AN AUTOSOMAL GENE THAT AFFECTS *X* CHROMOSOME EXPRESSION AND SEX DETERMINATION IN *CAENORHABDITIS ELEGANS*

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Manuscript received June 20, 1983
Revised copy accepted September 17, 1983

ABSTRACT

Recessive mutant alleles at the autosomal *dpy-21* locus of *C. elegans* cause a dumpy phenotype in *XX* animals but not in *XO* animals. This dumpy phenotype is characteristic of *X* chromosome aneuploids with higher than normal *X* to autosome ratios and is proposed to result from overexpression of *X*-linked genes. We have isolated a new *dpy-21* allele that also causes partial hermaphroditization of *XO* males, without causing the dumpy phenotype. All *dpy-21* alleles show hermaphroditization effects in *XO* males that carry a duplication of part of the *X* chromosome and also partially suppress a transformer (*tra-1*) mutation that converts *XX* animals into males. Experiments with a set of *X* chromosome duplications show that the defects of *dpy-21* mutants can result from interaction with several different regions of the *X* chromosome. We propose that *dpy-21* regulates X chromosome expression and may be involved in interpreting *X* chromosome dose for the developmental decisions of both sex determination and dosage compensation.

ANIMALS that have heteromorphic sex chromosomes pose a paradox, recognized long ago by MULLER (1950) but still poorly understood. If the two chromosomes differ in function, then either the homogametic sex should overproduce sex-linked gene products or the heterogametic sex should underproduce them. MULLER proposed that some dosage compensation mechanism makes the expression of most or all sex-linked genes equivalent. Dosage compensation has been demonstrated in many vertebrates, including species with heterogametic females and species with heterogametic males. It has also been demonstrated in Drosophila and is proposed to occur in all animals with sex chromosomes that are functionally distinct (OHNO 1967).

In Drosophila, the paradox is compounded by the observation that sex determination depends upon the ratio of *X* chromosomes to autosomal sets (*X:A* ratio), not upon the presence or absence of a *Y*. Somehow, therefore, the organism must be able to distinguish between *X:A* ratios of 0.5 (normal male) and 1.0 (normal female) and, yet, also equalize the expression of most sex-linked genes in *1X* and *2X* animals. In Drosophila, dosage compensation is accomplished by increasing rates of *X* chromosome transcription in *1X* animals (BELOTE and LUCCHESI 1980).

The free-living soil nematode *Caenorhabditis elegans* has two sexes: hermaphrodites and males. There is no *Y* chromosome, and as in Drosophila, sex is determined by the *X:A* ratio. Normal males have one *X* chromosome (*X:A* = 0.5) and hermaphrodites have two (*X:A* = 1.0). Ratios other than 0.5 and 1.0 have been studied in polyploids (MADL and HERMAN 1979). *X:A* ratios of 0.67 or less lead to male development. *X:A* ratios of 0.75 or more lead to hermaphrodite development. Ratios between 0.67 and 0.75, produced by adding *X* chromosome duplications to *2X:3A* triploid males, result in intersexes. As in Drosophila (DOBZHANSKY and SCHULTZ 1934), no single region on the *X* seems to be solely responsible for sex determination, which depends instead on the ratio of total *X* chromosome material, from any of at least several regions, to autosomal sets.

In both *C. elegans* and Drosophila (BAKER and RIDGE 1980), the *X:A* ratio determines sex through the action of only a few autosomal genes. Mutations in these genes result in a complete or partial sex reversal of one sex or the other. There are at least four genes involved in sex determination in *C. elegans*. Recessive mutations in three transformer genes (*tra-1 III*, *tra-2 II* and *tra-3 IV*) cause *2X* animals to become pseudomales but have no effect on *1X* animals. Recessive mutations in the fourth gene, *her-1 V*, cause *1X* animals to become hermaphrodites but have no apparent effect on *2X* animals. These four genes have been placed in a single pathway based on their epistatic interactions (HODGKIN and BRENNER 1977; HODGKIN 1980).

We assume that dosage compensation must occur in *C. elegans*, but this assumption so far is based only on genetic evidence that males hemizygous for an *X*-linked hypomorphic mutation are similar in phenotype to homozygous hermaphrodites (P. M. MENEELY unpublished results, see also argument in MENEELY and HERMAN 1979). Compelling molecular or biochemical evidence for dosage compensation is lacking. However, increases in *X* chromosome dose do have striking effects on both morphology and viability. Diploid animals with three *X* chromosomes have a dumpy (Dpy) morphology, and diploids with four *X* chromosomes are inviable (HODGKIN, HORVITZ and BRENNER 1979). Both the Dpy phenotype and the inviability can result from hyperploidy for any one of several different sets of *X*-linked genes (see RESULTS).

Dosage compensation of *C. elegans* could be accomplished by turning up *X* expression in *1X* animals or turning down *X* expression in *2X* animals. In either case, mutants with defects in dosage compensation that result in overexpression of *X* in *2X* animals should show phenotypes in diploids similar to those of *X* chromosome aneuploids. HODGKIN (1980, 1983b) has shown that mutations in the autosomal gene *dpy-21* have such an effect. Mutants with defects in the process by which *X:A* ratio is measured might be expected to affect both dosage compensation and sex determination. For example, mutants that overestimate the *X:A* ratio should have hermaphroditizing effects on *1X* animals as well as making *2X* animals Dpy or inviable. Furthermore, since sex determination does not depend on a single *X* chromosome region, the phenotypes of such mutants should not be affected by the dosage of only a single *X* chromosome locus, but rather by the total amount of *X* chromosome material from any of several

regions. In short, they should respond to *X* dose in a manner that is qualitatively normal (unlike the sex determination mutants) but quantitatively abnormal. In this paper we show that mutations in the *dpy-21* gene meet these criteria. We postulate that this gene is required for the correct measurement and interpretation of *X* chromosome dose.

MATERIALS AND METHODS

General procedures: Media and culture techniques for *C. elegans* var. Bristol (wild type designated N2) are described by BRENNER (1974). Incubations were at 20°, except as noted. Animals were routinely observed and handled with the aid of a Wild M5A dissecting stereomicroscope. More detailed morphological characterization was done with a Zeiss Universal microscope equipped with Nomarski optics. Nomenclature for genes and rearrangements conforms to accepted usage (HORVITZ *et al.* 1979).

