprp, are often transformed to execute an anterior ABxxa fate. In such cases for instance ABarp-derived hypodermal cells either divide further or develop into a programmed cell death and show no signs of hypodermal differentiation (Fig. 2, row P_1 ablated). The pattern of cell deaths and further cell divisions adopted by the descendants of the ABarp blastomere is typical for the development of the descendants of the ABala blastomere indicating a fate transformation (Fig. 2, row P_1 ablated). In contrast, ABp-derived blastomeres that are affected, e.g. ABplp, start producing hypodermal cells instead of differentiating mainly into nerve cells. However, this also indicates a transformation into anterior fate, because the pattern of hypodermal cells produced is characteristic for the development of the ABpla blastomere (Fig. 2, row P_1 ablated). Transformed blastomeres executed either the ABala-fate or the ABpla-fate depending on whether they were derived from the ABa or the ABp blastomere. An interpretation of these observations is that ABxxp blastomeres are not specified autonomously, but depend on an induction from either P_1 or its descendants. In contrast to these results ABxxa fates are unaffected by the laser ablations.

The P_1 blastomere itself is the source of the induction

One could think of different reasons why the ABxxp-fates are not specified correctly in P_1-ablated embryos. Either P_1 or its descendants could be involved directly in an interaction specifying the ABxxp-fates or these fate changes could be secondary effects of the ablation due to the altered topography of the embryo.

To distinguish between these possibilities we laser-ablated all descendants of P_1 at later stages. This should leave the environment for the AB descendants very similar to the P_1 ablation itself (Fig. 3). After ablating the daughters of P_1, EMS and P_2, at the 4-cell stage the lineage analysis again revealed a breakdown of the left-right asymmetry due to the elimination of MS. However, the ABxxp-fates were specified correctly (Figs 1D and 2, row EMS/P_2 abl.). This indicates that P_1 itself is the source of the signal and that the induction occurs at the 2-cell stage. The ABxxp blastomeres that are affected by this induction are only present at the 12-cell stage and are therefore not specified directly by the induction.

To examine the alterations in the environment of the AB descendants after the ablations we analysed the topography, that is the cell-cell contacts in normal, P_1-ablated and EMS/P_2 ablated embryos. The neighbourhood relations among the AB descendants are normal before the 8-AB cell stage (normally 12-cell stage; data not shown). A detailed analysis at the 8-AB cell stage, the first stage where the AB descendants whose fates were affected are present, revealed no difference between P_1- and EMS/P_2-ablated embryos (Fig. 3). Most of the cell-cell

contacts found in P_1-ablated embryos are also found in normal embryos. Among the AB descendants only 10% (13 of 134) of the contacts are new in the ablated embryos. Furthermore, in the ablated embryos 24% (32 of 134) of the normal contacts are missing, especially between the left and right AB descendants (28 of 32), because the large undivided ablated blastomere often occupies a central position. However, there is no correlation between the presence of certain cell-cell contacts and the presence of the posterior ABxxp-fates. The incomplete specification of the ABxxp-fates in P_1-ablated embryos therefore seems to be a direct consequence of the removal of a signalling centre and not due to an altered topography of the embryo.

Under certain conditions the P_2 blastomere is required for the proper development of the ABarp blastomere

