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Phil. Trans. R. Soc. Lond. B 1981 **295**, 539-551
doi: 10.1098/rstb.1981.0159

Phil. Trans. R. Soc. Lond. B **295**, 539–551 (1981)
Printed in Great Britain

Strategies for control of pattern formation in *Caenorhabditis elegans*

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In this paper, strategies for controlling pattern formation in *Caenorhabditis elegans* are reviewed. The somatic tissues of this small nematode develop, in large part, by invariant cell lineages, whereas the germ-line tissue arises primarily by a variable pattern of divisions. The spatial organization of the germ-line tissue depends on special regulatory cells, the distal tip cells, which appear to influence nearby germ cells to remain in mitosis. In somatic tissues, the problem of specifying that a cell in a particular position assumes a particular fate seems to be controlled by a number of different strategies. These include the production of non-equivalent cells in particular positions of the lineage tree, local interactions between apparently equivalent cells in close contact, and the influence of another special regulatory cell, the anchor cell, over certain neighbouring cells.

Introduction

During the development of a multicellular creature, repeated cell divisions produce cells that differ from one another and are arranged in a specific spatial pattern. How such a pattern is established is poorly understood. One approach to this problem is to study a simple organism that develops in a sufficiently invariant manner that cells can be identified, studied, and manipulated individually. *Caenorhabditis elegans*, a non-parasitic nematode, is such an organism. This primitive worm consists of a relatively small number of cells (954 somatic cells and about 2000 germ cells in the adult hermaphrodite) and is transparent throughout its life cycle of 3 days. These two features have permitted direct observation of each cell in the living animal, and have led to a detailed description of the anatomy and developmental behaviour of most of the individual cells in the worm.

Both mutational and physical intervention have proved useful to study the control of development in *C. elegans*. This particular nematode is suitable for genetic analysis because of its ease of maintenance, short life cycle, and small genome. Now, largely due to the efforts of S. Brenner, *C. elegans* is one of the most favourable multicellular eukaryotes for genetic analysis (Brenner 1974). The ability to physically perturb the environment of a cell during development relies on the use of a laser microbeam system originally designed by J. White. With the laser, single cells can be destroyed with no apparent damage to neighbouring cells, and the effects of this selective removal on the development of the remaining cells can be learnt by direct observation as the animal develops (Sulston & White 1980).

These two means of manipulating the development of *C. elegans* provide complementary information about the control of particular steps of development. The genetic approach has the advantage that developmentally critical genes are identified that should ultimately lead to the identification of gene products and the elucidation of the molecular mechanisms involved in controlling the process in question. So far, mutations specifically affecting cell lineages, early embryonic cleavage, and sexual transformation have been identified and characterized in *C. elegans*

(for a review see Herman & Horvitz 1980). The laser ablation approach has the advantage that the role of cell–cell interactions in development can be studied. This has led to the identification of interactions either between neighbouring cells or over some distance that influence the determination of cell fate (Sulston & White 1980; Kimble & White 1981; Kimble 1981). It has also led to the discovery of interacting groups of equivalent precursor cells (see, for example, Kimble *et al.* 1979) that appear to be analogous to polyclones in *Drosophila* (Crick & Lawrence 1975).

Like many invertebrates (e.g. nematodes, annelids, molluscs), much of the development in *C. elegans* occurs by essentially invariant cell lineages. Thus, a given precursor follows the same pattern of cell divisions in all animals, and the fate of each descendant of those divisions corresponds precisely to its position in the lineage tree. The cell lineage of *C. elegans* can be traced by direct observation of cells as they divide and differentiate in living animals (Sulston 1976). The embryonic lineage, currently described from the single cell of the egg to about 450 of the 553 cells present in the newly hatched hermaphrodite, is strictly invariant (Deppe *et al.* 1978; J. E. Sulston & E. Schierenberg, personal communication); and the post-embryonic lineage, in which about 50 somatic precursor cells generate an additional 456 cells (hermaphrodites) or 530 (males) during larval development, is also constant, with a few minor exceptions (Sulston & Horvitz 1977; Kimble & Hirsh 1979).

In contrast, the post-embryonic development of the germ-line tissue does not occur by invariant lineages (Kimble & Hirsh 1979). After hatching, the two germ-line precursor cells follow a division pattern that varies from animal to animal with respect to timing of divisions, orientations of cleavage planes, and relative positions of descendant cells. Thus, the somatic and germ-like tissues arise in quite different ways. Studies on the control of development in these tissues suggest that they make use of quite different strategies to control pattern formation among their constituent cells. This paper reviews those strategies.

Pattern formation in the developing germ-line tissue

A description of normal germ-line development

The organization of the germ-line tissue of *C. elegans* is similar to that of many invertebrates (see, for example, Beklemishev 1969). Mitotic germ cells, or stem cells, are located at one end of an elongate gonad, and meiotic germ cells occupy the remaining area of germ-line tissue. Within the meiotic region, cells are arranged in a gradient of maturation in which more and more mature cells are found further and further away from the stem cell end (Hirsh *et al.* 1976; Wolf *et al.* 1978) (figure 1).

