Controls of Postembryonic Germ Line Development
in Caenorhabditis elegans

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The development of a multicellular organism from one cell involves the
coordination of cell proliferation, morphogenesis, and differentiation
in multiple tissues. We have elected to study the control of development in
the germ line tissue of the nematode Caenorhabditis elegans. In this review,
we discuss our current knowledge of two controls of germ line develop-
ment—control over the decision between mitosis and meiosis and control
over sex determination. Our approach is to identify, by mutation, genes
central to each of these controls and to employ these mutations for genetic
and biochemical analyses of their function.

The nematode Caenorhabditis elegans offers several advantages for dis-
secting the developmental controls of higher organisms. Although simple, C.
elegans possesses the basic body plan and tissue types found in higher
eukaryotes. Genetic manipulation of C. elegans is particularly easy because
its life cycle is only 3½ days and individuals can reproduce by either self-
fertilization or cross-fertilization. In addition, C. elegans is transparent and
is composed of relatively few cells, so its development can be examined as it
occurs [Sulston, 1976]. Moreover, the cloning of selected genes from C.
elegans has become more routine because of the recent discovery of a transposable element [Emmons et al., 1983; Moerman and Waterston, 1984;
Eide and Anderson, 1985].

Development of the germ line tissue of C. elegans differs from that of
somatic tissues. The development of the germ line occurs by a variable
pattern of divisions [Kimble and Hirsh, 1979]. Furthermore, the ability to
continue cell divisions in the germ line is controlled by interaction with a
somatic regulatory cell, the distal tip cell [Kimble and White, 1981]. In stark
contrast, the somatic tissues, i.e., hypodermis, nerves, muscle, gut, gonadal
epithelium, develop by a fixed cell lineage in which, generally, the differen-
tiation of individual cells is not affected by cell interactions. Instead, cell fate
is almost always correlated strictly with cell ancestry [Sulston and Horvitz, 1977; Sulston and White, 1980; Kimble, 1981; Sulston et al., 1983]. The analysis of development in the germ line might therefore reveal mechanisms of control that are different from those operating in the soma.

Development of the germ line is particularly accessible to analysis in C. elegans for several reasons. As in other organisms, the germ line is dispensable, so mutants with drastic alterations of the tissue, though sterile, can survive for examination. Furthermore, the sterility of the mutants can be used for selection of suppressors to identify additional genes in the pathway of control. However, unlike most other organisms commonly used for experimentation, the germ line of C. elegans is easily visible because of the transparency and small size of the organism. This means that changes in the germ line can be observed in living animals by simple inspection. In addition, the syncytial nature of the C. elegans germ line permits microinjection for functional assay of molecules of potential interest [Kimble et al., 1982]. Finally, in C. elegans most of the known sex-determination genes affect both somatic and germ line tissues [Hodgkin, 1980], whereas in Drosophila these genes appear to control sex only in somatic tissues [e.g., Baker and Belote, 1983]. Thus, although C. elegans is commonly considered best suited for studies of the invariant cell lineage typical of its somatic tissues, it is also an excellent model organism for study of the genetic control of development in the germ line.

**REVIEW OF NORMAL GERM LINE DEVELOPMENT IN C. ELEGANS**

During embryogenesis, one cell arises that will generate the germ line tissue of C. elegans [Sulston et al., 1983]. This progenitor cell divides once before hatching, and its daughters, called Z2 and Z3, become located in the gonadal primordium. This primordium also contains two somatic progenitor cells, called Z1 and Z4, that will give rise to the somatic structures of the gonad. The embryonic development of the germ line and the morphology of the gonadal primordium are identical in the two sexes of C. elegans. The sexual dimorphism of the gonad arises after hatching, as the gonad develops during the four larval stages that occur before adulthood.

