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Molding Immortality From A Plastic Germline

Amelie A. Raz[1,2,*], **Yukiko M. Yamashita**[1,2,3,*]

[1]Whitehead Institute for Biomedical Research, Cambridge, MA

[2]Howard Hughes Medical Institute, Cambridge, MA

[3]Massachusetts Institute of Technology, Cambridge, MA

Abstract

Germ cells are uniquely capable of maintaining cellular immortality, allowing them to give rise to new individuals in generation after generation. Recent studies have identified that the germline state is plastic, with frequent interconversion between germline differentiation states and across the germline/soma border. Therefore, features that grant germline immortality must be inducible, with other cells undergoing some form of rejuvenation to a germline state. In this review, we summarize the breadth of our current interpretations of germline plasticity, and the ways in which these fate conversion events can aid our understanding of the underlying hallmarks of germline immortality.

Introduction

Unlike somatic cells, which age and die with the individual they comprise, the germline is an immortal lineage that connects all living individuals and is necessary for the continuation of all sexually reproducing life. Early models hypothesized perfect cellular continuity of immorality, passed from gametes to primordial germ cells (PGCs) immediately following fertilization[1]. This direct-inheritance model was supported by observations of germline segregation during the first divisions of development in many classical model systems[2]. However, more recent data suggests that germline specification through inductive signaling, in which PGCs arise from a more homogenous cell population after reception of cell signaling information, is the dominant developmental strategy in the Metazoa[3,4]. Furthermore, evidence of somatic contribution to germline lineages in adults has been found in many species[5–8]. Identification of germline specification mechanisms has even allowed for *in vitro* reprogramming of soma to germline[9].

Such results question the model of a strictly guarded immortal germline with a rigid, unbroken, and unidirectional developmental path. However, if the germline is not an unbroken lineage of specifically immortal cells, what are the necessary components that can induce immortality where it did not previously exist? How is the “perfection” of the germline genome maintained if the genome passes through other states or fates? Can only

*Corresponding Authors.

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somatic cells with continuous germline-level “privileges” generate germ cells, or can a cell without that history nevertheless be rejuvenated to an immortal state? Here, we will review the acquisition and loss of immortal germline fate in the context of adult germline fate plasticity, and highlight the many open questions still remaining.

Processes that confer immortality to germline

Several key features must accompany immortality. First, the germline must be under *protection* from injury in order to continue to give rise to new individuals indefinitely. All cycling cells face threats to genomic integrity from unstable regions, including shortening telomeres, moving transposons, and repetitive regions prone recombination-based gain and loss[10]. Mechanisms that protect against these threats include the piRNA pathway, a conserved small-RNA-based silencing pathway that protects against selfish, repetitive genomic elements (Figure 1A). Second, because protection will be imperfect, there must be a mechanism for *selection* of the least damaged genomes and cells. Because the germline is not essential for viability, elimination of slightly subpar cells or cellular components (thus enriching for the least damaged cells) does not sacrifice individual fitness. For example, stringent checkpoints keep germline cells highly sensitive to DNA damage; germline cells that receive DNA damage will frequently die rather than continue cycling with mutatations[11] (Figure 1B). Damaged mitochondrial genomes are likewise selected against in the germline through mitochondrial fragmentation, followed by mitophagy of individual mitochondria with deleterious mtDNA[12]*. Finally, because of inevitable failures in protection and selection, or because of germline injury or loss, the germline must be capable of *repair* and *restoration*. This can occur by replacing lost elements: telomerase activity can restore lost telomere length[13] (Figure 1C), and lost copies of repetitive regions (e.g., rDNA loci) can likewise be restored[14]. Cellular restoration can also occur by evacuating damaged materials, as has been modeled in budding yeast. Yeast mitotic divisions give rise asymmetrically to a mother cell that retains age-related damage and a restored daughter cell devoid of damage[15], and symmetric meiotic divisions restore all resulting offspring through sequestration and removal of damaged nuclear materials[16]*.

