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Two maternal genes, *apx-1* and *pie-1*, are required to distinguish the fates of equivalent blastomeres in the early *Caenorhabditis elegans* embryo

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SUMMARY

In a 4-cell *Caenorhabditis elegans* embryo, two sister blastomeres called ABa and ABp are born with equivalent developmental potential, but eventually produce distinct patterns of cell fate. The different fates of ABa and ABp are specified at least in part by inductive interactions with neighboring blastomeres. Previous studies indicate that, at the 4-cell stage, a signal from the posterior-most blastomere, P_2, is required for ABp to produce at least one of its unique cell types. This P_2/ABp interaction depends on *glp-1*, a putative receptor for intercellular interactions.

To investigate this early induction further, we isolated mutants in which ABp developed abnormally. We describe the effects of recessive mutations in *apx-1*, a maternal gene that appears to be required for P_2 to signal ABp. In embryos from mothers homozygous for mutations in *apx-1* (*apx-1* embryos), ABp fails to produce its characteristic cell types. Instead, ABp from *apx-1* embryos develops more like its sister ABa: it produces ABa-like pharyngeal cells and it recapitulates ABa-like cell lineages. Because mutations in *apx-1* affect the development of only the ABp blastomere, we suggest that the wild-type gene encodes a component of the P_2/ABp signalling pathway. To explain the observation that ABp in *apx-1* embryos adopts an ABa-like fate, we propose a model in which the P_2 signal is required to break the initial equivalence of ABa and ABp. We performed two independent tests of this model. First, we examined ABp development in *pie-1* mutant embryos, in which P_2 adopts the identity of another blastomere. We find that, in *pie-1* embryos, ABp fails to produce its characteristic cell types and instead adopts a fate similar to that of ABa. We conclude that the changed identity of P_2 in *pie-1* embryos prevents the P_2/ABp interaction. As a second test, we examined ABp development in wild-type embryos after physically removing P_2. These operated embryos produce extra pharyngeal cells, consistent with our proposal that a signal from P_2 breaks the initially equivalent developmental state of ABa and ABp. We discuss the possibility that *apx-1* acts as a ligand in this *glp-1*-dependent signalling pathway.

Key words: *apx-1*, *pie-1*, *glp-1*, *Caenorhabditis elegans*, equivalence groups, cell signalling, maternal gene, fate map, blastomere

INTRODUCTION

In a developing organism, cells initially equivalent in developmental potential become influenced to adopt different fates by interacting with neighboring cells (for a review, see Greenwald and Rubin, 1992). Studies of the nematode *Caenorhabditis elegans* led to the first demonstrations that certain pairs or groups of cells, called equivalence groups, are born with the potential to express either of two alternative fates (Kimble and Hirsh, 1979; Kimble, 1981; Sulston and White, 1980). Cell interactions are then required to determine which cell adopts which fate. In some cases, variability is observed in the decision, but in other cases precursors always make the same choice, even though they initially are capable of expressing more than one fate (e.g. Sulston et al., 1983; Bowerman et al., 1992a; Chamberlin and Sternberg, 1993). Studies in the grasshopper (Taghert et al., 1984; Kuwada and Goodman, 1985; Doe and Goodman, 1985), leech (Weisblat and Blair, 1984), and more recently zebrafish (Eisen et al., 1990; Eisen, 1992) have demonstrated that equivalence groups are widely conserved as a developmental strategy.

The earliest example of an equivalence group in *C. elegans* is found at the 4-cell stage of embryogenesis. Two sister blastomeres called ABa and ABp are born equivalent but ultimately express very different patterns of cell fate (Fig. 1; Sulston et al., 1983, Priess et al., 1987). For example, the anteriorly positioned blastomere ABa generates cells that form the pharynx, an organ used for feeding. In contrast, the dorsal-most blastomere ABp produces no pharyngeal cells, but does contribute many specialized cell types not made by ABa. These ABp-specific fates include a group of cells that form the excretory system, several cell types that form parts of the anus and rectum, and a variety of specialized neurons (Sulston et al., 1983). In spite of their ultimately divergent patterns of development, ABa and ABp initially are capable of replacing each other if their positions are switched at the beginning of

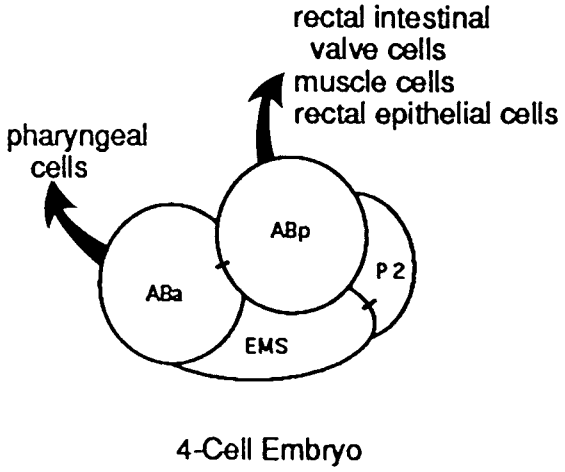

Fig. 1. A 4-cell stage *C. elegans* embryo. The two sister blastomeres, ABa (anterior) and ABp (dorsal), are born with equivelant developmental potential (Priess and Thomson, 1987). Ultimately however, only ABa will contribute descendants to the pharynx, while only ABp will generate non-pharyngeal muscles, intestinal-rectal valves and rectal-epithelial cells. While there are many differences in the cell fate patterns generated by these two blastomeres, ABa and ABp both produce many of the epidermal cells and neural cells made during embryogenesis in *C. elegans* (Sulston et al., 1983).

the 4-cell stage (Priess and Thomson, 1987). Thus ABa and ABp constitute an equivalence group in the early *C. elegans* embryo, and the specification of their different identities is essential for establishment of the nematode body plan.

The initial equivalence of ABa and ABp suggests that cell interactions with neighboring blastomeres might be reponsible for distinguishing their eventual fates. A likely source for an inductive signal that breaks the ABa/ABp equivalence is the P_2 blastomere. Because P_2 touches ABp but not ABa, a non-diffusible signal from P_2 would influence only ABp (see Fig. 1). Indeed, a previous analysis has shown that if P_2 is destroyed, ABp is unable to produce at least one cell type it normally makes, the intestinal-rectal valve cells (Bowerman et al., 1992a). However, the ultimate fate of ABp in the absence of P_2 signalling, beyond the absence of valve cells, has not been addressed. In this paper, we show that mutations in a newly identified maternal gene, *apx-1*, and in the maternal gene *pie-1*, appear to prevent the P_2/ABp interaction. In *apx-1* and in *pie-1* mutant embryos, ABp fails to produce its normal cell types and instead develops more like ABa. We also show that after removal of P_2, operated wild-type embryos produce extra pharyngeal cells and no intestinal-rectal valve cells, consistent with both ABa and ABp adopting ABa-like fates in the absence of a P_2 induction. We propose that the P_2/ABp interaction breaks the equivalence of ABa and ABp, and we discuss models in which *apx-1(+)* encodes a component of the P_2/ABp signalling pathway.

MATERIALS AND METHODS

Strains and culture

N2 was used as the standard wild-type strain and cultured as previously described (Brenner, 1974).The following alleles were used: *dpy-18 (e364), pie-1(zu127); pie-1(zu154), glp-1(zu24), unc-25 (e156)* LG III; *lag-2(s1486), dpy-11(e224), unc-46(e177)* LG V. The balancers *DnT1* (translocation balancer for LG IV and V), *eT1* (translocation balancer for LG III and V), *qC1* (inversion balancer for LG III) were used, as well as the deficiencies *sDf70* and *lag-2(q387)*. *apx-1* embryos were obtained from homozygous *apx-1* mothers produced by *apx-1*/*DnT1* heterozygotes. The *glp-1* allele *zu24* was isolated in a previous screen for maternal-effect mutants (B. Bowerman and J. Priess, unpublished data). *zu24* resembles the weak *glp-1* allele *e2142* (Priess et al., 1987; Kodoyianni et al., 1992) in that many *zu24* embryos hatch (89/112) but lack an anterior pharynx. Double mutant *glp-1;apx-1* embryos were obtained from homozygous hermaphrodites produced by *unc-32 glp-1/eT1 III; dpy-11 apx-1(or3)/eT1* parents. *pie-1* embryos were obtained from the strains *dpy-18 pie-1(zu127)/qC1* and *pie-1(zu154) unc-25/qC1*.

