The *dpy-30* Gene Encodes an Essential Component of the *Caenorhabditis elegans* Dosage Compensation Machinery

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ABSTRACT

The need to regulate *X* chromosome expression in *Caenorhabditis elegans* arises as a consequence of the primary sex-determining signal, the *X*/A ratio (the ratio of *X* chromosomes to sets of autosomes), which directs 1*X*/2A animals to develop as males and 2*X*/2A animals to develop as hermaphrodites. *C. elegans* possesses a dosage compensation mechanism that equalizes *X* chromosome expression between the two sexes despite their disparity in *X* chromosome dosage. Previous genetic analysis led to the identification of four autosomal genes, *dpy-21*, *dpy-26*, *dpy-27* and *dpy-28*, whose products are essential in *XX* animals for proper dosage compensation, but not for sex determination. We report the identification and characterization of *dpy-30*, an essential component of the dosage compensation machinery. Putative null mutations in *dpy-30* disrupt dosage compensation and cause a severe maternal-effect, *XX*-specific lethality. Rare survivors of the *dpy-30* lethality are dumpy and express their *X*-linked genes at higher than wild-type levels. These *dpy-30* mutant phenotypes superficially resemble those caused by mutations in *dpy-26*, *dpy-27* and *dpy-28*; however, detailed phenotypic analysis reveals important differences that distinguish *dpy-30* from these genes. In contrast to the *XX*-specific lethality caused by mutations in the other *dpy* genes, the *XX*-specific lethality caused by *dpy-30* mutations is completely penetrant and temperature sensitive. In addition, unlike the other genes, *dpy-30* is required for the normal development of *X*O animals. Although *dpy-30* mutations do not significantly affect the viability of *X*O animals, they do cause them to be developmentally delayed and to possess numerous morphological and behavioral abnormalities. Finally, *dpy-30* mutations can dramatically influence the choice of sexual fate in animals with an ambiguous sexual identity, despite having no apparent effect on the sexual phenotype of otherwise wild-type animals. Paradoxically, depending on the genetic background, *dpy-30* mutations cause either masculinization or feminization, thus revealing the complex regulatory relationship between the sex determination and dosage compensation processes. The novel phenotypes caused by *dpy-30* mutations suggest that in addition to acting in the dosage compensation process, *dpy-30* may play a more general role in the development of both *XX* and *X*O animals.

DOSAGE compensation is an essential regulatory process found in organisms whose primary sex-determining mechanisms cause males and females to differ in their relative dose of sex chromosomes (BULL 1983). By equalizing sex-linked gene expression, dosage compensation mechanisms prevent the sex-specific lethality that arises as a consequence of a twofold difference in sex chromosome dosage (CLINE 1993; DELONG *et al.* 1987; DONAHUE *et al.* 1987; MENEELY and WOOD 1987; MEYER and CASSON 1986; MULLER 1950). Studies of dosage compensation mechanisms in mammals, Drosophila and *Caenorhabditis elegans* have revealed that the strategies used to achieve dosage compensation are diverse, differing not only in the mechanisms by which they modulate sex-linked gene expression, but also in their relationship to the sex determination process, and in the nature of the regulatory hierarchies that control them (BAKER and BELOTE 1983; BORSANI and BALLABIO 1993; CLINE 1993; GRANT and CHAPMAN 1988; GUBBAY *et al.* 1990; HSU and MEYER 1993; KOOPMAN *et al.* 1991; SINCLAIR *et al.* 1990). An important aspect of these studies has been the identification of essential dosage compensation genes and the elucidation of their roles in normal development. The genetic analysis has provided, and will continue to provide, the background necessary for the molecular characterization of the mechanisms underlying this global regulatory process and will help clarify the similarities and differences among the diverse dosage compensation strategies.

In *C. elegans*, the genetic analysis of sex determination and dosage compensation has identified many components essential for the wild-type functioning of these processes. In recent years, it has been possible to assemble these components into a genetic regulatory hierarchy that details how both sex determination and dosage compensation are triggered in response to the primary sex determining signal, the *X*/A ratio (Figure 1) (VILLENEUVE and MEYER 1990a). Like Drosophila, both processes share common early regulatory steps, although these processes are ultimately implemented by

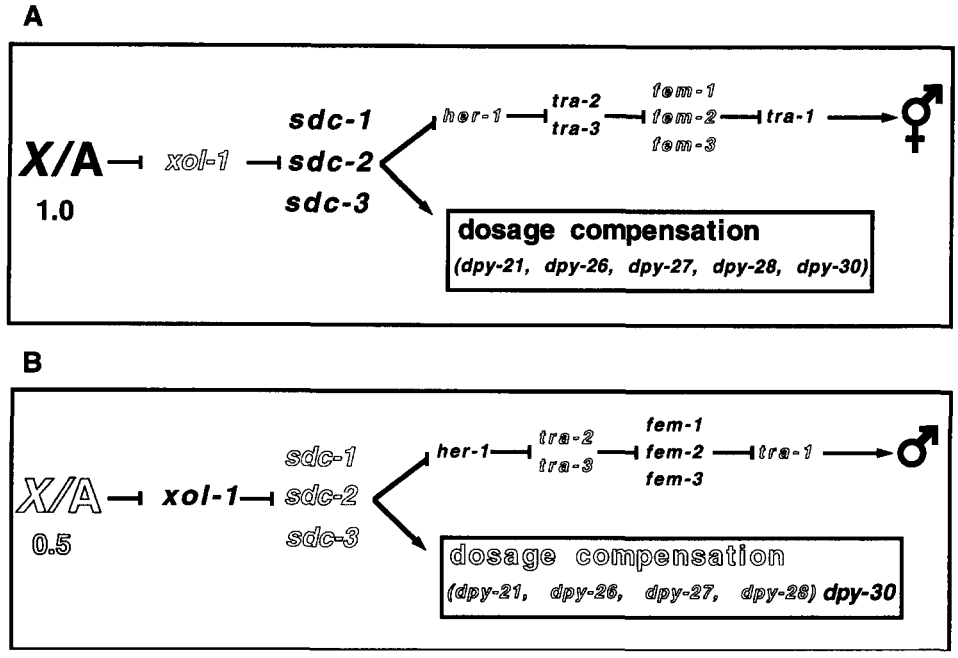

FIGURE 1.—The regulatory hierarchy controlling somatic sex determination and dosage compensation in *C. elegans*. This diagram shows the activity states of genes that act in a regulatory cascade to control both processes in hermaphrodites (*A*) and in males (*B*). These genes can be classified into three different groups; genes that control both sex determination and dosage compensation (*xol* and *sdc*), genes that implement dosage compensation (*dpy*), and genes that control sex determination (*her, tra, fem*). Solid lettering represents a high activity state, and outlined letters represent a low activity state. A bar indicates a negative regulatory interaction, whereas an arrow indicates a positive regulatory interaction. In *XO* males, *xol-1* negatively regulates the *sdc* genes, thus inactivating the hermaphrodite-specific dosage compensation *dpy* genes and activating the male-specific *her-1* gene. *her-1* inhibits *tra-2* and *tra-3*, allowing the *fem* genes to inhibit *tra-1* and promote male development. In *XX* animals, *xol-1* activity is low allowing the *sdc* genes to promote hermaphrodite development by negatively regulating *her-1* and by activating (either directly or indirectly) the hermaphrodite-specific dosage compensation *dpy* genes. The *dpy* genes either act alone or in conjunction with the *sdc* genes to promote the hermaphrodite mode of dosage compensation [reviewed in HODGKIN (1990), KUWABARA and KIMBLE (1992) and VILLENEUVE and MEYER (1990a)].

more specialized genes that control either sex determination or dosage compensation (BAKER and BELOTE 1983; VILLENEUVE and MEYER 1990a). Unlike Drosophila, the relationship between these two processes is complex: not only are they coordinately controlled, but a feedback mechanism operates such that a disruption in dosage compensation affects sexual fate (DELONG *et al.* 1993). At present, four genes are known to act in the coordinate control of these two regulatory processes. In *XO* animals, this coordinate control requires the activity of a single gene, *xol-1* (*XO lethal*) that acts as an essential early genetic switch to specify the male modes of both sex determination and dosage compensation (MILLER *et al.* 1988), (N. R. RHIND and B. J. MEYER, unpublished results). Mutations in *xol-1* cause *XO*-specific lethality, reduced *X*-linked transcript levels and feminization, all due to the failure to implement the *XO* modes of both processes. In contrast, the choice of the hermaphrodite fate and the hermaphrodite mode of dosage compensation requires the activities of three *sdc* (*s*ex determination and *d*osage *c*ompensation) genes whose activities are negatively regulated by *xol-1*. Mutations in *sdc-1*, *sdc-2* or *sdc-3* shift both sex determination and dosage compensation processes to their male modes of expression, resulting in masculinization, elevated *X*-linked transcript levels, and in the cases of *sdc-2* and *sdc-3*, *XX*-specific lethality (DELONG *et al.* 1993; NUSBAUM and MEYER 1989; VILLENEUVE and MEYER 1987).

The *C. elegans* dosage compensation process is implemented by at least four genes, *dpy-21*, *dpy-26*, *dpy-27* and *dpy-28* that are required for proper *X* chromosome expression in *XX* animals and are dispensable in *XO* animals (HODGKIN 1983; PLENEFISCH *et al.* 1989). Mutations in *dpy-26*, *dpy-27* and *dpy-28* cause a highly penetrant, maternal-effect lethality in *XX* animals, presumably due to a lethal disruption in the dosage compensation process. Rare survivors of the lethality have a dumpy (Dpy) morphology and exhibit elevated *X*-linked transcript levels relative to wild-type *XX* animals. Mutations in *dpy-21* also cause elevated transcript levels in *XX* animals, but they do not result in significant lethality, suggesting that the dosage compensation disruption caused by loss of *dpy-21* activity is less severe than that caused by other *dpy* mutations. In contrast to the *sdc* genes, the dosage compensation *dpy* genes do not have a direct role in the control of sex determination, and they have been proposed to act after the point at which the sex determination and dosage compensa-

tion pathways diverge (MILLER *et al.* 1988; PLENEFISCH *et al.* 1989). The combined analysis of the dosage compensation *dpy* genes and the upstream *xol* and *sdc* genes suggests that dosage compensation is achieved by a mechanism that reduces expression of each *X* chromosome in *XX* animals by half, allowing these animals to have a level of *X*-linked gene expression equivalent to that found in *XO* animals (HODGKIN 1983; MEYER and CASSON 1986; PLENEFISCH *et al.* 1989).

In this report, we describe the identification and characterization of a new, essential component of the dosage compensation machinery, the *dpy-30* gene. In addition to demonstrating *dpy-30*'s role in dosage compensation and its similarity to the other maternally provided dosage compensation genes *dpy-26*, *dpy-27* and *dpy-28*, we demonstrate that mutations in *dpy-30* affect male development and cause unexpected phenotypes in *XX* animals. The combined phenotypes in *XX* and *XO* animals suggest that *dpy-30*, unlike the other dosage compensation genes, may have a more general role in *C. elegans* development. The dosage compensation process may share components important to other processes required in both sexes.

MATERIAL AND METHODS

The standard methods described by BRENNER (1974) were used for culturing *C. elegans* strains. All experiments were performed at 20° unless otherwise noted. The strain referred to as wild type is the *C. elegans* variety Bristol, strain N2. In experiments where both maternal and zygotic genotypes are important, the abbreviations m and z are used to indicate the maternal and zygotic genotypes, respectively; the superscripts $^{+}$ or $^{-}$ refer to the presence or absence of a wild-type copy of the gene. For example, $m^{+}z^{-}$ refers to the homozygous mutant progeny of heterozygous mothers. The genetic nomenclature used in this paper conforms to that established in HORVITZ *et al.* (1979). The following strains and chromosomal aberrations were used in this study:

*Linkage group (LG) I: spe-9(hc52*ts*).*
LG II: y2; rol-6(e187).
*LG III: dpy-27(y57, y49); sup-5(e1464*ts*); unc-32(e189); tra-1(e1835e1816e1575).*
*LG V: dpy-11(e224); her-1(hv1y101*sd*, y101*sd*); unc-42(e270); daf-11(m47*ts*); sma-1(e30); dpy-30(y130, y228*am*); myo-3(st378); sqt-3(sc63*ts*); rol-4(sc8); him-5(e1490); sdc-3(y113, y129); unc-76(e911); dpy-21(e428); unc(n754*dm*); let(m435).*
*LG X: lon-2(e678); flu-2(e1003); xol-1(y9); dpy-6(e14); sdc-2(y202*ts*); y63; lin-15(n765*ts*).*
Chromosomal aberrations: ctDf1 V; yDf12 V; nT1(IV;V).

Isolation of *dpy-30* mutations and *yDf12*, a deficiency spanning the *dpy-30* locus: *dpy-30(y130)* was isolated as a maternal-effect suppressor of the *xol-1* *XO*-specific lethality (MILLER *et al.* 1988). Hermaphrodites of genotype *him-5; xol-1* were mutagenized with ethyl methanesulfonate (EMS), and the F_3 progeny were screened for the presence of males (M. SOTO, personal communication). To obtain additional alleles of *dpy-30*, we performed a non-complementation screen for mutations that failed to complement the zygotic dumpy (Dpy), egg-laying defective (Egl) phenotypes associated with *dpy-30(y130)*. For this screen, *unc-42 rol-4; lon-2 xol-1* hermaphrodites were mutagenized with EMS (BRENNER 1974), and mated with + *dpy-30(y130)* +/*unc-42* + *rol-4* *XO* males at 25°. Hermaphrodite cross progeny heterozygous for *dpy-30(y130)* were identified on the basis of their non-Unc non-Rol phenotype. Slightly Dpy Egl non-Unc non-Rol hermaphrodites were picked as candidates potentially carrying a new *dpy-30* allele.

We knew this screen would allow the recovery of *dpy-30* null mutations, since reconstruction experiments had already shown that *ctDf1/dpy-30 XX* animals of *ctDf1*/+ hermaphrodites are viable. Moreover, the *y130* allele could be recovered from the *y130/ctDf1* animals by mating them with wild-type males, and allowing the small number of cross progeny animals to self.

