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CHAPTER 8

Developmental Genetics of *Caenorhabditis elegans*

Judith Kimble and Timothy Schedl

THE DEVELOPMENT of a multicellular organism from a single-celled egg involves the coordinated control of many cells and tissues. How are cells specified to develop as one cell type rather than another, in one position rather than another, and at one time rather than another? What is the molecular basis of the spatial and temporal cues necessary to direct development of the organism? The information for this developmental feat is stored in the egg—either in *its* genome or in products of the *maternal* genome contributed to that cell. Developmental genetics provides a powerful way to investigate that information.

The nematode, *Caenorhabditis elegans*, has proven to be an excellent model organism for analysis of genes that control development. Not only is genetic manipulation of this simple and primitive worm particularly easy, but the organism's development is accessible at the level of individual cells from the one-cell to the adult stage. Numerous techniques have been developed over the past 10 years to facilitate developmental analyses of *C. elegans*. These include genetic methods, for example, generation of genetic mosaics (17) and use of transposable elements for gene cloning (14), and physical methods, such as laser ablation (39) and microinjection techniques (30). Together, these techniques provide a powerful battery of tools to dissect *C. elegans* developmental controls.

In this chapter we focus on genetic methods of analysis of *C. elegans* development. First we briefly review *C. elegans* development and some of the genetic tools available for its analysis. Next we discuss, in broad terms, the approach taken to analyze genes that specify developmental decisions. The sex determination genes and their analysis are used to exemplify the various steps discussed. Finally we describe recent studies that investigate sex determination in a single tissue, the germ line.

Overview of *C. elegans* Development

The development of *C. elegans* begins in a single-celled zygote. The events of embryogenesis are described in detail in Chapter 9. Briefly, the zygote cleaves by a fixed pattern of divisions to generate a cluster of cells. Then the embryo undergoes a period of morphogenesis, during which it elongates and cells differentiate to form a tiny worm folded up inside the eggshell.

After hatching, the worm develops through four larval stages (L1–L4) into adulthood. During larval development, the animal grows by an increase in cell number of its primary tissues (hypodermis, nervous system, muscle, gut), and matures sexually. The somatic structures of *C. elegans* develop by a cell lineage that is essentially invariant from animal to animal (29, 37, 38). In an attempt to observe an influence of cell-cell interactions on cell fate in somatic development, individual cells have been ablated by a laser microbeam. With only a few exceptions, cell fates are not affected by ablation of their neighbors, suggesting that cell-cell interactions play a limited role in the specification of cell fate in the somatic tissues of *C. elegans* (27, 37, 38, 39). Development of the germ line differs fundamentally from that of somatic tissues. Proliferation of the germ line from its embryonic precursor cell, P_4 (see Chapter 9), occurs by a variable pattern of divisions that continue throughout adulthood (29). Furthermore, laser ablation experiments show that germ-line divisions are under the control of a somatic regulatory cell, the distal tip cell (31).

Figure 1 diagrams the two sexes of *C. elegans* as young adults. These two

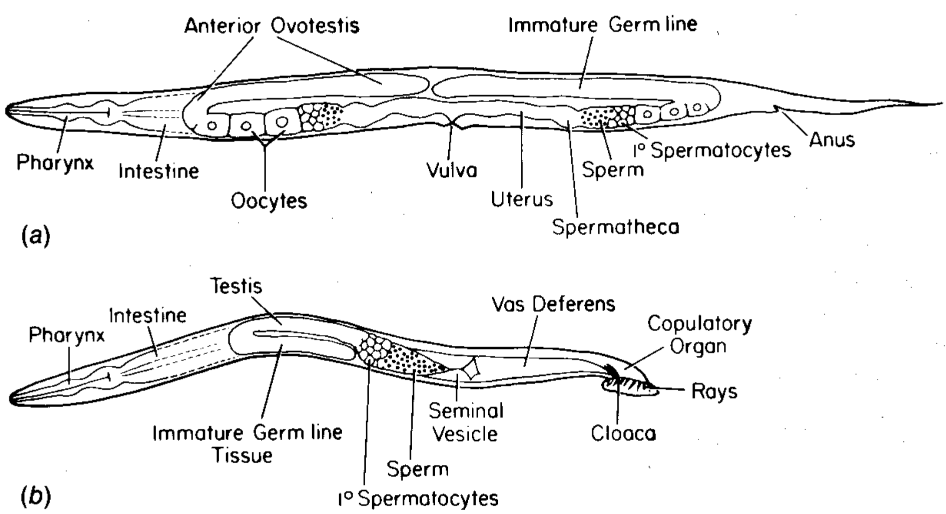

Figure 1 Schematic drawings of the anatomy of a young adult hermaphrodite (a) and a male (b). Notice the sexually dimorphic gonads (two ovotestes in the hermaphrodite, one testis in the male) and tails (a whip-like tail in the hermaphrodite, a tail specialized for copulation in the male).

sexes are the self-fertilizing hermaphrodite and the male. The hermaphrodite can be considered as a female that makes a limited number of sperm to permit self-fertilization. Thus, the hermaphrodite soma is essentially female, whereas the hermaphrodite germ line is part male (sperm) and part female (oocytes). Most tissues of *C. elegans* exhibit some sexual dimorphism (Table 1). In hermaphrodites, the gonad consists of two equivalent ovotestes that each produce sperm and then oocytes. In males, there is a single testis that makes only sperm. Secondary sexual structures (e.g., vulva in the hermaphrodite sex, copulatory apparatus in the male sex) develop from assorted precursor cells of hypodermal, neural, and muscle origin. The intestine, though morphologically identical in the two sexes, synthesizes yolk only in hermaphrodites.

