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REVIEW

Repression of somatic cell fate in the germline

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Abstract Germ cells must transmit genetic information across generations, and produce gametes while also maintaining the potential to form all cell types after fertilization. Preventing the activation of somatic programs is, therefore, crucial to the maintenance of germ cell identity. Studies in *Caenorhabditis elegans*, *Drosophila melanogaster*, and mouse have revealed both similarities and differences in how somatic gene expression is repressed in germ cells, thereby preventing their conversion into somatic tissues. This review will focus on recent developments in our understanding of how global or gene-specific transcriptional repression, chromatin regulation, and translational repression operate in the germline to maintain germ cell identity and repress somatic differentiation programs.

Keywords Reprogramming · Totipotency · Differentiation · Polar granules · Histones · Teratoma

Abbreviations

PGCs	Primordial germ cells
RBPs	RNA-binding proteins
E	Embryonic day
BMP	Bone morphogenetic protein
RNA Pol II	RNA polymerase II
CTD	C-terminal domain
Ser2	Serine2
GSC	Germline stem cells
MAPK	Mitogen-activated protein kinase
DTC	Distal tip cell
EGA	Embryonic gene activation

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Introduction

In all metazoans, germ cells have the unique property of being immortal, in that they are passaged from one generation to the next, and totipotent, having the potential to give rise to all tissue types. Recent genetic studies in model organisms have allowed significant advances in understanding the mechanisms that normally prevent the loss of germ cell identity. In addition, the development of new technologies, including high-throughput sequencing, has allowed the characterization of gene expression profiles from small amount of tissues, including germ cells. Altogether, these studies have contributed to an understanding of the essential role that transcriptional repression and chromatin modifications occupy during primordial germ cell (PGCs) specification during embryogenesis. In later stages of germline development, RNA-binding proteins (RBPs) have emerged as additional key players in the repression of somatic genes, thereby preventing reprogramming of germ cells into somatic cell types. An understanding of the mechanisms at work in reprogramming germ cells into somatic cells will answer fundamental questions about how germ cell identity and plasticity are maintained. These studies have implications beyond the field of germ cell research, since germ cells are closely related to the extensively studied in vitro pluripotent stem cell models. This review is focused on different mechanisms involved in repressing somatic gene expression, both during the specification of PGCs in embryogenesis, and in the adult germline. We summarize genetic control of germ cell specification, proliferation and differentiation in

Caenorhabditis elegans, *Drosophila melanogaster* and mouse, underlying similarities and differences in the strategies these animals use to protect germline identity.

PGC specification mechanisms

Segregation of germline precursors from somatic cells occurs early in embryogenesis, when PGCs segregate from the surrounding mitotic blastomeres (Fig. 1). In model organisms, two distinct mechanisms have been identified that specify germ cell fate. In flies and worms, maternally synthesized germ plasm organized in specialized ribonucleoprotein organelles is deposited into the egg during oogenesis (reviewed in [1]). These organelles, known as P granules in *C. elegans* and polar granules in *Drosophila,* carry germline-specific, mRNA–protein complexes required for multiple aspects of germ cell fate. In contrast, in mammals, germ cells are specified independently of preexisting maternal information and in response to instructive signaling during embryonic development [2–8].

Suppression of somatic fate during germ cell specification

In the *C. elegans* one-cell embryo (P_0), P granules are maternally inherited and dispersed throughout the cytoplasm (Fig. 1). During the first four asymmetric divisions of the embryo, the P granules asymmetrically segregate into the P lineage (reviewed in [9]). Germ cell specification is completed at approximately the 100-cell stage when the germline founder cell P_4 forms equivalent PGCs, Z2 and Z3, through a symmetric division. Z2 and Z3 resume mitosis once the L1 larvae start feeding, expanding their number before entering meiosis and thus allowing the production of a large number of gametes in the adult. Most

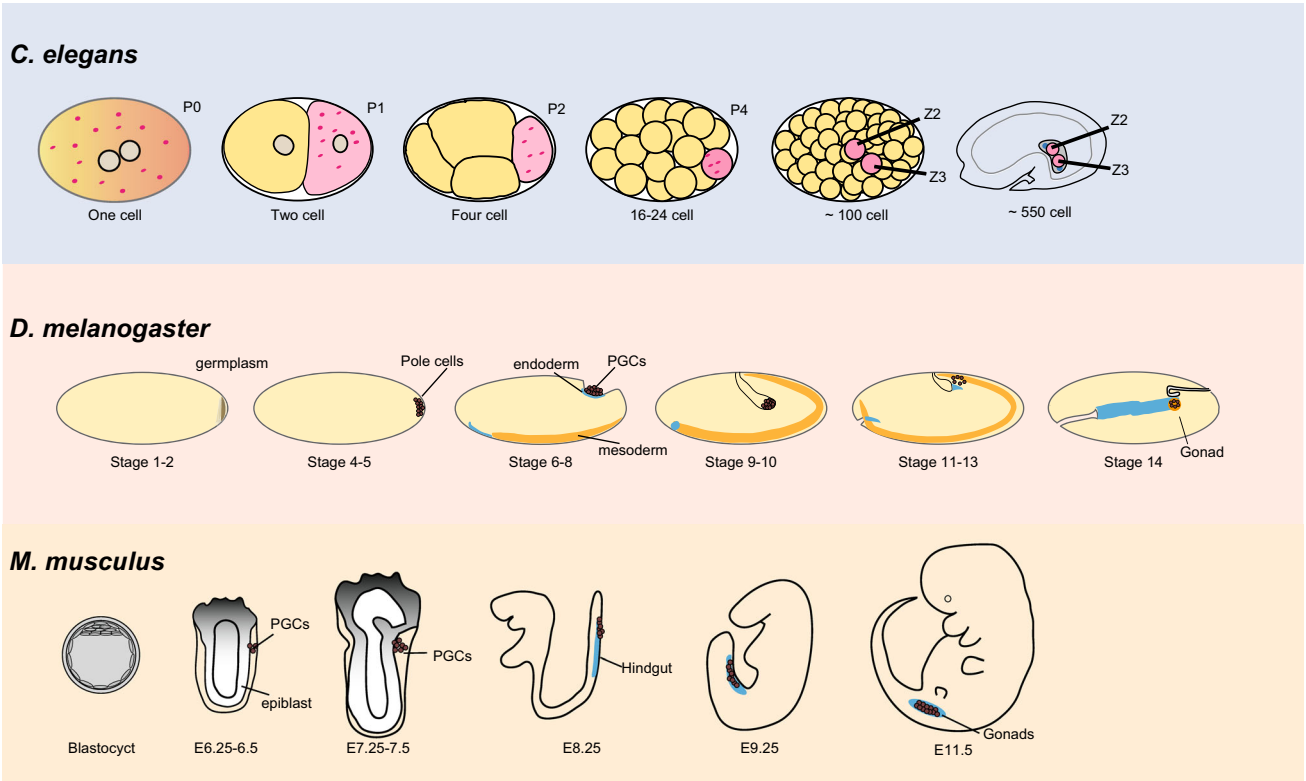

Fig. 1 Overview of germ cell development in *C. elegans*, *Drosophila* and mice. In *C. elegans*, P granules (*red speckles*), consisting of RNA-binding proteins and RNA of maternal origin asymmetrically segregates in the one-cell blastomere. Asymmetric partitioning, established within the P1 blastomere (*pink*), is maintained through successive divisions until P4 divides symmetrically to give rise to Z2 and Z3. In *Drosophila*, germ plasm is formed during oogenesis and assembles in the posterior pole region. This maternal plasm is incorporated into pole cells, which actively cross through the midgut epithelium, migrate towards the mesoderm, and join with somatic gonadal cells to form the final gonads. In mice, the germline is induced by extra-embryonic ectoderm signaling, as well as signals from the visceral endoderm to a set of epiblast cells. This signaling induces Blimp1 expression, leading to PGC proliferation and migration to the posterior extra-embryonic mesoderm. PGCs then migrate back into the embryo with the endgut and continue this movement eventually taking residence in the genital ridge (somatic gonad)

P-granule components are RBPs, including the germline helicases GLH-1-4, related to *Drosophila* Vasa, the P granule assembly proteins PGL-1, PGL-2 and PGL-3, and the meiotic regulators OMA-1 and OMA-2 [9] (Table 1). Depletion or loss of P granule components in parents results in sterile progeny, although frequently two or more redundant family members must be eliminated to achieve complete penetrance [10–12]. However, mutant embryos that fail to segregate P granules asymmetrically during embryogenesis, but instead segregate specific germ plasm components both into somatic and germ cell lineages, develop into fertile adults [13]. Therefore, while germ plasm components are central to germline development, germline-specific segregation of P granules is not essential to distinguish germline from soma in *C. elegans*.

In *Drosophila*, the location of germ cells in the embryo is already established during oogenesis, when maternally synthesized germ plasm (also referred to as pole plasm) components assemble in polar granules at the posterior pole of the oocyte (Fig. 1) [14, 15]. Polar granules guide both abdomen patterning and germ plasm assembly during embryogenesis. During nuclear division cycles 8–10, the nuclei migrate to the cortex, where they continue to divide. Shortly after reaching the cortex, nuclei that have migrated into the germ plasm initiate a budding of the plasma membrane that encapsulates them and the surrounding cytoplasm to form the "pole cells," or germ cell progenitors [16]. The polar granule component Oskar is necessary and sufficient to assemble germ plasm and induce germ cell fate through the recruitment of additional maternally provided polar granule proteins, including Vasa, Tudor and Valois [15, 17]. These, in turn, regulate the localization and translation of a second class of pole plasm components including *nanos* and *polar granule component* (*pgc*) that are required for pole cell formation, specification and function (Table 1) [15, 17–20].