Genetics

Three mutant alleles (*or3, or15 and or22*) of *apx-1* (anterior pharynx excess) were isolated in a genetic screen (see Kemphues et al., 1988) of roughly 28,000 haploid genomes, in which we searched for mutants with altered ABp development. For the first allele, we searched for embryos with unusual patterns of epidermal cells. Hypodermal cells in wild-type embryos at the 'bean stage' of embryogenesis form stereotyped rows of ventral, lateral and dorsal hypodermal cells (Sulston et al., 1983). Inspection of *apx-1* mutant embryos at the bean stage indicates that they often have rows of what resemble dorsal hypodermal cells but no recognizable patterns of lateral or ventral hypodermal cells (not shown). After isolating the first allele, we subsequently looked for embryos with extra pharyngeal cells. The frequency at which we found the alleles (1×10^{-4}) is similar to that at which reduction of function mutations in other maternal genes have been found. For example, in this same screen, we isolated one allele of *glp-1* and three alleles of *skn-1*. The three *apx-1* alleles map to chromosome V, between *lag-2* and *unc-46* [2/102 Unc nonLag recombinants from *apx-1(or3)/lag-2 unc46* did not pick up *or3*]. The alleles *or15* and *or22* were shown to be linked to chromosome V and failed to complement *apx-1(or3)*. The three alleles result in indistinguishable embryonic phenotypes (data not shown) and are completely penetrant for embryonic-lethality [e.g. 0/2340 embryos from 14 homozygous *apx-1(or3)* mothers hatched]. The *apx-1* allele *zu215* (C. Mello) fails to complement *or3*, and Mello et al., 1994, have reported a similar phenotypic description of *apx-1* mutant alleles. *apx-1/Df* animals were made by mating *apx-1/+* males into *lag-2(q387)/DnT1* (dominant Unc) hermaphrodites. Instead of 1/2 of the nonUnc outcross hermaphrodite adults being *apx-1/Df* and producing dead embryos, only 1/5 of the nonUnc adults produced *apx-1* mutant embryos (10/50). Other nonUnc animals died as larvae, most with apparently abnormal rectums and were presumably hemizygous for *apx-1*. Mutations in the *apx-1* homologue *lag-2* (Tax et al., 1994) cause rectal defects (Lambie and Kimble, 1991b), but the defects in hemizygous *apx-1* animals appear different, often having anal openings connected to the posterior end of the intestine.

Embryo manipulations

The monoclonal antibodies 5.6 and 9.2.1 (Miller et al., 1983), and an anti-intermediate filament antibody (Pruss et al., 1981), were used to detect the production of non-pharyngeal muscle cells, pharyngeal muscle cells, and pharyngeal marginal cells, respectively. The monoclonal antibody J126, from S. Strome, shows a staining pattern identical to that of the antibody 2CB7 described in Bowerman et al., 1992a (B. Bowerman and S. Mango, unpublished data) and was used to detect intestinal-rectal valve cells and pharyngeal gland cells. The monoclonal antibodies 3NB12, NE2/1B4 and NE8/46C.3 were isolated in a screen for cell-type-specific monoclonal antibodies (see Okamoto and Thomson, 1985). 3NB12 (Priess and Thomson, 1987) was used to detect pharyngeal muscle cells in Fig. 4, and in Fig. 6. NE2/1B4 (Okamoto and Thomson, unpublished data) was used to

detect epidermal seam cells produced by ABa and ABp in *apx-1(or3)* embryos. NE8/46C.3 (Okamoto and Thomson, unpublished data) was used to stain and count the body wall muscle cells produced by P3 and C in *apx-1(or3)* embryos. Because the muscle cells often are in clusters, it is difficult to count cell numbers precisely. The monoclonal antibody OIC1D4 (from S. Strome) was used to detect P-granules. The promoter trap strain UL8 (Hope, 1991; Young and Hope, 1993) was used to score for the production of rectal-epithelial cells (Mango et al., unpublished data) by staining with X-GAL (Fire, 1992).

Cell lineage analysis was done as described previously (Sulston et al., 1983; Mango et al., 1991), using Nomarski optics with a video-recording system (Hird and White, 1993). Blastomere names reflect their lineage. For example, ABa and ABp are the anterior and posterior daughters, respectively, of the 2-cell stage blastomere AB. Laser ablations, embryo fixation and antibody staining procedures, microscopy, and photography were performed as described previously (Bowerman et al., 1992b).

To analyze the production of ABp-specific cell types in wild-type, *apx-1*, and *pie-1* embryos (Fig. 2 and Tables 1 and 2), all blastomeres except ABp were killed in late 4-cell stage embryos, shortly before the next set of embryonic cleavages. This was done to allow any interaction between P_2 and ABp to occur (one can prevent the P_2/ABp interaction only by killing P_2 immediately after it is born (see Bowerman et al., 1992a). The development of the blastomeres P_3, C, and EMS in *apx-1(or3)* embryos were analyzed by killing all other blastomeres besides P_2 or EMS at the 4-cell stage (and then one of the daughters of P_2 to analyze P_3 and C).

To test the ability of ABa and ABp to produce pharyngeal cells in response to a signal from MS in wild-type, *apx-1*, and *pie-1* embryos (Fig. 3, Tables 1 and 2), all blastomeres other than either ABa and EMS, or ABp and EMS, were killed at the 4-cell stage, the E daughter of EMS was killed at the 8-cell stage, and the MS daughter of EMS was killed at the 12-cell stage, by which time one can no longer prevent the MS-dependent induction of ABa pharyngeal cells in wild-type embryos (Hutter and Schnabel, 1994; Mango et al., unpublished data). Laser ablation experiments to assay the production of induced pharyngeal cells by individual descendants of ABa and ABp are described in the legend to Table 1.

P_2 was removed from 4-cell stage wild-type embryos through a hole in the eggshell, according to S. Gendreau et al., manuscript in preparation. Embryos were allowed to develop 20 hours at 15°C, and then stained either with J126 (for intestinal-rectal valves), or first with 3NB12 (for pharyngeal muscle), and second with J126. In most embryos, different J126+ cell types could be distinguished by their different cellular morphology, making it possible to score for the presence of intestinal-rectal valve cells and intestine. We scored pharyngeal cell production only in embryos that made intestinal cells.

The ability of each ABp grand-daughter to produce epidermal seam cells (see Sulston et al., 1983) in *apx-1(or3)* embryos was analyzed by killing P_2 and EMS at the 4-cell stage, and all AB descendants but one ABp grandaughter at the 12-cell stage. After developing to the equivalent of hatching, the operated embryos were fixed and stained with the seam cell-specific antibody NE2/1B4 (see above). Neither ABpla or ABpra produced differentiated descendants that stained positively (0/17 and 0/13 operated embryos, respectively). When ABprp was allowed to develop, only 4/27 operated embryos stained positively. In contrast, 13/18 operated *or3* embryos stained positively when ABplp was allowed to develop.

RESULTS

The *apx-1* locus

To identify genes required for the P_2/ABp cell interaction, we screened for maternal-effect, embryonic-lethal mutations that result in abnormal ABp development (see Materials and Methods). We isolated three recessive alleles that map to the left arm of chromosome V and fail to complement each other. These alleles define the gene *apx-1* (anterior pharynx excess; also see Mello et al., 1994). Mutations in *apx-1* are strictly maternal in that sperm from wild-type males do not rescue the embryonic lethality [0/431 *apx-1(or3)*/+ embryos hatched after mating +/+ males into homozygous *apx-1(or3)* mothers purged of their own maternal supply of sperm] and do not cause any apparent change in the phenotype of the mutant embryos (data not shown). While the frequency with which we isolated these alleles and their recessive nature argue that the mutations reduce gene function, they may not be null. Essentially all homozygous *apx-1(or3)* animals [from *apx-1(or3)*/+ parents] survive to adulthood (212/213) and produce dead embryos, but over 50% of hemizygous animals [*apx-1(or3)/Deficiency*] die during larval development (see Materials and Methods), possibly due to abnormalities in the structure of the rectum (data not shown). We see a low frequency (<1%) of similar rectal defects in animals homozygous for any of the three alleles we have isolated (data not shown). Hemizygous mothers that survive to adulthood produce dead embryos with a phenotype indistinguishable from that of embryos produced by homozygous *apx-1(or3)* mothers (as judged by morphological criteria using Nomarski optics to score 50 mutant embryos for the presence of excess pharyngeal cells, as well as for the presence of neurons, epidermal cells, body wall muscles, intestinal cells, germ cells, and cell deaths). We conclude that the embryonic phenotype represents a loss of *apx-1* function, but *apx-1* may have additional function(s) late in embryogenesis or during larval development.

ABp fails to produce its characteristic cell types in *apx-1* mutants

In a wild type embryo, the ABp blastomere produces two intestinal-rectal valve cells, three rectal-epithelial cells and four muscles (Sulston et al., 1983). To determine the fate of ABp in *apx-1* embryos, we examined whether ABp from *apx-1* mutants produces these specific cell types. All blastomeres but ABp were destroyed with a laser microbeam late in the 4-cell stage. ABp was then allowed to develop to the equivalent of hatching and analyzed for the production of ABp-specific cell types by staining for cell-type-specific markers (see Materials and Methods). Whereas almost all ABp blastomeres from wild-type embryos produce intestinal-rectal valve cells, rectal epithelial cells and muscle cells, ABp from *apx-1* mutants fails to produce each of these ABp-specific cell types (Fig. 2; Table 1). Thus, *apx-1(+)* activity is required for the proper development of the ABp blastomere.