Overview of Genetic Manipulations in *C. elegans*

Genetic analyses of *C. elegans* are facilitated by its short life cycle (3 days), its large brood size (about 350), and its ease of maintenance (similar to *Escherichia coli*). *C. elegans* can reproduce either by self- or by cross-fertilization. The hermaphrodite (XX) has a diploid set of six chromosomes: five pairs of autosomes and one pair of X chromosomes. The male (XO) has five pairs of autosomes and a single X chromosome. The haploid genome is about 8×10^7 base pairs (bp) (36).

Over 600 genes have been identified in *C. elegans* by mutation. For the most part, individual genes have been defined by mapping of a mutation to a specific site in the genome and by complementation tests between alleles of that locus. Genetic mapping relies on standard techniques: segregation analy-

Table 1 Sexual dimorphism in *C. elegans*

Tissue	Hermaphrodite	Male
Chromosomes	XX; 5AA = 12	XO; 5AA = 11
Hypodermis	Vulva	Copulatory bursa, spicules, rays
Nerve	Neurons for egg laying	Neurons for mating
Muscle	Muscles for egg laying	Muscles for mating
Intestine	Yolk synthesis	—
Somatic gonad	Two ovotestes, uterus, spermathecae	One testis, seminal vesicle, vas deferens
Germ line	Sperm, then oocytes	Sperm
Total somatic nuclei	959	1031
Total germ-line nuclei	About 2500	About 1000

sis to learn linkage and recombination analysis with flanking markers to learn map position (7). Mutations used for morphological markers cause changes in the shape or movement of the animal. In the standardized genetic nomenclature of *C. elegans*, a gene is specified by a three-letter code indicating its mutant phenotype and a number, for example, *fem-1*. Mutant alleles are named by a letter specifying the laboratory of its origin and a number, for example, *e1*, the first allele isolated by S. Brenner in England; and mutant phenotypes are given a three-letter code, for example, Fem for feminized.

More recently, genes have been identified solely by molecular techniques. Cloned sequences can be mapped by standard segregation and recombination analysis using restriction fragment length polymorphisms as markers (13) and by *in situ* hybridization (1). A handful of genes [e.g., major sperm proteins (8), vitellogenins (6)] have been identified in this way.

Numerous chromosomal rearrangements have been isolated in *C. elegans* (18). These rearrangements include duplications, deficiencies, and complex intrachromosomal rearrangements. Duplications can exist in tandem, as free chromosomal fragments, or as translocated chromosomal segments. The centromeres of *C. elegans* are distributed throughout the chromosome rather than being localized to one position (2). Duplications existing as free chromosomal fragments can therefore be maintained at mitosis for multiple regions of any chromosome. Chromosomal rearrangements have many uses, including the balancing of lethal or sterile mutations, alteration of gene dosage, and generation of genetic mosaics.

An elegant genetic strategy has recently been devised to deduce the tissue in which an individual gene functions (17). This method takes advantage of both the invariant cell lineage and the availability of free duplications in *C. elegans*. Although free duplications are generally stable at mitosis, they are lost at a low rate. Due to this instability, genetic mosaics are found among animals derived from eggs containing two chromosomes that carry a given mutation plus a free duplication, which in turn carries a wild-type copy of that gene. To detect loss of the duplication in a certain lineage, a cell autonomous marker must also be covered by the duplication. Presence or absence of the duplication in a particular lineage is determined by the cell autonomous marker, and then the phenotype with respect to the gene of interest is assessed.

Suppressor mutations have been identified in *C. elegans* that encode amber suppressor tRNAs and promote readthrough of amber (UAG) terminator codons of many genes (42). Mutations of five different genes provide a battery of amber suppressors with varying suppressor strength (23, 40, 41). These suppressors serve as important genetic tools for identification of amber alleles.

Another genetic tool that has improved our ability to manipulate the *C. elegans* genome is the transposable element, Tc*1* (12, 32). Mutations caused by insertion of Tc*1* have already been used successfully for cloning genes by transposon tagging (14), and may prove useful in genetic transformation of *C. elegans*. (See Chapter 10 for a more detailed discussion of Tc*1*.)

Genetic Analysis of Developmental Control Genes

A genetic approach to the analysis of developmental control genes involves their identification by mutation and the genetic characterization of mutations in an attempt to elucidate their wild-type function. For this discussion, a developmental control gene is defined as one that, when mutant, results in the transformation of the fate of a cell, lineage, tissue, or organism to another fate that normally is found in a different position, at a different time, or in the opposite sex. Such fate transformations result in "normal," albeit inappropriate, development. This definition is meant to exclude genes that simply affect a given developmental event; the loss of such genes would be expected to result in developmental arrest rather than developmental transformation.

Tables 2 and 3 provide a selected list of developmental control genes in *C. elegans*. Table 2 lists the seven known genes that regulate the sexual phenotype in multiple tissues of the animal, the so-called global sex determination genes. Table 3 introduces representative genes of other developmental decisions that currently are under analysis. The global sex determination genes will be used to exemplify the various steps of genetic analysis discussed below. The process of dosage compensation has been relegated to Table 3. A discussion of recent results suggesting that sex determination and dosage compensation may be controlled together in *C. elegans* are beyond the scope of this chapter.

Identification of Developmental Control Genes

Two complementary strategies can be used to identify developmental control genes. First, screens can be done for mutants that affect a given developmental process. Then, among the mutants isolated, those with fate transformations can be identified. Second, extragenic suppressors of mutations in a control gene can be isolated. Such suppressors may identify other control genes in the same developmental process. The isolation of suppressor mutations is particularly useful for the identification of mutants with an unexpected phenotype. For example, dominant suppressors can identify redundant genes that have no phenotype when deleted.