Earlier observations indicated a requirement of P_2 for some

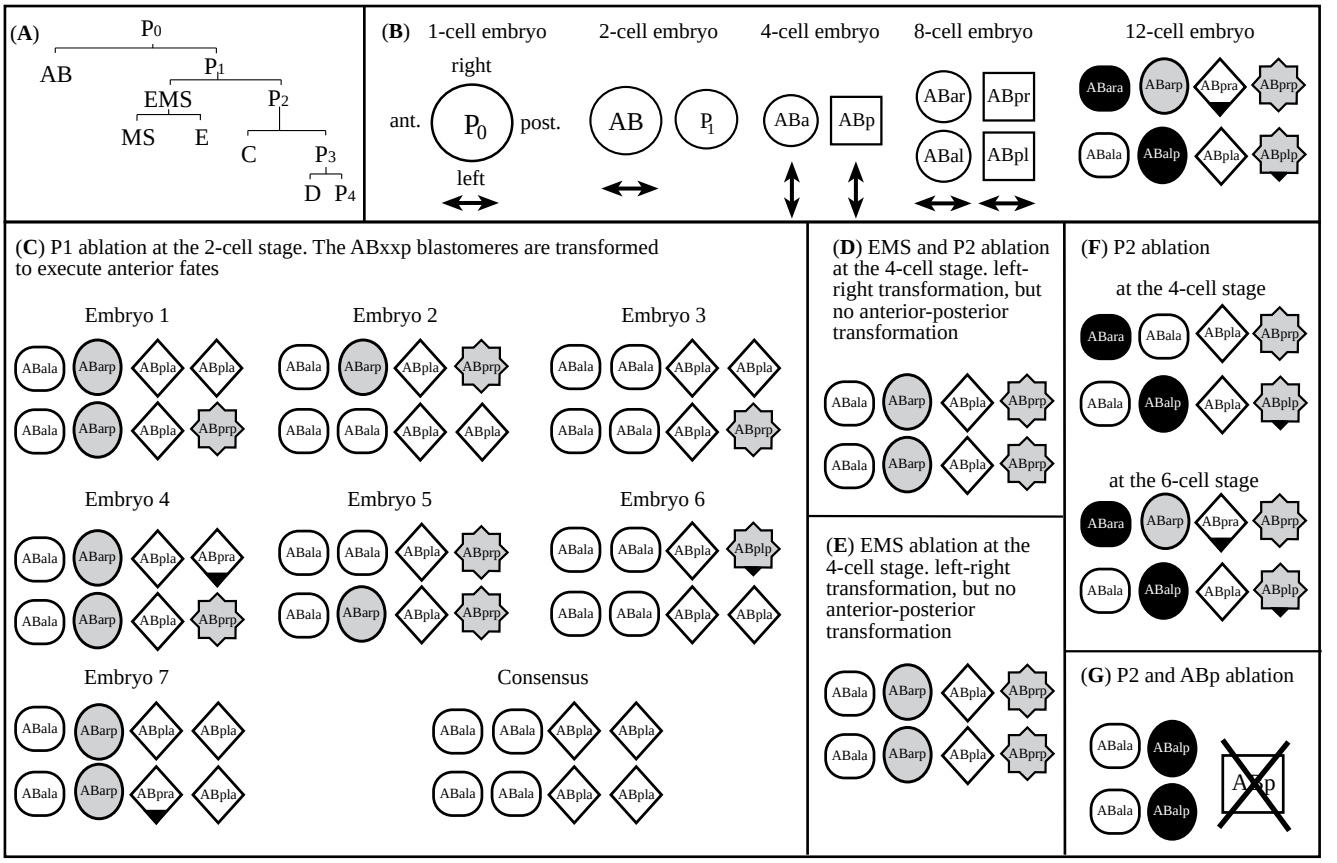

Fig. 1. Summary of the phenotypes of ablated embryos. A diagram of the early lineage producing the somatic founder cells is shown in A. The descendants of P_1 are not considered further in the figure. (B) Normal development. The anterior blastomere of the 2-cell stage, AB, divides three times up to the 12-cell stage of the embryo, creating 8 AB descendants. These 8 cells all have different fates (Sulston, 1983) which are indicated by different shapes and shadings. The fates shown in the diagram were assigned using extensive lineage analysis as shown in Fig. 2. The first and third divisions of AB or its descendants occur along the anterior-posterior axis whereas the second occurs along the left-right axis. (C) Ablation of P_1 removes the differences along the left-right axis, which depend on an intact MS blastomere, the grand-daughter of P_1 (Hutter and Schnabel, 1994). In addition, this ablation affects the difference between the anterior (ABxxa) and posterior (ABxxp) blastomeres after the division from 4 to 8 AB cells. It was not possible to block the induction completely in an individual embryo, probably because the induction has already started by the time of the ablation. In the consensus derived from the seven ablated embryos all posterior blastomeres are transformed with similar frequency and execute the fates of their anterior sisters. (D) When both daughters of P_1, EMS and P_2, are ablated at the 4-cell stage, the ABxxp-fates are specified correctly indicating that the induction occurs at the 2-cell stage. (E) If EMS is ablated alone only the left-right asymmetry is disturbed due to a lack of the left-right induction from the MS blastomere, the daughter of EMS (Hutter and Schnabel, 1994). (F) Ablation of P_2 at different stages. After the ablation of P_2 at the 4-cell stage most of the fates are normal. Concerning anterior-posterior differences only one blastomere, ABarp, is still affected. This appears to be due to the special direction of cleavage of this blastomere (see Fig. 4). A small part of the ABpra lineage is also affected. If P_2 is ablated at the 6-cell stage the AB lineage develops normally indicating that the induction has been completed. (G) When ABp and P2 are ablated at the 4-cell stage the anterior-posterior difference between the ABara and ABarp blastomeres, which disappears after ablation of P_2 alone, reappears. The ablation of ABp, like the ablation of EMS and P_1, alters the direction of cleavage of ABar which now divides in parallel to its counterpart ABal (Fig. 4). The change in the cleavage direction of ABar after the ablation of ABp causes a change of the normal cell-cell contacts at the 12-cell stage. ABara loses its contact with MS whereas ABarp now gains contact with MS (Fig. 4D). Consequently ABara is not induced by MS and executes the ABala-fate whereas ABarp now is induced by MS and executes the ABalp-fate. This further confirms that cell-cell contact with MS is necessary for the left-right induction to occur (Hutter and Schnabel, 1994). The fates shown in D-F are each a consensus of several embryos (see Fig. 2).

ABarp-derived fates (Schnabel, 1991). To see whether the observed aberrations correspond to fate transformations we laser-ablated the P_2 blastomere at different times during development and scored the embryos for fate transformations. After ablation of P_2 at the 4-cell stage the proper development of only one of the four ABxxp blastomeres, ABarp, was disturbed. It adopted the ABala-fate in all three analysed embryos (Figs 1F and 2, row P_2 early abl.). This extends an earlier observation that the ABarp blastomere develops aberrantly after ablation of P_2 (Schnabel, 1991). The left-right

