Isolation of Dominant *XO*-Feminizing Mutations in *Caenorhabditis elegans*: New Regulatory *tra* Alleles and an *X* Chromosome Duplication With Implications for Primary Sex Determination

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Manuscript received April 12, 1995
Accepted for publication July 7, 1995

ABSTRACT

A strain of *Caenorhabditis elegans* was constructed that permits selection of dominant or sex-linked mutations that transform *XO* animals (normally male) into fertile females, using a feminizing mutation, *tra-2(e2046gf)*, which by itself does not sexually transform *XO* males. Twenty-three mutations were isolated after chemical mutagenesis and found to fall into both expected classes (four dominant *tra-1* mutations and eight recessive *xol-1* mutations) and novel classes. The novel mutations include 10 second-site mutations of *tra-2*, which are called *eg* mutations, for *enhanced gain-of-function*. The *tra-2(gf, eg)* alleles lead to complete dominant transformation of *XO* animals from fertile male into fertile female. Also isolated was a duplication of the left end of the *X* chromosome, *eDp26*, which has dominant *XO* lethal and feminizing properties, unlike all previously isolated duplications of the *X* chromosome. The properties of *eDp26* indicate that it carries copies of one or more numerator elements, which act as part of the primary sex-determination signal, the *X*:A ratio. The *eDp26* duplication is attached to the left tip of the *X* chromosome in inverted orientation and consequently can be used to generate unstable attached-*X* chromosomes.

SEX determination in the nematode *Caenorhabditis elegans* has been the subject of extensive genetic, molecular and developmental investigations (for reviews see HODGKIN 1988, 1990; VILLENEUVE and MEYER 1990; KUWABARA and KIMBLE 1992; PARKHURST and MENEELY 1994). It offers an opportunity to trace a major developmental process in its entirety, from its initiating point, which is the primary sex determination signal, through to specific differentiative and morphogenetic events involved in sexual maturation and reproduction. Much progress to this end has been achieved. This paper describes the selection and analysis of two new kinds of feminizing mutation, which shed light on primary sex determination and on regulatory interactions among downstream control genes.

The two natural sexes of *C. elegans* are the male and the self-fertilizing hermaphrodite, which is essentially a female that has a limited capacity for spermatogenesis in addition to oogenesis. Primary sex determination depends on *X* chromosome dosage: diploid animals with two *X* chromosomes (*XX*) develop into hermaphrodites, while diploids with one *X* chromosome (*XO*) develop into males. Experiments with triploid and tetraploid strains have indicated that *X* dosage is assessed by the ratio of *X* chromosome to autosomes, rather than by the absolute number of *X* chromosomes. Thus, triploids and tetraploids with two *X* chromosomes (2*X*:3A and 2*X*:4A) develop into males, while tetraploids with three *X* chromosomes (3*X*:4A) develop into hermaphrodites (MADL and HERMAN 1979). The nature of what is measured in assessing *X*:A ratio has hitherto been the least understood part of the sex determination process in *C. elegans*.

This enigmatic primary signal acts on a set of at least four regulatory genes (*xol-1, sdc-1, sdc-2, sdc-3*), which have a dual role (VILLENEUVE and MEYER 1987; MILLER *et al.* 1988; NUSBAUM and MEYER 1989; KLEIN and MEYER 1993). On the one hand they regulate subordinate genes that are involved in somatic sex determination and germ line sex determination, and on the other hand they regulate subordinate genes required for *X* chromosome dosage compensation. These two sets of subordinate genes appear to function independently: mutations affecting sex determination do not usually affect dosage compensation and vice versa. Figure 1 provides an overview of the major genes involved in these processes and the predicted interactions between them.

C. elegans, like most other organisms with dimorphic sex chromosomes, actively compensates for the difference in *X* chromosome dosage between the two sexes (MEYER and CASSON 1986; MENEELY and WOOD 1987; HSU and MEYER 1993). A set of at least five genes, called the *dosage compensation dumpy* genes, is specifically required for *X* chromosome dosage compensation (*dpy-21, dpy-26, dpy-27, dpy-28* and *dpy-30*). These all appear to be required for downregulating the *X* chromosome activity in *XX* animals, so as to equalize expression levels

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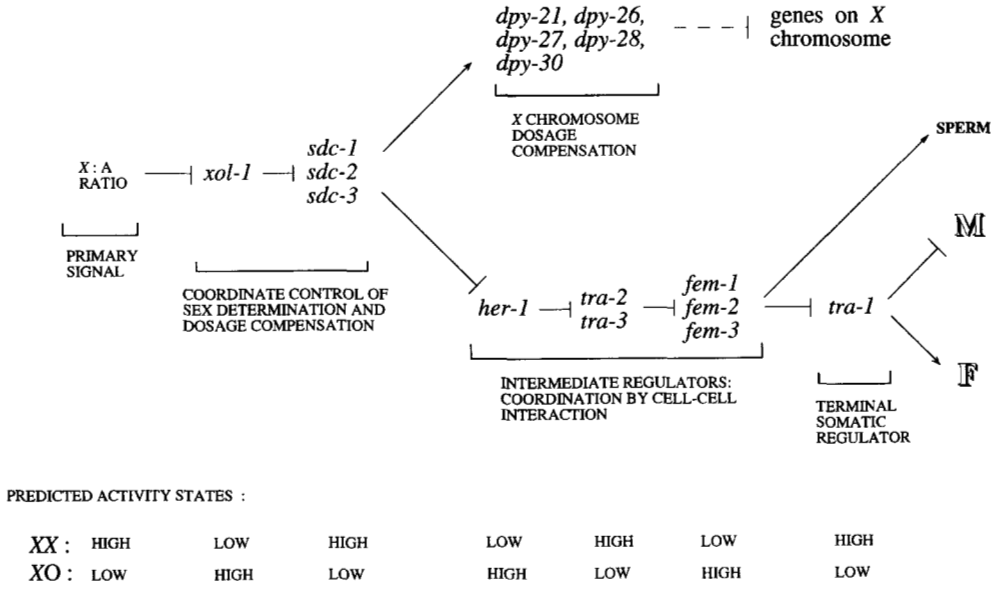

FIGURE 1.—Simplified overview of genes controlling sexual phenotype and dosage compensation in *C. elegans*, and the proposed interactions between them in the two sexes. Pointed arrows indicate predicted positive regulation, barred arrows indicate predicted negative regulation. The two lines at the bottom show the proposed activity states for the various genes in the two sexes. Somatic sexual development is dictated by the final gene in the pathway, *tra-1*, that directs female development when active at a high level, and male development when active at a low or zero level. The *fem* genes appear to have a dual function: in the soma they promote male development indirectly, by downregulating *tra-1* in *XO* animals, and in the germ-line they direct spermatogenesis, being essential for this process. Additional genes and interactions are involved in germ-line sex determination in both sexes and are not shown here (see SCHEDL 1991 for review). Various postulated minor interactions and feedbacks have also been omitted for the sake of simplicity. For more extensive description and discussion, see the reviews cited in the text.

of sex-linked genes between *XX* and *XO* karyotypes. Eliminating their activity has no effect on *XO* animals but is usually lethal to *XX* animals, presumably because of *X* chromosome overexpression (HODGKIN 1983, 1987b; PLENEFISCH *et al.* 1989; HSU and MEYER 1994). Conversely, inappropriately expressing these genes in *XO* animals (via a *xol-1* mutation) leads to lethality from presumed underexpression of the *X* chromosome (MILLER *et al.* 1988). Alterations in the dosage compensation process do not normally affect sexual phenotype, only viability.

The other set of genes, those required for sex determination but not for dosage compensation, have been extensively studied; the seven genes in Figure 1 have now all been cloned and sequenced. The last gene in the pathway, *tra-1*, encodes TRA-1A, a DNA-binding protein that directs female development in all somatic tissues (HODGKIN 1987a; ZARKOWER and HODGKIN 1992; D. ZARKOWER and J. HODGKIN, unpublished data). In the soma, all upstream regulatory genes act only to achieve the correct activity state of TRA-1A: active (feminizing) in *XX* animals and inactive (masculinizing) in *XO* males. Events and gene interactions in the germ line are more complicated and will not be discussed in detail here (for review, see SCHEDL 1991).

Molecular investigations of the genes upstream of *tra-1* have revealed an apparent signal-transduction system that ultimately controls the activity of this gene. The current model suggested by these studies is as follows. The *her-1* gene, expressed only in *XO* animals, encodes an apparent secreted protein (TRENT *et al.* 1991; PERRY *et al.* 1993), which probably acts as a repressive ligand for a predicted transmembrane receptor protein, TRA-2A, encoded by the *tra-2* gene (KUWABARA *et al.* 1992). Consequently, TRA-2A is inactive in *XO* animals. In *XX* animals, the absence of HER-1 means that TRA-2A is active and able to inhibit or sequester one or more of the FEM proteins, encoded by *fem-1*, *fem-2* and *fem-3*. These proteins act as direct or indirect inhibitors of TRA-1A: the net result is that in *XX* animals, TRA-1A is not inhibited by FEMs and can direct female development. In *XO* animals, FEMs are not inhibited by TRA-2A, so they are free to downregulate TRA-1 activity, permitting male development.

The continuing investigations of this system raise new questions and provide new tools. The present work was prompted in part by the molecular characterization of *tra-2* (KUWABARA *et al.* 1992). If TRA-2A is a receptor for HER-1, then one might expect to find mutations of *tra-2* encoding a protein that would fail to interact with HER-1 and consequently remain constitutively active in both *XX* and *XO* animals. Such mutations should be dominant and transform *XO* animals from males into hermaphrodites.

Two classes of dominant regulatory mutations of *tra-2* have been identified in previous work (DONIACH 1986; HODGKIN 1986; SCHEDL *et al.* 1989), but neither of these has the properties expected for mutants insensitive to

inhibition by HER-1. Both types have a dominant feminizing effect, primarily on the germ line of *XX* animals. One class, known as the *mx* alleles, leads to feminization of the *XX* germ line but has an opposite, weakly masculinizing effect on the *XX* soma, and no effect on *XO* animals (hence *mx*, for *mixed character*). The other class (the *gf*, or *gain-of-function* alleles) appears to be hypermorphic for *tra-2* activity (DONIACH 1986). *XX* animals carrying such mutations are female; *XO* animals are usually fertile males but may exhibit some signs of feminization such as limited oogenesis in the germ line and yolk production by the intestine. Recent molecular data support the interpretation of the *gf* alleles as hypermorphs (GOODWIN *et al.* 1994). All mutations in this class affect one or both copies of a 28-bp repeat in the 3′ untranslated region (UTR) of *tra-2* transcripts and probably result in increased synthesis of TRA-2 proteins. This suggests that the *gf* mutations affect a separate regulatory control, which acts normally to reduce synthesis of these proteins. This control may be necessary only to ensure that *tra-2* activity remains low enough to respond properly to specific masculinizing signals in the *XX* germ line and in the *XO* soma and germ line.

One possible explanation for the failure, in previous screens, to obtain a strong feminizing dominant mutation of *tra-2* was that any mutation affecting the predicted binding site for the HER-1 ligand might also affect the folding or stability of the TRA-2A protein. Therefore, elevating levels of *tra-2* products by means of a *tra-2(gf)* mutation might be a necessary prerequisite for obtaining a constitutive mutation of this type. Also, *tra-2* is known to be haploinsufficient, so strong feminization by a single dose of a constitutive mutation might also depend on elevated levels of *tra-2* products.