In *C. elegans*, the hermaphrodite gonad possesses two U-shaped arms of germ-line tissue, whereas the male gonad possesses only one. Meiosis begins at only one end of each U-shaped arm (the proximal end). This occurs at a fixed time during larval development (Kimble & White 1981) and is the first indication of polarity among the germ-line cells. The maturation gradient observed later in development follows the same polarity, since the most mature or differentiated germ cells are located most proximally. In males, gamete maturation produces sperm only. In hermaphrodites, about 150 sperm are made from the germ cells located most proximally; oocytes begin to differentiate from germ cells located at the distal edge of the sperm-containing region (figure 1). Thus, hermaphrodite germ-line development involves the production of two kinds of cells in a position-specific manner.

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Experiments concerning the control of germ-line pattern

Laser ablation experiments have demonstrated that two somatic cells, the distal tip cells, are required to establish and maintain the germ-line pattern described above (Kimble & White 1981). If the distal tip cells are destroyed, in either sex at any stage of gonadal development, all the germ cells normally in mitosis enter meiosis and complete gametogenesis (figure 2). During the larval period of germ-line proliferation, such a switch from mitosis to meiosis stops the

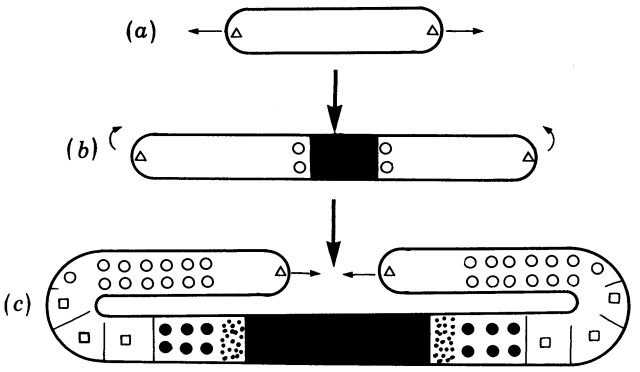

FIGURE 1. Development of pattern in the hermaphrodite germ-line tissue. (*a*), (*b*), (*c*) The morphology of the developing gonad at early, middle, and late stages of post-embryonic development, respectively. As development progresses (large arrows), the gonad grows both anteriorly and posteriorly (small arrows, (*a*)) and then reflexes to grow back in the other direction (small arrows, (*b*) and (*c*) (Hirsh *et al.* 1976; Klass *et al.* 1976). The developing somatic structures are shown in solid black; the positions of the distal tip cells are indicated by triangles, △. Germ-line tissue is represented by clear areas where cells are in mitosis. Other symbols include: ○, nuclei in pachytene; ●, primary spermatocytes; dots, mature sperm; □, oocyte nuclei.

growth of germ-line cells and enlargement of the gonad; during the reproductive adult stage, this operation ultimately results in the transformation of all the stem cells into gametes. In males, all the descendants of the germ-line precursors differentiate as sperm when the distal tip cells are killed. In hermaphrodites, the type of gamete produced after ablation of the distal tip cells depends on the time of development at which the cells are killed. After early ablation, all germ cells differentiate as sperm (figure 2*a*), whereas after later ablation, the more proximal germ cells differentiate as sperm and the more distal cells differentiate as oocytes (figure 2*b*, *c*). Thus, the normal *ratio* of sperm to oocytes is not maintained; instead the *number* of sperm appears to be critical. Oocytes are made only when the divisions of the germ line cells have produced more descendants than necessary to make the normal number of sperm.

An alteration in the *position* of the distal tip cells confirms the idea that they are essential for keeping germ cells in mitosis (Kimble & White 1981). In males, if two somatic gonadal cells other than the distal tip cells are killed, the distal tip cells can assume their positions as usual at the posterior end of the gonad or they can be relocated at the anterior end of the gonad. Moreover, after the same operation the two distal tip cells can be separated so that one occupies each end of the gonad or both can be displaced from the ends towards the middle. These changes in position of the distal tip cell result in corresponding shifts in the *axial polarity* of the germ line tissue (figure 3). Thus, the germ cells in proximity to the relocated distal tip cell remain mitotic and the germ cells further away enter meiosis. In these gonads, the meiotic cells do not exhibit the orderly gradient of maturation seen in the normal pattern, although the more mature stages of spermatogenesis are usually furthest away from the distal tip cell. This change is probably

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due to the deletion of a cell, a 'leader' cell, which normally directs the elongation of the developing gonad away from the distal tip cells.

It is also possible to reduce the *size* of the developing gonad by ablating one of the two germ-line precursors at hatching (Kimble & White 1981). After this operation, the gonad consists of about half the normal complement of germ cells at any given stage of gonadal development.