Duplication strains: All of the *X* chromosome duplications used, with the possible exception of *ctDp1*, are stably attached to an autosome. The properties of the *X* duplications designated *mnDp* are described in detail by HERMAN, MADL and KARI (1979). The duplication *stDp2* was isolated by R. WATERSTON as carrying *unc-6*$^+$. It is apparently attached to linkage group II (LGII) (R. WATERSTON, personal communication). The duplication *ctDp1* was generated by irradiating N2 males at the L4 larval stage with about 6000 r of γ rays from a Cs^{137} source. These irradiated males were mated, as adults, to *unc-1(e94) X* hermaphrodites; the mated animals were transferred to fresh plates on each of the next 2 days. From these matings, Unc males were counted to estimate the number of outcross progeny and non-Unc males were picked as possibly carrying *unc-1*$^+$ duplications. From five different irradiations, about 4100 Unc males were counted and seven non-Unc males were found. Each non-Unc male was mated singly to *unc-1* hermaphrodites. Six of the males sired no non-Unc male progeny; the seventh male had non-Unc male and non-Unc hermaphrodite progeny. A non-Unc hermaphrodite of putative genotype *Dp(unc-1*$^+$*)/unc-1/unc-1* was picked to maintain the stock. Upon self-fertilization, this hermaphrodite segregated both non-Unc and Unc hermaphrodite progeny. Because Unc hermaphrodites were more than a quarter of the progeny, the duplication is inferred to be free, but this has not been proven cytologically, nor has the duplication been tested for linkage with any of the autosomes. The duplication was outcrossed three times by mating N2 males to non-Unc hermaphrodites. The resulting non-Unc male progeny were then mated to *unc-1* hermaphrodites, and from this cross, a single non-Unc hermaphrodite was picked to maintain the stock.

The extent of the duplication in this stock, designated *ctDp1*, was tested by mating duplication-bearing males to *dpy-3(e27) X*, *unc-2(e55) X* and *unc-20(e112) X* and looking for nonmutant male progeny. The results showed that *ctDp1* does not carry *dpy-3*$^+$, *unc-2*$^+$ or *unc-20*$^+$. No other *X*-linked markers between *unc-1* and *dpy-3* have been tested.

Duplications of other parts of the *X* chromosome were looked for by similar procedures with the following results: *dpy-23(e840) X*, one non-Dpy male among 932 cross-progeny; *unc-6(e78) X*, 38 non-Unc males among about 35,200 cross-progeny; *unc-18(e81) X*, two non-Unc males among about 5500 cross-progeny; *dpy-22(e652) X*, 16 non-Dpy males among 1301 cross-progeny; and *vab-3(e648) X*, seven non-Vab males among 1305 cross-progeny. None of these putative duplication-bearing males sired nonmutant progeny when backcrossed to mutant hermaphrodites.

Strains carrying *him-5 V*, *dpy-21 V* and an *X* duplication were constructed as follows. First, *Dp/+; him-5(e1467); unc* strains were constructed, in which *Dp* is the *X* duplication and *unc* is an *X*-linked marker balanced by the duplication. For *mnDp1*, *mnDp8*, *mnDp9*, *mnDp10*, *mnDp25* and *mnDp27*, the *X*-linked marker was *unc-3(e151)*. For *stDp2*, the *X*-linked marker was either *unc-6(e78)* or *unc-18(e81)*; since the *unc-6* strain was more fertile, it was used more often. For *mnDp33*, the *X*-linked marker was *unc-20*; for *ctDp1*, it was *unc-1*. Because of *him-5*, these strains segregate males. Strains homozygous for a duplication, even though fertile in some cases, were observed to segregate almost no males; the reason for this was not investigated. The non-Unc males from duplication heterozygotes were then mated to *him-5 dpy-21*; *unc* hermaphrodites. The non-Unc males from this cross, of genotype *Dp/+; him-5 dpy-21/him-5*; *unc/0*, were mated to *him-5 dpy-21*; *unc* hermaphrodites, and a Dpy non-Unc hermaphrodite was picked to maintain the stock.

Strains carrying two different duplications were constructed as in the following example of *mnDp8/+; mnDp33/+; him-5 dpy-21; unc-20 unc-3/0 (unc-20* and *unc-3* have distinct phenotypes; however, an *unc-20 unc-3* double mutant is nearly indistinguishable from *unc-3*). First, *him-5 dpy-21; unc-20 unc-3* was constructed. These hermaphrodites were then mated to *mnDp8/+; him-5 dpy-21; unc-3/0* males, and Dpy non-Unc hermaphrodite progeny were picked; these had the genotype *mnDp8/+; him-5 dpy-21; unc-20 unc-3/unc-3.* Upon self-fertilization, they segregated Dpy Unc-20 hermaphrodites of genotype *mnDp8/+; him-5 dpy-21; unc-20 unc-3.* This stock was maintained by picking Dpy Unc-20 hermaphrodites. A male carrying both *mnDp8* and *mnDp33* was constructed by mating *mnDp33/+; him-5 dpy-21; unc-20/0* males to *mnDp8/+; him-5 dpy-21; unc-20 unc-3* hermaphrodites. Outcross progeny that were non-Unc-20 and non-Unc-3 and identified as "males" under the dissecting microscope by their tail morphology and movement were scored as Dpy or non-Dpy with the dissecting microscope and as intersexual or normal males with the Nomarski microscope.

Other strains with two *X* duplications were constructed by a similar procedure, that is, by first constructing a strain that has a duplication balancing the more severe of the two *X*-linked Unc mutants. The more severe Unc mutants used were *unc-3, unc-6* and *unc-18*; the less severe mutants used were *unc-1,* unc-*7(e139)* and *unc-20.* The *unc-7* mutant was used in constructing a strain carrying both *stDp2* and *mnDp8.*

Isolation and outcrossing of dpy-21(ct16): A new allele of *dpy-21,* designated *ct16,* was isolated from a mutagenesis designed to look for *X*-dependent Dpy mutants. *him-5* hermaphrodites were mutagenized with 0.25 M ethyl methanesulfonate (BRENNER 1974), and worms from both parental and F_1 generations were placed individually on plates. From among the F_2 progeny, Dpy individuals were picked to separate plates and their male F_3 progeny scored. Most of the Dpy animals were either triplo-*X* (recognized by few male and many non-Dpy hermaphrodite progeny) or were not *X*-dependent mutants (recognized by Dpy male progeny). In three isolates, the Dpy hermaphrodites consistently segregated non-Dpy males. Two of these mutant isolates failed to complement each other, mapped to LGIV and failed to complement the *dpy-26(n199)* allele described by HODGKIN (1983b). The third, designated *ct16,* failed to complement *dpy-21(e428).* This complementation test was done by mating *dpy-21(e428)* males to *ct16 unc-76(e911)* hermaphrodites; the non-Unc hermaphrodite progeny were Dpy. Heterozygous *ct16/+* males were also crossed to *dpy-21(e428)unc-76* hermaphrodites, and about half of the non-Unc hermaphrodite progeny were Dpy. The *ct16/+* males were used because *ct16/ct16* males are sterile (see RESULTS).

