A genetic analysis of the sex-determining gene, *tra-1*, in the nematode *Caenorhabditis elegans*

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The normal sexes of *Caenorhabditis elegans* are the self-fertilizing hermaphrodite (XX) and the male (XO). The autosomal gene *tra-1* is a major switch gene controlling sexual phenotype. Mutant phenotypes of 43 loss-of-function (*lf*) *tra-1* alleles and 22 gain-of-function (*gf*) *tra-1* alleles are described and discussed. The *tra-1(lf)* alleles are recessive and, in general, masculinizing. The most severe mutations (such as seven out of eight identified amber alleles) can transform XX animals into fertile males. These mutations have little effect on XO animals (which are male already) but lead to some abnormalities in XO gonadal development, indicating that *tra-1* has functions in normal development of both sexes, although its major function is confined to the XX hermaphrodite. Weaker *tra-1(lf)* alleles lead to incomplete masculinization of XX animals, resulting in a variety of intersexual phenotypes. The *tra-1(gf)* alleles are dominant and have an opposite, feminizing effect. Six out of 22 can transform XO animals into fertile females or hermaphrodites, whereas the remainder cause partial feminization. All 22 transform XX animals into fertile females. Limited intragenic mapping indicates that the gene is large and that *gf* alleles map to a location different from *lf* alleles. The results suggest that the *tra-1* gene has several roles in wild-type sexual development. First, *tra-1* activity dictates female, as opposed to male, development in all nongonadal tissues of XX animals. Second, *tra-1* activity dictates female development in the somatic gonad of XX animals. Third, a high level of *tra-1* activity may act to inhibit spermatogenesis in the XX germ line, thereby assisting the switch from spermatogenesis to oogenesis in the hermaphrodite. These three functions are all feminizing and specific to the XX animal; the gene also has minor functions in the XO animal, which are to assist normal male somatic gonad development and to promote abundant spermatogenesis in males. A low level of both spermatogenesis and oogenesis can occur in the absence of *tra-1* activity. Both the function and the regulation of this gene must be complex.

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The genetic analysis of development in *Caenorhabditis elegans* has tended to focus on "switch genes," that is, loci with genetic properties indicating that they control important choices during development (Hodgkin 1984; Sternberg and Horvitz 1984). For example, the *lin-14* gene appears to govern the relative timing of cell-division patterns in various tissues (Ambros and Horvitz 1987), and the *lin-12* gene is involved in determining the spatial distribution of certain cell lineages (Greenwald et al. 1983). The analysis of sexual development has also yielded good examples of switch genes that control the choice between male and female pathways of development.

In normal development of *C. elegans*, animals with a single X chromosome (XO) develop into males, and animals with two X chromosomes (XX) develop into hermaphrodites, which are essentially modified females. Mutations in at least seven different autosomal genes can cause partial or complete alteration of the normal process, either masculinizing XX animals or feminizing XO animals. For one of these genes, *tra-1*, ("trans-

former"), complete sexual transformations of both types have been demonstrated: loss-of-function (*lf*) alleles cause XX animals to develop into fertile males, and gain-of-function (*gf*) alleles cause XO animals to develop into fertile females. As a result, it is possible to construct stable strains in which sex is controlled by the state of the *tra-1* locus, not by X chromosome dosage (Hodgkin 1983b). This gene therefore appears to be a "master regulator" for sexual differentiation: *tra-1* gene activity is both necessary and sufficient to promote female development, and lack of *tra-1* activity is necessary and sufficient for male development.

The properties of the other six genes and the epistatic interactions between them and *tra-1* suggested that their main wild-type role is to mediate between the primary sex-determining signal [X chromosome dosage or, more accurately, the X chromosome-to-autosome ratio (Madl and Herman 1979)] and the key gene *tra-1* (Hodgkin 1980). In an XO animal (X/A ratio low), these regulatory genes were proposed to render the *tra-1* gene inactive ("OFF"), thereby permitting male development in both

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soma and germ line. In an XX animal (X/A ratio high), *tra-1* was proposed to be "ON" in the soma, promoting female development. In the XX germ line, *tra-1* would initially be OFF (permitting some spermatogenesis) but, later in development, would be ON (promoting oogenesis). The net result would be production of both sperm and oocytes in a female soma, resulting in a self-fertile hermaphrodite phenotype. The regulatory interactions leading to these different activity states have been studied extensively at a genetic level, and the current model for the whole process is quite detailed (Hodgkin et al. 1985).

This paper reports an extensive mutational analysis of the *tra-1* gene, based on the properties of 43 *lf* (masculinizing) alleles and 22 *gf* (feminizing) alleles. The results indicate that correct *tra-1* gene function is crucial to normal sexual development of all tissues and that this gene has a complex function. The simple model originally proposed (XX: *tra-1* ON, XO: *tra-1* OFF) is shown to require modification, because *tra-1* appears to have a minor role in XO animals, as well as its major role in XX animals.

In characterizing these mutations, five main aspects of the sexual phenotype have been examined: germ line, somatic gonad anatomy, gross tail anatomy, yolk production, and overall fertility (either self-fertility, in the case of the hermaphrodite, or cross-fertility, in the case of males). The anatomical features are shown in Figure 1.

Results and discussion

Recessive (masculinizing) alleles

Mutations of *tra-1* were first recognized by the appearance of masculinized animals in mutagenized self-ferti-

lizing hermaphrodite stocks (XX). Some mutants appeared to be masculinized completely and were shown to carry and transmit two X chromosomes, thereby demonstrating that they were XX animals transformed into fertile males (Hodgkin and Brenner 1977). General screens for a variety of *C. elegans* mutants have continued to yield recessive *tra-1* alleles, to a total of seven to date.

Masculinizing mutations have also been deliberately sought by two methods: screening for extragenic suppressors of a gene *her-1* (Hodgkin 1980) and by a general screen for mutations causing masculinization of XX animals (see Materials and methods). Both of these methods yielded *tra-1* mutants. This gene appears to be the only locus in *C. elegans* that can be mutated easily to yield fertile XX males. Two related genes, *tra-2* and *tra-3*, have also been identified, but *tra-2* and *tra-3* XX mutant males are masculinized incompletely and never fertile. The general screen for masculinizing mutations yielded several alleles of both *tra-1* and *tra-2*, permitting an estimate of forward mutation frequency for these loci: about 2×10^{-4} for *tra-1* and 10^{-3} for *tra-2*. These mutations are therefore relatively frequent and likely to represent loss of function for the respective loci.

A large number of additional masculinizing alleles of *tra-1* have been obtained by reverting a dominant, feminizing allele of *tra-1*. As explained in Materials and methods, this reversion was carried out in two steps: The strong dominant allele *e1575* was modified by the introduction of a linked modifying mutation, *e1816*, and the double-mutant allele *e1816e1575* (which is still feminizing) was used to select for *cis*-acting intragenic suppressors that would eliminate the dominant feminizing effect. A total of 27 recessive masculinizing alleles were obtained, ranging in strength from those causing

Figure 1. Major anatomical differences between hermaphrodite and male. The intestine of the adult hermaphrodite is specialized for the synthesis of large amounts of yolk proteins.

complete XX masculinization (the majority class) to those causing only slight masculinization. These alleles are distinguished from those isolated previously by the suffix *tr*, to indicate that they are triply mutant at the *tra-1* locus. In most cases, the effects of the original dominant mutation are masked completely by the recessive masculinizing mutation.