Mouse PGCs arise from epiblast cells that are not lineage restricted, as they give rise to PGCs as well as somatic cells, including the extra-embryonic mesoderm [2]. PGC specification occurs early during development in the proximal epiblast cells, a subset of otherwise pluripotent progenitors of the early embryo, at embryonic day (E) 6.25 just before the onset of gastrulation (Fig. 1, reviewed in [21, 22]). Bone morphogenetic protein (BMP) signaling from the extra-embryonic tissue to the proximal epiblast initiates the germ cell-specific program by switching on the expression of a transcriptional network including BLIMP1, PRDM14 and AP2γ (Table 1). By E7.25, approximately 30–40 founder PGCs are established. Irrespective of the apparent differences in how the germ cell lineage becomes distinct from somatic cells, one conserved aspect of germ cell specification during early embryogenesis is the active repression of programs of somatic differentiation. This is achieved by (1) global transcriptional repression through inhibition of RNA polymerase II (RNA Pol II) in *C. elegans* and *Drosophila*, (2) transcriptional reprogramming by a tripartite transcription factor network composed of BLIMP1, PRDM14 and AP2γ in mouse and (3) chromatin-based repression mechanisms in all three species.

C. elegans PGCs inhibit RNA Pol II through OMA1/2 and PIE-1

In *C. elegans*, after each asymmetrical division of the early embryo, embryonically transcribed RNAs are detected in somatic, but not P lineage germline blastomeres [23, 24]. This difference is due to transcriptional repression in the germline blastomeres. Their somatic sisters, by contrast, undergo rapid transcriptional activation and lineage-specific differentiation, requiring that any repressive mechanism operating in the P lineage be readily reversible. PGCs remain transcriptionally silent until approximately the 100-cell stage, after gastrulation begins [23]. Transcriptional repression in the P lineage depends on two groups of maternally supplied, zinc finger proteins, OMA-1/2 and PIE-1, whose partially redundant functions are temporally and spatially restricted by their respective expression patterns.

OMA-1 and OMA-2 inhibit TAF-4

OMA-1 and -2 are closely related cytoplasmic proteins detected in P_0 and P_1, and rapidly degraded after the first mitotic division [25, 26]. Combined depletion of OMA-1 and OMA-2 leads to oocyte maturation defects and embryonic lethality. In P_0 and P_1, OMA-1 and OMA-2, globally repress transcription initiation by binding to TAF-4, a key component of the RNA Pol II pre-initiation complex [27]. OMA-1/2 binding interferes with TAF-4 and TAF-12 dimer formation and results in TAF-4 sequestration to the cytoplasm, thereby repressing transcription. This interaction occurs only in embryos, where it is facilitated by phosphorylation of OMA-1 and OMA-2 by the MBK-2 kinase. Phosphorylation also marks both OMA proteins for subsequent degradation [26]. This releases TAF-4 for binding by TAF-12, followed by translocation of the TAF-4/12 heterodimer to the nucleus and relief of transcriptional repression (Fig. 2).

PIE-1 inhibits RNA Pol II

The PIE-1 zinc finger protein, although present at high levels from P_0 to P_4, is only essential for transcriptional repression in P_2 and P_3, and partially in P_4 [23, 24]. *pie-1* mutant embryos produce too many pharyngeal and

Table 1 Proteins and complexes involved in somatic repression in germ cells

Organism/developmental stage	Name	Molecular identity/function	Functional description	References
C. elegans/developing germline	OMA-1/OMA-2	*C. elegans*-specific Zn finger proteins; redundant paralogs	Globally repress transcription initiation by sequestering the RNA Pol II pre-initiation complex subunit TAF-4 in the cytoplasm	[27]
	PIE-1	*C. elegans* specific Zn finger protein	In *pie-1* mutant embryos, the P2 blastomere is transformed into a somatic blastomere and aberrant transcription is observed; PIE-1 inhibits the kinase complex required for RNA Pol II phosphorylation and activation	[23, 24, 28–30, 32]
C. elegans/adult germline	MES-2/6 (PRC2)	H3K27 methyltransferase; required for H3K27me2 and –me3 in the germline and soma	RNAi knock-down results in germline expression of somatic genes following ectopic expression of CHE-1 or HLH-1	[155, 156]
	LIN-53 (RbAp48)	Histone chaperone; required for H3K27me3 in the germline	RNAi knock-down results in germline expression of somatic genes following ectopic expression of CHE-1 or HLH-1	[154]
	SPR-5 (LSD1)	H3K4 demethylase	Null mutant show transgenerational loss of fertility and germline transdifferentiation in sterile animals	[157]
	SET-2 (SET1)	H3K4 methyltransferase; required for H3K4me2 and -me3 in the germline	Null mutants show transgenerational loss of fertility and germline transdifferentiation in sterile animals; required for germline repression of somatic genes including transcription factors involved in terminal differentiation of neuronal cell fate; reorganization of the epigenetic landscape in *set-2* mutant germlines	[74, 114]
	WDR-5.1 (WDR5)	Required for H3K4me2 and -me3 in the germline	Null mutants exhibit transgenerational loss of fertility and germline transdifferentiation into soma; required for germline repression of somatic genes including transcription factors involved in terminal differentiation of neuronal cell fate	[114, 158]
	HRDE-1	Ago family protein/nuclear RNAi component	Triggers heterochromatinization through the deposition of H3K9me3 at targeted loci; null mutants show transgenerational loss of fertility and germline transdifferentiation in sterile animals	[114, 159]
	GLD-1	RNA-binding protein with KH domains/translational regulation	Required for meiotic progression of differentiating germ cells and translational repression of *pal-1* and *cye-1* mRNA; precocious onset of embryonic gene activation (EGA) in *gld-1* mutant germlines	[112, 116]
	MEX-3	RNA-binding protein with KH domains/translational regulation	Translational repression of *pal-1; t*ransdifferentiation observed only in *gld-1mex-3* germlines	[112]
	LIN-41	TRIM-NHL family protein/translational regulation	Precocious onset of embryonic gene activation in *lin-41* mutant germlines; PAL-1 is required for transdifferentiation of *lin-41* mutant germlines into muscle	[113]

Table 1 continued

Organism/developmental stage	Name	Molecular identity/function	Functional description	References
Drosophila/developing germline	Su(var)3-3 (LSD1)	H3K4 demethylase	Mutants show increased H3K4me2; required to maintain repression of developmental genes	[69, 83]
	Pgc	Drosophiladae specific	Inhibits the kinase complex required for RNA Pol II phosphorylation and activation	[24, 42, 49]
	Gcl	Nuclear pore-associated protein	Transcriptional repression by an unknown mechanism	[43]
	Nanos (NANOS1,-2,-3)	RNA-binding protein	Pole cells in *nos* mutant embryos fail to establish and/or maintain transcriptional quiescence; partially conserved role in repressing somatic program in mammalian counterparts	[49, 168]
Drosophila/adult germline	E(Z) (EZH2)	H3K27 histone methyltransferase	Excessive number of cells expressing the somatic marker Zfh-1 in E(Z) mutant testes, non cell-autonomous role in somatic gonadal cells, E(Z) might regulate signalling pathways involved in the proper communication between the germline and its cellular microenvironment	[118]
Mouse/developing germline	BLIMP1/PRDM1	Transcriptional repressor with a SET domain and Zn finger motifs	Involved in a complex transcriptional reprogramming during PGC specification that results in expression of pluripotency genes and repression of somatic genes. Directly binds to its targets	[53–56]
	PRDM14	Transcription factor with multiple Zn finger motifs	Repression of the somatic mesodermal program in PGCs	[59, 60]
	AP2γ/TFAP2c	Transcription factor	Downstream target of BLIMP1	[61]
Mouse/*a*dult germline	DND1	RNA-binding protein	Dramatic increase in testicular teratomas when mutated; abnormal expression of meiotic proteins and pluripotency factors found to correlate with teratoma formation	[133–135]
	DAZL	RNA-binding protein	Binds to and suppresses expression of pluripotency genes including suz12 (core component of PRC2)	[116, 137]

For conserved proteins or complexes, the mammalian counterpart is given in parenthesis below the *C. elegans* or *Drosophila* name

