Assessment of X Chromosome Dosage Compensation in Caenorhabditis elegans by Phanotypic Analysis of lin-14

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Manuscript received July 15, 1987
Revised copy accepted September 5, 1987

ABSTRACT

Caenorhabditis elegans compensates for the difference in X chromosome gene dose between males (XO) and hermaphrodites (XX) through a mechanism that equalizes the levels of X-specific mRNA transcripts between the two sexes. We have devised a sensitive and quantitative genetic assay to measure perturbations in X chromosome gene expression caused by mutations that affect this process of dosage compensation. The assay is based on quantitating the precocious alae phenotype caused by a mutation that reduces but does not eliminate the function of the X-linked gene lin-14. We demonstrate that in diploid animals the lin-14 gene is dosage compensated between XO and XX animals. In XXX diploid animals, however, lin-14 expression is not compensated, implying that the normal dosage compensation mechanism in C. elegans lacks the capacity to compensate completely for the additional X chromosome in triplo-X animals. Using the lin-14 assay we compare the effects of mutations in the genes dpy-21, dpy-26, dpy-27, dpy-28, and dpy-22 on X-linked gene expression. Additionally, in the case of dpy-21 we correlate the change in phenotypic expression of lin-14 with a corresponding change in the lin-14 mRNA transcript level.

In the nematode Caenorhabditis elegans, males (XO) and hermaphrodites (XX) possess different doses of X-linked genes, a consequence of the primary sex-determining signal, the X/A ratio (the ratio of the number of X chromosomes to sets of autosomes). C. elegans compensates for this difference in X chromosome gene dose through a mechanism that equalizes the levels of X-specific mRNA transcripts between the two sexes (Meyer and Casson 1986). How the genetic control of X chromosome dosage compensation is achieved in C. elegans remains a fundamental and unresolved question.

Previous studies have established the involvement of at least five genes, dpy-21, dpy-26, dpy-27, dpy-28 and sdc-1, in the process of dosage compensation (Hodgkin 1983a, 1987; Menneely and Wood 1984, 1987; Meyer and Casson 1986; Villeneuve and Meyer 1987; J. Plenefisch and B. Meyer, unpublished observations). The potential role of these genes in determining the correct level of X chromosome expression was inferred initially from the finding that mutant phenotypes are dependent on X chromosome dosage (more specifically, the X/A ratio) but not on sexual phenotype. Whereas XO mutant animals are essentially wild type, XX animals are either dumpy (short and fat) or inviable, phenotypes postulated in these cases to result from inappropriate levels of X-linked gene expression. Subsequently, it was demonstrated that mutations in these genes disrupt dosage compensation, resulting in overexpression of X-linked genes in XX animals. The extent of overexpression in dpy-21, dpy-27, dpy-28 and sdc-1 mutant animals is reflected by a two- to threefold increase in the levels of X-specific mRNA transcripts (Meyer and Casson 1986; Villeneuve and Meyer 1987).

To facilitate the genetic dissection of dosage compensation we found it valuable to develop a rapid and sensitive genetic assay for analyzing X-linked gene expression. A genetic assay is an expedient means to explore the interactions among the known dosage compensation genes in C. elegans and to establish the role of newly characterized genes in this process. Such an assay permits comparisons of levels of X-linked gene activity among animals mutant in the different dosage compensation genes, whether or not mutant animals are amenable to direct analysis of transcript levels. For this purpose we adopted the approach, originally conceived by Muller (1950), of using the severity of mutant phenotypes caused by X-linked hypomorphic mutations (which reduce but do not eliminate gene function) to indicate the level of gene expression. Indeed, Muller first discovered the phenomenon of dosage compensation in Drosophila using this approach. He observed that the phenotypes produced by these X-linked mutations were equivalent in XX and XY individuals, indicating similar levels of X-linked gene products in both sexes despite the difference in gene dose. In our assay suppression of the mutant phenotype reflects an increase in X-linked gene expression, whereas enhancement of the mutant phenotype reflects a reduction in X-linked gene expression.
The assay is based on the quantitation of a phenotype caused by a mutation that reduces but does not eliminate the function of the X-linked gene lin-14, one of several genes that specify the temporal pattern of development in C. elegans (AMBROS and HORVITZ 1984, 1987). lin-14 fulfills the genetic and molecular criteria required for a gene to be optimal for a genetic assay of X-linked gene expression. We demonstrate that 1) lin-14(n179ts) is dosage compensated between XO and XX animals. It is essential that a gene used to monitor effects on dosage compensation be subject to the regulation normally associated with the process. 2) The lin-14 mutant phenotype can be quantitated to allow precise comparisons between strains. 3) The mutant phenotype associated with lin-14(n179ts) is sensitive to small changes in the level of gene product; hence small perturbations in X-linked gene expression caused by mutations in the dosage compensation genes are reflected in the lin-14 mutant phenotype. 4) A correlation can be made between the severity of the mutant phenotype and the level of lin-14 mRNA, since the wild-type gene has been cloned (G. RUHKUN, V. AMBROS and R. HORVITZ, personal communication). We have determined that the degree of suppression of the lin-14(n179ts) phenotype is correlated with the increase in the lin-14 mRNA level, a critical finding validating the view that this assay represents changes in X-linked mRNA transcript levels. The effects on lin-14(n179ts) expression that we measure are likely to reflect the state of X-linked gene expression in the L1 stage, because the temperature sensitive period of lin-14(n179) and the time of maximum lin-14 mRNA accumulation occur during the L1 (AMBROS and HORVITZ 1987; G. RUHKUN, V. AMBROS and R. HORVITZ, personal communication). The lin-14 assay thus provides an opportunity to examine gene expression at a specific early time.

Using the lin-14 assay, we analyze the effects of mutations in the X dosage-dependent dpy genes on the level of X-linked gene expression in XO and XX animals, enabling us to confirm and extend our understanding of the involvement of these genes in the process of dosage compensation. Moreover, we compare the levels of X-linked gene expression in these mutants to the level found in XXX animals. Finally, we explore the potential role of dpy-22 (HODGKIN 1983a; MENEELY and WOOD 1987) in the control of X-linked gene expression in both XX and XO animals.

MATERIALS AND METHODS

Strain maintenance and nomenclature: The techniques used for the maintenance and handling of C. elegans were as described by BRENNER (1974). Experiments were performed at 20°C unless otherwise noted. The wild-type background for all strains used in this study was the C. elegans variety Bristol (designated N2), obtained from S. BRENNER. The following mutations are referred to in the text:

- LG III: dpy-27(chr18, y49), unc-32(e189), unc-69(e587), dpy-28(y1, s999), vab-7(e1562), dpy-18(e364)
- LG IV: dpy-20(e1282), unc-22(e66, s7), dpy-26(n199, y6), unc-30(e191), dpy-4(e1166), unc(n752) (which is linked to nT1 and confers a recessive Unc phenotype)
- LG V: dpy-11(e224), her-1(e1520), unc-42(e270), ali-1(e1934), him-5(e1490), dpy-21(e428, e459, y47, y50)
- LG X: dpy-6(e14), dpy-22(e652), lin-14(n179ts)

chromosomal rearrangements: szT1(1;X), nT1(IV;V), yDp1(IV;V), nDp19(X).

dpy-27(chr18) was isolated by E. HEDGECOCK and mapped by J. HODGKIN (personal communication). dpy-27(y49) was isolated as a suppressor of the XO-specific lethality of rol-1(y9) (L. MILLER and B. MEYER, unpublished observations). dpy-28(y1) was isolated and characterized by J. PLENIFISCH and B. MEYER (unpublished observations). y39, a gift from D. BALLIE, was shown to be an allele of dpy-28 by its failure to complement dpy-28(y1) (J. PLENIFISCH and B. MEYER, unpublished observations) and by its map position between unc-32(e189) and vab-7(e1562) (data not shown). dpy-26(n199) is described in HODGKIN (1983a), y6, a gift from E. WOLINSKY, was shown to be an allele of dpy-26 by its failure to complement dpy-26(n199) and by its map position between unc-22(e66) and unc-30(e191) (data not shown). dpy-21(e428, e459) and dpy-22(e562) are described in HODGKIN and BRENNER (1977). dpy-21(y47, y50) were isolated as suppressors of the XO-specific lethality of rol-1(y9) (L. MILLER and B. MEYER, unpublished observations). lin-14(n179) and ndf19 are described in AMBROS and HORVITZ (1984). nT1 and unc(n752) are described in FERGUSSON and HORVITZ (1985). yDp1 is described in this work. Nomenclature conforms to the standard usage described in HORVITZ et al. (1979).

