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Germline stem cells: stems of the next generation

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Abstract

Germline stem cells (GSCs) sustain gametogenesis during the life of organisms. Recent progress has substantially extended our understanding of GSC behavior, including the mechanisms of stem cell self-renewal, asymmetric stem cell division, stem cell niches, dedifferentiation, and tissue aging. GSCs typically are highly proliferative, due to organismal requirement to produce large number of differentiated cells. While many somatic stem cells are multipotent, with potentially multiple differentiation pathways, GSCs are unipotent. For these relatively simple characteristics (e.g. constant proliferation and unipotency), GSCs have served as ideal model systems for the study of adult stem cell behavior, leading to many important discoveries. Here, we summarize recent progress in GSC biology, with an emphasis on evolutionarily conserved mechanisms.

Introduction

Germline stem cells (GSCs), like other somatic adult stem cells, are long-lived, often surviving throughout the life of an organism. GSCs provide a continuous supply of differentiated cells that sustain fertility. Division of a GSC produces two daughter cells: a stem cell and a differentiating cell. Unlike somatic stem cell lineages, where differentiated cells are supposed to be relatively dispensable (in that they will be worn out soon or later anyways), neither of GSC daughters is dispensable. GSCs must be protected to prevent tumorigenesis and tissue degeneration, while differentiating cells must be protected to accurately transmit genetic information to the next generation. The mechanisms by which GSCs fulfill these possibly contradictory requirements are not understood.

Recent research has revealed many features of GSCs to be consistent with their identity as stem cells. These characteristics include mechanisms of stem cell self-renewal, asymmetric division as a means of tissue homeostasis, and influence of the stem cell niche. These findings show that many aspects of stem cell biology are conserved in GSCs. These results, combined with the simple characteristics of GSCs, including unipotency and a high proliferation rate, have advanced the study of adult stem cell biology.

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Daughters of GSCs must decide whether to either retain the same characteristics as stem cells (self-renewal) or to initiate differentiation. The determination of cell fate is critical for long-term tissue homeostasis, maintaining the pipeline of gamete production while preserving the stem cell population. During steady-state tissue homeostasis, asymmetric stem cell division can contribute to this balance. Also, as is the case for many other stem cells, GSCs are often found in the stem cell niche, a microenvironment that specifies stem cell identity. Here we summarize recent findings on these topics regarding GSCs, mainly from *Drosophila*, *C. elegans*, and mouse, in the framework of important concepts in the adult stem cell biology.

Signaling pathway for maintaining stemness; self-renewal

The term “stem cell self-renewal” refers to the phenomenon that upon division, stem cells produce stem cells with their original characteristics. Unlike many other adult stem cells, GSCs are unipotent, producing only either sperm or eggs. Although there are striking similarities in GSCs among different species, the signaling pathways that govern their stemness appear to be divergent.

In *Drosophila*, GSCs typically divide asymmetrically to produce one stem cell and one differentiating cell. The differentiating cell then undergoes exactly four transit-amplifying divisions, yielding 16 interconnected germ cells, before entering meiosis (Figure 1). In *Drosophila* male GSCs, the JAK (Janus kinase)-STAT (Signal transducer and activator of transcription) pathway is essential for self-renewal of GSCs [1] (Figure 1a). Loss-of-function mutations of components of this pathway lead to rapid loss of GSCs in a cell-autonomous manner. However, the activation of the JAK-STAT pathway in GSCs is not sufficient for self-renewal. GSCs require inputs from surrounding cyst stem cells (CySCs) for stem cell identity [2].

In female GSCs, the BMP signaling pathway executes stemness through transcriptional repression of Bam, a key differentiation-promoting factor [3]. In the past few years, many factors have been identified that regulate GSC identity via regulation of Bam, including a chromatin remodeling factor ISWI [4], a nuclear membrane protein Otefin [5], eIF4A [6], and HOW [7]. These studies illuminate the importance of Bam in germline differentiation. Other studies identified additional factors necessary for female GSC identity, including the Histone H2B ubiquitin protease Scrawny [8], Pelota [9], and Effete E2 ubiquitin-conjugating enzyme [10]. Microarray analyses have identified genes highly expressed in GSCs [11,12]. Functional analysis on such genes identified in microarray may reveal unidentified signaling networks toward stemness.

