nounced blocking effect on crosslinking of NGF to $p75^{NGFR}$. This suggests that the peptide interfered with post-binding conformational changes of ligand or $p75^{NGFR}$ or both, thereby blocking activation of death-related pathways. The suggestion that such post-docking events determine the type of downstream signaling from $p75^{NGFR}$ emerges from the finding that, although all neurotrophins can bind to $p75^{NGFR}$, only NGF activates NF-κB through $p75^{NGFR}$ in Schwann cells (*4*).

In conclusion, we present in vivo evidence that $p75^{NGFR}$ mediates apoptosis of developing cholinergic basal forebrain and neostriatum neurons, and that pharmacological modulation of $p75^{NGFR}$ can promote neuronal survival. These findings are relevant to the observation that cholinergic basal forebrain neurons are involved in learning and memory, and degenerate in Alzheimer's disease.

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19. We synthesized a conformationally constrained peptide containing the V1 loop region of NGF, which participates in its binding to $p75^{NGFR}$ [C. F. Ibanez *et al.*, *Cell* **69**, 329 (1992)] and affects NGF-mediated neurite outgrowth [F. M. Longo *et al.*, *Cell Regul.* **1**, 189 (1990)], but has not been implicated in a direct TrkA interaction. The linear peptide Cys-Ala-Thr-Asp-Ile-Lys-Gly-Lys-Glu-Cys was synthesized [E. Atherton and R. C. Sheppard, *Solid Phase Peptide Synthesis* (IRL Press, Oxford, 1989)] and purified with high-performance liquid chromatography (HPLC). The sequence and composition was confirmed by sequence analysis and mass spectroscopy. The peptide was cyclized by way of disulfide bonds between the Cys residues to the depsi-cyclic product dc28-36 [K. K. Lee, J. A. Black, R. S. Hodges, in *High Performance Liquid Chromatography of Peptides and Proteins*, C. T. Mant and R. S. Hodges, Eds. (CRC, Boca Raton, FL, 1991), pp. 389–398] and purified by HPLC. The peptide nature was confirmed by appropriate changes in the HPLC retention time of the peptide and by mass spectroscopy.
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23. All animal procedures were performed according to the Canadian Council for Animal Care guidelines. Mouse brains were processed for histological analysis as described for rats (*15*). Every third 30-μm coronal section through the medial septum was immunoreacted with polyclonal antibodies to ChAT (AB143, Chemicon, Temecula, CA). The two sets of adjacent sections were stained with polyclonal antibodies to TrkA or $p75^{NGFR}$ (antiREX) (L. F. Reichardt, Univ. of California, San Francisco). The number of immunoreactive neurons in one half of the medial septum was counted in all of the six to eight sections per marker for each animal, and expressed as an average per section. Numbers were corrected according to the formula [M. Abercrombie, *Anat. Rec.* **94**, 239 (1946)] $N = n \times T/(T+D)$, where N = total number, n = counted profiles, T = section thickness, and D = longest cell diameter. The size of the neurons in control and $p75^{NGFR}$-deficient mice showed an increase up to P15, followed by a decrease to adult values as is seen in rats [E. Gould, N. J. Woolf, L. L. Butcher, *Brain Res. Bull.* **27**, 767 (1991)], but was similar for both groups at each age.

24. We are grateful for the $p75^{NGFR}$ and TrkA antibodies from L. F. Reichardt and the excellent technical support from J. Bunker, M. Netherton, and D. Sadi. We thank N. Tatton for guidance with the Apoptag method, J. Hebb for suggesting the peptide experiment, and E. M. Johnson, Jr. (Washington University, St. Louis, MO) and E. M. Shooter (Stanford University, CA) for their helpful input on the manuscript. Supported by Allelix Biopharmaceuticals, Mississauga, Ontario, Canada, and the Network of Centres of Excellence (Canada) (R.J.R. and T.H.) and a Scholarship from the Medical Research Council of Canada (T.H.).

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DPY-26, a Link Between Dosage Compensation and Meiotic Chromosome Segregation in the Nematode

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The DPY-26 protein is required in the nematode *Caenorhabditis elegans* for X-chromosome dosage compensation as well as for proper meiotic chromosome segregation. DPY-26 was shown to mediate both processes through its association with chromosomes. In somatic cells, DPY-26 associates specifically with hermaphrodite X chromosomes to reduce their transcript levels. In germ cells, DPY-26 associates with all meiotic chromosomes to mediate its role in chromosome segregation. The X-specific localization of DPY-26 requires two dosage compensation proteins (DPY-27 and DPY-30) and two proteins that coordinately control both sex determination and dosage compensation (SDC-2 and SDC-3).

