Molecular Mechanisms of Germline Stem Cell Regulation

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germline stem cells, self-renewal, differentiation, niche, asymmetric cell division

Abstract

Germline stem cells (GSCs), which can self-renew and generate differentiated progeny, are unique stem cells in that they are solely dedicated to reproduction and transmit genetic information from generation to generation. Through the use of genetic techniques in Drosophila, Caenorhabditis elegans, and mouse, exciting progress has been made in understanding molecular mechanisms underlying interactions between stem cells and niches. The knowledge gained from studying GSCs has provided an intellectual framework for defining niches and molecular regulatory mechanisms for other adult stem cells. In this review, we summarize recent progress and discuss conserved mechanisms underlying GSC self-renewal and differentiation by comparing three GSC systems. Because GSCs and other adult stem cells share “stemness,” we hope this review will help define fundamental principles of stem cell regulation and provide further guidance for future studies of other adult stem cells.
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INTRODUCTION

With their remarkable ability to self-renew and undergo differentiation, stem cells are crucial to development and tissue homeostasis (68, 88). Interest in stem cell research has burgeoned since the successful culture of human embryonic stem cells (hESCs), which are able to generate various differentiated cell types (81, 92). In addition to ESCs, stem cells in a variety of adult tissues are also able to generate
one or several differentiated cell types throughout an individual’s lifetime. Germline stem cells (GSC) are dedicated to producing gametes for transmission of genetic information from generation to generation and, therefore, are true “immortal stem cells.” The sterility resulting from GSC loss can be easily recognized, and it facilitates the identification of extrinsic signals and intrinsic factors in genetic model systems such as *Drosophila*, *C. elegans*, and mouse. Furthermore, GSCs are easily identified in the anatomically simple *Drosophila* ovary and testis and have enabled the first elucidation of relationships between stem cells and their microenvironment or “niche” (48, 95, 105). The investigation of the GSC niche and the regulatory mechanisms of stem cell self-renewal in *Drosophila* has provided guiding principles for study of adult stem cells in other systems, because relationships between stem cells and their niches are conserved. Cultured mouse GSCs and stem cell transplantation make it feasible to elucidate molecular regulatory mechanisms of mammalian GSCs (44, 53). This review summarizes our current understanding of GSC regulation, highlights conserved molecular mechanisms, and predicts future challenges.

**REGULATORY MECHANISMS OF GERMLINE STEM CELL SELF-RENEWAL AND DIFFERENTIATION IN THE DROSOPHILA OVARY**

**General Features of the Drosophila Ovarian GSCs and their Niche**

The identification of stem cells poses unique challenges particularly in mammalian systems because stem cells are rare and indistinguishable from early differentiated progeny (68, 88). The *Drosophila* ovarian GSC system circumvents this problem by virtue of its simple anatomy, unique molecular markers, and a linear arrangement between stem cells and their differentiated progeny (30, 60, 102). At the tip of the gerarium, the anterior end of the ovarioles, 2 to 3 GSCs can be identified by their anteriorly located spectrosome (spherical fusome) and anchorage to cap cells through DE-cadherin-mediated cell adhesion (Figure 1, Table 1) (87). The fusome is a germ cell–specific organelle rich in membrane skeletal proteins such as Spectrins and Hu-li tai-shao (Hts) (21) (62). The cap cells form a niche that regulates the behavior of GSCs, perhaps with some contributions from terminal filament (TF) and inner germarial sheath (IGS) cells close to GSCs (105). These GSCs undergo asymmetric division: The daughter that remains in the niche retains GSC identity, while the other daughter cell moves away from the niche to differentiate into a cystoblast. The cystoblast also has a spectrosome and undergoes four rounds of synchronous division with incomplete cytokinesis to form a 16-cell cyst containing a branched fusome and ring canals connecting individual cystocytes (21).

The well-defined morphology and the linear fashion in which GSCs and their progeny progress throughout the gerarium, along with available genetic tools in *Drosophila*, have facilitated the investigation of GSC maintenance and differentiation. For example, by using a heat-inducible FLP (flippase, a DNA recombinase)-FRT (FLP recognition target)-mediated recombination technique to produce a marked mutant GSC clone that can be compared with the control GSC clone side-by-side in the same gerarium, we can determine the role of a particular gene in stem cell self-renewal, differentiation, and division (104). With a well-characterized niche and readily identifiable GSCs, the *Drosophila* ovary represents an excellent model system to investigate stem cell biology in vivo at the molecular and cellular level (30, 60, 102, 103).

**BMP, Piwi, and Yb Function in the Niche to Regulate the Maintenance and Division of GSCs**

The GSC asymmetric cell division can be partially attributed to the extrinsic factors emanating from the niche. Regulatory molecules,
Figure 1
Major signaling pathways and intrinsic factors for GSC self-renewal and differentiation in the Drosophila ovary. The BMPs (Dpp and Gbb), Yb, Piwi, and Hh are expressed in the TF/cap cells and control GSC self-renewal. In the GSC, Pum/Nos and Vasa are required for controlling self-renewal, and Piwi regulates GSC division. GSCs are anchored to their niche by DE-cadherin-mediated cell adhesions. Gap junctions formed by Zpg are important for GSC maintenance and CB differentiation. Bam is required for cystoblast differentiation but is repressed in GSCs by Mad/Med complexes. However, this repression of Bam is overcome in CB, partially through Smurf.