Although it was thought that male and female GSCs depend on distinct signaling pathways, emerging evidence suggests that male and female GSCs may share greater similarities than previously appreciated. JAK-STAT signaling in somatic support cells plays a dominant role in both male [2] and female [13] GSC identity, likely via regulation of BMP signaling [14].

In *C. elegans*, all developing germ cells exist as syncytium until mature sperm and eggs are pinched off. GSCs reside at the distal end of the gonad, where they are surrounded by processes of a distal tip cell (DTC). Maintenance of GSCs is controlled by the Notch signaling pathway, involving the GLP-1 Notch receptor [15] (Figure 1c). FBF-1, FBF-2, and PUF (Pumilio and FBF) are RNA-binding proteins that serve as the downstream targets of GLP-1 [16]. FBF-1 and FBF-2 are the major regulators of GSC self-renewal. *fbf-1 fbf-2* double mutant germ cells enter meiosis without self-renewal [17].

In mouse testis, undifferentiated spermatogonial cells are classified by their morphology or connectivity. Cells are classified as A_s (single cell spermatogonia), A_{pr} (paired

spermatogonia), A_{al} (4–16 spermatogonia are aligned with connected cytoplasm). These cells eventually differentiate into spermatozoa. Generally, A_s is regarded as the most primitive progenitors, e.g. spermatogonial stem cells (SSCs), although recent work challenged this view [18]. Furthermore, it has been suggested that a population that functions as stem cells in unperturbed tissues might be different from the population that can colonize the host testes upon transplantation. This calls into question the gold standard of stemness as being capable of tissue reconstitution upon transplantation [19].

A series of studies have suggested that *Plzf*, *Bcl6b*, *Etv5*, *Lhx1*, *Oct4*, *Nanos2*, *Ngn3*, *GFR α 1*, *RET*, *FGFR2*, and *Nodal* are intrinsically required factors for the maintenance of SSCs and/or are highly expressed in SSC populations [20–26] (Figure 1d). β 1 integrin is required for homing of SSCs [27], although its functional significance in unperturbed tissues remains unclear.

It is now widely accepted that Piwi homologs are required throughout germline development to suppress transposable elements and protect genome integrity [50]. However, the founding member of this family, Piwi, was originally identified as being required for GSC maintenance [49]. In the *Drosophila* germline, Piwi contributes to GSC behavior through cell autonomous and non-autonomous manners [49]. Studies from other organisms, including *C. elegans* [49,51,52], mouse [50], and zebrafish [53][54], have not revealed specific functions of Piwi homologs in GSC maintenance.

Germline stem cell niche: a home for GSCs

Many stem cells, including somatic stem cells and GSCs, are known to reside in a special microenvironment called the stem cell niche. The niche provides essential factors to maintain stem cell identity and proliferation capacity, as well as to protect stem cells from the onset of a differentiation program. The niche mechanism probably contributes to tumor suppression, since it is unlikely that stem cells undergo unlimited tumorigenic division if “stem cell factors” must be provided from the environment.

In *Drosophila*, hub cells in the testis secrete the signaling ligand Unpaired (Upd) to activate the JAK-STAT pathway, while cap cells in the ovary secrete the BMP-like ligands *dpp* and *gbb* to activate the BMP signaling, leading to GSC identity [1] (Figure 1a, b). Additional somatic cells (cyst stem cells (CySCs) in the testis and escort stem cells (ESCs) in the ovary) also contribute to the GSC niche [1]. *Egfr* signaling plays a critical role in the proper association between GSCs and CySCs/ESCs, thus regulating GSC maintenance [28–31]. In addition to these signaling factors, both male and female GSCs require cadherin- and/or integrin-based cell adhesion to remain in the stem cell niche [32–34]. Recent reports illuminated the importance of the glypicans Dally and Dally-like in defining the range of niche signaling [35,36].

In *C. elegans*, the distal tip cell (DTC) provides the stem cell niche by secreting LAG-2 and APX-1, two DSL (Delta/Serrate/Lag-2) ligands, to activate the GLP-1/Notch receptor within the neighboring GSCs [15] (Figure 1c). DTC extends long cell processes to surround germ cells. This association of germ cells with the DTC process appears to specify GSC identity [37,38]. Activation of Notch signaling in germ cells is necessary and sufficient for GSC identity. It seems that any cells expressing the DSL ligand can function as an ectopic or latent niche, leading to GSC tumor [39].

