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Cell contact regulates neuroblast formation in the *Caenorhabditis elegans* lateral epidermis

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SUMMARY

A single line of epidermal seam cells lies along each side of the nematode *C. elegans*. During normal development, one of these cells, V5, produces a neuroblast that will give rise to a sensory structure, the postdeirid. If seam cells located either anterior or posterior to V5 are ablated however, this neuroblast formation is blocked. Because of this requirement for the presence of adjacent seam cells, we have asked whether V5's ability to produce a neuroblast depends on direct contact with its seam cell neighbors. We find that direct contact between seam cells is required for commitment to neuroblast production. Seam cells lose and reform their contacts with each other as they go through rounds of cell division during larval development. Signaling required for neuroblast formation occurs when the seam cells make contact after their first round of division. If this contact is prevented, no neuroblast is made; when it is delayed, the time of signaling is also delayed. The characteristics of these signals suggest that a seam cell must be part of a continuous epithelium in order to develop normally and that signaling may occur via a cell recognition/cell adhesion pathway. The effect of seam cell ablations on neuroblast formation is altered in *mab-5(–)* animals, suggesting that this HOM-C gene is part of the pathway by which seam cell signaling controls the decision to make a postdeirid neuroblast.

Key words: *Caenorhabditis elegans*, cell contact, neuroblast, lateral epidermis, seam cell

INTRODUCTION

The cell adhesion and intercellular signaling that occur as a result of contacts between cells in an epithelial sheet are required to create tissues and control the morphology and behavior of individual cells (reviewed by Hynes and Lander, 1992). Molecules involved in cell adhesion are also thought to have a role in pattern formation during development (Detrick et al., 1990; Fehon et al., 1990; Fujimori and Miyatani, 1990; Peifer and Wieschaus, 1990). We are interested in understanding the role of these adhesive cell contacts in specifying cell fate. We have begun to examine the pattern of cell contact and signaling that occurs within a small group of epithelial cells, the seam cells of the *C. elegans* lateral epidermis. Previous work had suggested that maintenance of contact between these cells might be necessary for their normal development (Sulston and White, 1980; Waring and Kenyon, 1990, 1991; Waring et al., 1992).

The seam cells are arranged in a line that extends from head to tail along each side of the worm (Sulston and Horvitz, 1977). A set of six seam cells, V1-V6, are found in the main body region of the worm. Individual V cells give rise to a precise pattern of neuronal structures. In both hermaphrodites and males, the seam cell V5 produces a neuroblast that generates a sensory structure called the postdeirid. In males, the seam cells V5 and V6 also give rise to sensory rays used during mating.

Experiments in which cells were ablated using a laser microbeam have shown that signaling between the seam cells is required to produce the normal pattern of seam cell-derived neuronal structures (Sulston and White, 1980; Waring et al., 1992; Waring and Kenyon, 1990). The effect of seam cell ablations on the decision of whether or not to make a postdeirid has been examined in detail. It was found that, by the time of hatching, V5 is the only seam cell with the potential to give rise to a postdeirid. In experiments where V5 was ablated, no postdeirid was made (Sulston and White, 1980). However, the presence of neighboring seam cells is also required for V5 to carry out this fate: when seam cells either posterior or anterior to V5 were ablated, the postdeirid was not made (Sulston and White, 1980; Waring et al., 1992). This requirement for both anterior and posterior seam cells suggested the hypothesis that for V5 to develop normally and produce a postdeirid it must receive signals from both its anterior and posterior neighbors.

The pattern of neural structures produced by the seam cells is controlled by the genes *lin-22* and *pal-1*. In the *pal-1*; *lin-22* double mutant, all six V cells can make a postdeirid (Horvitz et al., 1983; Waring et al., 1992). These ectopic postdeirids are affected by seam cell ablations in the same way as the V5 postdeirid: for each V cell, killing either anterior or posterior seam cells prevents postdeirid formation. The effect of seam cell ablations on the formation of ectopic postdeirids, along with the results of seam cell ablations in wild-type animals, indicated that the signals required for postdeirid

formation occur between all seam cells (Waring et al., 1992). Because these signals occur between all seam cells, and because both anterior and posterior signals are required to make a postdeirid, we were interested in the possibility that in order to produce a postdeirid neuroblast, V5 or its descendants must be in direct contact with the neighboring seam cells on both sides; that is, it must be part of a continuous epithelium.

We have investigated the nature of these signals that occur between the seam cells, using postdeirid formation as an indicator of signaling. We began by asking whether signaling between the seam cells requires direct cell contact. Interestingly, we have found although seam cells are in contact with each other at hatching, this contact is not continuous during development. When the seam cells divide, contact is temporarily lost and is then actively reformed by cell growth. We have found that signaling required for postdeirid formation occurs when the seam cells contact each other after their first round of cell division. When seam cell contact is prevented, signaling does not occur; when seam cell contact is delayed, signaling is delayed as well.

We have also asked what downstream events are elicited by signaling between the seam cells. Our results suggest that one pathway by which seam cell signaling may control the decision to make a postdeirid involves the HOM-C gene *mab-5*. In the absence of *mab-5* activity, postdeirid formation can occur even after disruption of seam cell signaling.

MATERIALS AND METHODS

General methods and strains

Worms were cultured as described in Brenner (1974). Except where noted, all experiments used *Caenorhabditis elegans* var. Bristol, strain N2 hermaphrodites. The following mutations were used: *mab-5(e1239)* III, *mab-5(e1936)* III, *egl-5(n945)* III, *lin-39(n1760)* III and *him-5(e1490)* V. These mutations are described in Chisholm (1991), Clark et al. (1993), Hodgkin et al. (1979) and Kenyon (1986). In addition, mutations that have been shown to affect intercellular signaling in *C. elegans* were tested for their effect on postdeirid formation (see below). All experiments were performed at 20°C.

Larvae were staged by collecting newly hatched animals at 30 minute intervals. The beginning of each hatching interval was defined as t=0. To observe seam cell development, animals were placed on a 2% agarose pad and examined using Nomarski differential interference contrast optics. The developmental stage of larvae was determined using epidermal and gonadal markers (Sulston and Horvitz, 1977). Under the conditions used in these experiments, we found that by 5 hours after hatching the seam cells V2-V6 had divided (V1 generally divided later than the other V cells); at 6 hours the seam cell T had gone through two rounds of division; at 7 hours the nuclei of some or all of the anterior ventral epidermal cells (P1-P6) had descended into the ventral cord; at 8 hours the nuclei of some or all of the posterior ventral epidermal cells (P7-P12) had descended into the ventral cord; at 9 hours the nuclei of all 12 P cells were in the ventral cord and P1-P10 had gone through at least one round of division.

Postdeirid formation was scored in late L2 or early L3 larvae, 24 hours after hatching. The neurons and support cells of the postdeirid were identified based on morphology (small, compact nuclei) and position (between the epidermal layer and the basement membrane). Animals were scored as having a normal postdeirid, a seam cell lineage or a reiterated lineage based on cell morphology and position. A group of 4 cells (2 neurons and 2 support cells) of appropriate morphology with no associated extra seam cells was scored as a postdeirid; an extra seam cell with no associated neuron or support cells was scored as a seam cell lineage; and an extra seam cell associated with a group of one or more neurons or support cells was scored as a reiterated lineage.

Ablation of cells using a laser microbeam

Individual cells were killed using a laser microbeam as described in Waring et al. (1992). The laser used in these experiments was a VSL 337 nitrogen-pumped dye laser with 7-amino 4-methyl coumarin dye, which produces a wavelength of 440 nm. Approximately 1 mM sodium azide was added to the agarose pad to anesthetize the animals. The level of azide was titrated to a level where movement of the animals was slowed down but not stopped, in order to minimize developmental delays due to anesthesia. In general, laser microsurgery produced only a small delay in development (≤1 hour). Animals in which greater developmental delays were observed were discarded.