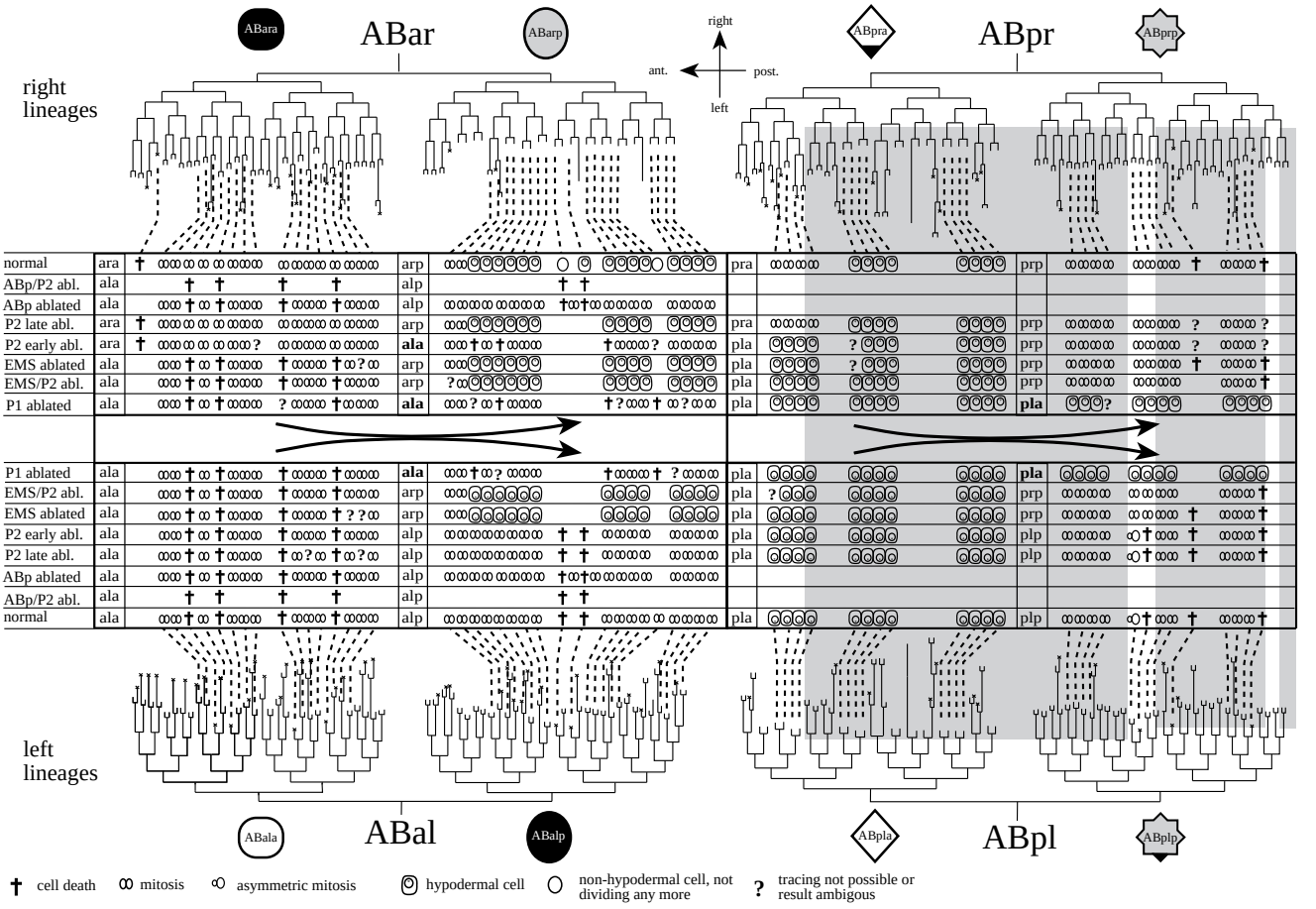

Fig. 2. Lineage analysis of ablated embryos. The figure shows the complete AB lineage (Sulston, 1983). The symmetric parts of the lineage are shaded. Dashed lines indicate the cells whose fate was traced. Fates were selected to allow a clear discrimination between the different blastomere identities corresponding to the 8-AB cell stage. The fate of each of the 8 AB-derived blastomeres is indicated in front of the rows showing the fates of cells. Anterior-posterior transformations are indicated by bold print. All P_1-ablated embryos show blastomere identity transformations resulting from the lack of two different inductions. The breakdown of the left-right asymmetry is caused by the absence of the MS blastomere normally inducing the left-right asymmetry of the embryo (Hutter and Schnabel, 1994). This blastomere is not born if either the P_1 blastomere or the EMS blastomere were ablated, since MS is derived from these lineages. Only the P_1-ablated embryos show in addition a breakdown of the differences between the fates of the posterior blastomeres ABxxp and the anterior blastomeres ABxxa born in the third cleavage round of the AB lineage. The posterior ABxxp blastomeres are transformed to execute the fates of ABxxa blastomeres. The arrows indicate the direction of the transformation. The identity of the anterior ABxxa blastomeres was never affected. In the different embryos at least one of the four ABxxp blastomeres was not transformed to an anterior fate (see Fig. 1). These blastomeres executed a posterior fate and developed as in the EMS-ablated embryos, where only the left-right asymmetry is absent (row EMS ablated). After ablation of EMS and P_2 at the 4-cell stage (row EMS/P_2 abl.) the ABxxp-fates are specified correctly. After ablation of P_2 at the 4-cell stage (row P_2 early abl.) only the ABarp blastomere shows an anterior-posterior fate transformation and executes an ABala fate. This no longer occurs when either P_2 is ablated at the 6-cell stage (row P_2 late abl.) or when ABp is ablated in combination with P_2 (row ABp/P_2 abl.). When ABp is ablated (rows ABp ablated and ABp/P_2 abl.) a change in the direction of cleavage of ABar leads to a change in cell-cell contacts to MS at the 12-cell stage (see Fig. 4). ABala and ABara execute the ABala-fate, whereas both ABalp and ABarp are induced by MS and therefore execute the ABalp-fate. In the seven embryos where P_1 was ablated a total of 784 lineages were analysed. 115 of these could not be scored and 40 developed aberrantly with respect to the fates expected according to the general identity deduced from the other fates in that lineage. Data for the other ablations are: EMS and P_2 ablated at the 4-cell stage: 336 fates analysed (3 embryos), 23 not scored, 16 aberrant; EMS ablated at the 4-cell stage: 456 fates analysed (4 embryos), 52 not scored, 14 aberrant; P_2 ablated at the 4-cell stage (P_2 early abl.): 351 fates analysed (3 embryos), 37 not scored, 5 aberrant; P_2 ablated at the 6-cell stage (P_2 late abl.): 351 fates analysed (3 embryos), 22 not scored, 6 aberrant; ABp ablated at the 4-cell stage: 408 fates analysed (6 embryos), 63 not scored, 18 aberrant; ABp and P_2 ablated at the 4-cell stage: 60 fates analysed (5 embryos), 16 not scored.

asymmetry was normal in these embryos apart from a minor part of the ABpra lineage. Ablation of P_2 at the 6-cell stage no longer affected the development of the AB lineage which appeared completely normal in all three analysed embryos (Figs 1F and 2, row P_2 late abl.). This suggests that in contrast to the result where P_2 was ablated together with EMS (see above), P_2 still has some function in the specification of the ABxxp-fates.

