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Germ cell development in *Caenorhabditis elegans*: a brief review

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Most multicellular eukaryotes possess a distinct group of germ-line cells that produces oocytes in one sex and sperm in the other. The production of adult germ cells appears to involve several developmental steps. First, during early embryogenesis, one or a few cells are committed to become germ precursor cells. Secondly, after a period of proliferation, some or all germ line descendants of the germ precursor cell leave the mitotic cell cycle and enter meiotic prophase. Thirdly, the meiotic germ cell matures as either a sperm or an oocyte. In this paper, I will review our knowledge of how each of these steps might be controlled in the small non-parasitic soil nematode, *Caenorhabditis elegans*.

Introduction to the organism

Caenorhabditis elegans has several major advantages for a detailed analysis of development. First, this organism is extremely favourable for genetic studies because of its small genome, short life cycle, and ease of maintenance (Brenner, 1974). Genes that are critical to particular developmental events can therefore be identified and genetically manipulated (for a review, see Herman & Horvitz, 1980). Secondly, *C. elegans* is physically well suited for examination of developmental events at the level of individual cells. The animal is transparent throughout its life cycle and consists of a small number of cells (959 somatic and about 2000 germ cells in the adult of one sex, the self-fertilizing hermaphrodite, and 1031 somatic and about 1000 germ cells in the adult of the other sex, the cross-fertilizing male). These features mean that cells can be observed directly in the living animal as it develops from the single-celled egg

to the mature adult form. Resolution sufficient to distinguish individual nuclei within the animal is achieved simply by using a light microscope equipped with Nomarski differential interference optics. Thirdly, cells of the somatic tissue of *C. elegans* as well as the two germ line precursor cells (Z2 and Z3) originate by means of a cell lineage that is invariant from animal to animal. The behaviour of individual cells is therefore reproducible throughout development with respect to division, migration and differentiation so that subtle differences in the developmental pattern can be detected. This invariance of much of *C. elegans* development and the transparence and small size of the animal have led to a detailed description of the anatomy and development of the entire organism (Albertson & Thomson, 1976; White *et al.*, 1976; Sulston & Horvitz, 1977; Deppe *et al.*, 1978; Kimble & Hirsh, 1979; Sulston *et al.*, 1980).

The extremely rigid development of *C. elegans* has been manipulated both genetically and physically in an attempt to learn how cells are instructed during development to follow their normal fates. Isolation of mutants has been used to identify genes that are essential to the specification of cell fate. Mutations in certain genes, for example, result in the transformation of an animal from one sex to the other (Hodgkin & Brenner, 1977; Hodgkin, 1980); these genes are considered to be essential to sex determination. A laser microbeam has been used to destroy individual cells during development so as to examine the role of cell interactions on their normal developmental behaviour. In most experiments using this technique, destruction of a given cell had no effect on the development of the remaining cells (Sulston & White, 1980; Kimble, 1981). However, in a few cases, specific cell interactions were discovered and one of these, critical to the control of mitosis and meiosis in germ line cells, will be discussed here.

Embryonic determination of the germ line

Boveri (1899) traced the early embryonic cleavages of the parasitic nematode *Ascaris megalocephala* to discover that the lineage is fixed and that the germ line originates from a single precursor cell. The earliest divisions give rise successively to one precursor cell of somatic tissue and a P cell (Fig. 1). The fourth P cell, P_4, is the ancestor of all germ line cells. It divides once during embryogenesis

to generate the two germ line precursor cells present in the gonadal primordium. Later work has shown that the embryonic germ line precursor cell of all nematodes examined (*T. aceti*, *C. elegans* and *P. redivivus*) arises in the same lineal position (Deppe *et al.*, 1978; J. E. Sulston, personal communication). Furthermore, in *C. elegans* P4 divides again to generate Z2 and Z3 during embryogenesis, the two germ line precursor cells present at hatching (J. E. Sulston, personal communication).