In the mouse, SSCs are believed to be located at the basement membrane of seminiferous tubules. In this niche, SSCs receive glial cell line-derived neurotrophic factor (GDNF) [40] and FGF2 [25], which are produced by somatic Sertoli cells, for their self-renewal [24] (Figure 1d). GDNF functions through the activation of *GFR α 1* and the *RET* receptor

complex in SSCs. This receptor complex is highly expressed in undifferentiated spermatogonia and required for SSC maintenance, as described above [23,40]. Interestingly, Sertoli cells also produce activin A, BMP4 and SCF, which promote differentiation. Therefore, other factors must exist in addition to those from Sertoli cells that define stem cell niche function [24]. Indeed, direct visualization of SSCs suggests that these cells tend to cluster at the basal membrane near blood vessels that run through between seminiferous tubules [41]. Thus, SSCs may be receiving factors from the vasculature in addition to those from Sertoli cells. Also, signaling between Leydig cells, which express CSF1, and SSCs, which express CSF1 receptor, is of particular interest [42].

Dedifferentiation

Dedifferentiation is a process by which differentiating cells revert to stem cell identity. In the *Drosophila* gonad, both female and male germ cells can regain stem cell identity after initiation of differentiation [43,44]. Thus, germ cell fate is plastic. In males, dedifferentiation occurs in unperturbed tissue and increases with aging [45]. This suggests that dedifferentiation is a mechanism by which stem cell numbers can be maintained during aging, although it is not known whether blockade of dedifferentiation can affect the maintenance of stem cell number with age.

Actin-based protrusions have been observed in germ cells that are induced to dedifferentiate [46], suggesting that dedifferentiation might be an active process that is guided by long-range cell-cell signaling to recruit new GSCs. While *Drosophila* male germ cells lose the capability to dedifferentiate when they become spermatocyte [44], *C. elegans* germ cells appear to maintain the capability to revert back to mitosis (and GSC fate) even after completing premeiotic prophase [47]. Transplantation experiments have shown that mouse testis contains actual and potential stem cell populations, the latter of which likely corresponds to spermatogonia capable of dedifferentiation [18,19,48].

Asymmetric division to balance self-renewal and differentiation

Stem cells can divide either asymmetrically to preserve stem cell number and create differentiating cells during steady-state tissue homeostasis, or symmetrically to increase stem cell number during development and tissue repair. Asymmetric cell division in the niche is a simple strategy that adult stem cells employ to balance their self-renewal and differentiation.

Drosophila GSCs normally divide asymmetrically (Figure 1a, b), generating one GSC and one differentiating cell. Such asymmetric stem cell division is achieved by the combination of local niche signaling and intracellular machinery that orients the mitotic spindle. In *Drosophila* female GSCs, the spindle is oriented via anchorage of one spindle pole to the spectrosome, a germ cell specific ER-like organelle, which is always located at the apical side of GSC [55].

In *Drosophila* male GSCs, the spindle orientation is set up by centrosome positioning, where the mother centrosome is always anchored to the apical side of the GSC by astral microtubules to adherens junctions formed between the hub and GSCs [56,57]. In addition to such elaborate cellular mechanisms that position centrosomes and orient spindles, male GSCs may possess a checkpoint that monitors the correct centrosome orientation prior to commitment to mitosis [45]. Such a mechanism could be similar to spindle position checkpoint (SPOC) observed in *S. cerevisiae* [58].

In *C. elegans*, GSCs do not appear to have any particular spindle orientation [37]. Instead, GSC fate solely depends on contact with the niche component distal tip cell (DTC), which

provides a parallel to the *Drosophila* GSCs and can be used to extend our understanding of how the mechanisms that govern GSC fate and number are regulated in divergent organisms.

In the mouse, spindle orientation during SSC division has not been examined. Recently, it was suggested that Plzf and ubiquitin carboxy-terminal hydrolase (UCH-L1) display asymmetric expression levels in A_{pr} spermatogonia, implying potential asymmetric SSC divisions [59]. It is tempting to speculate that some GSC populations may utilize spindle orientation in making the decision of symmetric vs. asymmetric stem cell division.

