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The Polycomb group in *Caenorhabditis elegans* and maternal control of germline development

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SUMMARY

Four *Caenorhabditis elegans* genes, *mes-2*, *mes-3*, *mes-4* and *mes-6*, are essential for normal proliferation and viability of the germline. Mutations in these genes cause a maternal-effect sterile (i.e. mes) or grandchildless phenotype. We report that the *mes-6* gene is in an unusual operon, the second example of this type of operon in *C. elegans*, and encodes the nematode homolog of Extra sex combs, a WD-40 protein in the Polycomb group in *Drosophila*. *mes-2* encodes another Polycomb group protein (see paper by Holdeman, R., Nehrt, S. and Strome, S. (1998). *Development* 125, 2457-2467). Consistent with the known role of Polycomb group proteins in regulating gene expression, MES-6 is a nuclear protein. It is enriched in the germline of larvae and adults and is present in all nuclei of early embryos. Molecular epistasis results predict that the MES proteins, like Polycomb group proteins in *Drosophila*, function as a complex to regulate gene expression. Database searches reveal that there are considerably fewer Polycomb group genes in *C. elegans* than in *Drosophila* or vertebrates, and our studies suggest that their primary function is in controlling gene expression in the germline and ensuring the survival and proliferation of that tissue.

Key words: *Caenorhabditis elegans*, *mes* genes, Polycomb group

INTRODUCTION

Germ cells are crucial for the propagation of species and are unique in their apparent totipotency and immortality: germs cells that unite at fertilization produce all of the somatic cells in an organism, as well as more germline cells for future generations. The molecular mechanisms that distinguish the germline from the soma remain, largely, unknown. To identify early acting products required uniquely for germline development in *Caenorhabditis elegans*, we screened for maternal-effect sterile (*mes*) mutations that result in sterile but otherwise healthy offspring. Our screens identified four genes, *mes-2*, *mes-3*, *mes-4* and *mes-6*, whose maternal contribution is both necessary and sufficient for normal postembryonic germline development (Capowski et al., 1991). Mutations in these *mes* genes result in essentially identical phenotypes: embryogenesis appears normal but, during hermaphrodite larval development, germ-cell proliferation is reduced, germ cells degenerate and gametes do not form (Capowski et al., 1991; Paulsen et al., 1995; Garvin et al., 1998).

To better understand the role of the *mes* genes in germline development, we have analyzed *mes-2*, *mes-3* and *mes-6* at a molecular level. *mes-3* encodes a novel protein (Paulsen et al., 1995) and thus did not provide any immediate insights into MES protein function. Analysis of *mes-2* provided significant clues (Holdeman et al., 1998) – *mes-2* encodes the worm homolog of Enhancer of zeste [E(z)], a member of the Polycomb group (Pc-G) of transcriptional regulators in *Drosophila* and vertebrates. In this paper, we present analysis of *mes-6*, which encodes the worm homolog of Extra sex combs (Esc), another member of the Polycomb group (Pc-G). Our finding that at least two of the *mes* genes (*mes-4* has not been cloned) encode Pc-G proteins predicts that the essential role of the MES proteins in the germline is to regulate gene expression by a mechanism similar to that used by the Pc-G of proteins. Pc-G proteins are required to maintain homeotic genes in a repressed state and are thought to act as multimeric complexes that locally alter chromatin structure (see Discussion). Consistent with a similar mechanism of action of the MES proteins, MES-6 and MES-2 are localized in nuclei and depend upon each other for proper nuclear localization (this paper and Holdeman et al., 1998), and the *mes* genes participate in germline-specific silencing of genes in extrachromosomal arrays (Kelly and Fire, 1998). Eight Pc-G genes have been cloned and sequenced from *Drosophila*, and there are mammalian homologs of all eight. In contrast, *mes-6* and *mes-2* are the only recognizable homologs of Pc-G genes in the ~90% of the *C. elegans* genome sequenced to date. This finding has important implications for thinking about the functions of Pc-G/MES protein complexes in insects, vertebrates and worms, and about the evolution of Pc-G genes.

MATERIALS AND METHODS

Alleles and strain maintenance

Worms were maintained and manipulated as described by Brenner (1974). All mutant strains were derived from wild-type N2 variety Bristol. The following mutations, deficiencies and balancers were used.
LGI: *mes-3(bn35, bn86), glp-4(bn2ts)*
LGII: *mes-2(bn11, bn27, bn48, bn72, bn76)*
LGIV: *mes-6(bn38, bn64, bn66, bn69), unc-24(e138), dpy-20(e1282ts), eDf18 DnT1[unc(n754) let] (IV,V)*
LGV: *mes-4(bn58, bn67), dpy-11(e224), DnT1[unc(n754) let] (IV,V)*

Germ cell counts

The developmental stages of larvae were determined by examining the ventral hypodermal cells and the vulval cells with Nomarski DIC optics (Sulston and Horvitz, 1977). The number of germ nuclei present in larvae and adults was determined by counting DAPI-stained germ nuclei as described in Capowski et al. (1991).

Transformation rescue

YAC DNA was isolated using a variation of Paulsen et al. (1995). Instead of running low-melt agarose gels with TBE buffer, genetic technology grade agarose (ICN Biomedicals) was used with TAE buffer. DNA was isolated from gel slices with a modification of the protocol from the GeneClean kit (Bio101). Gel slices were incubated for 30 minutes in NaI with 20 µl glassmilk and eluted in 100 µl ddH20. Two GeneClean extractions were combined for each YAC preparation and this was further concentrated by an additional GeneClean using the standard protocol. Cosmid DNA was prepared using a standard alkaline lysis method (Sambrook et al., 1989).

Transgenic lines were created by germline transformation using the technique described by Mello et al. (1991). Clones and restriction fragments containing DNA of interest were coinjected with 100 µg/ml pRF4, a plasmid containing the *rol-6* marker. YACs were injected at approximately 100 µg/ml, while cosmids and restriction fragments were injected at 10-20 µg/ml. Transformants were generated by injecting *mes-6(bn66)/unc-24 dpy-20*. Rescue of the Mes-6 sterile phenotype was assayed by looking for fertile worms among the progeny of homozygous *mes-6* mothers. Rescue was achieved with the YAC Y59H11, the cosmid T12D3, and various restriction fragments of T12D3 (a mixture of two overlapping fragments [17 kb *Pst*I and 7.4 kb *Eco*RV], the 12 kb *Bgl*II fragment, and the 6.5 kb *Xba*I fragment).

cDNA cloning and RNAi analysis

Two overlapping restriction fragments that rescue *mes-6* (17 kb *Pst*I and 7.4 kb *Eco*RV fragments of cosmid T12D3) were used to screen a λZAP mixed-stage *C. elegans* cDNA library (Barstead and Waterston, 1989). We recovered two classes of cDNAs, which correspond to the 2.1 and the 0.9 kb transcripts (see Fig. 2). Each class of cDNA hybridizes to an overlapping subset of the six transcripts detected by the 6.5 kb *Xba*I probe: cDNAs to the 2.1 kb transcript hybridize to the 2.9, 2.1, 1.6, 1.3 and 0.65 kb transcripts, and cDNAs to the 0.9 kb transcript hybridize to the 2.9, 1.3 and 0.9 kb transcripts on northern blots. RNA-mediated interference with gene function (RNAi) was done as described by Holdeman et al. (1998), using a 0.5 kb RNA generated from the 5′ end of the 2.1 kb cDNA and full-length RNA generated from the 0.9 kb cDNA.

