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Cell lineage and the control of *Caenorhabditis elegans* development

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This paper provides a brief summary of the *Caenorhabditis elegans* cell lineage, the evidence for both intrinsic and extrinsic cell specification, and experiments that suggest mechanisms for cell differentiation and patterning.

Introduction

If simple production rules operate to generate a multicellular organism from a fertilized egg, then we might expect to detect them from patterns that emerge in sequences of cell divisions and differentiations. Furthermore, with a knowledge of the relative contributions of cell ancestry and environment in determining cell fates, we might hope to describe these rules in precise formal terms.

For one organism, the soil nematode *Caenorhabditis elegans*, we are in a position to look for informative patterns. We know the complete cell lineage, and whether certain sections of it are likely to be generated by cell-intrinsic or extrinsic control mechanisms. In fact, patterns do emerge, but simple rules that might account for their existence in one part of the lineage seem to be broken in others. Indeed, much of the lineage is devoid of any obvious regularity.

However, the irregularities themselves are significant, and indicate that certain developmental strategies are much more probable than others. Here I summarize the lineage, and discuss the evidence that some sequences of cell division and differentiation are controlled autonomously while others require extrinsic signalling. Throughout, implications for the logical structure of the control circuitry are discussed. Developmental genetics is covered by Greenwald (this symposium).

General description of *C. elegans*

C. elegans exists in two sexual forms; the self-fertile hermaphrodite and the male. The adult hermaphrodite has 959 cell nuclei (some cells are multinucleate); males have 1031. In spite of this small cell number, the animal contains a rich diversity of cell types.

Development occurs in two distinct stages, embryonic and postembryonic. Embryogenesis lasts about 13 h and produces a 558-cell (male, 560) juvenile (figure 1).

The juvenile proceeds to the adult stage via four moults. Of the cells generated during embryogenesis, roughly 10% divide after hatching. The descendants of these cells either join existing tissues or form the reproductive structures, which are generated almost entirely during postembryonic development.

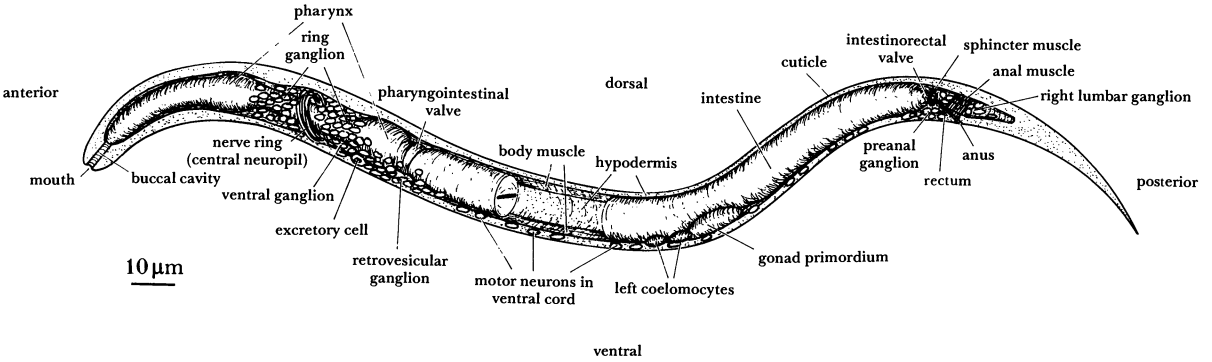

FIGURE 1. General anatomy of the newly hatched juvenile. Labelled cell types and tissues include those whose lineal origins are described in the text (from Sulston *et al.* 1983).

THE CELL LINEAGE

It was possible to determine the *C. elegans* cell lineage for three reasons. First, the cell number is small. Second, the pattern of cell divisions and differentiations, and also the relative positions of cells are essentially invariant, so data from many individuals can be combined. Third, because *C. elegans* is transparent, individual nuclei can be seen in living animals with Nomarski microscopy.

The cell lineage was completed in three parts. Sulston & Horvitz (1977) determined the postembryonic non-gonadal cell lineages, Kimble & Hirsh (1979) determined the gonadal cell lineages, and, subsequently, Sulston completed a seemingly impossible task, and determined the embryonic cell lineage (Sulston *et al.* 1983).

The patterns that emerge from these studies have been discussed in the (highly recommended) original papers, and elsewhere (Sulston 1983; Kimble 1981*b*; Hedgecock 1985). In addition, Sternberg & Horvitz (1981, 1982) have determined the postembryonic cell lineages of the related nematode *Panagrellus redivivus*, and have discussed their developmental and evolutionary implications.

Overview

During early embryogenesis, the zygote undergoes a series of unequal cleavages to generate blastomeres called founder cells: AB, MS, E, D, and P_4, and four granddaughters of the C blastomere (figure 2). Each founder cell undergoes a series of equal cleavages with a characteristic cell cycle periodicity. AB and two granddaughters of C produce largely ectodermal cell types (hypodermis and neurons); MS and D and the other granddaughters of C produce largely mesodermal cell types (muscles, glands, somatic gonad); E produces the intestine; and P_4 produces the germ cells.

At the turn of the century, embryologists thought that these initial divisions channelled cells into one of several alternative pathways, with founder cells producing strictly ectodermal (for example, skin, neurons), mesodermal (for example, muscles) or endodermal (for example, intestine) cell types. Their conclusions fitted a prevailing paradigm that development proceeded through a series of choices; each choice restricting the array of fates available to a cell until ultimately only a single choice remained. Moreover, it was generally assumed that the order in which potential was restricted was central to the mechanism of development; for

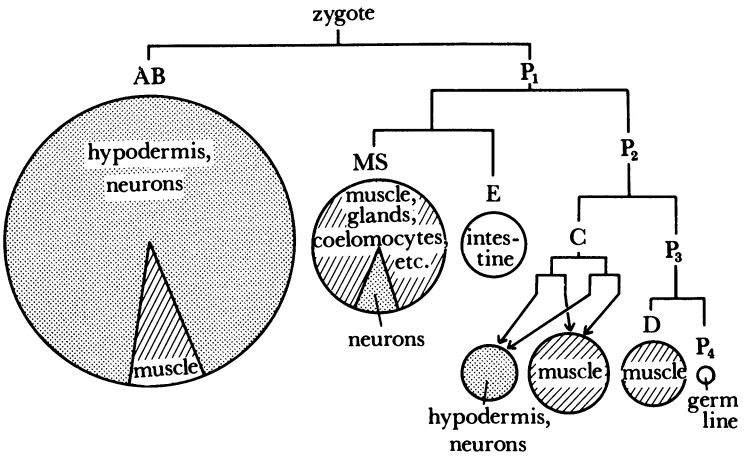

FIGURE 2. Generation of the embryonic founder cells. The areas of circles are proportional to the number of cells produced. Stippling: ectodermal cell types; stripes: mesodermal cell types (from Sulston *et al.* 1983).

example, cells would be restricted first to the production of ectodermal or mesodermal cell types, and ectodermal cells would subsequently be restricted to neuronal or epidermal pathways.