Each new *dpy-30* candidate animal was allowed to lay eggs for a few hours and was then transferred to a fresh plate and mated with N2 or *him-5* males at 20°. The following day, the first plates were examined; if healthy larvae and few unhatched eggs were observed, the candidate was eliminated from further consideration due to the lack of maternal-effect lethality. If the first plates had few or no hatched larvae, then the outcross progeny of the mating with wild-type males were picked to individual plates and allowed to self at 25°. Animals heterozygous for the mutagenized *unc-42 rol-4* chromosome were identified by the presence of Unc Rol progeny in their broods. To determine whether a new maternal-effect lethal mutation had been induced on the *unc-42 rol-4* chromosome, Unc Rol progeny were picked and the viability of their progeny was examined. Approximately 30,500 F_1 hermaphrodites of the genotype *dpy-30(y130)/** (where * represents a mutagenized chromosome) were screened in this manner, leading to the identification of a single new allele *dpy-30(y228*am*)*. When retested, this allele failed to complement both the zygotic and maternal-effect phenotypes displayed by *dpy-30(y130) XX* animals.

Importantly, the *dpy-30* non-complementation screen also yielded a chromosomal deficiency, *yDf12*, that spans the *dpy-30* locus. Hermaphrodites heterozygous for this mutation segregated approximately one quarter dead eggs, no Rol progeny and only occasional Unc progeny, suggesting that the mutation might be a deletion rather than a point mutation. Additional analysis of this lesion revealed that it behaves like a chromosomal deficiency, failing to complement tightly linked genetic markers (described below).

The frequency of *dpy-30* mutations obtained (1 new allele and 1 deficiency in 30,500 haploid genomes), is significantly lower than the average frequency of mutations induced by EMS (1/2000) (BRENNER 1974). This is probably due to two complicating factors. (1) The *dpy-30* gene is extremely small (<1 kb in length), thus providing a small target for mutagenesis (D. R. HSU and B. J. MEYER, manuscript in preparation). (2) At 25°, *dpy/dpy* progeny of *dpy*/+ mothers, are viable, but unhealthy; when crossed to wild-type males approximately 40% of these animals do not produce any cross progeny. Thus, it is quite possible that additional alleles were obtained; however, they might not have been recovered from the F_1 parent.

Genetic map position of *dpy-30*: *dpy-30(y130)* was initially mapped to LG V on the basis of linkage to *dpy-11(e224)*. Subsequently, *dpy-30* was mapped to the 0.4-map unit interval between *sma-1* and *myo-3* (Figure 2). This map position was assigned on the basis of the following three factor recombination data: 7/7 Dpy-11 non-Unc progeny of *dpy-30(y130)/dpy-11 unc-42* hermaphrodites segregated *dpy-30*; 0/7 Unc-42 non-Dpy progeny of *dpy-30(y130)/dpy-11 unc-42* hermaphrodites segregated *dpy-30*. These data place *dpy-30* close to or to the right of *unc-42*. Three of five Unc non-Sqt progeny of *dpy-30(y130)/unc-42 sqt-3* hermaphrodites segregated *dpy-30*; 1/11 Sqt-3 non-Unc progeny of *dpy-30(y130)/unc-42 sqt-3*

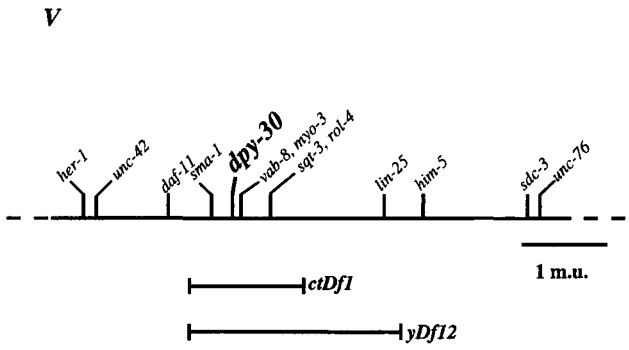

FIGURE 2.—Genetic map position of *dpy-30*. The *dpy-30* gene lies in the 0.4-map unit interval between *sma-1* and *myo-3* on chromosome *V*. The surrounding genes are described in HODGKIN *et al.* (1988). Chromosomal deficiencies *ctDf1* and *yDf12* are drawn below the map. These deficiencies fail to complement *dpy-30* and the genes flanking it.

hermaphrodites segregated *dpy-30*, thus indicating that *dpy-30* was located between *sqt-3* and *unc-42*. Nine of twelve Sma non-Myo-3 progeny of *dpy-30(y130)/sma-1 myo-3* segregated *dpy-30*; these data place *dpy-30* in the interval between *sma-1* and *myo-3*. It was not possible to identify Myo-3 non-Sma recombinants because *myo-3* mutations cause embryonic lethality. Additionally, *dpy-30(y228)* was mapped to the interval between *unc-42* and *sqt-3*.

Mapping the extent of *yDf12*: To determine the extent of *yDf12*, males heterozygous for a single mutation in *daf-11*, *sqt-3*, *sma-1*, *vab-8* or *lin-25* were mated with *unc-42 yDf12/nT1(n754*dm) hermaphrodites. *nT1(n754*dm) is a reciprocal translocation that balances *yDf12* and carries the dominant *unc(n754*dm) mutation (FERGUSON 1985). Cross progeny, identified on the basis of their non-Unc phenotype, were scored for the presence of mutant phenotypes, indicating a failure to complement the mutation being tested. *yDf12* fails to complement mutations in *sqt-3*, *sma-1*, *vab-8* and *lin-25*. *yDf12* complements mutations in *daf-11*. In addition, *yDf12/him-5 XX* animals do not segregate males, thus *yDf12* complements *him-5*. Based on these data, the endpoints of *yDf12* must lie between *daf-11* and *him-5*.

Amber suppression assays: Both alleles of *dpy-30* were tested for suppression by the *sup-7(st5)* tRNA amber suppressor (WATERSTON 1981; WILLS *et al.* 1983) at 20° and 22.5°. To test *dpy-30(y130)* for amber suppressibility, the following strategy was used. *sup-7*/O males were mated with *unc-42 y130 him-5* hermaphrodites. Hermaphrodite progeny of the genotype *unc-42 y130 him-5/+++; sup-7/+*, identified on the basis of their non-Unc phenotype, were picked and allowed to self. Sixteen homozygous *unc-42 y130 him-5* progeny, identified on the basis of their Unc phenotype, were picked and examined for their ability to produce viable self progeny. Of these animals, approximately one half should be *sup-7/+*, and approximately one quarter should be *sup-7/sup-7*. At both temperatures examined, the majority of these animals produced no viable progeny. Animals that were fertile were shown to be recombinants that had lost the *dpy-30* mutation. Thus, *sup-7* does not appear to suppress the *y130* allele. To test *y228* for amber suppression, *unc-42 y228 rol-4/+++* males were mated with *sup-7* hermaphrodites. Outcross male progeny (of which one-half were assumed to possess the genotype *unc-42 y228 rol-4/+++; sup-7*) were again crossed to *sup-7* hermaphrodites. Hermaphrodite progeny from these matings were picked to individual plates and were allowed to self. Some broods of these animals segregated Unc Rol progeny, thus the genotype of the parent must have been *unc-42 y228 rol-4/+++; sup-7. unc-42 y228 rol-4; sup-7* animals, identified on the basis of their Unc Rol phenotype were picked and examined for their ability to produce viable self-progeny. At 22.5°, no viable self progeny were identified; at 20°, the animals produced a small number of variably Dpy, sick animals. These animals, though slow growing, were fertile and produced small numbers of self progeny, thus indicating that at 20°, two copies of *sup-7* slightly suppresses the *y228 XX*-specific lethal phenotypes. At 20°, *sup-7* causes a cold-sensitive, embryonic lethality (WATERSTON 1981) thus, it is difficult to distinguish between the relative effects of the *y228* and *sup-7* mutations on *XX* viability. In similar experiments, *y228* was tested for amber suppression with an additional amber suppressor, *sup-5(e1464)*. No suppression of the *y228 XX*-specific lethality was observed in animals of the genotype *unc-32 sup-5; dpy-30 rol-4*.

Determining the karyotype specificity of the *dpy-30* phenotypes: To demonstrate the karyotype specificity of the *dpy-30* phenotypes, we examined the viability of *dpy-30 XX* and *XO* animals whose phenotypic sex had been reversed by mutations in the *her-1* and *tra-1* sex determination genes. The *her-1* gene is required for male sexual development (HODGKIN 1980; TRENT *et al.* 1991). Recessive mutations in *her-1* transform *XO* animals into fertile, wild-type hermaphrodites. To determine the viability of *her-1(hv1y101) dpy-30(y130) XO* hermaphrodites, we mated *her-1(hv1y101) dpy-30(y130)/++ XO* males with *her-1(hv1y101) dpy-30(y130); dpy-6 XX* hermaphrodites. The *X*-linked *dpy-6* marker allows the unambiguous identification of hemizygous *dpy-6*/O *XO* cross progeny on the basis of their Dpy phenotype. This cross generated 380 *XO* Dpy hermaphrodite cross progeny of the genotype *her-1(hv1y101) dpy-30(y130); dpy-6*/O and 420 *XO* Dpy male cross progeny of the genotype *her-1(hv1y101) dpy-30(y130)/++; dpy-6*/O. In this mating, no Dpy self progeny arose due to the maternal-effect lethality caused by *dpy-30(y130)*. Compared to the control *her-1 dpy-30/++ XO* animals, 90.5% of *her-1 dpy-30 XO* animals survive to adulthood, thus, *dpy-30 XO* hermaphrodites are not subject to the highly penetrant lethality seen in *XX* animals. Although the loss of *dpy-30* function does not cause significant lethality in *XO* animals, examination of the *dpy-30 her-1 XO* (m^-z^-) animals revealed that they were not wild type. To better characterize the phenotypes of *her-1 dpy-30 XO* animals, we examined *her-1 dpy-30 XO* animals lacking the *dpy-6* marker. *her-1 dpy-30 (y130) +/++ sqt-3(sc63)* males were mated with *her-1 dpy-30(y130) XX* hermaphrodites at 25°. The *sqt-3(sc63)* mutation causes a dominant roller phenotype at 25°. In addition, at 25°, all *dpy-30 XX* animals are inviable. Thus, all non-Rol hermaphrodite progeny of this mating should be *her-1 dpy-30 XO* animals. The phenotypes of these animals are described in the RESULTS.

Recessive mutations in the *tra-1* gene transform *XX* animals into phenotypic males (HODGKIN 1987; HODGKIN and BRENNER 1977). To obtain *XX* animals doubly mutant for *tra-1* and *dpy-30*, *tra-1(e1835e1816e1575)* males were mated with *dpy-30(y130) XX* animals. Cross progeny heterozygous for both genes were allowed to self; the broods of these animals were expected to contain approximately one-quarter homozygous *dpy-30* animals. Individual self progeny were picked and shifted to 15°. *dpy-30(y130)* homozygotes were identified by the fact that they produced many dead eggs, and a small number of Dpy adult progeny in their broods. The broods of *dpy-30(y130)* homozygous animals were then screened for the segregation of male progeny, indicating that the parental genotype was *tra-1/+; dpy-30(y130)*. The *XX* male progeny produced by these animals are Dpy and display numerous morphological defects (see RESULTS). Furthermore, the number of

these males was approximately one-quarter of the total number of viable *XX* adult animals; if the *tra-1* mutation was able to suppress the *dpy-30* lethality, then we would have predicted that the number of *tra-1; dpy-30 XX* male progeny from a *tra-1/+; dpy-30* mother would be approximately equal to one-quarter of the total number of zygotes. These results, taken together, indicate that the *tra-1* mutation does not suppress the *dpy-30 XX*-specific lethality.

The *dpy-30* Egl phenotype is not due to sexual transformation: The absence of any overtly masculinized structures in *dpy-30 XX* animals suggests that *dpy-30* is not directly involved in the control of sex determination. However, it was possible that the Egl phenotype of *dpy-30 XX* survivors was due to a subtle sexual transformation toward the male fate. Mutations that masculinize *XX* animals often cause the programmed cell death (male fate) of the HSNs (hermaphrodite specific neurons required for egg laying) and hence an Egl phenotype (TRENT *et al.* 1983, 1988). However, examination of *dpy-30 XX* animals using Nomarski optics revealed that the HSN neurons are present (data not shown), indicating that the Egl phenotype is not caused by a sexual transformation. Mutants defective in other dosage compensation *dpy* genes are also Egl, despite possessing HSN neurons, suggesting that this phenotype is a consequence of a dosage compensation disruption (PLENEFISCH *et al.* 1989).

***dpy-30/+* *XX* and *XO* progeny of homozygous mutant mothers are fully viable:** To ascertain whether zygotically supplied *dpy-30*(+) activity is sufficient to ensure the complete viability of *dpy/+* progeny of *dpy/dpy* mothers, we determined the viability of *dpy/+* progeny of a mating between wild-type males and *spe-9; unc-42 dpy-30(y228)* hermaphrodites at 25°. *spe-9(hc52*ts) is a temperature-sensitive mutation that affects sperm development (L'HERNAULT *et al.* 1988). At 25°, this mutation results in the formation of defective sperm, preventing essentially all self fertilization. Any embryos laid are the result of cross fertilization and must be of the genotype *spe-9/+; unc-42 dpy-30/++*. From these matings, 195 embryos were recovered; these embryos developed into 90 adult males and 100 adult hermaphrodites. Thus, there is insignificant strict maternal-effect lethality associated with the *dpy-30(y228)* mutation. However, there do appear to be strict maternal-effect phenotypes in some of the *dpy-30/+* *XX* and *XO* (m^-z^+) progeny of these matings. The *XX* hermaphrodite progeny of these matings were variably Dpy, and occasionally Egl and/or Pvul. A few of the *XO* male progeny appeared scrawny and unhealthy.

Viability analysis of *dpy-27; ctDf1/+* *XX* animals: To obtain *dpy-27(y49); ctDf1/+* *XX* animals, *dpy-27(y49)* males were mated with *unc-42* hermaphrodites. Non-Unc male progeny of the genotype *dpy-27/+; unc-42/+* were then mated with *ctDf1/nT1(n754*dm) hermaphrodites. The *nT1(n754*dm) chromosome is a reciprocal translocation carrying a dominant Unc mutation. All non-Unc cross progeny from matings with these hermaphrodites carry the *ctDf1* deficiency. Non-Unc hermaphrodites were picked and allowed to self. Some broods contained both Dpy non-Unc and Dpy Unc progeny, indicating that the genotype of the parent must have been *dpy-27/+; unc-42/ctDf1*. From these broods, 4 Dpy non-Unc hermaphrodites of the presumed genotype *dpy-27; unc-42/ctDf1* were picked and selfed. These animals laid 790 embryos that produced 36 Unc adult hermaphrodites and 27 non-Unc adult hermaphrodites. The viability of the Unc progeny is 18% according to the formula: viability = 4(Unc hermaphrodites)/(total embryos). The viability of the non-Unc hermaphrodites was 6.8% according to the formula: viability = 2(non-Unc hermaphrodites)/(total embryos). Thus, the viability of the *dpy-27; ctDf1/unc-42 XX* animals is lower than expected. Since the *ctDf1* chromosome shows no segregation distortion at 20° (see Figure 6 legend), this result indicates that one copy of the *ctDf1* chromosome enhances the lethality normally caused by the *dpy-27(y49)* mutation.

