DPY-30, a nuclear protein essential early in embryogenesis for

Caenorhabditis elegans dosage compensation

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SUMMARY

DPY-30 is an essential component of the *C. elegans* dosage compensation machinery that reduces X chromosome transcript levels in hermaphrodites (XX). DPY-30 is required for the sex-specific association of DPY-27 (a chromosome condensation protein homolog) with the hermaphrodite X chromosomes. Loss of *dpy-30* activity results in XX-specific lethality. We demonstrate that *dpy-30* encodes a novel nuclear protein of 123 amino acids that is present in both hermaphrodites and males (XO) throughout development. DPY-30 itself is not associated with the X chromosomes, nor is its pattern of expression perturbed by mutations in the gene hierarchy that controls dosage compensation. Therefore, DPY-30 is a ubiquitous factor that is likely to promote the hermaphrodite-specific association of DPY-27 with X by affecting the activity of a sex-specific dosage compensation gene. In XO animals, DPY-30 is required for developmental processes other than dosage compensation: coordinated movement, normal body size, correct tail morphology and mating behavior. We demonstrate that rescue of both the XX-specific lethality and the XO-specific morphological defects caused by *dpy-30* mutations can be achieved by inducing *dpy-30* transcripts either in the mother or in the embryo through the end of gastrulation. *dpy-30* appears to be cotranscribed in an operon with a novel RNA-binding protein.

Key words: dosage compensation, X chromosome, *Caenorhabditis elegans*, gene expression, RNA-binding protein

INTRODUCTION

Dosage compensation is a chromosome-wide regulatory process that controls the expression of numerous genes related solely by their linkage to the same sex chromosome. The need for dosage compensation arises in organisms whose primary sex-determining mechanisms cause males and females to differ in their relative dose of X chromosomes. Dosage compensation mechanisms equalize expression of X-linked genes between the sexes and prevent the sex-specific lethality that would otherwise result from the two-fold difference in X-linked gene dose. The mechanisms used to achieve dosage compensation are diverse. In mammals, dosage compensation is accomplished by random inactivation of one of the two female X chromosomes, thereby reducing the effective X chromosome dose to that of males (XY) (reviewed in Migeon, 1994). In *Drosophila*, the dosage compensation machinery acts to transcribe the single X chromosome of males (XY) at twice the rate as each of the two X chromosomes in females (XX) (reviewed in Baker et al., 1994). In *C. elegans*, XX hermaphrodites reduce the transcript levels produced by each X chromosome to achieve the same levels produced by the single X of males (XO) (reviewed in Hsu and Meyer, 1993).

Recent molecular characterization of genes essential for dosage compensation in mammals, flies and worms has greatly advanced our understanding of these unique global regulatory mechanisms. Studies in mammals have identified a large, non-coding transcript (*Xist*) from within the *cis*-acting X chromosome inactivation center that is specific to the inactive X and may play a role in initiating X chromosome inactivation (Brockdorff et al., 1992; Brown et al., 1992; Brown and Willard, 1994; Kay et al., 1993). In *Drosophila*, the proteins essential for elevated transcription of the male X (MLE, a putative RNA helicase, MSL-1 and MSL-3, two novel proteins, and MSL-2, a ring finger protein) are sex-specifically localized to the male X, a finding consistent with their direct involvement in X chromosome hypertranscription (Kuroda et al., 1991; Palmer et al., 1993; Gorman et al., 1995; Kelley et al., 1995; Zhou et al., 1995). The association of these proteins with X requires the functional products of all the *msl* and *mle* genes, suggesting that they form a multimeric complex. In addition, an acetylated isoform of histone H4 colocalizes with the MSL and MLE proteins on X in a manner dependent on their activities (Turner et al., 1992; Bone et al., 1994), suggesting that the male dosage compensation apparatus modulates gene expression through modification of X chromatin structure. In *C. elegans*, insight into how X chromosome expression is reduced in hermaphrodites has been gained through analysis of the hermaphrodite-specific *dpy-27* dosage compensation gene (Chuang et al., 1994). Immunolocalization studies of DPY-27 showed that it is expressed in the nuclei of both sexes, but is sex-specifically localized to only the hermaphrodite X chromosomes, consistent with its role in down regulating X chromosome expression. DPY-27 belongs

to the newly identified SMC gene family whose members are involved in multiple aspects of chromosome dynamics, including chromosome segregation (Strunnikov et al., 1993, 1994; Saka et al., 1994) and chromosome condensation (Hirano and Mitchison, 1994). The similarity of DPY-27 to these proteins suggests that the dosage compensation machinery effects global changes in X chromosome expression by condensing its higher order chromosome structure.

The sex-specific dosage compensation process in *C. elegans* is controlled by a regulatory cascade that is set to the male or hermaphrodite mode in response to the X:A ratio, the primary sex-determining signal. Two classes of genes within this hierarchy are required for proper X chromosome expression in hermaphrodites, those that coordinately control both sex determination and dosage compensation (*sdc-1*, *sdc-2* and *sdc-3*) (DeLong et al., 1993; Miller et al., 1988; Nusbaum and Meyer, 1989; Villeneuve and Meyer, 1987), and those that control dosage compensation but play no direct role in sex determination (*dpy-21, dpy-26, dpy-27, dpy-28* and *dpy-30*) (Hodgkin, 1983; Hsu and Meyer, 1994; Meyer and Casson, 1986; Plenefisch et al., 1989). Mutations in all these genes cause elevated X chromosome expression in XX animals and, with the exception of *dpy-21* and *sdc-1*, cause extensive XX-specific lethality. In contrast, mutations in these genes have no effect on the viability of XO animals. In addition to their effects on dosage compensation, the *sdc* mutations cause severe masculinization of XX animals. In XO animals, the activities of *sdc* and *dpy* genes are repressed by *xol-1*, a gene required for male development (Miller et al., 1988). *xol-1* is the first gene of the hierarchy and acts as an early developmental switch to set the choice of sexual fate and the level of X chromosome expression in response to the X:A ratio (Rhind et al., 1995). Mutations in *xol-1* activate the hermaphrodite program of development and cause the feminization and death of XO animals from inappropriately low X chromosome expression. As expected, DPY-27 is localized to the X in dead *xol-1* XO animals (Chuang et al., 1994). Central to understanding the mechanism of dosage compensation is determining how DPY-27 becomes localized to X. Since the protein is expressed in both sexes, other components of the regulatory hierarchy must be involved in conferring the sex-specificity in X chromosome binding. Analysis of DPY-27 localization in *sdc* and *dpy* mutant strains revealed that *dpy-30* is one of the components essential for DPY-27's X chromosome localization (P.-T. Chuang and B. J. Meyer, unpublished). To understand the role of *dpy-30* in dosage compensation, we performed an extensive molecular characterization.

Previous genetic analysis had shown that *dpy-30* mutations cause phenotypes similar to those caused by mutations in *dpy-26, dpy-27* and *dpy-28* – maternal-effect, XX-specific lethality correlated with elevated X chromosome expression (Hsu and Meyer, 1994). However, there are important differences that distinguish *dpy-30* from the other *dpy* genes (Hsu and Meyer, 1994). Unlike the other *dpy* mutations, *dpy-30* mutations cause XX-specific lethality that is completely penetrant and temperature sensitive. At the partially permissive temperature, the XX mutants that escape lethality are egg-laying defective (Egl), dumpy (Dpy) and have a protruding vulva (Pvul), phenotypes commonly caused by a disruption in dosage compensation (Plenefisch et al., 1989). *dpy-30* mutations have no effect on either the viability of XO animals or their X chromosome

expression, but they do cause XO animals to have numerous morphological and behavioral abnormalities, including a delay in development, a small body size, an inability to mate and a defect in male tail structures. The pleiotropic phenotypes caused by *dpy-30* mutations suggest that in addition to acting in dosage compensation, *dpy-30* plays a more general role in the development of both XX and XO animals.

We show that *dpy-30* encodes a novel nuclear protein ubiquitously expressed throughout the development of both XX and XO animals. In contrast to DPY-27, DPY-30 is not associated with the X chromosome, nor is its pattern of expression perturbed by mutations that disrupt dosage compensation, or by changes in the X:A ratio. Therefore, DPY-30 is likely to be required for the activity of sex-specific dosage compensation factors that down regulate X-linked gene expression in XX animals. Our studies also indicate a requirement for DPY-30 during early embryogenesis in XX animals, consistent with the need for an active dosage compensation process early in development. XO animals have a similar temporal requirement for DPY-30. Lastly, *dpy-30* appears to be cotranscribed in an operon with a novel RNA-binding protein.

MATERIALS AND METHODS

C. elegans cultures

To obtain large populations of nematodes of all developmental stages for RNA extractions, animals were grown in liquid culture and isolated as described in Klein and Meyer (1993). Adult XO males were isolated by the Nytex screening procedure described by Meyer and Casson (1986).

