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The Development of Germline Stem Cells in *Drosophila*

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Summary

Germline stem cells (GSCs) in *Drosophila* are a valuable model to explore of how adult stem cells are regulated in vivo. Genetic dissection of this system has shown that stem cell fate is determined and maintained by the stem cell's somatic microenvironment or niche. In *Drosophila* gonads, the stem cell niche—the cap cell cluster in females and the hub in males—acts as a signaling center to recruit GSCs from among a small population of undifferentiated primordial germ cells (PGCs). Short-range signals from the niche specify and regulate stem cell fate by maintaining the undifferentiated state of the PGCs next to the niche. Germline cells that do not receive the niche signals because of their location assume the default fate and differentiate. Once GSCs are specified, adherens junctions maintain close association between the stem cells and their niche and help to orient stem cell division so that one daughter is displaced from the niche and differentiates. In females, stem cell fate depends on bone morphogenetic protein (BMP) signals from the cap cells; in males, hub cells express the cytokine-like ligand Unpaired, which activates the Janus kinase-signal transducers and activators of transcription (Jak-Stat) pathway in stem cells. Although the signaling pathways operating between the niche and stem cells are different, there are common general features in both males and females, including the arrangement of cell types, many of the genes used, and the logic of the system that maintains stem cell fate.

Keywords

Drosophila; germline stem cell; GSC; PGC; primordial germ cell; stem cell fate; stem cell niche

1.1 Introduction

Stem cells are defined by their capacity to self-renew and to generate daughters that differentiate into one or more terminal cell types. Proper regulation of this property is critical for animal development, growth control, and reproduction. Understanding stem cell function is potentially very important for future developments in gene therapies and regenerative medicine. Research in *Drosophila* on germline stem cells (GSCs) has been instrumental in defining the important function of the stem cell's somatic microenvironment or niche in the control of its division and self-renewal (1,2). The *Drosophila* germline is an excellent model of stem cell biology because the system is genetically tractable, the sterility and rudimentary gonads resulting from GSC loss are easily recognized, and the stem cells can be easily identified based on molecular markers and position in the gonad. Germline morphology and development, from embryogenesis to the differentiation of gametes in adult flies, has been well characterized and offers a solid basis for the study of GSC fate determination, maintenance, and differentiation.

Drosophila GSCs are derived from embryonic pole cells, the first cell type defined in the embryo. They migrate from the posterior to meet the somatic gonadal precursors (SGPs) and form the embryonic gonad, a simple structure made up of about ten primordial germ cells (PGCs) intermingled with and surrounded by mesodermal cells. PGCs divide and remain in an undifferentiated state until the stem cell niche develops in the anterior of the gonad. The

niche acts as a signaling source that maintains the undifferentiated state of PGCs near the niche, thereby maintaining their capacity to populate the niche as GSCs. During the final phase of niche development, adherens junctions develop between the niche and the GSCs, thereby orienting stem cell division and holding the stem cells close to the maintenance signals of the niche.

1.2 Primordial Germ Cell Formation

Germline stem cells are derived from a population of PGCs, a distinctive lineage of cells set aside from the soma early in development. Germ cells are distinguished from somatic cells by the expression of germline-specific molecular markers, the most widely used of which is the DEAD-box RNA helicase Vasa (3). Although the mechanisms used to define the germline vary, the determinant role of localized germplasm has been described in many animal species, including *Drosophila*, *Caenorhabditis elegans*, *Xenopus*, and zebrafish (4–6). In mammals, PGC fate is specified in a subset of the epiblast through a fundamentally different mechanism, involving an inductive bone morphogenetic protein (BMP) signal from the extraembryonic ectoderm (7,8). Epigenetic modes of germ cell specification may be the more widely distributed in evolution (6).

PGCs in *Drosophila* begin their lives as pole cells, products of the first cellularization event in the syncytial embryo. Pole cells are committed to the germ cell fate at the time of their formation through the cytoplasmic inheritance of maternally deposited pole plasm, or germplasm, which is sufficient for germline determination (4,9). The pole plasm contains polar granules, electron-dense fibrous aggregates of RNA, and protein, which are closely associated with dividing pole bud nuclei and are specifically inherited by the PGCs on cellularization (see Fig. 1.1).