The actual *C. elegans* lineage provides a dramatic and conclusive demonstration that this paradigm is incorrect, at least with respect to production of ectodermal and mesodermal cell types. Both AB and MS generate ectodermal as well as mesodermal cells. In fact the separation can occur as late as the last cell division: in three cases, neurons are sisters of muscle cells. This is significant, because it demonstrates that *C. elegans* development does not proceed strictly through a hierarchical series of restrictions on final cell type (although other sorts of hierarchies may exist) and raises the possibility that the repertoire of differentiated fates available at any stage may actually be quite large.

The origins of tissues and organs

To acquaint the reader with the lineage, I will discuss briefly the origins of some major tissues and organs in the animal, proceeding in order of increasing lineal complexity. Cells in *C. elegans* are named by their ancestry; for example, ABpra is the anterior daughter of the right daughter of the posterior daughter of AB.

(i) *Germ line*. The germ line is derived exclusively from the P_4 founder cell, which produces no other cell types.

(ii) *Intestine*. The intestine is generated by the E founder cell, which produces no other cell types.

(iii) *Body muscle*. The body muscles, which are arranged in rows along the body axis, are produced by the D, C, MS and AB founder cells (figure 3). D produces only body muscles, as do two graddaughters of C, while MS also produces many other cell types. AB generates a single body muscle.

Like many anteroposterior compartment boundaries in *Drosophila*, lineal boundaries within the rows of muscles are not apparent morphologically. In spite of their different origins, the body muscles express the same myosin genes (A. Miller, F. Stockdale and J. Karn, personal communication) and have the same ultrastructural features. This is a good example of a recurrent feature of the *C. elegans* lineage, the production of apparently identical cell types at unrelated lineal positions.

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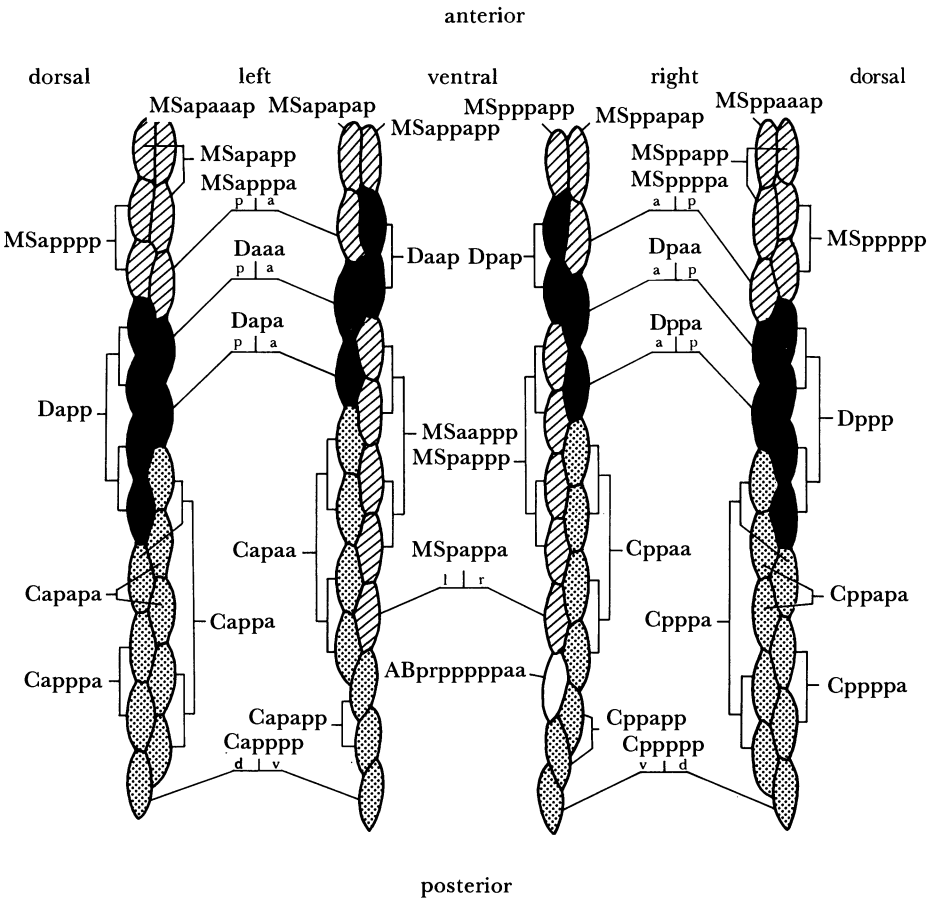

FIGURE 3. Ancestries of body muscles. Individual body muscles names are included to indicate precise lineal origins. MS-derived cells are striped; D-derived cells are filled; C-derived cells are stippled; and the single AB-derived cell is blank.

Most of the body muscles arise as small clones, suggesting that a determinative event has been followed by simple proliferation. Curiously, 16 out of 17 divisions that separate body muscle from non-muscle cells exhibit the same polarity: muscle cells arise as the posterior sisters of non-muscle cells (the exception is the sole AB-derived body muscle). This is a suggestive phenomenon, but it does not obviously generalize to other cell types.

(iv) *Somatic gonad.* The male and hermaphrodite somatic gonads are generated by two MS descendants, Z1 and Z4. These two cells arise in homologous positions in similar MS sublineages.

The gonad displays three features that recur in the lineage. The first is a stem cell lineage pattern, in which a precursor divides to produce one apparent copy of itself, plus a second cell (here a seminal vesicle cell).

The second are cases in which pairs of cells appear to have opposite polarities, as defined by the orientations of the lineages they generate. In hermaphrodites, Z1 and Z4 each produce one of two symmetrical gonad arms that extend in opposite directions from the vulva. Not surprisingly, the Z1 and Z4 lineages exhibit opposite polarities. In males, where a single gonad arm develops, part of the Z4 lineage exhibits reversed polarity compared with the hermaphrodite Z4 lineage.

The third are cases in which cells of a single type (uterine (u), vas deferens (vd), seminal vesicle (sv) cells) arise as clonal descendants of a single precursor. Interestingly, the sisters of u, vd or sv clonal precursors also generate one or a few u, vd, or sv cells, respectively. Possibly information for generating u, vd, or sv cells is unequally partitioned at the division that generates the clonal precursor.

(v) *Hypodermis*. The hypodermis of the newly hatched juvenile consists of several multinucleate syncytia (produced by cell fusion) and several longitudinal rows of mononucleate cells, many of which divide during postembryonic development.