Many sexually reproducing organisms, including *C. elegans*, rely on a chromosome-counting mechanism for determining their sex. In such organisms the two sexes differ in the dosage of their X chromosomes. These organisms have evolved strategies to compensate for this difference in gene dose and thereby prevent a lethal imbalance in gene products. The dosage compensation mechanisms involve chromosome-wide regulation of gene expression (*1*). In *C. elegans*, hermaphrodites (XX) reduce the level of transcripts from each of their two X chromosomes to equalize X-chromosome gene expression with that of males (XO) (*2–4*). A regulatory gene hierarchy controls dosage compensation in *C. elegans*, and hermaphrodites deficient in these genes die from overexpression of their X-linked genes (*5*, *6*). Dosage compensation is implemented by a protein complex that includes products of at least two genetically defined dosage compensation *dumpy* genes, *dpy-26* and *dpy-27* (*7*). The *dpy-27* gene product, DPY-27, associates specifically with hermaphrodite X chromosomes and is a member of a highly conserved family of proteins involved in chromosome condensation and segregation (*4*, *8*). These properties suggest that dosage compensation is achieved through changes in X chromosome structure (*4*).

Here, we present a study of the *dpy-26* gene, whose protein product, DPY-26, not only forms a complex with DPY-27 to achieve dosage compensation, but also functions independently of DPY-27 in meiosis. Unlike *dpy-27* mutations, *dpy-26* mu-

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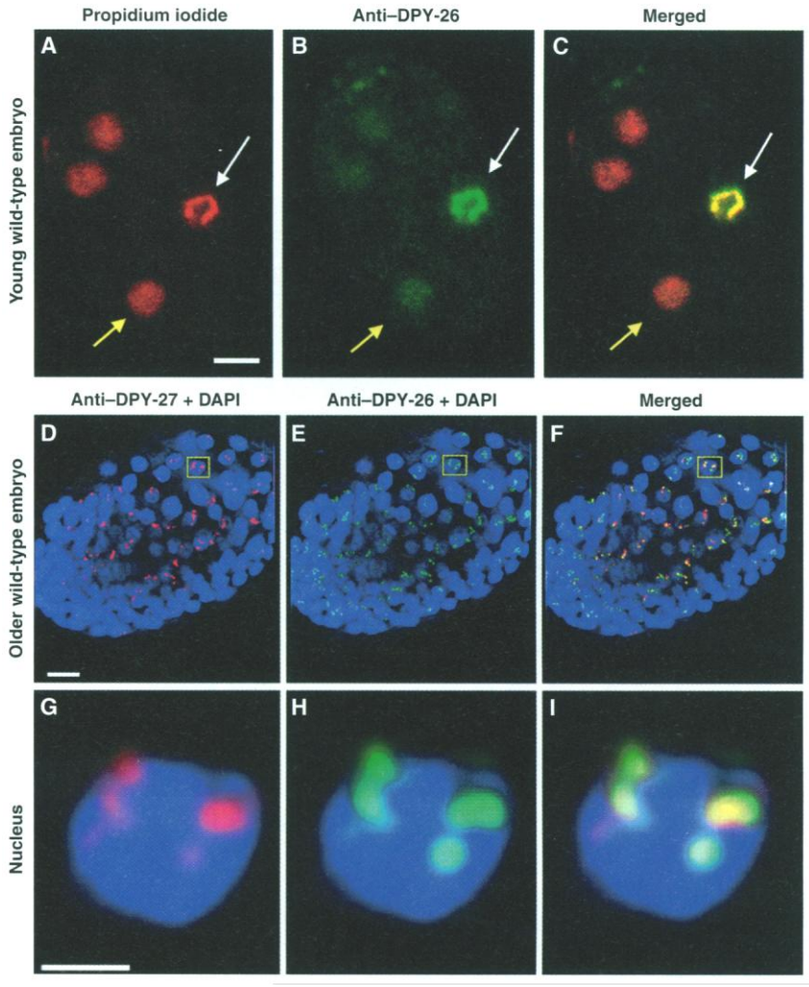