To eliminate extraneous mutations, backcrosses were performed with strains carrying *unc-76(e911) V* and *unc-51(e369) V.* These genes are the closest known visible markers to *dpy-21, unc-76* being about 4 map units to the left and *unc-51* about 10 map units to the right (P. M. MENEELY and W. B. WOOD, unpublished results). First, *unc-76 dpy-21(ct16)* and *dpy-21(ct16) unc-51* double mutants were constructed. Each of these hermaphrodites was mated with N2 males, and the *unc-76 dpy-21(ct16)/+* or *dpy21(ct16) unc51/+* hermaphrodites were picked and allowed to self-fertilize. From the progeny of each, Dpy non-Unc (*ct16/ct16*) animals were picked. A second set of backcrosses was then performed, in which the *dpy-21(ct16)* mutations extracted from the *unc-76* and *unc-51* backgrounds were used to construct *dpy-21(ct16) unc-51* and *dpy-21(ct16) unc-76,* respectively. The resulting double mutants were again mated with N2 males, and *dpy-21(ct16)* was reextracted without regard to its phenotype in males. Both backcrossed alleles retained the phenotypic properties of the original isolate.

Testing dpy-21 *alleles for suppressibility by* sup-7: Mutations in *sup-7 X* suppress UAG nonsense (amber) alleles of many different genes (WATERSTON 1980; WILLS *et al.* 1983). To test the effect of *sup-7* on *dpy-21(e428), dpy-21(e459)* and *dpy-21(ct16),* heterozygotes of the form *unc-13(e450)/+; dpy-21/+; sup-7(st5)/+* were constructed. The suppressible *unc-13(e450) I* marker was included because its phenotype is indicative of the number of copies of *sup-7* present. From among the self-progeny of heterozygotes, sluggish Dpy hermaphrodites were picked; these are expected to have the genotype *unc-13; dpy-21; sup-7/+,* because *unc-13; sup-7/+* is sluggish. Because sluggish Dpy animals were found for *dpy-21(e428), dpy-21(e459)* and *dpy-21(ct16),* none of the three alleles is apparently suppressed by a single copy of *sup-7.* The sluggish Dpy hermaphrodites gave paralyzed Dpy, sluggish Dpy and non-Unc Dpy progeny in about a 1:2:1 ratio corresponding to zero, one and two copies of *sup-7,* respectively. There were no obvious differences in the Dpy phenotypes of the three classes, again suggesting that none of the three *dpy-21* alleles is suppressed by *sup-7.*

Double mutants carrying defects in dpy-21 *and transformer genes*: Double mutants carrying *tra-1(e1099) III* and *dpy-21* were made by first mating *tra-1(e1099)* pseudomales to *unc-32(e189); dpy-21* hermaphrodites and picking the non-Unc non-Dpy F_1 progeny. From these animals, the Dpy F_2 self-progeny were picked and scored for production of Dpy non-Unc, and Dpy Unc hermaphrodite progeny and Dpy non-Unc pseudomale progeny; those that gave all three classes were concluded to be *tra-1/unc-32; dpy-21*. The Dpy pseudomales were examined by Nomarski optics. Since *tra-1(e1076) III* and *tra-2(e1095) II* pseudomales are not fertile as homozygotes, double mutants carrying these alleles were made by mating heterozygous *tra/+* males to *unc-32; dpy-21* (for *tra-1*) or to *unc-4(e120); dpy-21* (for *tra-2*) hermaphrodites and then isolating *tra; dpy-21* pseudomales as before.

The *tra-3(e1107); dpy-21* pseudomales were made by mating *dpy-21(e428)* males to *unc-30(e191) tra-3* hermaphrodites and picking the non-Unc F_1 hermaphrodites. From self-fertilization of these animals Dpy F_2 hermaphrodites, then Unc Dpy F_3 hermaphrodites, were picked. The Unc Dpy F_3 hermaphrodites are of genotype *unc-30 tra-3; dpy-21* and will segregate only Unc Dpy pseudomales (unless *tra-3* has been lost by recombination). These Unc Dpy pseudomales were examined and compared to *unc-30 tra-3* pseudomales by Nomarski microscopy.

RESULTS

Characteristics of a new dpy-21 *allele*: Diploid animals homozygous for mutations in the autosomal gene *dpy-21 V* exhibit the short phenotype known as dumpy (Dpy), whenever two *X* chromosomes are present. When only one *X* chromosome is present, *dpy-21* homozygotes are non-Dpy. HODGKIN (1980) has shown, by constructing strains carrying mutations in *dpy-21* and a sex transformer gene (*tra-1* or *her-1*), that the Dpy phenotype is dependent on the number of *X* chromosomes present and independent of sexual phenotype.

Two alleles of *dpy-21*, *dpy-21(e428)* and *dpy-21(e459)* have been described previously (HODGKIN 1980). We have isolated a third allele, designated *dpy-21(ct16)*, with the same morphological phenotype dependent on *X* chromosome dose: *2X* animals are Dpy and 1X animals are non-Dpy. All three alleles are recessive, and none of the three is visibly suppressed by the amber suppressor *sup-7*. However, the *dpy-21(ct16)* allele differs from the other two in affecting the sexual phenotype of *XO* animals. Unlike *dpy-21(e428) XO* and *dpy-21(e459) XO* males, which are fertile (HODGKIN 1983a), all *dpy-21(ct16) XO* animals are sterile and show a partial hermaphroditization of the male tail (Figure 1), although sperm are produced and the gonad is male-like.

One possible explanation for this effect is that the *dpy-21(ct16)* strain carries two mutations, a *dpy-21* allele and a separate hermaphroditizing (*her*) mutation which causes the tail abnormalities. However, the *her* mutation would have to be closely linked to *dpy-21*, because the Dpy phene and the Her phene segregated together in three outcrosses, and two attempts to separate them by recombination using the spanning markers *unc-76* and *unc-51* failed to do so (see MATERIALS AND METHODS). These findings and the observations that follow that *dpy-21(e428)* under some conditions also affects *XO* sexual phenotype, support the view that the *dpy-21(ct16)* allele itself causes hermaphroditization of *XO* animals.