Genetic screens have revealed many important constituents of the pole plasm. Loss of some polar granule components can cause maternal lethality, because the pole plasm is essential for stable localization of the essential posterior morphogen Nanos. Other mutations affecting pole plasm components cause a grandchildless phenotype, in which viable embryos are produced that lack germ cells. A critical component for polar granule assembly in *Drosophila* is Oskar (Osk), with accumulation in the posterior oocyte cytoplasm that is necessary and sufficient for the recruitment of Vasa and Tudor, thus initiating the polar granule assembly pathway (4,9). A second class of pole plasm components does not affect somatic development and is involved only in germ cell development. This class includes the RNAs *gcl* and *pgc*, which are discussed in **Subheading 1.2.1**, and Piwi, which has been implicated in gene silencing by RNA interference (10,11). Reducing the maternal contribution of Piwi, or the RNA-induced silencing complex (RISC) components Dicer1 or Fragile X mental retardation protein (FMRP), drastically reduces the number of pole cells without affecting posterior somatic patterning (11). In addition to their roles in maintaining GSC fate and controlling GSC division in the adult (12–14), Piwi and other components of the microRNA machinery may regulate germline fate determination through microRNA-mediated translational control in the pole plasm.

Polar granules are thought to regulate the translation of maternal messenger RNAs (mRNAs) that are required for germ cell determination and early germ cell behavior. The pole plasm is assembled and stabilized at the posterior pole of the developing oocyte before fertilization (4,9). Its constituents are produced by nurse cells during oogenesis and pass into the oocyte via large cellular interconnections called ring canals. Polar granules share components (including the translational regulators Vasa and Aubergine) with the perinuclear nuage, electron-dense ribonucleoprotein bodies near the germline cell nuclear pores. Regardless of how they are specified, a common characteristic of germ cells is the presence of these extranuclear organelles (e.g., nuage, P-granules, or chromatoid bodies), which are possibly

sites of messenger ribonucleoprotein (mRNP) biosynthesis and germline-specific translational control.

1.2.1 Transcriptional Quiescence of PGCs

The PGCs are transcriptionally quiescent until the onset of gastrulation, when they initiate zygotic transcription of germline-specific genes such as *Vasa* (15). PGC transcriptional quiescence correlates with reduced levels of active RNA polymerase II (RNAP II) that is phosphorylated on its C-terminal domain (CTD), suggesting that transcriptional repression might be established through regulating RNA Pol II function (16,17). The maternally supplied mRNAs *gcl*, *nos*, and *pgc* contribute to the transcriptional quiescence of PGCs, apparently through independent mechanisms that repress the transcription of different sets of genes (18).

nanos mRNA is maternally loaded into the pole plasm and is translated after fertilization. It forms a posterior-to-anterior protein gradient and directs posterior somatic patterning by shaping the anterior Hunchback morphogen gradient. Nos protein is inherited by the PGCs, in which it maintains transcriptional quiescence until they begin to migrate through the midgut epithelium (15,16,19). In embryos produced by *nos* mutant females, PGCs abnormally transcribe a subset of somatic genes, such as *Sex-lethal* (*Sxl*), and the segmentation genes *fushi tarazu* and *even skipped* (19–21). They do not complete their migration into the embryonic gonad, fail to repress mitosis, lose germ cell fate, and are eliminated by apoptosis (20,22–24). The cell cycle effects caused by loss of Nos can be explained by a direct role for Nos and the RNA binding protein Pumilio (Pum) in repressing the translation of *CyclinB* mRNA (25,26). *CyclinB* mRNA is another pole plasm component, and its translation is normally repressed in migrating PGCs until they reach the gonad. CyclinB normally promotes the division of PGCs, and its premature translation in *nos* or *pum* mutants allows precocious mitosis in migrating PGCs (25,27). Because Nos is a translational repressor, it presumably acts indirectly to repress transcription in wild-type PGCs by regulating the translation of another, unidentified factor.

Another gene that affects transcriptional quiescence of PGCs is *gcl*. Maternally supplied *gcl* mRNA is translated at the posterior pole of the embryo and is specifically inherited by the PGCs (28). The level of RNAP II CTD phosphorylation is increased in pole buds (precursors to PGCs) of embryos derived from *gcl* mutant mothers, bringing it to a level similar to that of the surrounding somatic nuclei. This correlates with the misexpression of somatic genes, such as *sisterless-a* and *scute*, in the pole buds of *gcl* mutant embryos (17). Conversely, ectopic *gcl* expression at the anterior of an otherwise wild-type embryo is sufficient to downregulate somatic expression of *sisterless-a* and *tailless* in anterior cells (17). Thus, *gcl* is implicated in establishing transcriptional quiescence in newly formed pole buds. Although its mechanism of action remains to be determined, *gcl* protein associates with the nucleoplasmic surface of pole cell nuclear envelopes, where it might affect chromatin compaction (29). Even though most *gcl* mutant embryos do not form PGCs, *gcl* is not absolutely required for this process; some embryos form a few PGCs and develop into fertile adults, suggesting redundancy in the mechanisms that silence transcription in the germline.