The processes of protection, selection, and restoration may vary across germline differentiation states. Adult germline differentiation frequently begins with germline stem cells (GSCs) that can both self-renew and generate differentiating cells through mitotic divisions. After multiple rounds of mitoses, germline cells will undergo meiosis to produce mature gametes. Unique differentiation-stage-specific features may help different germline cells achieve different aspects of germline immortality. For example, the Piwi family protein Aubergine acts canonically to suppress transposon activity and DNA damage in *Drosophila* female GSCs, but does not have this protective role in more differentiated germ cells[17]. Likewise, in mammalian spermatogenesis, less differentiated cells display high restorative telomerase expression, while differentiating spermatogonia and mature gametes have low/undetectable telomerase activity[13,18]. Asymmetric divisions occur in some germline cells and provide a distinct opportunity for rejuvenation of one cell. *Drosophila* GSCs almost always divide asymmetrically to retain only certain cellular components in the mother cell[19–21], which may help GSCs retain features of immortality and/or eliminate damages. By contrast, differentiating cells often undergo transit-amplifying symmetric divisions, and may

contribute to protection of GSCs by reducing the number of divisions GSCs must undergo. Transit-amplifying germ cells are often connected due to incomplete cytokinesis, which has been indicated to increase the sensitivity of germ cells to DNA damage and thus may serve as a selection mechanism[22] (Figure 1B). Because meiotic cells are programmed for DNA double strand breaks during recombination, compartmentalization of a sensitive DNA damage checkpoint may be necessary. Examination of these and other protective, selective, and restorative features in the context of germline fate transitions will provide us with an understanding of the necessary and sufficient features of germline immortality.

Interconversion of differentiation state within the germline

GSCs have a unique role in maintaining germline immortality: as a founder of many gametes, GSCs must maintain the highest quality so as not to produce subpar gametes. Interestingly, the germline division and differentiation path is not exclusively unidirectional: dedifferentiation of partially differentiated cells is found in many germline contexts. After passing through a differentiation state without the specific protective or selective mechanisms found in GSCs, are these dedifferentiated cells fully capable of reassuming their GSC immortality-preserving behavior, and, if so, how does this restoration occur? Examples of germline dedifferentiation in several systems are beginning to address these questions.

A striking example of germline differentiation state interconversion has been observed in the mouse (Figure 2A). The least differentiated cells of the adult rodent male germline are spermatogonial stem cells and individual spermatogonia, collectively known as A_{single} cells. A_{single} cells undergo mitosis with incomplete cytokinesis to generate interconnected spermatogonia, called $A_{aligned}$ cells. Live imaging of $A_{aligned}$ cells revealed that they can fragment into A_{single} cells, including reversion to a gene expression state found more frequently in A_{single} cells[23]. SSCs can be regenerated after ablation, and lineage tracing experiments determined that ~80% of such regenerated SSCs arose from $A_{aligned}$ fragmentation[23]. Although fragmentation of some $A_{aligned}$ cells appears to happen constantly in homeostatic conditions, fragmentation of $A_{aligned}$ cells with a more differentiated gene expression signature will only occur after SSC ablation[24] and requires specific transcriptional regulation[25]. Because of the limits of both lineage tracing techniques and specific markers, the extent to which divisions and gene expression changes represent true steps of differentiation is uncertain[26]. Single-cell RNA sequencing (scRNA-seq) experiments have recently further elucidated the (de)differentiation states of mammalian spermatogenesis[27]. A scRNA-seq dataset of the human testis revealed that early spermatogonial cell transcriptomes subcluster to form a loop, rather than a linear path, which was speculated to indicate interconversion between cell states within the loop[28]*. Much is still unknown about how interconversion between mammalian germline states is regulated, including if regenerated SSCs are fully equivalent to other SSCs after passing through a more differentiated state and, if so, how dedifferentiating cells are restored to an SSC state.

Germline dedifferentiation has also been described in *C. elegans*[29], though it does not typically give rise to normally functioning GSCs (Figure 2B). Somatic Notch signaling promotes germline expression of self-renewal and differentiation factors, including the RNA

binding protein PUF-8. In conditional *puf-8* mutants, spermatocytes, which would typically undergo meiotic divisions, instead dedifferentiate to a mitotically dividing early germ cell[30]. However, these dedifferentiated cells fail to return to normal GSC division/differentiation, instead forming a germline tumor[30]. Some upstream[31] and downstream[32,33]* components of PUF-8 activity have been identified, and in each case, germline tumors, rather than functional GSCs, are formed by dedifferentiation. These data thus suggest that, in *C. elegans*, the specific immortality-regulating behavior of GSCs may not be replicable by state changes of more differentiated cells.

