

Genetic Control of Sex Differences in *C. elegans* Neurobiology and Behavior

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ABSTRACT

As a well-characterized, genetically tractable animal, the nematode *Caenorhabditis elegans* is an ideal model to explore the connections between genes and the sexual regulation of the nervous system and behavior. The two sexes of *C. elegans*, males and hermaphrodites, have precisely defined differences in neuroanatomy: superimposed onto a “core” nervous system of exactly 294 neurons, hermaphrodites

and males have 8 and 89 sex-specific neurons, respectively. These sex-specific neurons are essential for cognate sex-specific behaviors, including hermaphrodite egg-laying and male mating. In addition, regulated sex differences in the core nervous system itself may provide additional, though poorly understood, controls on behavior. These differences in the nervous system and behavior, like all known sex differences in the *C. elegans* soma, are controlled by the master regulator of *C. elegans* sex determination, *tra-1*. Downstream of *tra-1* lie specific effectors of sex determination, including genes controlling sex-specific cell death and a family of regulators, the DM-domain genes, related to *Drosophila doublesex* and the vertebrate DMRT genes. There is no central (i.e., gonadal) regulator of sexual phenotype in the *C. elegans* nervous system; instead, *tra-1* acts cell-autonomously in nearly all sexually dimorphic somatic cells. However, recent results suggest that the status of the gonad can be communicated to the nervous system to modulate sex-specific behaviors. Continuing research into the genetic control of neural sex differences in *C. elegans* is likely to yield insight into conserved mechanisms of cell-autonomous cross talk between cell fate patterning and sexual differentiation pathways. © 2007, Elsevier Inc.

I. INTRODUCTION

A. *Caenorhabditis elegans* as a model for sex differences in nervous system structure and function

Coordinating the development and function of the multiple attributes that distinguish the sexes of a given species is a significant challenge to the genetic programs that govern metazoan development. Organisms must make a decision, usually early in development, about which sex to become; the outcome of this decision then instructs the development of multiple sex-specific structures and guides the sex-specific modification of common structures. This process is particularly complex in the nervous system, where sexual information must interface with the intricate genetic mechanisms that pattern the nervous system and regulate the properties of neural circuits.

In vertebrates, the sexual differentiation of the nervous system and the control of sex-specific behavior have been thought to rely completely on the action of gonadal steroid hormones (reviewed by [Morris et al., 2004](#)). However, a series of recent findings has demonstrated that this model is oversimplified: some sex differences are cell-intrinsic, depending on sexual karyotype in the nervous system itself rather than on the influence of gonadal cues (reviewed by [Arnold, 2004](#) and herein). However, in neither the hormonal nor the cell-intrinsic pathways are the links between sex determination, neural differentiation, and

behavior well understood. It is in this regard that invertebrate model systems, such as the nematode *C. elegans* and the fruit fly *Drosophila*, can make their most significant contributions, bringing about fundamental new insights into the genetic mechanisms that regulate complex biological processes like sex determination and differentiation.

Since Sydney Brenner's "taming" of the *C. elegans* as a genetic model system in the 1960s, there has been great interest in exploiting the unique experimental tractability of this system to dissect the genetic underpinnings of neural development and behavior (Ankeny, 2001; Brenner, 1974; Brown, 2003). Indeed, the most common class of mutants isolated in Brenner's original screens were the so-called *unc* (*uncoordinated*) mutants that exhibited defects in locomotion, the most obvious *C. elegans* behavior (Brenner, 1974). Subsequent studies of these mutants have led to a series of fundamental insights into neurobiology: the identification of the *unc-6*/Netrin family of axon guidance cues and their receptors, *unc-40*/DCC and *unc-5* (Chan *et al.*, 1996; Hamelin *et al.*, 1993; Hedgecock *et al.*, 1990; Ishii *et al.*, 1992); characterization of *unc-86*, a founding member of the POU-HD family of transcriptional regulators of neural fate (Baumeister *et al.*, 1996; Finney and Ruvkun, 1990; Finney *et al.*, 1988); identification of *unc-13*, a conserved regulator of synaptic vesicle fusion (Lackner *et al.*, 1999; Maruyama and Brenner, 1991; Richmond *et al.*, 1999); and many others.

At the same time, sex determination and sexual differentiation were also the subject of intensive early genetic analysis in *C. elegans* (Doniach and Hodgkin, 1984; Hodgkin and Brenner, 1977; Kimble *et al.*, 1984; Klass *et al.*, 1976; Madl and Herman, 1979; Nelson *et al.*, 1978). Beginning with the isolation and study of many mutants with completely or partially sex-reversed phenotypes, including the *tra* (transformer), *her* (hermaphroditization), and *fem* (feminization) mutants, a robust genetic pathway has been elucidated that links differential chromosome content (see below) to dosage compensation and sexual fate in the germ line and soma (for reviews see Ellis and Schedl, 2006; Meyer, 2005; Zarkower, 2006). Interestingly, several genes with sequence similarity to *D. doublesex* (the DM-domain genes *mab-3*, *mab-23*, and *dmd-3*) have been found to be terminal effectors of the *C. elegans* sex determination pathway, indicating that some components of sex determination are likely to be shared across metazoa (Lints and Emmons, 2002; Raymond *et al.*, 1998; Shen and Hodgkin, 1988; Yi *et al.*, 2000; D. A. Mason and D. S. P., unpublished data). As we describe here, understanding the relationships between these two complex regulatory networks—patterning and regulation of neural circuitry on one hand, sex determination and differentiation on the other—is the focus of active current investigation that is likely to lead to critical insights into the mechanisms that control sex-specificity in neural development and behavior throughout the animal kingdom.

II. MAIN TEXT

A. Sex determination and differentiation in *C. elegans*

C. elegans is an androdioecious species. As shown in Fig. 1.1A, its two sexes are hermaphrodite and male. The hermaphrodite is essentially a modified female that produces and stores some sperm that can be used to self-fertilize its own oocytes. Animals of this sex lack male genital structures; thus, *C. elegans* hermaphrodites are unable to cross-fertilize each other. In contrast, the male produces only sperm, and males can reproduce only by cross-fertilizing a hermaphrodite. Hermaphroditism is a recent evolutionary innovation in *C. elegans*, as its nearest phylogenetic neighbors are gonochoristic (i.e., male-female) species (Kiontke *et al.*, 2004), indicating that the hermaphrodite is generated from minor modification of an ancestral female developmental program. In self-fertilizing hermaphrodite populations, males arise very infrequently (<0.3%). Despite the relative rarity of the male, its developmental program is maintained in the genome, indicating that males may be required to provide outcrossing. However, the rate of outcrossing in wild populations seems to be very low, and over evolutionary time the role of males in the species may be dwindling (Barriere and Felix, 2005; Chasnov and Chow, 2002; Stewart and Phillips, 2002). Owing to its ability to self-fertilize, the hermaphrodite offers experimental advantages that have led to a much more thorough characterization of its neuroanatomy and development; in several regards, the biology of the *C. elegans* male is much less well understood than that of the hermaphrodite.

As in most animals, sex determination in *C. elegans* is chromosomal: embryos with two sex chromosomes (XX) develop as hermaphrodites; those with only one (X0) become males (Brenner, 1974; Nigon and Dougherty, 1949). There is no heteromorphic (Y) sex chromosome in *C. elegans*. Interestingly, the number of X chromosomes per se is not the primary sex-determining cue; rather, the sex chromosome to autosome ratio X:A is assessed according to the relative copy numbers of specific autosomal and sex chromosome “signal element” genes (Carmi *et al.*, 1998; Madl and Herman, 1979; Powell *et al.*, 2005). Downstream of these signal element genes lies a complex regulatory hierarchy that independently controls both dosage compensation (the reduction of gene expression from each hermaphrodite X chromosome by half) (reviewed by Meyer, 2005) and sexual differentiation (Fig. 1.1B). This latter pathway relies on successive repressive interactions to control the activity of the gene *tra-1*, the master sexual regulator in *C. elegans*. XX animals carrying a null mutation in *tra-1* develop as somatic males, whereas X0 animals are essentially unaffected, indicating that *tra-1* functions in hermaphrodites to promote female and/or repress male fates (Hodgkin, 1987; Hodgkin and Brenner, 1977). *tra-1* also has a role in the development of the gonad