Fig. 1. (**A** to **C**) DPY-26 is associated with all condensed mitotic chromosomes in young wild-type XX embryos before the onset of dosage compensation. A wild-type XX embryo (<30-cell stage) was stained with propidium iodide, a nucleic acid–intercalating molecule (red), in (A) and with anti–DPY-26 (green) [as in (*4*)] in (B). A merged image is shown in (C) (white arrows, condensed mitotic chromosomes in anaphase; yellow arrows, interphase nuclei). Scale bar, 10 μm. (**D** to **F**) DPY-26 is localized to the X chromosomes of XX animals that have activated dosage compensation. A wild-type XX embryo (>200-cell stage) was triply stained with the DNA-intercalating molecule diamidophenylindole (DAPI) (blue), with anti–DPY-27 (red) in (D), and with anti–DPY-26 (green) in (E). A merged image is shown in (F). Scale bar, 5 μm. (**G** to **I**) Enlargements of the boxed nuclei in (D) through (F), respectively. In this optical section both X chromosomes are visible, and the coincident positions of DPY-26 and DPY-27 on the X chromosomes are apparent. Scale bar, 1 μm. Embryos in (D) through (F) were analyzed by wide-field fluorescence microscopy and deconvolution software (*23*). Images in (A) through (C) and in all subsequent figures were obtained by confocal microscopy.

tations cause a recessive defect in the segregation of all meiotic chromosomes in both sexes (*3*, *9*, *10*). The most obvious manifestation of this defect is that *dpy-26* mutant XX animals maternally rescued for lethality produce 4% male self-progeny, 20 times the wild-type frequency.

To investigate how *dpy-26* modulates gene expression and mediates chromosome segregation, we isolated (*11*) and sequenced (*12*) the *dpy-26* gene. The deduced amino acid sequence revealed that DPY-26 is an acidic protein of 1263 amino acids (predicted isoelectric point, 4.71) with no similarity to any protein in current databases. The mutation associated with the canonical null allele *dpy-26(n199)* is a C → T transition (*13*) predicted to cause premature termination of translation at codon 525, consistent with *n199* causing complete loss of *dpy-26* function.

We then prepared polyclonal antibodies (*14*) to two nonoverlapping regions of DPY-26 and determined the staining pattern of DPY-26 in XX animals by confocal microscopy. In wild-type XX embryos at a stage (<30 cells) before the activation of dosage compensation, DPY-26 was distributed diffusely in interphase nuclei; DPY-26 was also associated with all chromosomes as they condensed and underwent mitosis (Fig. 1, A to C). In contrast, in older embryos at a stage (>30 cells) in which dosage compensation is activated, the DPY-26 staining pattern in interphase was subnuclear and punctate (Fig. 1E), and this pattern persisted in somatic cells throughout development. Costaining of XX embryos with anti–DPY-26 and anti–DPY-27 antibodies revealed that DPY-26 colocalizes with DPY-27 on the X chromosome throughout the cell cycle (Fig. 1, D to I). Once DPY-26 was