For these reasons, we selected for feminizing mutations in a strain homozygous for a *gf* allele of *tra-2, e2046,* which carries a deletion of one 28-bp repeat in the 3′ UTR of *tra-2* transcripts. Using a *tra-2(gf)* background has a separate advantage in such a selection, which is that strains homozygous for *tra-2(e2046)* are necessarily male/female, obligate crossing strains. Consequently any feminizing mutations recovered must be dominant or sex-linked. Previous screens and selections for feminizing mutations have been biased toward the recovery of recessive mutations, such as those in *her-1* and the three *fem* genes (HODGKIN 1980, 1986). To select for *XO* feminization, we combined *tra-2(e2046)* with a temperature-sensitive mutation in a dosage compensation gene, *dpy-28(y1),* which leads to the death of *XX* individuals at restrictive temperature but has no effect on *XO* animals (PLENEFISCH *et al.* 1989).

MATERIALS AND METHODS

Culture conditions: Standard conditions for growth, maintenance, mutagenesis and genetic crosses of nematodes were used (SULSTON and HODGKIN 1988).

Nomenclature: Standard nomenclature is used (HORVITZ *et al.* 1979) with minor modifications as follows. First, part of this work led to the isolation of dominant enhancing mutations of *tra-2,* isolated on chromosomes that already carried a *gf* mutation of *tra-2, e2046.* For clarity, these additional mutations are given the descriptive suffix, or abbreviation, *eg,* standing for *enhanced gain-of-function.* The resulting doubly mutant alleles are consequently referred to as *tra-2(gf, eg)* alleles.

Second, some of the experiments generated animals apparently carrying attached *X* chromosomes, that is, two *X* chromosomes joined at their left ends. This attachment is symbolized by a caret symbol (^). Thus, an *XX* hermaphrodite animal carrying attached *X* chromosomes, both marked with *lon-2,* has a genotype written *lon-2*^*lon-2,* as distinct from a normal diploid, *lon-2/lon-2.*

Cytogenetics: Fluorescent *in situ* hybridization (FISH) was carried out as previously described (ALBERTSON 1993). Probes derived from YAC clones Y47C4, Y51E2, Y8D1, Y42C9, Y29F10, Y46A5, Y5E3 and Y23A3 all gave duplicated hybridization signals on *eDp26* and *eDp27.* Probes from eight YAC clones further to the right (Y48C6, Y70E6, Y40H5, etc.) gave only a single signal.

Strains: The following mutations were used in this work.

*LGII: dpy-10(e128), tra-2(e1095), tra-2(e2046*gf*), tra-2(q276), unc-4(e120), mnC1.*

*LGIII: dpy-27(rh18), dpy-28(y1), tra-1(e1099), tra-1(e1575*gf*).*

*LGIV: unc-17(e245), unc-5(e53), fem-1(hc17*ts*), mor-2(e1125), unc-24(e138), fem-3(q20*gf*), him-8(e1489), dpy-20(e1282), dpy-26(n199), tra-3(e1767).*

*LGV: her-1(n695*gf*), him-5(e1490), dpy-21(e428).*

*LGX: egl-17(e1313), unc-1(e719), unc-1(e1598*dm*), dpy-3(e27), unc-2(e55), unc-20(e112), lon-2(e678), dpy-8(e130), dpy-7(e88), unc-10(e102), xol-1(y9), dpy-6(e14), sdc-2(y15), unc-7(e5), mnDp57, mnDp65, mnDp66, mnDp67, mnDp68, mnDp70, mnDp73.*

Except where noted, these alleles and rearrangements can be found listed in HODGKIN *et al.* (1988). The *tra-2* allele *q276* is an unusual allele isolated by T. SCHEDL (unpublished data), which leads to fertile male development in homozygous *tra-2(q276) XX* animals, in contrast to most *lf* (loss-of-function) *tra-2* alleles, which lead to slightly abnormal, infertile male development. The *X* chromosome duplications utilized in this paper are described by HERMAN and KARI (1989).

For examining patroclinous *XO* individuals, a standard strain CB4017 = *fem-1(hc17*ts*) him-8(e1489); unc-1(e1598*dm*)* was used (ZARKOWER and HODGKIN 1992). Animals of this strain were raised during larval growth at 25°, to eliminate self-progeny; they were then crossed, and progeny examined at 20°. The *fem-1(hc17)* mutation is both temperature sensitive and fully recessive (NELSON *et al.* 1978), so it has no effect on the phenotype of progeny from such crosses. The *him-8* mutation leads to extensive production of nullo-*X* oocytes, and the *unc-1(e1598)* mutation is dominant, so the non-Unc progeny from such crosses will be those that have received *X* chromosomes only from the male parent (patroclinous progeny).

Selection of feminizing mutations: The strain CB4919 = *tra-2(e2046*gf*); dpy-28(y1*ts*)* was constructed using standard methods. Partly synchronized cultures of this strain, containing numerous L4 individuals, were grown at 15° and mutagenized for 4 hr with 0.05 M EMS, using standard conditions. After mutagenesis, cultures were washed and plated on 10–20 large plates and then grown for approximately one generation at 15°. Plates were examined at this time to estimate the total F1 progeny for each mutagenesis experiment. The plates were then shifted to room temperature (20–22°). Under normal growth conditions, the strain rapidly becomes asynchronous. The presence of very large numbers of males leads to extensive burrowing in the culture plates, especially after shift-up, when many thousand frustrated adult males are scouring

the depths of each plate in search of the few remaining moribund females. To extract fertile females from such plates, each 9-cm plate was soaked in 10 ml M9 buffer for several hours, during which most of the motile worms emerged from the agar and became trapped in the overlying liquid. The resulting worm suspension was then removed; worms were permitted to settle and were pipetted in a small volume to a fresh culture plate, which was then incubated for additional days at room temperature. This procedure facilitated recovery of viable female individuals.

Characterization of mutants: The following strategies, or minor variants thereof, were adopted for preliminary characterization of the female survivors obtained by this selection. Individual lines were established by picking sib-mated females and propagated at 20° to confirm viability. Only one line was established from each original selection plate to ensure independence. Females from each line were then crossed with *dpy-28* males for at least two generations, to separate (if possible) *tra-2(e2046)* from the new mutation. After this step, 14 out of 24 resulting lines were still male/female, indicating linkage to *tra-2*, or else the induction of an unlinked mutation that feminizes both *XX* and *X*O animals, such as a strong *tra-1(gf)* allele. To distinguish between these possibilities, females were crossed with *dpy-10 unc-4/++* males, and single female progeny crossed again with *dpy-10 unc-4/++* males. As expected, half of these crosses produced Dpy Unc progeny. Males from these crosses were counted: 10 out of 14 produced wild-type and DpyUnc males in equal numbers, rather than the 3:1 ratio expected for an unlinked dominant feminizing mutation. This indicated the generation of a dominant mutation on LGII, which causes the death or sexual transformation of *X*O animals. Further crosses established, in all 10 cases, the presence of a dominant mutation tightly linked to *dpy-10(+)*, which transforms *X*O animals into phenotypic females. For each of the 10 cases, rare Unc non-Dpy recombinants were also picked from the test-crosses and crossed with *fem-3(q20*gf) *dpy-20* males. The *q20* mutation is a masculinizing mutation of *fem-3* (BARTON *et al.* 1987) that suppresses the feminization of *XX* animals caused by *tra-2(e2046)*, and therefore permitted the construction, in all 10 instances, of homozygous animals of genotype *tra-2(gf,eg) unc-4; fem-3(gf) dpy-20*. These animals grow as viable fertile Unc Dpy hermaphrodites at all culture temperatures. The viability of these lines demonstrates the absence of any recessive deleterious properties associated with each new mutation.

The other four male/female lines seemed likely to carry new *tra-1(gf)* alleles, especially since test-crosses generated *X*O females with truncated tail-spikes, characteristic of strong *tra-1(gf)* alleles. Further crosses established very tight linkage to the *tra-1* locus for all four by establishing *tra-1(gf)/tra-1(lf)* female/male lines in each case (as in HODGKIN 1987). These segregated only female and male individuals and no hermaphrodites. These four mutations were therefore assumed to be *tra-1(gf)* alleles.

For 10 out of the original 24 lines selected, the crosses with *dpy-28* yielded animals that were self-fertile *X*O hermaphrodites, rather than males or females. These hermaphrodites produced self-progeny broods of viable *X*O animals, ranging in phenotype from male through intersex to hermaphrodite. Eight of 10 proved to carry *xol-1* alleles. This was established by the following crosses: rare fertile male *X*O animals were crossed with *unc-10 dpy-6* hermaphrodites, and the resulting hermaphrodite progeny were crossed with wild-type males. In all eight cases, this cross generated no wild-type males but >100 DpyUnc males, plus occasional Dpy or Unc recombinant males. This demonstrated the presence of a *xol* mutation tightly linked to *dpy-6*, which is the same location as *xol-1*. In addition, strains of genotype *tra-2(e1095)/dpy-10 unc-4; xol-1* were constructed in each case and segregated Tra-2 *XX* males that were competent to mate, as expected for *tra-2(e1095); xol-1 XX* males. These males were crossed with hermaphrodites of genotype *tra-2(e1095)/mnC1; unc-5; xol-1(y9)* and fertile non-Unc *XX* male cross progeny were produced, demonstrating that each of the eight new *xol* mutations fails to complement the reference allele *y9* with respect to *tra-2(e1095)* enhancement.

The other two lines did not carry *xol-1* mutations. One carried a duplication of the left end of the *X* chromosome, *eDp26*, described in the RESULTS section. The other proved to be extremely difficult to propagate and cross, segregating only rare self-fertile hemaphrodites. Analysis of this mutation has not yet been pursued.

Reversion of *tra-2(e2046e2531)*: The double mutant *tra-2(e2046e2531)* exerts dominant feminization of both *XX* animals and *X*O animals. The two aspects of the phenotype (feminization of *XX* and feminization of *X*O) were both reverted, using a strain CB4940, genotype *tra-2(e2046e2531) unc-4/mnC1*. This strain is barely self-fertile, because almost all animals of this genotype are female, but some individuals produce a few self-progeny, so the strain can be propagated with patience. It segregates Unc animals, homozygous for *tra-2(e2046e2531)*, which are invariably female. When crossed with wild-type males, these females produce no male progeny, only females and occasional weakly self-fertile hermaphrodites. The reversion was carried out as follows: an asynchronous culture of CB4940 was mutagenized with EMS and distributed over 12 large plates. Forty-eight P_0 L4 Unc-4 females were picked from these plates and crossed with wild-type males in sets of four. A total of about 9400 F_1 progeny was produced. Three of the 12 crosses yielded one or more fertile males, which were crossed with marked hermaphrodites. The resulting hermaphrodite progeny were selfed, and in each case some of the hermaphrodites segregated Unc-4 Tra-2 *XX* males. For two of these cases, the mutagenized chromosome marked with *unc-4* exhibited neither dominant feminization of *X*O nor dominant feminization of *XX* and instead exhibited recessive masculinization due to new loss-of-function alleles of *tra-2* (designated *e2046e2531e2543* and *e2046e2531e2545*). This indicates that both dominant mutations had been simultaneously reverted in *cis*. In the third case, a temperature-sensitive loss-of-function allele of *tra-2* had been induced (*e2544*). At 25° *XX* animals of genotype *tra-2(e2046e2531e2544)* are Tra-2 pseudomales, at 20° they are variably masculinized hermaphrodites, and at 15° they are females, consistent with expectation.