In some cases XO males were generated by using the mutation him-5(e1490) (HODGKIN, HORVITZ and BRENNER 1979). The original isolate of him-5(e1490) contains the linked mutation ali-1(1934), which eliminates L1-specific alae (C. KENYON, personal communication). Although ali-1 does not affect production of adult alae, it does have a minor effect on the severity of the mutant phenotype in lin-14(n179) strains. (At 20°C, ali-1 him-5; lin-14(n179) males are H0% mutant, whereas him-5; lin-14(n179) males are 50% mutant.) We ensured that our him-5 strains did not contain this mutation either by using ali+(+) him-5 (a gift from C. KENYON) in their construction or by examining them after construction to ensure that ali-1 had been lost through recombination.

Standard method of scoring the lin-14 assay: The lin-14 mutant phenotype scored in this assay is described in results. Lin-14(n179) is a recessive, hypomorphic mutation with a temperature-sensitive period during the L1 (AMBROS and HORVITZ 1984, 1987). All stocks were maintained at 20°C where lin-14(n179) fertility and viability are good. Before scoring, unstarved L4 hermaphrodites were transferred to plates at 20°C or 24°C and at least 20 animals from their progeny were assayed for the degree of mutant phenotype. To minimize any possible bias in selecting animals for scoring, each plate was divided into sectors by placing a grid beneath it. All animals of the correct stage were picked or their progeny were assayed for the degree of mutant phenotype by scoring, each plate was divided into sectors by placing a grid beneath it. All animals of the correct stage were picked or their progeny were assayed for the degree of mutant phenotype.
divisions were complete and the pairs of sister nuclei were still easy to identify. For some mutant strains in which the L3 molt occurs prematurely, resulting in the completion of seam cell nuclear divisions after the molt, animals were picked in L3 lethargus and monitored individually to identify when seam cell nuclear divisions were complete. In all cases the extent of gonad and vulva development (KIMBLE and HIRSH 1979) was used to confirm the developmental stage and sex of the animal. In general animals were immobilized for microscopy by mounting them with 20 mm sodium azide, a modification of the SULSTON and HORVITZ (1977) procedure.

Although, in lin-14 mutant animals, cessation of seam cell nuclear divisions is associated with the production of precocious adult lateral alae, the presence or absence of alae is not always a reliable marker for scoring the developmental fate of a seam cell nucleus. For example, in lin-14(n179) animals raised at 20°, the less severely mutant animals can produce alae that are so faint as to be unscorable. Thus, nuclei were scored as mutant if they failed to divide, whether or not they produced scorable precocious alae. A nucleus was scored as divided only if there were clearly identifiable sister nuclei, or, in a few instances, when it was clearly in the process of division. This procedure eliminates any bias in scoring that could otherwise arise from the occasional undetected alae.

Sources of variability: In this assay, the fates of the midbody seam cell nuclei [those lying between the posterior bulb of the pharynx and the anus, the H(1,2), V(1-6) descendants (SULSTON and HORVITZ 1977)] were scored. Wild-type hermaphrodites have between 11 and 13 midbody seam cell nuclei due to some positional (not lineage) variability. Wild-type males have 10 midbody seam cell nuclei and appear to have no positional variability. Hermaphrodites with a dumpy phenotype frequently have 11 midbody seam cell nuclei.

lin-14(n179) animals grown at 20° frequently have additional (ectopic) seam cell nuclei (14 or more total midbody seam cell nuclei). These are believed to be the result of some seam cells undergoing a precocious (mutant) doubling in the L1 followed by a wild-type doubling in the L2 (AMBROS and HORVITZ 1987). Only rarely is an animal found with fewer than the expected number of seam cell nuclei. Thus, the complementary combination (wild-type L1 followed by precocious L2) must occur less frequently.

Additional lin-14 phenotypic markers: Mutations in lin-14 are pleiotropic. Therefore, several phenotypes besides the fates of seam cell nuclei can be scored with Nomarski optics and used for easy and unambiguous identification of all homozygous lin-14(n179) animals or lin-14(n179)/nDf19 animals from plates that also contain lin-14(n179)+/+, nDf19/+ , and +/- siblings. These phenotypes ensure the accuracy of diagnosing the genotypes in the strain constructions described below. At 20° lin-14(n179) animals that appear wild type on the basis of their L3 seam cell nuclear divisions always have ectopic seam cell cell nuclei (14 or more total midbody seam cell nuclei), a phenomenon which clearly differentiates them from true wild-type animals. Two additional phenotypes are evident in severely mutant lin-14 animals. 1) Seam cell nuclei in these animals frequently deviate from a straight line, sometimes straying as far away as the ventral cord. 2) Hermaphrodites usually have abnormal gonad morphology.

Construction and scoring of lin-14(n179)/nDf19: him-5; lin-14(n179) males, raised at 15°, were mated with szT1/nDf19 hermaphrodites at 20°. Random hermaphrodite progeny were scored without regard to the Lin phenotype observable at the dissecting microscope level. The animals clearly fell into two distinct classes; there were no animals with intermediate phenotypes. Lin hermaphrodites, inferred to be him-5/+; lin-14(n179)/nDf19, had extensive precocious alae, aberrant gonad morphology and aberrant positioning of the seam cell nuclei. Non-Lin hermaphrodites, inferred to be either szT1/nDf19 self-progeny or him-5/+; szT1/lin-14(n179) cross-progeny, had 11 to 13 pairs of sister nuclei, normal gonad morphology and normal positioning of seam cell nuclei. Two facts make it certain that all lin(n179)/nDf19 animals can be distinguished on the basis of the additional Lin phenotypes: 1) lin-14(n179) is a hypomorphic allele (see RESULTS) such that n179/Df animals are more mutant than n179/n179 animals, and 2) all lin(n179) animals have the additional Lin phenotypes described above. Moreover, no animals with phenotypes intermediate between the two classes were found, as would be the case if the experiment were wrong. Thus all animals of the desired phenotypic class (Lin) obtained in these strain constructions and the ones described below should represent all animals of the desired genotypic class.

Construction and scoring of XO her-1; lin-14(n179)/0: To address the question of whether there is any sex-specific difference in the expression of the lin-14 mutant phenotype, we examined the effect of transforming XO lin-14(n179)/0 animals into hermaphrodites. her-1/+ males were mated with dpy-11 her-1; lin-14(n179)/0, had precocious alae, nontending and ectopic seam cell nuclei. Non-Lin hermaphrodites, inferred to be dpy-11 her-1/+; her-1; lin-14(n179)/+, had 11 to 13 pairs of sister nuclei, normal gonad morphology and normal positioning of seam cell nuclei. The results obtained for XO her-1; lin-14(n179) hermaphrodites were consistent with the results obtained using the X-linked marker unc-3 to identify XO hermaphrodites in a separate experiment (data not shown). The unc-3(e151) mutation results in a slight suppression of the lin-14(n179) mutant phenotype in males and hermaphrodites raised at 20°. It was therefore more accurate to make the appropriate comparisons between strains that did not carry unc-3. This suppression by unc-3 is not seen at 24°, the temperature at which the most reliable and accurate suppression studies are conducted.