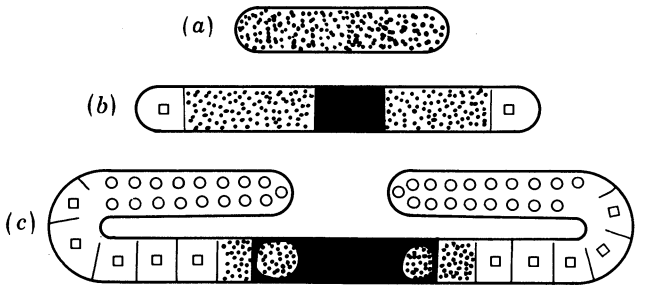

FIGURE 2. Effects of laser ablation of distal tip cells on germ cell development. All symbols are as in figure 1. (*a*) Early ablation; all germ cells stop mitosis soon after the operation and all germ cells differentiate as sperm. (*b*) Ablation midway through larval development; any germ cells in mitosis enter meiosis soon after the operation. Most germ cells differentiate as sperm, but a few distally located germ cells become oocytes. (*c*) Late ablation; all germ cells in mitosis enter meiosis and proximally located germ cells differentiate as sperm (some sperm are shown in the somatic structures). All distal germ cells ultimately become oocytes; the more distal germ cells become oocytes over a period of time, as they move proximally down the gonadal tube (being in a more proximal position seems to be necessary for oocyte differentiation).

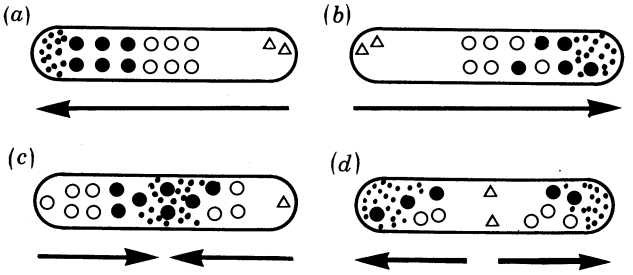

FIGURE 3. Changes in the axial polarity (arrows) of the germ-line tissue that accompany alterations in the position of the distal tip cells. Each drawing represents a different morphology observed in adult male gonads after ablating two somatic gonadal precursor cells at a very early stage of gonadogenesis. This operation leaves the distal tip cells intact, but their position varies from animal to animal. The drawings are schematics of camera lucida drawings published elsewhere (Kimble & White 1981). All symbols are as in figure 1. (*a*) Distal tip cells (△) occupy the same position as normal (for male) and axial polarity is normal. (*b*) Distal tip cells become relocated to the opposite end of the gonad, and the polarity is reversed. (*c*) Distal tip cells occupy positions at each end during the first half of development and a bipolar gonad results. The distal tip cell at the left end became displaced from the pole in this animal, which has led to the germ cells at that end entering meiosis. (*d*) Distal tip cells have assumed positions in the middle of the gonad, and a bipolar gonad results which is of opposite polarity to that shown in (*c*).

This alters the distance between the distal tip cell and the most proximal cells throughout development. The time of onset of meiosis, as judged by the first appearance of chromosomes with pachytene morphology in Feulgen stained preparations of gonads, normally occurs at a fixed time in development (at 33–34 h after hatching, where all of larval development takes 45 h from hatching). However, when the size of the gonad is reduced, the onset of meiosis is delayed by 5–10 h depending on the individual experiment. Control experiments (described in Kimble & White 1981) suggest that this delay is not an artefact of the operation but instead is a consequence of the influence of the distal tip cells over germ cells located in a proximal position.

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Model for control of pattern among germ cells

The strategy used to control pattern and cell fate in the germ-line tissue involves the influence of somatic regulatory cells over a population of apparently equivalent germ cells. The distal tip cells inhibit meiosis (or stimulate mitosis) in nearby germ cells and thereby establish and maintain the polarity of the germ-line tissue. The gradient of maturation observed normally among meiotic germ cells appears to derive from a combination of the localized distal tip cell activity

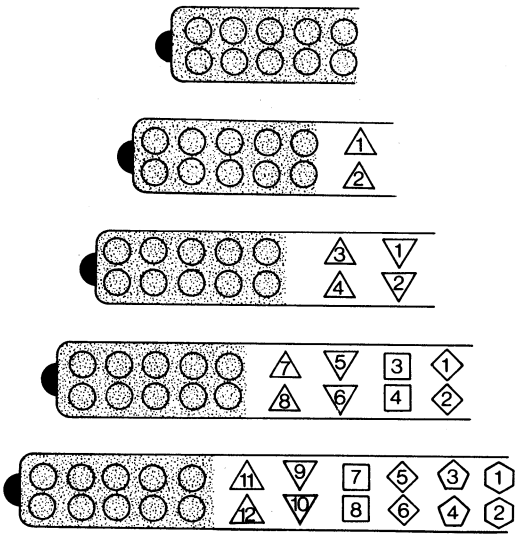

FIGURE 4. Generalized model for establishing a gradient of maturation based on the distal tip cell control over meiosis. The control element (solid half circle) is localized at one end of a growing tube, and the controlling activity acts over a distance (stippled area). Owing to proliferation of the target cells, some escape the controlling influence and begin to differentiate. Numbers identify individual cells; shapes represent stages of maturation, from least mature (△) to most mature (○). Reproduced with permission from Kimble & White (1981).

and the directed growth of germ cells away from that site. As the number of germ cells increases, cells progressively escape the influence of the distal tip cell. Therefore, germ cells initiate meiosis and gametogenesis in a gradient that reflects their time of escape (figure 4).