The basic features of postembryonic germ line development are essentially the same in the two sexes, except for the type of gamete produced (Figure 1). Z2 and Z3 divide mitotically to generate about 2,000 nuclei in hermaphrodites and about 1,000 nuclei in males. These nuclei are located at the periphery of the germ line cytoplasm, each partially enclosed by a membrane

![Diagram](image)

**Fig. 1.** Postembryonic development of the germ line in C. elegans. A) Z2 and Z3, the two germ line progenitor cells, are located in a gonadal primordium in the newly hatched worm of either sex. Postembryonically, all germ line cells divide mitotically to proliferate until the third larval stage. B) In L3, certain germ line nuclei enter the meiotic cell cycle. During the fourth larval stage, the mitotic nuclei begin gametogenesis. C) Hermaphrodites make sperm first and then begin oogenesis. Males (not shown) make only sperm. The somatic distal tip cell regulates the decision between mitosis and meiosis in the germ line. The U-shape of the gonad is not shown.

[Hirsh et al., 1976]. The germ line tissue, then, is actually a syncytium. Some germ line nuclei remain mitotic throughout the life of the animal; these are located at one end (the distal end) of the germ line tube. The other germinal nuclei, located more proximally, enter meiosis and mature through the stages of meiosis and gametogenesis as they progress proximally [Klass et al., 1976]. This means that the germ line tissue possesses a polarity of maturation with the least mature nuclei at one end (distal end), nuclei in meiotic prophase in the middle, and maturing gametes at the other end (proximal end). The male testis produces sperm continuously from the mid-L4 stage throughout adulthood; each hermaphrodite ovotestis makes about 150 sperm during L4 lethargus and then makes oocytes throughout adulthood.

**CONTROL OF THE DECISION BETWEEN MITOSIS AND MEIOSIS**

As described above, some germ line nuclei in C. elegans enter meiosis, whereas others remain in mitosis. Two somatic cells, the distal tip cells,
regulate entry into meiosis [Kimble and White, 1981]. In hermaphrodites, one distal tip cell occupies the distal end of each of the two equivalent ootestes; in males, both distal tip cells occupy the distal end of the single testis. The distal tip cells are descendants of the progenitor cells of the somatic gonad, Z1 and Z4. They arise and assume their distal tip position during the first larval stage [Kimble and Hirsh, 1979].

The regulatory role of the distal tip cell was discovered by a series of laser ablation experiments [Kimble and White, 1981]. When both distal tip cells are destroyed by a laser microbeam, all germ line nuclei enter meiosis after only a few (one to four) mitotic divisions (Fig. 2). The meiotic cells then complete gametogenesis. The type of gamete produced after distal tip cell ablation depends on the sex of the animal and, in hermaphrodites, on the stage of development at which the ablation was performed [for more detail,

A. WILD TYPE AND INTACT

\[ \text{distal tip cell} \]

\[ \text{mitosis} \quad \text{meiosis} \]

B. WILD TYPE AND LASER ABLATED

\[ \text{meiosis} \]

C. \textit{glp-1} AND INTACT

\[ \text{meiosis} \]

Fig. 2. Control over the decision between mitosis and meiosis. A) Once meiosis has begun, germ line nuclei in wild-type, intact gonads are in the mitotic cell cycle distally and in the meiotic cell cycle proximally. B) After ablation of the distal tip cell with a laser microbeam, all germ-line nuclei enter meiosis. C) In animals homozygous for \textit{glp-1}, all germ line nuclei enter meiosis, but the distal tip cell is present in its correct position. The U-shape of the gonad has not been shown in these diagrams. However, gonads in A and C are U-shaped because of the presence of the morphogenetic (leader) function of the distal tip cell, and the gonad in B is ball-shaped because of the absence of the distal tip cell.

see Kimble and White, 1981]. Thus, in the intact animal, the activity of the distal tip cell prevents germ line nuclei located near it from entering into meiosis. This activity is not only essential to germ line proliferation during larval growth but it also maintains a group of mitotic nuclei in the adult to serve a stem cell function.

