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Genes That Implement the Hermaphrodite Mode of Dosage Compensation in *Caenorhabditis elegans*

John D. Plenefisch, Leslie DeLong and Barbara J. Meyer

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT

We report a genetic characterization of several essential components of the dosage compensation process in *Caenorhabditis elegans*. Mutations in the genes *dpy-26*, *dpy-27*, *dpy-28*, and the newly identified gene *dpy-29* disrupt dosage compensation, resulting in elevated *X*-linked gene expression in *XX* animals and an incompletely penetrant maternal-effect *XX*-specific lethality. These *dpy* mutations appear to cause *XX* animals to express each set of *X*-linked genes at a level appropriate for *XO* animals. *XO dpy* animals are essentially wild type. Both the viability and the level of *X*-linked gene expression in *XX* animals carrying mutations in two or more *dpy* genes are the same as in animals carrying only a single mutation, consistent with the view that these genes act together in a single process (dosage compensation). To define a potential time of action for the gene *dpy-28* we performed reciprocal temperature-shift experiments with a heat sensitive allele. The temperature-sensitive period for lethality begins 5 hr after fertilization at the 300-cell stage and extends to about 9 hr, a point well beyond the end of cell proliferation. This temperature-sensitive period suggests that dosage compensation is functioning in *XX* animals by mid-embryogenesis, when many zygotically transcribed genes are active. While mutations in the *dpy* genes have no effect on the sexual phenotype of otherwise wild-type *XX* or *XO* animals, they do have a slight feminizing effect on animals whose sex-determination process is already genetically perturbed. The opposite directions of the feminizing effects on sex determination and the masculinizing effects on dosage compensation caused by the *dpy* mutations are inconsistent with the wild-type *dpy* genes acting to coordinately control both processes. Instead, the feminizing effects are most likely an indirect consequence of disruptions in dosage compensation caused by the *dpy* mutations. Based on the cumulative evidence, the likely mechanism of dosage compensation in *C. elegans* involves reducing *X*-linked gene expression in *XX* animals to equal that in *XO* animals via the action of the *dpy* genes.

DOSAGE compensation is an important aspect of sexual dimorphism that equalizes the expression of sex-linked genes between males and females, whose relative dose of such genes differs as a consequence of the primary sex-determining signal. While the mechanisms used to achieve dosage compensation are diverse, they share the property of coordinately modulating the expression of an array of genes related simply by their location on a sex chromosome. To ensure that animals of different sexes express their sex-linked genes at the correct level, the developing zygote must choose and implement an appropriate mode of dosage compensation.

In *Caenorhabditis elegans* both the proper mode of dosage compensation and the choice of sexual fate (male or hermaphrodite) appear to be set in response to the same primary sex-determining signal, the *X/A* ratio (the ratio of *X* chromosomes to sets of autosomes) (NIGON 1951; MADL and HERMAN 1979; VILLENEUVE and MEYER 1987; MILLER *et al.* 1988). In *XX* animals the genes *sdc-1* (VILLENEUVE and MEYER 1987) and *sdc-2* (C. NUSBAUM and B. MEYER, unpublished observations) initiate the hermaphrodite modes of both sex determination and dosage compensation, which are then implemented through separate pathways (Figure 1) [see HODGKIN (1987a) for review of the sex determination pathway]. While mutations in either *sdc-1* or *sdc-2* have no apparent effect in *XO* animals, in *XX* animals they shift both the sex determination and dosage compensation processes to the male modes, resulting in masculinization and elevated levels of *X*-linked gene transcripts. *XX sdc-2* mutants are inviable, suggesting that the failure to achieve proper *X* chromosome expression can be lethal. At least part of the machinery that ensures proper dosage compensation of *X*-linked genes in *XX* animals includes the genes *dpy-21*, *dpy-26*, *dpy-27*, *dpy-28*, and the newly identified gene *dpy-29* (HODGKIN 1983; MEYER and CASSON 1986; MENEELY and WOOD 1987; DELONG, CASSON and MEYER 1987). These dosage compensation *dpy* genes will hereafter be referred to as *dpy* genes. Mutations in *dpy-26*, *dpy-27*, *dpy-28* and *dpy-29* elevate *X*-linked gene expression in *XX* animals and result in an *XX*-specific maternal-effect lethality, presumably due to a lethal disruption of dosage compensation. Infrequent *dpy XX* animals escape the lethality and mature

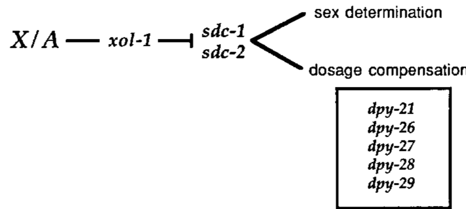


FIGURE 1.—Proposed model for the control of dosage compensation and sex determination in *C. elegans*. According to this previously proposed model (MILLER *et al.* 1988), *xol-1* controls the male (*XO*) modes of both dosage compensation and sex determination by negatively regulating the *sdc-1* and *sdc-2* gene activities. Mutations in *xol-1* shift both processes to the hermaphrodite modes, resulting in the feminization and death (presumed to be caused by the observed reduction in *X*-linked gene transcript levels) of *XO* animals. *sdc-1* (VILLENEUVE and MEYER 1987) and *sdc-2* (C. NUSBAUM and B. MEYER, unpublished observations) control the hermaphrodite (*XX*) modes of sex determination and dosage compensation; mutations in these genes shift both processes to the male modes, resulting in masculinization and elevated *X*-linked gene transcript levels in *XX* animals. *XX* animals carrying strong *sdc-2* mutations are inviable. The genes *dpy-21*, *dpy-26*, *dpy-27*, *dpy-28* and *dpy-29* are essential for the hermaphrodite mode of dosage compensation (HODGKIN 1983; MEYER and CASSON 1986; MENEELY and WOOD 1987; DELONG, CASSON and MEYER 1987; this report) and appear to act downstream of the point where the two pathways diverge (MILLER *et al.* 1988). The temperature-sensitive period we demonstrate for *dpy-28* is later than the temperature-sensitive period for *sdc-1* (A. VILLENEUVE, personal communication), consistent with the proposal that *sdc-1* acts prior to *dpy-28* to activate the hermaphrodite mode of dosage compensation mediated, in part, through *dpy-28*. While we can demonstrate that the *dpy* genes act in a common process, we cannot as yet place them in a gene hierarchy.

into fertile, dumpy hermaphrodites. *XO* animals are essentially wild type. Mutations in *dpy-21* result in *XX* animals that are dumpy in phenotype and exhibit an elevation in *X*-linked transcripts. Unlike the *dpy-26*, *dpy-27*, *dpy-28*, or *dpy-29* mutant *XX* animals, the *dpy-21* mutant *XX* animals are mostly viable. Moreover, although *dpy-21 XO* animals appear phenotypically wild type, they exhibit altered *X*-linked gene expression, unlike the other *dpy XO* animals.

In *XO* animals the male modes of both sex determination and dosage compensation are coordinately controlled at least in part through the gene *xol-1* (MILLER *et al.* 1988). Mutations in *xol-1* shift both processes to the hermaphrodite mode, resulting in feminization and reduced *X* chromosome expression, the presumed cause of death of *xol-1 XO* animals. Genetic data suggest that *xol-1* controls the male modes by negatively regulating the gene activities known to direct the *XX* modes of sex determination and dosage compensation (*sdc-1* and *sdc-2*). In *xol-1 XO* mutants inappropriate expression of the hermaphrodite-specific genes that affect dosage compensation and sex determination (*sdc-1* and *sdc-2*) or dosage compensation (*dpy* genes) results in lethality.

To understand more completely the dosage compensation process in *C. elegans* and ultimately the nature of the early events that coordinately control both sex determination and dosage compensation, we have performed a genetic analysis of genes essential for the hermaphrodite mode of dosage compensation including *dpy-26*, *dpy-27*, *dpy-28* and the newly identified gene *dpy-29*. The analysis involves a detailed characterization of the phenotypes and time of action of these dosage compensation *dpy* genes and the interactions of these genes with each other or with other genes involved in *X* chromosome expression or sex determination. The identification and genetic characterization of these *dpy* genes provides the background for a molecular analysis to determine how the dosage compensation process is established and implemented in *C. elegans.*

MATERIALS AND METHODS

Strains and maintenance: Techniques used for culturing *C. elegans* were as described by BRENNER (1974). All experiments were performed, except where noted, at 20°. Bristol strain N2 was the wild-type parent for all strains except *dpy-21(y87)*, which was derived from TR403, a wild isolate provided by the laboratory of P. ANDERSON (personal communication). In experiments where maternal and zygotic genotypes are important we occasionally use the terms m^+, m^-, z^+, or z^-, where *m* indicates maternal genotype, *z* indicates the zygotic genotype, and + or − indicates the presence or absence of the wild-type allele of a given gene. For example, m^+z^- animals refer to homozygous mutant progeny of a heterozygous mother. We designate karyotypes using the form *nX;mA*, where *n* and *m* are the number of copies of *X*-chromosomes and sets of autosomes respectively; this format facilitates comparison of the different *X/A* ratios in these animals. All other nomenclature used in this paper conforms to that of HORVITZ *et al.* (1979). The following mutations and chromosomal aberrations were used in the present study:

LG *III*: *dpy-1(e1)*; *unc-93(e1500, e1500n234am)*; *dpy-27 (rh18, y42, y44, y49, y56am, y57)*; *emb-1(hc57)*; *emb-2 (hc58)*; *emb-5(hc61)*; *emb-7(hc66)*; *emb-24(g40)*; *emb-32 (g58)*; *emb-33(g60)*; *unc-103(e1597)*; *dpy-17(e164)*; *unc-36 (e251)*; *unc-32(e189)*; *emb-9(hc70)*; *emb-16(g19)*; *emb-25 (g45)*; *emb-30(g53)*; *emb-34(g62)*; *unc-50(e306)*; *dpy-28 (y1ts, s939)*; *vab-7(e1562)*; *tra-1(e1099, e1835e1816e1575)*; *dpy-18(e364am, e499)*; *eDp6 (III;f)*; *mnDp37(III;f)*; *eDf2*.

LG *IV*: *unc-22(e66)*; *dpy-26(n198, n199, y6, y65)*; *unc-30(e191)*; *yDp1(IV,V;f)*.

LG *V*: *her-1(e1520, n695sd, y8, y10, y14, y69, y71)*; *unc-42(e270)*; *sqt-3(sc63)*; *him-5(e1490)*; *unc-61(e228)*; *dpy-29 (y100am)*; *unc-76(e911)*; *dpy-21(e428, e459, y47am, y87, y88)*.

LG *X*: *xol-1(y9, y70, y95)*; *lin-14(n179)*; *sup-7(st5)*.

mnDp37 was isolated and provided by R. HERMAN and C. KARI (personal communication). *dpy-27(rh18)* was isolated by E. HEDGECOCK; the genetic mapping and initial analysis were performed by J. HODGKIN (personal communication). The isolation and characterization of *dpy-21(e428)* and *dpy-26(n198, n199)* are described in HODGKIN (1983). *dpy-26(y6)* is described in DELONG, CASSON and MEYER (1987). The characterization of *xol-1(y9, y70, y95)* is in MILLER *et al.* (1988). The other genes and alleles can be found in HOGDKIN *et al.* (1988).

Isolation of mutants: *dpy-27(y42, y44, y49, y56, y57)*, *dpy-26(y65)*, *dpy-21(y47, y88)*, and *dpy-29(y100)* were isolated as ethyl methanesulfonate (EMS) induced suppressors of the

XO-specific lethality of *xol-1(y9)* (MILLER *et al.* 1988). *dpy-21(y87)* arose spontaneously in a *him-5 xol-1(y9)* derivative of TR403, a mutator strain in which transposition of several nematode mobile elements is active (J. COLLINS and P. ANDERSON personal communication). The *dpy-27* mutations were shown to be allelic with *dpy-27* by their failure to complement *dpy-27(rh18)* and by their map position in the *unc-93 dpy-17* interval on LG *III*. *y65* was shown to be an allele of *dpy-26* by its failure to complement *dpy-26(n199)* and its map position between *unc-22(e66)* and *unc-30(e191)* on LG *IV*. The *dpy-21* mutations were shown to be allelic to *dpy-21* by their map position and their failure to complement *dpy-21(e428)*.

dpy-28(y1) was isolated in a screen for mutations with sex-specific phenotypes. L4 hermaphrodites homozygous for *him-5(e1490)*, which results in the production of 30% *XO* male progeny due to meiotic nondisjunction of the *X* chromosome (HODGKIN, HORVITZ and BRENNER 1979), were mutagenized with EMS (BRENNER 1974), picked onto individual plates, and allowed to produce self progeny. F_1 L4 hermaphrodites (2500) were picked onto individual plates and 6 F_2 hermaphrodites from each F_1 were subsequently picked onto separate plates. Approximately 15,000 F_2 broods, equivalent to 4110 haploid genomes, were screened for evidence of mutants with sex-specific phenotypes. This calculation takes into account the fact that mutants would be found only if the F_2 mother were homozygous for a particular mutation. *dpy-28(y1)* was isolated from a brood in which all the hermaphrodites were dumpy and the males wild type. This screen also yielded one allele of *sdc-1(y4)*, one allele of *her-1(y8)*, and a maternal-effect *XO*-specific lethal mutation *y2II*. *dpy-28(s939)* was isolated by D. BAILLIE and subsequently shown to be an allele of *dpy-28* by its failure to complement *dpy-28(y1)* and its map position between *unc-50* and *dpy-18* on LG *III*.