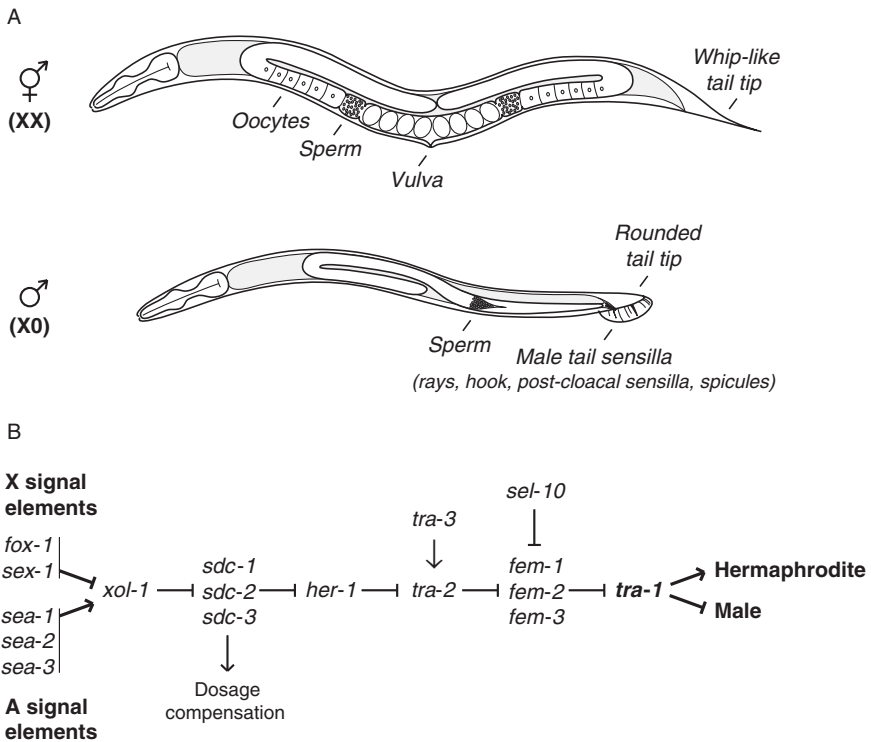


Figure 1.1. Sex determination and differentiation in *C. elegans*. (A) The primary sexual dimorphisms in *C. elegans*. Adult hermaphrodites (above) are XX and have a tapered, whiplike tail, a vulva, and a bilobed gonad that produces both oocytes and sperm. Adult males (below) are X0, slightly smaller in size, and have a rounded tail tip, several classes of tail sensilla, and a single-armed gonad that makes sperm. Modified from Zarkower (2006). (B) The somatic sex determination pathway. The X:A ratio is read by signal-element genes on the X chromosomes and autosomes; these converge onto the regulator *xol-1*, which is active only in X0 animals (Rhind *et al.*, 1995). Downstream of the *sdc* genes, dosage compensation (not shown) reduces gene expression from the X chromosome by half and is controlled independently from the differentiation of somatic characteristics (Meyer, 2005). Essentially all somatic characteristics are controlled through a series of repressive interactions that regulate the master sex-determining gene *tra-1*. In XX animals, *tra-1* is ON, repressing male fates and perhaps promoting hermaphrodite fates. In X0 animals, *tra-1* is OFF, allowing male development to proceed.

in both sexes; this is thought to be an ancestral function that is separate from the role of *tra-1* in sex determination (Hodgkin, 1987; Mathies *et al.*, 2004). Interestingly, neither *tra-1* nor its upstream regulators have conserved functions in sex determination outside nematodes, consistent with the idea that upstream events in sex determination evolve rapidly (Wilkins, 1995; Zarkower, 2001).

Through alternative splicing, *tra-1* encodes two Zn-finger proteins with sequence similarity to Ci/GLI transcription factors, indicating that a *hedgehog*-like pathway may have been co-opted in the worm to carry out a sex determination function (Zarkower and Hodgkin, 1992). The longer of these forms, called TRA-1A, is thought to be the active form, as the shorter is unable to bind DNA *in vitro* (Zarkower and Hodgkin, 1993). TRA-1A has been shown to act as a transcriptional repressor to block the expression of male-specific genes in XX animals (Conradt and Horvitz, 1999; Yi *et al.*, 2000); TRA-1A may also act to promote hermaphrodite gene expression. Genetic analysis has suggested that the sex-specific regulation of *tra-1* activity comes about posttranslationally (de Bono *et al.*, 1995). Consistent with this, it has been found that sex-specific proteolysis may be critical in generating a hermaphrodite-specific active form of TRA-1A (Schwarzstein and Spence, 2006). The mechanisms by which the *fem* genes, the most downstream genetic regulators of *tra-1*, control sex-specific TRA-1A activity remain an area of active investigation.

Elegant genetic studies have shown that *tra-1* acts cell-autonomously in the specification of nearly all sexually dimorphic cell fates in the *C. elegans* soma (Hunter and Wood, 1990). This stands in stark contrast to vertebrate sex determination mechanisms, in which sex determination events in the early gonad conscript peripheral tissues to adopt sex-specific characteristics through the influence of gonadal steroids (Morris *et al.*, 2004). In *C. elegans*, most sex-specific extragonadal characteristics do not depend on the gonad, and gonadal precursor cells can be completely removed by laser ablation very early in development with essentially no effect on sex-specific somatic development (Kimble, 1981; Klass *et al.*, 1976). Instead of relying on a gonadal cue, the sexual fate of somatic cells in *C. elegans* begins with cell-autonomous establishment of the X:A ratio early in embryonic development (Rhind *et al.*, 1995). A series of repressive interactions reinforces this decision, leading to local non-cell-autonomous activity of the secreted protein HER-1, which acts through the FEM proteins to regulate TRA-1A activity (Hodgkin, 1986; Hunter and Wood, 1992). Thus, the status of TRA-1A activity in any given somatic cell is sufficient to determine whether that cell adopts a male (*tra-1* OFF) or hermaphrodite (*tra-1* ON) fate. The only known exception to this is the hermaphrodite vulva, whose development relies on an inductive signal from the hermaphrodite somatic gonad (Hunter and Wood, 1992; reviewed by Sternberg, 2005).

Therefore, the genes repressed or activated by TRA-1A are thought to wholly account for the somatic characteristics that distinguish hermaphrodites from males, although recent evidence has suggested that there may be some relatively minor *tra-1*-independent functions that are necessary for the full complement of sex-specific differences (Grote and Conradt, 2006; van den Berg *et al.*, 2006). While some genes downstream of *tra-1* are known, the genetic mechanisms that link *tra-1* to sex-specific differentiated characteristics—particularly in the

nervous system—remain incompletely described. As described below, two genes controlled by *tra-1*, *egl-1* and *ceh-30*, regulate the sexual phenotype of the nervous system by controlling sex-specific programmed cell death. The Hox genes *mab-5* and *egl-5* are also regulated at least indirectly by *tra-1* and have important roles in shaping male-specific cell lineages and neural cell fates (Chisholm, 1991; Chow and Emmons, 1994; Costa *et al.*, 1988; Kenyon, 1986). Finally, three related genes, *mab-3*, *mab-23*, and *dmd-3*, control partially overlapping subsets of male-specific development and function in the nervous system and elsewhere, though their relationship to *tra-1* is complex and not fully understood (Lints and Emmons, 2002; Shen and Hodgkin, 1988; D. A. Mason, K. H. Lee, R. M. Miller, and D. S. P., unpublished data). As mentioned above, these genes encode proteins harboring one or more DM (*doublesex/mab-3*) domains, the only known conserved molecular link between effectors of sex determination in metazoans (reviewed in Zarkower, 2001). The importance of these factors in the sexual differentiation of the *C. elegans* nervous system has led to speculation about the potential for the vertebrate relatives of these genes, the DMRT family, to be regulators of sexual dimorphism in the central nervous system (CNS).

B. The *C. elegans* nervous system

The *C. elegans* nervous system simultaneously exhibits a minimalist simplicity and an astonishing complexity. The adult hermaphrodite nervous system comprises exactly 302 neurons, compared to 383 in the adult male. The identity and developmental history of each of these neurons have been completely described and are essentially identical between individuals. Moreover, through the painstaking work of John White and colleagues, the complete neuroanatomy of the adult hermaphrodite, including patterns of synaptic connectivity, has been reconstructed from serial electron micrographs (White *et al.*, 1986). This “wiring diagram” for the *C. elegans* nervous system provides a substrate for understanding the neural control of behavior unrivaled in any system.

The worm nervous system is organized into several major ganglia in the head and tail (Hall *et al.*, 2006; White *et al.*, 1986). Sensory information is received through several classes of ciliated sensory neurons, mostly in the head, that synapse onto integrating interneurons. Most neural processing is carried out in the nerve ring, a circumferential neuropil that surrounds the isthmus of the pharynx. Locomotory behavior is controlled by the activity of a small set of command interneurons that regulate the function of several classes of motor neurons in the ventral nerve cord.

In the laboratory, *C. elegans* exhibits a variety of behaviors, including regulated forward and reverse locomotion, response to touch, temperature, food availability, and a large variety of chemosensory cues. As discussed below, hermaphrodite egg-laying and male mating are the primary sex-specific behaviors in

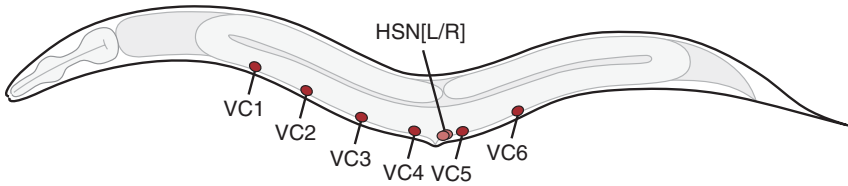
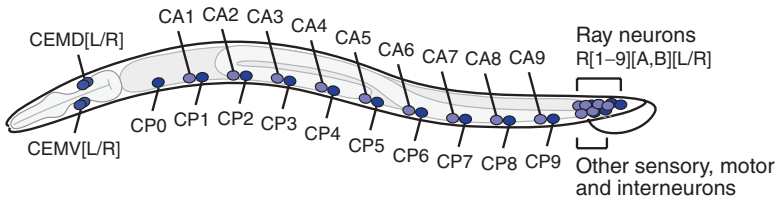
this species. None of these behaviors is essential for laboratory viability, and all have been subjected to genetic analysis. Indeed, it is this tractability across the molecular, cellular, circuit, and systems levels that makes *C. elegans* such an attractive model for the genetic studies of behavior (reviewed by Rankin, 2002; Whittaker and Sternberg, 2004). The worm nervous system has also been shown to be capable of several forms of plasticity, including habituation to mechanical stimuli, adaptation to the presence of chemoattractants, and associative learning of chemosensory cues coupled to food availability and quality (Kuhara and Mori, 2006; Tomioka *et al.*, 2006; Zhang *et al.*, 2005; reviewed by Giles *et al.*, 2006; Tsalik and Hobert, 2003). The study of non-sex-specific behavior in this organism has made many important contributions to understand the mechanisms by which genes control neural development, circuit function, and behavior; more information may be found in the excellent reviews on this topic (Bargmann, 2006; Goodman, 2006; O'Hagan and Chalfie, 2006; Sengupta, 2007; Von Stetina *et al.*, 2006).