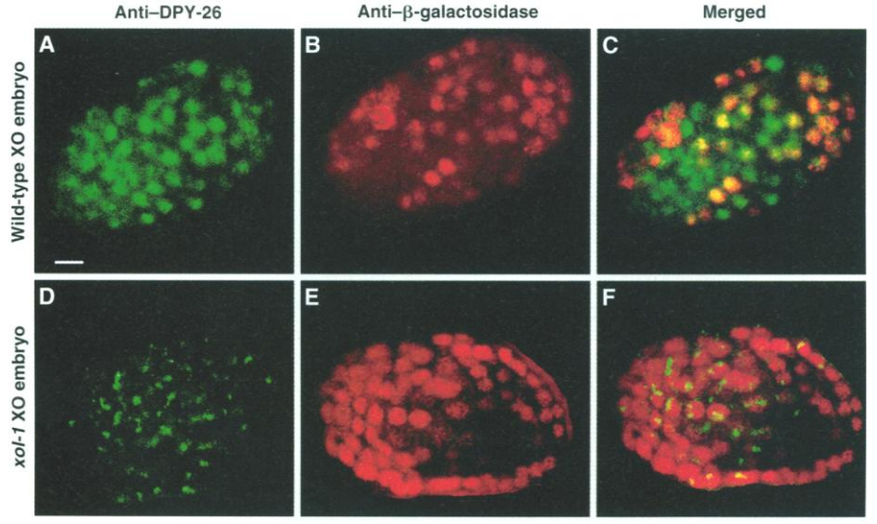

Fig. 2. DPY-26 is not associated with the X chromosome of wild-type XO embryos but is associated with the X of *xol-1* mutant XO embryos that activate dosage compensation. (**A** to **C**) *him-8 yIs2(xol-1::lacZ)* XO embryo double-stained with anti–DPY-26 (A) and anti–β-galactosidase (B). The *xol-1::lacZ* fusion is a reporter transgene expressed exclusively in XO embryos; its product is restricted to the nucleus (*14*). A merged image is shown in (C). (**D** to **F**) *him-8 yIs2(xol-1::lacZ); xol-1(y9)* XO embryo double-stained with anti–DPY-26 (D) and anti–β-galactosidase (E). A merged image is shown in (F). Scale bar, 10 μm.

fully recruited to X at approximately the 64-cell stage of embryogenesis, it was no longer associated with mitotic autosomes, although it remained associated with mitotic X chromosomes. Thus, the affinity of DPY-26 for all chromosomes appears to be eliminated once the dosage compensation complex is assembled on X.

