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REVIEW ARTICLE

Launching the germline in *Caenorhabditis elegans*: regulation of gene expression in early germ cells

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SUMMARY

One hundred years after Weismann's seminal observations, the mechanisms that distinguish the germline from the soma still remain poorly understood. This review describes recent studies in *Caenorhabditis elegans*, which suggest that germ cells utilize unique mechanisms to regulate gene expression. In particular, mechanisms that repress the production of mRNAs appear to be essential to maintain germ cell fate and viability.

Key words: PIE-1, MES-2, MES-6, transcriptional repression, P granules, germline, *Caenorhabditis elegans*

INTRODUCTION

The mechanisms that set the germline apart from the soma have fascinated biologists since the work of Weismann in the late 1800s (Weismann, 1893). Several developmental characteristics distinguish germ cells from somatic cells: during early development, germ cells show relative mitotic inertness compared to somatic cells; later, they are the only cells to undergo meiosis and gametogenesis. Because the germline is the only lineage to contribute its genetic material to the next generation, it is often referred to as an immortal and totipotent lineage, capable of "outliving" its somatic host to regenerate an entirely new organism (e.g. Wylie, 1999). These unique characteristics have led some biologists to wonder whether germ cell specification may involve molecular processes fundamentally different from those used by somatic cells. For example, examination of germ cell development across species has shown that primordial germ cells are often formed in locations and/or at times that appear to exclude them from the inductive events that specify the fates of somatic cells (Dixon, 1994). These observations have suggested that "protective mechanisms" that shield germ cells from somatic signals may be crucial for the proper establishment of the germline. In this review, we describe recent studies in *Caenorhabditis elegans* which suggest that such "protective mechanisms" indeed exist, and that these mechanisms function, at least in part, by repressing transcription in developing germ cells.

GERMLINE DEVELOPMENT IN *C. ELEGANS*

In the nematode *C. elegans*, the germline separates from the soma during the first four embryonic cleavages (Sulston et al., 1983) (Figs 1, 2). The zygote P_0, which can be considered the first germline blastomere, divides unequally into a large somatic blastomere AB and a smaller germline blastomere P_1. The AB blastomere divides equally to generate somatic daughters with equivalent developmental potential. In contrast, the P_1 germline blastomere divides unequally to give rise to the next germline blastomere P_2 and the somatic blastomere EMS. Unequal germline blastomere divisions continue until four somatic blastomeres (AB, EMS, C and D) and the primordial germ cell P_4 are formed. P_4 is the progenitor of the entire germline. At about the 100-cell stage, it divides symmetrically to generate two primordial germ cells (Z2 and Z3). These cells cease cell division and are joined by two somatic gonadal cells (Z1 and Z4) in mid-embryogenesis. Z2 and Z3 resume proliferation in the first larval stage to form the more than 1000 germ cells present in the adult germline. Separation of the germline from the soma and postembryonic germline development are reviewed in Kemphues and Strome (1997) and Schedl (1997).

GERMLINE BLASTOMERES LACK NEWLY TRANSCRIBED mRNAs

A difference in transcriptional activity between somatic and germline blastomeres was first observed in in situ hybridization experiments aimed at characterizing patterns of gene expression in early embryos (Fig. 3; Seydoux and Fire, 1994; Seydoux et al., 1996). A survey of 16 different early transcripts showed that newly synthesized mRNAs can be detected in somatic nuclei as early as the 4-cell stage; these