Genetic map positions of *dpy-28* and *dpy-29*: *dpy-28(y1)* was mapped to LG *III* on the basis of linkage to *unc-32* (data not shown). The map position for *dpy-28*, shown in Figure 2, was assigned on the basis of the following three factor recombination data: 4/4 Dpy-17 non-Unc recombinants from *dpy-17 unc-50/dpy-28* hermaphrodites segregated *dpy-28*; 11/11 Unc non-Dpy recombinants from *dpy-17 unc-50/dpy-28* hermaphrodites failed to segregate *dpy-28*, thus placing *dpy-28* to the right of *dpy-17*. Five of eight Unc non-Dpy recombinants from *unc-50 dpy-18/dpy-28* hermaphrodites and 2/7 Dpy-18 non-Unc recombinants from *unc-50 dpy-18/dpy-28* hermaphrodites segregated *dpy-28*, placing *dpy-28* between *unc-50* and *dpy-18*. Nine of nine Unc non-Vab recombinants and 2/13 Vab non-Unc recombinants from *unc-32 vab-7/dpy-28* hermaphrodites produced broods that segregated *dpy-28*, placing *dpy-28* to the left of *vab-7*. In addition, *dpy-28 dpy-18*; *eDp6* animals have a Dpy-28 non-Dpy-18 phenotype, whereas *dpy-28 dpy-18/eDf2* animals have a Dpy-18 non-Dpy-28 phenotype, indicating *dpy-28* is to the left of the endpoints of both *eDf2* and *eDp6*.

dpy-29(y100) was mapped to LG *V* on the basis of linkage to *unc-76* (data not shown). *dpy-29* was positioned between *unc-61* and *unc-76* (Figure 2) on the basis of the following three-factor data: 2/9 Unc Rol recombinant progeny of *him-5 dpy-29/sqt-3 unc-76* animals segregated *dpy-29* animals, and 3/5 Unc-61 non-Unc-76 recombinant progeny of *unc-61 unc-76/dpy-29* hermaphrodites segregated *dpy-29*.

A number of temperature-sensitive, maternal-effect lethal mutations, hereafter referred to as *emb* mutations, are located on LG *III*. To eliminate the possibility that mutations in *dpy-27* or *dpy-28* are allelic with mutations in any of these *emb* genes, complementation tests were performed between *dpy-27* and *emb-1(hc57)*, *emb-2(hc58)*, *emb-5(hc61)*, *emb-7(hc66)*, *emb-24(g40)*, *emb-32(g58)*, and *emb-33(g60)* and between *dpy-28* and *emb-9(hc70)*, *emb-16(g19)*, *emb-25(g45)*, *emb-30(g53)*, and *emb-34(g62)*. *unc-93(e1500) dpy-27/+ dpy-27* or *unc-32 dpy-28/+ dpy-28* males were mated with the respective *emb* hermaphrodites at 15° (the permissive temperature). Individual progeny of these animals were allowed to lay eggs for 24 hr at 15° and then shifted to 24° (the restrictive temperature for the *emb* mutations) to lay the remainder of their eggs. We identified *emb/dpy* cross progeny by the presence of Unc animals in the broods; the broods of these cross progeny were scored at 24° for evidence of either the *dpy* or *emb* phenotypes, *i.e.*, lethality or dumpiness. In all cases it was possible to maintain a hearty *emb/dpy* strain at 24°, indicating that the *embs* and *dpy-27* or *dpy-28* represent separate genes.

Genetic assay for effect on *X* chromosome gene expression: The *lin-14* assay is used to measure perturbations in *X*-chromosome expression and is described in detail in DELONG, CASSON and MEYER (1987); a brief summary is given here. *lin-14(n179)* is an *X*-linked hypomorphic mutation that results in the precocious occurrence of certain developmental events (AMBROS and HORVITZ 1987). For the purposes of this assay we examine the seam cells, a set of lateral hypodermal cells. In wild-type animals the seam cells divide during the L3 larval molt, cease cell division in the late L4 larval stage, and generate adult cuticular alae. In *lin-14(n179)* animals, many seam cells fail to divide in late L3 and instead produce precocious adult alae. Mutations that alter *X*-linked gene expression change the fraction of cells in each animal that express the mutant phenotype. The assay is quantitated using Nomarski optics to determine the fraction of cells in each animal that fail to divide following the L3 molt. For each animal scored we calculate the fraction of seam cell nuclei expressing the mutant phenotype (numbers of seam cell nuclei that fail to divide)/(total number of seam cell nuclei). Mutant strains are compared statistically using the Mann-Whitney *U* test (SOKAL and ROHLF 1981) to compare the scores obtained for individuals from each strain. In addition, for each strain we calculate the mean value of seam cells expressing the mutant phenotype (percent mutant nuclei) by combining all values from individual animals. In the *lin-14* assay, increased *X*-linked gene expression is reflected by a decrease in the fraction of cells expressing the mutant phenotype; reduced *X*-linked gene expression is reflected by an increase in this fraction. Suppression of *lin-14(n179)* is measured at 24° where the mutant phenotype is strong, and enhancement is measured at 20° where the mutant phenotype is weak.

dpy-26 and *dpy-28* homozygous hermaphrodite progeny from heterozygous mothers were identified as Unc progeny from the respective strains *dpy-28(y1* or *s939) unc-32/++*; *lin-14* and *dpy-26(y6* or *n199) unc-30/++*; *lin-14*. Scored animals were transferred to individual plates at 20° and their genotype confirmed by the phenotypes of their progeny. For *dpy-21*, *dpy-27* and *dpy-29*, random progeny from the strains *dpy-21(e428)/+*; *lin-14* or *dpy-27(y49)/+*; *lin-14* or *dpy-29(y100)/unc-76*; *lin-14* were scored for their *lin-14* phenotype and then transferred to individual plates at 20°. The genotype of each animal was inferred from the phenotypes of the brood it produced. This method eliminated any phenotypic bias in selecting animals to be scored.

Hermaphrodites of genotype *dpy-28(y1)*; *dpy-21(e428)*; *lin-14* and *dpy-27(rh18) dpy-28(y1)*; *lin-14* and *dpy-26(n199)*; *dpy-21(e459)*; *lin-14* undergo ecdysis prior to the completion of seam cell nuclear division. Thus, these animals were picked onto individual slides and monitored until seam cell divisions were complete.

dpy-29(y100); *lin-14* males were obtained by mating *dpy-*

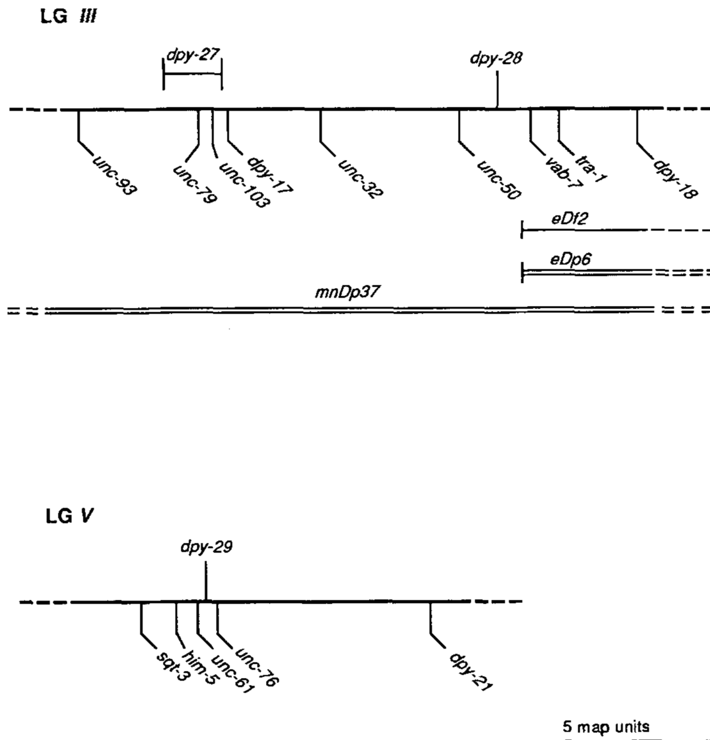


FIGURE 2.—Genetic map positions of *dpy-28* and *dpy-29*. The maps represent the portions of LG *III* and LG *V* that contain *dpy-28* and *dpy-29*. The map positions of *dpy-28* and *dpy-29* are based on the data provided in the text. The relative positions of the surrounding genes are from HODGKIN *et al.* (1988). Chromosomal deficiencies (*eDf2*) and duplications (*eDp6* and *mnDp37*) are drawn below the map.

29(y100) males with Dpy hermaphrodites from the strain *dpy-29(y100)/unc-76; lin-14*. The genotype of the parental hermaphrodites was confirmed by checking to be sure that only Dpy hermaphrodite cross progeny grew up on the mating plate. *dpy-27(rh18); dpy-21(e428); lin-14* males were obtained by mating *dpy-27(rh18); dpy-21(e428)* males with *dpy-27(rh18); dpy-21(e428); lin-14* hermaphrodites. *lin-14* males were obtained by mating N2 males with *lin-14* hermaphrodites. All other males were obtained by using spontaneous males to establish mating male/hermaphrodite strains at 20°.

***sup-7* suppression assays:** All *dpy-27*, *dpy-28*, and *dpy-29* alleles were tested for suppression by the amber suppressor tRNA mutation *sup-7(st5)* (WATERSTON 1981, WILLS *et al.* 1983) at 24°. *sup-7* males were mated with *unc-93 dpy-27*, or *unc-32 dpy-28*, or *dpy-29 unc-76* hermaphrodites. Outcross hermaphrodites were picked onto separate plates and their progeny were scored for the presence of Unc non-Dpy animals. *dpy-27(rh18, y42, y44, y49, y57)* and *dpy-28(y1, s939)* were not suppressed by *sup-7*. This was demonstrated by the fact that *unc dpy*/++; *sup-7*/+ hermaphrodites produced many Unc Dpy progeny but rare Unc non-Dpy progeny. These rare Unc non-Dpy hermaphrodites were shown to be recombinants. The Unc Dpy animals produced arrested L1 larvae and infrequent Unc Dpy progeny. *unc-93 dpy-27(y56)*/++; *sup-7*/+ animals, however, segregated Unc non-Dpy progeny, indicating that *sup-7* suppresses the Dpy phenotype of *dpy-27(y56)*. These potential *unc-93 dpy-27(y56); sup-7* hermaphrodites were mated with *dpy-27(y56)* males; the resulting outcross hermaphrodites were semi-Dpy, suggesting that a single copy of *sup-7* suppresses *dpy-27(y56)* only weakly at 24°. These semi-Dpy hermaphrodites produced both Dpy and non-Dpy animals. The non-Dpy animals were picked onto individual plates and those failing to segregate Unc animals became the founders of *dpy-27(y56); sup-7* strains. *dpy-27(y56); sup-7* L4s were shifted to 20° for progeny counts; 277 of 693 zygotes (40%) survived to adulthood, and were wild type in length. Thus *dpy-27(y56)* appears to be an amber mutation strongly suppressible by two copies of *sup-7*. [The *dpy-27(y56); sup-7* zygotes that did not survive to adulthood displayed a terminal phenotype unlike that seen in *dpy-27* (arrested L1 larvae) but similar to that reported for *sup-7* (dead eggs), which causes a cold-sensitive lethality (WATERSTON 1981). Thus the likely cause of death in the *dpy-27; sup-7* strain is the deleterious effects of *sup-7*.]

Unc and Dpy Unc progeny of *dpy-29(y100) unc-76*/++; *sup-7*/+ were picked onto individual plates. While 35/45 of the Unc animals produced broods consisting of greater than 15 adult non-Dpy Unc progeny, the Dpy Unc progeny produced >95% inviable animals. These results suggest that one copy of *sup-7* suppresses at least the Dpy phenotype of *dpy-29(y100)*. We constructed a *dpy-29; sup-7* strain by mating *dpy-29/unc-76; sup-7* males with *unc-76; sup-7* hermaphrodites and picking the non-Unc (*dpy-29/unc-76; sup-7*) hermaphrodite progeny. These animals segregated both Unc and non-Dpy non-Unc progeny; the non-Dpy non-Unc hermaphrodite progeny were picked onto separate plates. *dpy-29; sup-7* animals were identified as those that did not segregate the *unc* marker in their broods. The *dpy-29; sup-7* animals are wild type in appearance and viable, demonstrating that *sup-7* in two copies suppresses the Dpy and lethal phenotypes of *dpy-29(y100)*.

The newly isolated alleles of *dpy-21(y47, y87, y88)* were also tested for suppression by *sup-7(st5)* at 22.5°. *dpy-21/unc-76; sup-7* hermaphrodites were constructed by mating *dpy-21/unc-76; sup-7* males with *unc-76; sup-7* hermaphrodites and picking the non-Unc (*dpy-21/unc-76; sup-7*) hermaphrodite progeny. *dpy-21(y87* or *y88)/unc-76; sup-7* animals segregated Unc hermaphrodites, non-Dpy non-Unc animals, and Dpy non-Unc hermaphrodites; the latter class only segregated Dpy hermaphrodites and were thus presumed to be *dpy-21(y87* or *y88); sup-7*. Thus *dpy-21(y87, y88)* are not suppressed by *sup-7*. In contrast, *dpy-21(y47)/unc-76; sup-7* animals segregated only Unc hermaphrodites and non-Dpy non-Unc hermaphrodites. The non-Dpy non-

Unc hermaphrodites were picked onto individual plates and 7/28 failed to segregate Unc animals. Complementation tests revealed that *dpy-21(y47)* was homozygous in these non-Dpy non-Unc strains, indicating that *dpy-21(y47)* is suppressible by *sup-7*.

Temperature-shift experiments: At 15° 80% of the hermaphrodite self progeny of *dpy-28(y1)* homozygotes reach adulthood; at 20° only 18% survive (see Table 2). The temperature-sensitive period of *dpy-28(y1)* was determined by shifting synchronized embryos of various stages from either permissive to restrictive temperature or *vice versa*. For the downshift experiments, adult *dpy-28(y1)* hermaphrodites were raised at 20°. Animals were placed on fresh plates and allowed to lay eggs for 1 hr, after which the parent animals were removed. The eggs were incubated at 20° for defined periods of time and then shifted to 15°, at which time the eggs were counted and their developmental stages noted. A group of eggs was defined as being at a particular developmental stage when half the eggs were at or beyond that stage and half prior to the stage. In general, the eggs of any given cohort differed by no more than 3 hr (*i.e.*, all the eggs on any given plate passed through comma stage or hatched within three hours of each other). Shift times are expressed relative to fertilization; the time of fertilization was estimated to be seven hours prior to comma stage based on the data of SULSTON *et al.* (1983). At 20° the length of time between comma stage and hatching is the same for wild-type and *dpy-28* animals, indicating that their developmental clocks are the same. Since *dpy-28(y1)* animals are Egl and thus usually release eggs late, cohorts shifted prior to two hours of development were obtained by shifting the zygotes while still *in utero*. To obtain these time points, *dpy-28(y1)* hermaphrodites raised at 20° were shifted to 15°. At defined times after the shift these animals were transferred to fresh plates (still at 15°) and allowed to lay eggs for 1 hr. All cohorts were kept at 15° after the shift, and the percentage of resulting viable adults was determined.