A Gene Central to the Mitotic/Miotic Decision: \textit{glp-1}

The biochemical mechanism of distal tip cell control is not yet directly accessible to investigation. The regulatory activity is restricted to two small cells in each animal, and no biochemical assay for activity is available. Given the amenability of \textit{C. elegans} to genetic study, the most direct route to an understanding of the mechanism of distal tip cell control is by identification of genes essential to this control. Based on this reasoning, a screen for mutants altering distal tip cell function was performed. Parents were mutagenized with ethyl methane sulfonate, and 5,000 F1 hermaphrodites were picked to separate Petri dishes. Sterile animals among the F2 were then examined for a mutant phenotype similar to that of an animal in which the distal tip cell had been destroyed. In this way, one gene, \textit{glp-1}, was identified that appears to be essential to the control of germ line proliferation (Kimble and Austin, in preparation). Three recessive alleles of this locus were isolated among the 5,000 F1 clones examined. This frequency is typical for loss-of-function mutations in an average-sized gene [Brenner, 1974]. The three alleles of \textit{glp-1} fail to complement each other and map to the same position of chromosome III.

The germ line in animals homozygous for \textit{glp-1} is similar to that observed in animals after laser ablation of the distal tip cell in the L1 stage (Fig. 2C). Z1 and Z3 are found in the gonadal primordium of the newly hatched \textit{glp-1} homozygote, as is usual. However, little mitosis occurs during larval development, and all germ line descendants enter meiosis and become sperm. The somatic gonad, in contrast, is normal, the distal tip cells are present and morphologically normal, and the sperm produced are functional. In fact, a second, morphogenetic function of the hermaphrodite distal tip cells is not altered by mutation of \textit{glp-1}. The hermaphrodite distal tip cell in both wild-type and \textit{glp-1} animals causes the ootestis to develop as an elongate U-shaped tube. Thus \textit{glp-1} affects the state of germ line nuclei specifically and must be essential to the distal tip cell control.

It is intriguing that only one gene was discovered in our broad search for mutants that alter the distal tip cell control over the germ line. Surely more gene products are involved in this control. The inability to isolate other mutants with a Glp phenotype suggests that elimination of other gene prod-
CONTROL OF SEX DETERMINATION IN THE GERM LINE: THE SPERM/OOCYTE DECISION

Background to Sex Determination in C. elegans

Somatic and germ line sex in wild-type animals. C. elegans exists as one of two sexes. Diploid animals with two X chromosomes (XX) are self-fertilizing hermaphrodites; with only a single X chromosome (XO), they are males. The two sexes differ substantially in morphology, biochemistry, and behavior. Hermaphrodites possess a gonad with two equivalent ovotestes that meet centrally at a vulva. Males have a gonad with a single testis that opens to the exterior posteriorly in the tail. This male tail has a sex-specific elaboration of nerve, muscle, and hypodermis required to form a copulatory apparatus for mating with the hermaphrodite. The intestine is biochemically but not morphologically different in the two sexes. Only the hermaphrodite intestine produces yolk proteins [Kimble and Sharrock, 1983].

The hermaphrodite soma is essentially female; the hermaphrodite germ line is part male and part female. A hermaphrodite animal that produces no sperm, e.g., animals homozygous for a fem mutation (see below), is a functional female [Nelson et al., 1978]. Furthermore, the development and anatomy of the C. elegans hermaphrodite soma are strikingly similar to those of the Panagrellus redivivus female [Sternberg and Horvitz, 1981, 1982]. However, although the hermaphrodite soma is female, its germ line produces both male and female gametes. In each ovotestis, the most proximal germ cells produce sperm, and the rest become oocytes. The sperm and oocytes of C. elegans are distinct cells morphologically [Hirsh et al., 1976] and biochemically [Ward and Klass, 1982]. As in most other metazoa, sperm are tiny cells specialized for motility and oocytes are huge cells specialized for

embryogenesis. Since both male and female gametes are made in an otherwise female soma, it seems likely that the control of sex determination in somatic and germ line tissues is not identical.

