Analysis of the Role of *tra-1* in Germline Sex Determination in the Nematode *Caenorhabditis elegans*

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ABSTRACT

In wild-type *Caenorhabditis elegans* there are two sexes, self-fertilizing hermaphrodites (*XX*) and males (*XO*). To investigate the role of *tra-1* in controlling sex determination in germline tissue, we have examined germline phenotypes of nine *tra-1* loss-of-function (*lf*) mutations. Previous work has shown that *tra-1* is needed for female somatic development as the nongonadal soma of *tra-1(lf) XX* mutants is masculinized. In contrast, the germline of *tra-1(lf) XX* and *XO* animals is often feminized; a brief period of spermatogenesis is followed by oogenesis, rather than the continuous spermatogenesis observed in wild-type males. In addition, abnormal gonadal (germ line and somatic gonad) phenotypes are observed which may reflect defects in development or function of somatic gonad regulatory cells. Analysis of germline feminization and abnormal gonadal phenotypes of the various mutations alone or in *trans* to a deficiency reveals that they cannot be ordered in an allelic series and they do not converge to a single phenotypic endpoint. These observations lead to the suggestion that *tra-1* may produce multiple products and/or is autoregulated. One interpretation of the germline feminization is that *tra-1*(+) is necessary for continued specification of spermatogenesis in males. We also report the isolation and characterization of *tra-1* gain-of-function (*gf*) mutations with novel phenotypes. These include temperature sensitive, recessive germline feminization, and partial somatic loss-of-function phenotypes.

THE nematode *Caenorhabditis elegans* normally exists as either of two sexes, self-fertilizing hermaphrodite or male. The two sexes differ morphologically, biochemically and behaviorally (reviewed by HODGKIN 1988). The hermaphrodite soma is female (DONIACH 1986a; HODGKIN 1986; SCHEDL and KIMBLE 1988); its germline produces sperm briefly during the fourth larval stage (L4) and then makes oocytes continuously throughout adulthood. Males have a male soma and a germline that produces sperm continuously from the L4 stage throughout adulthood.

The initial signal for sex determination is the *X/A* ratio (MADL and HERMAN 1979). Diploid *XX* animals are hermaphrodites, while diploid *XO* animals are males. The *X/A* ratio is transduced by genes that direct both sex determination and dosage compensation (VILLENEUVE and MEYER 1987; MILLER *et al.* 1988; NUSBAUM and MEYER 1989). These transducing genes in turn specify sexual fate by regulating seven autosomal sex determination genes (*tra-1*, *tra-2*, and *tra-3*, *fem-1*, *fem-2*, and *fem-3*, and *her-1*).

Regulation of sex determination in somatic tissues is different from that in the germline. Of particular importance to this paper are the epistatic relationships of the *fem* genes and *tra-1*. In the soma, loss-of-function mutations in *tra-1* are epistatic to loss-of-function mutations in each of the *fem* genes, while in the germline, the converse is true: loss-of-function mutations in each of the *fem* genes are epistatic to loss-of-function mutations in *tra-1* (DONIACH and HODGKIN 1984; HODGKIN 1986). Thus, in the soma, six sex determination genes (*her-1*, *tra-2* and *-3*, and the three *fem* genes) act in a cascade of negative regulation to control the state of *tra-1*, which, when "ON," specifies the female fate (HODGKIN 1980, 1986, 1988). However, in the germ line, *her-1*, *tra-2* and *tra-3* act, again in a cascade of negative regulation, to control the state of the *fem* genes, which, when "ON," specify the male fate.

A second difference between the regulation of somatic and germline sex is its timing during development. In general, somatic sexual dimorphism is the result of sex-specific cell lineages which are executed during larval development. In contrast, the choice between spermatogenesis and oogenesis is made continuously throughout late larval and adult life in both hermaphrodites (BARTON, SCHEDL and KIMBLE 1987) and males (NELSON, LEW and WARD 1978; KLASS, WOLF and HIRSH 1979; KIMBLE, EDGAR and HIRSH

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1984; P. SCHEDIN and W. WOOD, personal communication). Therefore, the germ line may require activities to maintain commitment to a given sexual pathway over time in addition to activities that specify a particular sexual fate.

The *tra-1* gene plays a central role in specifying the female fate in somatic tissues (HODGKIN 1980, 1983, 1987), but its role in germline development is unclear. In this paper, we report studies with *tra-1* that are directed toward understanding its role in controlling the differentiation of a germ cell as sperm or oocyte. Our results complement and extend to work of HODGKIN (1987).

MATERIALS AND METHODS

General methods for culturing and handling *C. elegans* strains were as described by BRENNER (1974). Ethyl methanesulfonate (EMS) mutagenesis was also as described by BRENNER (1974) except that EMS was used at a final concentration of 0.0125 M for 5 hr. Experiments were performed at 20° unless otherwise indicated. For all experiments, worms were under continuous growth conditions and were not starved or recovering from the dauer state.

Nomenclature and strains: We employ the abbreviations *gf* for gain-of-function and *lf* for loss-of-function mutations. For numerically designated alleles, the suffix *gf* is used, while alleles without a suffix are assumed to be loss-of-function unless indicated to the contrary. Where necessary, maternal and zygotic genotypes are indicated by m( ) and z( ), respectively. All other nomenclature follows HORVITZ *et al.* (1979).

The *C. elegans* variety Bristol isolate N2 (BRENNER 1974) is defined as wild type. Most of the mutations used in this study are described in HODGKIN *et al.* (1988). The *tra-1* alleles (*e1076, e1099, e1781, e1835tr* and *e1575gf*) are described in HODGKIN (1987). Both *e1781* and *e1835tr* are amber alleles. The phenotypes of sex determination mutants are described and referenced in Table 2 and the text. The following mutations were used [*dpy* (dumpy), *fem* (feminization), *fog* (feminization of germline), *her* (hermaphroditization), *him* (high incidence of XO males), *lon* (long), *tra* (transformer), *unc* (uncoordinated)]:

Linkage group (LG) *II*: *dpy-10(e128), tra-2(e1095, e1425, q122gf), unc-4(e120).*
LG *III*: *unc-32(e189), dpy-19(e1259), dpy-18(e364, e499).*
LG *IV*: *unc-24(e138), fem-3(q20gf, q95gf, q96gf), dpy-20(e1282), unc-30(e191), tra-3(e1107).*
LG *V*: *him-5(e1490).*
LG *X*: *lon-2(e678), unc-3(e151).*

Chromosomal rearrangements are as follows: *eT1(III, V)* [a translocation that suppresses recombination in the region of *tra-1* and markers *dpy-19, unc-32,* and *dpy-18* (ROSENBLUTH and BAILLIE 1981)], *eT1[glp-1(q50)]* [a *glp-1* sterile mutation within the *eT1* chromosome (AUSTIN and KIMBLE 1987)], *qC1(III)* [a crossover suppressor for the region around *tra-1* and markers *unc-32* and *dpy-18* which has a *glp* sterile mutation (J. AUSTIN, personal communication)], *eDp6 (III)* a free duplication for the right arm of chromosome *III*, and *eDf2(III)* [a deficiency within the right arm of chromosome *III* including *tra-1* and *dpy-18*; it is covered by *eDp6*; HODGKIN 1980)]. All four rearrangements used, *eT1, eT1[glp-1(q50)], qC1* and *eDp6* contain *tra-1(+)*.

Isolation of *tra-1*(lf) alleles: Loss-of-function mutations in *tra-1* were obtained in three ways. (1) Two alleles (*q88* and *q165*) were discovered when *XX* males segregated out of unrelated mutant stocks, more than five generations following the initial EMS mutagenesis. Both are apparently spontaneous mutations isolated in the Bristol genetic background. (2) Two alleles were obtained as masculinizing mutations linked to the chromosome *III* markers *dpy-19* and *unc-32*. L4 hermaphrodites (*dpy-19/eT1* or *dpy-19 +/+ unc-32*) were mutagenized with EMS and F_1 progeny were placed individually on agar filled petri dishes. The F_2 generation was screened for sex determination mutants. From a total 5966 F_1 hermaphrodites, two alleles, *q106* and *q165* were obtained. (3) Four alleles were obtained as intragenic revertants of the *tra-1* gain-of-function mutation *q183gf* (see below). The *q183gf* mutant shows dominant, temperature sensitive feminization of the *XX* germline; *q183gf/+* animals are self-fertile at 15° but 100% female at 25° (also see Table 3). An intragenic mutation which reduces or eliminates *q183gf* activity in heterozygotes will restore self-fertility. L4 *unc-32 tra-1 (q183gf)/eT1[glp-1(q50)]* animals raised at 15° were mutagenized with EMS and shifted to 25°. The F_1 generation was screened for self-fertile animals and these segregated masculinized *unc-32 XX* animals in the F_2. From an estimated 7000 F_1 animals, four *tra-1* mutations were obtained (*q183q314, q183q315, q183q316* and *q183q338*). Of these four alleles, *q183q315* shows complete masculinization of the *XX* nongonadal soma while the other three show variable and incomplete masculinization. Only *q183q315* has been analyzed in detail (see RESULTS). All new alleles were outcrossed at least twice to wild type, and where necessary, linked markers were removed by two factor crosses.

The new mutations were shown to be *tra-1* alleles by failure to complement the masculinized nongonadal soma phenotype of canonical *tra-1* alleles (*e1099* and/or *e1781*) and by three factor mapping with respect to *unc-32* and *dpy-18* (*tra-1* maps between *unc-32* and *dpy-18*; HODGKIN *et al.* 1988) (data not shown). The new mutations were not tested for suppression by amber suppressor tRNA alleles. The frequency at which these *tra-1* mutants with a masculinized nongonadal soma arise following EMS mutagenesis is consistent with their causing loss of gene function; the frequency obtained from the screen of progeny from F_1 hermaphrodites (3×10^{-4} per haploid genome) and from the intragenic reversion of *q183gf* (6×10^{-4}) is similar to that obtained by HODGKIN (1987) (2×10^{-4}), and similar to that observed for loss-of-function mutations in other *C. elegans* genes (10^{-3} to 10^{-4}; BRENNER 1974; GREENWALD and HORVITZ 1980; HODGKIN 1986). Further, although our EMS mutagenesis conditions differed slightly from other laboratories (see above), the screen of progeny from F_1 hermaphrodites yielded these *tra-1* mutations and loss-of-function mutations in *glp-1* (AUSTIN and KIMBLE 1987), *fog-2* (SCHEDL and KIMBLE 1988), *tra-2* and *fem-3* (S. MAPLES and J. KIMBLE, personal communication) at similar frequencies.

