ACTIVITY OF THE SEX-DETERMINING GENE *TRA-2* IS MODULATED TO ALLOW SPERMATOGENESIS IN THE *C. ELEGANS* HERMAPHRODITE

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ABSTRACT

In the nematode *C. elegans*, there are two sexes, the self-fertilizing hermaphrodite (*XX*) and the male (*XO*). The hermaphrodite is essentially a female that makes sperm for a brief period before oogenesis. Sex determination in *C. elegans* is controlled by a pathway of autosomal regulatory genes, the state of which is determined by the *X:A* ratio. One of these genes, *tra-2*, is required for hermaphrodite development, but not for male development, because null mutations in *tra-2* masculinize *XX* animals but have no effect on *XO* males. Dominant, gain-of-function *tra-2* mutations have now been isolated that completely feminize the germline of *XX* animals so that they make only oocytes and no sperm and, thus, are female. Most of the *tra-2(dom)* mutations do not correspondingly feminize *XO* animals, so they do not appear to interfere with control by *her-1*, a gene thought to negatively regulate *tra-2* in *XO* animals. Thus, these mutations appear to cause gain of *tra-2* function in the *XX* animal only. Dosage studies indicate that 5 of 7 *tra-2(dom)* alleles are hypomorphic, so they do not simply elevate *XX* *tra-2* activity overall. These properties suggest that in the wild type, *tra-2* activity is under two types of control: (1) in males, it is inactivated by *her-1* to allow male development to occur, and (2) in hermaphrodites, *tra-2* is active but transiently inactivated by another, unknown, regulator to allow hermaphrodite spermatogenesis; this mode of regulation is hindered by the *tra-2(dom)* mutations, thereby resulting in *XX* females.

THE *C. elegans* hermaphrodite is essentially a female that makes sperm. Its soma is female, but its germline can undergo both male and female gametogenesis: first, sperm are made during a brief period at the time of the final molt into adulthood, and then oocytes are made throughout adulthood. The male makes only sperm in a male body.

From previous genetic studies, it is clear that the major developmental decision in sex determination in *C. elegans* is not between male and hermaphrodite development, but between male and female development. In this view, hermaphrodite development represents a modification of this decision by having a limited amount of male germline development, *i.e.*, spermatogenesis, occur in a female soma.

Consistent with this view, it has been shown that three genes that control

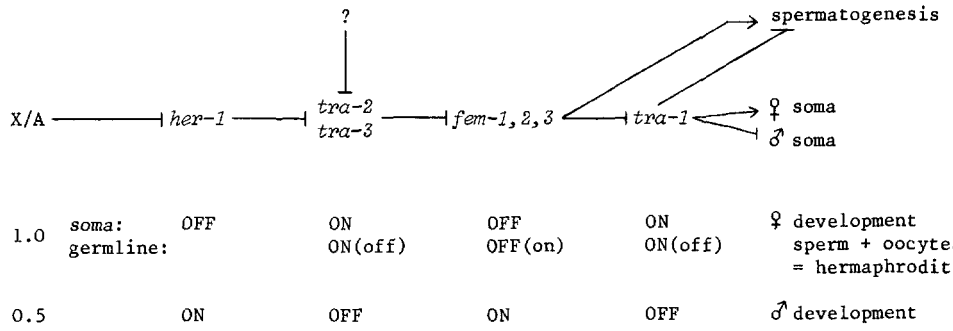

FIGURE 1.—The current simplified model for sex determination in *C. elegans* [see HODGKIN, DONIACH and SHEN (1985) for a more detailed version]. The *X* chromosome to autosome (*X/A*) ratio depicted is the normal diploid case, with *AA;XX* being hermaphrodite and *AA;XO* being male. All interactions between genes in the pathway are negative (⊣, negative effect; →, positive effect); all genes (or their products) are active (*on*) unless inactivated (turned *off*). The negative effect of *tra-1* on spermatogenesis has been inferred from the fact that dominant *tra-1* mutations, thought to result in constitutive *tra-1* expression, produce *XX* and *XO* females, rather than hermaphrodites (HODGKIN 1980). The "?" represents the hypothetical gene that modulates *tra-2* activity to allow sufficient *fem* activity to promote spermatogenesis in hermaphrodites (*XX*).

TABLE 1

Phenotypes of sex-determining mutants

	XX	*XO*
Wild type	Hermaphrodite	Male
her-1(null)	Hermaphrodite	Hermaphrodite
her-1(dom)	Masculinized hermaphrodite	Male
tra-2(null)	Incomplete male	Male
tra-2(dom)	Female	Male
tra-3(null)	Incomplete male	Male
fem-1(null) (*fem-2* and *fem-3* are similar)	Female	Female
tra-1(null)	Male	Male
tra-1(dom)	Female	Female

These phenotypes are summarized from HODGKIN (1980), HODGKIN and BRENNER (1977), TRENT, TSUNG and HORVITZ (1983), DONIACH and HODGKIN (1984) and HODGKIN (1986).

male spermatogenesis and male somatic development (the *fem* genes) also promote spermatogenesis in the hermaphrodite [*fem-1* (NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984), *fem-2* (KIMBLE, EDGAR and HIRSH 1984; HODGKIN 1986) and *fem-3* (HODGKIN 1986)]. This has been inferred from the fact that, if the activity of any of these genes is eliminated, both *XX* and *XO* animals develop as females, *i.e.*, animals with oocytes and no sperm in a female soma. Thus, the *fem* genes can control the decision between male and female development in both the soma and the germline.

It is believed that the *fem* genes form the penultimate step in a regulatory pathway controlling sex determination (schematized in Figure 1). This pathway has been inferred from the properties of mutations in seven autosomal sex-determining genes (summarized in Table 1), and the interactions between them. The genes in the pathway appear to act as binary switches, so the

pathway can exist in two alternative states, determined by the ratio of *X* chromosomes to autosomes (*X:A* ratio): a ratio of 1.0 leads to hermaphrodite development, whereas 0.5 results in male development (NIGON 1949; MADL and HERMAN 1979). Mutations in any of these genes can override the *X:A* ratio. The final step in the pathway is the *tra-1* gene, which acts to promote female somatic development and to inhibit spermatogenesis. Thus, this gene (or its product) is active (*on*) in the *XX* animal and inactive (*off*) in the *XO* animal. This has been inferred from the fact that recessive, lack of function mutations in *tra-1* lead to male somatic development in *XX* animals and have no effect on *XO* animals, while dominant, constitutive mutations result in *XX* and *XO* females (HODGKIN and BRENNER 1977; HODGKIN 1980, 1983). Presumably *tra-1* regulates subordinate genes that carry out sexual differentiation.

According to the model, the reason that the *fem* genes promote male somatic development is because they are "*on*" in *XO* animals and act to turn *tra-1* "*off*." In the *XX* animal, the *fem* genes are turned *off* by the negative action of *tra-2* and *tra-3*, thus allowing female somatic development. However, there must be some *fem* activity in the *XX* animal in order to allow hermaphrodite spermatogenesis. How is this permitted? There are two simple possibilities: (1) an activator of the *fem* genes could override *tra-2* and *tra-3*, or (2) *tra-2* and *tra-3* activity could be modulated (transiently repressed). In this paper, a novel class of *tra-2* mutations is described that has properties supporting the second hypothesis.

MATERIALS AND METHODS

Worms were grown in Petri dishes on nutrient agar seeded with *Escherichia coli* (strain OP50) as described by BRENNER (1974), at 15°, 20°, room temperature (22°) or 25°, as specified in the text.

Strains: The nomenclature used follows HORVITZ *et al.* (1979). Bristol N2 (BRENNER 1974) was used as the standard wild-type strain. The following mutations were used [*dpy* (dumpy), *egl* (egg-laying defective), *fem* (feminization), *fer* (fertilization defective), *her* (hermaphroditization), *mor* (morphological), *sup* (suppressor), *tra* (transformer), *unc* (uncoordinated), *vab* (variable abnormal)]:

Linkage group (LG) II: *tra-2(e1095, e1425)* (recessive, masculinizing alleles), *tra-2(e1939, e1940, e1941, e2019, e2020, e2021, e2046)* (dominant alleles), *vab-9(e1744)*, *fer(e1947)*, *mnC1*, *unc-4(e120)*.

LG III: *dpy-1(e1)*, *tra-1(e1099)* (recessive, masculinizing allele), *tra-1(e1946, e2013, e2014, e1575)* (dominant, feminizing alleles), *eDp6*.

LG IV: *fem-1(e1965)*, *mor-2(e1125)*, *fem-3* (*e1950, e2018*), *tra-3(e1767)*.

LG V: *her-1(e1518)* (recessive, hermaphroditizing allele), *her-1(n695)* (semidominant, masculinizing allele), *unc-42(e270)*, *egl-41(e2055, n1074, n1077)*.

LG X: *unc-7(e5)*, *unc-18(e81)*, *sup-7(st5)*.

These mutations are listed in SWANSON, EDGLEY and RIDDLE (1984), except for alleles of *egl-41* (this paper; C. DESAI, personal communication), *fem-1* (DONIACH and HODGKIN 1984), *fem-3* (HODGKIN 1986), *fer(e1947)* (this paper), *her-1(n695)* (TRENT, TSUNG and HORVITZ 1983), the balancer, *mnC1* (HERMAN 1978), and *tra-2(dom)* (HODGKIN, DONIACH and SHEN 1985; this paper). Phenotypes of the sex-determining mutations are shown in Table 1.