Genetics of sex determination. Seven genes have been identified as sex-determination control genes in C. elegans. These genes apparently respond to the initial signal for sex determination in C. elegans, the X:autosome ratio [Madl and Herman, 1979], and specify sex. Three genes, tra-1, tra-2, and tra-3, are necessary for hermaphrodite development; three genes, fem-1, fem-2, and fem-3, are required for male development (this includes formation of sperm in XX hermaphrodites and development of all tissues in XO males), and one gene, her-1, is necessary for male development in XO animals. The phenotypes caused by mutations in these genes are summarized in Table I. A null mutation in any of the three tra genes causes XX animals to develop along the male pathway of development but does not affect XO animals [Hodgkin and Brenner, 1977]. A loss-of-function mutation in any of the three fem genes feminizes both XX and XO animals [Kimble et al., 1984; Doniach and Hodgkin, 1984; Hodgkin et al., 1985]. A recessive mutation of her-1 transforms XO animals into hermaphrodites [Hodgkin, 1980] but does not alter hermaphrodite development in XX animals. The epistatic relationships observed in strains harboring more than one sex determination mutation have led to a formal model of genetic regulation of sex determination [Hodgkin, 1980; Doniach and Hodgkin, 1984].

Most of the master sex-determining genes appear to function in both somatic and germ line tissues. However, a clear difference in the genetic control of sex determination in somatic and germ line tissues was observed

<p>| TABLE I |
|---|---|---|
| Loss-of-Function Phenotypes of the Sex-Determination Genes in C. elegans |   |</p>
<table>
<thead>
<tr>
<th>XX</th>
<th>XO</th>
</tr>
</thead>
<tbody>
<tr>
<td>tra-1</td>
<td>Male&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>tra-2</td>
<td>Male&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>tra-3</td>
<td>Male&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>fem-1</td>
<td>Female</td>
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<tr>
<td>fem-2</td>
<td>Female</td>
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<tr>
<td>fem-3</td>
<td>Female</td>
</tr>
<tr>
<td>her-1</td>
<td>Hermaphrodite</td>
</tr>
</tbody>
</table>

<sup>a</sup>Somatic tissues are male, although some allele specificity is observed as to which tissues are completely transformed. Germ line contains sperm and oocytes.

<sup>b</sup>Male tail is not completely transformed.

<sup>c</sup>Male tail and gonad are not completely transformed.
in a mutant strain homozygous for recessive mutations at both the \textit{tra-1} and \textit{fem-1} loci. As mentioned above, \textit{tra-1} homozygotes develop as males and \textit{fem-1} homozygotes develop as females. However, the \textit{tra-1 fem-1} double mutant strain is male somatically but female in its germ line [Doniaich and Hodgkin, 1984]. Results of such experiments indicate that \textit{tra-1} is the primary sex-determining gene in the somatic tissues, whereas the \textit{fem} genes are primary in the germ line (see below). The role of \textit{tra-1} in the germ line is not yet understood. The apparent null phenotype of \textit{tra-1} in XX animals is to transform the sexual phenotype of XX animals from hermaphrodite to male [Hodgkin and Brenner, 1977]. However, in the germ line, both sperm and oocytes are made (Schedl, unpublished). Thus \textit{tra-1} might be a sex determination gene that specifies sex only in the somatic tissues.

Genetic Selections for Sex-Determining Mutants in the Germ Line

Most sex-determining genes in \textit{C. elegans} have been identified by their mutant effect on somatic tissues [e.g., Hodgkin, 1980]. In this section, we describe genetic selections for mutations that alter the sexual phenotype of the germ line. The idea of these selections is diagrammed in Figure 3. Basically, a hermaphrodite is self-sterile if it produces only sperm or only oocytes, but if it is self-fertile if both sperm and oocytes are made. Therefore, mutants that feminize the hermaphrodite germ line so that only oocytes are made (or that masculinize it so that only sperm are made) can be used to select for mutations that act as suppressors to reinstate self-fertility of the hermaphrodite. Thus any gene that can be mutated to effect a masculinization of the germ line might suppress a feminizing mutation and vice versa.

