INTRODUCTION

In all multicellular organisms, different cell types are generated from a few multipotential precursor cells. A long-standing problem in development is the mechanism by which a single cell gives rise to a variety of different cell types. The use of a small number of regulatory processes in various combinations appears to be a common mechanism by which cell type diversity is established. For example, a limited set of genes is used combinatorially to generate unique segmental identities in the Drosophila embryo (e.g., St. Johnston and Nüsslein-Volhard, 1992). Similarly, in the nematode Caenorhabditis elegans, specification of a number of cell types results from the action of multiple genes that function in various combinations (e.g., Wang et al., 1993; Clark et al., 1993; Mitani et al., 1993; Horvitz and Sternberg, 1991; Sternberg and Horvitz, 1989).

The generation of diverse cell types during embryonic development in C. elegans appears to be controlled both by cell-autonomous regulatory processes and cell-cell interactions. Early in embryonic development six ‘founder’ cells are born, each which gives rise to a unique set of cell types (Sulston et al., 1983). Several lines of evidence suggest that differences between the founder cells arise by the unequal distribution of developmental determinants (Laufer et al., 1980; Cowan and McIntosh, 1985; Edgar and McGhee, 1986). Although molecular identification of such determinants has not been reported, at least one candidate for such a regulatory molecule has been described (Bowerman et al., 1992a, 1993). While much of embryonic development in this animal appears to be regulated by cell-autonomous mechanisms (e.g., Sulston et al., 1983), cellular interactions in the early embryo are known to control the fates of many cells (Priess and Thomson, 1987; Wood, 1991; Schnabel, 1991; Bowerman et al., 1992b; Goldstein, 1992, 1993).

Cellular interactions are particularly important in regulating the fates of cells made by the AB founder cell, the anterior daughter of the zygote. Priess and Thomson (1987) showed that the daughters of AB, ABA and ABp, are initially equivalent in potential to their right-side homologues (Wood, 1991). Left/right differences in the fates of their descendants are established by an inductive interaction between the MS founder cell and specific AB great-
and differentiation of the remaining blastomeres in isolation.

2 or EMS at very early times, while reproducibly supporting growth

Partially embryos are sensitive to the osmotic strength of the culture

2. The unique identity of an AB cell is dictated by the

particular combination of these regulatory inputs that it

receives. Each combination initiates a developmental program

that appears to be largely intrinsic to each AB cell.

MATERIALS AND METHODS

Strains and culture

Nematode strains were maintained according to Brenner (1974). The wild-type strain used was C. elegans var Bristol or N2. The following mutants were used in this study: LGIII glp-1(q224ts, q415ts, e2142ts, e2072), unc-32(e189), dpy-19(e1259ts) and unc-69(e587). Experiments were conducted at 20°C, except where noted.

Laser ablations

Eggs were cut from gravid adults in M9 buffer (Sulston and Hodgkin, 1988). Embryos homozygous for temperature-sensitive glp-1 mutations were shifted to 25°C at least 30 minutes prior to dissection. 2-cell embryos were collected, placed on a 4% agar pad, covered with a coverslip, and sealed with Vaseline according to Sulston et al. (1983). Ablations of embryonic blastomeres were performed according to Sulston and White (1980) and Avery and Horvitz (1989), on a VSL-337 nitrogen laser. A DLM-110 dye laser module and coumarin 440 dye were supplied by Laser Science, Inc. Following ablations, embryos were incubated overnight at 20°C, transferred to poly-L-lysine-coated slides and processed for immunofluorescence.

Immunofluorescence

Fixation of embryos with methanol and acetone, and antibody staining procedures, were carried out as described by Albertson (1984) and Sulston and Hodgkin (1988). Slides were treated with a solution of 0.1% poly-L-lysine obtained from Sigma Chemical Co. Embryos were incubated with monoclonal antibody NE31B4 (Schnabel, 1991), which was used as a marker of epidermis (Gendreau et al., 1994), and IC84 (Okamoto and Thomson, 1985) as a marker of IL2 neurons and intestinal valve cells. (The reactivity of IC84 to intestinal valve cells is similar to that seen with 2CB7, described by Bowerman et al., 1992b.) The neuron-specific anti-unc-33 antibody was kindly provided by J. Shaw. In intact embryos, anti-unc-33 recognizes an antigen present in neuron cell bodies and processes (J. Shaw, personal communication). Fluorescein (DTAF)- and rhodamine (TRITC)-conjugated secondary antibodies from Jackson ImmunoResearch Laboratories, Inc. were used at a 1:50 dilution.

Embryos were mounted in glycerol mounting medium supplemented with 1.4 diazobicyclo-[2,2]-octane (DABCO) as described (Sulston and Hodgkin, 1988). 0.2 μg/ml 4’6-diamidino-2-phenolindole (DAPI, Sigma Chemical Co.) was added to the mounting medium to allow visualization of nuclei.

Blastomere removal

Gravid adult hermaphrodites were dissected in M9 buffer to harvest 2-cell embryos, or embryonic culture medium (Cowen and McIntosh, 1985) to harvest 1-cell embryos. The embryos were gathered with an eyelash and transferred to etched ring slides (Clay Adams, cat. no. 3032) containing 60 μl of embryonic culture medium. Blastomere extrusions were performed on a Nikon Diaphot inverted microscope with a Hoffmann modulation contrast optics system at 400x magnification. Two MN2 3-D Narashige manipulators were used, each equipped with a Narashige MO-202 joystick hydraulic manipulator. One manipulator controlled a needle, which was used to puncture a hole in the eggshell. The second manipulator controlled a holder, which held the embryo stationary by pulling or sucking on the eggshell. Fisher coagulation tubing was pulled on a Narashige PB-7 puller to a very fine and closed end and used as a needle. A needle that was broken and polished to an inner diameter of 15 μm with a Sutter beveler was used as the embryo holder. The pulling force exerted by the holder was controlled with a Hamilton threaded plunger syringe (cat. no. 81242), which was attached to the holder with Tygon tubing. The syringe and tubing were filled with silicon oil (Fisher, s159-500) and the beveler with DABCO (Sigma Chemical Co.). P2 or EMS was extruded very early in their cell cycles by first puncturing the eggshell and allowing the blastomere to bleb out partially. The embryo was released from the holder following the puncture and flipped around. The holder was then used to suck on the punctured end of the embryo. As soon as the blastomere was completely extruded, the resultant partial embryo was released into the medium. For the P2-removal, EMS-enucleration experiments, the posterior of the eggshell was punctured immediately after the formation of the EMS-P2 cleavage furrow. At this early stage, EMS and P2 are not completely separated (see above); therefore, extruding all of P2 usually resulted in the concomitant removal of the EMS nucleus.