BMP: bone morphogenetic protein

bone morphogenetic proteins (BMPs) and Piwi, are produced from cap cells to modulate ovarian GSC maintenance and division via intracellular signaling pathways (Figure 1; Table 1). Two BMPs, Dpp and Gbb, are expressed primarily in cap cells (86, 104) and serve as short-range signals to activate BMP signaling in GSCs through type I (Tkv and Sax) and type II (Punt) BMP receptors to nuclear complexes, Mad [a founding member of SMA and MAD (SMAD) family proteins; a BMP-specific SMAD], and Medea (SMAD4, also known as Co-SMAD) to control their self-renewal and division (42, 104). Mutations in dpp, gbb, or downstream components lead to GSC loss by premature differentiation and slower division rates (86, 104, 105). Overexpression of dpp, but not gbb, completely blocks cystoblast differentiation, resulting in proliferation of GSC-like tumors throughout the germarium (86, 104). These studies indicate that BMP signaling is necessary and
Table 1  Extrinsic signals and intrinsic factors that are required for regulating GSC function

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<th>Functions</th>
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<td>BMP: dpp, gbb (niche)</td>
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<td>GSC self-renewal (45, 85)</td>
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<tr>
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<td>CB differentiation (10, 11, 13)</td>
<td>Unknown</td>
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<tr>
<td>arm (CB)</td>
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<td>JAK/STAT: upd (niche)</td>
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<td>GSC self-renewal (16)</td>
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<td>EGF: stet (germline cells)</td>
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<td>Translational regulation: pum/neo (GSC)</td>
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<tr>
<td>vasa (germline cells)</td>
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<td>bam/hgcn (CB)</td>
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<tr>
<td>glp-1, lag-1, lag-3 (GSC)</td>
<td>GSC self-renewal and proliferation (19, 57, 100, 109)</td>
<td>Promoting meiosis (24, 35, 38, 40, 41, 55, 63, 97)</td>
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<tr>
<td>Translational regulation: bfl-1/bfl-2 (GSC)</td>
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<tr>
<td>gla-1, gla-2, gla-3, nos-3 (differentiated germline cells)</td>
<td>Unknown</td>
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<td><strong>M. musculus</strong></td>
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<td>GDNF: GDNF (Sertoli cells)</td>
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<tr>
<td>Ret, GFRa1 (GSC)</td>
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<td>BMP: BMP4, BMP7, BMP8a, BMP8b (germline cells)</td>
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<td>SCF/c-Kit: SCF (Sertoli cells)</td>
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<td>c-Kit (differentiated spermatogonia)</td>
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<td>Transcriptional regulation: Plzf (spermatogonia)</td>
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<td>Transcriptional regulation: nos-2 (germline cells)</td>
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GSC, germline stem cell; PGC, primordial germ cell; CB, cystoblast.
sufficient for GSC self-renewal. Since Gbb and Dpp likely use common signal transducers, it remains to be determined why dpp, but not gbb, is sufficient to block germ line cell differentiation when overexpressed (34, 46).

Although GSCs normally undergo asymmetric cell division, they are also capable of dividing symmetrically to generate two GSCs when both of the daughters remain in the niche (105). Surprisingly, partially differentiated cells such as 2-, 4-, and 8-cell cysts revert back to GSCs when the niche signal Dpp is provided (43). It remains to be determined how Dpp signaling can completely turn off the active differentiation program in cystocytes. Understanding this phenomenon would provide more insight into how GSC self-renewal and differentiation is regulated, and it may allow the regeneration of stem cells from differentiated cells in future regenerative medicine, if such cell fate reversal also exists in mammalian stem cells.

Like BMPs, Piwi and Yb are expressed in niche cells and are also involved in GSC maintenance (Figure 1; Table 1). Loss-of-function mutations in piwi and Yb cause rapid loss of GSCs, while Yb and piwi overexpression increases GSC-like or cystoblast-like germ cells (16, 17, 51, 52, 61). Yb is a novel intracellular protein that regulates piwi and hh (bridgehead) expression (51, 52), whereas Piwi is the founding member of the piwi family genes containing conserved PAZ and Piwi domains that bind to RNAs (16). Ih signaling appears to play a redundant role with Piwi to control GSC maintenance (52). Although the requirement of Piwi in the niche for GSC maintenance is well established, how this is accomplished remains unclear.

As a cystoblast moves away from the cap cells, it becomes surrounded by cellular processes of IGS cells, raising a possibility that IGS cells regulate cystoblast differentiation. Indeed, a study on stet function has revealed the link between IGS cells and germ cell differentiation (80). In stet mutant ovaries, the cellular processes of IGS cells are severely reduced, and spectroscopic-containing single germ cells accumulate, suggesting that stet is required for cystoblast differentiation. stet, expressed in germ cells, encodes a membrane protease similar to Rhomboid that can cleave and release EGF ligands (96). Supporting a role of EGF signaling in IGS cells, the activated MAP kinase accumulates in wild-type IGS cells but is reduced in stet mutant IGS cells. However, it remains to be determined which EGF ligand is activated by Stet in germ cells and how IGS cells control germ cell differentiation.

**Intrinsic Factors Play Essential Roles in GSC Maintenance and Differentiation**

Two classes of intrinsic factors govern GSC self-renewal or differentiation: self-renewing factors and differentiation-promoting factors (Figure 1; Table 1). Pumilio (Pum) and Nanos (Nos) are defined as intrinsic self-renewing factors because mutations in these genes cause premature GSC loss (25, 31, 61, 98). They are RNA binding proteins that form protein complexes that repress translation of mRNAs in Drosophila embryos (2). vasa (vas), encoding a Drosophila homologue of eukaryotic initiation factor 4A, is also likely required for ovarian GSC self-renewal, because vas mutant germinaria contain few degenerate or growth-arrested germ cells (89). These studies indicate that translational regulation plays a critical role in GSC self-renewal.

