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REGULATIVE DEVELOPMENT IN THE POST-EMBRYONIC LINEAGES OF CAENORHABDITIS ELEGANS

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INTRODUCTION

In many invertebrates, cell lineages are apparently invariant from individual to individual. A given precursor cell follows a specific pattern of cell divisions, and its descendants follow fates that correspond to their respective positions in the lineage tree. Such a reproducible sequence of events provides an excellent system for studying how cells come to pursue particular fates during development. We have been interested to know if a cell's fate is specified by factors intrinsic to the cell, or if it is influenced by interactions between the cell and its environment.

Caenorhabditis elegans is a particularly suitable organism for lineage studies because it is transparent throughout its life cycle, and because it consists of relatively few cells. Furthermore, *C. elegans* is a favorable organism for genetics, so the control of cell lineages can be studied by characterizing mutations that are defective in known lineages.

The cell lineages of *C. elegans* have been described in the embryo to the 182 cell stage[1] and after hatching[2,3]. Approximately 50 cells resume divisions post-embryonically. In the somatic tissues, the number of cells (or nuclei) is increased from about 550 to about 950 in hermaphrodites and to about 1025 in males. These post-embryonic lineages are essentially invariant from worm to worm. As the worm enlarges and matures sexually, cells (or nuclei) are added to previously existing tissues (hypodermis, muscle, gut, and nervous system), and structures necessary for reproduction are elaborated. The latter include a gonad in both sexes, a vulva in hermaphrodites, and a tail specialized for copulation in males.

This paper summarizes the results of laser ablation experiments performed on cells in the post-embryonic lineages of *C. elegans*. In particular, we focus on those experiments that demonstrate a regulative capacity in the cells of this predominantly invariant system. The post-embryonic lineages have the practical advantage for these studies that they can be easily traced by direct observation of the cells as they divide and assume their final fate. The regulative response, therefore, can be described at a level of cellular detail that has not been possible in other deletion studies. Our aim in performing these experiments is to infer how cells are controlled during normal development from their behavior in an abnormal situation.

MATERIALS AND METHODS

C. elegans var. Bristol of wild type was maintained on agar-filled Petri plates seeded with *E. coli* as described by Brenner[4].

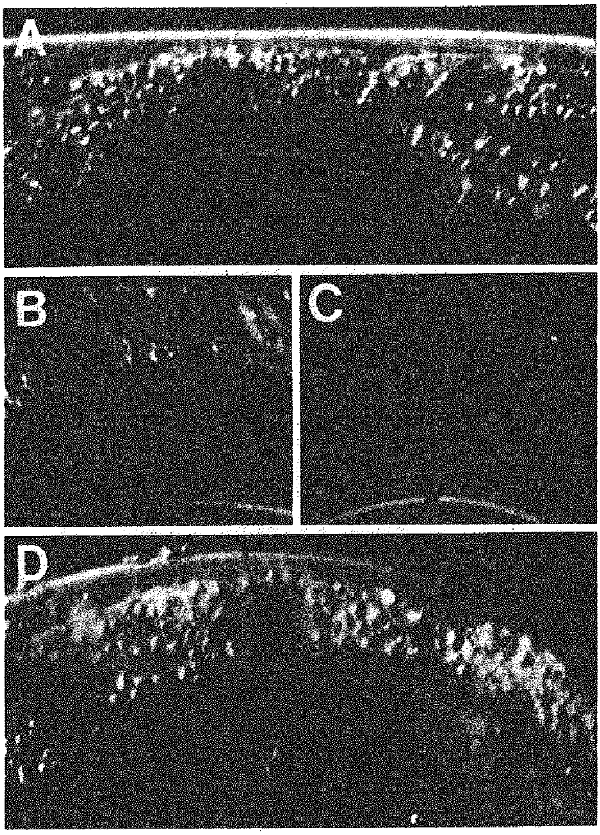

Fig. 1. Nomarski photomicrographs of an anchor cell before ablation (A), within 10 minutes after ablation (B and C), and six hours after ablation (D).

The details of the laser ablation procedure will be published separately (Sulston and White, in preparation). Briefly, individual worms are anaesthetized, transferred to a thin slab of agar, and covered with a coverslip. A target cell is identified and centered for ablation at 1000X with Nomarski optics (Figure 1A). The cell is killed over a period of 5 - 10 minutes (Figure 1B and 1C) by multiple shots of a coumarin 2 dye laser microbeam. A few hours after the cell ablation, the worm is observed in order to validate the success of the operation (Figure 1D). Animals that have suffered visible damage to neighboring cells or that retain the target cell are discarded. Each result reported here was obtained in at least two, and usually more, individuals.