The control of the germ-line tissue by distal tip cells seems to be a primitive and extremely simple solution to the problem of controlling polarity and pattern in a multicellular tissue. It seems possible that the same strategy may underlie more complex problems in pattern formation such as the control of limb formation by the apical ectodermal ridge (Wolpert *et al.* 1975). A simple gradient of maturation might be used, for example, to impose a polarity on a group of cells. In the nematode, such polarity seems to be exploited to assign cells as sperm or oocytes; perhaps in a more complex system like the development of the limb bud, a similar polarity might be used as an initial step to restrict a population of cells to an as yet undefined 'more proximal' pathway.

PATTERN FORMATION AND INVARIANT CELL LINEAGES

Correlation of cell lineage, position and fate

In the development of *C. elegans*, all somatic cells (as well as the two germ-line precursor cells present at hatching) arise in essentially fixed positions in the animal's lineage tree. Only minor

points of variability are observed. In a few cases, two cells each follow one of two alternative lineages, and, in a few other cases, a cell can either divide or not and thereby produce either one or two cells of the same type. Quite often cells with the same fate, e.g. a specific type of neuron, arise in identical branches of lineage trees of homologous precursor cells. This correlation of lineage and fate suggests that a cell's fate may be a direct consequence of its ancestry. However, a correlation between cell position in the animal and cell fate also exists. Since few cells migrate during the development of *C. elegans*, a cell occupies a reproducible position within the animal as a result of its birth. Thus, an invariant lineage may either produce equivalent daughters that become different owing to positional cues, or non-equivalent daughters that are placed in particular positions in the animal by controlling the orientation of the cleavage plane. Evidence from experiments using laser microsurgery or mutational analysis suggests that both strategies are used during the somatic development of *C. elegans*.

Equivalence groups and pattern formation

In several tissues, the ablation of a precursor cell leads to its replacement by a neighbouring cell. Cells that can abandon their own fate and assume the fate of a neighbour in such an experiment delineate discrete groups of homologous cells. The cells in such a group seem to be equivalent in developmental potential, but to assume different fates in the unoperated animal owing to interactions among the cells of the group. These groups have been called *equivalence groups* (Sulston & White 1980).

The *equivalence* of cells in one group (the vulval equivalence group in the hermaphrodite ventral hypodermis) is documented by both laser ablation and mutational studies. All six cells in this group can be induced to follow the same vulval lineage, by replacement after laser ablation (Sulston & White 1980), or to follow the same hypodermal lineage by ablating a gonadal cell that is required to induce the vulva (Kimble 1981). Furthermore, all six cells follow the same vulval lineage in certain mutants with a *multivulva* phenotype or the same hypodermal lineage in other mutants with a *vulvaless* phenotype (Sulston & Horvitz 1981). In other equivalence groups, the proposed equivalence of cells has been revealed solely by replacement after laser ablation. Since reciprocal replacement has never been observed, this does not show true equivalence. However, it seems likely, by analogy with the vulval equivalence group, that these cells, too, are actually equivalent in developmental potential.

The fates of the precursor cells in an equivalence group, however, do not seem to be equivalent. Instead, these cells seem to assume fates according to a hierarchy. Thus, if a cell in an equivalence group is killed, it is always replaced by a cell that normally follows a fate lower in the hierarchy, and never by a cell that normally follows a higher fate. Thus, the spatial pattern of descendants that arises from precursor cells in an equivalence group appears to be a reflection of this hierarchical information. A precursor cell in a certain position assumes the highest fate and other precursor cells in the group assume lower fates, depending on their position relative to that primary cell. In the vulval equivalence group, the cell that assumes the highest fate appears to be selected by its proximity to a regulatory cell (the anchor cell) situated outside the group; in other equivalence groups, the basis for choosing that cell seems to be a function of the cell's position within the group (e.g. the most anterior cell in the group).

Equivalence groups have certain features reminiscent of compartments and polyclones in *Drosophila* development (Garcia-Bellido *et al.* 1973; Crick & Lawrence 1975). They delineate equivalent cells that interact among themselves to specify fates within the group according to a

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particular pattern. They do not arise as a clone of cells, but instead arise 'polyclonally' in similar branches in the trees of homologous precursor cells (Kimble *et al.* 1979; Sulston & White 1980). Furthermore, the boundaries of the tissues derived from each group do not conform to any distinct anatomical boundaries of the adult animal. And finally, certain lineage-defective mutants (i.e. *multivulva* and *vulvaless* previously mentioned) differentially alter the lineages of cells in equivalence groups. However, equivalence groups are obviously not *identical*