To determine if blastomeres other than ABp develop normally in *apx-1(or3)* embryos, we examined the cell types produced by P_2, EMS and ABa. We used a laser ablation strategy similar to the one described above to isolate the development of each blastomere. To examine the fate of P_2 in *apx-1* embryos, we analyzed the development of its two daughters, P_3 and C. As in wild-type embryos (Sulston et al., 1983), P_3 produces about 20 body wall muscle cells: 18-21 muscle cells could be counted in each of 8/8 operated embryos stained with an antibody specific for body wall muscle. C, which normally produces epidermal cells and 32 body wall muscle cells (Sulston et al., 1983), also appears to develop normally in *apx-*

1 embryos. In 6/6 operated *apx-1* embryos, C produced epidermal cells (scored by their characterstic morphology using Nomarski optics) and body wall muscle cells (26-34 cells stained positively in each operated embryo). Similar numbers of body wall muscle cells were produced by P_3 and C in operated wild-type embryos (data not shown). We also analyzed the development of the P_2-derived germ-line precursors by staining a mixed-stage population of *apx-1(or3)* embryos with antibodies that recognize germ-line-specific P-granules (Strome and Wood, 1983). During wild-type embryogenesis, P-granules are segregrated to P_2, and subsequently to two descendants of P_2 that give rise to the germ line postembryonically (Strome and Wood, 1983). In *apx-1(or3)* embryos, P-granules segregation was normal (data not shown), and two germ-line precursors were present in all late stage embryos (*n*=163; scored by staining with antibodies that recognize P-granules). Thus the development of P_2 appears unaffected by mutations in *apx-1*. These results suggest that the altered development of ABp in *apx-1* mutant embryos is not due to generalized defects in P_2 identity leading to a loss of P_2 signalling (see below).

We next examined the fate of the EMS blastomere, which normally produces pharyngeal cells, body wall muscles and intestine (Sulston et al., 1983). Similarly, in 24/24 operated *apx-1(or3)* embryos, EMS produced pharyngeal muscle and body wall muscle cells, as scored by antibody staining; and 26/26 operated embryos generated intestinal cells, as scored by their production of birefringent gut granules. Finally, we examined the development of ABa. We found that ABa from *apx-1* embryos produces a normal pattern of pharyngeal cells (see below and Table 1) and specialized epidermal cells called seam cells (5/5 operated embryos stained positively for seam cells). These results suggest that the 4-cell stage blastomeres P_2, EMS, and ABa develop normally in *apx-1* embryos.

ABp produces ectopic pharyngeal cells in *apx-1* embryos

Because ABa and ABp are born equivalent in a wild-type embryo, we wanted to test the possibility that in the absence of *apx-1* function ABp might adopt a fate more like that of ABa. One important difference in the fates of these two blastomeres is that ABa, but not ABp, normally produces pharyngeal cells (Sulston et al., 1983). From visual inspection using Nomarski optics, it appeared likely that *apx-1* mutant embryos produce extra pharyngeal cells, an observation confirmed by staining intact *apx-1* embryos with an antibody that recognizes pharyngeal muscles (Fig. 4). Indeed, if all blastomeres other than ABp are destroyed with a laser microbeam, ABp, like ABa, can produce pharyngeal cells in *apx-1* mutant embryos (Fig. 3).

In a wild-type embryo, ABa and one other 4-cell stage blastomere, EMS, produce pharyngeal cells (Sulston et al., 1983). Pharyngeal cells produced by ABa differ from those made by EMS with respect to the specific cell types made and the developmental pathways used to generate them. For example, only EMS generates pharyngeal gland cells, while ABa and EMS both produce pharyngeal muscles and support cells called pha-

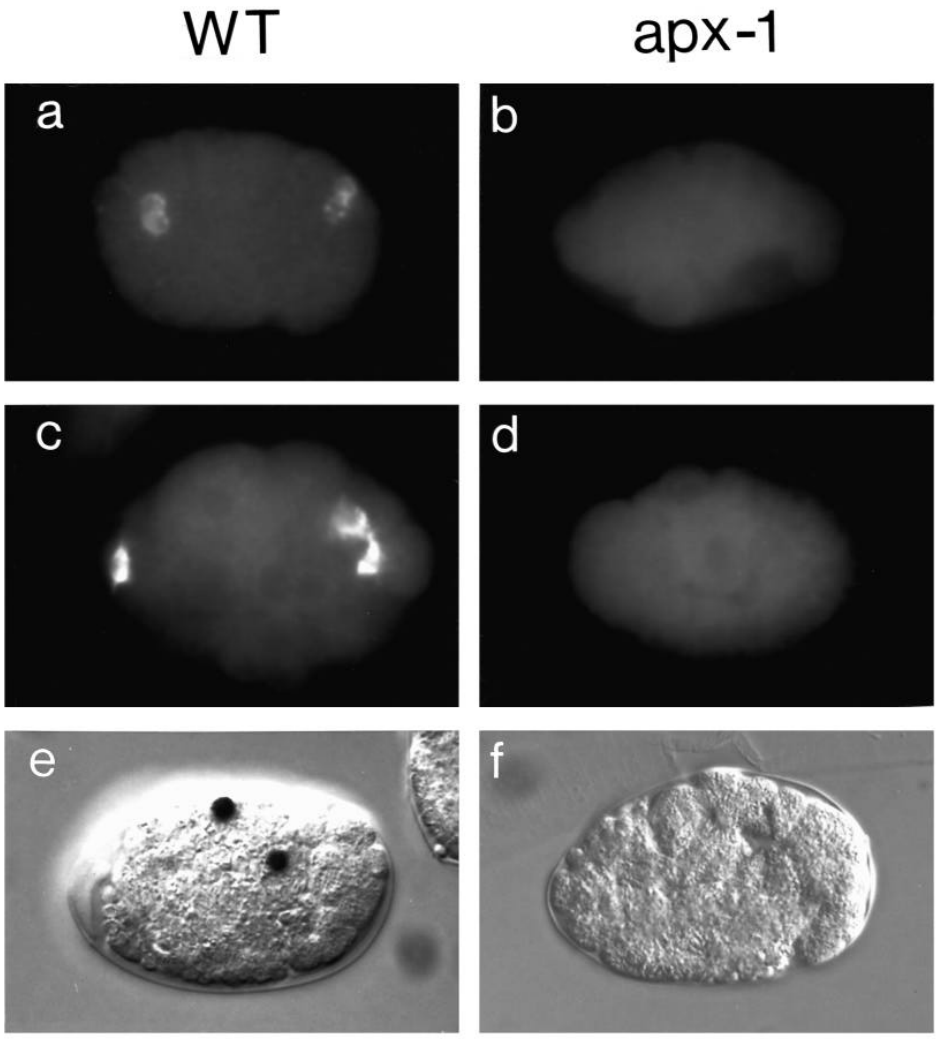

Fig. 2. *apx-1* mutant embryos fail to produce ABp-specific cell types. After laser ablation of all blastomeres except ABp, wild-type (left column, WT) and *apx-1(or3)* embryos (right column) were analyzed for the production of three differentiated cell types normally made by ABp and not by ABa: intestinal-rectal valve cells (a and b), muscle cells (c and d) and rectal-epithelial cells (e and f). In these experiments, no inductive signalling from MS was possible because the parent of MS (EMS) was killed with a laser microbeam. Therefore, the failure of ABp to produce its characteristic cell types in *apx-1* mutant embryos is not due to the MS induction causing ABp lineages that would otherwise produce ABp-specific cell types to instead produce pharyngeal cells.

ryngeal marginal cells (Sulston et al., 1983). In *apx-1(or3)* embryos, ABp produces muscle and marginal cells, but no gland cells, as does ABa from either wild-type or *apx-1(or3)* animals (Fig. 3). Moreover, approximately the same number of pharyngeal muscle cells are produced by ABp from *apx-1(or3)* as are made by ABa from either wild-type or *apx-1(or3)* embryos (12-16 cells, see Fig. 3). Thus, ABp produces the number and types of pharyngeal cells characterstic of ABa, not EMS.

Another characteristic feature of pharyngeal cell production by ABa is that it depends on cell signalling. Specifically, a signal from the MS daughter of EMS at about the 12-cell stage induces two grand-daughters of ABa to produce pharyngeal cells (see Fig. 7; Priess and Thomson, 1987; Hutter and Schnabel, 1994; Mango et al., submitted). Because of the requirement for cell signalling, ABa does not produce pharyngeal cells if MS is destroyed at the 8-cell stage. However, laser ablation of MS at the 12-15 cell stage cannot block ABa from producing pharyngeal cells (Hutter and Schnabel, 1994; Mango et al., unpublished data). Similarly, the production of pharyngeal cells by ABp in *apx-1(or3)* embryos depends on MS: neither ABa nor ABp produces pharyngeal muscles if MS is killed at the 8-cell stage (0/11 operated embyos stained positively with antibodies that recognize pharyngeal muscle), but they both produce pharyngeal cells if MS is killed at the 12-cell stage (Fig. 3 and Table 1). We conclude that ABp in *apx-1* embryos depends on an interaction with MS to produce pharyngeal cells.

In a wild-type embryo, the interaction between MS and ABa descendants depends on *glp-1*, a putative receptor for cell signals (Austin and Kimble, 1987; Priess et al., 1987; Austin and Kimble, 1989; Yochem and Greenwald, 1989; Evans et al., 1994; Crittenden et al., in press). Like ABa in *glp-1* mutant embryos, neither ABa nor ABp produce pharyngeal muscle cells in embryos doubly mutant for both *apx-1* and *glp-1* (Fig. 4). In summary, the production of pharyngeal cells by ABp in *apx-1* embryos depends on the MS blastomere and on *glp-1* activity, as does the production of pharyngeal cells by ABa in both wild-type and *apx-1(or3)* embryos. These data suggest that ABp in *apx-1* mutants develops more like its sister ABa.