Similar to *gcl*, PGCs in embryos that lack maternally contributed *pgc* have elevated levels of RNAP II CTD phosphorylation and express somatic genes such as *zerknüllt*, *tailless*, and *slam* (18,30,31). Loss of *pgc* leads to increased levels of methylated histone H3, which is associated with increased transcriptional activity (18). *pgc* mRNA is localized to the germplasm and is inherited by PGCs, and its disappearance correlates with the onset of zygotic transcription in the germline (31). A small 71-amino acid protein that is transiently expressed in the PGCs of stage 4–6 embryos is encoded by *pgc* (A. Nakamura, personal communication). Although the functions of *gcl* and *pgc* in transcriptional repression have not been defined, these results suggest that the undifferentiated state of germline precursors is maintained during embryogenesis by preventing transcriptional responses in PGCs to somatic patterning signals.

1.3 The Embryonic Gonad

1.3.1 Primordial Germ Cell Migration

In *Drosophila* as well as many vertebrate systems, PGCs and the somatic cells of the gonad are specified at different locations, so the germ cells must migrate to reach the somatic part of the gonad (*see* Fig. 1.1). PGC migration is a process involving several discrete steps, controlled by genetically separable mechanisms (32–34). During gastrulation, the germ band extends dorsally, and the PGCs are carried into the lumen of the invaginating posterior midgut (PMG). Once inside the PMG lumen, the PGCs lose close association with one another, become amoeboid, and migrate through the PMG epithelium. Migrating PGCs use filopodia to contact each other and their substrate; in the absence of Jak-Stat (Janus kinase-signal transducer and activator of transcription) signaling, the filopodia are reduced, and PGCs are frequently mislocalized (35). This is the first active step in PGC migration, and it involves the G protein-coupled receptor (GPCR) encoded by *trapped in endoderm 1* (*tre1*; 36). *tre-1* mRNA is maternally encoded and acts in the germline, but the ligand for this receptor has yet to be identified. Once the PGCs traverse the PMG epithelium, they move dorsally along the midgut and split into two groups that move laterally, away from the midline. PGCs migrate toward the mesoderm to contact three bilateral clusters of SGPs in parasegments 11 and 12 (37). During germ band retraction, the resulting groups of somatic and germline cells migrate anteriorly until they coalesce into a tight ball to form the embryonic gonad (*see* Subheading 1.3.2).

Migration of PGCs away from the ventral side of the PMG, and later away from the dorsal midline, is driven by repulsive cues provided by two phospholipid phosphatases, Wunen and Wunen2 (*see* Fig. 1.1 and refs. 38 and 39). The two Wunens, which are believed to have similar functions, are expressed together in tissues flanking the migration route, where they appear to provide a repulsive migration cue to PGCs. Wunens are expressed ventrally in the PMG, guiding PGC migration dorsally (*see* Fig. 1.1E and refs. 38 and 39). Expression of Wunens in the central nervous system (CNS) then drives PGC migration laterally, away from the midline (*see* Fig. 1.1F and ref. 40). Although Wunen expression in the mesoderm is sufficient to override the normal attractive cues of the SGPs, in the absence of both Wunens, germ cells migrate randomly through the embryo. The lipid phosphate hydrolysis activity of the two Wunens is localized to the extracellular environment. Although Wunen substrates in the embryo are unknown, their predicted enzymatic activity has led to the suggestion that Wunens act as an attractant sink instead of a repellent source (41,42). Wunens could locally hydrolyze and thus destroy a phospholipid attractant, and the PGCs would migrate toward higher levels of that attractant.

In addition to the Wunen cues, PGCs are guided toward the SGPs by attractive signals, including Hedgehog (Hh) and one or more products of a lipid metabolism pathway involving 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Hmgcr). Localized overexpression of either Hh or Hmgcr is sufficient to alter the migration path of PGCs, and both are expressed at high levels in SGPs as well as in other tissues, where they play vital roles in development (43–46). Although the Hmgcr pathway attractant itself has yet to be identified, geranyl-geranyl diphosphate, an isoprenoid product of the pathway that is attached to a variety of proteins, is crucial, implicating a protein modified by this lipid as the PGC guidance cue (45). The Hh signaling pathway is potentiated by Hmgcr, suggesting that the Hh signal emanating from the SGPs may be modified by Hmgcr, providing a mechanism for the migrating germ cells to distinguish Hh produced by the SGPs (46,47). Hmgcr and geranyl-geranyl protein modifications have also been implicated in zebrafish germ cell migration, suggesting evolutionary conservation of signals that guide migrating germ cells in spite of wide divergence in anatomical structure (48,49).