The identification of genes that control the sexual phenotype of *C. elegans* has relied on both mutant screens and genetic selections. The first sex determination mutants were isolated in a screen for mutations that transform XX animals, normally hermaphrodite, into males (25). This screen revealed three genes, *tra-1*, *tra-2* and *tra-3* (Table 2). Each of the *tra* genes is necessary for female development (a hermaphrodite is essentially a female that makes some sperm and then oocytes). In the absence of any one of the *tra* genes, XX animals develop along the male pathway. In a continued effort to find genes that direct the decision between hermaphrodite and male development, Hodgkin isolated mutations that transform XO animals into her-

TABLE 2 Properties of global sex determination genes of *C. elegans*[a]

Gene/allele type[b]		Mutant phenotype[c] XX	Mutant phenotype[c] XO	Probably wild-type function
Wild-type	Germ line	Sperm, then oocytes (self-fertile)	Sperm	
	Soma	Female	Male	
fem-1(lf) or *fem-2(lf)* or *fem-3(lf)*	Germ line	Oocytes	Oocytes	XX: Needed for spermatogenesis. No essential function for female somatic development XO: Needed for spermatogenesis. Needed for male somatic development
	Soma	Female	Female[d]	
fem-3(gf)	Germ line	Sperm	Sperm	
	Soma	Female	Male	
tra-1(lf)[e]	Germ line	Sperm, then oocytes. (extent of oogenesis is variable)	Sperm, then oocytes	XX: Function in germ line unclear. Needed for female somatic development XO: Needed for continued spermatogenesis; no essential function for male nongonadal somatic development
	Soma	Male (gonad often abnormal)	Male (gonad often abnormal)	
tra-1(gf)	Germ line	Oocytes	Oocytes	
	Soma	Female	Female	
tra-2(lf)	Germ line	Sperm	Sperm	XX: Needed for oogenesis. Needed for female somatic development XO: No essential function for spermatogenesis or male somatic development

	Soma	Incomplete male (tail incomplete, no mating behavior)	Male	
tra-2(gf)	Germ line	Oocytes	Sperm[f]	
	Soma	Female	Male[f]	
tra-3(lf)	Germ line	Sperm, then oocytes (extent of oogenesis variable)	Sperm	XX: Function in germ line unclear. Needed for female somatic development XO: No essential function for spermatogenesis or male somatic development
	Soma	Incomplete male (tail incomplete, no mating behavior, yolk synthesis)	Male	
her-1(lf)	Germ line	Sperm, then oocytes (self-fertile)	Sperm, then oocytes (self-fertile)	XX: No essential function for germ line or for female somatic development XO: Needed for continued spermatogenesis. Needed for male somatic development
	Soma	Female	Female	
her-1(gf)	Germ line	Sperm, then oocytes (self-fertile)	Sperm	
	Soma	Masculinized (variable and incomplete male tail, egg laying defective)	Male	

[a]See text for references.
[b]lf, loss-of-function allele (description is for putative null alleles); gf, gain-of-function allele.
[c]All phenotypes are of homozygotes
[d]We consider the soma of an XX hermaphrodite to be female; see text.
[e]Null phenotype of *tra-1* in germ line is not known; see text.
[f]Some *tra-2(gf)* alleles show oogenesis and yolk synthesis in old adult XO males.

TABLE 3 Some other developmental control genes in *C. elegans*[a]

Gene	Number of Alleles	Developmental Process Affected	Reference
ced-3	4	Programmed cell death	16, 26
daf-2	13	Entry into dauer stage of life cycle	34
dpy-21	3	Dosage compensation	22
glp-1	5	Maintenance of stem cell population in germ-line tissue	4
lin-12	10 (gf), 32 (lf)	Specification of cell fate	14, 15
lin-14	2 (gf), 19 (lf)	Progression through life cycle	3
unc-86	25	Neural cell lineages	9

[a]The genes listed here represent each of the major developmental decisions under analysis, other than sex determination.

maphrodites (19). From this search, a single gene, *her-1,* was discovered. The *her-1* gene is necessary for male development; in its absence, XO animals develop as hermaphrodites.

The *fem* genes were not discovered in either of the screens discussed above. Mutations of either *fem-1* or *fem-2* cause both XX and XO animals to develop as females (spermless hermaphrodites). Thus, the *fem* genes are required for male development (Table 2); in their absence, no male development occurs in either XX or XO animals. Mutations with this unexpected phenotype were isolated in broad searches conducted for mutants affecting fertility (28, 33). However, once the Fem phenotype was known, Hodgkin devised a clever selection for *fem* mutants, based on epistatic interactions among the sex determination genes, and was able to isolate multiple alleles of *fem-1* (11) and *fem-2* (24) and to discover a third gene required for male development, *fem-3* (24).

Thus, by a combination of screens directly for sex determination mutants, screens for fertility mutants, and selections for mutants that have a specific epistatic interaction with known sex determination mutants, seven genes —*tra-1, tra-2, tra-3, fem-1, fem-2, fem-3,* and *her-1*—have been identified that determine the sexual phenotype of the worm. These seven genes are the global sex determination genes.

Isolation of Multiple Alleles of a Gene of Interest

The isolation of multiple mutant alleles of a gene provides essential tools for its further characterization and manipulation. Two basic types of allele can be isolated. Loss-of-function alleles result from a lowered activity of a gene or its product. Such alleles are usually recessive, and are identified as loss-of-function by a comparison of their phenotype with that of a known chromosomal deficiency for the locus. Loss-of-function mutations can be dominant if the gene's function is sensitive to the dose of gene product made. Such loci are called haplo-insufficient, because one wild-type allele cannot satisfactorily complete the function. In contrast, gain-of-function alleles may result from increased activity, inappropriate activity, or interference with the activity of the wild-type gene (or its product). Such alleles are usually dominant, and are identified by reversion to a loss-of-function allele of the same locus. Both loss-of-function and gain-of-function alleles provide important clues about the function of the wild-type gene.

LOSS-OF-FUNCTION ALLELES

New loss-of-function (lf) alleles of a known genetic locus can be isolated by several methods. One method is to screen the F2 progeny of mutagenized parents for animals with the desired mutant phenotype. (The F3 must be screened if there is a maternal rescue effect; see Chapter 9.) This is a simple and straightforward method, but it is tedious and suffers from the fact that a search for animals with a certain mutant phenotype may yield a biased set of mutations.