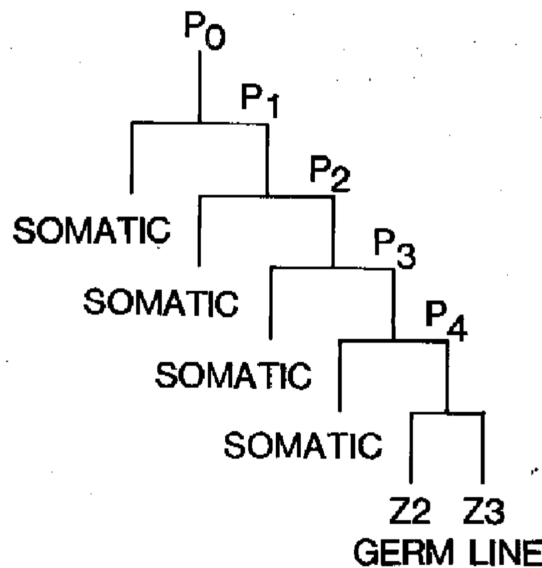

Fig. 1. Embryonic ancestry of the germ line precursor P_4. The fertilized egg, P_0, divides to generate P_1 and a somatic precursor cell. This pattern is reiterated until the embryonic precursor of the germ line P_4 is produced. P_4 divides once during embryogenesis to give rise to Z_2 and Z_3, the germ line progenitor cells present in the gonadal primordium at hatching. Z_2 and Z_3 produce all germ line cells by postembryonic proliferation.

The fixed origin of precursor cells in the embryonic cell lineage of *Ascaris* raised the question of how such an invariant ancestry is controlled. Examination of *Ascaris* eggs obtained after centrifugation or resulting from fertilization by more than one sperm demonstrated that any nucleus that acquires a position in the posterior region of the egg cytoplasm does not undergo the chromatin diminution typical of somatic precursor cells in this nematode (Boveri, 1910; Hogue, 1910). This result led to the idea that some substance must be present in the posterior region of the egg cytoplasm that channels the nuclei in that region into a germ line pathway of development.

No 'cytoplasmic determinant' for germ cell differentiation has

been biochemically identified to date. Yet, special 'granules' are observed in cytoplasm of the posterior pole in eggs of many phyla, and UV-irradiation of this area can result in an animal with no germ cells (for a review, see Eddy, 1975). The most convincing evidence for a determinative germinal cytoplasm involves the transfer of cytoplasm from the region normally occupied by germ line cells in *Drosophila* embryos to another region of the *Drosophila* egg. This transfer caused germ cells to be formed by cells from the new region (Illmensee & Mahowald, 1974).

In *C. elegans*, the idea that germ cells are determined by influence of some special determining substance has been investigated in several ways. The embryonic germ line precursor cell, P_4, has been killed by ablation with a laser microbeam to learn whether some other precursor cell might be able to generate the germ line instead. Animals operated upon in this way develop without germ cells, suggesting that no other precursor cell can substitute for P_4 (J. E. Sulston, personal communication).

A second experiment was designed to test directly the possibility of germ cell determinants in the *C. elegans* egg (Laufer & von Ehrenstein, 1981). A hole was introduced with a laser microbeam into the egg shell during early stages of embryonic cleavage and pressure was applied. In this way, about 15% of the cytoplasm from either the anterior or posterior pole of P_0, or 15–60% from P_1, P_2 or P_3, was extruded. This experiment resulted in the removal of the posterior region of the egg cytoplasm where the putative germ line determinant might be expected to reside. However, no effect on the development of the embryo or production of germ cells was observed in the resultant animal. This result suggested that germ cells may not be determined by pre-localized cytoplasmic determinants. It remains possible, however, that such determinants might not have been released by the manipulation or that they might have been replenished after the operation.

The pre-localization of granules that segregate specifically to the embryonic germ line precursor cell has recently been observed using fluorescein-conjugated antibodies with *C. elegans* eggs during the early stages of embryogenesis (Strome & Wood, 1982). An antibody preparation has been discovered that binds cytoplasmic structures that are segregated successively into blastomeres P_1, P_2, P_3 and P_4 during early cleavage of the embryo. The binding appears granular in P_0, P_1, P_2 and P_3 and therefore these structures are

called P granules. Binding is limited during the rest of embryogenesis to structures associated with the outside of the nucleus in P_4 and its daughters Z2 and Z3; after hatching, descendants of Z2 and Z3 continue to exhibit binding. The role of P granules in germ cell determination is unknown; but the existence of antibodies to P granules may provide a tool for study of the problem. These antibodies should facilitate biochemical isolation of the antigen, permit analysis of the presence and distribution of P granules in laser experiments or in mutants that alter the early stages of embryogenesis or germ cell determination, and may allow manipulation of the process of germ cell determination by microinjection.