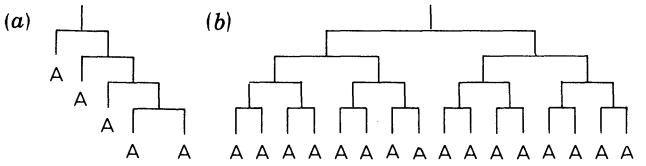

FIGURE 5. Simple reiterative patterns of division in which all cells produced are of the same cell type: type A. This lineage and all lineages shown in the remaining figures are diagrammed as follows. Each vertical line represents a cell and each horizontal line represents a cell division. Furthermore, left-hand and right-hand branches of each division represent positions in space; the left-hand branch is the anterior daughter and the right-hand branch is the posterior daughter of a division, by convention (Sulston & Horvitz 1977) unless noted otherwise on the lineage tree.

to polyclones. There is no evidence, as yet, for homoeotic transformation of one equivalence group into another, and clonal analysis does not generate overlapping clones within an equivalence group. Yet these groups may represent the worm's equivalent to compartments with respect to their role in building an animal. A more formal analogy between the two must await further genetic analysis of currently identified equivalence groups and the identification of other equivalence groups.

Speculations on the control of cell lineages

Although certain cells seem to be equivalent in potential to follow several lineages (previous section), most cells show no such equivalence (Sulston & Horvitz 1977; Laufer *et al.* 1980; Sulston & White 1980; Kimble 1981). Thus, most cells appear to assume a particular position and state of differentiation by a mechanism intrinsic to the lineage. If this is true, the problem of pattern formation becomes one of how the cell lineage is controlled. On first sight of the nearly completed lineage of *C. elegans* (Sulston & Horvitz 1977; Deppe *et al.* 1978; Kimble & Hirsh 1979; J. E. Sulston & E. Schierenberg, personal communication), this problem seems infinitely complex. However, if the lineage is viewed as a hierarchy of sublineages, such that one sublineage generates a finite number of precursor cells and each of these in turn follows its own sublineage, the problem becomes more approachable. The question of lineage control can be posed by asking whether each sublineage requires separate instructions, or whether there are one or a few fundamental sets of instructions that can be modified to generate many unique lineages.

Evidence that modifications of a simple lineage may generate complex lineages is beginning to emerge both from genetic analysis (Chalfie *et al.* 1981) and from laser ablation studies (Kimble 1981). Two kinds of simple lineage seem to be involved (figure 5). One is an asymmetric repeating pattern in which one daughter of each division continues to divide and the other daughter assumes a particular fate, called 'A' (figure 5*a*). The other is a symmetric repeating pattern in which each daughter of each division continues to divide to produce a clone of identical cells (figure 5*b*).

Chalfie *et al.* (1981) have characterized the phenotype of mutations in two genes that seem

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to be involved in the control of cell lineage. Mutations in either gene lead to a transformation of specific lineages from a more complex to an apparently simpler form (figure 6). Chalfie *et al.* (1981) suggest that instructions to follow a simple repeating pattern of division may underlie many lineages that appear complex in the wild-type animal. A corroboration of this possibility is found in the results of one group of laser ablation experiments. When a precursor cell that

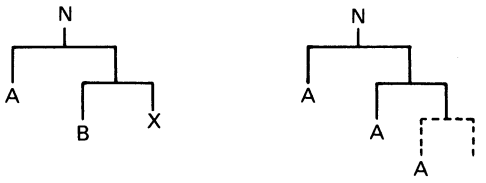

FIGURE 6. Effect of mutation in the *unc-86* gene on the lineage of certain neuronal precursor cells (N). A and B represent specific fates that may either involve further division or not; X represents cell death. The wild-type lineage (left) produces three cells, which each follow a unique fate; the *unc-86* lineage (right) produces three or more cells of identical fate by an asymmetric pattern of division. Reproduced, with the permission of the copyright holders, Massachusetts Institute of Technology, from Chalfie *et al.* (1981).

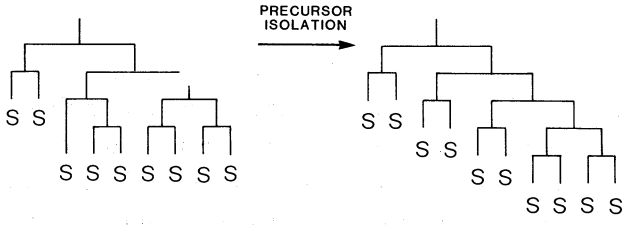

FIGURE 7. Effect of isolation of a precursor cell by laser ablation on the sublineage of one of its descendants. The normal pattern of divisions of the spermathecal precursor cell is shown at the left; the basic pattern of divisions followed by the same cell after the removal of its normal neighbours is shown at the right. Both patterns of division produce only spermathecal cells (S). The pattern shown at the right was followed precisely in one animal. In other animals, under either similar or identical conditions, this pattern appeared to serve as a 'backbone' of divisions. All followed this pattern, but a single extra division occurred in one or more non-stem cell daughters so that three instead of two spermathecal cells could be generated in these branches.

normally follows a pattern of division bearing only meagre resemblance to a repeating pattern is isolated, the lineage of that cell can change either to approximate or to follow precisely a repeating pattern (figure 7) (Kimble 1981).