Sequence analysis

All sequencing was performed using a Li-Cor 400L automated fluorescent sequencer. DNA was prepared using the Qiagen Plasmid Mini Kit. Sequencing reactions were prepared with the Sequitherm Long Read kit (Epicenter). The sequence of *mes-6* was determined from overlapping subclones of pIK8, a full-length cDNA, using T3 and T7 primers in a Bluescript (Strategene) vector. Genomic sequence was obtained in much the same manner but from subclones of the rescuing 6.5 kb *Xba*I fragment of T12D3. In addition, the *C. elegans* genome sequencing consortium sequenced cosmids in the region. Mutant alleles of *mes-6* were sequenced using the methods described in Holdeman et al. (1998). Four sets of primers were used for *mes-6*: IK5 and IK6 (5′ACGACACATATGAAGCTG and 5′GTCGGGAATGTTGCGAG), IK7 and IK8 (5′AAGATATTCCGTATGTTTCG and 5′GAACCACCAGCTCCACCG), IK9 and IK10 (5′GGAGATTTGGGCCACC and 5′AAGTTGCTGTCGACTGTC) and IK11 and IK12 (5′ATCATTCCTTCTCACCTTC and 5′GTGACATGAACGTGACGG). These covered all but the 5′ most 85 amino acids of MES-6. The GenBank accession number for the *mes-6* operon is AF016224.

Northern hybridization analysis

Northern hybridization analysis was carried out as in Holdeman et al. (1998). The 6.5 kb rescuing fragment was used as a probe against poly(A)+ RNA extracted from sychronous populations of wild-type worms (Conrad et al., 1991), and against adult worms that essentially lack a germline (*glp-4[bn2ts]* hermaphrodites raised at the restrictive temperature; Beanan and Strome, 1992). The transcript from the *C. elegans* ribosomal protein gene, *rp21* (recently named *rpp-1*; Evans et al., 1997) was used as a loading control (Paulsen et al., 1995). Transcript levels of the 2.9 kb, 2.1 kb, 0.9 kb and 0.65 kb transcripts from the *mes-6* region were determined using a Molecular Dynamics PhosphorImager. The 1.6 and 1.3 kb transcripts were not quantified because they are relatively rare and consequently the signal-to-noise ratio was problematic.

Antibody production and immunostaining

Amino acids 1-450 of MES-6 were fused to six histidines in the pET28a vector (Novagen). Procedures described in Holdeman et al. (1998) were used for expression of the fusion protein in *E. coli*, purification using nickel columns, injection into rabbits, blot affinity purification of anti-MES-6 antibodies and immunostaining of western blots and of fixed worms and embryos. In *mes* mutants that fail to accumulate MES-6 protein in nuclei, it is difficult to judge by immunofluorescence whether MES-6 is absent, or is present but dispersed in the cytoplasm. Thus, failure of nuclear accumulation may reflect defects either in MES protein stability or in transport and retention in nuclei.

Bioinformatics

Cosmid and YAC positions were obtained from the *C. elegans* genome consortium (Wilson et al. 1994) and viewed in ACeDB (see Waterston et al., 1997). Overlapping sequences were assembled using the lineup program from the Genetics Computer Group software package (GCG, Madison, Wisconsin). DNA Strider was used for routine sequence analysis (viewing restriction sites, displaying open reading frames, translating nucleotide sequence, etc.). Database searches were performed using BLAST (Altschul et al., 1990) and WU-BLAST (Warren Gish, personal communication, http://blast.wustl.edu). Protein sequences were aligned with SeqPup (D. Gilbert, personal communication, ftp://iubio.bio.indiana.edu/molbio/seqpup). NCBI entrez was used for sequence retrieval (http://www.ncbi.nlm.nih.gov).

RESULTS

The primary defect in *mes-6* mutants is diminished postembryonic germline proliferation

Capowski et al. (1991) showed that hermaphrodite offspring of *mes-2*, *mes-3* and *mes-4* mothers are defective in germ cell proliferation. To determine if this is also the case in *mes-6* mutants, offspring of *mes-6* mothers were synchronized at the L1 stage, and their germ nuclei were counted at progressively later stages of larval and adult development. Like *mes-2*, *mes-3* and *mes-4* mutants, *mes-6* mutants have underproliferated

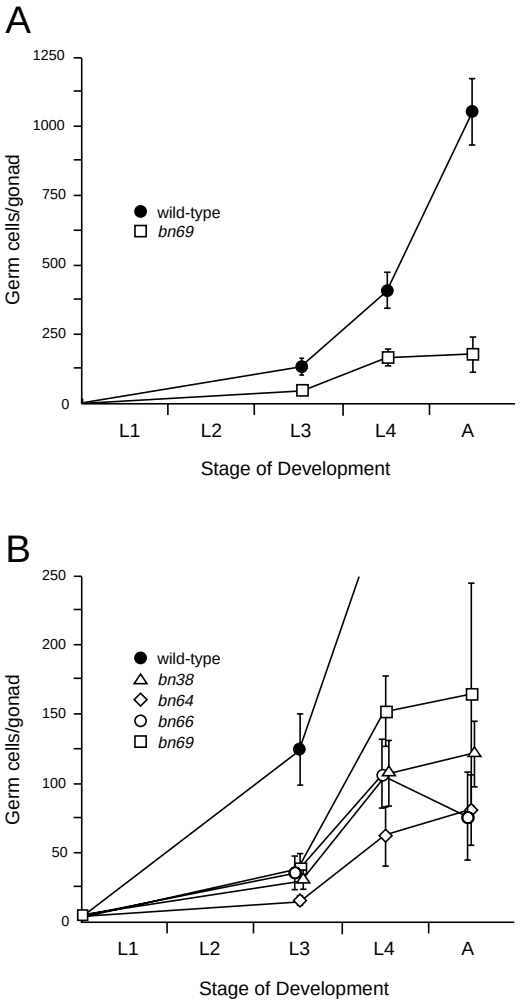

Fig. 1. Number of germ cells in *mes-6* mutants. Each data point represents the average of 11-15 worms. The top panel shows the number of germ cells from wild-type hermaphrodites (taken from Capowski et al., 1991) and *bn69* hermaphrodites. The bottom panel shows all *mes-6* alleles.