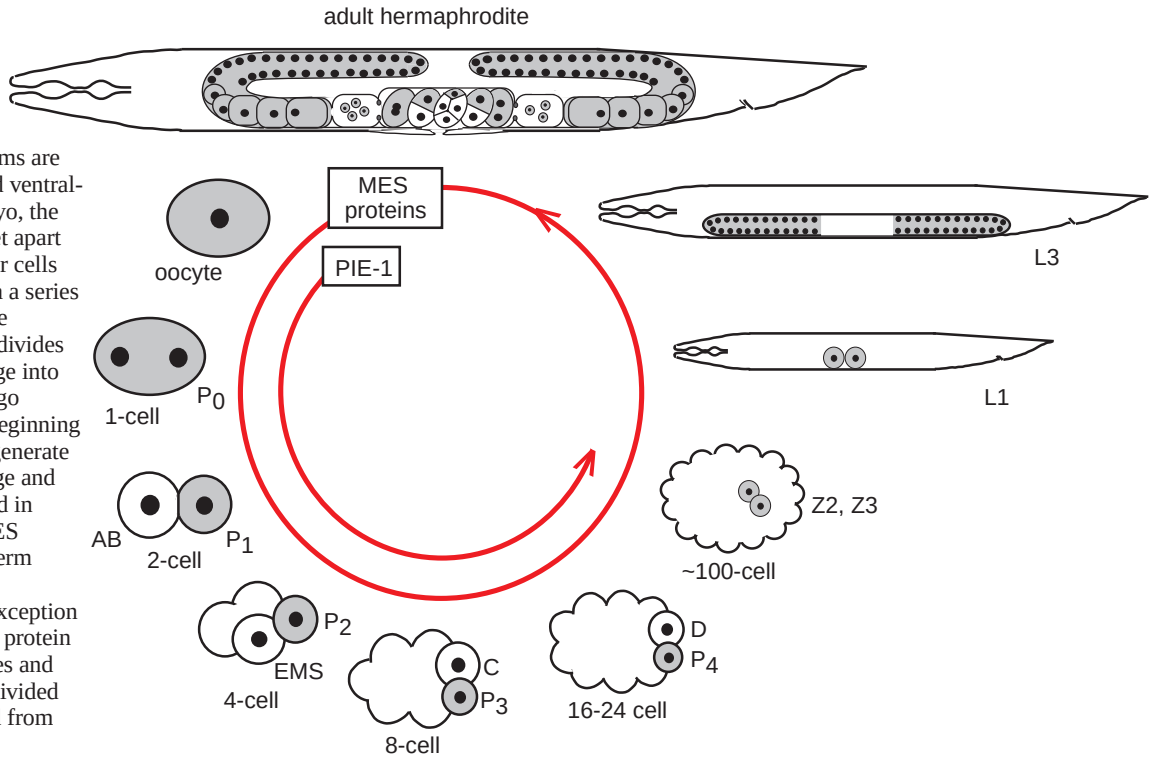

Fig. 1. The germline cycle. Embryos and worms are oriented anterior-left and ventral-down. In the early embryo, the germ lineage (grey) is set apart from the somatic founder cells (AB, EMS, C and D) via a series of unequal divisions. The primordial germ cell P_4 divides at about the 100-cell stage into Z2 and Z3, which undergo extensive proliferation beginning in the first larval stage, generate sperm during the L4 stage and oocytes during adulthood in hermaphrodites. The MES proteins are present in germ nuclei at all stages of development (with the exception of mature sperm). PIE-1 protein is first detected in oocytes and disappears after P_4 has divided into Z2 and Z3. Adapted from Strome et al. (1995).

transcripts, however, are never detected in the nuclei of germline blastomeres. The survey included mRNAs expressed in the germline of adult animals, suggesting that their absence from germline blastomeres might involve mechanisms specific to the early germline. These initial observations were consistent with either a block in mRNA synthesis or a decrease in mRNA stability in germline blastomeres. Evidence in favor of the former possibility came from analyzing the distribution of a specific phosphoepitope on RNA polymerase II. This phosphoepitope (RNAPII-H5) is defined by the monoclonal antibody H5, which recognizes repeats (YSPTSPS) in the carboxy-terminal domain (CTD) of the large subunit of RNA polymerase II that are phosphorylated on the first serine (Warren et al., 1992; Bregman et al., 1995; Kim et al., 1997; Patturajan et al., 1998). Phosphorylation of the CTD is thought to occur during the transition from the initiation phase to the elongation phase of transcription (Dahmus, 1996). Consistent with this, the RNAPII-H5 epitope can be found on polymerase subunits engaged in transcription in mammalian cells (Zeng et al., 1997), and its abundance increases in cells undergoing global changes in gene expression such as occur during stress response (Patturajan et al., 1998). Whether the phosphorylation event (or phosphorylated residues) recognized by H5 confers enhanced or novel activity on RNAPII subunits, however, remains unknown. In *C. elegans* embryos, the RNAPII-H5 epitope first appears in somatic nuclei in the 4-cell stage coincident with the onset of transcription, but remains absent from germline nuclei until the division of P_4 into Z2 and Z3 (~100-cell stage; Seydoux and Dunn, 1997). These observations are consistent with the idea that RNA polymerase II activity is reduced, or perhaps even absent, in germline blastomeres, and that mRNA synthesis does not begin until the 100-cell stage in the germ lineage. Not all transcription, however, is shut off in germline blastomeres. Newly transcribed rRNAs are readily detected in germline blastomeres, indicating that at least RNA polymerase I is active in these cells (Seydoux and Dunn, 1997). Whether

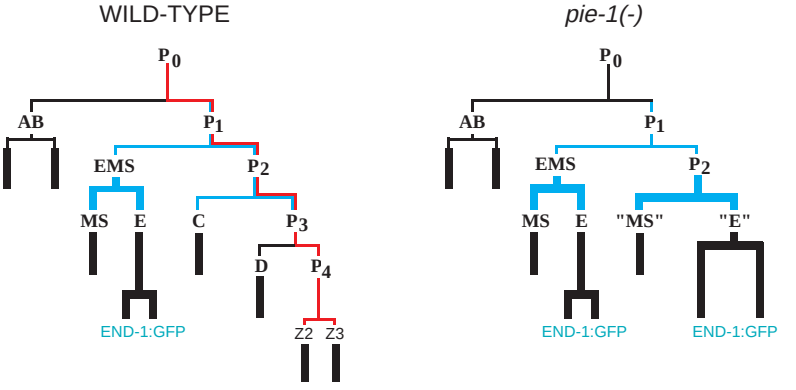

Fig. 2. Early embryonic lineage in wild-type and *pie-1* mutants. This tree diagram shows the series of divisions (horizontal lines) of the zygote (P_0) into somatic (AB, EMS, C, D) and germline (P_1, P_2, P_3, P_4) blastomeres, and the division of P_4 into Z2 and Z3. In *pie-1* mutants (no PIE-1 protein), transcription is activated in the germ lineage, and SKN-1 causes P_2 descendants to adopt fates similar to those adopted by EMS descendants. Thin vertical lines: cells that do not appear to produce mRNAs. Thick vertical lines: cells that produce mRNAs. Red lines: cells containing PIE-1 protein. Blue lines: cells containing SKN-1 protein.