Gentle pressure applied to embryos when cells were in the process of cleaving, or within 4 minutes after a boundary could be seen between cleaving cells, usually caused the cleavage margin to retract. This made it difficult to remove the eggshell without puncturing a microneedle. A similar observation has been reported by Goldstein (1993). Apparently the dividing cells are not completely separated at this time, although they appear so by light microscopy. We removed blastomeres between 5 and 7 minutes following the appearance of a boundary between the newly divided cells. This corresponds to 1.3 minutes after the removed cell was physically distinct from its sister, on the basis of a stable boundary between the cells. The operated embryo was either transferred to a poly-L-lysine-coated coverslip for lineage analysis, or allowed to develop in 80 μl of culture media on a 60 mm plate immersed with PBS-buffered Fisher light mineral oil. We report data only from experiments with no observable fragment of EMS or P2 mutations.

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Partial embryos are sensitive to the osmotic strength of the culture medium. Reproductive capacity was achieved only at a narrow range of salt concentrations. Proper osmolality was achieved as described by Priess and Hirsh (1986). Under other culture conditions, following rough treatment, or at elevated temperature, the partial embryos arrested without completing differentiation and often with too few cells. Cell deaths could be clearly seen in such embryos, but the embryos appeared incompletely differenti-ated for larvae. An additional factor was that occasional differentia-tion markers examined and often showed variable arrest in cell division. Our culture and handling conditions allowed removal of all traces of P2 or EMS at very early times, while reproducibly supporting growth and differentiation of the remaining blastomeres in isolation.
**Lineage analysis**

Lineage analysis (Sulston et al., 1983) was performed by 4-D time-lapse analysis (Schnabel, 1991; Hird and White, 1993) on a Nikon Microphot-SA microscope equipped with Nomarski differential interference contrast optics at 600x. A Hamamatsu Newvicon video camera was used to record images. The 4-D time-lapse system consists of a computer-controlled Bio-Rad focusing drive motor and a Sony LVR-5000/LVS-5000 optical disk recorder. Embryos with temperature-sensitive *glp-1* mutations were recorded at 25°C.

Intact embryos were mounted on a 4% agar pad and recorded for at least 7 hours of development. Blastomere-extruded partial embryos were pressure-sensitive and could not be sandwiched between an agar pad and a coverslip without rupturing them. Such operated embryos were mounted on a poly-L-lysine-coated coverslip, inverted and placed in the ring of an etched ring slide (Clay Adams, cat. no. 3032) with approximately 40 µl culture medium. The coverslip was sealed with Vaseline.

Cell divisions were reported as terminal only if the nuclei of the resulting cells could be followed through at least 3 hours of subsequent development without dividing. If a nucleus was lost, the lineage of that cell and its descendants were terminated (dotted vertical line on lineage diagrams). The number and pattern of cellular divisions, and morphology of epidermal cells were used for lineage comparisons. Cells with granular cytoplasm, large nuclei with smooth nucleoplasm and a large nucleolus were scored as epidermal. Such cells were usually located on the surface of the embryo. Cell deaths were readily scored as described by Sulston and Horvitz (1977).

**RESULTS**

**Background**

The AB blastomere constitutes the majority of the mass of the 2-cell *C. elegans* embryo. Of the six embryonic founder cells, it gives rise to the greatest number and variety of cells (Sulston et al., 1983). Its descendants are distributed primarily to three major organ types: the epidermis, nervous system, and the neuromuscular feeding organ, or pharynx. Different AB descendants contribute to different organs. The earliest stage at which AB descendants contribute to primarily one organ type is the AB^8^ cell stage (i.e., when there are eight AB great-granddaughters). This can be seen in a fate map of the AB^8^ cells (Fig. 1A). All AB^8^ cells produce at least some neurons. However, only two of the AB^8^ cells (arbitrarily numbered 2 and 3 in Fig. 1A) produce pharynx cells. We refer to these blastomeres as pharynx precursors. Three other AB^8^ cells (cells numbered 4, 5 and 6 in Fig. 1A) generate nearly all of the epidermis (also called hypodermis); we will call these blastomeres epidermal precursors (see also Fig. 1 legend). Finally, the remaining three AB^8^ cells produce neither pharynx nor substantial amounts of epidermis. For simplicity, we call such AB^8^ cells neuronal precursors since they generate mostly or exclusively nervous system. In addition to differences in the tissue types produced by the AB^8^ cells, a unique cell lineage pattern is associated with each of them (indicated by numbers in Fig. 1A). However, the lineages of the two posterior granddaughters of ABp are nearly identical (and both are therefore numbered 7/8 in Fig. 1A). The goal of this study was to determine how the wild-type pattern of AB^8^ cell fates arises during embryonic development.

**Mutations in *glp-1* alter the distribution of ABp-derived epidermal precursors**

In a separate study (Gendreau et al., 1994), we found that the distribution of ABp-derived epidermal precursors varies with a defined polarity in wild-type embryos, as indicated by the horizontal arrows: the central four cells are all epidermal precursors (shading) and the outer four cells are neuronal precursors (unshaded), resulting in a mirror-symmetric pattern. This figure illustrates the AB^8^ fate map in the absence of MS induction. Note that in intact embryos, MS induces two of the AB^8^ cells to become pharynx precursors (black cells in part A).
ability of AB descendants to produce abundant epidermis becomes restricted to one daughter of each AB granddaughter (AB^8 cell), as summarized in Fig. 1B. The reproducibility of this event is revealed when induction of AB^8 cells by MS is prevented (Gendreau et al., 1994; H. Hutter and R. Schnabel, personal communication). The sisters of each of these epidermal precursors produce primarily neurons instead. We will refer to AB^8 cells that can make large amounts of epidermis as having ‘epidermal potential.’ The process that restricts epidermal potential is autonomous to AB (Gendreau et al., 1994); the mechanism of this process has not been elucidated.