Germline dedifferentiation is frequent in *Drosophila melanogaster* (Figure 2C). Here, GSCs give rise to interconnected differentiating germ cells (called spermatogonia in the male and cystocytes in the female) through mitoses with incomplete cytokinesis. Both spermatogonia[34] and cystocytes[35] are capable of dedifferentiating to a GSC state by fragmenting into single cells and migrating back to the niche. In the male germline, spontaneous dedifferentiation is frequent: 40% of GSCs in aged male flies will have arisen from a dedifferentiation event[36]. Frequent dedifferentiation is also observed in young flies after recovery from GSC death[37]. Several signaling pathways, including the JAK-STAT[34], JNK[37], and E-Cadherin[38] pathways, have been implicated in dedifferentiation function. Though dedifferentiated GSCs can give rise to apparently normal gametes, these GSCs display several different behaviors. Dedifferentiated GSCs will more frequently initially misorient centrosomes relative to the division plane[36]. After a dedifferentiated GSC divides, the still-interconnected GSC-daughter cell pair will frequently “swivel” such that both daughter cells contact the niche, generating a symmetric division outcome[39]. Unlike native GSCs, which will non-randomly segregate X and Y sister chromatids, dedifferentiated GSCs will randomly segregate chromatids[19]. It remains unclear if these behavioral changes indicate a lineage memory of dedifferentiation – i.e., a “scar” left from passing through a state lacking the protection present in normal GSCs.

Acquisition of germline fate by post-embryonic somatic cells

Germline fate not only is plastic within the germ lineage, as demonstrated by interconversion between germline differentiation states, but can also be induced in cells that were not previously germline. During development of cricket[40], mouse[41], salamander[42], (notably) humans[43], and many others, germline fate is induced via cell signaling, rather than inherited from gametes during fertilization, and can occur quite late in development[44*,45]. Germline fate can also be acquired from somatic cells in an artificial context: mammalian induced pluripotent stem cells or embryonic stem cells can be *in vitro* reprogrammed to a germline fate[9]. Several emerging model systems have demonstrated similar inductive capabilities for generation of germline cells from somatic cells in adults, *in vivo*. These studies open the door for an understanding of the necessary components that confer immortal germline fate to any cell.

Regeneration of all body parts, including the germline, is common in annelid worms[46] and has been specifically examined in *Pristina leidyi*[47] and *Capitella teleta*[6,48]. *P. leidyi* gonads, including the germ cells, can be regenerated from somatic tissue after loss due to segment amputation or starvation[47]. *P. leidyi* gonad size is not compromised by undergoing

regeneration; to the contrary, gonad size is equivalent or bigger after regeneration than in steady-state[47]. The germline of *Capitella* normally arises from a single cell in the 64-cell embryo[49] (Figure 3A). After ablation of this cell, embryos will continue to develop but most (87%) will lack germ cells in the larval state[6]. Nevertheless, two weeks after metamorphosis, all juveniles will contain some germ cells, and when raised to adulthood, most animals were fertile[6]. Prior to appearance of germline cells in regenerating animals, *Capitella* larvae had significant ectopic expression of *vasa* and *nanos* in somatic larval tissue[6]. Somatic *vasa/nanos*$^+$ transition-state cells could be undergoing a process of selection and/or genomic restoration such that only the most stable somatic cells are capable of regenerating the germline. These results demonstrate the ability of somatic cells to dynamically acquire germline fate during post-embryonic development, despite stereotyped germline fate acquisition in only one cell during embryogenesis.

Planarian flatworms are famously capable of regenerating any lost body part, which, in sexually-reproducing animals, includes the germline[8]. Somatic stem cells, known as neoblasts, serve as the cellular source for all new adult tissues. After amputation to remove all germ cells, a subset of neoblasts expressing *nanos* can give rise to new germ cells[50] (Figure 3B). Inhibition of *nanos* results in a failure to regenerate germ cells[50], and transcriptional profiling has identified multiple *nanos* targets that are likewise required for germline specification by neoblasts[51]. A somatic cell type that specifically expresses the transcription factor *dmd-1* is found in close proximity to germ cells[52]. After ablation of *dmd-1*$^+$ cells, animals subsequently fail to regenerate germ cells[52], suggesting that somatic cell signaling is necessary to direct germline specification by neoblasts. Like planarians, the cnidarian *Hydra* can regenerate the germline from somatic multipotent stem cells (called i-cells in *Hydra*) (Figure 3C)[53,54]. A recent *Hydra* scRNA-seq dataset identified the first GSC markers[55]*, which should help enable the study of their specification. In the related cnidarian *Hydractinia*, the transcription factor AP2 is both necessary and sufficient for i-cells to adopt germline fate[56]*. Notably, both somatic planarian neoblasts and *Hydra* i-cells are characterized by expression of a group of genes, including *vasa* and *piwi*, that can promote genomic protection and are typically found in germ cells[57,58]. Additionally, like germ cells, neoblasts are constantly selected for undamaged cells: rather than survive and proliferate with potential mutations, neoblasts with DNA damage will die[59]. This suggests that somatic stem cells that can give rise to germ cells may undergo the same mechanisms of protection and selection as germ cells, and could have an equivalent immortal “quality”.