After ablation of EMS, the other daughter of P_1, only transformations along the left-right axis were observed (Fig. 2, row EMS ablated). The ABxxa/ABxxp difference was undisturbed (Figs 1E and 2), indicating that EMS is not involved in the specification of the ABxxp blastomeres.

Direction of cleavages and the effects of P_2 ablations

The ablation of P_2 affects the anterior right blastomere ABarp but not the other three ABxxp blastomeres. An asymmetric feature of the early embryo that is compatible with this result is the direction of cleavage of the mother of ABarp, ABar, which is almost perpendicular to that of the other three AB descendants (Fig. 4A,C,E). The importance of this difference in the direction of cleavage in specifying cell fate can be demonstrated by altering the cleavage direction of the blastomere ABar experimentally. Ablation of the blastomere ABp as well as ablation of EMS or P_1 results in a steric hindrance causing ABar to cleave in parallel with the other blastomeres (Fig. 4B,D,F) without affecting the specification of the ABxxa/ABxxp difference (Fig. 2, row ABp ablated). By ablating both ABp and P_2 at the 4-cell stage we show that ABarp now becomes independent of the activity of P_2 (Figs 1G and 2, row ABp/P_2 abl.). The direction of cleavage appears therefore to be important for the correct specification of cell fates.

DISCUSSION

Previously two inductions have been described (Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994) which induce complex lineage patterns in the *C. elegans* embryo (Hutter and Schnabel, 1994). Both inductions create differences in pairs of equivalent blastomeres of the AB lineage first along the anterior-posterior axis and then along the left-right axis. In this work we identified a third induction also specifying lineage programs in equivalent AB blastomeres leading to a further subdivision of fates along the anterior-posterior axis. Taken together the three inductions give an outline of how all the anterior, i.e. AB-derived, blastomeres are specified between the 2-cell and the 12-cell stage embryo.

	ala	**alp**	ara	**arp**	pla	**plp**	pra	**prp**	P_1
ala		1 2 3 4 5 6 7 a b c	1 4 5 6 7 a b c	1 4 6 7 b c	1 2 3 4 5 6 7 a b c	3			1 2 3 4 5 6 7 a b c
alp	10/10 13/13	2,3,6		1 2 3	1 2 4 5 6 7 a b c	1 2 3 4 5 6 7 a b c			1 2 3 4 5 6 7 a b c
ara	8/10 13/13	0/10 13/13		1 2 3 4 5 6 7 a b c	1 5 a	5	1 2 3 4 6 7 b c	3 4 6 7 b c	1 2 3 4 5 6 7 a b c
arp	6/10 0/13	3/10 0/13	10/10 13/13	3,5,6	4 5 6 7 a b c	2 3 5 6	1 4 5 6 7 a b c	1 2 5 a c	1 2 3 4 5 6 7 a b c
pla	10/10 13/13	9/10 8/13	3/10 13/13	7/10 13/13		1 2 3 4 5 6 7 a b c	1		1 2 3 4 5 6 7 a b c
plp	1/10 5/13	10/10 13/13	1/10 8/13	4/10 13/13	10/10 13/13	2,6,7	1 5 6		1 2 3 4 5 6 7 a b c
pra			8/10 13/13	8/10 13/13	1/10 0/13	3/10 0/13		1 2 3 4 5 6 7 a b c	1 2 3 4 5 6 7 a b c
prp			6/10 7/13	5/10 5/13			10/10 13/13	1,3, 4,7	1 2 3 4 5 6 7 a b c
P_1	10/10 0/13	10/10 13/13	10/10 13/13	10/10 13/13	10/10 0/13	10/10 13/13	10/10 13/13	10/10 13/13	

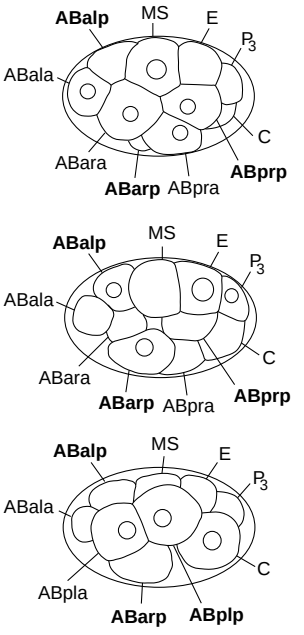

Fig. 3. Cell-cell contacts at the 12-cell stage in laser ablated and normal embryos. The upper right half of the matrix shows all the cell-cell contacts in 10 P_1- or EMS/P_2-ablated embryos at the 12-cell stage. Numbers 1 to 7 correspond to the seven P_1-ablated embryos (same numbers as in Fig. 1). Letters a, b and c correspond to the three EMS/P2-ablated embryos. All contacts between AB and P_1 descendants are summarised in the row P_1. The lower left half of the matrix compares the ablated embryos with normal embryos. Bold squares indicate the cell-cell contacts in 13 normal embryos. Most of the cell-cell contacts were found to be invariant and present in all embryos. Two cell-cell contacts were found to be variable, indicated by stippled inlets: a contact between ABara and ABplp existed only in 8 embryos and the contact between ABarp and ABprp was only found in 5 embryos. Two pairs of contacts were found to be mutually exclusive: either there was a contact between ABala and ABplp (5 of 13 embryos) or there was a contact between ABalp and ABpla (8 of 13 embryos) and either there was a contact between ABara and ABprp (7 of 13 embryos) or there was a contact between ABpra and MS (6 of 13 embryos; not included in the matrix, but shown in the drawing of the embryo beside the matrix). All combinations of these two pairs of contacts were found with similar frequency. Numbers within the boxes of the lower left half of the matrix indicate the fraction of embryos where the contact was present, summarised separately for ablated (upper line) and normal (lower line) embryos. The numbers of embryos where ABxxp transformations are found are listed along the diagonal to allow a comparison with the cell-cell contacts. To get an impression of the different strengths of the various contacts three focal planes of a normal 12-cell stage embryo are shown beside the matrix. The major conclusions of the comparison of cell-cell contacts are: first, as expected there is no significant difference between P_1-ablated and EMS/P_2-ablated embryos. Second, most cell-cell contacts found in P_1-ablated embryos (121/134) are also found in normal embryos. Because the large undivided P_1 blastomere pushes the AB descendants somewhat apart some of the normal contacts, especially between the left and right sides are missing in individual ablated embryos. Note that for example the contact between ABala and ABarp, which is found in P_1-ablated embryos and not in normal embryos, can be explained by a small change of the position of blastomeres. The major point we deduce from the analysis of the cell-cell contacts, however, is that no correlation between certain cell-cell contacts and the presence or absence of the posterior ABxxp-fates is found.