Characterization of *tra-1* phenotype: Sexually dimorphic structures in mutants were examined by Nomarski microscopy at 630X to determine if they were male, female, or other. The structures include tail, vulva, somatic gonad, and type and position of gametes in the germline. A wild-type hermaphrodite and male are shown in Figure 1, A and B. For a more detailed description of wild-type sexually dimorphic anatomy see HIRSCH, OPPENHEIM and KLASS (1976), KLASS, WOLF and HIRSH (1976), KIMBLE and HIRSH (1979), WHITE (1988), and HODGKIN (1988). The presence of yolk was scored qualitatively by Nomarski microscopy as refractile droplets which begin to accumulate in the pseudocoelom after the L4 molt into adulthood (KIMBLE and

SHARROCK 1983). The ability of males to sire cross progeny, and their *X*-chromosome constitution, was determined by mating single males with *XX* hermaphrodites or females [using *fem-1(ts)*] that have the *X*-linked marker *unc-3*. *XX* males produce only non-Unc hermaphrodite cross progeny while *XO* males produce both non-Unc hermaphrodites and Unc male cross progeny.

To score *tra-1(lf)* phenotypes, animals of the desired genotypes were generated as follows (data in Table 1 and Figure 1). For *tra-1(lf) XX*, self-progeny with a masculinized nongonadal soma from *tra-1(lf)/eT1* stocks were analyzed. For *tra-1(lf)/eDf2 XX*, *tra-1(lf) XX* males were mated with *eDf2/eDp6* hermaphrodites, and cross progeny with a masculinized nongonadal soma were analyzed. For *tra-1(e1076)/eDf2 XX*, single *eDf2/+ XO* males were mated with *tra-1(e1076)/eT1*; *lon-2* hermaphrodites (*lon-2* is an *X*-linked morphological marker), and non-Lon (therefore *XX* cross progeny) with a masculinized nongonadal soma were analyzed. For *eDf2/+ XO*, wild-type males were mated with *eDf2/eDp6* hermaphrodites and male cross progeny, one-half of which are *eDf2/+ XO*, were analyzed. For *tra-1(lf)/+ XO* and *tra-1(lf) XO* (both *q183q315* and *e1781*), single *tra-1(lf)/+ XO* males were mated with *tra-1(lf)/tra-1(e1575gf)*; *lon-2 XX* females. Lon (therefore *XO*) male progeny, one-half of which are *tra-1(lf)/+* and one-half *tra-1*(lf), were analyzed. Animals inheriting *tra-1(e1575gf)* are female (HODGKIN 1983, 1987; Table 3). Control experiments indicate that the presence of *tra-1(e1575gf)* in the mother does not contribute to the *tra-1(lf)* phenotype (our unpublished observations). For *tra-1(e1076)/+ XO*, *tra-1(e1076)*; *eDp6* hermaphrodites were mated with wild-type males and progeny with wild-type male tails, one-half of which are *tra-1(e1076)/+ XO*, were analyzed. For *tra-1(e1076) XO*, *tra-1(e1076) dpy-18/eT1* hermaphrodites were mated with *tra-1(e1076) dpy-18/+* males and Dpy animals with a wild-type male tail [*tra-1(e1076) XO* homozygotes, HODGKIN 1980] were analyzed. Desired animals at the L4 stage were transferred singly or in small groups to separate plates (away from hermaphrodites with which they can mate) and examined 24 to 48 hr later. Where possible, all *tra-1* progeny from a brood were analyzed.

***tra-1(lf)* double mutant construction:** A summary of single mutant phenotypes and the alleles used in the double mutants is shown in Table 2. The linked mutation *dpy-18* was used to mark *tra-1*; *unc-4*, and *dpy-10* to mark *tra-2*; and *dpy-20* to mark *fem-3(gf)*. Double mutants were characterized as described above for *tra-1(lf)*. For each construction, more than 25 animals of the predicted genotype were analyzed as described above. In most cases, recombinant animals could be identified by progeny testing.

tra-2(lf); tra-1(lf) XX: tra-1(lf)/eT1 hermaphrodites were purged (allowed to exhaust all their self sperm) and crossed with *+ tra-2(lf) unc-4/dpy-10 tra-2(q122gf) + XO* males. Individual *XX* cross progeny were transferred to separate plates and self-fertile animals [*i.e.*, that do not have *tra-2(gf)*] which segregate both non-Unc [*tra-1(lf)*] and Unc [*tra-2(lf) unc-4*] with a masculinized nongonadal soma were identified. Of the masculinized Unc animals, one-fourth are expected to be homozygous for both *tra-1(lf)* and *tra-2(lf)*. For six alleles we observed about one-fourth with a phenotype similar to the respective *tra-1(lf)* allele alone (Table 2C). However, for three alleles (*q183q315*, *e1781*, and *e1835tr*), approximately three-fourths of Unc animals displayed a wild-type male nongonadal soma. To clarify this phenomenon, Unc Dpy *tra-1(lf) dpy-18*; *tra-2(lf) unc-4* and non-Dpy *tra-1(lf) dpy-18/++*; *tra-2(lf)* animals were generated for each of these alleles by crosses similar to those described above. Homozygous (Unc Dpy) *tra-1(lf) dpy-18*; *tra-2(lf) unc-4* animals showed the same phenotype as each of the three *tra-1(lf)* alleles alone. Heterozygous (non-Dpy) *tra-1(lf) dpy-18/++*; *tra-2(lf)* animals for these three alleles displayed a wild-type male phenotype for both the germ line and soma. Crosses showed them to be capable of siring cross progeny, to be *XX* and confirmed their genotype. This dominance of *tra-1(lf)* is not specific to the *tra-2(lf)* allele as it was observed when either *e1425* or *e1095* was used. Finally, when *eDf2/+*; *tra-2(lf)* animals were examined, the *tra-2(lf)* phenotype was observed.

tra-2(lf); tra-1(lf) XO: *tra-2(lf) unc-4/++*; *tra-1(lf)*; *lon-2/+* hermaphrodites were purged and crossed to single *tra-1(lf)/+*; *tra-2 unc-4/++ XO* males. Of the Lon (*XO*), Unc [*tra-2(lf)*] cross progeny, one-fourth are expected to be *tra-1(lf)* homozygotes. Approximately one-fourth of Lon Unc progeny were observed with the *tra-1(q183q315* or *e1781)* phenotype.

tra-1(lf); tra-3(lf) XX: *tra-3(lf)* mutants exhibit maternal rescue such that m(−/+), z(−/−) worms are wild-type and segregate an entire brood of m(−/−), z(−/−) animals with a masculinized phenotype (Table 2A). Dpy self-progeny from *tra-1(lf) dpy-18/++*; *tra-3*[m(−/+), z(−/−)] hermaphrodites were analyzed.

tra-1(lf); fem-3(gf) XX: *fem-3(gf)* mutants are self-fertile at 15° while they produce a vast excess of sperm and no oocytes at 25° (Mog phenotype; Table 2B; BARTON, SCHEDL and KIMBLE 1987). *tra-1(lf)/+; fem-3(q20gf) dpy-20* stocks were established at 15°. Young adult hermaphrodites were shifted to 25° and progeny with a masculinized soma were analyzed.

tra-2(lf); tra-3(lf) XX: Unc self-progeny from *tra-2(lf) unc-4/++*; *tra-3*[m(−/+), z(−/−)] hermaphrodites were analyzed.

Isolation of *tra-1(gf)* alleles as extragenic suppressors: *tra-1* gain-of-function alleles were isolated as suppressors of *fem-3(q96gf)*. Homozygous *fem-3(q96gf) dpy-20* animals raised at 15° were mutagenized with EMS and shifted to 25°. Dominant suppressors of the *fem-3(q96gf)* Mog phenotype were obtained as self-fertile animals in the F_1 generation (M. K. BARTON, unpublished observations). Among EMS-induced suppressors, three *tra-1(gf)* alleles (*q183gf*, *q184gf* and *q185gf*) were obtained. New mutations were outcrossed to remove *fem-3(q96gf) dpy-20*. An additional mutant, *tra-1(q245gf)*, was obtained as a spontaneous *fem-3(q96gf)* suppressor in the TR403 mutator strain (COLLINS, SAARI and ANDERSON 1987). To remove any mutations linked to *tra-1* that may have arisen in the mutator strain, *q245gf* was out-crossed ten times to wild type and the linked marker *dpy-18* was recombined on and off chromosome *III*.