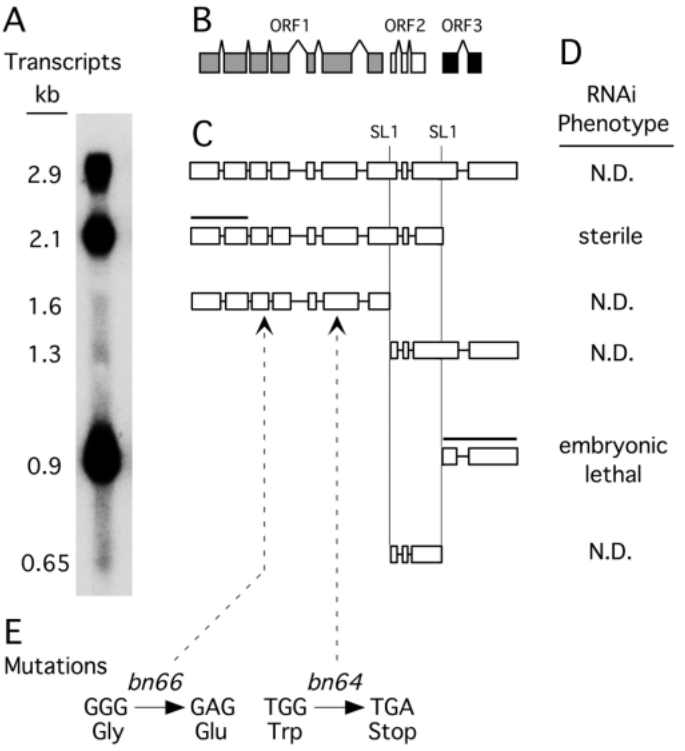

Fig. 2. Identification of the *mes-6* operon and cloning of *mes-6*. (A) The 6.5 kb *Xba*I fragment of cosmid T12D3 that rescues *mes-6* detects six transcripts. (B) Our sequence analysis and sequence annotations from the *C. elegans* Genome Sequencing Project indicated that the rescuing fragment contains three closely spaced open reading frames. (C) Differential processing of a 2.9 kb polycistronic RNA at two interior *trans*-splice sites may generate the smaller transcripts detected by northern hybridization. (D) Antisense RNA was prepared from the 5′ end of the cDNA corresponding to the 2.1 kb transcript, or from all of the cDNA corresponding to the 0.9 kb transcript (antisense RNAs shown as solid bars above transcripts in C) and injected into the germline of wild-type adult hermaphrodites. The RNAi phenotypes of the progeny of the injected worms are shown. (E) Genomic DNA corresponding to over 80% of ORF1 and the entirety of ORF2 was sequenced from all four alleles of *mes-6*. Molecular lesions were found only in ORF1, demonstrating that it is *mes-6*. The nucleotide changes and corresponding amino acid changes are indicated (also see Fig. 4). The results demonstrate that ORF1 encodes MES-6. ORF2 encodes a novel product, and ORF3 encodes a homolog of yeast and vertebrate Cks1.

gonads. Compared to wild type, the number of germ cells is reduced in the L3 and L4 larval stages and only reaches 5-10% of wild type in young adults (Fig. 1). On average, the gonads of *mes-6* mutants have more germ cells than other *mes* mutants (see Capowski et al., 1991). Also, whereas the numbers of germ cells in other *mes* mutants reach a peak during the L4 stage and then decline (Capowski et al., 1991), in three of the four alleles of *mes-6*, the number of germ cells does not decline from L4 to young adult. Nevertheless, *mes-6* germlines show signs of germ cell degeneration, as documented by Paulsen et al. (1995) for *mes-3* and observed in *mes-4* mutant worms as well (C. Garvin and S. S., unpublished).

To test whether maternal-effect sterility is the loss-of-function phenotype of the *mes-6* locus, we compared *mes-6/*deficiency worms to *mes-6* homozygous worms. *mes-6(bn66) dpy-20/eDf18* mothers produced 25% dead embryos; 100% of adult progeny were sterile (*n*=5 broods). Control *unc-24 dpy-20/eDf18* mothers produced 27% dead embryos, presumably embryos homozygous for *eDf18*. Thus, greatly reducing or eliminating *mes-6*$^+$ function did not enhance embryonic lethality, providing genetic evidence that maternal-effect sterility is the null phenotype.

Maternal expression of *mes*$^+$ product is both necessary and sufficient for the fertility of hermaphrodite offspring, since both *mes/+* and *mes/mes* offspring of *mes/mes* mothers are sterile (Capowski et al., 1991). Nevertheless, it is worth noting that zygotic expression of the wild-type gene in *mes/+* offspring does improve germline proliferation. For example, after mating *mes-6(bn66) dpy-20* mothers to wild-type males, Dpy self-cross progeny contained 25 ± 23 germ cells and non-Dpy outcross siblings contained 220 ± 179 germ cells (*n*=20-23 worms). This suggests that the *mes* genes are expressed in the nascent germline and that expression of a wild-type gene

can improve germline health, although such expression does not rescue germline fertility in the absence of maternally supplied *mes*$^{+}$ product.

mes-6 is in a complex operon of a new class

The *mes-6* locus was three-factor mapped between *unc-5* and *fem-3*, and positioned 0.3 map unit to the left of *fem-3* on linkage group IV (Capowski et al., 1991). A physical map consisting of overlapping YAC and cosmid clones exists for this region and most of the *C. elegans* genome (Coulson et al., 1986, 1988; Waterston et al., 1997). To identify DNA containing the *mes-6* gene, clones and restriction fragments in the region left of *fem-3* were tested for their ability to rescue the maternal-effect sterility of *mes-6* using the technique of germline transformation rescue (Mello et al., 1991). We were able to rescue *mes-6* sterility with the YAC Y59H11, the cosmid T12D3 and a 6.5 kb *Xba*I fragment of T12D3 (data not shown). In a typical rescuing line, ~70% of the homozygous *mes-6* mothers produced 10-40% fertile progeny.

Using the 6.5 kb *Xba*I fragment as a probe on northern blots, we found that there are six transcripts produced from this region (Fig. 2A). The transcripts appear to be coordinately expressed: by northern hybridization analysis, all transcripts have similar developmental profiles and are enriched ~15-fold in animals containing a germline compared to animals lacking a germline (not shown). Sequence analysis of genomic DNA in the region indicated that the rescuing 6.5 kb *Xba*I fragment probably contains an operon: there are three closely spaced open reading frames (ORFs), each transcribed in the same direction and each separated by a *trans*-splice site (Fig. 2B). In worm operons, genes are transcribed as polycistronic precursors and processed to monocistronic RNAs by a combination of *trans*-splicing of the downstream gene, and cleavage and polyadenylation of the upstream gene (Blumenthal and Steward, 1997). To determine if the *mes-6* region is organized in an operon, we compared cDNA sequence to genomic sequence (Fig. 3) and assayed for the presence of polycistronic RNA and for *trans*-splicing (C. Williams, D. Zorio, L. Xu, I. K., S. S. and T. Blumenthal, unpublished data). Our