Like the muscle, the hypodermis is a mosaic structure. Most cells are produced by AB, but C contributes half of the nuclei within a large syncytium that covers the dorsal body surface, and also one mononucleate cell.

The hypodermal cells (or nuclei) of the newly hatched nematode fall into six repeat units (figure 4). Each repeat unit contains a bilateral pair of dorsal syncytial nuclei, a pair of lateral 'seam' cells (also called V cells) and a pair of ventral cells (P cells; unrelated to $P_{(1-4)}$).

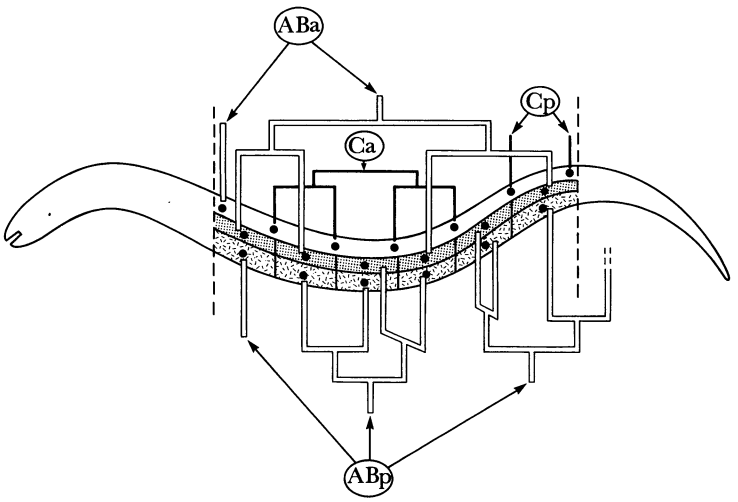

FIGURE 4. Lineal origins of the six hypodermal repeat units on the left side of the juvenile. Only terminal branches of the cell lineages derived from the indicated early blastomeres are shown. Solid lines represent C-derived syncytial nuclei. Open lines represent AB-derived nuclei (either in cells or syncytial). Lateral seam cells are stippled and ventral P cells are hatched. Notice that most of the seam and P cell lineages diverge at the first division of the AB founder cell, but that two seam cells are sisters of P cells. The second most posterior seam cell divides just before hatching to generate a seam cell plus a neuroblast (not shown).

Postembryonically, V seam cells exhibit a stem cell division pattern, in which posterior daughters are seam cells and anterior daughters become either seam cells, neuroblasts, or fuse with the hypodermal syncytium. In contrast, each P cell divides to produce a neuroblast and a hypodermal cell (figure 5). For the most part, the sublineages generated by the V cells are identical, as are those generated by the P cells. However, certain P or V descendants adopt fates that differ from those of their homologues, particularly in regions of reproductive specialization.

Given the striking morphological and developmental periodicity within the hypodermis after hatching, we might expect to find a corresponding periodicity in the lineages that generate the members of the repeat units. However, as described in figure 4, this is not the case. The large syncytium contains both the clonal descendants of C-derived cells, plus some AB descendants.

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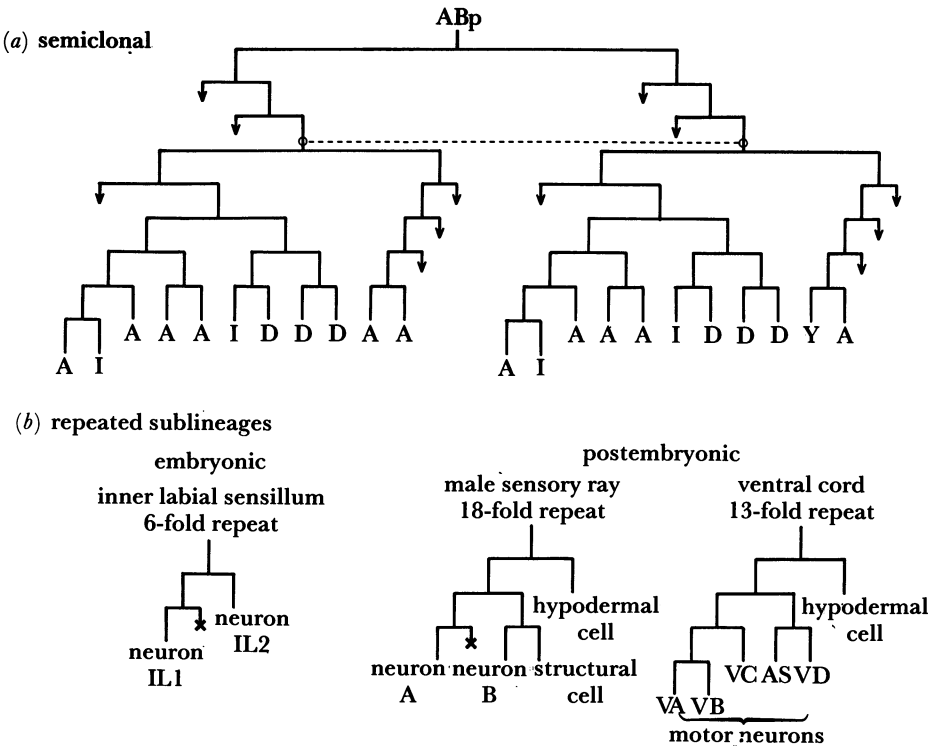

FIGURE 5. Examples of neurons within a class that arise semiclonally and in homologous positions in recurrent sublineages (*a*). A, class DA embryonic motoneurons; D, class DD embryonic motoneurons; I, interneurons; Y, hermaphrodite motoneuron and male blast cell. (*b*) Repeated sublineages generating inner labial sensilla and male ray sensilla are shown. Lineages labelled 'ventral cord' show the sequence of cell divisions generated by each hypodermal P cell during early postembryonic development; in certain lineages particular P descendants adopt fates that differ from those indicated (from Sulston 1983).

The majority of V cell lineages diverge from P cell lineages at the first division of AB, but, in striking contrast, a few V and P cells are sisters, diverging seven cell generations later.

(vi) *Nervous system. C. elegans* contains a small number of neurons (302; male, 381) but a relatively large number of distinguishable neuronal classes (118 in hermaphrodites) (White *et al.* 1986). Neurons are generated by AB, MS and C.

Members of neuronal classes arise in three quite different arrangements within the lineage: as clonal or semiclonal descendants of a single precursor, in equivalent positions in similar sublineages, and in unrelated lineal positions.

Relatively few neurons within a class are sisters, the major examples being some juvenile motoneurons (figure 5*a*).

More frequent are recurring sublineage patterns that produce similar cell types (figure 5*b*). For example, motoneurons generated postembryonically arise in equivalent positions in the P cell lineages. Certain embryonic neurons, such as the 6 IL1 and 6 IL2 neurons, arise within identical sublineages. In males, 18 identical sublineages produce 18 copulatory ray sensilla (each comprising two neurons and a structural cell).