Furthermore, E-cadherin-mediated cell adhesion and Cyclin B also participate in controlling GSC self-renewal (Figure 1; Table 1). E-cadherin and its interacting partner Armadillo (Arm, β-catenin), expressed in GSCs and cap cells, form adherens junctions, which anchor GSCs to cap cells during niche formation and help recruit GSCs to the niche (87). The main function of adherens junctions is to keep GSCs in close proximity to niche cells to receive maximal BMP signaling for self-renewal. Cadherin-mediated cell
adhesion represents a conserved mechanism for anchoring stem cells in the niche in a variety of systems (26). Cyclin B is specifically required in germ cells for promoting division of PGCs and GSCs and possibly for controlling GSC maintenance (99). Presumably, Cyclin B promotes PGC and GSC division through regulating cell-cycle progression by activating CDK1. However, it remains unclear whether it controls GSC maintenance indirectly by regulating cell-cycle progression or directly by interacting with the GSC maintenance machinery. Taken together, different classes of intrinsic factors play distinct roles in controlling GSC self-renewal and proliferation.

Several differentiation-promoting factors are involved in cystoblast differentiation in the ovary; these include Bam (Bag of marbles), Bgc (Benign gonial cell neoplasm), Orb, and Sxl (Sex lethal) (4, 14, 58, 59, 64, 69). Among these, Bam, a novel protein, and Bgc, related to DExH-box RNA binding proteins, are essential differentiation factors: Mutations in bam and bgcn germline cells abolish cystoblast differentiation, leading to a cystoblast-like germ cell tumor phenotype (28, 65, 69); overexpression of bam triggers GSC differentiation and consequently germ cell depletion in the ovary (70). Genetic interactions between bam and bgcn suggest that their gene products may form protein complexes that regulate the mRNA stability or translation. Mutations in orb and Sxl, encoding proteins involved in the regulation of mRNA polyadenylation and translation, respectively, cause an accumulation of GSC-like or cystoblast-like cells mixed with early differentiated cysts, implying that these genes play unessential roles in cystoblast differentiation (4, 14, 58). In addition, a gap junction connexin, Zpg (Zero population growth), is present in cytoplasmic membranes of GSCs and their differentiated progeny in the ovary and testis and is required to maintain GSCs and to promote germ cell differentiation (29, 91). Loss of zpg function results in partial GSC loss due to cell death and accumulation of a few undifferentiated single cells. Because Zpg is a gap junction component, it likely helps transport small molecules from supporting somatic cells to control the survival of GSCs and the differentiation of their progeny. zpg may function in parallel with bam to regulate germ cell differentiation (29). Bam and Nos control GSC self-renewal through repressing a Bam-independent differentiation pathway (12, 31, 90). bam transcription is not upregulated in pum and nos mutant GSCs, although bam mutant germ cells that are also mutant for pum can differentiate. In summary, different classes of differentiation factors work synergistically to drive cystoblast differentiation by negatively regulating functions of self-renewing factors.

**Extrinsic Signals Regulate the Function of Intrinsic Factors to Control GSC Self-Renewal and Differentiation**

Recent studies on Drosophila ovarian GSCs have revealed that extrinsic signals impinge on intrinsic factors to control their functions. In GSCs, bam is actively repressed through a transcriptional silencer in the bam promoter (13). BMP signals, Dpp and Gbb, from niche cells activate BMP signaling, leading to formation of SMAD complexes, which directly bind to the bam silencer to repress bam expression in GSCs (11, 86). Since BMPs function as short-range signals, cystoblasts with insufficient BMP signaling fail to repress bam expression and consequently upregulate bam to promote differentiation. Similarly, via an unidentified mechanism, the niche Piwi is required to repress bam in GSCs for their self-renewal (12, 90). As both the BMP- and Piwi-mediated signaling pathways are essential for controlling GSC self-renewal, Piwi may converge with BMP signaling by regulating the stability, production, processing, and/or activation of BMP molecules in the niche or BMP signal transduction in GSCs to repress bam expression (12, 90). The observation that a mutation in smurf, encoding a ubiquitin E3 ligase
that regulates degradation of phosphorylated Mad, can rescue a *pisi* GSC loss phenotype indicates that Piwi-mediated signaling and BMP signaling must intersect at the level of, or above, *smurf* (12). Therefore, extrinsic and intrinsic factors work in a coordinated manner to promote GSC fate over cystoblast fate, when instructive signals are received from the niche.

In cystoblasts, Bam initiates a differentiation program and promotes cyst formation. It is proposed that Bam interacts with Bgcn and represses Pum/Nos complexes to promote differentiation (Figure 1) (12, 90). Although Dpp and Gbb only activate BMP signaling in GSCs due to their short-range action, there is also a backup system for actively repressing BMP signaling in the cystoblast. Namely, upregulated Bam in the cystoblast can serve as a negative regulator of BMP signaling, and Smurf, which functions in CBs and descendants, negatively regulates BMP signaling activities by targeting phosphorylated Mad for degradation (10). Therefore, precise control of BMP signaling in GSCs and cystoblasts is crucial for these cells to achieve a critical balance between self-renewal and differentiation.