Post-embryonic development of the germ line

The essential features of post-embryonic germ line development are shown schematically in Fig. 2. The daughters of P_4, Z2 and Z3, show a variable pattern of divisions after hatching and produce approximately 2000 cells in hermaphrodites and 1000 cells in males. Certain descendants of Z2 and Z3 remain mitotic throughout the life of the worm and are located at one end of an elongate tube of germ line tissue. Other descendants enter meiosis and occupy the remaining area of the tube. Within the meiotic region, cells at the end opposite the mitotic end differentiate into gametes. The germ line tissue thus possesses a polarity of maturation with least mature cells at one end (distal end) and most mature cells at the other end (proximal end). (Proximal refers to proximity to the external opening of the gonad – the vulva in hermaphrodites and the cloaca in males. The hermaphrodite gonad possesses two equivalent U-shaped arms of germ line tissue, each of which has a proximo-distal axis along the length of the U, whereas the male gonad possesses a single U-shaped arm.)

During larval development, germ cells enter meiosis at a fixed time (33 h after hatching in the hermaphrodite) and in a fixed place – in the most proximal position in the germ line tube. The initiation of the most proximal germ cells into meiosis is the first indication of polarity in the germ line tissue. Gametogenesis also begins at a fixed time (42 h after hatching in the hermaphrodite) and in a fixed place – again in the most proximal position in the germ line tube. In males, gamete maturation produces sperm only. In hermaphro-

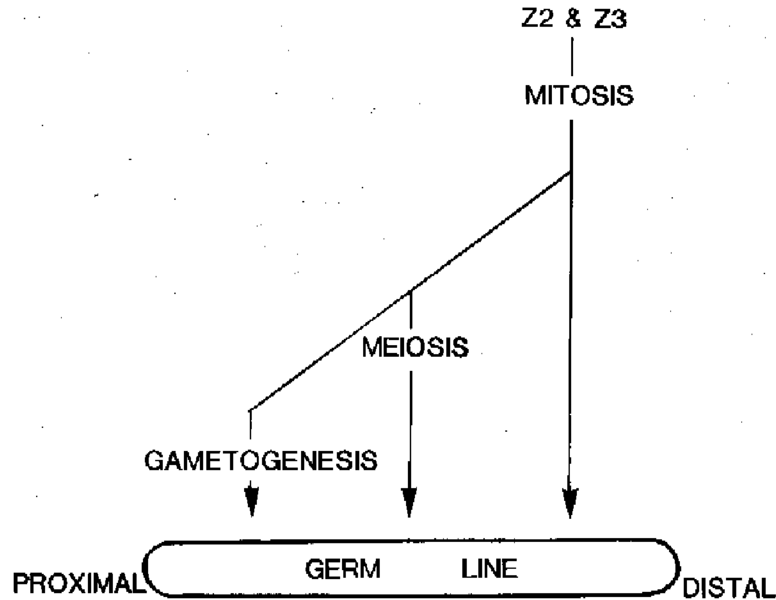

Fig. 2. Schematic view of postembryonic germ line development in *C. elegans*. The two germ line precursor cells, Z2 and Z3, begin to proliferate soon after hatching. Midway during larval development, descendants of Z2 and Z3 enter meiosis at the proximal end of the growing gonad. As the gonad grows further, more and more germ cells become meiotic. In the last larval stage, germ cells at the most proximal end of the gonad begin to differentiate. In males, germ cells differentiate in this region as sperm. In hermaphrodites, germ cells in this region differentiate as sperm and, subsequently, the adjacent germ cells more distally begin to differentiate as oocytes. See Hirsh *et al.* (1976) and Klass *et al.* (1976) for a more complete description of hermaphrodite and male gonadogenesis.

dites, 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.

Two somatic regulatory cells, called the distal tip cells, are required to maintain germ line nuclei in mitosis (Kimble & White, 1981). The hermaphrodite gonad, with two equivalent germ line tubes, has one distal tip cell at the end of each, whereas the male has both distal tip cells at the distal end of its single germ line tube. If both distal tip cells are destroyed in either sex at any stage of gonadal development, all the germ cells normally in mitosis enter meiosis (Fig. 3*a*,*b*). If one distal tip cell is killed in hermaphrodites, all germ cells occupying the tube of the ablated cell undergo the transition from mitosis to meiosis. In contrast, if all somatic cells in the gonad except the distal tip cells are destroyed, no effect on the mitotic/meiotic state of the germ cells is observed. In addition, germ cells at the distal end of the gonadal tube or extragonadal somatic cells in close proximity to the distal end of the gonad can be

ablated without causing germ cells to enter meiosis. The distal tip cells therefore uniquely control the mitotic/meiotic state of the germ line nuclei.