The distribution of epidermal precursors along the anterior-posterior axis is reversed in the ABp lineage relative to that in the ABa lineage, resulting in a mirror-symmetric pattern (Fig. 1B, horizontal arrows). Since differences between the ABa and ABp lineages arise from cellular interactions (Press and Thomson, 1987), we speculated that this pattern may be controlled by a cellular interaction. Certain properties of the glp-1 gene suggested that it might participate in such an interaction. Maternal glp-1 product is required for cellular interactions in the early embryo (Press et al., 1987; Austin and Kimble, 1987). Strong alleles of glp-1 lead to severe defects in epidermal morphogenesis, possibly reflecting a role for glp-1 in the establishment of the epidermal precursors. Moreover, the glp-1 product is present specifically in AB cells during the time that epidermal potential is restricted (Evans et al., 1994). These considerations led us to ask whether mutations in the glp-1 gene alter the distribution of epidermal precursors at the AB^8 cell stage.

Only the four central AB^8 cells (cells 3-6 in Fig. 1A) have epidermal potential in wild-type embryos (Fig. 1B). To address whether this distribution was altered, we killed the four normal epidermal precursors with a laser and analyzed expression of an epidermal marker in the operated embryos in glp-1 mutant embryos (Fig. 2). (Throughout this manuscript, ‘glp-1 embryos’ refers to embryos produced by homozygous glp-1 mothers.) To simplify interpretation of these experiments, all non-AB cells were also eliminated (Fig. 2 legend). Appearance of the epidermal marker in the operated embryos would indicate that epidermis was made ectopically from AB^8 cells that normally cannot produce epidermis. An antibody specific for seam epidermis (Schnabel, 1991) was used as a marker of epidermal fate; this marker recognizes only epidermis arising from the epidermal precursors (see Fig. 1 legend). A neuronal marker was used as a control for differentiation and permeabilization of embryos (see Materials and Methods; Gendreau et al., 1994).

As predicted from the wild-type fate map (Fig. 1B), the epidermal marker was never detected in wild-type embryos in which the four epidermal precursors were ablated (Fig. 2; Table 1). Operated embryos mutant for the weak glp-1(e2072) allele, which does not dramatically perturb morphogenesis (Press et al., 1987), also did not produce the marker (Table 1). In contrast, operated embryos mutant for any of three strong loss-of-function glp-1 alleles (q224, q415 and e2142) nearly always produced the epidermal marker (Fig. 2C; Table 1). All three of these alleles result in severe morphogenetic defects (Table 1; Press et al., 1987; Austin and Kimble, 1987; Kodoyianni et al., 1992). Thus, ectopic production of epidermis at the AB^8 cell stage correlated with aberrant morphogenesis in glp-1 mutants. This suggests that the altered distribution of epidermal precursors might be the cause of this morphogenetic defect.

We next asked which AB^8 cells ectopically produced the epidermal marker in glp-1 embryos. Individual AB^8 cells were isolated by ablating P2, EMS, and the four central AB^8 cells (ABalp, ABarp, AB pla and AB pra; see Fig. 1A for nomenclature) in embryos of the indicated genotype. The ablated embryos were allowed to develop 20 hours postfertilization at 20°C. All embryos were stained with antibody NE21B4 as a marker of epidermis (detected with fluorescein-conjugated second antibody) and anti-unc-33 (detected with rhodamine-conjugated second antibody) as a marker of neurons. Only embryos that had fully differentiated, and were therefore positive for the neuronal marker, were counted.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Strength</th>
<th>No. expressing seam marker</th>
<th>No. expressing neuronal marker</th>
<th>% With ectopic epidermis</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>glp-1(q224)</td>
<td>strong</td>
<td>31</td>
<td>36</td>
<td>86</td>
</tr>
<tr>
<td>glp-1(q415)</td>
<td>strong</td>
<td>6</td>
<td>9</td>
<td>87</td>
</tr>
<tr>
<td>glp-1(e2142)</td>
<td>strong</td>
<td>8</td>
<td>9</td>
<td>89</td>
</tr>
<tr>
<td>glp-1(e2072)</td>
<td>weak</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

The wild-type non-epidermal AB^8 precursors (see Fig. 1B) were isolated by laser-ablating P2, EMS, and the four central AB^8 cells (ABalp, ABarp, AB pla and AB pra; see Fig. 1A for nomenclature) in embryos of the indicated genotype. The ablated embryos were allowed to develop 20 hours postfertilization at 20°C. All embryos were stained with antibody NE21B4 as a marker of epidermis (detected with fluorescein-conjugated second antibody) and anti-unc-33 (detected with rhodamine-conjugated second antibody) as a marker of neurons. Only embryos that had fully differentiated, and were therefore positive for the neuronal marker, were counted.

The altered distribution of ABp-derived epidermal precursors among ABp descendants is altered to resemble that of the wild-type ABa descendants (Fig. 2D). One possible explanation for this effect is that ABp may adopt an ABa-like fate in this mutant. If this is indeed the case, then an excess of ABa-
specific cell types might be made. We addressed this possibility by analyzing one ABa-specific cell type, the IL2 neurons (Fig. 3D; Okamoto and Thomson, 1985). We found that glp-1(q224) mutants indeed contain excess IL2 neurons (Fig. 3).