RESULTS

Ablation of certain cells in the post-embryonic lineages of C. elegans results in an alteration in the expected lineage of one or more of the remaining cells. Such regulation has been observed among three types of precursor cells (Figure 2) and their progeny. Within each of these precursor types, cells are similar in morphological appearance and in prospective fate. Regulation is limited to subsets of the precursors, and each type displays unique features of regulation.

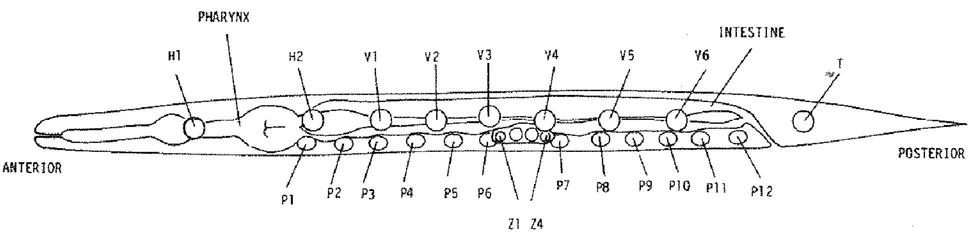

Fig. 2. Locations of three precursor cell types in a hypothetical young worm. The precursors do not coexist in these positions in a real animal due to differences between them in timing of divisions and migrations. Precursors of the ventral hypodermis, P1-P12, are found ventrally. Precursors of the lateral hypodermis, H1-H2, V1-V6, T are shown on one side only. Precursors of the gonadal somatic tissues, Z1 and Z4, are found in the mid-ventrally located gonadal primordium.

Ventral hypodermis

Twelve precursor cells, P1-P12, (Figure 2) give rise to cells of the ventral hypodermis and neurons in the ventral nerve cord. Regulation occurs in two groups of ventral hypodermal cells (P3.p-P8.p in hermaphrodites and

P9.p–P11.p in males, where Pn.p is the posterior daughter of Pn). In the intact animal, most Pn.p either become hypodermal cells, or divide once and produce two hypodermal daughters. In hermaphrodites, P5.p, P6.p, and P7.p divide further to make the vulva, and in males, P10.p and P11.p divide further to contribute cells to the copulatory male tail.

For brevity, only the results of ablation experiments pertaining to the male P9.p–P11.p group are presented here (Table 1).

TABLE 1

REGULATION IN THE VENTRAL HYPODERMIS

The first line shows the fates followed by each cell in the intact animal. Subsequent lines show the fates followed by cells after ablation of the cells indicated in the left column.

Cell Ablated	Cell Pn.p and its Fate				
	P8.p	P9.p	P10.p	P11.p	P12.p
–	P8.p	P9.p	P10.p	P11.p	P12.p
P8.p	–	P9.p	P10.p	P11.p	P12.p
P9.p	P8.p	–	P10.p	P11.p	P12.p
P10.p	P8.p	P10.p	–	P11.p	P12.p
P11.p	P8.p	P10.p	P11.p	–	P12.p
P12.p	P8.p	P9.p	P10.p	P11.p	–
P9, P10	P8.p	–	–	P11.p	P12.p
P10.p, P11.p	P8.p	P11.p	–	–	P12.p
P9.p, P10.p, P11.p	P8.p	–	–	–	P12.p

If P11.p is ablated, P10.p assumes the position of P11.p and follows the lineage normally followed by P11.p, and P9.p assumes the position of P10.p and follows the P10.p lineage. If P10.p is ablated, P9.p replaces P10.p, but P11.p is not affected. If P10.p and P11.p are killed P9.p replaces P11.p. Ablation of all three cells, P9.p, P10.p, and P11.p effects no change in lineage of the neighboring cells, and conversely, ablation of neighboring cells P8.p or P12.p effects no response in P9.p, P10.p, or P11.p

The main features of regulation in the male P9.p–P11.p group also apply to the hermaphrodite P3.p–P8.p group:

1) The regulative response involves the precise replacement of one precursor cell for another. The adult animal is therefore missing the progeny of those cells, that are recruited into another lineage and are not replaced

themselves.