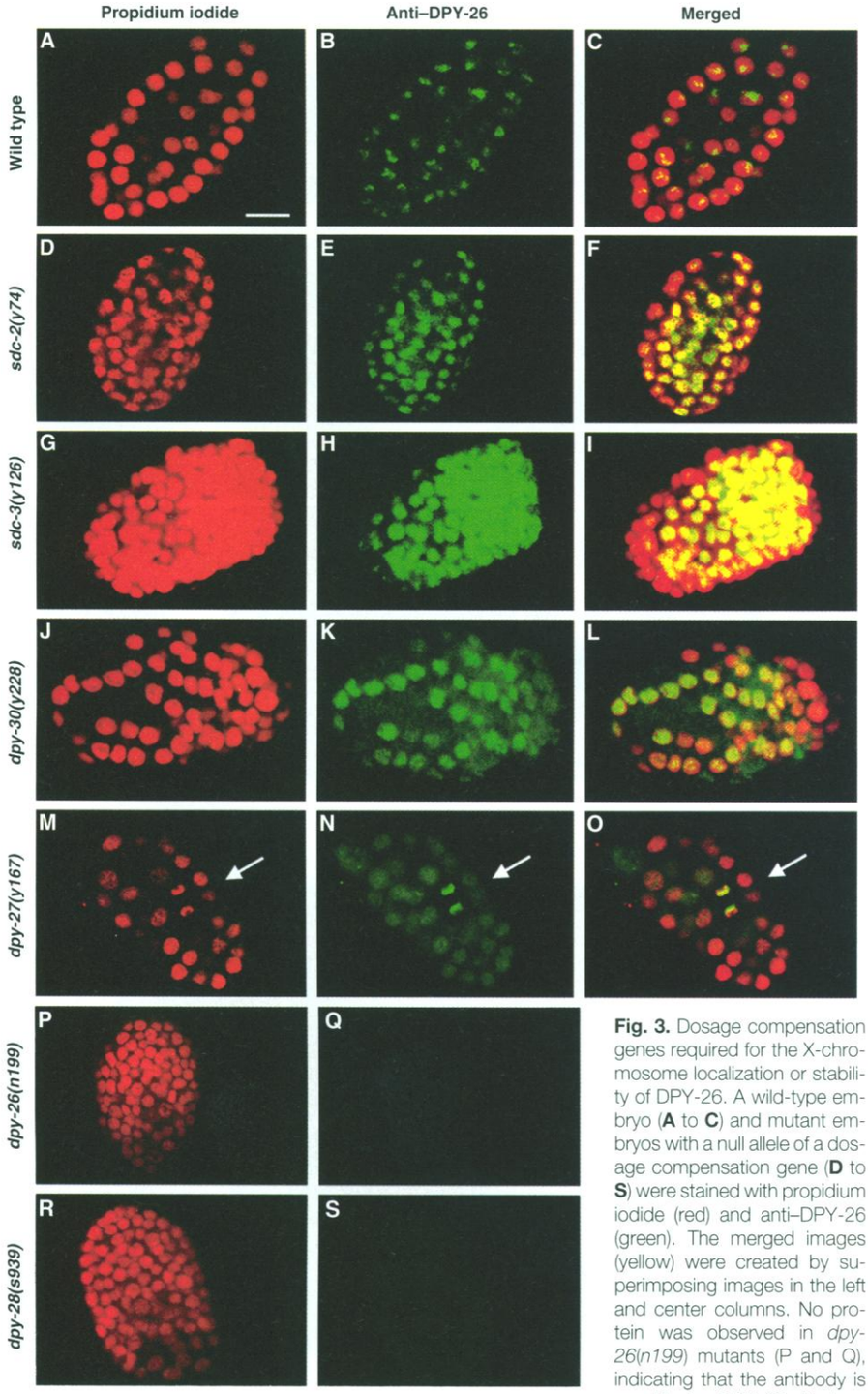

Fig. 3. Dosage compensation genes required for the X-chromosome localization or stability of DPY-26. A wild-type embryo (**A** to **C**) and mutant embryos with a null allele of a dosage compensation gene (**D** to **S**) were stained with propidium iodide (red) and anti–DPY-26 (green). The merged images (yellow) were created by superimposing images in the left and center columns. No protein was observed in *dpy-26(n199)* mutants (P and Q), indicating that the antibody is specific for DPY-26. X chromosome–specific localization of DPY-26 is dependent on two genes that coordinately regulate sex determination and dosage compensation (*sdc-2* and *sdc-3*) and two genes required for dosage compensation (*dpy-30* and *dpy-27*). The dosage compensation genes *dpy-27* and *dpy-28* are required for the stability of DPY-26. In mutants that fail to assemble a dosage compensation complex on X, DPY-26 still associates with mitotic chromosomes, as indicated by the white arrows (M to O). Scale bar, 10 μm.

Dosage compensation in C. *elegans* is essential for the viability of XX animals but is lethal if implemented in males. To determine how the hermaphrodite specificity of *dpy-26* action is conferred, we next examined the DPY-26 staining pattern in XO animals. We found that DPY-26 was localized in the nucleus of XO animals but was not exclusively associated with the male X chromosome at any time during development (Fig. 2, A to C). However, as in young XX embryos, DPY-26 associates with condensed mitotic chromosomes in male embryos (*15*). How is DPY-26 prevented from associating specifically with the male X chromosome? In males, the switch gene *xol-1* (XO lethal) is activated and represses the genes that implement dosage compensation in hermaphrodites (5). XO animals that have lost *xol-1* function through mutation are forced into the hermaphrodite modes of sex determination and dosage compensation and die from inappropriately low X-linked gene expression. We found that in dying *xol-1* XO embryos, DPY-26 was mislocalized to the X chromosome in interphase (Fig. 2, D to F). Therefore, in wild-type XO embryos, *xol-1* expression prevents the exclusive association of DPY-26 with X.

To investigate the mechanism by which DPY-26 associates specifically with the X chromosomes of hermaphrodites, we examined the pattern of anti–DPY-26 staining in mutants defective in the hermaphrodite-specific genes that control and implement dosage compensation. These genes fall into two classes: (i) those that coordinately control sex determination and dosage compensation (*sdc-1*, *sdc-2*, and *sdc-3*) (6) and (ii) those that regulate dosage compensation only (*dpy-21*, *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-30*) (*3*, *9*, *16*). Null mutations in all of these genes except *sdc-1* and *dpy-21* cause >95% lethality in XX animals. Using the approach in (7), we found that mutations in *sdc-2*, *sdc-3*, *dpy-27*, and *dpy-30* prevented selective association of DPY-26 with X. Diffuse nuclear staining was present during interphase, and condensed chromosome staining was present during mitosis (Fig. 3, A to O). In these mutant embryos, staining of condensed chromosomes was persistent, perhaps as a consequence of DPY-26's inability to localize specifically to X (Fig. 3, M to O). The X-chromosome localization of DPY-26 was unaffected by mutations in *sdc-1* or *dpy-21* (*15*). In every case, the effect of mutations on the localization of DPY-26 to X was the same as the effect on DPY-27 localization (7), further supporting the assertion that DPY-26 and DPY-27 function together in a complex.