Immunofluorescence

Animals were fixed and stained by a variation of the method described in Kenyon (1986). Animals were attached to a polylysine-coated glass microscope slide, covered with a coverslip and frozen on dry ice for at least 5 minutes. The coverslips were pried off and slides were fixed in −20°C methanol (5 minutes) and −20°C acetone (5 minutes). Samples were rehydrated in an ethanol series (90%, 60%, 30%) followed by PBS. Slides were incubated with MH27 monoclonal antibody (provided by R. Barstead and R. Waterston) for 1 hour at 37°C, rinsed and incubated with rhodamine-conjugated goat anti-mouse IgG for 1 hour at 37°C. All incubations were in PBS and included 1% BSA and 1% goat serum. Slides were mounted in 2% N-propyl gallate, 80% glycerol in PBS.

Visualization of seam cell outlines using sodium dodecyl sulfate (SDS)

Animals were incubated for 2-3 minutes in a solution of 0.25% SDS (Sigma no. L-5750) at room temperature, placed on a 1.75% agarose pad and viewed immediately using Nomarski optics.

Postdeirid formation in signaling mutants

Mutations in genes previously shown to be required for other intercellular signaling pathways in *C. elegans* were tested for their effect on postdeirid formation. Animals that were homozygous for the following mutations were scored for the presence of the postdeirid: *lin-12(n137 n720)*, *lin-12(n676 n909)* (Greenwald et al., 1983); *glp-1(q46)* (Austin and Kimble, 1987); *lag-2(q420ts)* (Lambie and Kimble, 1991); *lin-3(n378)*, *lin-3(n1058)* (Ferguson and Horvitz, 1985); *let-23(sy97)*, *let-23(sy10)* (Aroian and Sternberg, 1991); *let-60(sy100dn)*, *let-60(s1155)* (Clark et al., 1988; Han et al., 1990).

The function of the *lin-12* and *glp-1* genes has been shown to overlap and the *lin-12 glp-1* double has a stronger phenotype (L1 lethality) than either single mutant. *lag-2(q240ts)* animals grown at 25°C show many of the phenotypes of the *lin-12 glp-1* animals. To score postdeirid formation in *lag-2(q240ts)* animals, *lag-2(q240ts)* homozygotes were grown at 15°C and their eggs were shifted to 25°C after the temperature-sensitive period for early larval lethality (the 4- to 28-cell stage of embryogenesis).

Animals homozygous for null alleles of *lin-3*, *let-23* or *let-60* arrest as L1 larvae; therefore, postdeirid formation was scored in animals homozygous for hypomorphic alleles of each gene. *let-60(sy100dn)* is a dominant-negative allele of *let-60*: postdeirid formation was scored in the *let-60(sy100dn)* homozygous progeny of *let-60(sy100dn)/let-60(n1046gf)* heterozygotes.

For each mutation, at least 25 homozygotes were scored for presence of the postdeirid. In all cases normal postdeirid formation was observed.

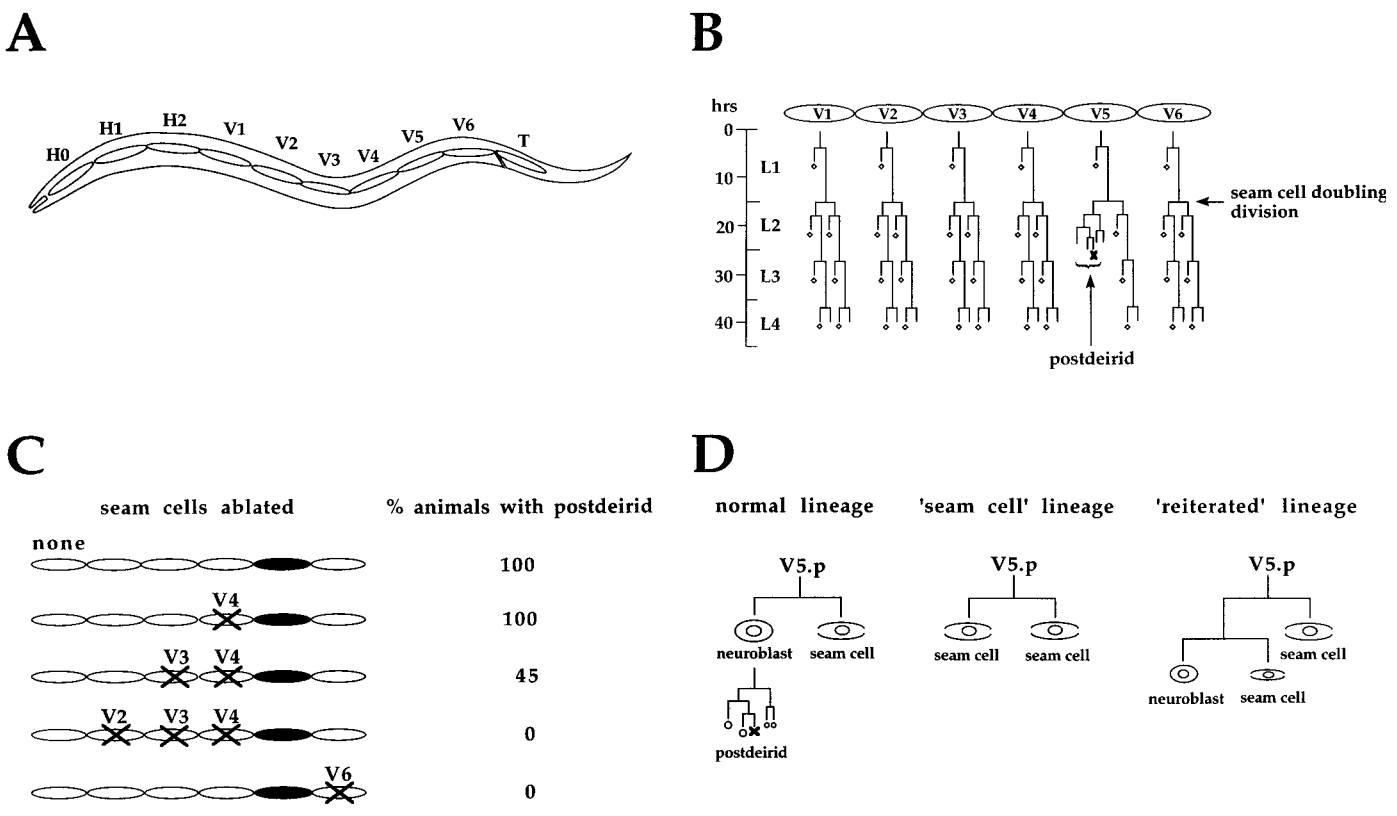

Fig. 1. (A) The *C. elegans* lateral epidermis early in the first larval stage (L1). Three seam cells, H0-H2, are in the head region, V1-V6 span the main body region and T is in the tail. At hatching an additional cell, the Q neuroblast, is found between V4 and V5. One to two hours after hatching, the Q neuroblast delaminates from the epidermal layer; V4 and V5 contact each other at this time. (B) V1-V6 cell lineage. ✕, cell death; ◇, cells that fuse with the syncytial epidermis. (C) Formation of the postdeirid after seam cell ablations. Cells were ablated approximately 1 hour after hatching (n=10-15 animals per ablation). (D) V5.p lineages. Left, normal lineage: V5.pa becomes the postdeirid neuroblast. Further divisions of this neuroblast generate the postdeirid neuron (V5.paaa), sheath (V5.papp) and socket (V5.papa) cells as well as a touch receptor (V5.paapa) and a cell death (V5.paapp) (Sulston and Horvitz, 1977). Middle and right, abnormal lineages observed after seam cell ablations: middle, V5.pa becomes a seam cell; right, V5.pa goes through an extra round of division to make a neuroblast and a seam cell.