XX hermaphrodites of normal self-fertility were also obtained in the reversion: one of the Unc-4 female × wild-type male crosses yielded such a hermaphrodite, which when selfed segregated Tra-2 Unc-4 pseudomale progeny. Again, this was found to be a new *tra-2(lf)* allele, *e2046e2531e2546*. Also, the 12 original P_0 plates were examined for the appearance of hermaphrodites of increased fertility. Three plates yielded such animals: two segregated Tra-2 Unc-4 pseudomales (new alleles *e2046e2531e2557* and *e2046e2531e2558*) and one carried an extragenic modifier, as yet incompletely characterized, that leads to increased spermatogenesis in *e2046 XX* animals. Complementation tests were carried out for some of these new *tra-2* alleles using the reference allele of *tra-2, e1095*. These tests confirmed that *e2543, e2544* and *e2558* are all loss-of-function alleles of *tra-2*.

Construction of *eDp26 sdc-2*: *XX* and *X*O animals of genotype *eDp26 sdc-2* were constructed as follows. Hermaphrodites of genotype *unc-1(e719) dpy-7/sdc-2* were selfed and Unc non-Dpy recombinants were picked. These were selfed to confirm genotype (*unc-1 sdc-2/unc-1 dpy-7*), then crossed with *X*O males of genotype *dpy-26; eDp26*. Single hermaphrodite prog-

eny were picked and crossed with wild-type males. Five out of ten hermaphrodites gave progeny male ratios with Unc males greatly in excess of wild-type males (total score: 137 Unc, 16 wild), consistent with a parental genotype *dpy-26/+; unc-1 sdc-2/ eDp26.* Fifteen of these wild-type male progeny (putative genotype *eDp26 sdc-2 XO*) were crossed singly with *unc-1 dpy-7* hermaphrodites. Hermaphrodite progeny from each cross were selfed for 24 hr and then crossed with wild-type males in excess to ensure complete outcrossing. Of seven hermaphrodites that produced both abundant self-progeny and abundant cross-progeny, all segregated self-progeny that included ~25% Sdc dead eggs or larvae, and cross-progeny males consisting of approximately equal numbers of wild-type and UncDpy males, some Unc males, and zero Dpy males (for example, one brood had 35 wild, 29 UncDpy, 8 Unc and 0 Dpy). Cross-progeny included occasional recognizable Xol larvae, presumably *eDp26 dpy-7 XO* recombinants. The conclusion from these crosses is that *sdc-2* is fully epistatic to *eDp26,* that is, *eDp26 sdc-2 XO* is a viable fertile male, and *eDp26 sdc-2 XX* is an inviable Sdc animal (sex not determined).

Construction of *eDp26 xol-1:* A chromosome of genotype *eDp26 xol-1(y9)* was constructed as follows: *tra-2(e1095); xol-1 XX* males were crossed with *unc-1 (e719) dpy-3(e27)* hermaphrodites. Unc non-Dpy recombinant progeny hermaphrodites were crossed again with *tra-2; xol-1 XX* males, and the resulting non-Unc males crossed with *eDp26 dpy-7(e88).* Wild-type hermaphrodite progeny from this cross were genotyped by progeny testing to identify those of genotype *tra-2/+; eDp26 dpy-7/unc-1 xol-1.* Wild-type progeny from these animals were picked to separate plates and again genotyped by progeny testing. Six of 88 were *tra-2/+; eDp26 xol-1/eDp26 dpy-7,* and 3/88 were *tra-2/+; eDp26 xol-1/unc-1 xol-1.* Tra-2 *XX* male progeny from these broods were crossed with *unc-1 dpy-7,* to yield wild-type hermaphrodite progeny of presumed genotype *tra-2/+; eDp26 xol-1/unc-1 dpy-7.* Six of these hermaphrodites were checked by selfing for 24 hr, then crossing with wild-type males. All produced self-progeny including wild-type and UncDpy hermaphrodites, and cross progeny, including numerous UncDpy males, but no Unc, Dpy, or wild-type males. This demonstrates that both *eDp26* and *xol-1* were present on the unmarked chromosome. Individual hermaphrodite self-progeny were selfed to identify homozygous *eDp26 xol-1* individuals. One of these was crossed with *dpy-26* males to obtain (from the F_3 progeny of this cross) an *XO* hermaphrodite line of genotype *dpy-26; eDp26 xol-1.*

Construction of a free derivative of *eDp26:* In *XO* individuals of genotype *dpy-26; eDp26,* wild-type *X* chromosomes are generated at a significant frequency, implying loss of the attached *X* chromosome and regeneration of an intact *X* chromosome. Crosses were carried out to recover the detached duplication as a free derivative, by crossing hermaphrodites of genotype *him-8; dpy-3 lon-2 unc-7* with *dpy-26 eDp26* males. The hermaphrodite parent is expected to produce ~8% diplo-*X* oocytes, as a result of nondisjunction caused by the *him-8* mutation (HODGKIN *et al.* 1979). If such an oocyte is fertilized by a nullo-*X* sperm carrying a free duplication derived from *eDp26,* then a non-Dpy Lon Unc hermaphrodite will result. In one set of crosses, progeny included 267 wild-type hermaphrodites and four non-Dpy Lon Unc hermaphrodites. Two of the latter were further propagated, and both genetic and cytogenetic data showed that they carried free duplications of the left end of the *X* chromosome, covering markers from *unc-1* to *unc-2.* Both appeared similar in properties, so only one of these was selected for detailed analysis and designated *eDp27.*

RESULTS

The strain CB4919, genotype *tra-2(e2046); dpy-28(y1),* was constructed and behaves as expected from the properties of its constituent mutations (HODGKIN 1986; PLENEFISCH *et al.* 1989). At 15° the temperature-sensitive mutation *y1* has only a slightly deleterious effect and the strain grows as well as a population of 50% female, 50% male animals, as a result of the *XX*-feminizing properties of *tra-2(e2046).* When shifted to 20° or higher, the strain becomes inviable, because of the deleterious effects of *y1* on all *XX* animals. The *XX* animals all die or become moribund, but the *XO* animals remain normal males in phenotype. A shift in temperature from low to high therefore constitutes a powerful selection for mutations that transform *XO* animals into females or hermaphrodites, because only these animals will be able to produce any progeny. A selection using *dpy-28(y1)* alone has been carried out previously by PLENEFISCH *et al.* (1989), which yielded only recessive *her-1* and *xol-1* mutations. No mutations leading to *XX* viability were obtained. The present selection was biased against the isolation of recessive mutations, because the strain is propagated by outcrossing, so it was expected that only dominant mutations (such as *tra-1(gf)* alleles) or hemizygous recessive mutations (such as *xol-1* alleles) would be recovered. Both of these classes were indeed obtained, but two additional kinds of feminizing mutation were also isolated.

A total of eight separate mutagenesis experiments were carried out, involving 94 large plates and an estimated total F_1 of ≥200,000 mutagenized genomes. Accurate assessment of the total is difficult because the large number of males in the strain lead to extensive burrowing in the agar plate, so this estimate should be regarded as a minimum. In all, 24 independent fertile lines were selected, all of which grew more or less well at 20°, as male/female strains. As described in MATERIALS AND METHODS, these lines were subjected to a series of test-crosses, which defined the nature of the feminizing mutation in all but one case. Ten of the lines carried enhancing mutations of *tra-2,* the *tra-2(gf, eg)* alleles. Four lines carried strong dominant mutations of *tra-1.* Eight lines carried mutations of *xol-1.* One line carried a duplication of the *X* chromosome, which has dominant *XO*-lethal and feminizing effects. These classes are discussed in order.

***tra-2(gf, eg)* mutations:** The first mutation analysed was given the allele number *e2531.* Preliminary crosses established that the *XO* sexual transformation by *e2531* cosegregated with the *XX* feminization caused by *e2046*gf. This suggested that an enhancing mutation in the *tra-2* gene had been induced, which (either alone or in combination with *e2046*) is able to transform an *XO* animal from male into female. The cosegregation is consistent with this explanation but does not prove it, especially since both mutations are strongly dominant. A reversion experiment was carried out to test this point. If both mutations lie in the *tra-2* gene, then introducing a *tra-2(lf)* mutation in *cis* should simultaneously revert both dominant phenotypes, *XX* feminiza-

tion and *X*O feminization. As described in MATERIALS AND METHODS, *cis*-revertants of both phenotypes were readily obtained, and in both cases these exhibited all the properties of standard *tra-2(lf)* alleles, with a Tra-2 masculinized phenotype in *XX* homozygotes and no dominant feminizing properties in either *XX* or *X*O. In summary, reversion of the *XX* dominant feminization (due to *e2046*) also reverts the *X*O dominant feminization (due to *e2531*) and vice versa, indicating that the *e2531* mutation lies in *tra-2*. Molecular characterization of the *tra-2(eg)* mutations has confirmed that they are indeed alterations in the *tra-2* sequence (P. E. KUWABARA, unpublished results).

The properties of *tra-2(e2046e2531)* were briefly investigated to compare the *X*O feminization by this genotype with that caused by other mutations in the sex determination pathway, such as loss-of-function mutations in *her-1, fem-1, fem-2,* or *fem-3,* or gain-of-function mutations of *tra-1*. Effects on *XX* animals appear to be similar to those of *tra-2(e2046)* alone: *XX* animals carrying either one or two doses of *tra-2(e2046e2531)* develop as fertile females. Suppression of *tra-3(e1767)* was also examined: the masculinizing effects of *tra-3(e1767)* are largely suppressed by *tra-2(e2046),* and, in fact, *tra-2(e2046)* was originally isolated because of this property (DONIACH 1986; HODGKIN 1986). The enhanced allele, *tra-2(e2046e2531),* appeared to be equally effective in suppressing *tra-3,* suggesting that the hypermorphic properties of *tra-2(e2046)* have not been reduced. Interactions with the masculinizing mutation *her-1(n695*gf) (TRENT *et al.* 1988) were not examined, because *tra-2(e2046)* alone is able to suppress *her-1(n695)* (DONIACH 1986).

Sexual transformation of *X*O individuals from male into female is implicit in the original isolation of the *tra-2(gf, eg)* mutations, but it was desirable to examine this transformation in the absence of other sex determination or dosage compensation mutations. *X*O individuals carrying a single dose of *tra-2(e2046e2531)* were constructed by first establishing a stable *XX* hermaphrodite line CB5055, of genotype *tra-2(e2046e2531); fem-3(q20*gf) *dpy-20; unc-7*. The *fem-3(gf)* mutation suppresses *XX* feminization caused by *tra-2(e2046),* and the *unc-7* marker is a sex-linked recessive mutation used as a standard marker for identifying hemizygous *X*O individuals (HODGKIN 1980, 1986). When CB5055 hermaphrodites were crossed with wild-type males, all Unc non-Dpy progeny [necessarily of genotype *tra-2(e2046e2531)/+; fem-3(q20) dpy-20 /++ ; unc-7/*O] were either female or (rarely) hermaphrodite in phenotype, showing no sign of masculinization.