One recessive lethal allele of *tra-1* was obtained by this means, which is probably a small deficiency, as it fails to complement an essential gene adjoining *tra-1* (see Materials and methods). A large *tra-1* deficiency, *eDf2*, has been described previously (Hodgkin 1980); this and the corresponding free duplication, *eDp6*, have been used for varying gene dosage of *tra-1* and for balancing *tra-1* alleles, because *eDp6* carries *tra-1*(+).

Null phenotype of *tra-1*

Superficially, most recessive alleles of *tra-1* appear to cause complete masculinization of XX animals. All non-gonadal structures examined so far have a male phenotype, behavior is indistinguishable from that of a wild-type male, and the XX animals are capable of cross-fertilizing hermaphrodites. The complete transformation of sexual phenotype in the tail is illustrated in Figure 2B (*tra-1* XX, indistinguishable from the wild-type XO male tail illustrated in Fig. 2A). Weaker recessive alleles, which cause only partial masculinization, have also been obtained (see Fig. 2C–G). Stronger masculinization is seen when one of these alleles (*e1076*, Fig. 2F) is placed in *trans* with either *eDf2* or with the strong allele *e1099* (Fig. 3). For this reason, it appeared possible that *e1099* might represent a complete or almost complete loss of function.

For all of the strong alleles, XX male fertility is much lower than that of wild-type XO males, indicating that the germ line and/or somatic gonad is not normal in the *tra-1* XX males, as compared to wild-type XO males. Gonadal phenotypes have been examined for a number of these strong alleles and found to fall into three main phenotypic classes (A1, A2, and A3 in Table 1). As discussed below, it is not certain which (if any) of these corresponds to a complete lack of *tra-1* function, that is, a *tra-1* null. Three alleles are described in detail, typifying each of the three classes: *e1099*, *e1781*, and *e1835tr*. Mutant gonads are illustrated in Figure 4, and histograms summarizing data are shown in Figure 5.

For *tra-1*(*e1099*) (class A1), the somatic gonadal phenotype is variable, ranging from a well-formed but undersized male gonad (Fig. 4A) to a disorganized mass (Fig. 4B). Oocytes or oocytelike cells are rarely seen in these gonads. In single male mating tests, 10/46 males were fertile, siring 2–262 progeny (mean 43). Wild-type XO males are much more fertile: In one test, 6/6 males were fertile, siring 213–2871 progeny (mean 1792).

In contrast, *tra-1*(*e1781*) XX males (class A3) always have a large well-formed male somatic gonad, containing few or no sperm, and many large oocytelike cells (Fig. 4C). In mating tests, 3/36 males were fertile, siring 15–63 progeny (mean 27).

Figure 2. *tra-1* mutants of *tra-1*: tail phenotypes, viewed ventrally. Bar, 25 μ . (A) wild-type XO (male); (B–G) *tra-1* XX; (H) wild-type XX (hermaphrodite). The *tra-1* alleles are *e1099* (B); *e1488* (C); *e1928* (D); *e1929* (E); *e1076* (F); *e1732* (G) (see also Table 1).

The third allele, *e1835tr* (class A2), is intermediate between these two: Some animals resemble *e1099* in phenotype, but most resemble *e1781* (Fig. 4D). Fertility is higher than that for either of the other two alleles: 15/34

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Figure 3. Tail phenotypes, semilateral view. Bar 25 μ . (A) *e1076/eDf2*; (B) *e1076/e1099* (cf. Fig. 2F).

males were fertile in mating tests, siring 1–125 progeny (mean 44).

Thus, none of these *tra-1* alleles, nor any of 10 other strong alleles examined, causes perfect masculinization of the gonad in an XX animal: Either the soma or the germ line is abnormal. The division of these mutations into three classes, as in Table 1, is somewhat artificial, because there seems to be a spectrum of gonadal phenotypes in which the A1 and A3 types represent the extremes and the A2 class includes all intermediates.

All of the strong alleles cause complete masculinization of tail structures and also of the intestine: None of those examined produces any yolk protein. It is likely this completely male nongonadal phenotype is the null phenotype of *tra-1*, for several reasons. First, strong alleles are found at gene knockout frequency (Greenwald and Horvitz 1980). Second, 37 alleles have been tested for suppressibility by the amber suppressor *sup-7* (Waterston 1980), and eight amber alleles were identified. Seven of the eight are strong alleles, and the eighth is only slightly less masculinized than these (Hodgkin 1985). Third, as described above, strong alleles and the deficiency *eDf2* behave similarly in complementation tests (Fig. 3).

For the gonadal phenotype, matters are more complicated. The three types described above all occur fairly frequently. The availability of amber alleles does not provide useful information, because all three classes are represented among the amber alleles. The *e1099* allele is nonamber, and the *e1781* allele is amber. Seven of the triple alleles obtained by *tra-1(gf)* reversion are amber, and these can be compared with each other: Two resemble *e1099* (*e1828tr* and *e1849tr*), one resembles *e1781* (*e1838tr*), and four are intermediate (*e1825tr*, *e1835tr*, *e1844tr*, and *e1860tr*).

Complementation tests using the *eDf2* deficiency gave the results shown in Figure 5: *e1099/DF* resembles *e1099/e1099*, and *e1781/Df* resembles *e1781/e1781*; however, *e1781/e1099* resembles *e1781/Df*. This result makes it possible to argue that *e1781* does not correspond to the deficiency but that *e1099* does and, therefore, that *e1099* is the “true null.” Nevertheless, it is still possible to make alternative arguments in favor of either *e1781* or *e1835tr* being a true null.

It is unlikely that there is a completely different null phenotype such as lethality. First, although the set of amber alleles is heterogeneous, none of them affects viability, and most amber mutations in *C. elegans* are either null or close to null (Waterston 1980). Second, the experiments inducing *lf* alleles by reverting the dominant *gf* allele yielded no recessive lethal alleles when a point mutagen [ethyl methane sulfonate (EMS)] was used, but one lethal (*e1855*) was found when X-rays were used. This lethal fails to complement both *tra-1* and an essential gene mapping close to *tra-1*, *pha-1(e2123)* (H. Schnabel, pers. commun.), so it is likely that *e1855* is a deficiency for both genes. Thus, lethals could have been recovered with EMS, but none was found. A dominant lethal null phenotype for *tra-1* is excluded, because *eDf2/+* is viable.

In conclusion, it is probable that the question of the true null will not be settled in the absence of molecular analysis, and maybe not even then. In dealing with a complex gene such as *tra-1*, this is perhaps not surprising.

Recessive alleles: XO phenotype

Initial experiments (Hodgkin and Brenner 1977) suggested that neither strong nor weak recessive *tra-1* alleles had any effect on XO animals, which appeared to be normal males. A test to compare the fertility of wild-type and *tra-1* XO males was carried out by selfing a hermaphrodite of genotype *tra-1(e1099)/+;him-5 dpy-21*. The *him-5* mutation leads to the production of XO self-progeny, and the *dpy-21* mutation permits distinction between XX and XO animals, independent of sexual phenotype (Hodgkin et al. 1979; Hodgkin 1980). All XO progeny were male; 36 individual males were picked and mated exhaustively with *unc-17* hermaphrodites, in order to measure maximum fertility (Hodgkin 1983a). Individual male genotypes were determined retrospectively, by progeny testing: 8/36 were *+/+*, siring 103–807 progeny; 16/36 were *tra-1/+*, siring 80–1006 progeny; 5/36 were *tra-1/tra-1*, siring 69–715 progeny. Therefore, it appeared that at least some *tra-1* XO males could exhibit high fertility, within the wild-type XO male range. However, it was possible that fertility might be enhanced by the *dpy-21* mutation; it was also possible that some *tra-1* XO males might be completely infertile.