It is common for neurons within a class to arise in unrelated lineal positions. A favourite example is the generation of the four RME neurons (figure 6). Two are posterior daughters of sister cells. One arises in a different lineal context as the sister of a unique neuron. The fourth,

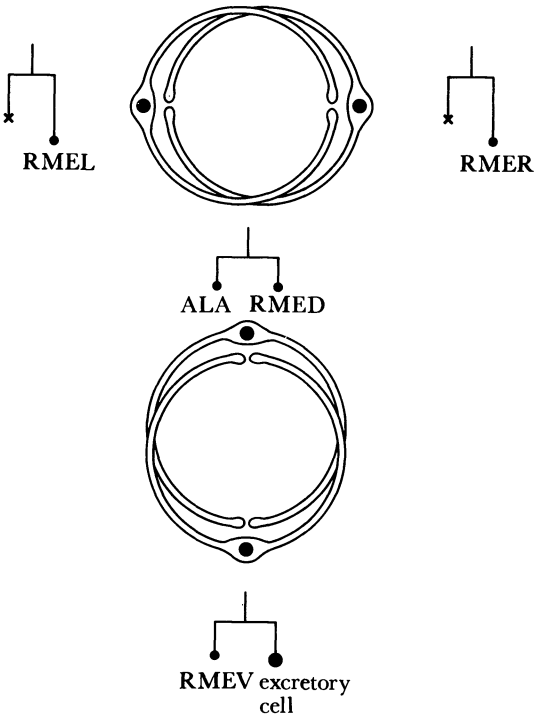

FIGURE 6. Disparate origins of class RME motoneurons. Sister cells generate the indicated RMEL (ABalaaaarlp) and RMER (ABalaaaarrp) sublineages (X-programmed cell death). RMEV (ABplpappaaa) and RMED (ABalapppaap) neurons are generated by more distantly related cells.

remarkably, arises in an unequal cleavage as the smaller sister of the (non-neuronal) excretory cell.

Also striking are the disparate origins of two pairs of deirid sensilla. Each deirid sensillum contains a dopaminergic neuron and two structural cells. The anterior deirids arise during embryogenesis. Both are composed of distantly related descendants of ABa and ABp. In contrast, each posterior deirid arises from a single neuroblast produced postembryonically during the stem cell lineages of a bilateral pair of hypodermal V cells.

(vii) *Pharynx*. The pharynx is a neuromuscular organ containing five cell types: muscles, glands, epithelial cells, neurons, and so-called marginal cells (Albertson & Thomson 1976). Pharyngeal cells are derived from MS and AB. Both produce multiple pharyngeal cell types, but AB (which is anterior of MS) produces the anterior half of the pharynx and MS produces the posterior half. Pharyngeal cells are produced in 'clones' consisting of 5–16 cells, with each 'clonal precursor' generating several pharyngeal cell types. Possibly, the generation of diverse pharyngeal cell types by single precursor cells evolved to facilitate cell positioning, since midway through embryogenesis, each pharyngeal precursor leaves the surface of the embryo and descends into the interior where the future pharynx will be located.

The pharynx has threefold rotational symmetry, so we might expect to find pharyngeal sublineages repeated three times (a situation which does occur in the generation of the threefold symmetrical vas deferens in the male gonad). However, in the pharynx, the two lateral sections arise from bilaterally symmetrical lineages that also generate non-pharyngeal cells, while cells within the third section have unique origins. Pharyngeal evolution has apparently exploited existing bilaterally symmetrical programmes for two sections, but has built the third piecemeal.

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Origin of bilateral symmetry

A surprising feature of the *C. elegans* cell lineage is that bilaterally symmetrical cells in the adult often do not arise by left–right divisions of the embryonic founder cells. Instead, as shown in figure 7, pairs of precursor cells that do generate identical sublineages to produce bilaterally symmetrical elements often arise in unrelated positions in the lineage.

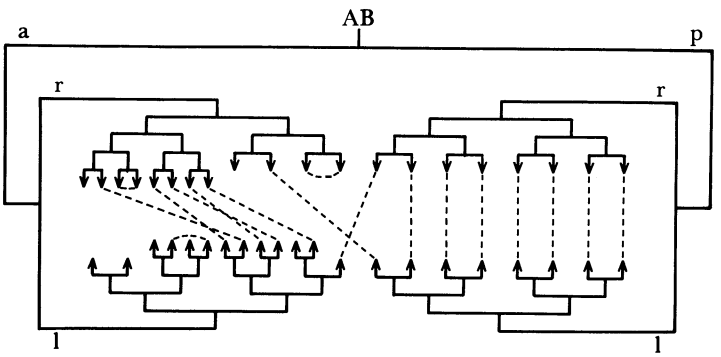

FIGURE 7. Origin of bilateral symmetry within the AB lineage. Cells that generate similar or identical sublineages producing bilaterally symmetrical elements are connected by dotted lines. Although they can occupy disparate positions in the lineage, in general cells that produce bilateral pattern elements are positioned symmetrically within the embryo (from Sulston 1983).

Summary of the lineage

There are three prominent features of the cell lineage that must be accommodated by any model of *C. elegans* development. First, many identical cell types arise in small or large clones from a single precursor cell. These include germ cells, intestinal cells, and certain muscle, hypodermal and gonadal cells. Clones imply a mechanism for the proliferation of a determined cell type.

Second, stem cell lineage patterns (defined here as a cell that repeatedly divides to produce a copy of itself plus a second cell) do occur, albeit infrequently. The two best examples of stem cells are gonadal seminal vesicle precursors and the hypodermal seam cells. However, there is evidence that stem cell lineage patterns may underlie more complex sublineages: in the absence of *unc-86* gene activity, several normally complex neuronal sublineages instead display simple stem cell lineage patterns (Chalfie *et al.* 1981). Thus a stem cell programme may represent one simple production rule whose effects are often masked by the operation of other aspects of the control circuitry.

Third, and perhaps most important, a wide variety of cells that appear to be in the same regulatory state arise in disparate lineal contexts. (Although cells that look the same could in fact differ, many morphologically similar cells are known to express the same cell-specific genes or antigens, and so are likely to be in the same state.) With the possible exception of the clonally derived intestine and germ line, there appears to be little restriction on the type of division pattern that can generate a particular cell type, or on the types of cells that can be close lineal relatives. This generalization applies not only to terminally differentiated cells (such as body muscles, motoneurons, RME neurons, and syncytial hypodermal nuclei), but also to sublineage precursors (such hypodermal V and P cells, and precursors of bilateral analogues). The simplest explanation for this phenomenon is that the instructions for the execution of a particular

terminal cell fate or sublineage are in some sense self-contained modules called subprogrammes (see Sternberg & Horvitz 1984), and that a particular subprogramme can be invoked at virtually any position in the lineage.