Germline transformation

Germline transformation rescue experiments were carried out as described in Mello et al. (1991) by microinjecting *dpy-30(y130)* young adults with a solution containing both test DNA (10 μg/ml) and marker DNA (plasmid pRF4, 100 μg/ml), which carries the dominant *rol-6(su1006)* marker and confers a roller (Rol) phenotype. Healthy *dpy-30(y130)* homozygous animals were obtained for injection as the non-Unc progeny of heterozygous *dpy-30(y130) / nT1(n754*dm*)* mothers. Cosmids and subclones were assayed for their ability to rescue the XX-specific lethality by examining broods of injected *dpy-30* XX animals for viable F₁ roller progeny. If F₁ rescue was obtained, rescued animals were picked to individual plates to establish stably transmitting lines carrying the rescuing construct. Constructs were eliminated from consideration if injections of 50 to 100 hermaphrodites failed to produce any viable progeny. Initial transformation experiments with the cosmids C02C8, C08F12, C09F3, C27A7, C35G11, C51B3 and F29D2 (from A. Coulson and J. Sulston) demonstrated that C09F3, located 50 kb to the left of the *myo-3*-cosmid C02B11, rescued the *dpy-30* XX transgenic animals. Stable arrays made from subclones of C09F3 (Fig. 1) were used to delineate the minimal genomic region required for rescue of the *dpy-30* mutant phenotypes. For each rescuing construct, at least two independent transmitting lines were scored for rescue of the *dpy-30* XX-specific phenotypes. The smallest rescuing clone, pDH38, also rescues the XX-specific lethality and the XO-specific morphological defects caused by the *dpy-30(y228)* null allele.

Isolation of RNA and northern hybridization experiments

Total RNA and poly(A)⁺ RNA were made as described by Klein and Meyer (1993) and Hsu and Meyer (1994). Northern analysis was performed as described by Meyer and Casson (1986). The *dpy-30*-specific probe was the 1.3 kb *Hinc*II to *Sty*I fragment from pDH13

that contains the entire *dpy-30* rescuing region. After probing with *dpy-30*, blots were rehybridized with a probe for the *act-1* gene (Krause et al., 1989).

Sequence analysis of *dpy-30* and *rnp-1*

Sequence analysis of the 4 kb genomic DNA that contains the *dpy-30* rescuing region, was performed according to Sambrook et al. (1989), using pDH13 and its reverse orientation clone pDH14. Additional genomic sequence 3′ to *dpy-30* was obtained from pDH8, a clone containing the entire *rnp-1* genomic region (see Fig. 1). Nucleotide number 1 in the genomic sequence listed in Fig. 3 is the first base of the *Xba*I site at the beginning of the insert of pDH38 (Fig. 1C).

Three independent *dpy-30* cDNA clones were obtained by probing N2 mixed-stage libraries (S. Kim and S. Lichtsteiner) and a *him-8* embryonic library (L. Miller) with either a ^{32}P- labeled 1.6 kb *Hinc*II fragment from pDH12 or a ^{32}P-labeled 1.3 kb *Hinc*II *Sty*I fragment from pDH13. pDH42, the largest cDNA clone, begins at nucleotide 672 and ends at nucleotide 1323, with a poly(A)$^+$ tail. pDH19 begins at nucleotide 826 and ends at nucleotide 1322, with a poly(A)$^+$ tail. pDH20, begins at nucleotide 665 and continues to the *Eco*RI site at nucleotide 1057. The 5′ end of pDH20 contains a 12 nucleotide sequence (5′ CCCAAGTTTGAG 3′) identical to the SL1 *trans*-spliced leader sequence found at the 5′ end of many *C. elegans* mRNAs (Blumenthal, 1995). The *trans*-splicing pattern of *dpy-30* was confirmed by PCR amplification of cDNA (Klein and Meyer, 1993) using oligonucleotides complementary to SL1 or SL2 and an oligonucleotide internal to *dpy-30*, DH-6 (5′ CTGTTGTCCTCCATTG-GCAG 3′). Using SL1 and DH-6, this amplification reaction produced an expected product of approximately 200 bp. No product was obtained using SL2 and DH-6.

The *rnp-1* cDNA pDH18 was isolated fortuitously because the 1.6 kb *Hinc*II genomic probe used to isolate *dpy-30* cDNAs also contained the first two exons of the *rnp-1* transcript. This cDNA begins at nucleotide 1421 with the ATG initiation codon and ends with a poly(A)$^+$ tail at nucleotide 3511 in the extended genomic sequence. The *trans*-splicing pattern of *rnp-1* was determined by PCR amplification of cDNA using oligonucleotides complementary to SL1 or SL2 and an oligonucleotide internal to *rnp-1*, DH-9 (5′ GCGGTCG-GTCGCAATTTTGAAG 3′). The amplification reaction produced a product of approximately 300 bp using SL2 and DH-9. DNA sequence analysis of the cloned product showed that SL2 is spliced directly to the initiator ATG at nucleotide 1421. No product was obtained using SL1 and DH-9. Genomic DNA clones were sequenced on both strands; cDNA clones were sequenced on a single strand.

Sequence analysis of *dpy-30* mutations

dpy-30 DNA was obtained from four homozygous *dpy-30(y130* or *y228)* adult animals using the PCR method described by Klein and Meyer (1993) and the oligonucleotides DH-1 (5′ CGAC-GAACAGTTTGGATGGCATC 3′) and DH-2 (5′ GTCTAGA-CACGCGTGTAACGTAC 3′) to amplify *dpy-30* products. These conditions yielded a single 1.4 kb product, consistent with the predicted size of the genomic fragment that should be obtained with DH-1 and DH-2. The DNA sequence of at least two independent clones derived from each amplification reaction was determined.

Construction of insertion mutations in the *dpy-30* coding region

The insertion mutation within pDH43 was made by cutting pDH38 with *Bcl*I, filling in the 4 nucleotide overhang with *E. coli* DNA polymerase I large fragment (Klenow) (Sambrook et al., 1989) and recircularizing the linear blunt-ended DNA. The presence of the 4 base pair insertion at the *Bcl*I site was confirmed by DNA sequence analysis. pDH39, which contains a frameshift mutation at the *Eco*RI site in *dpy-30*, was made and confirmed in similar fashion.

Sequence similarity searches

The initial analysis of the *dpy-30* and *rnp-1* nucleotide and protein sequence was performed using DNA Strider (Marck, 1988). Similarity searches for the *dpy-30* predicted protein were performed using either the BLAST algorithm (Altschul et al., 1990) or the FASTA algorithm (Devereux et al., 1984).

Recombinant DPY-30 protein, DPY-30 antibodies and immunocytochemistry

Expression of full-length recombinant DPY-30 protein and generation and analysis of DPY-30 antibodies were performed as in Chuang et al. (1994). For in situ antibody staining, embryos, larvae and adults were prepared as described in Chuang et al. (1994) from the following strains: wild-type XX, *sdc-1(n485)* XX, *dpy-21(e428)* XX, *sdc-3(y129)* XX, *dpy-27(y167)* XX, *dpy-28(y1*ts) XX, *dpy-28(s939); him-5(e1490); xol-1(y9)* XO, *dpy-26(y65); him-5(e1490); xol-1(y9)* XO, *sdc-2(y202*ts) XX, *her-1(hv1y101); xol-1(y9) sdc-2(y82) unc-9(e101)* XO, *her-1(hv1y101); xol-1(y9) sdc-2(y74) unc-9(e101)* XO and *him-8(e1489) yIs2[xol-1::lacZ, rol-6(su1006)]* XX. To obtain XX embryos homozygous for either *dpy-30(y228*ts) or *dpy-28(y1*ts), homozygous mutant XX animals were first grown for several generations at the partially permissive temperature (15°C) and then shifted to the non-permissive temperature (20°C) for at least one generation. The gravid hermaphrodites were then treated with hypochlorite. The same regime was used for the *sdc-2(y202*ts) strain, except the temperature shift was from 20° to 25°C. *dpy-27(y167)* XX embryos were obtained as described in Chuang et al. (1994). The procedures for antibody staining, epifluorescence and confocal microscopy are as described in Chuang et al. (1994).

hsp16-41::dpy-30 fusion

To determine the time in development when *dpy-30* transcription is essential for the viability of XX animals, *dpy-30(y228)* embryos or oocytes carrying an integrated heat-inducible *hsp16-41::dpy-30* fusion (*yIs15*) were heat shocked at different stages of their development (Rhind et al., 1995; Sulston et al., 1983). For heat shocks applied to embryos younger than comma stage, plates containing 30-40 gravid, non-Egl *yIs15; dpy-30(y228)* young adult hermaphrodites from *dpy-30/+* mothers were incubated at 20°C for 3 to 12 hours. These plates, which contained mixed-stage embryos, were then shifted from 20°C to 30°C for 30 minutes. Subsequently, comma-stage embryos were collected for 30 minute intervals to plates pre-cooled to 20°C. Animals collected by this method vary in age by approximately 45 minutes. For heat shocks applied after comma stage, comma-stage cohorts were collected and allowed to develop for defined intervals before applying the heat shocks. The time of heat shock relative to comma stage was defined as the time between the midpoint of the 30 minute heat-shock period and the midpoint of the 30 minute collection interval. Heat-shock times were subsequently expressed relative to first cleavage. 5 days after embryos were collected, the plates were scored for the presence of adult hermaphrodite progeny. For most time points, between 50 and 175 embryos were scored. Percent viability was calculated according to the formula: Viability = 100 (number of viable adult hermaphrodites)/(total number embryos). The baseline viability of *dpy-30(y228)* XX animals in the absence of heat shocks is approximately 12% (Hsu and Meyer, 1994). When survivors were present, they were also scored for the severity of the Dpy, Egl and Pvul dosage compensation phenotypes. The percent of the survivors that were wild type in length was calculated as follows: percent wild type = 100 (number of wild-type length survivors)/(total number of survivors).