Selection for masculinizing mutations. To select for mutations that masculinize the hermaphrodite germ line, temperature-sensitive (ts) alleles of either \textit{fem-1} (formerly called \textit{ixs-1} [Nelson et al., 1978]) or \textit{fem-2} [Kimble et al., 1984] have been used. Animals homozygous for a ts allele at either locus are self-fertile at permissive temperature but self-sterile at restrictive temperature because only oocytes are produced. Dominant suppressors of \textit{fem-1} and dominant suppressors of \textit{fem-2} have been isolated (Barton et al., in preparation). These suppressors, when removed from the \textit{fem-1} or \textit{fem-2} background, masculinize the hermaphrodite germ line so that sperm but no oocytes are made.

Among the dominant suppressors of \textit{fem-1} and \textit{fem-2}, a group of nine independently isolated suppressors are dominant gain-of-function alleles of \textit{fem-3} (Barton et al., in preparation). This identity has been established by mapping, intragenic reversion, and a cis-trans test. Hermaphrodites homozygous for any of these \textit{fem-3} (gain-of-function) alleles make a vast excess of sperm but no oocytes. No alteration of the hermaphrodite soma or of the XO male animal is observed. All of the gain-of-function alleles of \textit{fem-3} are temperature-sensitive, a feature that has been exploited for selection of feminizing mutations, as is described below. The strength of the alleles varies when penetrance and dominance of the phenotype are examined. Thus some are so weak that a few homozygous animals at restrictive temperature make oocytes and others are so strong that only a few heterozygous animals make oocytes at permissive temperature.

Two other dominant suppressors isolated in this selection, \textit{sup(q62)} and \textit{sup(q93)}, map to chromosome I. Both suppressors, when isolated away from \textit{fem-1} or \textit{fem-2}, transform the sexual phenotype of the hermaphrodite germ line so that vast excess of sperm are produced. These two suppressors may identify a new sex-determination locus that was previously unknown.

Selection for feminizing mutations. To select for mutations that cause feminization of the germ line, one of the temperature-sensitive gain-of-
function alleles of fem-3, q20, has been used. All animals homozygous for q20 are self-sterile at restrictive temperature; sperm but no oocytes are made in an otherwise hermaphrodite animal.

Dominant suppressors of q20 include both intragenic loss-of-function alleles of fem-3 and extragenic gain-of-function alleles of tra-2. In addition, another dominant feminizing suppressor has been mapped on chromosome III, sup(q81), and seems to identify another new sex-determination locus. The phenotype of the gain-of-function alleles of tra-2 is similar to that observed for gain-of-function alleles of tra-2 isolated by other means [Hodgkin et al., 1985]. Such alleles cause feminization of XX and XO animals when removed from the q20 background (T. Rosenquist, unpublished). XX animals are functional females, but XO animals are only slightly feminized. Some oocytes are observed in older XO animals, some appear to make yolk, and some have partially feminized tails. As described earlier, the null phenotype of tra-2 is masculinization of the XX animal [Hodgkin and Brenner, 1977]. Thus, the mutant phenotypes of gain-of-function and loss-of-function alleles of tra-2 have reciprocal effects on sexual differentiation. Other similar gain-of-function alleles of tra-2 have also been observed by Doniach (personal communication).

Recessive suppressors of q20 include loss-of-function alleles of fem-1, fem-2, and a previously unknown gene, fog-2 (for feminization of the germ line). A preliminary characterization of fog-2 suggests that this gene is required for the short burst of spermatogenesis typical of the hermaphrodite germ line before the onset of oogenesis. Thus a mutation of fog-2 affects spermatogenesis only in hermaphrodites. XX animals homozygous for fog-2 are females; XO animals homozygous for fog-2 are males. (It should be noted that by mutation of a single gene, q20, C. elegans can be changed from a hermaphroditic to a male/female species.) To determine whether the fog-2 mutant phenotype was specific to XX or to hermaphrodite animals, the effect of fog-2 on XO hermaphrodites was examined. As described above, animals homozygous for her-1, whether XX or XO, develop as hermaphrodites. In a double mutant strain homozygous for both her-1 and fog-2, both XX and XO animals are transformed into females. Thus fog-2 affects hermaphrodites regardless of their X:autosome ratio. Our working hypothesis for the function of fog-2 in germ line sex determination is that it negatively regulates tra-2 briefly in the hermaphrodite germ line to permit some spermatogenesis in an otherwise female animal (Schedl, unpublished).