Two dosage compensation proteins, DPY-27 and DPY-28, appear to be required

for the stability of DPY-26. DPY-26 was not detectable in *dpy-28* mutant embryos (Fig. 3, R and S). In *dpy-27* embryos, DPY-26 staining was diffuse and weaker than in wild-type embryos and persisted for a shorter time; no DPY-26 was detectable in the somatic cells of *dpy-27* mutant adults (*15*). Northern (RNA) blot analysis (*15*) revealed that in both *dpy-27* and *dpy-28* mutants, a *dpy-26* transcript of normal length was produced at levels comparable to those in wild-type animals, indicating that the *dpy-27* and *dpy-28* mutations affect either the translation or the stability of DPY-26. Combined with the observations that DPY-27 forms a complex with DPY-26 and that *dpy-26* and *dpy-28* are both necessary for the stability of DPY-27 (*7*), these results suggest that DPY-28 stabilizes DPY-26 by participating in the somatic dosage compensation complex.

Finally, we stained sexually mature adult animals with anti–DPY-26 to study how DPY-26 might affect meiotic chromosome segregation in the germline. DPY-26 colocalizes with all chromosomes in the germ cell nuclei of hermaphrodites as they condense and enter pachytene (Fig. 4, A to D). This colocalization persists throughout the first and second meiotic divisions. The germline distribution of DPY-26 was unaffected by mutations in any *sdc* or dosage-compensation *dpy* gene except for *dpy-28* (*15*). Thus, the meiotic function of DPY-26 is implemented independently of its function in dosage compensation. This notion is consistent with our observation that DPY-26's protein complex partner in somatic cells, DPY-27, was not expressed in germline cells of wild-type animals (Fig. 4, E and F). The absence of DPY-27 from the germline may permit DPY-26 to perform its germline-specific function. That function requires *dpy-28*, a gene also required for proper meiotic chromosome segregation (*3*). In *dpy-28* mutant adults, DPY-26 was absent from the germline (*15*). Because *dpy-26* appears to require *dpy-28* for its germline stability and both genes are required for proper meiosis, DPY-26 and DPY-28 may form a complex in the germline to ensure the fidelity of meiotic chromosome segregation.

The developmental regulation of *dpy-26* transcripts is consistent with the function of DPY-26 in dosage compensation and meiosis. The single ~4-kb *dpy-26* transcript is abundant in embryos, becomes depleted in the second and third larval stages, and reappears in the fourth larval stage and in adults (*15*). Dosage compensation is activated early in embryonic development, and the reappearance of *dpy-26* transcripts in the fourth larval stage and adulthood coincides with a period of rapid germline proliferation and the onset of meiosis.

Our results show that DPY-26 executes its roles in dosage compensation and meiosis independently—through its association with X to achieve reduced X chromosome gene expression, and through its association with germ cell meiotic chromosomes to ensure proper chromosome segregation. The association of DPY-26 with mitotic chromosomes in young embryos raises the issue of whether DPY-26 also plays an active role in mitosis. The specificity of DPY-26's chromosome association appears to be conferred through its interaction with specific protein partners and through its regulation by sex-specific genes. Initially, DPY-26 associates with all mitotic chromosomes; however, once dosage compensation is activated, that association is abolished. DPY-26 then becomes selectively localized to X in hermaphrodites. The sex and tissue specificity of DPY-26's role in dosage compensation may be conferred by SDC-2 and DPY-27, respectively. Both are required for the association of DPY-26 with X; however, SDC-2 is present only in hermaphrodites (*17*), whereas DPY-27 is present only in somatic cells. Neither is required for the role of DPY-26 in meiosis.

Chromosome association unifies the roles of DPY-26 in gene expression and chromosome segregation, suggesting that related structural requirements underlie both processes. Precedents exist for such a structural link. The *Drosophila* heterochromatin binding protein HP1 mediates transcriptional repression of genes adjacent to heterochromatin (*18*). Mutations in HP1 cause aberrant mitotic chromosome segre-