C. Sexual dimorphism in the *C. elegans* nervous system

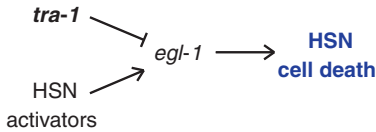
The sex differences exhibited by the *C. elegans* nervous system are of two general forms. First, and more conspicuous, is the presence of sex-specific neurons in both hermaphrodites and males (Fig. 1.2A; Table 1.1). This sex-specific component of the nervous system comprises 8 neurons in hermaphrodites and 89 in males, all of which are thought to function in circuits dedicated to sexually dimorphic behavior. In both sexes, the sex-specific nervous system is overlaid onto the 294 neurons common to both sexes, referred to here as the “core” nervous system. Owing to the experimental accessibility of *C. elegans*, the cellular composition of the sex-specific nervous system of both sexes is precisely known (Sulston and Horvitz, 1977; Sulston *et al.*, 1980, 1983; White *et al.*, 1986). In contrast, a second and much less well-understood dimension of sexual dimorphism in the nervous system is sex differences in the core nervous system itself (Table 1.1). In principle, these differences could encompass alterations in morphology, gene expression, neurophysiology, synaptic connectivity, and synaptic strength in shared neurons.

Interestingly, the existence of these two forms of sexual dimorphism is also characteristic of more complex organisms. As in *C. elegans*, vertebrate species have sex-specific neural structures, such as those that innervate the genitalia. In contrast, sex differences in the *C. elegans* core nervous system may be more analogous to subtler sexual dimorphisms in the vertebrate CNS, such as the difference in size of the hypothalamic medial preoptic area in mammals (Raisman and Field, 1971) and the high-vocal center in songbirds (Nottebohm and Arnold, 1976). Though, unlike *C. elegans* core nervous system differences, these structures likely have sex differences in cell number, in both cases these can be seen as modulations in the properties of neural structures common to both sexes.

A

Hermaphrodite-specific neurons**Male-specific neurons**

B



C

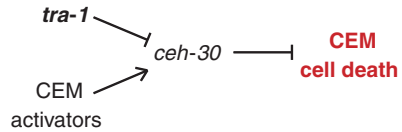


Figure 1.2. Sexual dimorphism in the *C. elegans* nervous system. (A) The adult hermaphrodite (above) has eight sex-specific neurons: six VC neurons in the ventral cord and two HSN motor neurons. These neurons regulate hermaphrodite egg-laying behavior. The adult male (below) has 89 sex-specific neurons. Four of these, the CEMs, are sensory neurons in the head and are thought to have a role in detecting hermaphrodite pheromone cues (see text). The male ventral cord contains the CA and CP motor neurons; both of these are implicated in specific steps of male mating behavior (Loer and Kenyon, 1993; Schindelman *et al.*, 2006). The male tail contains a variety of sensory, motor, and interneurons; the largest class of these is the RnA and RnB ray sensory neurons. (B) Sexual dimorphism in the presence of the HSN is regulated by differential programmed cell death. In XX animals, *tra-1* is active and represses *egl-1* expression in the HSNs. In X0 animals, unknown activators promote *egl-1* expression in the HSNs, leading to cell death (Conradt and Horvitz, 1999). (C) Differential cell death also underlies the sex difference in the CEM neurons. In XX animals, *tra-1* prevents the expression of the survival factor *cheh-30* in the CEM neurons, leading to their death. In X0 animals, *cheh-30* prevents HSN death; whether this occurs through regulation of *egl-1* is unknown (see text for details).

Table 1.1. Sex Differences in *C. elegans* Neuroanatomy

Cells	No.	Description
Hermaphrodite-specific neurons(8)		
VC[1-6]	6	Cholinergic motor neurons
HSN[L,R]	2	Serotonergic motor neurons
Male-specific neurons (89)		
CA[1-7]	7	Ventral cord motor neurons
CA[8,9]	2	Neuron-like cells; lack obvious synapses
CEM[D,V][L,R]	4	Cephalic sensory neurons
CP[0-7]	8	Ventral cord motor neurons; express serotonin
CP[8,9]	2	Tail interneurons
DV[E,F]	2	Tail interneurons
DX[1-4]	4	Tail interneurons
EF[1-4]	4	Tail interneurons
HO[A,B]	2	Hook sensory neurons
PC[A-C][L,R]	6	Postcloacal sensilla sensory motor neurons
PDC	1	Tail interneuron
PGA	1	Tail interneuron
PV[V,Z]	2	Tail motor neuron
PV[X,Y]	2	Tail interneuron
R[1-9][A,B][L,R]	36	Ray sensory neurons
SPC[L,R]	2	Spicule sensory motor neurons
SP[D,V][L,R]	4	Spicule sensory neurons
Sexually dimorphic core neurons		
ADF[L,R]	2	Amphid sensory neuron; expresses <i>srd-1</i> in males only
AIM[L,R]	2	Ring interneuron; expresses <i>srl-54</i> in males only
PDA	1	Motor neuron (hermaphrodite Y, male Y.a); sexually dimorphic connectivity
PDB	1	Motor neuron; sexually dimorphic connectivity
PHC[L,R]	2	Tail sensory neuron (striated rootlet in male); sexually dimorphic connectivity
PVP[L,R]	2	Called PVU and PVS in male; sexually dimorphic connectivity

As such, the genetic mechanisms that impart sex differences to these structures could share common elements, especially given recent evidence that downstream effectors of sexual differentiation may be conserved in animals (reviewed in [Bratus and Slota, 2006](#); [Zarkower, 2001](#)).

1. The hermaphrodite-specific nervous system

The *C. elegans* hermaphrodite-specific nervous system comprises just eight neurons: one pair of hermaphrodite-specific neurons (HSNs) and six VC motor neurons ([Table 1.1](#)). These two neuron classes regulate egg-laying, the primary hermaphrodite-specific behavior (reviewed in [Schafer, 2006](#)). The developmental basis for HSN sex-specificity has been elucidated through a series of elegant genetic studies; in contrast, the mechanisms underlying hermaphrodite-specificity of the VC neurons are not completely clear.

a. The HSNs

The HSNs (so-called even though they are not the only neurons specific to this sex) are a bilateral pair of serotonergic motor neurons that innervate the vm2 vulval muscles to stimulate egg-laying. The HSNs are essential for normal egg-laying behavior: genetic or microsurgical ablation of these cells renders animals unable to lay eggs ([Trent *et al.*, 1983](#)). As a result, many genes important for HSN development and function have been identified in genetic screens for egg-laying defective (Egl) mutants ([Desai and Horvitz, 1989](#); [Desai *et al.*, 1988](#); [Trent *et al.*, 1983](#)).

The sexual dimorphism in the presence of the HSNs occurs through differential cell death. In embryos of both sexes, the HSNs are born in the tail region and subsequently migrate to their final position in the midbody ([Sulston *et al.*, 1983](#)). In hermaphrodites, the HSNs remain quiescent until L4. Presumably in response to an extrinsic cue, they then extend short branched processes to innervate vulval muscles and long anterior processes that form synapses in the nerve ring. In contrast, in males the HSNs undergo programmed cell death during migration to the midbody ([Sulston and Horvitz, 1977](#); [Sulston *et al.*, 1980](#)), accounting for the sex-specificity of this cell type in adults.

HSNs born in males undergo programmed cell death through a canonical mechanism that is shared with nearly all other developmental cell deaths in the *C. elegans* soma: activation of the BH3-only factor EGL-1 blocks CED-9/Bcl-2 to activate CED-4/Apaf-1 and the caspase CED-3, triggering apoptosis ([Conradt and Horvitz, 1998](#); [Desai *et al.*, 1988](#); reviewed in [Conradt and Xue, 2005](#)). Interestingly, a series of experiments have demonstrated that the male-specificity of this cell death results from the sex-specific activation of *egl-1* itself. In wild-type males, *egl-1* is activated in the embryonic HSNs, triggering their

death; in contrast, *egl-1* is repressed in hermaphrodites, allowing their survival and eventual differentiation (Conradt and Horvitz, 1998). Important insights into the mechanism of differential *egl-1* activation arose from the study of an unusual dominant *egl-1* allele, *egl-1(n1084)*, in which HSNs undergo inappropriate (i.e., male-like) apoptosis in hermaphrodites (Desai *et al.*, 1988; Trent *et al.*, 1983), suggesting that *egl-1* is inappropriately activated in hermaphrodite HSNs by the *egl-1(n1084)* mutation. Molecular analysis showed that *n1084* is a single-nucleotide change in a conserved *cis*-acting regulatory element 5.6 kb downstream of *egl-1*-coding sequence. Using a combination of genetics and biochemistry, Conradt and colleagues demonstrated that this element is in fact a binding site for TRA-1A (Conradt and Horvitz, 1999). In wild-type hermaphrodites, TRA-1A binds to this regulatory element to repress *egl-1* expression in HSNs; in contrast, the lack of TRA-1A activity in males allows the activation of *egl-1* expression in the HSNs through unknown factors (Fig. 1.2B). The disruption of this element in *n1084* prevents the repression of *egl-1* by TRA-1A in hermaphrodite HSNs, causing them to adopt the male-like fate of programmed cell death. Thus in this case, *tra-1* acts to directly repress male-specific gene expression in the hermaphrodite. This mechanism, therefore, provides a striking example of a well-understood genetic pathway that regulates sexual dimorphism in the *C. elegans* nervous system.

b. The VC neurons

The VC neurons are cholinergic motor neurons that have synaptic connections to the HSNs and the vm2 vulval muscles (White *et al.*, 1986). The VCs have a more subtle effect on egg-laying behavior, acting to negatively regulate vulval muscle contraction. This function is mediated by their release of acetylcholine, which may inhibit the HSN-mediated stimulation of vulval muscle contraction (Bany *et al.*, 2003).

