Development 120, 3325-3338 (1994)
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Combinatorial specification of blastomere identity by *glp-1*-dependent cellular interactions in the nematode *Caenorhabditis elegans*

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SUMMARY

Most somatic cells in the nematode *Caenorhabditis elegans* arise from AB, the anterior blastomere of the 2-cell embryo. While the daughters of AB, ABa and ABp, are equivalent in potential at birth, they adopt different fates as a result of their unique positions. One such difference is that the distribution of epidermal precursors arising from ABp is reversed along the anterior-posterior axis relative to those arising from ABa. We have found that a strong mutation in the *glp-1* gene eliminates this ABa/ABp difference. Furthermore, extensive cell lineage analyses showed that ABp adopts an ABa-like fate in this mutant. This suggests that *glp-1* acts in a cellular interaction that makes ABp distinct from ABa. One ABp-specific cell type was previously shown to be induced by an interaction with a neighboring cell, P_2. By removing P_2 from early embryos, we have found that the widespread differences between ABa and ABp arise from induction of the entire ABp fate by P_2. Lineage analyses of genetically and physically manipulated embryos further suggest that the identities of the AB great-granddaughters (AB^8 cells) are controlled by three regulatory inputs that act in various combinations. These inputs are: (1) induction of the ABp-specific fate by P_2, (2) a previously described induction of particular AB^8 cells by a cell called MS, and (3) a process that controls whether an AB^8 cell is an epidermal precursor in the absence of either induction. When an AB^8 cell is caused to receive a new combination of these regulatory inputs, its lineage pattern is transformed to resemble the lineage of the wild-type AB^8 cell normally receiving that combination of inputs. These lineage patterns are faithfully reproduced irrespective of position in the embryo, suggesting that each combination of regulatory inputs directs a unique lineage program that is intrinsic to each AB^8 cell.

Key words: *C. elegans*, cellular interactions, epidermis, cell lineage, *glp-1*, blastomere identity

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. The extensive differences between their descendants are created by cellular interactions. One of these differences, production of intestinal valve cells by ABp, occurs as a result of induction by a neighboring cell, called P_2 (Bowerman et al., 1992b). Similarly, the granddaughters of AB on the left side of the embryo 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-

granddaughters ('AB^8 cells'; Priess and Thomson, 1987; Wood, 1991; Gendreau et al., 1994; H. Hutter and R. Schnabel, personal communication). The product of the *glp-1* gene, a putative cell surface receptor molecule (Austin and Kimble, 1989; Yochem and Greenwald, 1989), is required for induction of AB^8 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).

In this report, we address how the diversity of cell types is established during the development of AB descendants. We show that the *glp-1* gene is required to make the fate of ABp different from that of ABa. We also extend the findings of Bowerman et al. (1992b) by demonstrating that virtually all ABp-specific development requires an interaction with the P_2 blastomere. In the absence of this interaction, ABp adopts an ABa-like fate. The diversity of cell types produced by AB appears to arise from the combined action of the induction of ABp fate by P_2, the subsequent induction of particular AB^8 cells by MS, and an event that controls the underlying potential (either epidermal or neuronal; Gendreau et al., 1994) of each AB^8 cell. The unique identity of an AB^8 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^8 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). Adults 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 NE2\1B4 (Schnabel, 1991), which was used as a marker of epidermis (Gendreau et al., 1994), and 1CB4 (Okamoto and Thomson, 1985) as a marker of IL2 neurons and intestinal valve cells. (The reactivity of 1CB4 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). Fluoroscein (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 (Cowan 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 400× 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 holder with Flourinert (Sigma, F-9755). P_2 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 P_2-removal, EMS-enucleation experiments, the posterior of the eggshell was punctured immediately after the formation of the EMS-P_2 cleavage furrow. At this early stage, EMS and P_2 are not completely separated (see below); therefore, extruding all of P_2 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 occurred even when the eggshell was not punctured by 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 P_2.

Partial embryos are sensitive to the osmotic strength of the culture medium. Reproducible growth and differentiation of partial embryos was achieved only at a narrow range of salt concentrations. Proper osmolarity 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 differentiated by Nomarski microscopy, failed to express any differentiation markers examined and often showed variable arrest in cell division. Our culture and handling conditions allowed removal of all traces of P_2 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 600×. A Hamamatsu Newvicom 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

