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# Cell interactions involved in development of the bilaterally symmetrical intestinal valve cells during embryogenesis in *Caenorhabditis elegans*

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## Summary

**We describe two different cell interactions that appear to be required for the proper development of a pair of bilaterally symmetrical cells in *Caenorhabditis elegans* called the intestinal valve cells. Previous experiments have shown that at the beginning of the 4-cell stage of embryogenesis, two sister blastomeres called ABa and ABp are equivalent in developmental potential. We show that cell interactions between ABp and a neighboring 4-cell-stage blastomere called P2 distinguish the fates of ABa and ABp by inducing descendants of ABp to produce the intestinal valve cells, a cell type not made by ABa. A second cell interaction appears to occur later in embryogenesis when two bilaterally symmetrical descendants of ABp, which both have the potential to produce valve cells, contact each other; production of the valve cells subsequently becomes limited to only one of the two descendants. This second interaction does not occur properly if the two symmetrical descendants of ABp are prevented from contacting each other. Thus the development of the intestinal valve cells appears to require both an early cell interaction that establishes a bilaterally symmetrical pattern of cell fate and a later interaction that breaks the symmetrical cell fate pattern by restricting to only one of two cells the ability to produce a pair of valve cells.**

Key words: bilateral symmetry, *Caenorhabditis elegans*, cell fate, embryogenesis, equivalence groups, induction, lateral signaling.

## Introduction

At the beginning of the 4-cell stage of embryogenesis in the nematode *Caenorhabditis elegans*, two sister blastomeres called ABa and ABp appear to have equivalent developmental potentials: it is possible to interchange the positions of these blastomeres just after they are born with no discernible effect on subsequent development (Priess and Thomson, 1987). Nevertheless, descendants of these two blastomeres eventually undergo very different patterns of development (Sulston et al., 1983). For example, ABa produces several different cell types that form part of the pharynx, a neuromuscular organ that pumps food into the intestine (Albertson and Thomson, 1976). ABp does not produce any pharyngeal cells, but does produce other kinds of cells not made by ABa, such as four cell types that form the excretory system, a group of cells that appear to maintain osmotic balance in *C. elegans* (Nelson et al., 1983; Nelson and Riddle, 1984). Because ABa and ABp initially appear to be equivalent, subsequent cell interactions are thought to be responsible for their different patterns of development (Priess and Thomson, 1987; Schnabel, 1991; Wood, 1991; Wood and Kershaw, 1991). Many of these interactions probably occur within the next few cell divisions, because the different fates of several descendants of ABa and ABp appear to be determined by the 48-cell stage of embryogenesis (Sulston et al., 1983; Priess and Thomson, 1987).

Although early cell interactions are assumed to be involved in distinguishing the fates of ABa and ABp, the induction of ABa descendants to produce pharyngeal cells represents the only such interaction that has been characterized. Interactions between descendants of ABa and descendants of a neighboring 4-cell-stage blastomere called EMS are required for ABa to produce pharyngeal cells. If, at the 4-cell stage, EMS is removed or destroyed, ABa no longer makes pharyngeal cells and instead appears to develop more like its sister cell, ABp (Priess and Thomson, 1987). The interactions between descendants of EMS and ABa that commit ABa descendants to producing pharyngeal cells occur between the 4-cell and 28-cell stages of embryogenesis (Priess and Thomson 1987) and require maternal expression of the *glp-1* gene (Austin and Kimble, 1987; Priess et al., 1987). In embryos from mothers homozygous for mutations in *glp-1*, ABa no longer produces pharyngeal cells. The *glp-1* gene encodes a transmembrane protein (Yochem and Greenwald, 1989; Austin and Kimble 1989) related to the proteins encoded by the *C. elegans* gene *lin-12* (Greenwald 1985; Yochem et al., 1988) and the *Drosophila* gene *Notch* (Artavanis-Tsakonas

et al., 1983), both of which are known to participate in several different cell interactions (Greenwald et al., 1983; Ferguson and Horvitz, 1985; Xu et al., 1990; Greenwald and Rubin, 1992).

Antibodies that specifically recognize pharyngeal cells were used to define the *glp-1*-dependent cell interactions that result in ABa descendants producing pharyngeal cells. Although previous experiments have shown that the patterns of hypodermal and neuronal cells produced by both ABa and ABp are influenced by neighboring blastomeres (Schnabel, 1991), cell interactions presumed to be required for the production of unique cell types by ABp have been more difficult to identify, partly because markers for these cell types have only recently become available. In this paper, we use a previously described monoclonal antibody (Bowerman et al., 1992) that recognizes the intestinal valve cells, a pair of cells produced only by ABp, to ask how ABa and ABp adopt different fates. We show that ABp appears to acquire the ability to produce valve cells through interactions with other early blastomere(s). This early interaction appears to be distinct from the *glp-1*-dependent interaction between descendants of EMS and ABa that causes ABa descendants to produce pharyngeal cells: the induction of ABp to produce valve cells does not appear to require EMS or its descendants and occurs normally in *glp-1* mutant embryos. Although in normal development only one ABp descendant produces valve cells, we find that two bilaterally symmetrical descendants of ABp initially acquire the ability to produce valve cells as a result of the early interaction. A second interaction, occurring later in embryogenesis, appears to restrict the ability to produce valve cells to only one of these two descendants.

## Materials and methods

### *Strains and alleles*

N2 Bristol strain was used as the standard wild-type strain. The basic methods of *C. elegans* culture, genetics and nomenclature were as described in Brenner (1974). Alleles used in this study were *dpy-13(e184sd), glp-1(e2141ts), glp-1(e2142ts), lag-2(n1255n1323), lag-2(q387), lag-2(q431), let-23(mn23), let-23(n1045), lin-3(n1058), lin-12(n952d), lin-12(n137n720), lin-15(n765), skn-1(zu67)* and *unc-8(n491sd)*. *lag-2(q387)* and *lag-2(q431)* were kindly provided by Eric Lambie and Judith Kimble. Other strains were provided by the *C. elegans* Genetics Center.