The *lin-15* assay of *X*-linked gene expression: We used the *lin-15* assay to demonstrate that *dpy-30* mutations cause elevated *X* chromosome expression. *lin-15(n765*ts) is an *X*-linked temperature-sensitive hypomorphic mutation that results in the formation of *mu*ltiple pseudo*v*ulvae (Muv) (FERGUSON and HORVITZ 1985). At 15°, *lin-15(n765*ts) animals appear wild type. At 20°, homozygous *lin/lin* progeny of *lin/lin* mothers are Muv. Previous genetic analysis has demonstrated that this locus is dosage compensated; furthermore, the Muv phenotype caused by *lin-15(n765)* is suppressed by the elevated *X* chromosome expression caused by mutations in *dpy-21* and *dpy-26* (MENEELY and WOOD 1987).

Two different experimental protocols were used to assay the Muv phenotype of all the appropriate wild-type and *dpy-30* mutant strains. This strategy was necessitated by the fact that at 20°, the temperature at which this assay is scored, *dpy-30 XX* (m^-z^-) animals produce no viable *dpy-30 XX* progeny. For the first protocol, used in cases where the animals to be scored are normally viable at 20°, the parents of the animals to be scored were raised at 15° until the L4 larval stage, which is past the temperature-sensitive period of *lin-15(n765)* (FERGUSON *et al.* 1987). L4 hermaphrodites of the appropriate genotype were shifted to 20° and were either allowed to self or were mated with males of the appropriate genotype. The progeny of these animals were scored for the penetrance of the Muv phenotype. Hermaphrodite progeny were scored as Muv if they possessed two or more ventral protrusions; male progeny were scored as Muv if they possessed one or more ventral protrusions. The percent Muv of each class is represented as the ratio of (number of Muv animals of a genotype/total number of animals of that genotype).

To perform the *lin-15* assay on animals such as *dpy-30 XX* (m^-z^-), which are dead at 20°, we took advantage of two observations. (1) Some *dpy-30 XX* (m^-z^-) embryos raised at 15° can survive to adulthood when shifted to 20° relatively late in embryogenesis. (2) The temperature-sensitive period (TSP) for *lin-15(n765)* lasts from the L1 larval stage to the late L2 larval stage (FERGUSON *et al.* 1987). In addition, examination of the upshift curve obtained from temperature-shift experiments indicates that animals shifted to restrictive temperatures as late as the mid-L2 stage are usually mutant. Based on these observations, the following procedure was adopted. *unc-42 dpy-30/nT1(m435); lin-15* hermaphrodites were allowed to self at 15°. From their broods, progeny of the genotype *unc-42 dpy-30; lin-15* were identified on the basis of their Unc phenotype and were picked to individual plates. These animals were allowed to self overnight at 15°. The following day, each parent was transferred to a fresh plate and the embryos laid the previous night were transferred to 20°. This procedure was repeated until the hermaphrodites stopped laying eggs. This procedure produced a small number of viable embryos that were scored for the Muv phenotype. The control animals of genotype *unc-42; lin-15* were treated identically. Since approximately 100% of the control animals were Muv, any variation in the penetrance of the Muv phenotype in experimental animals was not likely to be due to the animals being shifted to non-permissive temperatures after the *lin-15* TSP.

Preparation of total RNA: Mixed-stage populations of nematodes were cultured at 15° [or 20° for *dpy-27(y57) XX* populations] on 90-mm NGM agar plates (SULSTON and HODGKIN 1988). When plates were nearly starved, cultures were washed off the plates with M9, rinsed several times in M9, and frozen at −80°. Frozen worm pellets (50–200 μl) were resuspended in digestion buffer (50 mM Tris-HCl, pH 8.0, 10 mM

EDTA, 100 mM NaCl) to a volume of 400 µl, and digested in the presence of 100 µg proteinase K and 0.6% sodium dodecyl sulfate for 2–4 hr at 65°. Following digestion, samples were phenol/chloroform extracted 3–6 times and ethanol precipitated at −20°. The nucleic acid pellet obtained was resuspended in 500 µl TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). To precipitate total RNA, an equal volume of 5 M LiCl was added. Following a 2–4-hr incubation on ice, the sample was pelleted by centrifugation at 4° and the RNA pellets were resuspended in TE plus 0.1% SDS. The RNA was ethanol precipitated again and stored in 70% ethanol at −80°. If RNA samples were impure (as determined by OD_{260}/OD_{280}), additional LiCl precipitations were performed.

RNase protection assays: RNase protection assays designed to measure the relative level of *X*-linked gene expression in wild-type and mutant animals used gene-specific probes for the autosomal gene *myo-1* and the *X*-linked genes *myo-2* (Dibb *et al.* 1989) and *lin-14* (Ambros and Horvitz 1987; Wightman *et al.* 1991). Gene-specific probes for *myo-1* and *myo-2* were cloned into pBluescript using standard techniques (Sambrook *et al.* 1989). The *myo-1* probe was a 171-bp *Bcl*I to *Sac*I fragment cloned into pBluescript SK(−) digested with *Bam*HI and *Sac*I (pTY765). Linearized template DNA for transcription was prepared by digesting pTY765 with *Eco*RV. This template produces a run-off transcript 213 nucleotides in length, which yields a protected fragment of approximately 171 nucleotides. The *myo-2* probe was a 117 bp *Eco*RI to *Eco*RV fragment cloned into pBluescript SK(−) digested with *Eco*RI and *Eco*RV (pTY766). Linearized template DNA for transcription was prepared by digesting pTY766 with *Eco*RI. This template produces a run-off transcript 175 nucleotides in length, which yields a protected fragment of approximately 117 nucleotides. The *lin-14* probe was the cDNA clone 518 (Wightman *et al.* 1991). Template DNA was prepared by digesting the 518 clone with *Fsp*I. This template produces a run-off transcript 275 nucleotides in length, which yields a protected fragment of approximately 215 nucleotides.

^{32}P-labeled antisense RNA probes were synthesized by incubating 0.5–2 µg of linearized template DNA in a 20-µl reaction mixture containing 40 mM Tris-HCl, pH 7.9, 6 mM $MgCl_2$, 10 mM NaCl, 2 mM spermidine, 10 mM dithiothreitol, 0.5 mM ATP, GTP, and CTP, 12.5 µM UTP, 20 units RNasin (Promega), 25 units of T3 or T7 RNA polymerase, and 50–200 µCi [α-^{32}P]UTP (>3000 Ci/mmol) for 1–2 hr at 37°. Following this incubation, 1 unit of RQ1 DNase (Promega) was added, and the reactions were incubated for an additional 15 min at 37°. Once the reactions were complete, 100 µl of H_2O were added to each reaction, the mixtures were extracted with phenol/chloroform, and RNAs were precipitated with the addition of 20 µl 5 M ammonium acetate and 250 µl ethanol. RNAs were stored in this 70% ethanol solution at −20°. All solutions used for RNA synthesis were treated with 0.2% diethyl pyrocarbonate to inactivate RNases.

For protection assays, 1–10 µg total nematode RNA was coprecipitated with 20 µg of yeast tRNA and ^{32}P-labeled antisense RNA probes specific for *myo-1*, *myo-2* and *lin-14*. The amount of probe required was determined empirically. RNA pellets were resuspended and hybridized overnight at 43° using standard conditions (Sambrook *et al.* 1989). Digestions and subsequent gel electrophoresis of reaction products were carried out as described by Sambrook *et al.* (1989), with the following modifications. (1) Digestion mixtures contained only RNase T1 (1000 units/reaction). (2) Digestions were performed at 20°. (3) Digestion reactions were stopped with phenol/chloroform extractions; no proteinase K was added.

Band intensities of the protection products for *myo-1*, *myo-2* and *lin-14* were quantified with a phosphor-imaging device using ImageQuant software (Molecular Dynamics). To ensure that the assay was linear in the range examined, multiple experiments using different concentrations of total RNA were quantified. Normalized band intensities for *X*-linked transcript levels were calculated as the ratio of (*X*-linked band intensity)/(autosomal band intensity). The relative level of *X*-linked gene expression between *dpy-30* and N2 samples was calculated as the ratio of (normalized *X*-linked band intensity *dpy-30*)/(normalized *X*-linked band intensity N2).

Analysis of variance (ANOVA) was performed on data from protection assays to determine whether the variance between samples of two different genotypes was statistically different from the variance between samples of the same genotype (Bryant 1966; Leon Avery, personal communication). For each RNA preparation, the normalized *X*-linked band intensity from two repetitions of the protection experiment was averaged; ANOVA was then performed comparing data from N2 and *dpy-30* RNA preparations. In order to simplify error calculations, analysis was performed using the natural log of the band intensities. The *F* test was used to compare the within-genotype variance with the between-genotype variance. Standard errors were computed using an estimate of the sample variance derived from analysis of variance of the logarithms of the measured band intensities.

Construction of strains doubly mutant for *dpy-30* and other mutations involved in sex determination and/or dosage compensation: The following strategy was used to demonstrate that *dpy-30(y130)* suppresses the masculinization caused by *her-1(y101*sd), a gain-of-function mutation that causes masculinization of *XX* animals (Trent *et al.* 1988, 1991; A. Villeneuve, personal communication). To construct the *dpy-30(y130) her-1(y101*sd) double mutant, *unc-42 dpy-30(y130)/++* males were mated with *her-1(y101*sd) hermaphrodites. Individual hermaphrodite progeny were picked and allowed to self at 25°. Hermaphrodite cross progeny of genotype *unc-42 + dpy-30(y130)/ + her-1(y101*sd) + were identified by the presence of Unc progeny in the next generation. The broods of these heterozygous animals were screened for the presence of slightly Dpy non-Unc recombinants. Because these recombinants were homozygous for *dpy-30(y130)*, and thus would produce no viable self-progeny, they were immediately mated with wild-type males to recover the recombinant chromosome, which was subsequently balanced by the *nT1(n754*dm) translocation. The presence of both mutations was confirmed by the observations that non-Unc progeny of putative *dpy-30(y130) her-1(y101*sd)*/nT1(n754*dm) hermaphrodites are often slightly Dpy, masculinized, and fail to produce viable progeny. When grown at 15°, these *dpy-30(y130) her-1(y101*sd) animals produced a brood consisting of only 2.1% masculinized animals.

The following strategy was used to demonstrate that *dpy-30(y130)* suppresses the masculinization caused by the *sdc-3(y113*Tra) mutation. The *sdc-3* gene controls sex determination and dosage compensation in *XX* animals (DeLong *et al.* 1993). *sdc-3(y113*Tra) disrupts only the sex determination function, causing masculinization of *XX* animals. To construct the *dpy-30(y130) sdc-3(y113)* double mutant, *dpy-30(y130) unc-76/++* males were mated with *sdc-3(y113)/nT1(n754*dm) hermaphrodites. Non-Unc progeny were allowed to self at 25°; approximately one-half of these animals produced broods containing slightly Dpy Unc progeny, thus indicating that their genotype must have been *dpy-30(y130) + unc-76/+ sdc-3(y113) +*. Slightly Dpy non-Unc recombinants from these broods were picked and mated with wild-type males to recover the recombinant *dpy-30(y130) sdc-3(y113)* chromosome, which was subsequently balanced over the *nT1(n754*dm) translocation. The presence of both mutations was confirmed by the observations

that at 25° non-Unc progeny of putative *dpy-30(y130) sdc-3(y113)/nT1(n754*dm*)* animals are generally masculinized and that the few non-Unc hermaphrodite progeny produce no viable progeny. However, when the non-Unc hermaphrodites are immediately shifted to 15°, they produce >95% hermaphrodite progeny.

The following strategy was used to demonstrate the inviability of the *dpy-30(y130) sdc-3(y129)* double mutant. The *sdc-3(y129)* mutation disrupts both the *sdc-3* sex determination and dosage compensation functions, causing a maternal-effect, *XX*-specific lethality, but no masculinization of *XX* mutants (DeLong *et al.* 1993). The *dpy-30(y130) sdc-3(y129)* double mutant was constructed by mating *dpy-30(y130) unc-76/++* males with *sdc-3(y129)* hermaphrodites, picking the non-Dpy progeny from these matings, and following the same strategy described above. At 20°, *dpy-30(y130) sdc-3(y129) XX* progeny of heterozygous parents are viable, Dpy hermaphrodites. When these Dpy animals were picked, they produced no viable self progeny, consistent with the absolute lethality caused by a *dpy-30* mutation.

The following strategy was used to demonstrate the total lethality of the *dpy-30(y228); sdc-2(y202)* double mutant. The *sdc-2* gene is a zygotically acting gene that controls sex determination and dosage compensation in *XX* animals (Nusbaum and Meyer 1989). *sdc-2(y202)* causes a temperature-sensitive dumpiness and lethality in *XX* animals. At 15°, approximately 90% of *sdc-2(y202) XX* animals survive as Dpy adults. At 20°, 50% of these animals survive to adulthood; these animals are very Dpy and unhealthy (T. Davis, personal communication). To construct the *dpy-30(y228); sdc-2(y202)* double mutant, *unc-42 dpy-30(y228)/++* males were mated with *sdc-2(y202)* hermaphrodites at 20°. Male progeny of this cross (one half of which were presumed to be *unc-42 dpy-30(y228)/++; sdc-2(y202)*) were again mated with *sdc-2(y202)* hermaphrodites at 15°. Because we could not distinguish between cross and self progeny in this mating, a large number of hermaphrodite progeny were picked and allowed to self. Some animals produced Unc hermaphrodites of the presumed genotype *unc-42 dpy-30; sdc-2*. The Unc animals were picked and allowed to self at either 15° or at 20°. At 15°, a small number of these animals produced viable progeny; these animals are very Dpy, Egl and slow growing, appearing similar to *dpy-30 XX* animals raised at the same temperature. At 20°, the animals picked produced no viable self progeny.