Characterization of *tra-1(gf)* alleles: New *tra-1(gf)* mutations were three-factor mapped with respect to markers *unc-32 dpy-18*. Animals were scored as self-fertile or female as previously described (SCHEDL and KIMBLE 1988), and examined by Nomarski microscopy as described above. For scoring phenotypes, animals that contain *tra-1(gf)* mutations (data in Table 3 and Figure 4) were obtained as follows. For *tra-1(gf) XX* heterozygotes and homozygotes, self-progeny from heterozygous or homozygous stocks were analyzed. For *tra-1(q183gf)/+ XO*, *tra-1(q183gf)/eT1*[*glp-1(q50)*]; *lon-2 XX* females were crossed with wild-type males and Lon cross progeny analyzed. For *tra-1(q183gf) XO*, masculinized *unc-32* self-progeny from a *unc-32 tra-1(q183gf)/q50*; *him-5* stock were analyzed. For *tra-1(q245gf* or *q185gf)/+ XO*, *tra-1(gf)*; *lon-2* females were crossed with wild-type males and Lon cross progeny analyzed. For *tra-1(q245gf* or *q185gf) XO*, *tra-1(gf)*; *lon-2* females were crossed with *tra-1(gf)/+ XO* males and Lon cross progeny analyzed. For *tra-1(gf)/tra-1(lf) XX* (*e1099* or *e1781*), *tra-*

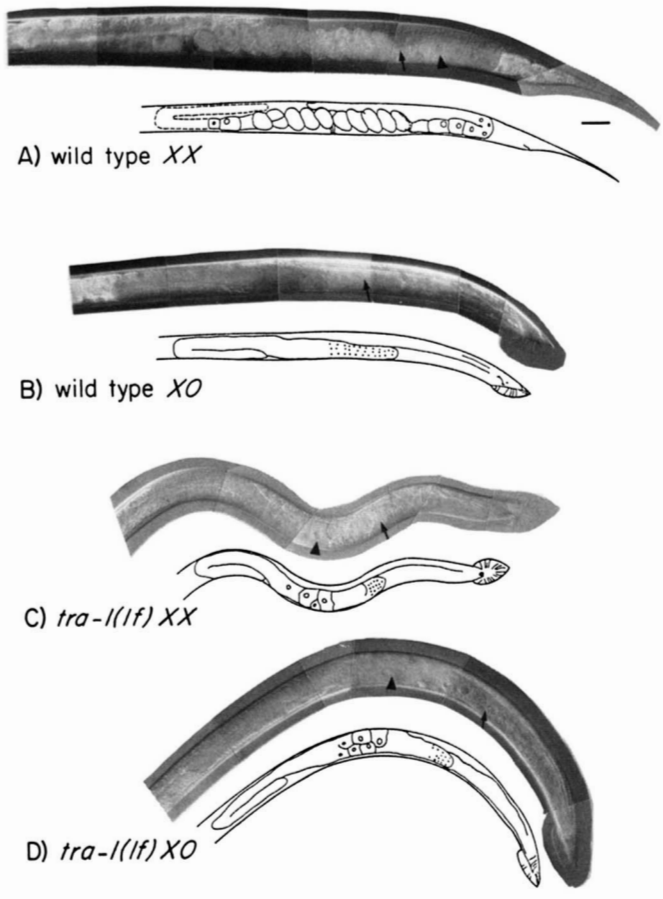

FIGURE 1.—Wild-type and *tra-1(lf)* *XX* and *XO* phenotypes. Composite photomicrographs using Nomarski microscopy. Posterior two-thirds of young adults are shown with the focal plane adjusted to maximize view of germline and somatic sexually dimorphic structures. Line drawings are shown below to illustrate gamete type and gonad/body shape. (A) Wild-type *XX* hermaphrodite; lateral view. The soma is female, with two symmetrical, U-shaped gonad arms, a vulva and a pointed tail spike. The germline is hermaphrodite, with first sperm (→) and then oocytes (▶). In adult hermaphrodites, sperm reside in the spermatheca where they fertilize oocytes derived from the same gonad arm. Embryos remain in the uterus until they are laid through the vulva. Mature oocytes (most proximal in each gonad arm) are large cells, with a large smooth nucleus and a granular cytoplasm. Immature oocytes (more distal), are smaller in size, have a nucleolus and a granular cytoplasm. Early stages of oogenesis are represented by a central cytoplasmic core region within each distal gonad arm that is filled with granules like those in oocytes (out of the plane of focus in this animal). Sperm are small. with a tiny elevated nucleus. (B) Wild-type *XO* male; lateral view. The soma is male, with a single U-shaped gonad arm which connects, via the vas deferens, to a fan shaped tail with sensory rays. The hypodermis lacks a vulva. The germline is male with sperm most proximally, and a thin cytoplasmic core that lacks granules more distally. Spermatogenesis continues throughout adulthood. (C) *tra-1(lf)* *XX*; ventral view. (D) *tra-1(lf)*; *lon-2*/O *XO*; lateral view. For both, allele = *q183q315*. The phenotypes of *XX* and *XO* *tra-1(lf)* males are similar (also see text and Table 1). The soma (nongonadal and gonadal) is male, like that in wild-type *XO* males (*B*). The germline is feminized. The most proximal gametes are sperm (→). However, spermatogenesis does not continue as in wild-type *XO* males (B). Instead, subsequent germ cells undergo oogenesis. Distal to the sperm are large cells which resemble mature oocytes (▶; compare with oocytes in A), followed by smaller cells which resemble immature oocytes, and then followed by a granular cytoplasmic core region that is enlarged relative to a wild-type male. These are functional sperm as *tra-1(lf)* males (*XX* and *XO* like those shown in C and D) can sire cross progeny. The ability of these oocytes to produce embryos can not be assayed. However, the oocytes in older adult *tra-1(lf)* mutants become polyploid, and thus resemble unfertilized oocytes in hermaphrodites which have passed through the spermatheca. The *tra-1(lf)* oogenesis phenotype is also similar to germline phenotypes observed in males that are incompletely feminized by leaky mutations in *fem-1*, *-2*, and *-3* (NELSON, LEW and WARD 1978; KIMBLE, EDGAR and HIRSH 1984; DONIACH and HODGKIN 1984; HODGKIN 1986; our unpublished observations). Based on morphological similarity to both oogenesis in hermaphrodites and to leaky feminized phenotypes, we propose that germ cells in *tra-1(lf)* are transformed from the male (spermatogenesis) to the female (oogenesis) fate. Note that in some *tra-1(lf)* animals, there are germ cells which may be intersexual (resemble 1° spermatocytes but are enlarged and have a granular cytoplasm). Scale bar = 40 μm.

1(gf) *XX* females were crossed with *tra-1(lf)* *XX* males and cross progeny analyzed. For *tra-1(gf)/eDf2* *XX*, *tra-1(q245gf* or *q185gf)*; *lon-2* females were crossed with single *eDf2*/+ *XO* males and non-Lon cross progeny analyzed.

Construction of *tra-1(gf)*; *fem-3(gf)* double mutants. The following strains were established at 25°: three self-fertile strains (1) *tra-1(e1575gf)*/+; *unc-24 fem-3(q95gf) dpy-20*, (2) *tra-1(e1575gf)*/+; *fem-3(q95gf) dpy-20*; *him-5*, and (*3*) *unc-32 tra-1(q183gf)*; *fem-3(q95gf) dpy-20*, and the stable *XO* male–*XX* female/Mog strain *tra-1(e1575gf)*/; *fem-3(q20gf)dpy-20*; *him-5*. Hermaphrodite, female and Mog phenotypes were determined as previously described (SCHEDL and KIMBLE 1988). Genotypes of hermaphrodites, females and males with normal morphology were determined by examination of self-progeny or progeny following a cross to wild type. *XO* animals were analyzed by Nomarski microscopy for suppression of the *tra-1(e1575gf)*/+ germline and somatic phenotypes.

RESULTS

Phenotype of *tra-1* loss-of-function (*lf*) mutations: Wild-type hermaphrodites and males are sexually differentiated in all tissue types (Figure 1, A and B). Most recessive mutations in *tra-1* lead to a masculinization of *XX* animals and are thought to be loss-of-function mutations (HODGKIN and BRENNER 1977; HODGKIN 1980, 1983, 1987; this paper, see DISCUSSION). Here we report our findings with nine *tra-1(lf)* mutations, some also examined by HODGKIN (1987). Our results are shown in Table 1A for *XX* and Table 1B for *XO* animals.

For all nine *tra-1(lf)* alleles examined, the nongonadal soma of *XX* homozygotes is masculinized with little or no variation for a given allele in the extent of sexual transformation (Table 1A; Figure 1C). For seven alleles (*q183q315*, *e1781*, *e1835tr*, *q106*, *e1099*, *q159* and *q165*), masculinization of the *XX* nongonadal soma is complete. This transformation is unaltered when any of these seven alleles is placed in *trans* to a *tra-1* deficiency (*eDf2*, Table 1A). In contrast, two other alleles, *e1076* and *q88*, do not fully masculinize the *XX* nongonadal soma (Table 1A). *e1076* *XX* homo-

TABLE 1

Phenotype of *tra-1* loss-of-function alleles in adult *XX* and *XO* animals

Genotype[a]	Percent animals with male nongonadal soma[b]	Percent animals with male somatic gonad[c]			Percent animals with abnormal somatic gonad[d]	No. of animals scored
		Spermatogenesis only[e]	Spermatogenesis then oogenesis[f]	Abnormal germline[g]		
A. *XX* ANIMALS						
q183q315	100 (complete male)	0	99 (62)	1	0	76
q183q315/eDf2	100 (complete male)	0	96 (59)	4	0	49
e1781	100 (complete male)	2	95 (74)	2	1	108
e1781/eDf2	100 (complete male)	5	88 (72)	4	3	100
e1835tr	100 (complete male)	0	84 (84)	16	0	50
e1835tr/eDf2	100 (complete male)	0	90 (90)	10	0	61
q106	100 (complete male)	0	93 (90)	5	2	67
q106/eDf2	100 (complete male)	0	92 (57)	6	2	51
e1099	100 (complete male)	27	17 (11)	36	20	66
e1099/eDf2	100 (complete male)	32	13 (6)	34	21	96
q159	100 (complete male)	23	41 (14)	22	14	47
q159/eDf2	100 (complete male)	16	49 (24)	21	14	56
q165	100 (complete male)	1	76 (74)	22	1	75
q165/eDf2	100 (complete male)	0	86 (86)	14	0	50
e1076	100 (incomplete male, truncated tail spike)	0	100 (100)	0	0	51
e1076/eDf2	100 (almost complete male tail)	1	80 (70)	16	3	78
q88	100 (incomplete male, makes yolk)	6	94 (40)	0	0	66
q88/eDf2	100 (incomplete male, makes yolk)	4	96 (66)	0	0	74
B. *XO* ANIMALS						
Wild type	100 (complete male)	100	0	0	0	>100
eDf2/+	100 (complete male)	100	0	0	0	65[h]
q183q315/+	100 (complete male)	100	0	0	0	43[h]
q183q315	100 (complete male)	0	98 (77)	2	0	47[h]
e1781/+	100 (complete male)	100	0	0	0	55[h]
e1781	100 (complete male)	0	98 (69)	2	0	55[h]
e1076/+	100 (complete male)	100	0	0	0	36[h]
e1076	100 (complete male)	4	88 (64)	8	0	48

Phenotypes were scored by Nomarski microscopy. See Figure 1 and text for further details.