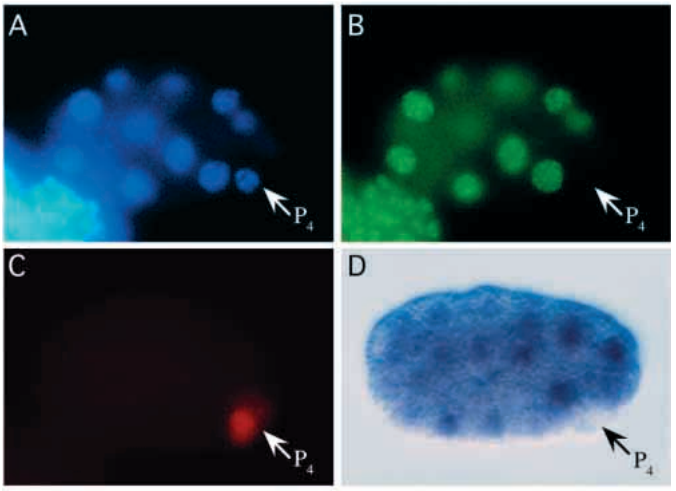

Fig. 3. Early germ cells contain PIE-1 and do not appear to produce mRNAs. (A-C) 28-cell embryo triply stained for DNA (blue), RNAPII-H5 (green) and PIE-1 (red). (D) Another 28-cell embryo hybridized to a *vet-5* probe (purple). *vet-5* is an embryonically transcribed RNA observed in all blastomeres except for the germline blastomeres.

there exist other genes besides those coding rRNAs that escape repression remains to be determined.

PIE-1: A MATERNAL FACTOR ESSENTIAL FOR TRANSCRIPTIONAL REPRESSION IN GERMLINE BLASTOMERES

The *pie-1* locus was first identified in a screen for maternal-effect mutations that disrupt the fates of embryonic blastomeres (Mello et al., 1992). In embryos derived from *pie-1* mutant mothers (hereafter referred to as *pie-1* embryos), descendants of the germline blastomere P_2 adopt fates similar to those of cells in the EMS lineage (Fig. 2). These fate transformations result in a lack of P_2-derived tissues, including a lack of primordial germ cells (Mello et al., 1992). Subsequently, *pie-1* mutants were shown to have abnormal patterns of gene expression in germline blastomeres (Seydoux et al., 1996). In *pie-1* embryos, transcription of several mRNAs and expression of the CTD phosphoepitope RNAPII-H5 are activated inappropriately in germline blastomeres (Seydoux et al., 1996; Seydoux and Dunn, 1997). These observations have suggested that an important function of *pie-1* is to keep mRNA transcription off in early germ cells. This proposed function is consistent with the fate transformations observed in *pie-1* mutants when the role of the transcription factor SKN-1 is considered (Fig. 2). SKN-1 is a maternally encoded transcription factor present in the blastomeres EMS and P_2 (Bowerman et al., 1993). In wild-type embryos, SKN-1 functions only in EMS to specify its fate (Bowerman et al., 1992), and is prevented from also acting in P_2 by PIE-1 (Mello et al., 1992). Presumably, PIE-1 prevents SKN-1 from functioning in P_2 by generally keeping mRNA transcription off in the germ lineage (Seydoux et al., 1996). In *pie-1* mutant embryos, transcription is turned on in the germ lineage and SKN-1 is free to activate its targets and cause P_2 descendants to adopt an EMS-like fate. Consistent with this interpretation, *end-1*, a gene activated in part by SKN-1 and transcribed only in EMS descendants in wild type (Zhu et al., 1997; J. Rothman, personal communication), is also transcribed in P_2 descendants in *pie-1* mutants (Fig. 2; J. Rothman, personal communication; C. Tenenhaus and G. S., unpublished observations). SKN-1, however, is not the only transcription factor whose activity is inhibited by PIE-1, since *skn-1;pie-1* double mutants still make no primordial germ cells (Mello et al., 1992) and since PIE-1 inhibits the expression of transcripts that do not depend on SKN-1 for expression (Seydoux et al., 1996).

These data indicate that *pie-1* is required to inhibit the production of new mRNAs in germline blastomeres, but is PIE-1 itself directly mediating this inhibition? The sequence of PIE-1 does not place it among one of the known families of transcriptional repressors. PIE-1 is a novel 38 kDa protein containing two zinc fingers of the C_3H class (Mello et al., 1996). Other C_3H zinc finger proteins (e.g. the 30 kDa subunit of Cleavage and Polyadenylation Specificity Factor (CPSF), Suppressor of Sable, Tristetraprolin (TTP), U2AF35) have been shown to bind RNA and have been implicated in mRNA cleavage, processing and/or turnover (Bai and Tolias, 1996, 1998; Barabino et al., 1997; Murray et al., 1997; Carballo et al., 1998; Rudner et al., 1998), but none yet have been implicated in transcriptional control. Several lines of evidence, however, suggest that PIE-1 may inhibit transcription directly.