The development of four blastomeres of the 12-cell stage depends on an induction from the P_1 blastomere at the 2 cell stage

The induction we describe here is required for the correct development of four blastomeres of the 12-cell stage embryo. The four blastomeres affected are the posterior cells ABxxp created during the third cleavage of the AB blastomere (Fig. 1B). Normally the fate of the ABxxp blastomeres is different from that of their anterior sisters ABxxa. Ablation of P_1, the posterior blastomere of the 2-cell stage, leads to a failure of the development of the ABxxp blastomeres. They now develop like their anterior sisters ABxxa, leading to a duplication of lineage programs (Fig. 2, row P_1 ablated). This demonstrates the equivalence of the developmental potential of these four pairs of blastomeres. The transformations lead to a loss of hypodermal cells from the ABa lineage and to the production of additional hypodermal cells from the ABp lineage. Therefore the fate changes are not obvious in immunostainings using hypodermal markers (Schnabel, 1991), but can only be detected by a detailed lineage analysis at the single cell level which is able to identify changes in lineage pattern. The ablation of P_1 interferes with directional information along the anterior-posterior axis. However, since the reproducible production of complex lineage patterns is still observed in P_1-ablated embryos there must be additional directional cues unaffected by the ablation of the P_1 blastomere.

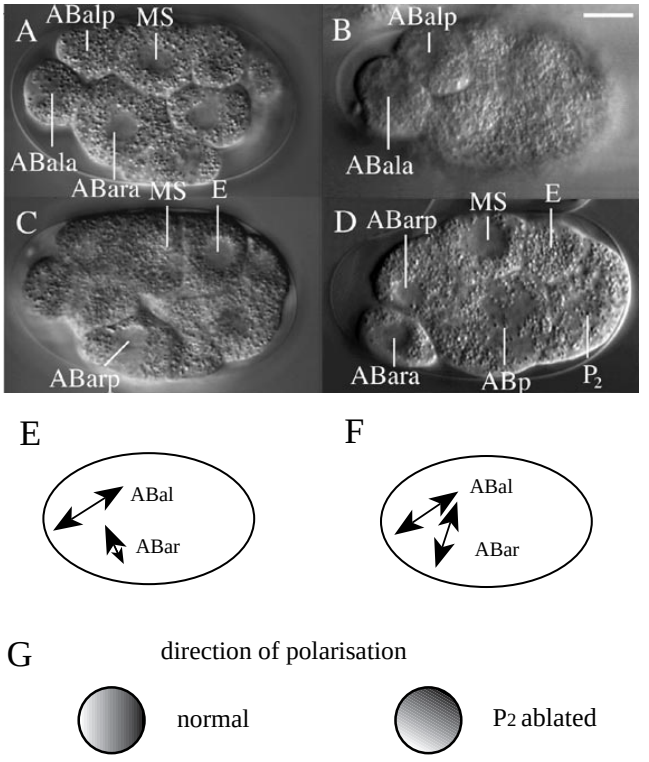

Fig. 4. Direction of cleavages after different ablations. The figure illustrates the difference in the directions of cleavage of ABal and ABar in embryos where different blastomeres were ablated. (A,C,E) In normal and in P_2-ablated embryos ABar divides mainly along the dorsoventral axis with a small anterior-posterior component. ABal and also ABpl and ABpr (not shown) in contrast divide essentially along the anterior-posterior axis. If one assumes that these blastomeres become polarised along the anterior-posterior axis by the interaction with P_1 (G, see also Fig. 5) this difference in direction of cleavage could mean that the polarisation is more completely transferred into a difference between the anterior and posterior daughter of ABal compared to ABar. Therefore ABar would be more susceptible to a disturbance of its polarisation than ABal. This is the effect observed after ablation of P_2 (Figs 1 and 2). (B,D,F) When ABp is ablated the large undivided ABp blastomere alters the direction of cleavage of ABar which now divides like its counterpart ABal. Therefore ABar is now less susceptible to an incomplete polarisation. And indeed after ablation of P_2 in ABp-ablated embryos ABarp is no longer transformed into ABala (Figs 1 and 2). Scale bar 10 μm.

The four ABxxp blastomeres were each affected with a frequency of 50% in the embryos analysed (Fig. 1C). Our interpretation of this variability is that the time of the ablation is very close to or even overlaps with the time of induction. Such a variability has also been observed after ablations of MS around the time of the left-right induction (Hutter and Schnabel, 1994) and during laser ablation studies of vulval induction (Sternberg and Horvitz, 1986). The ablation of precursors of the inducing cell instead of the inducing cell itself could avoid the problem of incomplete inactivation (Hutter and Schnabel, 1994) but in this instance the precursor of the inducing blastomere, P_0, is also the precursor of the induced blastomere.