To examine further whether the glp-1(q224) mutation transforms the ABp fate into an ABa-like fate, we analyzed cell lineages in several glp-1(q224) mutant embryos (see Materials and Methods). A summary of these lineage data are presented in Fig. 4 and Table 2. We found that the lineage of ABa was altered in only one respect: the lineages of the two ABa-derived AB8 cells that are normally pharynx precursors (ABara and ABalp, see Fig. 1A) resembled the lineages of their uninduced bilateral homologues (indicated by arrows on the left side of Fig. 4A and summarized in Fig. 4B). This result was expected: glp-1 mutations block induction of these two AB8 cells by MS (Priess et al., 1987; Austin and Kimble, 1987; Gendreau et al., 1994; S. Mango, personal communication; H. Hutter and R. Schnabel, personal communication) and it has been show that all MS-induced AB8 cells give rise to a lineage characteristic of their uninduced bilateral homologues in the absence of this induction (Gendreau et al., 1994; H. Hutter and R. Schnabel, personal communication). No other significant alterations in the ABa lineage were observed (Fig. 4; Table 2). In contrast, the lineages of all ABp descendants were completely transformed (Fig. 4). The entire ABp lineage in this mutant was altered to resemble the ABa lineage in the absence of MS induction (Fig. 4; Table 2). As indicated by the arrows on the right side of Fig. 4A, all AB8 descendants from ABp showed a lineage pattern that was strikingly similar to that of the corresponding uninduced ABa-derived AB8 cell. None of these lineage patterns were similar to any of the normal ABp lineages. Moreover, consistent with the results of the experiments described above (Fig. 2D), the positions of the AB8 epidermal and neuronal precursors were reversed relative to that in wild-type. Thus, those AB8 cells that are normally epidermal precursors gave rise to neuronal lineage patterns (explained in Fig. 4 legend) and their sisters gave rise instead to lineage patterns characteristic of epidermal precursors. We present a quantitative comparison of the glp-1(q224) AB8 cell...
to produce one ABp-specific cell type. We sought to test whether the widespread differences between the ABa and ABp 2 is required to maintain the wild-type lineage is presented in Table 1. Quantitative comparison of lineage data obtained from three manipulations made by ABa in the absence of MS induction. A quantitative comparison of lineage data obtained from three manipulations made (Fig. 5A; Table 3) revealed no ABp-specific lineage patterns were made. Instead, the lineage of ABp was apparently replaced by the lineage of a P1 descendant that maintained these axes in the AB descendants (Fig. 3G). It is possible that the EMS soma might retain the ability to produce an inductive signal. However, removal of the EMS nucleus prevented EMS from dividing and this manipulation appeared to be transformed to that of NT, no apparent transformation.

A P1 descendant is required to specify the entire ABp fate

The ABp gene is required for ABp to adopt a distinct fate from that of ABa, as described above. This suggests that a glp-1-dependent cellular interaction is responsible for this difference. Priess and Thomson (1987) showed that the differences in the development of ABa and ABp are a direct result of their unique positions. In addition, Bowerman et al. (1992b) demonstrated that an inductive interaction with a non-AB cell, P2, is required to produce one ABp-specific cell type. We sought to test whether the widespread differences between the ABa and ABp lineages arise from a single inductive interaction with a non-AB cell. To address this question, we removed blastomeres from early embryos and characterized the development of AB descendants in isolation.

First, we asked whether any ABp-specific lineages are made when all P1 descendants were absent. It was not possible to address this simply by removing P1, the sister of AB, from 2-cell embryos. This manipulation eliminates all embryonic axes (Gendreau et al., 1994) and therefore it is not possible to compare the lineage pattern of such an AB-derived embryo with the lineage of normal embryos. However, we found that we could closely approximate an embryo derived from AB alone by performing manipulations at the 4-cell stage. The 4-cell embryo consists of the two AB daughters and two P1 daughters, called EMS and P2 (Fig. 3A). We found that the anterior-posterior and dorsal-ventral axes of the embryo could be preserved if we removed P2 and the nucleus of EMS. In such embryos, the soma of EMS provided a physical constraint that maintained these axes in the AB descendants (Fig. 3G). It is possible that the EMS soma might retain the ability to produce an inductive signal. However, removal of the EMS nucleus prevented EMS from dividing and this manipulation effectively blocked the induction normally caused by MS, a daughter of EMS (see below).

Lineage analysis of the manipulated embryos revealed that no ABp-specific lineage patterns were made (Fig. 5A; Table 3). Instead, the lineage of ABp was apparently replaced by the lineage made by ABa in the absence of MS induction. A quantitative comparison of lineage data obtained from three manipulated embryos with the wild-type lineage is presented in Table 2.

<table>
<thead>
<tr>
<th>AB8 blastomere lineage*</th>
<th>Total no. descendants lineaged†</th>
<th>WT AB8 blastomere compared</th>
<th>No. of descendants with WT behavior‡</th>
<th>% Match to wild-type§</th>
<th>Proposed AB8 transformation compared¶</th>
<th>No. of descendants with transformed behavior**</th>
<th>% Match to proposed transformation††</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABala (2)</td>
<td>35</td>
<td>ABala</td>
<td>35</td>
<td>100%</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>ara (2)</td>
<td>31</td>
<td>ara</td>
<td>24</td>
<td>77</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>alp (2)</td>
<td>48</td>
<td>alp</td>
<td>6</td>
<td>13</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>arp (2)</td>
<td>31</td>
<td>arp</td>
<td>31</td>
<td>100</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>pla (2)</td>
<td>44</td>
<td>pla</td>
<td>11</td>
<td>25</td>
<td>ala</td>
<td>44</td>
<td>100</td>
</tr>
<tr>
<td>pra (2)</td>
<td>15</td>
<td>pra</td>
<td>3</td>
<td>20</td>
<td>ala</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>plp (3)</td>
<td>56</td>
<td>plp</td>
<td>4</td>
<td>7</td>
<td>arp</td>
<td>56</td>
<td>100</td>
</tr>
<tr>
<td>prp (3)</td>
<td>45</td>
<td>prp</td>
<td>1</td>
<td>2</td>
<td>arp</td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

Lineage data were obtained from 4-D time-lapse recordings of five glp-1(q224) embryos (see Materials and Methods). The table summarizes data for all descendants followed through the eighth sequential AB division. The left portion of the table shows the number of descendants lineaged from each AB8 blastomere. The middle portion shows a comparison of these experimental lineages with the wild-type AB8 lineages. The right portion shows a comparison of the experimental AB8 cell lineages with the wild-type AB8 cell lineages that most closely matched. We scored whether each descendant stopped at the eighth AB division (characteristic of an epidermal cell; Sulston et al., 1983; Gendreau et al., 1994), proceeded through a ninth division, or underwent programmed cell death. We then compared this behavior to the behavior of the descendant arising by the same lineage pattern from a wild-type AB8 blastomere. The total number of descendants in the experimental embryos that showed wild-type behavior is indicated. As an example, for the blastomere “alp,” indicated at the left of the table, 48 descendants were followed. Of these 48 descendants, 100% of the corresponding descendants from the wild-type alp blastomere. In contrast, all 48 descendants behaved the same as the corresponding descendants from the wild-type arp blastomere. Thus, the fate of alp appears to be transformed to that of NT, no apparent transformation.