**REGULATORY MECHANISMS OF GERMLINE STEM CELL SELF-RENEWAL AND DIFFERENTIATION IN THE DROSOPHILA TESTIS**

**General Features of the Drosophila Testicular GSCs and their Niche**

As in the *Drosophila* ovary, GSCs and their niche are also well defined in the *Drosophila* testis by virtue of its simple anatomy and availability of molecular markers (48, 95, 106, 107). Seven to nine spectrosome-containing GSCs are located at the apical tip of the testis and directly contact the hub cells that function as a GSC niche (106). Similar to ovarian GSCs, they divide asymmetrically and give rise to one GSC that remains in the niche and one differentiated gonialblast that moves away from the niche. The gonialblast, a counterpart of the cystoblast, also undergoes four rounds of synchronous division with incomplete cytokinesis to form a 16-cell cluster with a branched fusome (27, 47). Each gonialblast or its descendant is surrounded by two branched cyst cells (SCCs) that control its continuous differentiation (32, 36). These cyst cells are functionally similar to IGS cells in the ovary and are important for germ cell differentiation (49, 80, 93).

**Niche Signals, BMP and Unpaired, Control Testicular GSC Self-Renewal**

In the *Drosophila* testis, Janus Kinase-Signal Transduction and Activator of Transcription (JAK-STAT) and BMP signaling are indispensable for GSC self-renewal (45, 48, 85, 95) (Figure 2; Table 1). Upd produced by the hub acts as a short-range signal to activate JAK (Hopscotch, Hop) and STAT (STAT92E) downstream components in GSCs. Removal of either *hop* or *stat92E* causes GSC differentiation by disrupting self-renewal, whereas ectopic expression of Upd increases the number of GSC-like or gonialblast-like cells in the testis, indicating that JAK-STAT signaling is both sufficient and necessary for GSC renewal. Therefore, JAK-STAT signaling plays an instructive role in GSC self-renewal in the testis similar to that of the Dpp/BMP signaling pathway in the ovary. Remarkably, differentiated germ cells revert back into GSCs when *stat* function is restored in a temperature-sensitive *stat* mutant (5). As in the ovary (43), this study suggests that niche signals maintain GSCs not only by preventing differentiation but also by reprogramming differentiated cells back into stem cells when all GSCs are lost from the niche. It is important to identify downstream target genes of JAK-STAT signaling in GSCs to understand how it controls GSC self-renewal.

*gbb* appears to have a stronger effect on GSC maintenance than *dpp* in the testis,
Figure 2

Major signaling pathways and intrinsic factors for testicular GSC self-renewal and differentiation in the Drosophila testis. The GSCs are likely anchored to the niche via DE-cadherin-mediated adhesions and are enveloped by SSCs. Gbb/Dpp and Upd, expressed in hub cells (HC), activate BMP and JAK-STAT signaling for GSC self-renewal, respectively. Bam is repressed in GSCs and gonialblasts by Gbb/Dpp signaling and is expressed in spermatogonial cells. In the GSC, APC1, APC2, and Cnn are required for correct spindle orientation. Gap junctions formed by Zpg are important for GSC maintenance. EGFR signaling in somatic cyst cells (SCC) is activated by an unknown ligand and is important for gonialblast differentiation and spermatogonial cell (SG) proliferation.

which is consistent with their relative expression levels in the hub and SCCs (45, 85). Removal of BMP downstream components (punt, tkv, mad, and Med) causes severe GSC loss similar to the disruption of JAK-STAT signaling in the testis, indicating that both BMP and JAK-STAT signaling play essential roles in controlling male GSCs either by regulating each other or working in parallel. On the other hand, dpp overexpression only partially suppresses differentiation of gonialblasts, whereas gbb overexpression has no obvious effect. Therefore, BMP signaling plays a permissive role for GSC self-renewal in males.

Although Piwi-mediated signaling is also required for GSC maintenance in the testis, it remains unclear which cells require its function for GSC self-renewal and whether it interacts genetically with JAK-STAT and BMP pathways. piwi is expressed in early germ cells, hub cells, and somatic cysts of the adult testis, and mutations in piwi lead to premature loss of GSCs (61). As discussed earlier, Piwi-mediated signaling may interact with the BMP pathway potentially through Smurf and thereby work cooperatively to sustain testicular GSCs (12).

The IGS equivalent cells, SCCs, are also involved in regulating gonialblast differentiation (49, 80, 93). An unidentified SCC signal received by the gonialblast requires the EGFR and Raf-mediated MAP kinase signaling cascade in SCCs (49, 93) (Figure 2; Table 1). Genetic mosaic
analysis reveals the requirement of Egfr and raf functions in SCCs for regulating gonialblast differentiation/proliferation. The GSC population in the Egfr or raf mutant testes remains active longer than wild type. Finally, stet also functions in the germ cells to control gonialblast differentiation since stet mutant testes have more gonialblast-like cells than wild type (80). Because EGFR signaling is involved in regulating functions of IGS cells and SCCs, it will be interesting to see whether the same EGF ligand in the germ cells is responsible for activating EGFR signaling in both IGS cells and SCCs and what IGS/SSC signal(s) controls cystoblast or gonialblast differentiation.

Different Classes of Intracellular Factors Regulate Testicular GSCs

As for the ovarian GSCs, E-cadherin and β-catenin also accumulate between GSCs and hub cells (107), and are likely involved in anchoring GSCs (Figure 2). The accumulated E-cadherin at the GSC and hub interface may serve as a platform for binding APC2, a Drosophila Adenomatous Polyposis Coli (APC) homolog, to the GSC cortex to orient the mitotic spindles perpendicular to the niche (107). APC2 and two integral centrosome components, centrosomin (cnn) and APC1, are required for the orientation of the stem cell spindle. Loss-of-function mutations in apc1, apc2, and cnn result in mispositioned centrosomes and misoriented spindles, giving rise to symmetric cell division and the accumulation of more GSCs around the hub (107). This study demonstrates that intrinsic control of spindle orientation is crucial for maintaining a stable number of stem cells in the niche in addition to extrinsic niche signals. It remains to be seen whether this intrinsic mechanism of orienting the stem cell spindle is a universal mechanism for ensuring that only one of the two stem cell daughters maintains stem cell identity in other stem cell systems.