[a] Using the classification of HODGKIN (1987, in Table 1), *q183q315* and *q106* are probably A2 or A3 alleles, while *q159* and *q165* are probably A1 alleles. Note that a low frequency of *q106 XX* males have a swollen tail, although these animals sire cross progeny efficiently.

[b] Complete male nongonadal soma has a male hypodermis (fan tail with sensory rays, no vulva), no yolk in the pseudocoelom and the ability to sire cross-progeny.

[c] Male somatic gonad has a one-armed, U-shaped gonad connected to the tail via the vas deferens.

[d] Abnormal somatic gonad has a variable shape, usually a ball or oval, and does not connect with the tail. The germ cells usually fail to undergo gametogenesis.

[e] For all *XX* mutants except *q88*, the number of sperm produced is less than in wild-type males (see text).

[f] Oogenesis includes mature oocytes, immature oocytes, and/or early stages of oogenesis. Number represents percent of animals showing any oogenesis. Number in parentheses indicates percent of animals making mature oocytes.

[g] Abnormal germlines have a changed location and/or number of sperm, spermatogenesis and germline stem cells. Three types of abnormal germline phenotype were observed: (1) All germ cells undergo gametogenesis producing a few to several hundred sperm (like *glp-1*, see AUSTIN and KIMBLE, 1987). (2) Spermatogenesis occurs in both proximal and distal arms of the gonad. (3) Undifferentiated germ cells occupy the most proximal part of the gonad followed by spermatogenesis more distally.

[h] Represents the number of animals showing these phenotypes, which is approximately the expected one-half of total.

zygotes have a hermaphrodite-like tail spike, while *q88 XX* homozygotes make yolk. When *e1076* is placed in *trans* to a *tra-1* deficiency (Table 1A), or in *trans* to *tra-1(lf)* alleles *e1781* or *e1099* (data not shown; HODGKIN 1987), the nongonadal soma is further masculinized so that the tail is almost fully male. Although yolk production was not eliminated in *q88/eDf2* animals, a decrease in yolk production (increased masculinization) could not be scored by our assay (see MATERIALS AND METHODS). The seven mutations that completely masculinize the nongonadal soma are therefore the strongest of the *tra-1(lf)* alleles.

The effect of *tra-1(lf)* on the somatic gonad is similar to its effect on the nongonadal soma. The somatic gonad in most *tra-1(lf) XX* animals examined is transformed from female to male (Table 1A, all nine alleles; Figure 1C). Yet, in some animals, morphogenesis of the somatic gonad is defective rather than sexually transformed. Though the extent of such defects is widely variable, we collectively call these "abnormal somatic gonads." In a severe case, the gonad develops as a small oval mass with few germ cells and no recognizable somatic structures. A significant fraction of *e1099* or *q159* animals have an abnormal somatic gonad, but no or few animals with this phenotype were detected for the other five alleles

that show complete masculinization of the nongonadal soma (Table 1A).

A simple expectation based on the sexual transformation of the *XX* soma from female to male is that the *XX* germline would also be masculinized. However, the *tra-1(lf)* *XX* germline is almost never the same as a wild-type *XO* male germline (Table 1A, all nine alleles; Figure 1C). Indeed, one of three unexpected germline phenotypes may be observed. One phenotype is the production of oocytes after a period of spermatogenesis. The extent of oogenesis ranges from formation of an enlarged granular core region, which is indicative of early stages of oogenesis, to the production of oocytes. Alleles comparable in their effect on the nongonadal soma vary in the extent of feminization observed. For example, 99% of *q183q315* animals show signs of oogenesis and most make oocytes, whereas only 17% of *e1099* germlines show signs of oogenesis with about half limited to early stages of oogenesis.

A second *tra-1(lf)* germline phenotype is production of sperm with no sign of subsequent feminization. Although superficially similar to the germline of a wild-type *XO* male, these animals produce far fewer sperm than wild-type animals and have a markedly reduced number of undifferentiated germ cells (also see HODGKIN 1987). Alleles vary in how many animals show this phenotype (Table 1A). For example, 27% of *e1099* homozygotes make only sperm in a reduced germline, but no *q183q315*, *e1835tr*, or *q106* homozygotes show this phenotype (Table 1A). An exception is *q88*: *q88* germlines that produce sperm only (6%, Table 1A) make as many sperm and undifferentiated germ cells as wild-type *XO* males.

A third *tra-1(lf)* germline phenotype is an alteration in the presence or location of germline stem cells. Stem cells may be absent, reduced in number, and/or located abnormally. Because this effect is widely variable, these germlines are collectively scored as "abnormal" (Table 1A). Some animals with the "abnormal germline" phenotype were detected among homozygotes of all seven alleles that completely masculinize the nongonadal soma. The frequency is very low for *q183q315*, *e1781*, and *q106*, and progressively higher for *e1835tr*, *q165*, *q159*, and *e1099* (Table 1A).

Although alleles showing similar proportions of the various germline and somatic gonadal phenotypes can be grouped together (Table 1A), it is unclear how to order these alleles (phenotypes) to reflect the amount of remaining *tra-1* activity. In an attempt to deduce such an order and infer the phenotype in the absence of *tra-1* activity, animals homozygous for a given allele were compared to that allele in *trans* to the *tra-1* deficiency *eDf2*. However, homozygotes and hemizygotes of a given allele do not significantly differ in the proportion of individuals with the various germline and somatic gonad phenotypes (Table 1A). With a smaller *tra-1* deficiency (*e1855*, J. HODGKIN, personal communication), similar results were obtained (data not shown). Thus we are unable to order the alleles or infer a phenotypic endpoint with respect to *tra-1* germline and somatic gonad function. This contrasts with the nongonadal soma, where partially masculinizing alleles become further masculinized in *trans* to a deficiency (*e1076*; Table 1A, our unpublished observations; HODGKIN 1987) and where complete masculinization is the phenotypic endpoint (HODGKIN 1987).

The production of sperm and then oocytes is the normal pattern of hermaphrodite gametogenesis. This pattern in *tra-1(lf)* *XX* mutant germlines might have been explained by *tra-1* playing no role in germline sex determination. However, the phenotype of *tra-1(lf)* *XO* animals is similar to that of *XX* animals (Table 1B; Figure 1D). Not only does the *tra-1(lf)* *XX* germline produce oocytes but the *tra-1(lf)* *XO* germline does as well. Even for *e1076*, which causes only partial masculinization of the nongonadal soma, 88% of *XO* homozygotes display germline feminization. Furthermore, while the *XO* nongonadal soma is always male, the somatic gonad may be male or "abnormal" and the germline may be "abnormal" as defined above. The proportion of *XO* homozygotes with each phenotype is allele-specific and is similar to that observed in *XX* animals (Table 1B; also see HODGKIN 1987). These phenotypes are recessive in heterozygotes and *eDf2/+* *XO* males are unaffected. Therefore *tra-1(lf)* can feminize the *XO* germline, suggesting that *tra-1(+)* may play a role in germline sex determination in males (see DISCUSSION).

Interaction of *tra-1(lf)* with mutations in other sex-determination genes: The variable defects of *tra-1(lf)*, particularly the germline feminization, allow this phenotype of *tra-1(lf)* to be distinguished from that of other *tra* genes. We therefore constructed double mutants homozygous for *tra-1(lf)* and either *tra-2(lf)* or *tra-3(lf)* to determine the epistasis of the germline phenotypes.

The *tra-2(lf)* germline is indistinguishable from that of wild-type *XO* males; both *XX* and *XO* *tra-2(lf)* homozygotes produce an abundance of sperm in a male somatic gonad (Table 2A; Figure 2A). However, *tra-1(lf)*; *tra-2(lf)* *XX* double mutants show the feminized germline phenotype of *tra-1(lf)* (Table 2C, Figure 2B). The range of effects depends on the *tra-1* allele used and is essentially the same as that of the *tra-1* allele alone (Table 1A). For example, double mutants with alleles *q183q315*, *e1781*, *e1835tr*, *q106*, *e1076* and *q88* show a high frequency of germline feminization, while alleles *e1099* and *q159* show a lower frequency. Feminization of the *tra-2(lf)* *XX* germline by *tra-1(lf)* indicates that the *XX* germline phenotype, like the *XO* phenotype, is feminization,

TABLE 2

Single and double mutant phenotypes for some sex determination mutants

Genotype		Sexual phenotype: Germline	Somatic gonad	Nongonadal soma	Pattern of Epistasis[a]
Wild type	*XX*	Sperm then oocytes (self-fertile)	Female	Female	
	XO	Sperm	Male	Male	
A. Loss-of-function.					
tra-1[b]	*XX*	Sperm then oocytes[c]	Male or abnormal[c]	Male	
	XO	Sperm then oocytes[c]	Male or abnormal[c]	Male	
tra-2[d]	*XX*	Sperm	Male	Incomplete male	
	XO	Sperm	Male	Male	
tra-3[e,f]	*XX*	Sperm or sperm then oocytes	Male or intersexual	Incomplete male	
	XO	Sperm	Male	Male	
fem-1[f,g] or *fem-2*[f,h] or *fem-3*[f,h]	*XX*	Oocytes	Female	Female	
	XO	Oocytes	Female	Female	
her-1[i]	*XX*	Sperm then oocytes (self-fertile)	Female	Female	
	XO	Sperm then oocytes (self-fertile)	Female	Female	
B. Gain-of-function.					
tra-1(gf)[j]	*XX*	Oocytes	Female	Female	
	XO	Oocytes	Female	Female	
fem-3(gf)[k]	*XX*	Sperm	Female	Female	
	XO	Sperm	Male	Male	
C. Double Mutants.[l]					
tra-1(lf); tra-2(lf)[m]	*XX*	Sperm then oocytes	Male	Male	*tra-1(lf)* > *tra-2(lf)* germline and soma
	XO	Sperm then oocytes	Male	Male	*tra-1(lf)* > *tra-2(lf)* germline
tra-1(lf); tra-3(lf)[n]	*XX*	Sperm then oocytes	Male	Male	*tra-1(lf)* > *tra-3(lf)* germline and soma
tra-1(lf); fem-3(gf)[o]	*XX*	Sperm[p]	Male	Male	*fem-3(gf)* > *tra-1(lf)* germline[p] *tra-1(lf)* > *fem-3(gf)* soma
tra-1(lf); fem-1(lf)[q] or *fem-2(lf)*[h] or *fem-3(lf)*[h]	*XX*	Oocytes	Male	Male	*fem(lf)* > *tra-1(lf)* germline *tra-1(lf)* > *fem(lf)* soma
tra-2(lf); tra-3(lf)[r]	*XX*	Sperm	Male	Incomplete male	*tra-2(lf)* > *tra-3(lf)* germline and soma

[a] a > b means the A phenotype is epistatic to the B phenotype.