*Name of AB8 blastomere lineaged. Number of embryos analyzed is indicated in parentheses. Only portions of the AB lineages from each embryo were followed.
†The total number of descendants that were followed through at least the eighth AB division round.
‡The number of descendants followed in the experimental embryo that showed wild-type behavior.
§Percentage of descendants lineaged that matched the wild-type behavior, calculated from the ratio of the data in column 4 to the data in column 2.
¶Name of the wild-type AB8 cell whose lineage best matches the observed lineage of the AB8 blastomere shown in column 1. (The apparent transformations are also indicated in Fig. 4).
**Number of descendants lineaged that behaved the same as the corresponding descendants from the lineage of the wild-type AB8 blastomere indicated in column 6.
††Percentage of descendants lineaged whose behavior matched the wild-type behavior of the AB8 cell indicated in column 6. This was calculated from the ratio of the data in column 7 to the data in column 2.
3. These comparisons show that the ABp lineages of manipulated embryos matched poorly (0-44% match) with the corresponding wild-type ABp lineages. In contrast, they showed a near-perfect match (98-100%) with the corresponding uninduced ABa lineages in all cases. These lineage results were confirmed by examining production of cell-type-specific specific intestinal valve cells (arrow) were observed in all EMS-removed partial embryos (9/9 embryos), consistent with laser ablation experiments of Bowerman et al. (1992b). (G,H) P2-removed, EMS-enucleated partial embryos. The ABa-specific IL2 neurons (arrowhead) and ABp-specific intestinal valve cells (arrow) were observed in all EMS-removed, EMS-enucleated partial embryos (6/6). Most embryos showed excess (greater than 6) IL2 neurons (4/6 embryos). However, intestinal valve cells were not observed in any embryos (0/6 embryos), consistent with the laser ablation experiments of Bowerman et al. (1992b).
Fig. 4. AB lineages in *glp-1(q224)* mutants (A) Representative lineages of all AB\(^8\) cells in *glp-1(q224)* embryos are shown and compared to lineages of the wild-type AB\(^8\) cells. For simplicity, lineages are indicated only from the seventh to the ninth sequential AB divisions. The left-right order of the lineages follows the convention of Sulston et al. (1983). Above each lineage is indicated the name of the AB\(^8\) cell from which it was derived. In the case of the wild-type lineages, the numbers in parentheses following these names correspond to the convention used in the wild-type fate map (Fig. 1A). The lineage of each AB\(^8\) blastomere represents data from a single *glp-1(q224)* embryo and all data shown came from the analysis of two different embryos. The first and second columns are AB\(^a\) lineages and the third and fourth columns are AB\(^p\) lineages. Lineages of mutant embryos are shown in the first and third columns. The corresponding wild-type lineages, taken from Sulston et al. (1983) are shown in the second and fourth columns. Cells with epidermal morphology, when unambiguously scored (see Materials and Methods), are indicated by an 'E', an 'X' indicates a programmed cell death, a dashed line indicates a cell that was lost or not followed, and vertical lines ending in arrowheads indicate that the cell divided at a later time. Cell deaths occurring after the ninth AB division round are not shown. Time is indicated on the scale bar at the right in minutes from first cleavage. Proposed lineage transformations are shown by large arrows that link experimental lineages to wild-type lineages. Cell deaths that are circled in the experimental lineages also occur in the lineage of the wild-type AB\(^8\) cell to which there is an apparent cell fate transformation. (B) Summary of AB\(^8\) cell transformations observed in *glp-1(q224)* embryos. Lineage patterns associated with each of the wild-type AB\(^8\) cells are numbered using the convention in Fig. 1A. In the *glp-1(q224)* mutant, each AB\(^8\) cell is given the number of the wild-type AB\(^8\) cell whose fate it appears to have adopted. Left-right asymmetry is abolished since the *glp-1* mutation blocks MS induction. In the AB\(^a\) lineage, MS normally induces the wild-type cells 2 and 3. In the absence of induction, these cells take on the fate of their bilateral homologues (cell 1 and 4; Gendreau et al., 1994; H. Hutter and R. Schnabel, personal communication). Wild-type cells 5 and 6 are epidermal precursors (Fig. 1A). In this mutant, the fates of these cells were transformed to the fate of a neuronal precursor (wild-type cell 1). The pair of wild-type cells 7/8 are neuronal precursors (Fig. 1A). In the mutant, these cells showed the fate of an epidermal precursor (cell 4).
molecular markers. The manipulated embryos produced an excess of the ABa-specific IL2 neurons (Fig. 3H). Further, they did not produce the ABp-specific intestinal valve cells (Fig. 3H), consistent with the laser ablations of P2 reported by Bowerman et al. (1992b).

These data demonstrate that removal of P2 and the nucleus of EMS completely transforms the fate of ABp to an ABa-like fate, implying that a P1 descendant induces the ABp fate. This manipulation produced a phenocopy of the glp-1(q224) mutant (compare Fig. 4B and Fig. 5C) suggesting that the glp-1 product participates in the cellular interaction identified by this manipulation.