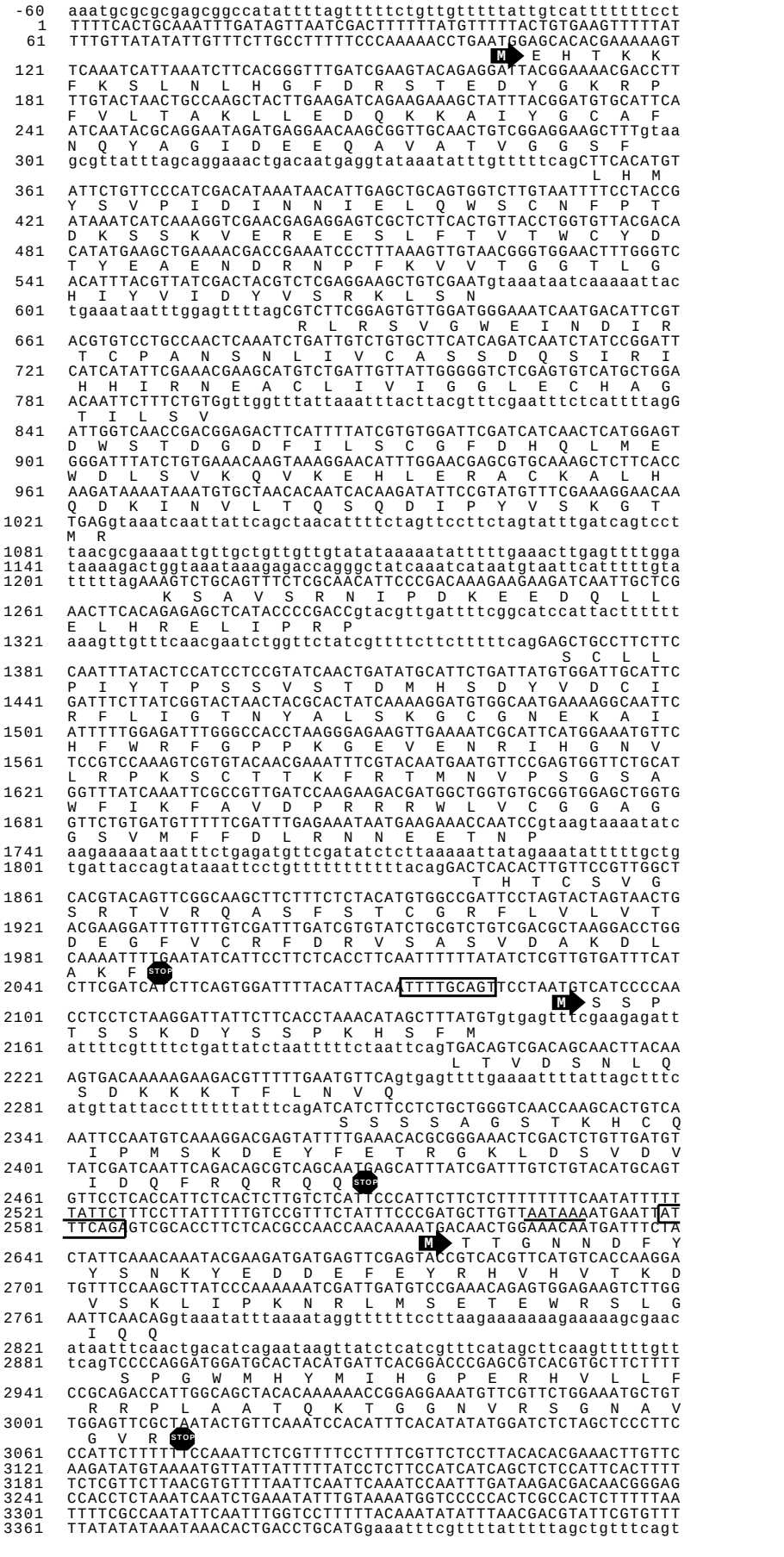

Fig. 3. Sequence of the *mes-6* operon. Lower case letters indicate non-transcribed regions (at the 5′ and 3′ ends) and introns. Upper case letters indicate the mature tricistronic mRNA. Two *trans*-splice sites (boxed) separate the three ORFs. Predicted amino acid sequences are shown below the nucleotide sequence. Arrows and stop signs indicate the initiator methionines and stop codons of each ORF. The polyadenylation consensus (AAUAAA) between ORF2 and ORF3 is underlined. *mes-6* operon has GenBank accession number AF016224.

findings demonstrate that the *mes-6* rescuing region contains a tricistronic operon; differential *trans*-splicing of the tricistronic RNA at two distinct SL1 sites may produce the six transcripts from the region (Fig. 2C). This operon resembles the *cyt-1/ced-9* operon (Hengartner and Horvitz, 1994) in that transcripts are separated by zero to only a few nucleotides, and the downstream genes are *trans*-spliced specifically to the SL1 splice leader. Thus, the *cyt-1/ced-9* and *mes-6* operons define a new class of worm operons.

We used RNA-mediated interference (RNAi; see Rocheleau et al., 1997) to determine which of the ORFs in the operon corresponds to *mes-6*. We injected wild-type hermaphrodites with antisense RNA complementary to two of the transcripts in the operon and examined their progeny for defects (Fig. 2D). Antisense RNA to the 5′ end of the 2.1 kb transcript (contains ORF1 and ORF2) resulted in production of sterile worms, while antisense RNA to the 0.9 kb transcript (contains ORF3) caused production of dead embryos with apparent cell cycle defects. To determine whether ORF1 or ORF2 corresponds to *mes-6*, we sequenced genomic DNA from worms bearing *mes-6* alleles (Fig. 2E). We found lesions in two alleles, *bn64* and *bn66*, in ORF1. No mutations were found in ORF2. This proves that ORF1 corresponds to *mes-6*. ORF2 codes for a novel product and is not yet known to be translated. ORF3 encodes a homolog of the yeast and vertebrate cyclin kinase regulatory subunit, Cks1 (E. Polinko, I. K., S. S., unpublished). The sequence of the entire operon is shown in Fig. 3.

MES-6 is homologous to Extra sex combs

Sequence analysis of the *mes-6* cDNA revealed that it encodes a 459 amino acid protein similar to *Drosophila* Extra sex combs (Esc) (Simon et al., 1995; Gutjahr et al., 1995; Sathe and Harte, 1995) and the murine homolog of Esc, termed Eed for Embryonic ectodermal development (Schumacher et al., 1996). Similarity searches against ~90% of the entire worm genomic sequence indicates that Esc is more similar to MES-6 than any other sequence in the worm genome, and therefore MES-6 likely represents the worm ortholog of Esc. MES-6, Esc and Eed share regions of sequence similarity that line up in register along the entire length of the proteins (Fig. 4). These regions contain WD-40 repeats, which are thought to be involved in protein-protein interactions (Neer et al., 1994). WD-40 repeats within a protein are often very dissimilar. In contrast, positionally equivalent repeats in homologous proteins are more highly conserved (Neer et al., 1994). Indeed, positionally equivalent WD-40 repeats in MES-6, Esc and Eed are more similar to each other than to any non-equivalent repeats compared within or between proteins. The crystal structure of the beta subunit of heterotrimeric G protein has revealed that its seven WD-40 repeats fold into a propeller-like conformation, with each ‘blade’ of the propeller composed of a WD-40 repeat and adjacent linker region (Wall et al., 1995; Sondek et al., 1996). Recently, Ng et al. (1997) compared Esc homologs from fruitfly, housefly, butterfly and grasshopper, and used homology modeling to predict that Esc also has seven WD-40 repeats that adopt a propeller structure similar to that of G beta. MES-6 also appears to contain seven WD-40 repeats, so it also may adopt a propeller-like tertiary stucture. Interestingly, the gly-to-glu change found in the *bn66* allele of *mes-6* maps to one of the loops that is predicted to project from the top of the Esc propeller and, based on sequence conservation among insect *esc* genes, is suggested to be important for protein-protein interactions.

MES-6 is localized in nuclei

To gain insight into the function of MES-6, we determined the spatial and temporal expression of MES-6 by staining worms with affinity-purified antibodies that were raised against the entire MES-6 protein fused to six histidine residues. As predicted by the similarity of MES-6 to Esc, MES-6 is localized in nuclei (Fig. 5). In wild-type adults, MES-6 staining is most prominent in the germline, but is also detectable in intestinal nuclei (Fig. 5B). A maternal load of protein is seen in the nuclei of oocytes. In early embryos, MES-6 is present in the nuclei of all cells (Fig. 5G). As embryogenesis proceeds, staining gradually fades in somatic cells. In late embryos and L1 larvae, MES-6 remains faintly visible in a number of cell types, including the intestine, but is most prominent in Z2 and Z3, the primordial germ cells (not shown). Nuclear staining is diminished or reduced below detectability in worms bearing any of the four mutant alleles of *mes-6* (Fig. 5F,K). This finding

Fig. 4. MES-6 is similar to *Drosophila* Esc and *mouse* Eed. Sequences were aligned using WU-BLASTP 2.0 and SeqPup. Boxes indicate the seven WD-40 repeats present in each protein. Residues identical in all three proteins are shaded. Note that residues that are not identical are often chemically similar to each other and/or match the WD-40 consensus. The positions of the *bn66* gly-to-glu and the *bn64* trp-to-stop mutations are indicated by dots over the MES-6 sequence.