Construction and scoring of her-1; lin-14(n179)/nDf19; lin-14(n179)/nDf19 animals are frequently sterile or have severely reduced brood sizes, so the strain construction was designed to allow unambiguous identification of hermaphrodites of the desired genotype at the L3 molt. dpy-11 him-5 males were mated with her-1 unc-42; lin-14(n179)/0 hermaphrodites at 15° to give mating males of the genotype + her-1 unc-42/+; dpy-11 +/+ + him-5; lin-14(n179)/0. (unc-42 was included in the her-1; lin-14(n179) strain to improve the mating efficiency of the dpy-11 males.) The cross-progeny males were mated with hermaphrodites of genotype dpy-11 her-1; szT1/nDf19 at 20° and the non-Dpy cross-progeny hermaphrodites were analyzed. These non-Dpy hermaphrodites clearly fell into two distinct classes with no intermediate phenotypes. Lin hermaphrodites, inferred to be + her-1 unc-42/dpy-11 her-1 +, + lin-14(n179)/nDf19 had extensive precocious alae, aberrant gonad morphology and aberrant positioning of seam cell nuclei. Non-Lin hermaphrodites, inferred to be + her-1 unc-42/dpy-11 her-1 +; szT1/lin-14(n179), had 11 to 13 pairs of sister nuclei, normal gonad morphology and normal positioning of seam cell nuclei.

Construction and scoring of XXX lin-14(n179): When animals were raised at 24° we could not reliably distinguish
between XX lin-14(n179) (sick, short) and XXX lin-14(n179) (less sick, Dpy) animals on the basis of phenotype, so the genotype of each animal scored was inferred from the phenotype of the progeny. In the brood of homozygous him-5(e1490) hermaphrodites (Hodgkin, Horvitz and Brenner 1979). However, at 24°C both XX lin-14(n179) and XXX lin-14(n179) hermaphrodites frequently have small broods, often causing a distortion of the progeny ratios. Thus in a him-5 background, the relative frequencies of either XXX Dpy progeny or XO male progeny will not be a reliable indicator of the maternal genotype in all cases. Therefore, a non-Him lin-14(n179) XX strain was used so that the presence of Dpy hermaphrodite progeny would be diagnostic for a triplo-X parent. To construct such a strain, a Dpy hermaphrodite of genotype XXX him-5; lin-14(n179) was mated with XO lin-14(n179) males that had been raised at 15°C. Dpy F1 progeny were cloned and grown at 20°C. Dpy F2 progeny were cloned from plates that had no males [the F1 hermaphrodite must have been XXX him-5/+; lin-14(n179)]. Subcloning of Dpy hermaphrodites continued until a strain was obtained that only produced males at the spontaneous rate. The strain was propagated at 20°C by cloning Dpy hermaphrodites, because a triplo-X strain will produce both XX (wild-type length) and XXX (Dpy) progeny. For scoring, animals raised at 24°C were mounted individually onto slides without sodium azide, analyzed, and then transferred to individual plates at 20°C to examine their progeny.

Construction and scoring of dpy-28(y1, s939); lin-14(n179) hermaphrodites: dpy-28(y1); lin-14(n179) hermaphrodites were maintained at 24°C for a few generations before enough animals were obtained for scoring. dpy-28(s939); lin-14(n179) cannot be maintained as a homozygous strain. Homozygous hermaphrodites for scoring were obtained by cloning approximately 80 non-Unc L4 hermaphrodite progeny of + dpy-28(s939)/unc-69 +; lin-14(n179) animals to individual plates at 24°C. Progeny from homozygous dpy-28 mothers were scored. Seam cell nuclear divisions in dpy-28 hermaphrodites occurred approximately two hours after the animals emerged from the L3 molt. It should be noted that dpy-26, dpy-27 and dpy-28 hermaphrodites develop much more slowly than wild-type hermaphrodites. All developmental events take much longer, including lethargus, which may last for many hours. Thus, the timing of events in these animals cannot be compared directly to the timing of the same events in wild-type hermaphrodites. When scoring dpy-26, dpy-27 and dpy-28 hermaphrodites the length of time between cloning an animal and scoring nuclear divisions was minimized by cloning animals onto individual slides and scoring for the presence of a large cuticular plug blocking the pharynx. This plug is characteristic of the end of lethargus. Only animals in the late stages of L3 lethargus were kept for scoring the fates of seam cell nuclei.

Scoring of dpy-27(r18, y49); lin-14(n179) hermaphrodites: dpy-27(r18); lin-14(n179) hermaphrodites were maintained at 24°C for two to three generations before enough animals were obtained for scoring. dpy-27(y49); lin-14(n179) hermaphrodites were scored one generation after shifting the strain to 24°C. Seam cell nuclear divisions in dpy-27 hermaphrodites occurred around the time that the animals emerged from the L3 molt.

Construction and scoring of dpy-26(n199, y6); lin-14(n179); dpy-26(n199); lin-14(n179) and dpy-26(n199); lin-14(n179) could be maintained as homozygous strains for only a few generations. Homozygous hermaphrodites for scoring were obtained by cloning approximately 80 non-

Unc L4 hermaphrodite progeny from dpy-26(n199) +/+ unc-30; lin-14(n179) and from dpy-26(n199) +/+ unc-30; lin-14(n179) animals onto individual plates at 24°C. Progeny of homozygous dpy-26(n199); lin-14(n179) mothers were scored immediately. Progeny of homozygous dpy-26(n199); lin-14(n179) mothers were maintained at 24°C for two to three generations before animals were scored. Seam cell nuclear divisions in dpy-26 hermaphrodites occurred 2-4 hr after the animals emerged from the L3 molt.

Scoring of XO her-1 dpy-21(e428); lin-4(n179) hermaphrodites: In the strain her-1 him-5 dpy-21(e428); lin-14(n179) XX hermaphrodites are dumpy and XO hermaphrodites are wild type in length at 20°C. At the L3 molt we could not accurately and reproducibly differentiate between dumpy and non-dumpy animals. To overcome this difficulty, animals were picked individually onto slides, scored, and then repicked onto separate plates. They were subsequently scored for their dumpy or wild-type phenotype when they reached adulthood. Two animals were of intermediate length; their karyotypes were determined from the phenotypes of their offspring.

Construction and scoring of XX dpy-21(e428); lin-14(n179)/nDf19 hermaphrodites: dpy-21(e428); lin-14(n179) males (raised at 15°C) were mated with unc-30; dpy-21(e428); szT1/nDf19 hermaphrodites at 20°C and at 24°C. Non-Unc Dpy hermaphrodite progeny were scored without regard to the Lin phenotype observable at the dissecting microscope level. The animals clearly fall into two distinct classes with no intermediate phenotypes. Non-Unc Dpy Lin hermaphrodites, inferred to be unc-30/+; dpy-21(e428); lin-14(n179)/nDf19, had precocious alae, aberrant gonad morphology and aberrant positioning of the seam cell nuclei. Non-Unc Dpy non-Lin hermaphrodites, inferred to be unc-30+/+; dpy-21(e428); lin-14(n179)/szT1, had 11 to 15 pairs of sister nuclei, normal gonad morphology and normal positioning of seam cell nuclei.

Production of XO dpy-28(y1, s939); lin-4(n179) males: Males of genotype dpy-28(y1); lin-14(n179) were generated by mating dpy-28(y1) males with dpy-28(y1); lin-14(n179) hermaphrodites. Males of genotype dpy-28(s939) +/dpy-28(s939)/unc-69; lin-14(n179) were generated at 20°C by mating dpy-28(s939)/unc-69 males (raised at 15°C) with dpy-28(s939) unc-69; lin-14(n179) hermaphrodites. dpy-28 hermaphrodites produce a very small number of nullo-X oocytes (0.5%), however there appeared to be no males that had received the lin-14(+)/paternal X chromosome.