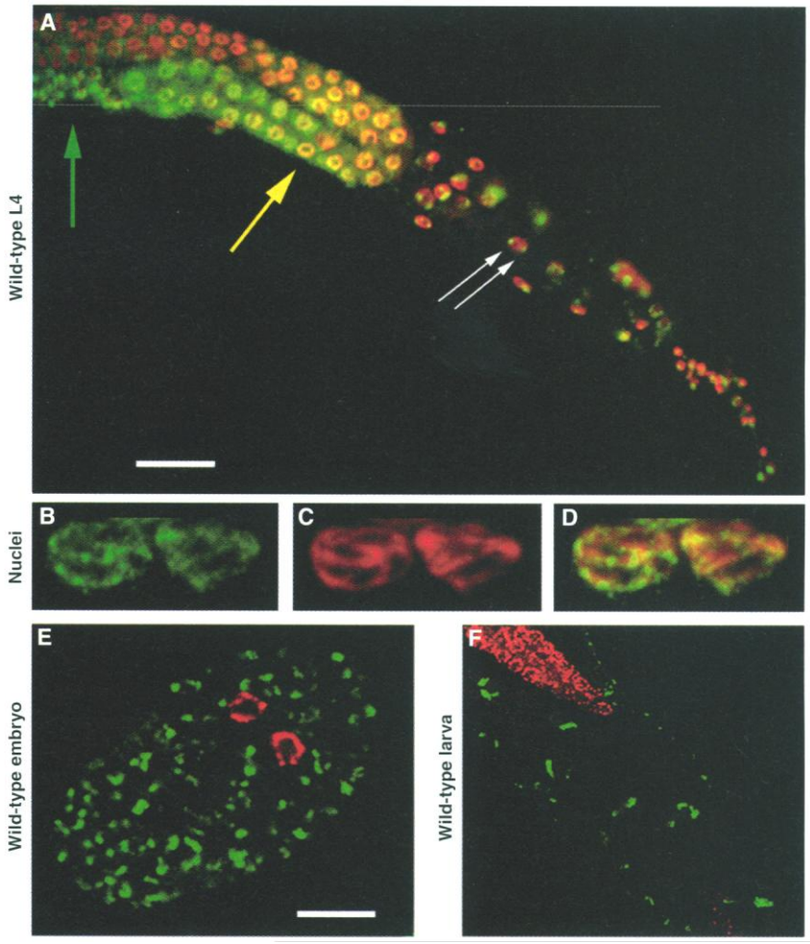

Fig. 4. DPY-26, but not DPY-27, is present in the germline and is associated with meiotic chromosomes. (**A**) The posterior half of a wild-type XX L4 worm stained with propidium iodide (red) and anti–DPY-26 (green) to create the merged image shown. The left half of the image shows the gonad. DPY-26 colocalizes with all chromosomes in the germ cell nuclei (yellow arrow) but is localized only to the X chromosomes (white arrows) in somatic cells. Maturing sperm (green arrow) indicate the proximal end of the gonad. Scale bar, 20 μm. (**B** to **D**) Germ cell nuclei in pachytene were stained with anti–DPY-26 (green) (B) and propidium iodide (red) (C). A merged image is shown in (D). (**E** and **F**) A wild-type XX embryo (E) and a wild-type XX larva (F) were stained with anti–DPY-27 (green) and anti–P-granule (red) specific to germline precursor cells and germ cells (*24*) to show that DPY-27 is absent from the germline. Scale bar, 10 μm.

gation, perhaps through defects in chromosome condensation (*19*). The *Schizosaccharomyces pombe swi6* gene maintains transcriptional repression at centromeres and silent mating-type loci. Mutations in *swi6* not only interfere with silencing, but also cause a high rate of chromosome loss during mitosis (*20*, *21*) correlated with lagging centromere migration during anaphase (*22*). The colocalization of Swi6p with heterochromatin-like domains of centromeres, telomeres, and the silent mating-type loci suggests a structural role for Swi6p in gene expression and chromosome segregation (*20*). The composition of a *C. elegans* dosage compensation complex (*7*) suggests that it modifies the structure of X chromosomes to reduce gene expression. DPY-26 may similarly exert its effects on meiotic chromosome segregation through changes in chromosome structure.