The alterations in lineage observed more generally in laser ablation studies (Sulston & White 1980; Kimble 1981) suggest a number of modifications that might be used to transform a simpler lineage into a more complex one. These alterations, shown schematically in figure 8, include (1) changes in the *type* of cell produced in a particular branch; (2) reversals in the *polarity* of a lineage or of a division; (3) changes in the *number* of cells produced in a particular branch, and (4) *duplications* of a lineage. These same changes in lineage can be inferred by comparing lineages of homologous cells in a single animal, between sexes, or between species (Sulston & Horvitz 1977; Kimble & Hirsh 1979; Sternberg & Horvitz 1981). This supports the idea that the control of these aspects of lineage may be used during development to convert one lineage into another.

Each of these lineage alterations must affect the pattern in the final structure. If one considers these changes in the light of the now classic 'French flag problem' in pattern formation (Wolpert 1969), a change in cell type would change the colour of a section of the flag, a change in the number of cells produced would alter its size, a reversal in polarity would rearrange the

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sections, and a duplication would duplicate all or part of the pattern. Reversals in polarity of a cell at an early point in a lineage can also have dramatic effects on the symmetry of the developing structure (Kimble 1981; Sternberg & Horvitz 1981).

The simplest form of the asymmetric repeating pattern (figure 5*a*) is not observed in wild-type lineages. However, by invoking the modifications already discussed (figure 8) to change

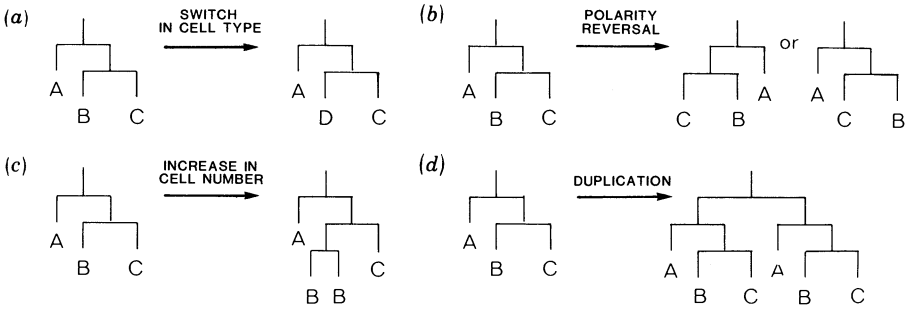

FIGURE 8. Summary of lineage modifications observed in laser ablation experiments and also inferred by comparing lineages of homologous cells. Each modification might be controlled by interactions with surrounding cells or by some mechanism internal to the cells involved. Lineages are drawn as explained in the legend to figure 5. Cell fates are represented by letters; the relative position of a cell in the animal corresponds to the position of the branch in the lineage diagram. (*a*) The fate of a cell arising in a particular branch can be switched to a different fate. Here, the cell that normally differentiates as 'B' differentiates as 'D' instead. (*b*) A polarity reversal can either reverse an entire lineage (reversing the positions of all three cells) or of a single division (reversing the positions of cells B and C). Polarity reversals appear to be used to control the position of a particular cell in the final structure, and take part in controlling the symmetry of that structure. (*c*) Increases in cell number can occur by generating more cells in one branch of a lineage tree as shown here, or by extending a stem cell backbone as shown in figure 7. (*d*) Another way of increasing cell number is by duplication of an entire lineage. Duplication often produces identical cells in either bilaterally symmetrical positions or mirror symmetric positions, and therefore may be used to generate a particular multicellular symmetry.

this pattern, certain lineages seen in the wild-type animal can be generated (figure 9). The seminal vesicle lineage is the simplest, since only a change in cell type is required to convert the 'primitive' pattern to the authentic one (figure 9*a*). The lateral hypodermal lineage is nearly as simple. Here, only changes in cell type and a duplication are required (figure 9*b*). The vas deferens lineage, on the other hand, is more intricate. In this case, modifications of three types must be used to transform the simple pattern to obtain the actual vas deferens pattern (figure 9*c*).

The mechanisms by which these lineage changes are mediated are not understood. Reversals in lineage polarity and duplications are often accompanied by visible changes from normal in the first cell division of the altered lineage. Thus, sometimes the first division is asymmetric, producing, for example, a larger anterior daughter and a smaller posterior daughter. If so, a polarity reversal in lineage is always foreshadowed by a polarity reversal of the first division (Kimble 1981). This suggests that a reversal in *lineage* polarity is caused by a reversal in the *cell* polarity of that initial cell. A duplication, similarly, is often accompanied by a shift in the usual plane of cleavage (Sulston & Horvitz 1977; Kimble 1981). Duplications are seen rarely in laser ablation experiments that otherwise cause polarity reversals (Kimble 1981). This suggests that the two may rely on the same cellular machinery to mediate their respective alterations of lineage.