Therefore, *tra-1* XO males of the three strong alleles described above were constructed explicitly (see Materials and methods), and gonadal phenotypes were examined by Nomarski microscopy. As shown in Figures 4 and 5, these phenotypes are frequently abnormal, and in

Table 1. XX phenotypes of *tra-1(lf)* alleles

Class	Alleles ^a	Phenotype
A1	<i>e1099</i> , <i>e1728</i> , <i>e1729</i> , <i>e1783</i> , <i>e1828tr*</i> , <i>e1849tr*</i>	fully male tail; somatic gonad small, variable, lacking oocytes; often fertile
A2	<i>e1516</i> , <i>e1835tr*</i> , <i>e1844tr*</i> , <i>e1860tr*</i>	fully male tail; somatic gonad variable, often containing oocytes; often fertile
A3	<i>e1781*</i> , <i>e1774</i> , <i>e1838tr*</i>	fully male tail; somatic gonad large, male, usually containing oocytes; infrequently fertile
A1–3	<i>e1730</i> , <i>e1783</i> , <i>e1822tr</i> , <i>e1829tr</i> , <i>e1832tr</i> , <i>e1833tr</i> , <i>e1834tr</i> , <i>e1836tr</i> , <i>e1845tr</i> , <i>e1856tr</i> , <i>e1857tr</i> , <i>e1858tr</i>	fully male tail; gonad uncharacterized; sometimes fertile
A4	<i>e440</i> , <i>e1766</i> , <i>e1830tr</i>	fully male tail; gonad abnormal; infertile; fertile in <i>trans</i> with <i>e1099</i>
A5	<i>e1825tr*</i> , <i>e1850tr</i>	slightly abnormal male tail (variable); somatic gonad variable, sometimes containing oocytes; infertile; infertile in <i>trans</i> with <i>e1099</i>
A6	<i>e1928</i> , <i>e1847tr</i> , <i>e1859tr</i>	fan and rays reduced; somatic gonad usually male, containing sperm and oocytes; never fertile
A7	<i>e1929</i> , <i>e1837tr</i> , <i>e1840tr</i> , <i>e1853tr</i>	fan and rays very reduced; gonad as A6
A8	<i>e1076</i> , <i>e1843tr</i>	rays absent, fan almost absent, tail spike present; gonad as A6
A9	<i>e1732</i> , <i>e1846tr</i>	almost fully hermaphrodite tail; gonad as A6; yolk present
B1	<i>e1488</i>	fully male tail, variable hermaphrodite gonad, often self-fertile; yolk present
B2	<i>e1764</i> , <i>e1831tr</i>	variable male tail; variable hermaphroditic gonad, some vulval induction; never self-fertile; yolk present

^a (*) Amber alleles.

general, XX and XO phenotypes are rather similar for each of these alleles. Some *e1099* XO males have very abnormal gonads, like some *e1099* XX males; others have a more normal male gonad, and oocytes have not been seen in *e1099* XO males. However, apparent oocytes are seen in XO males of both *e1781* and *e1835tr*. Male fertility for these alleles is low. Therefore, it appears that at least some aspect of *tra-1* activity is required for full male fertility and probably for normal development of the male somatic gonad.

The requirement for *tra-1* activity in the XO seems to be low and almost dispensable. XO male fertility has also been examined for a weaker *tra-1* allele, *e1488*, and found to be similar to wild type.

Weaker recessive alleles of *tra-1*: At least two allelic series

As well as the strong alleles, which cause almost complete masculinization of XX animals, a number of *tra-1* mutations causing incomplete masculinization have been identified and studied. The phenotypes of these alleles are significant because they demonstrate that different aspects of the sexual phenotype can be varied independently and that tissues of opposite sexual phenotype can coexist in the same body.

Most of the weak alleles fall into one allelic series (Table 1, classes A4–A9). The weakest members of this series have a male (one-armed) somatic gonad, containing both sperm and oocytes, but the nongonadal anatomy is almost completely hermaphrodite (e.g., *e1732*, illustrated in Figs. 2G and 6A). Intermediate alleles show increasing levels of masculinization, as illustrated for the tail phenotype in Figure 2. Yolk protein production (Fig. 7) is detectable in mutants carrying the weakest alleles, but little or no yolk is made by any of the stronger mutants.

Several other recessive alleles of *tra-1* have pheno-

types that cannot be fitted into this allelic series (Table 1, classes B1 and B2). The most remarkable is the *e1488* phenotype: XX animals homozygous for *e1488* have a male tail anatomy and exhibit mating behavior, but the gonad (both soma and germ line) is essentially hermaphrodite (Figs. 2C and 6B). As a result, *e1488* XX strains can be grown as homozygous self-fertile stocks. Only about half of the XX animals produce self-progeny (31/51), and brood sizes are low: 1–28, mean 12. Eggs are never laid (in part, because the sex-specific musculature is male, so egg-laying muscles are absent), and consequently, the self-fertile hermaphrodites end up as “bags of worms”—empty carcasses surrounding their progeny, which eventually break free. The low self-fertility cannot be ascribed to the egg-laying defect, because other kinds of egg-laying defective mutants usually produce higher numbers. For example, six *lin-3* bags were examined and found to contain 69–79 progeny each [*lin-3* mutant hermaphrodites have defective vulval development (Sternberg and Horvitz 1984)]. Therefore, gonad function is not normal in the *e1488* mutant. Vulval development also appears to be abnormal, because most *e1488* XX individuals cannot be cross-fertilized by males. Cross progeny have been obtained from occasional animals.

Two other alleles, *e1764* and *e1831tr*, exhibit phenotypes similar to that of *e1488*, as they often have hermaphroditic gonads and may exhibit partial vulval development. However, neither of these mutants is ever self-fertile. Both *e1488* and *e1764* make yolk proteins in abundance (Fig. 7). Another mutant that exhibits good male tail development but still makes yolk protein is the spontaneous allele *q88* (identified by T. Schedl, pers. commun.). In this mutant, the gonad has a male (A3) phenotype, and *q88* XX males are often fertile.

It is possible that more detailed examination of *tra-1* mutant phenotypes would reveal further complexities.

Figure 4. Gonadal phenotypes of wild-type and *tra-1* mutants. (Left) Nomarski micrographs; (right) sketches indicating gonad outlines and gametes. Anterior is left, dorsal is up. Bar, 25 μ . White arrows mark sperm, black arrows mark oocytes, arrowheads mark the level of the distal tip of the gonad (see Fig. 1). (A) *e1099 XX*: small well-formed male gonad. (B) *e1099 XX*: abnormal ovoid vacuolated gonad. (C) *e1781 XX*: male gonad containing sperm proximally, oocytes distally. (D) *e1835tr XX*: male gonad containing oocytes. (E) *lon-2 XO*: wild-type male gonad containing sperm. (F) *e1781 lon-2 XO*: male gonad containing oocytes. (G) *e1835tr lon-2 XO*: abnormal ovoid gonad containing some sperm.

Already the data suggest that the *tra-1* gene has a complex function: One class of partial mutant (A9) has a major effect on the gonad but little effect on the rest of the body, whereas another class (B1) has little effect on the gonad but a major effect on nongonadal anatomy. The two functions identified by these mutants are not wholly separable, because heterozygotes between mutations of the two classes exhibit an intermediate phenotype, rather than a wild-type phenotype. Heterozygous

e1732/e1488 XX animals are variable and usually have an intersexual phenotype in both tail and gonad; few self-progeny are ever produced.