To determine the time between 3 and 10 hours of embryonic development that transcription of *dpy-30* is required to rescue the *dpy-30* XO-specific morphological defects, groups of 20 *yIs15; dpy-30(y228) him-5(e1490)* young non-Egl adult hermaphrodites from *dpy-30/+* parents were placed on fresh plates pre-cooled to 20°C, allowed to lay embryos for 1 hour, and transferred to fresh pre-cooled plates. Cohorts

collected this way were allowed to develop for defined periods of time and then were heat shocked as described above. Embryos synchronized by these brood collections vary in age by up to 3 hours. 3-5 days after brood collections, adult males were scored for rescue of the small (Sma), uncoordinated (Unc) and disrupted tail phenotypes caused by the *dpy-30* mutation. The time of the heat shock was defined as the time between the midpoint of the hour long brood collection and the midpoint of the 30 minute heat shock. Heat-shock times were subsequently rescaled to reflect the time relative to first cleavage based on the observation that *yIs15; dpy-30(y228) him-5(1490)* embryos are laid approximately 4 hours prior to comma stage. To induce *dpy-30* transcription prior to 3 hours of embryonic development, non-Egl gravid *yIs15; dpy-30(y228) him-5(e1490)* hermaphrodites were shifted to 30°C for 30 minutes and their progeny subsequently collected at defined times and scored as described above. For most time points in both sets of experiments, between 22 and 53 males were scored. The percent wild-type males was calculated according to the formula: Percent wild type = 100 (number of wild-type males in a cohort)/(total number of males). In the absence of heat shocks, approximately 17% of the XO animals produced by *yIs15; dpy-30(y228) him-5(e1490)* hermaphrodites resemble wild-type XO males.

RESULTS

Molecular cloning of the *dpy-30* dosage compensation gene

We cloned *dpy-30* using germline transformation rescue experiments in conjunction with *C. elegans* genetic and physical map data. Genetic analysis had placed *dpy-30* near the center of chromosome *V,* approximately 0.1 map units to the left of the previously cloned myosin heavy chain gene *myo-3* (Miller et al., 1986) and to the right of the actin gene cluster *act-1, 2, 3* (Krause et al., 1989), in a 600 kb region of the genome that is represented by a contiguous set of overlapping cosmid clones (Coulson et al., 1986; Hsu and Meyer, 1994) (Fig. 1A). Cosmids to the left of *myo-3* were assayed for the presence of *dpy-30*(+) by testing their ability to rescue the *dpy-30* XX-specific maternal-effect lethality in germline transformation experiments (Materials and Methods). These experiments identified a single cosmid, C09F3, that rescues the XX-specific lethality caused by the *dpy-30(y130)* allele (Fig. 1B). Additional transformation experiments assaying subclones and deletions of C09F3 further delimited the *dpy-30* rescuing region to a small, 921 bp interval contained within pDH38 (Fig. 1B,C).

The rescue obtained with all subclones of this region was qualitatively similar to that obtained using the entire C09F3 cosmid, suggesting that most, if not all, of the essential elements of the *dpy-30* gene are contained within the 921 bp region. Subsequently, we determined that pDH38 also rescues animals homozygous for the *dpy-30(y228)* null mutation. In addition, pDH38 rescues the uncoordinated (Unc), small (Sma) and disrupted tail phenotypes associated with *dpy-30* XO males (data not shown).

dpy-30 produces a single small transcript that is expressed throughout development

To determine the temporal pattern of *dpy-30* mRNA expression in XX animals, we performed northern hybridization experi-
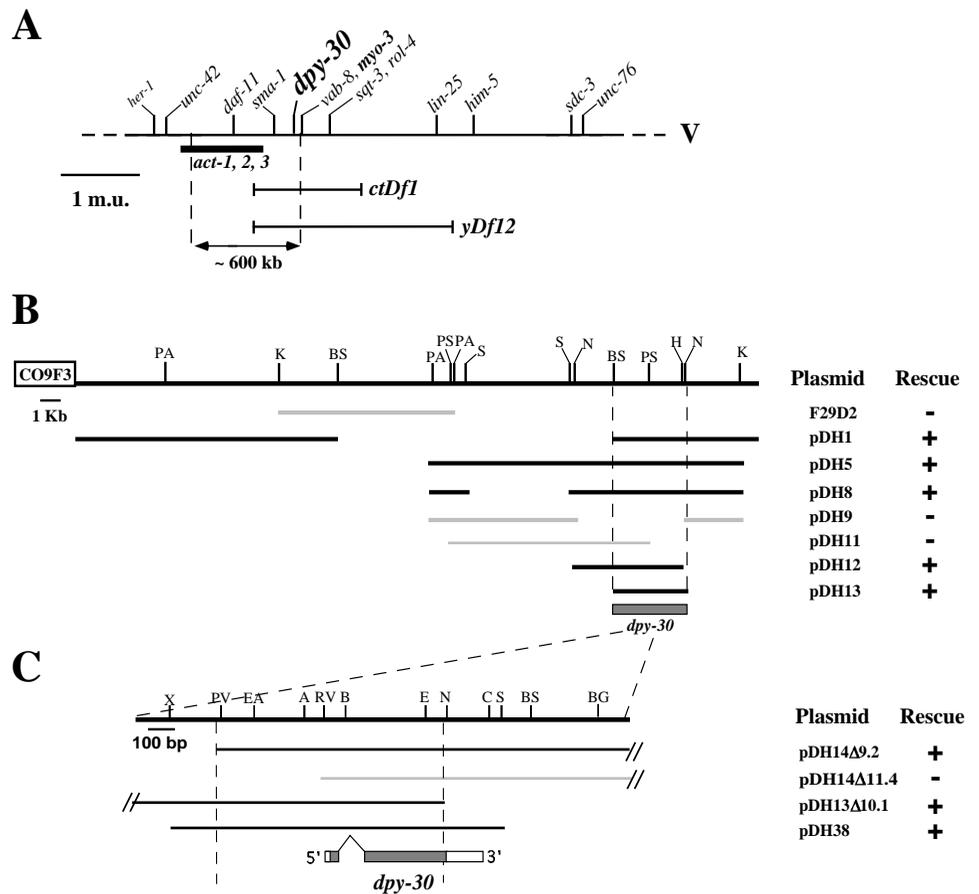

Fig. 1. Genetic and physical location of *dpy-30*. (A) Genetic map of the center of chromosome V. *dpy-30* lies in the 0.4 map unit interval between *sma-1* and *myo-3* (Hsu and Meyer, 1994). The flanking gene *myo-3* (bold lettering) and the *act-1, 2, 3* gene cluster (bar below map) define a region of approximately 600 kilobases that must contain *dpy-30*. Chromosomal deficiencies *yDf12* and *ctDf1* are drawn below the map. (B) Rescue of *dpy-30* mutant phenotypes with subclones of cosmid C09F3. The top line shows a partial restriction map of *dpy-30* rescuing cosmid C09F3. Horizontal lines under the restriction map show the extents of various subclones of this genomic region. The ability of each subclone to rescue *dpy-30* mutants is indicated by a plus and a black line (rescue) or a minus and a gray line (failure to rescue). The dashed vertical lines and the gray filled rectangle indicate a 4 kb region of genomic DNA encoding *dpy-30* rescuing activity. Enzymes shown are: *Pac*I (PA), *Kpn*I (K), *Bss*HII (BS), *Pst*I (PS), *Sal*I(S), *Nde*I (N), *Hpa*I (H). (C) Additional rescue experiments limit the *dpy-30* rescuing region to approximately 1 kb. pDH38, a 1.3 kb genomic clone, rescues all *dpy-30* phenotypes in XX and XO animals. The mature *dpy-30* transcript is shown; rectangles represent exons, lines represent introns and shaded regions indicate coding regions. Enzymes shown are: *Xba*I (X), *Pvu*I (PV), *Eag*I (EA), *Apa*LI (A), *Eco*RV (RV), *Bcl*I (B), *Eco*RI (E), *Nae*I (N), *Cla*I (C), *Sty*I (S), *Bsp*MI (BS), *Bgl*I (BG).

ments in which poly(A)$^+$ RNAs prepared from synchronized populations of wild-type embryos, the four larval stages and adults were probed with genomic DNA spanning the *dpy-30* locus. As shown in Fig. 2A, a single, relatively abundant 0.6 kb transcript that is expressed at approximately equal levels in all developmental stages was detected. This ubiquitous expression pattern is consistent with a role for *dpy-30* in the initiation and maintenance of the XX-mode of dosage compensation.

