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Germ Cells Are Forever

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Germ cells are the only cell type capable of generating an entirely new organism. In order to execute germline-specific functions and to retain the capacity for totipotency, germ cells repress somatic differentiation, interact with a specialized microenvironment, and use germline-specific networks of RNA regulation.

Germ cells are the founder cells of all sexually reproducing organisms. During development, they are set aside from all somatic cells of the embryo. In many species, germ cells form at the fringe of the embryo proper and then traverse through several developing somatic tissues on their journey to the emerging gonad. Once in the gonad, germ cells acquire sex-specific morphologies and the ability to undergo meiosis to generate egg and sperm. At fertilization, the haploid egg and sperm genomes unite and produce an entirely new organism, and the everlasting germline cycle continues from one generation to the next. The germline thus escapes the mortality that all somatic cells of an organism ultimately confront. What distinguishes germ cells from somatic cells and how these defining characteristics allow the germline to retain the ultimate developmental potential is a fascinating area of research.

In this Minireview, we focus on three defining features of the germline that are conserved among several species and that may together create, protect, and promote germ cell fate (Figure 1). First, transcriptional repression of somatic differentiation is essential for germ cell specification during embryogenesis. Second, cell-to-cell signaling between the somatic gonad and germ cells governs germ cell proliferation, maintenance, and differentiation. Third, evolutionarily conserved RNA regulatory networks acting in germ cells prevent somatic transdifferentiation while coordinating germline-specific processes. We propose that these three features contribute to the germ cell's unique ability to retain the totipotent potential necessary for the conception of an entirely new organism.

Transcriptional Repression Restricts Germ Cell Fate

Germ cell specification occurs through diverse means in different organisms. In worms and flies, a specialized set of maternally synthesized mRNAs and proteins is selectively incorporated into germ cells and provides the foundation for the germline program (for review see Seydoux and Braun, 2006). In mice, germ cells arise from the pluripotent epiblast layer of the embryo. A small number of epiblast cells receive a specific

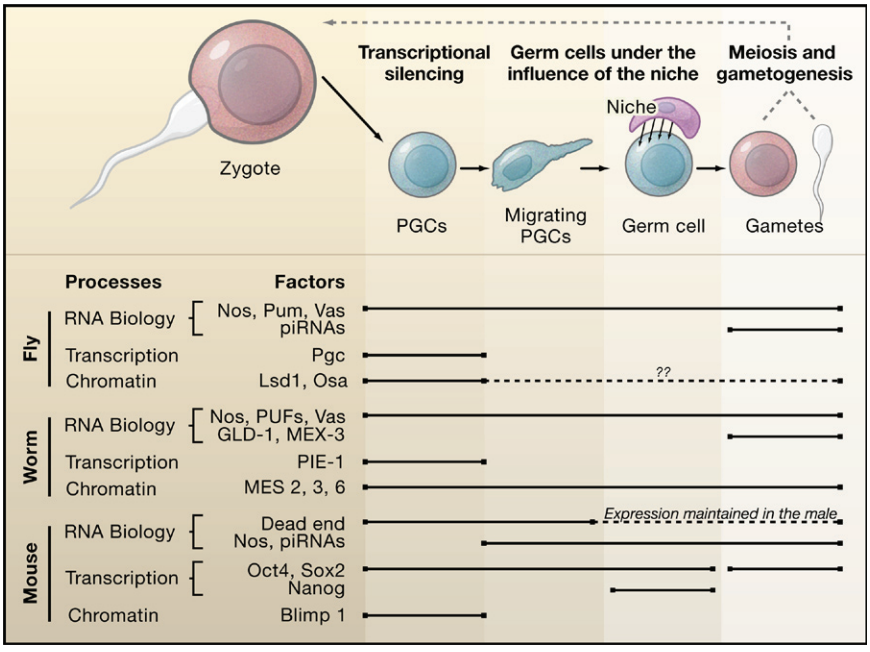

Figure 1. Conserved Programs in Germline Formation and Maintenance

(Top panel) The life cycle of a germ cell and the processes that affect germ cell formation and maintenance. Only those factors mentioned in the text are depicted. Transcriptional silencing is required for correct germ cell specification. Signaling from the somatic niche is required for their proliferation and differentiation into specialized gametes. (Bottom panel) Shown are the conserved processes involved in germline formation and maintenance and the expression of relevant factors at specific stages. RNA biology involves regulation of RNA translation, stability, and processing. Repression of transcriptional programs for somatic differentiation during germline specification is conserved among flies, worms, and mice, although different proteins and mechanisms mediate this repression in the different species. In the mouse, in addition to repression of somatic programs, the transcription factors Oct4, Sox2, and Nanog are expressed in the germline. Chromatin modifiers play important roles in germ cell maintenance (their roles in imprinting and X chromosome gene expression are not shown).

combination of signals from the extraembryonic ectoderm and visceral endoderm (Hayashi et al., 2007). Despite differences in modes of specification, all three organisms use similar mechanisms to establish and restrict the germ cell fate.

