

Chromatin regulation during *C. elegans* germline development

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Recent studies in *Caenorhabditis elegans* implicate PcG- and NuRD-like chromatin regulators in the establishment and maintenance of germline–soma distinctions. Somatic cells appear to utilize NuRD-related nucleosome-remodeling factors to overwrite germline-specific chromatin states that are specified through PcG-like activities. The germline, in turn, may rely on an asymmetrically inherited inhibitor to prevent chromatin reorganization that would otherwise erase pluripotency.

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Abbreviations

CTD	carboxyl terminal domain of RNA polymerase II
ESC	Extra Sex Combs
E(Z)	Enhancer of Zeste
GCL	Germ-Cell-Less
NuRD	nucleosome remodeling and histone deacetylase
PcG	Polycomb group
TrxG	Trithorax group

Introduction

Given the obvious importance of reproduction in all organisms, the specification and development of the reproductive cell lineage called the germline is surprisingly diverse. For example, in the mouse, the germline is not predetermined in the embryo but instead is induced by several overlapping signals originating from the extra-embryonic tissues [1–3]. In response, primordial germ cells activate key germline genes such as *fragilis* and *stella* [4], some of which may directly promote the retention of pluripotency whereas others may primarily mediate cell–cell interactions and signal transduction. In other organisms such as *Caenorhabditis elegans*, *Drosophila* and *Xenopus*, the germ cell lineage is restricted by the presence of a specialized cytoplasmic region called germplasm [5–7]. Germplasm is initially localized at the posterior (*C. elegans* and *Drosophila*) and the vegetal pole (*Xenopus*) of the early embryo and is sequentially inherited by germline blas-

tomeres. While the embryonic origin of the germline differs considerably among organisms, it remains possible that universally conserved features of germline development do exist and are yet to be fully uncovered.

One good place to look for mechanistic similarities is at the level of the most basic property of germ cells, their pluripotency. ‘Pluripotency’ refers to an ability to produce all cell types in the body during development. From the viewpoint of embryogenesis, germ cells can be said to ‘retain’ pluripotency, as this ability is inherited from the initial zygote. Somatic cells, on the other hand, exhibit a greatly limited developmental potential. How this ‘potential’ is encoded in cells and, more importantly, how it is modified during development are fundamental and as yet poorly understood issues. One key mechanism that appears to stably specify differences in developmental potential involves modifications in chromatin architecture. Proteins with chromatin binding and modifying activities can define transcriptionally active and inactive chromatin domains that are stably inherited through cell divisions. In this review, we focus on recent evidence suggesting that *C. elegans* germ cell potential is specified, at least in part, through the action of chromatin modifiers.

MES proteins and germline-specific chromatin organization

Several recent papers provide molecular clues to how the *C. elegans* germline organizes its chromatin differently from somatic cells. Combining data from microarray analysis and immunofluorescence staining of various histone modifications, Kelly *et al.* [8*] report that the *C. elegans* X chromosome is subject to selective repression during much of germline development. It is well accepted that chromatin is organized by linker histones and other binding proteins, which in turn respond to differential modification of core histones, including phosphorylation, acetylation and methylation of H3 and H4 [9]. In the adult germline, histone modifications that are generally associated with active transcription, including acetyl-K8 (Lysine 8) H4 and methyl-K4 (Lysine 4) H3, are absent on the X chromosome in the mitotic and early meiotic zones. Instead, the X chromosome is selectively stained with an antibody that recognizes methyl-K9 (Lysine 9) H3, an epigenetic mark typical of silenced chromatin. Consistent with these observations, very few X-linked genes are expressed in the mitotic and early meiotic regions of the adult germline [10]. X-linked genes that do get expressed are transcribed within a small temporal window during late meiosis of oogenesis, which correlates well with the appearance of acetyl-K8 H4 and methyl-K4 H3 epitopes [8*].