Although bam and bgcn are essential for cystoblast differentiation, they are dispensable for gonialblast differentiation because bam or bgcn mutant gonialblasts form germ cell cysts (33). However, bam alone is sufficient to cause male GSCs to differentiate because bam overexpression leads to GSC depletion (45, 79). In order to maintain GSC self-renewal in the testis, bam needs to be repressed. Indeed, it is actively repressed in testicular GSCs by BMP signaling initiated by Dpp and Gbb similar to the Drosophila ovary (Figure 2; Table 1). However, bam and bgcn play essential roles in restricting four rounds of cyst division as bam and bgcn mutant cysts continue to divide after the fourth division (33). In addition, communication between gonialblasts and their surrounding SCCs via Zpg-mediated gap junctions is also important for gonialblast differentiation and early germ cell survival (91). Although Pum and Nos in the Drosophila ovary, Pumilio homologs in C. elegans, and Nos in mice have been demonstrated to be required for controlling GSC self-renewal (19, 31, 94, 98), its role in GSC self-renewal in the Drosophila testis awaits determination. Furthermore, little is known about how niche signals control GSC self-renewal except for the involvement of BMP signaling in repressing bam expression in GSCs. The genetic and molecular relationships between extrinsic signals and intrinsic factors in the testis will require thorough-going investigation in the near future.

REGULATORY MECHANISMS OF GERM CELL FATE SPECIFICATION IN C. ELEGANS

General Features of the C. elegans GSCs and their Niche

The gonad of the C. elegans hermaphrodite consists of two symmetrical, U-shaped tubes that are connected to a common uterus. GSCs are located in the mitotic region (MR) that directly contacts the distal tip cell (DTC), although specific individual GSCs have not yet been precisely defined. GSCs and their
early progeny continue to proliferate in the MR, and the germ cells moving away from the DTC terminate their mitotic activities, enter meiosis, and eventually develop into mature eggs or sperm (20). The fact that only the germ cells that contact the DTC maintain GSC identity suggests that the DTC functions as a GSC niche. Consistent with this idea, the somatic DTC is shown to be required for maintaining germline mitosis by laser ablation experiments (50). Strikingly, the C. elegans gonad displays an arrangement of stem cells and differentiated cell types resembling those in the Drosophila ovary and testis. Powerful genetics and the simple gonadal anatomy have made C. elegans GSCs a productive system to study the regulation of stem cell self-renewal, proliferation, and differentiation.

A Notch-Like Signal from the DTC is Both Necessary and Sufficient for Controlling GSC Self-Renewal and Proliferation

Genetic studies have identified a Notch signaling cascade essential for maintaining GSCs in C. elegans (Figure 3; Table 1). The DTC expresses a Delta-like Notch ligand, LAG-2, whereas the mitotic germ cells express a Notch-type receptor, GLP-1 (20, 37). LAG-2 binding triggers proteolytic cleavage of GLP-1 to generate a truncated intracellular domain for transport to the nucleus, where it forms protein complexes with other transcription factors, LAG-1 and LAG-3, to control target gene expression. LAG-1 is a CSL (CBF-1, Su(H), Lag-1)-type transcriptional regulator, and LAG-3 is a glutamine-rich protein that possibly tethers LAG-1 and the cleaved GLP-1 intracellular domain (23, 75). Loss of GLP-1 and LAG-2 function causes GSC loss and consequently premature entry into meiosis (1, 37, 56). In contrast, constitutive GLP-1 activity prevents entry into meiosis and causes germ cell overproliferation (3). Together with the DTC ablation experiments, these studies show that the DTC functions as a GSC niche and GLP-1/Notch signaling activated by LAG-2 from the niche directly controls GSC self-renewal and proliferation. The direct targets of the GLP-1/Notch signaling pathway remain to be determined. Furthermore, it is unclear whether the C. elegans niche maintains GSCs through a population mechanism or a stereotypic asymmetric division mechanism as in Drosophila, although current data favor the former.

Intrinsic Factors Regulating RNA Stability and Translation Play Essential Roles in GSC Maintenance and Differentiation

As in Drosophila, two classes of intrinsic factors control GSC maintenance or differentiation (Figure 3; Table 1): self-renewing factors
and differentiation-promoting factors. Two nearly identical Pumilio-like RNA binding proteins, FBF-1 and FBF-2, control GSC self-renewal and proliferation. In fbf-1 fbf-2 double mutants, germline proliferation is initially normal, but GSCs are prematurely depleted owing to differentiation and entry into meiosis (19, 109). Pumilio-like proteins usually regulate protein translation by binding to the 3′ untranslated region (UTR) of a target mRNA to inhibit its translation (101). FBF1 functions redundantly with FBF2 to promote mitosis but has an opposite effect on fine-tuning the size of the mitotic region, reflecting a regulatory circuit for maintaining a GSC population (57). The size of the mitotic region is precisely controlled as FBF-1 and FBF-2 regulate each other’s expression, and this reciprocal repression is probably direct through FBF binding sites located in fbf-1 and fbf-2 3′ UTRs.