The following strategy was used to demonstrate the total lethality of the *dpy-27(y57); dpy-30(y228)* double mutant. The *dpy-27* gene is one of three known maternally supplied genes essential for the *XX* mode of dosage compensation (Plenefisch *et al.* 1989). To construct a *dpy-27; dpy-30* double mutant, we mated *unc-42 dpy-30(y228)/++* males with *dpy-27(y57) XX* hermaphrodites. The *y57* mutation is a weak allele of *dpy-27* that causes lethality in approximately 80% of *XX* animals. From the mating, non-Dpy hermaphrodites were picked and allowed to self. The genotype of animals that produced Unc progeny was inferred to have been *dpy-27/+; unc-42 dpy-30/++*. From plates that segregated Unc progeny, Dpy animals (of the presumed genotype *dpy-27; unc-42 dpy-30/++* or *dpy-27; +/+*) were allowed to self. Some broods contained Unc progeny, indicating that the genotype of the parent must have been *dpy-27; unc-42 dpy-30/++*. From these broods, Unc hermaphrodites were picked and were shifted to 15° or 20°. At both temperatures, the *dpy-27; unc-42 dpy-30 XX* animals appeared very Dpy, Egl and very unhealthy; these animals produced no viable progeny. This result is consistent with both the cold-sensitive nature of the *dpy-27* lethality (0.6% of *XX* animals viable at 15°) and the absolute lethality caused by *dpy-30* mutations at 20°.

The following strategy was used to establish the lethality of the *dpy-30(y130) dpy-21(e428)* double mutant. The *dpy-21* gene is a zygotically acting gene required for the *XX*-mode of dosage compensation. In *XX* animals, *dpy-21* mutations cause overexpression of *X*-linked genes, but not significant lethality (DeLong 1990; DeLong *et al.* 1987; Hodgkin 1983). To construct a *dpy-30; dpy-21* double mutant, *dpy-30(y130)* males were mated with *unc-76 dpy-21(e428)* hermaphrodites. Non-Unc cross progeny of the genotype *dpy-30 ++/+ unc-76 dpy-21* were allowed to self at 20°. Recombinant Dpy non-Unc progeny of the genotype *dpy-30 + dpy-21/+ unc-76 dpy-21* were picked from among the self progeny. These animals were mated with wild-type males to recover the *dpy-30 dpy-21* chromosome. Male cross progeny from these matings (one-half of which are presumed to be *dpy-30 dpy-21/++*) were individually mated with *unc-42 dpy-21* hermaphrodites to create a balanced strain. Some of these matings produced Dpy non-Unc progeny of the presumed genotype *+ dpy-30 dpy-21/unc-42 + dpy-21;* these animals were picked and allowed to self at 20°. They produced both Dpy non-Unc and Dpy Unc progeny. About one third of the Dpy non-Unc progeny were also extremely Egl, and appeared very unhealthy. When such individuals were picked and allowed to self, they produced no viable self-progeny. These animals were presumed to be *dpy-30 dpy-21* double mutants.

y63, a mutation that suppresses the *sdc-1* dosage compensation defect, does not suppress the *dpy-30* XX-specific lethality: *y63* is a mutation that suppresses the dosage compensation defect but not the sex determination defect caused by *sdc-1* mutations (Villeneuve and Meyer 1990b). We examined the ability of this mutation to suppress the *XX*-specific lethality caused by mutations in *dpy-30*. To obtain a strain doubly mutant for *dpy-30* and *y63*, *unc-42 dpy-30(y130* or *y228)/++* males were mated with *y63 lin-15* hermaphrodites at 15°. Male progeny from this mating (one-half of which are presumed to be heterozygous for *unc-42 dpy-30*) were again mated with *y63 lin-15* hermaphrodites. Numerous hermaphrodite progeny were picked to individual plates. The broods of these animals were screened for the presence of Unc progeny, indicating that the parental genotype must have been *unc-42 dpy-30/++; y63 lin-15*. From the broods of these animals, individual Unc animals were picked; some of these animals were shifted to 20° and some were kept at 15°. At 20°, most of these animals produced no viable progeny; rare Unc animals that produced progeny were determined to be Unc non-Dpy recombinants that had lost the *dpy-30* mutation. At 15°, most embryos were inviable; however, a small number of *XX* animals survived to become Dpy adults. Thus, *y63* does not appear to suppress the *XX*-specific lethality caused by *dpy-30* mutations.

Interactions between *dpy-30(y130)* and *y2*, a mutation that causes an XO-specific lethality: *y2* is a mutation that causes a maternal-effect, *XO*-specific lethality (Plenefisch *et al.* 1989). It was conceivable that this lethality was due to inappropriate activation of *dpy-30* in *y2 XO* animals. Alternatively, it was possible that the *XX*-specific lethality caused by *dpy-30* mutations was caused by inappropriate activation of *y2* in *dpy-30 XX* animals. If either model were true, then the naive expectation would be that in *y2; dpy-30* double mutants either the *XO*-specific lethality or the *XX*-specific lethality would be suppressed. *y2; dpy-30(y130)* double mutants were constructed by mating *y2 rol-6/++* males with *unc-42 dpy-30 him-5* hermaphrodites. Non-Unc cross progeny hermaphrodites were picked to individual plates and allowed to self. The broods of some of these animals contained approximately one-quarter Rol animals, indicating that the parental genotype was *y2 rol-6/++; unc-42 dpy-30 him-5/+++*. From these broods, animals of genotype *y2 rol-6; unc-42 dpy-30 him-5/+++* or *y2*

rol-6, identified on the basis of their Rol non-Unc phenotype, were picked to individual plates and allowed to self. In the next generation, from broods that contained approximately one quarter Unc progeny, individual Unc animals were picked; some of these animals were shifted to 15° and some were left at 20°. These Unc animals were presumed to be of the genotype *y2 rol-6; unc-42 dpy-30(y130) him-5*. *unc-42* and *him-5* are approximately 4.5 map units apart from each other thus most Unc animals should also be homozygous for *him-5*. At 15°, these animals produced a small number of Dpy Unc progeny and large numbers of dead eggs. There were no male progeny in these broods, despite the presence of the *him-5* mutation, which normally results in broods containing approximately 30% *X*O males (HODGKIN *et al.* 1979). At 20°, most Unc animals produced no viable progeny; those that did were determined to be recombinants that had lost *dpy-30(y130)*. Thus in *y2; dpy-30(y130)* animals, there does not appear to be suppression of either the *dpy-30 XX*-specific lethal or the *y2 X*O-specific lethal phenotypes.

RESULTS

Mutations in *dpy-30* result in *XX*-specific lethality: Using different strategies (see MATERIALS AND METHODS), we have isolated two alleles of *dpy-30*, *y130* and *y228*am, each of which affects the morphology and viability of *XX* animals in a manner characteristic of mutations that disrupt the *XX* mode of dosage compensation. The phenotypes caused by both *dpy-30* alleles are temperature sensitive and depend on maternal and zygotic genotypes (described in detail below). Homozygous *dpy-30 XX* progeny of *dpy-30/+* mothers are viable. When they are raised at the permissive temperature (15°), these animals are indistinguishable from wild-type *XX* hermaphrodites (Figure 3A). However, when raised at the nonpermissive temperature of 25°, they are slightly dumpy (Dpy), egg laying-defective (Egl), and often possess a protruding vulva (Pvul) (Figure 3B). In contrast, homozygous *dpy-30 XX* progeny of *dpy-30/dpy-30* mothers are generally inviable. When raised at a temperature of 20° or greater, all *dpy-30 XX* (m^-z^-) animals are inviable, dying as arrested embryos, or as necrotic, uncoordinated (Unc) and constipated (Con) L1 larvae. When raised at 15°, most *dpy-30* (m^-z^-) *XX* animals are also inviable and display the same terminal phenotypes. However, at this temperature, a small number of *dpy-30 XX* animals do survive and develop into Dpy, Egl and Pvul adults (Figure 3C). An Egl phenotype can be caused by a mild sexual transformation of the animal to the male fate; however, in *dpy-30* mutants, we find no evidence for such a defect (see MATERIALS AND METHODS). More likely, the Egl phenotype in *dpy-30 XX* animals results from their dosage compensation defect, as does the Egl phenotype caused by other dosage compensation mutations (HODGKIN 1983; PLENEFISCH *et al.* 1989).

To demonstrate that the *dpy-30* lethality is dependent on *X* chromosome dosage and not on sexual phenotype, we examined the viability of *dpy-30 XX* and *X*O animals whose phenotypic sex had been reversed by mutations in the *C. elegans* sex determination pathway. We used a mutation in the *her-1* gene to transform *dpy-30 X*O animals into hermaphrodites and determined that 90.5% of *her-1 dpy-30 X*O hermaphrodites are viable relative to their *dpy-30 her-1/++* siblings (see MATERIALS AND METHODS). Thus, *dpy-30* mutations do not specifically kill animals with a hermaphrodite sexual phenotype. Conversely, a mutation in the *tra-1* gene (HODGKIN 1987) that transforms *XX* animals into phenotypic males does not rescue *dpy-30 XX* animals. At 15°, most *tra-1; dpy-30 XX* animals are inviable. Rare surviving *tra-1; dpy-30 XX* animals are males that possess a Dpy morphology and malformed tails with severely reduced bursal fan size and abnormal or missing sensory rays. These phenotypes have previously been observed in *XX tra-1* males homozygous for other hermaphrodite-specific dosage compensation mutations (HODGKIN 1983; PLENEFISCH *et al.* 1989), suggesting that the morphological defects

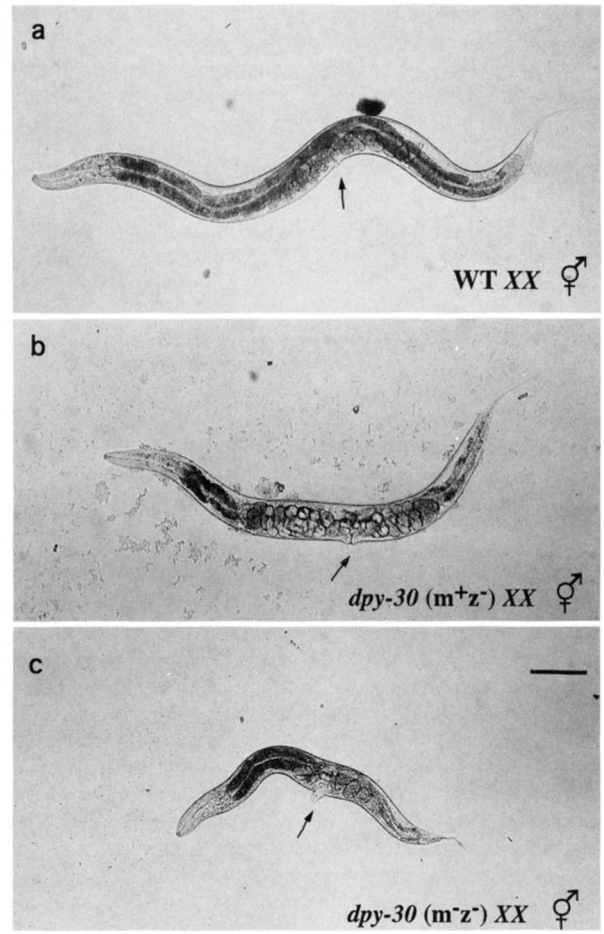

FIGURE 3.—Brightfield photomicrographs showing the characteristic phenotypes of *dpy-30 XX* mutants. In each micrograph, the arrow points to the vulva. (a) Wild-type *XX* hermaphrodite. (b) *dpy-30* (m^+z^-) *XX* hermaphrodite. This animal, raised at 25°, is slightly shorter than the wild-type *XX* hermaphrodite, possesses a slightly protruding vulva and retains late stage embryos. (c) *dpy-30* (m^-z^-) *XX* hermaphrodite. This animal, raised at 15°, is a rare survivor of the *dpy-30 XX*-specific lethality and displays severe Dpy and Pvul phenotypes. The scale bar is 100 μm.

TABLE 1

Phenotypes caused by mutations that disrupt the *XX* mode of dosage compensation

Gene[a]	Phenotype[b]		
	XO	$XX(m^+z^-)$[c]	$XX(m^-z^-)$[c]
dpy-21[d,e]	wt	Dpy, Egl	Dpy, Egl
dpy-26[e,f]	wt	Very slightly Dpy, Him	Inviable; escapers are Dpy, Egl, and Him
dpy-27[f]	wt	Dpy, Egl	Inviable; escapers are Dpy, Egl
dpy-28[f]	wt	Very slightly Dpy, Him	Inviable; escapers are Dpy, Egl, and Him
dpy-30	Slow growing, mating defective	Slightly Dpy, Egl, Pvul	Inviable; no escapers at 20° Dpy, Egl, Pvul escapers at 15°

[a] Genes such as *sdc-1*, *sdc-2* and *sdc-3* that control the hermaphrodite mode of sex determination as well as dosage compensation have not been included in this table.

[b] wt, wild type; Egl, egg-laying defective; Dpy, dumpy (short and fat); Pvul, protruding vulva; Him, high incidence of males.

[c] m refers to the maternal genotype; z refers to the zygotic genotype; the superscripts $^+$ and $^-$ indicate the presence or absence of a wild-type allele of a given gene. Thus, m^+z^- refers to homozygous *dpy* progeny of heterozygous mothers; m^-z^- refers to homozygous *dpy* progeny of homozygous mothers.

[d] HODGKIN (1983).

[e] DELONG *et al.* (1987).

[f] PLENEFISCH *et al.* (1989).

seen in *tra-1; dpy-30 XX* males arise as a consequence of elevated *X* chromosome expression. Taken together, these results demonstrate that the *dpy-30* lethality is correlated with the *X* chromosome dosage and not with sexual phenotype, as would be expected for a mutation that disrupts dosage compensation (see Table 1).

Either maternally or zygotically supplied *dpy-30*(+) activity is sufficient to ensure the complete viability of *XX* animals: Mutations in *dpy-30* display maternal and zygotic rescue such that *XX*-specific lethality only results when both mother and zygote are mutant for *dpy-30*. The maternal rescue is demonstrated by the observation that there is no lethality associated with the *dpy-30 XX* (m^+z^-) progeny of *dpy*/+ mothers (Figure 4, panel A). At 20°, the viability of these *XX* animals ranges from 97.7 to 99.2% (see figure legend for viability calculation). A similar absence of lethality is observed when these experiments are performed at 25°, the temperature at which the *dpy-30* (m^+z^-) morphological defects are most severe. In contrast, when both maternally and zygotically supplied *dpy-30*(+) activity is absent, extensive lethality of *XX* animals results (Figure 4, panel B). At 15°, approximately 12–14% of *XX dpy-30* (m^-z^-) animals are viable. As previously described, these rare survivors are Dpy, Egl and Pvul, phenotypes commonly associated with a disruption in the *XX*-mode of dosage compensation (HODGKIN 1983; PLENEFISCH *et al.* 1989). At 20°, there are no escapers of the *dpy-30 XX*-specific lethality. In contrast, putative null mutations in other dosage compensation *dpy* genes never cause complete lethality (PLENEFISCH *et al.* 1989). Despite the total lethality exhibited by the *dpy-30* (m^-z^-) *XX* animals, their *dpy-30 XO* (m^-z^-) siblings remain viable. The viability of *dpy-30* (m^-z^-) *XO* progeny of mutant mothers ranges from 84 to 94% for *y228* and *y130*, respectively, when compared to their *dpy*/+ siblings, indicating that these animals are essentially as viable as wild-type animals (Figure 4B). However, as will be discussed below, *dpy-30 XO* animals are not wild type, revealing that this gene is required for the development of *XO* animals.