2) This replacement occurs according to a hierarchy of fates. In males, P11.p occupies the primary, P10.p the secondary, and P9.p the tertiary position in this hierarchy.

3) Regulation is limited to a small group of cells. These cells are posterior daughters of similar precursor cells and therefore they are similar in ancestry.

Lateral hypodermis

Nine precursor cells of the lateral hypodermis, H1, H2, V1-V6, and T (Figure 2) are found on each side of the newly hatched worm. Regulation in the lateral hypodermis has been studied chiefly in the male where three cells, V5.p, V6.p, and T, normally generate one, five, and three sensory rays respectively for the copulatory apparatus of the male tail. V5 also gives rise to the cells of a special sensillum, the post-deirid.

Two types of regulation have been observed in the lateral hypodermis. The first type is similar in character to the regulation seen in the ventral hypodermis, but, in comparison, it is both incomplete and inexact:

1) Regulation occurs by replacement of the specialized structures normally made by the ablated cell (i.e. sensory rays), but is not reproducibly precise. If V6 is ablated, V5 can replace it exactly, or it can make an intermediate number of rays. V4, which normally does not make rays, can be recruited to do so, but V4 never replaces V5 or V6 fully. If V5 is ablated, V4 is recruited to make rays, and V6 remains unaffected.

2) Regulation occurs according to a hierarchy in that the regulating cell always makes more rays than normal.

3) Ray regulation occurs among a small group of cells, V4-V6. Although T also makes rays, no regulation is observed between V6 and T.

The second type of regulation involves a proliferative response without replacement of specialized structures. Several V cells must be ablated to invoke this response. For example, if V4-V6 are ablated, V3 undergoes extra divisions and produces extra hypodermal cells. If V1-V4 are ablated, V5 undergoes extra divisions. In this case, V5 produces supernumerary clusters of presumptive ray cells that do not differentiate into rays.

Gonad

Two precursors, Z1 and Z4, (Figure 2) give rise to the somatic cells of the gonad. Regulation in the gonadal lineages differs from regulation in the hypodermis in that the ability of a cell to alter its lineage correlates directly with a variability in lineage seen in the intact animal. This natural variability involves pairs of cells that follow one of two alternative fates in any given individual. The two members of each pair arise in equivalent branches of the Z1 and Z4 lineages.

The ancestry of two such pairs in the hermaphrodite (cells 1 and 4; cells 2 and 3) is shown in Figure 3a. These four cells, as a group, assume positions

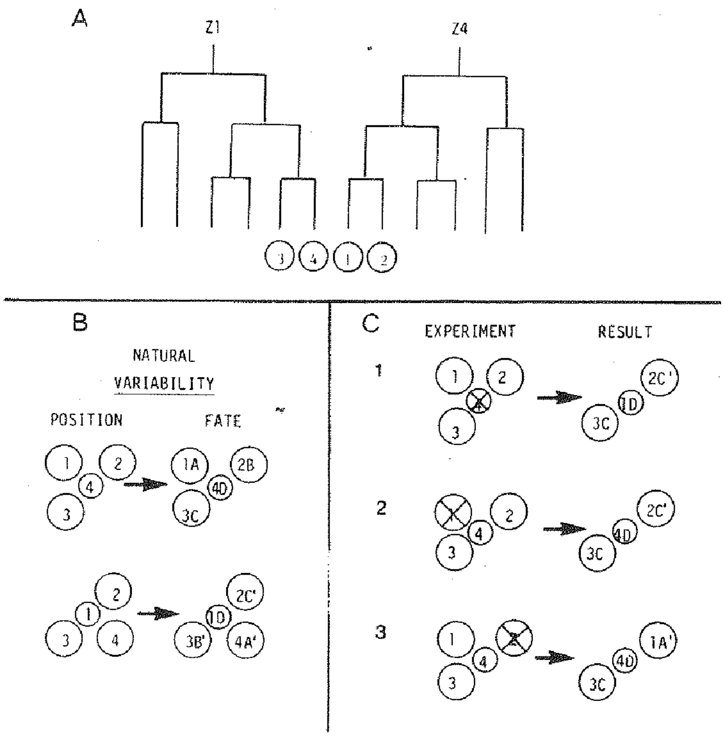

Fig. 3. Ancestry and fate of two cell pairs (cells 1 and 4; cells 2 and 3) in the developing hermaphrodite gonad. a) Members of each cell pair arise in equivalent points in the Z1-Z4 lineage. b) In intact animals, cells 1-4 assume one of two configurations and follow one of two alternative fates. The fate is represented by a letter and the polarity of the cell division pattern with respect to the worm's coordinates is indicated by the absence or presence of a prime (') next to the letter. c) Ablation experiments result in various types of regulation. An X shows the ablated cell. See text for details.