To address whether any sex-specific regulation of *dpy-30* is apparent at the transcript level, we also probed northern blots carrying either total RNA from purified populations of adult males or poly(A)$^+$ RNA prepared from a population of *him-8* embryos, consisting of approximately 37% XO embryos and 63% XX embryos (Hodgkin et al., 1979). The population of XX and XO embryos express the 0.6 kb *dpy-30* transcript at a similar level to that seen in XX embryos (Fig. 2A). XX and XO adults also express similar *dpy-30* transcript levels (Fig. 2B). The expression of *dpy-30* in animals of both karyotypes is consistent with genetic evidence demonstrating that *dpy-30* is required in both sexes (Hsu and Meyer, 1994). The absence of additional transcripts in XO animals indicates that the 0.6 kb *dpy-30* transcript is likely to encode a single protein that is required in both sexes.

The *dpy-30* gene encodes a small, novel protein

To determine the nucleotide sequence and splicing pattern of the *dpy-30* transcript, we analyzed three independent *dpy-30* cDNA clones and compared their DNA sequence to that of genomic DNA from the 4 kb rescuing clone pDH13 (Materials and methods). These partial, overlapping cDNA clones all correspond to the 0.6 kb transcript detected on northern blots. The 5′ end of the transcript was identified by virtue of one cDNA clone containing a short 12 nucleotide sequence 5′ CCCAAGTTTGAG 3′ that is absent from the genomic *dpy-30* DNA but is identical to part of the SL1 *trans*-spliced leader sequence found at the 5′ end of many *C. elegans* mRNAs (Blumenthal, 1995). To confirm that the mature *dpy-30* mRNA is *trans*-spliced, we performed RT-PCR using oligonucleotides for SL1 and a region internal to *dpy-30* near the 5′ end. This reaction produced a single product that corresponds to the size predicted by the existing *trans*-spliced cDNA clone. A similar experiment using an oligonucleotide for the SL2 *trans*-spliced leader and the *dpy-30* internal primer produced no product.

The mature *dpy-30* transcript consists of two small exons forming a single small open reading frame encoding a protein of 123 amino acids (Fig. 3). A BLAST (Altschul et al., 1990) comparison of the *dpy-30* protein sequence using the NCBI database revealed no significant similarities to known proteins or structural motifs, indicating that *dpy-30* encodes a novel protein. Because of the small size of this predicted protein, we explored the possibility that *dpy-30* might not encode a protein, but rather a regulatory or structural RNA that is required for the proper regulation of dosage compensation. To test this possibility, we engineered insertions of four nucleotides into the *dpy-30* coding region after codon 3 (at a *Bcl*I site) or codon 99 (at an *Eco*RI site) (Fig. 3) and assayed their ability to rescue *dpy-30* XX animals (see Materials and methods). These mutations, which change the reading frame and cause the new predicted proteins to be truncated after amino acids 19 or 103, prevent the altered genes from rescuing the *dpy-30* XX-specific
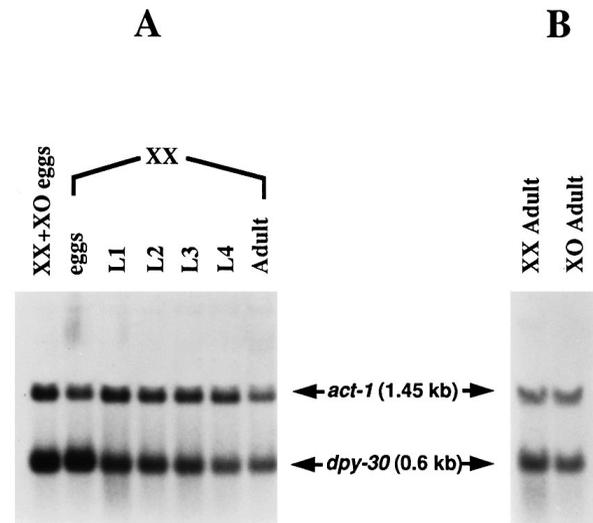

Fig. 2. Developmental and sex-specific regulation of *dpy-30* transcript levels. (A) Northern blot of poly(A)$^+$ RNAs (5 μg per lane) isolated from embryos, the four larval stages, or non-gravid adults. (B) Northern blot of total RNA prepared from wild-type, non-gravid adult XX hermaphrodites, or *him-5(e1490)* adult XO males. Both blots were hybridized with a 1.3 kb *dpy-30*-specific probe from pDH13 that detects a single 0.6 kb transcript and an *act-1*-specific probe that detects a 1.45 kb transcript.

lethality, suggesting that *dpy-30* does encode a protein. Additional evidence supporting this conclusion comes from the DNA sequence analysis of the two *dpy-30* alleles, which had been classified as complete or nearly complete loss-of-function mutations by genetic analysis (Hsu and Meyer, 1994). The *dpy-30(y130)* mutation is a G to A transition at nucleotide 1015, resulting in a Gly to Arg missense change at amino acid position 85 in the predicted protein (Fig. 3). The *dpy-30(y228)* mutation is a C to T transition at nucleotide 925 that creates an amber stop codon at amino acid position 55 (Fig. 3). This finding is consistent with the genetic analysis demonstrating that *y228* is partially suppressed by an amber tRNA suppressor. Molecular confirmation that *y228* is an amber allele that disrupts the coding capacity of the *dpy-30* gene provides strong evidence that *y228* is a null allele of *dpy-30*.

DPY-30 is a ubiquitous nuclear protein whose expression pattern is unaffected by mutations that disrupt dosage compensation

Activation of the hermaphrodite mode of dosage compensation requires the sex-specific localization of the DPY-27 protein to the X chromosomes in XX animals (Chuang et al., 1994). Immunofluorescence experiments have demonstrated that the DPY-30 protein is one of the components essential for the proper localization of DPY-27 to X (P.-T. Chuang and B. J. Meyer, unpublished). To gain insight into the role of DPY-30 in this activation step and to explore the temporal and spatial pattern of DPY-30 expression in wild-type XX and XO animals as well as XX animals harboring mutations that disrupt dosage compensation, we performed immunolocalization studies using polyclonal antibodies raised against DPY-30 and confocal microscopy to detect the in situ antibody staining pattern (Materials and methods). The distribution of DPY-30

was visualized by indirect immunofluorescence with fluorescein-labeled antibodies, and the morphology of interphase nuclei was visualized by staining with the nucleic acid-intercalating molecule propidium iodide (PI). Comparison of the DPY-30 staining pattern (Fig. 4A) with that of the PI pattern (Fig. 4B) reveals that the two patterns are identical in embryos of all ages (Fig. 4C), demonstrating that DPY-30 is localized to the nucleus, consistent with its role in the regulation of X-linked gene expression. In contrast to DPY-27, DPY-30 is not associated with the X chromosome, or with any of the autosomes (D. Albertson, personal communication). This diffuse nuclear staining pattern was also observed in embryos (Fig. 5A), hermaphrodite oocytes, larvae and adults (data not shown) using epifluorescence microscopy, a finding consistent with the ubiquitous expression of *dpy-30* mRNA and a possible requirement for *dpy-30* throughout development.

Two lines of evidence indicate that the observed staining pattern accurately reflects the normal distribution of DPY-30 in the embryo. First, preimmune serum does not stain wild-type embryos (data not shown). Second, DPY-30 staining is eliminated in embryos homozygous for the null mutation *dpy-30(y228)* (Fig. 5C,D). To rule out the possibility that the reduced staining in *y228* embryos is due to a general reduction in protein synthesis in these dying embryos, we also stained them with another antibody specific to the nuclear protein UNC-86 and obtained staining patterns similar to those of wild-type embryos (data not shown). These results indicate that the disappearance of DPY-30 antibody staining in homozygous *y228* embryos reflects a true reduction in the level of DPY-30 epitopes, possibly due to the instability of either the *dpy-30* mRNA or the truncated protein.

Since dosage compensation is a sex-specific process essential for the viability of only XX animals, we asked whether the pattern of DPY-30 expression differs in XX and XO animals, as does that of DPY-27. Previous genetic studies had demonstrated a role for *dpy-30* in XO animals and previous molecular studies indicated that the *dpy-30* transcript is expressed throughout development in both sexes, suggesting that DPY-30 expression is unlikely to be regulated in a sex-specific fashion. To identify XO embryos from a mixed population of XX and XO embryos, we took advantage of the sex-specific expression pattern of a *xol-1::lacZ* transgene, in which *lacZ* is under the control of the *xol-1* promoter. XO transgenic embryos carrying an integrated version of this transgene express *lacZ* at a much higher level than their XX siblings (Rhind et al., 1995). XX and XO embryos from a strain homozygous for both the *xol-1::lacZ* transgene and a *him-8* mutation, which increases X chromosome nondisjunction and thus the number of XO animals, were double

stained with an anti-β-galactosidase monoclonal antibody and the DPY-30 antibody. The DPY-30 staining pattern in embryos identified as XO by virtue of their intense staining with anti-β-galactosidase antibody is identical to that of equivalently staged XX embryos (Fig. 6A-C). Thus, at the level of protein expression and localization, DPY-30 does not appear to be sex-specifically regulated.