One conserved aspect of germ cell specification during early embryogenesis is the active repression of programs of somatic differentiation (Seydoux and Braun, 2006). In the germlines of worms and flies, repression of somatic gene expression initially occurs at the level of mRNA synthesis. In blastomeres of the worm germline, the zinc finger-containing protein PIE-1 prevents mRNA synthesis by globally inhibiting the ability of RNA Polymerase II (Pol II) to initiate and elongate until germ cell commitment is completed (Ghosh and Seydoux, 2008; Seydoux and Braun, 2006). Similarly, in fly germ cells, the small peptide Polar granule component (Pgc) prevents interaction of the RNA Pol II activating kinase complex P-TEFb with Pol II at promoters (Hanyu-Nakamura et al., 2008). Germline blastomeres lacking PIE-1 adopt a somatic cell fate, and germ cells lacking Pgc express transcripts characteristic of their somatic neighbors (Martinho et al., 2004; Seydoux and Braun, 2006; Seydoux et al., 1996). Thus it appears that both PIE-1 and Pgc prevent early germline cells from responding to the signals that promote somatic differentiation during early embryogenesis.

As germline development proceeds in both worms and flies, the factors that promote the global repression of transcription disappear and chromatin-based mechanisms of repression are implemented (Schaner et al., 2003). In worms, several of the *MES* (maternal effect sterile) genes encode orthologs of the Polycomb Group (PcG) family of repressors. This conserved histone H3 methyltransferase complex controls survival and proliferation of germ cells by silencing gene expression, in particular on the X chromosome (Bender et al., 2004). In flies, a definitive chromatin-based mechanism of transcriptional repression has yet to be identified. However, the *Drosophila* homolog of the LSD1 demethylase, which removes methyl marks from histone 3 lysine 4, is active in germ cells and the expression of somatic genes is observed in the germ cells of flies bearing mutations in Osa, a member of the SWI/SNF chromatin-remodeling complex (Martinho et al., 2004; Rudolph et al., 2007).

In mice, the transcriptional programs for somatic differentiation are also repressed during germ cell specification (Ohinata et al., 2005). The earliest marker of germ cells in mice is Blimp1, a SET domain and zinc finger-containing protein. Lineage tracing experiments have shown that cells expressing Blimp1 give rise to approximately 40 founder germ cells. In mice lacking Blimp1, germ cells are not properly specified; instead the cells normally destined to become germ cells express genes characteristic of the neighboring somatic cells. Blimp1 can associate with the arginine histone methyltransferase, Prmt5, and thus may be directly involved in setting a repressive chromatin state that precludes the expression of somatic differentiation programs. In contrast to worms and flies where new transcription can be blocked entirely due to maternal loading of factors specific to the germline, some genes such as *Oct4*, *Nanos3*, *Nanog*, and *Stella* escape transcriptional repression in mouse germ cells; however, the factors that promote expression of these genes remain to be identified.

Germ Cells Rely on Their Niche

Once germ cells reach the gonad, they take up residence in a unique somatic microenvironment, known as a niche, in which germ cell-soma interactions regulate germ cell behavior (see Review by S.J. Morrison and A.C. Spradling in this issue of *Cell*). The niche provides the necessary signals that regulate the balance between self-renewal and differentiation that is needed for proper progression through gametogenesis. For example, in worms, dividing germ cells are found at the distal tip of the niche, adjacent to a single somatic cell known as the distal tip cell. Notch signaling emanating from this cell prevents germ cells from entering into meiosis until they reach a critical distance from the distal tip cell. Similarly in the fly ovary, bone morphogenetic protein (BMP) ligands produced by somatic cells in the niche maintain the germline stem cells by repressing genes critical for differentiation. Additionally, in the gonad of the male fly, JAK/STAT signaling is critical for proper maintenance of the germline stem cell population (see Review by S.J. Morrison and A.C. Spradling). In mammals, germline stem cells are only found in the testis, whereas adult ovaries harbor a fixed number of meiotic oogonia that were generated in the embryonic gonad. It was only recently that a stem cell niche was identified for spermatogonial stem cells in the mouse. Sertoli cells may contribute to the niche given that they produce glial cell line-derived neurotrophic factor (GDNF), which regulates the renewal of spermatogonial stem cells (Meng et al., 2000). In addition, recent data suggest that the vasculature and associated Leydig cells form a niche for mouse spermatogonial stem cells in the interstitial areas between seminiferous tubules (Yoshida et al., 2007). The signals produced by these cells are unknown.