Loss of germ cell identity for acquisition of somatic fate

Whereas experiments demonstrating somatic cell transdifferentiation to a germline fate can help identify the *sufficient* factors required for germline identity, loss of germ cell fate can indicate *necessary* factors for germline identity. What, when lost, converts germ cells to a mortal somatic fate? In *C. elegans*, this process of adult germ cell conversion to somatic types has been extensively described[60]. Like many species in which germline fate is inherited in the first divisions of development, the *C. elegans* germline is specified by presence of germ granules (known in *C. elegans* as P granules), dense aggregates of RNA and RNA binding proteins. Loss of P granules in adult germ cells results in transdifferentiation of some germ cells to neurons and muscle[61]. P granule loss, and

concomitant transdifferentiation to a somatic fate, can be induced by modification of epigenetic state[62,63] (Figure 4A). For example, inhibition of the histone chaperone LIN-53 allows for adult reprogramming of germ cells to specific neuron types[62]. Likewise, loss of H3K27 trimethylation through removal of the Polycomb repressor complex 2, combined with overexpression of somatic-type specific transcription factors, results in germline conversion to those somatic types[63]. The chromatin state of germ cells can be inherited: offspring that arose from sperm lacking H3K27 trimethylation contain germ cells that lack that methylation state and are primed to lose germ cell identity and adopt a somatic fate[64*]. Another chromatin regulation complex, FACT, protects germline fate by decreasing chromatin accessibility for somatic transcription factors, and FACT loss results in germline conversion to soma[65]. Together, these results highlight the importance of epigenetic modifiers in suppressing somatic gene expression in adult germ cells to maintain a labile germline fate.

Chromatin modifiers likewise protect germline fate in *Drosophila*, but in a curiously cell non-autonomous manner. Activity of the Polycomb group component Enhancer of Zeste [E(z)] is necessary for H3K27 trimethylation. After inducible loss of *E(z)* in adults, GSCs will spontaneously transdifferentiate to somatic cells[66]. However, it is not *E(z)* in germ cells that results in this conversion event; rather, loss of *E(z)* in somatic cells specifically is sufficient for germ cell transdifferentiation to soma (Figure 4B). H3K27 trimethylation is likely required to regulate signaling from somatic cells that maintain germline fate. Notably, H3K27 trimethylation was frequently found at EGF pathway member loci, and partial removal of the EGF receptor gene *Egfr* suppresses the *E(z)* transdifferentiation phenotype, suggesting that EGF signaling may promote adoption of somatic fate by germ cells[66]. This particular example demonstrates a remarkable lability of germline fate: without the appropriate signaling environment, that fate, and its underlying immortality-conferring properties, can be spontaneously lost.

Conclusion

Germline fate has been shown to be plastic across many species and contexts, including conversion between more or less differentiated germline states and conversion across the germline/soma border. However, the key components that make and maintain germline fate during these transitions is still largely a mystery. Further study of each of these germline fate conversion events will significantly aid in our understanding of what underlies the unique and remarkable ability of the germline to remain immortal.