Production of XO dpy-26(n199, y6); lin-14(n179) males: Males of genotype dpy-26(n199) +/dpy-26(n199)/unc-30; lin-14(n179) and dpy-26(n199) +/+ dpy-26(n199) unc-30; lin-14(n179) were generated by mating the appropriate dpy-26 males with Unc hermaphrodites produced by the appropriate yDp1; dpy-26 unc-30; lin-14(n179) strain. dpy-26 hermaphrodites produce about 5% nullo-X oocytes. The dpy-26(n199) and dpy-26(n199) matings produced 2 and 3 males, respectively, that appeared to carry the lin-14(+)/paternal X chromosome; that is, they had 10 pairs of sister nuclei and normal positioning of seam cell nuclei. These animals were clearly different from the other males scored from these matings and were not included in the data sets for these strains.

Isolation of yDp1: yDp1(WV;J), a free duplication carrying portions of the right arm of chromosome IV and the left arm of chromosome V (Figure 1), was isolated as a "suppressor" of dpy-26(n199) following standard EMS mutagenesis of nT1(n752)/unc-22(7v) dpy-26(n199). Homozygous Unc-22 progeny of mutagenized hermaphrodites were cloned and examined for suppression of the maternal-effect lethal phenotype of dpy-26(n199). yDp1 fully suppressed the mutant phenotype of dpy-26(n199) and thus was suspected
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of being a duplication of dpy-26(+). Mapping experiments demonstrated that this was indeed the case. It is likely that yDpl arose as a result of a breakdown of the translocation nT1.

**yDpl is a free duplication:** yDpl was inferred to be a free duplication based on three characteristics commonly associated with free duplications (Herman, Madl, and Kari 1979). 1) The segregation of yDpl is inconsistent with it being translocated to a chromosome. In addition hermaphrodites carrying yDpl fail to produce the progeny ratio expected if yDpl had been translocated onto a chromosome and were homozygous lethal (50% suppressed:25% non-suppressed:25% lethal animals). Full progeny counts were performed on a hermaphrodite which was yDpl; dpy-26(n199), and all progeny were cloned to score for the dpy-26 maternal-effect lethal phenotype. Of 287 progeny, 143 (62%) were suppressed, 99 (34%) were non-suppressed, and 10 (4%) were lethal. More extensive viability counts on the broods of 8 yDpl; dpy-26(n199) hermaphrodites confirmed these results. Of 1946 eggs laid, only 3.4% failed to develop into adults. 2) The oocytes of hermaphrodites carrying yDpl were examined after staining with DAPI (Fixsen 1985) and found to have six chromosome pairs and a small extrachromosomal body characteristic of large free duplications. 3) yDpl segregates away from the X chromosome in male meiosis. yDpl; dpy-26(n199) unc-30 males were mated with unc-30; dpy-11 hermaphrodites and all non-Dpy cross-progeny were scored. Animals that had received a copy of yDpl were non-Unc. Of 347 Unc non-Dpy cross-progeny 270 were hermaphrodites and 77 were males; of 296 non-Unc non-Dpy cross-progeny, 66 were hermaphrodites and 230 were males. Thus, yDpl is much more likely to be carried in nullo-X sperm.

**RNA isolation and analysis:** The methods of isolating staged L1 hermaphrodites, for isolating RNA, for performing gel electrophoresis, for transferring and hybridizing RNA and for quantitating autoradiographs are described in Meyer and Casson (1986). The lin-14 probe used in this assay was a 3.8-kb EcoRI fragment from the carboxy terminus of the lin-14 locus provided by G. Ruvkun, V. Ambros and R. Horvitz (personal communication).

**Comparison of the normalized lin-14 transcript between XX wild-type L1 hermaphrodites and XX dpy-21 mutant L1 hermaphrodites:** Filter-bound total RNA derived from staged wild-type and dpy-21(e428) L1 larvae was probed simultaneously for the X-linked lin-14 transcript and the autosomal transcript act-1 V. Autoradiographs of five hybridization experiments similar to the one in Figure 8 were traced using a scanning laser densitometer. Normalized transcript levels for lin-14 were calculated as the ratio of peak areas for (the lin-14 X transcript band)/(the act-1 V transcript band). The relative lin-14 transcript levels in dpy-21 were calculated as the ratio of normalized transcript levels for dpy-21/wild type.

**RESULTS**

The **lin-14 assay:** Mutations in lin-14 affect the temporal pattern of specific developmental events in *C. elegans* (Ambros and Horvitz 1984, 1987). One such event, the formation of the cuticular structures known as the adult lateral alae, is the basis of our assay. During each developmental stage, *C. elegans* is covered by a characteristic cuticle that is produced by the hypodermis at the end of the preceding developmental stage (embryogenesis, L1, L2, L3 and L4). After each new stage-specific cuticle is formed, the old cuticle is shed. The cuticle made in the late L4 contains specialized adult structures, the lateral alae, generated by the hypodermal seam cells that underlie the cuticle. These specialized hypodermal cells extend laterally along the animal and undergo a series of cell divisions during the L1, L2, and L3 molts. In the late L4, the seam cells cease cell division and instead generate the adult lateral alae (Sulston and Horvitz 1977; Singh and Sulston 1978). Recessive mutations in the X-linked gene lin-14 cause the lateral hypodermal seam cells to express their fates precociously, resulting in the cessation of seam cell division and the formation of the adult lateral alae in the late L3.

In animals homozygous for the temperature-sensitive allele lin-14(n179), the fraction of seam cells expressing the precocious mutant phenotype is dependent on temperature, a parameter used to vary the severity of the mutant phenotype and thereby extend the range and sensitivity of the assay. A schematic representation of the phenotype of lin-14(n179) is presented in Figure 2. At the restrictive temperature (25°) lin-14(n179) animals are fully mutant; all seam cells fail to divide during the L3 molt and instead generate precocious alae. At the permissive temperature (15°) animals are essentially wild type; all seam cells divide during the L3 molt and generate no alae. At intermediate temperatures patches of precocious alae are produced as a consequence of some cells
expressing the mutant phenotype and some the wild-type phenotype (Figure 3). In the assay an increase in lin-14(n179) expression is reflected by suppression of the mutant phenotype, that is, a decrease in the fraction of seam cells generating precocious alae. Suppression of the lin-14 phenotype is measured at 24°, where the mutant phenotype of lin-14(n179) is severe but not null. A reduction in lin-14(n179) expression is reflected by an enhancement of the mutant phenotype, that is, an increase in the fraction of seam cells generating precocious alae. Enhancement is measured at 20° where the mutant phenotype of lin-14(n179) is weak.

The assay is quantitated using Nomarski optics to examine the individual fates of the midbody seam cell nuclei following the L3 molt. Our data indicate that
in parts (total midbody seam cell nuclei), are plotted against the percentage of individuals scored that had that value or lower. Therefore use each worm as a single statistic and choose their fates independently, as revealed by statistical analysis using the G test of independence. We hypothesize for this test is that the data sets are over-determined for any particular strain are reproducible, of the values from individual worms. The mean value is simply used as a shorthand way to refer to the degree (for example, see Figure 4A). For each strain we then calculate the mean value of seam cells expressing the mutant phenotype (% mutant nuclei) by combining all the values from individual worms. The mean value is simply used as a shorthand way to refer to the degree of lin-14(n179) expression in that strain. The values determined for any particular strain are reproducible, even when sets of animals are scored several months apart.

![Graphs A and B](https://academic.oup.com/genetics/article/117/4/657/5997416)

**Figure 4.**—The lin-14 locus is dosage compensated. The graphs in parts A and B are cumulative distribution plots of the data summarized in parts A and B, respectively, of Table 1. Scores for individual animals, represented as the fraction of (mutant midbody seam cell nuclei)/(total midbody seam cell nuclei), are plotted against the percentage of individuals scored that had that value or lower.