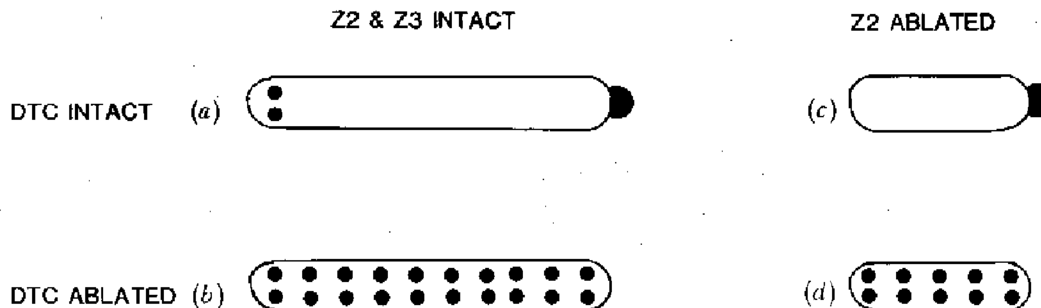

Fig. 3. Control of the mitotic/meiotic state of germ line nuclei by the distal tip cell (DTC). Each figure outlines a germ line tube at 33 h (the time of development at which pachytene figures are first observed in Feulgen-stained preparations of unoperated gonads). The large black ball at the distal (*right*) end depicts the distal tip cell; the small black dots show germ line nuclei that are in meiotic pachytene; the blank area represents a region occupied by germ cells that have not yet entered pachytene. (*a*) Nuclei at the proximal edge first enter pachytene in the intact animal. (*b*) If the distal tip cell is ablated early, all nuclei throughout the germ line tube enter pachytene at 33 h of development. (*c*) If Z2 is ablated just after hatching, the gonad is reduced in size and pachytene nuclei are not observed at 33 h. (*d*) If Z2 is ablated just after hatching and the distal tip cell is ablated subsequently, nuclei throughout the germ line tube enter pachytene at 33 h, despite its reduced size. See text for further explanation.

An alteration in the distance between the distal tip cell and the most proximal germ cells shows that the influence of the somatic cell over neighbouring cells acts over distance (Fig. 3*c*,*d*) (Kimble & White, 1981). If one of the two germ line precursor cells, Z2 or Z3, is destroyed by laser ablation in hermaphrodite larvae that have just hatched, the remaining precursor cell normally contributes half its descendants to one arm and half to the other arm of the gonad. The gonadal tubes in the animal which has been operated on contain about half the normal complement of germ cells at any given stage of gonadal development and therefore each tube is reduced in size. In hermaphrodites which have not been operated on, the most proximal germ cells enter meiosis 33–34 h after hatching (Fig. 3*a*). However, when the size of the gonad is reduced by ablation of Z2, the nuclei of the most proximal germ cells have clearly not entered pachytene at 33–34 h. Instead, the first appearance of pachytene nuclei is delayed by 5–10 h. (All of larval development in *C. elegans* takes only about 45 h.) Two control

experiments demonstrate that this delay is not simply an artefact of damage but instead that it is dependent on the presence of the distal tip cell and on the reduction in size of the gonad. Nuclei of a gonad reduced in size by ablation of Z2 display a pachytene morphology at 33–34 h if the distal tip cells are destroyed (Fig. 3*d*). In addition, in certain Z2 ablated animals, the descendants of Z3 are not distributed equally between both gonadal tubes, but instead are all incorporated into a single tube. In this case, the size of the gonad is normal, and the proximal germ line nuclei enter pachytene at 33–34 h as normal. These experiments suggest that the activity of the distal tip cells must have influence over a distance to affect proximal germ cells. However, since no good marker exists for the precise stage in which cells have switched from mitosis to meiosis, the distance over which the distal tip cells act cannot be accurately estimated.