The lack of sex-specific regulation of DPY-30 suggested the possibility that DPY-30 is a constitutively expressed protein whose pattern of expression is not influenced by other genes involved in dosage compensation. To test this hypothesis, we examined the DPY-30 staining pattern in mutant embryos defective in one of the several genes involved in the dosage
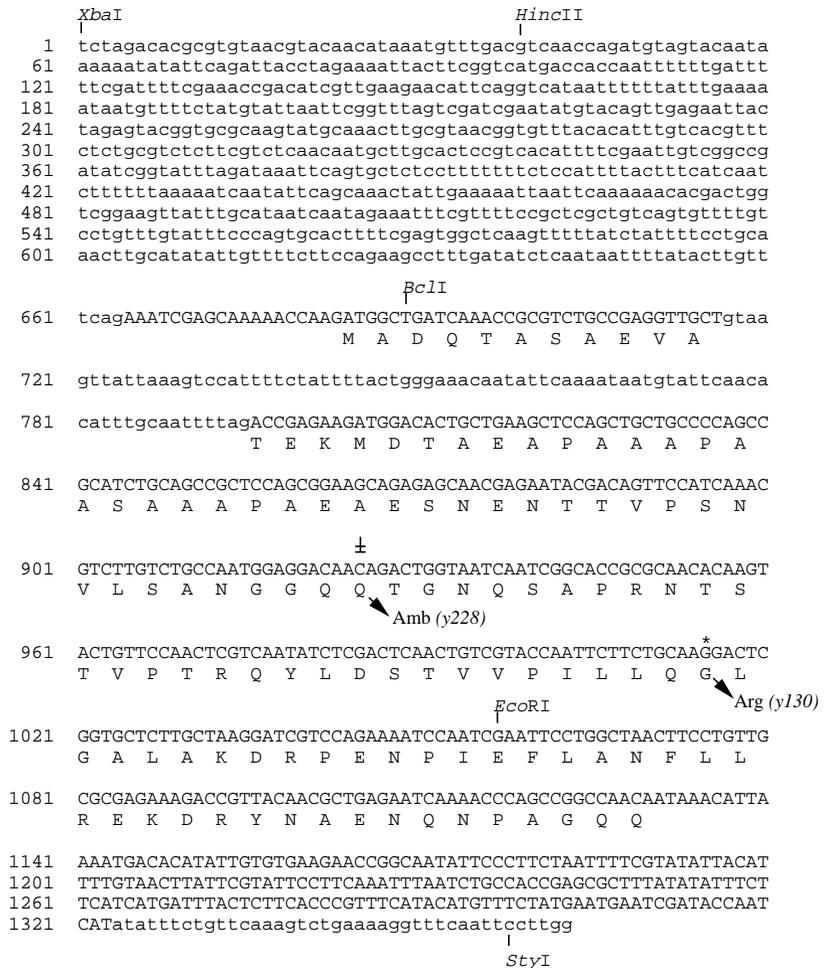
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       XbaI                                    HincII
       |                                       |
    1  tctagacacgcgtgtaacgtacaacataaatgtttgacgtcaaccagatgtagtacaata
   61  aaaaatatattcagattacctagaaaattacttcggtcatgaccaccaatttttgattt
  121  ttcgattttcgaaaccgacatcgttgaagaacattcaggtcataattttttatttgaaaa
  181  ataatgttttctatgtattaattcggtttagtcgatcgaatatgtacagttgagaattac
  241  tagagtacggtgcgcaagtatgcaaacttgcgtaacggtgtttacacatttgtcacgttt
  301  ctctgcgtctcttcgtctcaacaatgcttgcactccgtcacattttcgaattgtcggccg
  361  atatcggtatttagataaattcagtgctctcctttttttctccattttactttcatcaat
  421  cttttttaaaaatcaatattcagcaaactattgaaaaattaattcaaaaaacacgactgg
  481  tcggaagttatttgcataatcaatagaaatttcgttttccgctcgctgtcagtgtttttgt
  541  cctgtttgtatttcccagtgcacttttcgagtggctcaagttttttatctattttcctgca
  601  aacttgcatatattgtttttcttccagaagccttttgatatctcaataatttttatacttgtt

                             BclI
                             |
  661  tcagAAATCGAGCAAAAACCAAGATGGCTGATCAAACCGCGTCTGCCGAGGTTGCTgtaa
                   M  A  D  Q  T  A  S  A  E  V  A

  721  gttattaaagtccattttctattttactgggaaacaatattcaaaataatgtattcaaca

  781  catttgcaatttttagACCGAGAAGATGGACACTGCTGAAGCTCCAGCTGCTGCCCCAGCC
                     T  E  K  M  D  T  A  E  A  P  A  A  A  P  A

  841  GCATCTGCAGCCGCTCCAGCGGAAGCAGAGAGCAACGAGAATACGACAGTTCCATCAAAC
        A  S  A  A  A  P  A  E  A  E  S  N  E  N  T  T  V  P  S  N

                                  ±
  901  GTCTTGTCTGCCAATGGAGGACAACAGACTGGTAATCAATCGGCACCGCGCAACACAAGT
        V  L  S  A  N  G  G  Q  Q  T  G  N  Q  S  A  P  R  N  T  S
                                    Amb (y228)
                                             *
  961  ACTGTTCCAACTCGTCAATATCTCGACTCAACTGTCGTACCAATTCTTCTGCAAGGACTC
        T  V  P  T  R  Q  Y  L  D  S  T  V  V  P  I  L  L  Q  G  L
                                              Arg (y130)
                           EcoRI
                           |
 1021  GGTGCTCTTGCTAAGGATCGTCCAGAAAATCCAATCGAATTCCTGGCTAACTTCCTGTTG
        G  A  L  A  K  D  R  P  E  N  P  I  E  F  L  A  N  F  L  L

 1081  CGCGAGAAAGACCGTTACAACGCTGAGAATCAAAACCCAGCCGGCCAACAATAAACATTA
        R  E  K  D  R  Y  N  A  E  N  Q  N  P  A  G  Q  Q

 1141  AAATGACACATATTGTGTGAAGAACCGGCAATATTCCCTTCTAATTTTCGTATATTACAT
 1201  TTTGTAACTTATTCGTATTCCTTCAAATTTAATCTGCCACCGAGCGCTTTATATATTTCT
 1261  TCATCATGATTTACTCTTCACCCGTTTCATACATGTTTCTATGAATGAATCGATACCAAT
 1321  CATatatttctgttcaaagtctgaaaaggtttcaattccttgg
                                                |
                                               StyI
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Fig. 3. DNA sequence of *dpy-30*. Shown is the 1363 nucleotide genomic region of pDH38 that encodes *dpy-30*. The full-length *dpy-30* transcript, deduced from cDNA clones, is represented by DNA sequence listed in capital letters. The amino acid sequence of the predicted *dpy-30* translation product is listed below the DNA sequence. The ± symbol above nucleotide 925 and the * symbol above nucleotide 1015 mark the positions of the nucleotide changes associated with *dpy-30(y228)* and *dpy-30(y130)*, respectively. The *y228* lesion is a C to T transition that changes the glutamine codon (CAG) to an amber codon (TAG). The *y130* mutation is a G to A transition that changes the Gly codon (GGA) to an Arg codon (AGA). Insertion mutations at the designated *Bcl*I (nucleotide 689) and *Eco*RI (nucleotide 1057) restriction sites alter the wild-type *dpy-30* coding region after codons 3 and 99, respectively, and eliminate rescuing activity. The GenBank accession number for *dpy-30* is U21302.

compensation process. We found that XX embryos carrying null mutations in *sdc-1*, *sdc-2*, *sdc-3*, *dpy-21*, *dpy-26*, *dpy-27* or *dpy-28*, have a DPY-30 staining pattern that is indistinguishable from the pattern observed in wild-type embryos, indicating that DPY-30 expression does not depend on any of the known dosage compensation genes or master regulatory genes involved in the coordinate control of sex determination and dosage compensation (Fig. 5E-H and data not shown).

Transcription of *dpy-30* in the maternal germline or during gastrulation is sufficient to rescue both *dpy-30* XX and XO-specific phenotypes

To assess when transcription of *dpy-30* is essential to guarantee proper X chromosome expression, we constructed a heat-inducible *dpy-30* transgene that allowed us to supply wild-type *dpy-30* transcripts to mutant animals at different times in development. The heat-inducible transgene was created by fusing the promoter of the *C. elegans* small heat-shock protein *hsp16-41* (A. Fire, personal communication) to a wild-type copy of the *dpy-30* coding region. Animals carrying an integrated copy of the *hsp16-41::dpy-30* fusion (*yIs15*) were then used to determine the time during which *dpy-30* transcription could rescue the XX-specific lethality and the XO-specific morphological defects caused by *dpy-30* mutations. These experiments allowed us to determine that *dpy-30* transcription in the mother or in the embryo prior to the end of gastrulation is sufficient to fully rescue both the XX- and XO-specific defects caused by *dpy-30* mutations.