1.3.2 Gonad Coalescence

Gonad development involves the coordination of precursors from two distinct lineages: the SGPs of mesodermal origin and the PGCs that completed migration from the embryonic posterior pole. The SGPs are specified in bilateral clusters from part of the dorsolateral mesoderm of parasegments 10–12 (50). Differential expression of *abdominal A* (*abdA*) and *Abdominal B* (*AbdB*), homeotic genes that specify regional identity along the anterior–posterior axis of the embryonic abdomen, applies anterior, posterior, and male-specific SGP (msSGP) identities among the adjacent parasegmental clusters (37). SGPs can be identified by their expression of the 68–77 LacZ line or the nuclear proteins Eyes Absent (*Eya*) or Zfh-1 (51–53). On adherence of migratory PGCs and the gonadal precursors, they migrate anteriorly to form bilateral groups of cells in ps10, each including on average 12 PGCs and 30 mesodermal cells that coalesce into a round and compact gonad (37,54,55). After coalescence, SGPs intermingle with and individually ensheath PGCs in a process that requires the cell adhesion molecule E-Cadherin (E-Cad) and a novel transmembrane protein Fear-of-Intimacy (56,57). During coalescence, the PGCs are round and lack cellular extensions that would suggest an active role in the process; in contrast, the SGPs send cytoplasmic extensions that contact germ cells and other SGPs.

The Maf family transcription factor encoded by *traffic jam* (*tj*) is a key regulator of the morphogenetic movements that occur after gonad coalescence (58). Tj protein is expressed in the SGPs of embryonic gonads and in the somatic cells in contact with the germline at later stages of development. In *tj* mutants, SGPs are specified normally and ensheath PGCs but are restricted to the outside of the germline cluster instead of intermingling with the PGCs (58). This defect leads to the formation of small, disorganized gonads and sterility (58,59). Tj regulates the expression of the cell adhesion molecules E-Cad, Fasciclin 3 (*Fas3*), and Neurotactin, and the two-layer gonad formed in *tj* mutants is consistent with altered adhesive properties between the soma and germline causing a cell-sorting phenotype.

1.4 Female GSC Niche Development

The germarium houses three types of stem cells: GSCs, escort stem cells (ESCs), and somatic stem cells (SSCs) that produce follicle cells (see Table 1.1). The core of the female GSC niche is a group of five to seven nondividing somatic cap cells that physically anchor two or three GSCs to the anterior of each germarium (60–62). Anterior to each cap cell cluster, a single-file array of about eight terminal filament (TF) cells make up the TF that connects the germarium with the sheath cells that surround the ovariole. Both the cap cells and TF, which can be visualized with a *hedgehog-lacZ* enhancer trap (60, see Table 1.2 for a list of markers), are the sources of important stem cell maintenance signals such as Hh and Dpp, and the survival of GSCs is dependent on close association with these cells (see **Subheading 1.6.4** and ref. 63). To the posterior of the cap cells and surrounding the GSCs and newly formed cystocytes are the inner germarial sheath (IGS) cells, which can be identified by *ptc-lacZ* expression (64). IGS cells line the surface of the anterior half of the germarium and are thought to act as a niche for the SSCs (65). Cystoblast differentiation is supported by a subpopulation of IGS cells called *escort cells*, derived from ESCs that send thin cytoplasmic processes to insulate cystoblasts and cystocyte clusters prior to follicle formation, when they die and are replaced by follicle cells (66).

During larval stages, the female gonad can be divided into three regions: the anterior somatic cells, where the TF and cap cell precursors reside; the medial region, housing the PGCs and somatic interstitial cells; and the posterior region, housing the precursors of the basal stalk cells (see Fig. 1.2D). During the third larval instar, cells are added to each TF gradually and in a progressive manner from medial to lateral across the ovary until early pupal development (67,68). Cap cell differentiation occurs in early pupae as TF formation is completed. The niche

develops by cell rearrangement and cell recruitment, so that an individual niche is polyclonal in origin, technically limiting the ability to perform genetic loss-of-function studies in the niche (68,69). Advances in available genetic tools, such as the recent development of a method to target clone formation to the somatic gonadal cells (69), hold promise to expand the genetic analysis of niche morphogenesis and GSC maintenance.