First, PIE-1 is present at the right time and the right place to repress transcription in germline blastomeres. PIE-1 is maternally loaded and segregates with the germ lineage, where it accumulates in the cytoplasm and nucleus of each germline blastomere (Mello et al., 1996; Tenenhaus et al., 1998). PIE-1 disappears from the germ lineage shortly after the division of P_4 into Z2 and Z3, and, strikingly, this disappearance coincides with the appearance of RNAPII-H5 in these cells (Seydoux and Dunn, 1997). Thus, in wild-type embryos, the presence of PIE-1 correlates well with the absence of RNAPII-H5. This correlation also holds in mutants where PIE-1 expression is lost prematurely; in these mutants, RNAPII-H5 appears earlier in the germ lineage coincident with the loss of PIE-1 (Tenenhaus et al., 1998). In addition, ectopic expression of PIE-1 in somatic blastomeres significantly reduces the accumulation of certain mRNAs in those cells (Seydoux et al., 1996; Guedes and Priess, 1997). Together, these observations suggest that the presence of PIE-1 may be sufficient to interfere with RNA polymerase II activity.

More recently, evidence that PIE-1 can function directly as a transcriptional repressor has come from studies where PIE-1's effects on transcription were analysed in HeLa cell culture (Batchelder et al., 1999). By fusing different domains of PIE-1 to the DNA-binding domain of GAL4, Batchelder and colleagues have identified a region near the carboxy-terminal end of PIE-1 which can inhibit by 99-fold the expression of a promoter containing GAL4-binding sites (Batchelder et al., 1999). In this assay, the PIE-1 repressor domain appears similar in strength to strong repressor domains such as those found in the *Drosophila* repressors Knirps and Engrailed. These findings indicate that PIE-1 can act directly to repress transcription and that PIE-1 likely acts on a part of the transcriptional machinery that has been conserved between *C. elegans* and humans. Interestingly, the PIE-1 repressor domain contains a sequence (YAPMAPT) reminiscent of the repeated motif (YSPTSPS) that makes up the CTD of RNA polymerase

II. Non-conservative substitutions in this sequence eliminate the activity of the PIE-1 repressor domain in the GAL4 assay, and significantly reduce (but do not eliminate) the ability of a *pie-1* transgene to rescue a *pie-1* mutant (Batchelder et al., 1999). These observations have suggested that PIE-1 may inhibit transcription by targeting a CTD-binding complex (Batchelder et al., 1999). Since, as described above, PIE-1 is required in vivo to prevent the appearance of a specific phosphoepitope on the CTD of RNA polymerase II (RNAPII-H5), an attractive possibility is that PIE-1 interferes directly with the activity of a CTD kinase. Other more indirect models, however, are also possible, especially since it is not known whether the lack of RNAPII-H5 in germline blastomeres is a *cause* or a *consequence* of the apparent lack of RNA polymerase II activity in these cells. Which step in mRNA synthesis is inhibited by PIE-1 also remains a mystery. Transcriptional initiation, elongation and pre-mRNA processing are all possible candidates, since each of these steps has been shown to involve the CTD (Koleske and Young, 1994; Steinmetz, 1997; Corden and Patturajan, 1997; Neugebauer and Roth, 1997).

MES PROTEINS: MATERNAL FACTORS PREDICTED TO PARTICIPATE IN ESTABLISHING CORRECT PATTERNS OF GENE EXPRESSION IN THE NASCENT GERMLINE

After PIE-1 disappears and transcription begins in the germline, how are germline patterns of gene expression established? Four proteins, MES-2, MES-3, MES-4 and MES-6, are currently the best candidates for regulating this process. The *mes* genes were identified in screens for maternal-effect mutations that result in sterile offspring; the cause of sterility was found to be degeneration of the germline starting midway through larval development (Fig. 4D; Capowski et al., 1991; Paulsen et al., 1995). Thus, the *mes* genes encode maternally expressed regulators of some aspect of germline development required for survival of the germline. The hypothesis that the MES proteins are regulators of gene expression in the germline is supported by the similarity of certain MES proteins to known transcriptional regulators in *Drosophila* and the effect of *mes* mutations on expression of transgenes in the germline.

MES-2 and MES-6 appear to constitute the Polycomb group in *C. elegans*. The *Drosophila* Polycomb group genes, of which thirteen have been identified genetically, are best known for their role in maintaining transcriptional repression of homeotic genes (reviewed in Pirrotta, 1997). Patterns of homeotic gene expression are initially established in early *Drosophila* embryos by short-lived transcription factors encoded by the segmentation genes. Long-term maintenance of repression is mediated by Polycomb group proteins, which associate into multimeric protein complexes and associate with chromatin at distinct chromosomal sites (Franke et al., 1992; Rastelli et al., 1993; Carrington and Jones, 1996; Platero et al., 1996). A popular model of Polycomb group action is that protein complexes modify nucleosomes or higher order chromatin structure, leading to a heritably repressed state with reduced accessibility to at least some DNA-binding proteins (McCall and Bender, 1996; Pirrotta, 1997). MES-2 is homologous to the *Drosophila* Polycomb group protein, Enhancer of zeste (E[z]); both proteins and the mammalian and plant homologs contain a SET domain, which is shared by multiple chromatin-binding proteins, a cys-rich region adjacent to the SET domain, and five characteristically spaced cys residues found only in E(z) homologs (Jones and Gelbart, 1993; Carrington and Jones, 1996; Hobert et al., 1996; Goodrich et al., 1997; Holdeman et al., 1998). MES-6 is homologous to the *Drosophila* Polycomb group protein, Extra sex combs (Esc), which is composed of seven WD-40 motifs that are predicted to fold into a seven-bladed propeller configuration and to participate in protein-protein interactions (Gutjahr et al., 1995; Sathe and Harte, 1995; Simon et al., 1995; Ng et al., 1997; Korf et al., 1998).