Effects of intermediate X *dosage on the Dpy phenotype*: To determine whether the dependence of *dpy-21* phenotype on *X* chromosome dosage reflects interaction of *dpy-21* with one specific *X*-linked site or with any of several such sites, we have varied the *X* dosage using genetically defined duplications of various

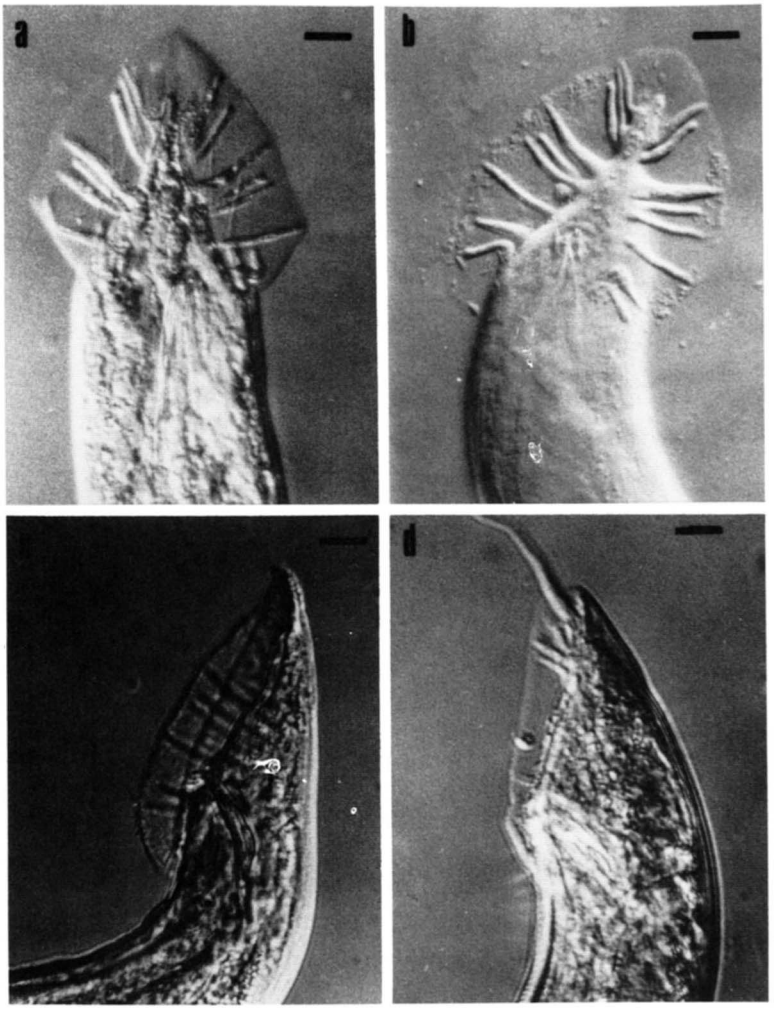

FIGURE 1.—Nomarski micrographs showing tail morphologies of wild-type and *dpy-21 XO* animals: a, N2; b and c, *dpy-21(e428)* top and side view, respectively; d, *dpy-21(ct16)*. The bar is 10 μm.

regions of the *X* chromosome. The experimental design, diagrammed in Figure 2, involved constructing strains homozygous for *dpy-21 V* and the nondisjunction mutant *him-5 V* and carrying three copies of a part of the *X*. Each strain is homozygous for a recessive *X*-linked *unc* marker [uncoordinated (Unc) phenotype] and has a third copy of the marked region carrying the unc^+ allele elsewhere in the genome. The genotypes of these strains are represented as *Dp*(unc^+)/+; *him-5 dpy-21/him-5 dpy-21 V*; *unc/unc X* (Figure 2). Phenotypically, they are Dpy non-Unc hermaphrodites: Dpy hermaphrodites because they have two *X* chromosomes and are homozygous for *dpy-21* and non-Unc because they carry the dominant unc^+ allele on the duplication. These animals will segregate

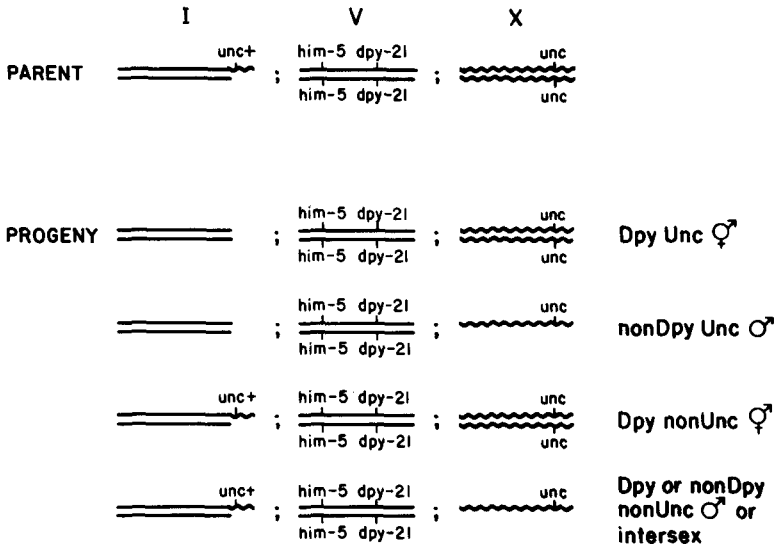

FIGURE 2.—Construction of *X* chromosome segmental aneuploids for analyzing effects of *X* dose in *dpy-21* mutants. The recessive *unc* on the *X* chromosome (wavy line) is balanced by the *X* duplication translocated to an autosome. The translocation is shown here as a wavy line on the end of LGI, although the actual autosome involved depends on the duplication. Positions shown for the duplication and for the markers *him-5*, *dpy-21* and *unc* on their respective linkage groups do not accurately reflect actual map locations. The duplication hermaphrodite segregates both haplo-*X* and diplo-*X* progeny due to the presence of the *him-5* mutation. About 25% of these progeny lack the duplication and are Unc; the remainder still carry the duplication and are non-Unc. The non-Unc haplo-*X* animals may be Dpy or non-Dpy, intersexual or male, depending on the duplication and the *dpy-21* allele.

both *1X* and *2X* progeny because of *him-5*, which causes *X* chromosome non-disjunction resulting in about 15% males (HODGKIN, HORVITZ and BRENNER 1979). In addition, some of the progeny will have the duplication and so will be non-Unc, whereas others will lack the duplication and will be Unc. Thus, there are four classes of progeny. Those lacking the duplication are Dpy Unc hermaphrodites and non-Dpy Unc males. Those carrying the duplication are Dpy non-Unc hermaphrodites and non-Unc males or intersexes. Males carrying the duplication have two copies of part of the *X* chromosome. If this region of the *X* interacts with *dpy-21*, the males (or intersexes) will be Dpy; if it does not interact with *dpy-21*, the males will be non-Dpy.