The selection for suppressors of q20 has proved to be useful for isolation of both dominant and recessive mutations that feminize the hermaphrodite germ line. Although most q20 suppressors are mutations of genes known before (e.g., fem-1, fem-2, tra-2), some new loci have been identified [e.g., fog-2, sup(q81)].

Role of fem-3 in Sex Determination

The fem-3 gene is essential to specification of the male developmental pathway in both hermaphrodites (sperm) and males (all tissues). Loss-of-function alleles of fem-3 transform both XX and XO animals into females [Hodgkin et al., 1985] (this laboratory, unpublished observations). This phenotype is identical to that of fem-1. No alleles of fem-2 have yet been isolated that completely transform an XO animal into a female [Hodgkin et al., 1985]. Moreover, the pattern of epistasis found for fem-3 places it in the same position in the genetic pathway of sex determination control as that of fem-1 and fem-2 [Hodgkin et al., 1985]. Thus, based on its null phenotype, fem-3 can be considered equivalent to fem-1. In the soma, the fem genes are thought to regulate tra-1 negatively, and therefore, tra-1 appears to be the final control in the somatic pathway of sex determination. However, in the germ line, the fem genes seem to be the final control in sex determination as positive regulators of spermatogenesis.

The phenotype of gain-of-function alleles of fem-3 strengthens the idea that fem-3 is a central control gene for sex determination. These gain-of-function alleles cause masculinization, whereas loss-of-function alleles lead to feminization. Many known swich-genes are similarly defined by gain-of-function and loss-of-function alleles that have opposite effects on cell fate: e.g., tra-1 [Hodgkin, 1983], lin-12 [Greenwald et al., 1983], Sex [Cline, 1978], and Antennapedia [Struhl, 1981].

In addition, the phenotype of animals homozygous for a fem-3 (gain-of-function) mutation suggests that fem-3 is regulated in a tissue-specific manner. One might have imagined that gain-of-function alleles would masculinize XX in both somatic and germ line tissues, since loss-of-function alleles feminize XO animals in both tissues. However, the hermaphroditic soma is not masculinized, even partially, in animals homozygous for gain-of-function alleles of fem-3. Since fem-3 exhibits a maternal effect [Hodgkin et al., 1985], it is likely that the fem-3 product is produced by the hermaphrodite germ line for deposition into oocytes. Perhaps the fem-3 product synthesized by the hermaphrodite germ line for the embryo is normally regulated so that it cannot affect the sex of the germ line in which it is synthesized. However, if the fem-3 gene product is rendered insensitive to this regulation, by mutation, it might then be able to act on the germ cells abnormally. A loss of such tissue-specific regulation would lead to a gain-of-function phenotype in which a vast excess of sperm is produced in the hermaphrodite germ line.
The temperature-sensitive period (TSP) of the gain-of-function alleles of fem-3 (Barton et al., in preparation) is consistent with the idea that the mutant gene product acts on the germ line as the tissue differentiates. If animals are maintained at restrictive temperature until early adulthood, so that excess sperm are already found in the hermaphrodite gonad, a shift to permissive temperature will result in subsequent oogenesis. If animals are maintained at permissive temperature until late L4, so that the normal number of sperm are made and oogenesis has begun as normal, a shift to restrictive temperature will result in a return to spermatogenesis from oogenesis. Since most of the germ line nuclei in the distal arm are in meiosis, this lability of gamete sex suggests that germ line nuclei can be specified as sperm or oocyte even after entry into meiosis. However, since mitotic nuclei are still present at the distal end of the gonad, the possibility remains that all meiotic nuclei are committed to one sex or the other and that only stem cell nuclei remain labile.