The mutant phenotype of alleles such as *e1488* and *e1732* also suggests that sexual phenotype in different parts of the body is determined autonomously, rather than by a diffusible factor such as a hormone. Laser ablation experiments have already provided evidence for a great deal of cellular autonomy in sexual development

(Sulston and White 1980), the only exception being the induction of vulval cell lineages, which is dependent on the somatic gonad (Kimble 1981).

gf (dominant) alleles

A total of 22 *gf* alleles have been isolated to date, using a variety of screens and selections (see Materials and methods). All cause dominant feminization, either partial or complete, in both XX and XO animals. All are tightly linked to the *tra-1* locus, being less than 0.5 map units from recessive *tra-1* alleles such as *e1099* or *e1781*. This shows that they must be *gf* mutations of some kind, because the deficiency *eDf2* has no dominant feminizing effect. The belief that all of these alleles are *tra-1(gf)* mutations rests on their map location and also

on experiments demonstrating that *e1575*, the first and best characterized of these alleles, is a *cis*-dominant *tra-1* mutation. Selections for revertants of *e1575* yielded recessive *tra-1* masculinizing mutations, including seven amber alleles. One of these, *e1838tr*, has a phenotype closely resembling that of the amber allele *e1781*, and these two amber mutations may, in fact, be identical. This permits a *cis-trans* test: If the *gf* mutations were in a gene separate from the *lf* mutations, a *cis* heterozygote *e1575e1816e1838/+ + +* should have an identical phenotype to a *trans* heterozygote *e1575e1816/e1781*. In fact, the latter genotype results in a female phenotype (both XX and XO), whereas the former leads to a hermaphrodite (XX) or male (XO) phenotype. This must be because the *cis*-dominant effects

Figure 5. Histograms summarizing gonad morphologies and gametogenesis, based on observations of 20–40 animals of each genotype. The bars show the proportions of animals with large, well-formed male gonads (L), small or thin well-formed gonads (S), and morphologically abnormal gonads (A); the last category includes a variety of anatomical derangements. The dotted region within each bar shows the proportion of gonads containing apparent oocytes; the stippled region shows the proportion with abundant spermatogenesis. XO individuals (G–J) were all hemizygous for *lon-2*, to distinguish them from XX animals. For G, H, and I, most or all of the animals with abundant spermatogenesis (5–10%) may have been *tra-1/+* recombinants (see text and Materials and methods for further explanation).

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Figure 6. Weak *lf* alleles of *tra-1*: bright-field photographs of whole worms. Bar, 25 μ . (A) *e1732* XX. (B) *e1488* XX. In A, note hermaphrodite tail and male somatic gonad, containing sperm trapped in a blocked vas deferens (white arrow) and oocytes more anteriorly (black arrow). In B, note male tail and eggs developing inside body (arrows marked e), as well as one hatched larva (single arrow); also rudimentary vulva (arrowhead).

Figure 7. Yolk protein synthesis by wild-type and *tra-1* mutant animals. Adults were labeled by feeding them on bacteria grown on [35 S]sulfate media, and total proteins were analyzed by gel electrophoresis (Kimble and Sharrock 1983).

Table 2. Dominant phenotypes of *tra-1(gf)* alleles

Class	Alleles	Phenotype of <i>tra-1(gf)/+ XO</i>
1	<i>e1575</i> , <i>e1937</i> , <i>e1946</i> , <i>e2009</i> , <i>e2014</i> , <i>e2109</i>	tail spike slightly truncated, gonad female, fertile (occasionally self-fertile)
2	<i>e1961</i> , <i>e1962</i> , <i>e2097</i>	tail spike very truncated, gonad female, infertile (occasionally self-fertile)
3	<i>e1993</i> , <i>e1997</i> , <i>e1998</i> , <i>e2098</i> , <i>e2071</i>	tail intersexual, gonad female, abnormal vulval induction
4	<i>e1951</i> , <i>e1953</i> , <i>e2067</i> , <i>e2198</i>	abnormal male tail, gonad intersexual, abnormal vulva; most animals rupture at L4 molt
5	<i>e2013</i> , <i>e2065</i> , <i>e2069</i>	abnormal, well-developed male tail, gonad intersexual, some vulval induction
6	<i>e2271</i>	normal male soma, germ line differentiates both sperm and oocytes

of *e1575* have been abolished entirely by the amber mutation *e1838*. The *e1575* lesion is demonstrably still present in the gene, because it can be recovered from the *e1575e1816e1838* compound allele by intragenic recombination (see below). Also, suppression of *e1838* by the amber suppressor *sup-7* restores dominant feminization, so that *e1575e1816e1838/+ + +*; *sup-7/0 XO* animals are intersexual rather than male. I conclude that *e1575* is a *gf* allele of *tra-1*. The other 21 *gf* alleles exhibit similar properties and map location to this allele, so I assume they are all *tra-1* mutations.

Although all of these mutations cause dominant feminization, they differ in the strength of this phenotype. As indicated in Table 2, only six of the alleles lead to a fertile female phenotype in *tra-1(gf)/+ XO* heterozygotes. Crosses demonstrating the fertility of *e1575/+ XO* animals have been reported previously; fertility is low, but this is a general characteristic of *XO* females or hermaphrodites (Hodgkin 1986). Yolk protein synthesis in these animals is shown in Figure 7. The other 16 *tra-1(gf)* alleles lead to an intersexual or abnormal male phenotype. Some tail phenotypes for this allelic series are shown in Figure 8. The strength of the *XO* phenotype is also correlated with the strength of the mutations as suppressors of *tra-3* in *XX* animals (Hodgkin 1986). For strong dominant alleles such as *e1575*, *tra-1(gf)/+*, *tra-3 XX* is female, but this genotype is hermaphrodite for weaker alleles. For very weak alleles, such as *e2013*, this genotype results in a hermaphrodite phenotype with a masculinized (truncated) tail, indicating that the somatic masculinizing effects of *tra-3* have been suppressed incompletely. The weakest of all these alleles, *e2271*, is a recessive suppressor of *tra-3* and, by itself, appears to cause feminization only of the germ line.

All but one of the *tra-1(gf)* alleles eliminate spermatogenesis in *tra-1(gf)/+ XX* animals, which therefore develop into fertile females. The exception is *e2271*, for which *e2271/+ XX* animals are hermaphrodites of reduced self-fertility; homozygous *e2271 XX* animals are female (at 25°C) or weakly self-fertile (at lower temperatures). None of the *tra-1(gf)* alleles appears to affect *XX* somatic development, in *tra-1(gf)/+*, *tra-1(gf)/tra-1(lf)*, or (where tested) *tra-1(gf)/tra-1(gf)* genotypes. This indicates that the somatic functions of *tra-1* in promoting female somatic development have not been altered. In

Figure 8. Tail phenotypes of *gf tra-1* alleles. All are *XO* animals; (A–C) viewed ventrally; (D–H) viewed laterally. Bar, 25 μ m. (A) *e1575/e1575*; (B) *e1575/+*; (C) *e1816e1575/+*; (D) *e1575/e1099*; (E) *e1993/+*; (F) *e1953/+*; (G) *e2013/+*; (H) wild-type male.

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contrast, most *tra-2(gf)* alleles appear to be partly defective in the somatic functions of *tra-2* (Doniach 1986). The *tra-1(gf)* mutations are therefore likely to be regulatory in nature. Nevertheless, it is conceivable that a *tra-1(gf)* phenotype might be caused by a truncated gene product. For this reason, 11 *tra-1(gf)* alleles were tested for suppression by the amber suppressor *sup-7*, with uniformly negative results.