The duplications used are shown in Figure 3. Their sizes have been estimated from recombination distances. With the exception of *ctDp1*, which is apparently a free duplication (see MATERIALS AND METHODS), each of these duplications is stably translocated to an autosome (HERMAN, MADL and KARI 1979; R. H. WATERSTON, personal communication).

The results with single duplications are shown in Table 1. Most of the duplications show no effect on *dpy-21* phenotype. However, two of the largest duplications, *mnDp25* and *mnDp10*, interact with *dpy-21* to produce Dpy *XO* animals with variable phenotype, ranging from nearly as Dpy as *dpy-21 2X*

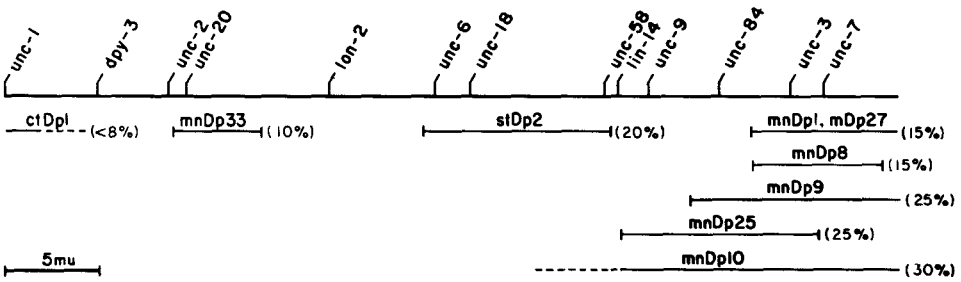

FIGURE 3.—A partial map of the *X* chromosome showing the genetic extents of several duplications. A dashed line indicates that the duplication has not been tested with all of the genes in that region, so that its extent is not accurately known. The number in parentheses to the right of each duplication is the approximate percent of the *X*-chromosome genetic map duplicated. The entire *X* map is about 50 map units long.

TABLE 1

Effect of X *duplications on morphological phenotypes of* dpy-21 XO *animals*

Duplication (% of *X* map)	*X:A* ratio	Morphology[a]
Single duplications		
ctDp1/+ (<8)	<0.54	Non-Dpy
mnDp33/+ (10)	0.55	Non-Dpy
mnDp8/+ (15)	0.58	Non-Dpy
mnDp1/+ (15)	0.58	Non-Dpy
mnDp27/+ (15)	0.58	Non-Dpy
stDp2/+ (20)	0.60	Non-Dpy
mnDp9/+ (25)	0.63	Non-Dpy or slightly Dpy
mnDp25/+ (25)	0.63	Variable; non-Dpy or Dpy
mnDp10/+ (30)	0.65	Variable; mostly Dpy
Double duplications		
ctDp1/+; *mnDp8*/+ (<23)	<0.62	Non-Dpy
mnDp33/+; *mnDp8*/+ (25)	0.63	Dpy
ctDp1/+; *stDp2*/+ (<28)	<0.64	Dpy
mnDp33/+; *stDp2*/+ (30)	0.65	Dpy
mnDp33/+; *mnDp9*/+ (35)	0.68	Dpy
mnDp8/+; *stDp2*/+ (35)	0.68	Dpy

[a] Phenotypes were determined using the dissecting microscope. All animals scored were superficially male; however, sexual phenotypes were not examined in detail.

hermaphrodites to nearly wild type. A third duplication, *mnDp9*, sometimes gives slightly Dpy *XO* animals. These results show that *X* duplications translocated to autosomes still interact with *dpy-21* as if *X* linked. The two *dpy-21* alleles tested, *dpy-21(e428)* and *dpy-21(ct16)*, apparently do not differ in their response to the duplications.

We considered two explanations for the ability of these three duplications to affect *dpy-21 XO* animals when other duplications do not. Because these are the largest duplications, the effect could be the result of cumulative *X* dose. Alternatively, because these duplications all have a substantial region of the *X*

chromosome in common, one or more loci that interact with *dpy-21* could be present on all three. To discriminate between these possibilities, we made strains with duplications from two different regions. These combinations, representing duplications covering about 60% of the *X* chromosome genetic map, are listed in Table 1. None of the duplications used in the combinations interacted singly with *dpy-21(e428)*, except for the weak and variable effect of *mnDp9*. However, duplications in all of the combinations tested were found to interact with *dpy-21*, if they represented together more than about 25% of the *X* chromosome. The effect of two duplications was apparently additive. These duplication results indicated that at least five different *X*-linked regions, near *unc-7*, *unc-9*, *unc-18*, *unc-20* and *unc-1*, can interact with *dpy-21* to produce Dpy *XO* animals.

Effects of dpy-21 *on lethality at high* X:A *ratios*: To test the effect of *dpy-21* on *3X* animals, we picked more than 100 Dpy hermaphrodites from a *him-5 dpy-21* stock to separate plates. Normally about 3% of the progeny of *him-5 (e1467)dpy-21*$^+$ are triplo-*X*, as indicated by a Dpy morphology and a low frequency of male self-progeny (HODGKIN, HORVITZ and BRENNER 1979). Since we could not use the Dpy phenotype to recognize triplo-*X* animals, we looked at the frequency of male self-progeny from these hermaphrodites. All of the Dpy animals we picked produced at least 15% males, indicating that none was triplo-*X*. We infer that *dpy-21* mutants are inviable when three *X* chromosomes are present. HODGKIN (1983b) has demonstrated the same effect.

We then tested the viability of *2X* animals carrying *X* duplications. None of the duplications is lethal in the heterozygous state in the presence of a *dpy-21* mutation, as shown by the construction of viable stocks for these experiments. Five duplications, *mnDp8*, *mnDp27*, *mnDp9*, *mnDp25* and *mnDp10*, are homozygous viable in *dpy-21*$^+$ strains, demonstrating that no part of these regions of the *X* is normally tetra-lethal (HERMAN, MADL and KARI 1979). However, *dpy-21* causes lethality in homozygotes of the larger duplications *mnDp10* and *mnDp25* and reduced viability in homozygotes of *mnDp8*, *mnDp9* and *mnDp27* based on the following results. A duplication homozygote is readily recognized by its lack of Unc self-progeny. We picked individual Dpy hermaphrodites from a *Dp/+; him-5 dpy-21/him-5 dpy-21; unc-3/unc-3* strain and looked for animals that gave no Dpy Unc self-progeny. These individuals should have four copies of a part of the *X* and be homozygous for *dpy-21*. Such strains were found only for the three duplications *mnDp8*, *mnDp27* and *mnDp9* (Table 2). None of these strains is very fertile, and only an *mnDp8* homozygote could be maintained. The two *dpy-21* alleles tested, *dpy-21(e428)* and *dpy-21(ct16)*, do not differ in their effect on *3X* viability (Table 2).