In searches of the now nearly completely sequenced *C. elegans* genome, MES-2 and MES-6 are the only recognizable homologs of the nine Polycomb group members that have been molecularly analyzed in *Drosophila* (Korf et al., 1998). This is surprising and intriguing, because vertebrates contain homologs of all of the molecularly characterized Polycomb group genes, revealing the evolutionary conservation of this group of transcriptional regulators (Pirrotta, 1997). Thus, the Polycomb group contains fewer genes in worms than in flies and vertebrates. Furthermore, the best understood role of the Polycomb group in flies and vertebrates is in anterior-posterior patterning in the soma, whereas the only essential role of the mini-Polycomb group in worms is in germline development. How MES-3 and MES-4 will fit into the Polycomb group story is unclear at present; MES-3 is a novel protein (Paulsen et al., 1995) and MES-4 contains motifs found in Polycomb group proteins but is not an obvious homolog of any particular protein in the group (Y. Fang and S. S., unpublished results).

As predicted by the sequence similarity of MES-2 and MES-6 to members of the Polycomb group, MES-2 and MES-6 are nuclearly localized (Fig. 4A; Holdeman et al., 1998; Korf et al., 1998). Also, similar to the Polycomb Group, the MES proteins probably function as multimeric protein complexes, since the normal nuclear localization of MES-2 requires wild-type *mes-6* function and vice versa, and the localization of both MES-2 and MES-6 requires wild-type *mes-3* function (Holdeman et al., 1998; Korf et al., 1998).

The MES proteins are enriched in, but not restricted to, the germline. In larvae and adults, *mes* gene product levels are highest in the germline but are detectable in somatic cells as well (Paulsen et al., 1995; Korf et al., 1998; Holdeman et al., 1998). In embryos, the MES proteins are present in all nuclei of early and mid stages (Holdeman et al., 1998; Korf et al., 1998); the levels of MES proteins gradually decline in somatic cells until, by the time of hatching, first-stage larvae contain detectable MES protein only in the two primordial germ cells, Z2 and Z3. Given the mutant phenotype (i.e. maternal-effect sterility) and the known role of the Polycomb group in flies, an attractive scenario is that maternally supplied MES proteins function in Z2 and Z3 and their descendants to modulate chromatin structure and regulate which genes are expressed and which genes are maintained in a repressed state in the early germline. In this scenario, death of the germline in mid-larval-stage *mes* mutants is caused by absence of an essential mechanism of repression, leading to altered patterns of gene expression.

Two lines of evidence support the view that the MES system is involved in control of gene expression in the

germline. One is the marked sensitivity of the Mes mutant phenotype to X chromosome dosage: *mes* mutants with one X chromosome (males) generally contain healthy-appearing germlines and gametes and are fertile, whereas *mes* mutants with two X chromosomes (hermaphrodites) show germline death, lack gametes and are sterile (Garvin et al., 1998). Germline defects are most severe in *mes* mutants with three X chromosomes. The sensitivity of the Mes phenotype to number of X chromosomes is reminiscent of the situation with genes that mediate dosage compensation (i.e. the process of equalizing X-chromosome gene expression in animals bearing one versus two X chromosomes) in the soma (Meyer, 1997). This raises the possibility that the MES system mediates dosage compensation in the germline of XX animals, by repressing X-chromosome gene expression, and that overexpression of X-linked genes in hermaphrodites leads to germline death. As discussed in Holdeman et al. (1998), we favor a model in which MES proteins serve a more general role in modulating chromatin structure and repressing gene expression from autosomal sites as well as sites on the X chromosome.

The most compelling evidence that the MES system influences gene expression in the germline comes from analysis of expression of transgenes. Transgenes present in many copies in extrachromosomal arrays can be efficiently expressed in somatic cells but are silenced in the germlines of wild-type worms (Fig. 4E; Kelly et al., 1997). This observation suggests that, unlike the soma, the germline may package repetitive sequences into transcriptionally silent chromatin, perhaps similar to heterochromatin. This view is supported by the finding that reducing the repetitive nature of extrachromosomal arrays (by placing transgenes in the context of complex DNA) can activate transgene expression in the germline (Kelly et al., 1997). Intriguingly, desilencing of transgenes in the germline is also observed when repetitive extrachromosomal arrays are introduced into a *mes* mutant background (Fig. 4F; Kelly and Fire, 1998). These findings suggest that MES proteins normally participate in keeping at least some genes silenced in the germline and that this is accomplished via an effect on chromatin state. Since the three transgenes studied by Kelly and Fire (1998) derive from autosomes, the MES system is not restricted to regulating genes on the X chromosome, at least in a transgene assay.