The phenotype of these animals, however, might have been affected by the dominant *fem-3(q20)* mutation. CB5055 hermaphrodites were therefore crossed twice with *mor-2* males to remove the *fem-3* mutation. Some of the resulting Mor females, of genotype *tra-2(e2046e2531)/+; mor-2; unc-7/+,* were crossed with wild-type males. Half of the Unc-7 progeny from these crosses were male, and half were completely female in somatic phenotype, indicating sexual transformation (Figure 2B). In particular, the tail spike shows complete transformation, in contrast to the incomplete feminization caused by a single dose of a *tra-1(gf)* mutation, such as *e1575* (Figure 2C). In this respect, therefore, the *tra-2(gf, eg)* alleles are the most potent feminizing mutations yet isolated. However, the fertility of these transformed *X*O individuals was low: of 15 Unc-7 female individuals mated with wild-type males, only eight produced eggs (range 1–9) and only one of these eggs hatched. It is possible that this poor fertility results from a dosage compensation effect, because the ancestral females, of genotype *tra-2(e2046e2531)/+; dpy-28(y1),* were significantly more fertile, otherwise the selection would not have worked. More direct and extensive evidence for such an effect has been obtained by P. E. KUWABARA (unpublished results), in further work on the *e2531* mutation. Also, two stable male/female strains have been constructed, using other dosage compensation mutations *dpy-26(n199)* and *dpy-27(rh18).* Each of these strains consists of *tra-2(gf, eg)/+ ; dpy X*O females and *dpy X*O males. These strains grow reasonably well, indicating substantial fertility in the *X*O females.

Interactions of *tra-2(gf,eg)* mutations with some of the

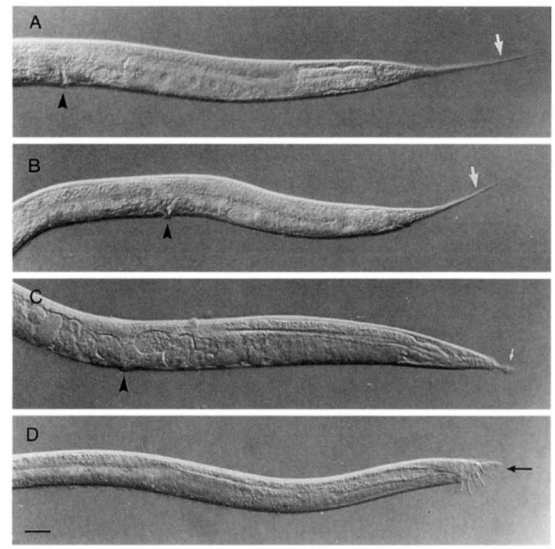

FIGURE 2.—Sexual transformation caused by dominant feminizing mutations. Photographs show the posterior regions of live worms, viewed by Nomarski optics. In all photographs anterior is to the left. Scale, 20 μm. Animals are viewed laterally unless otherwise indicated. (A) Wild-type *XX* hermaphrodite. Arrowhead indicates vulva, white arrow indicates long spike tail. (B) A *tra-2(eg, gf)/+ X*O female, showing complete transformation. For full genotype, see text. (C) A *tra-1(gf)/+ X*O female, showing almost complete transformation; truncated tail spike is indicated by small white arrow. (D) Wild-type *X*O male. Black arrow indicates characteristic male tail structure, with fully developed fan, rays, spicules and other distinctive structures.

other sex determination mutations were also examined, providing results consistent with the standard sex determination pathway model. It was particularly important to test the interaction of *tra-2(gf, eg)* with *tra-1(lf)* mutations in view of the strong feminization caused by these new alleles. The model predicts that the double mutant *tra-2(gf, eg); tra-1(lf)* should nevertheless be completely male in somatic phenotype. This is indeed the case and was demonstrated by constructing a stable strain consisting of *tra-2(e2046e2531); tra-1(e1099)/+ XX* females and *tra-2(e2046e2531); tra-1(e1099) XX* males. These males are indistinguishable from *tra-2(+); tra-1* males, exhibiting a male soma and low male fertility. This demonstrates that *tra-1(e1099)* is fully epistatic to *tra-2(e2046e2531)*. The presence of a dominant homozygous feminizing mutation in the males of this strain means that they should sire only female progeny when crossed with females or hermaphrodites from other strains. This prediction was tested in two crosses: first, to examine *XX* progeny, the males were crossed with marked hermaphrodites (homozygous for *unc-17*). All non-Unc progeny were female (33/33 tested). Second, to examine *XO* progeny, recipient females of genotype *fem-1 him-8; unc-1(dm)* were used. Non-Unc progeny from such females are patroclinous *XO* animals (see MATERIALS AND METHODS), and in control crosses with *tra-1(e1099) XX* males, the non-Unc progeny were invariably male (134/134). In contrast, in crosses with *tra-2(e2046e2531); tra-1(e1099) XX* males, the non-Unc progeny were invariably female or (rarely) hermaphrodite (238/238).

In this last cross, *tra-2(e2046e2531)* is supplied in the paternal gamete to oocytes produced by a homozygous *tra-2(+)* mother, but the patroclinous *tra-2(gf, eg)* allele nevertheless dictates fully female development. Therefore, maternal contributions from the *tra-2(gf, eg)* allele are not necessary to achieve complete feminization. A single dose of this allele, supplied zygotically, is sufficient to transform *XO* individuals from male to female in phenotype.

Nine other lines independently isolated from the selection all carried mutations that were found to be extremely similar to *e2531* in map position and in phenotypic effect. These nine mutations (*e2534, e2536, e2538, e2540, e2551, e2552, e2555, e2581, e2582*) were therefore assumed to be further *tra-2(eg)* mutations. Preliminary characterization revealed no obvious differences between any of these mutations and *e2531*, so further analysis of these lines was deferred.

***tra-1(gf)* mutations:** Four of the 24 lines carried feminizing mutations tightly linked to *tra-1*, which were assumed to be *tra-1(gf)* alleles (*e2535, e2537, e2554, e2579*). Each of these exhibited the dominant feminization characteristic of strong *tra-1(gf)* alleles. Both *XX* and *XO* animals are transformed by a single dose of *tra-1(gf)* to a fertile female phenotype. The two karyotypes remain distinguishable, however, because the *tra-1(gf)/+ XO* tail phenotype exhibits signs of residual masculinization, such as the absence of a tail spike (Figure 2C). This phenotype contrasts with that seen in wild-type *XX* hermaphrodites and in *tra-2(gf, eg)/+ XO* individuals, both of which have perfect tail spikes and show no signs of male development (Figure 2, A and B).

The majority of *tra-1(gf)* alleles analyzed hitherto (HODGKIN 1987) were isolated as mutations causing feminization of *XX* individuals or as suppressors of the *XX* masculinization caused by *tra-3*. Many of these are weak *gf* alleles, which cause incomplete feminization of *XO* animals. The present selection was biased toward the isolation of strong *gf* alleles (class 1, in the classification used in HODGKIN 1987 and DE BONO *et al.* 1995), because only these will cause efficient transformation of *XO* animals to a fertile female phenotype. It was therefore hoped that some of these new alleles might carry novel changes in *tra-1*. The sequence alterations in 29 *tra-1(gf)* alleles have now been determined (DE BONO *et al.* 1995), including these four. Almost all carry single missense alterations in a small stretch of the coding region, corresponding to a 16-amino acid sequence close to the predicted amino-terminus of *tra-1* proteins. This is likely to be the site for an inhibitory protein-protein interaction, which may prevent *tra-1* proteins from exerting a feminizing effect in *XO* individuals. All four alleles isolated in this work have changes identical to those seen in previously isolated class 1 *gf* alleles (H123Y for *e2535* and an identical P122S change in the other three alleles). The sequence changes confirm the interpretation of these alleles as *tra-1(gf)* alleles, but no novel *tra-1(gf)* changes were obtained in this selection, suggesting that saturation has been reached for EMS-induced changes of this class.

***xol-1* mutations:** Ten of 24 lines failed to exhibit dominant feminization of *XO* animals after outcrossing with *dpy-28* males, suggesting that they carried *xol-1* alleles or had a more complex origin. As summarized in MATERIALS AND METHODS, initial tests indicated that nine carried a *xol* mutation and one had a weaker and more variable phenotype. Eight of the nine *xol* mutations mapped to the *xol-1* locus on *LGX* and failed to complement *xol-1(y9)* with respect to enhancing the masculinization of *tra-2(e1095) XX* animals. Neither of these properties alone provides unambiguous proof of allelism, but taken together they justify the presumption that all eight are *xol-1* alleles. All appeared superficially identical in properties to the reference allele, *xol-1(y9)*, so they are presumed to be simple loss-of-function mutations of this gene.

An *XO* lethal duplication of the *X* chromosome: One mutation with a Xol phenotype behaved differently from the other nine. As explained below, this proved to be a duplication of the left end of the *X* chromosome, which exerts a dominant *XO* lethal and feminizing effect. This duplication is attached in inverted orientation to the left tip of the *X* chromosome and is called *eDp26*.

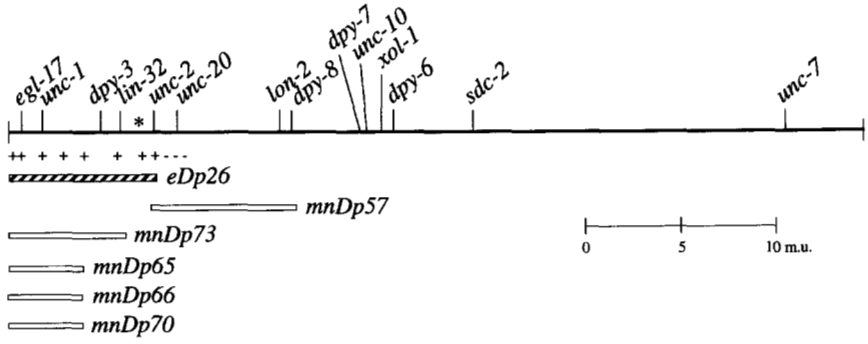

FIGURE 3.—Map of the *X* chromosome, showing the location of genes and rearrangements used in this study. The extent of the *X*O-lethal duplication, *eDp26,* was initially inferred from FISH analysis (see MATERIALS AND METHODS) and is indicated by candy-striping. Locations of probes inside the duplicated region are indicated by a plus sign, and probes outside by a minus sign. The *eDp26* duplication is attached to the end of the *X* chromosome in inverted orientation. *, predicted location of a major numerator site or region.

For consistency, it is given this name throughout this paper, although the nature of the mutation was not immediately apparent.

In all initial experiments, *eDp26* behaved like a *xol-1* mutation: a stable, homozygous viable mutation on the *X* chromosome with no recessive lethal effects on *XX* animals but complete lethality to *X*O animals. Closer inspection of *eDp26 XX* homozygotes revealed minor differences from wild-type *XX* animals: these homozygotes are slightly shorter than wild-type hermaphrodites and have somewhat smaller broods (mean brood size for 12 individuals was 234 hermaphrodites, 0 males, 3 inviable zygotes, as compared to the wild-type mean of 329 hermaphrodites, 0.2 males, 0.8 inviable zygotes (HODGKIN *et al.* 1979). In contrast, *xol-1* mutations do not affect the phenotype or fertility of *XX* hermaphrodites (MILLER *et al.* 1988). The lethal effects of *eDp26* were shown to be associated with *X*O karyotype, not maleness *per se,* by constructing animals of genotype *tra-2(q276); eDp26 XX* and *tra-1(e1099); eDp26 XX.* Both of these genotypes result in a viable male phenotype, and some of these males are able to sire progeny (fertility of *tra-2 XX* or *tra-1 XX* males is always low).