P2 is required to specify the entire fate of ABp
The above experiment shows that the entire ABp fate is induced by a signal requiring P2, EMS, or both. The results of Bowerman et al. (1992b) suggest that EMS is not likely to be required for this interaction: ABp-specific intestinal valve cells are made in embryos in which EMS was killed with a laser. We confirmed the findings of Bowerman et al. (1992b) using the more stringent test of blastomere removal: intestinal valve cells were always made when EMS was physically removed from 4-cell embryos (Fig. 3). Moreover, ABA-specific IL2 neurons were made in such EMS-removed embryos, showing that EMS is not required for production of this ABA-specific cell type.
The findings of Bowerman et al. (1992b), and the results presented here, suggest that P2 alone may be required to specify the entire ABp fate. This hypothesis was tested directly by removing P2 from 4-cell embryos and analyzing the AB lineages. In this experiment, EMS was left intact and its daughter, MS, was allowed to induce AB 8 cells. Removal of P2 caused the AB 8 cells to rearrange and thereby alter their contacts with MS. In such embryos, MS made homologous contacts with the ABa and ABp granddaughters (described in Fig. 5). In the P2-removed embryos, the lineage of ABp resembled the lineage of ABa (e.g., Fig. 5B) and no ABp-like lineages were seen (summarized in Fig. 5C). A ... (36-68% match), but did match the lineages of particular ABa descendants (100% match; Table 4). These results show that P2 is required to specify the entire ABp lineage pattern (summarized in Fig. 5C).

After our studies were completed, Mello et al. (1994) and Mango et al. (1994) reported that both daughters of AB produce pharynx cells when P2 is prevented from touching either of them, implying that ABp adopts an ABa-like fate in the absence of P2. These findings are consistent with the results reported here. Our analyses have led us to extend these findings by showing that the entire identity of AB 8 cells is controlled by this P2 to ABp interaction acting in combination with other regulatory inputs (see Discussion).

**DISCUSSION**

We report three findings. First, the glp-1 gene product is required for ABp to acquire a broadly different fate from that of ABa. Second, the P2 blastomere, by inducing the entire ABp fate, promotes widespread differences between the ABp and ABa lineages. A P2-dependent interaction that induces production of intestinal valve cells by ABp was first recognized by Bowerman et al. (1992b); here we show that a P2 to ABp interaction alters the identity of all of the ABp-derived AB 8 cells such that they give rise to distinct lineages (Figs 4, 5). Third, the P2/ABp interaction acts in combination with the MS inductive signal, and a
process that controls the underlying fate of AB\textsuperscript{8} cells to direct the final identity of each AB\textsuperscript{8} cell (see below).

**An early cellular interaction causes a polarity reversal at the AB\textsuperscript{8} cell stage**

The ability to produce epidermis becomes restricted to one cell of each pair of AB\textsuperscript{8} sisters (Gendreau et al., 1994; Fig. 1B). We have found that P\textsubscript{2} induction of ABp causes a reversal in the polarity of this restriction event among ABp descendants. Polarity reversals have been seen in a number of situations in *C. elegans*. Cellular interactions that control the polarity of lineages ensure the proper alignment of differentiated cells in the generation of terminal structures. For example, an interaction that inverts the polarity of the posterior vulval lineages (W. Katz and P. Sternberg, personal communication) results in a vulva that is mirror-symmetric around the central orifice (Horvitz and Sternberg, 1991). Cellular interactions also reverse the polarity of lineages within the developing gonad (Kimble, 1981). However, unlike these examples, the effect we have observed does not reverse the polarity of an entire lineage. Rather, the potential of certain ABp granddaughters to make epidermis instead becomes associated with their sisters. This P\textsubscript{2}-induced reversal causes epidermis to arise from the four central AB\textsuperscript{8} cells (Fig. 2D). This clustering together of the epidermal progenitors may facilitate assembly of a single epidermal sheet later in development. Consistent with this view, glp-1 mutations that block this polarity reversal cause embryos to undergo a catastrophic failure in epidermal morphogenesis (Priess et al., 1987; Austin and Kimble, 1987; Kodoyianni, 1992).

**The two functions of glp-1 in the early embryo**

The glp-1-encoded putative cell surface receptor is required for cellular interactions in the early embryo, the late embryo, and the germ line (Priess et al., 1987; Austin and Kimble, 1987; Lambie and Kimble, 1991). Multiple roles for other members of the glp-1 family have also been described, including lin-12 in *C. elegans* (Greenwald et al., 1983; Lambie and Kimble, 1991) and Notch in *Drosophila* (Cagan and Ready, 1989; Hartenstein et al., 1992; Xu et al., 1992). In addition to its requirement in MS induction (Priess et al., 1987; Austin and Kimble, 1987; Gendreau et al., 1994; H. Hutter and R. Schnabel, personal communication; S. Mango, personal communication), the experiments described here and elsewhere (Mello et al., 1994) implicate glp-1 in a second cellular interaction in the early embryo, the induction of the ABp fate. Since weak alleles block only the former induction, these two functions appear to be separable (Priess et al., 1987; Austin and Kimble, 1987; Mello et al., 1994).

GLP-1 protein may act directly as a receptor for inductive signals, or alternatively, may mediate lateral interactions between equivalent AB cells, in analogy to the function of *lin-12* (Seydoux and Greenwald, 1989). The results presented here and by Mello et al. (1994) and Mango et al. (1994) support the former model. No ABp-specific cell lineages were seen when P\textsubscript{2} was removed (Fig. 5; Tables 3, 4; Bowerman, 1992b), suggesting that an inductive signal is absolutely required for the ABp fate. In addition, we have found that an ABp-specific cell type, the intestinal valve cells, is made even when ABa is removed from the embryo (data not shown), implying that lateral interactions between the AB daughters are not required for ABp-specific differentiation. Finally, Mello et al. (1994) have shown that if P\textsubscript{2} is allowed to contact both ABa and ABp, both give rise to intestinal valve cells.

Similarly, our experiments suggest that glp-1 also acts to receive the MS inductive signal directly, rather than promoting lateral interactions between AB\textsuperscript{8} cells (Fig. 5C). Left and right AB\textsuperscript{8} homologues are initially equivalent (Wood, 1991); asymmetric induction by MS causes left/right differences in their fates (Gendreau et al., 1994; H. Hutter and R. Schnabel, personal communication). In embryos in which P\textsubscript{2} was removed, MS contacted and induced similar fates in bilateral homologues; no lateral inhibition was apparent (Fig. 5C).