Subsequently, the *mes-2*, *-3*, *-4* and *-6* genes were shown to encode components of the X-repression machinery [11**]. In the adult, the MES proteins are expressed in the germline, and mutants lacking maternal function of each *mes(+)* activity are defective in gametogenesis [12–14]. Interestingly, the *mes* sterile phenotype is exacerbated in animals carrying an extra copy of the X chromosome (XXX animals) whereas it is partially suppressed in those with a single X (XO animals) [15]. Fong *et al.* [11**] have found that one of the MES proteins, MES-4, is associated selectively with autosomes but not with X. Strikingly, MES-4 is found on all chromosomes in oocytes lacking *mes-2(+)*, *-3(+)* or *-6(+)* activity. Among the sterile animals that derive from these oocytes, some manage to produce early meiotic nuclei, in which X ectopically accumulates markers of active transcription, acetyl-K8 H4 and methyl-K4 H3 modifications. These results can be explained by proposing that, first, MES-4 inhibits the binding or function of yet unidentified repressors on autosomes (i.e. it acts as an activator) and, second, MES-2, *-3*, and *-6* prevent MES-4 from binding to X thereby leaving it unprotected and repressed. *mes-4* mutations cause the repressors to spread to all six chromosomes, which would in turn lead to the derepression of X if the repressors are limited in quantity. This model predicts that at the individual gene level, MES-4 and the other MES proteins may have distinct and even opposite effects on transcription. Consistent with this idea, mutations in *mes-2*, *-3* and *-6* but not *mes-4* can suppress male-specific developmental defects in certain mutant backgrounds [16**].

MES-2 and MES-6 are *C. elegans* homologues of *Drosophila* Enhancer of Zeste (E[Z]) and Extra Sex Combs (ESC), respectively, and together form a complex with MES-3, a novel protein [17]. E(Z) and ESC belong to PcG chromatin regulators, which participate in stable repression of Hox and other genes in *Drosophila* and vertebrates [18,19]. Similarly, *mes-2*, *mes-3*, and *mes-6* also repress Hox gene expression in *C. elegans* together with other global repression mechanisms [16**,20**]. Moreover, E(Z)/ESC-containing complexes isolated from fly and mammalian extracts exhibit histone H3 methyltransferase activity *in vitro* and promote the formation of heterochromatin domains *in vivo* [21–23]. These data, together with the uniform distribution of MES-2, *-3* and *-6* proteins in the germline nuclei, suggest that the MES-2/*-3*/*-6* complex also acts at selected autosomal loci to globally control transcriptional potential of the germline. These repressed domains may locally preclude MES-4 and/or additional transcriptional activators. Together, these processes may help to establish unique germline chromatin organization.