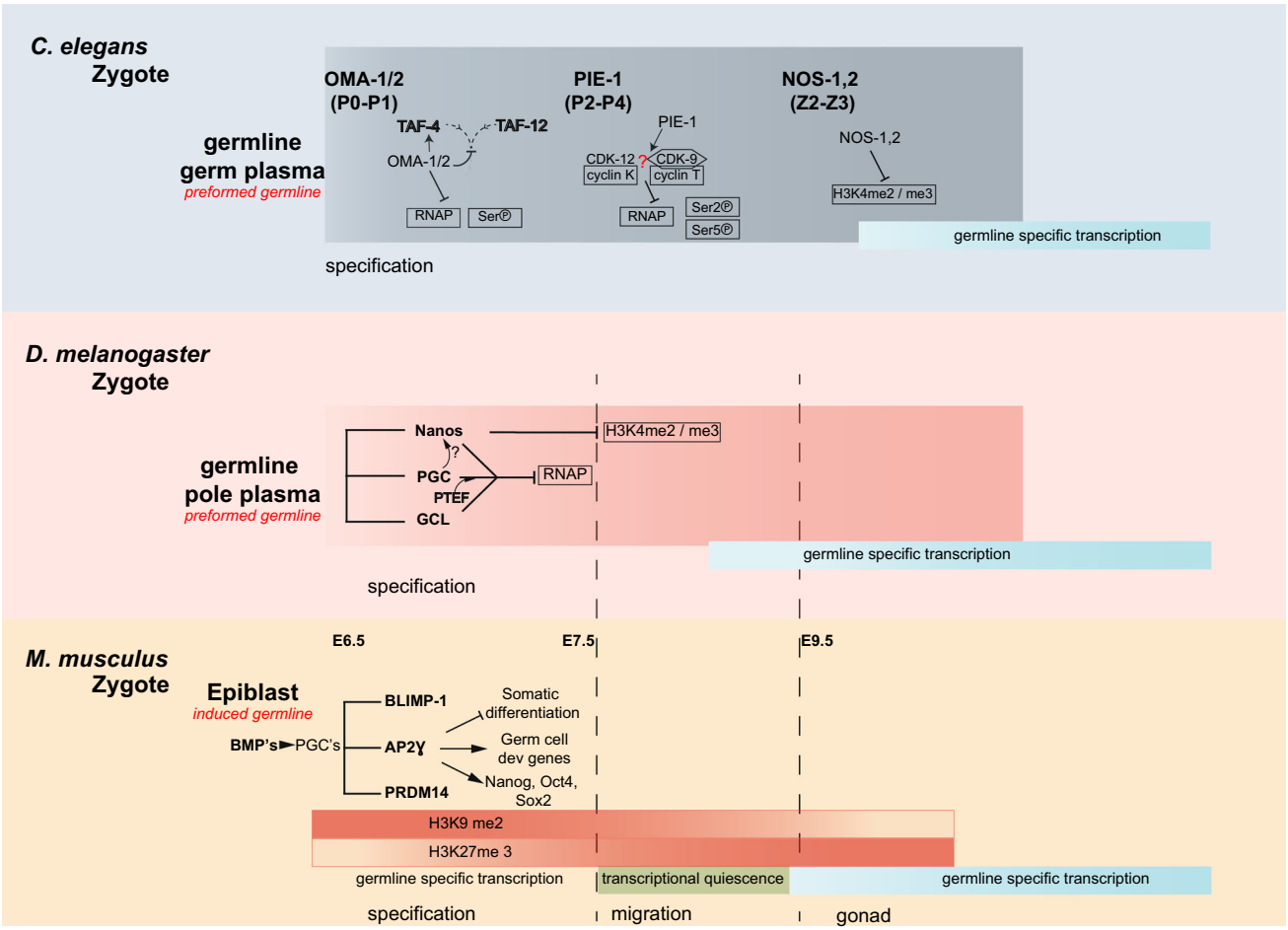

Fig. 2 Germ cell development requires precisely timed mRNA transcription as well as repression. In *C. elegans* and *Drosophila*, which have a preformed germline, the earliest steps in developmental specification rely upon transcriptional repression provided by the maternal elements delivered in the germ plasm and pole plasm. In the worm, this initial repression derives from the interaction of the proteins OMA-1 and 2 blocking TAF-4 dimerizing with TAF-12. Sequestering of TAF-4 inhibits RNA PolII initiation. OMA1/2 is degraded in P1, freeing TAF-4 to heterodimerize with TAF-12. This repression relay is taken up by PIE-1, which may act by inhibiting Pol II CTD phosphorylation. Whether PIE-1 acts through specific CDK complexes in the germline, and how this influences Ser-2P and Ser-5P, remains to be established (see text for details). Degradation of PIE-1 in Z2/Z3 correlates with the phosphorylation of Ser-2 and the re-establishment of transcriptional elongation. NOS-1/2, present in Z2 and Z3, inhibit methylation of H3K4 (a mark of transcriptionally active chromatin), contributing to the formation of repressive chromatin in these cells. In flies, three pole plasm proteins, PGC, nanos and GCL are the principle actors in transcriptional repression. PGC which interacts with P-TEF, thereby inhibiting transcription, may also be required to maintain adequate level of NOS in PGCs. NOS also inhibits the accumulation of H3K4me2 and me3 in migrating PGCs. The mechanism of action of GCL remains unclear. In mouse, the germline is induced by BMP signaling from extra-embryonic tissues, inducing specification of a subset of epiblast cells into PGC. Germline specification relies on a transcriptional network consisting of PRDM14, BLIMP1 and AP2ϒ, resulting in the repression of somatic transcripts, and activation of pluripotency genes and germline developmental genes. Later in development, as PGCs start migrating, large scale chromatin remodeling results in loss of H3K9me3, which is replaced by another repressive mark, H3K27me3

intestinal cells due to transformation of the P_2 germline blastomere into a somatic blastomere, like its sister EMS [28]. This cell fate transformation depends on the maternally inherited SKN-1 transcription factor, which is present in both P2 and the somatic blastomere EMS. Repression of transcription could, therefore, protect the germline blastomere from transcription factors like SKN-1 and somatic differentiation [23]. In embryos derived from *pie-1* mutants, zygotic transcripts are aberrantly present in the embryonic germ cell precursors, and the germline does not develop [23, 29], showing that PIE-1 mediated transcriptional silencing is in fact essential for germline specification.

Studies using *C. elegans* embryos as well as mammalian tissue culture cells have shown that PIE-1 can repress transcriptional elongation [30–32]. Progression from transcription initiation to elongation requires the p-TEFb kinase complex CDK-9/cyclin T [33], which binds to the

heptapeptide repeats of the RNA Pol II C-terminal domain (CTD), phosphorylating serine 2 (Ser2-P) of the CTD. A region of PIE-1 resembling the CTD heptapeptide sequence serves as a competitive inhibitor of Ser2-P [30, 32]. Based on the observation that PIE-1 is degraded at the birth of Z2/Z3, and degradation of PIE-1 in Z2/Z3 correlates with Ser2-P appearance in these cells, a model has been proposed whereby PIE-1 represses transcriptional elongation by interacting with TEFb and sequestering it away from RNA Pol II [30, 32]. However, recent data suggests that this model may need to be revised. Using immunofluorescence and genetic analysis, the Kelly lab [34] recently showed that while loss of CDK-9 from somatic cells abolishes Ser2-P in these cells, in the germline Ser2-P is mostly dependent on CDK-12, another Ser2 kinase which acts in complex with cyclin K upstream of CDK-9 [35, 36]. While loss of CDK-9 resulted in sterility, loss of CDK-12 and the disappearance of bulk Ser2-P specifically from germ cells had little impact on germline development or function. These results raise the possibility that neither CDK-12 nor bulk Ser2-P is crucial for germline transcriptional activity or development [34, 37]. However, it cannot be excluded that the small amount of Ser2-P (<5 %) remaining in the germline after CDK-12 depletion may represent CDK-9-dependent Ser2-P that occurs in a subset of loci that are essential for germ cell development and viability, explaining explain the requirement of CDK-9 for fertility. In addition, in the germline PIE-1 may regulate Pol II by inhibiting multiple CTD kinases, consistent with previous studies showing that PIE-1 also inhibits Ser5 phosphorylation by CDK-7 [31]. Future tissue-specific genome-wide studies should help to understand how Pol II regulation differs between the germline and soma.

Drosophila PGCs block transcription through inhibition of p-TEFb

Following precocious cellularization, *Drosophila* PGCs remain transcriptionally quiescent until the onset of gastrulation, when they associate with the gut primordium (stage 7, Fig. 1) [24]. The establishment of transcriptional quiescence in fly PGCs is mediated by at least three pole plasm determinants, *germ cell-less* (*gcl*), *polar granule component* (*pgc*), and *nanos* (*nos*) [38–42]. These three maternal factors contribute to the transcriptional quiescence of PGCs through independent or partially overlapping mechanisms.

Pgc is essential for repressing CTD Ser2-P in newly formed pole cells

Pgc is a short 71-amino-acid protein conserved only among *Drosophila* species [39, 42]. Like PIE-1 in *C. elegans*, Pgc inhibits Ser2-P of CTD repeats of RNA Pol II by physically interacting with the P-TEF kinase and inhibiting its recruitment at transcription sites [43]. In embryos lacking Pgc, newly formed pole cells are unable to silence the transcription of somatic genes. Conversely, ectopic expression of Pgc in the anterior of the embryo as well as in *Drosophila* S2 cells suppresses CTD Ser2-P and downregulates the transcription of terminal group genes such as *tailless* (*tll*) and *huckebein* [41, 43], showing that Pgc is sufficient to downregulate Ser2-P. *Pgc*$^-$deficient pole cells degenerate from stage 10 onwards, and few or no pole cells coalesce into the gonads. Consequently, the majority of *pgc*-deficient embryos develop into sterile adults. Inhibition of RNA Pol II phosphorylation may, therefore, represent a common mechanism during germ cell specification in *C. elegans* and *Drosophila*.

Germ cell-less (Gcl) is a nuclear pore-associated protein implicated in the earliest stages of transcriptional repression, before cellularization of the pole cells [41]. In embryos lacking maternally contributed *gcl* pole cells are completely absent, or greatly reduced in number [44]. The transcriptionally repressive effect of GCL does not appear to be global, but rather specific to a subset of somatic genes [41]. Therefore, whether Gcl acts directly on RNA Pol II activity remains an open question. In early stages, Ser2-P is detectable in interphase pole cells in both wild-type and mutant embryos, while at later stages, Ser2-P is repressed in the few pole cells that form in *gcl* mutants, indicating that Gcl does not act by suppressing Ser2-P. Since GCL localizes to the nuclear envelope [45], a hypothesis that remains to be tested is whether it could accomplish transcriptional repression by anchoring chromatin to the repressive environment of the nuclear periphery through protein binding partners [41, 46].