A second method is to screen for mutations that fail to complement an existing mutation. Such a "noncomplementation" screen is quick—F1 rather than F2 progeny of mutagenized parents are examined—and it assumes less about the mutant phenotype of the new allele than does the first screen described—for example, an allele may be viable in *trans* to a weaker allele of the same locus, but lethal as a homozygote. It should be noted, however, that only self- or cross-fertile mutants can be isolated in the F1 by this method.

A third method, intragenic reversion of a gain-of-function allele, has the advantages of the noncomplementation screen. In addition, since animals are screened for loss of a dominant phenotype, this method can be used in a heterozygote to isolate mutations that are homozygous nonviable or sterile. The only disadvantage of this method is that it yields double mutations—the original gain-of-function mutation closely linked to the newly induced loss-of-function mutation.

Loss-of-function alleles have varied uses. Most important, perhaps, is the insight such alleles provide into the wild-type function of the gene (e.g., Table 2). As discussed in the next section, the phenotype of a null allele corresponds to the effect of complete absence of the gene product; with this knowledge, the normal function can often be deduced. The existence of an amber allele shows that a gene encodes a protein. In addition, the phenotype of an amber allele often sheds light on the null phenotype. The range of mutant phenotypes provides an index for complexity of gene function (compare *fem-1* and *tra-1*, Table 2 and discussed in the section on null phenotypes). Alleles caused by deletion, insertion, or rearrangement of DNA at a locus are invaluable for molecular analyses of the cloned gene.

Maternal effect tests (see Chapter 9) usually rely on mutant alleles that lack gene function. For example, the maternal contribution of a wild-type allele is assessed in a homozygous mutant embryo derived from a heterozygous hermaphrodite. Since the embryo is assumed to lack the gene and its product, any rescue of the normal mutant phenotype must be from the maternal wild-type gene. Such tests can be done in several permutations, and results can lead to an idea about when and where the gene is expressed during development.

Loss-of-function temperature-sensitive alleles can be used to learn the time of development at which the wild-type gene is required (either its synthesis or function) (see Chapter 9 for a discussion of temperature-sensitive periods). In addition, temperature-sensitive alleles are particularly useful as tools in the case of genes that mutate to a sterile or lethal phenotype, since they can be grown as homozygotes at the permissive temperature.

GAIN-OF-FUNCTION ALLELES

The isolation of gain-of-function (*gf*) alleles by brute force screening is usually impractical due to their lower mutation frequency. [After ethyl methane sulfonate (EMS) mutagenesis, the mutation frequency for a loss-of-function allele of an average-sized gene in *C. elegans* is 5×10^{-4}/haploid genome (7), whereas the frequency for gain-of-function alleles occurs at a much lower frequency, e.g., 5×10^{-6}/haploid genome for *fem-3*(5).] However, several gain-of-function alleles have been isolated fortuitously in searches for mutants of a particular phenotype [e.g., *tra-2(gf)* (10)]. A more direct method is by their selection as dominant suppressors, e.g., *fem-3* (5).

Gain-of-function alleles have several uses. First, the mutant phenotype of gain-of-function alleles can support the identification of that gene as a regulatory gene. Many switch genes have gain-of-function and loss-of-function alleles that confer opposite mutant phenotypes (20, 35). By this criterion, all the genes listed in Tables 2 and 3 for which gain-of-function alleles have been isolated appear to be regulatory in nature.

A second use, already mentioned above, is to isolate new loss-of-function alleles of the same locus by intragenic reversion. A special use for such a reversion has been the isolation of alleles caused by insertion of the transposable element, Tc*1*. Such alleles can be used to clone the gene by transposon tagging. This approach has been successful in the cloning of *lin-12* (14) and *fem-3* (Rosenquist and Kimble, unpublished data).

The Null Phenotype

The null phenotype of a gene is the phenotype effected by complete absence of a functional gene product in the developing animal.The importance of this phenotype is that its basis is understood in molecular terms: The gene product(s) is absent. The function of the wild-type gene product can be inferred from the phenotype of animals that lack it.

The strongest genetic criterion for identification of a null allele is that it behave in all genetic tests like a deficiency of the locus. Other supporting lines of evidence include (a) the phenotype of an amber allele, (b) non-complementation with all other alleles of the locus, and (c) a strong mutant phenotype that is not temperature sensitive. Although none of these criteria alone is sufficient for identification of a null allele, if several are met, and none is transgressed, argument for nullity is reasonable.

For most genes, analysis of the null phenotype is fairly straightforward. For example, Doniach and Hodgkin isolated 21 recessive alleles of *fem-1* (11). Seven of the new alleles were not temperature sensitive and caused complete transformation of XX and XO animals into females; one of these was an amber. Fourteen of the new alleles were temperature sensitive and caused only incomplete feminization of XO animals. Doniach and Hodgkin suggested that the seven strongest alleles are null alleles (11). Isolation of a deficiency for *fem-1* would considerably strengthen their argument. Yet, the evidence for the null phenotype of *fem-1* is quite good, and is stronger than that used for many genes. The null phenotype of *fem-1* indicates that the wild-type *fem-1* function is to promote *all* aspects of male development.

For a few genes, identification of the null phenotype is more difficult. An example is *tra-1*. Numerous loss-of-function alleles of *tra-1* have been isolated (21, 24a, 25): Schedl and Kimble, unpublished data). These alleles cannot be ranked in a simple allelic series according to the strength of their effect on the sexual phenotype. Instead, they must be grouped into classes that differ in their effects on the sexual phenotype of various tissues. Alleles of one class masculinize the nongonadal soma of XX animals more than the gonad (somatic gonad plus germ line). Although XX males of this class can be cross-fertile with completely male nongonadal soma, fully male gonads have never been observed. Alleles of a second class masculinize the somatic gonad more than the nongonadal soma, though again, the gonad is not completely transformed.