[b] HODGKIN and BRENNER (1977); HODGKIN (1980, 1983, 1987); this report.

[c] The phenotype shown is that scored in the epistasis analysis. The abnormal germline and somatic gonad phenotypes in double mutants are discussed separately in the text.

[d] HODGKIN and BRENNER (1977); this report. *XX tra-2* males do not show mating behavior and cannot sire cross-progeny.

[e] HODGKIN and BRENNER (1977); HODGKIN (1986); SCHEDL and KIMBLE (1988); this report.

[f] Homozygous mutant from a homozygous mutant mother.

[g] DONIACH and HODGKIN (1984).

[h] HODGKIN (1986).

[i] HODGKIN (1980); TRENT, WOOD and HORVITZ (1988).

[j] HODGKIN (1983, 1987); this report.

[k] BARTON, SCHEDL and KIMBLE (1987).

[l] Double mutant constructions are described in MATERIALS AND METHODS.

[m] Alleles used for *XX* were *tra-1* (*q183q315*, *e1781*, *e1835tr*, *q106*, *e1099*, *q159*, *q165*, *q88* and *e1076*) with *tra-2(e1425)* and (*q183q315*, *e1781* and *e1099*) with *tra-2(e1095)*. For *XO tra-1* (*q183q315* and *e1781*) with *tra-2(e1425)*. *tra-2(e1095* and *e1425)* are putative null alleles. See also HODGKIN (1980).

[n] Alleles used were *tra-1(e1781* and *e1099)* with *tra-3(e1107)*. *tra-3(e1107)* is a putative null allele. All at 25°. See also HODGKIN (1980).

[o] Alleles used were *tra-1(e1781, q106, e1099, q159, q165, e1076* and *q88)* with *fem-3(q20gf)*.

[p] Epistatis is not complete as a low level of germline feminization is observed, see text.

[q] From DONIACH and HODGKIN (1984).

[r] Alleles used were *tra-2(e1425)* with *tra-3(e1107)*. At 25°. Double mutant shows the *tra-2* incomplete male nongonadal soma phenotype. See also HODGKIN (1980).

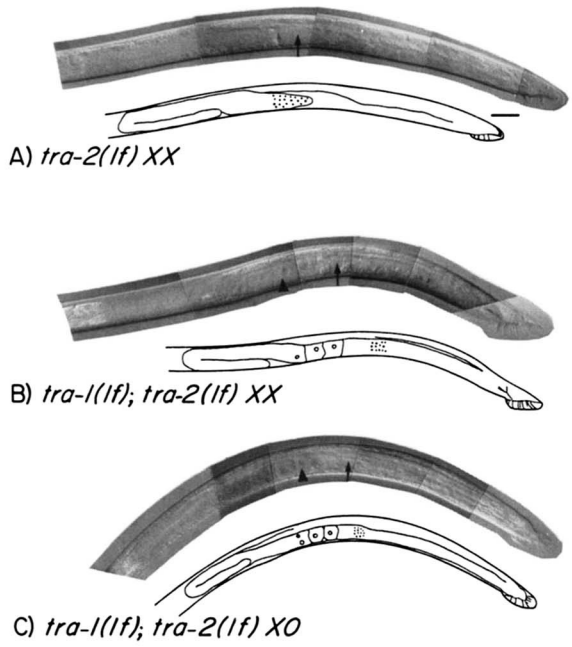

FIGURE 2.—*tra-1(lf)*; *tra-2(lf)* double mutants. (A) *tra-2(e1425)*; ventral view. The germline displays abundant sperm (→), with no evidence of oogenesis, just as in wild-type males. The soma is male, although incompletely transformed as the tail fan and sensory rays are reduced in size and animals do not show mating behavior. (B) *tra-1(e1781)*; *tra-2(e1425) unc-4 XX*; ventral view. (C) *tra-1(q183q315)*; *tra-2(e1425) unc-4*; *lon-2/O XO*; ventrolateral view. Both *XX and XO* double mutants show the *tra-1(lf)* phenotype. In the germline, there are a small number of sperm followed by oogenesis (▶). The soma is male, with a wild-type male tail (although obscured in C) by the ventrolateral view. Scale bar = 40 μm.

rather than a result of incomplete masculinization or execution of the hermaphrodite germline pattern in *XX* males.

The *tra-1(lf)*; *tra-2(lf)* double mutant phenotype was also examined in *XO* males (Table 2C). Because the double mutants are only one-fourth of the *XO* animals scored (see MATERIALS AND METHODS), two *tra-1* alleles with high frequency germline feminization (*q183q315* and *e1781*) were employed to maximize the possibility of observing a phenotypic change. As seen in *XX* animals, the *tra-1*; *tra-2 XO* double mutants show the *tra-1(lf)* phenotype (Table 2, Figure 2C). The simplest interpretation is that the continued spermatogenesis observed in *tra-2(lf) XX* and *XO* mutants is dependent on *tra-1(+)* activity.

The *tra-1(lf)* phenotypes were also found to be epistatic to those of *tra-3(lf)* (Table 2C). Among *tra-3(lf)* m(−/−),z(−/−) *XX* animals, about 40% make an amount of sperm typical of wild-type *XO* animals (in a male somatic gonad), about 40% make sperm and then oocytes (in a male somatic gonad), and about 20% have an abnormal gonad with variable amounts and types of gametes. In *tra-1(lf)*; *tra-3(lf)* m(−/−), z(−/−) double mutants, the phenotypes of *XX* animals is that of the *tra-1* allele alone. For example, when *tra-1(e1781)* was used, 98% of animals made sperm and then switched to oogenesis in a normal male somatic gonad. Therefore, the abundant spermatogenesis in *tra-3* mutants, like that in *tra-2*, may be dependent on *tra-1(+)* activity.

Since *tra-1* is epistatic to *tra-2* and because *tra-3* has a variable germline phenotype with some similarity to that of *tra-1*, we asked if *tra-3* was also epistatic to *tra-2*. We found that all *tra-2(lf)*; *tra-3(lf)* m(−/−),z(−/−) double mutant animals have a normal male germline with abundant and continued spermatogenesis and a normal male somatic gonad (Table 2C). Therefore, *tra-2* is epistatic to *tra-3* and we can conclude that the basis of the *tra-1(lf)* and *tra-3(lf)* germline phenotypes are distinct.

Finally, we examined double mutants carrying *tra-1(lf)* and gain-of-function (*gf*) mutations in *fem-3*. The *fem-3(gf)* mutations masculinize the *XX* germline so that sperm are produced continuously in an otherwise female soma (Table 2B; BARTON, SCHEDL and KIMBLE 1987). This phenotype is termed Mog for *m*asculinization *o*f the *g*ermline. *XO* males are unaffected in *fem-3(gf)* mutants. In *tra-1(lf)*; *fem-3(gf) XX* double mutants, the germline is masculinized relative to the *tra-1(lf)* alleles alone (Table 2C). For example, *tra-1(e1781)*; *fem-3(q20gf)* animals have 83% male and 10% feminized germlines and *tra-1(q106)*; *fem-3(q20gf)* have 89% male and 8% feminized germlines (*fem-3(q20gf) XX* is 100% Mog under these conditions). Therefore, the percentage of masculinized germlines in the double mutants is far greater than that observed in the *tra-1* single mutants (*e1781*, 2% sperm only and 95% feminized germlines; *q106*, 0% sperm only and 93% feminized germlines, Table 1A). We conclude that in the germline, *fem-3(q20gf)* is partially epistatic to *tra-1(lf)*. However, the *tra-1* somatic phenotypes as well as the abnormal germline phenotype were unaltered.

In making the double mutants with *tra-1(lf)* and *tra-2(lf)*, we uncovered an unexpected dominant activity of certain *tra-1(lf)* alleles. For three *tra-1* alleles (*q183q315*, *e1781* and *e1835tr*), the *tra-2(lf)*; *tra-1(lf)*/+ *XX* mutant animals are normal males (capable of mating). However, this was not observed for the remaining six alleles (see MATERIALS AND METHODS). Similarly, *tra-2(lf)*; *eDf2*/+ *XX* animals are incomplete males identical to *tra-2(lf) XX* alone. Thus, while these three *tra-1(lf)* alleles are recessive in a wild-type background, they have a dominant masculinizing activity in the absence of *tra-2* activity.