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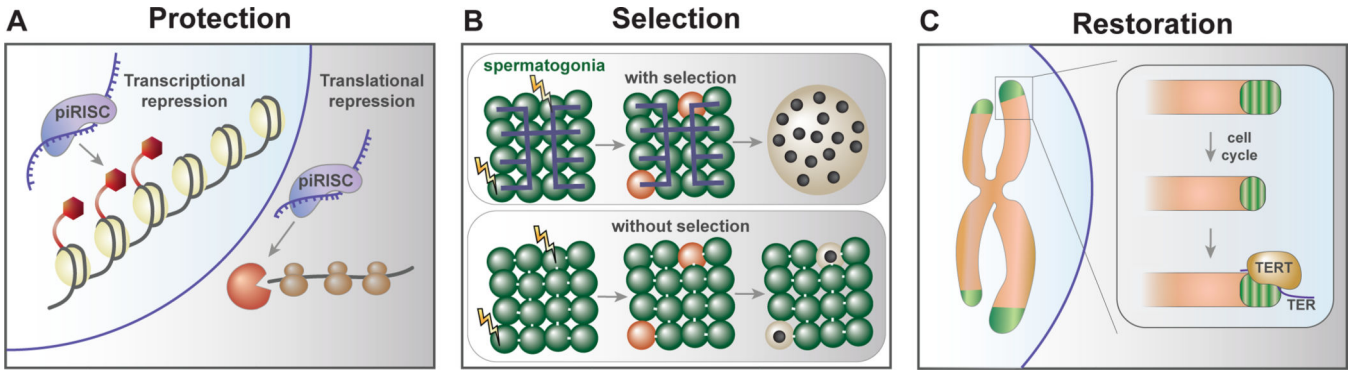

Figure 1. Mechanisms of germline immortality.

Immortality is conferred by several key features. **A.** *Protection* from dangers. Exampled here: piRNA activity, via the piRISC complex composed of Piwi protein and piRNA, protects the germline genome by suppressing transposon expression both on the transcriptional level by adding repressive epigenetic marks to transposon loci and on the translational level by destroying transposon mRNA transcripts. **B.** Immortality is conferred by *selection* of the best cells. Exampled here is one cell biological feature of germline that increases selectivity of cells. *Drosophila* spermatogonia are interconnected, and if any single spermatogonia receives DNA damage, all spermatogonia in that cyst will die (top). Without the fusome connection between spermatogonia, this extreme selection against potentially damaged cells is lost (bottom). **C.** Lost or damaged germline components must be capable of undergoing *restoration*. Exampled here: telomeres become shorter every cell cycle (end-replication problem). Telomere length is subsequently restored by telomerase activity, composed in most eukaryotes of a telomerase RNA (TER) and a telomerase reverse-transcriptase protein (TERT).

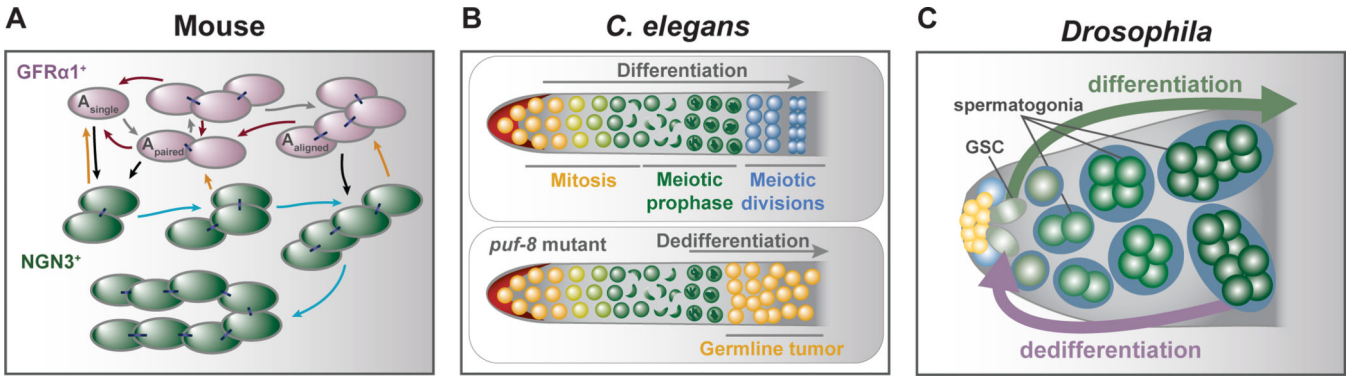

Figure 2. Interconversion of germline differentiation state.