### TABLE 1

The lin-14 locus is dosage compensated

<table>
<thead>
<tr>
<th></th>
<th>No. of animals scored</th>
<th>Percent mutant nuclei (mean)</th>
<th>Compared to XX lin-14/Df</th>
<th>No. of animals scored</th>
<th>Percent mutant nuclei (mean)</th>
<th>Compared to XX her-1; lin-14/Df</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XX lin-14</td>
<td>40</td>
<td>30</td>
<td>$P &lt; 10^{-6}$</td>
<td>XX her-1; lin-14</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>XO lin-14</td>
<td>40</td>
<td>46</td>
<td>$P &lt; 10^{-4}$</td>
<td>XO her-1; lin-14</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>XX lin-14/Df</td>
<td>20</td>
<td>84</td>
<td></td>
<td>XX her-1; lin-14/Df</td>
<td>20</td>
<td>82</td>
</tr>
</tbody>
</table>

All animals were raised at 20°C. The allele of lin-14 used is n179. XX lin-14 is significantly different from XO lin-14 ($P < 10^{-5}$). XX her-1; lin-14 is significantly different from XO her-1; lin-14 ($P < 0.02$). NS = $P > 0.05$ compared data sets are not significantly different; 0.05 > $P > 0.02$ compared data sets may be different; $P < 0.02$ compared data sets are significantly different.

a Df is nDf/19. The complete genotype of these animals is listed in MATERIALS AND METHODS.

The lin-14 locus is dosage compensated: lin-14(n179) is a hypomorphic allele by standard genetic criteria: in XX hermaphrodites the phenotype is fully recessive and becomes more severe in trans to a deficiency, which is itself fully recessive (AMBROS and HORVITZ 1984, 1987; L. DeLONG and B. J. MEYER, unpublished observations). Because the mutant phenotype of lin-14(n179) is sensitive to changes in gene dose in XX animals, it is possible to determine whether the locus is dosage compensated. In homozygous XX lin-14 hermaphrodites containing two mutant copies of lin-14, 30% of the seam cell nuclei display the mutant phenotype, whereas in hemizygous XX lin-14/Df hermaphrodites containing one mutant copy, 84% of the seam cell nuclei express the mutant phenotype. Without dosage compensation, the activity of lin-14 in the hemizygous lin-14/O male would be equivalent to that of the hemizygous hermaphrodite. However, if dosage compensation occurs, XO males will have a level of lin-14 activity similar to that of XX homozygous hermaphrodites but very different from XX hemizygous hermaphrodites. XO lin-14 males are only 46% mutant, indicating that XO males containing one mutant copy of the locus have a level of lin-14 activity similar to that of XX lin-14 hermaphrodites containing two copies but very different from that of XX lin-14/Df hemizygous hermaphrodites containing one copy (Figure 4A and Table 1A). Thus, the lin-14 locus is...
responsive to the general X chromosome dosage compensation mechanism in C. elegans.

To eliminate any possible complications in this analysis caused by purely sex-specific differences in lin-14 gene expression between males and hermaphrodites, we reexamined the lin-14 mutant phenotype in XO and XX animals of the same phenotypic sex. This was accomplished by using the sex transformation mutation her-1(e1320) to transform XO animals into hermaphrodites (Hodgkin 1980). Dosage compensation is again observed in the comparison of the lin-14 mutant phenotypes between XX her-1; lin-14/Df hemizygous hermaphrodites (82% mutant) and XO and XX her-1; lin-14 hermaphrodites (44% mutant and 25% mutant, respectively) (Figure 4B and Table 1B). The assay shows a small but statistically significant difference in lin-14 gene expression between XO and XX hermaphrodites, indicating that dosage compensation is not complete.

The degree of lin-14 suppression in dpy-21, dpy-26, dpy-27 and dpy-28 as a function of X chromosome number in diploid animals. *Maternal-effect XX-specific lethal. The escapers (the animals scored) are dumpy and comprise from 5 to 15% of the total brood, depending on the specific allele.

The equivalent extent of suppression in XXX animals and XX animals mutant in the dpy genes suggests that the increase in lin-14 expression may be equivalent. However, it is important to note that the assay may not be completely linear in this range, causing somewhat different levels of overexpression to result in the same level of suppression.

To show that the suppression of the lin-14 phenotype is not simply due to the dumpy phenotype of the animals, strains homozygous for various autosomal and X-linked dpy mutations not implicated in dosage compensation (Meneely and Wood 1987) were also scored. The phenotypes of these control dpy mutations are not dependent on X chromosome dosage (Hodgkin 1983b). The mutant values for XX dpy-4, XX dpy-6, XX dpy-18 and XX dpy-20 cluster around the mutant value for the XX lin-14 control strain (Table 2). Thus, dumpyness per se does not suppress the lin-14 mutant phenotype.

If, as we expect, the suppression of the lin-14 phenotype results from changes in expression of the lin-14 gene itself, then lin-14 alleles with very low or no lin-14 activity should not be suppressible. Our results fit this expectation. At 24° 100% of the seam cell nuclei in XX lin-14/Df animals express the mutant phenotype, and 98% of the seam cell nuclei in XX dpy-
changes in the level of the 

mals mutant in

from quantitation of 

seems highly likely that the suppression of the 

mutant hermaphrodites (see below).

<table>
<thead>
<tr>
<th>Animal</th>
<th>No. of animals scored</th>
<th>Percent mutant nuclei (mean)</th>
<th>Compared to XXX lin-14</th>
<th>Compared to XX lin-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
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<td>2</td>
<td>P &lt; 10^{-4}</td>
<td>P &lt; 10^{-6}</td>
</tr>
<tr>
<td>XXX lin-14</td>
<td>25</td>
<td>26</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>XX dpy-21(e428)</td>
<td>40</td>
<td>31</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>XX dpy-21(e459)</td>
<td>20</td>
<td>26</td>
<td>NS</td>
<td>P &lt; 10^{-6}</td>
</tr>
<tr>
<td>XX dpy-21(y77)</td>
<td>20</td>
<td>21</td>
<td>NS</td>
<td>P &lt; 10^{-6}</td>
</tr>
<tr>
<td>XX dpy-26(n199)</td>
<td>20</td>
<td>29</td>
<td>NS</td>
<td>P &lt; 10^{-6}</td>
</tr>
<tr>
<td>XX dpy-26(y6)</td>
<td>20</td>
<td>32</td>
<td>NS</td>
<td>P &lt; 10^{-6}</td>
</tr>
<tr>
<td>XX dpy-27(rh18)</td>
<td>20</td>
<td>29</td>
<td>NS</td>
<td>P &lt; 10^{-6}</td>
</tr>
<tr>
<td>XX dpy-27(y49)</td>
<td>20</td>
<td>29</td>
<td>NS</td>
<td>P &lt; 10^{-6}</td>
</tr>
<tr>
<td>XX dpy-28(y1)</td>
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<td>P &lt; 10^{-6}</td>
</tr>
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<td>XX dpy-28(939)</td>
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<td>39</td>
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<td>P &lt; 10^{-5}</td>
</tr>
<tr>
<td>XX lin-14</td>
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<td>77</td>
<td>P &lt; 10^{-6}</td>
<td>NS</td>
</tr>
<tr>
<td>XX dpy-4*</td>
<td>20</td>
<td>85</td>
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<td>NS</td>
</tr>
<tr>
<td>XX dpy-6*</td>
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<td>85</td>
<td>P &lt; 10^{-6}</td>
<td>NS</td>
</tr>
<tr>
<td>XX dpy-18*</td>
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<td>77</td>
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<td>NS</td>
</tr>
<tr>
<td>XX dpy-20*</td>
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<td>88</td>
<td>P &lt; 10^{-6}</td>
<td>NS</td>
</tr>
<tr>
<td>XX dpy-21; lin-14(Df+)</td>
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<td>98</td>
<td>P &lt; 10^{-6}</td>
<td>P &lt; 10^{-6}</td>
</tr>
<tr>
<td>XX lin-14(Df+)</td>
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<td>100</td>
<td>P &lt; 10^{-6}</td>
<td>P &lt; 10^{-6}</td>
</tr>
</tbody>
</table>

All animals were raised at 24°. Animals are homozygous for lin-14(n179) unless otherwise indicated.