### *Microscopy and laser ablation*

Light microscopy was performed using a Zeiss Axioplan microscope equipped for Nomarski optics and epifluorescence. For lineage analysis, gravid adult hermaphrodites were cut open under the dissecting microscope and their embryos transferred to agarose pads on microscope slides as described by Sulston et al. (1983). 28-cell-stage embryos with ventral surfaces facing up (a ventral presentation) were selected for lineage analysis under the Nomarski compound microscope, and the ABprpa and ABplpa blastomeres identified by comparing them with published anatomical charts (Sulston et al., 1983). ABprpa and ABplpa were lineaged simultaneously, following only the posterior part of the ABprpa and ABplpa lineages that produce the precursor to the valve cell pair and its bilaterally symmetrical relative, respectively. *skn-1* mutant embryos were obtained from the non-Dumpy, non-Uncoordinated progeny of *skn-1/dpy-13unc-8* hermaphrodites. *lag-2(n1255n1323)* mutant embryos were obtained from *lag-2;yDp1* hermaphrodites. About 50% of the embryos produced by these animals lose the *yDp1* duplication and express the mutant phenotype (F. Tax, unpublished observations). Homozygous *lag-2* mutant embryos could be distinguished from non-mutant siblings at about the 200-cell stage by abnormalities in the positions of embryonic cells. The genotype of the lineaged *lag-2* homozygous mutant embryos was confirmed by comparing their terminal phenotype with published descriptions of *lag-2* mutants (Lambie and Kimble, 1991b).

The monclonal antibody (mAb) 2CB7 stains three different cell types: the pharyngeal gland cells, the intestinal cells and the valve cell pair at the posterior end of the intestine (Bowerman et al., 1992). The intestinal cells and the intestinal valve cells begin to stain about 5 hours after fertilization when embryos are allowed to develop at 23°C. The pharyngeal gland cells first stain at about 7 hours (at 23°C) after fertilization. It is possible to limit 2CB7 staining to the valve cell pair only by killing the intestinal precursors (Ea and Ep; see Fig. 2) with a laser microbeam and staining the resulting embryos 6 hours (at 23°C) after fertilization.

Results from several of the experiments described in this paper suggest strongly that the antigen recognized by 2CB7 is expressed by the valve cells themselves. For example, *skn-1* mutant embryos that do not produce intestinal cells or pharyngeal gland cells show valve cell staining and no valve cell staining is observed when the parent of the valve cell pair is destroyed late in embryogenesis (see Tables 1 and 2, Fig. 3). The morphology of the stained cells is very reproducible; the small spherical cells are always present in characteristic pairs.

Development of the valve cell pair was examined in two temperature-sensitive alleles of *glp-1*. At the restrictive temperature, *e2142ts* is defective in the interaction between ABa and EMS (a maternally expressed function), while *e2141ts* is defective in both the zygotic and maternal functions of *glp-1* (Austin and Kimble, 1987; Priess et al., 1987). Homozygous *glp-1* adults were shifted to the restrictive temperature (25°C) for at least 12 hours before mutant embryos were collected for staining with mAb 2CB7. Gastrulation occurs normally at the restrictive temperature in nearly all embryos from mothers homozygous for these mutant alleles. To examine valve cell development in *lag-2*, *let-23*, *lin-3*, *lin-12* and *lin-15*, embryos were collected by allowing animals carrying these mutations to lay eggs and then the embryos were collected and stained. Two valve cell pairs were seen in embryos from animals with the mutations *lag-2(n1255n1323)*, *lag-2(q387)* and *lag-2(q431)*. No abnormalities in the valve cells were observed in embryos from animals with mutations in *let-23*, *lin-3*, *lin-12* and *lin-15*, with several hundred embryos examined for each strain. Because it is difficult to see the valve cell pair in every stained embryo and because some of these mutants are nonconditional zygotic lethals (in which only one quarter of the progeny produced by a heterozygous strain will be mutant), we cannot conclude that valve cell development is always normal in *let-23*, *lin-3*, *lin-12* and *lin-15* mutant embryos, or always abnormal in *lag-2* mutant embryos.

Laser ablation experiments were performed with a VSL-337 laser (Laser Science, Inc.) attached to a Zeiss Axioscope according to the procedure of Avery and Horvitz (1989). For experiments at or beyond the 28-cell stage, embryos with a ventral presentation were identified in the Nomarski compound microscope. The intestinal precursors (Ea and Ep) were ablated to prevent intestinal staining and then valve cell precursors were killed at the 28-cell stage or after waiting until either the 200-cell stage or the 350-cell stage. By first becoming familiar with the lineages of ABprpa and ABplpa up to the 350-cell stage in unoperated wild-type embryos, it was possible to identify the appropriate blastomeres at the 200-cell and 350-cell stages even after Ea and Ep

had been killed at the 28-cell stage (Ea and Ep still migrate inside the embryo after laser ablation). Laser ablations at earlier stages were done the same way, except 1-cell to 4-cell embryos were mounted. Early blastomeres (4-cell and 8-cell-stage embryos) were killed by directing between 50-100 pulses at both the nucleus and cytoplasm. Blastomeres at the 28-cell stage and later were killed with only 5-20 pulses directed at the nucleus. After the operated embryos developed at 23°C to the equivalent of 6 hours post-fertilization, they were transferred to polylysine-coated slides and stained with mAb 2CB7 as described by Bowerman et al. (1992).

## Results

### *The intestinal valve cells*

The two intestinal valve cells (which we will refer to as the valve cell pair) join the posterior end of the intestine to a ring of three rectal epithelial cells (Sulston et al., 1983). The terminal embryonic division that generates the valve cell pair in normal development occurs at about the 350-cell stage (Fig. 1). This division represents the conclusion of an essentially invariant sequence of embryonic cleavages (Sulston et al., 1983). Because cleavage patterns in *C. elegans* are highly reproducible, blastomeres are assigned names according to their relative positions. For example, ABa and ABp are the anterior and posterior daughters, respectively, of the 2-cell-stage blastomere called AB, and ABpr and ABpl are the right and left daughters, respectively, of ABp. The cell that normally produces the valve cell pair is a descendant of ABp called ABprpapppp (Fig. 1). As we have described previously, the monoclonal antibody 2CB7 first stains the valve cell pair at mid-embryogenesis (Bowerman et al., 1992) after the terminal division of ABprpapppp (see Materials and methods). The 2CB7 antibody therefore provides a marker for asking how the initially equivalent sister blastomeres ABa and ABp produce descendants with different fates.