We determined the temporal requirements for *dpy-30* transcription to rescue the XX-specific lethality by applying heat shocks to synchronized populations of *yIs15; dpy-30(y228)* XX embryos at different times and scoring hermaphrodite viability (Materials and methods). Fig. 7 shows that there are two distinct periods when *dpy-30* transcription can rescue the XX-specific lethality. Half hour heat shocks applied between 3 and 10 hours prior to first cleavage, or between 0.5 and 4 hours after first cleavage rescue greater than 80% of *dpy-30* XX animals. In contrast, heat shocks applied at 15.5 hours prior to first cleavage or between 6 hours of development and hatching fail to produce substantial numbers of viable hermaphrodites. Curiously, Fig. 7 also shows that heat shocks centered around the time of fertilization (−0.5 hours) fail to rescue. Since heat shocks immediately prior to or subsequent to fertilization prevent lethality, it seems unlikely that the failure of heat shocks during fertilization to overcome lethality reflects a time when *dpy-30* transcription cannot rescue XX *dpy-30* animals. Moreover, the heat-shock treatment itself cannot be responsible for the lack of viability (Rhind et al., 1995). The most plausible explanation is that this failure to rescue reflects a period in early embryonic development when the heat-shock promoter is not sufficiently active to generate the required level of *dpy-30* transcripts.

The heat-shock experiments with *yIs15; dpy-30(y228)* XX animals reveal a strong correlation between rescue of the XX-specific lethality and rescue of the XX-specific morphological defects (Dpy, Egl and Pvul) caused by the dosage compensation defect (Fig. 7). Cohorts with the highest viability also contained the highest proportion of wild-type hermaphrodites. In contrast, when rescue of viability was low, XX survivors appeared variably Dpy, Egl and Pvul. This close parallel between the rescue of viability and morphology is consistent with studies of other *C. elegans* dosage compensation mutants,

suggesting that the penetrance and severity of the Dpy, Egl and Pvul defects observed reflects the severity of their dosage compensation defect (Plenefisch et al., 1989). These heat-shock studies show that single heat shocks applied during gastrulation result in the production of completely wild-type hermaphrodites. We find no evidence for additional temporal requirements for heat-shock-induced *dpy-30* transcription.

We also determined the temporal requirement for *dpy-30* transcription to rescue the *dpy-30* XO-specific morphological defects by heat shocking *yIs15; dpy-30(y228) him-5* animals. The *him-5(e1490)* mutation causes X chromosome nondisjunction, resulting in the production of approximately 30% XO male progeny. Males produced in these experiments were scored for rescue of uncoordinated movement, small body size and malformed tail structures associated with *dpy-30* XO males. Fig. 7 shows that heat shocks applied to the mother during germline development or to the embryo during 2 to 6 hours of development is sufficient to rescue the *dpy-30* XO-specific morphological defects. As was the case for rescue of XX-specific lethality, little or no rescue of the XO defects is apparent in the hours centered around fertilization.

dpy-30 is probably cotranscribed with *rnp-1*, a gene encoding an unusual RNA-binding protein

The *C. elegans* genome contains many gene clusters that are cotranscribed, as in bacterial operons. The individual message for each gene is subsequently formed by polyadenylation and *trans*-splicing (Blumenthal, 1995). Genes that comprise these polycistronic transcription units share two major characteristics: they are in close proximity, typically separated by a distance of approximately 100 bp, and they have an unusual *trans*-splicing pattern. The upstream gene is generally *trans*-spliced to the 22 nucleotide SL1 *trans*-spliced leader sequence. The downstream gene is *trans*-spliced to the similar, but distinct, SL2 leader sequence. While the functional significance of these polycistronic transcription units is unknown, their existence suggests the possibility that the genes in these *C. elegans* operons could be functionally related, as they are in prokaryotes.

While cloning the *dpy-30* locus, we identified a new gene, *rnp-1,* whose proximity to *dpy-30* and *trans*-splicing pattern suggested that it might be cotranscribed with *dpy-30*. Comparison of the DNA sequence from genomic DNA and a *rnp-1* cDNA revealed that the *rnp-1* transcript starts within 100 bp downstream of the *dpy-30* polyadenylation site (Fig. 8A). Furthermore, an SL2 *trans*-spliced leader sequence is present at the 5′ end of *rnp-1,* as determined by the isolation of *rnp-1* cDNA clones using RT-PCR with an oligonucleotide complementary to SL2 and an oligonucleotide internal to *rnp-1* (data not shown). No product was obtained when an oligonucleotide complementary to SL1 was used in similar experiments. These observations, together with the fact that *dpy-30* is an SL1-accepting gene fulfill the criteria for genes known to be transcribed in *C. elegans* operons, suggesting that *dpy-30* and *rnp-1* may also be cotranscribed.

DNA sequence analysis revealed that the mature *rnp-1* transcript encodes a 305 amino acid protein with two distinct nucleic acid-binding domains. The first domain, encompassing approximately the first 80 amino acids of RNP-1, has significant similarity to the consensus RNA recognition motif (RRM) defined by a large and diverse family of proteins that bind pre-mRNA, mRNA, snRNA and pre-rRNA molecules (for review,
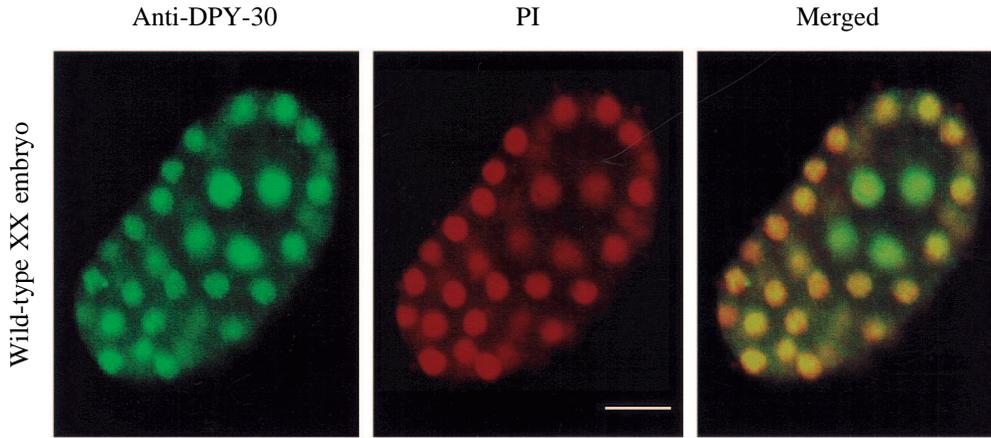
Anti-DPY-30   PI   Merged

Wild-type XX embryo

Fig. 4. Nuclear localization of DPY-30 in XX embryos. (A-C) Confocal images of a wild-type XX embryo stained with (A) anti-DPY-30 antibody (false green color) and (B) counterstained with the nucleic acid-intercalating dye propidium iodide (PI) (false red color). The merged image (C) (false yellow color), which was created by superimposing the images of A and B, shows that DPY-30 is diffusely distributed throughout the entire nucleus of each cell in this embryo. Scale bar, 10 μm.