Rather than sex-specific cell death, it is a cell fate change that underlies the sexually dimorphic presence of the VCs, and the genetic pathway mediating this switch is not well understood. As shown in Fig. 1.3, the six VCs are derived postembryonically from a set of ventral precursor cells, the Pn.a cells, that also produce several classes of core motor neurons (VA, VB, AS, and VD). The VCs themselves arise from the Pn.aap branch of the lineage; in males, the Pn.aap branch instead produces the male-specific CA and CP motor neurons (see below). (In *C. elegans* lineage nomenclature, *a* and *p* refer to anterior or posterior daughter cells; thus, Pn.aap is the posterior daughter of the anterior daughter of the anterior daughter of the Pn cell.) Thus, this sexual dimorphism—the production of VCs in hermaphrodites and CAs and CPs in males—arises from a change in the fate of the Pn.aap cells.

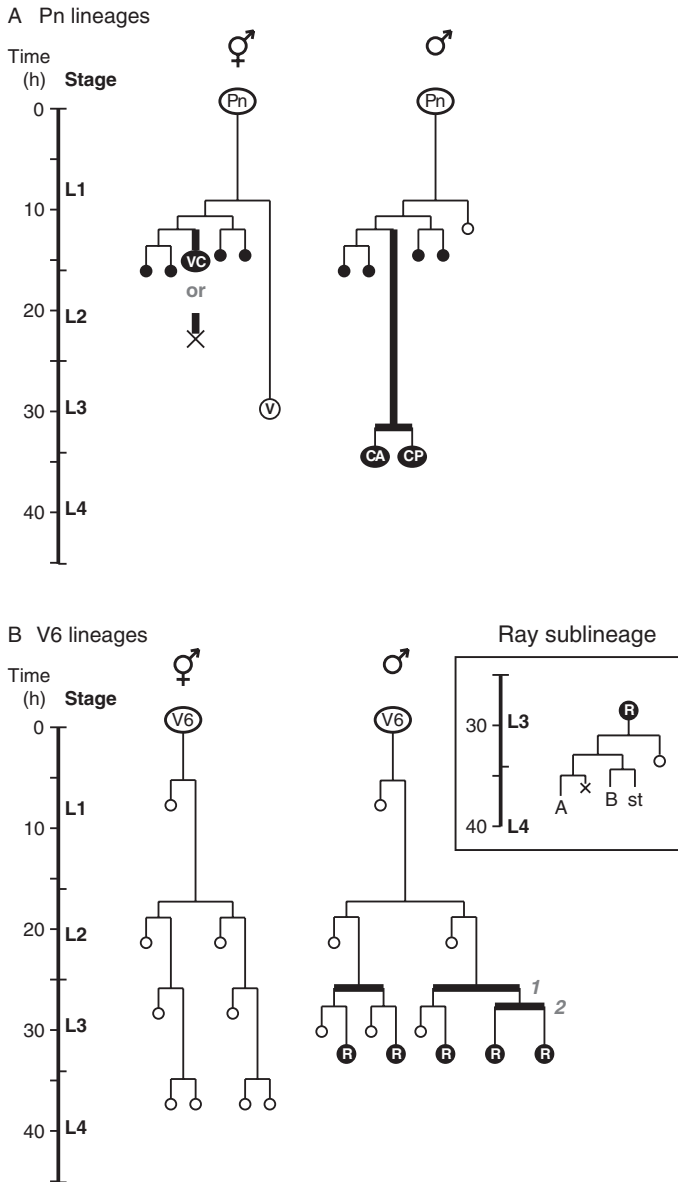


Figure 1.3. Sex differences in cell lineage give rise to differences in ventral cord and tail neurons. Developmental time is on the vertical axis, reading down; cell divisions are shown on the horizontal axis with anterior to the left. Open circles represent hypodermal cells, closed circles, neurons. Bold lines indicate differences between hermaphrodites (left) and males (right). On the left is shown larval stage and postembryonic developmental

In both sexes, the specification of neural fates in Pn.aap requires the midbody Hox cluster gene *lin-39*. In *lin-39* mutants, neither the hermaphrodite VC nor the male CA/CP neurons are generated (Clark *et al.*, 1993; Ellis, 1985; Loer and Kenyon, 1993). However, the fate of the Pn.aap cells themselves is still sexually dimorphic in *lin-39* mutants. In *lin-39* hermaphrodites, all of the Pn.aap cells that would become VC neurons undergo programmed cell death; in *lin-39* males, some of the Pn.aap cells die, some survive with small neuronal-like nuclei, and some divide but fail to give rise to fully differentiated motor neurons (Clark *et al.*, 1993; Ellis, 1985; Loer and Kenyon, 1993). Thus, it seems that the generation of sexual dimorphism in the Pn.aap lineage involves a mechanism of at least two steps: a *tra-1*-mediated propensity for cell death in hermaphrodites (perhaps through differential regulation of *egl-1*) and a sexually dimorphic response to *lin-39*. Efforts to understand the genetic basis for sex differences in Pn.aap-derived neurons are ongoing and should lead to interesting insights into the cross talk between sex determination and axial patterning pathways.

2. The male-specific nervous system

As mentioned above, the sex-specific nervous system of the *C. elegans* male is significantly more complex than that of the hermaphrodite, comprising a set of 89 sensory-, motor-, and interneurons that are connected to the core nervous system (Table 1.1; Fig. 1.2). However, unlike that of hermaphrodites, the ultrastructure of the male nervous system has not been fully determined so that a high-resolution integration of most of these cells into the worm “wiring diagram” is not yet possible. The primary obstacle to this project has been the complexity of circuitry in the male tail, thought to rival the complexity of the nerve ring, the main site of neural processing in the worm. However, with the help of recent

time in hours. V indicates a vulval precursor cell and R a ray precursor cell. (A) Both the hermaphrodite VC and the male CA and CP neurons arise from the Pn ventral cord blast cells. In XX animals, the Pn.aap branch of this lineage either differentiates into a VC neuron (in the P3-P8 lineages) or undergoes cell death (X in P1, P2, and P9-P12). In contrast, the Pn.aap cell in males remains undifferentiated until late L3, when it divides to give rise to one CA and one CP neuron. At the extreme ends of the P cell domain, there are exceptions to this (not shown) (Sulston and Horvitz, 1977; Sulston *et al.*, 1980). In P2, the lineage is altered such that Pn.aap does not divide and gives rise only to the CP0 neuron (there is no CA0). In P12, the Pn.aap cell becomes PVX, a male-specific tail interneuron. (B) The V6 lineage gives rise to rays 2–6 in males; in hermaphrodites, V6 produces tail hypodermal cells. Two male-specific doubling divisions (indicated by the bold horizontal lines labeled **1**) require *mab-5* (Kenyon, 1986; Salser and Kenyon, 1996) and account for the additional proliferation in V6. The division labeled **2** can also be considered a symmetrical doubling division; this requires the Hox gene *egl-5* (Chisholm, 1991). The inset shows the ray sublineage, the program by which a single Rn cell (R) gives rise to a hypodermal cell, an A-type and B-type neuron, a ray structural cell (st), and a cell death (X).

advances in computer-aided analysis, ultrastructural reconstruction of the male nervous system is now underway and has already led to interesting insights into male sensory circuits and the ways in which they are integrated into the core nervous system (S. W. Emmons, D. Hall, and M. Xu, personal communication).

Sensory neurons are the major constituent of the male-specific nervous system, accounting for 50 of the 89 male-specific neurons. This highlights the importance of sensory function in the male: unlike hermaphrodites, *C. elegans* males must locate potential mates in order to reproduce and must carry out a mating behavioral program that integrates multiple sensory cues. The majority of these sensory neurons are in the tail, innervating four classes of male-specific sensilla, the rays, hook, postcloacal sensilla, and spicules. Four additional sensory neurons, the CEMs, are present in the head of the male. The functions of many of these male-specific sensory neurons have been defined by laser ablation studies and the analysis of mutants (Barr and Sternberg, 1999; Liu and Sternberg, 1995); several excellent reviews address the roles of these neurons in mediating sensory behaviors (Barr and Garcia, 2006; Emmons, 2006).

The male also has sex-specific interneurons that are likely to be important for the integration of sensory information. All of these are located in the tail. Again, because the male nervous system is unreconstructed, potential connections between male sensory neurons, interneurons, and the core nervous system remain essentially unknown. Finally, the male tail and ventral cord contain several classes of sex-specific motor neurons. The CA neurons send processes to the dorsal nerve cord (White, 1988); they have also been implicated through an unknown mechanism in sperm-transfer behavior during mating (Schindelman *et al.*, 2006). The CP neurons are serotonergic and innervate the male-specific diagonal muscles that are important for controlling tail posture during mating (Loer and Kenyon, 1993). Some male-specific neurons (the PC [A,B,C] neurons of the postcloacal sensilla and the spicule SPC neurons) have both sensory- and motor functions (Sulston *et al.*, 1980).

For some of these male neurons, including the CEMs and the ray neurons, the mechanisms that impart sexual dimorphism are at least partly understood. For most others, however, essentially nothing is known about how *tra-1* effects alterations in cell lineage and fate to specify male neural development. Below we explore what is known for three classes of neurons present only in the male: the CEM head sensory neurons, the tail ray sensory neurons, and the CA/CP ventral cord motor neurons. For all other male-specific neurons, no specific link from *tra-1* to sexual dimorphism has yet been made.

a. The CEM neurons

The four CEM neurons, the so-called cephalic companion sensory neurons, are the only male-specific neurons in the *C. elegans* head. These cells have, by virtue of their location and morphology, been long thought to have a role in the response of males to secreted chemical cues (pheromones) produced

by hermaphrodites (Sulston *et al.*, 1980). A series of recent findings indicate that the CEMs are indeed important for the ability of males to detect hermaphrodite-secreted chemical cues (see below).