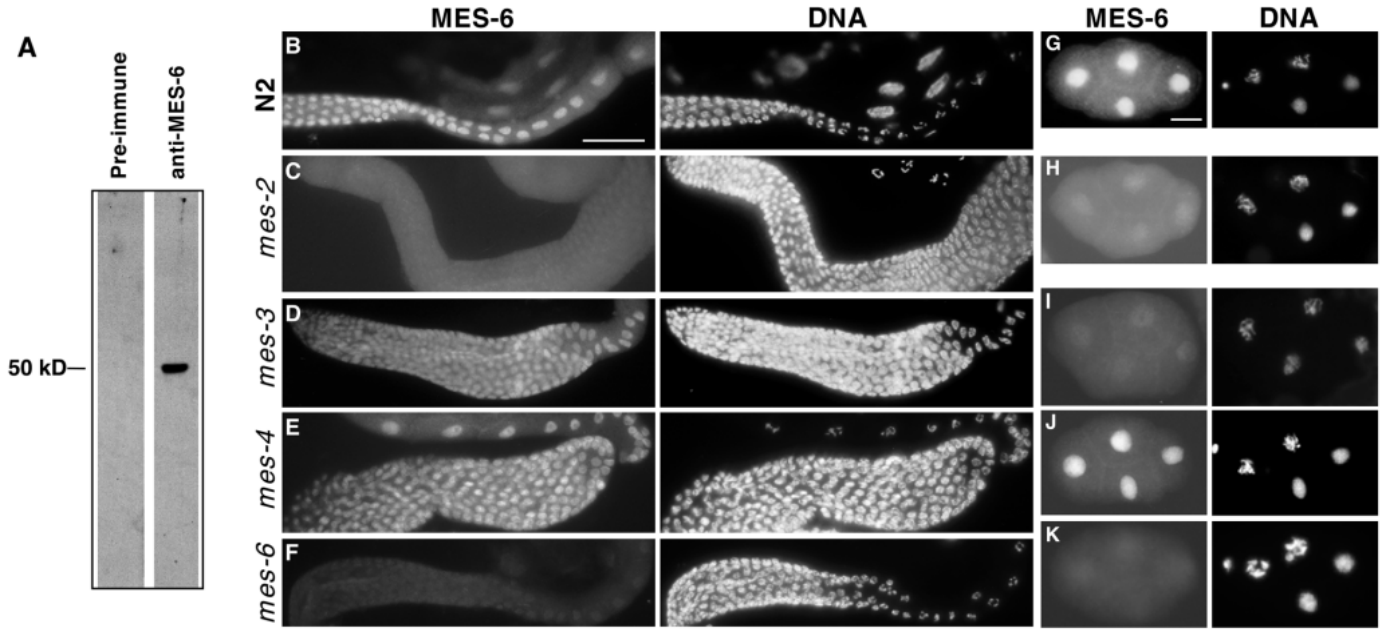

Fig. 5. MES-6 distribution in adults and embryos. (A) Affinity-purified rabbit anti-MES-6 antibody and preimmune serum from the rabbit were used to detect proteins from a *C. elegans* embryo homogenate on a western blot. Adult N2 or *mes* mutant hermaprodites (B-F) and their embryos (G-K) were stained with affinity-purified anti-MES-6 (left) and the DNA dye, DAPI (right). (B,G) MES-6 localizes to germ nuclei, including oocytes, in the gonads of wild-type adult hermaphrodites (B). MES-6 is also faintly detectable in the larger nuclei of the gut (upper nuclei in B). MES-6 is observed in all nuclei in wild-type embryos (G). (C,H) MES-6 staining is greatly reduced or undetectable in the germlines of homozygous *mes-2(bn11)* mutants (C) and in their embryos (H). (D,I) MES-6 staining appears wild type in the germlines of *mes-3(bn35)* homozygotes (D) but is greatly reduced or undetectable in their embryos (I). (E,J) MES-6 staining appears wild type in *mes-4(bn67)* mutant mothers (E) and in their embryos (J). (F,K) MES-6 staining is greatly reduced or undetectable in the germlines of *mes-6(bn38)* homozygotes (F) and in their embryos (K). Scale bars, 50 μm (B-F) and 10 μm (G-K).

and antibody recognition of a worm protein of the predicted size (~50 kDa) on western blots (Fig. 5A) demonstrate the specificity of the antibodies.

The normal distribution of MES-6 requires *mes-2*$^+$ and *mes-3*$^+$ function

The phenotypes of *mes-2*, *mes-3*, *mes-4* and *mes-6* mutants are nearly identical (Capowski et al., 1991; Garvin et al., 1998), suggesting that the gene products function in a similar process and may regulate or interact with one another. To determine if MES-6 distribution is altered in the other *mes* mutants, we stained animals bearing various alleles of *mes-2*, *mes-3* and *mes-4* with anti-MES-6 antibody (Fig. 5). We analyzed staining in the germline of fertile homozygous *mes* mothers and in their embryos, which would have grown up to be sterile adults. The results for MES-6 are compared to the results for MES-2 described by Holdeman et al. (1998) (Table 1). The three alleles of *mes-2* that abolish MES-2 staining (*bn11*, *bn27* and *bn76*) also disrupt MES-6 staining, while the two *mes-2* alleles that show normal MES-2 staining in germ and embryo nuclei (*bn48* and *bn72*) also display wild-type MES-6 staining. In both *mes-3* alleles tested (*bn35* and *bn86*), MES-6 staining appears normal in the germline, but is not localized in the nuclei of early embryos; MES-6 becomes somewhat enriched in nuclei in >16-cell embryos, but never reaches wild-type levels and disappears in older embryos. MES-2 displayed the same staining defects as MES-6 in *mes-3* mutants. *mes-4* mutants (*bn58* and *bn67*) displayed the wild-type pattern of MES-6, similar to the result obtained for MES-2. Thus, MES-6 and MES-2 depend on each other for proper nuclear accumulation, and the normal distributions of both MES-6 and MES-2 depend on *mes-3*$^+$ function but not on *mes-4*$^+$ function. Based on these results, it is likely that MES-6 and MES-2 and perhaps MES-3 interact.

MES-6 and MES-2 are the only recognizable Pc-G products in the *C. elegans* genome

In *Drosophila*, 13 Pc-G genes have been identified genetically and eight of these have been cloned (Table 2). Database searches have demonstrated that vertebrates possess homologs of all of the cloned Pc-G genes (see Pirrotta, 1997 and Gould, 1997 for reviews), indicating that this group of transcriptional regulators is conserved and likely existed in the last common ancestor of insects and vertebrates. We searched the ~90% of the *C. elegans* genome sequenced to date for worm homologs of the Pc-G. We did not find additional recognizable homologs of *esc* and *E(z)*, suggesting that *mes-6* and *mes-2* are likely to be the orthologous genes. Furthermore and quite surprisingly, except for *esc* and *E(z)*, we did not find recognizable nematode homologs of the other cloned Pc-G genes. Thus, based on analyzing eight out of the thirteen known Pc-G genes in *Drosophila*, the number of Pc-G genes in nematodes is considerably smaller than in flies and vertebrates.

DISCUSSION

The *mes-6* operon

The operon containing *mes-6* is the second member of what is

Table 1. Summary of MES-2 and MES-6 staining in *mes* mutants

Genotype	MES-2 staining		MES-6 staining	
	Germ nuclei	Embryo nuclei	Germ nuclei	Embryo nuclei
wild-type	+++	+++	+++	+++
mes-2(bn11)	–	–	–/+	–/+
(bn27)	–	–	–/+	–/+
(bn48)	+++	+++	+++	+++
(bn72)	+++	+++	+++	+++
(bn76)	–	–	–/+	–/+
*mes-3**	+++	–	+++	–/+
mes-4†	+++	+++	+++	+++
mes-6(bn38)	–	–	–/+	–/+
(bn64)	–	–	–/+	–/+
(bn66)	–/+	–/+	+	+
(bn69)	–	–	–/+	–/+

Staining results are summarized, with positive (+) and negative (–) results indicated for adult germline and embryonic nuclei. Results for MES-2 are from Holdeman et al. (1998).