Several differentiation genes have been identified for controlling the entry into meiosis: gld-1, gld-2, gld-3, and nos-3. gld-1 functions redundantly with gld-2 to promote meiotic entry and/or inhibit germ cell proliferation (41) (Figure 3; Table 1). Unlike other nos genes in Drosophila and mice, nos-3, one of three C. elegans nos genes, promotes differentiation by enhancing GLD-1 accumulation (35). gld-2 gld-1 and gld-2 nos-3 double mutants have a tumorous germline phenotype due to a defect in meiotic entry, whereas gld-1, gld-2, and nos-3 single mutants exhibit normal meiotic entry (35, 41). The GLD-1 and GLD-2 pathways are both thought to regulate expression of target genes at a posttranscriptional level on the basis of their molecular identities. GLD-1 is a STAR/KH domain translational repressor (38, 40, 78), while GLD-2 is a catalytic subunit of a poly(A) RNA polymerase (55, 97). GLD3, a Bicaudal-C homolog, genetically interacts with GLD-2 to promote meiosis by activating mRNAs critical for meiosis (24). Moreover, GLD-3 and GLD-2 form a heterodimeric enzyme that polyadenylates and may activate meiosis-promoting mRNAs such as gld-1 mRNA.

As expected, two classes of intrinsic factors regulate each other. For example, FBF2 regulates the size of the mitotic region by means of repressing the translation of meiosis-promoting gld-1 and gld-3 mRNAs (57). Similar to Drosophila, intrinsic factors that regulate mRNA stability and/or translation play key roles in controlling GSC maintenance and germ cell differentiation in C. elegans.

Interplay Between Notch-Like Signaling and Intrinsic Factors Is Critical for Controlling GSC Self-Renewal and Differentiation

In Drosophila, niche signals repress expression of differentiation-promoting genes and thereby maintain stem cell self-renewal. In C. elegans, GLP-1/Notch signaling also inhibits the activities of meiosis-promoting genes, gld-1, gld-2, and nos-3, although the mechanism currently is unclear (35, 41). Notch signaling may activate FBF translational repressors that repress the functions of differentiation-promoting genes (Figure 3) (24, 100, 109). In order for germ cells to differentiate, GLP-1/Notch signaling has to be shut off by differentiation genes. Indeed, GLD-1 facilitates meiosis through down-regulating GLP-1/Notch signaling in the distal region by binding to the 3′ UTR of glp-1 mRNA to block translation (63). Therefore, GLP-1/Notch signaling and intrinsic factors must precisely balance positive and negative regulatory actions to determine whether germ cells remain in the mitotic cycle or enter the meiotic cycle.

REGULATORY MECHANISMS OF GERMLINE STEM CELL SELF-RENEWAL AND DIFFERENTIATION IN MAMMALS

General Features of the Mouse GSCs and their Putative Niche

Stem cell transplantation, simple anatomy, and genetics make the mouse testis a powerful
model to study complex regulatory networks of mammalian GSCs and their niche. The spermatogonial stem cells (referred to as GSCs here for consistency) are a subset of single cells (A\textsubscript{single} or A\textsubscript{A}), which are located along the basement membrane of seminiferous tubules in the mouse testis (7). GSCs self-renew or produce a differentiated A\textsubscript{A} daughter that divides to form a pair of interconnected spermatogonial cells called A\textsubscript{pair}. The A\textsubscript{pair} spermatogonial cells can divide synchronously to form a chain of interconnected spermatogonial cells that subsequently become differentiating spermatagonia, spermatocytes, spermatids, and sperm cells, which is reminiscent of cyst formation in the Drosophila ovary and testis. GSCs and early differentiated spermatagonia are morphologically alike and thus indistinguishable. GSCs are very rare (1 in 5000) cells in the adult mouse testis based on transplantation studies (7). Fluorescence-activated cell sorting (FACS) in conjunction with the transplantation assay for GSCs has identified the antigenic profile of GSCs as α\textsubscript{6}-integrin\textsuperscript{−/−}dim α\textsubscript{6}-integrin\textsuperscript{+} Thy-1\textsuperscript{+/-} C-Kit\textsuperscript{−} (54).

Sertoli cells, the somatic cells in the seminiferous tubules that physically interact with GSCs, likely constitute a functional GSC niche by providing growth factors that promote GSC self-renewal and/or proliferation. Several studies support the idea that these cells regulate the maintenance of the stem cell pool. First, transplantation of GSCs into infertile male mice has shown that Sertoli cells can indeed support GSC maintenance and spermatogenesis (8). Second, Sertoli cells produce GDNF (glial cell line-derived neurotrophic factor) that controls GSC maintenance in a dosage-dependent manner (66). Furthermore, transplantation of Sertoli cells into the mouse testes that are defective in Sertoli cell function demonstrates that Sertoli cells are indeed essential for maintaining spermatogenesis (84), but it is still not clear whether and how Sertoli cells alone can constitute the GSC niche. A series of transplantation experiments in mice and rats suggest that the GSC niche in the mammalian testis is very dynamic during development (72, 73, 77, 82, 83). The most urgent issue in studying GSCs in the mouse testis is to define the physical structure of the niche and its associated signals.

Although most mammalian females were believed to lose the capacity for germ cell self-renewal during fetal life, a recent study argues that juvenile and adult mouse ovaries possess mitotically active germ cells that continuously replenish the follicle pool (39). Despite compelling experimental evidence, it remains to be seen whether these observations can be duplicated in other mammals. If those GSCs exist in the peripheral epithelial layer of the ovary, a new avenue will be opened to study GSCs in mammals and further investigate how GSCs are regulated differently in both sexes.