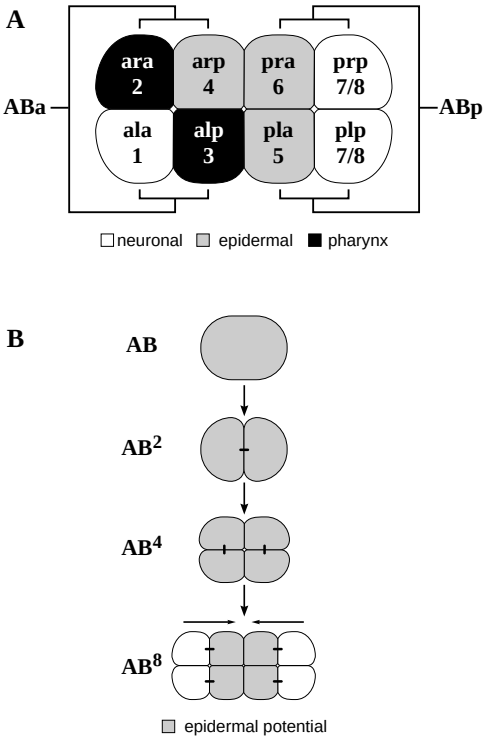


Fig. 1. Distribution of major cell types made by the AB^8 cells. (A) A dorsal view of the eight AB great-granddaughters (AB^8 cells) in wild-type embryos is presented schematically. The lineal relationships of these cells to ABa, the anterior, and ABp, the posterior daughter of AB are indicated. Anterior is to the left, right to the top. AB^8 cells are given numbers representing the unique lineage pattern that each produces. Since the lineage patterns of the posterior-most pair of cells are nearly identical, they are both given the number 7/8. These numbers relate to the conventional names for the AB^8 cells as indicated on the figure (e.g., 'alp' on the figure is ABalp). The three-letter nomenclature (Sulston et al., 1983) is invariant for each AB^8 cell even if its fate is altered: for example, ABalp is always the posterior daughter of the left daughter of the anterior daughter of AB. In contrast, the number given to an AB^8 cell varies depending on the lineage pattern that it actually generates in an experimentally manipulated embryo. Shading within each cell indicates the predominant tissue or organ type to which it gives rise: no shading indicates a neuronal precursor, black shading indicates a pharynx precursor and gray shading indicates an epidermal precursor. We refer to the epidermal cells made from the three epidermal precursors as major epidermal cells (Gendreau et al., 1994). A few 'minor' epidermal cells (Gendreau et al., 1994) are made by AB^8 cells that are not epidermal precursors. These are readily distinguished from the major epidermal cells by their cell lineage patterns. Major epidermal cells are always produced by eight sequential AB divisions, whereas minor (and essentially all other cell types) are produced by at least nine (Sulston et al., 1983; Gendreau et al., 1994). In addition, the molecular marker of epidermis that we have used in this work specifically recognizes seam epidermis, a major epidermal cell type. In this paper, 'epidermis' refers to major epidermis only. (B) This diagram summarizes findings of Gendreau et al. (1994). Cells that have the ability to produce epidermis are shaded. Short lines connect sister cells. AB, each of its daughters (AB^2 cells), and each of its granddaughters (AB^4 cells) can give rise to epidermis when isolated. However, only one daughter of each AB^4 cell can produce epidermis; the other produces primarily nervous tissue instead. This restriction in the ability to make epidermis occurs 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^4 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 (Priess 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 (Priess 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 (Priess 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; Priess 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.

Table 1. Epidermis is produced from ectopic AB^8 cells in strong *glp-1* mutants

Mutation	Strength*	No. expressing seam marker†	No. expressing neuronal marker‡	% With ectopic epidermis§
WT		0	18	0
glp-1(q224)	strong	31	36	86
glp-1(q415)	strong	6	9	67
glp-1(e2142)	strong	8	9	89
glp-1(e2072)	weak	0	5	0

The wild-type non-epidermal AB^8 precursors (see Fig. 1B) were isolated by laser-ablating P_2, EMS, and the four central AB^8 cells (ABalp, ABarp, ABpla and ABpra; 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 NE2\1B4 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.

*Strength of allele was defined according to Kodoyianni et al. (1992). All strong alleles cause severe defects in epidermal morphogenesis; the weak allele does not profoundly perturb morphogenesis.

†Number of embryos that stained positive for the seam epidermal marker.

‡Number of embryos that stained positive for the neuronal marker.

§Percent of all differentiated embryos that were positive for the seam epidermal marker.

We next asked which AB^8 cells ectopically produced the epidermal marker in *glp-1* embryos. Individual AB^8 cells were isolated by ablating all other cells in *glp-1(q224)* embryos and the ablated embryos were analyzed for the epidermal and neuronal markers (Fig. 2 legend). These experiments showed that epidermis arose ectopically from ABp-derived, but not ABa-derived AB^8 cells. Specifically, we found that the posterior pair of ABp-derived cells, normally neuronal precursors in wild-type, became epidermal precursors, whereas their anterior sisters, normally epidermal precursors, became neuronal precursors (summarized in Fig. 2). Thus, although the *glp-1(q224)* mutation does not prevent the restriction of epidermal potential per se, it does reverse the polarity of this event (Fig. 2D).

glp-1 is required to specify the entire ABp fate

The altered distribution of ABp-derived epidermal precursors (Fig. 2D) suggests that *glp-1* may play a role in controlling the ABp fate. Bowerman et al. (1992b) reported that *glp-1* mutations did not block production of one ABp-specific cell type, a pair of intestinal valve cells. In light of our results, we chose to re-examine the effects of a strong loss-of-function *glp-1* mutation on production of this ABp-specific cell type (Fig. 3). In contrast to the findings of Bowerman et al. (1992b) with other *glp-1* alleles, we found that intestinal valve cells were never made in the *glp-1(q224)* mutant (Fig. 3). This supports the view that the ABp fate is not properly specified in this mutant.

In *glp-1(q224)* mutants, the distribution of 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-