**mes-3* alleles tested were *bn21*, *bn35*, *bn86* and *bn88* for MES-2, and *bn35* and *bn86* for MES-6.

†*mes-4* alleles tested were *bn23*, *bn58*, *bn67*, *bn85* and *bn87* for MES-2, and *bn58* and *bn67* for MES-6.

now emerging as a novel class of operons. The first member is the *cyt-1/ced-9* operon (Hengartner and Horvitz, 1994). In these operons, transcripts are separated by zero to only a few nucleotides, the downstream genes are *trans*-spliced specifically to SL1 and polycistronic RNA is abundant. This is in marked contrast to most operons, where the intertranscript distance is 100 or more nucleotides, the downstream genes are *trans*-spliced to SL2 and polycistronic RNA is undetectable on northern blots (Blumenthal and Steward, 1997). It is possible that processing of SL1-specific operons is inefficient and that this leads to accumulation of the polycistronic precursors seen in the *cyt-1/ced-9* and *mes-6* operons. Comparison and experimental manipulation of specific regions of the *cyt-1/ced-9* operon, the *mes-6* operon and other recently identified SL1-specific operons offer the opportunity to identify the cues that specify SL1 versus SL2 *trans*-splicing and to explore the mechanistic relationship between 3′ end formation of upstream genes and *trans*-splicing of downstream genes in operons.

We observed that RNA-mediated interference with different genes in the *mes-6* operon resulted in distinct mutant phenotypes. This observation influences consideration of the mechanism by which injected RNA interferes with gene expression in *C. elegans*. Some of the surprising features of RNA-mediated interference in worms are that either sense or antisense RNA preparations can cause interference, double-stranded RNA is more potent than either single strand alone and only a few molecules of dsRNA per cell can result in interference (Guo and Kemphues, 1995; Fire et al., 1998). These and other observations have led to speculation that the injected RNA somehow acts catalytically at the level of the gene or its transcripts to inhibit expression. If the *mes-6* operon mRNAs are processed from a single polycistronic precursor RNA, then our finding of separable interference with different genes in an operon argues against an effect of injected RNA on initiation or elongation of transcription.

MES-6 is homologous to the Pc-G protein Esc

Sequence analysis of MES-6 indicates that it is the *C. elegans* homolog of *Drosophila* Esc. Esc is classified as a member of the Polycomb group (Pc-G). Pc-G genes are required for maintaining homeotic genes in an inactive state (Simon, 1995; Orlando and Paro, 1995). Esc is unique among Pc-G proteins in that it functions primarily during early embryogenesis, whereas other Pc-G proteins are required continuously (Struhl and Brower, 1982; Simon et al., 1995). Consequently, it has been proposed that Esc mediates the transition from short-term to long-term repression, perhaps by recognizing the initial repressed state of genes established by early acting transcription factors and recruiting Pc-G proteins into a repressive complex (Simon et al., 1995; Gutjahr et al., 1995; Jones et al., 1998). A candidate Pc-G protein to be recruited by Esc is Enhancer of Zeste (E[z]); Jones et al. (1998) recently demonstrated that *Drosophila* Esc and E(z) directly interact, as do the human homologs of Esc and E(z). This finding is especially intriguing in light of our finding that MES-2 is the worm homolog of E(z).

The Pc-G in *C. elegans*

The similarity of both MES-2 and MES-6 to Pc-G proteins, their similar staining patterns, the predicted interaction of MES proteins and the results of our database searches raise three important points.

(1) The crucial role of the MES proteins in ensuring germline survival and proliferation may be via control of patterns of gene expression, and this control may be at the level of nucleosome organization and/or higher-order chromatin structure. In *Drosophila*, expression patterns of the homeotic genes are established in the early embryo by short-lived

Table 2. Cloned Polycomb group genes in *Drosophila*, mouse and *C. elegans*

Drosophila genes		Protein motifs	Mouse homologs	*C. elegans* homologs
Enhancer of zeste	*E(z)*	SET domain, CXC domain	*Ezh1, Ezh2*	*mes-2*
extra sex combs	*esc*	WD-40 repeats	*eed*	*mes-6*
Polycomb	*Pc*	Chromodomain	*M33, MPc2*	–
polyhomeotic	*ph*	Zinc finger, SPM domain	*Mph1/Rae-28**	–
Sex comb on midleg	*Scm*	Zinc finger, SPM domain	*Mph1/Rae-28**	–
Posterior sex combs	*Psc*	RING finger	*Bmi1, Mel18*	–
Suppressor of zeste	*Su(z)2*	RING finger	*Bmi1, Mel18*	–
Polycomb-like	*Pcl*	PHD fingers	*M96*	–

The *Drosophila* and mouse genes are from three reviews (Simon, 1995; Pirrotta, 1997; Gould, 1997) and references therein. In addition to the mouse homologs shown, there are human homologs of some Pc-G genes as well. The *C. elegans* genes are from this study.

– indicates that no recognizable homologs have been found in the ~90% of the *C. elegans* genome sequenced to date.

**Mph1* and *Rae-28* are the same gene. There are two human homologs of *ph*.

transcription factors encoded by the segmentation genes. Long-term maintenance of these expression patterns requires the gene products of the Pc-G (for maintaining gene repression) and the Trithorax group (Trx-G) (for maintaining gene activation) (Simon, 1995; Orlando and Paro, 1995). Although the mechanism of Pc-G function in *Drosophila* is not known, a popular model is that Pc-G proteins modify nucleosomes or affect higher-order chromatin structure, leading to a heritably repressed chromatin state with reduced accessibility to at least some DNA-binding proteins (Simon, 1995; Orlando and Paro, 1995; McCall and Bender 1996; Pirrotta, 1997). Alternative models are that Pc-G repression is due to sequestration of chromatin in inactive regions of the nucleus, inhibition of enhancer-promoter interactions or even direct inhibition of transcription factors (McCall and Bender, 1996; Pirrotta, 1997). The nuclear localization of both MES-2 and MES-6 is consistent with their participating in control of gene expression patterns in the germline by any of the mechanisms described above.

(2) MES-2 and MES-6 may function in a protein complex. The correct pattern of MES-2 localization requires wild-type *mes-6* function and vice versa. This same localization dependence is observed with *Drosophila* Pc-G members: Pc-G proteins co-localize at many sites on polytene chromosomes in a manner that is dependent on the wild-type function of other Pc-G genes (Carrington and Jones, 1996; Rastelli et al., 1993; Platero et al., 1996). In addition, Pc-G proteins can be co-immunoprecipitated in multimeric protein complexes (Franke et al., 1992). Taken together with the demonstration of a direct interaction between *Drosophila* E(z) and Esc, and also between the human homologs of E(z) and Esc (Jones et al., 1998), we predict that MES-2 and MES-6 also are direct binding partners.

(3) *C. elegans* contains considerably fewer Pc-G genes than insects or vertebrates. Eight *Drosophila* Pc-G genes have been sequenced, and vertebrate homologs have been identified for all eight (see Table 2), revealing the high degree of conservation of this group of transcriptional regulators. The *C. elegans* genome contains homologs of only two of these eight genes: *mes-2* and *mes-6*, which are the orthologs of *E(z)* and *esc*, respectively.