An alteration in the position of the distal tip cells establishes the idea that these somatic cells act locally to keep neighbouring germ cells in mitosis and that the position of this activity is responsible for establishing the spatial organization of the germ line tissue (Kimble & White, 1981). In males, if the sister cells of the distal tip cells are killed, the positions of the distal tip cells are not always normal (at the posterior end of the gonad). If their position is normal, the germ cells remain in mitosis at the posterior end (Fig. 4*a*). However, if the distal tip cells become located at the anterior end of the gonad, the germ cells at the anterior end remain mitotic instead, and the polarity of the germ line tissue is reversed (Fig. 4*b*). If the distal tip cells are separated so that one cell occupies each end of the gonad, or are both displaced towards the middle, germ cells in mitosis are again located near the abnormally positioned distal tip cell (Fig. 4*c*,*d*). Since germ cells in proximity to the distal tip cell remain mitotic and the germ cells further away enter meiosis, these changes in position of the distal tip cells result in corresponding shifts in the axial polarity of the germ line tissue.

Control of the germ line tissue by regulatory somatic cells seems to be a primitive and extremely simple solution to the problem of controlling both the state of the germ line nuclei and the polarity of the germ line tissue. One might imagine that the same strategy is used by other gonads. For example, the organization of gonads in many invertebrates is quite similar to that of *C. elegans* (see Beklemishev, 1969) and it seems plausible that the establishment of that organization might be controlled in a conserved manner among

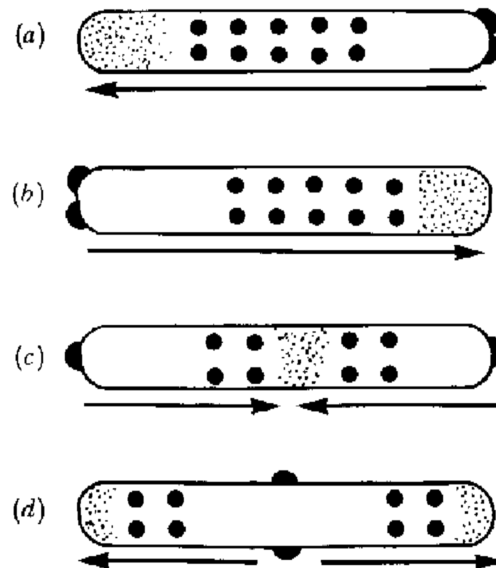

Fig. 4. Control of polarity in the germ line tube by distal tip cell position. Each drawing outlines a germ line tube observed in the young adult male if the sisters of the distal tip cell were ablated soon after hatching. (This operation causes distal tip cells to assume abnormal positions in the developing gonad.) The large black spheres outside the boundary of the germ line tube depict the distal tip cells; small dots show mature sperm, and larger dots represent germ nuclei in pachytene. The arrow beneath the gonad indicates the polarity of the gonad in question. (*a*) The distal tip cells occupy the posterior end of the germ line tube as normal and the polarity is not changed. (*b*) The distal tip cells occupy the anterior end of the germ line tube and the normal polarity is switched. (*c*) The distal tip cells each occupy one end of the germ line tube, and a bipolar gonad results. (*d*) The distal tip cells are displaced toward the middle of the germ line tube, and a bipolar gonad of the opposite polarity to that observed in (*c*) results. See text for further discussion.

invertebrate phyla. Among higher phyla, the organization of germ cell states can be easily explained in terms of a localized somatic control over mitosis and meiosis. The radial polarity of the mammalian testis, for example, might be due to an activity similar to that of the distal tip cell in the epithelial cells surrounding each seminiferous tubule. However, the universality of such a somatic control is purely a matter of speculation at the present time and must await further experimentation.

Control over the decision between sperm and oocyte formation

In hermaphrodites, germ cells first produce about 150 sperm in each gonadal arm and then switch to oocyte production for the remainder of gametogenesis. In males, germ cells differentiate only

as sperm. To learn about the control of the sperm/oocyte decision, mutations in genes controlling sex determination and the laser ablation of cells in the developing gonad have both been used.

Normally in *C. elegans*, sex is determined by the X : autosome ratio (Madl & Herman, 1979). In a wild-type diploid strain, XX animals are hermaphrodites and XO animals are male. A mutation in one of several genes effectively overrides the usual control, suggesting that in the wild-type animal, the X : autosome ratio operates through these genes to determine sex. Mutations in any of three *transformer (tra)* genes divert XX animals from the hermaphrodite to the male pathway of development; mutation in the one *hermaphroditization (her)* or in one of the two *feminization (fem)* (formerly *isx*) genes channels XO animals from the male to the hermaphrodite pathway (Hodgkin & Brenner, 1977; Nelson *et al.*, 1978; Hodgkin, 1980; J. Kimble & T. Doniach, unpublished data).