**Signals from Sertoli Cells Control the Maintenance and Differentiation of GSCs**

One extrinsic factor involved in GSC self-renewal and proliferation is GDNF, which is released from Sertoli cells (66) (Figure 4, Table 1). GDNF binds two heterologous receptors, Ret and GFR-α1 which are expressed in spermatogonial cells. gdnf\textsuperscript{+/-} mice lose their GSCs prematurely in testes, indicating that GDNF is essential for GSC self-renewal. Overexpression of GDNF causes accumulation of GSC-like cells. Two recent studies show that male mouse GSCs can be cultured and expanded in vitro in the presence of GDNF for more than 6 months and can reconstitute long-term spermatogenesis and restore fertility when transplanted to sterile recipient mice (44, 53). These findings support the hypothesis that Sertoli cells must contribute to the function of the GSC niche. In addition to GDNF signaling, BMP signaling also has a role in GSC maintenance (76, 110). Multiple BMPs, BMP4, BMP7, and BMP8 are expressed in male germ cells, while BMP4 is also expressed in Sertoli cells (76, 110), which is in...
Extrinsic and intrinsic factors for GSC self-renewal/differentiation in the mouse testis. The putative niche cell, Sertoli cells, which exhibit functional polarity, express GDNF, BMPs, and SCF to promote self-renewal and differentiation by binding to their receptors GDFα1/Ret, BMPs and C-Kit, respectively. The myoid cell may assist the niche function. Intrinsic factors for self-renewal include Plzf and Nos-2.

In contrast to restricted expression of BMPs only in somatic niche cells in both the Drosophila ovary and testis. Intriguingly, targeted disruption of these genes has revealed that they all play important but redundant roles in maintaining the viability of germ cells, including GSCs (76, 110). It remains unclear whether they are required for GSC self-renewal as well. Therefore, from C. elegans, Drosophila to mice, extrinsic signals from their niches play instructive roles in controlling GSC self-renewal.

Differentiation of GSC progeny in mice also depends on extrinsic signals. The stem cell factor (SCF) produced by Sertoli cells activates c-Kit, a tyrosine kinase receptor for SCF, to promote the differentiation of GSC progeny (22, 71, 108) (Figure 4; Table 1). Loss-of-function mutations in c-kit cause an arrest in an early step of spermatogonia differentiation, suggesting that the SCF/c-Kit pathway is required for germ cell differentiation and survival (22, 71, 108). The identification of immediate target genes controlled by SCF/c-Kit signaling will be crucial for understanding how germ cell differentiation is regulated in mice.

Different Classes of Intrinsic Factors Control GSC Maintenance and Differentiation in the Mouse Testis

Two different intrinsic self-renewing factors have been identified in mice, nanos2 and Plzf (promyelocytic leukemia zinc-finger) (9, 15, 94). nanos2 is expressed predominantly in male germ cells, and a nanos2 mutation results in complete GSC loss (94). In contrast, nanos3 is expressed in primordial germ cells (PGCs) of both sexes, and a nanos3 mutation leads to complete loss of germ cells in both sexes. As discussed earlier, one nanos gene...
maintains PGCs as well as GSCs in the *Drosophila* ovary, which is in contrast to two genes sharing these roles in mice. It would be interesting to see whether *pumilio*-like genes are also required for maintaining PGCs and GSCs in mice since its *Drosophila* counterpart functions in the same protein complex with Nanos to repress translation and maintain GSCs. *plzf*-null mice have lost their ability to maintain GSCs in the testis, and its function is required only in GSCs (9, 15). *Plzf*, a member of the POK (POZ and Krüppel) family of transcriptional repressors, is expressed in early undifferentiated spermatogonial cells. It can potentially recruit members of the mammalian Polycomb family, such as BMI1, to link epigenetic modifications to transcriptional control. Since BMI1 has recently been shown to be required for self-renewal and proliferation of hematopoietic and neural stem cells (67, 74), it would be worthwhile to see whether BMI1 is also required to maintain GSCs in the testis. It is equally important to know how *Plzf* and *Nanos2* interact with the known GDNF signaling pathway to control GSC self-renewal in the testis and to understand how the transition from a GSC to a differentiating A, is regulated intrinsically.

### SUMMARY AND FUTURE DIRECTIONS

A comparative analysis of GSC systems in *Drosophila*, *C. elegans*, and mice has elucidated some fundamental principles and strategies for GSC self-renewal and differentiation (Figure 5). First, GSCs are situated in specialized regulatory niches to ensure self-renewal. Cap cells function as a GSC niche in the *Drosophila* ovary, hub cells form a GSC niche in the *Drosophila* testis, and the DTC is a GSC niche in *C. elegans*, as demonstrated by cell biological and genetic studies (18, 106). In the mouse testis, GSC transplantation experiments have revealed the existence of a GSC niche but its structure has not yet been defined (6). Second, GSC niches in *Drosophila* and *C. elegans* exhibit structural asymmetry to ensure that the GSC daughters remaining inside the niche self-renew, and the others outside the niche generate differentiated cells. Third, short-range niche signals prevent GSCs from differentiation and thereby maintain stem cell self-renewal. Niche signals, BMPs in the *Drosophila* testis and ovary, Upd in the *Drosophila* testis, and LAG-2 in *C. elegans* gonad all function over a short distance to maintain GSCs. However, it is unknown whether GDNF is also a short-range signal that acts specifically on GSCs in the mouse testis. Fourth, intrinsic factors regulating mRNA stability and/or translation are conserved for their ability to regulate GSC self-renewal and differentiation. In *Drosophila* and *C. elegans*, the majority of intrinsic factors are related to regulation of mRNA stability and/or translation. Notably, Pum and Nos are conserved translational repressors that are involved in GSC self-renewal from *Drosophila*, *C. elegans* to mice. Finally, the interplay between extrinsic signals and intrinsic factors is critical for GSC self-renewal. In the *Drosophila* ovary and testis, BMP signaling directly represses expression of a differentiation-promoting

![Figure 5](https://www.annualreviews.org/doi/10.1146/annurev.genet.40.051805.104517)
factor, *bam*, in order to maintain GSCs, whereas, in *C. elegans*, GLP-1/Notch signaling is implicated in repressing functions of differentiation-promoting genes, such as *gld* genes. It will be interesting to see whether these conserved GSC regulatory mechanisms are also utilized by other adult stem cells.