Dosage studies

Gene dosage experiments were carried out to investigate further the nature of *tra-1(gf)* alleles. If these alleles result simply in an elevated level of *tra-1* gene product, one would expect their phenotypic effects to be enhanced by additional copies of *tra-1(+)*. Two aspects of the phenotype were examined: the self-fertility of XX animals, and the tail phenotype of XO animals.

As shown in Table 3, varying the dosage of the wild-type allele from one to three has no effect on XX self-fertility. The large free duplication *eDp6*, carrying *tra-1(+)*, causes a reduction in fertility, but this seems to be due to a general effect on viability not caused by *tra-1* itself. For two *tra-1(gf)* alleles, the feminizing effects (i.e., loss of sperm) are enhanced in *tra-1(gf)/tra-1(lf)* relative to *tra-1(gf)/+*, which is the opposite of what is expected for a simple increase in the level of *tra-1* gene product. It is more likely that these *tra-1(gf)* alleles are unresponsive to the normal control mechanism in the XX animal. The fact that an additional wild-type allele acts in a masculinizing direction in this context is puzzling, though explicable by, for example, models in which the *tra-1* gene is capable of autoregulation.

For the male tail phenotype, varying wild-type gene dosage from one to three has little effect. XO males car-

rying *eDp6* [i.e., three copies of *tra-1(+)*] are of low fertility, but again, this is probably a nonspecific effect caused by the large size of this duplication, and there is no sign of anatomical derangement that could be ascribed to excess *tra-1* activity (data not shown). For the *gf* allele *e1575*, tail phenotypes are similar in *tra-1(gf)/+* and *tra-1(gf)/tra-1(lf)* XO animals (Fig. 8B,D). In one dose, *e1575* does not cause complete feminization of the tail, which carries a truncated tail spike. In two doses, a complete tail spike is made (Fig. 8A), indicating that this allele is capable of causing a complete transformation of an XO animal. None of the other *tra-1(gf)* alleles isolated so far is any stronger, indicating that none of them results in as high a level of *tra-1* activity as is found in an XX animal, a *her-1* XO animal, or a *fem* XO animal (Hodgkin 1986). In all of these genotypes, a single copy of *tra-1(+)* is sufficient for a perfect female tail spike; therefore, it appears that none of the *tra-1(gf)* alleles is fully deregulated. It may be that a very complex mutation would be required to achieve complete deregulation; alternatively, such a mutation might have damaging pleiotropic effects. There is some reason to believe that premature *tra-1* expression could have damaging consequences during embryogenesis (Hodgkin 1986).

In conclusion, it appears that the *gf* allele *e1575* does not cause overproduction of *tra-1* product but, instead, renders the gene (or its product) insensitive to the normal regulatory mechanism, presumably mediated by the negative action of *fem* gene products (Hodgkin 1986). Consequently, the *tra-1* product remains at a high level of activity in both XX and XO animals, resulting in feminization in both cases. This allele can therefore be regarded as a "constitutive" for *tra-1* activity, rather than an "overproducer." Other *gf* alleles have not been analyzed to the same extent as *e1575*, but there is no reason to believe that they would behave very differently. Genetic observations such as these do not indicate whether the inappropriate regulation of *tra-1* in these mutants occurs at DNA, RNA, or protein levels.

Intragenic mapping

A limited set of mapping experiments, using a variety of protocols, have yielded the following results. First, the *gf* allele *e1575* maps to the right of all eight independently isolated amber alleles and of other recessive alleles. Second, approximate intragenic map distances vary from 0.02 map units for the distance *e1828-e1575*, to 0.15 map units for *e1825-e1575*. Third, the modifying mutation *e1816* maps either very close to *e1825* or further to the left. Fourth, five *gf* alleles all map to the right of *e1099* and, in one case, to the right of *e1575*. In sum, the gene is large, and the structural part of the gene (identified by the amber alleles) is probably separate from the regulatory part of the gene. A map of the *tra-1* region is shown in Figure 9A, and a preliminary intragenic map of *tra-1*, in Figure 9B.

The most informative experiments involved dissection of the triply mutant alleles such as *e1825tr*, that is, *e1816e1825e1575*. From a heterozygote *vab-7 + dpy-18/ + tra-1(e1825tr) +*, 134 Vab recombinants were

Table 3. Effect of *tra-1* gene dosage on XX self-fertility

<i>tra-1</i> genotype	Dosage of <i>tra-1(+)</i>	Broods counted	Average number of progeny ^a
+/+ (wild type)	2	12	329
+/ <i>eDf2</i>	1	7	219
+/ <i>e1099</i>	1	4	356
+/ <i>e1076</i>	1	4	355
+/+; <i>eDp6</i>	3	6	263
+/ <i>eDf2</i> ; <i>eDp6</i>	2	8	339
<i>e1099/eDf2</i> ; <i>eDp6</i>	1	6	285
<i>e1099/e1099</i> ; <i>eDp6</i>	1	6	253
<i>e1575/+</i>	1 + <i>gf</i>	12	0
<i>e1816e1575/+</i>	1 + <i>gf</i>	24	6
<i>e1816e1575/e1099</i>	0 + <i>gf</i>	12	0
<i>e1816e1575/e1781</i>	0 + <i>gf</i>	30	0
+/+ (15°C)	2	6	336
<i>e1816e1575/+</i> (15°C)	1 + <i>gf</i>	12	77
<i>e1816e1575/e1099</i> (15°C)	0 + <i>gf</i>	12	1
<i>e1816e1575/e1781</i> (15°C)	0 + <i>gf</i>	30	1
<i>e2013/+</i>	1 + <i>gf</i>	15	45
<i>e2013/e1099</i>	0 + <i>gf</i>	15	0
<i>e2013/e1781</i>	0 + <i>gf</i>	15	0

^a Progeny counts included both viable progeny and unhatched eggs.

Figure 9. Genetic maps of *tra-1* region and fine structure of *tra-1*, showing markers used in this study. (A) The region shown is about one-quarter of LGIII; *unc-32* is centrally located on LGIII. Both *eDf2* and *eDp6* extend to the right-hand end of LGIII. The gene *pha-1* was mapped by H. Schnabel (pers. commun.). For a more complete map of LGIII, see Swanson et al. (1984). (B) Rough intragenic map of *tra-1*; scale expanded 67-fold relative to A. (*) Amber alleles. The order and spacing of the alleles above the line is tentative, because it is based on few events and on estimates of recombination frequency, rather than exact measurements.

picked: 65 carried *vab-7 e1825tr*, 8 carried *vab-7 e1575*, and 61 carried *vab-7 +*. Also picked were 92 Dpy recombinants: 46 carried *+ dpy-18*, and 46 carried *e1825tr dpy-18*. The two-factor distance between *vab-7* and *dpy-18* was also measured in this experiment and found to be 2.5 map units. Thus, *e1825* maps to the left of *e1575*, and the distance between the two mutations is $8/134 \times 2.5$, that is, about 0.15 map units. In contrast, in analogous crosses using a different amber allele (*e1828tr*) only 1/68 recombinants in the *tra-1-dpy-18* interval carried *tra-1(gf)*, showing that *e1828* is much closer to *e1575*. One may speculate that the *e1825* mutation, which clearly has residual *tra-1* function, lies close to the 3' end of the coding portion of *tra-1*.