Characterization of sexual phenotype: Sexual phenotype of mutant animals was compared to that of wild type by scoring the morphology of the tail, gonad, vulva, presence or absence of hermaphrodite-specific neurons (HSNs, which are present in the

hermaphrodite but undergo programmed cell-death in the male (SULSTON and HORVITZ 1977)) and type and position of gametes. As shown in Figure 2a, the wild-type hermaphrodite adult has a long, pointed tail (spike) and a gonad that consists of two reflexed arms connected to a uterus that opens ventrally at the vulva, midway down the body. Germ cells undergo maturation in the portion of each arm proximal to the uterus, first forming approximately 170 sperm per arm around the time of the final (L4) molt and then forming oocytes throughout adulthood. The sperm are stored in the spermathecae, and they fertilize oocytes as they pass through to the uterus (for further details, see HIRSH, OPPENHEIM and KLASS 1976). Animals are described as females if they are identical to hermaphrodites but lack sperm. Sperm and oocytes are best defined functionally, but have characteristic appearances using Nomarski optics (see Figures 2 and 3) that serve as criteria for identifying gametes in animals that are unable to produce progeny: oocytes are very large cells that have a grainy cytoplasm and a large, smooth nucleus that contains a relatively large nucleolus when the oocyte is immature. Sperm are small, round and compact, with a tiny, shiny nucleus.

The adult male (Figure 3a) is smaller than the hermaphrodite and has a tail specialized for copulation. The tail structures scored were the shape and size of the sensory rays (of which there are nine pairs), bursal fan and copulatory spicules (for further details, see SULSTON, ALBERTSON and THOMSON 1980). The male gonad consists of a single reflexed arm that is connected via the vas deferens to the cloaca in the tail. In the gonad, spermatogenesis occurs in the germ cells next to the vas deferens, beginning in the L4 and continuing throughout adulthood.

The tail is a sensitive indicator of sexual phenotype and is readily judged at the dissecting microscope level. Degree of *XX* masculinization can range from a slight truncation of the tail spike to a male tail. Conversely, the feminized *XO* tail can range from having a reduced bursal fan size to the absence of a fan and the presence of the spike normally found in hermaphrodites.

Hermaphrodite-specific neurons (HSNs) were scored in young L1 larvae or L3 to L4 larvae using a 100× objective with Nomarski optics (total 1250×). Normally (as described in SULSTON and HORVITZ 1977), in the L1 hermaphrodite the HSNs lie laterally, one on each side, on the same focal plane as, and usually just ventral to, the excretory canal, over the primordial gonad. Their nuclei are round and compact and have a typically neuronal grainy texture. Often the HSN on the far side of the animal was difficult to see and, therefore, was not usually scored. The canal cell nucleus served as a useful marker for the correct focal plane because it has a distinctive oval shape and lies on the same plane as the HSN, just above the excretory canal and usually anterior to the HSN. In the L3/L4, on each side of the animal, the HSN usually lies just posterior to the developing vulva, on the highest focal plane that nuclei are seen, in a similar focal plane to the seam cells. The nucleus is perfectly round, with fine sharp edges and a small nucleolus, and is larger and smoother than in the L1. Its grainy cell body appears to rest on the body wall muscle.

The B blast cell was also scored in L1s as above. This cell gives rise to male-specific lineages in males, but not hermaphrodites, and is often used as an indicator of sex in the newly hatched L1. As described in SULSTON and HORVITZ (1977), the nucleus of this cell is located on a central focal plane immediately posterior to the rectum; it is small, with a small nucleolus in the L1 hermaphrodite, but distinctly larger, with a larger nucleolus in the L1 male.

Isolation of dominant *tra-2* alleles: These mutations were found in a general screen for dominant feminizing mutations. Using a dissecting microscope, females were searched for among the self-progeny (F_1) of hermaphrodites (P_0) that had been mutagenized as L4 larvae with 0.05 M ethyl methanesulfonate (EMS) for 4 hr (BRENNER 1974). For each screen, 5 P_0's per plate (× 24 plates) were transferred daily for 3–4 days at room temperature. The F_1 potential females found in this screen were put on separate plates overnight; those animals that produced no progeny after at least 8 hr were crossed individually with several wild-type (N2) males. Females carrying dominant

feminizing mutations would be expected to produce 50% female F_1 *XX* progeny. To determine this, several (5–20) F_1 *XX* larvae from each cross were placed on a separate plate overnight (to ensure virginity) and were inspected for femaleness. The parent plate was also examined for the presence of intersexual *XO* progeny, which might be expected if the mother were carrying a *tra-1(dom)* mutation.

Mapping: Since it was likely that the dominant feminizing ("*dom*") mutations were alleles of the *tra* genes, the new mutations were tested for linkage to null mutations of *tra-1 III* and *tra-2 II* as follows: (1) one to three F_1 females from each outcrossed female isolate were crossed with either *tra-1 XX* or *tra-2 XO* males (for *tra-1*, *XX* males were obtained from a strain of *tra-1;Dp6*; for *tra-2*, *XO* males were from a strain of *tra-2;tra-1(e1575dom)*/+ ♀ × *tra-2* ♂); (2) the female progeny (*dom*/+;*tra*/+ or *dom*/*tra*) were then crossed again with respective *tra* males, and their progeny were examined for the presence of females and/or hermaphrodites. If the dominant mutation is linked to the *tra* being tested, cross (2) will produce only females and males, and no hermaphrodites. The three *tra-1(dom)* alleles, *tra-1(e1946)*, *tra-1(e2013)* and *tra-1(e2014)*, were mapped in this way to LG III, and the dominant *fer* mutation, *e1947*, to LG II. The *fem-3(e2018)* mutation was shown to be allelic with *fem-3* by its failure to complement *fem-3(e1950)* (data not shown).

By this test, the dominant *tra-2* alleles proved to be unlinked to *tra-1* (data not shown) and linked to *tra-2*. Unexpectedly, five of seven *tra-2(dom)* alleles produced self-fertile intersexual *XX* animals when in *trans* with *tra-2(e1095)* (these were *e1939*, *e1940*, *e1941*, *e2019* and *e2021*). Linkage of these mutations to *tra-2* was demonstrated by the three types of progeny (all *XX*) produced by these intersexes, which were self-fertile: incomplete male (=*tra-2(e1095)*), intersex (=*tra-2(dom)*/(*e1095*)) and female (=*tra-2(dom)*), proving linkage to *tra-2*, because no wild-type hermaphrodites were produced. For *tra-2(e2020)* and *tra-2(e2046)*, the *XX trans* heterozygotes were female, so these were crossed again with *tra-2(e1095)* males. The *XX* progeny from this cross were either female (*tra-2(dom)*/(*e1095*)) or Tra (*tra-2(e1095)*), thus proving linkage to *tra-2* (because no hermaphrodites were produced).

***tra-2(dom)* reversions:** To prove that these dominant mutations are alleles of *tra-2*, several *cis* mutations that eliminate the dominant feminization phenotype and fail to complement *tra-2(1095)* were obtained for three representative alleles, *tra-2(e1940)*, *tra-2(e2020)* and *tra-2(e2046)*.

For *tra-2(e2020)*, this was done by crossing 15–20 *tra-2(dom) vab-9 XO* males [that had been mutagenized with EMS] with eight to 10 *fem-1 mor-2;unc-7 XX* females × 24 plates per screen. These mated females (P_0) were then transferred daily for 3–4 days at room temperature. [*vab-9*, a recessive mutation approximately 1 map unit away from *tra-2*, was used to mark *tra-2(dom)*, and *fem-1* mothers were used because they do not give self-progeny and also reduce the leakiness in self-fertility of the *tra-2(e1940) XX* progeny at 25°; *mor-2*, a recessive mutation *ca.* 0.5 map units away from *fem-1*, was used to distinguish *fem-1* females from *tra-2(dom)* females.]

This procedure selected for revertants of the *tra-2(dom)* phenotype, *i.e.*, self-fertilizing hermaphrodites, because they were the only animals that could propagate under these conditions. [In such crosses, all F_1 progeny were cross-progeny, because the mothers were Fem; of these, all *XO* progeny were Unc because of the maternal *X*-linked *unc* and, therefore, could not mate (except for rare non-Unc males from maternal nullo-*X* ova resulting from *X* nondisjunction); and all *XX* progeny were female and, therefore, gave no progeny, unless they carried a suppressor, in which case they were hermaphrodite.] Two types of suppressor were expected: (1) if the paternal *tra-2(dom)* had been inactivated by a second (loss of function) mutation within the *tra-2* gene (with a recessive incomplete male (Tra) phenotype), or (2) a dominant suppressor of *tra-2(dom)* had been induced in another gene. In those cases where Tra Vab progeny were seen, in order to prove that these recessive mutations were *tra-2* alleles and not some other linked *tra* mutation, complementation tests were done as follows: putative revertant *tra-2 vab-9*/++; *unc-7(e5)* hermaphrodites (which had segregated from the self-progeny of the orig-

inal F_1 hermaphrodite) were crossed with *tra-2(e1095)/mnC1 XO* males [*mnC1* is a balancer for linkage group II (HERMAN 1978)]. If approximately one-quarter of the *XX* cross-progeny (*i.e.*, non-Unc, non-Vab) were Tra-2 *XX* males, then the new mutation was considered to be an allele of *tra-2*. Progeny from this cross were cloned to obtain balanced strains of *tra-2 vab-9/mnC1*.

For *tra-2(e2020)*, eight independent recessive *tra-2* mutations were isolated from approximately 14,000 F_1 *XX* animals (these are *e2047, e2048, e2049, e2050, e2051, e2052, e2053* and *e2054*). In this screen, 12 F_1 hermaphrodites were picked, of which eight carried *cis* recessive *tra-2* mutations; the remaining four gave *XO* male progeny, so these must have been mated by rare non-Unc *XO* brothers.

The other two *tra-2(dom)* alleles, *e1940* and *e2046*, were reverted using the same scheme, except that they were not marked with *vab-9* and that 25° was used for *e1940*. For *tra-2(e1940)*, six independent recessive *tra-2* mutations were isolated from approximately 17,000 F_1 *XX* animals (these are *e2022, e2023, e2024, e2025, e2026* and *e2029*). In this screen, at 25°, a total of 18 F_1 hermaphrodites were picked, of which six carried *cis* recessive *tra-2* mutations. One carried a dominant extragenic suppressor of *tra-2(dom)*, an allele of *egl-41 V, e2055*. The remaining 12 either gave male progeny (*i.e.*, were nonvirgin) or did not breed true (*i.e.*, were the result of the slight leakiness of *tra-2(e1940)*). For *tra-2(e2046)*, five F_1 hermaphrodites were picked from approximately 3000 F_1 *XX* progeny, of which one gave *XO* male progeny and four carried recessive *tra-2* alleles; these are *e2113, e2114, e2115* and *e2116*) (J. HODGKIN, personal communication).