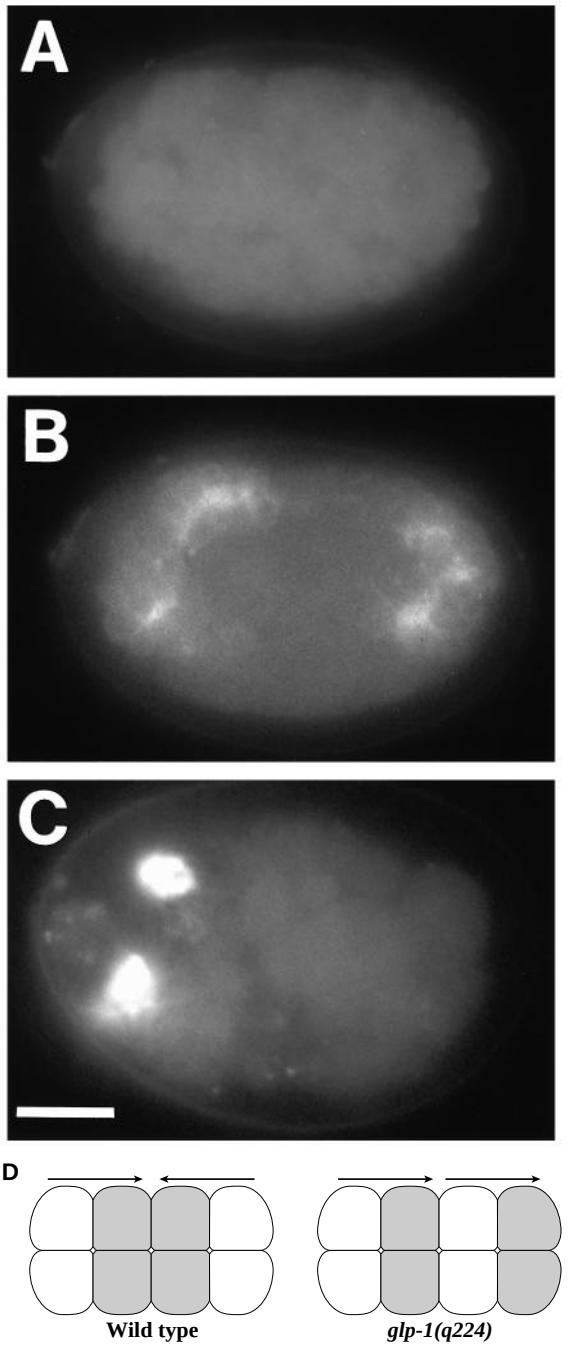


Fig. 2. The distribution of epidermal precursors is altered in a strong *glp-1* mutant. (A-C) Immunofluorescence micrographs of laser-operated embryos. Embryos were analyzed for production of the epidermal seam antigen (recognized by antibody NE2\1B4) and the neuronal antigen (recognized by anti-*unc-33* antibody) following the ablation of P_2, EMS and the four AB^8 epidermal precursors (ABalp, ABarp, ABpla, ABpra; Gendreau et al., 1994). (A,B) The same ablated wild-type embryo stained for epidermis and neurons, respectively. Wild-type embryos never produced the epidermal antigen (A and Table 1) but always produced the neuronal marker (B). In contrast, a similarly ablated *glp-1(q224)* embryo produced epidermis, which must have arisen from ectopic AB^8 cells (panel C). Bar, approximately 10 μm. (D) Comparison of the epidermal fate maps in wild-type and *glp-1(q224)* embryos. Isolation of individual AB^8 cells that descend from ABp showed that the *glp-1(q224)* mutation causes a reversal in the distribution of epidermal precursors from ABp, as schematized here. *glp-1(q224)* embryos in which the anterior granddaughters of ABp (denoted cells 5 and 6 in Fig. 1A) were individually isolated by ablating P_2, EMS and seven of the AB^8 cells never produced the epidermal marker (0/8 and 0/6 embryos with cell 5 and cell 6 isolated, respectively). In wild-type embryos, these cells produced this marker when isolated by a similar ablation (Gendreau et al., 1994). *glp-1(q224)* embryos in which the posterior granddaughters of ABp (denoted cells 7/8 in Fig. 1A) were individually isolated always produced the epidermal marker (3/3 and 5/5 embryos with the left cell 7/8 and the right cell 7/8 isolated, respectively). In wild-type embryos these cells never produced epidermis when isolated by a similar ablation (Gendreau et al., 1994). In contrast, the epidermal fate map of the ABa descendants appeared normal. Arrows are used to illustrate the polarity reversal in the distribution of epidermal and neuronal precursors. Note that in *glp-1(q224)* embryos, the distribution of epidermal precursors is identical for ABa and ABp descendants, and mimics the wild-type ABa distribution.

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). (The criteria used to compare the lineages of wild-type and mutant embryos are described in Fig. 4). 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 AB^8 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 AB^8 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 AB^8 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 AB^8 descendants from ABp showed a lineage pattern that was strikingly similar to that of the corresponding uninduced ABa-derived AB^8 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 AB^8 epidermal and neuronal precursors were reversed relative to that in wild-type. Thus, those AB^8 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)* AB^8 cell

Table 2. Comparison of lineages of *glp-1(q244)* embryos with wild-type lineages

AB^8 blastomere lineaged*	Total no. descendants lineaged†	WT AB^8 blastomere compared	No. of descendants with WT behavior‡	% Match to wild-type§	Proposed AB^8 transformation compared¶	No. of descendants with transformed behavior**	% Match to proposed transformation††
ABala (2)	35	ABala	35	100%	NT	NT	NT
ara (2)	31	ara	24	77	ABala	31	100%
alp (2)	48	alp	6	13	arp	48	100
arp (2)	31	arp	31	100	NT	NT	NT
pla (2)	44	pla	11	25	ala	44	100
pra (2)	15	pra	3	20	ala	15	100
plp (3)	56	plp	4	7	arp	56	100
prp (3)	45	prp	1	2	arp	45	100

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 AB^8 blastomere. The middle portion shows a comparison of these experimental lineages with the wild-type AB^8 lineages. The right portion shows a comparison of the experimental AB^8 cell lineages with the wild-type AB^8 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 AB^8 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. 6 of these descendants behaved the same as 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 arp. NT, no apparent transformation.

*Name of AB^8 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 AB^8 cell whose lineage best matches the observed lineage of the AB^8 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 AB^8 blastomere indicated in column 6.

††Percentage of descendants lineaged whose behavior matched the wild-type behavior of the AB^8 cell indicated in column 6. This was calculated from the ratio of the data in column 7 to the data in column 2.

lineages with those of wild-type in Table 2. These comparisons show that the mutant ABp lineages matched very poorly (2-25% match) with the corresponding wild-type ABp lineages. In contrast, they showed a perfect match with the corresponding uninduced ABa lineages in all cases. This lineage analysis implies that ABp in its entirety takes on an ABa-like fate in a *glp-1(q224)* mutant. After our studies were completed, Mello et al. (1994) and Hutter and Schnabel (personal communication) also reported that *glp-1* is required to establish ABa/ABp differences.

This analysis shows that the development of AB is greatly simplified in *glp-1(q224)* mutants. Instead of the seven distinct lineage patterns normally generated by the eight AB^8 cells, there appear to be only two unique patterns in this mutant (summarized in Fig. 4B), one characteristic of a neuronal precursor and the other of an epidermal precursor. This implies that there are only two distinct AB^8 cell types made in the absence of *glp-1* function.