PIE-1 and transcriptional silencing in the germline

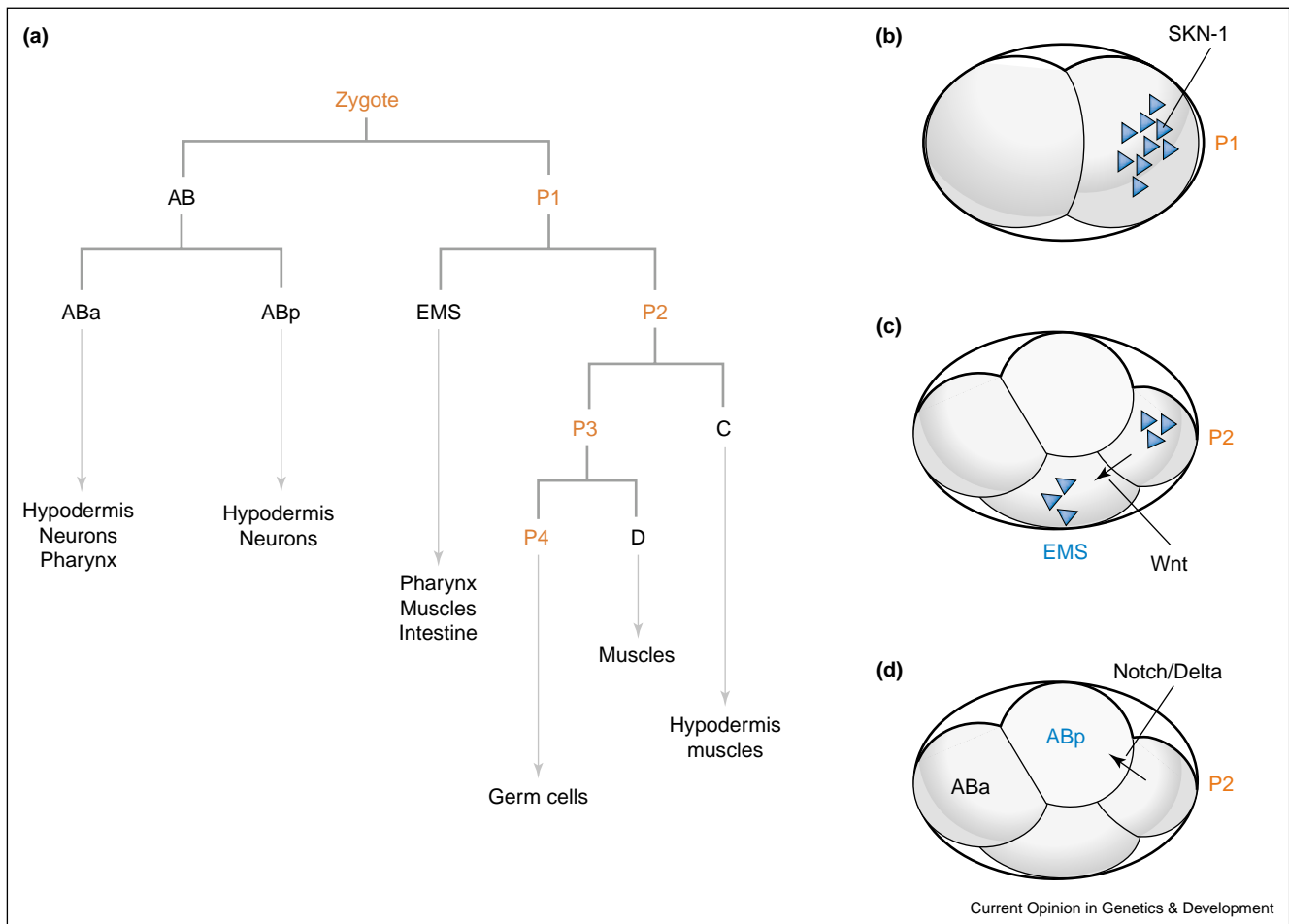
Once embryogenesis begins, the early *C. elegans* germline cells act in a manner analogous to stem cells; like stem

cells [24,25], these cells undergo asymmetric divisions to produce one daughter cell destined for differentiation and one daughter that retains germline potential. Interestingly, the early germline cells play an important role in directing the development of the neighboring somatic cells. For example, the 4-cell-stage germline cell called P2 signals via the Notch/Delta signaling pathway to specify the fate of the dorsal blastomere ABp (Figure 1) [26–28]. At the same time, P2 signals via a Wnt pathway and phosphotyrosine signaling pathway to direct the division orientation and differentiation of the ventral cell, called EMS (Figure 1) [29–33]. In addition to cell-surface signaling components, the early germline cells also contain nuclear-localized transcriptional regulators, which direct differentiation in somatic daughters of these cells. For example, the transcription factor SKN-1 is first synthesized in the germline cell P1, but its function is to promote somatic differentiation in the P1 granddaughter EMS [34,35]. The presence of differentiation-promoting factors in germline cells highlights the importance of mechanisms that protect the germline against inappropriate activation of these factors.

Among the candidates for factors that specify and protect the germline potential are the P granules and the PIE-1 protein, both of which are localized exclusively to germline blastomeres during early development. The P granules are composed of mRNAs as well as several protein components and thought to constitute *C. elegans* germlasm [36–39]. These cytoplasmic–perinuclear structures share conserved components with *Drosophila* polar granules, including VASA-like putative RNA helicases, whose homologues are essential for germ cell development in multiple organisms [40]. PIE-1 is a Cys-3-His zinc-finger protein that localizes to the early embryonic germline [41–43]. In *pie-1* mutants, the germline cell P2 differentiates into somatic tissues in response to transcriptional activators (e.g. SKN-1) that are normally present but inactive in the wild-type germline [41], indicating that PIE-1 plays an important role in protecting germline pluripotency.