Further results indicating that *eDp26* is not a simple loss-of-function allele of *xol-1* were as follows: first, that it fails to confer male mating competence on *tra-2(lf) XX* animals and second, anomalous mapping behavior. A separate property of *xol-1* mutations, distinct from the *X*O lethality, is that *tra-2(lf); xol-1(lf) XX* males are fertile mating males (MILLER *et al.* 1988), unlike *tra-2(lf); xol-1(+) XX* males, which are slightly abnormal in male anatomy and do not mate (HODGKIN and BRENNER 1977). As noted above, all eight *xol-1* mutations isolated in this study enhance *tra-2(lf)* in this way. In contrast, *tra-2(lf); eDp26 XX* males do not exhibit mating behavior, and their tail anatomy resembles that of *tra-2(lf); xol-1(+) XX* males. Confusingly, however, *eDp26* fails to complement *xol-1(y9)* with respect to this property. That is, *tra-2(lf); xol-1/eDp26 XX* animals are fertile mating males. This result initially raised the possibility that *eDp26* might be associated with an unusual mutation of *xol-1,* comparable to the *xol-1(mn467)* allele described by RHIND *et al.* (1995), which also leads to *X*O lethality without enhancing *tra-2(lf).* The mapping data described below preclude this possibility. An alternative explanation is that *eDp26* may lead to some downregulation of *xol-1(+)* in *xol-1/eDp26 XX* heterozygotes.

The *X*O lethality of *eDp26* was mapped, indicating a genetic location at the left end of the *X* chromosome. A three-factor cross with the markers *egl-17* and *unc-1* indicated that the *X*O lethality maps left of *egl-17,* the leftmost marker on the current genetic map. A location clearly distinct from *xol-1* was established, and wild-type *X* chromosomes, with no Xol property, were readily recovered by recombination from *eDp26/xol-1* heterozygotes. Therefore, the *eDp26* chromosome is not defective in *xol-1.*

The *X*O lethality of *eDp26* might also have been due to loss or mutation of material at the left end of the *X* chromosome. Therefore, attempts were made to rescue the *X*O lethality using duplications that were found to complement both *egl-17* and *unc-1* and are therefore likely to include the entire left end of the *X* chromosome (Figure 3). None of the duplications *mnDp65, mnDp66, mnDp67, mnDp68* or *mnDp70* were able to rescue the *X*O lethality of *eDp26.*

Two recombinational anomalies became apparent in the course of this mapping. First, *eDp26* causes a dominant partial suppression of recombination on the left end of the *X* chromosome. For example, the *dpy-3 lon-2* interval was reduced from 10.0 map units (control) to 3.9 map units in *eDp26/+* heterozygotes (chi-squared probability $\ll 0.01$). The *unc-2 dpy-7* interval was reduced from 15.4 map units to 8.1 map units (chi-squared probability $\ll 0.01$). In contrast, recombination elsewhere on the *X* chromosome did not appear to be affected: the interval *lon-2 unc-7* was measured as 30.1 map units in *eDp26/+* heterozygotes, similar to control values.

The second anomaly was that no recombinants car-

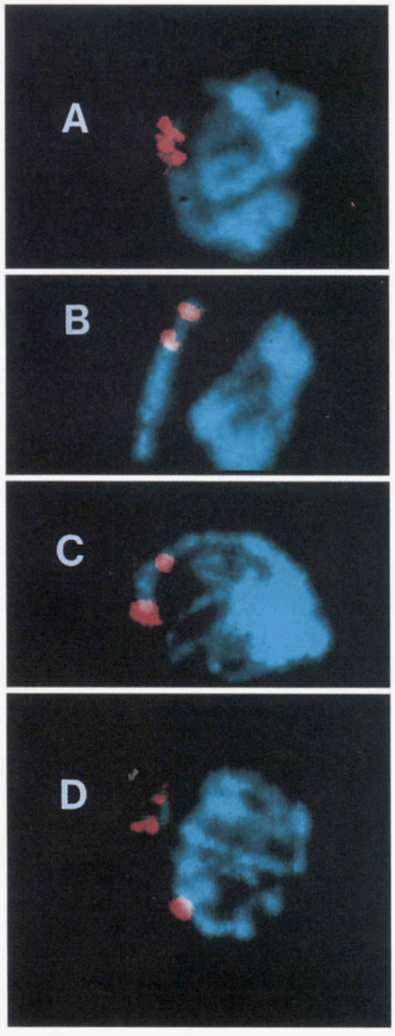

FIGURE 4.—FISH images: hybridization of labeled YACs to bivalents from *XX* hermaphrodites carrying *eDp26* (A–C) or *eDp27* (D). The site of hybridization of rhodamine dUTP-labeled YACs (red false color) was visualized on DAPI-stained chromosomes (blue false color). Probes shown are Y51E2 (A), Y8D1 (B) and Y46A5 (C and D), with locations indicated by the first, third and sixth + symbols in Figure 3. A–C demonstrate that *eDp26* is attached in inverted orientation to the left end of the *X* chromosome, because a probe covering *lin-32* (Y46A5) shows two separated spots of hybridization, whereas a probe close to the left end (Y51E2) shows two almost coalescent spots. D shows that *eDp27* is a free double duplication with two spots of hybridization; a single spot of hybridization to the normal *X* bivalents is also visible.

rying both *eDp26* and markers left of *unc-2* could be recovered, although recombinants with markers further to the right, such as *lon-2*, were readily obtained.

Subsequent experiments, described below, also revealed that *eDp26* is unstable, reverting to a wild-type *X* chromosome under certain conditions. The instability, together with the abnormal mapping properties, suggested that *eDp26* might be a duplication of the left end of the *X* chromosome. FISH analysis was therefore carried out, using a series of YAC probes covering the left end of the *X*. Probes up to and including Y23A3 (the probable location of *unc-2*) gave two signals on *eDp26* chromosomes, while probes further to the right gave only one signal. Furthermore, the FISH patterns showed that *eDp26* is attached in inverted orientation to the left tip of the *X* (see Figure 4). No evidence for rearrangement, deletion or duplication within *eDp26* was obtained, so cytogenetically it appears to be a simple duplication of the left end of the *X* chromosome, ~6.5 map units or 2100 kb (estimated from correlations between the genetic and physical maps of the genome) (COULSON *et al.* 1991; ALBERTSON 1993).

The *eDp26* duplication is unstable, giving rise to a wild-type *X* chromosome under certain conditions. However, the instability of *eDp26* was not immediately apparent. The penetrance of the *X*O lethal phenotype was tested by crossing *dpy-10 unc-4; eDp26* homozygous hermaphrodites with wild-type males. In all, 3162 cross-progeny (non-Dpy non-Unc) hermaphrodites were obtained, together with large numbers of unhatched eggs and arrested L1 larvae, and no males. This indicates *X*O lethality in excess of 99.9%. To explore *X*O lethality further, a strain of genotype *him-8; eDp26* was constructed. In this strain, males were occasionally seen, though vastly fewer than the 35–40% normally seen in an *him-8* strain (HODGKIN *et al.* 1979), and the males were often small or otherwise abnormal. Twelve complete self-progeny broods were counted for *him-8; eDp26 XX* hermaphrodites, yielding a total of 1442 hermaphrodites and five males. Each of these males was crossed with hermaphrodites carrying sex-linked markers [*unc-1(e719) dpy-3(e27)*]. One male failed to mate, but the other four sired wild-type hermaphrodite progeny. These hermaphrodites were then test-crossed with wild-type males. In two of the four cases, equal numbers of UncDpy males and wild-type males (entirely normal and fertile in phenotype) were produced, indicating that a normal *X* chromosome had been generated in the parental male, presumably by detachment of the duplicated tip of the *X* chromosome, leaving behind an intact normal chromosome. In the other two cases, UncDpy males but no wild-type males were generated from the test cross, indicating that the parental male had transmitted an unaltered *eDp26* chromosome. Thus, it appears that on rare occasions an *eDp26 X*O genotype is compatible with normal male development. Further examples of this effect are described below.

The reversion to a wild-type *X* chromosome is significant because it confirms that *eDp26* is attached to an otherwise unaltered *X* chromosome, and therefore that its *X*O lethal effects are dominant. The fact that reversion to wild type appears to happen at a significant frequency in a *him-8* background but not a wild-type background can be ascribed to the effect of *him-8* on meiosis in *XX* hermaphrodites. In the *him* background, recombinational events on the *X* chromosome are abnormal (HODGKIN *et al.* 1979; BROVERMAN and MENEELY 1994), so exchanges or other interactions between sister

FIGURE 5.–Variable sexual phenotypes of *X*O animals of genotype *dpy-26; eDp26.* Presentation as in Figure 2. (A) Hermaphrodite. Note spike tail (white arrow), vulva (arrowhead), developing eggs and oocytes. (B) Intersex (ventral view). Note vulva (arrowhead), egg and oocytes, and abnormal male tail (small black arrow). (C) Male. Note fully developed male tail (black arrow); compare with Figure 2D.

chromatids are likely to occur more often than in a wild-type background.

In addition to *X*O lethality, *eDp26* has a strong feminizing effect. This is implicit in the original isolation of this mutation, in the form of *dpy-28; eDp26 X*O hermaphrodites. The viability of these animals indicates that *dpy-28* is able to rescue the *X*O lethality, which can therefore be ascribed to incorrect dosage compensation. In contrast to normal *dpy-28 X*O individuals, which are invariably male, most of the *dpy-28; eDp26 X*O animals are either self-fertile hermaphrodites or feminized intersexes, demonstrating that *eDp26* is both lethal and feminizing. Similar properties are seen in *xol-1* mutants (MILLER *et al.* 1988). Double mutants of *eDp26* with other dosage-compensation mutants, *dpy-26(n199)* and *dpy-27(rh18),* were also constructed. In both cases the dosage compensation mutation restores viability to *eDp26 X*O animals, and these surviving animals are often transformed to a fertile hermaphrodite phenotype. One of these strains, *dpy-26(n199); eDp26 X*O was examined in more detail and compared with a strain of genotype *dpy-26; xol-1(y9) X*O. In both cases the phenotype is variable, ranging from self-fertile hermaphrodite through sterile or fertile intersex to fertile male (Figure 5). Feminization caused by a null allele of *xol-1* is significantly stronger than that caused by *eDp26,* but still incomplete (Table 1).

The male animals in a strain of genotype *dpy-26; eDp26 X*O are of two types. Some are indistinguishable from *dpy-26 X*O males, and others are distinctly smaller. Genetic evidence, obtained by crossing the males with marked recipient hermaphrodites, also indicates that there are two types of males. Some (most or all of the larger males) are revertants, which have lost *eDp26* and carry only a normal *X* chromosome. Others (most or all of the smaller males) appear to carry and transmit an unaltered *eDp26* chromosome. Thus, in a *dpy-26* background, *eDp26* can lead to fertile male development, as can *xol-1.* The reversion to wild type in the progeny of *X*O hermaphrodites is not surprising, given the previous evidence for increased reversion in a *him-8 XX* background.