Dissection of the triple alleles was also achieved by constructing strains of genotype *unc-32 vab-7 + dpy-18/unc-32 + tra-1(tr) +*. In the Unc-32 phenotypic background, females [carrying *tra-1(gf)*] are much more conspicuous than those in a wild-type phenotypic background and can be detected in large populations (10^3 – 10^4 animals per plate). Using this semiquantitative method, seven amber alleles were shown to lie to the left of *e1575*. The allele *e1825* recombined most readily of the

set (14 events recovered), consistent with the previous result; the allele *e1838* also recombined readily. Out of a total of 22 recombinants for *e1825* and 21 for the other six alleles, all carried *e1575* rather than *e1816e1575*. Consequently, the *e1816* modifying mutation must lie well to the left of *e1575*, and probably left of *e1825*.

An estimate for the distance from *e1099* to *e1575* was obtained by picking non-Unc Tra(lf) recombinants from hermaphrodites of genotype *unc-47 tra-1(e1099)/+tra-1(e1816e1575); tra-3(e1107)*. These recombinants may be either Tra-1(lf) or Tra-3(lf), which are distinguishable, but a Tra-3 phenotype can only be seen in a *tra-1(+)* background, because both *tra-1(lf)* and *tra-1(gf)* mutations are epistatic to *tra-3* (Hodgkin 1980). Of these, 3/327 were Tra-3 and 324/327 were Tra-1, giving a distance of about one hundredth of the distance from *unc-47* to *tra-1*, that is, 0.05 map units.

To determine the relative order of other dominant and recessive *tra-1* alleles, a different protocol was used. Male/female strains consisting of *tra-1(lf)* males and *tra-1(gf)/tra-1(lf)* females can be stably propagated for several generations (Hodgkin 1983b), but if the two mutations are recombinationally separable, a *tra-1(+)* allele

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will eventually be generated. This will result in the appearance of hermaphrodites that will take over the population rapidly. Inclusion of linked outside markers (*unc-32*, *dpy-18*, or *sup-1*) on either of the two original chromosomes permits relative ordering. Establishment of male/female strains of this type was used routinely to demonstrate tight linkage of all *tra-1(gf)* alleles to *tra-1(e1099)*, and 10 of these strains were maintained for 4–34 additional generations. All eventually produced a *tra-1(+)* recombinant, usually within the first 10 generations (representing $<10^5$ individuals). In four cases where an outside marker was included, the *tra-1(gf)* allele was shown to lie to the right of *tra-1(e1099)* (alleles *e1951*, *e2009*, *e2013*, and *e2014*). In initial experiments, the recessive alleles *e1099*, *e1516*, and *e1781* were shown to lie to the left of *e1575*, the reference *gf* allele.

One exception to the rule that *tra-1* recessive alleles are recombinationally separable from *tra-1(gf)* alleles is the unusual allele *e1488*. A male/female strain *e1488/e1575*; *him-5* was constructed and grown for over 350 generations, without yielding a *tra-1(+)* recombinant. Eventually one did appear, at approximately generation 400, but it is clear that *e1488* and *e1575* must be extremely tightly linked. The *e1488* mutation does not appear to interfere with recombination, because recombination between the flanking markers *unc-47* and *dpy-18* was found to be the same in *trans* with both *tra-1(+)* and *tra-1(e1488)*.

Male/female strains can also be used to test for recombination between different *tra-1(gf)* alleles, by using a triple allele as the *tra-1(lf)* allele. For example, a strain of *tra-1(e1828tr)* males and *tra-1(e1961)/tra-1(e1828tr)* females was established but yielded a *tra-1(+)* recombinant after 12 generations, indicating that *e1575* and *e1961* are separable, with *e1961* probably to the right (assuming that this was not a gene conversion event). However, another strain of the same type, using *e1962* as the *tra-1(gf)* allele, failed to yield a *tra-1(+)* recombinant after more than 50 generations, suggesting that *e1962* lies to the left of *e1575* or is inseparable from it.

These mapping experiments indicate that the total recombinational length of the *tra-1* gene is at least 0.15 map units. This is much larger than the lengths estimated for two large muscle genes, *unc-22* and *unc-54*, which are less than 0.04 map units in extent (Moerman and Baillie 1979; Waterston et al. 1982). Either *tra-1* is a very big gene, or it experiences considerable map expansion. The relationship between physical distance and recombinational distance in the *C. elegans* genome is known to vary considerably, from less than 300 kb/map unit to at least 900 kb/map unit (Greenwald et al. 1987).

Conclusion

The main conclusions from this study are that activity of the *tra-1* gene is the major factor controlling the sexual phenotype of the soma and that it also plays a significant role in controlling germ-line phenotype. The gene appears to be large, at least in terms of recombinational map distance, and to have a complex function.

As far as nongonadal aspects of sexual phenotype are concerned, the role of *tra-1* appears to be simple: Activity directs female development, and inactivity directs male development. This follows from the observations that most *tra-1(lf)* alleles result in completely male development of the tail, intestine, and so on, in both XX and XO; therefore, *tra-1* activity must be necessary for normal female somatic development in wild-type XX animals. Furthermore, *tra-1* activity is also sufficient to direct female development in XO animals.

The weaker alleles of *tra-1* (classes A4–A9, B1–B2) have properties suggesting that *tra-1* has a complex function in the nongonadal parts of the soma, because mutants have phenotypes consisting of mixtures of male, female, or intersexual tissues. Some of these phenotypes may result from threshold effects: A partly defective *tra-1* allele may have sufficient activity to direct female development in one tissue but not in another, which therefore develops in a male or intersexual pattern. Threshold effects cannot explain why the mutants do not all fall into one allelic series, so it is likely that there is more than one component to *tra-1* function. Class A and class B mutants do not fully complement each other, suggesting that these components are partly overlapping. Ambros and Horvitz (1987) have found similar behavior in the heterochronic *lin-14* gene. Exhaustive characterization of mutant phenotypes would probably subdivide these classes further but would require a great deal of work, in view of the dozens of sexually dimorphic features that have been documented so far (Sulston and Horvitz 1977; Kimble and Hirsh 1979; Sulston et al. 1980).

The role of *tra-1* in the somatic gonad is more complicated. Female gonadal development requires *tra-1* activity, but some part of *tra-1* activity is also required for completely normal male gonadal development. This is inferred from the fact that many *tra-1(lf)* alleles (classes A1 and A2) lead to a variably abnormal somatic gonad in both XX and XO. This could be a secondary consequence of germ-line abnormality, but this explanation is unlikely because wild-type XO animals whose germ cells have been diminished by laser ablation do not exhibit abnormalities of this type (Kimble and White 1981). The requirement for *tra-1* activity is not absolute, because many mutant males have a functional male somatic gonad, although many do not. The variability of the somatic gonad in these mutants stands in contrast to the invariant nongonadal phenotype.