Upshifts were performed in a manner similar to downshifts except for the following differences: the *dpy-28(y1)* adults were raised at 15°; each cohort consisted of eggs laid within a 2-hr interval; the eggs were shifted to 20° and maintained at that temperature. The upshift data were normalized to the downshift data by scaling the period between comma stage and hatching for the upshift cohorts to that for the downshift cohorts. All shift points are described in terms of development at 20°; this yields an approximately 2:1 ratio between the 15° and 20° developmental clocks.

The use of cohorts was required because the manipulation of individual eggs tended to result in high levels of nonspecific lethality. The use of cohorts has the effect of smoothing the temperature-shift curves but has no effect on the position of the curves relative to the time axis.

The cold-sensitive period for *dpy-27(y57)* was determined by a procedure similar to that for *dpy-28(y1ts)*.

Construction of strains with multiple mutations in dosage compensation genes: *dpy-27 dpy-28*: Dpy-27 non-Unc recombinants were picked from among the progeny of *dpy-27 unc-32/dpy-28* heterozygotes. Dpy non-Unc progeny of these recombinants were picked to establish potential *dpy-27 dpy-28* double mutant strains. Strains that segregated males at a high frequency (Him phenotype characteristic of *dpy-28* mutants) and no Unc animals were tentatively identified as homozygous *dpy-27 dpy-28* strains. The genotypes were confirmed by complementation tests with *dpy-27* and *dpy-28*.

dpy-27; *dpy-29* or *dpy-28*; *dpy-29* animals were constructed by picking Dpy non-Unc progeny from hermaphrodites of genotype *dpy-27 unc-32/++*; *dpy-29/+* or *unc-32 dpy-28/++*; *dpy-29/+*. Individual Dpy Unc progeny of these Dpy animals were picked to establish potential *dpy-27 unc-32*; *dpy-29* and *unc-32 dpy-28*; *dpy-29* strains. The genotypes of these strains were confirmed by complementation tests with *dpy-29* and *dpy-27* or *dpy-28*.

dpy-28; *dpy-26* animals were constructed by picking slightly Dpy, Egl, non-Unc segregants from heterozygous hermaphrodites of genotype *dpy-28/+*; *dpy-26 unc-30/++*. The Dpy Unc progeny in their broods were picked as potential *dpy-28*; *dpy-26 unc-30* animals. Animals from the resulting strains were tested for failure to complement *dpy-28* and *dpy-26*.

dpy-27; *dpy-26* animals were constructed by picking Dpy progeny from *dpy-27/+*; *dpy-26/+* hermaphrodites. Animals that displayed a Him phenotype (characteristic of *dpy-26* mutants) were shown to be of the correct genotype by complementation tests with *dpy-27* and *dpy-26*.

To construct *dpy-27*; *dpy-21* and *dpy-28*; *dpy-21*, and *dpy-27 dpy-28*; *dpy-21* animals we used the following scheme. *dpy-21* hermaphrodites marked on LG *III* with either *unc-93* or *unc-103* were mated with either *dpy-27*, *dpy-28*, or *dpy-27 dpy-28* males. Dpy progeny from cross-progeny hermaphrodites were picked onto individual plates. From those that segregated Unc progeny, the non-Unc siblings were picked. Strains descended from animals that no longer segregated the Unc marker were maintained as potential *dpy-27*; *dpy-21* or *dpy-28*; *dpy-21*, or *dpy-27 dpy-28*; *dpy-21* strains. These assignments were confirmed by complementation tests with *dpy-21* and *dpy-27* and/or *dpy-28*. *dpy-26*; *dpy-21* strains were constructed in a similar manner using *unc-30* as the opposing marker. In all these double and triple mutant stocks sufficient escapers arose to maintain the strains.

Construction of strains with *dpy-29*; *mnDp37* and *dpy-28*; *yDp1*: *mnDp37* is a free duplication of most of LG *III*, including wild-type copies of *dpy-1*, *dpy-27*, *unc-36*, and *dpy-28* (R. HERMAN and C. KARI, personal communication, and data not shown). *unc-36/+*; *dpy-29* males were mated with *mnDp37*; *dpy-1 unc-36* hermaphrodites. Non-Dpy-1, non-Unc F_1 hermaphrodites were picked onto individual plates; those that segregated many non-Dpy-1 Unc progeny must have been *mnDp37*; *dpy-1 unc-36/+ unc-36*; *dpy-29/+*. These animals segregated large numbers of non-Unc Dpy-29 hermaphrodites, indicating that extra wild-type copies of both *dpy-27* and *dpy-28* had no effect upon the Dpy phenotype of *dpy-29*. Broods of non-Unc Dpy-29 showed no evidence suggesting rescue of the *dpy-29* maternal-effect lethality by the additional copies of *dpy-27* and *dpy-28* on *mnDp37*.

dpy-28 animals carrying an extra wild-type copy of *dpy-26* provided by the free duplication *yDp1* (DELONG, CASSON and MEYER 1987) were also constructed. *unc-30*; *yDp1* males were crossed with *dpy-28*; *unc-30* hermaphrodites, and non-Dpy non-Unc F_1 hermaphrodites were picked. Random non-Unc progeny were picked from *dpy-28/+*; *unc-30*; *yDp1* animals. The broods of many included only Dpy Egl, non-Unc [*dpy-28(y1)*; *unc-30*; *yDp1 XX*] animals that were phenotypically indistinguishable from *dpy-28(y1)*. There appeared to be no difference in the viability of the *dpy-28*; *unc-30*; *yDp1* hermaphrodites compared to *dpy-28* hermaphrodites.

dpy-26; _dpy-22_ m^-z^- and _dpy-27_; _dpy-22_ m^+z^- and _dpy-28_; _dpy-22_ m^+z^- _XX_ animals are inviable: Males of genotypes *dpy-27 unc-32/dpy-27 +* or *unc-32 dpy-28/+ dpy-28* were mated with *dpy-22* hermaphrodites. Individual F_1 hermaphrodites were picked and numerous Dpy-22 segregants of the *unc-32* carrying F_1 hermaphrodites were picked onto

individual plates; these animals segregated only Dpy-22 animals. Only rare Unc animals were found among their progeny, as would be predicted if the only viable Unc animals were recombinants. The Unc Dpy-22 animals were shown to be recombinants by complementation tests with *dpy-27* or *dpy-28*.

In contrast, some *dpy-26*; *dpy-22* m^+z^- animals are viable, but they produce no viable m^-z^- hermaphrodite progeny and only rare male progeny. *dpy-26 unc-30/++*; *dpy-22* hermaphrodites segregated 376 non-Unc and 30 Unc hermaphrodites (a ratio of 12.5:1). None of these presumptive *dpy-26 unc-30*; *dpy-22* hermaphrodites produced viable hermaphrodite progeny. [Control *unc-30/+*; *dpy-22* hermaphrodites segregated 418 non-Unc and 127 Unc hermaphrodites (a ratio of 3.3:1).]

dpy-28; *dpy-22* and *dpy-27*; *dpy-22 XO* animals are viable and Dpy-22-like. Males of genotypes *dpy-27 unc-32/dpy-27* + or *unc-32 dpy-28/+ dpy-28* were mated with *dpy-27 unc-32/+*; *dpy-22/+* or *unc-32 dpy-28/+*; *dpy-22/+* hermaphrodites, respectively. In both matings the outcross Unc males were equally represented by Dpy-22 and non-Dpy-22 animals. The Unc Dpy-22 males (presumed to be *unc-32 dpy-27*; *dpy-22* or *unc-32 dpy-28*; *dpy-22*) displayed characteristic *dpy-22 XO* male phenotypes.

Isolation of polyploid strains: *dpy-28* strains (as well as *dpy-21*, *dpy-26*, *dpy-27* and *dpy-29* strains) occasionally segregate large, slightly Unc hermaphrodites. These animals segregate large, slightly Unc hermaphrodites and males as well as Dpy hermaphrodites and wild-type males and are presumably polyploid. A large, slightly Unc *dpy-28* hermaphrodite was picked; continued selection for large, slightly Unc hermaphrodite descendants over several generations resulted in a stable polyploid strain composed of large, slightly Unc hermaphrodites and occasional large, slightly Unc males. Staining with DAPI (Fixen 1985) revealed that these large, slightly Unc hermaphrodites contained oocytes with 12 tetravalents, confirming their tetraploid status (Madl and Herman 1979).

In a separate strain construction a nonmutant tetraploid strain was generated by outcrossing an EMS-induced *4X;4A her-1(n695)* strain to remove *her-1(n695)*.

Construction of strains containing *dpy-27*, *dpy-28*, or *dpy-29* and various sex determination mutations: To construct a strain with *dpy-27* or *dpy-28* in combination with *her-1(n695)*, Unc progeny were picked from hermaphrodites of genotype *dpy-27/+*; *her-1(n695) unc-42/++* or *dpy-28/+*; *her-1(n695) unc-42*. Dpy Unc or slightly Dpy Unc animals were picked from among the progeny of the Unc hermaphrodites to establish *dpy-27*; *her-1(n695) unc-42* and *dpy-28*; *her-1(n695) unc-42* strains. Because *unc-42* is less than 0.1 map unit from *her-1* (Hodgkin 1980), it is unlikely that *her-1(n695)* would have been lost through recombination. *her-1(695) unc-42 dpy-21(e428)* was a gift from A. Villeneuve.

her-1(e1520) is a recessive mutation that transforms *XO* animals into fertile hermaphrodites (Hodgkin 1980). To construct the strains *dpy-28*; *her-1(e1520)* and *dpy-27*; *her-1(e1520)*, males of the genotype *dpy-27*; *unc-42/+* or *dpy-28*; *unc-42/+* were mated with *her-1(e1520)* hermaphrodites. Potential cross progeny were picked onto individual plates. In the next generation Dpy or slightly Dpy hermaphrodite progeny were picked from broods that also segregated Unc animals. Dpy F_2 animals that did not segregate Unc animals in the next generation were *dpy-27*; *her-1(e1520)* or *dpy-28*; *her-1(e1520) XX* animals; eventually all the strains established from these animals produced rare non-Dpy hermaphrodites. These non-Dpy hermaphrodites always produced both non-Dpy and Dpy, Egl progeny. To prove that these non-Dpy hermaphrodites were indeed *XO* animals they were mated with males bearing the *X*-linked marker *lin-14*. The progeny from these matings contained large numbers of males hemizygous for the patroclinous *X* chromosome, confirming that the wild-type animals were indeed *XO*.

Recessive mutations in *tra-1* cause the transformation of *XX* animals into males (Hodgkin and Brenner 1977; Hodgkin 1987b). *dpy-27 tra-1* was constructed by mating *tra-1 XX* males with *dpy-27 unc-32* hermaphrodites. Dpy non-Unc recombinants in the broods from the non-Unc cross progeny hermaphrodites were picked onto individual plates and their broods were screened for the presence of non-Unc males. All the males produced by such *dpy-27 unc-32/dpy-27 tra-1* heterozygotes were dumpy and had various morphological defects in the male tail structure (see results). *dpy-28 tra-1* was constructed by mating *tra-1 XX* males with *dpy-28 dpy-18* hermaphrodites. Slightly Dpy, Egl non-Dpy-18 recombinants in the broods from the non-Dpy-18 cross progeny were picked onto individual plates. Approximately half of these recombinants segregated Dpy males (*dpy-28 tra-1 XX* animals) in their broods; half segregated no males. All the *dpy-28 tra-1 XX* males had morphological defects in their tail structure (see results).

tra-1; *dpy-29 XX* pseudomales were obtained as segregants from *tra-1/+*; *dpy-29/+* animals. The F_1 heterozygotes produced both Dpy and non-Dpy males: all the Dpy males had morphological defects in their tail structure, whereas the non-Dpy males did not. We also picked Dpy hermaphrodites from the F_1 heterozygotes; about two-thirds of the F_2 Dpy hermaphrodites segregated Dpy males with morphological defects in their tails.

Reversion of *dpy-28(y1)*: *dpy-28(y1)* L4 hermaphrodites raised at 15° were mutagenized with EMS as described in Brenner (1974) and picked onto individual plates at 15°. These animals were kept at 15° for 5 days, sufficient time to produce a brood of about 10 to 15 F_1 hermaphrodites. These broods were shifted to 25°. The plates were screened for the presence of non-Dpy hermaphrodites 4–7 days later. From the progeny of approximately 11,000 F_1 hermaphrodites we recovered five wild-type *XO* hermaphrodites; two contained alleles of *xol-1(y70, y95)*, and three contained alleles of *her-1(y14, y69, y71)*. [In *dpy-28(y1) XX* animals, *X* chromosome nondisjunction occurs at a greater frequency than in wild-type *XX* animals (Table 2) and results in a considerable number of *XO* animals.] In addition, two spontaneous wild-type hermaphrodites arose in the *dpy-28* background. One carried *xol-1(y9)*, and one carried *her-1(y10)*.

RESULTS

Mutations in *dpy-27*, *dpy-28*, and *dpy-29* result in *XX*-specific lethality: The phenotypes of mutations in *dpy-27*, *dpy-28*, and *dpy-29* are dependent on *X* chromosome dosage but not on sexual phenotype. While diploid *XO* animals are essentially wild type, diploid *XX* animals are generally inviable. The majority of the *dpy XX* animals arrest and persist as uncoordinated L1 larvae for up to several weeks prior to dying. These L1 animals appear morphologically normal, albeit dumpy, when examined by Nomarski microscopy; however, they appear starved despite being maintained on plates with abundant food. Consistent with this observation is the finding that *dpy-28(y1)* larvae hatched on plates containing black ink (Avery and Horvitz 1987), accumulate ink particles

TABLE 1

Phenotypes caused by mutations that disrupt dosage compensation in *XX* animals[a]

Genotype	Phenotype[b]		
	XO	*XX* (m^+z^-)[c]	*XX* (m^-z^-)
dpy-26[d]	wt	Slightly Dpy, Egl, Him	Inviable; Dpy, Egl, Him escapers
dpy-27	wt	Dpy, Egl	Inviable; Dpy, Egl, escapers
dpy-28	wt	Slightly Dpy, Egl, Him	Inviable; Dpy, Egl, Him escapers
dpy-29	wt	Dpy, Egl	Inviable; Dpy, Egl escapers
dpy-21[e]	wt	Dpy, Egl	Dpy, Egl

[a] Genes such as *sdc-1* and *sdc-2* that control the hermaphrodite mode of both dosage compensation and sex determination have been omitted from this table.

[b] wt: wild type, Egl: egg-laying defective, Dpy: dumpy (short and fat), Him: high incidence of males. The Egl animals all possess the HSN motor neurons that are required for egg laying.