RESULTS

Background - seam cell signals are required for postdeirid formation

At hatching, a row of seam cells extends from head to tail along each side of the *C. elegans* first stage larva (Fig. 1A). V1-V6, the seam cells in the body region, go through a characteristic series of divisions during larval development (Sulston and Horvitz, 1977; Fig. 1B). Each V cell divides once per larval stage in a stem-cell pattern, producing an anterior daughter that fuses with the multinucleate epidermal syncytium, hyp7, and a posterior daughter that will become the new seam cell. At the beginning of the second larval stage, an additional division occurs in which the seam cells divide symmetrically, producing two seam cell daughters. In the V5 lineage however, this division is asymmetric; V5.p (the posterior daughter of V5) divides to make a neuroblast (V5.pa) and a seam cell (V5.pp). This neuroblast undergoes a series of divisions, which result in the production of the neuron and two support cells of the postdeirid as well as a mechanosenory neuron (Sulston and Horvitz, 1977; Way and Chalfie, 1988; Fig. 1D, 'normal lineage'; Fig. 2A). In the present paper, this group of four cells will be referred to as the postdeirid.

Formation of the postdeirid by V5 depends on the presence of other seam cells. In animals where V6, the seam cell posterior to V5, is killed by laser ablation, the postdeirid is not formed. The same result is seen when V2, V3 and V4, a set of seam cells anterior to V5, are ablated (Sulston and White, 1980; Waring et al., 1992; Fig. 1C). In these animals, V5 generally develops in a pattern similar to that seen for the other V cells: V5.p divides to produce two seam cell daughters instead of a neuroblast and a seam cell (Fig. 1D, 'seam cell lineage'; Fig. 2B). Occasionally V5.pa, the cell that would normally become the postdeirid neuroblast, instead goes through an extra round of division to make a seam cell and a neuroblast (Fig. 1D, 'reiterated lineage'). This neuroblast divides to produce a variable number of neurons and support cells (Waring et al., 1992; data not shown).

These results indicated that signaling occurs between the seam cells or their descendants. Two characteristics of this signaling stand out. First, ablation of seam cells either anterior or posterior to V5 affects postdeirid formation, suggesting that signals from both directions are required. Second, ablation of anterior seam cells has a graded effect. Killing just V4, the seam cell immediately anterior to V5, does not prevent postdeirid formation. Removal of two cells (V3, V4) has an inter-

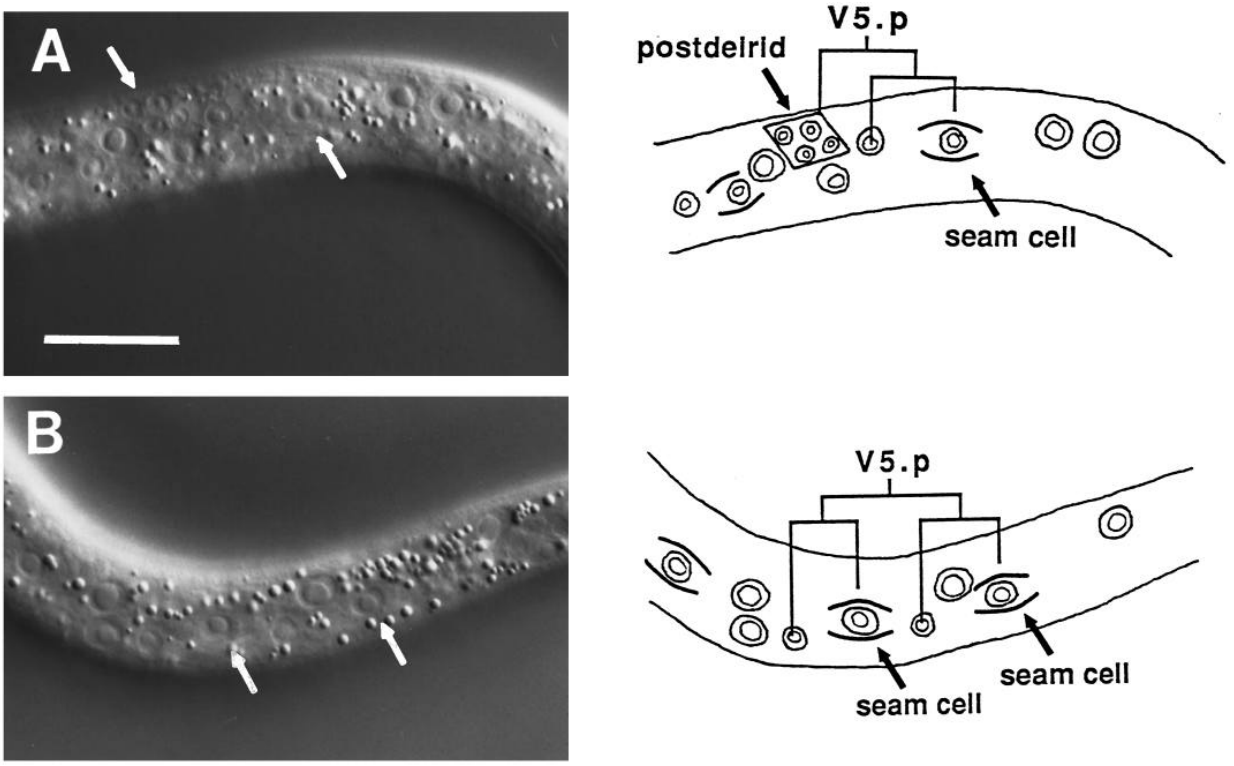

Fig. 2. Nomarski photomicrographs of L2 larvae showing nuclei of cells descended from V5.p. (A) Unablated control; V5.p divides to make a seam cell and the postdeirid neuroblast. (B) Animal in which V6 was ablated 1 hour after hatching: an extra seam cell is present in place of the postdeirid. Scale bar is 10 μm.

mediate effect, while ablation of three cells (V2, V3, V4) completely prevents postdeirid formation. This result suggested that cells other than the immediate neighbors of V5 can provide the signals required for postdeirid formation.

Seam cells are not in continuous contact with each other

As a first step toward understanding the relationship between seam cell contact and signaling, we determined the pattern of contacts between the seam cells. We focused on the period between hatching and the birth of the postdeirid neuroblast, during which the decision of whether or not to make a postdeirid is made. To determine when and where seam cells were in contact, animals were stained with MH27, an antibody that stains the apical junctions between epithelial cells in *C. elegans*, thus allowing the outlines of the seam cells to be seen (Francis and Waterston, 1991).

In larvae stained with MH27 at different times after hatching, we found a changing pattern of seam cell contacts (Fig. 3). Initially, V1-V6 formed a connected line of cells (Fig. 3A). After the first round of seam cell division, the new seam cells, V1.p-V6.p, were separated by their sisters that subsequently fuse with the epidermal syncytium (Fig. 3B). The result of these cell fusions was a line of seam cells surrounded by the epidermal syncytium (Fig. 3C, D). At this time, V1.p-V6.p extended anterior and posterior cell processes that contacted those of the neighboring seam cells at about eight hours after hatching (Fig. 3E). An analogous cycle of loss and reformation of cell contacts has been seen at the later rounds of stem-cell division by the seam cells (data not shown; Podbilewicz and White, 1994).

Thus seam cell contact is not continuous between hatching and the time that the postdeirid neuroblast, the granddaughter of V5, is born. V5 is in contact with its neighbors V4 and V6, but its daughter V5.p is not initially in contact with V4.p and V6.p. Instead, V5.p actively forms contacts with its neighbors and then goes on to divide and produce a postdeirid neuroblast daughter.