Many fundamental questions about MES targets and mechanism remain to be addressed. Does MES regulation in the germline operate at the level of individual genes, domains of chromosomes or the entire genome? If at the level of individual genes, which genes are the natural targets of MES regulation? What is the nature of the repressed state of chromatin thought to be induced by MES complexes? Do MES proteins also function in somatic cells? The absence of obvious somatic defects in *mes* mutants suggests that the *mes* genes serve an essential role only in the germline (Capowski et al., 1991). However, the findings that under certain conditions *mes* mutations can alter somatic sex determination (Garvin et al., 1998), and that *mes* mutants display low penetrance homeotic transformations of certain somatic cells (J. Maloof and C. Kenyon, personal communication; see Holdeman et al., 1998) suggest that the *mes* genes serve non-essential roles in the soma as well.

FUNCTIONAL AND EVOLUTIONARY SIGNIFICANCE OF THE PIE-1 AND MES MECHANISMS OF TRANSCRIPTIONAL REPRESSION

Why inhibit transcription in developing germ cells? The phenotype of *pie-1* mutants suggests that the complete, or nearly complete, inhibition of mRNA production protects early germ cells from transcription factors (e.g., SKN-1) that promote somatic development. The phenotype of *mes* mutants suggests that, later in development, when germ cells have initiated transcription, chromatin-mediated repression of a subset of genes is essential for germ cell viability. Thus, one simple hypothesis is that transcriptional repression is essential to maintain the fate (early) and survival (later) of developing germ cells in *C. elegans*. This hypothesis relies on the assumption that the main function of PIE-1 and of the MES proteins is to regulate transcription in germ cells. This assumption appears well founded for MES-2 and MES-6, two nuclear proteins with significant homology to known regulators of transcription. In the case of PIE-1, as described above, there is good in vivo and in vitro evidence that at least one role of the protein is to repress transcription. However, PIE-1 resides both in the nucleus and cytoplasm of germline blastomeres, raising the possibility that PIE-1 has additional functions besides inhibiting transcription. Consistent with this possibility, *pie-1* mutants fail to express at least one protein (NOS-2) that is translated in P_4 from maternal mRNA (K. Subramaniam and G. S., unpublished observations). This observation suggests that PIE-1 may regulate the stability and/or translation of maternal RNAs, in addition to zygotic transcription, in the early germline. A future challenge, therefore, will be to determine whether PIE-1's requirement for germ cell fate is directly linked to PIE-1's ability to repress transcription or also involves other activities of the protein. Now that the region of PIE-1 involved in transcriptional repression has been defined (Batchelder et al., 1999), it should be possible to address this question by analyzing the germline phenotype of mutants that specifically disrupt this domain.

Are similar strategies of transcriptional repression utilized in the germ lineage of other animals? Little is known about whether regulation of chromatin structure, predicted to be the role of the MES proteins, occurs in germ cells in other species. The results of clonal analysis experiments done in *Drosophila* suggest that most of the Polycomb group genes are not required for germline development in that organism (Haynie, 1983; Breen and Duncan, 1986; Soto et al., 1995). An exception is E(z), which appears to have an essential germline role (Phillips and Shearn, 1990; A. Shearn, personal communication), raising the possibility of conservation of a chromatin-level mechanism of regulating gene expression in germ cells.

Several observations in *Drosophila* suggest that a mechanism similar to the one mediated by PIE-1 is operating in that insect. Like *C. elegans* germline blastomeres, early *Drosophila* germline "pole cells" lack the RNAPII-H5 phosphoepitope and do not accumulate newly transcribed mRNAs, although they express rRNAs (Zalokar, 1976; Lamb and Laird, 1976; Kobayashi et al., 1988; Seydoux and Dunn, 1997; Van Doren et al., 1998). In addition, supplying a potent transcription factor (VP16) to early pole cells is not sufficient to activate mRNA transcription, suggesting that their inability to transcribe mRNA is not simply due to a shortage of