Unusual *tra-1* gain-of-function (*gf*) alleles obtained by suppression of *fem-3(gf)*: A large number of *tra-1(gf)* mutations have been isolated previously which have the general properties of dominant, nonconditional, feminization of the germline and soma of *XX* and *XO* animals (HODGKIN 1980, 1987). The phenotype of the cononical *tra-1(gf)* allele, *e1575gf*,

TABLE 3

Summary of phenotypes for *tra-1(gf)* alleles isolated as *fem-3(q96gf)* suppressors

Allele/genotype[a]	*XX* phenotypes[b]	*XO* phenotypes
Canonical allele[c]		
e1575gf/+	Female germline; female soma	Female germline; female soma–tail spike slightly truncated
e1575gf/lf	Female germline; female soma	Female germline; female soma–tail spike slightly truncated
Suppressor alleles[d]		
q183gf/+	63% self-fertile, 37% female germline; female soma (15°); 100% female germline; female soma (25°)	Some self-fertile, most with sperm then oocytes; female somatic gonad with partial vulval induction, incomplete male tail (15° and 25°)
q183gf	100% female germline; female soma (15° and 25°)	100% female germline; female somatic gonad with partial vulval induction, incomplete male tail (15° and 25°)
q183gf/lf	3% self-fertile, 97% female germline; female soma (15°)	ND
q245gf/+	100% self-fertile,[e] female soma	73% male germ line and somatic gonad, 27% intersexual gonad; 100% male nongonadal soma
q245gf	2% self-fertile, 98% female germline; female soma with protruding vulva[f]	Sperm then (variably) oocytes; female or intersexual somatic gonad, partial vulval induction, incomplete male tail (sterile)
q245gf/lf	Random sperm and oocytes; intersexual somatic gonad, partial vulval induction, incomplete male tail (sterile)	ND
q185gf/+	100% self-fertile[e]; female soma	56% sperm, 44% sperm then oocytes; male somatic gonad (low frequency intersexual gonad)
q185gf	29% self-fertile, 71% female germline; female soma	Random sperm and oocytes; intersexual gonad, partial vulval induction, incomplete male tail (sterile)
q185gf/lf	89% self-fertile, 11% female germline; female soma with a slightly truncated tail spike and protruding vulva	ND

At 20°C unless indicated. See MATERIALS AND METHODS and text for further details. ND is not determined. Animals with a female or intersexual somatic gonad and partial vulval induction tend to burst as young adults. Therefore, extent of self-fertility may be an underestimate and scoring of yolk in the pseudocoelom could not be determined reliably. When (sterile) is indicated, animals are neither self-fertile nor cross-fertile.

[a] For *e1575/lf*, *lf* is *eDf2*, *e1099* or *e1781*. For *q183gf/lf*, *lf* is *e1099*. For *q245gf/lf* and *q185gf/lf*, *lf* is *eDf2* or *e1781*.

[b] A female germline produces only oocytes; a self-fertile germline produces first sperm then oocytes.

[c] From HODGKIN (1983, 1987) and our observations.

[d] *XX* (*q183gf/+*, *q183gf*, *q245gf/+* and *q185gf/+*) animals were segregants from a heterozygous self-fertile mother. *XX* (*q245gf* and *q185gf*) animals were segregants from a homozygous self-fertile mother.

[e] Brood size was not determined, although it was not obviously smaller than parental *eT1* heterozygotes.

[f] The protruding vulva of *q245gf* is illustrated in Figure 4A.

is shown in Table 3. We have isolated three gain-of-function mutations in *tra-1* as extragenic suppressors of the *fem-3(q96gf)* Mog phenotype (see MATERIALS AND METHODS) which exhibit properties distinct from previously described *tra-1(gf)* mutations (HODGKIN 1980, 1987). Table 3 summarizes the phenotypes of *tra-1(q183gf, q245gf,* and *q185gf)* as heterozygotes, homozygotes, and in *trans* to *tra-1(lf)*. These mutations are defined as gain-of-function because they are dominant (*q183gf* in both *XX* and *XO* animals, *q245gf* and *q185gf* only in *XO* animals; Table 3) and because they, like *tra-1(e1575gf)*, feminize the germline of *XX* and *XO* animals and feminize the soma of *XO* animals. The *q183gf* mutation was shown to be allelic to *tra-1* by isolation of intragenic *tra-1(lf)* revertants (see MATERIALS AND METHODS) and by map position. The *q245gf* and *q185gf* mutations were shown to be *tra-1* alleles by failure to complement *tra-1(lf)* (see below) and by map position.

Unlike previously examined *tra-1(gf)* alleles, *tra-1(q183gf)* shows some temperature sensitivity. Heterozygous, but not homozygous *XX* are temperature sensitive; neither heterozygous nor homozygous *XO* animals are obviously temperature sensitive (Table 3). Temperature shift experiments were performed to determine the time during development that *q183gf* mutant activity causes germ cells to develop as oocytes instead of sperm in the *XX* germline (Figure 3). The up-shift curve has a sharp transition from mutant to wild type during the L2 molt, but the down-shift curve changes gradually from wild type to mutant. The *q183gf/+* temperature sensitive period (TSP) derived from Figure 3 is from the L1 molt to the L4 molt. This TSP is similar to that found for *fem-3(e2006)* (HODGKIN 1986); it begins slightly after and extends

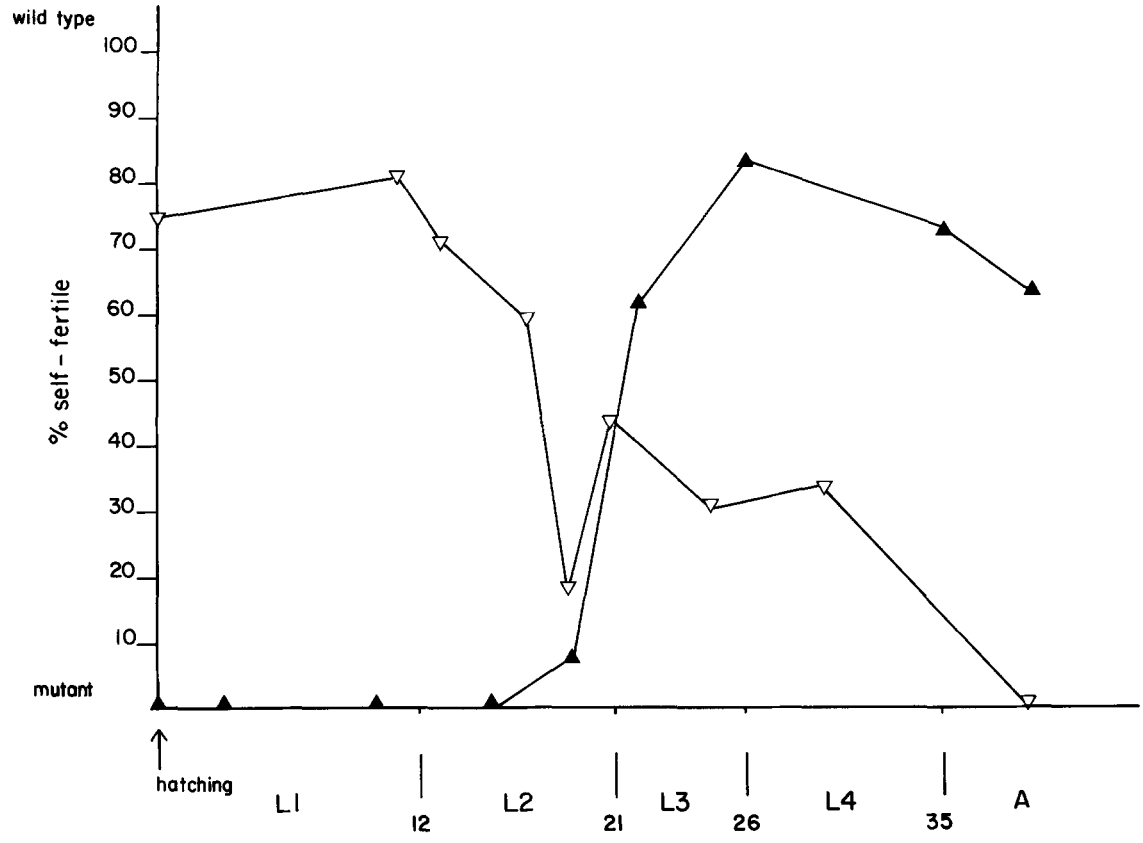

FIGURE 3.—Temperature-sensitive period of *tra-1(q183gf)/+* germline feminization. Percentage of animals that are self-fertile is plotted *vs.* the stage at which animals were shifted. (▽) shifts from restrictive to permissive temperature; (▲) shifts from permissive to restrictive temperature. Temperature shifts of *unc-32 tra-1(q183gf)/qC1* were performed essentially as described previously by BARTON, SCHEDL and KIMBLE (1987). Heterozygous animals (non-Unc and non-*qC1*) were scored as hermaphrodite if they produced self-progeny or female as judged by a characteristic phenotype using the dissecting microscope and the lack of self-progeny. The abscissa represents the developmental stage of individual animals, determined by Nomarski microscopy, at the time of temperature shift. The number of shifted animals is greater than ten for each time point. The vertical lines below the *x*-axis represent larval molts. All time points have been translated to 25° hours (HIRSH and VANDERSLICE 1976).

longer than the TSPs of *fem-1(hc17)* (NELSON, LEW and WARD 1978) and *fem-2(b245)* (KIMBLE, EDGAR and HIRSH 1984); and it precedes the TSP for *fem-3(q20gf)* (BARTON, SCHEDL and KIMBLE 1987). The simplest interpretation is that the *tra-1(q183gf)* mutant activity acts approximately during the same developmental period as *fem-1*, *-2*, and *-3*. In addition, we have found that *tra-1(q183gf)* has a weak maternal effect on *XX* self-fertility at 15° (Table 4). Heterozygous progeny produced by homozygous (female) mothers are significantly more feminized than if produced by heterozygous (female) mothers.