Epistatic interactions of *eDp26* with other mutations affecting sex determination were investigated. As described above, *eDp26* does not interfere with the complete *XX* masculinization caused by *tra-2(q276)* or *tra-1(e1099).* A double mutant with a dominant masculizing allele of *her-1* (TRENT *et al.* 1988) was also constructed. Homozygous animals of genotype *her-1(n695*gf*); eDp26 XX* were found to exhibit variable masculinization, like *her-1(n695*gf*) XX* alone. Finally, a double mutant of *sdc-2* and *eDp26* was constructed (see MATERIALS AND METHODS), and both *XX* and *X*O phenotypes were examined. The *eDp26 sdc-2 XX* animals are not viable, having an Sdc phenotype, and the *X*O animals are viable fertile males, like *sdc-2* alone. Thus, both the feminization and the *X*O lethality of *eDp26* are fully suppressed by a mutation in *sdc-2.* This is consistent with action at a very early step in the sex determination pathway, at a level equivalent to, or preceding, *xol-1.*

Interactions of *eDp26* with *xol-1* itself are not so easily tested or interpreted, because both mutations have similar phenotypic effects. A double mutant chromosome, *eDp26 xol-1* was constructed as described in MATERIALS AND METHODS. This was found to be homozygous viable in an *XX* strain, with a phenotype similar to *eDp26,* but *X*O lethal, like either mutation alone. Feminization by the double mutant was examined by constructing a

TABLE 1

Sexual transformation of *dpy-26 X*O animals

Genotype	Hermaphrodite	Intersex	Male	% Hermaphrodites
dpy-26	0	0	>100	0
dpy-26; xol-1	209	45	50	69
dpy-26; eDp26	151	92	68	49
dpy-26; eDp26 xol-1	378	3	4	98

Data for the first line refer to progeny of *dpy-26 XX* hermaphrodites (HODGKIN 1983). Data for lines two, three and four were obtained by counting complete viable progeny produced by 12 separate *X*O hermaphrodites (picked as L4's), for each genotype. Numerous unhatched eggs or dead young larvae were also produced, as is usual with *dpy-26 X*O hermaphrodites.

strain of genotype *dpy-26; eDp26 xol-1*. This strain shows significantly stronger feminization than either mutation alone (Table 1).

The preceding data suggest that the properties of *eDp26* arise from duplication of key numerator elements, which act as primary sex determinants for female development. If this is so, then they should act zygotically. In the experiments above, the duplication was always present in the maternal germ line, so it is conceivable that both lethality and feminization could have been exacerbated by maternal effects. Experiments introducing *eDp26* zygotically, via sperm, were carried out using *tra-1(e1099); eDp26 XX* males. Such males were crossed with *fem-1 him-8; unc-1(dm)* females, which generate nullo-*X* oocytes and can therefore be used to generate patroclinous *XO* individuals. One series of crosses yielded 1092 Unc hermaphrodites, five non-Unc hermaphrodites, two Unc males, eight non-Unc males, and numerous unhatched eggs and arrested Xol larvae. A control cross with *tra-1 XX* males yielded 216 Unc hermaphrodites and 134 non-Unc males. Consequently, the expected number of patroclinous *XO* progeny in the cross with *tra-1; eDp26 XX* males is 677 (1092 × 134/216). Only eight of these survived, as normal or slightly abnormal males. The five non-Unc hermaphrodites were unexpected and might have been transformed *XO* progeny, but they produced self-progeny inconsistent with such a genotype. Instead, they were probably *XX* animals carrying attached *X* chromosomes, which can be generated from *eDp26* (see below). The eight males were test-crossed to establish whether they still carried *eDp26* or not. Three were not fertile, one was a revertant to a wild-type *X* chromosome, and four transmitted apparently unaltered *eDp26* chromosomes. Therefore, *eDp26 XO* males can occasionally survive, at a frequency of ~0.6%, if the duplication is supplied paternally. This survival rate is higher than seen if the duplication is present maternally, implying that there is a slight maternal enhancement of the *XO* lethality. However, the zygotic *XO* lethality is still in excess of 99%, demonstrating that zygotic presence of *eDp26* is sufficient to kill almost all *XO* progeny.

A duplicated free derivative of *eDp26*: The reversion to wild type of *eDp26* chromosomes, apparently by loss of the duplicated part of the chromosome, raised the possibility that this duplication could be recovered as a free fragment, which would be useful for further manipulations and dosage studies. Crosses to recover such a free derivative were carried out as described in MATERIALS AND METHODS. Several candidates were recovered with apparently similar properties, and one of these, *eDp27*, was examined in more detail. Genetically it behaves as a free duplication covering all tested markers on the left end of the *X* chromosome, up to and including *unc-2*. It retains the Xol property of *eDp26*, consistent with expectation. Animals carrying *eDp27* were examined by FISH analysis, and the presence of a free duplication of the left end of the *X* chromosome was confirmed. However, the FISH data demonstrated that *eDp27* is not a simple duplication; instead, it carries two copies of the left end of the *X* chromosome, in inverted orientation (Figure 4D). A free duplication with this structure could be generated from an *eDp26* chromosome by aberrant meiotic pairing, as shown in Figure 6A, so in retrospect it is not surprising that a duplication such as *eDp27* was recovered.

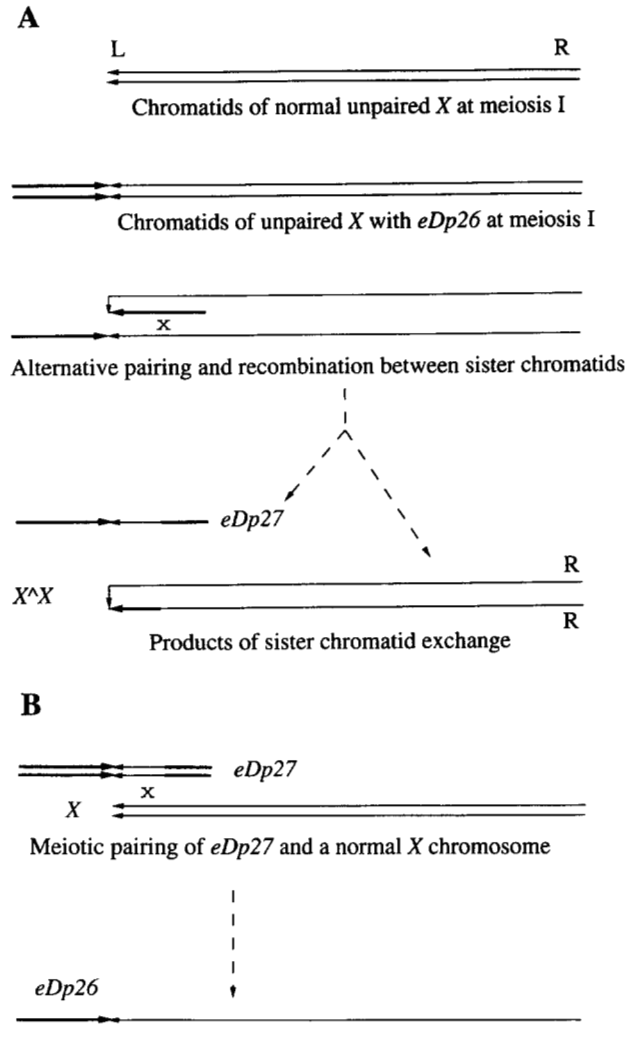

FIGURE 6.—Diagrams illustrating the structures of *eDp26* and *eDp27*, and proposed recombination events involving these duplications. The left telomere of the *X* chromosome is marked by an arrowhead, and the duplicated material in *eDp26* is shown as a thicker line. (A) In an *XO* individual carrying *eDp26*, alternative sister chromatid pairings are possible. Recombination between the sister chromatids can then give rise to a double duplication, *eDp27*, and to an attached *X* chromosome as the reciprocal product. The same products could also be generated by meiosis in an *XX* individual homozygous for *eDp26*, as a result of end-to-end pairing (not illustrated). (B) Recombination between *eDp27* and a normal *X* chromosome can regenerate an attached duplication, presumably identical in structure to the original *eDp26*.

This duplication, *eDp27,* carries two copies of the left end of *X,* so it might be expected to have stronger effects than *eDp26.* This was examined by constructing animals of genotype *dpy-26; unc-2; eDp27 X*O. These are viable hermaphrodites, segregating self progeny consisting of many dead eggs, some viable hermaphrodites and intersexes, as well as many Unc-2 males (which have presumably lost the duplication). Feminization is comparable or stronger than with *eDp26,* because most *dpy-26; unc-2; eDp27 X*O animals appear to be hermaphrodite in phenotype (8/11 in one brood). However, they are rather small and sickly. It is not possible to maintain a stable strain of this genotype, because healthier and more fertile hermaphrodites arise spontaneously, and outgrow the parental hermaphrodite strain. These hermaphrodites do not segregate Unc-2 progeny and therefore probably result from recombination between *eDp27* and the *X* chromosome, which will generate an attached single duplication, similar or identical in structure to *eDp26.* Such an event is diagrammed in Figure 6B.

Generation of apparent attached *X* chromosomes: The event giving rise to *eDp27* should also give rise, as a reciprocal meiotic product, to an attached *X* chromosome ($\hat{XX}$), that is, two *X* chromosomes joined at their left ends (Figure 6A). Rare patroclinous hermaphrodites were indeed generated in the cross described in an earlier section (cross between *tra-1; eDp26 XX* males and *fem-1 him-8; unc-1(dm)* females), and it seemed likely that these might have been $\hat{XX}$ individuals. Attached chromosomes have not been generated in any previous work on *C. elegans,* so it was of interest to see if convincing evidence of such a genotype could be obtained and to examine the behavior of an attached *X* structure.

To generate marked $\hat{XX}$ chromosomes, males of genotype *dpy-26; eDp26 lon-2 X*O were crossed with *fem-1 him-8; unc-1(dm)* females. Rare (fewer than 1%) Lon non-Unc hermaphrodites were seen among the progeny of this cross. One of these was selfed and then crossed with wild-type males. The self-progeny included many dead eggs as well as Lon hermaphrodites, a few Lon males and some shorter hermaphrodites with phenotypes similar to *lon-2/lon-2/lon-2* 3*X* hermaphrodites (HODGKIN *et al.* 1979). The cross yielded predominantly wild-type males, Lon hermaphrodites and 3-*X* hermaphrodites. Both self-progeny and cross-progeny are therefore consistent with an attached *X* genotype. The 3-*X* cross-progeny were selfed, yielding predominantly 3-*X* hermaphrodites and Lon hermaphrodites. Several of the latter were selfed and produced broods like the original Lon hermaphrodite. These were crossed against wild-type males for four more generations, to ensure removal of the original marked chromosomes carrying *fem-1 him-8,* or *dpy-26.*

One of the Lon hermaphrodites resulting from this series of crosses was used to found a strain CB5042, of presumed genotype $\hat{lon\text{-}2lon\text{-}2}$. Complete broods for five animals from this strain were scored, giving average progeny of 90 Lon hermaphrodites, nine Lon males, six 3-*X* hermaphrodites, and 84 inviable zygotes. This brood composition is very different from that of normal *lon-2* hermaphrodites from strain CB678 (*lon-2(e678)*) (average single brood for five animals: 277 Lon hermaphrodites, 25 inviable zygotes). The production of Lon males, and also of 3-*X* hermaphrodites, suggests that the putative attached *X* chromosome structure breaks down at a significant frequency, generating normal *X* chromosomes, and consequently to Lon male self-progeny. Two such males were crossed with *unc-1(e719)* hermaphrodites, siring wild-type hermaphrodite progeny. Some of these were selfed and produced a normal hermaphrodite brood consisting of wild-type, Unc and Lon hermaphrodites, no males, and very few inviable zygotes. The Lon segregants were examined, and none behaved like the parental CB5042 strain; instead, they appeared identical to CB678 hermaphrodites. Thus, it appears that the attached *X* structure can break (presumably at the attachment site at the left end), regenerating a normal *X* chromosome.

Meiotic chromosomes of hermaphrodites expected to carry attached *X* chromosomes were examined cytogenetically and by FISH analysis. The meiotic figures showed substantial levels of abnormal chromosomes and chromosome breakage. Definitive evidence of an attached *X* structure was not obtained, but this structure seems to be the simplest explanation of the genetic phenomenology described above.