Commitment occurs when V5.p contacts its seam cell neighbors

Having found that the seam cells were not in continuous contact during the time that the decision to make a postdeirid must occur, we wanted to know whether the signaling required for this decision takes place at a time when seam cells are in contact. To determine when signaling required for postdeirid formation occurs, we ablated seam cells anterior or posterior to V5 at different times during the first larval stage. Seam cell ablations before completion of the required signaling should block postdeirid formation. In contrast, if there is a point at which signaling is complete, ablating seam cells after this time should have no effect.

We found that there was a dramatic shift in the effect of seam cell ablations on the decision to make a postdeirid, midway through the first larval stage, at about 8 hours after hatching. Ablating the seam cells V6 or V6.p before 8 hours prevented postdeirid formation, whereas ablation of V6.p after

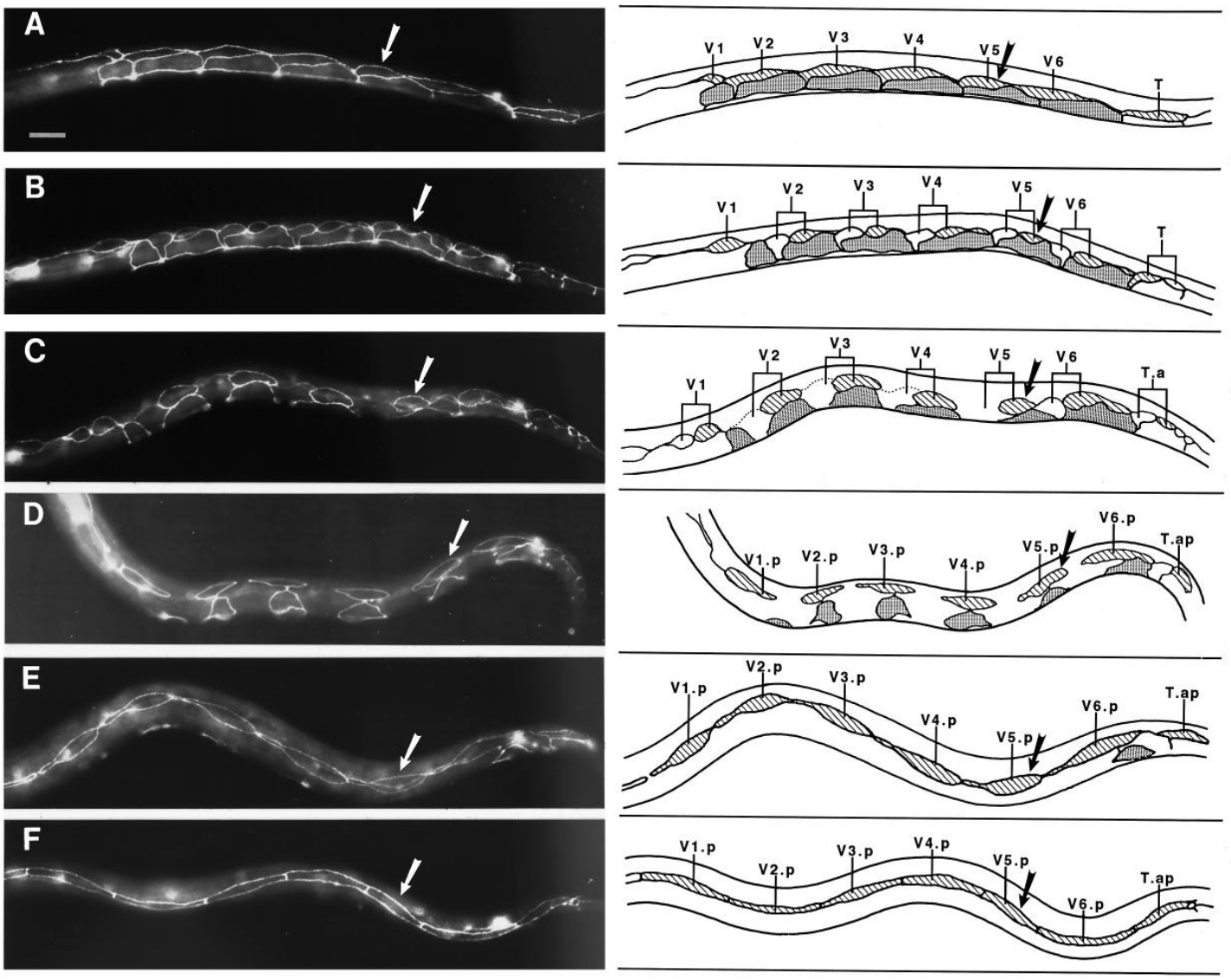

Fig. 3. Pattern of seam cell contacts in L1 larvae. Animals were stained at different times after hatching with MH27, a monoclonal antibody that recognizes the apical junctions of epithelial cells in *C. elegans* (Francis and Waterston, 1991). In each picture, the seam cells V5 or V5.p are indicated with an arrow. In the interpretive drawings, seam cells are marked with diagonal lines; ventral epidermal cells (P cells) are shaded. (A) 3 hours; (B) 5 hours; (C) 6 hours; (D) 7 hours; (E) 8 hours; (F) 9 hours. Scale bar is 10 μm.

8 hours had no effect (Fig. 4, open symbols). A similar result was seen after ablation of seam cells anterior to V5. Ablating either V2, V3, V4 or V2.p, V3.p, V4.p prior to 8 hours after hatching blocked postdeirid formation. When V2.p, V3.p, V4.p were ablated after 8 hours however, V5.p developed normally (Fig. 4, filled symbols).

These results show that V5.p, the mother of the postdeirid neuroblast, requires the presence of its anterior and posterior neighbors in order to develop normally but that this requirement is transient. At about 8 hours after hatching, signaling required for postdeirid formation has occurred and V5.p has become committed to postdeirid formation. After this time, seam cell ablations have no effect. Comparison of this result with the pattern of seam cell contacts described above, indicates that V5.p's commitment to postdeirid formation occurs soon after it contacts the neighboring seam cells V4.p and V6.p. In addition, the sharp change in the effect of cell ablations before and after this time argues that signaling, critical for V5.p's normal development, occurs at the time that these seam cell contacts are formed.

Seam cell contact is required for the decision to make a postdeirid

The correlation between the time of seam cell contact and the time that V5.p becomes committed to making a postdeirid suggested that these cell contacts are required for V5.p to develop normally. In this case, one might predict that cell ablations that prevent formation of seam cell contacts by V5.p should prevent postdeirid formation, while cell ablations that do not affect V5.p's ability to contact its seam cell neighbors should not.

To test this prediction, we wanted to determine whether V5.p is capable of forming new seam cell contacts when its normal neighbors have been removed by cell ablation. Because

in these experiments it was necessary to examine contacts between the seam cells in single animals, we used a different technique to visualize the seam cell outlines. We had found that when animals were incubated in 0.25% SDS, the seam cells become visible using Normarski optics (see Materials and Methods). The patterns of seam cell contact observed after treatment with SDS were similar to those observed after staining with MH27 (Fig. 5A-D). Using this technique, we examined the seam cell contacts formed by V5.p after seam cell ablations.

We found that V5.p is able to form new seam cell contacts after ablation of its normal neighbors. After the first round of division, at the time of normal seam cell process extension, seam cells can extend cell processes across a gap produced by cell ablation to form new contacts. In animals in which one (V4) or two (V3, V4) seam cells were ablated, V5.p was able to make contact with the next intact anterior seam cell (Fig. 5E; Fig. 6A). However, there was a limit to the extent of cell growth. When three seam cells (V2, V3, V4) were ablated, cell processes were extended by both V1.p and V5.p, but process growth stopped before they made contact (Fig. 6A).