### *Cell interactions in the early embryo are required for ABp to produce valve cells*

During wild-type embryogenesis, ABp produces a valve cell pair but ABa does not (Sulston et al., 1983). Because ABa and ABp are equivalent at the beginning of the 4-cell stage (Priess and Thomson, 1987; see Introduction), some subsequent event must be responsible for only ABp producing valve cells. Both ABa and ABp initially might be committed to produce valve cells, with subsequent cell interactions inhibiting valve cell production by ABa. Alternatively, neither ABa nor ABp could be committed to produce valve cells initially, with neighboring blastomeres inducing only ABp to make them. To distinguish between these two possibilities, we asked when in development blastomeres acquire the ability to produce valve cells (Table 1). We used a laser microbeam to kill early embryonic blastomeres, allowed the remaining blastomeres to develop for the same amount of time normally required for valve cell differentiation, and then assayed for valve cells by staining the partial embryos with the antibody 2CB7 (see Materials and methods). We find that if all 4-cell-stage blastomeres except either ABa or ABp are killed, no valve cells are produced, suggesting that intially neither ABa nor ABp are committed to produce valve cells. Valve cells are produced when ABp is allowed to develop after killing only ABa, but no valve cells are produced when ABa is allowed to develop after killing only ABp. These results indicate that after the 4-cell stage, ABa and ABp are no longer equivalent, presumably as a result of cell interactions with other early blastomeres.

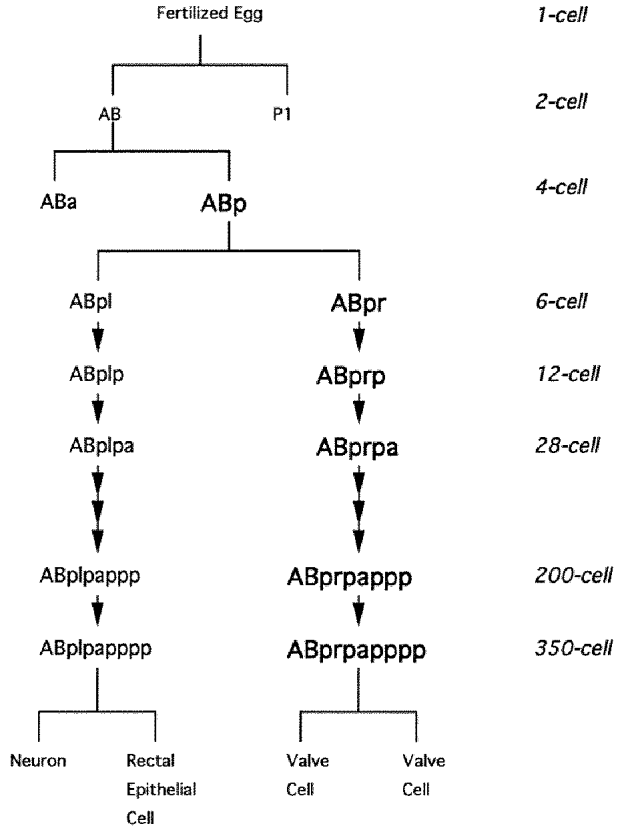


**Fig. 1.** The intestinal valve cell lineage. Abbreviated embryonic lineage diagram depicting the origins of the valve cell pair in wild-type development. The total number of embryonic cells is indicated to the right, and each arrowhead between named blastomeres corresponds to one cell division. At the 6-cell stage, two bilaterally symmetrical blastomeres called ABpl and ABpr result from the division of ABp. Although many of the bilaterally symmetrical descendants of ABpr and ABpl adopt identical patterns of differentiation, ABpr produces the valve cell pair while ABpl instead produces a neuron and a rectal epithelial cell. See Fig. 2 for a diagram of the positions of some of these blastomeres. See Sulston et al. (1983) for a complete description of the embryonic lineage.

We used similar laser ablation experiments to determine if other blastomeres induce ABp to produce valve cells (Table 1). In addition to ABa and ABp, the 4-cell embryo contains blastomeres named EMS and P2. ABa and ABp both contact EMS, but only ABp contacts P2 (Fig. 2). Embryos in which EMS is killed always produce valve cells. Indeed, embryos in which either EMS or ABa are killed always produce more than the normal number of valve cells (Table 1 and Fig. 3; see below). In contrast, almost no embryos in which P2 is killed produce any valve cells, suggesting that ABp descendants acquire the ability

**Table 1.** *Development of the valve cell pair in wild-type embryos after laser ablation of blastomeres at the 4-cell and 8-cell stages*

| Blastomere(s) killed | Embryos with no valve cell pairs | Embryos with one valve cell pair | Embryos with two valve cell pairs |
|---|---|---|---|
| ABp, EMS and P2 | 13/13 | 0/13 | 0/13 |
| ABa, EMS and P2 | 11/11 | 0/11 | 0/11 |
| EMS | 0/18 | 0/18 | 18/18 |
| ABa | 0/9 | 0/9 | 9/9 |
| ABa and EMS | 0/12 | 0/12 | 12/12 |
| ABp | 10/10 | 0/10 | 0/10 |
| P2 | 14/16 | 1/16 | 1/16 |
| P3 and C | 0/8 | 0/8 | 8/8 |

Development of valve cell pairs was analyzed after killing specified blastomeres with a laser microbeam and allowing the operated embryos to develop at 23°C to about six hours postfertilization. The operated embryos were then fixed and stained with mAb 2CB7 (see Materials and methods). The numbers in each column indicate the fraction of operated embryos that produced no valve cell pairs, one valve cell pair, or two valve cell pairs. To facilitate scoring of valve cell production in experiments in which EMS was not killed, Ea and Ep, two grandaughters of EMS that produce all of the intestinal cells, were killed to eliminate intestinal cell staining. As described in the text, when ABa, EMS, or P3 and C are killed, gastrulation and morphogenesis are severely disrupted, and two valve cell pairs are always produced. Because it is difficult to kill these early, large blastomeres with a laser microbeam, they were ablated immediately after they were born using about 50 to 100 pulses of laser light aimed at both the nucleus and the cytoplasm. When the blastomere P2 is killed later in the cell cycle, a higher fraction of the operated embryos produce one or two pairs of valve cells (data not shown). See Fig. 3 for examples of operated embryos.

to produce valve cells through interactions with P2 or descendants of P2 (Table 1 and Fig. 3). To determine when ABp or its descendants become committed to produce valve cells, we killed the two daughters of P2. The resulting embryos always produced valve cells, indicating either that ABp requires interactions with P2 itself, or that the laser-induced damage to the P2 daughters is insufficient to prevent interactions from occurring.