[c] *m* indicates the maternal genotype; *z* indicates 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 *dpy* mothers.

[d] HODGKIN (1983) and present study.

[e] HODGKIN (1983) and DELONG, CASSON, and MEYER (1987). See text for discussion of *dpy-21* phenotype.

throughout the pharynx but not in the gut, suggesting that these animals have difficulties in feeding. *XX* animals that escape the lethality are dumpy (Dpy) and egg-laying defective (Egl) (Table 1).

The lethality and dumpiness caused by mutations in these *dpy* genes is *XX*-specific rather than hermaphrodite-specific since *dpy XO* animals transformed into hermaphrodites by a mutation in the sex-determining gene *her-1* (HODGKIN 1980) are wild type in length and fully viable, whereas *her-1*; *dpy XX* animals are Dpy and Egl or dead. Similarly, a *tra-1* mutation capable of transforming *XX* animals into fertile mating males (HODGKIN 1987b) fails to rescue the *XX* animals mutant in *dpy-27, dpy-28* or *dpy-29* (see MATERIALS AND METHODS). All the surviving *dpy tra-1 XX* animals are Dpy and, unlike *tra-1 XX* animals, have malformed male tails. These tails have shortened or missing rays, reduced bursal fan size, and often contain crumpled spicules (Figure 3). The phenotypes of mutations in these three *dpy* genes resemble the phenotypes caused by mutations in *dpy-26*, a gene required for the *XX* mode of dosage compensation (HODGKIN 1983; MENEELY and WOOD 1987; DELONG, CASSON and MEYER 1987).

The *XX*-specific lethality depends on both the maternal and zygotic genotype: The *dpy-27, dpy-28*, and *dpy-29* mutants exhibit a maternal-effect such that *XX* (m^-z^-) homozygous mutant progeny of homozygous mutant mothers are generally inviable, while the *XX* (m^+z^-) homozygous mutant progeny of heterozygous mothers are fully viable and Egl. All the m^+z^- *dpy-27* or *dpy-29* animals are Dpy, but the m^+z^- *dpy-28* animals are only slightly Dpy and not reliably distinguishable from wild type [as is *dpy-26* (HODGKIN 1983)] (Table 1). The recently isolated alleles of *dpy-27(y49, y56), dpy-28(s939)*, and *dpy-29(y100)* cause a high degree of *XX*-specific lethality (approximately 95%), an amount similar to that caused by the *dpy-26(n199)* allele (Table 2), which is thought to be null or nearly null (HODGKIN 1983). The viability of different *dpy-27* mutants at 20° falls into a series (Table 2); one of the alleles that results in the greatest lethality (*y56*), is suppressed by the amber suppressor *sup-7(st5)*. *dpy-27(y56)*; *sup-7(st5)* animals are non-Dpy and viable (see MATERIALS AND METHODS). These results suggest that the null phenotype of *dpy-27* might be close to that of the most severe alleles, *y56* or *y49*. Similarly, the strongly mutant *dpy-29(y100)* allele is also suppressed by *sup-7(st5)*; the *dpy-29(y100)*; *sup-7(st5)* animals are non-Dpy and viable (see MATERIALS AND METHODS).

The data summarized in Table 3 reveals that at 20° *dpy-27, dpy-28*, and *dpy-29*(m^+z^-) homozygous progeny of heterozygous *dpy*/+ mothers are fully viable. Similar results were obtained for *dpy-27, dpy-28* or *dpy-29* m^+z^- animals grown at 15° (data not shown). The absence of zygotic lethality was deduced from the observation that Unc slightly Dpy (*unc-32 dpy-28*) self progeny of *unc-32 dpy-28(y1* or *s939)*/++ mothers represent approximately 25% of the total progeny, the result expected if *dpy-28* (m^+z^-) animals are fully viable. Similar results were obtained for *unc-93 dpy-27(y57)* homozygous progeny of *unc-93 dpy-27(y57)*/++ mothers. Since all the *unc-93 dpy-27* progeny are Dpy, it is possible to assess the viability of *dpy-27(y57* or *y56)* (m^+z^-) animals that do not carry a marker in *cis* by scoring the relative proportion of Dpy progeny from the heterozygous *dpy* mother. A similar approach was used to determine that m^+z^- *dpy-29(y100)* animals (Dpy) are fully viable. HODGKIN (1983) has shown that *dpy-26* progeny of heterozygous mothers are also completely viable at 20°.

The *XX*-specific lethality caused by mutations in *dpy-26, dpy-27, dpy-28*, and *dpy-29* is also fully rescued

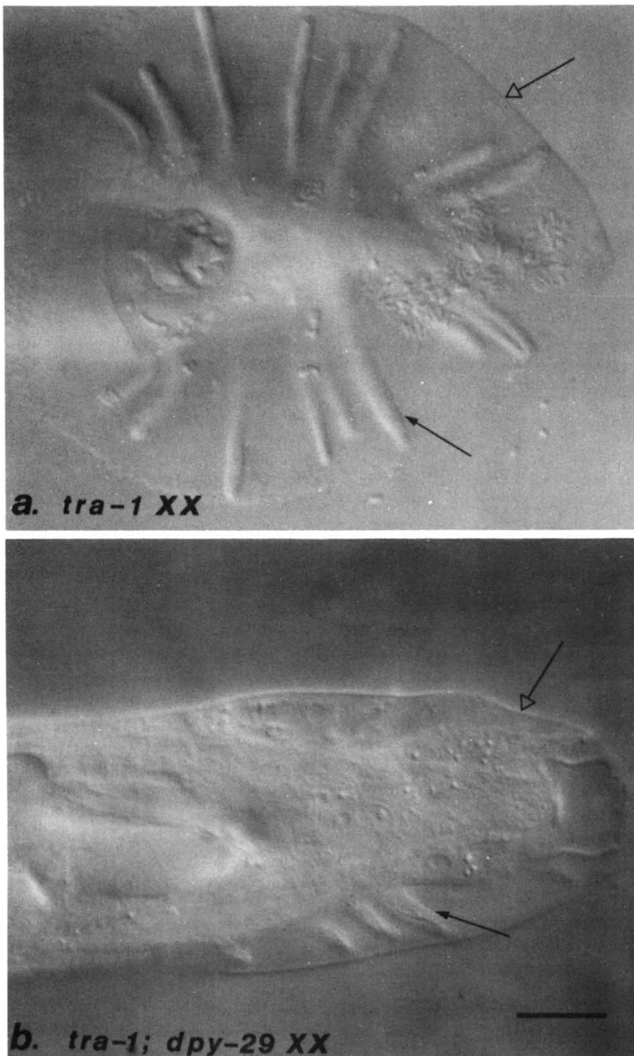


FIGURE 3.—Nomarski photomicrographs showing ventral views of *tra-1 XX* and *tra-1; dpy-29 XX* male tails. a, the tail anatomy of *tra-1 XX* animals is identical to that of wild type *XO* males. The open arrow indicates the bursal fan, the closed arrow indicates sensory rays. b, The *tra-1; dpy-29 XX* male tail is malformed, displaying shortened and/or missing rays and a reduced bursal fan size. There is no evidence of the reappearance of hermaphrodite tail structures (such as a tail whip) in these *tra-1; dpy-29 XX* animals, consistent with the proposal that the effects of the *dpy* genes on *tra-1* male tail structure is a consequence of the disruption of dosage compensation. Scale bar equals 10 μm.

by a wild-type allele of the appropriate gene introduced into the zygote from the father (Table 4). This conclusion was drawn from the observation that *dpy*/+ hermaphrodite cross progeny and *dpy*/+ male cross progeny are equally represented in the progeny of matings between wild-type males and marked *dpy-26*, *dpy-27*, *dpy-28*, or *dpy-29* hermaphrodites. HODGKIN (1983) demonstrated a similar zygotic rescue for *dpy-26(n199)*. Consistent with the complete zygotic rescue of m^-z^+ *XX* animals is the observation that in *dpy-26* and *dpy-28* m^-z^+ animals *X*-linked gene expression is also wild type (see below and Table 5).

The extent of *XX*-specific lethality is temperature dependent: The viability of homozygous *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29 XX* animals is highly sensitive to the temperature at which they are raised. At 20° the viability of different mutants ranges from 3% to 20%. At 15°, 12 of the 13 known alleles of *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29* result in a cold-sensitive phenotype such that viable animals comprise less than 1% of the total zygotes (Table 2). The possibility that each individual mutation results in a cold-sensitive mutant product seems unlikely. The remarkable uniformity in degree of lethality at 15° suggests instead that mutations in *dpy-26*, *dpy-27*, *dpy-28* and *dpy-29* reveal the presence of a cold-sensitive process required for viability of *XX* animals.

dpy-28(y1ts), on the other hand, is likely to be a mutation that results in a thermolabile *dpy-28* product, which masks the cold-sensitive phenotype. At 20°, 20% of the *XX* animals reach adulthood; at 15°, 80% of the *XX* animals reach adulthood and are somewhat less dumpy and egg-laying defective than *dpy-28(y1ts)* animals raised at 20° (Table 2).

***XO* animals mutant in *dpy-26*, *dpy-27*, *dpy-28* or *dpy-29* are fully viable:** To show that the homozygous *dpy XO* progeny from homozygous *dpy XX* hermaphrodites are fully viable we compared the viability of *dpy/dpy*, *dpy*/+ and appropriate control males in the crosses of Table 4. Males of genotype *dpy-26/unc-30*, *dpy-27/unc-32*, or *dpy-28/unc-32* were mated with hermaphrodites of genotype *dpy-26 unc-30*, *dpy-27 unc-32*, or *unc-32 dpy-28*, respectively. Equal numbers of wild-type males (*dpy/dpy*) and Unc males (*unc dpy/unc*) were obtained, indicating that homozygous *dpy* males are as viable as heterozygous *dpy* males. Additionally, males of genotype *dpy-26/unc-30* were mated with *unc-30* hermaphrodites, and males of genotype *dpy-27/unc-32* or *dpy-28/unc-32* were mated with *unc-32* hermaphrodites; equal numbers of wild-type males (*dpy/unc*) and Unc males (*unc/unc*) were produced. Together, these results indicate that homozygous *dpy-26*, *dpy-27* or *dpy-28 XO* progeny of homozygous *dpy-26*, *dpy-27* or *dpy-28* mothers are fully viable. Similar experiments with *dpy-29(y100)* indicate that *dpy-29* males are also completely viable (Table 4).

Mutations in *dpy-28* increase *X* chromosome nondisjunction: In general, mutations in *dpy-26*, *dpy-27*, *dpy-28* and *dpy-29* have similar phenotypes. However, mutations in *dpy-26* (HODGKIN 1983) or *dpy-28* but not mutations in *dpy-27* or *dpy-29* increase *X* chromosome nondisjunction and result in a Him (high incidence of males) phenotype (Table 2). All mutant alleles of *dpy-28* and *dpy-26* (and appropriate heteroallelic combinations) but no mutant alleles of *dpy-27* and *dpy-29* are Him. The dumpy and lethal phenotypes have never been separated from the Him phenotypes; thus, these three phenotypes are likely to be caused by the mutations in *dpy-26* or *dpy-28*, although

TABLE 2

Viability of *dpy-26*, *dpy-27*, *dpy-28* and *dpy-29* homozygous mutant *XX* progeny of homozygous *dpy* mothers

	15°			20°		
Genotype	Percent viability[a]	No. of zygotes (broods)	Percent males	Percent viability[a]	No. of zygotes (broods)	Percent males
dpy-26(y65)	0.4 ± 0.2	793 (10)	4.2	8.7 ± 0.8	1255 (8)	3.5
(n199)	0.2 ± 0.1	2309 (6)	2.2	5.5 ± 0.5	1938 (15)	4.0
(n198)	0.9 ± 0.3	1368 (10)	1.5	5.3 ± 0.7	1160 (7)	2.2
(y6)	0.4 ± 0.2	1047 (6)	2.4	3.2 ± 0.5	1366 (6)	3.0
dpy-27(y57)	0.6 ± 0.3	781 (13)	0.3	20.3 ± 1.0	1503 (9)	0
(rh18)	0.3 ± 0.2	668 (11)	0	11.4 ± 1.3	982 (19)	0
(y44)	0.2 ± 0.2	660 (12)	0	8.6 ± 0.5	2906 (20)	0.1
(y42)	0.8 ± 0.2	1503 (10)	0	7.6 ± 0.6	2201 (16)	0.2
(y56am)	0.2 ± 0.2	560 (10)	0	5.1 ± 0.5	1829 (18)	0.2
(y49)	0.4 ± 0.2	1589 (9)	0	4.5 ± 0.5	1873 (21)	0.1
dpy-28(y1)	79.1 ± 0.7	3666 (19)	0.2	18.1 ± 0.3	2822 (34)	0.4[b]
(s939)	0.5 ± 0.3	763 (9)	3.3	6.7 ± 0.5	2440 (18)	4.2
dpy-29(y100am)	0.6 ± 0.3	649 (7)	0	3.4 ± 0.5	1464 (12)	0

[a] Viability is calculated for hermaphrodite (*XX*) progeny only ± σ (standard deviation). The viability of the hermaphrodite progeny was calculated in accord with the formula $H/(Z - M)$ where H is the number of adult hermaphrodites, Z the number of zygotes, and M is the number of adult males. *XO* males are 100% viable (see RESULTS). σ is calculated in accord with the formula $\sigma = (p*q/k)^{1/2}$, where p and q are the probabilities of an animal reaching adulthood (p) or failing to reach adulthood (q), and k is the sample size minus the number of males.
[b] At 24° *dpy-28(y1)* produces 3% males.