A P_1 descendant is required to specify the entire ABp fate

The *glp-1* 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, P_2, 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 P_1 descendants were absent. It was not possible to address this simply by removing P_1, 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 P_1 daughters, called EMS and P_2 (Fig. 3A). We found that the anterior-posterior and dorsal-ventral axes of the embryo could be preserved if we removed P_2 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

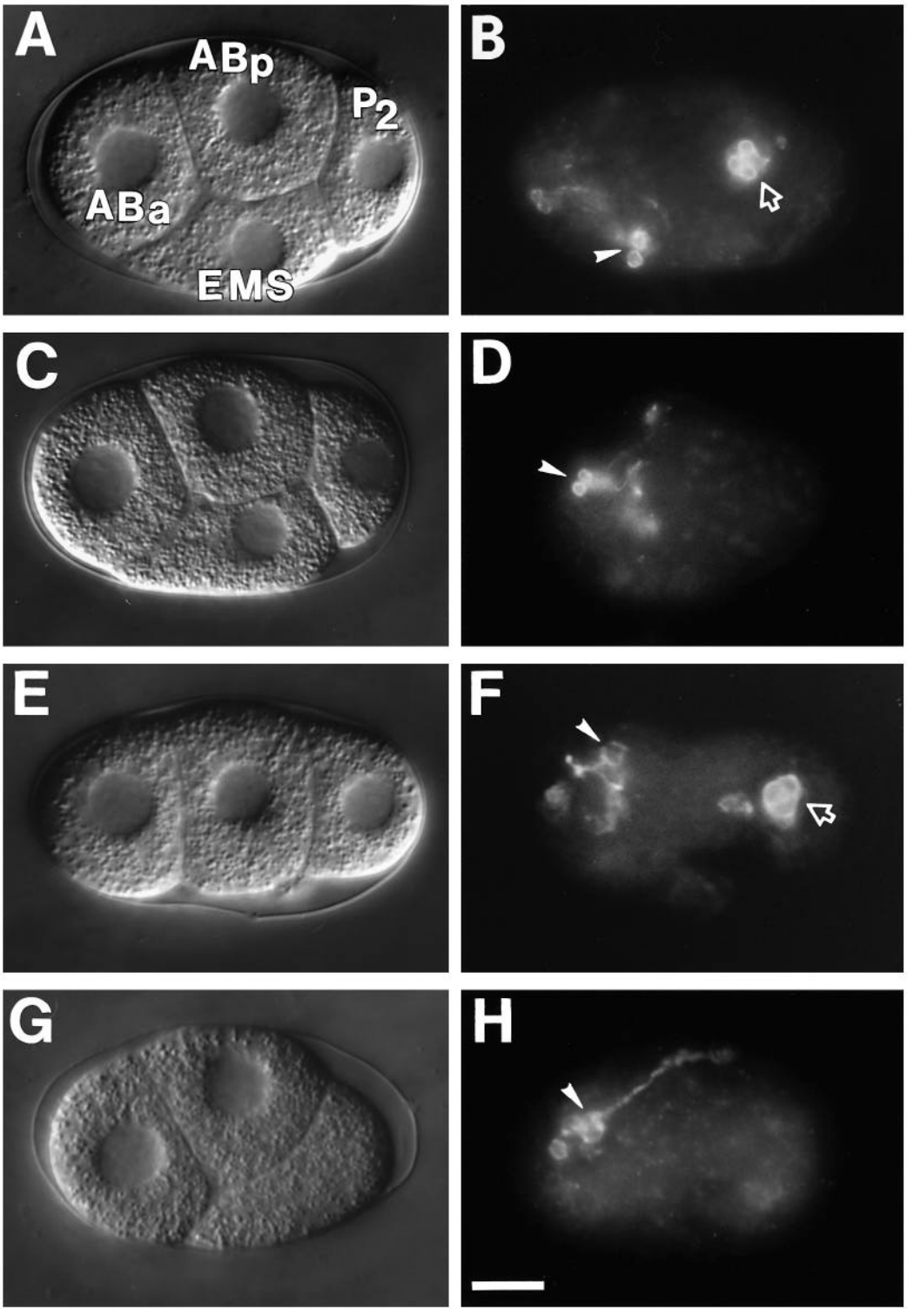
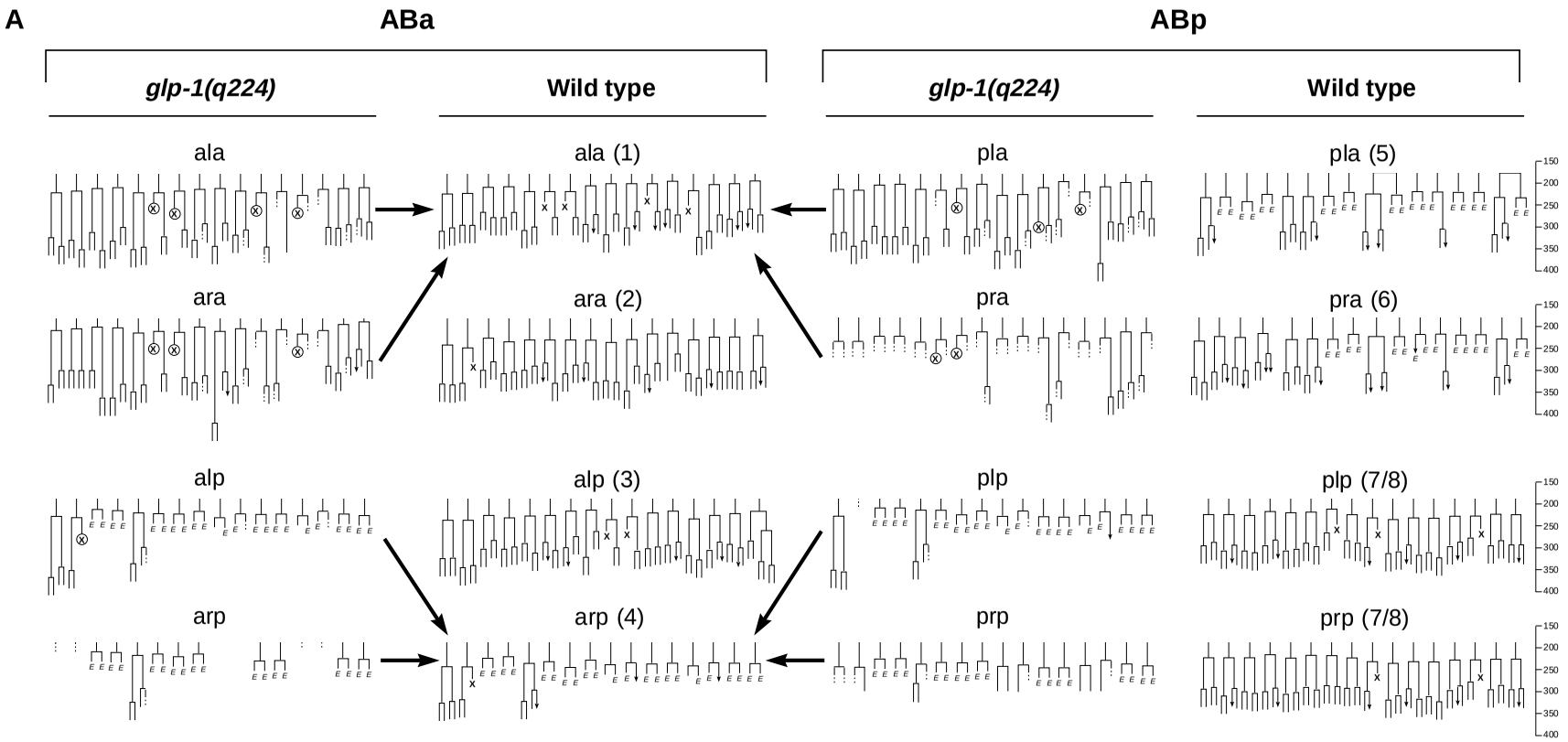