Pgc maintains Nos levels in germ cell precursors

While in *pgc* and *gcl* mutants the number of PGCs is substantially reduced, functional germline stem cells (GSC) are formed. By contrast, in *nos* mutants, PGCs fail to maintain PGC identity, and never develop into functional GSCs [47, 48]. Pole cells in *nos* mutant embryos fail to establish and/or maintain transcriptional quiescence and ectopically express the sex determination gene Sex-lethal (*sxl*) and the segmentation genes *fushi tarazu* and *even-skipped* [40]. *nos* mutant PGCs can be partially rescued by mutations in *sxl*, further supporting the importance of *nos*-dependent transcriptional silencing. While Pgc specifically inhibits transcription elongation-dependent Ser2-P, *nos* appears to downregulate transcription in PGCs at an earlier step, since in addition to Ser2-P, Ser5-P phosphorylation, associated with Pol II initiation, is also elevated in *nos* mutant PGCs [49]. Recent reexamination of the function of

pgc in germline development has shown that a substantial fraction of the PGCs in *pgc* mutant embryos have greatly reduced levels of Nos protein. This correlates with elevated Ser-5P, and several other characteristic of *nos* mutant PGCs, including failure to form GSC. These results suggest that *pgc* may play an important role in ensuring the functioning of *nos* in germline development [50]. Consistent with such a function, PGCs in *pgc* mutant embryos were partly rescued by supplying Nos. Whether maintaining high levels of Nos activity in a subset of PGCs is the primary role pf *pgc*, or whether *pgc* has additional independent functions that are important for the specification and development of PGCs remains an open question.

Transcriptional repression of the somatic program in the mouse germline

Unlike *C. elegans* and *Drosophila*, mouse PGC specification does not depend on global transcriptional repression, and PGCs remain positive for Ser2-P and Ser5-P. Instead in mouse, PGC specification relies on selective inhibition of somatic transcriptional programs by mammalian specific factors.

Blimp1 is a key regulator of primordial germ cell (PGC) specification

Single-cell analysis of cDNA isolated from founder PGCs (E7.5) and their somatic neighbors first showed that PGCs are characterized by exclusive expression of the nuclear cytoplasmic shuttling protein stella (also known as *Dppa3* or *Pgc7*) and the transmembrane protein fragilis *(*also known as *Ifitm3* or *mil-1)*, and specific repression of *Hox* genes, which are highly upregulated in somatic mesodermal neighbors [51, 52]. Subsequently, *Blimp1* (B lymphocyte induced maturation protein, also known as *Prdm1*), encoding a transcriptional repressor with a SET domain and Krüppel-type zinc fingers, was identified as a key factor marking the origin of PGCs [53, 54]. In *Blimp1* mutants, only a few PGC-like cells are formed, and these show inconsistent repression of Hox genes and activation of some of the PGC-specific genes such as *stella*, indicating that transcriptional control mediated by BLIMP1 plays a critical role in PGC specification [53]. In wild-type animals at early stages (E7.25), a proportion of *Blimp1*-expressing cells show developmental heterogeneity, expressing *Hoxb1* but not *Sox2*, a key pluripotency gene [55]. Since at later stages (E8.25) *Blimp1*- and *stella*-positive PGCs instead show consistent repression of the *Hox* genes and expression of *Sox2*, the Saitou lab proposed that *Blimp1*-positive cells may initially have properties more similar to somatic mesodermal neighbors, but specifically turn off the somatic program and concomitantly reacquire pluripotency, a salient feature of PGCs [55]. The Saitou lab then carried out more comprehensive genome wide analysis of single-cell cDNAs derived from PGCs at different stages of germ cell specification (E6.25-E8.25 with 12 h intervals) to confirm this model [56]. They showed that in PGCs, Blimp1 is required both for repressing somatic genes, including those associated with mesodermal development (e.g., *Hoxb1*, *Snail*), and promoting expression of pluripotency genes (*Sox2* and *Nanog*) as well as germ cell development genes (*Dnd1*, *Kit*, *Blimp1*). In addition, BLIMP1 was also found to promote S phase and DNA methylation.

The fact that in somatic cells, BLIMP1 acts as a potent transcriptional repressor by binding to specific regulatory sequences [57] suggested that it could also play a direct role in repressing somatic genes in PGCs. However, the fact that PGCs are relatively rare, difficult to culture, transfect and manipulate, hindered biochemical approaches to directly test how BLIMP1 functions in the repression of somatic genes in PGCs. By expressing a BLIMP1–EGFP fusion protein in an in vitro specification system consisting of P19 embryonal carcinoma cells (P19EC), which originate from postimplantation epiblast cells, the Surani lab was able to carry out ChIP sequencing (ChIP-seq) experiments using an EGFP antibody to identify BLIMP1 bound genes [58]. These studies revealed BLIMP1 binding peaks for 4389 protein-coding and 313 non-coding genes. Notably, BLIMP1 was found to bind to *T-Brachyury*, *Eomes* and the entire *Hox* gene loci, genes previously identified as upregulated in the absence of BLIMP1 [53, 56]. Functional category analysis revealed a striking enrichment of BLIMP1 binding to genes encoding transcriptional regulators and genes regulating developmental processes, revealing that BLIMP1 binds directly to repress somatic and cell proliferation genes.

BLIMP1 acts with PRDM14 and TFAP2c in a tripartite network of transcriptional repressors required for PGC specification

Repression of the somatic mesodermal program in PGCs was subsequently found to also depend on PRDM14, a close relative of BLIMP1 with exclusive expression in the germ cell lineage and pluripotent cell lines [59]. *Prdm14*-deficient PGCs are specified, but fail to proliferate and are eventually lost during migration towards the genital ridges [59]. Absence of PRDM14 was shown to result in diminished expression of germ-cell specification genes and regulators of pluripotency, but correct repression of mesodermal specification genes. Immunostaining experiments further suggested that *Prdm14*-deficient cells undergo the repression of the mesodermal specification program apparently normally. However, in a more recent

study using single cell expression profiling of *Prdm14*-deficient PGCs, derepression of *HoxB1* and *HoxA1* was observed [60]. These studies further suggested that *Prdm14* protects cells from acquiring somatic fates partly by attenuating mitogen-activated protein kinase (MAPK) signaling, thereby stabilizing a naive pluripotent state. Both studies reported that *Prdm14* also represses the DNA methyltransferase machinery, further promoting naive pluripotency [59, 60].

The current model for PGC specification stipulates that combined expression of *Blimp1*, *Prdm14*, and transcription factor activator protein-2 (*Tfap2c*, also known as AP2γ) a downstream target of *Blimp1* [61], is required to execute changes in different branches of the PGC gene expression program, including repression of somatic genes. Forced expression of these three factors is sufficient to confer PGC fate when expressed at the correct developmental time point in in vitro specification systems [58, 62]. Using P19EC cells, the Surani lab found that combined ectopic expression of BLIMP1, AP2γ and PRDM14 is sufficient to induce PGC specification, resulting in extensive repression of somatic and cell cycle regulators [58]. By differentiating ES cells into epiblast-like cells (EpiLCs) competent to induce the PGC fate [63], the Saitou lab also showed that BLIMP1, PRDM14, and TFAP2c act synergistically to suppress ongoing somatic differentiation and drive re-expression of pluripotency and germ cell-specific genes [62]. Although in this system, overexpression of *Prdm14* alone was sufficient for the induction of PGC-like cells at a low frequency, this was accompanied by the induction of endogenous *Blimp1* and *Tfap2c*. While BLIMP1 was found mainly enriched at transcriptional start sites, in ESC PRDM14 was found predominantly bound to enhancers [64], suggesting parallel mechanisms in repressing transcription. AP2γ, on the other hand, was found both on distal regulatory elements as well as on promoters [58]. A requirement for all three factors to induce PGC specification is consistent with genetic experiments showing that they are all essential for PGC development in vivo [53, 59, 61]. Therefore, contrary to what is observed in *Drosophila* and *C. elegans*, PGC specification in mouse does not rely on global repression of mRNA transcription, but on a complex transcriptional reprogramming that represses or activates specific target genes.

A recent study suggests that SALL4, a critical transcription factor for pluripotency in embryonic stem cells (ESCs), may be an additional component of the transcriptional repressor network required for mouse PGC specification [65]. In PGC progenitors carrying a *Sall4* conditional knockout, somatic genes (*Hoxa1* and *Hoxb1*) were expressed, while expression of the stem cell program was not altered. Furthermore, in differentiated ESC cells, SALL4 was found to bind the same loci reported to bind PRDM1 in EC cells [58], including *Hoxa1*, *Hoxb1/b2*, and *Dnmt3b*, suggesting that SALL4 may bind target genes in conjunction with PRDM1 to facilitate the repression of the somatic cell program during PGC specification. Consistent with an important role for SALL4 in PGC specification, conditional inactivation of *Sall4* during PGC specification led to a reduction in the number of PGCs in embryonic gonads.