The *tra-1* alleles *q245gf* and *q185gf* have properties of both gain-of-function and loss-of-function *tra-1* mutations (Table 3; Figure 4). Both alleles feminize the germline and somatic tissues of *XX* and *XO* animals; this feminization is dominant in *XO* animals but, unlike previously described *tra-1(gf)* alleles, is recessive in *XX* animals. In addition both alleles masculinize *XX* animals when placed in *trans* to *tra-1(lf)* (Table 3 and Figure 4C). This failure to complement the somatic defects of *tra-1(lf)* indicates a partial loss of *tra-1* function. Previous *tra-1(gf)* alleles were isolated primarily on the basis of dominant *XX* or *XO* feminization or suppression of an *XX tra-3* masculinized soma (HODGKIN 1980, 1986, 1987; DONIACH 1986a). Alleles such as *q245gf* and *q185gf*, which show recessive *XX* germline feminization and are partially defective in female somatic development, have not been isolated by these previous schemes.

TABLE 4

Maternal effect of *tra-1(q183gf)* on *XX* spermatogenesis

Maternal genotype		Zygotic genotype	Phenotype at 15°		
			Percent female	Percent self-fertile	
q183gf/+	× wild type♂	*q183gf/+*	79	21[a]	(n = 169)
q183gf	× wild type♂	*q183gf/+*	89	11[a]	(n = 166)

Experiments were conducted simultaneously and on the same shelf of the incubator.

[a] Results are significantly different from each other [$P < 0.05$] by z-test (FREUND 1973) and Yates corrected chi-squared test (ROSNER 1986).

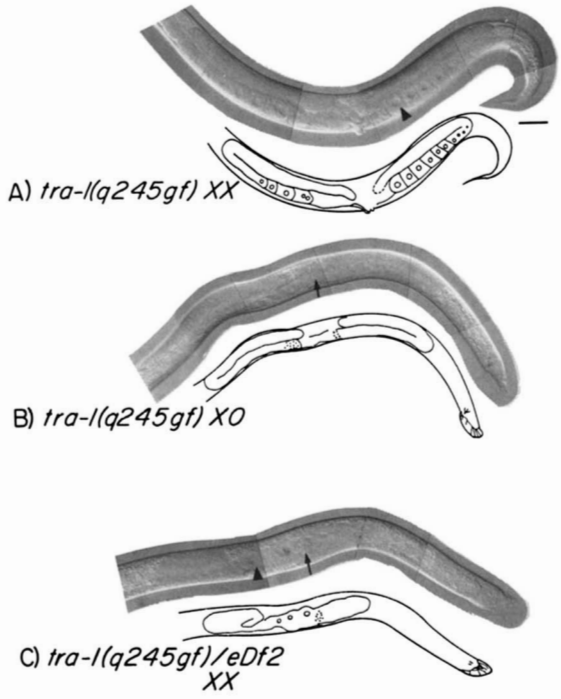

FIGURE 4.—Phenotypes of *tra-1(q245gf)*. (A) *tra-1(q245gf) XX*; lateral view. The germline is female: the first germ cells develop as oocytes (▶) rather than sperm. The soma is essentially female. However, *q245gf XX* animals have a protruding vulva phenotype (compare A with Figure 1A). As *tra-1(q185gf)/tra-1(lf) XX* animals also have a protruding vulva phenotype (Table 3), it is possible that this vulval phenotype is the result of partial loss-of-*tra*-1-function in the soma. (B) *tra-1(q245gf); lon-2*/O XO. An adult just after the L4 molt, lateral view. The germline contains sperm (→). Animals which survive to become older adults can undergo oogenesis. The *XO* soma is partially feminized. The somatic gonad has two U-shaped arms and a partially formed uterus. During adult life, such animals usually rupture at the position of the incompletely formed vulva. The tail has the male characteristics of a fan, rays and spicules. (C) *tra-1(q245gf)/eDf2* (non-Lon) *XX*. Ventral view. The arrangement of sperm and oocytes is disorganized. The *XX* soma is partially masculinized, indicating that *q245gf* cannot supply the normal level of *tra-1(+)* female somatic activity. The tail is partially masculinized exhibiting a fan and rays of reduced size and spicule material. The somatic gonad is disorganized being neither male nor female shaped with gametogenesis occurring primarily in the central region. Vulval formation is only rudimentary. Scale bar = 40 μm.

Interactions of *tra-1(gf)* with *fem-3(gf)*: Analysis of the *XX* phenotype of double mutants carrying both *tra-1(gf)* germline feminizing activity and *fem-3(gf)* germline masculinizing activity shows that they produce sperm followed by oocytes and thus are self-fertile (Table 5). This cosuppression is not the result of selected, allele-specific interactions. For example, *tra-1(e1575gf)/+; fem-3(q95gf)* is self-fertile even though *q95gf* was obtained as a suppressor of *fem-1(lf)* and not as a suppressor of *tra-1(gf)*. The extent of cosuppression correlates with phenotypic strength. The stronger *fem-3(gf)* allele *q95gf* suppresses *e1575gf/+* while the weaker allele *q20gf* does not (Table 5). Similarly, the stronger *tra-1(gf)* allele *e1575gf*, as a homozygote, is fully epistatic to *q95gf*

TABLE 5

Interaction of *tra-1(gf)* and *fem-3(gf)* in the *XX* germline

Allele		Phenotype at 25°			
tra-1(gf)	*fem-3(gf)*	Percent female	Percent self-fertile	Percent Mog[a]	
+	*q20gf*	0	0	100	($n > 200$)[b]
+	*q95gf*	0	0	100	($n > 200$)[b]
e1575gf/+	*q20gf*	100	0	0	($n > 100$)
e1575gf/+	*q95gf*	89	11	0	($n = 75$)
e1575gf	*q95gf*	100[c]	0	0	($n = 21$)
q183gf	*q95gf*	0	56	44	($n = 54$)

[a] Masculinization of the germline.
[b] Data from BARTON, SCHEDL and KIMBLE (1987).
[c] Identified as females, which when crossed to wild-type males, produce broods of only female (*XX* and *XO*) cross progeny.

while the weaker allele, *q183gf*, only partially suppresses *q95gf* (Table 5). Thus, there is a balancing of *fem-3(gf)* masculinizing and *tra-1(gf)* feminizing activities that can result in self-fertility. An analogous balancing is observed between *fem-3(gf)* and *tra-2(gf)* (BARTON, SCHEDL and KIMBLE 1987; SCHEDL and KIMBLE 1988). It should be noted that *fem-3(gf)* mutations stronger than *q95gf* probably could not have been isolated by the original method (BARTON, SCHEDL and KIMBLE 1987) as they would have been unconditionally sterile (Mog).

Analysis of the *XO* phenotype of *tra-1(gf); fem-3(q95gf)* double mutants reveal a partial suppression of *tra-1(gf)* in both germline and soma. Heterozygous *e1575gf/+ XO* animals have a female germline and a female soma with a truncated whip tail (Figure 5A). However, *fem-3(q95gf); tra-1(e1575)/+ XO* animals have a masculinized germline and a partially masculinized soma–an incomplete male tail with fan, rays and spicules (Figure 5B). Somatic suppression by *q95gf* was also observed for *tra-1(q183gf) XO* animals which have a wild-type male tail in the homozygous double mutant (data not shown) rather than the incomplete male tail of the *q183gf* single mutant (Table 3). By contrast, *fem-3(q20gf)* did not show any suppression of *tra-1(e1575gf)* phenotypes. The *XO* somatic masculinization observed with *q95gf* in a *tra-1(gf)* background reveals that, at least for this allele, the *fem-3(gf)* activity is not limited to the germline as previously thought (BARTON, SCHEDL and KIMBLE 1987).

DISCUSSION

This paper describes our investigation of the role of *tra-1* in germline development in *C. elegans*. Table 6 summarizes the phenotypic characterization of both loss-of-function and gain-of-function *tra-1* mutations. The striking observation is that while the soma of *XX* and *XO tra-1(lf)* mutants is masculinized, the germ line of *XX* and *XO tra-1(lf)* mutants is feminized.

The nine somatic masculinizing mutations of *tra-1*

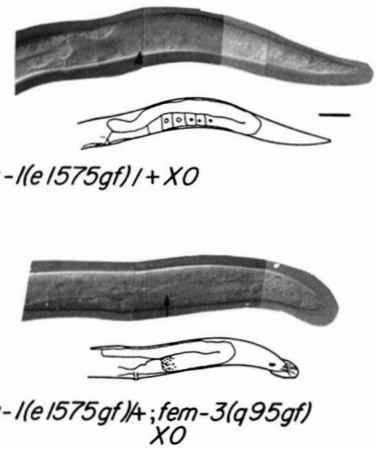

FIGURE 5.—Suppression of *XO tra-1(e1575gf)/+* germline and somatic feminization by *fem-3(q95gf)*. Posterior one-half of young adult animals; lateral view. (A) *tra-1(e1575gf)/+ XO*. Almost complete feminization of *XO* by *e1575gf/+* (Table 2 and 3; HODGKIN 1983, 1987). The germline contains only oocytes (►). The soma is female, with a truncated tail spike. The tail lacks a fan, rays and spicules. (B) *tra-1(e1575gf)/+; fem-3(q95gf) dpy-20 XO*. The *tra-1(gf) XO* feminization is partially suppressed. The germline contains sperm (→). The tail has a fan and rays (of reduced size and number) and spicule material. Animals still have a female shaped somatic gonad. The vulva appears normal although most animals rupture at the vulva during adulthood. Scale bar = 40 μm.

that we have examined are likely to be loss-of-function mutations (also see HODGKIN 1987). Two are amber mutations (HODGKIN 1987), and most amber suppressible mutations in *C. elegans* are null or nearly null (WATERSTON 1981). In addition, they (including the deficiency) are recessive, arise after EMS mutagenesis at a frequency typical of loss-of-function mutations in other *C. elegans* genes, and are obtained as intragenic revertants of dominant *tra-1* alleles (see MATERIALS AND METHODS; HODGKIN 1987). Finally, alleles that only weakly masculinize the nongonadal soma become stronger when placed over a deficiency or over one of the more severe alleles. Therefore, masculinization of the nongonadal soma by these mutations clearly results from a loss of *tra-1* function and they can be referred to as *tra-1(lf)* alleles. Given this loss-of function phenotype, *tra-1(+)* is postulated to direct female development in the *XX* nongonadal soma (HODGKIN 1980, 1983, 1987).