Although inviability in these homozygotes is correlated with the size of the duplication, we cannot rule out the possibility that a single site on the *X* near *unc-9* is responsible for the inviability with *mnDp25* and *mnDp10*, and that a single site in the region of *unc-3* and *unc-7* results in a reduction of fertility in *mnDp8*, *mnDp27* and *mnDp9*. Both of these hypothetical sites would have to exert their effect on *dpy-21* only when present in four copies.

All three dpy-21 *alleles affect sexual phenotype at intermediate* X *doses*: As de-

TABLE 2

Effects of homozygous X *duplications on viability and fertility of* dpy-21 XX *animals*

Duplication homozygote (% of *X* map in duplication)	*X:A* ratio	Viability, fertility
mnDp8/mnDp8 (15)	1.15	Viable, low fertility
mnDp27/mnDp27 (15)	1.15	Viable, sterile
mnDp9/mnDp9 (25)	1.25	Viable, sterile
mnDp25/mnDp25 (25)	1.25	Inviable
mnDp10/mnDp10 (30)	1.30	Inviable

scribed before, the *dpy-21(ct16)* allele causes partial hermaphoditization of *XO* animals, whereas the *dpy-21(e428)* and *dpy-21(e459)* alleles do not. To test whether *dpy-21(e428)* might also exert a hermaphroditizing effect too weak to observe in *XO* animals using the dissecting microscope, we examined the sexual phenotypes of *dpy-21(e428) XO* animals carrying *X* duplications at higher magnification using Nomarski optics. As shown in Table 3, no duplication that represents less than about 20% of an *X* chromosome has an effect on sexual phenotype in *dpy-21(e428)* males; in fact, several of these males proved fertile in stock constructions. However, the three largest duplications, *mnDp9*, *mnDp25* and *mnDp10*, which have no effect on wild-type males, cause partial and variable hermaphroditization of the *dpy-21(e428)* male tail. Moreover, among these animals, the Dpy and intersexual phenotypes appear to be correlated. For example, some *mnDp25/+; e428/e428 XO* animals are Dpy and some are not. The non-Dpy animals are fertile, phenotypically normal males. The Dpy animals, recognized as males in the dissecting microscope by their movement and tail morphology, are decidedly intersexual with regard to four sexually dimorphic characteristics: the presence or absence of a pseudovulva, the shape of the somatic gonad, the germ cells and the tail. All four of these characteristics are variable in the Dpy *XO* animals. The shape of the somatic gonad is correlated with appearance of the vulva; a pseudovulva is seen in all individuals with an hermaphrodite somatic gonad but in no individuals with a male gonad. This observation is not surprising, because presence of the anchor cell of the hermaphrodite somatic gonad is both necessary and sufficient to induce formation of a vulva-like structure (KIMBLE 1981). However, the other characteristics apparently vary independently; we commonly observed animals with fairly normal male tails and male somatic gonads containing oocytes. We do not know the full range of intersexuality that is possible, since all individuals studied were first identified as "males" in the dissecting microscope.

The *mnDp9* and *mnDp10* duplications in *dpy-21(e428) XO* animals gave results much like those obtained with *mnDp25*. The effects of *mnDp9* were more subtle than those of *mnDp25*, the most common intersexual characteristic being a partial hermaphroditization of the tail; as with *mnDp25*, only the Dpy *XO* animals had intersexual tails. Among the Dpy *XO* animals produced by *mnDp10*, the most common phenotype was characterized by an intersexual tail, a male-like somatic gonad with no vulva and presence of both sperm and oocytes.

TABLE 3

Effects of X *duplications on morphological and sexual phenotype of* dpy-21(e428) XO *animals*

Duplication (% of *X* map)	*X:A* ratio	Morphology, sex[a]
ctDp1/+ (<8)	<0.54	Non-Dpy, male
mnDp33/+ (10)	0.55	Non-Dpy, male
mnDp27/+ (15)	0.58	Non-Dpy, male
stDp2/+ (20)	0.60	Non-Dpy, male
mnDp9/+ (25)	0.63	97% non-Dpy, male; 3% slightly Dpy, intersex
mnDp25/+ (25)	0.63	85% non-Dpy, male; 15% Dpy, intersex
mnDp10/+ (30)	0.65	Dpy, intersex

[a] Animals picked as males using the dissecting microscope were examined at higher magnification using Nomarski optics to determine sexual phenotypes.

Similar results were obtained with *mnDp10* in strains carrying the *dpy-21(e459)* allele.

The fraction of intersexual progeny in these strains varies and tends to decrease as the strain is maintained in culture. We also noted that the Dpy intersexes are less viable than their non-Dpy male siblings and are more fragile in handling and transfers.

To determine whether smaller duplications in combination could produce these effects, we analyzed *dpy-21(e428)* animals carrying both *mnDp33* and *mnDp8*. These two duplications together represent about 25% of an *X* chromosome, which is the threshold found for the Dpy phenotype. The effects on sexual phenotype were similar to those described for *mnDp10*: the degree of hermaphroditization was partial and variable and was correlated with the Dpy phenotype. Similar results were observed among *e428 XO* animals carrying both of the duplications *mnDp8* and *stDp2*. Therefore, duplications of several different regions on the *X* can interact with *dpy-21(e428)* to affect sexual differentiation of *XO* animals. We conclude that the hermaphroditization phenotype of *dpy-21(ct16)* is not allele specific or strain specific. Rather, it is a common property of the *dpy-21* alleles but can be seen with *dpy-21(e428)* and *dpy-21(e459)* only at intermediate *X* doses.

The effects of duplications on *dpy-21(ct16) XO* animals, which already have hermaphroditized tails, were similar to those found with *dpy-21(e428)*. Only *mnDp25* and *mnDp10* resulted in additional hermaphroditization, and their effects on *dpy-21(ct16)* resembled their effects on *dpy-21(e428)*: a few *XO* animals had hermaphrodite gonads and a vulva and made oocytes regardless of the morphology of the somatic gonad.