Fig. 3. Production of ABa- and ABp-specific cell types in *glp-1* and manipulated embryos. IL2 neurons, a cell type specifically derived from the ABa lineage, and intestinal valve cells, a cell type specifically derived from the ABp lineage, were analyzed in wild-type, *glp-1(q224)*, and manipulated partial embryos. Representative Nomarski micrographs of intact (A,C) or operated (E,G) embryos at the 4-cell stage are shown in the left set of panels. The identity of each of the four blastomeres is indicated in A. Representative immunofluorescence micrographs of the 1CB4 staining pattern are shown in the right set of panels. The antibody 1CB4 recognizes both cell types as well as some cells derived from the EMS blastomere (pharynx glands). To facilitate the scoring of IL2 neurons and intestinal valve cells, EMS was ablated in all embryos. Embryos were then stained with 1CB4 after 20 hours of development postfertilization at 20°C (see Materials and Methods). Bar, approximately 10 μm. (A,B) Wild-type embryos. Both ABa-specific IL2 neurons (arrowhead) and ABp-specific intestinal valve cells (arrow) are apparent (IL2 neurons in 16/16 embryos and intestinal valve cells in 14/16 embryos) in wild-type embryos in which EMS was ablated. (C,D) *glp-1(q224)* embryos. ABa-specific IL2 neurons (arrowhead) are apparent; however, no ABp-specific intestinal valve cells are seen in this embryo. Excess IL2 neurons (greater than 6) were seen in almost all cases (16/17 embryos) and intestinal valve cells generally were not observed (16/17 embryos). (E,F) EMS-removed partial embryo. Both ABa-specific IL2 neurons (arrowhead) and ABp-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) P_2-removed, EMS-enucleated partial embryos. The ABa-specific IL2 neurons (arrowhead) are apparent; however, no ABp-specific intestinal valve cells are seen. IL2 neurons were observed in all P_2-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).

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



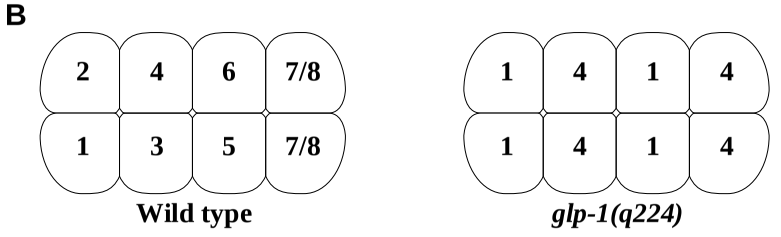


Fig. 4. AB lineages in *glp-1(q224)* mutants (A) Representative lineages of all AB^8 cells in *glp-1(q224)* embryos are shown and compared to lineages of the wild-type AB^8 cells. For simplicity, lineages are indicated only from the seventh to the ninth sequential AB divisions. The left-right order of 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 ABa lineages and the third and fourth columns are ABp 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 ABa 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).