How does PIE-1 repress somatic fates? A partial answer to this question comes from the finding that PIE-1 is essential for global transcriptional silencing in the early germline [43,44]. In wild-type embryos, a transcriptionally engaged form of RNA polymerase II — phosphorylated on Ser2 within the CTD repeats — is not detectable in the early germline, suggesting that mRNA transcription is largely absent in these cells. The germline eventually resumes transcription after the onset of gastrulation, and by this time, PIE-1 is no longer present in these cells. In the embryos lacking the *pie-1* function, the elongating form of polymerase II accumulates in early germline, as early as the 4-cell stage, accompanied by ectopic expression of zygotic mRNAs. Although molecular mechanisms by which PIE-1 elicits repression is yet to be identified,

Figure 1



C. elegans blastomere names and cleavage patterns during early embryogenesis. **(a)** In this abbreviated cell-lineage chart, branched bars represent cell divisions, and arrows indicate adult tissues that each cell lineage ultimately produces. Somatic blastomere names are indicated in black while the germline is indicated in red. Each of the germline divisions is asymmetric and produces one somatic daughter and one germline daughter. PIE-1 segregates with the germline daughter at each division. **(b–d)** The germline directs somatic differentiation through cell-autonomous and signal-induced mechanisms. The synthesis of the transcription factor SKN-1 (blue triangles in [b]) is activated in P1 from the maternally provided mRNA at the two-cell stage. SKN-1 then equally segregates between the two daughters of P1 (c) and directs mesodermal and endodermal differentiation in EMS but not in P2. At the four-cell stage (c), P2 controls the orientation and the outcome of the EMS division through a complex signaling mechanism, which includes MOM-2, a Wnt/Wg homolog, and SRC-1, a Src-related cytoplasmic tyrosine kinase. In response, EMS divides along the anterior–posterior axis of the embryo and produces two daughters of distinct fates. (d) P2 also expresses the Delta homolog APX-1, which functions through the GLP-1/Notch receptor present on the neighboring cell to activate the ABp-specific fate. This signal produces the asymmetry between the initially equivalent AB daughters, ABa and ABp.

recent evidence suggests that PIE-1 may target an early phase of transcription elongation, which depends on the activity of the P-TEFb CTD Ser2 kinase [45,46].

Germline cells inherit maternally produced mRNAs from the oocyte, which are sufficient to direct the germline-specific signaling, asymmetric-division and development. Thus, global transcriptional silencing in the zygotic germline is an efficient way to deter a variety of somatic differentiation signals that early embryonic germ cells may experience.

Chromatin regulation in somatic cells

If the zygote is pre-programmed for germline potential by MES proteins, its somatic descendants must establish chromatin states that favor somatic differentiation. This chromatin remodeling process should erase any pre-existing epigenetic marks that define the germline fate. Unhavaithaya *et al.* [47••] have identified a likely candidate for this chromatin-rewriting activity. This complex consists of at least three polypeptides encoded by the *hda-1*, *let-418* and *mep-1* genes. HDA-1 and LET-418 are highly homologous to subunits of the mammalian NuRD

(nucleosome remodeling and histone deacetylase) complex, the histone deacetylase HDAC1 and the ATP-dependent nucleosome remodeling factor Mi-2, respectively [48–50]. MEP-1 contains multiple Krüppel-class zinc fingers and has a close *Drosophila* homologue [51•]. Embryos deficient for *hda-1(+)* activity show poor embryonic differentiation and fail to hatch [52]. By contrast, embryos lacking *mep-1(+)* or *let-418(+)* activity are able to complete embryogenesis but die during the first stage (L1) of larval growth. Despite this difference, zygotic loss of *hda-1(+)*, *mep-1(+)* or *let-418(+)* each cause similar defects in vulval/hypodermal and germline development [47•,53,54], suggesting that the MEP-1, LET-418 and HDA-1 function together, at least some of the time.

Strikingly, the dying *mep-1* and *let-418* larvae contain numerous endodermal and ectodermal cells that express characteristics of germ cells [47•]. These include morphological similarities in cell shape and nuclear architecture as well as ectopic expression of at least three P-granule components, including a putative RNA-binding protein PGL-1 [55]. In the *mep-1* and *let-418* mutants, *pgl-1* and other germline-specific genes become ectopically activated during late embryogenesis, which coincides with the normal timing for the transcriptional activation of these genes in the germline. These observations suggest that somatic cells in the *mep-1* and *let-418* mutants retain germ cell potential and inappropriately respond to developmental cues that normally regulate germ cell specific gene expression.