Despite the strong case for loss-of-function described above, the *tra-1(lf)* alleles are probably not "simple" loss-of-function mutations. The first indication of complexity is the differences in germline and somatic gonad phenotypes among the seven alleles which display a complete male nongonadal soma. For example, almost all *tra-1(q106)* mutants have a feminized germline and a male somatic gonad; by contrast, few *tra-1(e1099)* mutants have a feminized germline and they often display abnormal germline and somatic gonad phenotypes. The differences in phenotype observed among the various *tra-1(lf)* alleles cannot simply be attributed to differences in allele strength. If, for example, either *q106* or *e1099* were just a weaker allele, the phenotype of one of them would be expected to change and become more similar to the stronger allele when placed in *trans* to a deficiency. However, the allele specific germline and somatic gonad phenotypes do not significantly change in hemizygotes (see RESULTS). Thus the germline and somatic gonad phenotypes of the various alleles can not be ordered in a graded series and do not converge to a single phenotypic endpoint. This is distinct from the nongonadal soma, where alleles converge to a male phenotypic endpoint (HODGKIN 1987).

The second indication of complexity is the difference observed among the *tra-1(lf)* alleles in *tra-1(lf)/+; tra-2(lf)* double mutants. Although seven *tra-1(lf)* alleles are indistinguishable in their effect on the nongonadal soma as homozygotes, the alleles differ dramatically in their effect on the nongonadal soma in the absence of *tra-2(+)*. Three alleles (*q183q315*, *e1781* and *e1835tr*) show a dominant masculinization of the nongonadal soma in the absence of *tra-2(+)*, while all the other alleles examined show no such dominance. The dominance is not due to a haploinsufficiency for *tra-1*, because the deficiency does not exhibit similar dominance.

What does it mean that *tra-1(lf)* mutations are not "simple" loss-of-function mutations? We envision two possibilities. First, the *tra-1* locus may make more than one product. These products may be differentially affected by the mutations resulting in the allele specific phenotypes. Second, the *tra-1* product(s) may regulate synthesis or activity of itself or another *tra-1* product. Positive autoregulation might produce equivalent amounts of *tra-1* product(s) in hemizygotes and homozygotes resulting in the similar phenotypes. Either possibility, or a combination of the two, can explain the genetic data available (HODGKIN 1987; this paper). The possibility of multiple *tra-1* functions and of *tra-1* autoregulation has also been suggested from analysis of somatic phenotypes in *tra-1(lf)* mutants and analysis of germline phenotypes in *tra-1(gf)* mutants (HODGKIN 1987; SHEN and HODGKIN 1988).

Feminization of the *XX* and *XO* germ line observed in *tra-1(lf)* animals was unexpected given that the soma is masculinized (Table 6). Loss-of-function mutations in each of the other autosomal sex-determining loci (*her-1*, *tra-2*, *tra-3*, *fem-1*, *fem-2* and *fem-3*) cause the same sexual transformation in all tissues for that particular locus. For example, *tra-2(lf)* masculinizes all tissues (HODGKIN and BRENNER 1977) and *fem-1(lf)* feminizes all tissues (DONIACH and HODGKIN 1984). If the *tra-1(lf)* alleles are not "simple" loss-of-function alleles (as discussed above), might the feminization of the *tra-1(lf)* germline be due to the aberrant production of a *tra-1* product? For example, perhaps loss of

TABLE 6

Summary of sexual transformations in *tra-1* mutants

Allele	Phenotype	
	Soma	Germline
tra-1(lf)	Masculinized[a]	Feminized[b]
tra-1(gf)	Feminized[c]	Feminized[d]

See text, Tables 1 and 2, and HODGKIN (1987) for further details. Since it is unclear whether the abnormal germline and somatic gonad phenotypes are the result of sexual transformation, they have not been included (see RESULTS).

[a] *XX* soma.

[b] *XX* and *XO* germline. Feminization refers to a brief period of spermatogenesis followed by oogenesis.

[c] *XO* soma.

[d] *XX* and *XO* germline. Feminization refers to production of oocytes only.

one *tra-1* product leads to the synthesis of a novel/inappropriate product. If true, then that product (or a normal product produced at an abnormal time or in an abnormal tissue) may direct feminization of the germ line. The primary argument against the feminization being caused by an aberrant *tra-1* function is the fact that this phenotype is recessive, unlike feminization by *tra-1(gf)* mutations (HODGKIN 1987; this report). In *XO* animals, where dominant feminization of the germ line would be easily detected, the *tra-1(lf)* feminization is always recessive. Although recessive gain-of-function mutations do exist (*e.g.*, *fem-3(q22gf)*; BARTON, SCHEDL and KIMBLE 1987), they are unusual.

Two opposing models can explain the germline feminization by *tra-1(lf)*. If *tra-1(lf)* leads simply to the loss of *tra-1* germline function, then the wild-type *tra-1* product is required for continued specification of spermatogenesis in the male germline. Although it seems counterintuitive that *tra-1* would have opposite roles in sex determination, it is not without precedent. For example, the *dsx* gene of Drosophila specifies both male and female somatic fates (BAKER and BELOTE 1983). On the other hand, given the complexity of *tra-1* genetics, we cannot exclude the possibility that these mutations lead to the production of a novel/inappropriate *tra-1* product which causes feminization. To explain the recessive nature of this effect, one can postulate that in *tra-1(lf)*/+ heterozygotes, the novel/inappropriate product is negatively regulated by *tra-1*(+), but in the *tra-1(lf)* homozygote the novel/inappropriate product is able to function.

Feminization of the *XX* and *XO* germline in certain *tra-1(lf)* mutants (*e.g.*, *e1099*) occurs at a low frequency. However, oogenesis may be precluded in these animals due to defects in gonadal morphogenesis and in the germline stem cell population. When very few germ cells are produced, gametogenesis is limited to spermatogenesis even in wild type (KIMBLE and WHITE 1981). The abnormal somatic gonad and germline stem cell defects observed in some *tra-1(lf)* homozygotes are reminiscent of the elimination of regulatory cells in the somatic gonad: the somatic gonadal "leader" cell is responsible for gonadal morphogenesis and the somatic gonadal "distal tip cell" is responsible for the existence and position of the germline stem cell population (KIMBLE and WHITE 1981). Therefore if the lineages of the somatic gonad are defective in *tra-1(lf)* homozygotes, the generation and function of these regulatory cells may be variably affected, resulting in the variable phenotypes.

What can we deduce about the role of *tra-1*(+) in male germline development if we assume that *tra-1(lf)* leads to loss of *tra-1* germline function? Since both sperm and oocytes are produced in mutant animals, *tra-1* is not necessary for specification of sperm or oocytes *per se* (DONIACH and HODGKIN 1984; HODGKIN 1986). Our experiments indicate that the continuous spermatogenesis observed in *XO* males, *XX* and *XO* *tra-2(lf)* and *XX* *tra-3(lf)* mutants is dependent on *tra-1*(+) activity. Thus, while *tra-1*(+) is not necessary *per se* for specification of spermatogenesis, it may act to maintain commitment to the male germline pathway once it has been initiated. Since *tra-1(lf)* is epistatic to *tra-2(lf)* and *tra-3(lf)*, *tra-1* probably does not act through *tra-2* and *tra-3*, but instead acts independently and/or downstream of them. The spermatogenesis that does occur in *tra-1(lf)* mutants is dependent on *fem-1*, *fem-2* and *fem-3* gene activity (DONIACH and HODGKIN 1984; DONIACH 1986b; HODGKIN 1986). *tra-1*(+) may thus promote continuous spermatogenesis in males by maintaining *fem* gene activity.

The genetic complexity of *tra-1* is reinforced by the phenotypes of *tra-1(gf)* alleles. Previously described *tra-1(gf)* mutants display dominant feminization of the germline and soma of *XX* and *XO* animals (HODGKIN 1980, 1983, 1987; Table 6). Hence, in the soma, *tra-1(lf)* mutations are masculinizing while *tra-1(gf)* mutations are feminizing (Table 6). This reciprocal transformation of somatic sexual fate indicates that *tra-1* acts as a genetic switch: the presence of *tra-1* activity specifies the female somatic fate, the absence of *tra-1* activity specifies the male somatic fate (HODGKIN 1983). By contrast for germline phenotype, both *tra-1(lf)* and *tra-1(gf)* feminize (Table 6). The disparate consequences of the state of *tra-1* on germline and somatic phenotype suggest that the function and/or regulation of *tra-1* in directing germline and somatic sex is different.

Two *tra-1(gf)* alleles (*q185gf* and *q245gf*) described here are novel in that the feminized germline phenotype of *XX* animals is recessive and that a partial loss-of-function phenotype is observed for *XX* somatic characteristics (see RESULTS). These two alleles may define a domain of the *tra-1* locus that is distinct from

that defined by previous *tra-1(gf)* mutations.

The role of *tra-1(+)* in hermaphrodite germline development is uncertain. It may not be valid to extend to the germline of hermaphrodites the hypothesized role of *tra-1(+)* in maintaining continued spermatogenesis that was deduced from the germline phenotype of *tra-1(lf) XX* and *XO* animals with a male soma. The *tra-1(gf)* phenotype demonstrates that *tra-1* mutations can affect germline sex of animals with a female (hermaphrodite) soma. In addition, self-fertility can be restored to *tra-1(gf)* mutants by a cosuppressive interaction with *fem-3(gf)*. However, deducing the role of *tra-1(+)* in the hermaphrodite germline based on *tra-1(gf)* alleles may be misleading since the mechanism(s) of their inappropriate function is not known.

The genetic complexity of *tra-1* mutants reported here and previously (HODGKIN 1987; SHEN and HODGKIN 1988) can be explained by a number of models. The *tra-1* gene has recently been cloned (J. HODGKIN, personal communication). This will allow the basis of the genetic complexity to be determined, and allow predictions based on these models to be tested.

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