Unlike the phenotypes caused by mutations in other dosage compensation *dpy* genes, the *dpy-30* maternal-effect phenotypes are not fully suppressed by zygotically supplied *dpy-30*(+) activity. Despite possessing a wild-type copy of *dpy-30*, a small proportion of the *dpy-30*/+ *XX* progeny of mutant mothers appear slightly Dpy, uncoordinated, and unhealthy. However, there does not appear to be any significant lethality among the *dpy*/+ *XX* (m^-z^+) progeny of mutant mothers. At 25°, a mating between wild-type *XO* males and *dpy-30 XX* hermaphrodites produced 195 *dpy-30*/+ embryos that developed into 90 (46%) adult males and 100 (51%) adult hermaphrodites (see MATERIALS AND METHODS). Thus, *dpy-30*(+) supplied to the zygote from the male prevents lethality, but does not always ensure wild-type morphology. Comparison of the relatively mild phenotypes caused by the absence of maternally supplied *dpy-30*(+) product in m^-z^+ animals with the more severe phenotypes caused by the absence of zygotically supplied product in m^+z^- animals, reveals that the maternal and zygotic contributions are not equivalent.

***dpy-30* is required for the wild-type development of *XO* animals:** We discovered that unlike the other dosage compensation *dpy* genes, *dpy-30* is required for the wild-type development of *XO* animals even though it is generally not required for their viability. *dpy-30 XO* animals display several incompletely penetrant morphological and behavioral defects, which, like the *dpy-30 XX*-specific phenotypes, depend on both temperature and maternal genotype. At 25°, *dpy-30 XO* male progeny of mutant mothers are often small, scrawny, developmentally delayed and constipated (Con). In addition, the males are mating defective and have tails with a reduced number of sensory rays and a reduced bursal fan (Figure 5). At 20°, these phenotypes are less pronounced. *dpy-30 XO* (m^+z^-) progeny of *dpy-30*/+

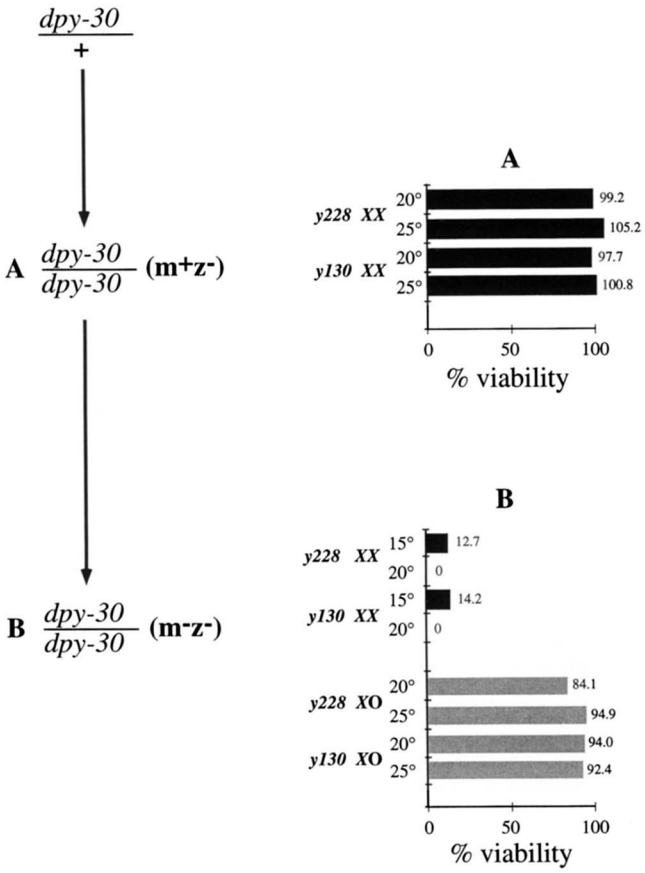

FIGURE 4.— *dpy-30* mutations result in a maternal-effect, *XX*-specific lethality. (A) *dpy-30* (m^+z^-) *XX* mutants display maternal rescue. *XX* animals of the genotype *unc-42 dpy-30/++* were allowed to self-fertilize at either 20° or 25°, and the number of Unc progeny was scored. The viability of homozygous *unc-42 dpy-30* animals was calculated according to the formula: viability = 4(Unc progeny)/(total progeny). For each experiment, at least 1000 progeny of heterozygous mothers were scored. (B) *dpy-30* mutations cause a maternal-effect lethality in *XX* animals. *dpy-30 XX* hermaphrodites were allowed to self fertilize at 15° or 20°, and the number of surviving progeny was scored. The viability of *dpy-30 XX* (m^-z^-) animals was calculated according to the formula: viability = $H/(Z - M)$, where H is the number of adult hermaphrodites, Z the number of zygotes, and M the number of adult *XO* males. For each experiment, at least 1000 embryos were scored for their ability to develop into viable adults. To determine the viability of *dpy-30 XO* (m^-z^-) animals, males of the genotype *dpy-30/unc-42* were mated with *unc-42 dpy-30* hermaphrodites at 20° or 25°. *dpy-30* (m^-z^-) males were identified on the basis of their non-Unc phenotype. Viability of these animals was calculated according to the formula: viability = (non-Unc males)/(Unc males), where 100% of the Unc (*unc-42 dpy-30/unc-42 +*) males are assumed to be viable.

mothers are less severely affected. It seems unlikely that these developmental defects caused by *dpy-30* mutations result from a transformation of *XO* animals toward the hermaphrodite fate, since *dpy-30 XO* animals transformed into hermaphrodites by a *her-1* mutation are also abnormal (see MATERIALS AND METHODS). *her-1 dpy-30 XO* hermaphrodites are often slightly Dpy, Unc and Pvul; occasionally, these animals are also constipated. The observations that the *XX* and *XO* phenotypes are both temperature sensitive and maternally rescuable as well as genetically inseparable strongly indicates that these phenotypes arise as a consequence of a mutation in a single locus. Unfortunately, the pleiotropic nature of these *dpy-30 XO* phenotypes does not provide a clear indication of the primary defect in these animals. A genetic assay of *X*-linked gene expression (described below) does not provide compelling evidence for a dosage compensation defect in *dpy-30 XO* animals, suggesting that the *dpy-30 XO* phenotypes might reflect an additional role for *dpy-30* in development.

Analysis of the *dpy-30* null phenotype: The completely penetrant XX-specific maternal-effect lethality associated with both *dpy-30(y130)* and *dpy-30(y228)* initially suggested that both alleles cause complete, or nearly complete, loss of *dpy-30* function. Furthermore, the *dpy-30(y228)* allele was isolated in a screen that would allow the recovery of *dpy-30* null mutations since it allowed the recovery of a deficiency spanning the *dpy-30* region *(yDf12)* (see MATERIALS AND METHODS). However, in the course of characterizing *dpy-30* phenotypes, two observations raised the concern that *dpy-30* alleles might not completely eliminate gene activity. First, the phenotypes associated with both alleles are temperature sensitive, suggesting the possibility that these alleles might result in the synthesis of partially functional, heat-labile *dpy-30* proteins rather than the simple elimination of gene function. Second, *dpy-30 XO* animals display incompletely penetrant phenotypes, suggesting the possibility that mutant *XO* animals still possess residual *dpy-30(+)* activity. To test if existing *dpy-30* alleles completely eliminate *dpy-30* activity, the viability and morphology of *XX* and *XO* animals carrying either allele in *trans* to *ctDf1*, a chromosomal deficiency that spans the *dpy-30* locus, were examined. We reasoned that if *y228* and *y130* cause only a partial loss of function, then *dpy-30/ctDf1* animals would display more severe zygotic and/or maternal-effect phenotypes than *dpy-30/dpy-30* animals. Under these stringent conditions, additional phenotypes revealing other *dpy-30* functions might also become apparent.

First, we examined the viability and morphology of *dpy-30/Df XX* and *XO* progeny of *Df/+* mothers to determine if the deficiency would result in a further reduction of *dpy-30* activity and cause zygotic lethality and/or a more severe Dpy phenotype. Figure 6 demonstrates that there is no zygotic lethality apparent when *dpy-30 XX* or *XO* animals are examined under these stringent conditions. At 25°, *dpy-30/ctDf1 XX* and *XO* progeny of *ctDf1/+* mothers are essentially as viable as wild-type control animals. Phenotypically, both *XX* and *XO* *dpy-30/ctDf1* (m^+z^-) animals are indistinguishable from the *dpy/dpy* progeny of *dpy/+* mothers. That is, the zygotic phenotypes are not enhanced by the deficiency and

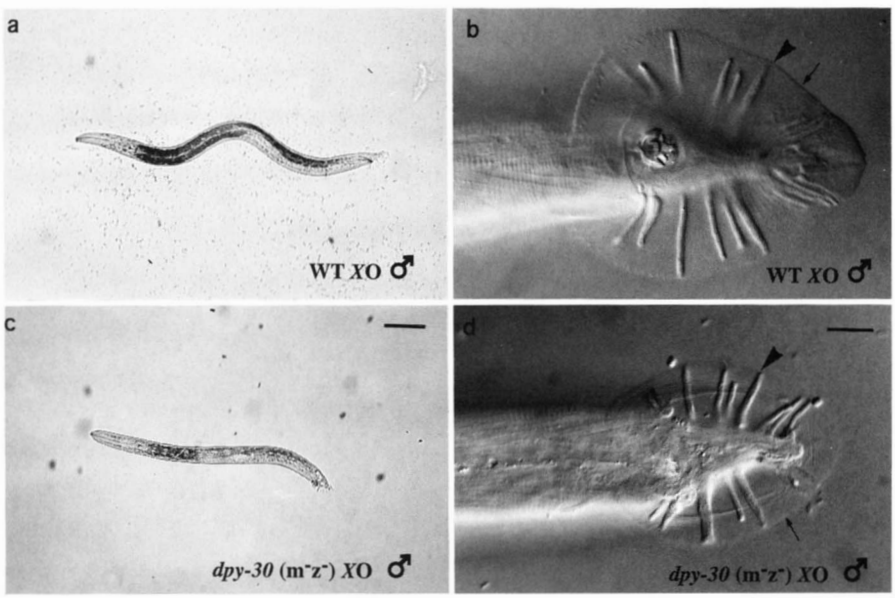

FIGURE 5.—*dpy-30* mutations affect the morphology of *XO* males. (a) Brightfield photomicrograph of a wild-type (N2) male. (b) Nomarski photomicrograph of a wild-type male tail, ventral view. (c) Brightfield photomicrograph of *dpy-30 XO* (m^-z^-) male. (d) Nomarski photomicrograph of *dpy-30 XO* (m^-z^-) male tail, dorsal view. Comparison of the brightfield photographs reveals that *dpy-30 XO* males are smaller than wild-type males. In addition, the Nomarski photomicrographs show the reduced bursal fan often seen in *dpy-30* males. In panels b and d, a sensory ray is indicated by an arrowhead, and the fan is indicated by an arrow. The scale bar for a and c is 100 μm; the scale bar for b and d is 10 μm.

no additional phenotypes are revealed by these deficiency experiments.

To examine the maternal-effect *dpy-30* phenotypes under the most stringent conditions, we determined the viability of the *XX* and *X*O mutant progeny of *dpy-30/ctDf1* mothers (Figure 6, panels B and C). At 20°, all of the *dpy/dpy* and *dpy/Df XX* progeny of *dpy/Df* mothers are inviable. At 15°, the viability of *dpy/dpy XX* progeny of *dpy/Df* mothers (7.9 and 13.3% for *y130* and *y228*, respectively) is not appreciably different from the 12–14% viability of the *dpy/dpy XX* progeny of *dpy-30* mothers (Figure 6, panel B). However, in these experiments, the viability of the *dpy/Df XX* progeny of *dpy/Df* mothers (1.5–2.5%) is lower than that of their *dpy/dpy* siblings (Figure 6, panel C). The reduced viability of the *dpy/Df* progeny from *dpy/Df* mothers suggests the possibility that both *dpy-30* alleles have residual dosage compensation activity. An alternative explanation is that the alleles are null alleles, with the additional lethality resulting from a non-specific dominant, dose-dependent interaction with a gene other than *dpy-30* that is included in the deficiency. This latter interpretation seems more plausible, since a single copy of *ctDf1*, which has no effect on the viability of otherwise wild-type animals, enhances the lethality caused by an unlinked homozygous *dpy-27* dosage compensation mutation. Whereas 18% of *dpy-27(y49); unc-42 XX* animals are viable, only 6.8% of their *dpy-27; ctDf1/unc-42* siblings are viable (see MATERIALS AND METHODS). A similar situation has been observed previously with the *sdc-3* gene. The lethality caused by *bona fide sdc-3* null mutations (KLEIN and MEYER 1993) is enhanced by a deficiency that spans *sdc-3*. This deficiency, *yDf9*, also enhances the lethality caused by an unlinked homozygous *dpy-27* mutation (DELONG *et al.* 1993). In view of these results, it seems likely that both *dpy-30* alleles cause complete or nearly complete loss of gene function.

A further, small reduction in the viability of *dpy-30/Df XO* animals compared to *dpy-30/dpy-30 XO* animals was also observed (Figure 6, panels B and C). However, these experiments failed to reveal any additional *X*O-specific *dpy-30* mutant phenotypes. The viability of *dpy/dpy XO* progeny of *dpy/Df* mothers is 80 and 100% for *y228* and *y130*, respectively, when compared to their *dpy/+ XO* siblings. The viability of the *dpy/Df XO* siblings of these animals is 80 and 74% for *y228* and *y130*, respectively, when compared to their *Df/+ XO* siblings. Although these results could be interpreted to mean that the existing *dpy-30* alleles are not null with respect to their *X*O phenotypes, the following two caveats temper that view and make it more plausible that both *dpy-30* alleles cause substantial, if not complete, loss of function. First, in these experiments, the only genetic markers available to identify different progeny classes were difficult to score with total confidence, resulting in a slightly inaccurate assessment of the number of animals in each progeny class. Second, the *ctDf1* deficiency may cause additional nonspecific lethality of *dpy-30/Df XO* animals, as it does of *dpy-30/Df XX* animals.