Five of the recessive *tra-2* mutations induced in *cis* to *tra-2(e2020)* (*e2048, e2049, e2050, e2052* and *e2054*), and all four of those in *cis* to *tra-2(e2046)* were tested for suppressibility by *sup-7 X*, an amber suppressor tRNA mutation (WATERSTON 1981; WILLS *et al.* 1983) by constructing *tra-2 vab-9/++;sup-7/+* or *tra-2 unc-4/++;sup-7/+* hermaphrodites and examining their marked (Vab or Unc) self-progeny for suppression of the Tra phenotype.

Mating efficiency: Tests were performed as in HODGKIN, HORVITZ and BRENNER (1979): six late L4 males of a given genotype were crossed with six late L4/young adult *dpy-11(e224)* hermaphrodites for about 24 hr, after which the males were removed and the hermaphrodites were transferred to fresh plates every day until they gave no more cross-progeny (4–5 days). The number of cross-progeny was converted into a percentage of a wild-type value of 2138. Mating efficiencies >50% are considered wild type. It should be noted that mating efficiency specifically measures the physical capability of males to mate, as well as their fertility in the first 24 hr of adulthood. It is not an absolute measure of how wild type given males are, illustrated by the fact that *tra-2(e2020)* animals have apparently wild-type mating efficiency but produce "oocytes" later in adulthood and, thus, are clearly not wild type.

***her-1* constructions:** The *tra-2(e2046)/+;her-1 XO* double mutant was constructed by crossing *her-1 XX* hermaphrodites with *tra-2(e2046) XO* males, crossing the F_1 males with *dpy-1;her-1;unc-7 XX* hermaphrodites (*unc-7* is *X*-linked) and looking at the phenotype of their Unc non-Dpy progeny. These should have been either *her-1/+* or *her-1 XO*, with 50% of them being *tra-2(e2046)/+*. The *tra-2(e2020);her-1* double mutant was made by crossing *tra-2(e2020) vab-9 XO* males with *her-1 unc-42* hermaphrodites, crossing the F_1 non-Unc (outcross) females and males together, and examining the resulting F_2 Vab Unc animals, half of which should have been *XO*.

To construct *tra-2(e2020)/(e1095);her-1(n695)/+* or *tra-2(e2046)/(e1095);her-1(n695)/+ XX* animals, *her-1(n695)* hermaphrodites were crossed with *tra-2(dom)* males, and F_1 females were crossed with *tra-2(e1095) XO* males. The *XX* progeny from this cross were picked onto a separate plate as L4s overnight and, if female, were crossed individually with wild-type (N2) males and, if self-fertile, were cloned on separate plates. The genotypes of these *XX* animals were deduced from the phenotypes of the resulting progeny.

***tra-3* constructions:** *tra-2(dom)/+;tra-3* animals were constructed by (1) crossing *tra-2(dom) XX* females with *tra-3 XO* males, (2) crossing the F_1 females (picked as larvae to

ensure virginity) with *tra-3 XO* males and then (3) crossing the F_1 female from (2) (either *tra-2(dom)/+;tra-3* or *tra-2(dom)/+;tra-3/+*) individually with *tra-3 XO* males. Because *tra-3* has a maternal effect, the *tra-2(dom)/+;tra-3 XX* homozygotes produced in step (2) will not express the *tra-3* mutation. However, all of their progeny will lack *tra-3(+)*; therefore, the *XX* progeny will be incomplete males unless *tra-2(dom)* can promote female development in the absence of *tra-3(+)*.

Because the stronger *tra-2(dom)* alleles, *e2020* and *e2046*, partially suppress *tra-3* mutations, *tra-2(dom)/+;tra-3 XX* animals (obtained by the method outlined above) are often self-fertile intersexes, especially at 15°. This made it possible to make doubly homozygous strains of *tra-2(dom);tra-3* by cloning these intersexes and picking the double homozygotes. For *tra-2(e2020);tra-3*, it was necessary to mark *e2020* with *vab-9 II*; a *tra-2(e2020) vab-9;tra-3* male/female strain was constructed at 15° by crossing *tra-2(e2020) vab-9/++;tra-3 XX* fertile intersexes with males of the same genotype and crossing the F_1 Vab male and female progeny (*i.e.*, the triple homozygotes) together. To obtain non-Vab *tra-2(e2020);tra-3* animals, female progeny from *tra-2(e2020)/+;tra-3* intersexes were crossed individually with *tra-2(e2020) vab-9;tra-3 XO* males, and crosses that produced no Tra animals were taken to be homozygous for *e2020*. For *tra-2(e2046)*, a doubly homozygous *XX* stock was made by cloning self-fertile F_1 *XX* progeny from *tra-2(e2046)/+;tra-3 XX* intersexes and choosing a clone that contained no Tra progeny. Progeny counts were done by cloning L4 *XX* animals on separate plates.

***fem-1;egl-41* construction:** *fem-1 mor-2 XX* females were crossed with *egl-41(e2055) XO* males, F_1 *XX* progeny (*fem-1 mor-2/++;egl-41/+*) were cloned and then their Egl self-progeny (*fem-1 mor-2/++;egl-41* or *++;egl-41*) were cloned. Homozygous *fem-1* animals were identified using the round-head phenotype of *mor-2*. Some of these *XX* animals were self-fertile because of maternal rescue by *fem-1(+)* (DONIACH and HODGKIN 1984); the phenotype of their self-progeny, which lacked *fem-1(+)* product, was scored in order to determine the epistasis of *fem-1* and *egl-41*.

RESULTS

Isolation of *tra-2(dom)*

Dominant *tra-2* mutations were found in a general screen for dominant feminizing mutations [see MATERIALS AND METHODS ("Isolation of dominant *tra-2* alleles") for details]. This involved visually screening for females amongst the self-progeny (F_1) of hermaphrodites that had been mutagenized with EMS. Adult females can be distinguished from hermaphrodites using a dissecting microscope: they lack fertilized eggs, so the uterus is empty and appears clear. Also, the unfertilized oocytes form stacks in the proximal gonad arms, giving a striped appearance to the ventral side of the animal.

The *tra-2(dom)* alleles were found at a frequency of approximately one per 25,000 chromosomes: out of approximately 75,000 F_1 *XX* self-progeny screened (from 390 P_0's), 74 were picked as potential females, of which 50 gave no self-progeny. From these, six independent *tra-2(dom)* alleles were found (*e1939, e1940, e1941, e2019, e2020* and *e2021*). This screen also yielded three *tra-1(dom)* alleles (*e1946, e2013* and *e2014*), one *fem-3* allele (*e2018*) (*fem-3* mutations are slightly semidominant and, therefore, can occasionally result in heterozygous females) and one dominant sperm-defective mutation, *e1947 II*. The remaining 40 candidates were either sterile (producing no self- or cross-progeny) or potential duplicates, which were discarded. The non-*tra-2* mutations will not be discussed further. A seventh *tra-2(dom)* allele, *e2046*, was selected as a suppressor of *tra-3* (HODGKIN 1986).

Demonstration of allelism with *tra-2*

Linkage: Linkage of the *tra-2(dom)* alleles to LG II was demonstrated by showing that all were linked to a putative null mutation of *tra-2, e1095* [see MATERIALS AND METHODS ("Mapping")]. In addition, a three-factor cross with *dpy-10* + *vab-9*/+ *tra-2(dom)* + animals gave both + *tra-2(dom) vab-9*/*dpy-10* + *vab-9* and + + *vab-9*/*dpy-10* + *vab-9* recombinants [using *tra-2(e1940), tra-2(e1941)* or *tra-2(e2020)*], indicating that the *tra-2(dom)* mutations show approximately the same map position as recessive *tra-2* mutations.

***tra-2(dom)* reversions:** It seemed likely that these dominant feminizing mutations on LG II were causing a gain of *tra-2* activity (at least in the *XX* animal), because the feminizing effect they have on *XX* animals is the opposite to that of recessive, loss-of-function mutations in *tra-2*, such as *e1095*, which masculinize *XX* males (HODGKIN and BRENNER 1977). To demonstrate this possibility, it was necessary to show that the dominant feminizing effect of these mutations could be reverted by inactivating the *tra-2* gene in *cis*. This was effectively a *cis-trans* test, in which the *tra-2(dom)* mutations were expected to be suppressed by a *tra-2* loss-of-function mutation in *cis*, but not in *trans*. (This would contrast with an extragenic but linked dominant suppressor of the dominant mutation that should act either in *cis* or in *trans*.)

The reversion procedure was a simple selection for self-fertility of *tra-2(dom)*/ + *XX* animals, normally female, in a situation where no mating could occur [see MATERIALS AND METHODS ("*tra-2(dom)* reversions")]. Revertants were found at a frequency of about 5×10^{-4} per haploid genome for three representative *tra-2(dom)* alleles, *e1940, e2020* and *e2046*. This is similar to the frequency with which null mutations arise at other loci (GREENWALD and HORVITZ 1980). All but one of these revertants were carrying recessive *tra-2* mutations that had been induced in *cis* to the *tra-2(dom)* mutations and failed to complement *tra-2(e1095)*, a lack-of-function allele, demonstrating that the three dominant mutations tested are *tra-2* alleles. The other four mutations, *e1939, e1941, e2019* and *e2021*, are assumed to be *tra-2* alleles also. The remaining revertant was carrying an extragenic suppressor, *egl-41(e2055)*, which will be discussed later.