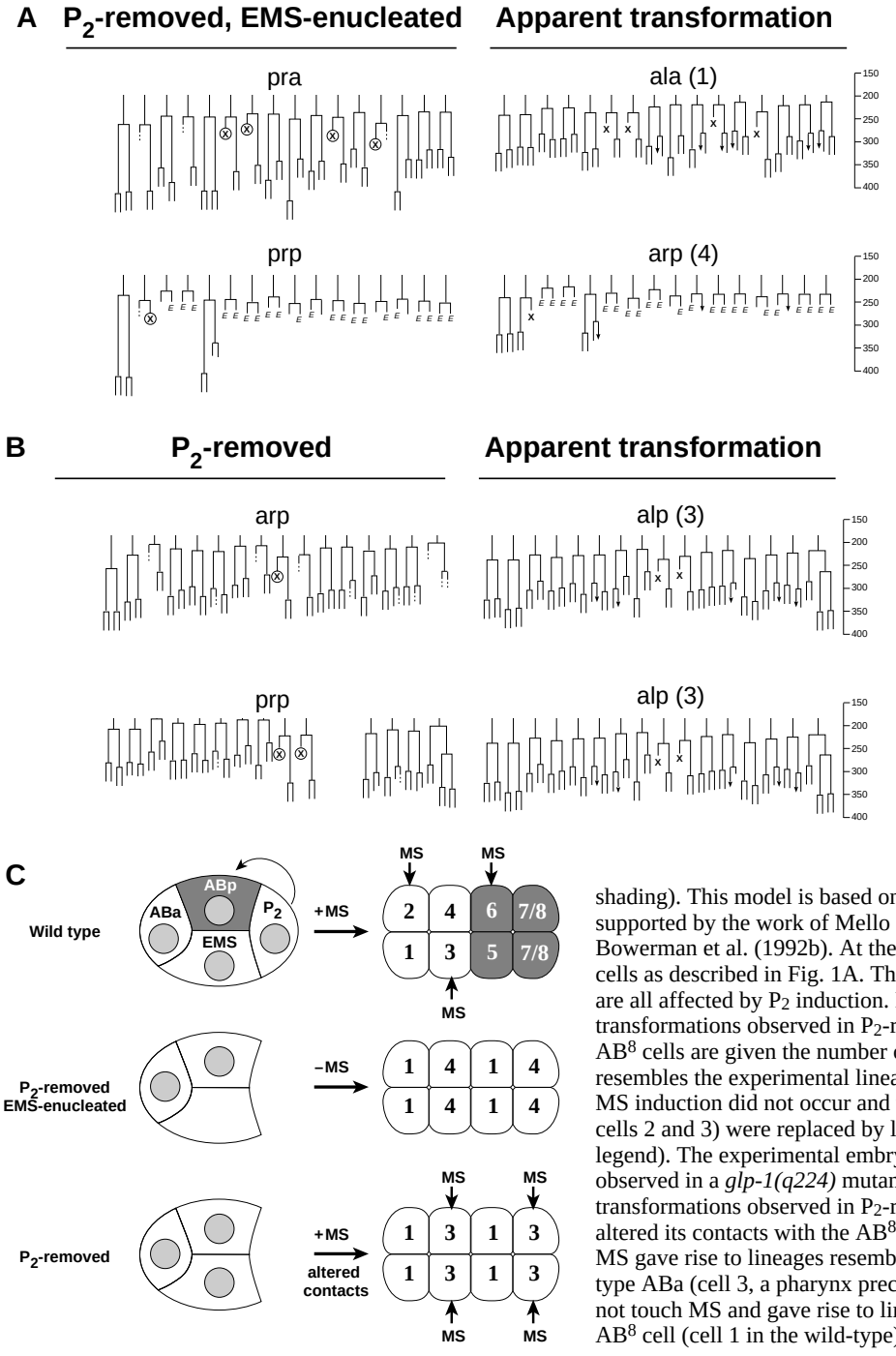


Fig. 5. AB lineages in manipulated embryos. (A) Representative lineage data from AB-derived partial embryos in which P_2 was removed and EMS was enucleated. Lineages are indicated only from the seventh to the ninth AB divisions. The first column shows selected lineages of the manipulated embryos and the second column, the corresponding wild-type AB^8 lineage to which there is an apparent transformation. Nomenclature and symbols are as described in Fig. 4. P_2 and the nucleus of EMS were removed by micromanipulation (see Materials and Methods). Embryos were recorded by 4-D time-lapse at 18°C. Each lineage represents data from a single embryo. Additional lineage results are presented in Table 3. (B) Lineage data obtained from partial embryos in which P_2 was removed by micromanipulation (see Materials and Methods). Embryos were recorded by 4-D time-lapse at 20°C. Format follows part A. Additional lineage results are presented in Table 4. (C) Summary of lineage transformations observed in manipulated embryos. Embryos are shown at the 4-cell stage to indicate the effects of P_2 induction. At the right are shown the lineage patterns produced by the AB^8 cells in the indicated embryos. AB^8 cells that are contacted and induced by MS are indicated. Top: model of 4-cell embryo showing induction of ABp fate by P_2. This interaction causes the entire ABp lineage to be altered (indicated by dark shading). This model is based on the findings reported here and is also supported by the work of Mello et al. (1994), Mango et al. (1994), and Bowerman et al. (1992b). At the right is shown the pattern of wild-type AB^8 cells as described in Fig. 1A. The fates of the four AB^8 cells derived from ABp are all affected by P_2 induction. Middle: summary of AB^8 cell lineage transformations observed in P_2-removed, EMS-enucleated partial embryos. The AB^8 cells are given the number of the wild-type AB^8 cell whose lineage resembles the experimental lineage. Because the nucleus of EMS was absent, MS induction did not occur and the normal MS-induced lineages (wild-type cells 2 and 3) were replaced by lineages 1 and 4, as expected (see Fig. 4 legend). The experimental embryo showed the same cell fate transformations as observed in a *glp-1(q224)* mutant (see Fig. 4). Bottom: Summary of AB^8 cell transformations observed in P_2-removed partial embryos. In such embryos, MS altered its contacts with the AB^8 cells as shown. The four AB^8 cells that touched MS gave rise to lineages resembling that of an MS-induced AB^8 cell from wild-type ABa (cell 3, a pharynx precursor in the wild-type). The other four cells did not touch MS and gave rise to lineages that were similar to that of an uninduced AB^8 cell (cell 1 in the wild-type).

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 P_2 reported by Bowerman et al. (1992b).

These data demonstrate that removal of P_2 and the nucleus of EMS completely transforms the fate of ABp to an ABa-like fate, implying that a P_1 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.