A. Mouse spermatogonia and spermatogonial stem cells (SSCs) undergo constant interconversion of differentiation state. GFRα1+ cells, which include SSCs, undergo incomplete divisions to generate longer spermatogonial syncytia (grey arrows), and also constantly fragment to generate single cells (red arrows). GFRα1+ cells differentiate to NGN3+ cells (black arrows), which is correlated with an increase in syncytia length. NGN3+ cells largely divide (blue arrows) and eventually undergo the next stage of differentiation, but can dedifferentiate to a GFRα1+ state (orange arrows) after ablation of A_{single} cells. **B.** (top) *C. elegans* germ cells can undergo differentiation, but do not return to a native GSC state. Undifferentiated mitotic germ cells, including GSCs (orange) reside in the niche near the distal tip cell (red). They differentiate as they migrate proximally, eventually entering meiotic prophase and subsequently undergoing meiotic divisions. (bottom) In *puf-8* mutants, cells that have entered meiotic prophase will spontaneously dedifferentiate to a GSC rather than proceeding to meiotic divisions. These dedifferentiated GSCs form a germline tumor. **C.** *Drosophila* male germ cells differentiate to spermatogonia and can dedifferentiate through fragmentation to GSCs. Dedifferentiation occurs to regenerate GSCs after mass GSC loss, and also occurs spontaneously to maintain the longevity of the germline.

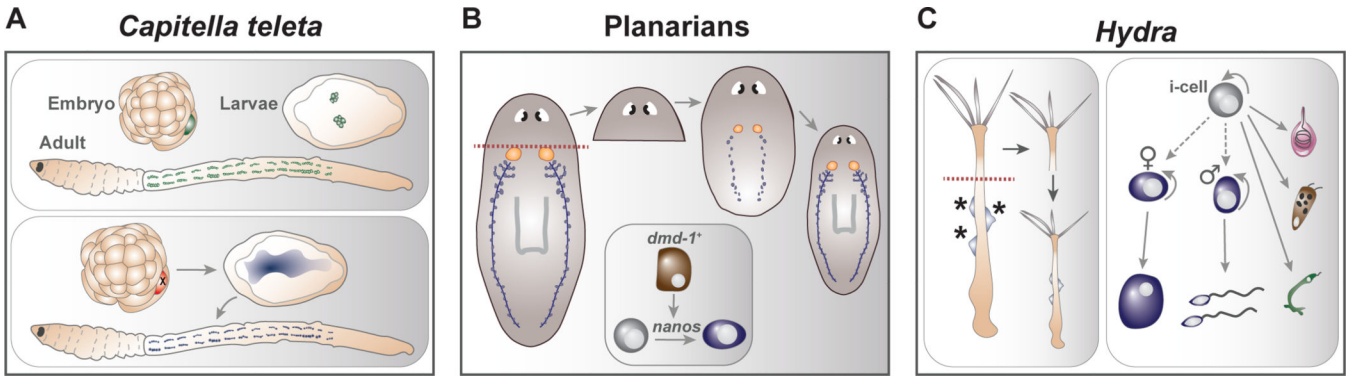

Figure 3. Acquisition of germline fate by post-embryonic somatic cells.

A. (top) Fate-mapping experiments in *Capitella* have determined that germ cells (green) in the larvae and adult typically arise from a single cell in the 64-cell stage embryo. (bottom) After ablation of this single cell, larvae will lack germ cells, but will display significant ectopic expression of *vasa* and *nanos* in somatic cells (blue). These animals will have fully normal *vasa/nanos*+ germ cells after development to adulthood, suggesting a fate transition event at some point between the larval and juvenile stages in which germ cells are specified from soma. **B.** Sexual planarians have both ovaries (orange) and testes (blue). After amputation, head pieces will lack germ cells and gonads, but they can be fully regenerated. (Box) The cellular source of new germ cells are somatic stem cells called neoblasts (grey), which, under the control of somatic *dmd-1*+ cells, will begin expressing *nanos* to differentiate to a germline fate. **C.** (left) *Hydra* regenerate gonads (asterisk) and germ cells after amputation. (right) The cellular source of regenerated germ cells are somatic stem cells called i-cells. After amputation, i-cells give rise to female germline stem cells (female symbol) and male germline stem cells (male symbol), which can give rise to eggs and sperm. i-cells also give rise to somatic lineages, including nematocytes (pink), gland cells (brown), and neurons (green). Germline stem cells are only regenerated from i-cells after germline loss, whereas somatic types are constantly replenished during homeostatic conditions.