Like the HSNs, sexually dimorphic programmed cell death establishes sex differences in the CEMs (Sulston *et al.*, 1983). In this case, however, the situation is reversed: these neurons die in the hermaphrodite embryo but survive in males. A critical event in this pathway is the sex-specific expression of the Bar-homeodomain transcription factor gene *ceh-30* (H. Schwartz and H. R. Horvitz, personal communication). This gene acts in the embryonic male CEMs to promote their survival: the CEMs die (i.e., they adopt a hermaphrodite-like fate) in males lacking *ceh-30* function. Interestingly, *ceh-30* seems to be a direct target of *tra-1*, such that TRA-1A activity represses *ceh-30* expression in the CEMs of hermaphrodites. In males, *ceh-30* expression (presumably via unknown CEM-specific activators) is thought to block the function of the canonical cell death pathway, though the mechanism through which this occurs is not known (Fig. 1.2C) (H. Schwartz and H. R. Horvitz, personal communication). Thus, the CEMs provide a second example of the regulation of sex-specific cell death in the nervous system by *tra-1*.

b. The CA and CP motor neurons

The only male-specific cells of the ventral nerve cord are the CA and CP motor neurons. Lineally, the CAs and CPs are analogous to the hermaphrodite VC neurons, as they arise from the division of the Pn.aap cells, which either become VC neurons or die in hermaphrodites (Fig. 1.3). In males, the Pn.aap cells do not die (this may be the default hermaphrodite fate), but instead remain undifferentiated until they divide during L3 to give rise to one CA and one CP. As discussed above, it is possible that both sexually dimorphic activation of the cell death program and differential responses to the activity of Hox genes may underlie the switch from VC to CA/CP fate.

c. The rays

The rays are sensory organs that protrude laterally from the male tail and are the most prominent among the several classes of sensory specializations with which the male is endowed. Each ray is an independent sensillum innervated by two distinct sensory neurons, called A-type and B-type (Sulston *et al.*, 1980). As adult *C. elegans* males have nine bilateral pairs of rays, the ray neurons account for 40% (36/89) of the male-specific nervous system. Though much is known about ray development (reviewed in Emmons, 2005), we do not yet have a full understanding of the genetic mechanisms that make ray formation male specific. We do know, however, that the mechanism is significantly more complex than those that underlie sex differences in the HSNs, CEMs, and ventral cord neurons, and involves multiple sexually dimorphic regulatory events.

Each ray forms through a self-contained developmental module, called the ray sublineage, that generates the three cell types of the differentiated ray (Sulston *et al.*, 1980). The ray sublineage is triggered in a set of nine bilateral pairs of ray precursor cells called the Rn cells, where *n* represents a number from 1 to 9, designating the precursor to a specific ray. The ray precursor cells themselves are lateral hypodermal cells that are part of the body “seam” that runs along each side of the animal from head to tail. During late larval development, each ray precursor cell executes the ray sublineage program to give rise to multiple differentiated cell types: two sensory neurons (called RnA and RnB, or simply A-type and B-type), an associated glial-like ray structural cell (Rnst), a cell that undergoes programmed cell death (Rn.aap), and a hypodermal cell (Rn.p). Despite their similar cellular composition, all rays are not identical: each has a unique identity that is defined by its position, morphology, patterns of gene expression, and perhaps neural connectivity. A gene that is critical for ray development is *lin-32*, an *atonal*/MATH-class bHLH gene that acts, together with its heterodimerization partner HLH-2, as the ray proneural gene (Portman and Emmons, 2000; Zhao and Emmons, 1995). Males null for *lin-32* function lack nearly all ray development (R. M. Miller and D. S. P., unpublished data) and ectopic *lin-32* expression can in some cases be sufficient to trigger ray development (Zhao and Emmons, 1995).

The sexual dimorphism in ray development does not come about through a simple mechanism (e.g., death of the ray precursor cells in hermaphrodites). Instead, the sex determination pathway impinges on male development in at least three ways to make the rays male specific. First, alterations in the lineage of the seam cell progenitors of the ray precursor cells occur in males to generate additional numbers of lateral hypodermal cells (Sulston *et al.*, 1980). Second, the expression of *lin-32* occurs in these tail hypodermal cells only in males (Zhao and Emmons, 1995). Third, the response of these cells to *lin-32* activation is sexually dimorphic, allowing *lin-32* to direct the formation of differentiated ray cells rather than other non-sex-specific cell types (Zhao and Emmons, 1995). In recent years, our understanding of the control of each of these three regulatory points by the sex determination pathway has become more complete, though several significant gaps remain.

The first of these sex-specific modifications is the alteration of seam cell lineages to produce nine ray precursor cells on each side of the larval male tail. The full complement of ray precursor cells arises only in males as a result of a set of male-specific proliferative divisions in the hypodermal V5, V6, and T seam cells lineages that give rise to the ray precursor cells (Sulston *et al.*, 1980). As a result, males have a row of 11 hypodermal seam cells in the L3 tail, 9 of which give rise to rays; hermaphrodites have only 4 such seam cells. In wild-type males, the ray precursor cell R1 arises from the V5 lineage, whereas R2 through R6 descend from V6, and R7 through R9 from T. As shown in Fig. 1.3, V6 undergoes an additional round of doubling divisions to generate extra progeny in the male; V5 and T undergo similar

but less extensive changes in cell lineage (Sulston *et al.*, 1980). In V5 and V6, this additional round of division is controlled by the *C. elegans* *ftz* orthologue, the Hox gene *mab-5* (Aboobaker and Blaxter, 2003; Costa *et al.*, 1988; Kenyon, 1986; Salser and Kenyon, 1996). In *mab-5* mutant males, the V5 and V6 lineages are identical to those in the hermaphrodite. Interestingly, they are also identical to the lineages of the more anterior seam cells V1–V4 in both sexes, indicating that *mab-5* imparts both spatial and sexual patterning. *mab-5* is known to bring about the patterning of these lineages cell-autonomously (Kenyon, 1986). Additionally, MAB-5 protein is detectable in both sexes in the early stages of seam cell development (when these lineages are sexually equivalent), but disappears from hermaphrodites and remains on in males once seam cell lineages diverge sexually in early L3 (Salser and Kenyon, 1996). However, the downstream targets of *mab-5* that bring about this symmetric doubling division are unknown. One attractive model is that *tra-1* acts together with a temporal cue to repress *mab-5* expression in the V5 and V6 lineages of L3 and L4 hermaphrodites (Fig. 1.4A); this possibility has not yet been tested directly. In contrast to V5 and V6, the lineage alterations in T are apparently not regulated by Hox genes; the pathway that controls sex differences in the T lineage remains undefined.

A second important sex-specific step in ray development is the activation of *lin-32*. As *lin-32* is not expressed in the tail seam of L3 hermaphrodites, this is clearly a sexually dimorphic event, though *lin-32* does not seem to be a direct target of *tra-1*. Instead, *lin-32* is activated in males through the combined effects of two pathways, a Hox pathway that involves *mab-5* and the *Abd-B* orthologue *egl-5*, and a second pathway involving the DM-domain gene *mab-3*. A series of elegant experiments has shown that *mab-5* acts to promote ray development late in the V5 and V6 lineages—after it promotes the earlier doubling divisions—presumably by activating *lin-32* (Salser and Kenyon, 1996). In addition, *egl-5* acts downstream of *mab-5* to allow *lin-32* expression in V6.ppppa, such that the two daughters of V6.pppp both produce rays (Chisholm, 1991). Thus, the full extent of *lin-32* activation in V5 and V6 requires both *mab-5* and *egl-5*. Acting in parallel with Hox genes is *mab-3*, which is expressed non-sex-specifically in all seam cells (Yi *et al.*, 2000). *mab-3* indirectly activates *lin-32* by blocking expression of the Hox-class bHLH gene *ref-1*, a repressor of *lin-32* (Ross *et al.*, 2005). As *mab-3* is expressed throughout the seam in both sexes, it does not act as an instructive factor for *lin-32* expression in the male tail. Instead, it seems that the combined function of *mab-3* and the Hox genes is to limit *lin-32* expression to the ray precursor cells (Fig. 1.4B), presumably functioning together with an L3-specific temporal cue. The means by which *tra-1* acts to make these events male specific is unclear, though it is again likely to occur through sex-specific regulation of Hox genes. As above, the mechanisms acting in the T-derived rays are likely to be different, as their development is largely independent of Hox genes and *mab-3* (Chow and Emmons, 1994; Shen and Hodgkin, 1988).

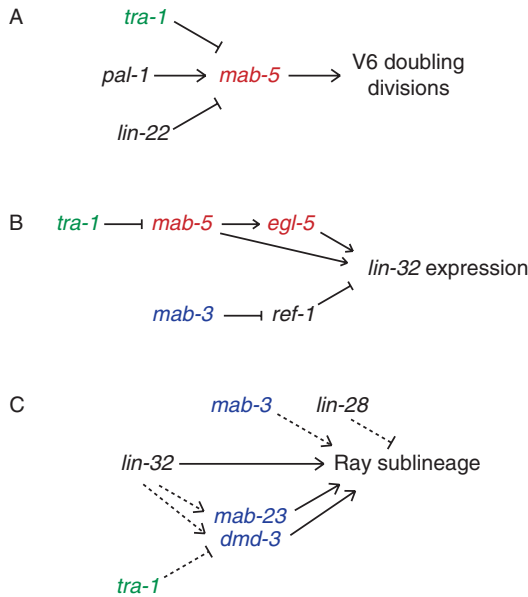


Figure 1.4. Sexually dimorphic steps in ray development. (A) The male-specific lineage alterations in V6 require the Hox gene *mab-5*. *mab-5* expression requires the caudal orthologue *pal-1* (Hunter *et al.*, 1999); its anterior boundary is limited by the bHLH gene *lin-22* (Kenyon, 1986; Wischnick and Kenyon, 1997). The male-specific maintenance of *mab-5* in the V6 lineage is likely to require *tra-1*, though the mechanism is not known. (B) Expression of *lin-32*, the ray proneural gene, requires the Hox genes *mab-5* and *egl-5* (Yi *et al.*, 2000; Zhao, 1995). In addition, the DM-domain gene *mab-3* promotes *lin-32* expression by inhibiting the action of the bHLH gene *ref-1* (Ross *et al.*, 2005). (C) The mechanisms that generate male-specific cell types (ray cells) from the ray sublineage are not well understood. *mab-3* is necessary for *lin-32* to efficiently drive ray production (Yi *et al.*, 2000), and *mab-23* and *dmd-3* have a role in the differentiation of the RnA neuron (Lints and Emmons, 2002; R. Lints and D.S. Portman, unpublished data). Whether *mab-23* and *dmd-3* act downstream of or in parallel with *lin-32* is not known, nor is whether *tra-1* may block *dmd-3* expression in the hermaphrodite seam. *lin-28* may act to confer temporal specificity to the ray sublineage, preventing a ray from being made in the L2 postdeirid lineage (Ambros and Horvitz, 1984).