Further support for the idea that the homozygous *dpy-30* mutant phenotypes result from complete loss of *dpy-30* function comes from the fact that the *dpy-30(y228)* allele is an amber mutation. Genetic evidence for this finding came from the observation that the *XX*-specific lethality caused by *y228* is weakly suppressed by the *sup-7(st5)* tRNA amber suppressor (WATERSTON 1981; WILLS *et al.* 1983). At 20°, a temperature at which *dpy-30 XX* animals are normally inviable, a small proportion of *dpy-30(y228); sup-7(st5) XX* (m^-z^-) animals are viable and produce small numbers of variably Dpy, Egl progeny. Molecular confirmation of this result has come from DNA sequence analysis of the *dpy-30* coding region of *y228* mutants. The *y228* mutation causes a glutamine to amber

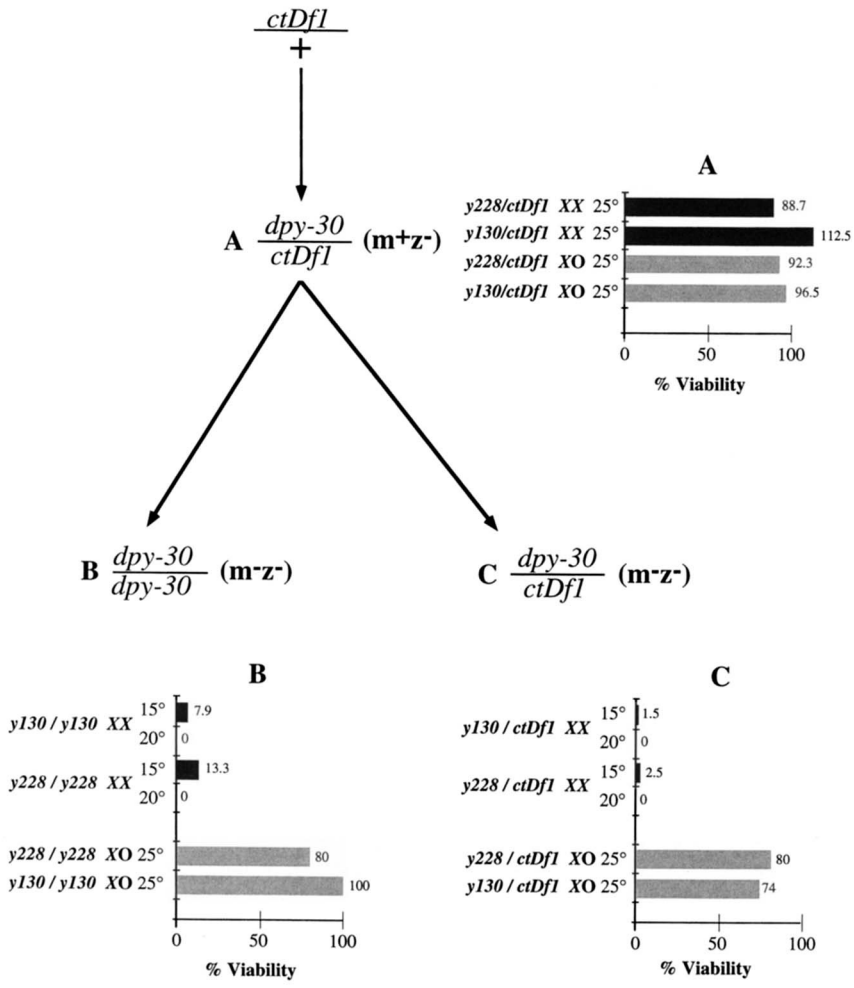

FIGURE 6.—Viability analysis of *dpy-30/Df XX* and *XO* animals. (A) *dpy-30/ctDf1 XX* and *XO* progeny of *Df/+* mothers are essentially as viable as wild-type animals. *dpy-30/ctDf1 XX* and *X*O animals were generated by mating *dpy-30 unc-42/++* males with *unc-42 ctDf1/+ sqt-3* hermaphrodites at 25°. The *dpy/Df* progeny were identified on the basis of their Unc phenotype. Viability of *dpy/Df* hermaphrodites (*XX*) and males (*X*O) was calculated according to the formula: viability = 2(Unc hermaphrodites or Unc males)/(*sqt-3/M* males), where *M* = *unc-42 dpy-30*, or *++*. This formula assumes that *sqt-3/M* animals, identified on the basis of the dominant roller phenotype conferred by the *sqt-3(sc63*ts*)* mutation, arise at twice the frequency of the other progeny classes. For *y130* and *y228*, these crosses produced a total of 375 and 503 *sqt-3/M* males, respectively. (B and C) Viability of the *dpy/dpy* and *dpy/Df XX* and *X*O progeny of *dpy/Df* mothers. *XX* animals of the genotype *dpy-30(y130) unc-76/unc-42 ctDf1* or *unc-42 dpy-30(y228)/ctDf1* were allowed to self at 15° or at 20°, and the number of adult progeny was scored. The viability of *dpy/dpy XX* progeny (panel B), identified on the basis of their Unc phenotype, was calculated according the formula: viability = 4(Unc hermaphrodites)/(total zygotes). The viability of the *dpy/Df XX* progeny (panel C), identified on the basis of their non-Unc phenotype, was calculated according to the formula: viability = 2(non-Unc hermaphrodites)/(total zygotes). The *ctDf1* homozygotes are included in the category of total zygotes. For each experiment, the results are based on the analysis of at least 1000 embryos from *dpy/Df* mothers. *dpy/dpy* and *dpy/Df XO* progeny of *dpy/Df* mothers were generated by mating *unc-42 dpy-30 +/++ sqt-3* males with *unc-42 dpy-30/+ ctDf1* hermaphrodites at 25°. The viability of *dpy/dpy X*O animals, identified as the Unc male progeny of this cross, was calculated according to the formula: viability = (Unc males)/(Rol males), where the genotype of Rol males is assumed to be *++ sqt-3/unc-42 dpy-30 +*. For *y228* and *y130*, these matings produced 166 and 177 *dpy/+ X*O animals, respectively. *dpy/Df X*O progeny were identified on the basis of their non-Unc non-Rol non-Dpy phenotype. (*sqt-3* has a recessive Dpy phenotype in addition to its dominant Rol phenotype; *sqt-3/sqt-3* and *sqt-3/Df* animals are Dpy.) The viability of the *dpy/Df X*O animals was calculated according to the formula: viability = (non-Unc non-Rol non-Dpy males)/(Dpy males), where the genotype of Dpy males is assumed to be *sqt-3/ctDf1*. For *y228* and *y130*, these matings produced 133 and 113 *Df/+ X*O progeny, respectively. At 20°, there is unlikely to be any segregation distortion associated with the *ctDf1* chromosome: the broods of *him-5 unc-42/ctDf1 XX* hermaphrodites contained 280 (24%) Unc progeny, 575 (49%) non-Unc progeny, and 313 (27%) dead zygotes. However, at 15°, there may be a slight segregation distortion associated with *ctDf1*. At 15°, the broods of *him-5 unc-42/ctDf1 XX* hermaphrodites contained 114 (20%) Unc progeny, 252 (45%) non-Unc progeny, and 198 (35%) dead zygotes.

TABLE 2

***lin-15* assay of *X*-linked gene expression**

Maternal genotype	Zygotic genotype	Percent Muv (n) [a]	
		Male progeny	Hermaphrodite progeny
unc-42; lin-15	*unc-42; lin-15*	69.4 (108) [b]	100 (614) [c]
unc-42 dpy-30(y130); lin-15	*unc-42 dpy-30(y130); lin-15*	98.9 (190) [d]	5.4 (357) [e]
unc-42 dpy-30(y130); lin-15	*unc-42 dpy-30(y130)/++; lin-15*	98.3 (294) [f]	99.6 (271) [g]
unc-42 dpy-30(y130)/++; lin-15	*unc-42 dpy-30(y130); lin-15*	ND	99.3 (566) [h]

[a] The phenotype of adults with the zygotic genotypes listed was scored. *lin-15(n765)* is an *X*-linked hypomorphic mutation that causes a temperature-sensitive *mu*ltivulva phenotype (Muv) (FERGUSON and HORVITZ 1985). This phenotype is suppressed by mutations that cause overexpression of *X*-linked genes (MENEELY and WOOD 1987). *XX* hermaphrodites are scored as Muv if they possess two or more ventral protrusions; *XO* males are scored as Muv if they possess one or more ventral protrusions. Percent Muv is expressed as the ratio of (no. of Muv animals of a particular genotype)/(total number of animals of that genotype). ND = not determined.

[b] These animals are the male progeny of *unc-42 him-5; lin-15* hermaphrodites raised at 20°. The *him-5(el490)* mutation causes *X* chromosome nondisjunction resulting in the production of broods containing approximately 30% *XO* males.

[c] These animals were treated identically to the *unc-42 dpy-30; lin-15* experimental animals.

[d] These animals were identified as the Unc male progeny from a cross between *unc-42 dpy-30/++* males and *unc-42 dpy-30; lin-15* hermaphrodites.

[e] In order to obtain viable animals of this genotype, *unc-42 dpy-30; lin-15* mothers were raised at 15° and transferred to fresh plates daily. After eggs were laid, they were shifted to 20°. This procedure produced a small number of *dpy-30 XX* progeny; the phenotype of these animals was scored.

[f] These animals were identified as the non-Unc progeny from a cross between WT *XO* males with *unc-42 dpy-30; lin-15 XX* hermaphrodites.

[g] These animals were identified as the non-Unc progeny from a cross between *lin-15(n765) XO* males and *unc-42 dpy-30; lin-15 XX* hermaphrodites.

[h] These animals were identified as the Unc self-progeny of *unc-42 dpy-30/++; lin-15* mothers.

change that prevents translation of approximately 50% of the *dpy-30* protein (D. R. HSU and B. J. MEYER, manuscript in preparation).

***dpy-30* results in overexpression of *X*-linked genes in *XX* animals, demonstrating a disruption in the dosage compensation process:** The karyotype-specific nature of the *dpy-30* mutant phenotypes, and the similarity of these phenotypes to those caused by other mutations that disrupt the *XX* mode of dosage compensation suggested that *dpy-30* is required for proper regulation of *X* chromosome expression in *XX* animals. To test this possibility, we used both genetic and molecular assays of gene expression that allowed us to examine the relative expression of several *X*-linked loci. The genetic assay uses an *X*-linked hypomorphic mutation, *lin-15(n765*ts), which causes a temperature-sensitive, *mu*ltivul*v*a (Muv) phenotype (FERGUSON and HORVITZ 1985). The basis of the genetic assay is that the severity of the *lin-15* mutant phenotypes caused by this loss-of-function mutation that reduces, but does not eliminate gene function, reflects the relative level of *lin-15* gene activity. In the *lin-15* assay, suppression of the Muv phenotype reflects an increase in the level of *lin-15* activity, whereas enhancement of the Muv phenotype reflects a decrease in activity. This assay has been used to demonstrate that mutations in *dpy-21* and *dpy-26* cause overexpression of *X*-linked genes (MENEELY and WOOD 1987). When examined with this assay, *dpy-30(y130) XX* animals display significant suppression of the *lin-15* Muv phenotype only in the absence of maternally and zygotically supplied *dpy-30(+)* activity (Table 2). Whereas 100% of the *lin-15* control animals were Muv, only 5.4% of the *dpy-30; lin-15 XX* (m^-z^-) progeny of mutant mothers were Muv. No suppression of the Muv phenotype was seen in the *dpy-30; lin-15 XX* (m^+z^-) progeny of *dpy-30/+; lin-15* mothers, or the *dpy-30/+; lin-15 XX* (m^-z^+) progeny of *dpy-30; lin-15* mothers. Because *dpy-30* mutations also affect the development of *XO* animals, the *lin-15* assay was performed on *dpy-30 XO* animals. No suppression of the *lin-15* Muv phenotype was detected in either the *dpy-30; lin-15 XO* (m^-z^-) progeny or the *dpy-30/++; lin-15 XO* (m^-z^+) progeny of mutant mothers. Instead, a slight enhancement of the Muv phenotype in the *dpy-30; lin-15 XO* (m^-z^-) progeny was detected; however, the modest nature of the effect makes its significance unclear. At the sensitivity level of this assay, we find substantial evidence for aberrant *X* chromosome expression in *dpy-30 XX* animals, but not in *dpy-30 XO* animals.