In summary, these experiments indicate that the *tra-2(dom)* mutations are indeed alleles of *tra-2*, because it was possible to eliminate their effects by inducing recessive *tra-2* mutations in *cis*, resulting in hermaphrodites instead of females. In contrast, the *trans* combination of *tra-2(dom)*/*(null)* does not and results in females or intersexes, as discussed below.

Some of these revertant *tra-2* alleles were tested for suppressibility by *sup-7(st5) X*, an amber suppressor tRNA mutation (WATERSTON 1981; WILLS *et al.* 1983) [MATERIALS AND METHODS ("*tra-2(dom)* reversions")]. None of the five *tra-2(e2020)* revertants tested was suppressed, but two of the four revertants of *tra-2(e2046)* were suppressed, indicating that they are amber mutations. The two amber alleles, *tra-2(e2046 e2115)* and *tra-2(e2046 e2116)*, differ in suppressibility: *e2115* is fairly well suppressed, so that *tra-2(e2046 e2115) XX* homozygotes carrying either one or two doses of *sup-7* are either self-fertile her-

maphrodites or (occasionally) females, instead of incomplete males. The other allele, *e2116*, is only weakly suppressed (J. HODGKIN, personal communication).

General phenotype of *tra-2(dom)* mutants

The *tra-2(dom)* mutations result in *XX* females, when heterozygous (*tra-2(dom)/+*) or homozygous. These mutations appear to feminize the germline of *XX* animals, rather than simply eliminating hermaphrodite spermatogenesis. That is, they appear to transform germline sex: examination of mutant *XX* animals with Nomarski microscopy shows that oogenesis begins around the L4 molt into adult, at the same time and place that spermatogenesis would normally occur in *XX* animals. This germline feminization is comparable to that seen in *fem* mutants (*e.g.*, NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984). In contrast, *XO* animals heterozygous or homozygous for all but one of the *tra-2(dom)* alleles (*tra-2(e2020)*) are apparently wild-type males.

In addition to this general feminization of the *XX* germline, some of these mutations also slightly masculinize the *XX* soma. Results described in the following sections indicate that this is because these alleles are hypomorphic. The different *tra-2(dom)* alleles are phenotypically heterogeneous, and their effects on the soma are not strictly correlated with those on the germline (as presented in more detail below and summarized in Table 2).

Properties of the *tra-2(dom)* alleles

Three properties of each *tra-2(dom)* allele were compared to wild type: (1) their effects on the soma and germline of *XX* and *XO* animals were determined, when homozygous or heterozygous; (2) in order to see whether the level of *tra-2* activity in the *tra-2(dom)* alleles was higher or lower than wild type, the phenotype of a single dose of each allele was compared to that of wild type; (3) in order to determine how the *tra-2(dom)* mutations affect the interactions of *tra-2* with other genes in the sex-determination pathway, the effects of the *tra-2(dom)* alleles were examined in combination with mutations in either *her-1* or *tra-3*.

***XX* phenotype:** All seven of the *tra-2(dom)* alleles produce *XX* females, but their penetrance and expressivity vary, depending on the allele, in both the germline and the soma. This variation gives some clues about the nature of these mutations.

In the germline, five of the alleles are leaky, such that *XX* animals heterozygous or homozygous for many of these alleles sometimes make some sperm and are therefore self-fertile. One of these alleles, *e1941*, is only self-fertile when heterozygous. The remaining two alleles, *tra-2(e2020)* and *tra-2(e2046)*, are exceptional in that they always produce females when heterozygous or homozygous. Table 2, columns A and B, shows the fraction of self-fertile *XX* animals heterozygous and homozygous for each of the alleles.

In the soma, *XX* animals homozygous for some of these alleles sometimes appear to be slightly masculinized. For example, the tail spikes of these animals are occasionally truncated, or snubbed (Table 2, column F). This effect is most often seen with *tra-2(e1939)* and *tra-2(e2021)*, and can be enhanced after star-

TABLE 2

Comparison of *tra-2(dom)* animals with wild type

	Germline: self-fertility						Soma		
	A		B		C		D	E	F
tra-2 allele	*dom/dom* %sf	Average brood when sf (range)	*dom/+* %sf	Average brood when sf (range)	*dom/null* no. sf	Average brood when sf (range)	HSNs: *dom/dom* %HSN(+)	HSNs: *dom/null* %HSN(+)	Tail spike: *dom/dom* %wt
Wild type	100	327 (274–374)	—	—	8/8	354 (288–426)	100	75	100
e2021	5	55 (26–80)	70	156 (46–273)	6/6	82 (13–236)	65	—	3
e1939	23	63 (23–160)	65	148 (17–255)	6/6	135 (11–266)	100	—	13
e1940	7	50 (16–83)	47	140 (50–262)	4/6	73 (42–102)	100	—	100
e1941	0	0	40	47 (15–100)	4/6	209 (87–310)	—	—	100
e2019	3	37	93	157 (17–252)	6/6	259 (237–286)	90	—	100
e2020	0	0	0	0	0/48	0	—	90	100
e2046	0	0	0	0	0/50	0	—	98	100

(Note: *dom* refers to a given *tra-2(dom)* allele; for the wild-type row, substitute + for *dom*.) A, Self-fertility of homozygous *XX* animals, *i.e.*, two doses of a given allele. *Left column*: %sf, percentage of animals that were self-fertile out of total number of animals tested (14 wild type were tested, 30–100 animals tested per *tra-2(dom)* allele) (animals were put on separate plates as larvae); *right column* is the average brood when self-fertile, with the range of brood sizes in brackets below. B, Self-fertility of heterozygous *XX* animals, *i.e.*, *tra-2(dom)/+*; same conventions as in A (20–100 animals tested per allele). C, Self-fertility of *XX* animals carrying one dose of a given allele, *e.g.*, *tra-2(dom)/(e1095)*. *Left column*: no. sf, number of animals that were self-fertile out of the total number tested; *right column*, as in A. D, Percentage of homozygous *XX* animals (*i.e.*, with two doses of a given allele) that had HSNs, scoring L1's (10–20 animals scored per allele). [See MATERIALS AND METHODS "Characterization of sexual phenotype" for HSN scoring]. E, Percentage of *XX* animals with one dose of a given allele that had HSNs, scored in L4's (10–19 animals scored per allele). F, Percentage of *XX* animals (two doses) with wild-type tail spikes; the remaining animals had tails that were snubbed to varying extents (20–45 animals scored per allele).

— = Not scored.

vation (Figure 2c). In addition, some animals show an egg-retaining (Egl) phenotype, most obviously seen in those animals that are self-fertile. This phenotype has been correlated with absent or malfunctioning hermaphrodite-specific neurons (HSNs) (TRENT, TSUNG and HORVITZ 1983); these neurons normally innervate the egg-laying muscles in the hermaphrodite, but undergo programmed cell-death in the male (SULSTON and HORVITZ 1977). Indeed, a significant portion of *tra-2(e2021)* *XX* animals lacked HSNs (Table 2, column D). Also, a proportion of *e1939* (four of 10) and most *e2021* (15 of 19) *XX* animals have enlarged B cells in the L1, indicating further masculinization of the soma [see MATERIALS AND METHODS ("Characterization of sexual phenotype") for HSN and B scoring]. These masculinization effects suggest that *tra-2* activity of these alleles is slightly too low to promote normal female somatic development, *i.e.*, lower than wild-type *tra-2*, or hypomorphic.

The interpretation that these mutations are hypomorphic is supported by two additional pieces of evidence: (1) the fact that *recessive* hypomorphic alleles, such as *tra-2(e1875)* and *tra-2(e1403)*, also produce *XX* animals with snub tails and egg-laying defects (TRENT, TSUNG and HORVITZ 1983; J. HODGKIN, personal communication) and (2) the results of the dosage experiments presented below.

Dosage effects: phenotype of *tra-2(dom)/(null)*: In order to see if the *tra-2(dom)* mutations alter the level of *tra-2* activity compared to wild type, the phenotype of the soma and germline of animals carrying a single dose of each allele was compared to that of wild type. This was done by making *trans* heterozygotes of each allele with *tra-2(e1095)* or *tra-2(e1425)*, putative null alleles (*e1425* is an amber allele of *tra-2*).

The wild-type *tra-2* gene itself is slightly haplo-insufficient in the soma: a proportion of *XX* animals carrying a single dose of *tra-2(+)* (*e.g.*, *tra-2(e1095)/+*) are slightly masculinized, such that the hermaphrodite-specific neurons (HSNs) sometimes die or are nonfunctional, resulting in an egg-retaining (Egl) phenotype (TRENT, TSUNG and HORVITZ 1983 and Table 2, column E). The germline of *tra-2(e1095)/+* *XX* animals appears to be slightly masculinized, as judged by a slight increase in self-fertility (Table 2, column C), but this increase is not statistically significant, given the small sample size (*t*-test).

In the germline, *XX* animals carrying a single dose of either *tra-2(e2020)* or *tra-2(e2046)* are equivalent to those with two doses of these alleles: these animals are always female (Table 2, column C). In the soma, these females have pointed tail spikes and generally show normal egg laying (when mated), and a higher proportion of these animals possess HSNs than do *tra-2(e1095)/+* hermaphrodites (Table 2, column E). Thus, a single dose of either of these alleles is as good, or better, at promoting female somatic development, including the survival of HSNs, than is a single dose of *tra-2(+)*. This suggests that the level of *tra-2* activity of these alleles may be higher than that of wild type (*i.e.*, they may be hypermorphic alleles).