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11. The *dpy-26* gene had been mapped to linkage group IV, between *unc-22* and *unc-31* (*9*). To map *dpy-26* more precisely, we used cosmids W03F2 and C03F9, which are flanked by *unc-22* and *unc-31*, as probes to identify polymorphisms between Bristol N2 and Bergerac N62 strains. We mapped *dpy-26* relative to these two polymorphisms by analyzing the DNA from Unc non-Dpy recombinants picked from the strains *dpy-26(n199) unc-31*/++N62 and *unc-22 dpy-26(n199)*/++N62. Of the *unc-31* recombinants, 20 had the N62 version of C03F9, and 5 had the N2 version. Of the *unc-22* recombinants, 26 had the N62 version of W03F2, and 4 had the N2 version. Thus, *dpy-26* mapped 0.18 map units (mu) to the left of C03F9 and 0.05 mu to the right of W03F2. Overlapping cosmids that form a contiguous link between C03F9 and W03F2 (100 μg/ml) were injected into gonads of *dpy-26(n199) unc-31(e169)/unc-22* heterozygotes with the dominant injection marker *rol-6* (100 μg/ml) [C. C. Mello, J. M. Kramer, D. Stinchcomb, V. Ambros, *EMBO J.* **10**, 3959 (1991)]. Lines that stably transmitted the dominant *rol-6* marker were isolated, and *dpy-26 unc-31* mutant offspring were tested for rescue of lethality. Overlapping cosmids C25G4 (7 of 7 lines) and C01G3 (6 of 6 lines) rescued the XX-specific lethality. The *dpy-26* rescuing region was narrowed to an 8.5-kb Sal I fragment of C25G4, pS2 (6 of 6 lines).
12. A 3.5-kb cDNA clone (pJL2) was obtained from a mixed-stage cDNA library screened with the 8.5-kb pS2 fragment. To obtain the 5′ end of the cDNA, we amplified the 5′ 375 base pairs (bp) by reverse transcriptase polymerase chain reactions (RT-PCR) using primers specific for the SL1 (5′-TCTAGAATTCCGCGGTTTAATTACCCAAGTTTG-3′) or SL2 (5′-TCTAGAATTCCGCGGTTTTAACCCAGTTACTC-3′) trans-splice leaders and a primer specific for *dpy-26*, JDL8 (5′-CCACTCGAGAGAGCTGAATCAAT-3′). The *dpy-26* transcript is trans-spliced to both SL1 and SL2 RNA leaders, suggesting that *dpy-26* may be a member of an operon [T. Blumenthal, *Trends Genet.* **11**, 132 (1995)]. The *dpy-26* genomic and cDNA clones were sequenced [M. Strathmann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1247 (1991)] and the *dpy-26* cDNA sequence in GenBank (accession number U43562) and genomic sequence (accession number Z70680) were deposited.
13. Cloned *Pfu* polymerase was used with primers JDL18 (5′-TTCTCCTGCTTTCTATTATCTAA-3′) and JDL19 (5′-CTTACTCATCCATCTGCTCAT-3′) in a PCR to amplify the first 2200 bp of *dpy-26* from single *dpy-26(n199)* homozygous mutant worms. PCR products from two independent reactions were cloned and sequenced on both strands.
14. Fragments of pJDL2 corresponding to amino acids 127 to 739 and 739 to 1263 of DPY-26 were subcloned into the T7 expression vector pRSET A (Invitrogen) fused to an epitope encoding six histidines. Expression of histidine-tagged DPY-26 fragments was induced in *Escherichia coli* BL21 pLys-S cells with 1 mM isopropyl-β-D-thiogalactoside, and the protein was purified by nickel-chelate chromatography and polyacrylamide gel electrophoresis. Mice received subcutaneous injections of 10 μg of protein mixed with Ribi adjuvant (RIBI ImmunoChem Research); rabbits received injections of 100 to 500 μg of protein mixed with Freund's adjuvant. All staining was performed with affinity-purified antibody. No staining was observed in wild-type embryos treated with preimmune sera.
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Sex-Specific Assembly of a Dosage Compensation Complex on the Nematode X Chromosome

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In nematodes, flies, and mammals, dosage compensation equalizes X-chromosome gene expression between the sexes through chromosome-wide regulatory mechanisms that function in one sex to adjust the levels of X-linked transcripts. Here, a dosage compensation complex was identified in the nematode *Caenorhabditis elegans* that reduces transcript levels from the two X chromosomes in hermaphrodites. This complex contains at least four proteins, including products of the dosage compensation genes *dpy-26* and *dpy-27*. Specific localization of the complex to the hermaphrodite X chromosomes is conferred by XX-specific regulatory genes that coordinately control both sex determination and dosage compensation.

Dosage compensation is an essential chromosome-wide regulatory process that modulates the expression of numerous genes related solely by their linkage to the same chromosome. The need for dosage compensation arises in organisms whose sex-determination mechanisms cause males (typically XO or XY) and females (typically XX) to differ in their dose of X chromosomes. Dosage compensation equalizes expression of most X-linked genes between the sexes despite the twofold difference in X-chromosome dose, thereby preventing the sex-specific lethality that would otherwise result from inappropriate amounts of X-chromosome products. This global modulation of gene activity is superimposed upon gene-specific regulation that allows X-linked genes to be expressed in specific tissues, at particular times, and in the correct sex (*1*).

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