Conserved RNA Regulators Control the Germ Cell Program

Posttranscriptional regulation plays a paramount role in the germline (Kimble and Crittenden, 2007). Not only are many RNA regulators specific to the germline where they control proliferation, survival, and differentiation of germ cells, but they also show striking evolutionary conservation. Indeed, finding homologs of the germline-specific RNA helicase Vasa led to the identification of germ cells in many animals, ranging from planaria to humans (Extavour and Akam, 2003). Consistent with the notion that RNA regulators represent the core of the "germline program," several conserved RNA regulators are expressed in germ cells throughout the life cycle of the germline where they control the translation, stability, and processing of RNA. For example, the Pumilio or PUF family of RNA-binding proteins and their binding partner Nanos are required for germ cell survival in fly, mouse, and worm embryos and also affect germline stem cell maintenance in the adult (Seydoux and Braun, 2006). Many of the known germline RNA regulators repress RNA translation and promote RNA deadenylation. They are often found in large germline-specific RNA-protein granules that resemble RNA processing or storage bodies found in other cells. It has been proposed that networks of these conserved RNA regulators control the timing and spatial distribution of specific targets throughout germline development

(Kimble and Crittenden, 2007). In addition to germline-specific functions, these targets may include somatic genes that need to be suppressed in the germline to prevent transdifferentiation (Ciosk et al., 2006).

The microRNA (miRNA) pathway is another form of RNA regulation that is required for germ cell maintenance in worms, flies, and mice. Targeted deletion of the miRNA-processing enzyme Dicer in germ cells of flies and mice results in altered patterns of germ cell-specific gene expression, changes in nuclear structure, and cell-cycle defects that lead to developmental arrest (see Minireview by B.M. Stadler and H. Ruohola-Baker in this issue). In zebrafish, some miRNAs are inactivated in germ cells. For example, miR-430 represses the translation of maternal *nanos* RNA in zebrafish somatic cells causing its degradation, whereas in germ cells, miR-430 is prevented from binding to *nanos* RNA by the RNA-binding protein *Dead-end (Dnd)* (Kedde et al., 2007). Dnd belongs to a conserved family of RNA-binding proteins that contain nucleic acid editing motifs. Mutations in Ter, the Dnd mouse ortholog, lead to loss of the germline or formation of tumors depending on the genetic background of the mouse (Youngren et al., 2005). Thus a role for Dnd may be conserved and its function adds another level of complexity to the regulation of gene expression by miRNAs in germ cells.

A new class of small RNAs, known as the piRNAs, are active in the gonads of flies and mice. piRNAs are thought to regulate selfish DNA elements and promote genomic stability during gametogenesis. Mutations in components of the piRNA pathway result in mobilization of transposable elements, defects in heterochromatin formation, and sterility in many organisms (Aravin et al., 2007; Brower-Toland et al., 2007). It remains to be seen if piRNAs in addition to protecting the genomic stability of germ cells also play an instructive role in germ cell biology. One possibility is that piRNAs somehow contribute to the assortment of the maternal and paternal genomes in meiotic germ cells. Thus, these small RNAs may not only help to generate the next generation but also may foster the evolution of a generation that is more genetically fit.

Germ Cells Restrict Their Totipotent Potential

Germ cells are often described as the ultimate totipotent stem cell. However, germ cells give rise to only sperm or eggs, and it is not until the fertilization of the egg or parthenogenesis that an entirely new organism arises. Therefore, germ cells are unipotent cells that retain a totipotent potential that is eventually transferred to the zygote at fertilization. This totipotent potential is evident in the mature eggs of many animals, where somatic gene products, including those required for the patterning of the early embryo, are present but not utilized until fertilization.