* Control dpy mutations.

\( ^{+} \) unc-30(+); dpy-21(e428); lin-14(n179)/nDf19.

\( ^{+} \) him-5(+); lin-14(n179)/nDf19.

21; lin-14/Df animals express the mutant phenotype (Figure 6 and Table 2). In view of these results, it seems highly likely that the suppression of the lin-14 phenotype in dpy-21 mutant animals occurs through changes in the level of the lin-14 gene product itself. More direct evidence in support of this notion derives from quantitation of lin-14 mRNA levels in dpy-21 mutant hermaphrodites (see below).

**Assessment of lin-14 overexpression in XX animals mutant in dpy-21:** In XX animals it is possible to assess the degree of overexpression of lin-14 caused by the dpy-21(e428) mutation. To accomplish this, the effect of dpy-21(e428) on the lin-14 phenotype in XX animals with one copy of lin-14 was compared with the lin-14 phenotype in XX dpy-21(+) animals with one or two copies of lin-14. XX dpy-21; lin-14/Df animals (one mutant copy) are 48% mutant at 20°. This value is close to the 30% mutant value seen in XX lin-14 animals (two mutant copies) and is very different from the 84% mutant value seen in XX lin-14/Df animals (one mutant copy). These results suggest that dpy-21(e428) increases lin-14 expression nearly, but somewhat less than, two-fold (Figure 7 and Table 3). This conclusion is supported by direct measurement of lin-14 mRNA transcript levels.

A mutation in dpy-21 increases the wild-type lin-14 mRNA transcript level by approximately two-fold: To validate our assumption that the suppression of the lin-14 mutant phenotype results from an increase in lin-14 mRNA levels, we determined the increase in the wild-type lin-14 mRNA transcript level resulting from the dpy-21(e428) mutation. In Northern hybridization assays, we compared lin-14 transcript levels in RNA preparations derived from staged L1 wild-type hermaphrodites and staged L1 dpy-21 mutant hermaphrodites. L1 animals were used as the source of RNA because the L1 period is the time of maximum lin-14 transcript accumulation (G. RUVKUN, V. AMBRÓS and R. HORVITZ, personal communication). For the comparison, each lin-14 transcript level was first normalized to the level of an autosomal transcript, act-1 V, which encodes a body-wall actin (KRAUSE and HIRSH 1984). The autoradiograph in Figure 8 is a typical example of the results obtained from such experiments. Comparison of the dpy-21 lane with the first wild-type lane, containing a similar amount of act-1 mRNA, reveals that the lin-14 transcript is elevated in the dpy-21 mutant. The similarity

![Graph showing the percentage of animals scored vs. fraction of mutant nuclei.](https://example.com/graph.png)
in level of the lin-14 transcript in the dpy-21 lane compared with that in the second wild-type lane, which contains two-fold more act-1 mRNA than the first lane, reveals that the lin-14 mRNA is enhanced approximately twofold. Quantitation by densitometry of five similar experiments indicates that the extent of overexpression is 1.9-fold (standard error of the mean is 0.01). This result indicates that the suppression of the lin-14(n179) phenotype caused by dpy-21(e428) is correlated with an increase in the level of wild-type lin-14 mRNA caused by dpy-21(e428). Based on this correlation it is reasonable to think that the lin-14(n179) mRNA levels would also be elevated approximately twofold by dpy-21(e428). The lin-14 assay appears to provide a reliable representation of changes in lin-14 mRNA transcript levels.

In XO animals, mutations in dpy-26, dpy-27 and dpy-28 have no detectable effect on lin-14 expression: XO animals mutant in dpy-26, dpy-27, and dpy-28 are essentially wild type. Consistent with this observation is our finding that the level of lin-14 expression in XO males is not altered by mutations in these genes. The percent of mutant seam cell nuclei characteristic of XO lin-14 males carrying dpy-26(y6, n199), dpy-27(yh18, y49) or dpy-28(y1, s939) is not different from the percent of mutant seam cell nuclei characteristic of XO lin-14 males (Table 4).

In XO animals, mutations in dpy-21 enhance the lin-14 mutant phenotype: Although mutations in dpy-21 have no effect on the visible phenotype of XO animals, they alter lin-14 expression in XO animals in a direction opposite to that found in XX animals. At 20° the percent of mutant seam cell nuclei in XO dpy-21(e428), XO dpy-21(e459), XO dpy-21(y47) and XO dpy-21(y50) animals (67–76%) is significantly greater than the percent for XO lin-14 animals (46%) and is similar to the value for XX lin-14/Df animals (84%) (Table 5). The enhancement was also seen in XO dpy-21(e428)/dpy-21(e459) animals (data not shown). The enhancement of the lin-14 mutant phenotype in males is not sex-specific, since XO animals transformed into hermaphrodites by the mutation her-1(e1520) exhibit the same effect (Table 5). These results imply that dpy-21 mutations reduce lin-14 expression in XO animals.

A mutation in dpy-22 enhances the lin-14 phenotype in XO animals but has little effect in XX animals: The role of the X-linked dpy-22 gene in dosage compensation has not been clearly determined. Hodgkin and Brenner (1977) reported that dpy-22(e652) has a sick, dumpy phenotype in XX hermaphrodites and a dumpy or lethal phenotype in XO males (many males die as larvae). The observed differences in phenotypes were a consequence of the X chromosome.
dosage rather than the phenotypic sex, since XX tra-1; dpy-22 males were more viable than XO dpy-22 males. (tra-1 mutations transform XX animals into fertile males.) The finding that males appear more affected than hermaphrodites is opposite to the observations with the other X dosage-dependent dpy genes. Thus, if dpy-22 were involved in dosage compensation, it might play a role opposite to that of dpy-21, dpy-26, dpy-27 and dpy-28.

We reexamined the effect of dpy-22(e652) on the viability of XO and XX animals after outcrossing the strain extensively. In contrast to the previous report, we failed to find any strong bias in viability between XX and XO animals. The viability of XX animals was determined directly by counting eggs from homozygous dpy-22 hermaphrodites and following their development through adulthood. Of the 569 eggs laid, 83% developed into sick, dumpy adult hermaphrodites. The viability of XO dpy-22 mutant males was determined by counting the progeny from a cross in which XX dpy-22 hermaphrodites were mated with wild-type XO males. The cross yielded 485 non-dumpy adult hermaphrodites (dpy-22/+) and 377 dumpy adult males (dpy-22/O), all of which were sick and very dumpy. Relative to dpy-22/+ hermaphrodites, dpy-22/O males are 78% viable. Thus, the viability of XO and XX dpy-22 animals is essentially the same (78% vs. 83%). Moreover, XX tra-1; dpy-22 males appear as sick and dumpy as XO dpy-22 males (J. PLENEFISCH and B. MEYER, unpublished observations). To further explore possible differences between the sexes in the dpy-22 phenotype, we investigated the effects of dpy-22(e652) on the expression of lin-14.