The Pc-G in *Drosophila* currently consists of 13 genes, which display homeotic transformations and enhance each others' mutant phenotypes. The results of large-scale screens for enhancers of Pc-G mutations suggest that most of the key Pc-G genes have been identified, but that ultimately up to 30-40 genes may be classified as belonging in the Pc-G (Jurgens, 1985; Landecker et al., 1994). As new fly and vertebrate Pc-G genes are molecularly characterized, it will be interesting to continue the search for *C. elegans* homologs and also determine whether *mes-3* and *mes-4* are Pc-G homologs.

The small number of Pc-G genes in *C. elegans* influences consideration of how Pc-G complexes assemble and function. Formation of a MES-2–MES-6 complex in the absence of other Pc-G proteins would suggest that Esc and E(z) may not require other Pc-G players to assemble into a functional complex in *Drosophila* (Jones et al., 1998). Early in *Drosophila* development, the transition from short-term to long-term repression of gene expression may be mediated by such a minimal Esc-E(z) complex. Subsequently, the composition of Pc-G protein complexes may vary between different chromosomal sites of gene regulation and in different tissues and developmental stages. The issue of heterogeneity among Pc-G complexes in *Drosophila* must be addressed by co-immunoprecipitation studies and by size fractionation of complexes and subsequent analysis of their composition, as done by Franke et al. (1992).

The germline role of the MES proteins

Our studies have shown that the wild-type functions of the *mes* genes are essential for normal germline development in *C. elegans*. Do Pc-G genes serve an essential role in germline development in *Drosophila*, in addition to their well-known roles in somatic development? Since Pc-G mutations are generally lethal, addressing this question has required generating mutant germline clones by pole cell transplantation or by induction of mitotic recombination in the germline. Most of the Pc-G genes tested appear not to be essential for female germline development (Haynie, 1983; Breen and Duncan, 1986; Soto et al., 1995). E(z) does appear to be essential in the germline, since certain temperature-sensitive alleles of E(z) are sterile (Phillips and Shearn, 1990), and transplanted pole cells mutant for a null allele of E(z) generate germlines indistinguishable from those in the temperature-sensitive alleles, indicating that the defect is germline autonomous (A. Shearn, personal communication).

The strict maternal-effect sterility caused by *mes* mutations reveals that a maternal supply of wild-type *mes* gene product is both necessary and sufficient for normal germline development in the next generation. With this in mind, we hypothesize that the MES proteins are required to maintain a germline-specific organization of chromatin from one generation to the next, and that this chromatin state is essential to initiate the correct pattern of gene expression in the germline. This hypothesis integrates both the maintenance function of Pc-G proteins and the initiation function and germline specificity suggested by the maternal-effect sterile phenotype of *mes* mutants.

Recent results from Kelly and Fire (1998) provide strong support for our hypothesis. Kelly and Fire previously observed that 'housekeeping' genes introduced into worms as transgenes and present in many tandem copies in extrachromosomal arrays are efficiently expressed in somatic cells but are specifically silenced in the germline of wild-type worms (Kelly et al., 1997). Remarkably, gene expression is desilenced in the germline of animals mutant for any of the four *mes* genes (Kelly and Fire, 1998). Gene expression also can be desilenced in the germline of wild-type worms by placing the test genes in the context of complex DNA in the array (Kelly et al., 1997). These findings indicate that gene expression in the germline is dependent on chromatin context and wild-type MES function, supporting the notion that the MES proteins repress gene expression through an influence on chromatin state.

We propose the following model of germline establishment and protection of immortality. The early germline requires two forms of silencing of gene expression. As the germline is being set apart from the soma in the early embryo, maternally supplied PIE-1 keeps the germline blastomeres transcriptionally quiescent, while surrounding somatic cells respond to differentiation factors and become transcriptionally active (Mello et al., 1996; Seydoux et al., 1996; Seydoux and Dunn, 1997). During this period, maternally supplied MES proteins provide an underlying 'memory' of the germline state

of chromatin, by maintaining nucleosomes or higher order chromatin in a particular conformation. When PIE-1 decays and the primordial germ cells become transcriptionally active (Mello et al., 1996; Seydoux et al., 1996; Seydoux and Dunn, 1997), the MES-regulated chromatin conformation maintains repression of certain genes or regions of the genome and selectively allows initiation of expression of germline-required genes. In the absence of a functional MES system, death of the germline could be due to ectopic expression of genes that are normally kept silent in the germline or alternatively due to altered levels or timing of expression of genes that are normally expressed in the germline.

The chromosomal sites of binding of the predicted MES complex are currently unknown. However, several observations suggest that a key feature of these sites may be the number of copies of target sequences. MES^+ function participates in keeping repetitive arrays of transgenes silenced in the germline, while non-repetitive arrays escape this silencing (Kelly et al., 1997; Kelly and Fire, 1998). Somewhat analogously, *Drosophila* Pc-G proteins repress expression of transgenes that are present in multiple copies (Pal-Bhadra et al., 1997). Interestingly, the requirement for MES^+ function is sensitive to chromosome dosage: animals with three X chromosomes absolutely require MES^+ function for fertility, while animals with one X show a reduced requirement (Garvin et al., 1998). This sensitivity to X-chromosome dosage may indicate participation of the MES proteins in dosage compensation in the germline, as discussed in Garvin et al. (1998) and Holdeman et al. (1998). Future identification of MES targets will enhance our understanding of the mechanism of Pc-G/MES control of gene expression and how this control contributes to the germline/soma dichotomy in *C. elegans*.

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REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410.

Barstead, R. J. and Waterston, R. H. (1989). The basal component of the nematode dense-body is vinculin. *J. Biol. Chem.* **264**, 10177-10185.

Beanan, M. J. and Strome, S. (1992). Characterization of a germ-line proliferation mutation in *C. elegans*. *Development* **116**, 755-766.

Blumenthal, T. and Steward, K. (1997). RNA processing and gene structure. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 117-145. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Breen, T. R. and Duncan, I. M. (1986). Maternal expression of genes that regulate the Bithorax complex of *Drosophila melanogaster*. *Dev. Biol.* **118**, 442-456.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Capowski, E. E., Martin, P., Garvin, C. and Strome, S. (1991). Identification of grandchildless loci whose products are required for normal germ-line development in the nematode *Caenorhabditis elegans*. *Genetics* **129**, 1061-1072.

Carrington, E. A. and Jones, R. S. (1996). The Drosophila *Enhancer of zeste* gene encodes a chromosomal protein: examination of wild-type and mutant protein distribution. *Development* **122**, 4073-4083.

Conrad, R., Thomas, J., Spieth, J. and Blumenthal, T. (1991). Insertion of part of an intron into the 5′ untranslated region of a *Caenorhabditis elegans* gene converts it into a *trans*-spliced gene. *Mol. Cell. Biol.* **11**, 1921-1926.

Coulson, A., Sulston, J., Brenner, S. and Karn, J. (1986). Toward a physical map of the genome of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **83**, 7821-7825.

Coulson, A., Waterston, R., Kiff, J., Sulston, J. and Kohara, Y. (1988). Genome linking with yeast artificial chromosomes. *Nature* **335**, 184-186.

Evans, D., Zorio, D., MacMorris, M., Winter, C. E., Lea, K. and Blumenthal, T. (1997). Operons and SL2 trans-splicing exist in nematodes outside the genus *Caenorhabditis*. *Proc. Natl. Acad. Sci. USA* **94**, 9751-9756.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S.E. and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806-811.

Franke, A., DeCamillis, M., Zink, D., Cheng, N., Brock, H. W. and Paro, R. (1992). Polycomb and polyhomeotic are constituents of a multimeric protein complex in chromatin of *Drosophila melanogaster*. *EMBO J.* **11**, 2941-2950.