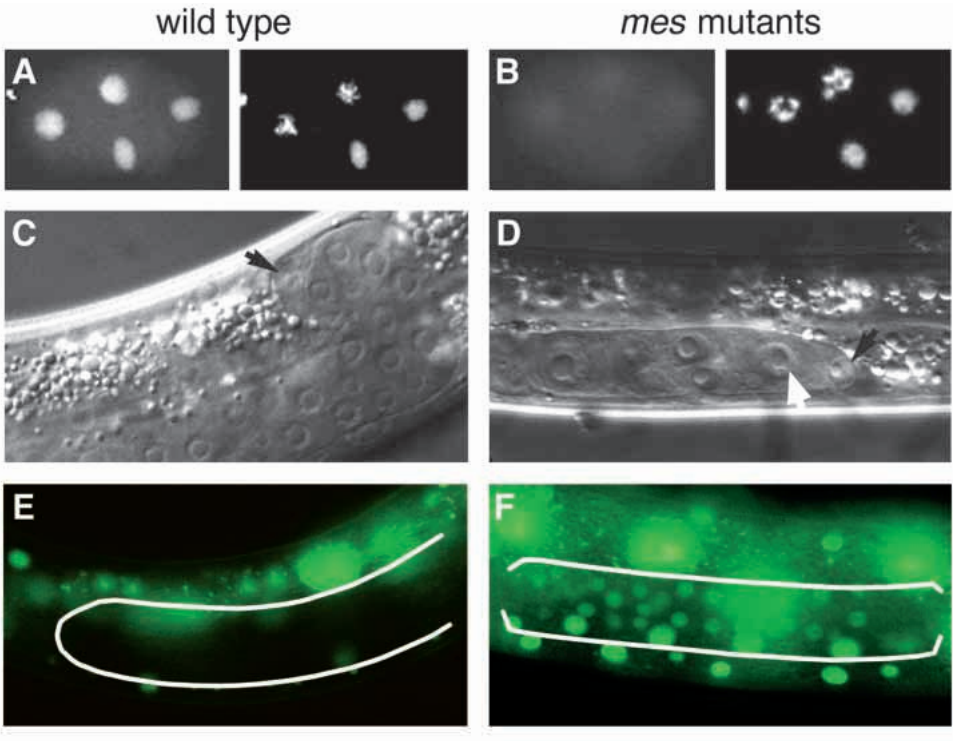

Fig. 4. *mes* mutant phenotypes. (A,B) Staining of MES-6 on the left and DNA on the right in a wild-type embryo (A) and a *mes-6* mutant embryo (B). The MES proteins are in all nuclei in early wild-type embryos. *mes-2*, *mes-3* and *mes-6* mutant embryos lack detectable nuclear staining of MES-2 and MES-6. C and D: Germline nuclei are uniform in size and evenly spaced in a wild-type larva (C) but are enlarged (white arrow) and surrounded by coagulated cytoplasm in a *mes-3* mutant larva (D), indicative of germ cell death. Black arrows show the distal tip of each gonad arm. (E,F) A GFP-tagged transgene (*let-858*) present in many copies in an extrachromosomal array is not expressed in the germline of a wild-type worm (E) but is expressed in the germline of a *mes-6* mutant worm (F), revealing that wild-type MES function participates in transgene silencing in the germline. The germline in each panel is outlined in white. Figure adapted from Korf et al. (1998), Paulsen et al. (1995) and Kelly and Fire (1998).

transcriptional activators (Van Doren et al., 1998). mRNA transcription begins in pole cells during gastrulation, when these cells are in the posterior midgut pocket inside the embryo, approximately 2 hours after somatic cells have begun transcription (Zalokar, 1976; Van Doren et al., 1998). Remarkably, in *C. elegans*, mRNA transcription appears to begin in the germ lineage at a similar stage: PIE-1 disappears and RNAPII-H5 first appears in the primordial germ cells Z2 and Z3 when these cells have entered the embryo and are associated with the gut primordium (Seydoux and Dunn, 1997). These similarities make it likely that a transcriptional repressor with properties similar to PIE-1 exists in *Drosophila*, although such a factor has yet to be described. The parallels between *Drosophila* and *C. elegans* also suggest that inhibition of mRNA transcription may be a commonly used mechanism to protect early germ cells from somatic influences.

Consistent with this possibility, recent evidence from mice suggests that mammals may also rely on transcriptional regulation to protect the totipotency of germ cells. Oct-4 (also called Oct-3) is a member of the POU family of transcription factors whose expression correlates with totipotency and germ cell fate (reviewed in Pesce et al., 1998). In early mouse embryos, Oct-4 initially is expressed in all cells, but becomes restricted to cells of the inner cell mass of the blastocyst and then to the epiblast (the stem cells that will form the embryo), and eventually is expressed only in primordial germ cells. This pattern of expression has suggested that Oct-4 may function to maintain an undifferentiated totipotent state in embryonic cells (Pesce et al., 1998). Consistent with this hypothesis, in Oct-4-deficient embryos, cells of the inner cell mass differentiate inappropriately into trophoblast cells and no epiblast is formed (Nichols et al., 1998). One possibility is that Oct-4 maintains totipotency by inhibiting the expression of genes that trigger somatic differentiation (Pesce et al., 1998). In that sense, Oct-4 may perform in mammals a role analogous to that fulfilled by PIE-1 in *C. elegans*. However, the mechanisms employed by the two proteins are likely to be different: PIE-1 generally inhibits the production of mRNAs, whereas Oct-4, like other POU-domain transcription factors, is thought to regulate the expression of specific genes (Pesce et al., 1998). It will be interesting to identify the targets of Oct-4 that are critical to maintain totipotency.

POST-TRANSCRIPTIONAL MECHANISMS UNIQUE TO THE GERMLINE?

In this review, we have described two mechanisms used by *C. elegans* germ cells to regulate mRNA production during development. An important question for the future will be to determine whether germ cells have also evolved unique mechanisms to regulate mRNA stability and translation. This possibility, first suggested by Mahowald (1968), is supported by the observation that germ cells in most species contain distinctive RNA-rich granules in their cytoplasm. These germ granules, referred to as P granules in *C. elegans*, polar granules in *Drosophila* and germ plasm in *Xenopus*, are unique to germ cells, and have been shown to be essential for germline development in *Drosophila* and more recently in *C. elegans* (Lehmann and Nusslein-Volhard, 1986; Ephrussi and Lehmann, 1992; Gruidl et al., 1996; Kawasaki et al., 1998).