Suppression of sex transformer phenotypes by dpy-21 *mutations*: The *tra* genes are involved in control of sex determination in response to the *X:A* ratio. The Tra mutant phenotype is transformation of *2X* animals into pseudomales; depending on the allele, the phenotype can range from nearly normal fertile males to weakly masculinized hermaphrodites (HODGKIN and BRENNER 1977; HODGKIN 1980).

The effects of *dpy-21* mutations on sex determination suggested that this gene might interact with the *tra* genes. To test this possibility, we examined the sexual phenotypes of double mutants carrying defects in *dpy-21* and either *tra-1*, *tra-2* or *tra-3*. Two *dpy-21* alleles (*e428* and *ct16*) and four *tra* mutants (a weak and a strong allele of *tra-1*, one allele of *tra-2* and one allele of *tra-3*) were tested.

Both *dpy-21* alleles noticeably suppress the weak *tra-1* allele *e1076*. The *tra-1(e1076); dpy-21*$^+$ *XX* animal is an incomplete male that has a male gonad, makes sperm and sometimes oocytes and has a variably male-like tail (HODGKIN and BRENNER 1977); we observed that eight of 30 animals made oocytes. By comparison, a *tra-1(e1076); dpy-21* animal is much more hermaphroditic. It has a male somatic gonad, but routinely makes both sperm and oocytes (30 of 30 animals), apparently following the hermaphrodite pattern of germ cell differentiation. The tail is more hermaphroditic than that of *tra-1(e1076)*. The effect of *dpy-21* on the strong *tra-1* allele *e1099* is much less pronounced. The *tra-1(e1099); dpy-21*$^+$ *XX* animal is often a fertile male of normal appearance. The *tra-1(e1099); dpy-21 XX* animal has a slightly more hermaphroditic tail, and the gonad is frequently intersexual, containing sperm but no oocytes. HODGKIN (1983b) has described the same effect and has noted that the phenotype of *tra-1* (*e1076*) triplo-*X* animals resembles *tra-1* (*e1076*); *dpy-21*.

Neither *dpy-21* allele suppresses *tra-2*, and *dpy-21(e428)* does not affect *tra-3*. (The effect of *dpy-21(ct16)* on *tra-3* was not tested.) The *tra-2; dpy-21* and *tra-3; dpy-21* animals appear indistinguishable from the respective single *tra* mutants by the criteria we have used. We have not examined other sexually dimorphic structures.

The effect of the X *duplications on* 2X *and* 3X dpy-21$^+$ *animals.* Diploid *dpy-21*$^+$ animals carrying three *X* chromosomes are Dpy, and diploid *dpy-21*$^+$ animals with four *X* chromosomes are inviable (HODGKIN, HORVITZ and BRENNER 1979). To complete our characterization of the *X* duplications, we carried out experiments to determine whether their effects on *dpy-21*$^+$ animals are similar to their effects on *dpy-21* animals in mimicking sex chromosome aneuploidy. The effects of *X* duplications in *2X* and *3X* hermaphrodites are shown in Table 4. None of the duplication heterozygotes is appreciably Dpy in *2X* animals. However, *mnDp9*, *mnDp25* and *mnDp10* duplication homozygotes are somewhat Dpy.

The duplications were assayed for lethal effects on *3X* animals by examining the progeny of *Dp/+; him-5/him-5; unc/unc X* hermaphrodites. About 3% of these progeny should be Dpy triplo-*X* animals, assuming that *him-5* is not affected by the duplications. Of these *3X* progeny, some should lack the duplication and be Dpy Unc. Such animals were found for all duplication stocks, in about the right frequency. If the duplication is not lethal, the progeny should also include Dpy non-Uncs. Only some of the duplication strains yielded such animals, which have four copies of part of the *X*. However, strains carrying the largest two duplications, *mnDp10* and *mnDp25*, did not yield Dpy non-Unc progeny, indicating that these duplications are lethal when present with three *X* chromosomes in a diploid. These are the same two duplications shown to affect the Dpy phenotype of *dpy-21* strongly in *XO* animals.

TABLE 4

Effects of X *duplications on morphology and viability of* dpy21$^+$ 2X *and* 3X *animals*

Duplication(s) (% of X map)	*2X* animals[a]		*3X* animals[b]	
	X:A ratio	Morphology	*X:A* ratio	Viability
ctDp1/+ (<8)	<1.04	Non-Dpy	<1.54	Viable
mnDp33/+ (10)	1.05	Non-Dpy	1.55	Viable
mnDp8/+ (15)	1.08	Non-Dpy	1.58	Viable
mnDp1/+ (15)	1.08	Non-Dpy	1.58	Viable
mnDp27/+ (15)	1.08	Non-Dpy	1.58	Viable
stDp2/+ (20)	1.10	Non-Dpy	1.60	Viable
mnDp9/+ (25)	1.13	Non-Dpy	1.63	Viable
mnDp25/+ (25)	1.13	Non-Dpy	1.63	Viable
mnDp10/+ (30)	1.15	Non-Dpy	1.65	Viable
mnDp8/mnDp8 (15)	1.15	Non-Dpy	1.65	Viable
mnDp27/mnDp27 (15)	1.15	Non-Dpy	1.65	Viable
mnDp9/mnDp9 (25)	1.25	Slightly Dpy	1.75	Viable
mnDp25/mnDp25 (25)	1.25	Slightly Dpy	1.75	Inviable
mnDp10/mnDp10 (30)	1.30	Slightly Dpy	1.80	Inviable

[a] All *2X* animals are viable.
[b] All viable *3X* animals are Dpy.

DISCUSSION

Our principal results can be summarized as follows. The phenotypes of animals mutant in the autosomal gene *dpy-21* depend on the number of *X* chromosomes: *1X* animals are non-Dpy, *2X* animals are Dpy and *3X* animals are inviable (HODGKIN 1980; 1983b). Using *X* chromosome duplications, we have shown that several regions on the *X* interact with *dpy-21* to produce the dose-dependent response. In fact, all regions tested (approximately 60% of the *X* chromosome genetic map) interact with *dpy-21* when there is at least an additional quarter of an *X* present. Both the Dpy and lethal phenotypes of *dpy-21* can be produced by the same duplications, suggesting that these phenotypes have a common physiological basis.