The glp-1 product first accumulates specifically in the AB daughters and persists until the AB\textsuperscript{16} stage (Evans et al., 1994). Thus, it is present in the appropriate cells and at the proper time to receive inductive signals from both P\textsubscript{2} and MS. If GLP-1 serves as a receptor for inductive signals, then its ligands might be expected to be expressed specifically in the inducing cells, P\textsubscript{2} and MS. Although no such ligands have been identified, candidates for signaling molecules in both interactions have recently been found (Mello et al., 1994; Mango et al., 1994; J. Priess, personal communication; B. Bowerman personal communication).

**The identities of the AB\textsuperscript{8} cells appear to be specified combinatorially**

Our lineage studies revealed striking reiterations of wild-type lineage patterns in ectopic positions (e.g., Figs 4, 5; Tables 2, 3, 4). These lineage patterns accurately reproduced not only the proper numbers of cell divisions and terminal cell morphology, but frequently the relative timings of terminal cell divisions as well. Thus, the lineages of the AB\textsuperscript{8} cells appear to represent sub-lineage modules or cassettes that can be executed as complete units. These findings suggest that the identity of each AB\textsuperscript{8} cell is directly reflected in the lineage pattern that it generates.

The two glp-1-dependent inductive interactions appear to control the identity of AB\textsuperscript{8} cells per se. Induction of the two ABa-derived pharynx precursors by MS (Fig. 1A; Priess and Thomson, 1987; Gendreau et al., 1994; S. Mango, personal communication; H. Hutter and R. Schnabel, personal communication) causes them not only to produce pharynx tissue (Priess and Thomson, 1987), but, in fact, causes them to adopt fates that are entirely distinct from those of their uninduced, opposite side homologues, as reflected in their lineage patterns (Gendreau et al., 1994; H. Hutter and R. Schnabel, personal communication).

The MS signal does not act alone to control the fate of ABa-derived blastomeres. The two MS-induced AB\textsuperscript{8} cells from ABa give rise to entirely different lineages (see Fig. 1A). The MS signal appears to act in combination with the underlying identity of each AB\textsuperscript{8} blastomere to specify a unique fate. The underlying identity of each AB\textsuperscript{8} cell is in turn controlled by a process that restricts epidermal potential to one daughter of each AB\textsuperscript{8} cell (Gendreau et al., 1994; Fig. 1). MS induces an ABa granddaughter with epidermal potential to produce a lineage pattern characteristic of one pharynx precursor (cell no. 3, Fig. 1A) and an ABa granddaughter without epidermal potential to produce a lineage pattern characteristic of the other pharynx precursor (cell no. 2. Fig. 1A).

The combinatorial effect of MS induction with the process that controls the underlying identity of AB\textsuperscript{8} cells is apparent when the results of the two P\textsubscript{2} removal experiments (Fig. 5)
The AB8 cells receive their normal regulatory inputs and these cells show essentially wild-type lineage patterns (Figs 4, 5B; Tables 2, 3). Two AB8 cells each showed lineage patterns characteristic of a single epidermal precursor (cell no. 4, Fig. 1A) from the wild-type embryo (Fig. 5C). When EMS was left intact, allowing these four AB8 cells to be induced by MS, each showed lineage patterns characteristic of a single AB8 pharynx precursor (cell no. 3, Fig. 1A) (Fig. 5C). Further, it is this wild-type pharynx precursor (cell no. 3, Fig. 1A) that becomes an epidermal precursor (cell no. 4, Fig. 1A) when MS induction is prevented in otherwise intact embryos (Fig. 1B; Gendreau et al., 1994; H. Hutter and R. Schnabel, personal communication). These results imply that the fate of an MS-induced AB8 blastomere is determined by MS induction in combination with its underlying identity.

Similarly, our lineage analysis suggests that induction of ABp by P2 does not only promote differentiation of certain ABp-specific cell types (e.g., intestinal valve cells; Bowerman et al., 1992b) but alters the entire fate of ABp, and thus all of its descendants. P2 induction alters the consequences of MS induction. Extra nervous system, but not pharynx, is made in the ABp-derived AB8 cell that is induced by MS (Fig. 1A). This implies that the two inductive signaling processes can act in concert to specify blastomere identities.

We propose a model that describes how the diversity of blastomere fates is generated within the AB lineage (Fig. 6). The figure shows schematic representations of the AB8 cells in wild-type and mutant or manipulated embryos. The results are based on the findings reported here and elsewhere (Gendreau et al., 1994; Mello et al. (1994); Mango et al. (1994); H. Hutter and R. Schnabel, personal communication). The fate of each AB8 cell is indicated by the number of the wild-type AB8 cell that shows the same fate. The three ovaries within each cell represent the three regulatory inputs that control AB8 cell fates. These three inputs are: (1) induction of the ABp fate by P (indicated by a P), (2) acquisition of epidermal potential (indicated by an E; Gendreau et al., 1994), and (3) induction by MS (indicated by an M). A black oval containing a letter indicates that the AB8 cell receives that input, an open oval indicates that it does not. The fate of all four ABp-derived AB8 cells is influenced by this induction. For simplicity, we have assumed that epidermal potential is controlled by a process that promotes epidermal development (e.g., the unequal inheritance of an epidermal determinant). An 'E' indicates that the cell has received this input and therefore has the potential to make epidermis; absence of an E indicates that the cell did not receive this input. Note that the AB8 cells from ABp that receive this input in the absence of P2 induction are the sisters of the AB8 cells that receive it following P2 induction. (A) In wild-type embryos, each blastomere receives a different combination of binary regulatory inputs, with the exception of the two cells numbered 7/8. Seven different lineage patterns are associated with these seven different combinations. The cell nos 1 and 4 fates appear to be the background fates in the absence of the P2 or MS inductions. Cell 4 represents the uninduced fate of a blastomere that has epidermal potential, whereas cell 1 represents the induced fate of a blastomere that does not. MS induction of a cell lacking epidermal potential results in a cell 2 fate. MS induction of a cell with epidermal potential results in a cell 3 fate. P2 induction affects the fate of all four ABp granddaughters. This induction causes an epidermal precursor to exhibit a cell 5 fate. MS induction further modifies this to promote a cell 6 fate. Finally, P2 induction causes a non-epidermal precursor to exhibit a cell 7/8 fate. Although MS contacts both cells 7/8, there is no evidence that MS affects their fates, as ablation of MS does not dramatically alter their lineages (Gendreau et al., 1994). This observation suggests that P2 induction may prevent non-epidermal precursors from responding to MS. We have therefore omitted the MS input from these two ABp descendants. (B) In embryos in which MS induction is prevented by laser-ablation of MS, the regulatory inputs in three AB8 cells are changed. Five AB8 cells receive the same set of regulatory inputs as their bilateral homologues (cells #1, 4, and 5) and their lineages show the corresponding transformations (Gendreau et al., 1994 and H. Hutter and R. Schnabel, personal communication). As noted above, MS does not appear to influence the posterior-most AB8 cells significantly, such that the fates of these cells are unchanged in the absence of MS. (C) In glp-1(q224) embryos or partial embryos in which P2 is removed and EMS is enucleated, both the P2 and MS inductions are prevented. The regulatory inputs in six AB8 cells are changed. Two AB8 cells receive their normal regulatory inputs and these cells show essentially wild-type lineage patterns (Figs 4, 5B; Tables 2, 3). Regulatory inputs of three AB8 cells are altered to those of the wild-type cell 1, while the inputs of the other three are altered to those of the wild-type cell 4. All six cells show lineage transformations consistent with the altered regulatory inputs. Note that the absence of P2 induction causes a reversal in the distribution of epidermal potential in the AB lineage. (D) In partial embryos lacking P2, P2 induction does not occur and MS changes its contacts with the AB8 cells. Only two AB8 cells (cells 1 and 3 in wild-type) receive their normal regulatory inputs. These two cells give rise to essentially wild-type lineage patterns. Regulatory inputs of three AB8 cells are altered to correspond to those of the wild-type cell 1, while the inputs of the other three are altered to those of the wild-type cell 3. All six cells display lineages consistent with the altered regulatory inputs. Again, the absence of P2 induction causes a reversal in the distribution of epidermal potential in the AB lineage.