During normal development the totipotent potential of the germ cell is suppressed. However, there are experimental scenarios in which the totipotent potential of germ cells is revealed prior to the fusion of egg and sperm. In these cases, one of the key features of germline transcriptional silencing, the niche, or translational regulation was manipulated. For example, mutations in the transcriptional silencers PIE-1 and Blimp1 "transform" embryonic cells destined to become germ cells into somatic cells, illustrating the importance of these regulators in preventing somatic gene expression. Using transplantation assays, Wylie et al. (1985) demonstrated that the niche environment is also critical for restricting the potential of germ cells. By removing germ cells from the frog gonad and placing them back into the embryonic blastocoel, germ cells differentiated into the three primary embryonic germ layers (Wylie et al., 1985). Similarly, when germ cells are removed from the mouse gonad and placed subcutaneously into another mouse, germ cells differentiate and form tumors, known as teratomas, which are composed of cell types representative of the primary germ layers. Finally, germ cell-specific RNA networks also suppress the potential of germ cells. Recently, teratoma formation was described in the gonads of worms (Ciosk et al., 2006). These tumors emerged in a mutant background in which two translational repressors, GLD-1 and MEX-3, were inactivated. Interestingly, GLD-1 alone controls entry into meiosis, whereas MEX-1 specifies the identity of early somatic blastomeres, supporting the notion that germline identity is maintained by the coordinated regulation of germline differentiation and programs for somatic repression. Taken together, the three features of the germline discussed above function in part to suppress the totipotent potential of the germ cell until fertilization. Understanding the mechanisms that suppress and reactivate the totipotent potential of germ cells may provide important insight into the reprogramming and programming of other stem cells.

Outlook

Germ cells possess a dual identity: they are both highly specialized and uniquely capable of forming an entirely new organism. Here, we summarize three features of the germline: broad repression of transcriptional programs for somatic differentiation, complex regulation of RNA targets that promotes germ cell maintenance and differentiation and represses somatic differentiation, and the reliance of germ cells on their niches for regulating germline development while blocking somatic signals.

Inhibition of somatic differentiation programs in the early germline is likely to be of paramount importance given that germ cells either are surrounded by or are migrating through developing somatic tissues during embryogenesis. It is perhaps surprising that the proteins and regulatory processes involved in this essential feature of the germline do not seem to be conserved between organisms. Moreover, although misexpression of PIE-1, Pgc, or Blimp1 alone represses transcription, these factors are not sufficient to specify germ cell fate. Thus, these repressors may have evolved independently due to the need for widespread silencing of "somatic" genes rather than as instructive "master regulators" of germ cell fate.

Evidence from model organisms also clearly points to the importance of signaling between the niche and the germline for the maintenance of germ cells in the adult. The specific signaling pathways (Notch, BMP, Jak/Stat, and GDNF) that regulate germline cells in different organisms are not unique to germ cells. In addition to providing an environment for the germline that is conducive to continued proliferation, survival, and protection from differentiation, it remains to be seen whether and how germ cell niches control germline-specific functions. One candidate for a regulator that is gonad specific is Piwi,

the piRNA-binding protein. Recently, Piwi was shown to activate piRNA expression in the *Drosophila* germ cell niche that is genetically linked to its role in germline stem cell maintenance (Yin and Lin, 2007). How Piwi and small RNAs affect niche function remains unclear. One possibility is that the effect of piRNAs on heterochromatin could modulate the interaction of the niche with the germline.

Of the three features discussed, the prominent role played by conserved regulators of RNA translation seems specific to germ cells. However, the fundamental question of "germ cell-ness" cannot easily be answered by outlining a hierarchical pathway of germ cell fate specification as can be done for somatic tissues. Despite a large number of regulators of germline RNAs, there is no evidence as yet that any one of them alone plays an instructive role in germ cell fate. Removing one of these factors from germ cells generally causes cell death rather than transdifferentiation, suggesting that multiple parallel pathways function to promote germ cell fate. It has been suggested that one role of the RNA-centric cytoplasmic regulation of gene expression in germ cells is to suppress the inherent potential of the germ cell genome to produce somatic cell fates (Seydoux and Braun, 2006). However, germ cells also have exquisitely orchestrated germ cell-specific functions, such as their migration in the embryo, their interactions with the niche, their ability to undergo meiosis, and their sexual differentiation into sperm and eggs. For flies and worms, RNA regulators have been implicated in each of these functions. It remains a challenge for the future to dissect how the network formed by these RNA regulators is controlled during the germ cell life cycle.

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