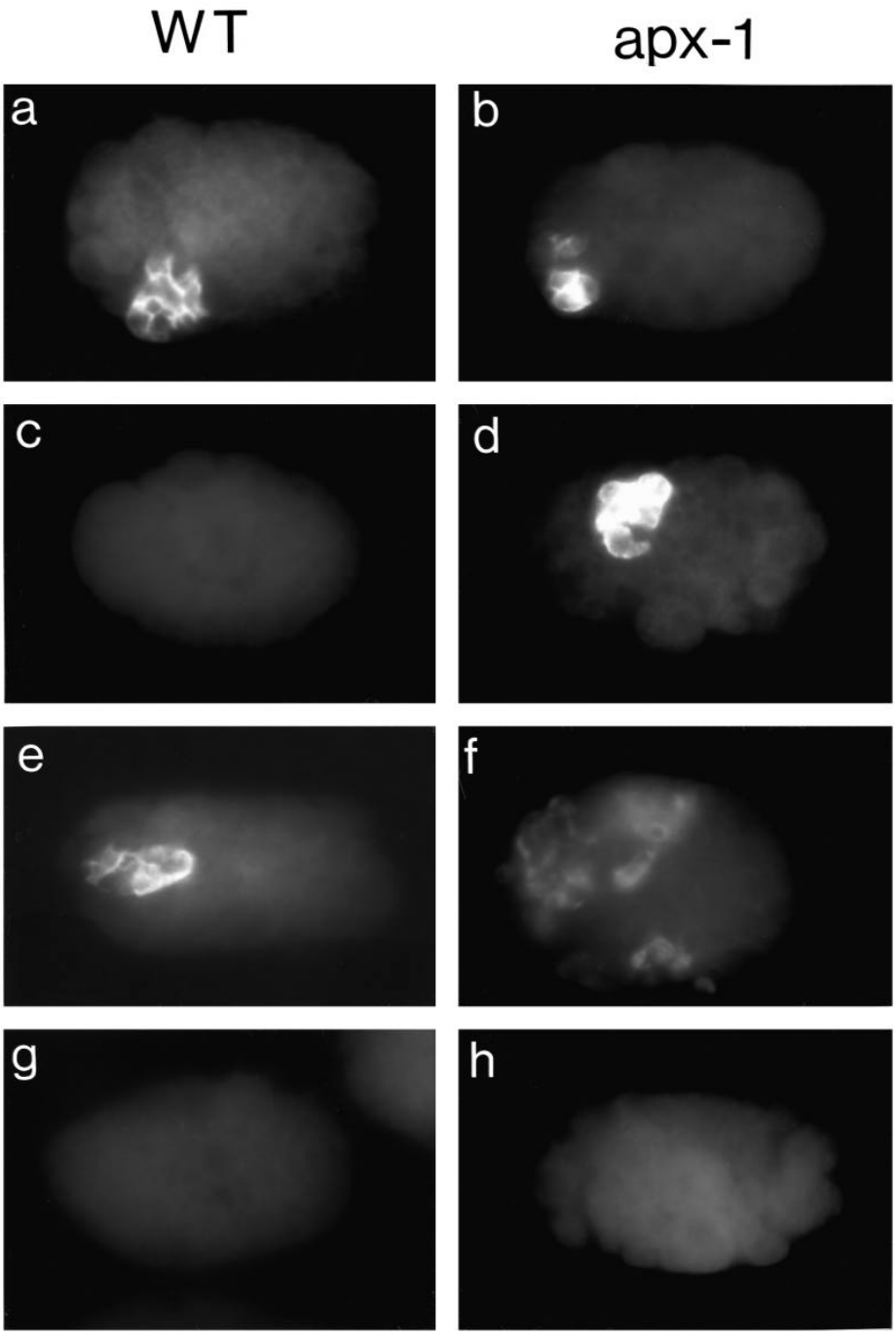

Fig. 3. ABp in *apx-1* mutant embryos produces ABa-like pharyngeal cells. ABa from wild-type (left column, WT) and *apx-1(or3)* mutant embryos produce pharyngeal muscle cells (a and b, respectively). In contrast, ABp produces pharyngeal muscle cells in *apx-1* embryos (d), but not in wild-type embryos (c). In e-h, descendants of both ABa and ABp were allowed to develop together. Normally, ABa produces pharyngeal muscles (a) and structural cells called pharyngeal marginal cells (e), but no pharyngeal gland cells (g). The presence of extra marginal cells in operated *apx-1* embryos (f) is consistent with both ABa and ABp producing marginal cells in *apx-1* mutants. However, neither ABa nor ABp in *apx-1(or3)* embryos produce gland cells (h). Therefore both ABa and ABp in *apx-1* embryos produce only pharyngeal cell types normally made by ABa in wild-type embryos.

Table 1. Development of ABa and ABp in wild-type and *apx-1* embryos

I.

	Fraction of operated embryos in which ABp made:		
	Intestinal valve cells	Muscle cells	Rectal epithelial cells
apx-1(or3)	0/18	0/16	0/25
apx-1(or15)	0/10	0/7	–
Wild type	10/10	14/14	13/14

II.

Blastomere allowed to develop after MS induction	Fraction of operated embryos in which the indicated blastomere made pharyngeal muscle cells: *apx-1(or3)* embryos	*apx-1(or15)* embryos	Wild type embryo
(1) ABa	11/11	8/9	14/15
(2) ABalpa	6/7	–	8/8
(3) ABalpp	0/27	–	0/7
(4) ABaraa	9/9	–	6/6
(5) ABarap	13/13	–	7/7
(6) ABp	14/15	6/6	0/17
(7) ABpla	3/26	–	–
(8) ABpra	2/18	–	–
(9) ABplpa	15/18	–	–
(10) ABplpp	1/21	–	–
(11) ABprpa	16/19	–	–
(12) ABprpp	3/26	–	–

I. The ability of ABp to produce its characteristic cell types was analyzed. ABp in wild-type embryos produces intestinal-rectal valve cells, non-pharyngeal muscle cells, and rectal-epithelial cells. In contrast, *apx-1* embryos fail to produce each of these ABp-specific cell types. **II.** The ability of ABa and ABp, and some of their descendants, to produce pharyngeal muscles in *apx-1(or3)* and in wild-type embryos was assessed. While in wild-type embryos only ABa (1) and not ABp (6) is induced to produce pharyngeal muscle cells, in *apx-1* embryos both ABa and ABp (1 and 6) respond to the MS induction by producing pharyngeal muscles. The ability of specific descendants of ABa and ABp to produce pharyngeal cells was also analyzed (see below for laser ablation protocols). These experiments show that the two posterior grand-daughters of ABp in *apx-1* embryos (ABprp and ABplp; see Figure 7 for a diagram showing the positions of these blastomeres) both develop like the wild-type grand-daughter of ABa called ABalp: only the anterior daughters of ABalp (2), ABplp (9), ABprp (11) produce pharyngeal cells in *apx-1* embryos, while their posterior daughters do not (3, 10 and 12). In contrast, both daughters of the ABa grand-daughter ABara produce pharyngeal cells in wild-type and *apx-1* embryos (4 and 5). Finally, the anterior grand-daughters of ABp (ABpla and ABpra) only rarely produce any pharyngeal cells in *apx-1* mutant embryos (7 and 8). Laser ablation strategies: If one waits until the 12-cell stage, or later, to destroy MS, it is possible to eliminate the production of MS-derived pharyngeal cells without preventing the MS induction of AB-derived pharyngeal cells (see Materials and Methods). In experiment 3, MSaa, MSpa, ABaraa, ABarap, ABalpa and all eight ABp great grand-daughters were killed with a laser microbeam between the 24-cell and 28-cell stages. No pharyngeal cells are produced, arguing that no other blastomeres produce pharyngeal cells in *apx-1* mutants. To assay the ability of ABpla and ABpra to produce pharyngeal cells (7,8), the same blastomeres were destroyed as in experiment 3, except either ABplaa and ABplap, or ABpraa and ABprap were allowed to develop. In experiments 2, 9 and 10, ABpr was killed at the 8-cell stage, MS at the 12-cell stage, and the remaining descendants of ABa and ABp (see experiment 3), except for the indicated blastomere, were killed between the 12-cell and 28-cell stages. In experiments 4, 5, 11 and 12, ABpl was killed at the 8-cell stage, MS at the 12-cell stage, and the remaining descendants of ABa and ABp (see experiment 3), except for the indicated blastomere, were killed between the 12-cell and 28-cell stages. Because the same ABa descendants in operated wild-type embryos (2-5) produce pharyngeal cells as in intact wild-type embryos (see Sulston et al., 1983), these ablation protocols appear not to disrupt cell contacts required for a normal patterning of pharyngeal cell fates.