GSCs can be defined as the only mitotically active germline cells that remain in the gonad and that continue to divide long after they are genetically marked. They are most commonly identified as the germline cells in contact with the cap cells (female) or hub cells (male) of the stem cell niche and that contain a particular cytoplasmic organelle known as a *spectrosome*, which in the female is invariably attached to the site of contact with the niche (*see* Fig. 1.2E and refs. 70 and 71). Viewed with antibodies recognizing an adducin-like protein (MAb1B1) or α -Spectrin, the spectrosome is an apical sphere that elongates and reaches into the daughter cell during mitosis, before it is partitioned unequally on cytokinesis, with the stem cell retaining a greater portion (72). Dividing GSCs are easily observed because of a prolonged G1 phase of mitosis, during which the mother and daughter cells are connected by an elongated spectrosome through a temporary cytoplasmic bridge. After division, the spectrosome retracts to the anterior of the GSC and regains its spherical shape. The spectrosome is the progenitor of the fusome, a meshwork of membranes and membrane skeletal proteins representing a germ cell-specific modification of the endoplasmic reticulum (73,74). During cyst divisions, the fusome grows and fuses to become an elongated, branched structure that extends throughout the cyst, where it coordinates mitosis and microtubule polarity.

In the female, PGCs proliferate during larval stages to produce a population of over 100 undifferentiated germline cells (75). PGCs proliferate with random division orientation, and clonal analysis indicated dispersion of daughter cells, so that there is little detectable relationship between clonal history and position (76). Lineage analysis of single, marked PGCs did not indicate early restriction of GSC fate among PGCs, although there is a tendency for PGCs in the anterior of the embryonic gonad to populate the adult niche (76). GSCs are anchored to the cap cells by adherens junctions, and the division plane of the GSC division is such that one daughter cell loses close contact with the cap cells and differentiates (77). Adherens junctions between presumptive GSCs and newly formed cap cells appear during early pupal stages, at the same time that differentiation is first apparent in PGCs away from the niche (using Bam expression or branched fusomes as markers) (77,78). The spectrosomes of the PGCs in closest proximity to the cap cells become anchored to the site of the newly forming adherens junction, thereby adopting the architecture that we use to recognize adult GSCs (78). Thus, after the completion of the stem cell niches, the nearby anterior PGCs become GSCs, and the rest of the PGCs directly enter the cyst differentiation pathway (77–79).

1.5 Male GSC Niche Development

In the testis, there are two types of stem cells that maintain spermatogenesis: the GSCs and the cyst progenitor cells, which produce somatic cyst cells (SCCs) to encapsulate differentiating germ cells (*see* Table 1.1). Approximately 12 nondividing somatic hub cells at the apical tip of the testis, commonly marked with Fas3 antibodies (80), maintain five to nine spectrosome-containing GSCs in a characteristic rosette pattern (*see* Fig. 1.3 and refs. 81 and 82). The hub cells make up the GSC niche, the cellular microenvironment that regulates, induces, and supports the development of GSCs. Each GSC is surrounded by two somatic cyst progenitor cells, which are also attached directly to the hub. During male GSC division, one spindle pole associates with the GSC–niche interface, and the GSC divides radially with respect to the hub. This orientation ensures that one of the two progeny cells remains in the niche as a GSC, and the other, the gonialblast, is displaced from the niche and differentiates (83). Each gonialblast is surrounded by two SCCs that support its differentiation. The behavior of the fusome and

ring canals during cyst divisions in testes is highly similar to that in ovaries, except that the male GSC spectrosomes are not specifically localized to the GSC–niche interface as in the female (*see* Fig. 1.3 and ref. 83).

Although striking similarities exist between the GSC niches of *Drosophila* males and females (66,84), sexual dimorphism in gonad morphogenesis is present soon after gonad coalescence. Markers such as Eyes absent (Eya) and the *Drosophila* homolog of Sox9 (Sox100B) are expressed at highest levels in the msSGPs at the posterior of the gonad (85,86). The msSGPs are initially specified in ps13 of both males and females but are subsequently lost in the female by apoptosis (85). At the anterior of the gonad, escargot (*esg*)-*LacZ* marks a different subset of SGPs in which the precursors of the male stem cell niche are located (87,88). The expression of *esg-LacZ* becomes further restricted to a subset of anterior SGPs when development of the male stem cell niche commences at the end of embryogenesis (88). It is not known how *esg-LacZ* expression and hub cell fate are restricted to a subset of anterior SGPs.

The embryonic male stem cell niche closely resembles that of the adult male: a tight cluster of SGPs that express *esg-LacZ*, Fas3, and the Jak-Stat ligand Unpaired, which is associated with a group of anterior PGCs in the same rosette pattern as that observed in the adult (81,88). The restriction of hub cell fate and the rosette pattern of PGCs around the hub suggest that a functional GSC niche forms in male embryos, much earlier than niche development in the female. The presence of spermatogonia in newly hatched larvae and cysts of spermatocytes before the second larval instar indicate that spermatogenesis has commenced before hatching (89) and suggest that the PGCs organized around the embryonic hub are already specified as GSCs. Although it is not clear how the newly formed hub recruits GSCs from the PGC population, the major GSC maintenance signals in the male, Unpaired and Glass-bottom boat (Gbb; *see* **Subheadings 1.6.3** and **1.6.4**), are both short-range signals, suggesting that anterior PGCs first need to be in close contact with the hub to receive these signals.