The dpy-22 mutation results in a substantial enhancement of the lin-14 mutant phenotype in XO males. The mutant values for XO dpy-22 males (79%) are significantly higher than the mutant values for XO lin-14 males (50%) and are equivalent to those seen in the hemizygous XX lin-14/Df hermaphrodites (84%) (Table 6), indicating that the dpy-22 mutation reduces lin-14 expression in XO males. As before, to show that the enhancement of the lin-14 phenotype is not simply due to the dumpy phenotype of the XO animals, strains homozygous for various dpy mutations were also scored. Mutations in these control dpy genes, dpy-4, dpy-6, dpy-18 and dpy-20, have no significant effect on the lin-14 mutant phenotype in XO males (Table 6). We believe that the enhancement seen in XO dpy-22

---

**TABLE 5**

In XO animals, mutations in dpy-21 enhance the lin-14 mutant phenotype

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of animals scored</th>
<th>Percent mutant nuclei (mean)</th>
<th>Compared to XO lin-14</th>
<th>Compared to XX lin-14/Df</th>
</tr>
</thead>
<tbody>
<tr>
<td>XO lin-14</td>
<td>40</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XO dpy-21(e428)</td>
<td>20</td>
<td>76</td>
<td>$P &lt; 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>XO her-1 him-5</td>
<td>20</td>
<td>72</td>
<td>$P &lt; 10^{-4}$</td>
<td>0.05 &gt; $P &gt; 0.02$</td>
</tr>
<tr>
<td>dpy-21(e428)*</td>
<td>20</td>
<td>73</td>
<td>$P &lt; 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>XO dpy-21(e459)</td>
<td>20</td>
<td>67</td>
<td>$P &lt; 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>XO dpy-21(y47)*</td>
<td>20</td>
<td>67</td>
<td>$P &lt; 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>XO dpy-21(y50)*</td>
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<td>70</td>
<td>$P &lt; 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>XX lin-14/Df*</td>
<td>20</td>
<td>84</td>
<td>$P &lt; 10^{-6}$</td>
<td></td>
</tr>
</tbody>
</table>

* The complete genotype of these animals is listed in MATERIALS AND METHODS.

All animals were raised at 20°. All animals are hemizygous for lin-14(n179).

---

**TABLE 4**

In XO animals, mutations in dpy-26, dpy-27 and dpy-28 have no detectable effect on lin-14 expression

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of animals scored</th>
<th>Percent mutant nuclei (mean)</th>
<th>Compared to XO lin-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>XO lin-14</td>
<td>40</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>XO dpy-26(n199)*</td>
<td>20</td>
<td>54</td>
<td>NS</td>
</tr>
<tr>
<td>XO dpy-26(y6)*</td>
<td>20</td>
<td>46</td>
<td>NS</td>
</tr>
<tr>
<td>XO dpy-27(rh18)</td>
<td>20</td>
<td>41</td>
<td>NS</td>
</tr>
<tr>
<td>XO dpy-27(y49)</td>
<td>20</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>XO dpy-28(y1)</td>
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<td>NS</td>
</tr>
<tr>
<td>XO dpy-28(y39)*</td>
<td>20</td>
<td>53</td>
<td>NS</td>
</tr>
</tbody>
</table>

All animals were raised at 20°. All animals are hemizygous for lin-14(n179).

* The complete genotype of these animals is listed in MATERIALS AND METHODS.
TABLE 6
In XO animals, a mutation in dpy-22 enhances the lin-14 mutant phenotype

<table>
<thead>
<tr>
<th></th>
<th>No. of animals scored</th>
<th>Percent mutant nuclei (mean)</th>
<th>Compared to XO lin-14</th>
<th>Compared to XX lin-14/1Df</th>
</tr>
</thead>
<tbody>
<tr>
<td>XO dpy-22</td>
<td>20</td>
<td>79</td>
<td>(P &lt; 10^{-3})</td>
<td>NS</td>
</tr>
<tr>
<td>XX lin-14/1Df</td>
<td>20</td>
<td>84</td>
<td>(P &lt; 10^{-3})</td>
<td></td>
</tr>
<tr>
<td>XO lin-14</td>
<td>20</td>
<td>50</td>
<td>(P &lt; 10^{-3})</td>
<td>(P &lt; 10^{-3})</td>
</tr>
<tr>
<td>XO dpy-4(a)</td>
<td>20</td>
<td>50</td>
<td>NS</td>
<td>(P &lt; 10^{-3})</td>
</tr>
<tr>
<td>XO dpy-6(b)</td>
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<td>59</td>
<td>NS</td>
<td>(P &lt; 10^{-2})</td>
</tr>
<tr>
<td>XO dpy-18(c)</td>
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<td>62</td>
<td>NS</td>
<td>(P &lt; 10^{-3})</td>
</tr>
<tr>
<td>XO dpy-20(d)</td>
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<td>61</td>
<td>NS</td>
<td>(P &lt; 0.02)</td>
</tr>
</tbody>
</table>

All animals were raised at 20\(^\circ\). All animals are hemizygous for lin-14(n179). All XO animals were generated by hermaphrodites homozygous for the mutation him-5.
\(a\) him-5/+; lin-14(n179)/nDf1Y.
\(b\) Control dpy mutations.

TABLE 7
In XX animals, a mutation in dpy-22 slightly enhances the lin-14 mutant phenotype

<table>
<thead>
<tr>
<th></th>
<th>No. of animals scored</th>
<th>Percent mutant nuclei (mean)</th>
<th>Compared to XX lin-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX lin-14/1Df</td>
<td>20</td>
<td>84</td>
<td>(P &lt; 10^{-6})</td>
</tr>
<tr>
<td>XX dpy-22</td>
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<td>41</td>
<td>(P &lt; 0.002)</td>
</tr>
<tr>
<td>XX lin-14</td>
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<td>XX dpy-4(a)</td>
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<td>12</td>
<td>(P &lt; 10^{-3})</td>
</tr>
<tr>
<td>XX dpy-6(b)</td>
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<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>XX dpy-18(c)</td>
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<td>NS</td>
</tr>
<tr>
<td>XX dpy-20(d)</td>
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<td>22</td>
<td>NS</td>
</tr>
</tbody>
</table>

All animals were raised at 20\(^\circ\). Animals are homozygous for lin-14(n179) unless otherwise indicated.
\(a\) him-5/+; lin-14(n179)/nDf1Y.
\(b\) Control dpy mutations.

DISCUSSION

The lin-14 assay provides a rapid and sensitive means by which to measure perturbations in X-linked gene expression caused by mutations that affect dosage compensation in C. elegans. Using this assay we demonstrated that in diploid animals the level of lin-14(n179) expression is compensated between XO and XX animals, although the compensation is not complete. Furthermore, we have used the lin-14 assay to confirm the involvement of various genes in the process of dosage compensation. Specifically, the dosage compensation of lin-14 is disrupted by mutations in dpy-26, dpy-27 and dpy-28, which result in elevated lin-14 expression in XX animals but unaltered lin-14 expression in XO animals. This result is consistent with the view that these genes are important in XX animals to achieve proper dosage compensation (MEYER and CASSON 1986). Mutations in dpy-21 cause an elevation of lin-14 expression in XX animals but a reduction in expression in XO animals. This finding suggests that dpy-21 is different from the other dosage compensation dpy genes and might be required in both sexes for proper dosage compensation, as previously proposed (MEYER and CASSON 1986; MENEELY and WOOD 1987). Last, although we cannot demonstrate differential viability between XO and XX animals mutant in dpy-22, we have observed that lin-14 expression in XO and XX dpy-22 animals is reduced.

With one exception, the results obtained with the lin-14 assay agree with those from an independent study measuring the effects of mutations in dpy-21, dpy-27 and dpy-28 on the level of specific X-linked mRNA transcripts (MEYER and CASSON 1986). Among the similarities are the findings that mutations in dpy-21, dpy-27 and dpy-28 result in a two- to threefold increase in the X-linked mRNA transcript levels in XX hermaphrodites, whereas mutations in dpy-27 and dpy-28 cause no change in the X-linked transcript levels of XO males. These results are consistent with both the degree of suppression of the lin-14 phenotype in dpy-21, dpy-27 and dpy-28 mutant XX animals, and with the twofold increase in the lin-14 mRNA transcript level in XX dpy-21 mutant animals. In addition the finding that XO males mutant in dpy-27 or dpy-28 exhibit no enhancement or suppression of the lin-14 mutant phenotype is consistent with the results from the X-linked transcript measurements.