Two ABp descendants develop like an ABa descendant in *apx-1* embryos

The observation that ABa and ABp in *apx-1* mutants produce similar numbers and types of pharyngeal cells suggests that ABp descendants may adopt fates similar or even identical to those of ABa descendants in a wild-type embryo. To test this hypothesis, we observed the patterns of cell division and programmed cell death produced by certain ABp descendants (see drawing in Fig. 7 showing the positions of ABp descendants). We found that two posterior ABp grand-daughters, ABplp and ABprp from *apx-1(or3)* embryos each generate a cell lineage pattern that is characteristic of the posterior wild-type ABa grand-daughter ABalp (Fig. 5). These results suggest that ABplp and ABprp in *apx-1* embryos adopt ABalp-like identities.

We also examined the patterns of differentiated pharyngeal cells produced by ABplp and ABprp from *apx-1(or3)* embryos. In a wild-type embryo, two ABa grand-daughters produce pharyngeal cells, ABalp and ABara. Both daughters of ABara generate pharyngeal cells, but only the anterior daughter of ABalp produces pharyngeal cells (Sulston et al., 1983). In *apx-1(or3)* embryos, only the anterior daughters of ABplp and ABprp produce pharyngeal muscles, similar to ABalp in wild-type (Fig. 5 and Table 1). These patterns of cell lineage and terminal cell fate argue that the two ABp grand-daughters ABplp and ABprp in *apx-1* embryos recapitulate the development of the ABa grand-daughter ABalp. We also note that because the cell lineages followed correspond to a portion of the ABalp lineage that does not generate pharyngeal cells, the similarity between ABplp and ABprp in *apx-1* mutants and ABalp in wild-type embryos extends beyond the production of pharyngeal cells.

ABp produces pharyngeal cells in *pie-1* embryos

The failure of ABp to produce its characteristic cell types in *apx-1* embryos suggests that the P_2/ABp interaction, shown previously to be required for the proper development of ABp (Bowerman et al., 1992a), may be defective in *apx-1* mutants. Based on our analysis of the *apx-1* phenotype, we suggest that in the absence of P_2/ABp signalling, the equivalence between ABa and ABp is not broken and therefore ABp develops more like ABa. As an independent test of this model, we examined the development of ABp in *pie-1* mutant embryos. In *pie-1* mutants, P_2 appears to adopt a fate identical to its sister EMS (Mello et al., 1992), thereby 'genetically ablating' the P_2 blastomere. Consistent with a loss of P_2 identity resulting in a loss of P_2/ABp signalling, ABp in *pie-1* embryos appears to develop similarly to ABa in wild-type embryos: neither intestinal-rectal valve cells nor muscle cells are produced by ABp, which instead makes pharyngeal cells in response to inductive signals from MS (Table 2). These results support a model in which signalling from P_2 breaks the equivalence of ABa and ABp.

ABa and ABp adopt similar fates after removal of P_2 in wild-type embryos

One prediction of our P_2/ABp interaction model is that removal of P_2 from wild-type embryos should result in the wild-type ABp blastomere producing pharyngeal cells. To test this prediction, P_2 was physically removed from wild-type embryos 4-

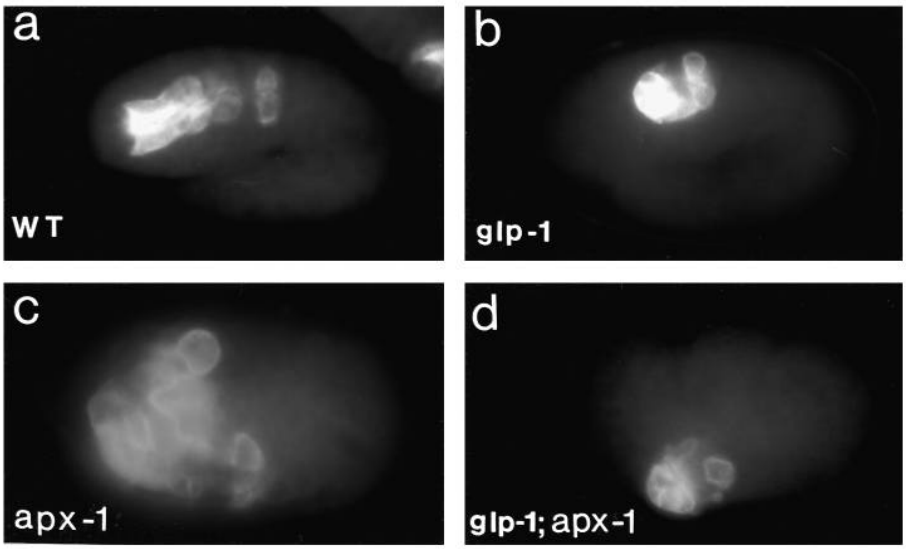

Fig. 4. The production of pharyngeal cells by ABa and ABp in *apx-1* mutants depends on *glp-1(+)* activity. (a) Wild-type (WT) embryo showing the normal complement of pharyngeal muscle cells as detected by staining with the antibody 3NB12. (b) A *glp-1(zu24)* mutant embryo, in which ABa fails to produce anterior pharyngeal cells, and only MS-derived pharyngeal cells are present. (c). An *apx-1(or3)* mutant embryo, showing the presence of extra pharyngeal cells in the anterior (left) part of the embryo. (d) a *glp-1; apx-1(or3)* double mutant embryo, in which neither ABa nor ABp produce pharyngeal cells and only MS-derived pharyngeal cells are present [0/12 *glp-1; apx-1(or3)* embryos produced pharyngeal muscles after killing MS at the 12-cell stage]. All embryos are shown with anterior to the left and dorsal to the top, at about 1000× magnification.

5 minutes after the P_1 blastomere divided into P_2 and EMS. These operated embryos fail to produce intestinal-rectal valve cells, indicating that the interaction between P_2 and ABp was prevented (3/3, data not shown). Significantly, these operated embryos often produce extra pharyngeal muscle cells (7/10; Fig. 6). The P_2/ABp interaction appears to occur early in the cell cycle, as operated embryos rarely produce extra pharyngeal cells if P_2 is removed more than 5 minutes after the division of P_1 (1/6; Fig. 6). We suggest that after removal of P_2 in wild-type embryos, ABp develops like its sister ABa.

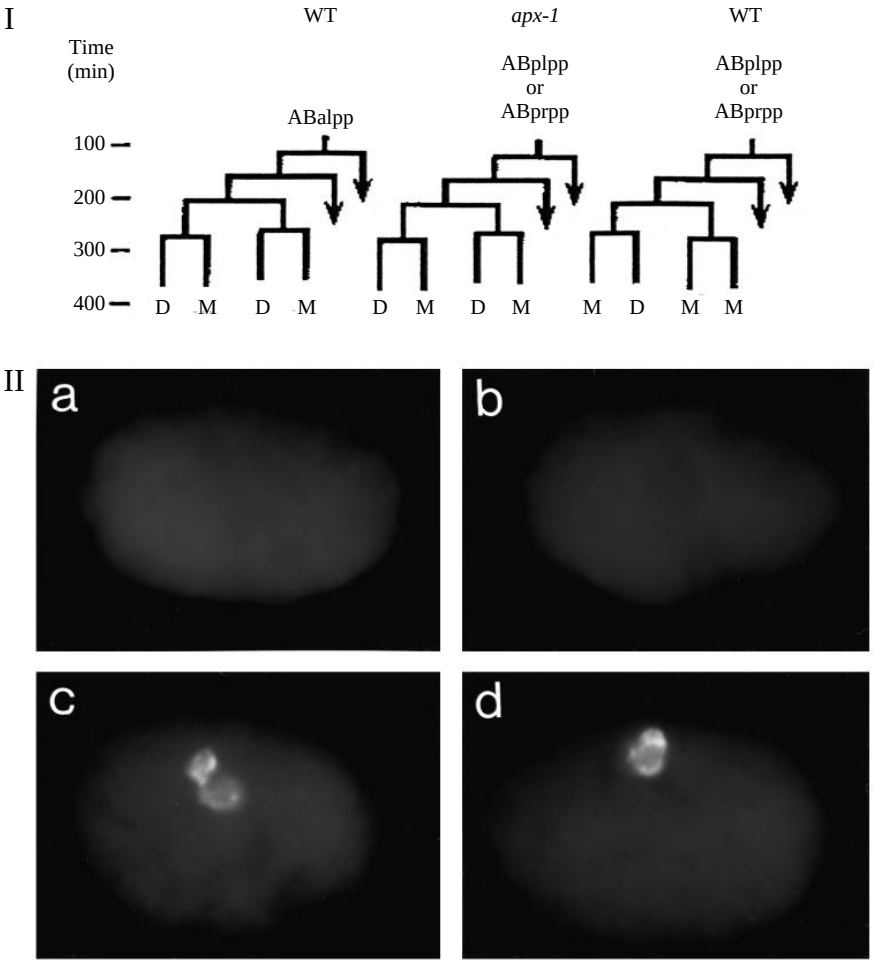

Fig. 5. Two ABp grand-daughters in *apx-1* mutants develop like a wild-type ABa grand-daughter. (I) Lineage diagrams showing the development of two ABp descendants (ABplpp and ABprpp) in *apx-1(or3)* mutant embryos (middle), the same ABp descendants in a wild-type embryo (right, from Sulston et al., 1983), and the wild-type ABa descendant ABalpp (left, Sulston et al., 1983). The pattern of cell division (M) and cell deaths (D) produced by both ABplpp (n=2) and ABprpp (n=3) in *apx-1* mutants resembles the pattern normally produced only by the wild-type ABa descendant ABalpp. In addition, ABalpp in *apx-1(or3)* embryos produces a cell lineage pattern indistinguishable from ABalpp in wild-type (data not shown). Horizontal lines represent cell divisions, and vertical lines indicate the approximate time between divisions (scale at left). (II) In *apx-1(or3)* mutant embryos, the production of pharyngeal cells by two ABp grand-daughters mimics that of the wild-type ABa grand-daughter ABalp. In wild-type embryos, ABalp divides into an anterior daughter that produces pharyngeal cells and a posterior daughter that does not (see Table 1; Sulston et al., 1983). Similarly, in *apx-1(or3)* embryos, the anterior daughters of both ABplp and ABprp (ABplpa and ABprpa) produce pharyngeal cells (c and d, respectively), while their posterior daughters (ABplpp and ABprpp) do not (a and b, respectively).