Intrinsic and extrinsic controls

We want to know how the information that determines cell fates is spatially and temporally distributed. If cell fate is determined intrinsically, then a large part of the answer will involve an apparatus that segregates regulatory information asymmetrically to daughter cells. On the other hand, if cell fate is determined by cell-extrinsic signals, then we must learn how regulatory information is localized extracellularly. Thus a first step toward understanding the control of development is to establish which sections of the lineage are generated cell-autonomously and which are determined by cell-extrinsic signals.

In *C. elegans*, we now have good evidence for both intrinsic and extrinsic specification during both embryonic and postembryonic development.

Evidence of intrinsic control

If a cell fails to adopt a different fate when its position or neighbourhood is changed, then its fate is considered to be specified intrinsically. Unfortunately, grafting experiments are not possible in *C. elegans*, so experiments generally involve changing the environment of cells by killing neighbours with a laser microbeam, or by attempting to culture blastomeres in isolation.

The first division of the zygote appears to create blastomeres with intrinsically different developmental potentials (Laufer *et al.* 1980; Sulston *et al.* 1983). AB has been partly isolated by lysing its sister Pl with gentle pressure or killing P1 with a laser microbeam. Under these conditions AB generated cells with ectodermal morphologies characteristic of normal AB descendants, but did not appear to produce cell types normally generated only by P1.

Conversely, when P_1 was partly isolated, it generated what appeared to be the remaining founder cells (by using criteria of cell size, and subsequent cleavage rate). Unlike isolated AB cells, isolated P1 cells produced embryos that twitched, indicating the presence of muscle cells, and produced intestine-specific granules.

In general, killing particular embryonic blastomeres does not change the fates of remaining cells (Sulston *et al.* 1983). Individual C and D descendants, second- to fourth-generation MS descendants, and fifth-generation AB descendants have been ablated. Following laser treatment, many MS blastomeres, and one AB blastomere failed to move inside the embryo during gastrulation, thus probably removing significant contacts and displacing remaining cells.

The apparently intrinsic nature of embryonic cell specification is nowhere as striking as in the production of the sixfold symmetrical pattern of anterior labial sensilla. Because much of this spatial symmetry is not reflected in the lineage, it seemed an excellent test case for extrinsic cell patterning. However, when any one of nine cells contributing descendants to the pattern was ablated, that cell's descendants were invariably subtracted from the final (otherwise fairly normal) pattern.

In all, changes in cell fate during embryogenesis were observed in only two cases, and these involved cells similar to P cells, known to exhibit a similar behaviour during postembryonic development (discussed below).

Later, during postembryonic development, the selected ablation of cells in many lineages

has also failed to change the fates of their neighbours. The most compelling evidence for intrinsic specification comes from ablations within the ray sublineages, in which multiple attempts to alter cell fates by ablating cells within the sublineage (singly or in combination) were unsuccessful (Sulston & Horvitz 1977; Sulston & White 1980).

There are important caveats associated with these ablation experiments. (i) In general, only one precursor cell was removed. Thus if more than one cell signalled another, or if the ablations did not change the coordinates of the remaining cells within fields of positional information, external signals existing would probably be missed. (ii) Because animals were examined specifically for the presence of cells normally generated by the ablated blastomere, alterations in cell fate that did not lead to the replacement of missing cell types may not have been observed. (iii) Although often early precursor cells were ablated, one cannot rule out the possibility that debris from the killed cells influenced survivors.

In summary, while we remain uncertain about the autonomy of much of the lineage, the evidence indicates that certain parts of it (including very early embryonic development, portions of late embryonic development, and portions of late postembryonic development) are generated intrinsically. Thus as a framework for designing and interpreting experiments, it may be useful to state explicitly some of the requirements that an intrinsic developmental programme will have to fulfil.

Requirements for the intrinsic control of development

Localization of regulatory information

(*a*) *A segregation apparatus*

In principle, a segregation apparatus need segregate information in only one direction during cleavage. The state of one daughter could be specified by the presence of a molecule, and the other by its absence. However, there could be mechanisms to segregate some substances to one daughter cell and other substances to the other daughter cell. In this case the two branches of the segregation apparatus must differ, so that passenger molecules can distinguish between them and segregate in the correct direction (figure 8).

A segregation apparatus could take many molecular forms: for example, microtubules are known to function in the shuttling of vesicles proximally or distally along axons (Schnapp *et al.* 1985); there is evidence that electric fields can segregate charged molecules in insects (see Jaffe 1981; Woodruff & Telfer 1980), and that an actin-based microfilament system segregates substances in *C. elegans* (discussed below). Whether another reasonable candidate, the dividing nucleus (including centrosomes and chromosomes) ever functions to segregate information differentially is unknown.

Recently, in a beautiful series of experiments, Strome & Wood (1983) have obtained direct evidence of a segregatory apparatus in *C. elegans*. By using monoclonal antibodies against particles called P granules, they have shown that P granules are segregated before mitosis of each early blastomere into the next germ line precursor. The function of P granules is unknown, but their behaviour clearly demonstrates that a segregatory apparatus, which in principle could position regulatory molecules, functions in *C. elegans*.

Drug inhibition patterns suggest that an actin-based microfilament system is necessary for P-granule segregation, and that microtubules are not involved. Furthermore, segregation does not require the mitotic spindle, because there are mutants with altered spindle and cleavage

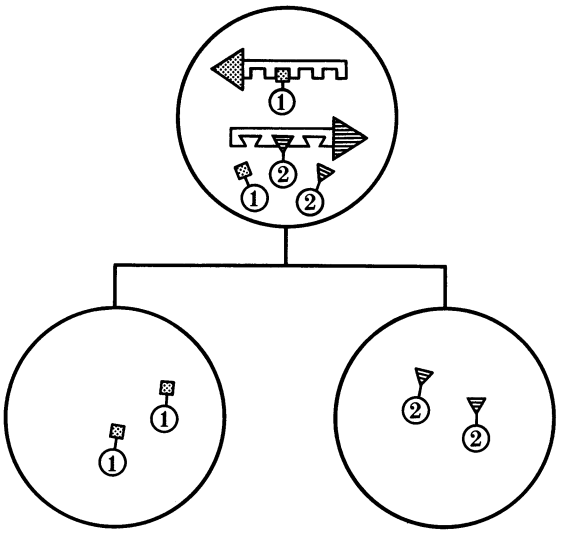

FIGURE 8. Formal representation of an apparatus that segregates information differentially to both daughter cells. Arrows pointing in opposite directions represent the two branches of the segregation apparatus, triangular and square notches represent binding sites with different specificities. Segregated regulatory molecules are depicted as having structurally distinct regulatory and segregatory domains (circles, and squares or triangles, respectively), although this need not be the case.

orientations that segregate P granules normally. Recently a set of genes necessary for the establishment of early asymmetries, including P granule segregation, has been identified (J. Priess and K. Kemphues, personal communication). Biochemical analysis of their products is of central importance.