A final layer of sex-specificity in ray development is the ray sublineage itself. Though this part of the mechanism is not well understood, several findings point to the importance of this step. First, *lin-32* has many other functions during *C. elegans* development, specifying the development of other neurons and neural structures in both embryos and larvae, including the touch cells and head sensory and interneurons (Chalfie and Au, 1989; Shaham and Bargmann, 2002; D. S. P., unpublished data). This indicates that some mechanism must give specificity to *lin-32* such that the ray sublineage is triggered when it is expressed in male seam

cells. Though ectopic expression of *lin-32* in the hermaphrodite can cause seam cells to produce neural structures (Zhao and Emmons, 1995), it is not clear whether these are rays or other neural cell types. Second, overexpression of *lin-32* is not able to efficiently rescue the ray loss in *mab-3* mutants, suggesting that *mab-3* may act in the male seam to potentiate *lin-32* function (Yi et al., 2000). Together, these findings indicate that the cellular context of the male seam, presumably comprising spatial, temporal, and sexual information, dictates the execution of the ray sublineage in response to *lin-32* expression.

Though no direct targets of *lin-32* in the rays are known, the four *lin-32*-dependent differentiated cells that descend from the ray precursor cell must require the activation of multiple, asymmetrically distributed cell type-specific regulatory factors (Portman and Emmons, 2000). The temporal component of the mechanism that gives ray specificity to *lin-32* may involve the heterochronic gene *lin-28*. In *lin-28* mutants of both sexes, the V5.pa seam cell generates a ray-like structure in place of the postdeirid, a non-sex-specific sensory structure that requires *lin-32* (Ambros and Horvitz, 1984). This indicates that *lin-28* may normally function to repress a male-specific *lin-32* cofactor in V5.pa. Hox genes and DM-domain genes are also likely to have important roles in this process. Both *mab-5* and *egl-5* have important roles in specifying ray identity, patterning the rays along the anterior–posterior axis. Additionally, the DM-domain genes *mab-23* and *dmd-3* have important roles in controlling cell fates in the ray sublineage itself, particularly in regulating differentiated properties in the RnA neurons (Lints and Emmons, 2002; R. Lints and D. S. P., unpublished data). One appealing model is that *mab-3*, *mab-23*, and/or *dmd-3* may act to “masculinize” the progeny of the ray precursor cell by specifying the development of male-specific neural types, rather than the non-sex-specific neural structures generated by *lin-32* function elsewhere (Fig. 1.4C).

3. Sex differences in the core nervous system

Relatively less attention has been given to the development and function of sex differences in the core nervous system itself, though this is likely to be of central importance to generating distinct male and hermaphrodite behaviors. Table 1.1 outlines our understanding of the extent of these differences, which are of two general types. First, some neurons that are lineally equivalent in both sexes show subtle differences in morphology and connectivity, such as the PDA pre-anal ganglion motor neuron. This may allow these cells to integrate information coming from the male-specific nervous system, or to allow the core nervous system to have output onto male-specific musculature. A second type of sex difference in sexually isomorphic neurons is at the level of changes in gene expression. As discussed below, these differences are only easily detectable through the use of fluorescent gene-expression reporter constructs, and only two examples of this sort are known.

a. Ultrastructural differences

Several examples of sex differences of the core nervous system were identified in the cell lineage and ultrastructural studies of the 1970s and 1980s. In one case, these differences are lineal: in the hermaphrodite, the tail cell called Y becomes the PDA neuron, a motor neuron in the pre-anal ganglion; in males, Y divides and its anterior daughter (Y.a) becomes PDA (Sulston *et al.*, 1980). Several core neurons also demonstrate differences in connectivity: this is the case for PDA and another pre-anal ganglion motor neuron, PDB, as it is for the PVP(R/L) interneurons (called PV(S/U) in males) and the PHC sensory neurons (Sulston *et al.*, 1980; S.W. Emmons, personal communication). The full extent of these synaptic differences awaits the completion of the male tail wiring project. The PHCs also differ ultrastructurally, having a striated rootlet only in the male (Sulston *et al.*, 1980). In general, the genetic mechanisms underlying these sex differences are unknown, as these differences are subtle and difficult or impossible to observe by light microscopy. Nevertheless, it is expected that these are in some way under the control of *tra-1*.

b. Molecular differences

In addition to these subtle structural differences, two examples of sex differences in gene expression in the core nervous system are known. First, the seven-transmembrane putative chemoreceptor gene *srd-1* has been found to be expressed in the ADF sensory neurons only in males, even though these cells are present and appear identical in both sexes (Troemel *et al.*, 1995; White *et al.*, 1986). A second serpentine receptor gene, *srj-54*, is expressed in the common AIM interneurons in males only (D. S. P., unpublished data). In neither of these cases is the functional significance of sexually dimorphic gene expression understood; however, it is noteworthy that both of these neurons are implicated in sensory behavior. The ADF neurons regulate entry into the long-lived dauer larva stage (Bargmann and Horvitz, 1991a), have secondary roles in sensing several classes of ions and small molecules (Bargmann and Horvitz, 1991b), and encode a learned aversion to pathogenic bacteria by expressing serotonin (Zhang *et al.*, 2005). Interestingly, males and hermaphrodites differ in their propensity to enter the dauer stage (Ailion and Thomas, 2000), suggesting that sex differences in ADF could have functional consequences. In contrast, no precise functions have been defined for the AIM neurons; however, they do synapse extensively onto the AIA interneurons, an important center for the integration of chemosensory information (Sengupta, 2007; White *et al.*, 1986). It is quite possible that these differences represent only a small glimpse of the true extent of sex differences in gene expression in the core nervous system, as *C. elegans* gene expression patterns are often examined only in hermaphrodites. Several microarray experiments designed to detect sex-specific gene expression in *C. elegans* have been carried out (Jiang *et al.*, 2001; Reinke *et al.*, 2004; Thoemke *et al.*, 2005), but

these may not be sufficiently sensitive to reliably identify expression differences in a small number of neurons. Further investigation of this area should lead to significant insight into the sex-dependent regulation of behavior, especially as sexual dimorphism in sexually isomorphic circuitry is a common theme in the nervous systems of more complex animals.

D. Sex differences in *C. elegans* behavior

The full extent of sex differences in *C. elegans* behaviors is only beginning to be understood. The more obvious sex-specific behaviors—that is, hermaphrodite egg-laying and male mating—are well characterized and, to a first approximation, controlled by the sex-specific nervous system. However, it is becoming increasingly apparent that behaviors common to both sexes—locomotion, for example, and a variety of sensory responses—also differ between the sexes. In some cases, more complex behaviors (e.g., male food-leaving; see below) may arise through the modification of basic parameters of behaviors common to both sexes. Here we review the known sex differences in *C. elegans* behavior and, to the extent to which it has been determined, discuss the genetic and neural underpinnings of these differences.

1. Hermaphrodite behavior

a. Egg-laying behavior

Under favorable conditions, hermaphrodites incubate early newly fertilized eggs (produced either by self- or cross-fertilization) in the uterus for several hours before they are laid. Egg-laying is a result of regulated contraction of the vulval muscles: on contraction, the vulva opens transiently and a single egg is laid. The rate of egg-laying is regulated by several factors, particularly the availability of food.

The genetic and neural control of egg-laying has been the subject of an extensive recent review by [Schafer \(2006\)](#). As described above, the HSNs are thought to be the primary mediator of egg-laying, promoting vulval muscle contraction through serotonergic innervation of these muscles. In addition, the VC motor neurons provide cholinergic input to the vulval muscles and HSNs to inhibit muscle contraction ([Bany et al., 2003](#)). Both the HSNs and VCs have extensive contacts with core nervous system neurons, which may allow the regulation of egg-laying behavior according to the state of the animal. The extent to which there may be anatomical and/or functional sex differences in the neurons that are pre- or postsynaptic partners of the HSNs and VCs is unknown.

b. Mating behavior

Historically, the *C. elegans* hermaphrodite has been considered to be a passive partner in mating behavior. However, recent evidence suggests that the hermaphrodite's response to male copulatory behavior may be regulated. Perhaps to promote the generation of self- rather than cross-progeny, young (i.e., self-fertile) hermaphrodites appear to express a locomotory "escape" response when a male attempts to copulate. In contrast, older animals—those that are no longer self-fertile owing to the depletion of self-sperm—may be more receptive to males (Kleemann, 2005). In addition, self-fertile hermaphrodites have been observed to actively expel sperm deposited by males, while those depleted of sperm show no such behavior (Kleemann, 2005). The genetic and neural mechanisms that regulate these behaviors are unknown. However, the dependence of hermaphrodite behavior on reproductive status suggests the existence of a pathway, perhaps endocrine, by which the state of the germ line and gonad can be communicated to the nervous system. It is quite possible that additional aspects of hermaphrodite behavior are regulated during mating; this remains relatively unexplored.

c. Avoidance of hermaphrodite-conditioned medium

It has recently been found that adult hermaphrodites may aversively respond to liquid medium conditioned by other hermaphrodites, possibly as a way to avoid competition for food or other resources (J. White and E. M. Jorgensen, personal communication). The neural and genetic bases for this behavior are unknown as is the identity of the secreted factor(s) to which animals respond. Interestingly, as described below, males display the opposite response to hermaphrodite-conditioned medium. This finding has interesting implications for the potential mechanisms that could bring about this sexually dimorphic response.