Table 2. ABp develops like ABa in *pie-1* mutant embryos

I.	Fraction of operated embryos in which ABp made:	
	Intestinal-rectal valve cells	Muscle cells
pie-1(zu127)	3/20	1/21
pie-1(zu154)	2/13	2/15

II.	Fraction of operated animals in which the indicated blastomere made pharyngeal muscle cells in response to MS induction:	
	ABa	ABp
pie-1(zu127)	10/10	14/15
pie-1(zu154)	8/8	14/16

ABp in *pie-1* mutant embryos fails to produce ABp-specific cell types (Part I) and instead develops more like ABa, producing pharyngeal cells in response to the MS induction (Part II). If P_2 and EMS are killed in *pie-1* mutant embryos at the 4-cell stage, neither ABa nor ABp produce pharyngeal cells (Mello et al., 1992). We also tested the ability of the C blastomere to induce pharyngeal cell production in *pie-1(zu127)* embryos. C is a daughter of P_2 (see Fig. 7) which in *pie-1* embryos develops like a wild-type MS blastomere (Mello et al., 1992). Consistent with C adopting an MS identity early in development, it can sometimes induce descendants of ABp, which touch C, to produce pharyngeal muscle cells (10/18 embryos). However, C cannot induce descendants of ABa, which are not in contact with C, to produce pharyngeal cells (0/11 embryos). Thus cell contact may be necessary for MS blastomeres to induce pharyngeal cell production (see Discussion). To analyze C induction, either ABa and EMS, or ABp and EMS, were killed with a laser microbeam at the 4-cell stage. C was then killed at the 12-cell stage.

DISCUSSION

We have shown that two maternal genes, *apx-1* and *pie-1*, are required for proper development of the ABp blastomere. In *apx-1* and *pie-1* mutants, ABp fails to produce its characteristic cell types and instead develops more like its sister ABa, producing pharyngeal cells in response to an inductive signal from MS. We can phenocopy the *apx-1* and *pie-1* production of extra pharyngeal cells by removing the P_2 blastomere from wild-type embryos. Therefore, we propose that the alteration in ABp fate observed in *apx-1* and *pie-1* embryos reflects a block in P_2/ABp signalling at the 4-cell stage. We suggest that normally in embryogenesis, the P_2/ABp interaction breaks the initial equivalence of ABa and ABp, leading to the production of ABp-specific cell types and rendering ABp incapable of producing pharyngeal cells in response to the subsequent signal from MS (see Fig. 7). While the effect of *pie-1* on the P_2/ABp interaction is probably indirect, due to the changed identity of P_2, we suggest that *apx-1* participates directly in the P_2/ABp signalling pathway.

apx-1 and blastomere signalling

The *apx-1* locus could play one of two possible roles during ABp specification. First, *apx-1* could encode a component of the P_2/ABp signalling pathway. Alternatively, *apx-1* could code for a localized factor, or determinant, that contributes to the proper specification of ABp (for a review, see Wood and Edgar, 1994). Given that ABa and ABp are born developmentally equivalent, mutations in such a 'determinant of AB fate' would be expected to affect both ABa and ABp. Because ABa

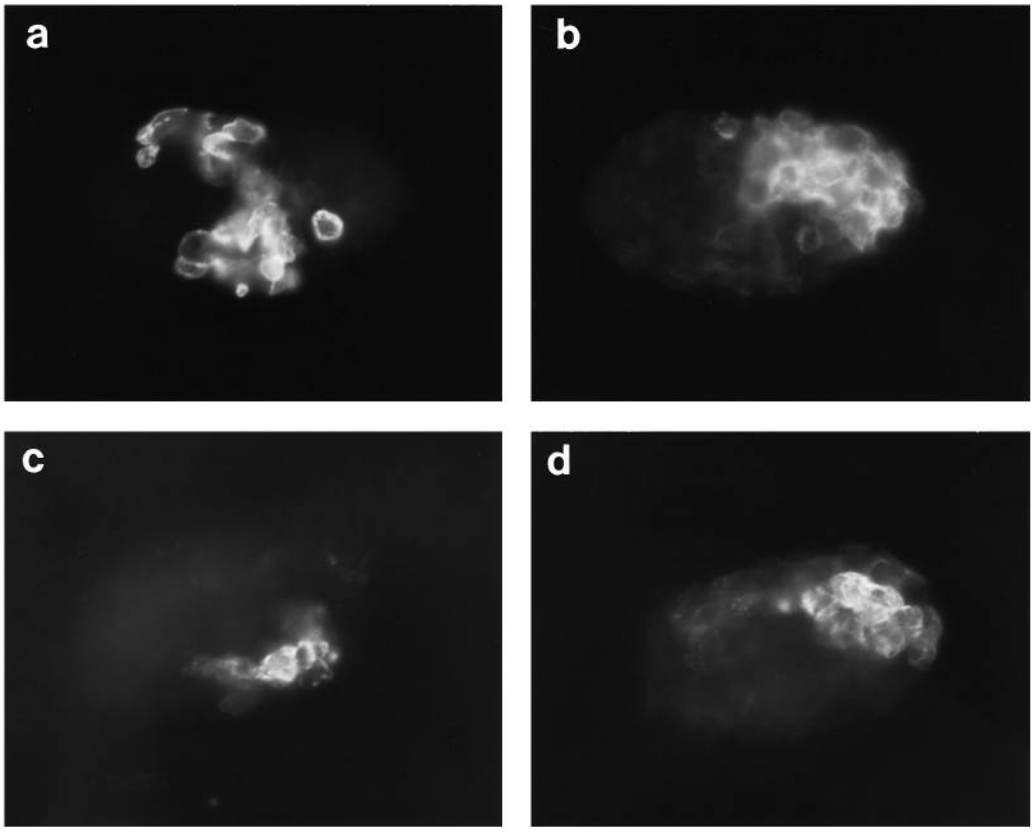

Fig. 6. Extra pharyngeal cells are produced by wild-type embryos after removal of the P_2 blastomere. P_2 was removed from wild-type embryos either 5 minutes (a,b) or >5 minutes (c,d) after the parent of P_2 had divided. Excess pharyngeal muscle cells are often produced from the embryos in which P_2 was removed early (a). We counted 26-28 muscle cells in each operated embryo, n=3. Embryos in which P_2 was removed later usually made normal amounts of pharyngeal muscle (c). Approximately 13-18 muscles were counted in these embryos, n=3. Both operated embryos produced intestinal cells (b, d). Therefore, the production of extra pharyngeal cells in (a) is not due to E adopting an MS-like fate (see Goldstein, 1992). Embryos were stained with 3NB12 (a,c) or J126 (b,d).