The derepression of germ cell potential in the *mep-1* and *let-418* larvae is consistent with a role for the MEP-1/LET-418/HDA-1 complex in reprogramming the germline chromatin in somatic descendants of the zygote. As discussed earlier, the MES proteins participate in the germline-specific chromatin organization in the zygote, which appears to be essential for germ cell development. Thus, the failure to remodel chromatin and to remove the effects of the MES activities could underlie the ectopic germline differentiation observed in embryos lacking *mep-1(+)* and *let-418(+)* activities. Consistent with this idea, mutants lacking both *mep-1(+)* and each of the *mes(+)* activities exhibit a strong suppression of the soma-to-germline transformation [47•]. The somatic cells in these animals are morphologically normal and no longer express PGL-1. Furthermore, many such double mutant animals reach adulthood, indicating that MEP-1 and LET-418 are largely dispensable for larval growth as long as the MES function is not available. These findings suggest that the MES proteins, in addition to their role in repressing X chromosome, also have a more general role in promoting germline-specific gene expression. The observation that the *mep-1/let-418* phenotype is suppressed by mutations in all *mes* genes raises the possibility that for the germline-specific genes, MES-4 and the MES-2/-3/-6 complex both play an activator role.

Alternatively, the involvement of the MES-2/-3/-6 complex may be primarily to facilitate proper localization and function of MES-4.

PIE-1 and chromatin regulation

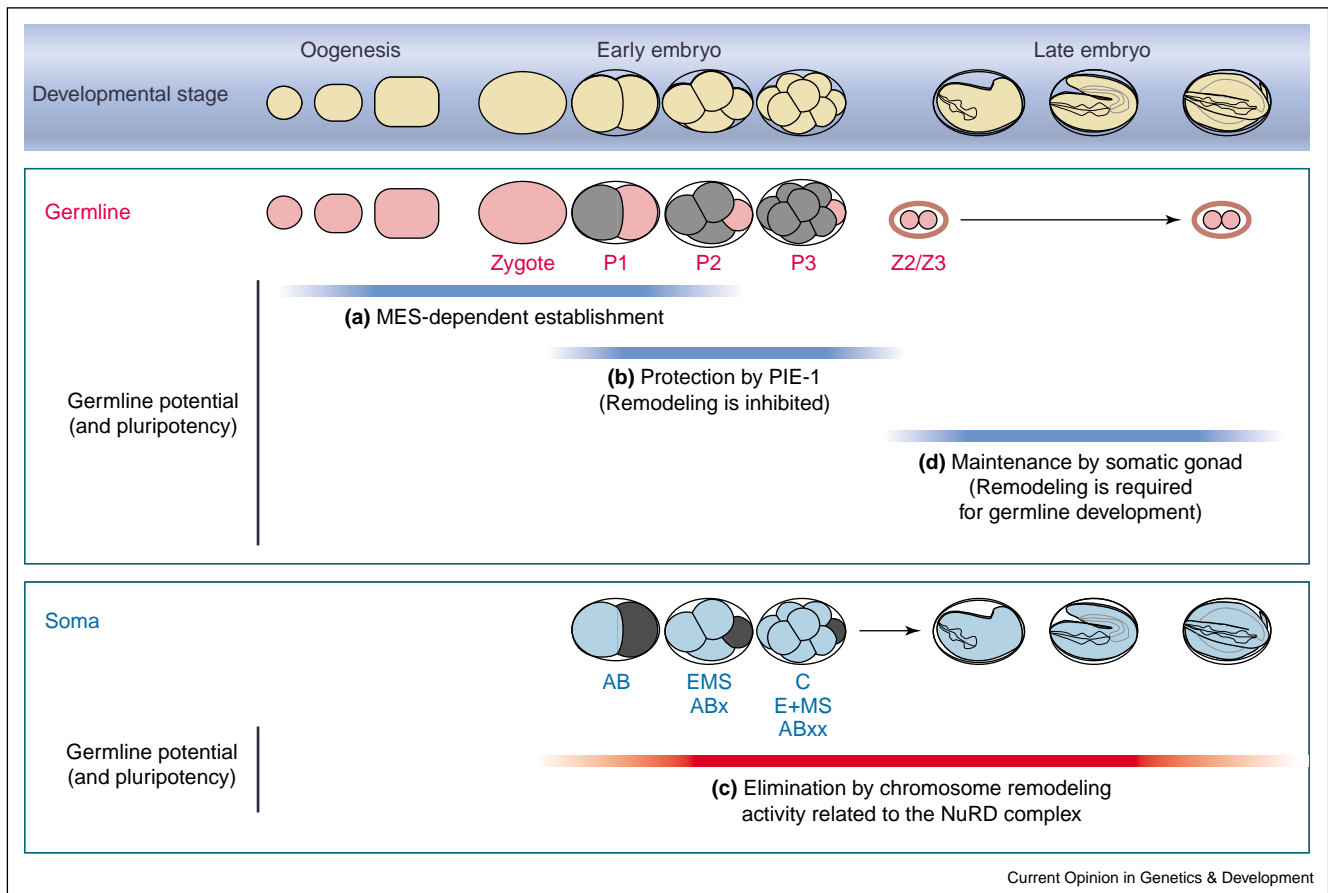
The observations described above suggest that the MEP-1/LET-418/HDA-1 complex prevents germline differentiation in somatic cells. Curiously, however, each subunit of this complex is also present in early germline cells. How, then, does the germline manage to escape chromatin remodeling? It is reasonable to assume that asymmetrically segregated factors in the germline provide ‘protection’ against the MEP-1/LET-418/HDA-1 complex. In fact, several lines of evidence suggest that none other than PIE-1 represses this complex [47•]. First, PIE-1 interacts with MEP-1 *in vitro* and associates with the MEP-1/LET-418/HDA-1 complex *in vivo*. Second, an *in vitro* histone deacetylase activity of HDA-1 can be inhibited by PIE-1. And third, expressing PIE-1 in somatic cells mimics several phenotypes of *mep-1* mutants, including the derepression of PGL-1 in the soma. Quantitative immunoprecipitation experiments suggest that the majority of MEP-1, LET-418 and HDA-1 proteins is complexed with PIE-1 in early germ cells. Thus, it is possible that PIE-1 can completely block the MEP-1/LET-418/HDA-1-dependent chromatin remodeling in the germline.