The mechanism by which switches in cell type occur is also not understood. The switching of mating type in yeast is an attractive possible paradigm, but consideration of *C. elegans* lineages

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according to rules derived from yeast lineages (Strathern & Herskowitz 1979) indicates that this model is insufficient. The proposed rules suggested by yeast lineages are (1) that sibling cells differ in their ability to switch (one can switch and one cannot), and (2) that the cell capable of switching produces an identical pair of cells (daughters of the switching cell are of the same cell type, which is different from the mother cell). The first rule cannot yet be tested

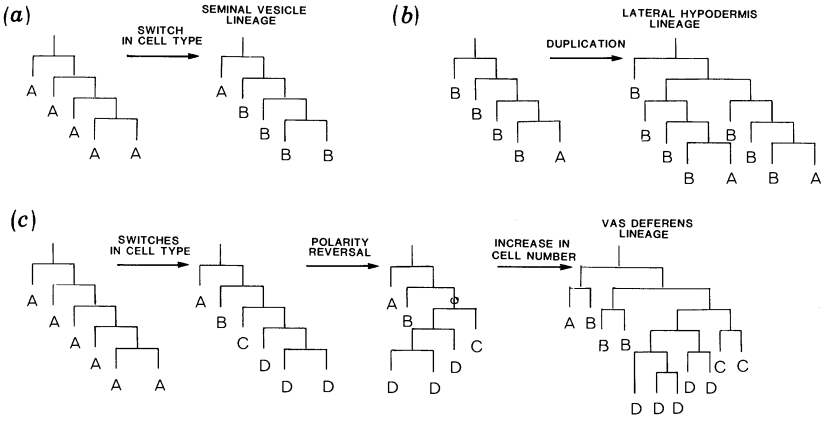

FIGURE 9. Generation of *C. elegans* lineages by invoking modification of a simple asymmetric pattern of divisions. Cell assignments in the seminal vesicle and vas deferens lineages have been made by comparing the light microscopic morphology of cells arising in particular branches of the lineage with their ultrastructural morphology observed by reconstruction from serial sections of a male gonad (J. E. Kimble & J. N. Thomson, unpublished observations). (*a*) A single type of modification can convert the simple pattern to the sublineage typical of the seminal vesicle precursor in the male gonad. 'A' cells here are flat epithelial cells, and 'B' cells are secretory. (*b*) Two modifications are required to produce the lineage typical of lateral hypodermal precursors (e.g. V1–V4, Sulston & Horvitz 1977). The first, a change in cell type, is not shown. The second involves a duplication after the first division of the lineage. 'A' refers to seam and 'B' to syncytial hypodermal cells (Sulston & Horvitz 1977). (*c*) Three types of modification must be invoked to generate the vas deferens lineage. The first, a change in cell type generates four different kinds of cells. 'A' cells are large, flat epithelial cells, 'B' cells are secretory, tiny, and make up the 'valve' region of the vas deferens, 'C' cells are secretory and form the ejaculatory duct at the proximal end of the male gonad; 'D' cells constitute the major secretory portion of the vas deferens. The 'B' and 'D' classes of cells may comprise two or three cell types based on the ultrastructure of their secretions; their classification together is based on features shared by members of each group such as a similar size and morphology during development, and their contribution to apparently discrete substructures of the vas deferens. The second modification is a polarity reversal of the lineage after the second division of the stem cell backbone (circular arrow). This reversal seems to be responsible for positioning 'C' cells at the proximal end of the vas deferens. The third modification involves a division of the non-stem cell daughter to produce two cells that in most cases are of the same type. In one case (the first non-stem cell division), sisters assume different fates. It is intriguing that in this case one of the daughters is similar to the first non-stem cell daughter of the seminal vesicle lineage, and the other daughter is similar to the daughters of the second non-stem cell division in the vas deferens lineage. The former observation suggests that the seminal vesicle and vas deferens lineages may be homologous; the latter observation fits the switching pattern suggested for the hermaphrodite early gonadal lineage (figure 10*d*).

in *C. elegans*, and the nematode lineage disobeys the second rule. In several cases in the worm, the daughters of a terminal division are not the same (e.g. figure 9*b*, *c*). In fact, sister cells can even differentiate as nerve and muscle in some cases (J. E. Sulston & E. Schierenberg, personal communication). Thus, the switching patterns in yeast cannot explain certain nematode lineages.

Yet the idea of generating a cell lineage from the reiteration of simple instructions, as occurs in yeast, remains an attractive one. Certain patterns of cell type switching observed in the nematode can be modelled in this way if one makes a few assumptions. First, each cell in a lineage may be considered to carry a particular state of determination. Boveri (1899) demonstrated

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in the early embryonic divisions of another nematode, *Ascaris*, that the chromosomes of one daughter at each division remain intact while those of the other daughters undergo chromosome diminution. This observation led to the idea of a stem cell lineage in which one daughter at each division retains the state of its mother. The lateral hypodermal lineage (figure 9*b*) provides a second example of the classical stem pattern. Here, the dividing daughter exhibits