By contrast, XO males mutant in dpy-21 behave differently in the two assays. Adult XO dpy-21 males exhibit an approximately 1.5-fold increase in X-linked mRNA transcripts, whereas XO dpy-21 males exhibit a reduction in lin-14 expression. The most conspicuous difference between the two assays is that the RNA assay measures transcript levels in adult males, whereas the lin-14 assay most likely reflects the state of lin-14 expression at a much earlier developmental

mutant males is a reflection of reduced lin-14 gene expression.

The dpy-22 mutation appears to cause a slight enhancement of the lin-14 phenotype in XX animals. The percent of seam cells expressing the mutant phenotype is 41% in XX dpy-22 animals compared to 30% in XX lin-14 animals. The control dpy mutations do not cause any significant enhancement of the lin-14 phenotype (Table 7). These results suggest that the dpy-22 mutation may reduce lin-14 expression slightly in XX animals. The mutation dpy-4(e1166) results in a significant suppression of the lin-14 phenotype at 20\(^\circ\) (Table 7) but not at 24\(^\circ\) (Table 2). Mutations that have been shown to affect dosage compensation by criteria other than the lin-14 assay suppress the lin-14 phenotype at 24\(^\circ\). The fact that dpy-4 fails to suppress the phenotype at 24\(^\circ\), the temperature at which the most reliable suppression tests are conducted, makes it extremely unlikely that dpy-4 is involved in dosage compensation or that the lin-14 assay is inaccurate.
period, the L1 larval stage. It is possible that dpy-21 mutations first cause an underexpression of X-linked genes and later an overexpression in XO animals. Alternatively, expression of the lin-14 locus in XO animals might be influenced by other X-linked genes that are affected by dpy-21 mutations. The resolution of this problem is not straightforward, since there is no significant accumulation of lin-14 mRNA in adults and there is as yet no method for isolating staged L1 males.

A different genetic assay utilizing hypomorphic mutations in the X-linked gene lin-15 has been devised by MENEELY and WOOD (1987) to study dosage compensation in C. elegans. The effects of mutations in dpy-21, dpy-22 and dpy-26 on lin-14 expression in XX animals are similar to those on lin-15 expression. The effects of dpy-26 mutations on lin-14 and lin-15 expression in XO animals might be different. While we observe no effect of mutations in dpy-26 on lin-14 expression in XO males, MENEELY and WOOD (1987) report variable results with the two lin-15 hypomorphic alleles examined. The phenotype of one allele is somewhat suppressed whereas the phenotype of the other is unchanged and therefore similar to the results with the lin-14 assay.

The results of the two assays do differ for XO dpy-21 males, however. In XO dpy-21 mutant males, the lin-15 mutant phenotype is suppressed, whereas the lin-14 mutant phenotype is enhanced. The lin-15 result is consistent with the results from direct measurement of mRNA transcript levels of other genes (MEYER and CASSON 1987). The difference in the two genetic assays might reflect different developmental profiles of lin-14 and lin-15 gene expression.

The involvement of dpy-22 in dosage compensation remains uncertain. Although lin-14 expression is affected by a mutation in dpy-22, no sex-specific difference in viability of dpy-22 mutant animals can be demonstrated, unlike the situation with other dosage compensation genes. In addition, no interactions have been demonstrated between dpy-22 and the known dosage compensation genes. For example, the dpy-22 mutation does not suppress the XX-specific lethality of mutations in dpy-26, dpy-27 and dpy-28; similarly mutations in these genes do not suppress the mutant phenotypes of dpy-22 (J. PLENEFISCH, L. DELONG and B. MEYER, unpublished results). Establishing a clear role for dpy-22 in dosage compensation must await further experiments, particularly defining the null phenotype.

It is extremely important to have a variety of genetic assays that allow a glimpse of X chromosome expression at different times in development and in different X chromosome regions. Such monitors of X chromosome expression may permit the identification of genes that are active in dosage compensation at different developmental stages and help establish whether all regions of the X chromosome respond to the dosage compensation process in a similar way. This point is illustrated in part by the example of the X-linked gene runt in Drosophila melanogaster. The runt gene is required for proper segmentation and is active around the blastoderm stage of development (NÜSSLIN-VOLHARD and WIESCHAUSS 1980). The locus is dosage compensated, and mutations in daught-erless and Sex-lethal, but not mutations in the msl (male-specific lethal) genes, interfere with its dosage compensation. These results indicate that at least some Drosophila genes are compensated by the blastoderm stage, and that daughterless and Sex-lethal, but probably not the msl genes, control X chromosome expression at that stage (GERGEN 1987). In similar fashion the lin-14 assay has contributed additional information to the understanding of dosage compensation in C. elegans by permitting the demonstration that compensation can occur at least as early as the L1 stage and that the X dosage-dependent dpy genes must be active before and/or during that time.

There appears to be an interesting difference between dosage compensation in C. elegans and that in Drosophila. In D. melanogaster there is some evidence that triplo-X females (metafemales, 3X/2A) exhibit dosage compensation (for a review, see LUCCHESI and MANNING 1987). The mechanism responsible for dosage compensation in these 3X/2A animals may not simply be an extension of the normal mechanism used in XY males, but rather an independent mechanism (DEVLIN, HOLM and GRIGLIATTI 1985). The situation in C. elegans seems quite different, since the lin-14 locus appears not to be compensated in 3X/2A animals. In fact 3X/2A animals have a particular dumpy phenotype characteristic of 2X/2A dpy-21 animals, implying that in general the X chromosome dosage compensation mechanism in C. elegans lacks the capacity to compensate completely for the extra X chromosome in triplo-X animals (HODGKIN 1983a). This view is supported by the finding that the lin-15 locus is also not compensated in 3X/2A animals (MENEELY and WOOD 1987).

Although the genetic and biochemical analysis of dosage compensation in C. elegans is well underway, numerous important questions remain unanswered. Both the resolution of these issues and the elucidation of similarities between the dosage compensation mechanisms in C. elegans and other organisms will be furthered by genetic assays such as the lin-14 assay.

We thank V. AMBROS for continuing advice and for providing nBfj9 and alleles of lin-14. We are especially grateful to W. FIXSEN and H. ELLIS for initial help in scoring seam cell nuclei. We are indebted to L. AVERY for advice about statistics and the computer programs used in performing the Mann Whitney U test and the G test for independence. We thank G. RUVKUN for providing the lin-14 clone and for sharing unpublished information. We are grateful
to M. Solomon, T. Cline, E. Ferguson and the members of the Meyer and Horvitz laboratories for useful discussions. We thank T. Cline, I. Greenwald, A. Villeneuve, M. Solomon and L. Miller for critical comments on the manuscript. This work was supported by grant GM30702 from the National Institutes of Health to B.J.M. L.D. was supported by Institutional National Research Service predoctoral training grant 5 T32 GM 07287.12 from the National Institutes of Health.

Note Added in Proof: n530, another temperature-sensitive, hypomorphic allele of lin-14 behaves similarly to lin-14(n179). At 20° lin-14(n530) exhibits partial dosage compensation. XX animals are 28% mutant, whereas XO animals are 42% mutant. The mutation dpy-21(e428) enhances the lin-14(n530) mutant phenotype in XO males enhanced to 20° (77% mutant); however, it suppresses the lin-14(n530) phenotype in XX hermaphrodites grown at 24° (20% mutant compared to 67% mutant in XX dpy-21(+); lin-14(n530) animals.

LITERATURE CITED


Communicating editor: R. K. HERMAN