X-linked transcript levels were also examined directly using an RNase protection assay. In this assay, *X*-linked transcript levels, normalized to the level of an autosomal control transcript, are compared between total RNA prepared from wild-type or *dpy-30 XX* animals. In these experiments, the two *X*-linked genes assayed were *myo-2*, encoding a myosin heavy chain isoform expressed in the pharynx (DIBB *et al.* 1989), and the heterochronic gene *lin-14* (AMBROS and HORVITZ 1987; WIGHTMAN *et al.* 1991). *X*-linked transcript levels were normalized to transcript levels of the autosomal *myo-1* myosin gene (DIBB *et al.* 1989). Comparison of normalized *X*-linked transcript levels in RNA prepared from several independent mixed-stage populations of wild-type (N2) and *dpy-30 XX* animals raised at 15° demonstrates that in *dpy-30 XX* animals, the transcript levels of both *myo-2* and *lin-14* are approximately 1.5 times higher than the

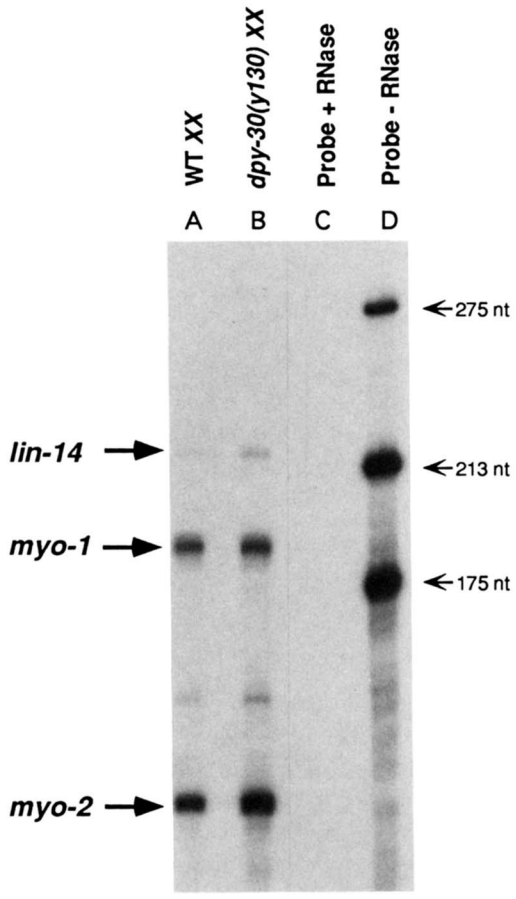

FIGURE 7.—RNase protection assay of *X*-linked gene expression. In lanes A and B, total RNA prepared from populations of wild-type (N2) or *dpy-30(y130) XX* animals grown at 15° was simultaneously hybridized to gene specific, ^{32}P-labeled RNA probes for the two *X*-linked genes, *lin-14* and *myo-2*, and a probe for the autosomal gene, *myo-1*. Following hybridization, the RNA samples were digested with RNase T1, which produces a single band corresponding to the transcript of each gene (labeled to the left of the figure). When lanes A and B are normalized to the *myo-1* signal, the intensity of the *lin-14* and *myo-2* signals is higher in *dpy-30* RNA than in N2 RNA, thus indicating that these genes are overexpressed in *dpy-30* animals. The results of several independent repetitions of these experiments are summarized in Table 6. In lane C, the same hybridization and digestion was performed in the absence of total RNA, thus leading to complete digestion of the probe. In lane D, no RNase was added to the digestion mixture, thus the *lin-14* (275 nucleotides), *myo-1* (213 nucleotides) and *myo-2* (175 nucleotides) probes remain intact.

levels found in wild-type *XX* animals (Figure 7, Table 3). As a positive control, we also compared *X*-linked transcript levels between the N2 RNA samples prepared for these experiments and RNA prepared from *dpy-27 XX* mixed-stage populations. In quantitative Northern hybridization experiments *dpy-27 XX* populations were previously found to possess elevated *X*-linked transcript levels relative to those found in wild-type *XX* animals (MEYER and CASSON 1986). In the RNase protection assay, *dpy-27 XX* animals express *myo-2* and *lin-14* at a level approximately 1.5-fold higher than wild-type levels (Table 3). Due to the relatively small difference in expression levels between *dpy-30*, *dpy-27* and N2 *XX* animals, normalized *X*-linked transcript levels obtained from the independent RNA preparations were compared by performing ANOVA. The ANOVA, comparing normalized *lin-14* and *myo-2* transcript levels, demonstrates that the difference in the expression of these genes in N2 and *dpy-30* or N2 and *dpy-27* backgrounds is highly significant ($P < 0.01$) (Table 3).

***dpy-30* functions downstream of *xol-1*, a gene required for sex determination and dosage compensation in *XO* animals:** To determine where *dpy-30* acts in the regulatory hierarchy that controls sex determination and dosage compensation in *C. elegans*, we examined the interaction between *dpy-30* and *xol-1*, the earliest-acting gene thus far identified in this pathway. *xol-1* is the only known gene required for the coordinate control of both processes in the male, and appears to function as a developmental switch to specify the choice between male (*XO*) or hermaphrodite (*XX*) development (N. R. RHIND and B. J. MEYER, unpublished results). Loss-of-function mutations in *xol-1* cause *XO*-specific feminization and lethality, due to the inappropriate activation of the *XX* modes of sex determination and dosage compensation. Because mutations in *xol-1* cause phenotypes opposite to those caused by genes required for the *XX* mode of dosage compensation, it has been possible to make double mutant combinations and order the action of the genes. To date, all mutations known to disrupt the *XX* mode of dosage compensation act as suppressors of the *xol-1 XO*-specific lethality, consistent with a model in which *xol-1* acts upstream of these genes as a negative regulator to prevent their activation in *XO* animals. In addition to positioning genes relative to *xol-1* in the control of dosage compensation, the analysis of *xol-1* suppressors often provides information about the role of these suppressors in the sex determination process. For example, mutations in *dpy-26*, *dpy-27* and *dpy-28* disrupt only the dosage compensation process and consequently suppress the lethality but not the feminization caused by *xol-1* mutations. In contrast, mutations in the *sdc* genes disrupt both sex determination and dosage compensation in *XX* animals, and they suppress both the lethality and feminization caused by *xol-1* mutations (MILLER *et al.* 1988).

The analysis of *dpy-30; xol-1* double mutants reveals that a mutation in *dpy-30* can suppress the *xol-1 XO*-specific lethality. Thus, like the other genes required for the *XX* mode of dosage compensation, *dpy-30* appears to act downstream of *xol-1* in the control of this process. Suppression of the *xol-1 XO*-specific lethality by a *dpy-30* mutation is highly dependent on both maternal and zygotic genotypes (Table 4). Whereas only 0.2% of *dpy-30(y228); xol-1 XO* progeny of *dpy-30(y228)/+; xol-1* mothers are viable, 76.9% of *dpy-30(y228); xol-1 XO* progeny of *dpy-30(y228); xol-1 XX* mothers are viable. In a similar experiment, 93.0% of *dpy-30(y130); xol-1 XO* progeny of *dpy-30(y130); xol-1* mothers were viable.

TABLE 3

RNase protection assays of X-linked gene expression

Animals compared	Stage	No. of RNA preps	*myo-2 X* Mean band intensity[a,c]	*myo-2 X* Relative expression[b,c]	*myo-2 X* Compared to wild-type XX[d]	*lin-14 X* Mean band intensity[a,c]	*lin-14 X* Relative expression[b,c]	*lin-14 X* Compared to wild-type XX[d]
A								
Wild-type *XX*	Mixed	3	164.6 ± 6.13	1	—	138.1 ± 7.8	1	—
dpy-30(y130) XX	Mixed	5	231.7 ± 6.69	1.41 ± 0.07	$P < 0.01$	212.8 ± 9.3	1.54 ± 0.11	$P < 0.01$
B								
Wild-type *XX*	Mixed	3	92.6 ± 1.5	1	—	128.0 ± 5.1	1	—
dpy-27(y57) XX[e]	Mixed	3	119.0 ± 2.0	1.29 ± 0.03	$P < 0.01$	219.9 ± 8.7	1.72 ± 0.1	$P < 0.01$

[a] These values represent the average of normalized X-linked transcript levels from different RNA preparations made from animals of the same genotype. Band intensities were normalized to the intensity of the autosomal control transcript *myo-1*. The values are expressed in arbitrary units.

[b] Relative expression is calculated as the ratio of (normalized X-linked gene expression of experimental sample)/(normalized X-linked gene expression of wild-type sample).

[c] Standard errors were computed using an estimate of the sample variance derived from analysis of variance of the logarithms of the measured band intensities.

[d] The *F* test was used to compare the variance between wild-type and *dpy-30* samples. For the comparison of the three wild-type and five *dpy-30* samples, $F = 52.55$ for *myo-2*, and $F = 36.97$ for *lin-14*, with 1 and 6 d.f. For the comparison of the same three wild-type samples and three *dpy-27(y57)* samples, $F = 117.0$ for *myo-2*, and $F = 93.1$ for *lin-14*, with 1 and 4 d.f.

[e] *dpy-27(y57) XX* animals are almost completely inviable at 15°, making it impossible to grow large populations of these animals. Therefore RNA was prepared from populations of these animals raised at 20°.

TABLE 4

***dpy-30* mutations suppress the XO-specific lethality and feminization caused by a *xol-1* mutation**

Maternal genotype	Zygotic genotype	Sex of XO animals: Percent hermaphrodites	Sex of XO animals: Percent intersexes	Sex of XO animals: Percent males	Percent viability of XO animals (n)[a]
dpy-30(y228)/+; lon-2 xol-1	*dpy-30(y228); lon-2 xol-1*[b]	0	0	100	0.2 (357)
dpy-30(y228); lon-2 xol-1	*dpy-30(y228); lon-2 xol-1*[c]	0	0.6	99.4	76.9 (454)
dpy-30(y228); lon-2 xol-1	*dpy-30(y228)/+; lon-2 xol-1*[d]	0	0	100	56.0 (454)
dpy-30(y130); flu-2 xol-1	*dpy-30(y130); flu-2 xol-1*[e]	1.1	2.2	96.7	93.0 (287)

This table lists the percent viability and sexual phenotypes of *dpy-30; xol-1* or *dpy-30/+; xol-1* XO rescued animals. Animals are scored as intersexual if they display a mixture of both male and hermaphrodite structures.

[a] Percent viability of XO animals is calculated as the ratio of (number of XO animals of the zygotic genotype listed)/(number of *dpy/+ XX* siblings). We have shown that 100% of *dpy/+ XX* animals are viable. n is the total number of *dpy/+ XX* progeny.

[b] These animals are the XO progeny of a cross between *dpy-30(y228)/sqt-3* XO males and *dpy-30(y228)/nT1(n754*dm); *lon-2 xol-1 XX* hermaphrodites at 25°. At this temperature, *sqt-3(sc63)* causes a dominant Roller phenotype that allowed the identification *dpy/sqt-3* cross progeny. *nT1(n754*dm) is a chromosomal translocation containing a dominant Unc mutation. The *lon-2* mutation causes animals to be longer than wild type (Lon phenotype). Thus, hemizygous *dpy-30; lon-2 xol-1/OXO* cross progeny could be identified by their Lon non-Unc non-Rol phenotype irrespective of their sexual phenotype.

[c] These animals are identified as the Lon non-Rol progeny of a cross between *dpy-30/sqt-3* males and *dpy-30; lon-2 xol-1* hermaphrodites.

[d] These animals are identified as the Lon Rol progeny of the cross described in (*c*).

[e] XO progeny of this genotype were identified as the Flu non-Unc progeny of a mating between *dpy-30(y130)/unc-42* males and *dpy-30(y130) unc-42; flu-2 xol-1 XX* hermaphrodites.

Curiously, for both alleles, viable *dpy-30; xol-1* XO animals are male, revealing that unlike mutations in the other maternal-effect *dpy* genes, mutations in *dpy-30* suppress both the lethality and feminization of *xol-1* XO animals. This result raises the possibility that *dpy-30* might act like the *sdc* genes and have a role in hermaphrodite sexual development. This possibility is further explored in the next section. Rescued *dpy-30; xol-1* XO animals are not wild type; they generally possess the scrawny, uncoordinated and malformed tail phenotypes commonly displayed by *dpy-30* XO males. In contrast to the other dosage compensation *dpy* genes, the absence of maternally supplied *dpy-30*(+) product is sufficient to rescue a significant proportion of *xol-1* XO animals even in the presence of zygotically supplied *dpy-30*(+) activity. Fifty-six percent of *dpy-30(y228)/+; xol-1* (m^-z^+) *XO* progeny of *dpy-30(y228); xol-1* mothers are viable males that reach adulthood. In this generation (m^-z^+), the rescue of *xol-1* XO animals is qualitatively different from that obtained when both mother and zygote are mutant for *dpy-30*. Viable *dpy-30/+; xol-1* XO (m^-z^+) males are slow to develop, possess highly malformed tails, and are very scrawny and Unc. It is unlikely that these phenotypes are simply due to the *dpy-30* mutation, since *dpy-30/+* XO animals are generally wild type. More likely, these phenotypes arise as a consequence of the incomplete rescue of the *xol-1* dosage compensation defect caused by the presence of zygoti-

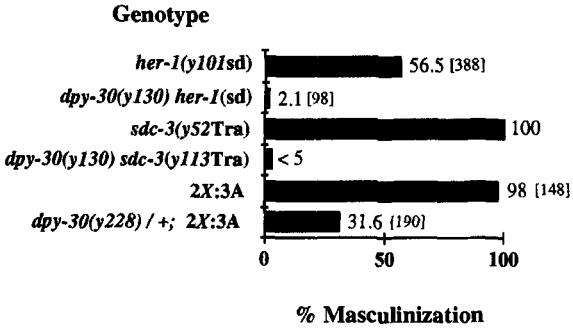

FIGURE 8.—Loss of *dpy-30* activity causes feminization. The bar graph shows the effects of *dpy-30* mutations on the sexual phenotypes of partially masculinized animals. The masculinization caused by the *her-1(y101sd)*, or by the *sdc-3(y52* or *y113Tra)* mutations or by a 2X:3A karyotype is suppressed by a *dpy-30* mutation. Masculinization was calculated as the percentage of adult animals that had one or more male-specific tail structures, or that lacked a hermaphrodite tail whip. Numbers in the square brackets indicate the total number of animals scored for each genotype. The *sdc-3(y52Tra)* data are from DELONG *et al.* (1993). The *sdc-3(y113)* and the *sdc-3(y52)* alleles cause the same nucleotide change (KLEIN and MEYER 1993) and behave indistinguishably (DELONG *et al.* 1993).

cally supplied *dpy-30*(+) activity.

Interactions between *dpy-30* and the sex determination process: To further assess the role of *dpy-30* in sex determination, we determined the effects of a *dpy-30* mutation on the sexual phenotype of *XX* animals masculinized by an intermediate *X*/A ratio (2*X*/3A karyotype), or by mutations in the sex determination genes *her-1* or *sdc-3*. These three genetic backgrounds result in *XX* animals possessing a sexually ambiguous identity that is easily perturbed by additional masculinizing or feminizing influences (DELONG *et al.* 1993; HODGKIN 1983; PLENEFISCH *et al.* 1989). In these backgrounds, mutations in the dosage compensation *dpy* genes, or increases in *X* chromosome dosage cause feminization. This feminization is thought to occur as a consequence of elevated *X* chromosome expression caused by the disruption in dosage compensation. In contrast, mutations in the *sdc* genes either fail to feminize, or actually enhance the masculinization normally observed, thus revealing the sex determination defect in *sdc* mutants. Thus, these genetic backgrounds provide a means for distinguishing between mutations that disrupt dosage compensation but do not affect sex determination from those that disrupt both processes. We reasoned that if *dpy-30* is required for hermaphrodite sexual development, as are the *sdc* genes, then the sex determination defect in *dpy-30* mutants might be revealed by examining the effects of *dpy-30* mutations in these genetic backgrounds.