The null phenotype of *tra-1* in the nongonadal somatic tissues appears to be complete transformation into a male (21). This null phenotype is based on the phenotype of an amber, the phenotype of various alleles over a deletion, and the strongest mutant phenotype. However, in the gonad, the null phenotype remains unclear. One amber allele of *tra-1*, *e1781*, does not transform the germ line of XX animals—oocytes can be observed in a male somatic gonad, and this allele does not behave like a deficiency in combination with other mutants (Schedl, unpublished data). Furthermore, for several loss-of-function alleles that affect gonadal differentiation in distinct ways, the gonadal phenotype of a hemizygous (allele/Df) animal is essentially identical to that of its respective homozygote (Schedl, unpublished data). Thus, by standard criteria (phenotype of an amber, phenotype of an allele over a deficiency, strongest phenotype), none of the alleles of *tra-1* appears to be null in the gonad. A plausible explanation of this complexity is that *tra-1* produces more than one product, and therefore will have more than one null phenotype.

A Pathway of Control Can Be Deduced from Double Mutants

The phenotype of strains that harbor mutations in more than one gene can be used to deduce the functional relationship between those genes and to refine ideas about their wild-type function. Specifically, for genes that function in a dependent pathway, double-mutant phenotypes often suggest a sequence of gene function. The rationale used to order the action of enzymes in a biosynthetic pathway or to order the assembly of subunits into a macromolecular structure has often been described. Here, we discuss the use of double-mutant phenotypes to understand a pathway of developmental regulation. Again, the sex determination genes are used to exemplify the basic approach.

Extensive experiments have been done to analyze the functional relationships among the sex determination genes and to propose and test a regulatory pathway of gene control (11, 19, 24). Many double-mutant strains have been constructed that harbor two mutants with distinct phenotypes (e.g., Tra and Fem) for examination of the double-mutant phenotype. If only one of the two mutant phenotypes is observed, that mutant is said to be epistatic to the other.

Figure 2 shows a simplified version of a pathway proposed by J. Hodgkin (24) for control of the sexual phenotype of the somatic tissues. The proposed pathway consists of a cascade of negative regulators that ultimately controls the state of *tra-1*. Gene activity is indicated as ON or OFF; this is a genetic formalism and is not meant to imply a molecular mechanism of regulation. Two steps in the pathway are mediated by more than one gene (*tra-2* and *tra-3* at one step, the three *fem* genes at another step). More sophisticated methods, for example, reciprocal shift experiments, are required to order genes with similar loss-of-function phenotypes. The temperature-sensitive periods of the five sex determination genes for which temperature-sensitive alleles have been isolated concur with this suggested order (20).

The pathway for regulation of the somatic sexual phenotype diagrammed in Figure 2 was deduced from epistasis experiments in which a clear rank order could be seen among the sex determination mutants. The somatic mutant phenotype of *tra-1* is epistatic to all others, suggesting that the state of *tra-1* dictates the sexual phenotype of the soma. The hierarchy of epistasis is: *tra-1* > *fem-1*, *fem-2*, *fem-3* > *tra-2*, *tra-3* > *her-1*. Several points can be made from these results. First, the genes alternate in the sequence between those

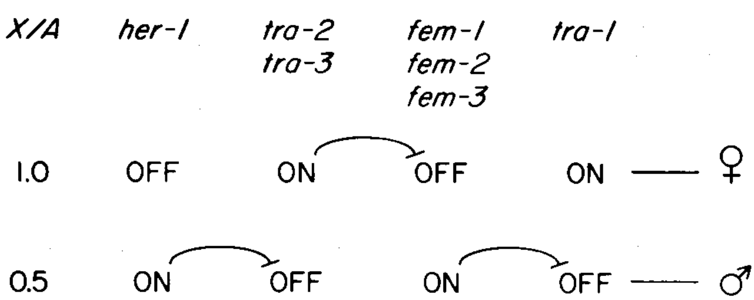

Figure 2 Diagram of the genetic pathway of control over the sexual phenotype of the soma of *C. elegans*. See text for explanation.

normally required for male development (*her-1* and the *fem* genes) and those required for female development (the *tra* genes). This result would not be expected for genes that encode enzymes of a biosynthetic pathway. Nor would it be expected for genes that encode components of a macromolecular structure. Second, only one gene, *tra-1* is essential for female somatic development; without *tra-1*, male somatic development always ensues.In contrast, the other genes, though normally required for development of a given sex, are not essential to that development. For example, on the basis of its null phenotype, *tra-2* is thought to be needed for female development. However, an animal homozygous for both *fem-1* and *tra-2* is female. Thus, in the absence of *fem-1*, *tra-2* is no longer required for female development. It follows that the function of *tra-2* in female development is negative regulation of *fem* activity.

It must be stressed that null mutations (or putative nulls) were used for the experiments described above. The interpretation of an epistasis experiment is based on the assumption that a gene (or its product) is absent. This is an invalid assumption in the case of weak alleles, and in fact, weak alleles can give opposite results in such tests. Epistatic relationships using gain-of-function alleles are difficult to interpret. For example, *fem-3(gf)* causes the XX germ line to make only sperm (5), whereas *fem-1*(null) causes it to make only oocytes (11). The *fem-3(gf) fem-1*(null) double mutant makes only oocytes (5). One can conclude from this experiment that the *fem-1* product is required for spermatogenesis. However, an order of gene function cannot be deduced. The *fem-1* gene may encode an obligate positive regulator of *fem-3*, or *fem-3* may positively regulate *fem-1*.

Genetics of the Sperm/Oocyte Switch in *C. elegans*

Why Study Sex Determination in the Germ Line?