A model for the regulation of germline potential and pluripotency

The new information described here suggests the following model for how germ cell potential is established and maintained in the *C. elegans* germline (Figure 2). During late oogenesis and early embryogenesis, the MES proteins establish stable chromatin domains that permit pluripotency and promote germ cell specific development at later times. In the germline, PIE-1 blocks the MEP-1/LET-418/HDA-1 activity thereby preventing chromatin remodeling antagonistic to germline potential. In this way, PIE-1 contributes to long-term preservation of the germline potential. PIE-1 also blocks immediate differentiation through global transcription silencing. This function is necessary because the germline in the early embryo is exposed to various differentiation signals both through cell autonomous mechanisms and through cell-cell signaling. In somatic cells, by contrast, cell-fate-specific transcription is activated in the absence of PIE-1. The MEP-1/LET-418/HDA-1 complex also becomes active and inactivates MES-protein-dependent germline potential in these cells.

After the completion of gastrulation, primordial germ cells become encased in the somatic gonad. This event is also promoted by PIE-1, partly through post-transcriptional activation of NOS-2 synthesis [56•]. NOS-2, whose mRNA is maternally provided, is a *C. elegans* homologue of *Drosophila* NANOS and is involved in some aspects of

Figure 2



A model for how germline-soma distinctions may be established and maintained in *C. elegans* germline. This model contains the following proposals. **(a)** During oogenesis and early embryogenesis, the PcG-like and SET domain-containing regulators (MES proteins) establish germline-specific chromatin state. **(b)** This state is protected in early germline by PIE-1, which inhibits the chromatin-remodeling activity of the NuRD-related complex. PIE-1 also inhibits transcription and thus blocks immediate differentiation. **(c)** Somatic descendants of the germline activate the NuRD-related complex, which incapacitates the germline potential and allows soma-specific development. As these cells lack PIE-1, the NuRD-related complex could become active immediately following the division. Alternatively, the activation may require additional developmental cues. **(d)** Later germ cells, Z2 and Z3, are incorporated into the somatic gonad (brown oval), a process also promoted by PIE-1. The somatic gonad provides additional signals and physical protection essential for the germ cell specific development and for the maintenance of pluripotency. Like somatic development, germ-cell development requires stage-specific transcription and, thus, depends on the chromatin-remodeling activity of the NuRD-related complex. P1, P2 and P3 denote early germline. AB, EMS and C are somatic sister of P1, P2 and P3, respectively. ABx and ABxx refer to the daughters and granddaughters of AB whereas the daughters of EMS are called E and MS.