SOX17, rather than BLIMP1, is the key regulator of human PGC fate

The recent development by the Surani and Hanna groups of a robust system allowing the specification of human PGC-like cells (hPGCLCs) from ground state naive hESC [66] having acquired germ cell competence [67] has made it possible to explore the similarities and differences between mouse and human PGC specification. Surprisingly, SOX17, a critical transcription factor for endoderm lineages, was shown to be the earliest marker of hPGCLC. Expression of SOX17 prior to BLIMP1 marks the onset of hPGCLC specification, a key difference between the specification of human and mouse germline fate. SOX17 null hPGCLCs failed to express PGC genes including *NANOS3*, *TFAP2C*, *DNS1*, *OCT4*, *NANOG* and importantly, *BLIMP1*. Instead, there was upregulation of mesodermal genes. Therefore, SOX17 acts upstream of BLIMP1 to initiate the human germ cell transcriptional network.

Additional experiments showed that *BLIMP1* acts downstream of *SOX17* to repress endodermal and other somatic genes during hPGCLC specification. *BLIMP1* knockout resulted in loss of PGC gene expression, as in mouse [56]. In addition, upregulation of mesodermal and *HOX* genes, as well as endodermal genes, including *GATA4*, *GATA6* and *FOXA1* was observed, suggesting a role for human BLIMP1 in suppressing endodermal and other somatic genes which may otherwise be induced by SOX17 during PGC specification. While in mouse BLIMP1 also represses somatic genes in PGCs [53, 54], endodermal genes are not upregulated following *Blimp1* inactivation [54, 56]. Furthermore, mouse BLIMP1 also play a central role with PRDM14 and TFAP2C in PGC specification. By contrast, SOX17 is essential for human PGC specification and is alone sufficient to induce germ cell genes in *SOX17* mutant cells. Although PRDM14 is critical for mouse PGC specification, its expression does not correlate with hPGCLC specification, perhaps reflecting differences in PRDM14 function in maintaining pluripotency in the two species [59, 60, 64, 68]. PRDM14 knockout in hPGCLC will be required to establish how it contributes to human germ cell specification.

A repressive chromatin landscape is established in *C. elegans* PGCs following the disappearance of PIE-1 in late embryos

In *C. elegans* embryos around the 100 cell stage, following the division of the germline blastomere P_4 into Z2 and Z3, PIE-1 disappears (Fig. 1) [69]. At this stage, RNA Pol II is transiently activated [24, 37]. Transcriptomic analysis performed on FACS-sorted Z2/Z3 cells revealed that specific expression programs, including those involved in oocyte differentiation, are activated in these cells [70]. The disappearance of PIE-1 in a cellular background permissive for transcription suggests that additional mechanisms intervene to prevent ectopic expression of somatic genes in these cells. At this stage, the chromatin of germline blastomeres, which is indistinguishable from that of somatic blastomeres during the period of OMA-1-, OMA-2- and PIE-1-dependent repression, changes dramatically [69, 71], suggesting that the establishment of a repressive chromatin structure could contribute to maintaining repression of somatic gene. High-resolution studies using light and electron microscopy have convincingly shown that changes in the chromatin organization of germline precursor cells first become apparent in P4, and are characterized by a greater chromatin compaction and an expansion of the interchromatin compartment [71]. Interestingly, however, the ultrastructure of individual chromatin domains does not differ between germline and somatic cells, pointing to a specific nuclear organization during the establishment of germ cell identity.

Immunofluorescence analysis showed that in Z2- and Z3-specific histone modifications associated with active chromatin, including H3K4me2/3 and H4acetylK8, specifically disappear, while remaining present in somatic embryonic cells. *nos-1* and *nos-2* (the homologues of *Drosophila nanos*) are required for loss of H3K4me2/3 in Z2 and Z3, and their inactivation results in sterility [69, 72, 73]. Although loss of H3K4 methylation in Z2 and Z3 has not been directly linked to a specific demethylase activity, absence of the SPR-5/LSD1 H3K4 demethylase across multiple generations results in the inappropriate retention of H3K4me2 in these cells, suggesting a heritable accumulation of this mark [73]. Intriguingly, H3K4me2 is also retained in Z2 and Z3 in animals lacking ASH-2, a conserved component of the H3K4 methyltransferase complex [74]. Together, these results suggest complex regulation of H3K4 methylation in PGCs.

Z2 and Z3 cells (and somatic embryonic cells), exhibit H3K27me3, a repressive histone mark that may be involved in the transgenerational memory of transcriptional repression [75]. In Z2/Z3 cells, H3K27me3 is catalyzed by the evolutionary conserved Polycomb Repressive Complex 2 (PRC2) consisting of the Enhancer of Zeste [E(Z)] homologue MES-2, the Extra Sex Comb (ESC) homologue MES-6, and the *C. elegans*-specific protein MES-3 [76–79]. *mes-2*, *mes-3* or *mes-6* mutants derived from heterozygous animals are fertile and produce viable but sterile progeny that fail to develop a germline, showing the importance of these genes in germline development [76]. Therefore, in Z2/Z3 cells, H3K27me3 could potentially contribute to the chromatin landscape involved in gene repression after PIE-1 disappearance [80]. The development of cell type-specific isolation techniques [35, 70, 81] will help to further examine, specifically in Z2 and Z3, the consequence of chromatin reorganization on the repression of somatic genes.

A repressive chromatin structure is also observed in *Drosophila* PGCs

In early *Drosophila* embryos, H3K4 methylation is first detected in somatic nuclei between nuclear division cycles 12 and 13 of the syncytial embryo, coincident with, or slightly preceding the time when transcription is broadly upregulated [82]. Significantly, high levels of H3K4me are maintained through the cellular blastoderm stage (stage 5), but are not detected in the pole cells or germ cells in early gastrulation stage through midgut invagination. These cells instead show intense staining for H3K9me2 and H3K9me3 [69, 83]. A global lack of H3K4me may, therefore, be a conserved feature of chromatin structure in transcriptionally inert germ cell nuclei. However, once the germ cells begin migrating away from the hindgut, H3K4me3 becomes readily detectable in germ cells, coincident with the onset of transcription at stage 9/10 [84, 85]. As in *C. elegans*, germ cell precursors lacking *nanos* or the *spr-5*/LSD1 histone demethylase homologue Suppressor of variegation 3–3 [*Su*(*var*)*3–3*] show increased levels of H3K4me2 and fail to appropriately maintain the repression of developmental genes [69, 83].

Both DNA methylation and histone marks mediate transcriptional silencing in mouse PGCs

During PGC specification in mouse, chromatin structure is likely to mediate at least some of the early BLIMP1-mediated transcriptional repression of somatic genes, since BLIMP1 binds and recruits several repressive chromatin proteins including the histone deacetylase HDAC2 [86, 87], the histone lysine methyltransferase G9a [88], and the protein arginine methyltransferase PRMT5 [89], presumably in a context-dependent manner [87]. Following

specification, PGCs proliferate and migrate towards the genital ridges, which they colonize by E10.5 (Fig. 2). PGC migration is accompanied by global epigenetic reprogramming events which include exchange of histone variants, loss of histone modifications, and erasure of DNA methylation [90, 91]. These events, which are thought to be completed around E13.5 in both male and female embryos, are likely to contribute to the repression of somatic fate. De novo DNA methylation is suppressed as the result of the downregulation of the DNA methyltransferases *Dnmt3b* and *Uhrf1* [56], resulting in a passive DNA demethylation. Global demethylation is pivotal for parental imprint erasure and X chromosome reactivation. Global profiling of DNA methylation in E13.5 PGCs showed promoter demethylation not only of germline-specific genes, but also somatic genes involved in various biological processes including hematopoietic differentiation and defense response, signaling at the membrane, and neuronal functions [92]. While these results suggest that promoter demethylation during PGC development may contribute in promoting the activation of the germline expression program, DNA methylation-independent mechanisms, including histone post-translational modifications, may contribute to repress these somatic genes in PGCs following specification.

Dynamic changes in H3K9me2 and H3K27me3, two marks associated with transcriptional repression, take place following PGC specification. During specification, PGCs harbor a high, genome-wide level of the repressive histone modification H3K9me2, similar to the surrounding somatic cells. This modification is gradually lost beginning at E7.75, as PGCs start migrating, and by E9.25 is undetectable in most PGCs, where it is replaced by H3K27me3 [90, 91]. It has been postulated that the loss of H3K9me2 is complemented by the gain of H3K27me3 to maintain a repressive chromatin state in PGCs [90]. The onset of H3K9me2 demethylation coincides with global repression of RNA Pol II dependent transcription in migrating PGCs ($\sim$E9.0). This loss is not due to the PGC-specific downregulation of RNA Pol II transcription itself, as the levels of RNA Pol II protein detected in PGCs are comparable to those detected in their somatic neighbors. Bromo-UTP incorporation experiments confirmed that mRNA synthesis is greatly reduced or absent in PGCs during this time [90]. Neither the functional significance, nor the mechanism of this repression is known. It may be that transcriptional repression is crucial to protect germ cells from deregulated transcription in the absence of major chromatin-based repressive mechanisms during epigenetic reprogramming. Consistently, this transcriptional repression persists until PGCs acquire high levels of H3K27me3 and is relieved gradually afterwards. PRMT14-deficient PGC-like cells that fail to repress the H3K9 methyltransferase GLP appropriately fail to reduce H3K9me2 at E8.5 and upregulate H3K27me3 at E9.5, suggesting that active repression of this essential enzyme by PRMT14 during specification may contribute to subsequent genome-wide epigenetic reprogramming [90]. H3K27 trimethylation is catalyzed by Ezh2, a subunit of PRC2, which has an established role in downregulating the expression of genes involved in somatic differentiation [93, 94]. Global analysis of histone modifications has shown that H3K27me3 is enriched at genes related to development and differentiation functions in E13.5 PGCs, suggesting that this mark may play a role in inhibiting differentiation of PGCs [95].