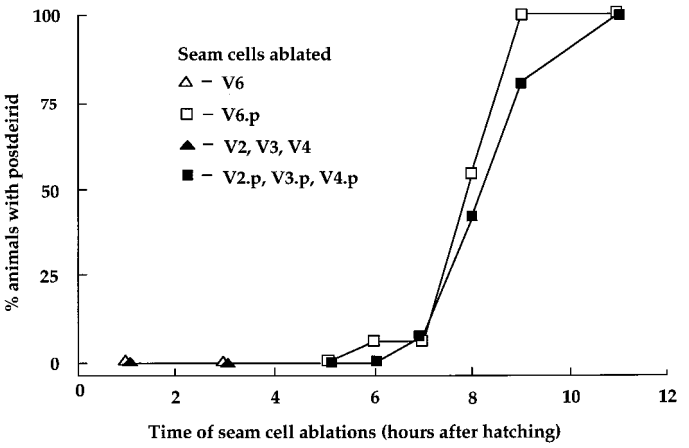

Fig. 4. Time of commitment to postdeirid formation. Seam cells were ablated at indicated time after hatching; animals were scored as late L2 larvae for presence of a normal postdeirid. In general, the seam cells (including V5) divided between 3 and 5 hours after hatching (see material and methods). $n \geq 10$ for each time point.

These results support the model that cell contact is required for postdeirid formation. After anterior seam cell ablations that do not prevent postdeirid formation (V4 or V3, V4, see Fig. 1C), V5.p forms a new cell contact. After anterior ablations that prevent postdeirid formation (V2, V3, V4), no new contact is formed.

Formation of a new seam cell contact is not always sufficient to allow postdeirid formation. After ablation of V3 and V4, although V5.p is able to contact V2.p, a postdeirid is sometimes, but not always, made (Fig. 1C; Fig. 6A). In addition, after ablation of V6, V5.p does not make a postdeirid even though it connects to a seam cell in the tail, T.ap (Figs 1C, 6B). This result suggests that additional factors, such as the time of seam cell contact, or the identity of the cell that V5.p makes contact with, also play a role in the decision whether or not to make a postdeirid.

Delayed seam cell contact delays postdeirid commitment

We had found that V5.p normally becomes committed to its normal developmental fate soon after the time at which it contacts its seam cell neighbors. We wanted to know whether this commitment decision was actually triggered by cell contact. If so, delaying the time of either anterior or posterior cell contact should also delay the time of commitment. As

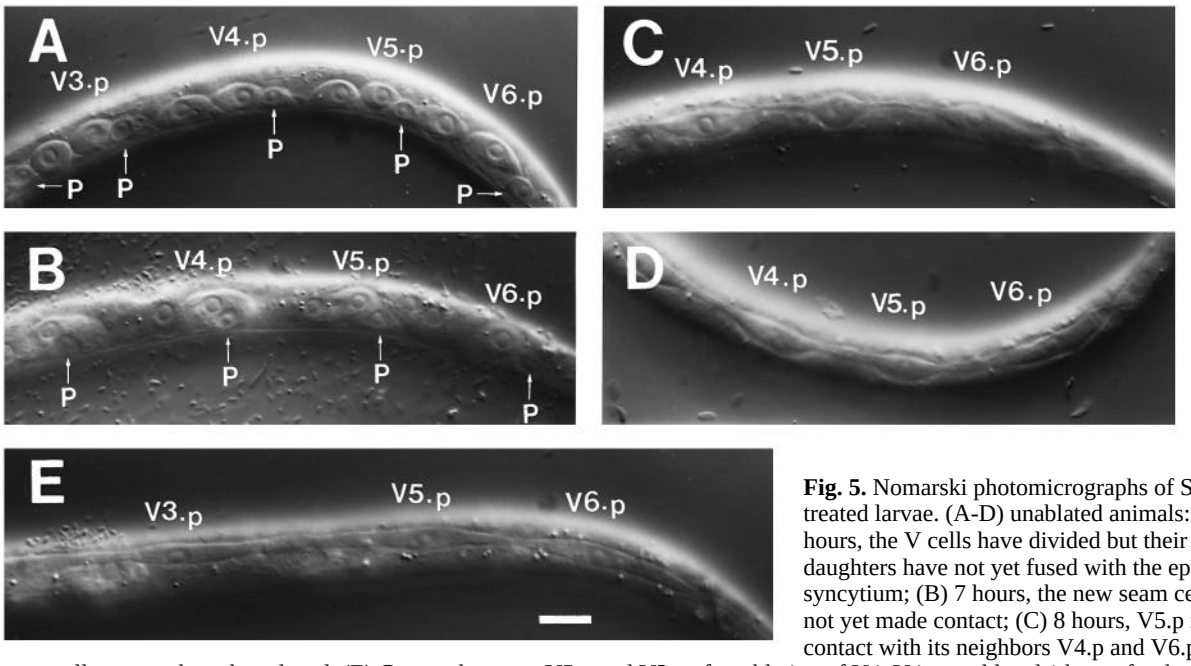

Fig. 5. Nomarski photomicrographs of SDS-treated larvae. (A-D) unablated animals: (A) 5 hours, the V cells have divided but their anterior daughters have not yet fused with the epidermal syncytium; (B) 7 hours, the new seam cells have not yet made contact; (C) 8 hours, V5.p is in contact with its neighbors V4.p and V6.p; (D) 9 hours, the seam cell contacts have broadened. (E) Contact between V5.p and V3.p after ablation of V4. V4 was ablated 1 hour after hatching and animal was photographed ~11 hours after hatching. Note that treatment with SDS does not make the boundaries between the seam cells visible. Scale bar is 10 μm.

described above, after ablation of V4, V5.p was able to contact V3.p and develop normally. The time of this contact however, was delayed about 2 hours beyond the time of normal contact between V5.p and V4.p (Fig. 6A). If V5.p's decision to develop normally occurs when it contacts V3.p, then the time of this decision should also be delayed.

To test this prediction, we determined the time of commitment to postdeirid formation after ablation of V4 (Fig. 7A). In the experimental animals, V4 was ablated at hatching and the anterior seam cells V2.p and V3.p were ablated at later times to determine when V5.p had become committed to making a postdeirid. In these animals, V5.p and V3.p should make contact 9-11 hours after hatching. If V5.p's commitment to postdeirid formation occurs when it contacts V3.p, then ablation of V2.p and V3.p before this time should prevent postdeirid formation while later ablations should not. In the control animals, we ablated V2 at hatching, to control for the effects of laser microsurgery, and then determined the time of postdeirid commitment by ablating V3.p and V4.p at later times.

We found that ablation of V4 shifted the time of V5.p's commitment to postdeirid formation (Fig. 7B). In control animals, the critical time for commitment to postdeirid formation was 8-10 hours after hatching. For animals in which V4 had been ablated, we found that the critical time for postdeirid formation was approximately 2 hours later, at 10-11 hours after hatching. Thus when ablation of V4 caused V5.p to contact V3.p, at a later time than it would normally contact V4.p, the final decision to make the postdeirid did not occur until after formation of the V5.p-V3.p contact. This result indicates that postdeirid commitment occurs as the result of seam cell contacts: when the time of contact is delayed, the time of commitment is delayed as well.