Regulation of GSC behavior by systemic factors and aging

In addition to the regulation of stem cells during steady-state tissue homeostasis as described above, the behavior of GSCs is further modulated by systemic factors and aging. While it is obvious that reproduction, and thus GSC behavior, should be modulated by the availability of nutrients and during the aging process, the underlying mechanisms are only beginning to emerge. The division and numbers of GSCs are affected by nutrients and insulin signaling in both *Drosophila* and *C. elegans* [60–64]. Moreover, age-related changes in the number of function of GSCs have been reported [45,65–67]. Interestingly, these changes appear to involve both GSC intrinsic factors as well as niche factors.

In *C. elegans*, it is known that germ cells, including GSCs, and insulin signaling help regulate the aging process [68]. However, it is not clear how GSCs change with age. In the mouse, SSC number, function, and niche function decreases with age ([69] and references therein). Understanding how systemic factors, such as nutrients and insulin signaling, merge onto core GSC regulatory networks awaits future investigation.

Conclusion

Substantial progress has been made in the past several years to better understand the regulation, function, and behavior of GSCs. GSC biology represents an intersection of stem cell biology and germ cell biology. GSCs must fulfill potentially contradictory requirements inherent in their shared identity as stem cells and germ cells. While GSCs must protect themselves from tumorigenesis and degeneration, GSCs must also protect their differentiating daughters to ensure successful transmission of genetic material to the next generation. Our understanding of GSC biology is still very limited, but future studies fueled by recent progress will broaden our view on GSCs.

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Figure 1. Anatomy of GSC niches

(a) *Drosophila* male GSC niche. GSCs and cyst stem cells (CySCs) are attached to hub cells via adherens junctions (purple crescents). GSCs divide asymmetrically to self-renew and produce a differentiating gonialblast (GB). The GB undergoes four synchronous, transit-amplifying divisions to yield 16 interconnected spermatogonia, through which the fusome (red lines) run. The spectrosome (red sphere) is the spherical version of the fusome found in GSCs. A pair of CySCs encapsulates the GSCs and provides the signals required for GSC identity. Similar to GSCs, CySCs are thought to divide asymmetrically to self-renew and produce cyst cells. Cyst cells exit the cell cycle, a pair of which encapsulates the GB and spermatogonia to promote differentiation. Hub cells secrete the Unpaired (Upd) ligand to activate the JAK-STAT signaling pathway in both GSCs and CySCs to specify their stem cell identity. The Zfh-1 transcription factor acts downstream of the JAK-STAT to specify CySC identity. EGFR signaling ensures the encapsulation of germ cells by cyst cells. The mitotic spindle in GSCs is oriented to ensure the asymmetric stem cell division by positioning a mother centrosome (yellow star) toward the hub and GSC interface. GB and spermatogonia can dedifferentiate to regain GSC identity.

(b) *Drosophila* female GSC niche. GSCs are attached to the cap cells via adherens junctions (purple crescents). GSCs divide asymmetrically to self-renew and produce a differentiating cystoblast (CB). The CB divides four more times to give rise to 16 germ cells interconnected by the fusome, only one of which becomes an oocyte. The remaining 15 cells become nurse cells. Escort stem cells (ESCs), the analog of CySCs, encapsulate the GSC, while their daughters, escort cells, encapsulate the developing germ cells. Cap cells secrete the BMP ligands (dpp and gbb) to repress the key differentiation-promoting factor, Bam, in GSCs to maintain stem cell identity. The JAK-STAT pathway in ESCs contributes to GSC identity. EGFR signaling also ensures the encapsulation of germ cells by cyst cells. The mitotic spindle in GSCs is oriented toward the cap cells via anchoring of one spindle pole to the spectrosome, which localizes consistently to the apical side of GSCs.

(c) *C. elegans* germline stem cell niche. The single distal tip cell (DTC) constitutes the niche for germline stem cells. DTC secretes LAG-2/APX-1 ligands to activate GLP-1/Notch signaling in GSCs. FBF-1 and FBF-2 are the direct downstream targets of GLP-1. No consistent spindle orientation was observed.

(d) Mouse spermatogonia stem cell (SSC) niche. Mouse SSCs are found in undifferentiated spermatogonial populations, which are classified as A_s (single cell spermatogonia), A_{pr} (paired spermatogonia), and A_{al} (4–16 aligned spermatogonia with interconnected cytoplasm). Spermatogonia are surrounded by Sertoli cells, which are believed to be a major niche component. Leydig cells and blood vessels may also contribute to the SSC niche. Sertoli cells are sealed with tight junctions (purple oval).