in one of two alternative configurations, and the choice of fate for each of the four cells corresponds to the configuration of the group (Figure 3b). Fates A, B, and C are unique with respect to the number of cell divisions, but are the same with respect to the kinds of cell produced. Fate D involves differentiation into the anchor cell - a special cell that does not divide and that appears to induce the vulva.

Ablation experiments show that these cells regulate by following their natural alternative fate (Figure 3c) in two cases. Three modes of regulation, however, have been found.

1) If the presumptive anchor cell (fate D) is ablated, the alternative anchor precursor replaces it (Expt. 1). Since the reciprocal replacement does not occur (Expt. 2), fate D is primary and fate A is secondary. In the male, regulation in a homologous pair of cells occurs in the same way.

2) The removal of cell 1, either by regulation (Expt. 1) or by ablation (Expt. 2), influences cell 2 to follow fate C' rather than B. This alteration of lineage restores the symmetry of the developing gonad.

3) Ablation of cell 2 influences cell 1 to reverse the polarity of its division pattern with respect to the worm's coordinates (switch from A to A', Expt. 3). We call this reversal vectorial regulation (Figure 4). Another example of vectorial regulation is seen at a different point in the hermaphrodite gonadal lineage.

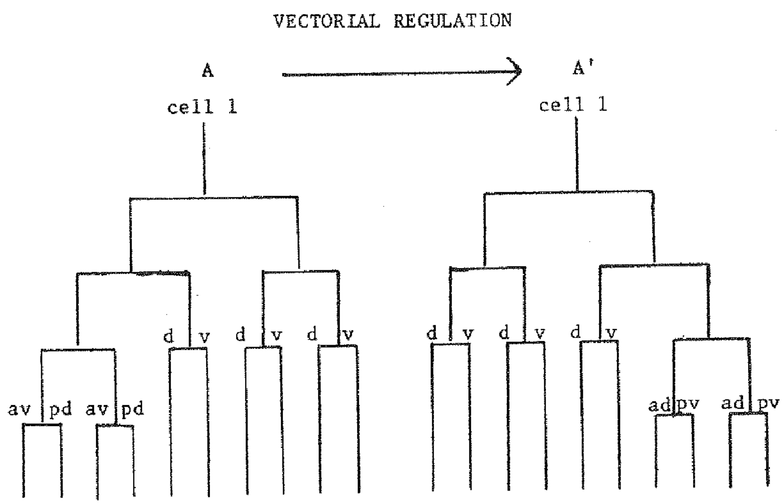

Fig. 4. Vectorial regulation. The lineage is changed by reversing the pattern of cell divisions with respect to the anterior-posterior axis of the worm. Anterior is to the left.

DISCUSSION

Despite the invariance of the post-embryonic lineages in C. elegans, some cells modify their lineages in response to the laser ablation of a neighboring cell. Such a change in the expected behavior of a cell appears to compensate for the deleted cell or cells, and is called regulation.

Three types of regulative response have been observed.

1) In the ventral hypodermis, lateral hypodermis, and gonad, specialized cells are replaced by the recruitment of a neighboring cell. Such replacement occurs according to a hierarchy of fates: if putative cell X is ablated, putative cell Y will replace it, but the converse replacement of cell Y by cell X is never observed. We refer to fate X (the fate typically followed by cell X) as the primary fate, and fate Y as the secondary fate in this hierarchy. In each case, the ablated cell and the recruited cell are similar in ancestry in that they arise in identical branches of the lineages of similar precursor cells.

2) In the lateral hypodermis, a proliferative response has been observed in which neighboring cells undergo extra divisions without replacement of specialized cells.

3) In the gonad, vectorial regulation, a reversal in the polarity of the pattern of cell divisions, without any change in the number or order of cell divisions, has been observed.