In *C. elegans*, P granules are segregated to the germline blastomeres (P_1, P_2, P_3, P_4) during the early unequal divisions that separate the germline from the soma (see Fig. 1), and remain present in germ cells throughout development (with the exception of mature sperm; Strome and Wood, 1982). Eight P-granule-associated proteins have been identified so far (see Table 1; Draper et al., 1996; Gruidl et al., 1996; Jones et al., 1996;

Table 1. Proteins present in the nuclei and/or on P granules in early germ cells

Protein	Motifs	Localization	References
MES-2	SET domain, CXC domain Similar to *Drosophila* E(z)	1 cell to 500-cell embryos: All embryonic nuclei Larvae and adults: Primarily germline nuclei	Holdeman et al., 1998
MES-6	7 WD-40 repeats Similar to *Drosophila* Esc	1-cell to 500-cells embryos: All embryonic nuclei Larvae and adults: Primarily germline nuclei	Korf et al., 1998
PIE-1	2 CCCH fingers	1-cell to 100-cell embryos: Cytoplasm and nuclei of germline blastomeres and of Z2 and Z3. Also on P granules. On centrosomes during mitosis Adults: Cytoplasm and nuclei of oocytes	Mello et al., 1996; Tenenhaus et al., 1998
MEX-1	2 CCCH fingers	1-cell to 100-cell embryos: Cytoplasm of germline blastomeres and on P granules. Also present transiently in the cytoplasm of the somatic blastomeres AB, EMS, C and D Adults: Cytoplasm of oocytes	Guedes and Priess, 1997
POS-1	2 CCCH fingers	2-cell to 28/100-cell embryos: Cytoplasm of germline blastomeres and on P granules. Also present transiently in the cytoplasm of the somatic blastomeres EMS, C and D	Tabara et al., 1998
MEX-3	2 KH domains	1-cell to 4-cell embryos: Cytoplasm of all blastomeres with preference for anterior (AB) blastomeres. Also on P granules 4-cell to 28-cell embryos: Disappears from AB descendants and persists transiently in cytoplasm of P_1 descendants. Also on P granules Adults: Cytoplasm of oocytes	Draper et al., 1996
GLD-1	KH domain Similar to mammalian Quaking and Sam68	4-cell to 28-cell embryos: Cytoplasm of germline blastomeres and on P granules. Also present transiently in the cytoplasm of the somatic blastomeres EMS, C and D 28-cell embryo to adult: Low level in cytoplasm of primordial and proliferating germ cells, higher level in cytoplasm of germ cells in early stages of meiosis, very low level in oocytes	Jones and Schedl, 1995; Jones et al., 1996
GLH-1	DEAD-box helicase motifs, 4 CCHC fingers Similar to *Drosophila* Vasa	1-cell embryo to adult: In germline cells, on P granules	Gruidl et al., 1996
GLH-2	DEAD-box helicase motifs, 6 CCHC fingers Similar to *Drosophila* Vasa	1-cell embryo to adult: In germline cells, on P granules	Gruildl et al., 1996
PGL-1	RGG box	1-cell embryo to adult: In germline cells, on P granules	Kawasaki et al., 1998

Mello et al., 1996; Guedes and Priess, 1997; Kawasaki et al., 1998; Tabara et al., 1998). All eight contain motifs implicated in RNA binding, consistent with the idea that P granules regulate some aspect of mRNA metabolism in the cytoplasm of germ cells. In particular, a subset of P-granule components have been implicated in translational control: (1) POS-1 is required for the expression of APX-1, a protein translated in the P_1 and P_2 germline blastomeres from maternal RNA (Tabara et al., 1998), (2) GLD-1 appears to act as a translational regulator in the maternal germline (Jones and Schedl, 1995; Jan et al., 1999) and (3) PGL-1 and the GLH proteins contain motifs found in Vasa, a component of *Drosophila* polar granules implicated in regulation of translation during oogenesis (Gruidl et al., 1996; Kawasaki et al., 1998; Styhler et al., 1998). A role for germ granules in translation is also supported by the observations that germ granules in *Drosophila* and *Xenopus* contain ribosomal RNA derived from mitochondria (Kobayashi et al., 1993, 1998) and that this ribosomal RNA is essential for germline formation in *Drosophila* (Iida and Kobayashi, 1998). P granules in *C. elegans* have been shown to contain poly(A)+ RNAs (Seydoux and Fire, 1994), but it is not yet known whether they also contain ribosomal RNAs. Clearly it will be important to pursue the genetic and molecular analysis of P-granule components to determine whether these mysterious organelles provide yet another unique mechanism for germ cells to regulate gene expression.

FUTURE PROSPECTS

Although our understanding of early germline development is still limited, the data accumulated so far support the idea that germ cells exploit unique mechanisms to regulate gene expression in order to establish their unique fate and maintain viability. Paradoxically, in *C. elegans*, these same mechanisms have hindered the molecular analysis of germline gene expression by making it difficult to express transgenes in the germline. The discovery by Kelly et al. (1997) that placing transgenes in the context of complex genomic DNA can circumvent this silencing (at least transiently) has made available transgenic approaches that previously were applicable only to genes expressed in somatic cells. In particular, using this approach, it is now possible to use mutant rescue assays to define functional domains of at least certain germline proteins, and to tag these proteins with green fluorescent protein to study their localization in vivo. Combined with traditional genetic and biochemical techniques, these new transgenic approaches promise to provide a wealth of new insights into the molecular mechanisms that launch the germline.

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