Maternally expressed *glp-1(+)* activity is required for the cell interactions between descendants of ABa and EMS that result in ABa descendants producing pharyngeal cells (Priess et al., 1987; Austin and Kimble, 1987). To determine if the cell interactions that result in ABp producing valve cells also require *glp-1(+)* function, we fixed and stained embryos from mothers homozygous for either of two *glp-1* mutations. All *glp-1* mutant embryos examined had a normal valve cell pair at the posterior end of the intestine (data not shown; see Materials and methods).

### *Two bilaterally symmetrical descendants of ABp initially have the potential to produce a valve cell pair*

*C. elegans* embryos normally produce only a single pair of valve cells during development. However, we have found that two valve cells pairs are produced when ABp and P2 are allowed to develop after killing other blastomeres (Table 1 and Fig. 3). Because our experiments on wild-type 4-cell-stage embryos indicate that only ABp can produce valve cells, either more than one descendant of ABp must be able to produce valve cells, or the single descendant of ABp that normally produces one valve cell pair must be able to produce an extra pair. To test the possibility that more than one ABp descendant has the potential to produce valve cells, we used a laser microbeam to kill, at the 28-cell stage, the only ABp descendant that normally produces valve cells, a blastomere called ABprpa. In all cases, the operated embryos still produced a valve cell pair, indicating that the ability to produce valve cells is not limited to ABprpa at the 28-cell stage (Table 2 and Fig. 3).

ABprpa, the normal precursor to the valve cell pair and a bilaterally symmetrical descendant of ABp called ABplpa in general produce bilaterally symmetrical patterns of differentiated cell types (Sulston et al., 1983; see Fig. 2). One exception is seen in the development of the valve cell pair: while a descendant of ABprpa normally produces the valve cell pair, the bilaterally symmetrical descendant of ABplpa produces a rectal epithelial cell and a neuron (Sulston et al., 1983; see Fig. 1). Because most of the bilaterally symmetrical descendants of ABprpa and ABplpa adopt identical fates, ABplpa seemed a possible source of the valve cell pair made after killing ABprpa. We find that when both ABprpa and ABplpa are killed at the 28-cell stage, no valve cells develop, whereas a single valve cell pair always develops when only ABplpa or only ABprpa is killed (Table 2). Therefore, the interaction between ABp and P2 (or their descendants) at about the 4-cell stage appears to result in two bilaterally symmetrical descendants of ABp acquiring the ability to produce a valve cell pair.

Because only a single descendant of ABp produces valve cells in normal development, one of the two ABp descendants that has the potential for making a valve cell pair must subsequently adopt an alternative fate. We asked when the potential for producing a valve cell pair becomes restricted to a single ABp descendant by killing the normal valve cell precursor at the 200- and 350-cell stages of embryogenesis (Table 2). Embryos can still produce a single pair of valve cells after killing the normal valve cell pair precursor, ABprpappp, at the 200-cell stage, but not after killing both ABprpappp and its bilaterally symmetrical relative (ABplpappp). If at the 350-cell stage the normal valve cell precursor (ABprpapppp) is killed immediately after it is born, about half of the embryos still produce a valve cell pair. However, when ABprpapppp is killed about 10 minutes after its birth, almost no embryos produce a valve cell pair. Thus two separate ABp lineages (ABp to ABprpapppp and ABp to ABplpapppp; see Fig. 1) retain the ability to produce a valve cell pair up until about the 350-cell stage, after which only ABprpapppp can make a pair of valve cells.