Although the global sex determination genes (Table 2) function in both somatic and germ-line tissues, their effects show some clear differences upon examination of individual tissues. These differences suggest that an understanding of the genetic control of sex determination will require analysis of individual tissues. In our laboratory, we have chosen to analyze sex determination in a single tissue—the germ line. The germ line provides several advantages for study of sex determination. Sex determination in the germ line involves a control over cell type—sperm versus oocyte differentiation—rather than control over production of complex sex-specific structures. Furthermore, genetic selections are available for germ-line sex determination mutants (5, and see below) and, once regulatory molecules have been obtained, the syncytial germ line can be microinjected for bioassays (30).

A hermaphrodite produces about 150 sperm in each of her two ovotestes, and then makes oocytes. A male produces only sperm Thus, sex determination in the *C. elegans* germ line not only involves a decision between spermatogenesis and oogenesis in one organism or another, but also requires regulation of that decision in a single organism to achieve a switch from one cell type to another. The sex of the somatic gonad does not seem to be crucial to the type of gametogenesis that occurs within it. The wild-type female (hermaphrodite) somatic gonad accommodates both sperm and oocytes. Certain mutants produce only oocytes and others produce only sperm in an otherwise normal XX female soma. In addition, in various single or double mutants, cells that, by morphological criteria, resemble oocytes can be produced in a male somatic gonad.

The requirements for control of sex determination in the germ line are different from those for its control in formation of sex-specific somatic structures. Sex determination in the germ line is an ongoing process. Germ cells are continuously generated throughout larval and adult life from a mitotic stem cell population (31). These immature germ cells are uncommitted, so that a choice between spermatogenesis and oogenesis must continually be made (5). The commitment to produce only one type of gamete may require a function to maintain that particular pathway. In contrast, somatic sex determination generally involves a decision between two sex-specific lineages—an irreversible commitment made early which is executed during larval development.

Isolation of Germ-Line Sex Determination Mutants

Mutants that specifically affect sex determination in the germ line have been isolated both in mutant screens and by genetic selection. The two phenotypes sought in screens were the Fog phenotype, for *f*eminization *o*f the *g*erm line, and the Mog, phenotype for *m*asculinization *o*f the *g*erm line (Fig. 3).

The idea behind the genetic selections used to isolate germ-line sex determination mutants is shown in Fig. 4. Basically, an XX hermaphrodite is self-sterile if it produces only sperm or only oocytes, but it is self-fertile if it produces sperm and then oocytes. Therefore, mutants that feminize the hermaphrodite germ line so that only oocytes are made (or masculinize it so that only sperm are made) can be used to select for suppressor mutations that reinstate self-fertility.

Mutant alleles of *fem-1* and *fem-2* that make only oocytes have been used to isolate suppressors that masculinize the germ line (5; Barton and Kimble, unpublished data). Among the dominant suppressors of *fem-1(ts)* or *fem-2(ts)* are a group of nine gain-of-function alleles of *fem-3*. These *fem-3(gf)* alleles, when separated from *fem-1(ts)* or *fem-2(ts)*, are temperature sensitive and show a dominant Mog phenotype. They have been identified as alleles of *fem-3* by reversion, mapping, and a cis/trans test. They are deduced to be gain-of-function alleles of *fem-3* by their dominance and by the fact that their effect on the sexual phenotype is opposite that of loss-of-function alleles of *fem-3*.

The gain-of-function alleles of *fem-3* that make only sperm have been used to isolate suppressors that feminize the germ line (Kimble laboratory, unpublished data). Selection of both masculinizing and feminizing suppres-