(*b*) *Segregated regulatory elements*

In a formal sense, segregated regulatory molecules will have two functional parts, a regulatory part and a segregatory part. Depending upon the mechanism of segregation, the segregatory part could take many forms (an actin-binding region, sequences causing incorporation in certain vesicles, a clustered or distributed charged area, affinity for one aster, and so on). An interesting possibility is that the parts of the molecules responsible for segregation and regulation will fall into two distinct domains. We may find that different regulatory molecules segregating in the same direction will have homologous segregation domains.

In *C. elegans*, there is indirect evidence that cytoplasmic regulatory molecules are segregated asymmetrically into the E blastomere (Laufer *et al.* 1980). Normally, the descendants of E produce fluorescent gut granules. When cytokinesis is blocked at the two-, four- and eight-cell stages, the precursor of E often produces gut granules. Thus the potential to produce these granules seems to be progressively segregated into E. More significantly, cytoplasts formed by extruding the nucleus of an E-precursor cell will stimulate granule production if they are fused with neighbouring blastomeres, suggesting that these regulatory molecules are cytoplasmic (E. Schierenberg, personal communication; Wood *et al.* 1984).

Molecular requirements for segregation might be defined by biochemical analysis of P granules. In addition, a large number of *C. elegans* developmental control genes have been identified (see Sternberg & Horvitz, 1984). Through molecular cloning and biochemical analysis, we may soon determine whether their products segregate asymmetrically, and if so learn how their regulatory and segregatory properties are structurally related.

Control of cell division

Cells have to know whether and when to divide. In *C. elegans*, the strikingly different cell cycle periods of founder cell lineages seem to be determined by the concentration of a regulatory substance (Schierenberg & Wood 1985): when cytoplasts are derived from different founder cells, their relative periodicities are maintained, irrespective of their size. Fusions of these cytoplasts to other blastomeres produce intermediate cleavage rates.

The cell division pattern must be coordinated with cell differentiation. An important question is whether the same regulators that determine states of differentiation also control cell division, or whether the two are controlled independently. Coordinate control is suggested by the finding that many mutations produce coordinate transformations in patterns of cell division and differentiation (see Sternberg & Horvitz 1984).

A mechanism for cell positioning

Cells are either born in the right positions or migrate to them. In *C. elegans*, the majority of cells (with a few striking exceptions) differentiate close to their birthplaces. This fact has implications for *C. elegans* development. As has been pointed out (Sulston *et al.* 1983), it may provide an explanation for the complexity of the lineage. Evolution may have selected for control circuitry that can invoke any subprogramme (specifying any terminal fate or sublineage) in any position in the developing animal, irrespective of its lineal context.

Although mechanical constraints and adhesive properties may help to position cells, blastomere isolation experiments suggest that intrinsic factors play a role in orienting the division axes, at least during early development. In isolation, certain blastomeres cleave orthogonally while others divide repeatedly in the same orientation to produce linear arrays (Laufer *et al.* 1980).

In addition to controlling the division axis, the polarity of the segregation apparatus must be determined, so that information is segregated into the correct daughter. Four lines of evidence suggest that segregation polarities are not immutable, but are under some form of (intrinsic or extrinsic) regulation: (i) certain sublineages expressed during normal *C. elegans* development differ only by polarity (they are mirror symmetrical); (ii) sublineages in certain mutants show polarity reversals with respect to wild type *C. elegans* (Sternberg 1984); (iii) certain *P. redivivus* and *C. elegans* sublineages differ only by polarity (Sternberg & Horvitz, 1981;1982); and (iv) the polarity of many lineages can be reversed by ablating neighbouring cells (Sulston & White 1980; Kimble 1981*a*). Mechanisms that switch the polarity of the segregation apparatus in particular cells during normal development may play an important role in cell patterning, and may add to the apparent complexity of the cell lineage.

The three requirements listed above will apply to any system of intrinsic determination. Three additional features are suggested by aspects of wild-type (and mutant) *C. elegans* cell lineages.

A mechanism for changing cell states in successive generations

Certain cells in *C. elegans*, such as stem cells, seem to enter the same regulatory states as their parents; that is, they resemble their parents morphologically and biochemically, and they produce the same types of descendants. Stem cell lineages could arise by the reiterated segregation of a regulatory molecule into one daughter cell. In the presence of this molecule, cells would remain stem cells; in its absence they would adopt different fates.

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However, in most *C. elegans* sublineages, daughter cells adopt fates that differ from those of their parents (in terms of the cell types they produce). This implies a mechanism for changing cell states at nearly every division. To understand how regulatory states are linked from generation to generation is a key developmental challenge.

A classical model for intrinsic development postulates that regulatory molecules known as determinants are progressively segregated or partitioned into particular descendants. Cells differ from their parents because they contain different combinations of regulatory molecules. Determinants are usually assumed to initiate pathways of terminal differentiation, but a reasonable alternative would be for them to invoke developmental subprogrammes involving more complex patterns of cell division and differentiation (perhaps as discussed below).

In *C. elegans*, P granules, which may or may not be determinants, provide an example of elements that are segregated asymmetrically through several cell divisions. In addition, the potential to form gut granules (discussed above) is also progressively segregated into the E blastomere; however, in this case we do not know whether the same molecular species is segregated at each division.

Considering the complexity of the lineage (and genetic data), it seems unlikely that all regulatory information is present in the zygote and segregated differentially into each terminal cell, or that it is prelocalized in the zygote such that each terminal cell inherits the proper portion. A more attractive possibility is that cell states are specified sequentially. Here, regulatory molecules segregated into daughter cells would act to produce new regulatory molecules with different specificities which, in turn, could segregate asymmetrically at subsequent cell divisions. In a sequential cascade, a simple production rule can produce a complex pattern, because at each stage it can specify a change in its own properties.

In a sequential process, segregating regulatory molecules must 'know' when to activate new regulators. A simple possibility would be the removal of inhibitory factors by differential segregation. In addition, various types of counting mechanisms (to record the number of cell cycle progressions) are conceivable. To illustrate more clearly the idea of sequential specification, one possible model is presented in figure 9.

Multiple ways to invoke the same state

As mentioned earlier, one of the most striking features of the *C. elegans* lineage is that similar cell types (terminally differentiated cells as well as precursor cells that produce the same sublineage) often arise in dissimilar lineages. There are at least three simple ways to activate the same subprogramme in different lineal contexts: (i) different activators could independently activate the same regulatory molecules in different cells (as in figure 9); (ii) molecules with the same regulatory properties could be segregated into lineally unrelated cells; (iii) different combinations of regulators could specify the same state.