In contrast, lowering the dosage of the other five *tra-2(dom)* alleles results in a strong somatic masculinization of *XX* animals: *XX trans* heterozygotes of *e1939*, *e1940*, *e1941*, *e2019* and *e2021* with *e1095* are somatically intersexual

(see Figure 2d for *tra-2(e1939)/(e1095) XX*). The extent of masculinization depends on the allele, although the expression of this masculinization phenotype is somewhat variable for a given allele. For example, *tra-2(e2019)/(e1095) XX* animals are only slightly masculinized, with 30% having wild-type tail spikes (n = 98) and the rest having slightly snubbed tails, whereas 100% of *tra-2(e2021)/(1095) XX* animals have masculinized tails. Nevertheless, it is possible to rank the phenotypes of the *trans* heterozygotes in order of increasing somatic masculinization as follows: *e2019* < *e1941* < *e1940* < *e1939* < *e2021* (data not shown). This masculinization effect indicates that a single dose of any of these alleles is less able to promote normal female somatic development than the wild-type *tra-2* allele. The simplest interpretation of this effect is that *tra-2* activity in these five *tra-2(dom)* alleles is lower than the wild-type level, *i.e.*, they are hypomorphic. In addition, the germline of these animals is also masculinized, because they produce sperm as well as oocytes and, thus, are self-fertile (Table 2, column C). Therefore, lowering the level of *tra-2* activity in these animals permits some spermatogenesis.

In summary, the results of the dosage experiments presented above indicate that five of seven *tra-2(dom)* alleles are hypomorphic and two of seven may be hypermorphic.

***XO* phenotype:** In contrast to *XX* animals, *XO* animals heterozygous and homozygous for all the *tra-2(dom)* alleles produce sperm. In fact, they are fertile males, as shown by the fact that strains consisting of homozygous *tra-2(dom)* males and females have been constructed and propagated for all seven alleles. In addition to being fertile, *XO* males homozygous and heterozygous for all of the alleles except for *tra-2(e2020)* are morphologically wild type (as judged by using Nomarski microscopy). Mating efficiency is also essentially wild type, ranging between 53–93% of wild type (MATERIALS AND METHODS). Thus, it appears that *XO* animals are unaffected by these mutations.

In contrast to the other alleles, although homozygous *tra-2(e2020) XO* animals can be fertile males as young adults and even have wild-type mating efficiency (80% of wild type), they can produce oocyte-like gametes when they become older (Figure 3b). This apparent feminization is not restricted to the germline, because these animals also produce yolk. Yolk is normally synthesized in the intestine, a somatic tissue (KIMBLE and SHARROCK 1983), by hermaphrodites but not males (KLASS, WOLF and HIRSH 1979). It is released from the intestine into the pseudocoelom and is taken up by the gonad for oogenesis. Thus, unincorporated yolk can be present in the pseudocoelom and can be seen using Nomarski microscopy. Yolk can also be detected using gel electrophoresis of whole worm extracts (KLASS, WOLF and HIRSH 1979; SHARROCK 1983). Using both of these methods, yolk was found in *tra-2(e2020) XO* animals, but was not detected in *tra-2(e1940)* or *tra-2(e2046) XO* animals (Figures 3b and 4).

In addition, the tails of *tra-2(e2020) XO* animals can be "feminized" to varying extents after recovery from starvation. This apparent feminization ranges from a reduced bursal fan size to the complete absence of a fan and the presence of a pointed tail spike characteristic of a female soma. Curiously, the

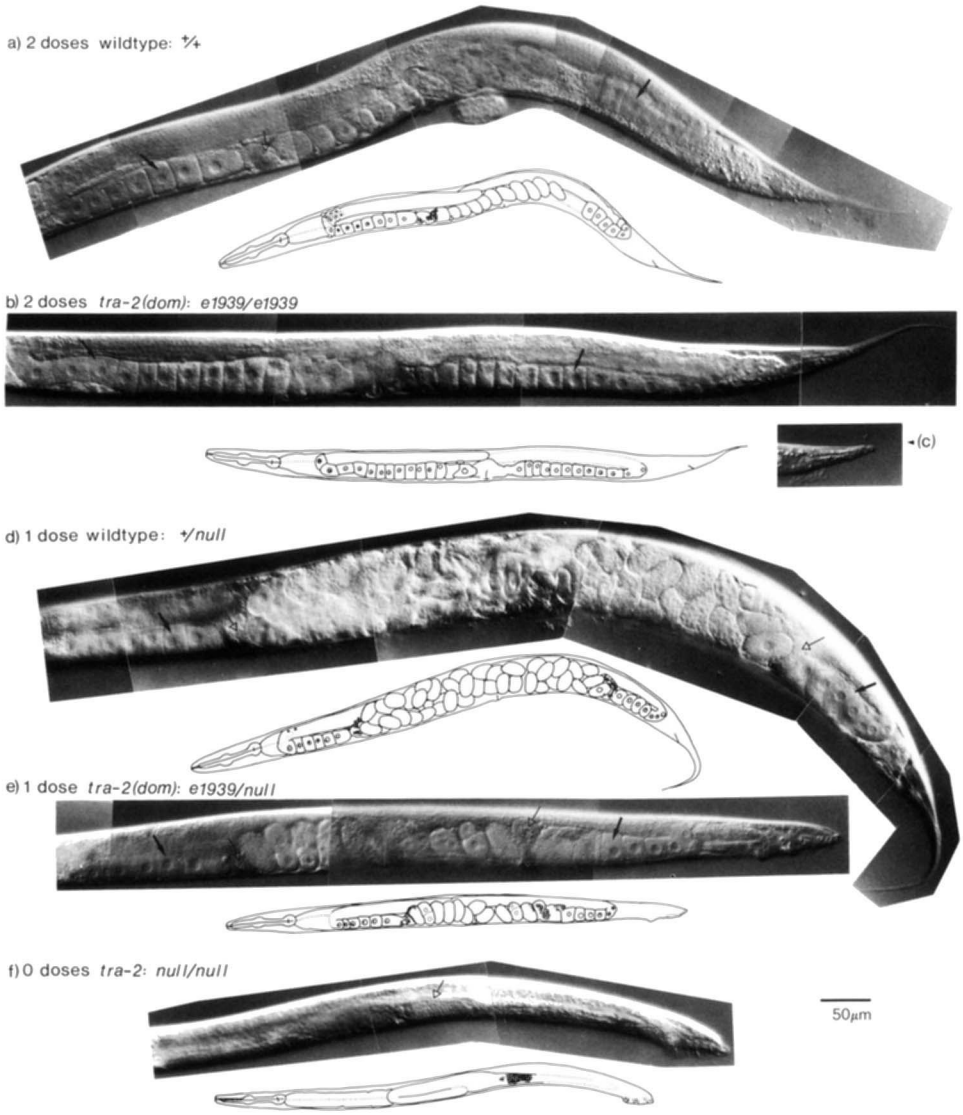

FIGURE 2.—Effects of varying gene dosage of *tra-2*(+) and the putative hypomorphic allele, *tra-2*(*e1939*) in the *XX* animal. ➡, oocytes; ▷, sperm. a, Two doses of *tra-2*(+) = wild-type hermaphrodite: note a pointed tail spike, a gonad consisting of two reflexed arms (posterior arm is partly obscured by intestine, see tracing), oocytes, sperm and fertilized eggs and normal egg-laying ability (with functional HSNs). b, Two doses of *tra-2*(*e1939*) = female: note two-armed gonad containing oocytes and no sperm; pointed tail is only present in about 13% of the animals (Table 2). c, Truncated tail of a *tra-2*(*e1939*) animal, indicating insufficient *tra-2* activity for wild-type development. d, One dose of *tra-2*(+), *i.e.*, *tra-2*(*e1095*)/+ = Egl hermaphrodite: *tra-2*(*e1095*) is a putative null allele and, thus, is assumed to be equivalent to a deficiency of *tra-2*. Note pointed tail spike, two-armed gonad, oocytes, sperm and fertilized eggs; the animal is impaired in egg laying because of defective or absent HSNs (Table 2). e, One dose of *tra-2*(*e1939*), *i.e.*, *tra-2*(*e1939*)/(*e1095*) = self-fertile intersex: note masculinized tail, two-armed gonad, eggs, sperm and fertilized eggs. f, Zero doses of *tra-2*, *i.e.*, *tra-2*(*e1095*) = incomplete male: note strongly masculinized tail and a one-armed, male-shaped gonad that produces only sperm. All animals are adult, viewed laterally with anterior to the left with heads removed, and are shown at the same scale, although the animal in d is older than the others and, thus, is slightly larger. The line drawings are simplified tracings of the photographs (including the heads) to illustrate morphology and gamete type and are all the same scale. The photographs were taken using Nomarski optics and a 40× objective, as in DONIACH and HODGKIN (1984).