The most straightforward interpretation of these regulative responses is that cell-cell interactions influence the lineage a particular cell will follow in the intact animal. Alternatively, the process of ablating a cell might release "determinants", and the neighboring cell might take them up and be reprogrammed. Or, secondary damage to a neighboring cell might destroy some component critical to its determined state and allow reprogramming. Although neither of these latter explanations has been ruled out, both seem unlikely. The specificity and reproducibility with which regulation occurs argue against a random event being the basis of regulation. Furthermore, cells have been observed to regulate in response to the removal of a neighbor, either by ablation of the neighbor or by regulation of the neighbor to a different fate. This argues against the hypothesis that the ablated cell releases determinants to reprogram its neighbor. Our bias, therefore, is that regulative behavior after ablation is indicative of processes that normally influence cell fate.

The various modes of regulation observed in these experiments suggest that lineage patterns can be influenced in a number of ways by cell-cell interactions:

1) The existence of a fate hierarchy indicates that the decision to follow

a secondary or tertiary fate depends on some kind of signal that the higher fates have been assigned.

2) The proliferative response observed suggests that the total number of divisions for a particular cell is subject to influence from its environment.

3) The reversal of the polarity of a cell's lineage suggests that local cell contacts are critical in establishing the orientation of a cell in a group of cells.

Equivalence groups

The form of regulation in which one cell replaces another by assuming its position and lineage is limited to small groups of cells. The cells in such a group are equally competent to follow a primary fate. In the ventral hypodermis, P3.p-P8.p can each follow the P6.p vulval lineage in hermaphrodites, and P9.p-P11.p can each follow the P11.p lineage in males. In the male lateral hypodermis, V5 can be recruited to the V6 lineage, and V4 can approximate the V5 or V6 lineage. In the gonad, either cell 1 or cell 4 (Figure 3) can become the anchor cell in hermaphrodites, and a homologous pair displays a similar regulative response in males. The reciprocal alteration of lineage, in which a cell follows a fate that is lower in the hierarchy, has been seen in the P3.p-P8.p group under two different circumstances. If the gonadal anchor cell is ablated, cells P3.p-P8.p all follow the tertiary fate typical of P3.p, P4.p, and P8.p. And, in a lineage defective (lin) mutant, lin-2 (e1309)X, P3.p-P8.p again all follow the tertiary fate, and no vulva is made (Horvitz and Sulston, unpublished results). We suggest that these groups of cells are equivalent in developmental potential, and call them equivalence groups.

Cells assigned to equivalence groups on the basis of ablation experiments correspond exactly to cells grouped according to two other considerations. First, a comparison of the lineages of P1-P12 in the intact animal reveals differences in lineage detail (e.g. cell deaths, extra divisions) that delineate three groups, P1-P2, P3-P8, and P9-P11 (see [2]). The members of each group share specific lineage features and differ from other Pn. Second, lineage defective mutants alter the lineages of precursor cells differentially. In one mutant, lin-1 (e1026) IV, only P3-P8 are affected. P3.p-P8.p all follow the primary vulval lineage of P6.p resulting in a multivulva phenotype (Horvitz and Sulston, unpublished results). In a second mutant, mab-5 (e1239) III, the V5 and V6 lineages are defective, whereas the T lineage is normal. This mutant honors the V6/T boundary of the lateral hypodermal equivalence group. The delineation of the same groups of cells by several techniques supports the idea that cells in equivalence groups are intrinsically different with respect to their developmental potential from cells outside equivalence groups.

The hypothesis that cells of equivalent developmental potential are set apart in groups raises intriguing questions about the mechanism by which such groups are established and the role played by such groups during development. As far as is known, cells in an equivalence group have a similar ancestry. Each arises in an identical branch of the lineage tree of similar cells (e.g. the posterior branch of the ventral hypodermal precursor cells). Furthermore, in the gonad, the ancestries of the members of an equivalence pair, both in hermaphrodites and males, are equivalent and unique. If the commitment of cells to an equivalence group occurs by a lineage mechanism, the embryonic ancestry of the ventral and lateral hypodermal precursors may reveal differences in the origin of the precursor cells that correspond to equivalence group boundaries.

The developmental significance of equivalence groups is purely a matter for speculation at the present time. One appealing hypothesis is that they are fundamental units of worm assembly analogous to polyclones in fly assembly[5]. One might imagine that distinct groups of cells are founded during embryogenesis that are uniquely responsible for construction of specific regions of the worm's anatomy. Elaboration of this idea, however, must await the isolation and characterization of more mutants that are equivalence group specific.

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