Anti-DPY-30   DAPI

Wild-type XX

dpy-30(y228) XX

sdc-2(y202) XX

dpy-27(y167) XX
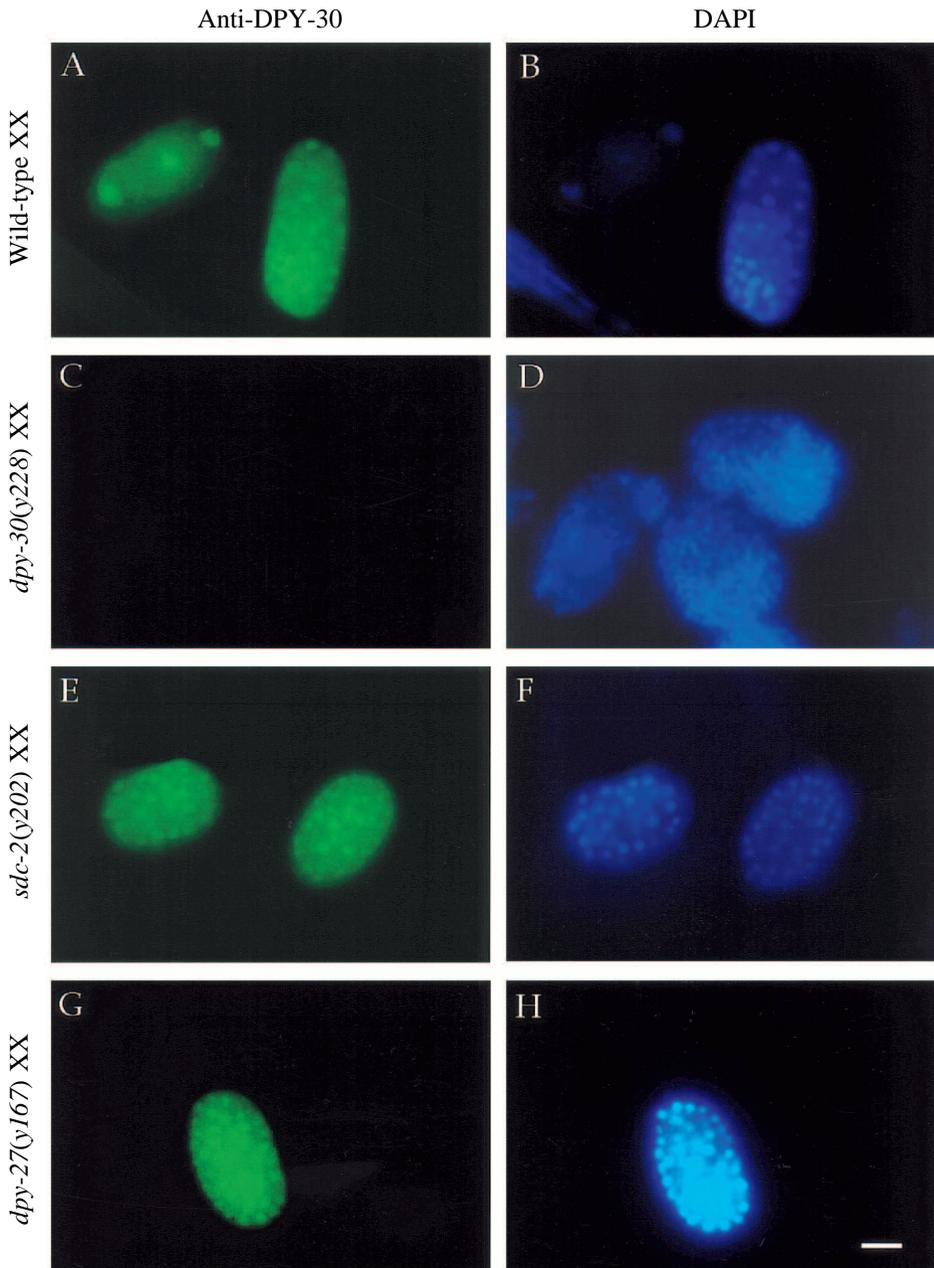
Fig. 5. Expression of DPY-30 in wild-type and dosage compensation defective embryos. Fluorescent photomicrographs of XX embryos stained with anti-DPY-30 antibody (A,C,E,G) and costained with DAPI to visualize nuclei (B,D,F,H). The wild-type nuclear staining pattern of DPY-30 is not perturbed by mutations that disrupt dosage compensation. Scale bar, 10 μm.

Fig. 6. Nuclear localization of DPY-30 in XO embryos. (A-C) Fluorescent photomicrographs of XX and XO *him-8 yIS2(xol-1::lacZ)* embryos triple stained with anti-DPY-30 antibody (A), anti-β-galactosidase antibody (B) and the nuclear stain DAPI (C) show that DPY-30 is localized to the nuclei of XO embryos. The *xol-1::lacZ* transgene is expressed exclusively in XO embryos and β-galactosidase protein is restricted to the nucleus by a nuclear localization signal. The embryo to the left in A was identified as an XO embryo by virtue of its staining with anti-β-galactosidase antibody (B, left side embryo). In contrast, the embryo to the right in A fails to stain with anti-β-galactosidase antibody (B, right side embryo), demonstrating that it is an XX embryo. Scale bar, 10 μm.

see Bandziulis et al., 1989) (Fig. 8B,C). This motif, found in single or multiple copies, is thought to be essential for the RNA-binding activities of these proteins. The alignment of the RNP-1 sequence with the RRM consensus shows that the RRM of RNP-1 has most of the conserved amino acids within this domain. Of particular importance is the fact that RNP-1 is nearly identical to the RRM consensus in two subdomains (RNP 1 and RNP 2) that are the most highly conserved portions of this RNA-binding motif (Fig. 8C).

Following the RRM, the *rnp-1* gene contains a second domain encoding a single zinc finger motif extending from amino acid positions 84 to 97. Using the BLAST algorithm, we compared this finger motif to other known zinc finger proteins in the GenBank database and found that it is most closely related to the nucleic acid-binding protein (NBP) class of zinc finger motifs defined by the consensus $X_2CX_2CGX_2GHX_2CX$ (Berg, 1986) (Fig. 8D). The NBP family of zinc fingers is derived from the *gag* genes of retroviruses, which contain one or two copies of this motif, and define a group of low molecular weight retroviral proteins capable of binding RNA or single-stranded DNA (Covey, 1986). In addition, seven copies of a similar motif are present in a cellular nucleic acid-binding protein (CNBP), which binds a sterol regulatory element (SRE) in a sequence and single strand-specific manner (Rajavashisth et al., 1989).

The presence of both the RRM and NBP-type zinc finger motif provide a strong indication that *rnp-1* acts as an RNA-binding molecule. Among RNA-binding molecules, RNP-1 is unusual in juxtaposing these two distinct RNA-binding motifs in a single protein. A BLAST search of both the PIR and GenBank databases revealed no other proteins with both RNA-binding domains. Thus, *rnp-1* appears to define a novel class of RNA-binding proteins.

DISCUSSION

Multiple roles of DPY-30 in development

C. elegans has adapted an evolutionarily conserved chromosome condensation mechanism to achieve dosage compensation
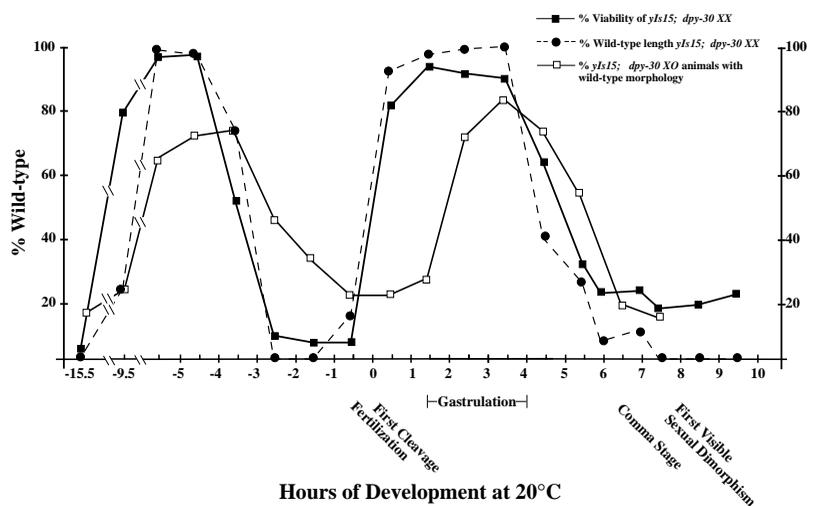

Fig. 7. Temporal requirements for *dpy-30* transcription. Shown are temperature-shift experiments demonstrating the time during which induction of *dpy-30* transcription can suppress the XX-specific lethality and XO-specific morphological defects of *dpy-30* mutants. The requirement for *dpy-30* transcription in XX animals (—■—) was determined by assessing the viability of synchronous populations of *yIs15[hsp16-41::dpy-30, rol-6(su1006)]; dpy-30(y228)* XX transgenic animals subjected to 30 minute heat-shock treatments at various times prior to fertilization or during embryonic development. Survivors of the XX-specific lethality were also scored for the severity of the Dpy, Egl and Pvul phenotypes characteristic of *dpy-30* escapers. The percent of survivors with a wild-type body length is shown (--●--). The temporal requirements for *dpy-30* transcription to alleviate the XO-specific morphological defects (—□—) were determined by scoring synchronous populations of *yIs15; dpy-30(y228) him-5(e1490)* males for rescue of their small body size, uncoordinated movement and abnormal tail structures after heat-shock treatments. These experiments demonstrate that *dpy-30* transcription either in the mother or in the embryo prior to the end of gastrulation is sufficient to alleviate the XX and XO-specific defects. Because different staging methods were used to synchronize the embryos in each experiment, it is difficult to know if the differences observed are significant.

(Chuang et al., 1994). It uses the X-chromosome-specific DPY-27 chromosome condensation protein to reduce expression of the hermaphrodite X chromosomes (Chuang et al., 1994). *dpy-30* plays a critical role in this process, since it is essential for the sex-specific localization of DPY-27 to the hermaphrodite X chromosomes (P.-T. Chuang and B. J. Meyer, unpublished). However, *dpy-30* is unique among dosage compensation genes in that it plays a more general role in *C. elegans* development than solely regulating X chromosome expression in hermaphrodites. Previous phenotypic analysis of *dpy-30* mutants exposed two major differences between *dpy-30* and the other maternal-effect dosage compensation genes, *dpy-26*, *dpy-27* and *dpy-28*. First, null mutations in the other genes kill most, but never all XX animals, suggesting that the absence of dosage compensation is not completely lethal (Plenefisch, et al. 1989). In contrast, null mutations in *dpy-30* cause complete XX-specific lethality, suggesting that other developmental processes are aberrant in animals lacking *dpy-30* activity (Hsu and Meyer, 1994). Second, while the other dosage compensation genes are dispensable in XO animals, *dpy-30* is required for the wild-type development of XO males. *dpy-30* XO mutants have wild-type X chromosome expression, but have numerous abnormalities including uncoordinated movement, reduced mating efficiency as well as abnormal tail and body morphology (Hsu and Meyer 1994). These pleiotropic phenotypes highlight the other developmental processes that require *dpy-30* activity. The potential involvement of DPY-30 in the morphology and behavior of XX animals is obscured by the severity of the phenotypes caused by the dosage compensation defects.