Functional interactions between regulatory molecules

Theoretically, a single regulatory species could determine the state of each cell (as in the sequential model shown in figure 9). However, if this were so, then mutations inactivating regulatory molecules should produce 'unspecified' cells that fail to adopt fates seen anywhere in the normal animal. Although certain mutations may fall into this category, mutations in many control genes cause cells to adopt fates characteristic of other cells (see Sternberg & Horvitz 1984). If these genes function intrinsically to determine cell fate, then this implies that

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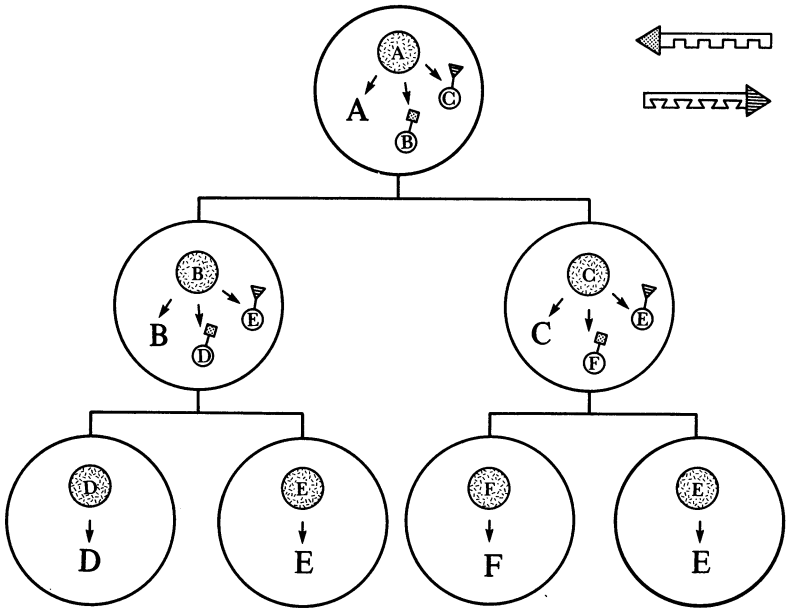

FIGURE 9. One form of a sequential control mechanism. In the version shown, we imagine that properties of regulatory molecules cause them to function one generation following production and differential segregation. Symbols are described in figure 8. Active regulatory molecules (hatched) establish the current cell state (large letters), and also specify the production of new regulatory molecules, drawn with segregation tags, but in an inactive (non-hatched form) to act in the following generation. The activation of the same regulatory function (E) by different upstream regulators (B and C) could lead to the expression of the same subprogramme in different lineal contexts.

Control molecules could function transiently as shown, or could persist and function in combination with new regulators. Combinatorial regulatory mechanisms could explain the transformations seen in many lineage mutants. As an example, the following is an interpretation of (recessive) *unc-86* mutations, which cause neuroblasts to generate stem cell lineages instead of complex lineage patterns (see Summary of the lineage, and Interactions between regulatory molecules).

In a combinatorial version of the cascade shown above, A might normally segregate rightwards and, in the subsequent generation, function combinatorially with C to define state AC. In this case, a mutation inactivating C would result in the repetition of state A (producing a stem cell lineage pattern, as seen in *unc-86* mutants).

In *unc-86* mutants, different complex sublineages are converted into different stem cell lineages. In the model, such a pattern would occur if C were normally activated by (and functioned combinatorially with) different regulators in different sublineages. Loss of C in different lineages would cause the repetition of different cell states, producing different stem cell lineage patterns.

lineages contain information (regulatory molecules) sufficient to specify more than one set of cell fates.

There are at least two ways for multiple regulatory molecules to produce one state. First, one regulatory molecule could suppress expression of the state otherwise specified by another. For example, the activity of the *lin-32* gene, which is required for certain cells to become neuroblasts instead of seam cells or syncytial hypodermal cells (E. Hedgecock and C. Kenyon, unpublished), probably directly or indirectly prevents the expression of an underlying hypodermal programme.

Second, one regulatory molecule could act in combination with another to produce a novel state. Mutations that cause different developmental transformations in different lineages provide evidence for combinatorial control. To illustrate: in the absence of *unc-86* gene activity, each of several neuroblasts produces a different stem cell lineage pattern (Chalfie *et al.* 1981). For example, one mutant neuroblast produces a line of dopaminergic neurons, and another

produces a line of migratory neuroblasts. The fact that these sublineages differ imples that they are under the control of different regulators. During normal development, when *unc-86* activity is present, each neuroblast produces a complex lineage instead of a stem cell pattern, but again, the neuroblast lineages differ from one another. This implies that *unc-86* activity functions in combination with other (unidentified) regulatory molecules to determine cell states. A simple way of explaining the *unc-86* phenotype in terms of a sequential regulatory mechanism in which cell states are specified combinatorially is described in the legend of figure 9.

Cell-extrinsic regulatory signals

In apparent contrast to embryogenesis, extrinsic signalling is widespread during early postembryonic development, and instances have been demonstrated in virtually all tissues. An incomplete list is presented here.

Natural variability

Most cells in *C. elegans* adopt invariant positions and fates. However, in a few cases cells can adopt alternative spatial configurations and alternative corresponding fates. For example, during the first juvenile stage each of six bilateral pairs of hypodermal P cells move into the ventral cord, one behind the other. The subsequent lineage is correlated with each cell's position (see below). Later, in gonadogenesis, a natural positional variability determines which of two cells becomes an anchor cell and which becomes a ventral uterine precursor cell. In both cases, laser ablation experiments have demonstrated that the fates of these cells is determined by extrinsic signalling (Kimble 1981*a*; Sulston & White 1980).

Germ cells divide with random orientation, and appear to have variable lineage patterns (Kimble & Hirsh, 1979). However, because sperm and oocytes occupy fixed spatial positions within the gonad it will be interesting to determine whether extrinsic signals define these two alternative fates.

Control of meiosis

At the distal end of each gonad arm is a somatic 'distal tip cell' that regulates germ cell state (Kimble & White 1980)(figure 10). Under normal conditions, germ cells form a maturation series along the proximal–distal axis of the gonad, consisting of gametes, then arrested meiotic cells, then mitotic cells. The distal tip cell behaves as a source of a gradient of mitogen: ablating it initiates a wave of meiotic maturation that progresses distally along the arm. Furthermore, when the position of the distal tip cell is changed, the maturation axis is realigned, with mitotic cells near the new distal tip cell position.

Control of vulva development

Ventral hypodermal cells are induced to generate vulval cells by the anchor cell of the underlying gonad (Kimble 1981*a*).