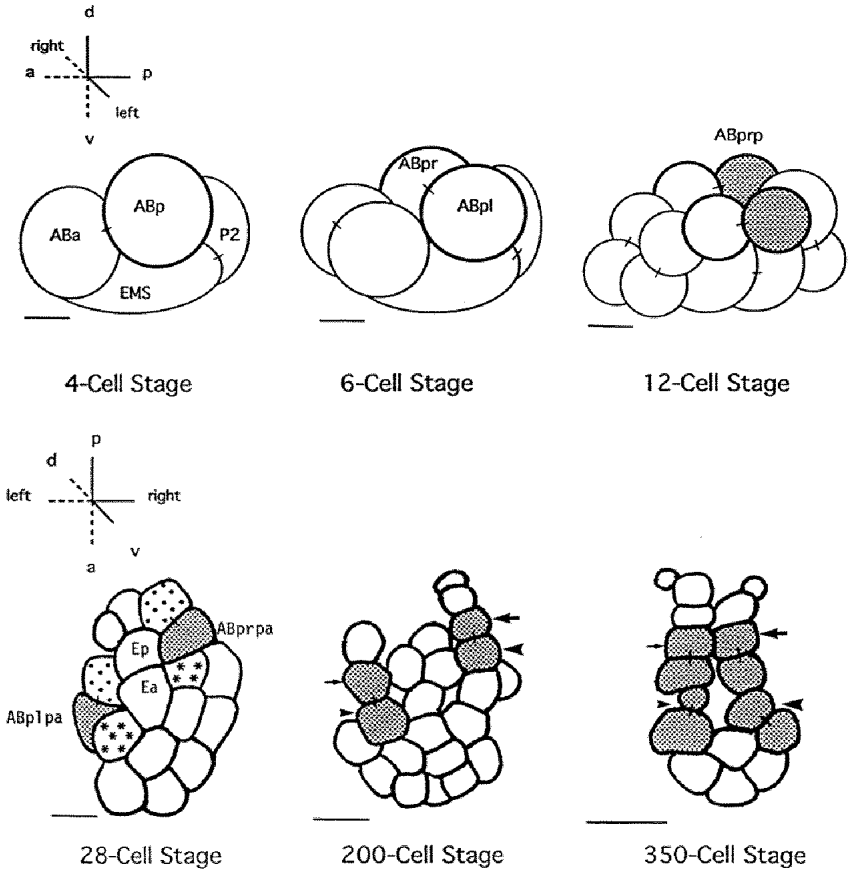


**Fig. 2.** Development of the intestinal valve cell pair. Schematic drawings of embryonic stages illustrating the relative positions of the valve cell precursors and neigboring cells. Blastomere names are indicated inside or near the borders of cells, and sister blastomeres are indicated by short connecting lines. ABp and its descendants at the 6-cell and 12-cell stages of embryogenesis are outlined in bold. The spatial orientations of the embryos are indicated by axes to the left of each row of drawings: a, anterior; p, posterior; d, dorsal; v, ventral. All blastomeres in a 4-cell-stage embryo are in a single plane; the next round of cleavage establishes a left-right axis. Two descendants of ABp (ABprp and ABplp; indicated by shading) at the 12-cell stage are the first two embryonic cells with nearly bilaterally symmetrical patterns of cell fate. For example, one descendant of ABplp becomes a left phasmid sheath cell and the bilaterally symmetrical descendant of ABprp becomes the right phasmid sheath cell (Sulston et al., 1983). To emphasize left-right symmetries, the ventral surface of the embryo is shown for the 28-cell, 200-cell and 350-cell stages. At the 28-cell stage, the valve cell pair precursor (ABprpa) and its bilaterally symmetrical relative (ABplpa) are indicated by shading. In addition to ABprpa and ABplpa, two other left-right symmetrical pairs of 28-cell-stage blastomeres are indicated by patterning. 134 of the 166 descendants of these three pairs of blastomeres have bilaterally symmetrical cell fates in normal development (Sulston et al., 1983). Posterior descendants of ABprpa and ABplpa (shaded in the 200-cell and 350-cell embryos) form two parallel rows of cells separated by other blastomeres. The intervening blastomeres eventually migrate inside the embryo during gastrulation, with the rows of ABprpa and ABplpa descendants moving medially and eventually contacting each other at the ventral midline. The blastomeres that have the potential to produce valve cell pairs at the 200-cell and 350-cell stages are indicated by arrows. After contacting each other at the ventral midline at the 350-cell stage, only the right-hand cell (ABprpapppp), large arrow, retains the potential to produce a valve cell pair (see text and Table 2), with the left-hand cell (ABplpapppp), small arrow, producing a rectal epithelial cell and a neuron. An earlier exception to the general pattern of bilaterally symmetrical fates is illustrated by the development of another pair of cells at the 200-cell stage (arrowheads). The left-hand cell divides unequally to produce a small daughter that undergoes cell death and a large daughter that later divides to produce the excretory cell and a neuron, while the bilaterally symmetrical right-hand cell divides equally (350-cell embryo). This earlier departure from bilateral symmetry suggests that mechanisms not requiring cell contact between bilaterally symmetrical cells at the ventral midline account for some left-right asymmetry in cell fates. Drawings are based on observation with the light microscope of living embryos . Bars to the lower left of each drawing represent 10 μm.

*A second cell interaction limits the ability to produce valve cells to a single descendant of ABp*

Although *C. elegans* embryos normally produce only one valve cell pair, we have observed three different situations in which embryos produce two valve cell pairs; presumably the events that normally restrict valve cell production to only one ABp descendant do not occur properly in such embryos. First, we noticed that embryos from mothers homozygous for maternal-effect mutations in the gene *skn-1* (Bowerman et al., 1992) usually contain two valve cell pairs (Fig. 3). We confirmed that these two valve cell pairs are produced by the same two ABp-derived lineages that have the potential to produce valve cells in wild-type embryos: no valve cell pairs are produced in *skn-1* embryos when both ABprpa and ABplpa are killed at the 28-cell stage, but a single valve cell pair is produced when only ABprpa, or only ABplpa, is killed (Table 3). Second, we found that mutations in the gene *lag-2* (thought to be involved in some cell interactions during wild-type embryogenesis; Lambie and Kimble, 1991a,b; see Discussion), reproducibly cause mutant embryos to have two valve cell pairs at the posterior end of the intestine (Fig. 3). We have examined the valve cell pair in strains with mutations in other previously identified genes that also appear to be required for certain cell interactions during embryonic and post-embryonic development (for reviews, see Sternberg, 1988; Horvitz and Sternberg, 1991; Lambie and Kimble, 1991a; Greenwald and Rubin, 1992). Valve cell development appeared normal in embryos from animals with mutations in the genes *let-23*, *lin-3*, *lin-12* and *lin-15* (data not shown; see Materials and methods for a description of the alleles analyzed). Finally, we have found that two valve cell pairs are always produced in wild-type embryos after killing either EMS or ABa at the 4-cell stage (Table 1 and Fig. 3).

**Table 2.** *Development of the valve cell pair in wild-type embryos after laser ablation of blastomeres at the 28-cell, 200-cell, or 350-cell stages of embryogenesis*

| Blastomere(s) killed | Embryos with no valve cell pairs | Embryos with one valve cell pair |
|---|---|---|
| Ea and Ep | 0/14 | 14/14 |
| ABprpa | 0/6 | 6/6 |
| ABplpa | 0/10 | 10/10 |
| ABprpa and ABplpa | 7/7 | 0/7 |
| ABprpappp | 1/12 | 11/12 |
| ABplpappp | 0/7 | 7/7 |
| ABprpappp and ABplpappp | 8/8 | 0/8 |
| ABprpapppp* | 2/5 | 3/5 |
| ABprpapppp** | 6/7 | 1/7 |
| ABplpapppp | 0/3 | 3/3 |
| ABprpapppp and ABplpapppp | 4/4 | 0/4 |

Development of valve cell pairs was analyzed after killing specified blastomeres with a laser microbeam (see Materials and methods). The numbers in each column indicate the fraction of operated embryos that produced no valve cell pairs or one valve cell pair. To facilitate identification of the valve cell pairs, Ea and Ep (see Fig. 1), 28-cell-stage blastomeres which produce all of the intestinal cells in *C. elegans* (Sulston et al., 1983), were killed in all experiments. If only Ea and Ep are killed, morphogenesis occurs fairly normally in about 90% of the operated embryos and blastomeres can still be identified by their relative positions in the embryo; for this analysis, valve cell development was scored only in operated embryos that underwent nearly normal morphogenesis. ABprpapppp was killed either immediately (*) or ten minutes after (**) it was born. See Fig. 3 for examples of operated embryos.