Poised chromatin in the mouse germline

Poised (bivalent) chromatin, defined by the simultaneous presence of H3K27me3 and H3K4me3, was first described at promoters of lineage-specific regulatory genes in ESCs [96–98]. In general, poised chromatin is correlated with pluripotency, and these domains tend to resolve toward an active or repressed state during differentiation [98–100]. A number of recent studies have shown that poised chromatin is found at promoters of key developmental genes at various stages of germ cell development in mouse, from the E11.5 stage to differentiated germ cells in both males and females [95, 101–107]. The poised state at developmental promoters appears to be maintained throughout germ cell development and retained in mature male gametes [106]. Data were not available to show whether this also holds true for the female gametes. Further analysis comparing chromatin binding studies to available expression profiling data shows that most of these poised genes are not expressed in the germline [108, 109]. Based on this data, Lesch and Page [109] have hypothesized that this poised state may play an essential role in the biology of germ cells. The authors propose three biological functions for poised chromatin: (1) antagonism of DNA methylation at developmental promoters, thereby preventing long-term repression of key developmental genes while at the same time preventing their activation in PGCs; (2) maintenance of germ cell identity by (marking) germ cells, thereby setting them apart from surrounding somatic cells and; (3) preparation for totipotency after fertilization by promoting rapid and efficient activation of poised promoters in the early embryo, as already proposed for ESCs [96]. The combined action of these three mechanisms would prevent the expression of somatic developmental genes in the germline. Validating the role of bivalent marks in maintaining germ cell identity in mouse will require functional studies and genome-wide chromatin profiling of early time points in PGC specification (E6.5–E7.5). As an alternative, the Saitou lab recently carried out genome-wide analysis of chromatin state dynamics during in vitro PGC specification, focusing on the transition from EpiLC epiblast-like

cells to primordial germ cell-like cells (PGCLCs) [110]. EpiLC cells were shown to contain abundant bivalent gene promoters characterized by low H3K27me3 and H3K4me3, consistent with a state primed for differentiation. PGCLCs initially lost H3K4me3 from many bivalent genes, but subsequently regained this mark with a concomitant increase in H3K27me3, particularly at developmental regulatory genes. H3K9me2 was also progressively lost in PGCLCs.

Preventing reprogramming of germ cells into somatic cell types in the adult germline

The wide developmental potential, or totipotency, of the adult germline is manifested in rare germline tumors, called teratomas, containing differentiated somatic cells representative of all three germ layers. Historically, teratoma formation has been studied in mouse model systems in which their incidence is much greater [111, 112]. The more recent discovery of teratomas in the *C. elegans* germline offers new perspectives into the molecular mechanisms regulating totipotency [113–116]. In both mouse and *C. elegans*, inactivation of the translational repressors has revealed important roles in maintaining germline totipotency, and in *C. elegans,* their absence alone is sufficient to form teratomas [113, 116, 117]. By contrast, while *Drosophila* RBPs play a conserved role in translational control of germ stem cell differentiation (reviewed in [118]), experimental evidence supporting a function in repressing somatic genes expression in the germline and/or promoting maintenance of germ cell fate is lacking. Instead, repressive histone marks appear to be essential for the repression of somatic gene expression in the male germline [119]. Recent studies suggest that additional mechanisms, including chromatin regulation and P granules are also required for the repression of somatic fate in the adult germline of *C. elegans* [9]. Therefore, it appears that multiple, redundant mechanisms operate in the germline to prevent reprogramming of germ cells into somatic cell types.

Translational repression of somatic cell fate in the *C. elegans* germline

During *C. elegans* post-embryonic development, Z2 and Z3 proliferate to form a pool of stem cells which occupies the distal region of the elongating gonad. Stem cell renewal is promoted and differentiation is repressed by a Notch signaling pathway activated in response to signalling from the distal tip cell (DTC), which is the somatic niche cell at the distal end of the gonad [120]. In germ cells close to the DTC, high levels of Notch signaling promote proliferation, while in cells further from the DTC, redundant RNA regulatory proteins promote entry into meiosis. These include GLD-1 and GLD-3, two RBPs containing KH domains, GLD-2, the catalytic subunit of a cytoplasmic the poly(A) polymerase, and NOS-3, a homolog of Nanos (Table 1). Meiosis results in the production of spermatocytes during the L4 larval stage and oocytes in young adults. As a result of this developmental process, the adult germline resembles an assembly line, with germ cells progressing from an undifferentiated stem cell fate in the distal end to a fully differentiated gamete at the proximal end (Fig. 3) [121].

Loss of translational repressors in the *C. elegans* germline results in conversion of germ cells into somatic cells

Evidence implicating translational regulation in repressing somatic fate in the germline of *C. elegans* first came from examining mutant germlines lacking the translational repressors GLD-1 and MEX-3, another KH-domain RBP protein [113, 122]. In these germlines, most meiotic germline nuclei lose their distinctive shape and morphologically resemble somatic nuclei. Combined use of microscopy, immunostaining and somatic reporter transgenes showed that cells present in *gld-1 mex-3* double mutant germlines differentiated into somatic cell types, including muscle, neurons and intestine, forming a teratoma-like structure. Most of the muscle cells present in *gld-1 mex-3* mutant germlines appeared to differentiate through a pathway dependent on PAL-1, a transcriptional regulator, and HLH-1, a homolog of the myoD transcription factor involved in muscle terminal differentiation. PAL-1 and HLH-1 are required for specification of muscle precursors during embryogenesis [123–125], and both MEX-3 and GLD-1 contribute to the translational repression of *pal-1* mRNA in wild-type germlines [126, 127]. Initial cytological analysis of *gld-1* null alleles showed that in the absence of GLD-1, germline stem cells enter meiotic prophase normally, but are unable to progress beyond the pachytene stage and instead return to mitosis [128, 129]. It was proposed that the combination of *pal-1* mRNA derepression and an abnormal meiotic progression are responsible for the induction of muscle cell fate in *gld-1 mex-3* germlines. However, *pal-1*(RNAi);*gld-1 mex-3* germlines still contain numerous neurons and pharyngeal cells, suggesting the involvement of additional, PAL-1-independent pathways of neuronal and pharyngeal differentiation that still need to be identified.

Translational repression of cyclin E by GLD-1 prevents precocious mitosis and embryonic gene activation

Differentiated somatic cells were also observed, although at a lower frequency, in the gonads of *gld-1*, but not *mex-3*

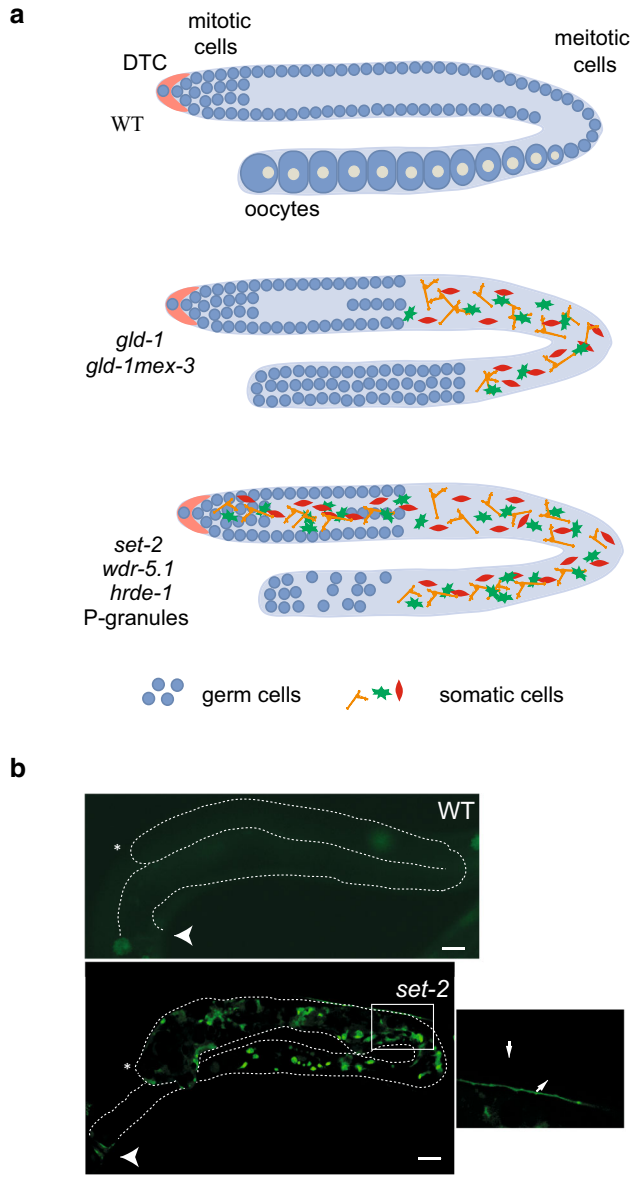

Fig. 3 Germline transdifferentiation in *C. elegans*. **a** The hermaphrodite germline is linearly organized with dividing stem cells at the distal end, differentiating meiotic cells in the central part, and differentiated oocytes at the proximal end. Germline identity is controlled both by translational factors and the epigenetic landscape. A wild-type (WT) and a mutant germline that has undergone transdifferentiation into somatic cells is represented. *DTC* distal tip cell. **b** Images of a wild-type and a *set-2* mutant germline from animals carrying a pan-neuronal reporter transgene (*Punc-119::GFP*). The *set-2* germline has transdifferentiated and expresses the pan-neuronal reporter transgene. The *asterisk* shows the germline distal end and the *arrowhead* indicates the position of the vulva. The *small panel* is a zoom of the *boxed in region* showing dendritic-like projections (*white arrows*)

single mutant germlines [113]. In the germline of *gld-1* mutant animals, cells exit meiosis to re-enter mitosis through an abnormal nuclear division [116]. GLD-1 represses *cye-1*/cyclin E, a key cell cycle regulator, through its association with specific binding elements located within the 3′ UTR of *cye-1* mRNA. In *gld-1* mutant germ cells, CYE-1 is expressed and regulates cyclin kinase CDK-2 activity, which in turn drives re-entry into mitosis of meiotic cells present in the central part of the gonad. Re-entry into meiosis after *cye-1* derepression in the absence of GLD-1 is associated with the precocious onset of embryonic gene activation (EGA), which normally takes place in early wild-type embryos for the acquisition of somatic identity [23, 130]. In worms, EGA is characterized by transcription of several very early transcripts, including *vet-1*, *vet-4*, and *vet-6*. In *gld-1* mutant gonads, both qRT-PCR and in situ hybridization showed that *vet-1*, *vet-4* and *vet-6* mRNA levels were much higher than in either wild-type or *gld-1*; *cye-1*(RNAi) gonads.