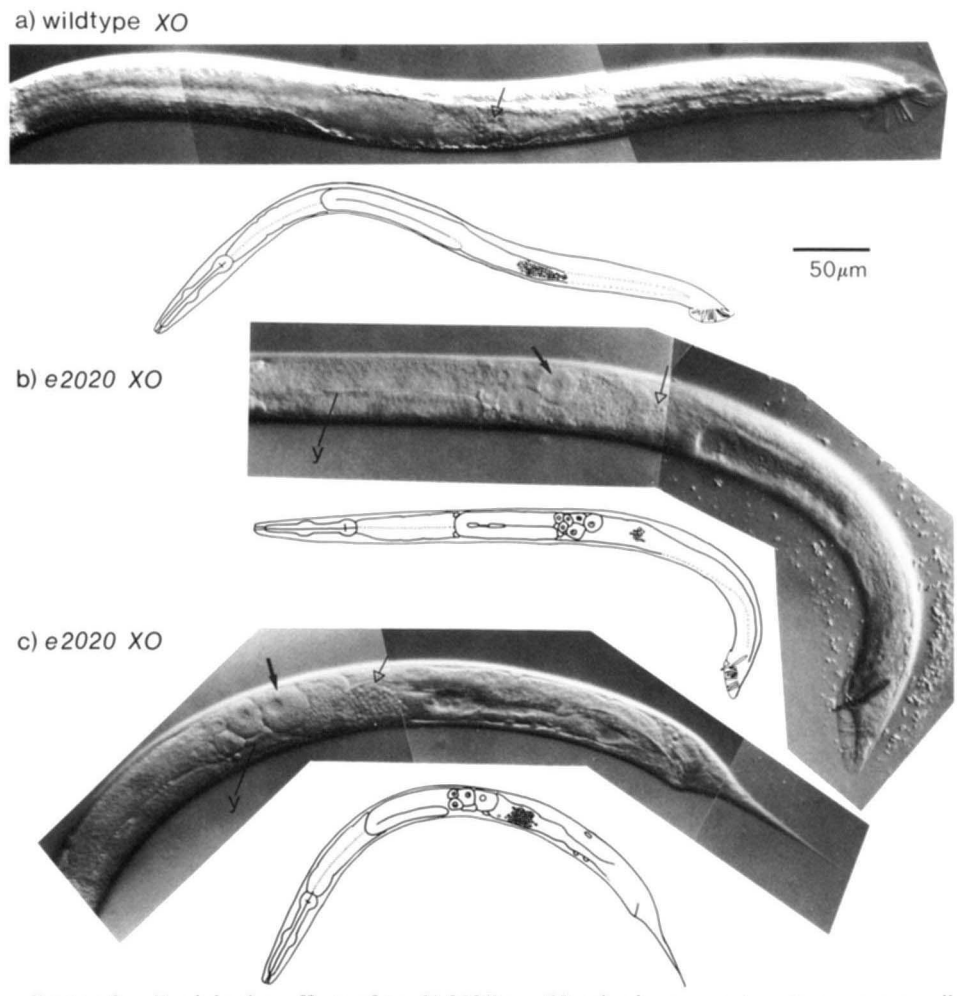

FIGURE 3.—Feminization effects of *tra-2(e2020)* on *XO* animals: ➡, oocytes; ▷, sperm; y, yolk. Same presentation as in Figure 2. a, Wild-type *XO* male: note shape of tail (see tracing), the absence of yolk in the pseudocoelom, gonad with one reflexed arm producing only sperm. b, *tra-2(e2020) XO* male, unstarved as a larva: note wild-type male tail and gonad, producing sperm and "oocytes"; such animals are cross-fertile when young, but never show self-progeny. Yolk is present in the pseudocoelom. c, *tra-2(e2020) XO* animal, recovered from starvation: note spike tail, characteristic of a hermaphrodite, male-shaped gonad, producing sperm and "oocytes," and yolk in the pseudocoelom.

whole soma is not uniformly feminized; although such animals can have female tails and produce yolk, they also have rudimentary spicules (normally present in the male tail) and always have male-shaped gonads (Figure 3c). Like the unstarved *e2020 XO* animals, the germline of these animals is also apparently feminized, making first sperm and then "oocytes". These results indicate that *tra-2(e2020)* causes some *tra-2* activity in the *XO* animal, where it is normally inactive. It suggests that this allele is partially insensitive to the negative control of *tra-2* activity by *her-1*.

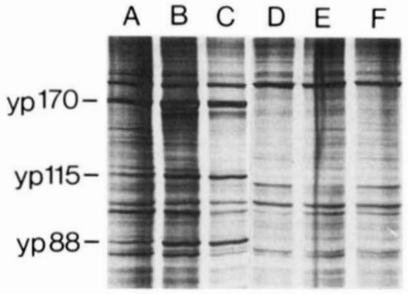

FIGURE 4.—Yolk proteins in *tra-2(dom) XO* animals compared to wild type (as in DONIACH and HODGKIN 1984): 7.5% SDS-PAGE of whole older adult worms (SHARROCK 1983), with 15–30 worms per lane, and silver-stained (OAKLEY, KIRSCH and MORRIS 1980). The three yolk proteins are labeled according to their molecular weights (SHARROCK 1983). A, Wild-type hermaphrodite (*XX*); B, *tra-2(e2020) XX* female; C, *tra-2(e2020) XO* animal (unstarved); D, wild-type male(*XO*); E, *tra-2(e2046) XO* male; F, *tra-2(e1940) XO* male.

The fact that *tra-2(e2020)* affects *XO* animals made it possible to look at the effect of a single dose of this allele in *XO* animals. In contrast to *tra-2(e2020) XO* homozygotes, *tra-2(e2020)/(e1095) XO* animals do not make oocytes (even when old) (zero of 37 had "oocytes," and three had some yolk, compared with *tra-2(e2020) XO*: ten of 32 had "oocytes," and 32 of 32 had yolk, as judged by Nomarski microscopy). Further, if the hypothesis that *tra-2*(+) is normally *off* in the *XO* animal is correct, then *tra-2(e2020)/+ XO* animals should have the same phenotype as *tra-2(e2020)/(e1095) XO* animals. This was the case (zero of 36 had oocytes, one of these had yolk).

In summary, under these conditions, six of seven *tra-2(dom)* alleles show no effects on *XO* males, but one of them, *tra-2(e2020)*, can partially feminize the germline and soma of *XO* males.

Interactions with *her-1*: *her-1* is thought to promote male development by turning off *tra-2* and/or *tra-3* (Figure 1; HODGKIN 1980): in the absence of *her-1*(+) activity, both *XX* and *XO* animals are hermaphrodite, and null mutations in *tra-2* and *tra-3* are epistatic to *her-1* mutations (HODGKIN 1980). Therefore, *tra-2(dom)* mutations are also expected to be epistatic to *her-1* mutations. If this is the case, then *tra-2(dom)/+;her-1 XO* animals should be female instead of hermaphrodite.

Two alleles, *e2046* and *e2020*, were tested. In one experiment, in which half of the *her-1(e1518);unc-7/0 XO* progeny were expected to be *tra-2(e2046)/+*, nine of 16 animals were female and the remaining seven of 16 were hermaphrodite; in another experiment, in which half of the *tra-2(e2020);her-1(e1518)* animals were expected to be *XO*, all were female (11 of 11 animals) [see MATERIALS AND METHODS ("*her-1* constructions") for constructions]. Thus, it appears that these *tra-2(dom)* alleles feminize the germline of *her-1 XO* animals.

These experiments also revealed that *her-1* can be haplo-insufficient under some conditions: *tra-2(e2020)/+;her-1(e1518)/+ XO* animals (16 of 16) make yolk and oocytes as well as sperm, and some of these animals appear to be partially feminized (seven of 16 had stunted bursal rays, a potential sign of feminization). This contrasts with *tra-2(e2020)/+ XO* animals (*i.e.*, in a *her-1*(+)

background), which are essentially normal males (this paper), as are *her-1(e1518)/+ XO* animals. This *her-1* haplo-insufficiency is also revealed with *tra-2(e2046)*, so that *tra-2(e2046)/+;her-1(e1518)/+ XO* animals often make oocytes and yolk (15 of 19 had oocytes, and all had yolk), although they are morphologically male. This result also indicates that *tra-2(e2046)* is partially insensitive to *her-1* negative control, but to a lesser extent than *tra-2(e2020)*, because *tra-2(e2046)* has no effect on *XO* animals in a *her-1(+)* background.

The effect of the dominant *her-1* allele, *her-1(n695)*, on *tra-2(dom)* was also examined. This mutation is thought to cause partial gain of *her-1* function, and results in semidominant masculinization of *XX* animals, varying from the absence of HSNs to masculinized tails and defective vulvae (TRENT, TSUNG and HORVITZ 1983). The putative hypomorphic *tra-2(dom)* alleles are strongly masculinized by *her-1(n695)*: doubly heterozygous *XX* animals of *tra-2(e1940)/+;her-1(n695)/+* are more masculinized than *her-1(n695)/+ XX* animals and resemble *tra-2(e1940)/(e1095) XX* animals (*i.e.*, they are self-fertile and have truncated or intersexual tails). This is consistent with the interpretation that *tra-2(e1940)* is a hypomorphic allele. Other putative hypomorphic alleles show similar responses, the relative extent of masculinization being consistent with the pattern seen in the dosage experiments.

In contrast, the two stronger alleles, *tra-2(e2020)* and *tra-2(e2046)*, are not affected by *her-1(n695)*, even when present in a single dose, so that *tra-2(e2020)/(e1095);her-1(n695)/+* and *tra-2(e2046)/(e1095);her-1(n695)/+ XX* animals are female in the soma and the germline, whereas *tra-2(e1095)/+;her-1(n695)/+ XX* animals are strongly masculinized.

Interaction of *tra-2(dom)* with *tra-3*: *tra-2* and *tra-3* are thought to promote female development by acting together to turn off the *fem* genes (Figure 1) (DONIACH and HODGKIN 1984). Neither alone is sufficient to do this, because when either is inactivated by mutation, *XX* animals are masculinized (HODGKIN and BRENNER 1977). There is evidence that suggests that *tra-3* plays a less important role than *tra-2* in female development (HODGKIN 1980). For example, *tra-3 XX* males are less masculine than *tra-2 XX* males, but the *tra-2;tra-3 XX* double mutant has a *tra-2* phenotype, indicating that *tra-2* can promote some female development independently of *tra-3*, but not vice versa. In addition, all *tra-3* alleles (three amber and one nonamber putative nulls) are slightly rescued at low growth temperatures (15° or less), so that about 20% of these *tra-3 XX* animals are slightly self-fertile (average brood is 17 progeny), although these animals are somatically intersexual (HODGKIN 1985). Two reasons could explain this rescue: either *tra-3* is partly dispensable under these conditions, or there is residual *tra-3* activity at low temperatures. The latter possibility is less likely: in order for there to be residual *tra-3* activity, the three independent amber mutations and the nonamber mutation would all have to result in the same degree of partial activity (because they all show the same phenotype) (J. HODGKIN, personal communication).

In any case, it was of interest to know whether the *tra-2(dom)* mutations improve *tra-2*'s ability to promote female development in the absence of nor-

mal *tra-3* function. If this were the case, then one would expect *tra-2(dom) XX* animals that lack *tra-3*(+) to be female, instead of male.