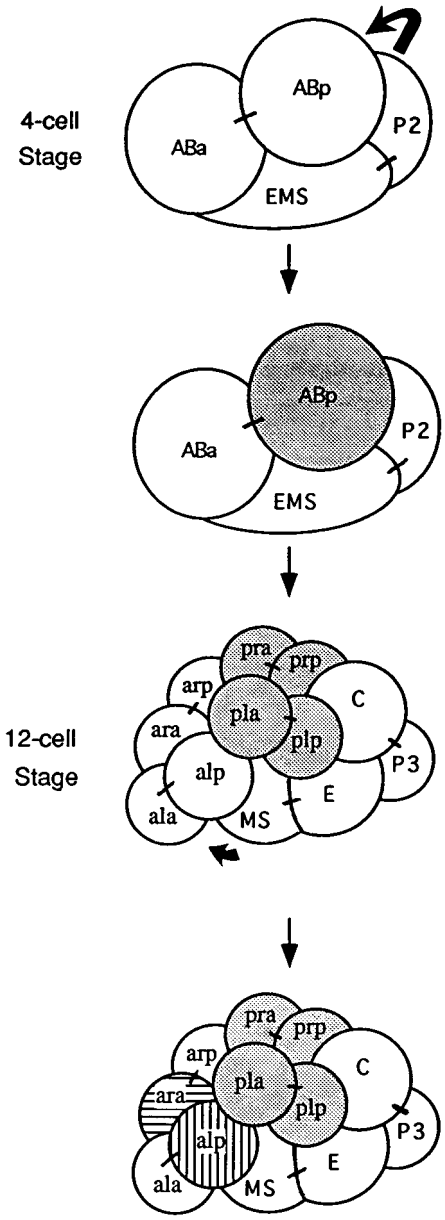

Fig. 7. A model in which two spatially and temporally distinct cell interactions distinguish the fates of ABa and ABp. In a wild-type embryo, ABa and ABp are born equivalent in developmental potential (Priess and Thomson, 1987). We propose that an interaction between P_2 and ABp during the 4-cell stage (large arrow) breaks the equivalence of ABa and ABp (indicated with shading). We suggest that the signal emanating from P_2 cannot diffuse throughout the embryo and therefore affects ABp and not ABa. A second, distinct interaction occurs between the 8- and 15-cell stages (small arrow), in which the MS blastomere signals ABa descendants to produce several cell types, including pharyngeal cells, indicated here by cross-hatching (Priess and Thomson, 1987, Hutter and Schnabel, 1994; Mango et al., submitted). These early interactions contribute to the specification of both the dorsal/ventral axis (by influencing the fate of ABp) and the anterior/posterior axis (by influencing the fate of ABa). Mutations in either *apx-1* or *pie-1* appear to disrupt the P_2/ABp interaction but not the MS induction. In both *apx-1* and *pie-1* mutants, ABp fails to produce its normal pattern of cell fates and instead develops more like ABa, producing pharyngeal cells in response to the MS signal. We suggest that *apx-1* acts directly in the P_2/ABp interaction, whereas *pie-1* may influence ABp development indirectly due to a loss of P_2 identity (P_2 in *pie-1* mutant embryos adopts a fate essentially identical to that of its sister, EMS; see Mello et al., 1992). Although in wild-type embryos ABp does not produce pharyngeal cells in response to MS, the MS signal does influence the cell fate patterns produced by ABp (Hutter and Schnabel, 1994). Thus, it appears that the P2/ABp interaction alters the response of ABp descendants to subsequent signalling from MS. For simplicity the response of ABp to MS is not illustrated in this schematic diagram.

development appears wild-type in *apx-1* mutants, we favor the first hypothesis, that *apx-1* is involved in P_2/ABp signalling. Moreover, the apparently normal development of P_2 in *apx-1* embryos suggests that *apx-1* functions directly in the P_2/ABp signalling pathway, and not in other processes that might indirectly affect signalling by altering the identity of P_2. These arguments are strengthened by recent sequence information that shows *apx-1* encodes a predicted transmembrane protein and is similar in sequence to *Delta* and *Serrate* in *Drosophila* and *lag-2* in *C. elegans* (Mello et al., 1994). These three genes encode candidate ligands for *Notch*-like receptors (Artavanis-Tsakonas et al., 1991; Tax et al., 1994; Henderson et al., unpublished data). The *glp-1* gene is a *Notch* homologue (Austin and Kimble, 1989; Yochem and Greenwald, 1989), and is required for the P_2/ABp interaction (Mello et al., 1994; Hutter and Schnabel, 1994; Moskowitz et al., unpublished data). Therefore, by analogy, *apx-1* may be a P_2-produced ligand for the *glp-1* receptor.

Two types of intercellular interactions have been shown to regulate *C. elegans* development: lateral signalling and inductive interactions (for a review, see Lambie and Kimble, 1991a). In lateral signalling, cells of equivalent developmental potential interact with each other to determine their fates. By contrast, inductive interactions involve non-equivalent cells, one signalling and the other receiving. The P_2/ABp interaction discussed here is an example of an inductive interaction, in which two equivalent cells, ABa and ABp, become different due to an interaction with a third non-equivalent cell, P_2. Because interactions that rely on lateral signalling can result in variable fate choices by individual cells (e.g. the AC/VU decision; see Kimble, 1981), it may be that the P_2/ABp inductive interaction prevents variability in the fates adopted by ABa and ABp.

In addition to the P_2 and MS inductions discussed here, other interactions may also be necessary to specify the different fates of ABa and ABp. Consistent with this idea, cell lineages of the two anterior ABp grand-daughters (ABpla and ABpra) in *apx-1(or3)* embryos did not appear to recapitulate ABa cell fates (S. Mango and B. Bowerman, unpublished observations). Specifically, the ABp descendant ABplapapaa in *apx-1(or3)* embryos did not appear to undergo programmed cell death (n=4), in contrast to the lineally equivalent descendant of ABa in wild-type embryos, ABalapapaa (see Sulston et al., 1983). Nor does ABpla produce pharyngeal cells or epidermal seam cells, as do other ABa descendants. Furthermore, although another ABp descendant, ABprp, generates a cell lineage pattern characteristic of ABarp, in the absence of MS induction (Mello et al., 1994), we have found that ABprp fails to produce at least one differentiated marker for ABarp fate (epidermal seam cells; see Materials and Methods). Thus, while *apx-1*

function, and by extension the P_2 interaction, appears to play a critical role in breaking the initial equivalence of ABa and ABp, additional interactions may influence the ultimate fates of ABa and ABp descendants.

glp-1, apx-1 and multiple cell interactions

The family of proteins with homology to *glp-1* and *Notch* are required for multiple cell interactions to specify many different cell fates (Austin and Kimble, 1987; Priess et al., 1987; Artavanis-Tsakonas et al., 1991). How do these proteins participate in so many different cell fate decisions? For example, how can ABa and ABp, which presumably are born with identical intracellular components, both use *glp-1* to specify completely different developmental pathways? One possibility is that P_2 and MS produce distinct ligands that interact with *glp-1* to trigger different downstream effects. Alternatively, the P_2 and MS inductions may act through distinct receptors that also require a common *glp-1* signalling pathway to specify different fates. The *Delta*-like sequence of *apx-1*, and the observation that *apx-1* is required for P_2 but not MS signalling, are consistent with either of these possibilities. Alternatively, P_2 and MS could send qualitatively identical signals, with ABa and ABp descendants responding differently depending on the time at which the signal is received. For example, a highly labile product that influences the response to the P_2 signal could be present in both ABa and ABp at the 4-cell stage when P_2 signalling occurs, but decay before the MS induction at the 12- to 15-cell stage. In this case, the specificity of *apx-1* for the P_2/ABp interaction may reflect a requirement for precise temporal and spatial control of the expression of a signalling molecule, and not a requirement for qualitatively different signals.

The P_2 blastomere is involved in two distinct cell signalling events

During wild-type embryogenesis, the P_2 blastomere is required for two inductive events: the P_2/ABp interaction discussed here, and an interaction between P_2 and EMS that is required for EMS to produce intestinal cells (Goldstein, 1992). Both interactions occur early in the 4-cell stage (Goldstein, 1992; Bowerman et al., 1992a; this work), and it is conceivable that P_2 might produce one signal that is interpreted differently by ABp and EMS. This scenario appears unlikely, however, as these two interactions are separable. In both *apx-1* and *pie-1* mutants, ABp development is disrupted, but EMS still generates its normal complement of intestinal cells. Moreover, after P2 removal, the operated wild-type embryos often produce intestinal cells, but not the ABp-derived intestinal-rectal valve cells. Therefore, we suggest that signalling between P_2 and ABp utilizes a different mechanism than does signalling between P_2 and EMS.

Cell contacts may be important for the P_2 and MS inductions

The orientation of the early embryonic cleavages and the rigidity of the chitinous eggshell in *C. elegans* result in ABa and ABp occupying anterior and dorsal positions, while the sisters P_2 and EMS occupy posterior and ventral positions, respectively (see Hyman and White, 1987, and Fig. 7). Thus, at the 4-cell stage, P_2 touches ABp, but not ABa. Because of this geometry, an inductive signal from P_2 that depends on cell contact would influence only ABp, providing a simple mechanism for breaking the ABa/ABp equivalence. The extent of contact between different blastomeres may be important at the 12-cell stage as well, when ABa descendants that are induced to produce pharyngeal cells have broad contacts with MS, whereas ABa descendants that do not produce pharyngeal cells have little or no contact. Similarly in *apx-1* embryos, only the two ABp grand-daughters that make extensive contact with MS are induced to produce pharyngeal cells. Another correlation between cell contact and the induction of AB descendants is observed in *pie-1* mutant embryos. In *pie-1* embryos, a daughter of P_2 (called C) develops like a wild-type MS blastomere (Mello et al., 1992). Consistent with C adopting an MS identity early in development, it can sometimes induce descendants of ABp, which touch C, to produce pharyngeal muscle cells. However, C cannot induce descendants of ABa, which do not contact C, to produce pharyngeal cells (see Table 2). These data argue that cell contact is necessary for the inductive interactions of P_2 and MS. Thus, in the early *C. elegans* embryo, as in the early *Drosophila* embryo, cell position appears to be critical for specifying cell fate. In *Drosophila*, the embryonic axes are specified in a syncytium, and identity appears to be determined largely by position within gradients of Bicoid, Nanos and nuclear Dorsal proteins (Driever, 1993; Chasan and Anderson, 1993; St. Johnston, 1993). In *C. elegans*, cell position is critical for determining cell contacts that may delimit which cells participate in different inductive interactions. Together, the early positioning of blastomeres and the inductive interactions that follow are crucial for properly establishing the nematode body plan.