P_2 is required to specify the entire fate of ABp

The above experiment shows that the entire ABp fate is induced by a signal requiring P_2, 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.

Table 3. Comparison of lineages of P_2-removed, EMS-enucleated partial embryos with wild-type lineages

AB^8 blastomere lineaged	Total no. descendants lineaged	WT AB^8 blastomere compared	No. of descendants with WT behavior	% Match to wild-type	Proposed AB^8 transformation compared	No. of descendants with transformed behavior	% Match to proposed transformation
ala (2)	6	ala	6	100%	NT	NT	NT
ara (2)	2	ara	0	0	ala	2	100%
alp (2)	4	alp	0	0	arp	4	100
arp (2)	2	arp	2	100	NT	NT	NT
pla (2)	2	pla	0	0	ala	2	100
pra (3)	41	pra	18	44	ala	40	98
plp (2)	31	plp	0	0	arp	31	100
prp (3)	30	prp	4	13	arp	30	100

Lineage data were obtained from 4-D time-lapse recordings of three P_2-removed, EMS-enucleated partial embryos (see Materials and Methods). Parameters for this table are the same as for Table 2. Recordings were performed at 20°C. P_2 was removed and EMS was enucleated within 2 minutes of the time that P_2 and EMS divided (see Materials and Methods).

Table 4. Comparison of lineages of P_2-removed partial embryos with wild-type lineages

AB^8 blastomere lineaged	Total no. descendants lineaged	WT AB^8 blastomere compared	No. of descendants with WT behavior	% Match to wild-type	Proposed AB^8 transformation compared	No. of descendants with transformed behavior	% Match to proposed transformation
ala (2)	18	ala	18	100%	NT	NT	NT
ara (2)	13	ara	7	54	ala	13	100%
alp (2)	14	alp	14	100	NT	NT	NT
arp (2)	30	arp	5	17	alp	30	100
pla (2)	16	pla	6	38	ala	16	100
pra (3)	50	pra	18	36	ala	50	100
plp (2)	58	plp	34	59	alp	58	100
prp (3)	63	prp	43	68	alp	63	100

Lineage data were obtained from 4-D time-lapse recordings of three P_2-removed partial embryos (see Materials and Methods). Parameters for this table are the same as for Table 2. Recordings were performed at 22°C. P_2 was removed within 4 minutes of the time that it was distinct from its sister (see Materials and Methods).

The findings of Bowerman et al. (1992b), and the results presented here, suggest that P_2 alone may be required to specify the entire ABp fate. This hypothesis was tested directly by removing P_2 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 P_2 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 P_2-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 quantitative comparison showed that the ABp lineages did not match the wild-type ABp lineages (36-68% match), but did match the lineages of particular ABa descendants (100% match; Table 4). These results show that P_2 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 P_2 is prevented from touching either of them, implying that ABp adopts an ABa-like fate in the absence of P_2. 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 P_2 to ABp interaction acting in combination with other regulatory inputs (see Discussion).

Contact with MS is necessary and sufficient to induce AB^8 cells

The position of cells in the wild-type embryo suggests that physical contact of AB^8 cells with MS is both necessary and sufficient for MS induction (Gendreau et al., 1994; Mello et al., 1994; Mango et al., 1994; H. Hutter and R. Schnabel, personal communication). The altered contacts between the AB^8 cells and MS in embryos lacking P_2 allowed us to test this hypothesis further. An AB^8 cell (wild-type cell 2 in Fig. 5C) that touches MS and is induced in wild-type embryos did not contact MS in these partial embryos. This cell was apparently not induced by MS, and gave rise to a lineage that was similar to the lineage of its normally uninduced opposite-side homologue (cell 1; Fig. 5C). This confirms that MS contact per se is necessary to specify an MS-induced fate. Another AB^8 cell (cell 4; Fig. 5C, wild-type) that does not contact MS and is not induced in wild-type embryos contacted MS in these partial embryos. This cell gave rise to a lineage that was similar to the lineage of its normally induced opposite-side homologue (cell 3; Fig. 5C). This result implies that MS contact is sufficient for induction.

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 P_2 blastomere, by inducing the entire ABp fate, promotes widespread differences between the ABp and ABa lineages. A P_2-dependent interaction that induces production of intestinal valve cells by ABp was first recognized by Bowerman et al. (1992b); here we show that a P_2 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 P_2/ABp interaction acts in combination with the MS inductive signal, and a

process that controls the underlying fate of AB^8 cells to direct the final identity of each AB^8 cell (see below).

An early cellular interaction causes a polarity reversal at the AB^8 cell stage

The ability to produce epidermis becomes restricted to one cell of each pair of AB^8 sisters (Gendreau et al., 1994; Fig. 1B). We have found that P_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_2-induced reversal causes epidermis to arise from the four central AB^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 blocks 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_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_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^8 cells (Fig. 5C). Left and right AB^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_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^{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_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_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^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^8 cells appear to represent sublineage modules or cassettes that can be executed as complete units. These findings suggest that the identity of each AB^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^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^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^8 blastomere to specify a unique fate. The underlying identity of each AB^8 cell is in turn controlled by a process that restricts epidermal potential to one daughter of each AB^4 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^8 cells is apparent when the results of the two P_2 removal experiments (Fig. 5)