2. Male behavior

The behavior of *C. elegans* adult males differs from that of hermaphrodites in several respects. Males exhibit some behaviors, such as mating, that are completely absent in the hermaphrodite; males also have generally increased activity and have a propensity to leave a food source devoid of potential mates. As two excellent reviews have focused on male behavior (Barr and Garcia, 2006; Emmons, 2006), the emphasis here is on the neural and genetic mechanisms that underlie sex differences in the expression of these behaviors.

a. Male mating behavior

As a stepwise behavior that must integrate several sensory cues and produce several types of coordinated motor output, male mating is thought to be the most complex behavior encoded in the *C. elegans* nervous system. As these steps are

likely hardwired into the male nervous system, male mating is considered to be an innate behavior. However, it is not unlikely that male mating is regulated by experience, though this remains unexplored.

Male mating behavior entails a series of distinct steps: response to hermaphrodite contact, turning, vulva location, spicule insertion, and sperm transfer (Liu and Sternberg, 1995). Each of these steps requires male-specific neuromuscular or gonadal structures. In the first step, response to contact, the male apposes the ventral surface of his tail against the hermaphrodite body and several coordinated changes in activity occur. The male tail assumes a characteristic “clenched” posture (a result of the contraction of tail diagonal muscles), allowing tail sensilla to efficiently detect cues on the hermaphrodite body. The male also ceases forward locomotion and head foraging movements, and initiates backward (tail-first) locomotion along the hermaphrodite body in search of the vulval opening. Response to contact can be triggered by several putative mechanosensory structures in the male tail, primarily the rays and hook, depending on where on the male’s body contact is first made (Liu and Sternberg, 1995). Response is mediated by signaling through the polycystin-class TRP channels LOV-1 and PKD-2, which may act as mechanosensors (Barr and Sternberg, 1999), though other sensory pathways are likely to be involved (Barr *et al.*, 2001; Peden and Barr, 2005; R. M. Miller and D. S. P., unpublished data). The sexual dimorphism in the initiation of mating behavior can, therefore, largely be explained by its reliance on male-specific sensory organs and muscles, though backward locomotion and the cessation of feeding almost certainly involve circuitry of the core nervous system.

The goal of the male’s reverse locomotion is to locate the hermaphrodite vulva. If the male has initiated response on the dorsal side of the hermaphrodite (or on the ventral side past the vulva), the male will need to turn at least once in order to search the other side of the hermaphrodite body. Turning involves continuous contact between the male tail and the hermaphrodite body and requires a tight curling of the tail mediated at least in part by the diagonal muscles. Serotonergic innervation of the diagonal muscles by the CP neurons is necessary for this step (Loer and Kenyon, 1993), as are the dopaminergic ray neurons, R5A, R7A, and R9A (Lints and Emmons, 1999; Liu and Sternberg, 1995). Ablation studies have shown that the most posterior rays (the so-called “T-rays,” rays 7, 8, and 9) are essential for coordinating turning behavior (Liu and Sternberg, 1995), which is thought to be triggered by a change in the shape of the hermaphrodite body at the head or tail (Liu and Sternberg, 1995; P. W. Sternberg and A. Whittaker, personal communication).

The location-of-vulva step of mating behavior is mediated primarily by the hook, a sensory organ on the ventral side of the male tail (Liu and Sternberg, 1995). Unknown mechanical cues are first sensed by the hook, causing the male to stop in the vicinity of the vulva; like response, this step also requires polycystin signaling (Barr and Sternberg, 1999). High-resolution location of the vulval slit

involves the postcloacal sensilla and spicules; the spicules rapidly prod the hermaphrodite body as the male slowly moves back and forth (Garcia *et al.*, 2001; Liu and Sternberg, 1995). Penetration of the vulva by the spicules occurs through cholinergic innervation of the spicule protractor muscles. This event is thought to be sensed by proprioceptive neurons in the spicules that regulate the transfer of sperm through the vas deferens and cloaca into the hermaphrodite uterus (Garcia *et al.*, 2001; Schindelman *et al.*, 2006). Pharyngeal pumping also slows dramatically when the spicules are inserted (Liu and Sternberg, 1995). The initiation of sperm transfer requires neurons in the male ventral cord, most likely the male-specific CA motor neurons (Schindelman *et al.*, 2006). Once sperm transfer is complete, the spicules are withdrawn and the mating behavioral program terminates.

As mentioned above, the execution of each of these steps depends critically on sex-specific components of the male nervous system. However, the neural and genetic control of these steps clearly also involves the core nervous system. The precise means by which these interactions are important for mating behavior are not clear, though some steps, such as response, must involve communication with the core nervous system at several levels to modulate locomotion and block pharyngeal pumping and foraging. It is also likely that regulatory events in the core nervous system modulate mating behavior in several ways. Males are reluctant to initiate mating behavior in the absence of food; in addition, feeding behavior is linked through the pharyngeal NSM neurons to the regulation of spicule protraction (Gruninger *et al.*, 2006). Recently it has been found that components of the core mechanosensory circuit are necessary for turning behavior (T. Liu, C. Li, and M. M. Barr, personal communication). Together these findings indicate that multiple connections between the male-specific nervous system and the core nervous system are likely to be important for the regulation and execution of mating behavior.

The sex-specificity of mating behavior has been thought to be completely under the control of *tra-1*. Though *tra-1* males have fertility defects, this was attributed to a role for *tra-1* in the development of the germ line, a function of *tra-1* that is thought to be non-sex-specific and separate from its role in sex determination (Hodgkin, 1987; Mathies *et al.*, 2004). However, evidence has indicated that *tra-1* XX pseudomales may also have defects in mating behavior, particularly in the response step of mating (Grote and Conradt, 2006; R. M. Miller and D. S. P., unpublished data). This may indicate that additional pathways act in parallel to *tra-1* to completely masculinize the male nervous system (Grote and Conradt, 2006; van den Berg *et al.*, 2006). Alternatively, it may be that existing alleles of *tra-1* are not truly null such that *tra-1* may still have residual feminizing activity in *tra-1* XX pseudomales. A third possibility is that the gonadal defects in *tra-1* animals could secondarily cause behavioral phenotypes, consistent with the speculation that the gonad may have some role in regulating neural function in the male (see below) (Lipton *et al.*, 2004).

Acting downstream of *tra-1*, the DM-domain gene *mab-23* has been shown to have an important role in mating behavior. *mab-23* mutant males have severe defects in turning behavior as a result of defects in the differentiation of diagonal muscles. The transfer of sperm is also blocked in *mab-23* males owing to defects in male-specific proctodeum or cloaca formation (Lints and Emmons, 2002). This is consistent with the idea that *mab-23* acts to masculinize both sex-specific and non-sex-specific tissues to bring about male behavior. Interestingly, two other DM-domain genes, *mab-3* and *dmd-3*, also have important roles in masculinizing neural circuits in the male-specific and/or core nervous system (see below). Understanding how these factors act downstream of *tra-1* to engender male-specific behavioral programs remains an important goal for the field.

b. Male food-leaving

In laboratory culture, males in the absence of hermaphrodites will leave a food source, often lethally. As this behavior is exhibited only by adult males, and is not expressed when a hermaphrodite is present, it has been interpreted as a male mate-searching or sex-drive behavior (Lipton *et al.*, 2004). While the specific signals that control this behavior have not been identified, one possibility is that the presence of a hermaphrodite inhibits a mate-searching drive state. Interestingly, the rate at which males leave food over time is constant, indicating that solitary males are constitutively in a mate-searching mode that can be expressed stochastically, possibly as result of random encounters with the edge of the food source. Recent evidence indicates that the ray sensory neurons are important for implementing food-leaving (A. Barrios and S. W. Emmons, personal communication), suggesting that tonic input from the male-specific nervous system regulates this behavioral state. Like the hermaphrodite escape response described above, food-leaving behavior in both sexes depends on the state of the gonad (Lipton *et al.*, 2004), consistent with the possibility of endocrine signals modulating the function of the nervous system. Recently, the steroid hormone receptor *daf-12* has been found to regulate food-leaving, suggesting the intriguing possibility that a steroid could be involved in the sex-specific expression of this behavior (G. Kleemann and S. W. Emmons, personal communication). Again, because expression of this behavior occurs ultimately through modulation of locomotory patterns, components of the core locomotory circuit (Gray *et al.*, 2005; Tsalik and Hobert, 2003; Wakabayashi *et al.*, 2004) may be actively regulated to bring about sex-specific outcomes. It is also known that the DM-domain genes *mab-3*, *mab-23*, and *dmd-3* are important for male food-leaving behavior (Yi *et al.*, 2000; L. Jia, G. Kleemann, and S. W. Emmons, personal communication; R. M. Miller and D. S. P., unpublished data), again indicating that this class of genes may have important roles in controlling male-specific behavior.

c. Response to hermaphrodite pheromones

The existence of hermaphrodite-produced pheromone(s) detected by males has long been postulated but has remained elusive. Recently, several groups have demonstrated that males can recognize culture medium conditioned by hermaphrodites (Simon and Sternberg, 2002; J. White and E. M. Jorgensen, personal communication; K. L. Chow, personal communication; A. Barrios and S. W. Emmons, personal communication) or by females from other *Caenorhabditis* species (Chasnov *et al.*, 2007). Depending on the assay conditions, males will linger in regions containing conditioned medium (Simon and Sternberg, 2002) or will display taxis toward pheromone signals. Interestingly, it has been found that both the sex-specific CEM sensory neurons as well as non-sex-specific sensory neurons of the head are required for males to be able to detect these signals, indicating that sex differences in the core nervous system, in addition to the male-specific nervous system, may generate male-specificity in the response to conditioned medium (J. White and E. M. Jorgensen, personal communication; W. K. So and K. L. Chow, personal communication). The genetic basis for these sex differences are unknown; however, recent evidence suggests that *dmd-3* males fail to recognize hermaphrodite-conditioned medium, indicating that *dmd-3* is necessary for some aspect of this sexually dimorphic behavior (R. Miller and D. S. P., unpublished data).