DISCUSSION

The strategy outlined in the Introduction, for selection of dominant feminizing mutations of *C. elegans,* evidently works effectively, yielding both known and novel kinds of mutation. Four separate components of this investigation will be discussed.

First, methodological points: the general approach used is likely to be relevant in other areas of *C. elegans* genetics. A major advantage of genetic analysis in this organism is the ability to apply powerful selections or efficient screens to very large numbers of individuals, to isolate suppressors or enhancers of a great variety of mutant phenotypes. *C. elegans* reproduces normally by meiotic self-fertilization, permitting isolation of both recessive and dominant modifiers. Sometimes selections will be swamped by common recessive suppressors, impeding the isolation of rare dominant modifiers, which are often uniquely informative. Carrying out selections *en masse* in an obligate male/female strain will focus attention on dominant mutations. In this investigation, the mutation *tra-2(e2046)* was used to ensure propagation as a male/female strain, but other mutations could be used instead, such as *fog-2* (which, like *tra-2(e2046),* results in a purely female germ-line in *XX* individuals) (SCHEDL *et al.* 1988) or either *spe-8* or

spe-12, mutations that lead to nonfunctional sperm in hermaphrodites although male sperm are normal (L'HERNAULT *et al.* 1988).

In addition, we used an approach that started with an already hypermorphic allele of *tra-2* and selected for enhancers, in the hope of identifying additional regulatory sites. Important developmental control genes may often be subject to multiple modes of regulation, as in the case of both *tra-2* and *tra-1* (DONIACH 1986; ZARKOWER *et al.* 1994), so selections of this type may be appropriate and effective elsewhere.

The second feature of this work is the isolation of *tra-2(eg)* mutations. Our results demonstrate that a *tra-2(eg, gf)* allele is able, in a single dose supplied by either female or male parent, to completely transform an *X*O individual from the normal male fate to a functional female fate, in all somatic and germ-line tissues. Observations on these alleles confirm and extend previous work defining *tra-2* as a major switch gene acting at this level in the sex determination pathway (DONIACH 1986; HODGKIN 1986; KUWABARA *et al.* 1992). Ten independent *tra-2(eg, gf)* mutations were obtained with apparently identical properties. The existence of these new alleles raises new questions and possibilities for the further investigation of *tra-2.* The data reported here are consistent with the possibility that the *tra-2(eg)* mutations render TRA-2A insensitive to inhibition by HER-1. However, proper assessment of the properties of the *tra-2(eg)* mutations entails their separation from the antecedent *tra-2(gf)* allele. This separation and the further analysis of these mutations at both molecular and genetic levels has been carried out by P. E. KUWABARA (unpublished results).

The third significant feature of these experiments is the isolation of the unusual Xol duplication, *eDp26.* As documented in RESULTS, this duplication has the following properties: it is almost 100% lethal to *X*O individuals, but entirely viable in homozygous *XX* animals. Genetic analysis indicates that it is a duplication of the left end of the *X* chromosome, attached to the left tip of the *X.* Cytological observations confirm this and demonstrate that it is attached in inverted orientation, a property that would have been difficult to deduce from genetics alone.

Under most circumstances the attachment of *eDp26* to the *X* chromosome is very stable, but the duplication-bearing chromosome does revert to a wild type state on rare occasions, and more frequently if the *X* chromosome is unpaired. The reversion to wild type and the homozygous viability of *eDp26/eDp26* individuals demonstrates that no sequences have been lost from the *X* chromosome, and hence that the *X*O-lethal effects are likely to be dominant. This was confirmed by the isolation of *eDp27,* a free derivative of *eDp26,* which has comparable dominant *X*O-lethal effects.

The *X*O-lethality of these duplications (*eDp26* and *eDp27*) appeared to be phenotypically similar to that caused by loss-of-function mutations in the gene *xol-1.* Consistent with this, the *X*O lethality is rescued by mutations of dosage compensation genes such as *dpy-26, dpy-27* and *dpy-28.* When *eDp26 X*O animals are restored to viability by this means, they are transformed either partly or wholly into viable fertile hermaphrodites, although a minority develop as fertile males. Similar behavior is observed with *xol-1* mutations in combination with these dosage compensation genes (MILLER *et al.* 1988), although the feminizing effects of *xol-1* are significantly stronger than those of *eDp26* (Table 1). The doubly mutant *X* chromosome, *eDp26 xol-1,* is stronger still.

The similarity between *eDp26* and *xol-1* mutations is confirmed by the interaction between *eDp26* and *sdc-2.* The *eDp26* duplication has been combined with a loss-of-function mutation of *sdc-2,* which has opposite properties by itself: *X*O *sdc-2* animals are viable males, but *XX sdc-2* animals are inviable and masculinized (NUSBAUM and MEYER 1989). *X*O animals of genotype *eDp26 sdc-2* are normal fertile males, and *XX* animals are dead, demonstrating that the *sdc-2* mutation is fully epistatic to *eDp26.* Interactions with the other *sdc* genes, *sdc-1* and *sdc-3,* have not yet been examined, but mutations of these genes appear to have weaker and more complex propeties than *sdc-2* (VILLENEUVE and MEYER 1987; DELONG *et al.* 1993). The fact that an *sdc-2* mutation is epistatic to *eDp26* indicates that the duplication is acting at a very early step in the sex determination pathway.

The duplications *eDp26* and *eDp27* cover a region, between *lin-32* and *unc-2,* that has not been previously duplicated. There appear to be only a few such regions on the *X* chromosome (MENEELY 1994), though it is not possible to exclude the possibility that some of the existing duplications are discontinuous and therefore fail to cover some small interstitial regions. The contrast between *eDp26* and *mnDp73,* a duplication only slightly smaller than *eDp26,* is dramatic: *X*O males carrying *mnDp73* can develop as fertile males, whereas those carrying *eDp26* are inviable.

These results are most easily explained by proposing that *eDp26* duplicates one or more key sites for the assessment of *X*:A ratio, and that all its effects on viability and sexual phenotype arise from this. Furthermore, the extreme difference between *mnDp73* and *eDp26* suggests that a major numerator site is located between *lin-32* and *unc-2.*

As a result of these observations, a specific search was carried out for a dose-sensitive element in the interval between the right end-points of *mnDp73* and *eDp26* (HODGKIN *et al.* 1994). Cosmid clones from this region were identified and tested for the ability to confer an *X*O-lethal effect, by constructing transgenic lines carrying additional copies of each tested cosmid. One set of overlapping cosmids did have such an effect, conferring both *X*O lethality and feminization at much the same level as does *eDp26.* This is the first time such an

effect has been seen, although many transgenic lines carrying extra copies of other *X* chromosome clones have been onstructed by other laboratories, in the course of cloning specific genes (for example, ZHAO and EMMONS 1995) or in searches for numerator elements (MCCOUBREY *et al.* 1988). The molecular data define a region of <25 kb with lethal and feminizing properties, which we have named *fox-1* (standing for *f*eminizing locus *o*n *X*).

The observations on *fox-1* suggest, but do not prove, that most or all of the effects of *eDp26* are due to the extra dosage of this locus and that the rest of the duplication does not carry major numerator elements. Some contrasting data suggesting the existence of additional elements have been obtained by AKERIB and MEYER (1994). As a result of our observations on *eDp26,* these authors searched for additional duplications of parts of the left end of the *X* chromosome and tested existing duplications more stringently for numerator activity. They obtained evidence for a region (region 1) at the left end of the *X,* duplicated by *mnDp66,* which has some deleterious effect on *X*O animals when present in three doses. Duplications covering the *lin-32-unc-2* interval (region 3) exert a significant *X*O lethal effect (25% lethality) in a single extra dose, consistent with the prediction of a major site in this interval. This lethality is increased by the presence of an extra copy of an interval to the left of *lin-32* (region 2) and by *mnDp66.* Surprisingly, none of the duplications or combinations examined by these authors has *X*O lethality quite as high as seen with *eDp26.* At present, the most straighforward interpretation of the data obtained in our work (this paper and HODGKIN *et al.* 1994) and by AKERIB and MEYER (1994) is that there exists a major numerator site, probably *fox-1,* between *lin-32* and *unc-2,* plus a limited number of additional sites elsewhere on the *X* chromosome. Further genetic and molecular work will undoubtedly clarify the nature and importance of these various contributions to measuring *X* chromosome dose. However, it is reasonable to conclude now that a rather small number of sex-linked factors is involved, much as in Drosophila (CLINE 1993), and that most of the *X* chromosome material does not contribute significantly to assessment of the *X*:A ratio.

Molecular investigations of the effects of these duplications should also be rewarding. Both genetic and molecular experiments on *xol-1* (MILLER *et al.* 1988; RHIND *et al.* 1995) indicate that this gene plays a major role in the initial response to the *X*:A ratio. Loss-of-function mutations of *xol-1* lead to *X*O lethality and feminization, while overexpression of *xol-1* leads to *XX* lethality. Moreover, a *xol-1::lacZ* reporter transgene responds embryonically to the *X*:A ratio: *X*O embryos exhibit substantially higher *lacZ* staining than do *XX* embryos, suggesting *xol-1* is under sex-specific transcriptional regulation (RHIND *et al.* 1995). This reporter construct has also been used by AKERIB and MEYER (1994) to examine the effect of *X* chromosome duplications. Increased dosage of *mnDp66,* which covers the left end of the *X,* leads to reduced expression from this transgene in *X*O animals. In contrast, *yDp14,* a duplication that includes the *lin-32-unc-2* interval, had no apparent effect on expression, although its *X*O lethal effects are stronger. It may be that the reporter transgene does not accurately reflect regulation of the endogenous *xol-1,* or possibly there are additional genes as well as *xol-1* that act at this level in the hierarchy, as discussed below.

Two additional properties of *eDp26* are noteworthy in this context. First, it appears that *X*O animals carrying *eDp26* can sometimes survive to develop as normal fertile males, especially if the duplication is supplied via sperm. It is conceivable that these animals are in fact mosaic, having lost the duplication in the soma but retained it in the germ line, but this explanation seems less likely. One interpretation of the survival of these animals is that *X*:A ratio is assessed at an early point in development, but thereafter sexual identity is maintained by some other means. In the survivors, *eDp26* may have failed to switch the program into the female, *X*O-lethal mode of development at this early point, so they develop normally as healthy males.

Second, the fact that *eDp26* has an enhancing effect on the feminization caused by *xol-1* is significant, because it suggests that *eDp26* is exerting a feminizing effect over and above that caused by downregulation of *xol-1.* It might be argued that the *xol-1* mutation retains some residual activity, which can be further reduced by the action of *eDp26,* but it is known that *xol-1(y9)* is a deletion of ≥25 kb, which entirely removes the *xol-1* gene (RHIND *et al.* 1995), so this does not seem a possibility. The normal process of sex determination in the wild type may therefore entail some gene or genes in addition to *xol-1,* though it seems probable that *xol-1* activity is an important factor. It was postulated long ago that there may be minor effects of *X* chromosome dosage that bypass the main control genes (HODGKIN 1980).