Part of the mechanism used to capture GSCs involves the localization of high levels of E-Cad, a large homophilic transmembrane adhesion molecule, to the hub. E-Cad expression is initially increased in all SGPs at the time of gonad coalescence, but high-level E-Cad expression is refined to a pattern resembling that of *esg-lacZ*: first to the anterior portion of the gonad (57) and then to the hub cells (90,91). Although not yet demonstrated in the niche, *esg* positively regulates E-Cad expression during tracheal development (92). E-Cad is an integral component of the adherens junctions that maintain GSC fate in adults by ensuring physical contact between GSCs and the niche and that polarize GSC divisions perpendicular to the hub (77,83,84).

Drosophila gef26 encodes a guanine nucleotide exchange factor for the Rap guanine triphosphatase (GTPase) pathway that localizes to the hub–GSC interface (93,94). In *gef26* mutants, the hub is specified normally, but GSC fate is not maintained because of the absence of adherens junctions between hub cells and GSCs (93). Experiments in mammalian cell culture and *Drosophila* epithelia indicated a direct role for the Rap pathway in localizing E-Cad to newly formed intercellular contact sites, facilitating the development of mature adherens junctions (95,96). The interaction between the Rap pathway and E-Cad suggests that a Rap-mediated signal from the hub guides the formation of adherens junctions specifically at the interface of hub cells and nearby PGCs, effectively capturing GSCs from among the population of PGCs near the hub (93).

1.6 Niche–PGC Interactions

1.6.1 Egfr Signaling

After the gonad forms, regulatory interactions between the PGCs and gonadal mesoderm ensure that a proper ratio of germline to soma is maintained through larval stages. An example

illustrating the importance of this mechanism is the phenotype of *germcell-less* (*gcl*) mutants. Although most embryos produced by *gcl* mutant females do not form PGCs, some do form a few PGCs and develop into fertile adults (97). Examination of *gcl* mutant larval gonads, which in L1 have an average of one to three PGCs, revealed an increase in PGC division rate through larval development until their numbers reach near wild type (75). PGC number is likely to be regulated by the BMP family member Dpp because overexpressing Dpp in the somatic cells of larval gonads increases PGC number by promoting proliferation (78). PGC number is also regulated by the somatic activity of the Epidermal growth factor receptor (Egfr), which is probably activated by the Egf ligand Spitz from PGCs (75). Decreasing Egfr pathway activity in the soma, or decreasing Spitz expression in the PGCs, yields an expanded number of PGCs and a concomitant decrease in the number of somatic cells intermingled with the PGCs (58, 75). Conversely, constitutively high Egfr activity in the soma reduces the number of PGCs through an undefined inhibitory mechanism. Thus, a dividing population of PGCs appears to positively regulate the number of somatic support cells, which in turn keep PGC numbers in check during larval development by inhibiting further PGC division.

In addition to regulating mitosis, germline–soma communication affects the encapsulation of germline cells by somatic support cells. Stem cell tumor (Stet), a rhomboid-like transmembrane protease, is required in male and female germline cells to promote their enclosure by somatic cells (98). Rhomboid cleaves and activates the membrane-tethered Egfr ligand Spitz in the signaling cell, implicating Stet in Egfr pathway regulation. Without germline Stet, somatic support cells do not surround germ cells properly, leading to the accumulation of cysts at early stages of differentiation in adult gonads (98). The *stet* phenotype is consistent with disrupted signaling between the germline and somatic support cells because of the absence of normal intercellular contacts between the two cell types.

1.6.2 Notch Signaling

Notch signaling is involved in a feedback regulatory mechanism between GSCs and niche cells in the female. Notch and its ligands Delta and Serrate are large trans-membrane proteins with extracellular EGF-like repeats (99). Notch signaling operates by a direct mechanism by which, on activation, the intracellular domain of Notch is released from the membrane and accesses the nucleus. In the nucleus, it interacts with the DNA-binding protein Suppressor of Hairless [Su(H)] to activate expression of target genes, such as genes of the *Enhancer of split Complex* [*E(spl)-C*]. GSCs mutant for Delta or Serrate are not maintained in the niche, and Notch and Su(H) are dispensable in GSCs, indicating that Notch ligands signal from the GSCs to another cell type to maintain GSC fate (100). One target of the germline Delta signal are the cap cells because germline overexpression of Delta induces up to a tenfold increase in the number of cap cells at the anterior of the germarium (100). This increase in cap cell number increases the capacity of the stem cell niche because more cells with GSC markers, such as increased pMAD levels (*see Subheading 1.6.4*), are present after germline Delta overexpression. These results illustrate that a germline-to-niche signal affects the normal functioning of the niche, and that the flow of information between niche and stem cells is not unidirectional. The ESCs are also possible targets for the germline Delta signal, and further study is likely to result in a more elaborate model of signaling cross talk among the cells of the niche and the GSCs.