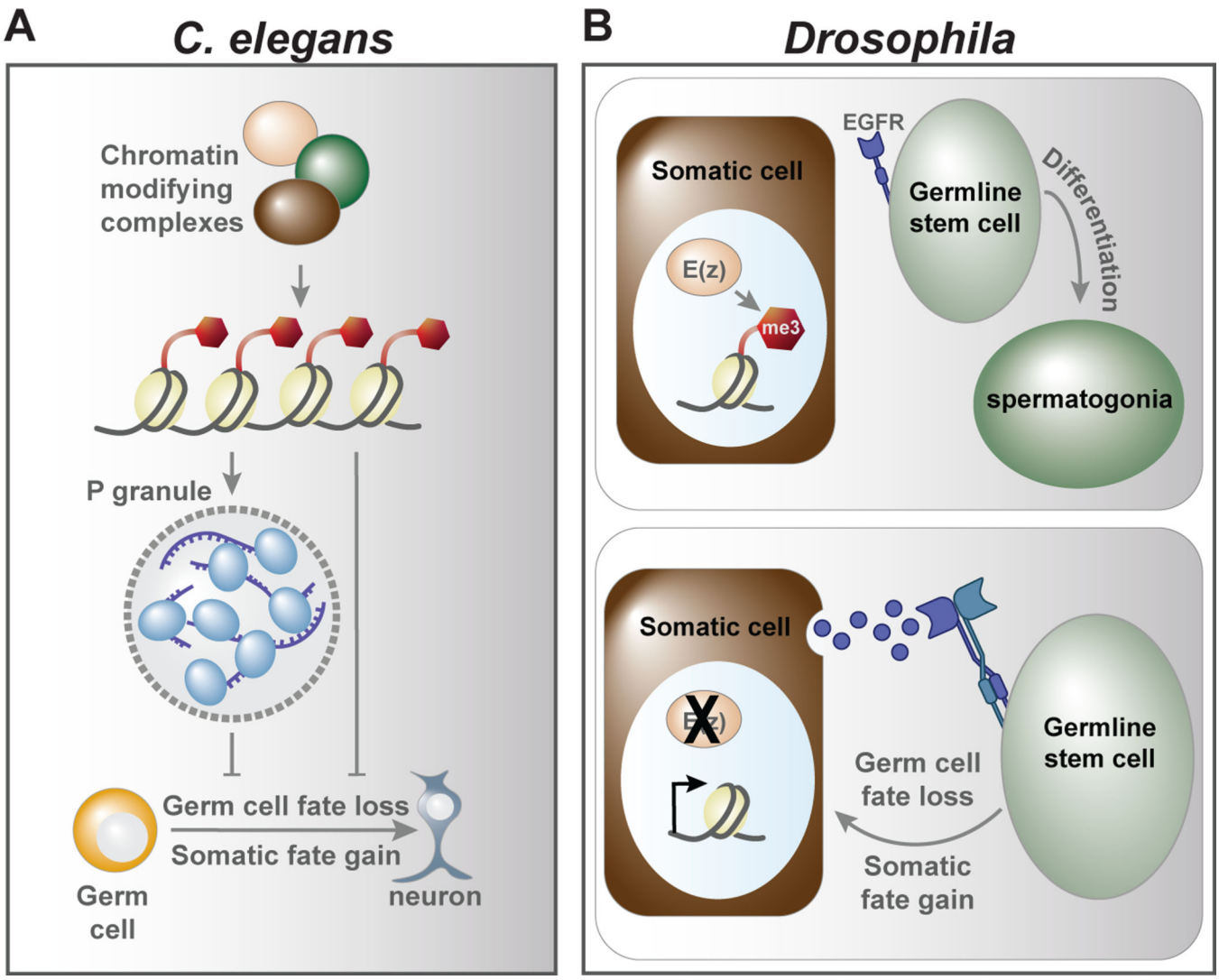

Figure 4. Loss of germ cell fate and gain of somatic cell fate.

A. Multiple chromatin modifying factors act in *C. elegans* germline to maintain fate. Chromatin modifications promote P granule formation and suppress acquisition of somatic fate. Without these factors, germ cells will lose germ fate and gain somatic fate, transdifferentiating to specific somatic types. **B.** (top) *Drosophila* germline fate is maintained cell non-autonomously by chromatin factors. Histone transferase E(z) generates H3K27 trimethylation, including at genes that encode for EGF pathway members. This signaling environment allows germline stem cells to maintain germline fate and differentiate normally to spermatogonia. (bottom) In the absence of E(z) activity, EGF ligands are secreted. Rather than differentiating, germline stem cells in this different signalling environment will lose germ cell fate and transdifferentiate to the somatic cell fate.