Two putative hypomorphic alleles, *tra-2(e1940)* and *tra-2(e1939)*, and the two stronger alleles, *tra-2(e2020)* and *tra-2(e2046)*, were tested [see MATERIALS AND METHODS ("*tra-3* constructions") for constructions]. At 20°, *tra-2(e1940)* did not suppress *tra-3* in the soma, and it had no significant effect on the germline: all *XX* animals examined were Tra; half of these should have been *tra-2(dom)/+;tra-3* and the other half *tra-3*. In the germline, for *tra-2(e1940)*, four of 21 *XX* animals had oocytes, compared to three of 31 for *tra-3* alone. Similar results were seen with *tra-2(e1939)* (not shown). In conclusion, *tra-2(e1940)* and *tra-2(e1939)* did not appear to suppress *tra-3 XX* animals in the soma or the germline.

In contrast, *tra-3* was significantly suppressed by *tra-2(e2020)* and *tra-2(e2046)* in the soma and the germline. When homozygous, *tra-2(e2020)* strongly suppresses *tra-3*, so that at 15°, *tra-2(e2020);tra-3 XX* animals are often almost female in the soma, with truncated tails but otherwise female bodies (28 of 29 had snub tails). In the germline, they are often female (and cross-fertile) (16 of 29 were female), sometimes sterile (11 of 29, *i.e.*, neither self- nor cross-fertile) and rarely self-fertile (two of 29, giving seven and 11 progeny, respectively). At 20°, these animals are sometimes fertile, but they are more intersexual in the soma, although they are more feminine than *tra-3 XX* animals. Further, *tra-2(e2020)* is a dominant suppressor of *tra-3*, so that at 15° and 20°, *tra-2(e2020)/+;tra-3 XX* animals are more somatically feminine than are *tra-3 XX* animals and can produce self- and/or cross-progeny (four of 30 were female, 18 of 30 were self-fertile and eight of 30 were sterile at 15°). The other potentially hypermorphic allele, *tra-2(e2046)*, also suppresses *tra-3* in the soma and germline (this allele was selected as a suppressor of *tra-3*). However, although the *XX* double homozygotes resemble *tra-2(e2020);tra-3 XX* animals in the soma at 15°, *tra-2(e2046);tra-3(e1767) XX* animals are often self-fertile (at 15°, 24 of 34 were self-fertile, averaging 38 progeny, with a range of nine to 102 progeny, and the rest (ten of 34) were sterile. These double homozygotes are also self-fertile at 20° (not shown). In conclusion, since the self-fertility of *tra-3 XX* animals at 15° is 20%, with an average brood size of 17, these data show that *tra-2(e2020)* and *tra-2(e2046)* significantly suppress the germline phenotype of *tra-3*, in addition to suppressing the somatic phenotype to a large extent. These results support the idea that the two mutations *e2020* and *e2046* have increased the ability of *tra-2* to function independently of *tra-3*(+).

In sum, all of these results indicate that (1) *tra-2(dom)* mutations feminize the germline of *XX* animals, with varying degrees of expressivity and penetrance; (2) two of seven alleles may raise the level of *tra-2* expression in the *XX* animal [*tra-2(e2020)* and *tra-2(e2046)*], whereas five appear to lower it [*tra-2(e1939)*, *tra-2(e1940)*, *tra-2(e1941)*, *tra-2(e2019)* and *tra-2(e2021)*]; and (3) five alleles show no effects in *XO* animals, whereas two [*tra-2(e2020)* and *tra-2(e2046)*] can feminize *XO* animals under some conditions.

egl-41, a dominant suppressor of *tra-2(dom)* hypomorphs

The selection for recessive *tra-2* mutations in *cis* with *tra-2(dom)* [MATERIALS AND METHODS ("*tra-2(dom)* reversions")] was also designed to yield dominant suppressors of *tra-2* that might identify new genes that regulate *tra-2*. In this selection, an allele of *egl-41*, *e2055*, was found as a dominant suppressor of *tra-2(e1940)*. Alone, *egl-41* mutations are semidominant and appear to have weak masculinizing effects on *XX* animals, such as the death of the HSNs, resulting in an egg-retaining (Egl) phenotype, and the enlargement or division of B and Y cells, blast cells that divide in males but not hermaphrodites [as also seen with the other three alleles, *n1069*, *n1074* and *n1077* (CHAND DESAI, personal communication)]. These masculinizing effects may explain why *egl-41* mutations act as suppressors of hypomorphic *tra-2(dom)* alleles, perhaps in a similar way that lowering the activity in the hypomorphs results in self-fertility [*e.g.*, *dom/null XX* animals are self-fertile (this paper)]. For example, *tra-2(e1940)/+;egl-41(e2055)/+ XX* animals are self-fertile, Egl hermaphrodites. Further masculinization is seen in *tra-2(e1940);egl-41(e2055)/+ XX* animals, which are self-fertile intersexes, and in *e1940;e2055 XX* animals, which are severely intersexual and usually sterile. Other hypomorphic *tra-2(dom)* alleles tested (*e1939* and *e1941*) gave similar results, with degrees of intersexuality that are consistent with their putative degrees of hypomorphy. Also, two of the other *egl-41* alleles (*n1074* and *n1077*) have similar effects on these alleles. However, the strong allele, *tra-2(e2020)*, is not made self-fertile by *egl-41(e2055)*, consistent with the idea that this allele is not hypomorphic. Finally, *fem-1* null mutations are epistatic to *egl-41(e2055)*, so that *fem-1(e1965);egl-41(e2055)* animals have HSNs, indicating that *egl-41* acts upstream from *fem-1* in the sex-determination pathway (HODGKIN, DONIACH and SHEN 1985) (see MATERIALS AND METHODS for construction). However, as yet, the null phenotype of *egl-41* and its role in sex determination remain unclear.

DISCUSSION

This paper describes seven dominant alleles of *tra-2* that eliminate spermatogenesis in *XX* animals, causing them to develop as females instead of hermaphrodites. These mutations are significant because they imply that *tra-2* plays a role in controlling spermatogenesis in the hermaphrodite.

The properties of the *tra-2(dom)* mutations are consistent with the model for sex determination proposed previously (DONIACH and HODGKIN 1984), but they also appear to clarify an inconsistency: according to the model, activity of the *fem* genes is required for spermatogenesis in *XX* hermaphrodites as well as *XO* males. However, in the model, the *tra-2* and *tra-3* genes act to prevent *fem* activity in the *XX* animal. The properties of the *tra-2(dom)* mutations appear to explain this contradiction, by indicating that *tra-2* activity is normally modulated (transiently repressed) to allow the *fem* genes to promote spermatogenesis in the hermaphrodite (Figure 1).

The following observations and arguments have led to this conclusion: *tra-2(dom)* mutations are dominant and have a feminizing effect, the opposite

phenotype to that of loss-of-function *tra-2* mutations; therefore, they are likely to be gain-of-function mutations. The simplest type of gain-of-function mutation in *tra-2* would be one that causes constitutive *tra-2* activity in both *XX* and *XO* animals; such a mutation might cause dominant feminization of *XO* animals, in the same way that *tra-1(dom)* mutations do (HODGKIN 1983). However, this is not the case here, because the majority of *tra-2(dom)* mutations have no effect on *XO* animals. Thus, they appear to cause gain of function in *XX* animals only, and so do not interfere with regulation by *her-1* (Figure 1). Furthermore, *tra-2(dom)* mutations do not appear to create a new type of *tra-2* function, because they promote the same processes as wild-type *tra-2*; namely, oogenesis and female somatic development. Finally, the phenotypes of the *tra-2(dom)* mutations are not explained by an *overall* increase in the level of *tra-2* activity in the *XX* animal, because five of seven alleles appear to reduce the overall levels of *tra-2* activity compared to wild type. Instead, the following model seems plausible: there is normally a time and/or place in the *XX* animal when *tra-2* is inactive, allowing sperm production; the *tra-2(dom)* mutations prevent this inactivation, and the resulting constant *tra-2* activity eliminates hermaphrodite spermatogenesis, by preventing *fem* activity in the *XX* animal.

How is *tra-2* activity modulated in the wild type? In the proposed model, there are two basic requirements for the modulation of *tra-2* activity to allow hermaphrodite spermatogenesis. First, *tra-2* activity must be turned *off*, or reduced below a threshold at an appropriate time and/or place, to allow the *fem* genes to promote spermatogenesis before oogenesis. Temperature shift experiments with recessive temperature-sensitive (*ts*) alleles of *fem-1(hc17)* and *fem-2(b245)* indicate that the *fem* activity is required approximately 24 hr before hermaphrodite spermatogenesis begins, roughly corresponding to the second larval stage (L2) (assuming that these *ts* periods represent time of function) (NELSON, LEW and WARD 1978; KIMBLE, EDGAR and HIRSH 1984). This window of *fem* requirement, which should correspond roughly to the period of *tra-2* modulation, will be referred to as the sperm critical period (SCP). In addition, temperature shift experiments using a recessive *ts* allele of *tra-2, b202*, indicate that *tra-2* activity is required from the end of the L2 to the adult for normal oogenesis (KLASS, WOLF and HIRSH 1976); this suggests that *tra-2* modulation should be over by the end of the L2.

Second, *tra-2* modulation must not interfere with female somatic development. Temperature shift experiments with *tra-2(b202)* indicate that *tra-2* activity is required throughout larval development for proper female somatic development to occur (KLASS, WOLF and HIRSH 1976). This suggests that either *tra-2* modulation occurs only in the germline or that a brief modulation during the L2 does not interfere with female somatic development.