Despite the commonalities of GSCs in different systems, obvious differences exist between them. First, a different combination of extrinsic signals is needed for different GSCs. For example, only one GLP-1/Notch signaling pathway is required for GSC self-renewal in *C. elegans*, Piwi and BMP signaling are needed for controlling GSC self-renewal in the *Drosophila* ovary, while Piwi, BMP and JAK-STAT signaling contribute to GSC maintenance in the *Drosophila* testis, and GDNF and, likely, BMPs work together to control GSC maintenance in the mouse testis. Because these different combinations of signaling pathways are sufficient to prevent GSC differentiation, these differences may reflect distinct developmental histories of different GSCs. Second, the same extrinsic signals exhibit some differences in their ability to regulate GSCs. One example is how Dpp plays slightly different roles in the *Drosophila* ovary and testis. Dpp plays an instructive role in controlling GSC self-renewal in the *Drosophila* ovary, but it plays a minor role in GSCs of the *Drosophila* testis. Third, some intrinsic factors are needed in only one GSC system but not in others to direct GSC self-renewal and differentiation. GSCs in *Drosophila*, *C. elegans*, and mice form differently during early development; their different developmental histories may confer GSCs distinct properties, which thus require the use of different intrinsic factors to control their self-renewal and differentiation. For example, *bam* has not been identified and shown to be required for germ cell differentiation in *C. elegans*, while many intrinsic factors identified in *C. elegans*, such as *gld* genes, have not been shown to be required for GSC differentiation in *Drosophila*. Together, these differences in the mechanisms regulating GSC behavior have revealed that different combinations of extrinsic signals and intrinsic factors can achieve the same purposes of controlling self-renewal and differentiation.

The knowledge gained from studies on GSCs and their niches in *Drosophila* and *C. elegans* has provided an intellectual framework for defining stem cells and their niches in mammalian systems. Moreover, the signaling pathways identified for controlling GSC self-renewal have also been shown to regulate different adult stem cell types in mammals. Although we have learned so much from studying GSCs in different systems, many questions remain. What constitutes a GSC niche in the mouse testis? How is GSC niche formation regulated in different species? How does signaling initiated by niche cells interact with intrinsic factors to control GSC self-renewal and differentiation? Whether and how do GSCs regulate their niche function? Is GSC aging due to intrinsic aging or niche aging? The answers to these questions will greatly advance our understanding of GSC regulation and will also provide insight into how adult stem cells are regulated in general.

**SUMMARY POINTS**

1. The regulatory microenvironment or “niche” directly controls GSC asymmetric division and self-renewal.

2. GSC niches exhibit structural asymmetry to ensure that only one of the two GSC daughters remains in the niche for self-renewal.
3. Niche signals function at short range to act directly on GSCs to prevent them from differentiation and thereby control self-renewal.

4. Different combinations of niche signals are needed for GSC self-renewal in different systems.

5. The same niche signal may function differently in different GSC systems.

6. Differentiation of GSC daughters is controlled by extrinsic signals from their surrounding somatic cells.

7. Several classes of intrinsic factors are involved in controlling GSC self-renewal and differentiation. Pumilio and Nanos families of proteins are conserved intrinsic factors for GSC self-renewal.

8. Intimate interplay between extrinsic factors and intrinsic factors is critical for GSC self-renewal.

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LITERATURE CITED


This paper reports that Nos and Pum may function together to repress PGC and GSC differentiation in a Dpp-independent manner.

This paper provides experimental evidence challenging the dogma that GSCs do not persist in postnatal mammalian ovary.

This study shows that differentiated germ cell cysts can revert back into GSCs in early female gonads and in the adult ovary. The GSCs regenerated from differentiated germ cells function normally.

This study shows that the hub cells function as a GSC niche in the *Drosophila* testis by demonstrating that they produce Upd that activates JAK-STAT signaling to control GSC self-renewal.
94. This paper shows that two mouse nanos genes are involved in regulation of PGC and GSC functions in mice. nano2 is required for GSC maintenance in the testis, while nano3 is required for PGC maintenance in both sexes.

95. This study shows that the hub cells function as a GSC niche in the Drosophila testis by providing Upd that activates JAK-STAT signaling in GSCs for self-renewal.

96. This study shows that nanos is required for preventing the differentiation of PGCs and GSCs in the Drosophila female gonads.

97. This study demonstrates that Dpp/BMP2-4 is essential for controlling GSC self-renewal and division. The first study using mutant clonal analysis to study gene function in the control of GSC self-renewal.


104. Xie T, Spradling AC. 1998. decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell 94:251–60


105. The first study directly demonstrating that GSCs are situated in the niche in the *Drosophila* ovary. A GSC can undergo symmetric cell division to produce two GSCs if both daughters remain in the niche.

107. This study demonstrates that regulation of GSC spindle orientation is critical for asymmetric GSC division in the *Drosophila* testis. The authors also show that APC-1, APC-2 and Centrosomin play crucial roles in orienting the GSC spindle.
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ERRATA

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