1.6.3 Jak-Stat Signaling

The Jak-Stat pathway is the major signaling pathway required for GSC maintenance in the male. Upd, a short-range signal expressed in the hub cells, maintains GSC fate by activating the Jak-Stat signaling pathway in GSCs (101,102). In *Drosophila*, Jak-Stat signaling initiates when Upd binds to the Domeless receptor and activates Hopscotch (the Janus kinase). Hopscotch activity results in phosphorylation of the Stat92E transcription factor, which

translocates into the nucleus and activates target gene transcription (103). GSCs mutant for *hop* or *Stat92E* cannot transduce the Upd signal and lose their ability to self-renew, whereas Upd overexpression is sufficient to induce an excess of GSC fate (101,102). These results indicate that Upd is both necessary and sufficient for GSC fate in the male. Understanding the mechanism by which GSC fate is controlled by the Jak-Stat pathway awaits the identification of relevant target genes.

In the female, the Jak-Stat pathway plays a less-important role in GSC maintenance. Upd overexpression in the cap cells and escort cells causes an increase in GSC proliferation without a striking effect on GSC number (66). Reducing *Stat92E* function specifically in the ESCs and escort cells affects the normal organization of the anterior germarium, leading to a reduction in stem cell number (66). These findings illustrate the complexity of the GSC–niche signaling network because the niche signals affect the stem cells as well as their support cells, and the support cells affect stem cell proliferation and differentiation.

1.6.4 Dpp Signaling and Bag of Marbles

Dpp signaling is the major GSC-maintaining pathway in females, fulfilling a function analogous to the Jak-Stat pathway in the male. Dpp, the *Drosophila* homolog of human BMP2, acts nonautonomously over the short range from the stem cell niche to the GSCs to repress the transcription of *bag of marbles* (*bam*) (104,105). *Bam* is the cystoblast determinant that is both necessary and sufficient for GSC differentiation in the female (106,107). As *Bam* contains no well-characterized functional motifs, the biochemical mechanism by which it regulates cyst formation remains unclear. *Bam* works together with Benign Gonial Cell Neoplasm (BgcN), an unusual RNA helicase-like protein, suggesting that a *Bam*:BgcN complex might regulate the translation of cystoblast promoting mRNAs (108).

Loss of *dpp* signaling in GSCs allows *bam* expression and cyst differentiation, and conversely, constitutive *dpp* signaling prevents *bam* transcription and blocks the differentiation of GSC daughter cells (104,109). It appears that *bam* is the principal Dpp target because *Bam* expression is sufficient to drive differentiation of GSCs or cystoblasts even in the presence of high levels of Dpp (109). The repression of *bam* by Dpp is direct as the Dpp pathway transcriptional corepressors Mothers against Dpp (Mad) and Medea (Med) bind to transcriptional silencer elements in the *bam* promoter (104,105,109).

In PGCs, *bam* is not expressed until early pupal stages, when the PGCs not in contact with the cap cells begin to differentiate. Forced expression of *bam* in PGCs during larval stages is sufficient to drive the differentiation of all PGCs, effectively eliminating PGC fate (110). Similarly, preventing Dpp signal transduction in PGCs by removing the Dpp receptor Tkv induces differentiation of PGCs, beginning as early as late embryogenesis (110). These data strongly support a model in which Dpp is continuously required throughout embryonic and larval development to repress *Bam* expression and PGC differentiation. In fact, active BMP signaling, measured with an antibody specific to the active phosphorylated form of the transcription factor MAD (pMAD), can be observed in embryonic pole cells and in all PGCs throughout larval stages (110–113). Once niche formation is completed in early pupae, a Dpp signal from the cap cells prevents anterior PGCs from differentiating, thereby maintaining their potential to become GSCs (78).

bam mutant ovaries fill with undifferentiated germline cells that resemble GSCs, confirming the critical role for *bam* in the differentiation of the stem cell's descendants (106,107). *bam* mutant germline cells can be maintained for long periods in the presence of Dpp and cultured somatic cells from the germarium (114). Under these conditions, they maintain their spectrosomes and even develop adherens junctions with somatic cells (114). Undifferentiated cells isolated from *bam* mutant ovaries, when injected into the dorsal mesoderm of embryos,

are able to populate the embryonic gonad and establish a dividing population of *bam* mutant stem cells in the adult (115). These cells retain the capacity to differentiate on transgenic rescue of *bam* expression. These results show that the *bam* mutant GSC-like cells are not committed to a post-stem-cell fate because they are able to revert to PGC fate and establish a stem cell population in the adult. They also imply a close relationship between the undifferentiated state of GSCs and PGCs.