3. Sex differences in common behaviors

Some behaviors are characteristically expressed in both sexes and are known or thought to emerge through the functions of sexually isomorphic circuits and gene activity. However, as discussed above, this view is likely to be an oversimplification, and the establishment of sex differences in the core nervous system may have significant implications for *C. elegans* behaviors, both those that are wholly sex-specific (i.e., egg-laying and mating) and those that occur through differential regulation of behaviors common to both sexes (e.g., the hermaphrodite escape response and male food-leaving).

a. Locomotion

As for all nematodes, locomotion in *C. elegans* is driven by the propagation of dorsoventral sinusoidal waves along the length of the body. Animals modify their direction of movement by turning, often in conjunction with multiple brief reversals, in a maneuver termed a “pirouette” (Pierce-Shimomura *et al.*, 1999). Though male and hermaphrodite locomotion is qualitatively similar, males have been observed to be generally more active under standard culture conditions and can be considered to be hyperkinetic compared to hermaphrodites. Recent more quantitative analysis of sex differences in locomotion has indicated that males differ significantly from hermaphrodites in their locomotory waveform, body

bend frequency, as well in the frequency and duration of spontaneous reversals (W. R. Mowrey and D. S. P., unpublished data). These differences could in principle reflect a modification of the core locomotory circuit or could result from the influence of male-specific circuitry on locomotory behavior. Males also display significant differences in their responses to drugs that interfere with cholinergic transmission (aldicarb, levamisole, and nicotine), suggesting specific differences in the characteristics of acetylcholine signaling at the neuromuscular junction (Matta *et al.*, 2007; W. R. Mowrey and D. S. P., unpublished data). As for other sex differences in behavior, these differences in locomotion are expected to be under the control of *tra-1*, but specific downstream effector genes that may act in this pathway have not yet been identified.

b. Olfaction

Chemosensory behavior, particularly olfaction, has been the subject of extensive analysis in the *C. elegans* adult hermaphrodite, revealing important insights into the genetic and neural control of sensory responses (for reviews see Bargmann, 2006; Sengupta, 2007). As described above, the putative chemoreceptor *srd-1* is male specifically expressed in the shared sensory neuron ADF, suggesting the possibility of sex differences in chemosensory behavior (Troemel *et al.*, 1995). Recently, it has been found that adult males are significantly less responsive than hermaphrodites to several attractive olfactory cues in standard olfaction assays. Males also display significantly different olfactory preferences when animals are simultaneously exposed to two odorants, indicating that the *C. elegans* olfactory system may be modified to generate characteristic sex-specific repertoires of sensory behavior (K. H. Lee and D. S. P., unpublished data). Again, these sex differences are downstream of *tra-1*, and may at least partially require *dmd-3* function, as *dmd-3* males display some partially feminized olfactory behavior characteristics (K. H. Lee and D. S. P., unpublished data). The specific cellular focus of *tra-1* and *dmd-3* action is not known; these genes may act autonomously in the olfactory system or indirectly either through modification of olfactory behavior by the male-specific nervous system or by influences from the gonad and/or germ line. Interestingly, at least some sex differences in *C. elegans* olfactory preference are developmentally regulated, suggesting that sex-specific maturation of the nervous system may underlie these changes (K. H. Lee and D. S. P., unpublished data). Understanding the genetic and neural control of sex differences in olfaction is likely to reveal important mechanisms by which sex modifies neural function in the worm.

c. Learning and memory

It has been recently reported that there are significant sex differences in the ability of *C. elegans* adults to associate a sodium chloride chemosensory cue with the lack of food. Under standard assay conditions, hermaphrodites form this associative memory quite efficiently, while males perform much more poorly

(Vellai *et al.*, 2006). Interestingly, insulin-like growth factor signaling (through the insulin receptor DAF-2 and its downstream target DAF-16/FOXO) is required for the formation of this memory, and mutations in this pathway abolish sex differences in this behavior, indicating that differential insulin-like signaling may underlie these sex differences (Vellai *et al.*, 2006). In a different paradigm, males and hermaphrodites have been shown to have similar nonassociative habituation responses to mechanical stimulation. However, male responses to tap stimuli were greater than those of hermaphrodites, perhaps because of the additional sex-specific mechanoreceptors present in the male tail (Mah and Rankin, 1992).

III. CONCLUSIONS

A. Genetics of sex-specific neural development

A number of important insights into genetic mechanisms have emerged from the study of sex differences in the *C. elegans* nervous system. During worm development, the master regulator *tra-1* acts cell-autonomously in many tissues to control sexual dimorphism. In the nervous system, one of the functions of *tra-1* is to effect two important sets of sex-specific cell deaths, those of the HSNs in males and the CEMs in hermaphrodites. Both of these occur through embryonic regulation of programmed cell death, such that sexual dimorphism in the nervous system is already present in newly hatched larvae. These pathways may provide paradigms for other systems in which the role of sex-specific cell death in shaping sex differences in the nervous system is not well known.

Other sex differences in the nervous system—for example, motor neurons in the ventral cord and sensory neurons in the male tail rays—arise through more complex developmental mechanisms. In the ventral cord, both cell death and Hox-dependent changes in cell fate are likely to be important. In the rays, Hox-dependent cell lineage alterations, differential expression of bHLH factors, and the specification of male-specific neural cell types are all ultimately under the control of *tra-1*. Though these pathways are not completely understood, two important principles are already apparent. First, as in *Drosophila*, the interface between Hox-dependent spatial patterning and sex determination is critical for the emergence of sex-specific cell types, highlighting the importance of understanding the interface between these pathways. Second, three genes of the DM-domain family are essential for male-specific cell lineage and fate in sensory ray development, again suggesting that these genes may be ancient regulators of sexual dimorphism and may have similar roles in other systems.

The most subtle sort of sex difference in the *C. elegans* nervous system, ultrastructural and molecular differences in the core nervous system, is just beginning to be investigated. Again, these dimorphisms are almost certainly

regulated by *tra-1*, but nothing is known about the genetic mechanisms by which they occur. Given the emerging evidence that nonhormonal, cell-autonomous mechanisms shape neural properties in the vertebrate CNS, further investigation of the pathways regulating these processes in *C. elegans* may lead to important insights into conserved genetic pathways that control sex differences in neural character in other organisms.

B. Genetics of sex-specific behavior

Considerably less is known about the genetic mechanisms that engender behavioral differences between the sexes in *C. elegans*. Like the developmental mechanisms discussed above, these differences are controlled by *tra-1*, though recent evidence suggests that this gene may not completely account for the full extent of sexual dimorphism. Again, DM-domain genes are proving to be critical regulators of this process, as three genes of this family in *C. elegans* are required for some aspects of male-specific behavior. Additionally, recent results have led to the surprising possibility that the status of the gonad regulates neural function and sex-specific behavior through unknown mechanisms. Further investigation to understand how the sex-specific nervous systems interface with the core nervous system, the nature of the neural circuits that control sex-specific behaviors (particularly in the male), and the ways in which shared behaviors are differentially modified to produce higher-order sex-specific outcomes is certain to be fruitful.

C. Relevance for vertebrate systems

How can research in a simple invertebrate illuminate the mechanisms that shape sex differences in the vertebrate nervous system? Though *C. elegans* has a strong history of defining conserved genetic mechanisms critical for neural development and function, the mechanisms used by vertebrates to effect sexual differentiation of the nervous system seem, at first glance, fundamentally different from those in *C. elegans*. As described above, gonadal hormones have long been considered to be the sole arbiter of these processes in vertebrates; their effects, particularly in mammals, are both powerful and well demonstrated. In contrast, steroid hormones have no obvious role in the sex-specific development of the *C. elegans* nervous system, though whether they regulate sex-specific behavior in the worm is an open question. However, the *C. elegans* genome contains no obvious orthologues of the androgen or estrogen receptors so that vertebrate gonadal steroids are unlikely to be involved.

A revision of this orthodox view has come from a series of recent studies in vertebrates that have demonstrated that cell-autonomous sex determination pathways, acting in parallel to gonadal hormones, function in the nervous system

to link sexual karyotype to the development of sex-specific characteristics. Arnold has proposed that regulatory genes on the X (that escape dosage compensation) and Y chromosomes may directly organize sex-specific CNS characteristics (Arnold, 2004). Indeed, there is some evidence that the Y-chromosome sex-determining gene *Sry* has such a role in the nervous system (Dewing *et al.*, 2006). However, such a model may capture only one aspect of this process. It is also quite possible that sexual karyotype controls much more complex regulatory networks, such as those characteristic of *C. elegans* and *Drosophila* sex determination, that read the sex-determining signal and set into motion a cascade of interactions that only very indirectly lead to sex-specific gene expression. The potential existence of such a pathway in the mammalian nervous system has intriguing implications for the mechanisms that bring about sex differences in neuroanatomy and neural function; moreover, genes in such a pathway could have central importance in the development of a wide variety of neurological and mental health conditions, such as autism, mental retardation, and anxiety disorders, that occur with strong sex bias in humans. As conserved regulators of sexual fate with critical functions in *C. elegans* sexual dimorphism, DM-domain factors could be important components of such a mechanism. Further genetic analysis of sex differences in *C. elegans* neural development and behavior is likely to lead to additional candidates regulating similar processes in much more complicated, less experimentally tractable organisms.

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