TABLE 3

***dpy-27*, *dpy-28* and *dpy-29* homozygous *XX* progeny of *dpy*/+ mothers are fully viable at 20°**

Maternal genotype	Unc progeny	Total progeny	Percent Unc progeny	No. of broods
Part A				
unc-32 dpy-28(y1)/+ +	453	1776	25.5%	6
unc-32 dpy-28(s939)/+ +	168	749	28.9%	7
unc-93(e1500) dpy-27 (y57)/+ +	259[a]	1112	23.3%	4
	Dpy progeny		Percent Dpy progeny	
Part B				
dpy-27(y57)/unc-93(e1500)	486	2080	23.3%	8
dpy-27(y56)/unc-93(e1500)	359	1527	23.5%	6
dpy-29(y100)/+	267	1083	24.6%	4

In Part A, the number of Unc progeny (*unc-32 dpy-28* or *unc-93 dpy-27* homozygotes) is compared to the total number of progeny to ascertain the viability of m^+z^- animals; in Part B, the number of phenotypically Dpy progeny (*dpy-27* or *dpy-29* homozygotes) is compared to the total number of progeny to ascertain the viability of m^+z^- animals. If the *dpy XX* (m^+z^-) animals are fully viable, the expected percentage of Unc animals in Part A or Dpy animals in Part B would be 25%.
[a] All nonrecombinant Unc animals were Dpy.

the formal possibility remains that the Him phenotype could be due to a simultaneous mutation in a neighboring gene. For both *dpy-26(y6)* and *dpy-28(y1)* the percent of nullo-*X* ova corresponds to the percent of males [for *dpy-26(y6)* at 25°: 2% nullo-*X* ova *vs.* 2% males (from approximately 1000 progeny); for *dpy-28(y1)* at 22.5°: 2% nullo-*X* ova *vs.* 2% males (from approximately 1000 progeny)]. (The percent of nullo-*X* ova was determined from the number of *XO* animals that inherited a paternal *X* chromosome in an appropriate cross.) A similar correspondence for *dpy-26(n199)* has been reported (HODGKIN 1983). These results are consistent with the Him phenotype being due to meiotic nondisjunction in these mutants.

***dpy-29(y100)* disrupts dosage compensation, resulting in the overexpression of an *X*-linked gene in *XX* but not *XO* animals:** Mutations in *dpy-26*, *dpy-27*, and *dpy-28* disrupt dosage compensation, resulting in the overexpression of *X*-linked genes in *XX* animals (MEYER and CASSON 1986; MENEELY and WOOD 1987; DELONG, CASSON and MEYER 1987). *XX* animals mutant in *dpy-27* or *dpy-28* exhibit a two to threefold elevation in *X*-linked transcript levels, while the *XO* animals mutant in *dpy-27* or *dpy-28* have wild-type *X*-linked transcript levels (MEYER and CASSON 1986). The *XX*-specificity of the *dpy-29(y100)* mutant phenotypes suggested that *dpy-29(y100)* might also disrupt dosage compensation and elevate *X* chromosome expression in *XX* animals. To test this hypothesis we employed the *lin-14* assay developed by DELONG,

TABLE 4

Both *dpy/+ XX* and *dpy/dpy XO* progeny of homozygous *dpy* mothers are fully viable at 20°

Maternal genotype	Paternal genotype	Male progeny[a]		Hermaphrodite progeny[a]	
		dpy/+[b]	*dpy/dpy*[c]	*dpy/+*[b]	*dpy/dpy*[d]
dpy-26(n199) unc-30[e]	*dpy-26(n199)/unc-30*	537	586 (109%)	571 (106%)	9 (2%)
dpy-26(y6) unc-30	+/+	287		284 (99%)	
dpy-27(y57) unc-32	*dpy-27(y57)/unc-32*	309	316 (102%)	320 (104%)	102 (33%)
dpy-27(y57) unc-32	+/+	344		337 (98%)	
unc-32 dpy-28(y1)	*dpy-28(y1)/unc-32*	151	131 (87%)	143 (95%)	37 (25%)
unc-32 dpy-28(y1)	+/+	276		280 (102%)	
dpy-29(y100) unc-76	*dpy-29(y100)/unc-76*	173	227 (131%)	151 (87%)	7 (4%)
dpy-29(y100) unc-76	+/+	558		571 (102%)	

[a] Total number of progeny in each phenotypic class is listed followed by the percent viability of that class relative to the viability of the *dpy/+* males (considered to be 100% viable). In the crosses with *dpy/unc* fathers, the *dpy/+* male progeny have an Unc phenotype; in the crosses with +/+ fathers, the *dpy/+* male progeny are wild type.

[b] In the crosses with *dpy/unc* fathers these animals were identified on the basis of their Unc non-Dpy phenotype; in the crosses with +/+ fathers these animals were identified on the basis of their wild-type phenotype.

[c] These males were identified on the basis of their non-Unc phenotype.

[d] These animals were identified on the basis of their Dpy non-Unc phenotype.

[e] There is unlikely to be any lethality associated with *dpy/+* males. A cross between *unc-30* hermaphrodites and *dpy-26(n199)/unc-30* males yielded 849 *dpy/unc* males and 735 *unc/unc* males. A cross between *unc-32* hermaphrodites and *dpy-27(y57)/unc-32* males yielded 228 *dpy/unc* males and 218 *unc/unc* males. A cross between *unc-32* hermaphrodites and *dpy-28(y1)/unc-32* males yielded 894 *dpy/unc* males and 858 *unc/unc* males. A cross of *unc-76/+* males and *unc-76* hermaphrodites yielded 967 *unc/+* males and 580 *unc/unc* males, indicating that the viability of the *unc/unc* males was 60% of the viability of *unc/+* males. A cross between *unc-76* hermaphrodites and *dpy-29(y100)/unc-76* males yielded 1305 *unc/dpy* males and 887 *unc/unc* males, indicating that the viability of the *unc/unc* males was 67% of the viability of *unc/dpy* males. These results predict that in the cross between *dpy-29(y100) unc-76* hermaphrodites and *dpy-29(y100)/unc-76* males, *dpy-29 unc-76/+ unc-76* outcross progeny should be 60–70% viable compared with *dpy/dpy* males if *dpy-29* has no effect upon the viability of m^-z^+ animals or homozygous males. This is what is observed, suggesting that *dpy/+* hermaphrodites, *dpy/+* males and *dpy/dpy* males are equally viable.

TABLE 5

Analysis of *X* chromosome expression using the *lin-14(n179)* phenotypic assay

Genotype	No. of animals scored	Percent mutant nuclei (mean)	Compared to *XXX lin-14*	Compared to *XX lin-14*
XX	60	79	$P < 10^{-6}$	
XXX[a]	25	26[b]		$P < 10^{-6}$
Homozygous *dpy* progeny of heterozygous mothers				
XX dpy-21(e428)	11	30	NS	$P < 10^{-6}$
XX dpy-27(y49)	20	25	NS	$P < 10^{-6}$
XX dpy-29(y100)	20	34	NS	$P < 10^{-6}$
XX dpy-26(n199)	20	61	$P < 10^{-3}$	$P < 0.02$
XX dpy-26(y6)	20	68	$P < 10^{-5}$	$P < 0.02$
XX dpy-28(y1)	20	51	$P < 10^{-3}$	$P < 10^{-5}$
XX dpy-28(s939)	20	52	$P < 10^{-3}$	$P < 10^{-4}$
Heterozygous *dpy/+* progeny of homozygous *dpy* mothers				
XX dpy-26(n199)/+[c]	20	74	$P < 10^{-5}$	NS
XX dpy-28(s939)/+[d]	20	69	$P < 10^{-5}$	NS
Homozygous *dpy XO* progeny of homozygous mothers				
XO	60	49		
XO dpy-29[e]	20	40		

This assay is described in DELONG, CASSON and MEYER (1987) and briefly in MATERIALS AND METHODS. For experiments in this table, all hermaphrodites were raised at 24° and all males at 20°; all animals carry *lin-14(n179)*. *P* is the probability that compared data sets are the same by the Mann-Whitney *U* test. NS = $P > 0.05$, compared data sets are not significantly different; $P < 0.02$, compared data sets are significantly different.

[a] From DELONG, CASSON and MEYER (1987).

[b] Suppression of *lin-14(n179)* in *3X;2A* animals is equivalent to that found in *dpy-21*, *dpy-26*, *dpy-27*, or *dpy-28* m^-z^- *XX* animals (DELONG, CASSON and MEYER 1987).

[c] Full maternal genotype is *dpy-26(n199) unc-30; lin-14*.

[d] Full maternal genotype is *unc-32 dpy-28(s939); lin-14*.

[e] Not significantly different from *dpy(+) lin-14 XO*.

CASSON and MEYER (1987), which is a sensitive measure of perturbations in *X*-linked gene expression. The assay is described briefly in MATERIALS AND METHODS. The basis of the assay is that the severity of the mutant phenotype caused by the *X*-linked *lin-14(n179)* hypomorphic mutation (which reduces but does not elimi-

nate gene function) is used to indicate the level of gene expression. In the assay, suppression of the *lin-14* mutant phenotype (premature cessation of seam cell division in the L3 larval stage and subsequent formation of adult alae) reflects an increase in *X*-linked gene expression, whereas enhancement of the mutant phenotype reflects a reduction in *X*-linked gene expression. *dpy-29* results in suppression of the *lin-14* phenotype in *XX* animals (Table 5), indicating that *dpy-29* causes overexpression of *lin-14*, and presumably other *X*-linked genes. The *dpy-29 XX* animals examined in the *lin-14* assay were the homozygous progeny of heterozygous *dpy-29*/+ mothers; the level of suppression seen in these animals (34% mutant nuclei) is equivalent to the maximum suppression of the *lin-14* phenotype caused by *3X;2A* animals (26% mutant nuclei) or homozygous *dpy* progeny of homozygous *dpy* mothers in *dpy-26*, *dpy-27*, or *dpy-28* strains (DeLong, Casson and Meyer 1987). In *dpy-29 XO* animals *lin-14* expression is unaffected (40% mutant nuclei compared to 49% mutant nuclei in *lin-14 XO* males), indicating that *X* chromosome expression is probably wild type. This result is consistent with the wild-type phenotype of *dpy-29* mutant *XO* animals. Similar results have been obtained previously for *XO* animals mutant in *dpy-26*, *dpy-27* or *dpy-28* (DeLong, Casson and Meyer 1987).

The *XX*-specific Dpy phenotype correlates with overexpression of *X*-linked genes: The observation that *dpy-29 XX* progeny of *dpy-29*/+ mothers are Dpy and suppress the *lin-14* mutant phenotype to the same extent as the *dpy*/*dpy* progeny (Dpy) of *dpy*/*dpy* mothers or *3X;2A* Dpy animals suggested a correlation between the Dpy phenotype and suppression of *lin-14* mutant phenotype. To test the extent of this correlation we examined the *lin-14* phenotype in *dpy-21*, *dpy-26*, *dpy-27*, or *dpy-28* homozygous mutant progeny of heterozygous *dpy* mothers. Since *dpy-27* and *dpy-21* animals are Dpy in this generation and *dpy-26* and *dpy-28* animals are only slightly Dpy, we predicted that full suppression of the *lin-14* phenotype should be evident in the *dpy-27* and *dpy-21* animals but only partial suppression should be evident in the *dpy-26* and *dpy-28* animals. Our results fulfill this expectation (Table 5), indicating a strong correlation between the degree of dumpiness and the extent of overexpression of the *X*-linked *lin-14* gene. For this correlation to be complete we expected that the non-Dpy *dpy-26*/+ or *dpy-28*/+ progeny of *dpy*/*dpy* mothers should exhibit wild-type *X*-linked gene expression. As anticipated, the *dpy-26*/+ and *dpy-28*/+ animals exhibit a *lin-14* phenotype indistinguishable from the *lin-14 XX* control (Table 5).

The viability of *dpy-28* heteroallelic zygotes: In order to interpret results of reciprocal temperature-shift experiments with *dpy-28(y1ts)* reported below, it was important to determine the relative contribution

TABLE 6

Relative contribution of the maternal genotype to heteroallelic *dpy-28* zygotes

Allele present at the *dpy-28* locus at 15°		Heteroallelic progeny		
Maternal allele[a]	Paternal allele	No. of males	No. of hermaphrodites	Percent hermaphrodite survival[b]
y1	+	359	370	103
y1	*y1*	308	244	79
y1	*s939*	293	220	75
s939[c]	*y1*	599	72	12

Males homozygous for *dpy-28*(*y1*, *s939*, or +) were mated into hermaphrodites homozygous for *unc-32 dpy-28*(*y1* or *s939*). All the non-Unc (heteroallelic) progeny were scored, and the viability of hermaphrodites was calculated relative to the viability of their male sibs, which is assumed to be 100%.

[a] Full genotype *unc-32 dpy-28*(*y1* or *s939*).

[b] Viability of hermaphrodites relative to their male sibs.

[c] Homozygous offspring of heterozygous mothers.

of the maternal and zygotic *dpy-28* products to the viability of *XX* homozygous *dpy-28* animals. Even though the *XX*-specific lethality of *dpy-28* mutations can be fully rescued by either a maternal or a zygotic *dpy*(+) allele, the maternally and zygotically provided *dpy-28* products may not have an equivalent ability to rescue *dpy-28* animals, especially under conditions where the total amount of *dpy-28* activity may be limiting. Either the zygote or the mother could be providing the majority of the *dpy-28* activity, and as long as the total activity is over some threshold level, the animals would escape the lethality. Using conditions where the total activity is suboptimal it should be possible to distinguish whether the majority of the *dpy-28* activity required for viability is maternally or zygotically provided. Heteroallelic combinations of *dpy-28*(*y1*) and *dpy-28*(*s939*) at 15°, where *dpy-28* (*s939*) strains are about 0.5% viable and *dpy-28*(*y1*) strains are 75% viable, provide the conditions to evaluate the relative maternal and zygotic contributions. Table 6 shows that the viability of *y1*/*s939 XX* animals is strongly dependent on the maternal genotype: *y1*/*s939* progeny of homozygous *y1* mothers are 75% viable, whereas *y1*/*s939* progeny of *s939* mothers are 12% viable. Table 7 shows that the zygotic contribution of *dpy-28* activity towards the rescue of lethality is equivalent to the maternal contribution: the viability of *y1* homozygous progeny of *y1*/*s939* mothers (78%) (Table 7) is the same as that for *y1*/*s939* progeny of *y1*/*y1* mothers (75%) (Table 6). Furthermore *s939 XX* homozygous progeny of *y1*/*s939* mothers and *y1*/*s939* *XX* progeny of *s939*/*s939* mothers also have similar viabilities (8% and 12%, respectively). These results strongly suggest that the maternal and zygotic contributions of *dpy-28*(*y1*) product are very similar.

Reciprocal temperature-shift experiments with *dpy-28(y1ts)* and *dpy-27(y57cs)* define temperature-sensitive periods in mid-embryogenesis: To define a

TABLE 7

Viability of *XX* progeny of heteroallelic *dpy-28* animals at 15°

Genotype of parent	No. of zygotes	No. of Unc adults	Percent viability of Unc offspring[a]
dpy-28(s939)/unc-32 dpy-28(y1)	2182	425	78
dpy-28(y1)/unc-32 dpy-28(s939)	900	19	8

All animals have a Dpy phenotype. The Unc marker identifies progeny that are homozygous for either *y1* (top line) or *s939* (bottom line). The *dpy-28(s939)/unc-32 dpy-28(y1)* parents also produced 572 non-Unc progeny, including both *s939* homozygotes and *y1/s939* heteroallelic animals. The *dpy-28(y1)/unc-32 dpy-28(s939)* parents also produced 284 non-Unc progeny, including both *y1* homozygotes and *y1/s939* heteroallelic animals.