Two exceptions to the correlation between cell contact and signalling are observed with MS and ABp descendants. First, the ABp grand-daughters ABplp and ABprp have extensive contacts with MS, but as yet MS has not been found to influence their wild-type development (e.g. Bowerman et al., 1992a). Thus *apx-1(+)* activity may render ABplp and ABprp completely insensitive to the MS signal. Second, ABpra depends on MS signalling to develop properly (Hutter and Schnabel, 1994), but makes relatively little contact with MS. These less extensive contacts may be sufficient for contact-mediated cell signalling. Alternatively, it is possible that ABpra is influenced by a diffusible MS signal, or that the effects of MS on ABpra are indirect.

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REFERENCES

Artavanis-Tsakonas, S., Delidakis, C. and Fehon, R. G. (1991). The *Notch*

locus and the cell biology of neuroblast segregation. *Ann. Rev. Genet.* **7**, 427-452.

Austin, J. and Kimble, J. (1987). *glp-1* is required for regulation of the decision between mitosis and meiosis in C. elegans. *Cell* **51**, 589-599.

Austin, J. and Kimble, J. (1989). Transcript analysis of *glp-1* and *lin-12*, homologous genes required for cell interactions during development of C. elegans. *Cell* **58**, 565-571.

Bowerman, B., Tax, F. E., Thomas, J. H., and Priess, J. R. (1992a). Cell interactions involved in development of the bilaterally symmetrical intestinal valve cells during embryogenesis in Caenorhabditis elegans. *Development* **116**, 1113-1122.

Bowerman, B., Draper, B. W., Mello, C. C., and Priess, J. R. (1992b). The maternal gene *skn-1* encodes a protein that is distributed unequally in early C. elegans embryos. *Cell* **74**, 443-452.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. *Genetics* **77**,71-94.

Chamberlin, H. M., and Sternberg, P. W. (1993). Multiple cell interactions are required for fate specification during male spicule development in Caenorhabditis elegans. *Development* **118**, 297-324.

Chasan, R. and Anderson, K. V. (1993). Maternal control of dorsal-ventral polarity and pattern in the embryo. In *The Development of Drosophila melanogaster* (eds. M. Bate and A. Martinez Arias), pp. 387-245. Cold Spring Harbor Laboratory Press.

Crittenden, S. L., Troeml, E., and Kimble, J. (1994). *glp-1* is localized to the mitotic region of the C. elegans germ line. *Development*, in press.

Doe, C. Q. and Goodman, C. S. (1985). Early events in insect neurogenesis. II. The role of cell interactions and cell lineage in the determination of neuronal precursor cells. *Dev. Biol.* **111**, 206-219.

Driever, W. (1993). Maternal control of anterior development in the Drosophila embryo. In *The Development of Drosophila melanogaster* (eds. M. Bate and A. Martinez Arias), pp. 301-324. Cold Spring Harbor Laboratory Press.

Eisen, J. S., Pike, S. H., and Romancier, B. (1990). An identified neuron with variable fates in embryonic zebrafish. *J. Neurosci.* **10**, 34-43.

Eisen, J. S. (1992). The role of interactions in determining cell fate of two identified motoneurons in the embryonic zebrafish. *Neuron* **8**, 231-240.

Evans, T. C., Crittenden, S. L., Kodoyianni, V. and Kimble, J. (1994). Translational control of maternal *glp-1* mRNA establishes an asymmetry in the C. elegans embryo. *Cell* **77**, 183-194.

Fire, A. (1992). Histochemical techniques for locating Escherichia coli β-galactosidase activity in transgenic organisms. *Genetic Analysis-Techniques and Applications* **9**, 5-6.

Goldstein, B. (1992). Induction of gut in *Caenorhabditis elegans* embryos. *Nature* **357**, 255-257.

Greenwald, I. and Rubin, G. M. (1992). Making a difference: the role of cell-cell interactions in establishing separate identities for equivalent cells. *Cell* **68**, 271-281.

Hird, S. N., and White, J. G. (1993). Cortical and cytoplasmic flow polarity in early embryonic cells of Caenorhabditis elegans. *J. Cell Biol.* **121**, 1343-1355.

Hope, I. (1991). 'Promoter trapping' in *C. elegans. Development* **113**, 399-408.

Hutter, H. and Schnabel, R. (1994). *iglp*-1 and inductions establishing embryonic axes in *C. elegans. Development* **120**, 2051-2064.

Hyman, A. A., and White, J. G. (1987). Determination of cell division axes in the early embryogenesis of Caenorhabditis elegans. *J. Cell Biol.* **105**, 2123-2135.

Kemphues, K. J., Priess, J. R., Morton, D. and Cheng, N. (1988). Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* **52**, 311-320.

Kimble, J. and Hirsch, D. (1979). The post-embryonic cell lineages of the hermaphrodite and male gonads in Caenorhabditis elegans. *Dev. Biol.* **70**, 396-417.

Kimble, J. (1981). Alterations in cell lineage following laser ablation of cells in the somatic gonad of Caenorhabditis elegans. *Dev. Biol.* **87**, 286-300.

Kodoyianni, V. Maine, E. M. and Kimble, J. (1992) Molecular basis of loss-of-function mutations in the *glp-1* gene of Caenorhabditis elegans. *Mol. Biol. Cell* **3**, 1199-1213.

Kuwada, J. Y. and Goodman, C. S. (1985). Neuronal determination during embryonic development of the grasshopper nervous system. *Dev. Biol.* **110**, 114-126.

Lambie, E. J., and Kimble, J. (1991a). Genetic control of cell interactions in nematode development. *Ann. Rev. Genetics* **25**, 411-436.

Lambie, E. J., and Kimble, J. (1991b). Two homologous regulatory genes, *lin-12* and *glp-1*, have overlapping functions. *Development* **112**, 231-240.

Mango, S. E., Maine, Eleanor, M., and Kimble, J. (1991). Carboxy-terminal truncation activates *glp-1* protein to specify vulval fates in *Caenorhabditis elegans. Nature* **352**, 811-815.

Mello, C. C., Draper, B. W., Krause, M., Weintraub, H. and Priess, J. R. (1992). The *pie-1* and *mex-1* genes and maternal control of blastomere identity in early C. elegans embryos. *Cell* **70**, 163-176.

Mello, C. C., Draper, B. W., and Priess, J. R. (1994). The maternal genes *apx-1* and *glp-1* and establishment of dorsal-ventral polarity in the early C. elegans embryo. *Cell* **77**, 95-106.

Miller, D. M., Ortiz, I., Berliner, G. C., and Epstein, H. F. (1983). Differential localization of two myosins within nematode thick filaments. *Cell* **34**, 477-490.

Okamoto, H. and Thomson, J. N. (1985). Monoclonal antibodies which distinguish certain classes of neuronal and supporting cells in the nervous tissue of the nematode Caenorhabditis elegans. *J. Neurosci.* **5**, 643-653.

Priess, J. R. and Thomson, J. N. (1987). Cellular interactions in early C. elegans embryos. *Cell* **48**, 241-250.

Priess, J. R., Schnabel, H. and Schnabel, R. (1987). The *glp-1* locus and cellular interactions in the early C. elegans embryo. *Cell* **51**, 601-611.

Pruss, R. M., Mirsky, R. Raff, M. C., Thorpe, R., Dowding, A.J., and Anderton, B.H. (1981). All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody. *Cell* **27**, 419-428.

St. Johnston, D. (1993). Pole plasm and the posterior group genes. In *The Development of Drosophila melanogaster* (eds. M. Bate and A. Martinez Arias), pp. 325-364. Cold Spring Harbor Laboratory Press.

Strome, S., and Wood. W. B. (1983). Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos. *Cell* **35**, 15-25.

Sulston, J. E. and White, J. G. (1980). Regulation and cell autonomy during postembryonic development of Caenorhabditis elegans. *Dev. Biol.* **78**, 577-597.

Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode Caenorhabditis elegans. *Dev. Biol.* **100**, 64-119.

Taghert, P. H., Doe, C. Q. and Goodman, C. S. (1984). Cell determination and regulation during development of neuroblasts and neurones in grasshopper embryo. *Nature* **307**, 163-165.

Tax, F. E., Yeargers, J. J. and Thomas, J. H. (1994). Sequence of C. elegans *lag-2* reveals a cell-signalling domain shared with *Delta* and *Serrate* of *Drosophila*. *Nature* **368**, 150-154.

Weisblat, D. A. and Blair, S. S. (1984). Developmental interdependency in embryos of the leech Helobdella triserialis. *Dev. Biol.* **101**, 326-335.

Wood, W. B., and Edgar, L. G. (1994). Patterning in the C. elegans embryo. *Trends in Genet.* **10**, 37-67.

Yochem, J. and Greenwald, I. (1989). *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in C. elegans encode similar transmembrane proteins. *Cell* **58**, 553-563.

Young, J. M and Hope, I. A. (1993). Molecular markers of differentiation in *Caenorhabditis elegans* obtained by promoter trapping. *Developmental Dynamics* **196**, 124-132.

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