Three possible mechanisms of control over the sperm/oocyte decision have been tested genetically, or by physical intervention, or both. First, it has been shown that the chromosomal sex of a cell or an animal is not essential to the sperm/oocyte decision. In the wild-type animal, oocytes are produced by XX cells and sperm are made by either XX or XO cells. Hodgkin (1980) demonstrated that functional oocytes are also made by XO germ cells in strains carrying *her-1*. Therefore, both sperm and oocytes can be made by either XX or XO cells.

Secondly, both genetic studies and laser ablation experiments argue that the sex or the presence of the somatic gonad of the animal is not critical to the specification of sperm or oocyte production by the germ line. Since sperm are made by both sexes, the production of oocytes by the male becomes the central question when considering the role of somatic sex on germ cell differentiation. Although functional oocytes have not been produced by a male gonad, cells that appear morphologically very similar to oocytes can be made by apparently male gonads (Klass *et al.*, 1976; Nelson *et al.*, 1978; J. A. Hodgkin, personal communication; L. Edgar & D. Hirsh, personal communication). Such oocyte-like cells are large and blocky in comparison to the tiny amoeboid sperm, and appear to contain yolk granules. Moreover, yolk-protein synthesis is initiated and the production of a sperm-specific protein arrested in these animals (Kimble & Sharrock, 1983). The most

striking example of apparent oocyte production in a male gonad is observed in a strain harbouring a temperature-sensitive allele of the gene *fem-2*. Here, the XO animal develops as a morphologically normal male at permissive temperature, but if it is raised to restrictive temperature as an adult, sperm formation is blocked, and germ cells begin to differentiate as blocky, yolk-filled cells (L. Edgar & D. Hirsh, personal communication).

The role of the somatic structures in sperm and oocyte formation has also been investigated by laser ablation (Kimble & White, 1981). In both hermaphrodites and males, all somatic structures can be deleted without blocking the initiation of sperm or oocyte formation as long as the distal tip cells are left intact. Therefore, the somatic structures do not seem to be necessary for the production of either sperm or oocytes. (The distal tip cells are required for germ cell proliferation as described in the previous section.) The oocytes produced in a gonad lacking somatic structures (or by a male gonad as described above) are smaller and irregularly shaped. It therefore seems likely that hermaphrodite somatic structures, in particular the epithelial sheath that surrounds the maturing oocytes, may be necessary for the normal maturation of the oocyte into a functional oocyte.

Thirdly, no role of cell lineage in the specification of precursor cells for sperm or oocytes has been found. The germ line precursor cells present at hatching, Z2 and Z3, were shown to follow a variable pattern of cell division unlike the somatic precursor cells (Kimble & Hirsh, 1979). In addition, evidence from both genetic and laser ablation experiments demonstrates that sperm and oocytes must share a common ancestor during early gonadogenesis. The first indication of this possibility relied on the fact that, during self-fertilization, sperm made by a single hermaphrodite fertilize oocytes made by the same individual. The probability of causing a mutation in the same gene in two precursor cells in a single animal is extremely low. Therefore, if sperm and oocytes differentiate from separate precursor cells, mutagenesis during early larval development would not be expected to cause recessive mutants visible in the first generation (F1). Yet F1 animals homozygous for such mutations have been observed (Hodgkin, 1974). This means that the mutagenic event must have taken place in a cell that gives rise to both sperm and oocytes. The second indication of a common ancestry for sperm and oocytes was that, after ablation of the distal

tip cells, descendants of the putative sperm precursor should differentiate as sperm and descendants of the putative oocyte precursor as oocytes. The expected composite gonad containing both sperm and oocytes is, however, not observed after ablations during the first half of larval development (Kimble & White, 1981). Instead, all germ cells differentiate as sperm if the distal tip cells are killed early before much germ line proliferation has occurred (Fig. 5*a*,*b*). 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. When sufficient germ line cells have been generated, germ cells differentiate to produce the typical number of sperm proximally and any excess germ cells differentiate as oocytes more distally after distal tip cell ablation (Fig. 5*c*,*d*). This experiment argues that sperm and oocytes have a common ancestor and that the number of sperm produced appears to be critical to the onset of oogenesis.