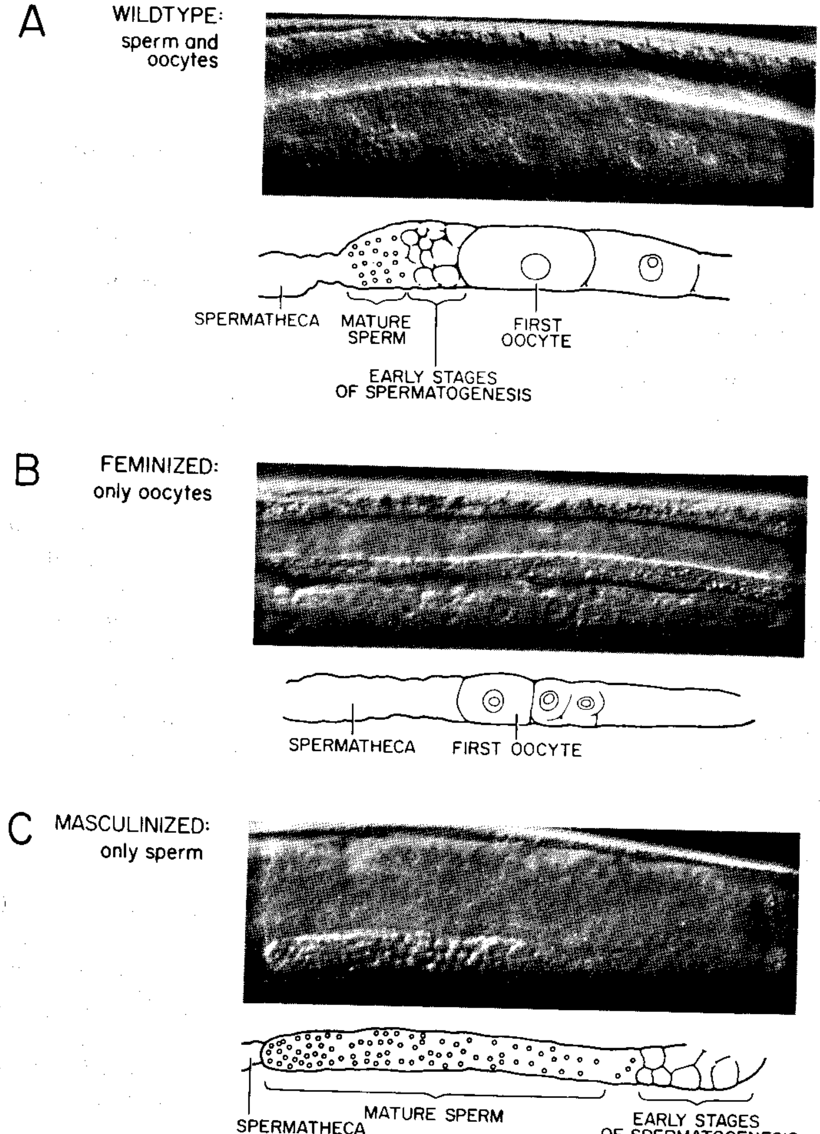

Figure 3 (A) The wild-type hermaphrodite germ line produces first sperm and then oocytes in each ovotestis. (B) The feminized hermaphrodite germ line produces only oocytes. This mutant phenotype is typical of loss-of-function mutations of *fem* and *fog* genes and gain-of-function mutations of *tra-1* and *tra-2*. (C) The masculinized hermaphrodite germ line produces only sperm. This mutant phenotype is typical of loss-of-function mutations of *mog* genes and gain-of-function mutations of *fem-3*.

sors has proved successful for isolation of dominant and/or recessive mutations both in previously known (*fem-1, fem-2, fem-3, tra-1, tra-2)* and novel [*sup(q62), sup(q80), sup(q81), fog-1, fog-2*] loci.

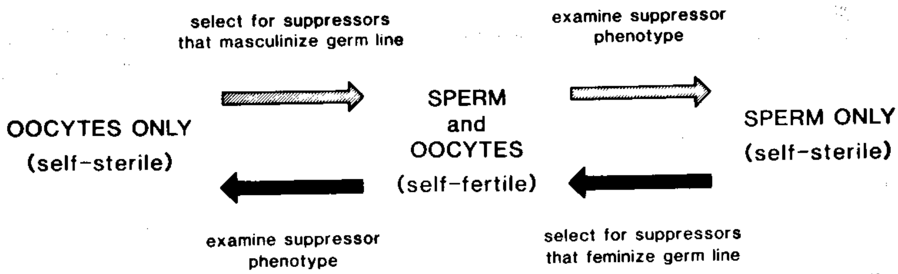

Figure 4 Basic idea behind genetic selections for mutants that affect germ-line sex determination. See text for explanation.

Global Sex Determination Genes with Germ-Line Specific Controls

Gain-of-function alleles of two global sex determination genes, *fem-3* (5) and *tra-2* (10), cause sexual transformation of the hermaphrodite germ line without affecting its soma. Neither affects the XO male sexual phenotype. A *tra-2(gf)* hermaphrodite makes oocytes but no sperm and is therefore female; a *fem-3(gf)* hermaphrodite makes sperm but no oocytes and is therefore Mog (Table 2). Loss-of-function alleles of either *tra-2* or *fem-3* cause sexual transformation of both soma and germ line (Table 2). The germ–line-specific sexual transformation of *fem-3(gf)* and *tra-2(gf)* suggests that each gene, in wild-type animals, is regulated to influence germ-line sex determination separately from somatic sex determination.

The activity of *fem-3* appears to be under at least two controls in wild-type hermaphrodites. The first control restricts its effect to the germ line. In wild-type hermaphrodites, the influence of *fem-3* is limited to the germ line—spermatogenesis, a *fem–3*-dependent male process, occurs without masculinization of the female (hermaphrodite) soma. In *fem-3(gf)* hermaphrodites, as in wild-type hermaphrodites, masculinization is confined to the germ line. The simplest explanation for this tissue restriction is that *fem-3* is produced in the germ line and not in the soma. Alternative possibilities include the activation of *fem-3* late in development, at a time when the somatic sex has been determined; a lower threshold for action of *fem-3* in the germ line compared with the soma; or regulation of another gene (or its product) upon when the activity of *fem-3* depends.

A second control of *fem-3* restricts its activity to production of a small number of sperm. In wild-type hermaphrodites, the activity of *fem-3* is necessary for spermatogenesis but must be curtailed to permit oogenesis. In *fem-3(gf)* hermaphrodites, sperm are made continuously and to vast excess (5). A likely explanation of this defect is that *fem-3(gf)* escapes a negative control that normally is imposed to begin oogenesis. A negative regulation of *fem-3* activity is also implied by the maternal effects observed for *fem-3(lf)* mutants (5, 24). If *fem-3* is produced in oocytes for sex determination in the embryo, it must be inactivated or the germ line will become masculinized. Such inactivation must be reversible so that *fem-3* can function during embryogenesis. Thus, negative regulation of *fem-3* activity is suggested both to permit the onset of oogenesis and to permit packaging of *fem-3* into oocytes.

Germ–Line-Specific Sex Determination Genes

Five genes that affect the sexual phenotype of the germ line specifically have been discovered by a combination of mutant screens and genetic selection. These include three *fog* genes and two *mog* genes. Mutations in *fog* or *mog* genes result in a transformation of cell fate. Germ cells that would normally undergo spermatogenesis in wild-type animals undergo oogenesis in *fog* mutants. Similarly, germ cells that would normally undergo oogenesis in wild-type animals undergo spermatogenesis in *mog* mutants. In XX animals, *fog* and *fem* mutants are identical. However, in XO animals, *fog* mutants are never feminized in the soma, whereas *fem* mutants are feminized in both soma and germ line. Therefore, the Fog phenotype is distinct from the Fem phenotype.

Mutation of either two *fog* genes, *fog-1* (25a; Barton and Kimble, unpublished data) or *fog-3* (Schedl, unpublished data), causes feminization of the germ line of both XX hermaphrodites and XO males. Mutation of one *fog* gene, *fog-2*, eliminates spermatogenesis specifically in hermaphrodites. XX animals homozygous for *fog-2* are female, whereas XO animals are male (Schedl, unpublished data). (Note that by mutation of this single gene, *C. elegans* can be changed from an hermaphroditic to a male/female strain.) Thus, the wild-type *fog-2* gene is required for the burst of spermatogenesis typical of the hermaphrodite germ line before the onset of oogenesis. The *fog-2* gene is not required for spermatogenesis per se, since male spermatogenesis is not affected.