Normally the vulva is formed from the three ventral hypodermal cells nearest the anchor cell. However, laser microsurgery has shown that vulval cells can be generated by any of six ventral hypodermal cells (the vulval equivalence group) (Sulston & White 1980). In animals in which particular ventral hypodermal cells have been ablated, the remaining hypodermal cells move toward the anchor cell and adopt fates that correlate with their final distance from

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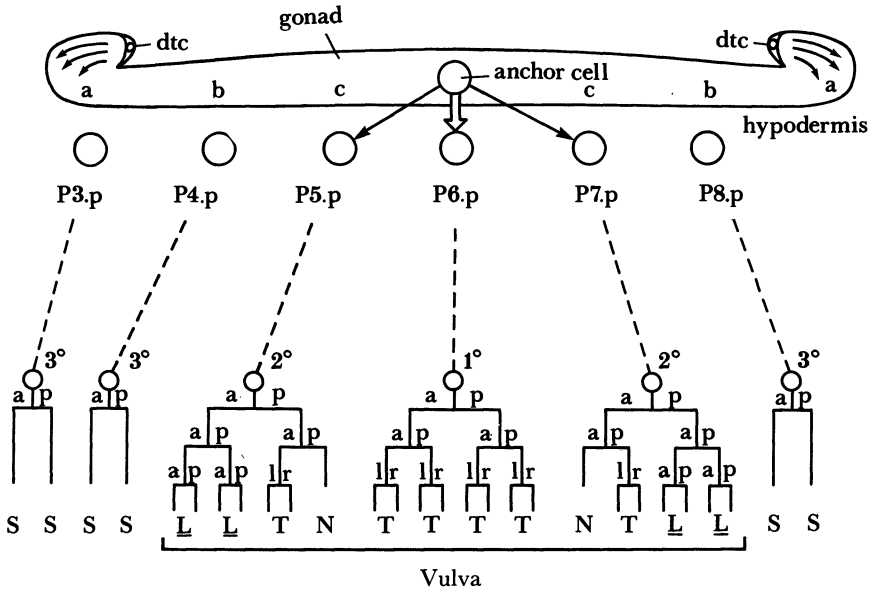

FIGURE 10. Two systems of cell–cell communication in the hermaphrodite. In this model for vulva development, proposed by Sternberg & Horvitz (1984), the anchor cell is postulated to specify the production of a graded signal whose level determines which of three fates is expressed by the underlying hypodermal P(3–8).p cells (posterior daughters of ventral P cells). Each fate involves the series of cell divisions and differentiations shown. S, fuses with the hypodermal syncytium; L, divides longitudinally and adheres to the ventral cuticle; T, divides transversely; N, does not divide. As indicated here, the gonadal distal tip cell (dtc) is thought to produce an inhibitor of meiosis (Kimble & White 1980). a, Region containing mitotic germ cells; b, meiotic zone; c, mature gametes (individual germ cells not shown). This figure is modified from Sternberg & Horvitz (1984).

the anchor cell. Interactions between the hypodermal cells do not seem to be required, because an isolated hypodermal cell still adopts the fate appropriate for its distance from the anchor cell (Sternberg & Horvitz 1984, and personal communication). The simplest interpretation of these results is that the anchor cell produces a graded signal that determines which of a set of alternative fates vulval precursors adopt (figure 10).

P cells outside of the vulval equivalence group compose different but analogous equivalence groups. It will be interesting to learn whether other regulatory cells provide a signalling function analogous to that of the anchor cell for these equivalence groups.

Evidence that positional information initiates posterior cell specialization in multiple tissues

During normal postembryonic development, many cells in the posterior of the body adopt fates that differ from their anterior homologues. Mutations inactivating the *mab-5* gene (Hodgkin 1983) specifically abolish posterior specialization in the hypodermis, nervous system, and mesoderm (C. Kenyon, unpublished). In general, cells instead adopt fates characteristic of their anterior homologues. Thus *mab-5* activity is necessary to create global anteroposterior differences. The affected cells are related only by position, suggesting *mab-5* is one component of a system of positional information that normally specifies posterior cell fates. This possibility is supported by the finding that the ablation of posterior cells causes anterior neighbours to shift posteriorly and adopt posterior cell fates (Sulston & White 1980).

The level of *mab-5* activity can determine which cells express posterior fates: decreasing *mab-5* activity levels can reduce the size of the area in which lateral hypodermal cells express

posterior-specific fates, and increasing *mab-5* levels can increase the size of this posterior domain. These observations are consistent with the possibility that a graded signal determines anteroposterior differences along the body axis.

Interactions between mesoderm and ectoderm

The postembryonic blast cell M produces sex myoblasts, gland cells and body wall muscles. If the overlying epidermal syncytium is slightly damaged with a laser microbeam, extra sex myoblast-like cells are generated (C. Kenyon, unpublished). This effect is seen even if the damage is inflicted at sites far from M. This suggests that during normal postembryonic development, function of the epidermis may be necessary for the correct establishment of certain mesodermal cell fates.

Extrinsic signals cause certain homologous cells to adopt different fates

Certain cells that occupy homologous positions in similar sublineages adopt different fates. The expression of some of these non-identical fates is known from laser microsurgery to depend on cell-extrinsic signals, and in some cases is known to require the correct level of activity of the *lin-12* gene (Greenwald *et al.* 1983). The *lin-12* protein is homologous to a set of related extracellular proteins that includes human epidermal growth factor (I. Greenwald, personal communication; see Doolittle *et al.* 1984). Thus *lin-12* protein might be a component of an intercellular signalling system that determines cell fates.

Interactions between intrinsic and extrinsic signals

In *C. elegans*, where both lineal and positional controls operate, it is important to understand how intrinsic and extrinsic signals are coordinated. It seems likely that in many cases lineal controls determine the responses of cells to environmental signals. For example, intrinsic signals may define the regulatory state that permits one cell to become a myoblast in the presence of *lin-12* protein and a gland cell in its absence. Other intrinsic signals could define the different regulatory state that permits a different cell to become a ventral uterine precursor in the presence of *lin-12* protein and an anchor cell in its absence. Thus intrinsic and extrinsic signals may specify final cell states combinatorially.

Conversely, some extrinsic signals seem to initiate intrinsically controlled subprogrammes. For example, a graded signal produced by the anchor cell is thought to determine which of three intrinsically controlled sublineages is expressed by hypodermal cells that generate the vulva (Sternberg & Horvitz 1984).

Another point at which intrinsic and extrinsic controls appear to intersect involves the intrinsic production of regulatory cells that produce extrinsic signals. For example, intrinsic controls seem to generate the distal tip cell, which then produces an extrinsic inhibitor of meiosis (Kimble & White 1980).

This is an interesting time for studying the control of development because many *C. elegans* genes that seem to play key regulatory roles have been identified and are now being isolated for molecular and biochemical analysis. In a short time we should have a much better understanding of the topics discussed here: the different components of intrinsic control systems, the structures and positioning of extrinsic signals, and finally the interplay between intrinsic and extrinsic control mechanisms.

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