**Table 3.** *Development of the valve cell pair in* skn-1 *mutant embryos after laser ablation of blastomeres at the 28-cell stage of embryogenesis*

| Blastomere(s) killed | Embryos with no valve cell pairs | Embryos with one valve cell pair |
|---|---|---|
| ABprpa | 1/15 | 14/15 |
| ABplpa | 0/12 | 12/12 |
| ABprpa and ABplpa | 7/7 | 0/7 |

Development of valve cell pairs was analyzed after killing specified blastomeres with a laser microbeam (see Materials and methods). The numbers in each column indicate the fraction of operated embryos that produced no valve cell pairs or one valve cell pair. As described previously, in *skn-1* mutant embryos EMS appears to adopt a fate similar but not identical to that of P2, with both blastomeres producing hypodermal cells and body wall muscle cells (Bowerman et al., 1992). The observation that only descendants of ABp produce valve cell pairs and the failure of EMS to induce ABa to make valve cells provide additional evidence that EMS and P2 are not identical in *skn-1* mutant embryos.

In all three types of embryos that produce two valve cell pairs, we detect visible abnormalities at the 350-cell stage in the relative positions of the two ABp descendants with the potential for making a valve cell pair. During wild-type embryogenesis, the positions of these two ABp descendants change considerably as blastomeres on the ventral surface of the embryo migrate into the body cavity during gastrulation (Sulston et al., 1983). Descendants of ABprpa and ABplpa initially are on the right and left sides of the embryo, respectively. The migration of ventral blastomeres into the interior eventually brings these descendants together in two parallel rows at the ventral midline, where the normal valve cell precursor, a descendant of ABprpa, first contacts its bilaterally symmetrical relative, a descendant of ABplpa (Fig. 2). We examined the development of ABprpa and ABplpa in four *skn-1(zu67)* mutant embryos using the light microscope (see Materials and methods). In all four *skn-1* embryos, the posterior descendants of ABprpa and ABplpa did not meet at the ventral midline. Instead, these cells were separated from each other by numerous descendants of two early blastomeres called Ea and Ep (see Fig. 2). As described previously (Bowerman et al., 1992), *skn-1* mutant embryos show defects in gastrulation. In particular, Ea and Ep fail to migrate to the interior of the embryos and their descendants remain on the ventral surface. The development of ABprpa and ABplpa also was observed with the light microscope in three *lag-2(n1255n1323)* mutant embryos (see Materials and methods). In the *lag-2* embryos, the normal valve cell parent was delayed in contacting its bilaterally symmetrical relative by about 20 minutes; in wild-type embryos the contact occurs immediately after the two cells are born (Fig. 2). Although the contact is delayed in *lag-2* mutants, after meeting, these two ABp descendants migrated into the interior of the embryo as they do in wild-type development. Finally, we find that in wild-type embryos killing ABa or EMS with a laser microbeam at the 4-cell stage severely disrupts gastrulation and morphogenesis, preventing the normal contacts between cells at the 350-cell stage.

## Discussion

### *At least two distinct cell interactions in the early* C. elegans *embryo distinguish the fates of ABa and ABp*

Although the sister blastomeres ABa and ABp have equivalent developmental potentials at the beginning of the 4-cell stage of embryogenesis (Priess and Thomson 1987), in normal development only ABp produces intestinal valve cells. We have considered two possible explanations for these different fates. First, perhaps neither ABa nor ABp is committed to produce valve cells initially, with other blastomeres specifically inducing only ABp to make them. Alternatively, both ABa and ABp might be committed to produce valve cells initially, with subsequent cell interactions specifically preventing ABa from making them. We have shown here that neither ABa nor ABp is capable of producing valve cells when all other 4-cell-stage blastomeres are killed. ABp can produce valve cells when an adjacent blastomere, called P2, is also allowed to develop, but when P2 is killed with a laser microbeam, ABp no longer produces valve cells. These results suggest that cell interactions between P2 and ABp, or their descendants, result in the production of valve cells by ABp, much as interactions between descendants of EMS and ABa result in the production of pharyngeal cells by descendants of ABa (Priess and Thomson, 1987). These early cell interactions between P2 and ABp, and between EMS and ABa, can both be thought of as inductive because they involve blastomeres that have very different fates.

Consistent with the hypothesis that ABp must interact with P2 to produce valve cells, ABp does not produce valve cells in strains with maternal-effect mutations in the gene *pie-1* (C. Mello, personal communication). In *pie-1* mutant embryos, the blastomere P2 adopts a fate nearly identical