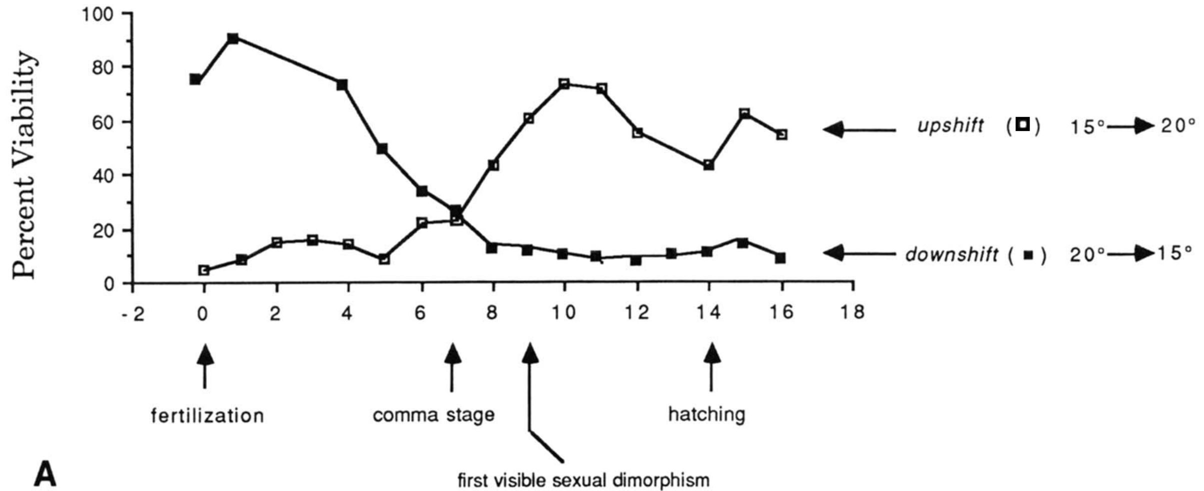
[a] If the Unc progeny were fully viable they would only constitute ¼ of the total of zygotes. Thus, the viability of the hermaphrodite progeny was calculated in accord with the formula $4H/(Z - M)$ where H is the number of adult Unc hermaphrodites, Z the total number of zygotes, and M is the total number of adult males.

potential time of action for the *dpy-28* gene we performed reciprocal temperature-shift experiments with a heat-sensitive allele. Cohorts of *dpy-28(y1)* embryos or early L1 larvae were shifted from either 15° to 20° or 20° to 15° at various stages of development, and the extent of viability was determined (as described in the MATERIALS AND METHODS). A graph depicting the viability of each cohort is given in Figure 4A. These curves define a 4–5-hr period of time centered around the comma stage of embryonic development during which *dpy-28* product is required for viability. The comma stage is a point in development immediately after embryonic cell proliferation during the onset of elongation and morphogenesis and is approximately 2 hr prior to the first evidence of sexual dimorphism (SULSTON *et al.* 1983). The temperature-sensitive period for *dpy-28(y1)* is much earlier than the time of larval arrest and occurs when many zygotically transcribed genes are active (HECHT, GOSSET and JEFFERY 1981; EDGAR and MCGEE 1986; and I. SCHAUER and W. WOOD, personal communication). Temperature pulse experiments confirm that the requirement for *dpy-28* is centered around the comma stage (Figure 4B). In these experiments, groups of embryos were either grown at 15° and shifted to 20° for approximately 3 hr or grown at 20° and shifted to 15° for approximately 3 hr. Only the 3-hr pulses coincident with the previously defined temperature-sensitive period for *dpy-28(y1)* had any effect on viability. The 20° pulses during comma stage resulted in only 35% viability, while 15° pulses during comma stage resulted in 55–60% viability. These temperature shift experiments are the first indication that any of the genes involved in dosage compensation might be required as early as 5 hr after fertilization and suggest that the dosage compensation process is functioning by mid-embryogenesis. For these conclusions to be valid, the temperature sensitive period of *dpy-28(y1)* must reflect the time of action of the *dpy-28* product and not the time of synthesis of a *dpy-28* product.

This experiment also demonstrates that the maternally provided *dpy-28(y1)* product must be thermolabile. If the mother were providing a thermostable product, the upshift curve would have an inflection point prior to fertilization, and shifting the animals to the restrictive temperature at any point after fertilization would have no effect on viability. This is not the result obtained. Moreover, if the maternally provided product were thermostable, the shape of the upshift curve in Figure 4A would have to be interpreted to mean that the maternal endowment is insufficient to rescue the zygotes shifted to restrictive temperature prior to comma stage. Since we know that the maternally contributed *dpy-28(y1)* product is sufficient for rescue and that the maternally and zygotically contributed products are functionally equivalent (Tables 6 and 7), the maternally provided *dpy-28(y1)* product must be thermolabile. This product could either be a thermolabile protein, or it could be RNA that is translated zygotically.

We also determined the temperature-sensitive period for the lethality caused by *dpy-27(y57)* at 15°. The cold-sensitive period is also centered around the comma stage (Figure 5). Because the cold-dependent lethality is a phenotype common to all *dpy-27* alleles, the temperature-sensitive period of *dpy-27(y57)* is probably more a reflection of a critical time in the putative cold-sensitive process uncovered by mutations in *dpy-27*, than it is a reflection of a temporal requirement for the *dpy-27* product. It is entirely possible that the requirement for *dpy-27* product occurs at any time prior to the point at which the lethality is most affected by low temperature.

Interactions between mutations in *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29*: If *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29* act in a common pathway to achieve dosage compensation in *XX* animals, *XX* animals carrying mutations in two different genes would probably be as viable as animals carrying a mutation in only one gene. If these genes act in parallel pathways, the effect of mutations in any two of the *dpy* genes would be expected to be combinatorial, resulting in *XX* animals with reduced viability compared to *XX* animals mutant in only one gene. We find that *XX* animals carrying mutations in *dpy-27* and *dpy-26*, or *dpy-28* and *dpy-26*, or *dpy-28* and *dpy-29*, or *dpy-27* and *dpy-28* are either as viable as the least viable single mutant in the pair or intermediate in viability (Table 8). Moreover, *XX* animals doubly mutant in *dpy-27* and *dpy-28* exhibit the same degree of *lin-14* suppression as the single mutants (Table 9). These observations indicate that *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29* probably act to-



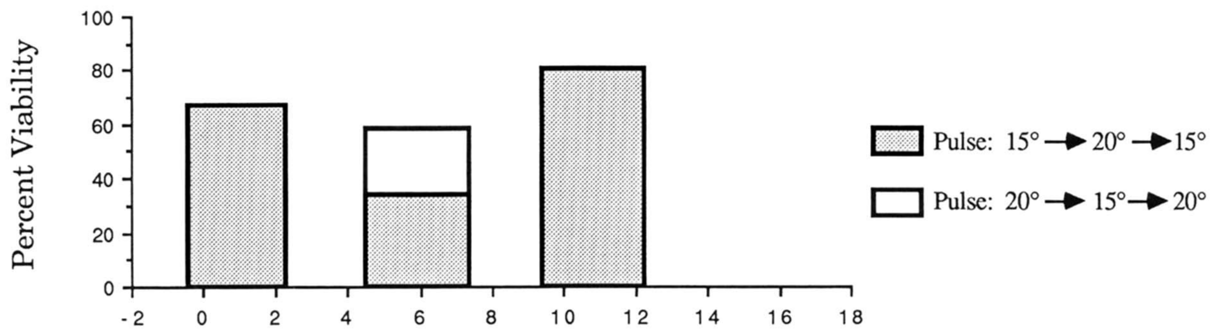


FIGURE 4.—Temperature-sensitive period for the viability of *dpy-28(y1) XX* animals is during mid-embryogenesis. A, the graph represents the results of reciprocal temperature shift experiments in which cohorts of *dpy-28(y1)* animals were shifted at various times in development from restrictive to permissive temperatures (downshift) or from permissive to restrictive temperatures (upshift) (described in MATERIALS AND METHODS). The viability of the *XX* animals in a given cohort of temperature-shifted embryos is plotted relative to the time at which the cohort was shifted. Percent viability is indicated on the vertical axis. The time after fertilization at which the temperature shift occurred is given on the horizontal axis. Important events during embryogenesis are noted below the horizontal axis. The temperature-sensitive period for *dpy-28(y1)* starts at about five hours after fertilization (300 cell stage) and extends through comma stage until about nine hours of development. B, Temperature pulse experiments: The bars represent groups of *dpy-28(y1)* embryos that were either grown at permissive temperature (15°) and briefly pulsed at restrictive temperature (20°, shaded bars), or grown at restrictive temperature and briefly pulsed at permissive temperature (the open bar). The width of the bar indicates the period during development at which the embryos were pulsed; the height indicates the percentage of viable hermaphrodite adults in a particular cohort. The pulses confirm a requirement for *dpy-28* within the temperature-sensitive period defined by the temperature-shift experiments shown in part A.

gether in a common process such that mutations in any one gene can disrupt this process, but no further disruption results from eliminating the function of a second gene. However, these genes probably do not encode products with redundant functions, because additional wild-type copies of one or more of these genes do not appear to substitute for the loss of the product of another of these genes. For example, one additional copy of *dpy-26* carried on the free duplication *yDp1* does not rescue *dpy-28* mutants; one additional copy of both *dpy-27* and *dpy-28* carried on the free duplication *mnDp37* does not rescue *dpy-29* mutants (see MATERIALS AND METHODS). It is likely that *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29* play distinct roles in the common process of executing the hermaphrodite mode of dosage compensation.

Interactions between mutations in *dpy-26*, *dpy-27*, *dpy-28*, or *dpy-29* and *dpy-21* and *dpy-22*: The gene *dpy-21* appears to be essential for proper dosage compensation, but mutations in this gene result in some phenotypes that are different from those caused by mutations in other *dpy* genes. Mutations in *dpy-21* cause overexpression of *X*-linked genes in *XX* animals but do not cause an *XX*-specific maternal-effect lethality. *XO* animals mutant in *dpy-21* exhibit altered *X* chromosome expression, unlike other *XO dpy* animals. (HOGDKIN 1983; MENEELY and WOOD 1987; MEYER and CASSON 1986; DELONG, CASSON and MEYER

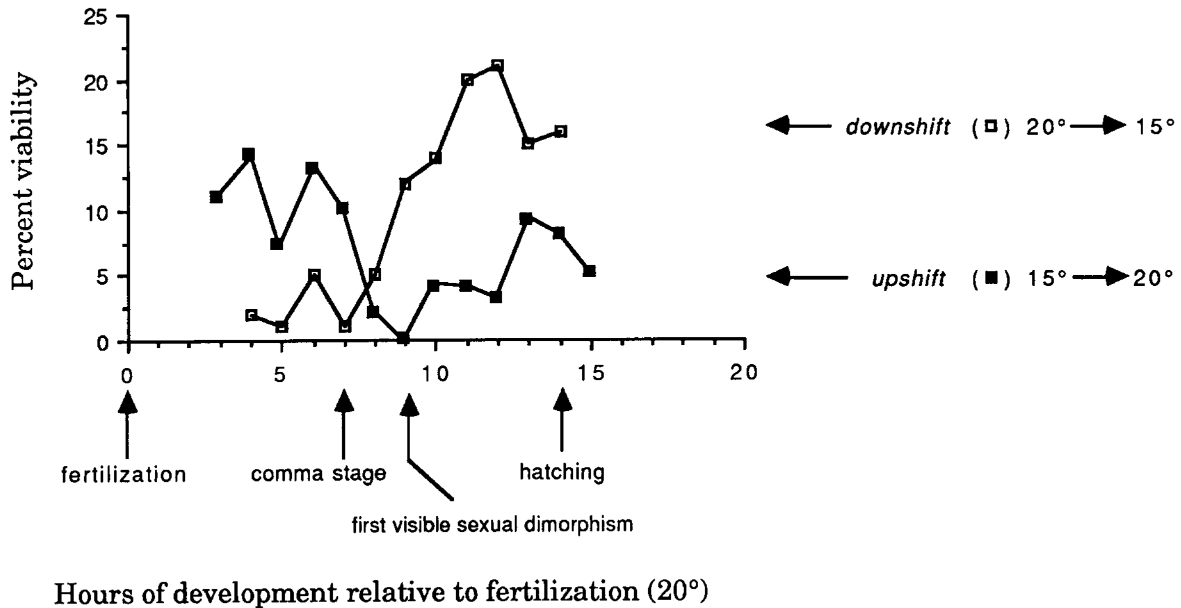


FIGURE 5.—Temperature-sensitive period for the cold-sensitive lethality revealed by *dpy-27(y57)*. The temperature-sensitive period for the cold-sensitive process revealed by the mutation *dpy-27(y57)* is approximately coincident with the temperature-sensitive period of *dpy-28(y1)*. These temperature-shift experiments were performed as described for *dpy-28(y1)*.

1987). We have demonstrated that mutations in *dpy-21* have a slight ameliorating effect on the lethality caused by mutations in *dpy-27*, *dpy-28*, and *dpy-27 dpy-28*. As shown in Table 8 strains containing *dpy-21* and different combinations of other *dpy* mutations are somewhat more viable than strains with mutations in only *dpy-27*, *dpy-28*, or *dpy-27 dpy-28*. We have observed this phenomenon with five different alleles of *dpy-21*, including an amber allele (*y47*) and a weak allele (*y88*), indicating that the interaction is not allele-specific. A similar effect was observed in the *dpy-26*; *dpy-21* double mutant (HODGKIN 1983). Combinations of various *dpy* mutations suppress the *lin-14* phenotype to the same extent as any of the individual *dpy-21*, *dpy-26*, *dpy-27*, or *dpy-28* mutations (Table 9). These results are an indication that *dpy-21* acts in a common process with *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29*. The lack of clear epistasis in *XX* animals precludes ordering *dpy-21* and *dpy-27*, or *dpy-21* and *dpy-28* in a hierarchical pathway.