Neither the ablation of P_1 nor P_2 affects the general identity of ABp (see Figs 1 and 2). This was surprising because there is evidence that an induction from P_2 is required for the specification of the ABp-fate (Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994). Under certain circumstances an ablated blastomere still seems to be able to signal. One reason might be that the ligand for the induction of the ABp-fate is already located in the cell membrane where it cannot be destroyed by the laser beam which is focused to the cytoplasm. The observation that the ABp-fate is present in P_2-ablated embryos conflicts with the view of others who have argued that ablation of P_2 leads to a general failure of the specification of the ABp-fate (Mello et al., 1994; Moskowitz et al., 1994; Priess, 1994; Tax and Thomas, 1994). They were lead to this conclusion by the observation that two ABp-derived valve cells fail to develop after the ablation of P_2 (Bowerman et al., 1992). The requirement of the P_2-lineage for proper valve cell development appears to be different from the induction of ABp-fate and more similar to the failure of development of several neuronal lineages derived form ABpraaap (see Fig. 2, row P_2 early abl.). As will be discussed below there appear to be different types of induction affecting the ABp lineage, one type that changes complex lineage patterns and serves to specify the identity of blastomeres and the other type that modifies these identities further and affects only very specific terminal lineages (H.H. and R.S. unpublished data).

Ablation of the two daughters of P_1, EMS and P_2, at the 4-cell stage no longer affects the development of the ABxxp blastomeres indicating that the induction occurs at the 2-cell stage and that P_1 is the source of the signal. The specification of the ABxxp-fates appears to be cell autonomous after this stage (but see below for the effects of the ablation of P_2).

Analysis of the cell-cell contacts in ablated and normal embryos (Fig. 3) revealed that most of the normal contacts are preserved in the ablated embryos. No correlation between the presence or absence of ABxxp-fates and the presence or absence of certain cell-cell contacts was found. The breakdown of ABxxp-fates therefore must be a direct consequence of the

ablation of P_1 rather than an indirect effect due to an altered topology in the embryo.

A model of the polarising induction

The induction described here appears to employ a completely different mechanism to induce complex lineage patterns than two previously described inductions in the early *C. elegans* embryo (Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994). The observation that the ABxxa/ABxxp difference is still present in embryos from mothers mutant for *glp-1*, where the two other inductions affecting blastomere identities in the AB lineage are disturbed (Hutter and Schnabel, 1994), also supports this view. Since the blastomeres affected are not yet born when the induction occurs, the embryo is faced with a unique signalling problem. We suggest that the solution to this problem lies in an inductive signal that serves to polarise the great-grandmother of the affected blastomeres rather than inducing fate changes directly in the blastomeres. This polarity is propagated and subdivided through several divisions and is only later transferred into the specification of cell fates.

Fig. 5 outlines a model of how an initial polarisation of the AB blastomere could be established and how this polarisation could then specify anterior-posterior differences of blastomeres at the 8-AB cell stage. In this model the posterior blastomere P_1 polarises its anterior sister AB in the anterior-posterior direction at the 2-cell stage (Fig. 5A). The AB blastomere then starts to divide in a dorsoventral direction perpendicular to the direction of polarisation (Fig. 5B), so that the polarisation is inherited by both daughters of AB. When P_1 also divides a few minutes later (Fig. 5C) the division axis of AB is skewed and the direction of polarisation is changed from anterior-posterior to anterior-dorsal/posterior-ventral. The ABa and ABp blastomeres next divide along the left-right axis, i.e. perpendicular to the direction of the polarisation which is again inherited by all daughter cells (Fig. 5E). The polarisation leads to differences between cells only in the next division of the AB descendants which occurs mainly in the direction of the polarisation and creates the 8 AB descendants of the 12-cell stage (Fig. 5F). This model is intended to demonstrate that an anterior-posterior polarisation of the AB blastomere indeed could be transmitted through the following two divisions, because the division axes are essentially perpendicular to the direction of the polarisation. The polarisation is represented in our model as a graded cytoplasmic content, but since the molecular basis for the polarisation is not known, other models e.g. linking the polarising principle to the cell membranes only are equally possible.

This situation is reminiscent of induction of gut fate in the EMS blastomeres by the neighbouring P_2 blastomere (Schierenberg, 1987; Goldstein, 1992, 1993). In this case P_2 polarises the EMS blastomere so that the posterior daughter of the next division, E, becomes different from her anterior sister MS and develops into intestine. When the induction is missing the E blastomere develops more like its anterior sister MS (Goldstein, 1993). In contrast to the induction described here, however, the polarisation of EMS is immediately transferred into fate changes in the following division.

The effects of the P_2 ablations on the development of the ABarp blastomere depend on the direction of cleavage of ABar

The ablation of P_2 at the 4-cell stage leads to a transformation of ABarp into ABala. This effect was no longer observed when P_2 was ablated at the 6-cell stage, indicating that it is due to the ablation itself and not due to a secondary effect.