Postdeirid formation can occur after seam cell ablations in *mab-5(–)* animals

How does formation of contacts between the seam cells control cell fate? What are the downstream events that occur within V5.p as the result of these contacts and are required for its normal development? One target of these signals may be the HOM-C gene *mab-5*. In *C. elegans*, as in *Drosophila* and other metazoan species, a cluster of genes encoding Antennapedia-class homeodomain proteins has been shown to be responsible for the specification of cell fate along the anteroposterior axis (Bürglin et al., 1991; Clark et al., 1993; Costa et al., 1988; Kenyon and Wang, 1991; Wang et al., 1993). Specification of cell fate in the posterior body region of *C. elegans* is controlled by the *mab-5* gene, while *lin-39* specifies cell fates in the middle body region, and *egl-5* specifies cell fates in the tail (Kenyon, 1986; Chisholm, 1991; Wang et al., 1993; Clark et al., 1993). Each of these genes is expressed in the body region in which it acts to specify cell fate (Costa et al., 1988; Wang et al., 1993).

V5 is located in the region where many cells express *mab-5*, therefore, we were curious to know what role this gene might play in the decision to make the postdeirid neuroblast. *mab-5* activity is not required for postdeirid formation: normal postdeirid formation is observed in *mab-5(–)* animals. In addition, although V5 and its daughter V5.p lie in the region of general *mab-5* expression, these cells do not express *mab-5* (Cowing and Kenyon, 1992; Salser and Kenyon, 1992; S. Salser and C. Kenyon, unpublished data). Previous experiments had

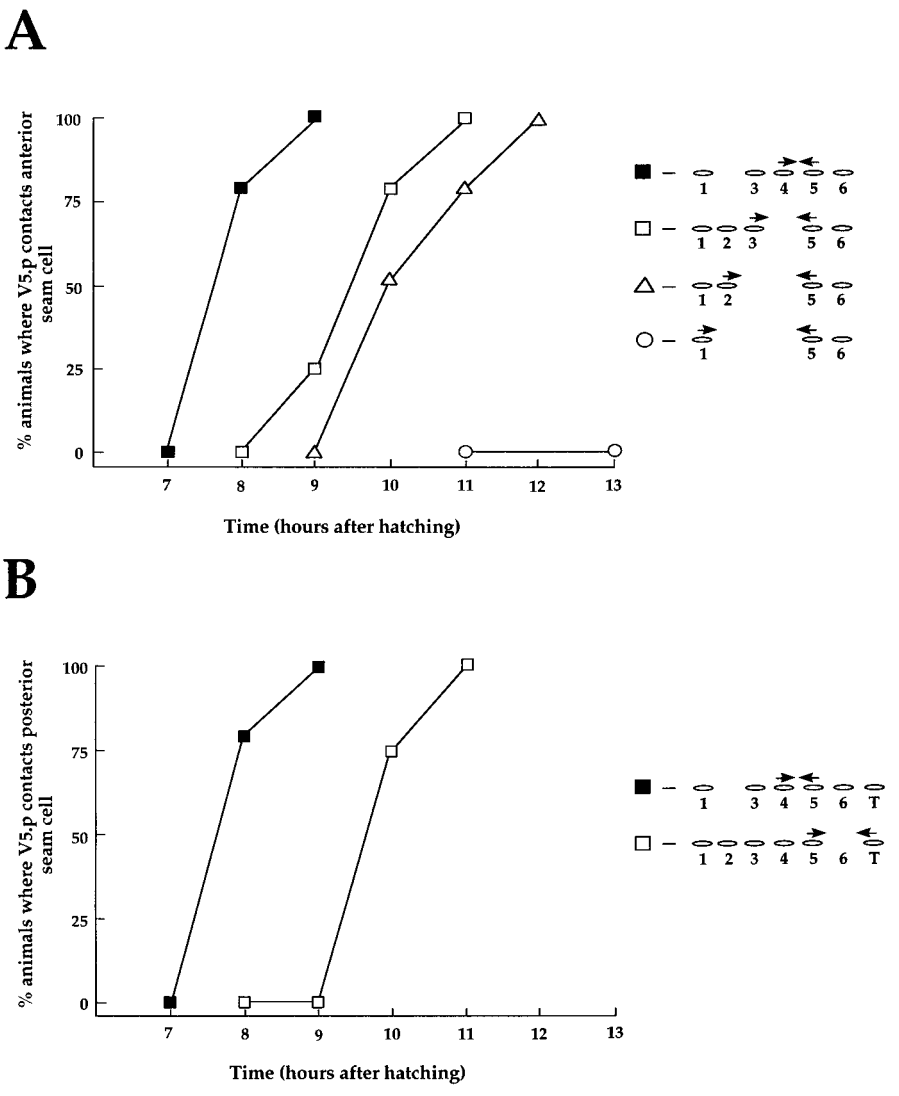

Fig. 6. Seam cell ablations delay or prevent contact between V5.p and its neighbors. Seam cells were ablated 1 hour after hatching; at 1 hour intervals between 7 and 13 hours after hatching, animals were treated with SDS to determine whether V5.p was in contact with its anterior and posterior neighbors. $n \geq 4$ for each time point. (A) Time of contact between V5.p and anterior neighbor. ■, V5.p-V4.p contact after V2 ablation (control). □, V5.p-V3.p contact after V4 ablation. △, V5.p-V2.p contact after V3, V4 ablation. ○, V5.p-V1.p contact after V2, V3, V4 ablation. (B) Time of contact between V5.p and posterior neighbor. ■, V5.p-V6.p contact after V2 ablation (control). □, V5.p-T.ap contact after V6 ablation.

suggested the model that *mab-5* expression or activity is inhibited in V5 or its descendants by signaling from their seam cell neighbors and that this inhibition can be relieved by ablation of the neighboring seam cells (Sulston and White, 1980; Waring and Kenyon, 1990 and see Discussion). This model suggested the possibility that *mab-5* might be involved in the inhibition of postdeirid formation after seam cell ablations.

If seam cell ablations inhibit postdeirid formation by activating *mab-5*, then these cell ablations should not prevent postdeirid formation in a *mab-5(−)* animal. To test this prediction, we ablated either V6 or V2, V3, V4 in *mab-5(1239)* animals. Based on genetic and molecular data, *mab-5(e1239)* appears to be a null allele (Kenyon, 1986; S. Salser, C. Kenyon, unpublished data).

We found that, in *mab-5(e1239)* animals, ablating the seam cells anterior or posterior to V5 was not effective in blocking postdeirid formation (Fig. 8). Whereas ablation of V6 inhibited postdeirid formation in wild-type animals, 60% of the *mab-5(e1239)* animals made a postdeirid after ablation of V6. After ablation of V2, V3, V4, 15% of the *mab-5(e1239)* animals also made a postdeirid. In addition, in many of the *mab-5(e1239)* animals that did not make a normal postdeirid after these seam cell ablations V5.pa appeared to undergo a reiterated lineage (see Fig. 1D, above). Similar results were seen when V6 or V2, V3, V4 were ablated in *mab-5(e1936)* animals (data not shown). In contrast, mutations in two other HOM-C genes, *lin-39* and *egl-5*, did not alter the effect of seam cell ablations on postdeirid formation (data not shown). The results of these experiments suggest that *mab-5* may be part of the pathway by which signaling between the seam cells determines whether or not a postdeirid will be made.

DISCUSSION

Seam cell signals commit V5.p to producing a neuroblast daughter

Previous studies showed that ablating seam cells anterior or posterior to V5 could alter its developmental fate and prevent it from forming the postdeirid neuroblast. This result indicated that some type of signaling between the seam cells is required for normal development. Our results indicate that it is V5.p, the mother of the postdeirid neuroblast, that requires signals from its seam cell neighbors in order to produce a neuroblast daughter. If these signals are disrupted, then V5.p will take on the fate of the analogous cells in the other V cell lineages and divide to produce two seam cell daughters. After signaling occurs between V5.p and its neighbors, seam cell ablations no longer affect V5.p's fate, indicating that it is now committed to its normal developmental fate.