In *XO* animals, mutations in *dpy-21* result in an enhancement of the *lin-14* mutant phenotype whereas mutations in the other *dpy* genes have no effect on the *lin-14* phenotype. In *XO* animals carrying mutations in *dpy-21* and *dpy-26*, *dpy-27* or *dpy-28*, the *lin-14* phenotype resembles that of *dpy-21 XO* animals. This result is expected since *dpy-26*, *dpy-27*, and *dpy-28* probably do not act in *XO* animals and mutations in these genes should not enhance or compensate for the defect caused by mutations in *dpy-21*.

The mutation *dpy-22(e652)* reduces *X* chromosome expression in *XX* and *XO* animals; both sexes are dumpy and somewhat inviable, and males have abnormal tails (MENEELY and WOOD 1987; DELONG, CASSON and MEYER 1987). We asked whether *dpy-22(e652)* could suppress the lethality caused by mutations in *dpy-26*, *dpy-27*, or *dpy-28*. We determined that *XX* animals carrying mutations in both *dpy-22* and *dpy-26*, *dpy-27*, or *dpy-28* are inviable, whereas *dpy-22* mutant *XO* animals carrying an additional mutation in *dpy-26*, *dpy-27*, or *dpy-28* are indistinguishable from *dpy-22(e652) XO* animals (MATERIALS AND METHODS). Although *dpy-22* clearly affects *X*-linked gene expression, it is probably not controlling or controlled by the activities of *dpy-26*, *dpy-27*, or *dpy-28*; rather it is probably involved in an independent process.

Effects of mutations in *dpy-27*, *dpy-28*, or *dpy-29* on the process of sex determination: Mutations in *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29* have no apparent effect on sex determination in otherwise wild-type diploid *XX* or *XO* animals. Although an egg-laying defect is sometimes associated with a subtle disruption in sex determination, the egg-laying defect caused by mutations in the *dpy* genes is instead probably an indirect consequence of disruptions in dosage compensation. The Egl phenotype associated with the sexual transformation caused by weakly masculinizing mutations in the sex determination pathway results from the loss of the hermaphrodite-specific HSN motor neurons required for egg laying (TRENT, TSUNG and HORVITZ 1983; TRENT, WOOD and HORVITZ 1988). The *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-29 XX* mutant animals all possess HSNs. Two other examples of an *XX*-specific Egl phenotype caused by disruptions in dosage compensation (rather than HSN loss) result from mutations in *sdc-1* (VILLENEUVE and MEYER

TABLE 8

Viability of *XX* animals carrying combinations of mutations in *dpy-21, dpy-26, dpy-27, dpy-28* or *dpy-29*

Genotype[a]	Percent viability[b]	No. of zygotes (broods)
dpy-27(rh18); dpy-26(n199)[c]	2.0 ± 0.2	507 (3)
dpy-27(rh18) dpy-28(y1)	10.7 ± 1.2	694 (16)
dpy-27(y57) dpy-28(s939)	3.2 ± 0.6	996 (6)
dpy-27(y57); dpy-29(y100am)[d]	16.4 ± 2.3	250 (10)
dpy-28(y1); dpy-29(y100am)[d]	12.7 ± 1.8	331 (8)
dpy-28(y1); dpy-26(y6)[e]	10.7 ± 1.4	484 (9)
dpy-26(n199); dpy-21(e459)	12.3 ± 0.9	1293 (17)
dpy-27(rh18); dpy-21(e428)[f]	26.6 ± 1.2	1175 (27)
dpy-27(y42); dpy-21(e428)	19.1 ± 2.3	298 (6)
dpy-27(y44); dpy-21(e428)	13.2 ± 1.1	874 (7)
dpy-27(y49); dpy-21(e428)	18.8 ± 2.1	342 (6)
dpy-27(y49); dpy-21(y47am)[g]	27.1 ± 1.3	1100 (10)
dpy-27(y49); dpy-21(y87)[h]	15.5 ± 2.3	251 (7)
dpy-27(y49); dpy-21(y88)[i]	20.2 ± 1.7	563 (10)
dpy-27(y56am); dpy-21(e428)	11.8 ± 1.6	422 (8)
dpy-27(y57); dpy-21(e428)	26.7 ± 1.4	1049 (8)
dpy-28(y1); dpy-21(e428)	49.5 ± 1.6	1022 (9)
dpy-27(rh18) dpy-28(y1); dpy-21(e428)	31.3 ± 1.8	645 (11)

Experiments were performed at 20°. At 15°, mutations in *dpy-26* do not rescue the cold-sensitive lethality of mutations in *dpy-26, dpy-27* or *dpy-28*.

[a] Progeny of homozygous mothers.

[b] Viability is calculated for hermaphrodite (*XX*) progeny only ± σ (standard deviation). The viability of the hermaphrodite progeny was calculated in accord with the formula $H/(Z - M)$ where H is the number of adult hermaphrodites, Z the number of zygotes, and M is the number of adult males. σ is calculated in accord with the formula $\sigma = (p{\bullet}q/k)^{1/2}$, where p and q are the probabilities of an animal reaching adulthood (p) or failing to reach adulthood (q), and k is the sample size minus the number of males.

[c] In the strain *dpy-27(y56am); dpy-26(n199)* some of the *XX* animals are viable.

[d] Hermaphrodite homozygous for the *unc-32* marker. In the strain *dpy-28(s939); dpy-29(y100)* some of the *XX* animals are viable.

[e] Hermaphrodite homozygous for the *unc-30* marker.

[f] Percent viability of *dpy-21(e428)* is 82.9 ± 1.4 (731 animals; 3 broods).

[g] Percent viability of *dpy-21(y47am)* is 86.0 ± 1.3 (721 animals; 3 broods).

[h] Percent viability of *dpy-21(y87)* is 93.5 ± 0.9 (692 animals; 3 broods).

[i] Percent viability of *dpy-21(y88)* is 99.7 ± 0.2 (765 animals; 3 broods).

1987) and *sdc-2* (C. NUSBAUM and B. MEYER, unpublished observations).

Mutations in the *dpy* genes have a feminizing effect on animals in which the primary sex-determining signal (*X/A* ratio) is ambiguous. Specifically, we demonstrate that a mutation in *dpy-28* causes the feminization of animals that have intermediate *X/A* ratios. Animals with an *X/A* ratio of 1.0 develop as hermaphrodites, while animals with a ratio of 0.5 develop as males. MADL and HERMAN (1979) demonstrated that *2X;3A* animals with an intermediate *X/A* ratio of 0.67 develop as males whose sexual phenotype is sensitive to slight increases in *X* chromosome material. For example, *2X;3A* animals carrying *X* chromosome duplications of varying sizes range from males to intersexual animals to hermaphrodites, depending on the amount of additional *X* chromosome material. We have found that mutations in *dpy-28* shift the sexual phenotypes of *2X;3A* animals from predominantly male to predominantly hermaphrodite (Table 10). The greatest effect is seen in *2X;3A* animals homozygous for a *dpy-28* mutation (a hermaphrodite to male ratio of 10). The effect of *dpy-28* mutations on *2X;3A* animals is strongly dependent on the maternal genotype. *dpy-28/+/+ 2X;3A* progeny from a mating between *dpy-28 2X;2A* hermaphrodites and *dpy(+) 2X;4A* males have a hermaphrodite to male ratio of 2.6. The proportion of hermaphrodites is much lower (ratio of 0.2) in the *dpy-28/dpy-28/+ 2X;3A* progeny from a mating between *dpy(+) 2X;2A* hermaphrodites and *dpy-28 2X;4A* males. HODGKIN (1987c) has reported that mutations in *dpy-21* and *dpy-27* have a similar effect on *2X;3A* animals.

Furthermore, mutations in the *dpy* genes can also feminize *XX* animals carrying a weak masculinizing mutation in a sex-determining gene. The semi-dominant sex transformation mutation *her-1(n695)* causes all *XX* animals to be Egl due to the death of the HSN neurons and about 30% of the animals to be further masculinized, displaying mail tail structures (TRENT, WOOD and HORVITZ 1988). These weak masculinizing effects of *her-1(n695)* are sensitive to small perturbations in the *X/A* ratio caused by additional *X*-chromosomal material. For example, *her-1(n695)* triplo-*X* animals rarely have masculinized tail structures (TRENT, WOOD and HORVITZ 1988). Duplications of the *X* chromosome, which have no phenotype in a *her-1(+)* background, also suppress the masculinization caused by *her-1(n695)* (A. VILLENEUVE, personal communication). This effect can be mimicked by mutations in *dpy-21* (Table 11) (TRENT, WOOD and HORVITZ 1988). Similarly, mutations in *dpy-27* and *dpy-28* also suppress the masculinization caused by *her-1(n695)* in *XX* animals, reducing the number of masculinized *XX* animals from 30% to <1–6% (Table 11). These results provide an additional demonstration that mutations in the *dpy* genes can have a feminizing effect in situations where the sex determination process has been genetically perturbed.

Extragenic suppressors of *dpy-28*: As a means of identifying other components of the dosage compensation process we sought extragenic suppressors of the *XX*-specific lethality caused by *dpy-28* mutations. We isolated two independent spontaneous wild-type length *dpy-28(y1)* hermaphrodites from liquid cultures (L. CASSON, personal communication) and five independent wild-type length *dpy-28(y1)* hermaphrodites following mutagenesis with ethyl methanesulfonate. (See MATERIALS AND METHODS for a description of the strategy.) In all cases, the wild-type length hermaphrodites were shown to be *dpy-28 XO* animals

TABLE 9

X-chromosome expression in animals containing mutant combinations of *dpy-21*, *dpy-26*, *dpy-27* or *dpy-28*

Genotype	No. of animals scored	Percent mutant nuclei (mean)	Compared to *XXX lin-14*	Compared to *XX lin-14*	Compared to *XO lin-14*
XX	60	79	$P < 10^{-6}$		
XXX[a]	25	26[b]		$P < 10^{-6}$	
XX dpy-27(rh18) dpy-28(y1)	20	29	NS	$P < 10^{-6}$	
XX dpy-26(n199); dpy-21(e459)	20	35	NS	$P < 10^{-6}$	
XX dpy-27(rh18); dpy-21(e428)	20	27	NS	$P < 10^{-6}$	
XX dpy-28(y1); dpy-21(e428)	20	26	NS	$P < 10^{-6}$	
XO	60	49[c]			
XO dpy-27(rh18) dpy-28(y1)	20	49			NS
XO dpy-21(e428)[a]	20	76			$P < 10^{-4}$
XO dpy-21(e459)[a]	20	73			$P < 10^{-4}$
XO dpy-26(n199); dpy-21(e459)	20	67[d]			$P < 10^{-2}$
XO dpy-27(rh18); dpy-21(e428)	20	72[d]			$P < 10^{-3}$
XO dpy-28(y1); dpy-21(e428)	20	67[d]			$P < 10^{-3}$
XO dpy-27(rh18) dpy-28(y1); dpy-21(e428)	20	64[d]			$P < 0.02$

This assay is described in DELONG, CASSON and MEYER (1987). For the experiments in this table all *XX* animals were raised at 24°; all *XO* animals were raised at 20°. All animals carry *lin-14(n179)*.

[a] Data from DELONG, CASSON and MEYER (1987).

[b] Suppression of *lin-14(n179)* in *3X;2A* animals is equivalent to that found in *dpy-21, dpy-26, dpy-27* or *dpy-28* m^-z^- *XX* animals (DELONG, CASSON and MEYER 1987).

[c] *dpy-27; lin-14* or *dpy-28; lin-14 XO* animals are not significantly different from *dpy(+); lin-14 XO* animals (DELONG, CASSON and MEYER 1987).

[d] Not significantly different from *dpy-21(e428* or *e459); lin-14 XO.*

TABLE 10

Sex of triploid *2X;3A dpy-28* animals at 20°

Parental genotype (karyotype)		Sex of progeny (*2X;3A*)			Ratio of hermaphrodites/ males and isx
Hermaphrodite (*2X;2A*)	Male (*2X;4A*)	Hermaphrodite	isx[a]	Male	
unc-32	+	3	1	84	0.03
unc-32	*dpy-28*	32[b]	2[b]	138[b]	0.2
unc-32 dpy-28	+	232[c]	3[c]	88[c]	2.6
unc-32 dpy-28	*dpy-28; lin-14*	72	4	3	10

[a] Intersexual animals are those which exhibit a combination of both male and hermaphrodite features, generally a hermaphrodite vulva and gonad and male copulatory tail structures. In addition, between 15% and 25% of the males from each cross exhibited some subtle hemaphrodite sexual characteristics, for example, a rudimentary tail whip or oocytes and/or yolk in animals with an obviously male gonad.

[b] Genotype of these animals is *dpy-28/dpy-28/+*.

[c] Genotype of these animals is *dpy-28/+/+*.

TABLE 11

Masculinization of *XX* animals by *her-1(n695sd)* is suppressed by mutations in *dpy-21*, *dpy-27* and *dpy-28*

Genotype	Percent masculinized hermaphrodites[a]	No. of viable adults scored (broods)
her-1(n695) unc-42	31.7	1529 (10)
her-1(n695) unc-42 dpy-21(e428)	1.8	805 (11)
dpy-27(rh18); her-1(n695) unc-42	<1[b]	195 (34)
dpy-27(y57); her-1(n695) unc-42	2	149 (6)
dpy-28(y1); her-1(n695) unc-42	6	78 (9)

[a] Animals lacking a hermaphrodite tail whip.

[b] 0/195.

transformed into hermaphrodites by mutations in either *her-1 V* or a newly defined gene *xol-1 X*. From 22,000 mutagenized haploid genomes screened we isolated no mutants in which the *XX*-specific lethality had been suppressed.

Both *her-1* and *xol-1* cause the feminization of *XO* animals (HODGKIN 1980; MILLER *et al.* 1988). *dpy-28; her-1* and *dpy-28; xol-1 XO* hermaphrodites are wild type in length, whereas *dpy-28; her-1* and *dpy-28; xol-1 XX* hermaphrodites are dumpy or dead. While the *her-1* gene is required for male sexual development, it does not appear to play a role in the dosage compensation process (VILLENEUVE and MEYER 1987; DELONG, CASSON and MEYER 1987). However, *xol-1*, although it was isolated on the basis of its effects on sex determination, is a complex gene that is required for the proper control of both sex determination and dosage compensation in *XO* animals (MILLER *et al.* 1988).