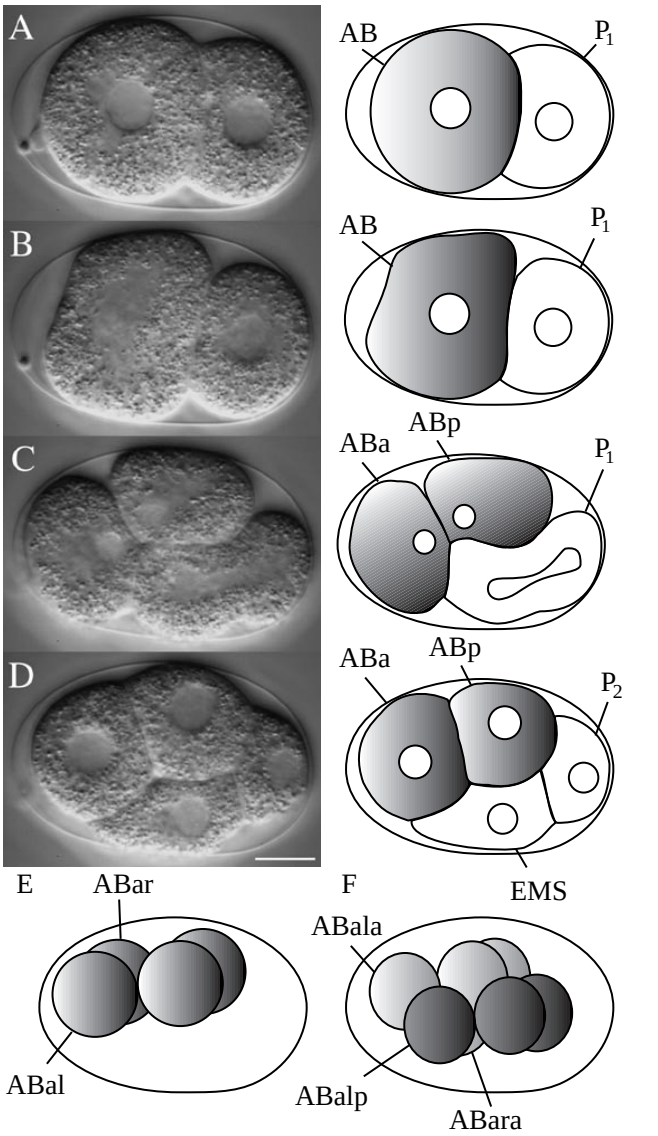

Fig. 5. A model showing that a polarisation of the AB blastomere by P_1 could be propagated to the 12-cell stage embryo where it is used for the specification of the four posterior ABxxp-fates. (A) At the 2-cell stage P_1 polarises AB along the anterior-posterior axis. (B) The AB blastomere starts dividing along the dorsoventral axis. The anterior-posterior polarisation therefore is transmitted to both daughter cells. (C) The division axis of AB tilts during the division of P_1 in the anterior-posterior direction. According to the model the AB daughters ABa and ABp are then polarised in an anterior-dorsal/posterior-ventral direction. (D) P_2 might be required to shift the polarisation into a more anterior-posterior direction. This is only essential for ABar because this blastomere divides almost perpendicular to the others (Fig. 4). (E) The following division of the AB descendants occurs along the left-right axis. The anterior-posterior polarisation can therefore be transferred to all daughter cells. (F) The next division, which occurs along the anterior-posterior axis, i.e. in the direction of the polarisation, creates a difference between anterior and posterior daughter cells which is then transferred into the specification of ABxxp-fates in the posterior daughters. Scale bar 10 μm.

The other ABxxp blastomeres were never affected. The observation that the blastomere ABar cleaves almost perpendicular to the other AB descendants (Fig. 4A, C and E) offered a possible explanation for the asymmetry of the observed effect. By experimentally altering the direction of cleavage of ABar to occur in parallel to its left sister ABal (Fig. 4B, D and F) we were able to make the development of the ABarp blastomere independent of P_2 (Figs 1 and 2). The importance of the direction of cleavage of the AB descendants for the proper specification of the ABxxp-fates fits well in our model which assumes that a prior polarisation must be transmitted through the divisions. In our specific model (Fig. 5) P_2 would be required to shift the polarisation of the AB descendants from the anterior-dorsal/posterior-ventral direction, after the division of AB, back to a more anterior-posterior direction (Fig. 5D) in order to allow the correct specification of the ABar blastomere, which cleaves in a direction different from the other AB descendants (Fig. 4), and therefore might be more sensitive to either a diminution or rotation of the polarisation.

Three successive inductions specify the AB lineage from the 2-cell stage to the 12-cell stage

The polarising induction described here explains the establishment of differential developmental fates between the four posterior and four anterior blastomeres created during the third cleavage of the AB blastomere. It has several aspects in common with two other inductions affecting descendants of the AB blastomere described previously (Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994). The induction induces complex lineage programs and thus blastomere identities rather than tissues. All three inductions serve to make pairs of equivalent blastomeres different. Taken together the three inductions form a hierarchical and interdependent decision process subdividing the AB-derived part of the embryo along the anterior-posterior and left-right axis. The combinatorial action of these three inductions explains how the AB lineage becomes successively divided into blastomeres with unique developmental potential. The first induction is the polarising induction described here which anticipates the establishment of the differences between the ABxxa and ABxxp blastomeres created three cleavage rounds later (Fig. 6A). The next induction specifies the difference between the anterior ABa blastomere and its posterior and more dorsally located sister ABp (Fig. 6B). This induction was called anterior-posterior induction (Hutter and Schnabel, 1994) since it specifies anterior-posterior differences in reference to the body plan of the hatching larva (Sulston et al., 1983). Note, however, that Mello et al. (1994) refer to this induction as specifying dorsal-ventral polarity in the embryo. This induction probably occurs during the 4- to 6-cell stage and the posterior blastomere P_2 appears to be the source of the signal (Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994). Finally, the left-right induction originating from the MS blastomere creates the differences between the left and right blastomeres of the AB lineage (Hutter and Schnabel, 1994; Fig 6C). This induction occurs during the 12-cell stage, i.e. one cleavage after the left and right AB descendants are born. It induces only 2 different ABa-derived fates, the ABara- and ABalp-fate. If the left-right induction does not occur ABara and ABalp execute the fates of their bilateral homologs ABala and ABarp respectively (Hutter and Schnabel, 1994). One aspect of these identity transformations, the expression of a hypodermal marker by ABalp descendants, was also found by Gendreau et al. (1994). The ABp descendants are not competent to respond to the left-right signal from MS after the anterior-posterior induction (Hutter and Schnabel, 1994). In the end, therefore, only 6 instead of 8 theoretically possible different fates are specified by the three inductions. This reflects the symmetry situation within the AB lineage. The 4 ABa-derived AB descendants execute completely asymmetric lineage patterns, whereas the 4 ABp-derived AB descendants execute only 2 different anterior or