Signaling occurs via seam cell contact

During larval development, the seam cells undergo cycles of loss and reformation of cell-cell contact. At hatching, each seam cell is in contact with its anterior and posterior neighbors. After each round of stem-cell division, the new

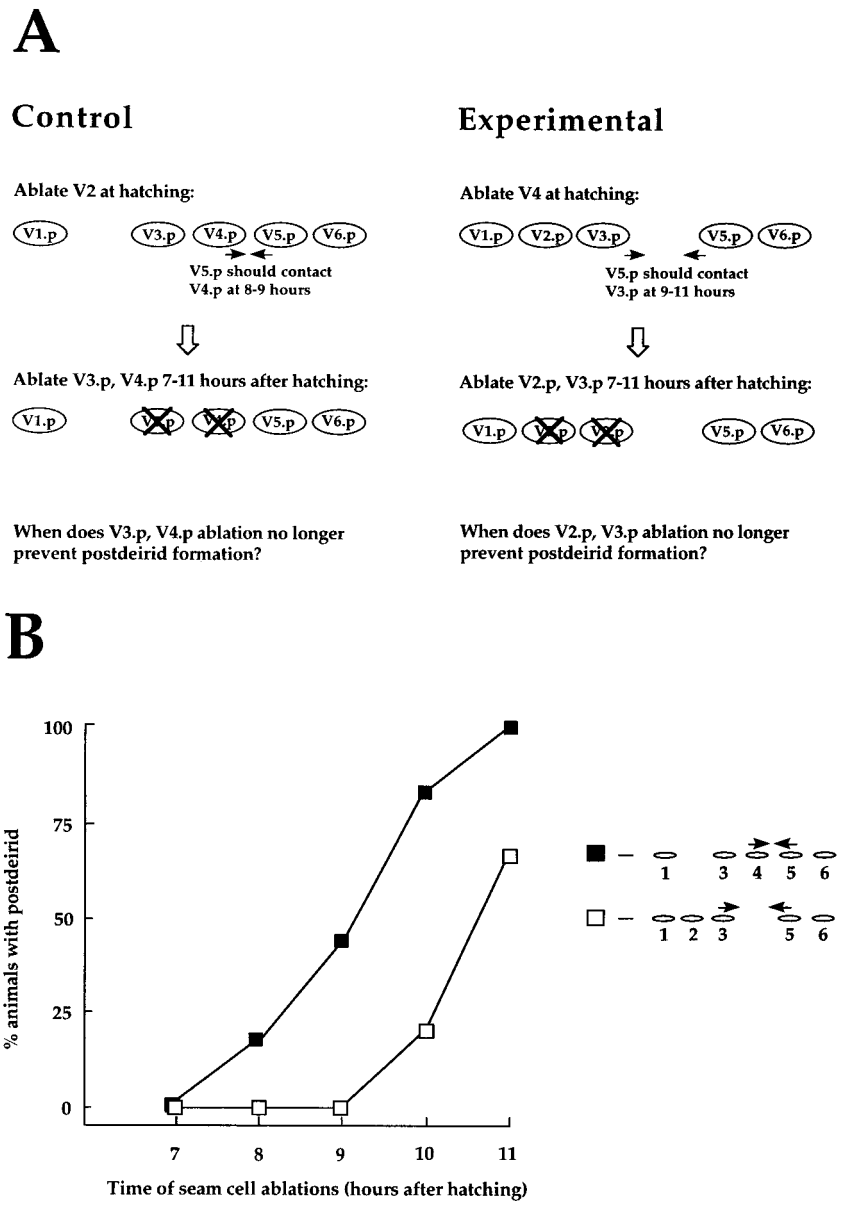

Fig. 7. Ablating V4 delays both seam cell contact and commitment to the postdeirid fate. (A) Experimental design. The time of commitment to the postdeirid fate in animals where V4 was ablated soon after hatching was compared to that seen in control animals where V2, a seam cell not adjacent to V5, was ablated instead. V2 ablations (control): V2 was ablated 1 hour after hatching, V4.p and V3.p were ablated 7-11 hours after hatching. V4 ablations: V4 was ablated 1 hour after hatching, V2.p and V3.p were ablated 7-11 hours after hatching. All animals were subsequently scored for postdeirid formation as described in Materials and Methods. Times shown for V5.p-V3.p and V5.p -V4.p contact are from experiments described in Fig. 6. (B) Timing of commitment to postdeirid formation after ablation of V4. □, animals in which V4 was ablated at hatching. ■, control animals in which V2 was ablated at hatching. *n*=5-7 for each time point.

daughter seam cells are separated by their sisters, which will fuse with the epidermal syncytium, hyp7. Contact between the seam cells is subsequently re-established by anterior-posterior extension of seam cell processes. We have found that signaling required for V5.p to produce a postdeirid neuroblast daughter occurs at the time that V5.p contacts its seam cell neighbors after the first round of division. Ablation of the seam cells anterior or posterior to V5.p just before this time prevents postdeirid formation; after contact is made these ablations have no effect.

We tested the correlation between seam cell contact and signaling in two ways. First, we asked whether V5.p is capable of forming new seam cell contacts after ablation of the adjacent seam cells. We found that, for anterior seam cell ablations that did not prevent postdeirid formation, V5.p contacted the next remaining seam cell. In contrast, killing the seam cells V2, V3 and V4 prevented both cell contact and postdeirid formation. Second, we found that after ablation of V4, when V5.p makes a delayed contact with V3.p, the time of signaling required for postdeirid formation is also delayed.

What are the molecules involved in seam cell signaling?

After each round of stem-cell division, the seam cells extend anterior and posterior processes that grow until they contact the next seam cell. This behavior suggests that formation of contacts between the seam cells generates a signal that inhibits further growth. When V5.p contacts its seam cell neighbors, it receives signals that control its fate. How are these signaling events mediated? Is signaling the result of a ligand-receptor pair specific for this cell fate decision or is it part of a more general process of cell recognition and adhesion?

The requirement for cell contact suggests that signaling between the seam cells involves an interaction between membrane-bound molecules. Moreover, some part of it must be seam-cell specific, since the seam cells are able to distinguish seam cells from other epidermal cell types. Because these signals appear to occur between all seam cells, they may be part of a system of seam cell-specific recognition and adhesion.

Intercellular signaling pathways that control cell fate have been identified in *C. elegans*. The *glp-1* and *lin-12* genes encode transmembrane proteins homologous to the *Drosophila* gene *Notch*, that act as receptors in cell interactions that control cell fate during embryonic and larval development (Austin and Kimble, 1987; Greenwald et al., 1983; Priess et al., 1987; Yochem and Greenwald, 1989; Yochem et al., 1988). The genes *lin-3* and *let-23* are respectively members of the EGF and EGF receptor families and, along with the ras homologue *let-60*, define a signal transduction pathway that controls the process of vulva induction (Aroian et al., 1990; Aroian and Sternberg, 1991; Ferguson and Horvitz, 1985; Han et al., 1990; Han and Sternberg, 1990; Hill and Sternberg, 1992). When animals carrying mutations in each of these genes were examined, no effect on postdeirid formation was observed. This result suggests that signaling between the seam cells does not involve either of these signal transduction pathways (see Materials and Methods).