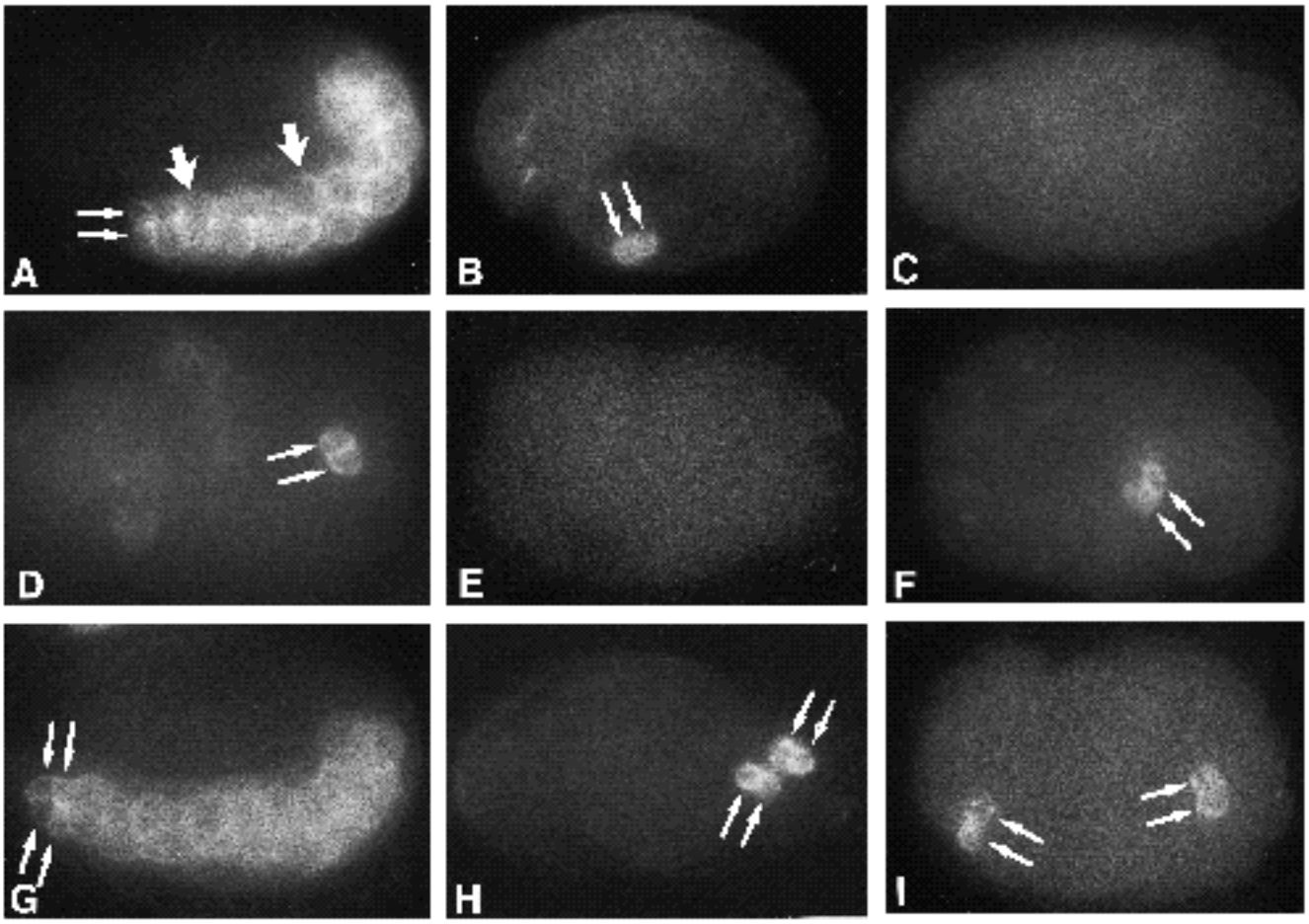


**Fig. 3.** Valve cell pair development in wild-type embryos and in *skn-1* and *lag-2* mutant embryos. Immunofluorescence micrographs of wild-type or mutant embryos stained with the 2CB7 antibody (see Materials and methods). (A) Wild-type embryos show staining of intestinal cells (large arrows) and the valve cell pair (small arrows). (B-F) In each of these wild-type embryos the intestinal precursors (Ea and Ep) were killed at the 28-cell stage to facilitate identification of valve cells (arrows). If only Ea and Ep are killed, morphogenesis is nearly normal and only one valve cell pair is produced (B, see Materials and methods). No valve cells are produced in wild-type embryos in which the P2 blastomere is killed at the 4-cell stage (C). One valve cell pair develops when at the 28-cell stage ABprpa, the normal valve cell precursor, is killed (D), but no valve cells develop when both ABprpa and its bilaterally symmetrical relative, ABplpa, are killed (E). One valve cell pair develops when only ABplpa is killed (F). These results suggest that at the 28-cell stage both the normal precursor to the valve cell pair and its symmetrical relative have the potential for producing a valve cell pair; results from these experiments are summarized in Table 2. (G-I) Two valve cell pairs are present in a *lag-2(n1255n1323)* mutant embryo (G), a *skn-1(zu67)* mutant embryo (H) and in a wild-type embryo after the EMS blastomere was killed at the 4-cell stage (I; also see Table 1). In *lag-2* mutant embryos, the 2CB7 antibody stains 20 intestinal cells (the same number as in wild-type embryos; Sulston et al., 1983) in addition to the two pairs of valve cells. As described previously, *skn-1* mutant embryos lack intestinal cells (Bowerman et al., 1992). *C. elegans* embryos are about 50 μm in length.

to that of its sister blastomere EMS (Mello et al., 1992); this change in fate appears to eliminate the ability of P2 to induce valve cell production by ABp.

The interactions between descendants of EMS and ABa that specify the production of pharyngeal cells require maternal expression of the *glp-1* gene (Priess et al, 1987; Austin and Kimble, 1987). In *glp-1* mutant embryos, ABa fails to produce pharyngeal cells and instead appears to develop more like ABp (Priess et al., 1987). However, development of the valve cell pair does not appear to be affected by *glp-1* mutations. Thus at least two different cell interactions, possibly involving different gene products, appear to distinguish the fates of ABa and ABp.

### *Cell interactions late in embryogenesis restrict the potential for producing valve cells*

We have shown that an early cell interaction appears to result in two bilaterally symmetrical descendants of ABp acquiring the ability to produce a valve cell pair. Because only one of the two ABp descendants retains this ability after the 350-cell stage, a second restrictive interaction must prevent the other descendant from making a valve cell pair. The time at which this restriction appears to occur is correlated with the time at which these ABp descendants first contact each other at the ventral midline of the embryo. After the two descendants have been in contact for several minutes, only one descendant retains the ability to produce

the valve cell pair. The experimental or mutant embryos we have analyzed that produce two valve cell pairs all have visible defects at the 350-cell stage in the positions of the two ABp descendants; instead of contacting each other at the ventral midline they are separated by other intervening cells. However, because multiple cell contacts are disrupted in these abnormal embryos, we do not know whether interactions between the two ABp descendants themselves are required to restrict the potential for producing valve cells in normal embryogenesis, or whether neighboring cells participate in this restriction.

The development of the anchor cell (AC) in *C. elegans* provides an example of a cell fate decision resulting from interactions between two initially equivalent cells (Kimble and Hirsh, 1979; Kimble, 1981; Hedgecock et al., 1990). In normal development, these two cells contact each other and undergo an interaction that restricts expression of the AC fate to only one of the cells. If either cell is killed with a laser microbeam, the remaining cell adopts the AC fate. The decision as to which cell becomes AC appears to be random, for in any given animal either cell can adopt the anchor cell fate. In contrast, the interaction that restricts the potential for making valve cells to one of two ABp descendants appears to be nonrandom; the right-hand descendant, ABprpapppp, usually if not always produces the valve cells. No variation in valve cell development was noted in the original description of the wild-type embryonic lineage (Sulston et al., 1983), and results from our experiments on 350-cell-stage embryos suggest that ABprpapppp always produces the valve cells. In each of 14 wild-type embryos that we examined at the 350-cell stage, ABprpapppp always migrated into the interior of the embryo ahead of its bilaterally symmetrical relative (ABplpapppp). This observation confirms our results with the mAb 2CB7 that indicate ABprpapppp always adopts the same fate, and shows that ABprpapppp and ABplpapppp are already different before the valve cells are born. Therefore, the two ABp descendants with the potential for producing valve cells either already have an intrinsic bias in their fates at the 350-cell stage, or alternatively reproducible differences in their contacts with or proximity to neighboring cells might account for the restriction of valve cell production always to ABprpapppp. Examples in *C. elegans* of both random and nonrandom decisions involving cells with initially equivalent fates (equivalence groups) have been described previously (Sulston and Horvitz, 1977; Sulston et al., 1980; Sulston and White, 1980; Kimble, 1981; Greenwald et al., 1983; Sulston et al., 1983; Sternberg and Horvitz, 1986; Sternberg, 1988; for reviews see Sternberg, 1988; Lambie and Kimble, 1991a; Greenwald and Rubin, 1992).