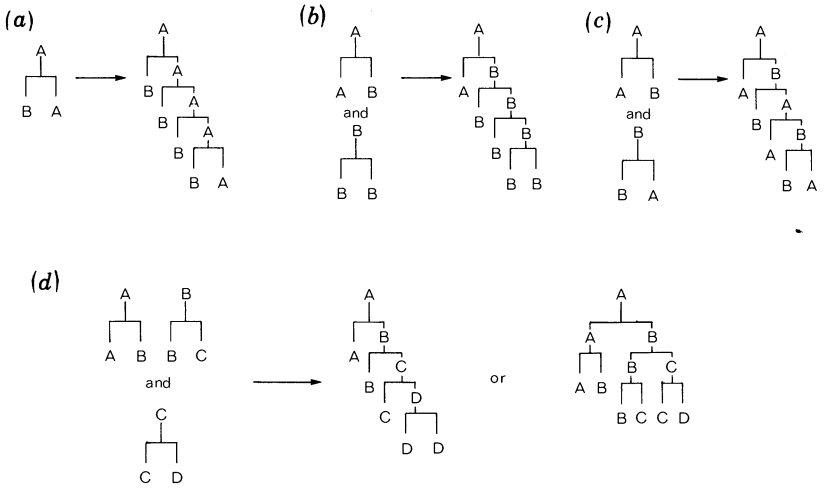

FIGURE 10. Patterns of switching cell type that are observed in *C. elegans* can be generated by assuming that one or a few simple instructions for division (shown at left in (*a*)–(*d*) is (are) reiterated in successive divisions. See the text for further explanation.

one state of differentiation, that of a seam cell, and the other daughter acquires a different state, that of a syncytial hypodermal cell (J. G. White, H. R. Horvitz & J. E. Sulston, personal communication). Based on these two examples, it seems useful to propose that cells in other lineages may be cryptically determined to a particular state. A second assumption that must be postulated is that a 'stem cell' property exists, which instructs a cell to divide repeatedly. If this property is passed on to only one daughter, an asymmetric pattern is produced. If it is segregated to both daughters at each division, a symmetrical division pattern results, which adds another level of complexity to the lineage.

Based on these assumptions, a number of switching patterns actually observed in *C. elegans* can be generated if one invokes the repetition of one or a few simple instructions. The first lineage (figure 10*a*) is produced by repeating a division in which A cells give rise to A and B cells, with A-ness being segregated to the stem cell side. This generates the basic stem cell pattern represented by the lateral hypodermal precursor cell (figure 9*b*). The second lineage (figure 10*b*) is produced if A cells give rise to A and B cells, B cells give rise only to B cells, and B-ness is segregated into the stem cell daughter. This generates the precise pattern of the seminal vesicle lineage (figure 9*a*). The third lineage (figure 10*c*) is based on the reiteration of divisions in which both A and B cells give rise to cells of both types, but with a reversed polarity. This alternating pattern mimics the 'grandparental reiteration' observed in certain lineages of mutants described by Chalfie *et al.* (1981).

The last two proposed lineages (figure 10*d*) extend the number of cell types made by postulating consecutive switches in cell type. Thus, A gives rise to A and B, B gives rise to B and C, and C gives rise to C and D. In the first case switching is limited to the stem cell; in the second case it is not. The first lineage closely approximates the vas deferens lineage as well as others

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not shown. The second example approximates the early gonadal lineage of hermaphrodites of *C. elegans* (Kimble & Hirsh 1979) and is precisely the pattern observed in the homologous early gonadal lineages of *P. redivivis* (Sternberg & Horvitz 1981).

It should be emphasized that this model for controlling lineages is purely speculative at present. Although the patterns of switching cell type presented in figure 10 generate lineages actually observed in *C. elegans*, many lineages do not conform as neatly to these patterns. However, the underlying assumption of this model – that complex lineages rely on the modification of a simple repeating pattern of division – is based on studying changes in lineages induced by laser ablation or by mutation. It is hoped that further mutational analyses and experimentation will extend and refine these preliminary ideas. In this way, it should be possible to understand whether the lineages are actually controlled as proposed and whether other lineages are also based on equally simple, though perhaps different, instructions.

Conclusions

Pattern formation is controlled by multiple strategies in *C. elegans*. Pattern in the germ-line tissue depends on somatic regulatory cells. These seem to influence the state of differentiation in nearby germ cells and thereby to establish and maintain the polarity and spatial differentiation of the germ cells. In contrast, pattern in the somatic tissues depends largely on invariant cell lineages. The lineage seems to control, in most cases, the position and state of determination of each descendant. However, groups of equivalent somatic cells have been identified in which each cell follows a specific lineage according to its position among the cells of the group.

I gratefully acknowledge M. Chalfie, I. Herskowitz, H. R. Horvitz, P. A. Lawrence, G. Struhl, J. E. Sulston and J. G. White for many stimulating discussions over the course of the last several years. P. A. Lawrence and J. E. Sulston have been critical to my thinking about analogies between equivalence groups and polyclones, and I. Herskowitz and J. G. White to my ideas about the control of cell lineages. I also thank Barbara Meyer, J. G. White, M. Wickens, and M. Wilcox for critical reading of the manuscript.

J. K. was a fellow of the Jane Coffin Childs Memorial Fund of Medical Research during the course of the work reviewed here.

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