These observations suggest that chromosomal sex, the presence

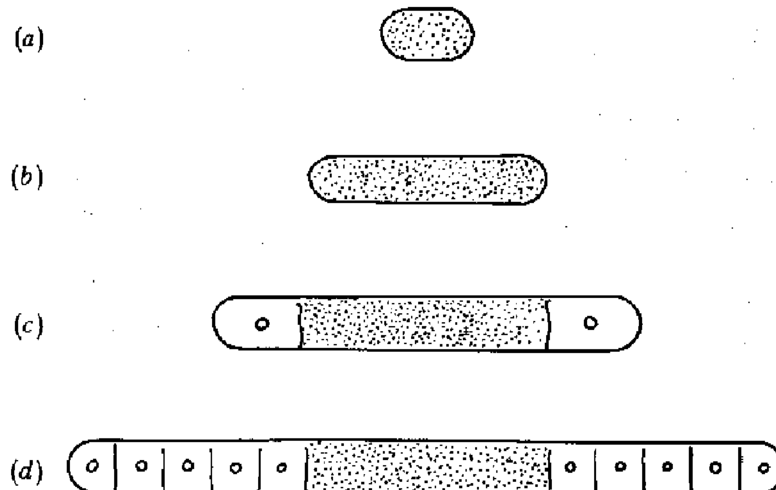

Fig. 5. Control of the sperm/oocyte decision. Each drawing outlines a germ line tube observed in the young adult hermaphrodite after ablation of the distal tip cell at different stages of development. In hermaphrodites, two germ line tubes are made with opposite polarities, so that proximal is in between the two. All germ line cells differentiate as sperm (small dots) or oocytes (open circles represent oocyte nuclei). (*a*) If the distal tip cells are ablated soon after hatching, mitoses are blocked early; the few germ cells that are made all differentiate as sperm. (*b*) If the distal tip cells are ablated somewhat later, more germ cells are made but again they all differentiate as sperm. (*c*) If they are ablated yet later, a sufficient number of germ cells has been generated to make the normal number of sperm and other cells differentiate as oocytes. (*d*) If ablated quite late, the normal number of sperm are again produced in a proximal position, and other cells differentiate as oocytes.

or sex of somatic gonadal structures, and the specific ancestry of germ line descendants are not critical to the decision of a germ cell to differentiate as a sperm or oocyte. Instead, the control over this decision seems to depend on the same genes that control the decision between hermaphrodite and male pathways of sexual differentiation by somatic cells. Thus, mutation of the *her-1* gene causes both somatic and germ line tissues of an XO animal to differentiate as a hermaphrodite and a self-fertile hermaphrodite results (Hodgkin, 1980). Mutation of the *tra-1* gene transforms an XX animal into a cross-fertile male (Hodgkin & Brenner, 1977). Furthermore, the *fem* genes can be inactivated by a shift of a temperature-sensitive *fem* mutant to restrictive temperature in the adult XO male animal, resulting in a block in spermatogenesis and the induction of oocyte-like cells (L. Edgar & D. Hirsh, personal communication); the same genes can be inactivated earlier in development to result in a transformation of the XO animal into a spermless hermaphrodite (J. Kimble, unpublished results).

Summary

The mechanisms of control of the three steps of germ line development have been discussed:

1. The mechanism by which a single cell is specified during embryonic cleavage to become a germ line precursor cell remains elusive. Evidence has been found both for and against the existence of cytoplasmic determinants in nematode development (Boveri, 1910; Laufer & von Ehrenstein, 1981). The recent discovery of an antibody preparation that recognizes 'P granules' may provide a tool for the further analysis of the possible role of cytoplasmic determinants in germ line determination (Strome & Wood, 1982).

2. The cellular mechanism by which certain germ line descendants are regulated to remain in mitosis involves a somatic regulatory cell, the distal tip cell (Kimble & White, 1981). This cell appears to act over a distance to prevent meiosis in neighbouring germ cells and thereby establishes the polarity of the germ line tissue. The molecular basis of distal tip cell activity is unknown.

3. The genetic control of the decision between oocyte and sperm differentiation appears to involve the same genes as are responsible for the decision between hermaphrodite and male development in the soma (Klass *et al.*, 1976; Hodgkin & Brenner, 1977; Nelson *et*

al., 1978; Hodgkin, 1980; J. Kimble, unpublished results). The molecular and cellular mechanism(s) by which these genes regulate differentiation of somatic and germ line tissues are unknown.