Contrary to the expectations for an *sdc*-like gene, the results summarized in Figure 8 show that a *dpy-30* mutation feminizes animals possessing a sexually ambiguous identity, thus revealing a greater similarity between *dpy-30* and the dosage compensation *dpy* genes than between *dpy-30* and the *sdc* genes. A single copy of a *dpy-30* mutation feminizes animals with an intermediate *X/A* ratio. Whereas 98% of 2*X*:3A animals are male, only 31.6% of *dpy-30*/+ 2*X*:3A animals are male. Additionally, loss of *dpy-30* activity feminizes *XX* animals carrying either the *sdc-3(y113*Tra) mutation or the *her-1(y101*sd) mutation, both of which normally cause extensive masculinization of *XX* animals (TRENT *et al.* 1991; DELONG *et al.* 1993; A. VILLENEUVE and B. J. MEYER, unpublished data). The *sdc-3 (113*Tra) mutation specifically eliminates the *sdc-3* sex determination function, causing nearly all of the *sdc-3(y113*Tra) *XX* progeny of heterozygous mothers to develop as males (DELONG *et al.* 1993). *dpy-30(y130) sdc-3(y113*Tra) animals display a high degree of feminization (≥95% hermaphrodite) and can be propagated as a self-fertile strain at 15°. *her-1(y101*sd) is a semidominant mutation in the *her-1* sex determination gene that causes approximately 57% of homozygous mutant animals to be masculinized. A *dpy-30* mutation reduces the masculinization of *her-1(y101*sd) *XX* animals to 2.1%. Taken together, these results reveal important similarities between *dpy-30* and the other dosage compensation *dpy* genes, while failing to provide evidence for a direct involvement of *dpy-30* in hermaphrodite sexual development.

DISCUSSION

The *dpy-30* gene acts in the dosage compensation process: The dosage compensation process in *C. elegans* has a complex relationship to the sex determination process. Initially, the two processes are coordinately regulated in response to the primary sex-determination signal. Subsequently, each process is regulated by a specialized set of genes that controls either sex determination or dosage compensation. In addition, there is an interplay between the two processes such that a feedback mechanism operates to allow a disruption in dosage compensation to affect the sexual fate of *XX* animals. The genetic characterization presented in this paper demonstrates that *dpy-30* encodes an essential component of the dosage compensation process, most likely acting as a specialized gene downstream of the genes that coordinately regulate sex determination and dosage compensation. Mutations in *dpy-30* also affect sex determination in a manner consistent with the operation of a feedback mechanism in *XX* animals. Not only does *dpy-30* play a specialized role as a dosage compensation gene, *dpy-30* also has properties that set it apart from the previously identified genes required for dosage compensation, *dpy-21*, *dpy-26*, *dpy-27* and *dpy-28*.

On the one hand, loss-of-function mutations in *dpy-30* cause a repertoire of phenotypes characteristically associated with a disruption in the *XX* mode of dosage compensation (HODGKIN 1983; PLENEFISCH *et al.* 1989). *dpy-30 XX* animals suffer from a highly penetrant, maternal-effect lethality that causes animals to

arrest development during embryogenesis or to die shortly after hatching. Rare *XX* survivors of the lethality are slow growing, dumpy, egg laying-defective hermaphrodites, which express their *X*-linked genes at higher than wild-type levels. These phenotypes are consistent with a role for *dpy-30* in reducing *X* chromosome expression in *XX* animals (HODGKIN 1983; PLENEFISCH *et al.* 1989). *dpy-30* appears to act downstream of *xol-1*, the master regulatory gene required for the male modes of both sex determination and dosage compensation, most likely at the same position in the regulatory hierarchy as the other dosage compensation *dpy* genes. It has not been possible to order *dpy-30* with respect to the other *dpy* genes, since animals carrying combinations of mutations in *dpy-30* and one of the other *dpy* genes are generally inviable (see MATERIALS AND METHODS).

On the other hand, several phenotypes caused by *dpy-30* mutations distinguish *dpy-30* from *dpy-26*, *dpy-27* and *dpy-28* and suggest that it is a unique member of this gene family. Most striking among these novel phenotypes is the observation that *dpy-30* mutations disrupt the normal development of *XO* animals, independent of their sexual phenotype. *dpy-30 XO* animals suffer from a wide array of developmental and behavioral defects including abnormal morphology, uncoordinated movement, and in *XO* males, a severe reduction in mating efficiency. Another difference between *dpy-30* and the other maternal-effect *dpy* genes is apparent in a direct comparison of the *XX*-specific lethality caused by these genes. Null mutations in *dpy-26*, *dpy-27* or *dpy-28*, either individually or in combinations, kill most, but not all *XX* animals. In contrast to the incompletely penetrant lethality caused by the other *dpy* mutations, *dpy-30* mutations cause complete lethality at the nonpermissive temperatures.

A puzzling aspect of the dosage compensation mutations is that they all have temperature-dependent phenotypes. All mutations, including null mutations in *dpy-26*, *dpy-27* and *dpy-28* (except one) cause more severe *XX*-specific lethality at 15° (>99% lethality) than at 20°, while the two *dpy-30* mutations cause more severe lethality at 20° than at 15°. A reasonable interpretation of the cold-sensitive lethality is that *XX* embryos severely defective in dosage compensation become dependent on an essential process that is deficient at cold temperatures (PLENEFISCH *et al.* 1989). If this model is correct, then why are *dpy-30* null mutations heat sensitive? Perhaps at 15° *dpy-30* null mutations do not disrupt dosage compensation to the same degree as mutations in other dosage compensation genes, and the additional lethality at 20° reflects a defect in an additional process that requires *dpy-30*. Alternatively, the absence of *dpy-30* causes the dosage compensation process itself to be temperature sensitive.

Is *dpy-30* involved in a second process?: Although the pleiotropic phenotypes caused by mutations in *dpy-30* highlight the fundamental differences between it and the other *dpy* genes, they fail to suggest the basis for the differences. It is formally possible that *dpy-30* mutations only affect *X* chromosome expression, but in a manner that affects both *XX* and *XO* animals. This possibility seems somewhat unlikely, since the *XX*-specific lethality caused by a *dpy-30* mutation is more extreme than the lethality caused by a null mutation in any single *sdc* or *dpy* gene. Moreover, we have been unable to demonstrate any significant alteration of *X* chromosome expression in *dpy-30 XO* animals. In contrast, the alteration of *X* chromosome expression in *dpy-21 XO* animals, which are mating proficient and appear phenotypically wild-type, was easy to demonstrate (DELONG *et al.* 1987; MENEELY and WOOD 1987; MEYER and CASSON 1986). An appealing alternative possibility is that the pleiotropies caused by *dpy-30* mutations might reflect *dpy-30*'s involvement in an additional process unrelated to dosage compensation. The additional lethality of *XX* animals and all the phenotypes of *XO* animals might be caused by the disruption of that second process. This process could be as global as general transcription or as specific as another developmental process. Precedent for the involvement of sex determination and dosage compensation genes in multiple developmental processes comes from the analysis of these two pathways in Drosophila. Molecular genetic studies of *sisterless-b*, *runt* and *deadpan*, all *X* chromosome elements of the *X*/A ratio, and of *daughterless* and *extramacrochaetae*, maternally supplied genes required for transduction of this primary signal, have revealed that these genes also have essential functions in neurogenesis or in the case of *runt*, in segmentation (CLINE 1989, 1993). The pleiotropic phenotypes caused by mutations in these Drosophila genes reflects the requirement for their wild-type activity in different tissues at different times in development. This observation begs the question of whether the pleiotropies associated with *dpy-30* mutations result from a need for *dpy-30*(+) activity at single or multiple times and places during development. Detailed studies examining the temporal and spatial expression of *dpy-30*, and the relationship of the expression patterns to the observed phenotypes will be essential in distinguishing among these possibilities and should provide insight into the basis of the *dpy-30*-associated pleiotropies.

Effects of *dpy-30* mutations on sexual phenotype: While *dpy-30* mutations do not affect the sexual development of otherwise wild-type animals, they can have a dramatic effect on the sexual fate of animals harboring a sex determination mutation or an intermediate *X*/A ratio. Paradoxically, depending on the genetic background, *dpy-30* mutations can cause either masculinization or feminization. The fact that *dpy-30* mutations can influence sexual fate raised the possibility that *dpy-30* might act directly in the sex determination process. However, for reasons that will be detailed in this

TABLE 5

A comparison of dosage compensation mutations that affect sexual fate

Mutation in gene class[a]	Genetic background			
	2X/3A	*her-1*(d)XX	*sdc-3*(Tra)*XX*	xol-1 *XO*
None	Masculinized	Masculinized	Masculinized	Variably feminized
sdc gene	Enhanced masculinization[b]	Enhanced masculinization[b,c,]	ND	Masculinized[c,d]
dpy-30	Feminized	Feminized	Feminized	Masculinized
Maternal-effect *dpy* gene	Feminized[e]	Feminized[c,e]	Feminized[c]	Feminized[d]

This table compares the effects of *dpy-30* mutations with the known effects of mutations in the *sdc* and the dosage compensation *dpy* genes on sexual phenotype.

[a] At least a single mutation in each gene class has been examined in the genetic backgrounds listed.

[b] Data from PLENEFISCH (1990).

[c] Data from DELONG *et al.* (1993).

[d] Data from MILLER *et al.* (1988).

[e] Data from PLENEFISCH *et al.* (1989).

section, we favor the hypothesis that these sex effects arise as an indirect consequence of the dosage compensation defect caused by *dpy-30* mutations, rather than as a reflection of a direct role for *dpy-30* in the sex determination process.

The masculinizing activity of *dpy-30* mutations was revealed through the analysis of *dpy-30; xol-1* double mutants. Like null mutations in the *sdc* genes, *dpy-30* mutations have the ability to suppress both the lethality and the feminization caused by a *xol-1* mutation, suggesting that loss of *dpy-30* function might cause a cryptic defect in the hermaphrodite mode of sex determination. Precedent for such a cryptic defect was recently established with the characterization of the *sdc-3* gene (DELONG *et al.* 1993; KLEIN and MEYER 1993). *XX* animals homozygous for *sdc-3* null mutations show no signs of masculinization despite eliminating both the sex determination and dosage compensation functions of the *sdc-3* gene. The cryptic sex determination defect in *sdc-3* mutants was established in part by the observation that *sdc-3; xol-1* XO animals are male. It was corroborated by the identification of *sdc-3* mutations that specifically affect sex determination and cause *XX* animals to be masculinized. In general, however, extensive characterization of *xol-1* suppressor mutations shows that the ability to masculinize *xol-1* XO animals is a necessary, but insufficient criterion to demonstrate a defect in the hermaphrodite mode of sex determination.

Several observations indicate that the sexual phenotype of viable *xol-1* XO animals can be influenced by factors possessing no direct relationship to the sex determination process. For example, the sexual phenotype of the rescued *xol-1* XO animals can be influenced by the degree to which the *xol-1* dosage compensation defect (*i.e.*, reduction in *X* chromosome expression) has been suppressed in XO animals (DELONG *et al.* 1993; MILLER *et al.* 1988). When *X* chromosome expression is restored to nearly wild-type levels by a mutation in *dpy-27*, all the *xol-1* XO animals are rescued, and all are hermaphrodite. In contrast, in *dpy-27/+; xol-1* XO animals, the *xol-1* dosage compensation defect is only weakly suppressed. As a consequence, very few *dpy-27/+; xol-1* XO animals are viable, and essentially all viable animals are masculinized (DELONG *et al.* 1993). In a similar vein, it is possible that *dpy-30* mutations do not fully correct the reduction in *X* chromosome expression caused by *xol-1* mutations and therefore indirectly cause *dpy-30; xol-1* XO animals to be male. As a second example, the *X* chromosome karyotype of a *dpy; xol-1* parent influences the sexual fate of its *dpy; xol-1* XO progeny; the proportion of masculinized *dpy; xol-1* XO progeny is greater if their parent is XO in karyotype (MILLER *et al.* 1988). While we do not know the mechanism by which the sexual fate of rescued *xol-1* XO animals is established, or why the *dpy-30; xol-1* XO animals are male, these examples clearly demonstrate the need for an independent means of establishing a sex determination defect in *dpy-30* mutants.

Additional experiments directed at assessing the role of *dpy-30* in the sex determination process failed to reveal masculinization of *dpy-30 XX* mutants, demonstrating instead that a *dpy-30* mutation causes feminization of *XX* animals with a sexually ambiguous identity. For example, we have shown that a *dpy-30* mutation feminizes animals with an intermediate *X*/A ratio. Whereas 2*X*/3A animals (*X*/A = 0.67) are generally male, most 2*X*/3A animals carrying a single copy of a *dpy-30* mutation are hermaphrodite. In addition, a *dpy-30* mutation strongly suppresses the masculinization caused by the *her-1*(sd) or the *sdc-3*(Tra) mutations. The feminizing activity observed in these genetic backgrounds distinguishes *dpy-30* mutations from *sdc* mutations, which tend to enhance masculinization, and further establishes the similarity between *dpy-30* and the other dosage compensation *dpy* genes (Table 5). The genetic characterization of *dpy-21*, *dpy-26*, *dpy-27* and *dpy-28* established considerable precedent for the ability of mutations that disrupt only dosage compensation to feminize 2*X*/3A, *her-1*(sd)*XX*, or *sdc-3*(Tra) *XX* animals (DELONG *et al.* 1993; HODGKIN 1983; PLENEFISCH *et al.*

1989; TRENT *et al.* 1988). Rather than revealing a direct role for these genes in the control of sex determination, the observed feminization most likely reveals a regulatory feedback mechanism that translates a disruption in dosage compensation into a change in sexual fate. Since *dpy-30* mutations disrupt dosage compensation in *XX* animals, it is likely that their feminizing activity occurs through the same mechanism. All evidence considered, however, *dpy-30* mutations behave neither entirely like *sdc* mutations nor entirely like maternal-effect *dpy* mutations, thus presenting an ambiguous picture of the relationship between *dpy-30* and the sex determination process.

In light of the paradoxical effects of *dpy-30* mutations on sexual fate, what may we conclude about the normal role of *dpy-30* in hermaphrodite sexual development? Based on the overall similarity between *dpy-30* and the other maternal-effect *dpy* genes, we favor the hypothesis that *dpy-30* is not required for the hermaphrodite mode of sex determination and that both the masculinizing and feminizing effects of *dpy-30* mutations arise as indirect consequences of their effects on *X* chromosome expression. Regardless of which model proves correct, the analysis of the effects of *dpy-30* mutations on sexual fate reveals that there is complex interplay between the dosage compensation and sex determination processes in *C. elegans* that has no precedent in other systems. Furthermore, the existence of these complexities suggests that the simple linear pathway proposed for the control of sexual development in *C. elegans* is not sufficient to account for all the interactions between the sex determination and dosage compensation processes.

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