With regard to the germ-line phenotype, *tra-1* function again appears to be complex. Both *gf* and some *lf* alleles (classes A2 and A3, at least) can lead to the production of oocytes in an XO animal (where they are never normally found). As suggested previously (Doniach and Hodgkin 1984), *tra-1* activity does not seem to be essential for the formation of either sperm or oocytes, but it has an important role in determining which gametes are made. It is striking that all *tra-1(gf)* alleles isolated so far eliminate spermatogenesis from XX animals, although most (17/22) were obtained by selections strongly biased toward maintenance of spermatogenesis. For this reason, it

is likely that *tra-1* can exert a negative effect on spermatogenesis. This conclusion is reinforced by the observation that *fem-3(gf)* mutations, which lead to constitutive spermatogenesis in mutant XX hermaphrodites (Barton et al. 1987), can be suppressed by *tra-1(gf)* mutations (K. Barton and J. Kimble, pers. commun.; J. Hodgkin, unpubl.). However, spermatogenesis is also reduced in *tra-1(lf)* mutants. A low level of spermatogenesis is to be expected in *tra-1(lf)* XX mutants, because *fem* activity is limited in an XX animal and spermatogenesis appears to be absolutely dependent on *fem* activity (Doniach and Hodgkin 1984). A low level is not expected in *tra-1(lf)* XO mutants, because the *fem* genes should be fully active, promoting abundant spermatogenesis. For class A1 alleles, it is conceivable that the low level of spermatogenesis is a secondary consequence of abnormality in the somatic gonad, but this seems less likely for class A3 alleles, because the somatic gonad in these mutants appears to be normal. One consistent correlation is that *tra-1(lf)* mutants with large, well-formed male gonads usually contain apparent oocytes. Formation of oocytes in class A1 mutants may therefore be inhibited because of the small size or abnormality of the gonad. A possible rationalization of all these observations is that both the somatic and the germ-line components of the male gonad are partly dependent on a low level of *tra-1* activity. In the partial absence of *tra-1* activity, reduced numbers of both sperm and oocytes are made; in the complete absence of *tra-1* activity, somatic gonadal abnormalities prevent formation of any late gametes, so only sperm are made.

The finding that *tra-1* has functions in both sexes of *C. elegans* (major function in XX; minor function in XO) is striking in view of the fact that the same is true for several of the major sex-determining loci of *Drosophila*: the *Sxl*, *tra-2*, and *dsx* genes all have functions in both male and female flies (Baker and Belote 1983; Maine et al. 1985).

From these data, it would appear that the *tra-1* gene has most of the genetic properties expected of a "complex gene," in the sense of *Drosophila* loci such as the Bithorax Complex, the scute-achaete complex, the *Sxl* and *dsx* genes, and so forth. Whether other features of *Drosophila* complex genes, such as large physical size and complex transcription pattern, are also found in *tra-1* must await molecular characterization of the locus.

Materials and methods

General

Standard methods of culture, manipulation, and examination were used (Brenner 1974; Doniach and Hodgkin 1984). Experiments were carried out at 20°C unless otherwise noted. Gamete types were determined by visual inspection using Nomarski microscopy: sperm are small, round cells with compact dotlike nuclei; oocytes are very large, round, or oblong cells with large nuclei and often with conspicuous nucleoli. Gonad and gamete examination was carried out on young adult males, 12–36 hr after the last larval molt. Between 20 and 40 individuals of each genotype were examined for the data in Figure 5.

Yolk protein synthesis was examined by visual inspection, using Nomarski microscopy, or by gel electrophoresis of ³⁵S-labeled whole adult worms, using the procedures of Kimble and Sharrock (1983).

Strains

The following mutations were used: LGII, *tra-2(e1209)*; LGIII, *unc-32(e189)*, *unc-47(e307)*, *unc-49(e382)*, *vab-7(e1562)*, *dpy-18(e499)*, *sup-1(e1955)*; LGIV, *unc-17(e245)*, *lin-3(e1417)*, *dpy-20(e2017)*, *dpy-26(n199)*, *tra-3(e1107,e1767)*; LGV, *him-5(e1490)*, *egl-41(e2055)*, *dpy-21(e428)*; LGX, *dpy-7(e88)*, *unc-18(e81)*, *sup-7(st5)*, *lon-2(e678)*. Alleles of *tra-1* are listed in Tables 1 and 2. The *sup-1* mutation is a dominant suppressor of *unc-17(e245)* but has no other phenotype (J. Hodgkin, unpubl.).

Isolation and maintenance of *tra-1(lf)* alleles

Seven *tra-1* alleles (*e440*, *e1076*, *e1099*, *e1488*, *e1516*, *e1928*, *e1929*) have been recovered during routine mutant screens, by S. Brenner, P. Anderson, E. Hedgecock, and J. Hodgkin. One of these (*e1488*) was isolated after acetaldehyde mutagenesis, and the remainder, after EMS mutagenesis.

Another *tra-1* allele, *e1728*, was isolated as an extragenic suppressor of *her-1* (Hodgkin 1980). Eight more were isolated, as described below. All have been maintained as stocks of genotype *tra-1*; *eDp6*, or *vab-7 + dpy-18/ + tra-1 +*.

A general screen for autosomal masculinizing mutations

A strain CB3609 of genotype *dpy-7 +/+ unc-18 X* was used for this screen. The two sex-linked markers *dpy-7* and *unc-18* are linked tightly to each other (recombination distance, ~0.4 map units), so that hermaphrodites of this genotype will segregate hermaphrodite self-progeny in the ratio 2 wild-type : 1 dumpy : 1 uncoordinated. The occasional XO self-progeny will be either dumpy or uncoordinated, and wild-type (non-Dpy, non-Unc) males are never seen in the population. Of strain CB3609, 213 hermaphrodites were mutagenized with EMS at L4 stage and placed on separate large (9-cm) NGM plates (Brenner 1974). In the F₁ generation, all produced the expected three classes of hermaphrodite progeny. Brood counts on animals picked in parallel showed that each mutagenized P₀ animal produced about 100 (mean 97) wild-type F₁ hermaphrodites, indicating that a total of 43,000 haploid autosomal genomes were screened. No F₁ wild-type male progeny were seen, but in the F₂ generation, 51/213 plates were found to produce several wild-type males or intersexes, which were presumably transformed XX animals. In 5/51 cases, these animals were fertile XX males, so the mutation responsible was recovered easily by mating the males with marked hermaphrodites. All five were *tra-1* alleles (*e1729*, *e1730*, *e1774*, *e1781*, *e1783*). In other cases, the males were not fertile, so each mutation had to be isolated by picking groups of hermaphrodites in smaller and smaller numbers in successive generations, until a single *tra/+* hermaphrodite could be identified. This somewhat laborious procedure was only used for 8/46 cases, because most (41/46) of the infertile males had the distinctive phenotype characteristic of *tra-2* XX males. To ascertain that these were *tra-2* mutants, 6/41 were isolated: 5/6 were indeed *tra-2*, and the sixth was a *tra-1* allele (*e1766*). After outcrossing, the phenotype of *e1766* resembled *tra-1* rather than *tra-2*, so the original stock probably carried modifier mutations that made it resemble a *Tra-2* phenotype. An additional two nonmating *tra-1* mutants were recovered (*e1732* and *e1764*), both of which had unusual phenotypes. Three further plates carried apparent *tra-1* mutants, but these

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were not recovered. Thus, out of 43,000 mutagenized genomes screened, 8–11 *tra-1* visible alleles were induced (8 recovered), and an estimated 40 *tra-2* alleles were induced (5 recovered), giving forward mutation frequencies of 2×10^{-4} for *tra-1* and 10^{-3} for *tra-2*. The F_3 population was screened cursorily for wild-type males on the 162 plates that segregated no F_2 males, in order to detect maternal-effect *tra* mutations. Each plate carried about 10^5 starved F_3 animals, so screening was not efficient. One maternal-effect mutant was recovered, *e1767*; this proved to be an allele of *tra-3* (Hodgkin 1985, 1986).