the germline-gonad interaction [38]. By controlling germ cell positioning, PIE-1 thus further promotes proper germ cell development and the maintenance of pluripotency.

PIE-1 is present in the germline until around the 100-cell stage, at which time transcription resumes in the germline. It could be expected that the MEP-1/LET-418/HDA-1 complex also becomes active in germ cells once PIE-1 is no longer available. The MEP-1/LET-418/HDA-1 complex may then positively contribute to germ cell development by permitting chromatin remodeling required for developmental transitions in the germline, for example, from mitosis to meiosis and from spermatogenesis to oogenesis. Consistent with the

latter possibility, the zygotic loss of *mep-1(+)*, *let-418(+)* or *hda-1(+)* causes sterile adults, with defects in the switch from spermatogenesis to oogenesis in hermaphrodites [51*].

Conclusions

Do these findings bring us closer to the universal germline mechanism? The finding that *mes* mutations suppress ectopic expression of germline genes in *mep-1* and *let-418* mutants is consistent with the model that the PcG-like MES-2/-3/-6 complex regulates chromatin structure both positively and negatively in the germline. Interestingly, although *Drosophila* PcG genes generally act antagonistically to *TrxG* genes and repress Hox gene

expression, several *PcG* mutations, including a loss-of-function mutation in the *mes-2* homologue *E(Z)*, can enhance phenotypes of some *TrxG* mutations [57], indicating that a subset of *PcG* proteins can promote gene expression under certain circumstances. Furthermore, a strong temperature-sensitive allele of *E(z)* causes decreased binding of both *PcG* and *TrxG* proteins to polytene chromosomes [58] and leads to either repression or derepression of the same Hox genes depending on the timing of the temperature shift [59]. Thus it will be interesting to determine whether *PcG* proteins in other animals may also positively regulate germline development.

Although PIE-1 contains conserved protein motifs, a functional homologue of PIE-1 has yet to be identified in other animals. Transcriptional silencing in the early germline is also observed in *Drosophila*, [44,60] that requires Germ-Cell-Less (GCL), an inner nuclear pore protein localized exclusively to the germline [60,61]. A mouse GCL homologue shows a transcriptional repressor activity [62,63], and nuclear pore proteins in *C. elegans* are also implicated in the regulation of germline potential [64], raising the possibility that some aspects of the silencing mechanism may be universally conserved in the germline of many animals. In these animals, transcriptional repression may be limited in its extent. For example, the cells selected as primordial germ cells in the mouse embryo selectively repress a set of *Hox* genes expressed in neighboring cells, and this repression is likely a key mechanism by which the germ cells escape from expressing the mesodermal fate [4].

The mechanisms of germline specification appear to have diverged considerably during evolution. Nonetheless, some molecular features may be universally important for the maintenance of pluripotency. One feature that is likely to be conserved in many organisms is the way in which chromatin is reorganized in early somatic cells. This chromatin-remodeling mechanism acts primarily to enforce stable and progressive differentiation during multicellular development but can also serve to erase pluripotency at the beginning of soma-germline separation. Consistent with this view, the repression of pluripotency in the *Arabidopsis* root requires the LET-418/Mi-2 homologue, PICKLE [65,66]. Remarkably, excised root tissues from the *pickle* mutant plant can reiterate embryonic development and produce an entire plant without any experimental manipulation. Thus, PICKLE, and presumably chromatin remodeling mediated by this factor, is critical for the suppression of the germline potential and pluripotency in somatic tissues of the plant. These findings indicate that the regulation of chromatin remodeling is extremely important for the maintenance of somatic differentiation and that loss of these activities can lead to reversion to a previously specified developmental potential.

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