SUMMARY OF GERM LINE SEX DETERMINATION

The choice between spermatogenesis and oogenesis is controlled by a small number of switch genes. Most of the somatic sex-determination control genes of C. elegans also function to specify germ line sex. However, these master control genes must be regulated in the hermaphrodite germ line to achieve the production of both male and female gametes in the hermaphrodite, essentially female, soma. We have exploited genetic selections to isolate both dominant and recessive mutations that influence the sex of the hermaphrodite germ line. These selections have uncovered several sex-determination genes that were previously unknown. They also have been useful for isolation of gain-of-function alleles of fem-3, tra-2, and other new sex determination loci. Such gain-of-function alleles provide information critical for understanding the functions of these control loci.

The gain-of-function alleles of fem-3 have provided evidence that this locus is of particular import in specification of the male sex. The reciprocal effect on sexual transformation observed for gain-of-function and loss-of-function alleles of fem-3 is supportive of its role as a switch-gene for sex determination. The masculinization phenotype and the late TSP of the gain-of-function alleles are consistent with the idea that the fem-3 gene product must be negatively regulated in the hermaphrodite germ line so that it can be packaged into oocytes for embryogenesis without transforming those oocytes into sperm.

A deeper understanding of the pathway of sex-determination control depends on identification of the genes central to the pathway and genetic analysis of several types of alleles of these genes. The genetic selections described above will aid in the identification of new sex-determination genes and in the isolation of multiple alleles of genes central to this pathway. Our focus on the control of germ line sex determination was based on the possibility, since proven to be fact, that tissue-specific controls influence sex in the germ line. Such tissue-specific controls, if present in all tissues, might complicate the analysis of sex determination when viewed at the organismal level. A molecular understanding of sex determination controls in general, and fem-3 function in particular, awaits the biochemical isolation of the sex-determination genes and their products. To this end, the selections described here are being used to isolate alleles of the sex-determination genes caused by insertion of the transposable element, TcI, for molecular cloning of those genes by transposon tagging.

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The Regulation of Xenopus laevis Oocyte Maturation

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Amphibian oocytes undergo an extended growth period while arrested in meiotic prophase. In Xenopus laevis, oogenesis is asynchronous such that oocytes of various sizes are present within the ovary at the same time. This oocyte growth period has been subdivided into six major stages, I–VI [Dumont, 1972]. Under normal circumstances, it is only the full grown stage VI oocytes (1,200–1,300 μm in diameter) that are capable of responding to hormonal stimulation and resuming meiosis. This response is called meiotic maturation, which prepares the full grown oocyte for fertilization and subsequent embryonic development. At the morphological level, meiotic maturation includes the migration of the oocyte nucleus (germinal vesicle) to the apex of the animal pole, dissolution of the nuclear envelope (germinal vesicle breakdown; GVBD), chromosome condensation, spindle formation, segregation of homologous chromosomes, and arrest at metaphase II.

In vivo, the process of meiotic maturation is normally triggered by gonadotropins that are released from the pituitary gland. The peptide hormones travel to the ovary via the circulatory system and stimulate a layer of somatic cells (follicle cells) that surround the full grown oocyte. The follicle cells in turn synthesize and release the steroid progesterone, which is ultimately responsible for triggering the resumption of meiosis in the full grown oocyte. Meiotic maturation can be induced in vitro by directly applying progesterone to stage VI oocytes in culture [for reviews, see Wasserman and Smith, 1978a; Masui and Clark, 1979; Maller 1983].

Unlike most steroid-target cell interactions, which involve the translocation of the hormone to the nucleus and altering gene transcription [for review, see Anderson, 1984], progesterone appears to be working at or near the plasma membrane of the oocyte. Microinjection of progesterone into full grown oocytes will not induce meiotic maturation [Masui and Markert, 1971; Smith and Ecker, 1971], whereas cell surface exposure successfully triggers the resumption of meiosis. In addition, progesterone will induce meiotic maturation even when it is covalently bound to polystyrene beads and cannot enter the oocyte [Ishikawa et al., 1977; Godeau et al., 1978]. Therefore, the steroid receptor seems to be located in the plasma membrane of the oocyte.