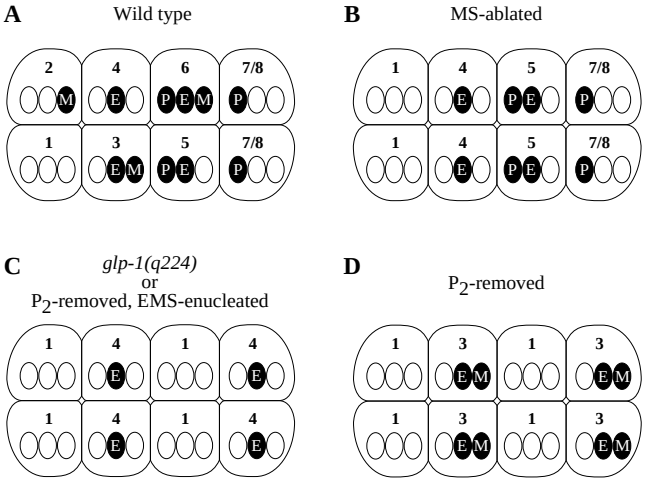


Fig. 6. Combinatorial model for AB^8 blastomere fate specification. The figure shows schematic representations of the AB^8 cells in wild-type and mutant or manipulated embryos. The results are based on the findings reported here and elsewhere (Gendreau et al., 1994; Mello et al. (1994); Mango et al. (1994); H. Hutter and R. Schnabel, personal communication). The fate of each AB^8 cell is indicated by the number of the wild-type AB^8 cell that shows the same fate. The three ovals within each cell represent the three regulatory inputs that control AB^8 cell fates. These three inputs are: (1) induction of the ABp fate by P_2 (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 AB^8 cell receives that input, an open oval indicates that it does not. Although induction of ABp by P_2 occurs two divisions before the AB^8 stage, the fates of all four ABp-derived AB^8 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 AB^8 cells from ABp that receive this input in the absence of P_2 induction are the sisters of the AB^8 cells that receive it following P_2 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 P_2 or MS inductions. Cell 4 represents the uninduced fate of a blastomere that has epidermal potential, whereas cell 1 represents the uninduced 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. P_2 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, P_2 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 P_2 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 AB^8 cells are changed. Five AB^8 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 AB^8 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 P_2 is removed and EMS is enucleated, both the P_2 and MS inductions are prevented. The regulatory inputs in six AB^8 cells are changed. Two AB^8 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 AB^8 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 P_2 induction causes a reversal in the distribution of epidermal potential in the AB lineage. (D) In partial embryos lacking P_2, P_2 induction does not occur and MS changes its contacts with the AB^8 cells. Only two AB^8 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 AB^8 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 P_2 induction causes a reversal in the distribution of epidermal potential in the AB lineage.

are compared. The only difference between these experiments was that the EMS nucleus was removed in one but not the other. When this nucleus was removed, preventing MS induction, four AB^8 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 AB^8 cells to be induced by MS, each showed lineage patterns characteristic of a single AB^8 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 AB^8 blastomere is determined by MS induction in combination with its underlying identity.

Similarly, our lineage analysis suggests that induction of ABp by P_2 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. P_2 induction alters the consequences of MS induction. Extra nervous system, but not pharynx, is made in the ABp-derived AB^8 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 identities is generated within the AB lineage (Fig. 6). The precise identity of individual AB^8 blastomeres appears to depend on three events: the two *glp-1*-dependent inductions, which involve sequential signaling by inducing cells, P_2 and MS, and the regulatory process that restricts epidermal potential to one daughter of each AB^4 cell. Each appears to be an independent regulatory input that controls a binary decision. These three regulatory inputs act in various combinations, and each unique combination appears to specify a unique AB^8 cell identity. In principle, 2^3, or eight, different combinations of regulatory inputs are possible. Of these eight possibilities, seven actually occur in the wild-type embryo based on cell contacts (Fig. 6). As predicted from the model, seven clearly distinct lineage patterns emerge from the AB^8 cells in wild-type embryos (Sulston et al., 1983; Fig. 1), reflecting the seven different combinations of inputs. Two of the AB^8 cells (cells

7/8) receive the same set of inputs and give rise to virtually identical lineage patterns.

This model is supported by the analysis of genetically and physically manipulated embryos (Fig. 6). When a particular combination of inputs occurs in an ectopic position, the AB^8 blastomere receiving the new set of inputs adopts a lineage that is strikingly similar to that of the wild-type AB^8 blastomere that normally receives that set of instructions. In sum, the code in seven of the eight AB^8 blastomeres has been experimentally altered (Fig. 6). In all cases, the expected changes in cell lineage have ensued. These lineage patterns are accurately reiterated irrespective of position in the embryo, suggesting that they are intrinsic to an AB^8 cell. This model is consistent with the ablations of Sulston et al. (1983) which showed that the fates of many cells appear to be determined cell autonomously by the AB^{32} stage. Thus, the fates of AB descendants are not fixed before the AB^8 cell stage. Once the AB^8 cells are specified, however, their fates appear to be largely fixed by cell-intrinsic developmental programs.

We are grateful to Judith Kimble and members of the Kimble laboratory for strains, use of the laser and much sage advice; to Michelle Sims and Neil First for assistance in the micromanipulation experiments and for allowing us to use their micromanipulation apparatus; and to Judith Kimble, Susan Mango and John White for useful discussions. We thank Bruce Bowerman, Harold Hutter, Susan Mango, Craig Mello, Jim Priess, Ralf Schnabel and Jocelyn Shaw for communicating results prior to publication and Jocelyn Shaw for providing anti-*unc-33* antibody. We are grateful to Judith Kimble, Jeff Hardin, Asako Sugimoto and Becky Terns, for critically reading the manuscript, and John White for comments on an earlier version. I. M. was supported by an NIH Cell and Molecular Biology Training Grant. This work was supported by the Chicago Community Trust/Searle Scholars Program, the Milwaukee Foundation Shaw Scientists Program and by a grant from the NIH (1RO1-GM48137).

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(Accepted 25 July 1994)

Note added in proof

The work cited throughout as H. Hutter and R. Schnabel has now been published as: **Hutter, H. and Schnabel, R.** (1994). *glp-1* and inductions establishing embryonic axes in *C. elegans*. *Development* **120**, 2051-2064. The work cited throughout as S. Mango, personal communication is now in press as: **Mango, S. E., Lambie, E. J. and Kimble, J.** (1994a). The *pha-4* gene is required to generate the pharynx primordium of *C. elegans*. *Development*, in press.