The finding that *lag-2* mutants produce two valve cell pairs is intriguing because of the role the *lag-2* gene product may play in other cell interactions in *C. elegans*. *lag-2* mutants resemble phenotypically *lin-12 glp-1* double mutant animals (Lambie and Kimble, 1991b). The *lin-12* and the *glp-1* genes encode predicted transmembrane proteins that are closely related (Greenwald, 1985; Yochem et al., 1988), and both *lin-12* and *glp-1* are required for multiple cell interactions during embryonic and post-embryonic development (Greenwald et al., 1983; Austin and Kimble, 1987; Priess et al., 1987). The observation that *lag-2* mutants resemble *lin-12 glp-1* double mutants (Lambie and Kimble, 1991b) and the observation that certain *lag-2* alleles can suppress *lin-12* mutations (F. Tax, J. Thomas and H. R. Horvitz, unpublished data), suggest that the *lag-2* gene product may function in several different cell interactions. The *lag-2* gene product could function directly in limiting valve cell production to only one of the two ABp descendants. However, because the contact between these two ABp descendants is delayed substantially in *lag-2* mutants, it is possible that the *lag-2* gene product functions only indirectly in this interaction, possibly by affecting cell positions during gastrulation.

### *Establishment and refinement of bilateral symmetry during embryogenesis in* C. elegans

Much of the *C. elegans* body plan is bilaterally symmetrical, with several different pairs of early blastomeres producing bilaterally symmetrical patterns of cell fates (Sulston et al., 1983; Wood, 1991; Wood and Kershaw, 1991; see Fig. 2). Development of the bilaterally symmetrical intestinal valve cell pair appears to require two different cell interactions during embryogenesis. First, an early interaction is required for the 4-cell-stage blastomere ABp to produce the valve cells. Although ABp and its sister blastomere ABa are initially equivalent in developmental potential (Priess and Thomson, 1987), the topography of the 4-cell-stage embryo results in these two blastomeres having asymmetrical contacts with other early blastomeres. In particular, only ABp contacts the posterior-most blastomere P2 and our results suggest that interactions between ABp and P2, or their descendants, induce ABp to produce the valve cells. Although it may not be required for the establishment of bilateral symmetry per se, this early interaction results in two bilaterally symmetrical descendants of ABp initially acquiring the potential to produce valve cells. Bilaterally symmetrical descendants of ABp retain this potential until about the 350-cell stage, at which time the left-hand and right-hand descendants of ABp meet at the ventral midline, where a second interaction appears to occur. This late interaction disrupts a latent symmetry in cell fate by restricting the potential to produce a valve cell pair to only one of the two ABp descendants. The right-hand ABp descendant divides to make the two valve cells that attach to the posterior end of the intestine and form a bilaterally symmetrical structure, while the two daughters of the left-hand ABp descendant differentiate as a rectal epithelial cell and a neuron that both contribute to asymmetrical structures (Sulston et al., 1983; Hall and Russell, 1991). Thus the development of a simple symmetrical structure, the valve cell pair, requires interactions that both establish and disrupt a symmetrical pattern of cell fate.

Previous analyses of development in *C. elegans* have identified pairs of bilaterally symmetrical cells with equivalent developmental potentials that subsequently adopt different fates postembryonically (Sulston et al., 1980, 1983). For example, there is a G2/W equivalence group in which one cell becomes G2, an ectoblast, and the other becomes W, a neuroblast. Like the valve cell pair precursor and its bilaterally symmetrical relative, these two cells also contact each other on the surface of the embryo at the ventral midline. Although both cells initially have the ability to

become an ectoblast, after the embryo hatches it is always the left-hand cell that expresses the ectoblast fate (Sulston et al., 1983). As described in this paper, both ABprpapppp and ABplpapppp have the ability to produce a valve cell pair. However, it is the right-hand cell (ABprpapppp) that always produces the valve cell pair during normal development. Thus not all symmetry breaking decisions at the ventral midline are biased in the same left/right direction. The valve cell decision also differs from previously described examples of equivalence groups in *C. elegans* in that the cell contact at the ventral midline occurs earlier in embryogenesis, and the fates of the two cells that touch each other are expressed before hatching.

The restriction of valve cell production to one of two bilaterally symmetrical cells may be one of many such cell fate decisions that occur during embryogenesis in *C. elegans*. Several pairs of bilaterally symmetrical cells that contact each other at the ventral midline subsequently express different fates during embryogenesis, even though they are descended from blastomeres that in general produce bilaterally symmetrical patterns of cell fates. For example, two pair of cells (ABprpapppa/ABplpapppa and ABprpppppa/ABplpppppa) make contact on the surface of the embryo at the ventral midline (one just anterior and one just posterior to the valve cell parent and its symmetrical relative, respectively; see Fig. 2) and then adopt asymmetrical fates (Sulston et al., 1983). Perhaps these and other pairs of cells that meet at the ventral midline are initially equivalent in developmental potential but undergo cell interactions late in embryogenesis that cause them to adopt asymmetrical fates. It will be interesting to determine how widespread these kinds of interactions are during embryogenesis in *C. elegans*, and if the different decisions involve common mechanisms of cell communication. A general theme in nematode development may be that early events, some of which appear to involve cell interactions, establish broad patterns of bilateral symmetry (Wood, 1991; Wood and Kershaw, 1991), with cell interactions at subsequent stages serving to refine the body plan by breaking otherwise symmetrical patterns of cell fates.

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