References

Albertson, D. G. & Thomson, J. N. (1976). The pharynx of *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond. B*, **275**, 299–325.

Beklemishev, W. N. (1969). *Principles of Comparative Anatomy of Invertebrates*, vol. 2. Edinburgh: Oliver & Boyd.

Boveri, T. (1899). *Die Entwicklung von* Ascaris megalocephala *mit besonderer Rucksicht auf die Kernverhaltnisse*. Jena: Gustav Fischer.

Boveri, T. (1910). Uber die Teilung Centrifugiter Eier von *Ascaris megalocephala*. *Arch. EntwMech. Org.*, **30**, 101–25.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics*, **77**, 71–94.

Deppe, U., Schierenberg, E., Cole, T., Krieg, C., Schmitt, D., Yoder, B. & von Ehrenstein, G. (1978). Cell lineages of the embryo of the nematode *Caenorhabditis elegans*. *Proc. natn. Acad. Sci. USA*, **75**, 376-80.

Eddy, E. M. (1975). Germ plasm and the differentiation of the germ cell line. *Int. Rev. Cytol.*, **43**, 229–80.

Herman, R. & Horvitz, R. (1980). Genetic analysis of *Caenorhabditis elegans*. In *Nematodes as Biological Models*, ed. B. Zuckerman, pp. 227–64. New York: Academic Press.

Hirsh, D., Oppenheim, D. & Klass, M. K. (1976). Development of the reproductive system of *Caenorhabditis elegans*. *Devl Biol.*, **49**, 200–19.

Hodgkin, J. A. (1974). Genetic and anatomical aspects of the *Caenorhabditis elegans* male. Ph.D. Thesis, University of Cambridge.

Hodgkin, J. (1980). More sex-determination mutants of *Caenorhabditis elegans*. *Genetics*, **96**, 649–64.

Hodgkin, J. A. & Brenner, S. (1977). Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. *Genetics*, **86**, 275–87.

Hogue, M. J. (1910). Uber die Wirkung der Centrifugalkraft auf die Eier von *Ascaris megalocephala*. *Arch. EntwMech. Org.*, **29**, 109–45.

Illmensee, K. & Mahowald, A. P. (1974). Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. *Proc. natn. Acad. Sci. USA*, **71**, 1016–20.

Kimble, J. (1981). Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. *Devl Biol.*, **87**, 286–300.

Kimble, J. & Hirsh, D. (1979). The post embryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Devl Biol.*, **70**, 396 417.

Kimble, J. & Sharrock, W. J. (1983). Tissue specific synthesis of yolk proteins in *Caenorhabditis elegans*. *Devl Biol.*, **96**, 189–96.

Kimble, J. E. & White, J. G. (1981). On the control of germ cell development in *Caenorhabditis elegans*. *Devl Biol.*, **81**, 208–19.

Klass, M. K., Wolf, N. & Hirsh, D. (1976). Development of the male

reproductive system and sexual transformation in the nematode *Caenorhabditis elegans*. *Devl Biol.*, **52**, 1–18.

Laufer, J. & von Ehrenstein, G. (1981). Nematode development after removal of egg cytoplasm: absence of localized unbound determinants. Science, **211**, 402–5.

Madl, J. E. & Herman, R. K. (1979). Polyploids and sex determination in *Caenorhabditis elegans*. *Genetics*, **93**, 393–402.

Nelson, G. A., Lew, K. K. & Ward, S. (1978). Intersex, a temperature sensitive mutant of the nematode *Caenorhabditis elegans*. *Devl Biol.*, **66**, 386–409.

Strome, S. & Wood, W. B. (1982). Cytoplasmic localization of germ line specific granules in embryos of *Caenorhabditis elegans*. *Proc. natn. Acad. Sci. USA*, **79**, 1558–62.

Sulston, J. E., Albertson, D. G. & Thomson, J. N. (1980). The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. *Devl Biol.*, **78**, 542–76.

Sulston, J. E. & Horvitz, H. R. (1977). Postembryonic cell lineages of the nematode *Caenorhabditis elegans*. *Devl Biol.*, **56**, 110–56.

Sulston, J. E. & White, J. G. (1980). Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans*. *Devl Biol.*, **78**, 577–97.

White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. (1976). The structure of the ventral nerve cord of *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond. B.*, **275**, 327–48.