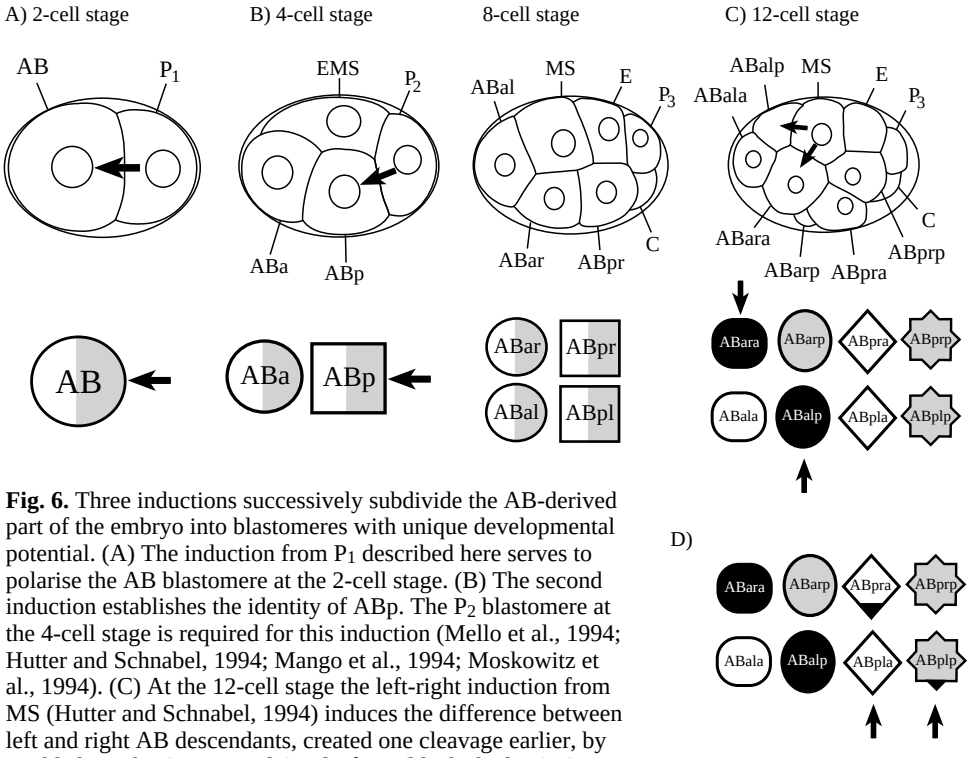

Fig. 6. Three inductions successively subdivide the AB-derived part of the embryo into blastomeres with unique developmental potential. (A) The induction from P_1 described here serves to polarise the AB blastomere at the 2-cell stage. (B) The second induction establishes the identity of ABp. The P_2 blastomere at the 4-cell stage is required for this induction (Mello et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994). (C) At the 12-cell stage the left-right induction from MS (Hutter and Schnabel, 1994) induces the difference between left and right AB descendants, created one cleavage earlier, by establishing the ABara- and ABalp-fates (black shading). At this stage also the original polarisation of the AB blastomere is transferred into anterior-posterior fate differences (grey shading). Together these three inductions form a hierarchy of binary decisions whose combined action specifies the 6 different blastomere identities of the 8 AB descendants at the 12-cell stage. (D) Further inductions modify these identities and create the minor asymmetries characteristic of ABp-derived blastomeres.

posterior fates almost symmetrically on the left and right sides (Sulston et al., 1983).

We propose that the minor left-right asymmetries in the ABp-derived blastomeres are specified by further inductions. There is evidence that two separable inductions modify the asymmetric parts of ABpra and ABplp (Hutter and Schnabel, 1994; H. H. and R. S. unpublished data). One induction from the MS-lineage after the 12-cell stage specifies the left-right differences between ABplp and ABprp and another induction among the AB-descendants establishes the difference between ABpla and ABpra (H. H. and R. S. unpublished data). The fate changes observed in the ABpra lineage after the ablation of EMS or P_2 (Figs 1 and 2) are therefore secondary effects due to the fate changes in other parts of the AB lineage.

This view of the early determination of the AB lineage is different from a model proposed by Moskowitz et al. (1994). Their model postulates three independent combinatorial inputs to specify 7 different fates of the 8 AB descendants of the 12-cell stage. Two of the inputs (inductions) are identical to the previously described anterior-posterior (Mello et al., 1994; Mango et al., 1994; Hutter and Schnabel, 1994) and left-right inductions (Hutter and Schnabel, 1994). The third input is the potential to form a certain tissue, the hypodermis (in their work renamed to epidermis), which is initially intrinsic to both ABa and ABp. They propose that this potential is segregated to ABalp, ABarp, ABpla and ABpra during the division to the 12-cell stage (Gendreau et al., 1994). Their model is inconsistent with the following observations. (i) Ablation of MS affects the development of the ABplp blastomere, but not the ABprp blastomere (Hutter and Schnabel, 1994). To retain a simple combinatorial code their model has to assume that ABplp and ABprp are not competent to receive input from MS and therefore should not be affected by ablation of MS. (ii) A cell-cell contact between MS and ABpra exists only in some of the normal embryos (Hutter and Schnabel, 1994; this work, legend to Fig. 3). Their model, however, depends on the invariant existence of such a contact. (iii) As described in this work after ablation of P_1 additional ABp-derived blastomeres execute hypodermal lineages, whereas ABa-derived blastomeres fail to execute hypodermal lineages. The model of Moskowitz et al. postulates a segregation of a hypodermal potential in the anterior-posterior direction in ABa and in the posterior-anterior direction in ABp. Even by assuming that the ablation of P_1 somehow affects this segregation, the concept of segregation of a hypodermal potential cannot explain our observation that a failure of correct specification of the ABa-derived blastomeres ABalp and ABarp is always correlated with a lack of hypodermal fates whereas the failure of the analogous ABp derived blastomeres, ABplp and ABprp, is correlated with the expression of additional hypodermal fates. Our model explains the changes in hypodermal fates as a reflection of changes in the general identity of the whole lineage. It therefore implies that there are different developmental pathways for the specification of a single tissue like the hypodermis. Blastomere identities and not tissues are induced during the three early inductions affecting the AB lineage of the *C. elegans* embryo.

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