Garvin, C., Holdeman, R. and Strome, S. (1998). The phenotype of *mes-2*, *mes-3*, *mes-4*, and *mes-6*, maternal-effect genes required for survival of the germline in *Caenorhabditis elegans*, is sensitive to chromosome dosage. *Genetics* **148**, 167-185.

Gould, A. (1997). Functions of mammalian Polycomb group and trithorax group related genes. *Curr. Opin. Genet. Dev.* **7**, 488-494.

Guo, S. and Kemphues, K. J. (1995). *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative ser/thr kinase that is asymmetrically distributed. *Cell* **81**, 611-620.

Gutjahr, T., Frei, E., Spicer, C., Baumgartner, S., White, R. A. H. and Noll, M. (1995). The Polycomb-group gene, *extra sex combs*, encodes a nuclear member of the WD-40 repeat family. *EMBO J.* **14**, 4296-4306.

Haynie, J. L. (1983). Maternal and zygotic roles of the gene *Polycomb* in embryonic determination in *Drosophila melanogaster*. *Genetics* **100**, 399-411.

Hengartner, M. O. and Horvitz, H. R. (1994). *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell* **76**, 665-676.

Holdeman, R., Nehrt, S. and Strome, S. (1998). MES-2, a maternal protein essential for viability of the germline in *C. elegans*, is homologous to a Drosophila Polycomb group protein. *Development* **125**, 2457-2467.

Jones, C. A., Ng, J., Peterson, A. J., Morgan, K., Simon, J. and Jones, R. S. (1998). The *Drosophila* esc and E(z) proteins are direct partners in Polycomb-group-mediated repression. *Molec. Cell Biol.* **18**, 2825-2834.

Jurgens, G. (1985). A group of genes controlling the spatial expression of the bithorax complex in *Drosophila*. *Nature* **316**, 153-155.

Kelly, W. G., Xu, S., Montgomery, M. K. and Fire, A. (1997). Distinct requirements for somatic and germline expression of a generally expressed *Caenorhabditis elegans* gene. *Genetics* **146**, 227-238.

Kelly, W. G. and Fire, A. (1998). Chromatin silencing and the maintenance of a functional germline. *Development*, **125**, 2451-2456.

Landecker, H. L., Sinclair, D. A. R. and Brock, H. W. (1994). Screen for enhancers of *Polycomb* and *Polycomblike* in *Drosophila melanogaster*. *Dev. Genetics* **15**, 425-434.

McCall, K. and Bender, W. (1996). Probes for chromatin accessibility in the *Drosophila* bithorax complex respond differently to Polycomb-mediated repression. *EMBO J.* **15**, 569-580.

Mello, C. C., Kramer, J. M., Stinchcomb, D. and Ambros, V. (1991). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**, 3959-3970.

Mello, C. C., Schubert, C., Draper, B., Zhang, W., Lobel, R. and Priess, J. R. (1996). The PIE-1 protein and germline specification in *C. elegans* embryos. *Nature* **382**, 710-712.

Neer, E. J., Schmidt, C. J., Nambudripad, R. and Smith, T. F. (1994). The ancient regulatory-protein family of WD-repeat proteins. *Nature* **371**, 297-300.

Ng, J., Li, R., Morgan, K. and Simon, J. (1997). Evolutionary conservation and predicted structure of the *Drosophila* extra sex combs repressor protein. *Mol. Cell. Biol.* **17**, 6663-6672.

Orlando, V. and Paro, R. (1995). Chromatin multiprotein complexes involved in the maintenance of transcription patterns. *Curr. Opin. Genet. Dev.* **5**, 174-179.

Pal-Bhadra, M., Bhadra, U. and Birchler, J. A. (1997). Cosuppression in *Drosophila*: gene silencing of Alcohol dehydrogenase by white-Adh transgenes is Polycomb dependent. *Cell* **90**, 479-490.

Paulsen, J. E., Capowski, E. E. and Strome, S. (1995). Phenotypic and molecular analysis of *mes-3*, a maternal-effect gene required for proliferation and viability of the germ line in *C. elegans*. *Genetics* **141**, 1383-1398.

Phillips, M. D. and Shearn, A. (1990). Mutations in *polycombeotic*, a *Drosophila* polycomb group gene, cause a wide range of maternal and zygotic phenotypes. *Genetics* **125**, 91-101.

Pirrotta, V. (1997). PcG complexes and chromatin silencing. *Curr. Opin. Genet. Dev.* **7**, 249-258.

Platero, J. S., Sharp, E. J., Alder, P. N. and Eissenberg, J. C. (1996). In vivo assay for protein-protein interactions using *Drosophila* chromosomes. *Chromosoma* **104**, 393-404.

Rastelli, L., Chan, C. S. and Pirrotta, V. (1993). Related chromosome binding sites for zeste, suppressors of zeste and Polycomb group proteins in *Drosophila* and their dependence on Enhancer of zeste function. *EMBO J.* **12**, 1513-1522.

Rocheleau, C. E., Downs, W. D., Lin, R., Wittmann, C., Bei, Y., Cha, Y.-H. Ali, M., Priess, J. R., and Mello, C. C. (1997). Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell* **90**, 707-716.

Sambrook, J. E., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd Edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sathe, S. S. and Harte, P. J. (1995). The *Drosophila* extra sex combs protein contains WD motifs essential for its function as a repressor of homeotic genes. *Mech. Dev.* **52**, 77-87.

Schumacher, A., Faust, C. and Magnuson, T. (1996). Positional cloning of a global regulator of anterior-posterior patterning in mice. *Nature* **383**, 250-253.

Seydoux, G., Mello, G. C., Pettitt, J., Wood, W. B., Preiss, J. R. and Fire, A. (1996). Repression of gene expression in the embryonic germ lineage of *C. elegans*. *Nature* **382**, 713-716.

Seydoux, G. and Dunn, M. A. (1997). Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in early embryos of *Caenorhabditis elegans* and *Drosophila melanogaster*. *Development* **124**, 2191-2201.

Simon, J., Bornemann, D., Lunde, K. and Schwartz, C. (1995). The *extra sex combs* product contains WD40 repeats and its time of action implies a role distinct from other Polycomb group genes. *Mech. Dev.* **53**, 197-208.

Simon, J. (1995). Locking in stable states of gene expression: transcriptional control during *Drosophila* development. *Current Biol.* **7**, 376-385.

Sondek, J., Bohm, A., Lambright, D. G., Hamm, H. E. and Sigler, P. B. (1996). Crystal structure of a G_A protein $\beta\gamma$ dimer at 2.1 Å resolution. *Nature* **379**, 369-374.

Soto, M. C., Chou, T.-B. and Bender, W. (1995). Comparison of germline mosaics of genes in the Polycomb group of *Drosophila melanogaster*. *Genetics* **140**, 231-243.

Struhl, G. and Brower, D. (1982). Early role of the *esc*$^+$ gene product in the determination of segments in *Drosophila*. *Cell* **31**, 285-292.

Sulston, J. E. and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110-156.

Wall, M. A., Coleman, D. E., Lee, E., Iniguez-Lluhi, J. A., Posner, B. A., et al. (1995). The structure of the G protein heterotrimer $G_{i\alpha 1}\beta_1\gamma_2$. *Cell* **83**, 1047-1058.

Waterston, R. H., Sulston, J. E. and Coulson, A. R. (1997). The genome. In *C. elegans* II (eds. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 23-45. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., et al. (1994). 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature* **368**, 32-38.