A different system in which signaling between cells in an epithelial sheet has been shown to control cell fate is determination of *Drosophila* segment polarity (Ingham, 1991). The segment polarity gene *armadillo* has been identified as a homologue of β-catenin (Peifer et al., 1992; Peifer and Wieschaus, 1990). In vertebrates, molecules of this class have been shown to associate with cadherin-based junctional complexes (Peifer et al., 1992) and a similar complex may exist in *Drosophila* (Oda et al., 1993). Although members of the cadherin family have not yet been identified in *C. elegans*, such a system of cell-type-specific adhesion and signaling would fit well with the characteristics of the system described here. It has been found that signaling between cells in the determination of segment polarity requires the *Wnt* gene family member *wingless* (Nüsslein-Volhard and Wieschaus, 1980; Rijsewijk et al., 1987). Two members of the *Wnt* gene family have so far been identified in *C. elegans* (Kamb et al., 1989; Shackleford et al., 1993) and would be possible candidates for the signaling molecules that act between the seam cells.

The HOM-C gene *mab-5* has a role in postdeirid formation

The HOM-C gene *mab-5* specifies the fates of many cell types in the posterior body region of the worm (Kenyon, 1986). It has been shown that *mab-5* gene expression is also restricted to this region (Costa et al., 1988; Cowing and Kenyon, 1992; Salser et al., 1993). However within this region *mab-5* expression is not uniform. Although the seam cells V5 and V6 both lie within the region of *mab-5* expression, only V6 expresses *mab-5* (Cowing and Kenyon, 1992; S. Salser, C. Kenyon, unpublished data).

Experiments that examined the production of the *mab-5*-dependent male sensory rays have shown that *mab-5* activity in the V5 and V6 lineages is subject to several types of regu-

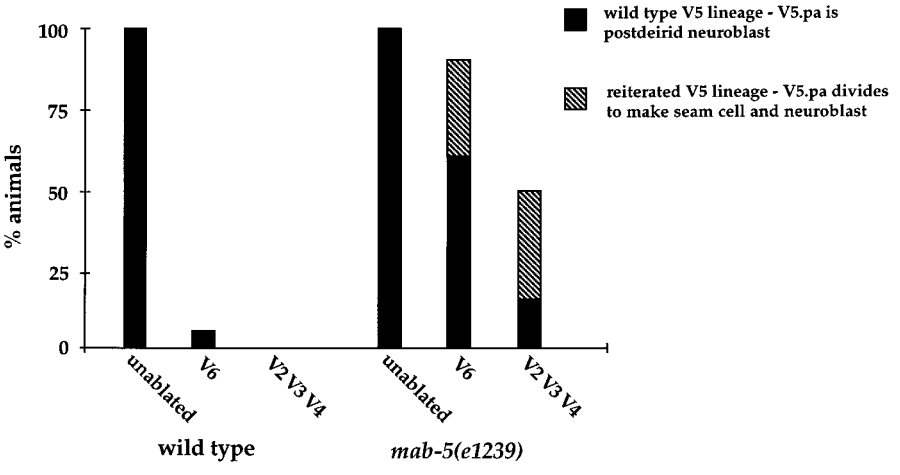

Fig. 8. Commitment to the postdeirid fate is inhibited by *mab-5* activity. The percentage of animals in which a postdeirid is made after anterior or posterior seam cell ablations was compared for wild-type and *mab-5(e1239)* animals. Both strains also contained *him-5(e1490)*, a mutation that increases the frequency of males. All seam cell ablations were done 1 hour after hatching. Wild type: n=20 for unablated control and V6 ablations; n=10 for V2, V3, V4 ablations. *mab-5(e1239)*: n=20 for unablated control, V6 ablations and V2, V3, V4 ablations.

lation (Sulston and White, 1980; Waring and Kenyon, 1990, 1991). Based on these results, the model was proposed that seam cell signals act to inhibit *mab-5* expression or activity in both V5 and V6 or their descendants, but that the *pal-1* gene in turn inhibits or overides this inhibition of *mab-5* in the V6 lineage (Waring and Kenyon, 1991).

The results of the present experiments indicate that control of *mab-5* activity may also be crucial to the normal development of V5.p and its decision to make the postdeirid. We have shown that in *mab-5(–)* animals V5.p can produce a postdeirid neuroblast daughter after seam cell ablations that would normally prevent postdeirid formation. One intriguing possibility suggested by these results is that *mab-5* expression is normally inhibited in V5.p by signaling from neighboring seam cells and that disruption of these signals by seam cell ablations allows aberrant *mab-5* expression in this cell, resulting in an altered cell fate. Alternatively, it is possible that in *mab-5(–)* animals, the requirement for seam cell signaling is altered in such a way that seam cell ablations no longer effectively inhibit postdeirid formation. Experiments to distinguish these possibilities are in progress.

Why do V4 and V6 ablations have different effects?

Both anterior and posterior seam cell ablations can alter the fate of V5.p. However, there is a difference in the effect of these two types of ablations. It is necessary to ablate two or more anterior seam cells in order to affect postdeirid formation; after ablation of only V4, V5.p is able to develop normally. In contrast, ablation of a single posterior seam cell, V6, alters V5.p's fate. A similar difference in the effect of V4 and V6 ablations on the production of *mab-5*-dependent male sensory rays by V5 has also been observed (Sulston and White, 1980).

What is the cause of this difference in the effect of anterior and posterior ablations? One possibility was that there are differences in the ability of V5.p to reconnect with another seam cell after ablation of V4 versus V6. However, we have found that this is not the case. V5.p is able to reconnect with the next neighboring seam cell after either V4 or V6 ablations. A second possible explanation is that T.ap, the seam cell in the tail that V5.p contacts after ablation of V6, is incapable of providing the appropriate signals. This also does not appear to be the case. In *pal-1*; *lin-22* animals, V6 produces a postdeirid: formation of this V6 postdeirid can be prevented by ablation of V6's posterior neighbor, the T seam cell (Waring et al., 1992).

Given that V5.p is able to connect to the seam cell T.ap and that this seam cell appears to be able to signal, why is production of the postdeirid still prevented by ablation of V6? One possibility currently under consideration is that *mab-5* expression in V5 switches on especially quickly or more strongly following ablation of its posterior neighbor V6. *mab-5* expression is normally restricted to the posterior body region (Costa et al., 1988; Cowing and Kenyon, 1992; Salser et al., 1993). It may be that V5.p extension in the posterior direction results in rapid expression of *mab-5* due to exposure to localized posterior signals that control *mab-5* expression. In this case, a short delay in the formation of the posterior seam cell contact might be sufficient to allow V5.p to express *mab-5*: a longer delay in the time of the anterior seam cell contact would be required to have the same effect.

Why are both anterior and posterior signals required?

On the basis of previous experiments it was proposed that V5 or its descendants require the presence of both anterior and posterior seam cells for normal development because they need to be part of a continuous line of cells (Waring et al., 1992). Our results lend credence to this hypothesis. We found that seam cells are initially in contact with one another and actively reform these cell contacts after rounds of cell division. In addition, after seam cell ablations, continued cell growth occurs over a distance several times that normally seen, that can result in reformation of these cell contacts. As the seam cells extend, they are constantly in contact with the epidermal syncytium. Therefore, this continued growth indicates that they are able specifically to recognize contact with other seam cells, and that the formation of these contacts generates a signal that results in a cessation of cell growth. Thus the extent of seam cell growth as well as developmental fate is controlled by signaling events that occur when seam cells make contact.

How does the cell discern the difference between seam cell contact in both the anterior and posterior directions and cell contact on one side only? One possibility is that there is an additive effect, such that the signals generated by two sites of seam cell contact are required for normal development. An alternative possibility however, is that the unconnected end of the seam cell is the source of a signal that acts to inhibit postdeirid formation. In this case, it would be the presence or absence of an unconnected cell process that determines the developmental fate of V5.p. Normally, an unconnected seam cell process would exist only for a short period of time. After seam cell ablations, however, the period of cell extension is artificially prolonged, resulting in a novel state for the cell that may alter preset patterns of gene expression.

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