Isolation of *tra-1(gf)* alleles

The first *tra-1(gf)* allele, *e1575*, was isolated during a general screen for mutations transforming XO animals into fertile hermaphrodites or females (Hodgkin 1980). Thirteen additional mutations were isolated as extragenic suppressors of *tra-3*, these alleles being *e1951*, *e1953*, *e1961*, *e1962*, *e1997*, *e1993*, *e1998*, *e2009*, *e2065*, *e2067*, *e2069*, *e2071*, and *e2097* (Hodgkin 1986). Reversion experiments on other weakly masculinizing mutations, *tra-2(e1209)* and *egl-41(e2055)*, have also yielded *tra-1(gf)* mutations: alleles *e2198* and *e2271* from the former, and *e2109* from the latter (J. Hodgkin, unpubl.; T. Doniach unpubl.). Screens for dominant XX feminizing mutations have yielded five alleles: *e1937*, *e1946*, *e2013*, *e2014*, and *e2098* (Doniach 1986, and unpubl.). These studies (Doniach 1986; Hodgkin 1986) suggest that the frequency of *tra-1(gf)* mutations is 2×10^{-5} or lower, which is about 10-fold less frequent than *tra-1(lf)* mutations.

Most *tra-1(gf)* alleles were propagated using strains homozygous for *tra-3(e1767)*: *tra-1(gf)/+; tra-3 XX* animals are either hermaphrodite (for weak alleles) or female (for strong alleles), so strains can be propagated by selfing, in the first case, or crossing with *tra-3(e1767)* XO males, in the second case.

The homozygous *tra-1(e1575)* XO animal shown in Figure 8A was generated by selfing an animal of genotype *e1575/+; dpy-26 XO* (Hodgkin 1983c).

Reversion of a *tra-1(gf)* allele

The allele *e1575* was used. A strain consisting of *tra-1(e1575)/+; tra-3(e1107)* females and *tra-3* males (XO) and intersexes (XX) was constructed and propagated as an obligate male/female strain. A population ($\sim 10^4$ individuals, mixed ages) was mutagenized with 0.05 M EMS, grown for one generation, and plated on 10 large NGM plates containing tetramisole (Brenner 1974). This drug prevents movement but not growth and reproduction by self-fertilization, so self-fertile animals were selected. Of 10 plates, one yielded self-fertile hermaphrodites, one of which was used to found a stock, self-fertile hermaphrodite genotype *tra-1(e1816e1575)/+; tra-3(e1107) XX*. Further growth at low temperature yielded a homozygous stock (CB3373), *tra-1(e1816e1575); tra-3*, which is weakly self-fertile at 15°C, almost completely female at 20°C, and completely female at 25°C. The partial revertant allele, *e1816e1575*, still has a strong dominant feminizing effect: At 25°C *e1816e1575/+ + XX* animals are female, and XO animals are sterile intersexes (see Fig. 8B,C). The CB3373 stock is only self-fertile because of the presence of the *tra-3* mutation (i.e., the *tra-1(gf)* and the *tra-3* mutations are mutually suppressive at 15°C). Consequently, intragenic revertants of *tra-1(e1816e1575)* were selected easily by crossing mutagenized CB3373 XX animals with wild-type males and shifting to 25°C. All F_1 cross progeny must be *tra-1(gf)/+; tra-3/+*, which are either female (XX) or intersexual (XO). F_2 progeny are only obtained if the F_1 population contains hermaphrodites or males; these can be generated by an intragenic event within *tra-1* or by an extragenic suppressor. A total

F_1 population of approximately 1.5×10^5 individuals was screened, derived from about 1600 P_0 females mutagenized with 0.05 or 0.075 M EMS, and crossed with wild-type males in sets of three or four females per small plate. Twenty-eight independent *tra-1(tr)* alleles were recovered, usually as XX hermaphrodites of genotype *tra-1(tr)/+*. Apparent males [*tra-1(tr)/+ XO*] were seen on a number of plates but were often unable to sire progeny. Two *tra-2* recessive alleles were also recovered, because *tra-2* null alleles have weak dominant masculinizing effects (Trent et al. 1983), and, therefore, *tra-2/+; tra-1(e1816e1575)/+ XX* animals are slightly self-fertile. Smaller-scale reversion CB3373 experiments were also carried out, using either diepoxyoctane (Anderson and Brenner 1982), ultraviolet light (Greenwald and Horvitz 1980), or X-rays [8000 roentgen (Herman et al. 1976)] as a mutagen. Only the last yielded any revertants: The recessive lethal *e1855* was recovered as an *e1855/+; tra-3/+ XO* male, out of about 2×10^4 F_1 progeny. Homozygotes for *e1855* die as late embryos, but *e1855/e1099 XX* heterozygotes are viable healthy transformed males. Attempts to separate the lethality from the *tra-1* mutation were made: A total of 136 Unc and 132 Dpy recombinants were picked from a heterozygote *unc-47 + dpy-18/+ e1855 +*, but none of these segregated F_2 Unc Tra or Dpy Tra progeny. Recombination between these flanking markers, *unc-47* and *dpy-18*, was slightly but significantly reduced in this heterozygote (linkage 4.5 map units as compared to 6.2 map units in a control heterozygote *unc-47 dpy-18/+ +*). Subsequently H. Schnabel (pers. commun.) has found that *e1855* fails to complement an embryonic lethal mutation, *pha-1(e2123)*, which maps between *vab-7* and *tra-1* and complements these two markers. It is likely that *e1855* is a small deficiency, but it is also possible that it is a small inversion.

Suppression tests

Recessive *tra-1* alleles were tested for amber-suppressibility by selfing hermaphrodites of genotype *unc-32 tra-1/+ +; sup-7/+* and examining tail phenotypes of Unc animals. In the absence of suppression, most (85%) of the Unc animals exhibit a Tra phenotype, and a few (15%) are completely non-Tra recombinants (*unc-32 +/unc-32 tra-1*). The appearance of many non-Tra Unc progeny, or intersexual Unc progeny, indicated suppression. All recessive *tra-1* alleles in Table 1 have been tested, except for *e1928*, *e1929*, *e1837tr*, *e1840tr*, *e1843tr*, and *e1853tr*.

gf tra-1 alleles were tested by crossing *tra-1(gf); tra-3(e1767) XX* females with *dpy-20/+; sup-7/0* males and crossing progeny XX females with more of the same males; XO progeny were examined, in order to see if any had a less feminized phenotype than control *tra-1(gf)/+ XO* progeny. Also, suppressed dumpy XX progeny could usually be recognized (Hodgkin 1985); the appearance of suppressed dumpy female XX animals was taken to indicate lack of suppression. Eleven *tra-1(gf)* alleles were tested, and none showed any sign of suppressibility (alleles *e1575*, *e1951*, *e1953*, *e1961*, *e1962*, *e1993*, *e2013*, *e2014*, *e2071*, *e2109*, and *e2198*).

Construction of *tra-1 XO* animals

Wild-type males, *tra-1 XX* males, and *tra-1 XO* males have almost identical phenotypes by dissecting microscope; therefore, marked *tra-1 XO* males were generated by the following crosses: *unc-32 vab-7 tra-1/unc-32 vab-7 + dpy-18* hermaphrodites crossed with *dpy-18 XO* males, yielding non-Dpy non-Unc males of genotype *unc-32 vab-7 tra-1 +/+ + dpy-18*. These were crossed with hermaphrodites of genotype *vab-7 + dpy-18/+ tra-1 dpy-18; lon-2 X*. Non-Vab, non-Dpy

Lon progeny of this cross will be almost all of genotype $+ + tra-1 dpy-18/unc-32 vab-7 tra-1; lon-2/0$, apart from rare recombinants (which may account for most or all of the rare fully male animals seen). XO males of *tra-1(e1099)* generated by this means were sometimes unusually thin, but *e1099* XX males were not thin, nor were XO males of *e1781* and *e1835tr*. The *lon-2* mutation leads to an abnormally long body, but does not significantly alter gonad morphology in control *lon-2/0* males (Figs. 4E and 5J). Other markers are recessive.

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