Fig. 6. Combinatorial model for AB8 blastomere fate specification. The figure shows schematic representations of the AB8 cells in wild-type and mutant or manipulated embryos. The results are based on the findings reported here and elsewhere (Gendreau et al., 1994; Mello et al. (1994); Mango et al. (1994); H. Hutter and R. Schnabel, personal communication). The fate of each AB8 cell is indicated by the number of the wild-type AB8 cell that shows the same fate. The three ovaries within each cell represent the three regulatory inputs that control AB8 cell fates. These three inputs are: (1) induction of the ABp fate by P (indicated by a P), (2) acquisition of epidermal potential (indicated by an E; Gendreau et al., 1994), and (3) induction by MS (indicated by an M). A black oval containing a letter indicates that the AB8 cell receives that input, an open oval indicates that it does not. Although induction of ABp by P2 occurs two divisions before the AB8 stage, the fates of all four ABp-derived AB8 cells are influenced by this induction. For simplicity, we have assumed that epidermal potential is controlled by a process that promotes epidermal development (e.g., the unequal inheritance of an epidermal determinant). An 'E' indicates that the cell has received this input and therefore has the potential to make epidermis; absence of an E indicates that the cell did not receive this input. Note that the AB8 cells from ABp that receive this input in the absence of P2 induction are the sisters of the AB8 cells that receive it following P2 induction. (A) In wild-type embryos, each blastomere receives a different combination of binary regulatory inputs, with the exception of the two cells numbered 7/8. Seven different lineage patterns are associated with these seven different combinations. The cell nos 1 and 4 fates appear to be the background fates in the absence of the P2 or MS inductions. Cell 4 represents the uninduced fate of a blastomere that has epidermal potential, whereas cell 1 represents the induced fate of a blastomere that does not. MS induction of a cell lacking epidermal potential results in a cell 2 fate. MS induction of a cell with epidermal potential results in a cell 3 fate. P2 induction affects the fate of all four ABp granddaughters. This induction causes an epidermal precursor to exhibit a cell 5 fate. MS induction further modifies this to promote a cell 6 fate. Finally, P2 induction causes a non-epidermal precursor to exhibit a cell 7/8 fate. Although MS contacts both cells 7/8, there is no evidence that MS affects their fates, as ablation of MS does not dramatically alter their lineages (Gendreau et al., 1994). This observation suggests that P2 induction may prevent non-epidermal precursors from responding to MS. We have therefore omitted the MS input from these two ABp descendants. (B) In embryos in which MS induction is prevented by laser-ablation of MS, the regulatory inputs in three AB8 cells are changed. Five AB8 cells receive the same set of regulatory inputs as their bilateral homologues (cells #1, 4, and 5) and their lineages show the corresponding transformations (Gendreau et al., 1994 and H. Hutter and R. Schnabel, personal communication). As noted above, MS does not appear to influence the posterior-most AB8 cells significantly, such that the fates of these cells are unchanged in the absence of MS. (C) In glp-1(q224) embryos or partial embryos in which P2 is removed and EMS is enucleated, both the P2 and MS inductions are prevented. The regulatory inputs in six AB8 cells are changed. Two AB8 cells receive their normal regulatory inputs and these cells show essentially wild-type lineage patterns (Figs 4, 5B; Tables 2, 3). Regulatory inputs of three AB8 cells are altered to those of the wild-type cell 1, while the inputs of the other three are altered to those of the wild-type cell 4. All six cells show lineage transformations consistent with the altered regulatory inputs. Note that the absence of P2 induction causes a reversal in the distribution of epidermal potential in the AB lineage. (D) In partial embryos lacking P2, P2 induction does not occur and MS changes its contacts with the AB8 cells. Only two AB8 cells (cells 1 and 3 in wild-type) receive their normal regulatory inputs. These two cells give rise to essentially wild-type lineage patterns. Regulatory inputs of three AB8 cells are altered to correspond to those of the wild-type cell 1, while the inputs of the other three are altered to those of the wild-type cell 3. All six cells display lineages consistent with the altered regulatory inputs. Again, the absence of P2 induction causes a reversal in the distribution of epidermal potential in the AB lineage.


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