The TRIM-NHL protein LIN-41 represses precocious embryonic gene activation in *C. elegans* oocytes

Using precocious onset of EGA as a tool to identify novel factors involved in regulating germline pluripotency, the Ciosk laboratory carried out a genetic screen using germline expression of a *Pvet-4::GFP* transgene as a readout of EGA [114]. Through this approach, they identified LIN-41/TRIM71, a member of the TRIM-NHL protein family and a component of the somatic heterochronic pathway that temporally controls the transition from larval to adult cell fates [131]. In the soma, LIN-41 represses translation of *lin-29* mRNA, encoding a transcription factor required to control the cessation of the molting cycle and the switch to adult hypodermal cell fates [131]. qRT-PCR analysis of *lin-41* mutant germlines revealed transcriptional derepression of somatic genes including *hlh-1/myoD*, *end-1* and *end-3*, which specify endodermal fate, *unc-120,* a muscle lineage marker, and several *hox* genes [114]. Immunostaining experiments and the use of somatic reporter transgenes revealed the presence of teratoma-like structures expressing both neuronal and muscle markers in the proximal region of *lin-41* gonads. Importantly, the germline expression of muscle markers depended on PAL-1, suggesting that muscle differentiation in *lin-41* gonads mimics, at least partly, the pathway driving muscle formation in embryos, and that the reprogramming of proximal germ cells in *lin-41* gonads results from the acquisition of an embryonic-like state. Reprogrammed proximal germ cells reentered mitosis, confirming that they behave as embryonic cells. LIN-41 functions autonomously in the germline, where it is expressed in the cytoplasm from late pachytene cells to oocytes. By contrast, *lin-29* mRNA is either poorly or not at all expressed in the germline, and genetic evidence suggests that LIN-41 may function in the germline and soma via distinct targets

and/or mechanisms. Specifically, it appears that LIN-41 function in the germline requires the NHL domain independently of its mRNA binding activity shown to be essential for LIN-41 somatic functions. A hypothesis that requires further investigation is that in the germline LIN-41 represses precocious EGA by interacting with additional proteins via its NLH domain. Recently, LIN-41 was shown to promote oocyte growth and meiotic progression [132]. Therefore, reminiscent of what is observed in the absence of GLD-1, precocious EGA occurs in a meiosis defective context in *lin-41* mutant germlines.

Mutations in the RNA-binding protein DND1 contribute to teratoma formation in mouse

Once they colonize the gonad (at E11.5), mice PGCs of both sexes exit their pluripotent, migratory state and acquire competence to initiate sexual differentiation and enter meiosis [22]. During this phase, known as licensing [133], the importance of translational regulation in repressing the expression of somatic genes becomes evident. The identification of mouse strains with high incidence of teratomas made it possible to genetically map loci that contribute to their formation. In the 129 mouse inbred strain background, mutations in the *Ter* locus, corresponding to the RNA-binding protein DND1 [134], cause a dramatic increase in testicular teratomas. Interestingly, when not in the 129 strain background, the *Ter* mutation also results in loss of PGCs. Failure to undergo mitotic arrest, and the abnormal expression of meiotic proteins and pluripotency factors were found to correlate with teratoma formation [135, 136]. Similar to the function of GLD-1 in repressing cell cycle regulators in *C. elegans* [116], DND1 was found to directly bind transcripts that encode negative regulators of the cell cycle, and may inhibit the cell cycle by protecting *p27* (a CDK2 inhibitor) mRNA from miRNA-mediated degradation [137].

DAZL prevents expression of pluripotency genes and apoptosis in mouse PGCs

DAZL (deleted in azoospermia-like) another germ cell-specific RBP in mouse, is also essential for developing PGCs [138]. Following migration, *Dazl*-deficient germ cells fail to express markers of male or female differentiation, do not enter meiosis, and eventually undergo apoptosis [133, 138]. Very recently, using an in vitro assay consisting of a *Dazl*-GFP reporter expressed in ESC line to obtain PGC-like cells, Chen et al. [117] were able to recover sufficient PGC-like cells for RNA-immunoprecipitation experiments. By this approach, DAZL was shown to bind and directly suppresses specific pluripotency genes, including *Sox2*, *Sall4*, and the PRC2 gene *Suz12*. All of these contain a *Dazl*-binding motif in their 3′ UTRs. Downregulation of pluripotency genes coincides with the transition of PGCs into germ cells and sexual differentiation to gametes around E13.5, and represents a key developmental point in germ cell development [139, 140]. PGCs that show continued expression of pluripotency markers and fail to differentiate into oocytes and spermatogonia are potentially at risk of somatic differentiation, the cause of teratomas in the adult animal. SUZ12 is a core component of PRC2 [93, 94] and is required for ES cell differentiation [141]. DAZL-mediated silencing of both pluripotency factors and PRC2 may therefore reduce the risk of teratoma formation by inhibiting the pluripotent program while simultaneously preventing somatic differentiation [117]. Interestingly, DAZL also associates with mRNAs of key Caspases to inhibit their translation [117], and *Dazl*$^{-}$-deficient germ cells show increased apoptosis [138]. This raises the possibility that loss of pluripotency regulation simultaneously triggers germ cell death and prevents germ cell tumor formation through an efficient fail-safe mechanism. This is consistent with the observation that abnormal gene expression in PGCs in which *Nanog* [142], Blimp1 [53, 56], Sall4 [65], or Nanos [143] have been inactivated, results in apoptotic cell death rather that conversion to somatic fate.

In culture, mouse PGCs can easily be reprogrammed into pluripotent embryonic germ cells (EGCs) in the presence of specific extracellular factors [144–147]. Activation of the AKT signalling pathway was shown to greatly increase PGC conversion to EGCs. This effect is likely to be mediated, at least in part, by AKT activation preventing apoptosis of PGCs, allowing more PGCs to initiate conversion into EGCs. When these EGCs were transplanted into nude mice, they produced teratomas composed of various differentiated cells [148, 149], suggesting that AKT signalling also acts to inhibit acquisition of pluripotency and teratoma formation.

Chromatin regulation contributes to totipotency in *Drosophila* testes and the *C. elegans* germline

Very little is known about the mechanisms involved in somatic fate repression in the *Drosophila* germline following PGC specification. The only study available so far points to a role of repressive chromatin in repressing expression of a somatic marker in *Drosophila* adult testes [119].

A role for E(Z)-dependent H3K27me3 in preventing germ cells from expressing somatic cell fate

In adult testes, GSCs interact with two populations of somatic gonadal cells known as the postmitotic hub and

cyst stem cells (reviewed in [150, 151]). During spermatogenesis, GSCs are encapsulated into somatic cyst cells to undergo differentiation into spermatogonia and spermatocytes. By combining in *trans* null and thermosensitive alleles of E(Z), which encodes an H3K27 methyltransferase [152], the authors demonstrated that loss of H3K27me3 in adult testes results in an excessive number of cells expressing the somatic marker Zfh-1. Using an UAS-GAl4 system to RNAi knock-down E(Z) in specific cell types, H3K27me3 was shown to be specifically required in cyst stem cells and cyst cells to prevent the accumulation of Zfh-1-positive cells in adult testes. Lineage-tracing experiments and morphological analysis of the extra Zfh-1-positive cells showed that they derived from germ cells, but had lost germ cell features.

The above results suggest that E(Z) and H3K27me3 may play a non-cell autonomous role to prevent germ cells from ectopically expressing somatic cell markers, thereby maintaining germ cell identity. In somatic gonadal cells, E(Z) might regulate signaling pathways involved in the proper communication between the germline and its cellular microenvironment. Supporting this hypothesis, ChIP-seq experiments revealed that, in somatic gonadal cells, H3K27me3 is enriched at genes involved in Wnt and epidermal growth factor (EGF) signaling pathways [119].