To determine how *fog-2* regulates spermatogenesis in hermaphrodites, strains have been constructed with null alleles of *tra-2*. The *tra-2; fog-2* double-mutant phenotype is identical to the *tra-2* null phenotype (Schedl, unpublished data). This suggests that *fog-2* may be a negative regulator of *tra-2*. The gain-of-function phenotype of *tra-2* is identical to the loss-of-function phenotype of *fog-2*: XX animals are female and XO animals, unaffected, are male. If *fog-2* negatively regulates *tra-2*, then *tra-2(gf)* may be defective in regulation by *fog-2*. This possibility predicts that *tra-2(gf)* and *fog-2* should behave identically in genetic tests and that the effects of *tra-2*(gf) and *fog-2* mutations should not be additive. These predictions are currently being tested.

Mutation of either of two *mog* genes results in the production of excess sperm with no signs of oogenesis in the XX hermaphrodite gonad (Schedl and Kimble, unpublished data). The *mog* genes have no effect on the sexual phenotype of XO animals. Unlike *fog* mutants, which make oocytes and therefore are cross-fertile, homozygous *mog* hermaphrodites are self-and cross-sterile. Genetically, the *mog* mutants are therefore more difficult to manipulate and have lagged behind the *fog* mutants in their characterization.

Conclusions and Future Prospects

A genetic analysis of developmental regulation allows the identification of control genes and gives insight into the wild-type function of those genes in development. The only prerequisite for applying this approach is the ability to isolate mutants with a phenotype clearly indicative of altered control. Transformation from an expected fate to an inappropriate, but nevertheless "normal," fate is a hallmark of developmental control genes; loss-of-function and gain-of-function alleles that confer opposite-fate transformations support the identity of a gene as a switch gene. Once genes of interest are identified, genetic experiments can be used to make predictions about the function of genes.

Studies of sex determination have been used in this chapter to exemplify the genetic analysis of developmental control genes in *C. elegans*. Identification of the global sex determination genes may now be complete, and a genetic mechanism by which these genes control the sexual phenotype has been proposed. Many major questions remain. What is the biochemical mechanism by which the sex determination genes regulate each other in the pathway proposed by genetic experiments? What is the mechanism by which the sex determination genes are regulated by the X/A ratio? How are the genes regulated to achieve a tissues-specific effect on sexual phenotype? Given the powerful genetic and molecular tools available for the analysis of these questions, it seems only a matter of time before we have the answers.

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Questions for Discussion with the Editor

1. *Now that the sex determination genes have been identified and a scheme proposed for the way they generate sex phenotypes, the next step appears to be elucidation of the regulatory mechanisms. What are some possible experimental strategies for identifying the actual trans-acting factors (e.g., regulatory proteins) involved in controlling the function of the various genes?*

We plan to pursue both the mechanisms by which the sex determination genes are regulated and the mechanisms by which they regulate the sexual phenotype. The biochemical elucidation of either regulatory mechanism requires that the sex determination genes be cloned. The genetic selections outlined in Fig. 3 can be employed to isolate mutations caused by insertion of Tc*1*, and such insertion mutations can then be used to clone the gene (transposon tagging). The *fem* genes are of particular interest, because genetic experiments indicate that their products negatively regulate *tra-1* and positively regulate spermatogenesis. So far, *fem-3* and its putative negative regulator, *tra-2*, have been cloned (Rosenquist, Okkema, and Kimble, unpublished data). With a cloned gene in hand, two questions can be asked. First, does the amino acid sequence of the gene provide any clues about its biochemical mode of action (a DNA-binding protein, a kinase, etc.)? Second, is the gene regulated at the level of transcription? Assays of transcript levels during development in wild-type animals and in DNA transformants carrying specific deletions of the gene sequence (constructed *in vitro*) will provide necessary information about transcriptional control. If *fem-3* transcription appears to be regulated, and if the *tra-2* sequence has the hallmarks of a DNA-binding protein, then *tra-2* is a reasonable candidate for a regulatory protein that acts to repress the *fem-3* gene. A biochemical test of this possibility requires purification of the *tra-2* protein and binding studies to the *fem-3* gene. A similar strategy can be envisaged for each of the sex determination genes and its putative regulators.

2. *The developmental "style" of* C. elegans*—rigid, cell-lineage-based format—contrasts markedly with the so-called "regulatory pattern" of many other animals (e.g., amphibia, mammals, etc.). What fundamental advantage to the species is provided by the* C. elegans *style?*

Let us first comment on the statement that the *C. elegans* style of development contrasts markedly with that of other animals. First, *C. elegans* is capable of both "styles" of development. The rigid cell-lineage-based format is typical of somatic structures, but the "regulatory pattern" is found in development of the germ line. Second, development by a rigid cell-lineage-based format is observed in animals of many phyla (e.g., mollusks, annelids, arthropods, and tunicates). The evidence that higher vertebrates (e.g., amphibia, mammals) develop according to a regulatory pattern does not exclude the possibility that some development in later stages of embryogenesis may occur by a fixed cell lineage.

One can only speculate about the advantages a rigid cell lineage might have for a species. One idea is that rigid cell lineages may require a minimum of information for generation of complex multicellular structures. The lineage pattern that generates the *C. elegans* soma is composed of many sublineages, and a sublineage may result from modification of a simpler lineage. Several such modifications (e.g., changing the number of cell divisions in a particular branch of the lineage) have been deduced from laser ablation experiments and from descriptions of cell lineage mutants. Development by strict cell lineages may be a mechanism that is more primitive than the use of complex gradients for establishment of complex patterns. (Note, however, that a simple gradient mechanism seems to be used to establish two different populations of cells in the germ line of *C. elegans*.) Another possible advantage of a fixed lineage is that it might be a mechanism that makes embryogenesis relatively independent of environmental fluctuations, for example, in temperature.