The molecular results described in this paper further distinguish *dpy-30* from other dosage compensation genes and are consistent with an involvement of DPY-30 in multiple processes. *dpy-30* encodes a novel protein that is ubiquitously expressed in the somatic and germline nuclei of both XX and XO animals throughout development. DPY-30 is not associated with the X chromosomes nor with any autosomes, indicating that DPY-30's role in dosage compensation is fundamentally different from that of DPY-27. Unlike DPY-27, expression of DPY-30 is not affected by mutations in the other dosage compensation genes or in the master regulatory genes that control both sex determination and dosage compensation. However, we have not yet ruled out the possibility that DPY-30 is subject to sex-specific post-translational modification that is important for its role in dosage compensation. The lack of sex specificity in expression and the lack of obvious regulation by other dosage compensation genes suggests that DPY-30 participates in the localization of DPY-27 to the X chromosome by affecting the activity of another gene more directly

involved in X chromosome binding. Likely candidates for such genes are *sdc-2* and *sdc-3*, which are also required for proper localization of DPY-27 to the X chromosome (P.-T. Chuang and B. J. Meyer, unpublished). We have further shown that rescue of both the XX-specific lethality and the XO-specific morphological defects have similar, if not identical, temporal requirements for *dpy-30* transcription, suggesting that *dpy-30* mediates its diverse roles in XX and XO animals by acting at a similar time in both sexes. DPY-30 might affect multiple functions in males and hermaphrodites through its involvement in a general process such as transcription, or instead through its effects on a small number of genes having multiple roles in development. *dpy-30* is not only unusual among the *C. elegans* dosage compensation genes, it is also different from the *Drosophila* dosage compensation genes (*msls*) that are required to elevate expression of the male X chromosome. All *msl* genes bind the hyperactivated male X and appear to play a direct role in elevating transcription (Baker et al., 1994).
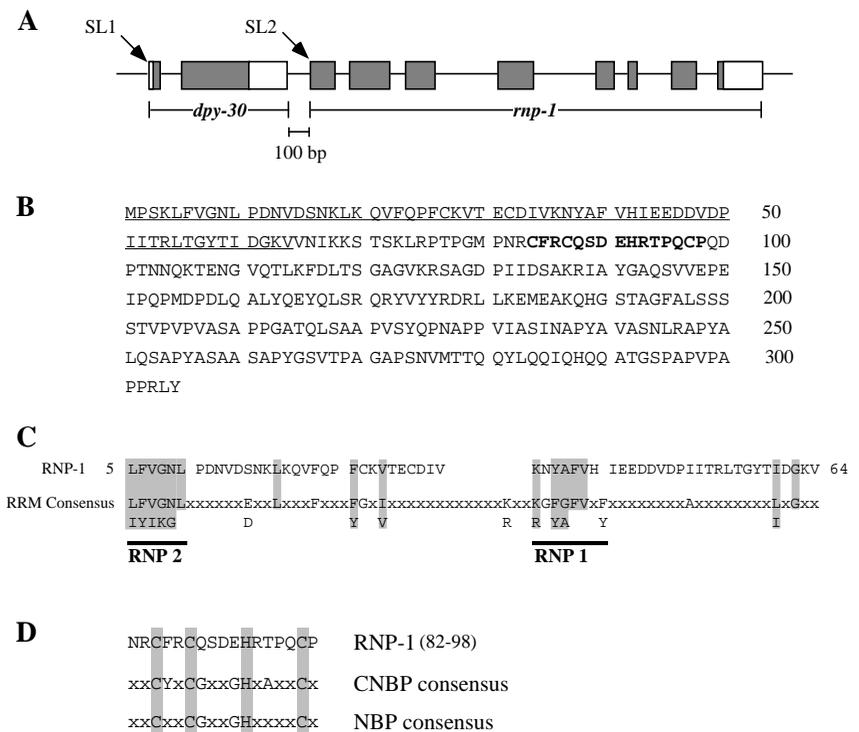

Fig. 8. Proposed *dpy-30* operon. (A) Genomic structure and splicing pattern of *dpy-30* and *rnp-1*. Analysis of both genomic and cDNA clones for *dpy-30* and *rnp-1* revealed that the 5′ end of the *rnp-1* transcript lies within 100 base pairs of the 3′ end of the *dpy-30* transcript. *dpy-30* is *trans*-spliced to the SL1 leader and *rnp-1* is *trans*-spliced to the SL2 leader. Rectangles represent exons in the mature transcript, lines represent introns and shaded regions indicate coding regions within each transcript. The extent of each gene is indicated underneath the genomic structure. (B) Predicted 305 amino acid translation product of the *rnp-1* gene, with the RNA recognition motif (RRM) underlined and the putative zinc finger domain indicated by bold lettering. The GenBank accession number for *rnp-1* is U21302. (C) Amino acid sequence alignment of the *rnp-1* RRM and the RRM consensus (Bandziulis et al., 1989), with the shaded boxes identifying conserved regions. *rnp-1* shares the greatest similarity to the consensus in the two most highly conserved subdomains of the RRM, RNP 1 and RNP 2, which are underlined. (D) Amino acid sequence alignment of the *rnp-1* zinc finger with consensus sequences for the CNBP and NBP families of zinc-fingers. Shaded rectangles indicate the conserved cysteine and histidine residues in these families. Numbers to the right of the RNP-1 sequence indicate the amino acid positions in the protein.

Timing of dosage compensation

Temperature-shift experiments revealed that *dpy-30* transcription is only required early, either maternally prior to fertilization or during the first few hours of embryogenesis, to completely rescue *dpy-30* mutants. These findings indicate that *dpy-30* has an essential role in initiation of the XX mode of dosage compensation. Other lines of evidence are consistent with a role for *dpy-30* in the activation of dosage compensation and further demonstrate that dosage compensation must be functional early in embryogenesis to ensure the viability of XX animals. The DPY-30-dependent localization of DPY-27 to the hermaphrodite X chromosomes, a key step in the activation of dosage compensation, occurs around the 30-cell stage (Chuang et al., 1994), the time when *dpy-30* transcription is essential. In addition, temperature-shift experiments using a heat-sensitive allele of the *dpy-28* dosage compensation gene established a requirement for *dpy-28* around mid-embryogenesis (following gastrulation) for the viability of XX animals (Plenefisch et al., 1989). Finally, transcription of *xol-1*, a master switch gene whose activity state determines whether an embryo develops into a male or hermaphrodite, is required during gastrulation (2-4 hours following fertilization) to set the XO mode of dosage compensation (Rhind et al., 1995). Transcription of *xol-1* in XX animals at the same early time causes nearly complete lethality because the hermaphrodite mode of dosage compensation cannot be activated. These results indicate that dosage compensation is functional soon after activation of zygotic transcription, which is thought to occur between the 8- and 16-cell stages (Edgar et al., 1994).

A question that remains unanswered by current studies is whether dosage compensation is required at any point after embryogenesis for wild-type development. Our studies indicate that *dpy-30* mRNA and protein are present throughout the lifetime of XX animals and raise the issue of whether *dpy-30* plays a role in dosage compensation throughout development. Biochemical studies have demonstrated that X-linked transcript levels are equalized between adult XO and XX animals, indicating that dosage compensation is active late in development (Meyer and Casson, 1986). Moreover, the sex-specific localization of DPY-27 to X persists throughout the lifetime of hermaphrodites (Chuang et al., 1994). This localization may require the continuous participation of DPY-30, since XX larvae and adults that lack wild-type DPY-30 fail to localize DPY-27 to X (P.-T. Chuang and B. J. Meyer, unpublished). Our results indicate that embryogenesis is a critical stage during which dosage compensation is essential for viability and raise the possibility that while dosage compensation occurs during later stages of development, it may not be essential for health and viability.

A *dpy-30* operon

The *dpy-30* and *rnp-1* genes have all the properties common to *C. elegans* genes that are cotranscribed in an operon (for review see Blumenthal, 1995). The *rnp-1* locus encodes a putative RNA-binding protein that is unusual in having both an RNA recognition motif (RRM) common to many RNA-binding proteins, as well as a single zinc finger motif similar to those from the nucleic acid-binding proteins (NBPs) of retroviruses. While the relationship of *rnp-1* to the dosage compensation process is unknown, the fact that it appears to be encoded in an operon with *dpy-30* presents the possibility that the two genes are functionally related and that dosage compensation may require aspects of RNA metabolism. Alternatively, evolutionary selection pressure on *C. elegans* might have caused a compaction of the genome, resulting in the clustering and co-regulation of genes that are related simply by their need for similar levels of expression in similar tissues (see Blumenthal, 1995).

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