A final feature of these experiments is the generation of apparent attached *X* chromosomes, by recombination events involving *eDp26* (diagrammed in Figure 6). Cytological observations on hermaphrodites predicted to carry such $\hat{XX}$ chromosomes provided evidence of abnormality, but no unequivocal proof of such a structure. However, it is hard to explain the transmission properties of these chromosomes in any other way, and the structure of *eDp26* makes it likely that they would be generated. In organisms such as Drosophila, attached chromosomes are very stable, making them useful as balancers and for chromosomal manipulations. In the situation we have examined, however, the apparent $\hat{XX}$ chromosomes appear to give rise to normal *X* chromosomes at a significant frequency, presumably by breakage at the attachment site during meiosis. They are therefore unlikely to be very useful as tools. Possibly

the attachment site in *eDp26* and the resulting $\hat{XX}$ chromosomes is unusually fragile, in which case stable $\hat{XX}$ chromosomes (or attached autosomes) might be generated by other means. More probably, the mechanism of meiotic segregation in *C. elegans* precludes the formation of any stable attached chromosomes. After exchange at meiosis I, chiasmata terminalize to the ends of paired chromosomes, and either end of each chromosome has the potential to act as a kinetochore (ALBERTSON and THOMSON 1993). In an attached *X* chromosome, if terminalization occurs toward the attachment site, then two interlocked chromosomes may result, with chromosomal breakage at disjunction as a likely consequence.

Some of the strains used in this study were provided by the Caenorhabditis Genetics Center, which is funded by the National Insitutes of Health Division of Research Resources. We are also grateful to CLAIRE KARI and BOB HERMAN for providing additional strains. PATRICIA KUWABARA, MARIO DE BONO and CATHERINE MILNE provided helpful comments on the manuscript. J.H. is an International Research Scholar of the Howard Hughes Medical Institute.

LITERATURE CITED

AKERIB, C. C., and B. J. MEYER, 1994 Identification of *X* chromosome regions in *C. elegans* that contain sex-determination signal elements. Genetics **138:** 1105–1125.

ALBERTSON, D. G., 1993 Mapping chromosome rearrangement breakpoints to the physical map of *Caenorhabditis elegans* by fluorescent *in situ* hybridization. Genetics **134:** 211–219.

ALBERTSON, D. G., and J. N. THOMSON, 1993 Segregation of holocentric chromosomes at meiosis in the nematode, *Caenorhabditis elegans*. Chromosome Res. **1:** 15–26.

BARTON, M. K., T. SCHEDL and J. KIMBLE, 1987 Gain-of-function mutations of *fem-3*, a sex determination gene in *Caenorhabditis elegans*. Genetics **115:** 107–119.

BROVERMAN, S. A., and P. M. MENEELY, 1994 Meiotic mutants that cause a polar decrease in recombination on the *X* chromosome in *Caenorhabditis elegans*. Genetics **136:** 119–127.

CHUANG, P.-T., D. G. ALBERTSON and B. J. MEYER, 1994 DPY-27: a chromosome condensation protein homolog that regulates *C. elegans* dosage compensation through association with the *X* chromosome. Cell **79:** 459–474.

CLINE, T. W., 1993 The *Drosophila* sex determination signal: how do flies count to two? Trends Genet. **9:** 385–390.

COULSON, A., Y. KOZONO, B. LUTTERBACH, R. SHOWNKEEN, J. SULSTON *et al.*, 1991 YACs and the *C. elegans* genome. Bioessays **13:** 413–417.

DE BONO, M., D. ZARKOWER and J. HODGKIN, 1995 Dominant feminizing mutations implicate protein-protein interactions as the main mode of regulation of the nematode sex determining gene *tra-1*. Genes Dev. **9:** 155–167.

DELONG, L., J. D. PLENEFISCH, R. D. KLEIN and B. J. MEYER, 1993 Feedback control of sex determination by dosage compensation revealed through *Caenorhabditis elegans sdc-3* mutations. Genetics **133:** 975–896.

DONIACH, T., 1986 Activity of the sex-determining gene *tra-2* is modulated to allow spermatogenesis in the *C. elegans* hermaphrodite. Genetics **114:** 53–76.

GOODWIN, E. B., P. G. OKKEMA, T. C. EVANS and J. KIMBLE, 1993 Translational regulation of *tra-2* by its 3′ untranslated region controls sexual identity in *C. elegans*. Cell **75:** 329–339.

HERMAN, R. K., and C. K. KARI, 1989 Recombination between small *X* chromosome duplications and the *X* chromosome in *Caenorhabditis elegans*. Genetics **121:** 723–737.

HODGKIN, J., 1980 More sex determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

HODGKIN, J., 1983 *X* chromosome dosage and gene expression in *Caenorhabditis elegans:* two unusual dumpy genes. Mol. Gen. Genet. **192:** 452–458.

HODGKIN, J., 1986 Sex determination in the nematode *Caenorhabditis elegans:* analysis of *tra-3* suppressors and characterization of *fem* genes. Genetics **114:** 14–52.

HODGKIN, J., 1987a A genetic analysis of the sex determining gene, *tra-1*, in the nematode *Caenorhabditis elegans*. Genes Dev. **1:** 731–745.

HODGKIN, J., 1987b Primary sex determination in the nematode *C. elegans*. Development **101** (Suppl.): 5–16.

HODGKIN, J., 1988 Sexual dimorphism and sex determination, pp. 491–584 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

HODGKIN, J., 1990 Sex determination compared in *Drosophila* and *Caenorhabditis*. Nature **344:** 721–728.

HODGKIN, J. A., and S. BRENNER, 1977 Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. Genetics **86:** 275–287.

HODGKIN, J., H. R. HORVITZ and S. BRENNER, 1979 Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

HODGKIN, J., M. EDGLEY, D. RIDDLE and D. G. ALBERTSON, 1988 Appendix 4: Genetics, pp. 491–584 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

HODGKIN, J., J. D. ZELLAN and D. G. ALBERTSON, 1994 Identification of a candidate primary sex determination locus, *fox-1*, on the *X* chromosome of *Caenorhabditis elegans*. Development **120:** 3681–3689.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

HSU, D. R., and B. J. MEYER, 1993 *X* chromosome dosage compensation and its relationship to sex determination in *C. elegans*. Sem. Dev. Biol. **4:** 93–106.

HSU, D. R., and B. J. MEYER, 1994 The *dpy-30* gene encodes an essential component of the *Caenorhabditis elegans* dosage compensation machinery. Genetics **137:** 999–1018.

KLEIN, R. D., and B. J. MEYER, 1993 Independent domains of the Sdc-3 protein control sex determination and dosage compensation in *C. elegans*. Cell **72:** 349–354.

KUWABARA, P. E., and J. KIMBLE, 1992 Molecular genetics of sex determination in *C. elegans*. Trends Genet. **89:** 151–156.

KUWABARA, P. E., P. G. OKKEMA and J. KIMBLE, 1992 *tra-2* encodes a membrane protein and may mediate cell communication in the *Caenorhabditis elegans* sex determination pathway. Mol. Biol. Cell. **3:** 461–473.

L'HERNAULT, S., D. C. SHAKES and S. WARD, 1988 Developmental genetics of chromosome I spermatogenesis defective mutants in the nematode *Caenorhabditis elegans*. Genetics **120:** 435–452.

MADL, J. E., and R. K. HERMAN, 1979 Polyploids and sex determination in *Caenorhabditis elegans*. Genetics **93:** 393–402.

MCCOUBREY, W. K., K. D. NORDSTROM and P. M. MENEELY, 1988 Microinjected DNA from the *X* chromosome affects sex determination in *Caenorhabditis elegans*. Science **242:** 1146–1151.

MENEELY, P. M., 1994 Sex determination in polyploids of *Caenohabditis elegans*. Genetics **137:** 467–481.

MENEELY, P. M., and W. B. WOOD, 1987 Genetic analysis of *X*-chromosome dosage compensation in *Caenorhabditis elegans*. Genetics **117:** 25–41.

MEYER, B. J., and L. P. CASSON, 1986 *Caenorhabditis elegans* compensates for the difference in *X* chromosome dosage between the sexes by regulating transcript levels. Cell **47:** 871–881.

MILLER, L. M., J. D. PLENEFISCH, L. P. CASSON and B. J. MEYER, 1988 *xol-1*: a gene that controls the male modes of both sex determination and *X* chromosome dosage compensation in *C. elegans*. Cell **55:** 167–183.

NELSON, G. A., K. K. LEW and S. WARD, 1978 Intersex, a temperature-sensitive mutant of the nematode *Caenorhabditis elegans*. Dev. Biol. **66:** 386–409.

NONET, M. L., and B. J. MEYER, 1991 Early aspects of *Caenorhabditis elegans* sex determination and dosage compensation are regulated by a zinc-finger protein. Nature **351:** 65–68.

NUSBAUM, C., and B. J. MEYER, 1989 The *Caenorhabditis elegans* gene *sdc-2* controls sex determination and dosage compensation in *XX* animals. Genetics **122:** 579–593.

PARKHURST, S. M., and P. M. MENEELY, 1994 Sex determination and

dosage compensation: lessons from flies and worms. Science **264:** 924–932.

PERRY, M. D., W. LI, C. TRENT, B. ROBERTSON, A. FIRE *et al.,* 1993 Molecular characterization of the *her-1* gene suggests a direct role in cell signalling during *Caenorhabditis elegans* sex determination. Genes Dev. **7:** 216–228.

PLENEFISCH, J. D., L. DELONG and B. J. MEYER, 1989 Genes that implement the hermaphrodite mode of dosage compensation in *Caenorhabditis elegans.* Genetics **121:** 57–76.

RHIND, N. R., L. M. MILLER, J. B. KOPCZYINSKI and B. J. MEYER, 1995 *xol-1* acts as an early switch in the *C. elegans* male/hermaphrodite decision. Cell **80:** 71–82.

SCHEDL, T., 1991 The role of cell-cell interactions in postembryonic development of the *Caenorhabditis elegans* germ line. Curr. Opin. Gen. Dev. **1:** 185–190.

SCHEDL, T., and J. KIMBLE, 1988 *fog-2,* a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans.* Genetics **119:** 43–61.

SCHEDL, T., P. L. GRAHAM, M. K. BARTON and J. KIMBLE, 1989 Analysis of the role of *tra-1* in germline sex determination in the nematode *Caenorhabditis elegans.* Genetics **123:** 755–769.

SULSTON, J. E., and J. HODGKIN 1988 Methods, pp. 587–606 in *The Nematode Caenorhabditis elegans,* edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

TRENT, C., W. B. WOOD and H. R. HORVITZ, 1988 A novel dominant transformer allele of the sex-determining gene *her-1* of *Caenorhabditis elegans.* Genetics **120:** 145–157.

TRENT, C., B. PURNELL, S. GAVINSKI, J. HAGEMAN, C. CHAMBLIN *et al.,* 1991 Sex-specific transcriptional regulation of the *C. elegans* sex-determining gene *her-1.* Mech. Dev. **34:** 43–56.

VILLENEUVE, A. M., and B. J. MEYER, 1987 *sdc-1:* a link between sex determination and dosage compensation in *C. elegans.* Cell **48:** 25–37.

VILLENEUVE, A. M., and B. J. MEYER, 1990 The regulatory hierarchy controlling sex determination and dosage compensation in *Caenorhabditis elegans.* Adv. Genet. **27:** 117–88.

ZARKOWER, D., and J. HODGKIN, 1992 Molecular analysis of the *C. elegans* sex-determining gene *tra-1:* a gene encoding two zinc finger proteins. Cell **70:** 237–249.

ZARKOWER, D., R. ARONOFF, M. DE BONO and J. HODGKIN, 1994 Regulatory rearrangements and *smg*-sensitive alleles of the *C. elegans* sex-determining gene *tra-1.* Dev. Genet. **15:** 240–250.

ZHAO, C., and S. EMMONS, 1995 A transcription factor controlling development of peripheral sense organs in *C. elegans.* Nature **373:** 74–78.

Communicating editor: R. K. HERMAN