Mutations in *dpy-21* have a consistent hermaphroditizing effect on sexual development. This was demonstrated in three ways. First, the new allele *dpy-21(ct16)* causes variable hermaphroditization of the tail in *XO* males. Second, homozygotes for *dpy-21(e428)* with one *X* chromosome and an *X* duplication are intersexual when the duplication represents about a quarter of an *X* chromosome. The mutant sexual phenotype, like the Dpy phenotype, does not depend on which region of the *X* is diploid. Third, *dpy-21* alleles partially suppress *tra-1* mutations, which make *XX* animals resemble males. However, *dpy-21* apparently does not affect either *tra-2* or *tra-3*. These results indicate a role for *dpy-21* in sex determination and also suggest that the gene is active in both *1X* and *2X* animals.

Our interpretation of these results depends upon observations with *X* chromosome aneuploids in *dpy-21*$^+$ strains: *1X* diploids are males, *2X* diploids are hermaphrodites, *3X* diploids are Dpy hermaphrodites and *4X* diploids are inviable (HODGKIN, HORVITZ and BRENNER 1979). The Dpy and inviable phe-

notypes are not due simply to the increased number of *X* chromosomes but rather to the altered *X:A* ratio, because in tetraploid stocks *3X* and *4X* animals are non-Dpy hermaphrodites (MADL and HERMAN 1979). Moreover, as we have shown here, the Dpy and inviable diploid phenotypes seem to be due to an excess of *X* chromosome material regardless of origin, rather than to extra copies of one specific *X* chromosome site. A reasonable assumption is that increasing dosage of *X*-linked genes leads to increased expression of these genes, which in turn produces first a Dpy and then an inviable phenotype.

Three recessive mutant alleles of *dpy-21* cause these same phenotypes to appear at lower *X:A* ratios. This result suggests that the *dpy-21*$^+$ gene functions as a regulator of *X* chromosome expression, and that this gene is likely to be involved in a mechanism for dosage compensation. We do not know the null phenotype of *dpy-21*. If, as seems likely, the three alleles so far isolated have reduced functions, then the function of *dpy-21*$^+$ is probably repression of the *X* chromosome, either directly or indirectly, for example by negative regulation of an activator of *X* expression.

Mutations in *dpy-21* appear to affect sex determination in a similar manner, lowering the *X:A* ratio at which hermaphrodite development is observed. In *dpy-21*$^+$ animals, when the *X:A* ratio is increased in small increments by addition of *X* duplications to *XO* animals, intersexual phenotypes begin to be seen at ratios of about 0.7 (MADL and HERMAN 1979). In *dpy-21(e428)* animals, intersexes are seen at a lower *X:A* ratio of about 0.65. At this ratio both Dpy and sexual phenotypes are variable and correlated: non-Dpy animals are normal males, whereas their Dpy siblings are intersexual. However, in a *dpy-21(ct16)* background the *XO* animals, with an *X:A* ratio of 0.5, are intersexual and non-Dpy, indicating that the two mutant phenotypes of *dpy-21* are not necessarily correlated.

HODGKIN (1980) has presented evidence that of four genes controlling sexual differentiation, *tra-1* and *her-1* are directly influenced by the *X:A* ratio, whereas *tra-2* and *tra-3* are not. Consistent with this evidence and the results presented here, *dpy-21* mutants suppress *tra-1* alleles but do not appear to interact with *tra-2* or *tra-3*. Moreover, C. TRENT (personal communication) has found that *dpy-21* mutations also suppress a dominant transformer allele of *her-1*.

In summary, *dpy-21* mutants behave as if *X* chromosome expression is abnormally high, and complete male sexual development is either prevented or is abnormally sensitive to additional *X* chromosome material in *XO* animals. The hermaphroditizing effects of two of the three *dpy-21* alleles, *e428* and *e459*, might be explained as a secondary consequence of *X* overexpression. However, the finding that *dpy-21(ct16) XO* animals are hermaphroditized but non-Dpy suggests that this allele directly affects sex determination without causing *X* overexpression. Therefore, we propose that the *dpy-21* gene may have two functions that are differently affected by the different *dpy-21* alleles: (1) to repress general *X* chromosome expression, perhaps as part of the mechanism of dosage compensation, and (2) to activate the pathway of male development, perhaps through interaction with the *her-1* gene. When the *X:A* ratio is about 1.0 or higher, function (1) is normally active, and function (2) is

inactive, leading to dosage-compensated hermaphrodite development. When the *X:A* ratio is below about 0.7, function (1) is normally less active or inactive, and function (2) is active, leading to dosage-compensated male development. In terms of such a model, the *dpy-21(ct16)* gene product would be defective such that function (2) is partially inactive even at *X:A* = 0.5. In *dpy-21(e428)* animals the gene product could be differently defective, or variably underproduced, such that, at *X:A* ratios between 0.6 and 0.7, some animals are defective for both functions and, therefore, are Dpy intersexes, whereas other animals are defective for neither and, therefore, are non-Dpy males.

C. elegans appears to be like Drosophila (LUCCHESI 1977) in that *X* to autosome translocations still are regulated as if *X*-linked, suggesting a gene by gene or region by region mechanism of compensation specific to genes of the *X* chromosome. No autosomal gene with the properties of *dpy-21* has been reported in Drosophila, although the sex-linked gene *Sxl* affects both dosage compensation and sex determination (LUCCHESI and SKRIPSKY 1981; SKRIPSKY and LUCCHESI 1982; CLINE 1983). Dosage compensation in Drosophila apparently involves turning up the single *X* chromosome in males to the *2X* level of expression (BELOTE and LUCCHESI 1980).

The mode of dosage compensation in *C. elegans* should be testable by assays at the molecular level using cloned *X* chromosome sequences to measure transcription rates of various *X*-linked loci in wild-type and mutant animals of both sexes. Further evidence on the role of *dpy-21* should be obtainable from such experiments as well as from further analysis of *dpy-21* interaction with other genes that have mutant phenotypes dependent on *X* chromosome dose, such as *dpy-22*, *dpy-23* and *dpy-26* (P. M. MENEELY and W. B. WOOD, unpublished results; HODGKIN 1983b).

This research was supported by a grant to W. B. W. from the National Institutes of Health (HD-11762) and by postdoctoral fellowships to P. M. from the American Cancer Society (PF-1840) and the National Institutes of Health (GM-07775). Some of the strains used were provided by the *Caenorhabditis* Genetics Center, which is supported by contract N01-AG-9-2113 between the National Institutes of Health and the Curators of the University of Missouri. We are grateful to J. HODGKIN, R. HORVITZ and his collaborators and R. WATERSTON for communication of unpublished results, to L. GOLDSTEIN, R. HORVITZ, K. KEMPHUES and S. STROME for suggestions on the manuscript and to DOUGLAS PATRON for technical assistance.

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