DISCUSSION

The relationship between *dpy-26*, *dpy-27*, *dpy-28* and *dpy-29* and the process of dosage compensation:

We have presented a genetic characterization of several essential components of the dosage compensation process in *C. elegans*, including the initial genetic analysis of *dpy-29*. Mutations in the genes *dpy-26* (HODGKIN 1983), *dpy-27*, *dpy-28*, and *dpy-29* result in remarkably similar phenotypes and appear to cause *XX* animals to express each set of *X*-linked genes at a level appropriate for *XO* animals. While *dpy XO* animals are fully viable and essentially wild type, *dpy XX* animals exhibit overexpression of *X*-linked genes (MEYER and CASSON 1986; DELONG, CASSON and MEYER 1987; MENEELY and WOOD 1987) and an incompletely penetrant maternal-effect lethality that often results in a distinctive L1 larval arrest. The alleles are uniformly cold-sensitive and exhibit greater than 99% lethality at 15°, suggesting they reveal an essential cold-sensitive process. Genetic analysis of the interactions among mutations in these genes indicates that both the viability and the level of *X*-linked gene expression in *XX* animals carrying mutations in two or more *dpy* genes are the same as in animals carrying only a single mutation. Taken together these results suggest that all four genes act in a common process to promote dosage compensation. However, the *dpy* genes probably play distinct roles within the dosage compensation process, since any single *dpy* mutation causes significant lethality, and additional wild-type copies of one or more *dpy* genes do not substitute for the loss of a different *dpy* gene.

Based on cumulative evidence, an appealing way to think about dosage compensation in *C. elegans* is that in *XX* animals the *X*-linked genes on each *X* chromosome are active but their level of expression is lowered via the action of the *dpy* genes to equal the level in *XO* animals (MILLER *et al.* 1988; MEYER and CASSON 1986; MENEELY and WOOD 1987; DELONG, CASSON and MEYER 1987; HODGKIN 1983). According to this model the basal level of *X* chromosome expression would be that associated with the single *X* chromosome in *XO* animals, whose *X* chromosome expression is apparently not regulated by the maternal-effect *dpy* genes. These *dpy* genes might achieve dosage compensation by reducing the basal level of *X* chromosome expression through a variety of mechanisms including acting as subunits of a multimeric complex that physically interacts with the *X* chromosome and affects its accessibility to transcription factors. Alternatively, the *dpy* genes might be part of a biochemical pathway that produces a factor required for the global regulation of *X*-linked genes in *XX* animals. According to either scenario, mutations in any one of the *dpy* genes would disrupt the *XX* mode of dosage compensation by failing to reduce the basal (*XO*) level of *X*-linked gene expression, resulting in the overexpression of *X*-linked genes in *XX* animals. An important caveat in thinking about any specific model for the action of the *dpy* genes in *XX* animals is that we cannot as yet eliminate the model that the *dpy* genes have a completely different function: to prevent inappropriate expression of putative *XO*-specific genes responsible for elevating *X* chromosome expression in *XO* animals. We have as yet been unable to find any evidence for such a class of *XO*-specific dosage compensation genes. They might reasonably have been isolated as suppressors of the *XX*-specific lethality of the *dpy-28* mutation.

One aspect to the regulation of *X* chromosome expression not accounted for by either model is the existence of various mutations that affect *X*-linked gene expression in both sexes. Mutations in *dpy-21* are known to elevate *X* chromosome expression in adults of both sexes, although *XX* animals are more severely affected (MEYER and CASSON 1986; MENEELY and WOOD 1987). There is evidence for the reduction in *X*-linked gene expression in *dpy-21 XO* L1 larvae (DELONG, CASSON and MEYER 1987). Because mutations in *dpy-21* fail to overexpress an *X*-linked gene that is not compensated, the effects of *dpy-21* mutations on *X* chromosome expression are probably caused through the involvement of *dpy-21* in dosage compensation rather than a more general transcription mechanism (MEYER and CASSON 1986). Mutations in *dpy-22* cause both sickness and reduction of *X*-linked gene expression in *XX* and *XO* animals (DELONG, CASSON and MEYER 1987; MENEELY and WOOD 1987). The mutation *y2* (MATERIALS AND METHODS) reduces *X* chromosome expression in both *XX* and *XO* *y2/y2* animals (from *y2/+* mothers) and results in a completely penetrant *XO*-specific maternal-effect lethality and 10% lethality in *XX* animals. *y2* does not suppress any of the *XX*-specific phenotypes caused by mutations in *dpy-28* or *sdc-2*, nor is the *XO*-specific lethality of *y2* suppressed by mutations in *dpy-28* or *sdc-2* (J. PLENEFISCH, unpublished observations). (The assays used to evaluate *X* chromosome expression in *dpy-22* and *y2* mutants were genetic assays that could not evaluate whether autosomal transcription is also affected.) Conceivably, one or more of these genes might be required in both sexes for general *X* chromosome transcription, proper dosage compensation, or for the control of transcription on all chromosomes.

Ultimately an informative way to discern the mechanism of dosage compensation (whether it involves turning down *X* chromosome expression in *XX* animals, turning up *X* chromosome expression in *XO* animals, or some combination of both) is to compare the transcript levels of autosomal genes in their normal locations to the transcript levels of these genes translocated to various regions of the *X* chromosome. The availability of a DNA transformation system in the nematode provides the means to begin this molecular dissection of dosage compensation (FIRE 1986).

Although the dosage compensation mechanisms used in *C. elegans* and *Drosophila melanogaster* both involve differential *X* chromosome expression, the

details of the mechanisms appear to differ. In nematodes dosage compensation appears to be implemented through a class of genes whose mutant phenotype is hermaphrodite-specific lethal; in flies, mutations in the class known to implement dosage compensation, *msl-1, msl-2, mle* and *mle(3)132*, result in a male-specific lethality. These genes are thought to achieve dosage compensation by turning up expression of the single *X* chromosome in males, since mutations in these genes result in the inappropriate reduction in *X* chromosome expression in *XY* males but have no apparent effect *X* chromosome expression in *XX* remales [for review see LUCCHESI and MANNING (1987)]. These genes are not sufficient for dosage compensation early in Drosophila development, since mutations in them fail to disrupt compensation of *runt*, a gene that is normally active and properly regulated by the blastoderm stage (GERGEN 1987).

The dosage compensation process is functional by mid-embryogenesis: To evaluate when the dosage compensation process functions, we exploited the temperature sensitivity of *dpy-28(y1)* to define a temporal requirement for this gene. The temperature-sensitive period for the lethality caused by *dpy-28(y1)* extends from 5 to 9 hr after fertilization and is centered around the comma stage. This period is just prior to the first evidence of sexual dimorphism, the programmed cell deaths of two hermaphrodite-specific motor neurons (HSNs) in the male and four male-specific sensory neurons in the hermaphrodite (SULSTON *et al.* 1983). If this temperature-sensitive period reflects the time of action of *dpy-28*, then the dosage compensation process is likely to be functioning at least by mid-embryogenesis, 5 hr after fertilization when many zygotic genes are active (HECHT, GOSSET and JEFFERY 1981; EDGAR and MCGEE 1986; and I. SCHAUER and W. WOOD, personal communication). The temperature-sensitive period probably marks the latest time the dosage compensation process begins, since the temperature-shift experiments measured lethality of *XX* animals rather than overexpression of *X*-linked genes. The temperature-sensitive period probably reflects a critical time in embryogenesis when overexpression of particular *X*-linked genes leads to larval lethality, rather than reflecting the earliest time overexpression of *X*-linked genes begins. The potential time of *dpy-28* action suggests two possible roles for *dpy-28* in the dosage compensation process. It might be required only early to initiate the hermaphrodite mode of dosage compensation (in response to the action of *sdc-1* and *sdc-2*), or it might be required continuously to maintain dosage compensation. If the latter case were true, the measured temperature-sensitive period might appear shorter than the actual time of *dpy-28* action, because the failure to regulate particular *X*-linked genes during comma stage leads to lethality. There are two caveats to the interpretation of the temperature-shift experiments. First, if the temperature-sensitive period reflects the time of synthesis of *dpy-28* product rather than the time of action, then the *dpy-28* product could be required later in development and the temperature-sensitive period would not be an indication of when the dosage compensation process is functional. Second, it is possible that the lethality caused by *dpy-28* is not the consequence of overexpression of *X*-linked genes, but rather reflects a second vital function of *dpy-28* that is unrelated to its role in dosage compensation.

With the exception of *dpy-28(y1)*, all available mutations in the *dpy* genes result in a cold-sensitive lethality. A reasonable explanation of this phenomenon is that the removal of the dosage compensation genes reveals a cold-sensitive process that is seriously affected by the overexpression of certain *X*-linked genes. Accordingly, the cold-sensitive period of *dpy-27(y57)* would represent the time in this putative cold-sensitive process that is most sensitive to disruptions in dosage compensation. It is intriguing that the cold-sensitive period for *dpy-27(y57)* is almost coincident with the temperature-sensitive period for *dpy-28(y1)*, suggesting that both mutations reveal processes (or a process) that are sensitive to upsets in dosage compensation. The cold sensitive period as well as the temperature sensitive period is likely to be a reflection that the dosage compensation process is functional at least by mid-embryogenesis.

Effects of *dpy* mutations on sexual phenotype: While mutations in one or more *dpy* genes have no apparent effect on sex determination in otherwise wild-type diploid *XX* or *XO* animals, they do have a feminizing effect on animals that have an intermediate primary sex-determining signal (an *X/A* ratio of 0.67). Animals with a *2X;3A* karyotype are predominantly male, but become predominantly hermaphrodite in combination with a mutation in *dpy-28*. This feminizing effect has a strong maternal component such that *2X;3A* animals that are themselves heterozygous for the *dpy-28* mutation are strongly feminized provided the mother was homozygous for the *dpy-28* mutation. The same feminizing effect on *2X;3A* animals has been observed with mutations in *dpy-21* and *dpy-27* (HODGKIN 1987c). Additionally, mutations in *dpy-21* shift diploid *XO* animals carrying a large *X* chromosome duplication from males to intersexes (MENEELY and WOOD 1984). We have also observed that mutations in *dpy-27* and *dpy-28* feminize *XX* animals carrying a weakly masculinizing mutation in a sex-determining gene [*her-1(n695)*], as do mutations in *dpy-21* (TRENT, WOOD and HORVITZ 1988). It seems unlikely that the *dpy* genes act to coordinately control both dosage compensation and sex determination since the *dpy*

mutations have feminizing effects on sex determination and masculinizing effects on dosage compensation. More likely the *dpy* genes are required to implement the hermaphrodite mode of dosage compensation and only indirectly affect sex determination through their effects on the dosage compensation process. In this respect the *dpy* genes are very different from *sdc-1* and *sdc-2*, both of which coordinately control the hermaphrodite mode of sex determination and dosage compensation, and *xol-1*, which controls the male modes of both processes. Mutations in *sdc-1* and *sdc-2* shift both processes in the same direction, to the male mode, while mutations in *xol-1* shift them to the hermaphrodite mode.

A plausible way disruptions in the dosage compensation process might cause feminization of animals whose sexual identity is ambiguous is through the overexpression of *X*-linked genes. One previously suggested possibility is that the overexpression caused by mutations in the *dpy* genes affects the perceived *X/A* ratio. According to this scenario *X* chromosome transcripts would comprise the numerator of *X/A* ratio and their level would be elevated by the *dpy* mutations, causing an increase in the perceived *X/A* ratio and a shift in sexual fate. This scheme would allow for a significant maternal contribution (HODGKIN 1987c). There are several other plausible scenarios. For example, overexpression of the *X* chromosome could lead to an elevation in the *X*-linked *sdc-1* and *sdc-2* transcript levels. Theoretically, overexpression of either of these two genes could result in the feminization of *2X;3A* animals. Since *sdc-1* has a strong maternal component, overexpression of the maternal *sdc-1* gene could cause feminization of the zygote, a scenario consistent with the known maternal contribution to the feminization of *2X;3A* animals. Alternatively, the feminization need not be due to the overexpression of a few genes with major effects in the sex determination pathway, but rather to the overexpression of a number of genes that serve to modulate the sex determination pathway. Individually these genes may have little or no effect on sex determination, however together they could have profound effects on sexual development. These genes need not be sex determination genes *per se* but could be any genes whose products are utilized in implementing sexual fate. While overexpression of these genes might not have any effect on the sexual development of wild-type animals, their overexpression could affect animals whose sexual identity is ambiguous.

It is also conceivable that the feminizing effects are not the result of *X* chromosome overexpression but rather the consequence of changes in *X* chromosome structure. For example, the absence of the *dpy* products might alter the accessibility of putative *X* chromosome sites (the numerator of the *X/A* ratio) and lead to an incorrect assessment of the *X/A* ratio.

An overview of dosage compensation: Based on our work and that of others, a broad outline of the control and execution of dosage compensation emerges (Figure 1) (VILLENEUVE and MEYER 1987; MILLER *et al.* 1988; C. NUSBAUM, personal communication). The *X/A* ratio appears to act as the primary signal to trigger either the hermaphrodite or male modes of both dosage compensation and sex determination. In *XO* animals, *xol-1* (and perhaps other genes) apparently controls the male modes of both processes by ensuring that genes (or gene products) directing the hermaphrodite modes of sex determination and dosage compensation (*sdc-1* and *sdc-2*) are inactive in *XO* animals. Inappropriate expression of these hermaphrodite-specific genes and those that affect the *XX* mode of dosage compensation (the *dpy* genes) is lethal to *XO* animals. In *XX* animals, *sdc-1* and *sdc-2* respond to a high *X/A* signal and initiate the hermaphrodite modes of both processes, which are then implemented independently through separate pathways. At least part of the machinery that ensures proper dosage compensation of *X*-linked genes in *XX* animals includes *dpy-21*, *dpy-26*, *dpy-27*, *dpy-28* and *dpy-29*, each of which probably plays a distinct role. In view of the small number of *dpy-26*, *dpy-27*, *dpy-28* and *dpy-29* alleles, it is likely there are more as yet unidentified genes in this class. Consistent with the proposal that *sdc-1* (and *sdc-2*) acts prior to *dpy-28* to activate the hermaphrodite mode of dosage compensation (mediated in part by *dpy-28*) is the temperature-sensitive period for *sdc-1*. The presence of *sdc-1* activity only appears to be required through the first half of embryogenesis to establish the *XX* mode of dosage compensation (A. VILLENEUVE, personal communication), while the requirement for *dpy-28* extends at least two hours beyond this point. The identification and genetic characterization of several essential components of the dosage compensation process provides the background to begin a molecular dissection of how dosage compensation is established and implemented in *C. elegans*.

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