Thus, in keeping with the requirements discussed above, there are four ways that *tra-2* activity could be modulated, three of which are illustrated in Figure 5 as graphs of hypothetical *tra-2* activity during development: (1) *tra-2* could be turned *off* briefly during the SCP in both the soma and the germline (without interfering with female somatic development); (2) it could be turned *off* briefly during the SCP in the germline only; (3) it could be *off* in the

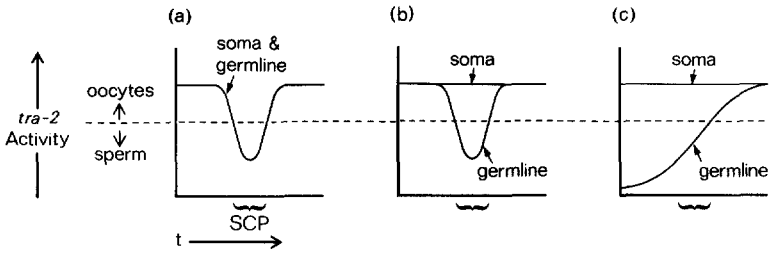

FIGURE 5.—Three models for *tra-2* modulation. Vertical axes = *tra-2* activity, horizontal axes = time; the dashed line represents the hypothetical threshold for the level of *tra-2* activity below which spermatogenesis occurs and above which, oogenesis. SCP = sperm critical period, representing the time during which *tra-2* activity must be below the sperm/oocyte threshold in order to allow the *fem* genes to promote correct hermaphrodite spermatogenesis (temperature shift experiments with the *fem* genes indicate that this period occurs around the L2 (NELSON, LEW and WARD 1978; KIMBLE, EDGAR and HIRSH 1984). a, Modulation of *tra-2* activity in both soma and germline; b, modulation of *tra-2* activity in the germline only, constant activity in the soma; c, slow increase of *tra-2* activity in the germline, constant activity in the soma.

germline alone until after the SCP; or (4) it could be *off* in only those germ cells that will become sperm.

It should be noted here that, although it is clear that the sex-determining genes can control germline sex, it is not clear whether they do so from within the germline itself or from within the soma. However, to simplify the discussion, gene activity that controls germline sex will be referred to as acting *in* the germline, and that which controls somatic sex as acting *in* the soma.

Interpretation of the *tra-2(dom)* mutations: The properties of the *tra-2(dom)* mutants are consistent with any of the models discussed above. For simplicity, the first model (Figure 5a) will be used to illustrate an interpretation of how the *tra-2(dom)* mutations alter *tra-2* activity in *XX* animals, as shown in Figure 6.

Figure 6a shows the hypothetical level of *tra-2* activity for one and two doses of wild-type *tra-2*. With two doses, there is sufficient *tra-2* activity to promote normal female somatic development, and *tra-2* activity falls below the sperm/oocyte threshold during the SCP. With one dose of wild-type *tra-2*, the overall level is lower and there is a slight masculinization of the soma (*e.g.*, death of HSNs) but essentially normal hermaphrodite spermatogenesis.

With the graphs in Figure 6, it is easy to see how the *tra-2(dom)* mutations might affect *tra-2* activity in the *XX* animal, using *tra-2(e1940)*, *tra-2(e2019)* and *tra-(e2020)* as examples. With two doses of the *tra-2(dom)* alleles, *tra-2* activity is always above the sperm/oocyte threshold, preventing spermatogenesis. It is also generally sufficient to promote female somatic development. However, the phenotypes of animals carrying single doses of these alleles indicate that these mutations alter the overall level of *tra-2* activity. Single doses of five alleles show insufficient activity for normal female somatic development, and they result in masculinized *XX* animals. This suggests that these alleles lower the level of *tra-2* activity overall, besides interfering with modulation [*i.e.*, they are hypomorphic (these are *e1939*, *e1940*, *e1941*, *e2019* and *e2021*)]. It is inferred that the greater the masculinization, the lower the level

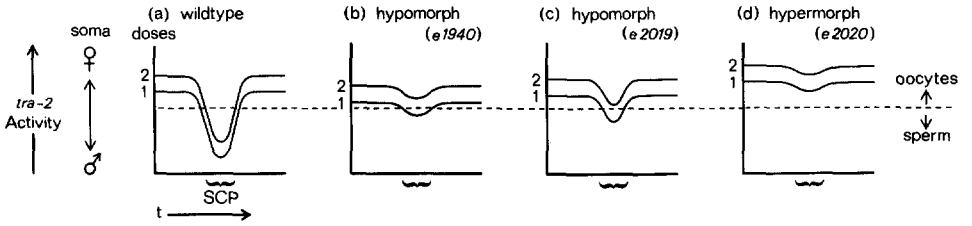

FIGURE 6.—Interpretation of the *tra-2(dom)* mutants in *XX* animals, including dosage effects (refer to Figure 2 for photographs of comparable animals): The model from Figure 5a has been used for simplicity, using the same conventions; in addition, "1" and "2" refer to 1 and 2 gene doses, and the somatic scale to the left of the graphs refers to the gradual range of somatic sexual phenotypes observed, from essentially male development (no *tra-2* activity) to female somatic development (high *tra-2* activity). a, Wild-type hermaphrodite: two doses of *tra-2*(+) are sufficient for female somatic development, including a spike tail and functional HSNs; with one dose, animals often have defective or absent HSNs. Activity of *tra-2*(+) is modulated so that the correct amount of sperm is made. b, *tra-2* activity in the putative hypomorph, *e1940* [similar to *tra-2(e1939)* (depicted in Figure 2b and c) and *tra-2(e2021)*, but with slightly higher activity]: two doses are sufficient for female somatic development, but *tra-2* activity is not modulated enough to fall below the sperm/oocyte threshold, so no sperm are made, thereby resulting in a female. In contrast, one dose is insufficient for female somatic development; instead, these animals are intersexual, with partially masculinized tails and defective vulvae; however, their gonads are usually two-armed. In addition, modulation of *tra-2* activity is sufficient to allow some spermatogenesis. c, *tra-2* activity in *tra-2(e2019)*: This allele appears to have reduced modulation without significantly altering the overall level of *tra-2* activity: two doses are sufficient for female somatic development, and there is insufficient modulation of *tra-2* activity to permit spermatogenesis. One dose appears to have only slightly lower *tra-2* activity than one dose of wild-type *tra-2*, with about 30% of animals having wild-type tail spikes, the rest having slightly snubbed tails (Table 2, column C); however, *tra-2* activity is modulated enough to permit a substantial amount of spermatogenesis. d, *tra-2* activity in the putative hypermorph *tra-2(e2020)* (similar to *tra-2(e2046)*): two doses are sufficient for female somatic development, with *tra-2* activity always above the sperm/oocyte threshold; one dose is better at promoting female somatic development than *tra-2*(+), as judged by the presence of HSNs.

of *tra-2* activity of a given allele. Interestingly, these animals are self-fertile, indicating that *tra-2* is still modulated in these mutants, although apparently to a lesser extent.

In contrast, single doses of two other alleles (*e2020* and *e2046*) are as good as, or better than, wild type at promoting female somatic development and, thus, may raise the overall level of *tra-2* activity (*i.e.*, they may be hypermorphic). In addition, these two *tra-2(dom)* mutations have improved the ability of *tra-2* to direct female development in the absence of wild-type *tra-3*, also consistent with hypermorphy. In the germline, animals with a single dose of either of these alleles are female, indicating that *tra-2* activity in these mutants does not fall below the sperm/oocyte threshold during the SCP. Nevertheless, there does appear to be some *tra-2* modulation in these alleles, because *e2020* and *e2046 XX* animals that lack *tra-3* can make sperm before oogenesis; thus, this *tra-2* modulation is only apparent when the effective *tra-2* level is reduced. Finally, these alleles can partially feminize *XO* animals under some circumstances, especially in the germline. This indicates that these alleles cause some *tra-2* expression in *XO* animals and therefore partially interfere with the negative

effect of *her-1*. (It is possible that the putative hypomorphs also interfere with *her-1* control but that this is obscured by their reduced activity.)

It is interesting that the different properties of the *tra-2(dom)* alleles appear to vary independently. For instance, some alleles are more leaky than others in terms of self-fertility in *XX* animals (Table 2, columns A, B and C), but this is not necessarily correlated with the inferred degree of hypomorphy (e.g., compare *e1939* to *e2021*). Further, although the combination of different properties is unique to each allele, the properties overlap, which is remarkable considering that each is likely to be a single mutational event [EMS generally induces point mutations and occasionally deletions or rearrangements (DIBB *et al.* 1985)]. These combinations may suggest that these "functions" physically overlap in the *tra-2* gene. For example, single mutations in the two alleles, *e2020* and *e2046*, appear to have interfered with modulation, possibly increased the level of *tra-2* activity and also, perhaps as a consequence, increased the ability of *tra-2* to function independently of *tra-3*, as well as interfering with *her-1* control. However, further understanding of these mutations awaits a molecular analysis of the *tra-2* gene.

What modulates *tra-2* activity? Since *her-1* is thought to be a negative regulator of *tra-2* and *tra-3*, one might ask whether *her-1* is responsible for modulating *tra-2* activity in the *XX* animal. However, this seems highly unlikely, because the *her-1* gene is not required for hermaphrodite spermatogenesis: *her-1* animals are hermaphrodites, whether *XX* or *XO*. What the *her-1* null phenotype does suggest is that the *tra-2* modulator can be active in both *XX* and *XO* animals; therefore, it may not be regulated in a sex-specific way in the wild type. Further, the observation that *tra-2(dom);her-1 XO* animals are female indicates that the *tra-2(dom)* mutations can interfere with *tra-2* modulation in *XO* animals as well as *XX* animals.

Considering the requirements for *tra-2* modulation outlined above, lack of function mutations in a *tra-2* "modulator" gene might be expected to be recessive and have the same phenotype as *tra-2(dom)* mutations—that is, to make *XX* animals female but not affect *XO* animals—and these mutations should be hypostatic to *tra-2* null mutations. Indeed, mutations with these properties have been found in the gene *fog-2* (T. SCHEDL and J. KIMBLE, personal communication), which is thus a candidate for the modulator gene.

Finally, it is of interest to note that the control of spermatogenesis in the *C. elegans* hermaphrodite represents a kind of "fine tuning" of a major developmental decision; this sort of mechanism may be important as a means of creating greater flexibility during the evolution of complex organisms.

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