Chromatin modifications protect the *C. elegans* germline from acquiring somatic cell fate

H3K27me3 also plays an essential role in repressing somatic fate and promoting germline identity and pluripotency in the *C. elegans* hermaphrodite germline. In this tissue, H3K27me3 marks are concentrated and uniformly distributed on the X chromosome and have a banded appearance on the autosomes [80]. Based on these initial observations, studies have focused on the role of H3K27me3 in the silencing of the X chromosome in the germline [80, 153, 154]. However, two recent studies suggest a wider role for this modification in protecting the germline against somatic differentiation. Reducing H3K27me3 either in the absence of the histone chaperone LIN-53 (RbAp46/48 in humans) [155] or by RNAi knock-down of the PRC2 subunits *mes-2*, *mes-3* and *mes-6* [156] was shown to drive transdifferentiation of germ cells into sensory-like neurons or muscle-like cells following ectopic expression of either the neuronal transcription factors CHE-1, or the MyoD orthologue HLH-1. Importantly, compromising H3K27 methylation through knock-down of PRC2 complex activity alone is not sufficient to drive somatic cell fate conversion, which is fully dependent on ectopic expression of transcription factors. Consistent with this observation, transcriptome analysis of *mes-2* mutant germlines further showed that while some somatic genes, including muscle- and neuronal-expressed genes, are derepressed in H3K27me defective germlines, expression of the key transcription factors known to be involved in cell fate determination is not affected in this background [157]. Altogether, these observations suggest that decreasing repressive histone H3K27 methylation may render germ cells more sensitive to reprogramming following the forced expression of transcription factors.

In contrast to the above studies, spontaneous conversion of germ cells into somatic cells, without the forced expression of tissue-specific transcription factors, was reported by two independent studies in which H3K4 methylation was altered. In the first study, expression of somatic fate was observed in the germline of animals in which the histone H3K4 demethylase SPR-5/LSD1 was inactivated [73, 158]. Loss of SPR-5 results in the inappropriate accumulation of H3K4me2 in PGCs over generations, which correlates with a transgenerational loss of fertility (known as the "mortal germline phenotype", Mrt) peaking at 28–30 generations. Kaser-Pebernard et al. [158] showed that the germline of late generation *spr-5* sterile animals, which appear stochastically within the population, expressed neuronal cell fate and showed accumulation of H3K4me3. Whether these changes correlate with changes in the germline expression profile was not tested in this study.

In apparent contrast with the above results, we have observed a similar phenotype following inactivation of conserved subunits of the H3K4 histone methyltransferase complex SET1 (also known as COMPASS; Table 1) [115, 159]. Both SET-2, the *C. elegans* orthologue of SET1, and the conserved WDR-5.1 subunit are responsible for global H3K4me2 and me3 in the germline, and their absence results in a temperature-sensitive Mrt phenotype peaking at generations 6–8 [74, 159]. Expression profiling of healthy, fertile *set-2* and *wdr-5.1* mutant germline showed wide expression of somatic genes, including homeodomain transcription factors involved in terminal differentiation of neuronal cell fate. In this context, the onset of sterility over subsequent generations is accompanied by expression of neuronal and muscle cell fates, indicating that loss of fertility correlates with germline transdifferentiation into soma. The observation that either increasing or decreasing H3K4 methylation results in a similar phenotype—loss of germline totipotency—can be reconciled by assuming that over several generations, additional changes in the epigenetic landscape ultimately lead to irreversible changes in germ cell fate. This appears to be the case in *set-2* mutant germlines, where loss of germline H3K4 methylation was accompanied by a general reorganization of repressive H3K9me3 [115]. In an attempt to identify additional factors involved in the establishment and maintenance of the epigenetic landscape responsible for germline identity, we

identified HRDE-1 and NRDE-4, two subunits of the nuclear RNAi machinery that mediates H3K9me3 deposition and transcriptional silencing at targeted loci [160–162]. Mutations in *hrde-1* or *nrde-4* result in a temperature-sensitive progressive sterility associated with expression of neuronal cell fate over generations [115]. These results are consistent with HRDE-1 contributing to the epigenetic landscape that maintains germline identity. Genetic analysis and expression profiling suggests that the SET-2/WDR-5.1 complex and the nuclear RNAi machinery may act in two independent parallel pathways to maintain germline identity through the repression of a common set of somatic genes in the germline. A better understanding of how the epigenetic landscape contributes to the repression of somatic genes in the *C. elegans* germline will require genome-wide chromatin profiling to look at the distribution of repressive and activating histone marks and their dependence on specific histone methylase and demethylase activities.

P granules are essential to repress somatic cell fate in the *C. elegans* germline

Strikingly, the transdifferentiation of *C. elegans* germ cells into somatic tissues was very often found to correlate with the disappearance of P granules [113–115, 155, 158]. The absence of P granules in transdifferentiated germlines in *C. elegans* suggested two possibilities. Either these structures are directly involved in the maintenance of germline totipotency and identity, or their loss in transdifferentiated germlines may simply be a consequence of loss of germline identity. To distinguish between these two possibilities, the Stome group used RNAi to simultaneously knock-down factors that nucleate P-granule formation and promote their perinuclear localization [163]. RNAi-treated animals in which P granules were no longer present expressed both neuronal and muscle cell fate, suggesting that P granules may play a direct role in repressing somatic cell fate in the *C. elegans* germline. Many RNAs are regulated by germline-expressed RNA-binding proteins that transiently associate with P granules [9]. P granules may, therefore, selectively degrade or impair the translation of mRNAs that promote somatic differentiation [163]. Whether their loss in *mex-3 gld-1*, *spr-5* or *set-2* mutant germlines is the cause or consequence of transdifferentiation remains an open question. The fact that neither P granule components, nor factors involved in P granule degradation were found to be misexpressed in the germline of animals lacking *set-2* or *wdr-5.1* argues against defective P granule synthesis or degradation being responsible for transdifferentiation in these animals [115].

Further studies are required to decipher the molecular networks that link translational regulators, P granules and the epigenetic landscape in the repression of somatic cell fate in the *C. elegans* germline. Based on the experimental evidence, it is tantalizing to consider each of these as independent levels of regulation. In support of such redundancy, transcriptomic analysis performed on *set-2*, *wdr-5.1* or *mes-2* dissected gonads showed that very early zygotic transcripts, including *vet-1*, *vet-4*, and *vet-6* are not upregulated. This suggests that, contrary to what is observed in *gld-1* or *lin-41* germlines, EGA is probably not misregulated in these germlines. Different requirements in the timing and sex specificity of teratoma formation following inhibition of P granules or translational regulators also suggest independent reprogramming processes. While germline transdifferentiation following P granule depletion occurs throughout the germline in both mitotic and meiotic cells and is observed in both hermaphrodites and males, reprogramming in *mex-3 gld-1* germlines requires entry into meiosis and is only observed in hermaphrodites. Nonetheless, the fact that the PRC2 subunit MES-3 is a direct target of GLD translational repression [164] suggests that the different regulatory networks involved in repressing somatic fate in the *C. elegans* germline might be somehow interconnected.

Stochasticity contributes to reprogramming of germ cells into somatic cell types

When H3K4 methylation is misregulated in the *C. elegans* germline, the presence of transdifferentiated cells is heterogeneous within the cell population, but only becomes apparent after several generations, and is often favored by higher temperatures. This suggests that acquisition of somatic cell fate may require a threshold effect of triggering factors, such as expression of somatic genes or accumulation of chromatin changes over time. Alternatively, or in addition, stochastic events at the single-cell level, whose frequency increases at higher temperatures or over generations, may be required to trigger ectopic expression of somatic cell fate over subsequent generations. Consistent with germline reprogramming being dependent on stochastic events, single-molecule fluorescence in situ hybridization (FISH) revealed that following disruption of P granules, specific neuronal transcripts are equally distributed throughout the germline, while protein expression is only observed in a subset of cells [163]. P granules may act to buffer germ cells from the effects of stochastic expression of somatic genes, thereby maintaining germline totipotency and preventing reprogramming of germ cells into somatic cell types. Likewise, during the

reprogramming of mammalian somatic cells, all cells are equally amenable to factor-mediated reprogramming, but have to go through a series of stochastic epigenetic events to acquire pluripotency [165, 166].

Concluding remarks

Studies in *C. elegans*, *Drosophila* and mouse point to the existence of multiple mechanisms ensuring that germ cells escape somatic fates both during their specification and in the adult germline. In *Drosophila* and *C. elegans*, germ cell precursors show global inhibition of RNA Pol II until germ cell fate is established. In mouse by contrast, selective inhibition of the somatic transcriptional program during germ cell specification is mediated by three key regulators: BLIMP1, PRDM14 and AP2γ. Following specification, PGCs in all three species show extensive chromatin remodeling. Although these changes in chromatin structure appear to be essential for inhibiting somatic differentiation, uncovering their functional significance is a future challenge. Another question that remains to be answered is how germline-specific genes escape repression and are specifically activated to promote germline development.

In the adult germline of both *C. elegans* and mouse, specific inhibition of translational regulation is associated with the development of teratomas, revealing an essential role in repressing somatic differentiation programs. In addition, in *C. elegans* chromatin regulation and germ–granule components are required to antagonize somatic fate. While the absence of translational regulators in *C. elegans* appears to provoke teratoma formation through early activation of the zygotic genome, it will be important to understand the molecular basis of teratoma formation following changes in the chromatin landscape and to identify the factors restraining the penetrance of germline transdifferentiation From a more global perspective, because germline cells are uncommitted pluripotent cells that are poised to differentiate shortly after gametogenesis, advances in the understanding of germ cell totipotency in *C. elegans* will provide insights into in vivo cell reprogramming, including the evolutionary conserved regulatory circuits that control stem cell totipotency, proliferation and differentiation. Using simple model systems to understand these fundamental processes will lead to more efficient and effective therapies that are capable of partial or complete recovery of diseased tissues, the key precept of regenerative medicine. In addition, molecular characterization of the mechanisms through which the chromatin landscape contributes to the maintenance of germ cell fate may also contribute to an understanding of epigenetic inheritance [167].

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