As in the female, the BMP ligands Dpp and Gbb play a role in male GSC maintenance (116–118). Loss of BMP pathway components leads to Bam expression in GSCs and loss of GSC fate. As in the female, overexpressing Bam is sufficient to drive GSC differentiation. However, unlike results with Upd in the male or Dpp in the female, overexpression of Dpp or Gbb is not sufficient to block the differentiation of gonialblasts in the male—Bam is not required for gonialblast differentiation but instead restricts the number of cyst divisions to four (116–119). These results indicate that Bam repression in both male and female GSCs is required to maintain GSC fate, but that Bam differentially affects the differentiation of cystoblasts and gonialblasts. The BMP and Jak-Stat signaling pathways control GSC self-renewal in both males and females, but the relative importance of the two pathways is reversed, perhaps reflecting differences in gonadal development.

1.6.5 Nanos

Nanos (Nos) is an evolutionarily conserved translational repressor that maintains the fate of germline progenitors in *C. elegans*, zebrafish, and mouse embryos (120–122). In *Drosophila*, Nos functions in a complex with Pumilio (Pum), the founding member of a widely conserved class of RNA-binding proteins (Puf domain proteins). By preventing their differentiation, *nos* promotes PGC fate during larval stages and GSC fate in adults (24,123, 124). If *nos* or *pum* function is removed during larval stages, a large proportion of PGCs, even those in contact with the stem cell niche, prematurely differentiate into cysts (124). Differentiation of *nos* mutant PGCs begins as early as the first larval instar, so that by the third instar, 16-cell cysts are already present. Similarly, loss of *nos* or *pum* from adult GSCs allows them to differentiate (110). Analogous to its role as a translational repressor of *hunchback* mRNA during embryogenesis (123), a Nos:Pum heterodimer likely prevents the activation of a PGC–GSC differentiation pathway by repressing the translation of factors important for germ cell differentiation.

Remarkably, loss of *pum* is sufficient to allow the differentiation of *bam* mutant germline cells, suggesting that Bam represses the Nos:Pum complex to allow the translation of differentiation-promoting mRNAs (125,126). In this model, Bam prevents the function of the Nos:Pum complex in cystoblasts and dividing cysts, allowing cells to differentiate if low Dpp signaling permits Bam expression. In GSCs, it is proposed that Dpp signaling prevents Bam expression, thus activating the Nos:Pum complex and maintaining an undifferentiated state (125,126).

If applied to PGCs, however, there are several observations that are not easily explicable within the framework of this model. First, in *nos* mutant larval gonads, differentiating cysts show active *bam* transcription (110). Bam is not normally expressed before pupation, and this is an unexpected result if Nos acts downstream of Dpp and Bam. Second, loss of *nos* or *pum* does not allow the differentiation of PGCs that are constitutively active for Dpp signaling (110). These results indicate that the Nos:Pum complex does not prevent PGC differentiation in a simple linear pathway downstream of Dpp signaling, underscoring the complexity of interactions between extrinsic somatic signals and factors intrinsic to PGCs that prevent differentiation.

1.7 Conclusion

Once the stem cell niche is established, it functions as a signaling center to recruit GSCs. A remarkable example illustrates this point. In the female germline, loss of Dpp signaling allows the GSCs to begin the cystoblast differentiation program. However, if Dpp signaling is reactivated in the cystoblast daughters, their limited differentiation can be reversed, and they can repopulate the niche as stem cells (127). Similarly, early stages of gonialblast differentiation can be reversed in the male on the return of Jak-Stat activity to the differentiating male germline cysts (128). Direct physical interaction between GSCs and their niche is essential for recruiting GSCs and for stem cell maintenance. GSCs are held in the niche by adherens junctions, and GSCs mutant for the cell adhesion molecules E-Cad or Armadillo (β -Catenin) lose their adherens junctions and differentiate (65,83). E-Cad accumulates between cap cells and putative GSCs from the beginning of niche establishment, and this is important in recruiting the GSCs to the niche and maintaining their proximity to stem cell maintenance factors. Localizing high levels of E-Cad expression to the niche might play a central role in recruiting nearby PGCs into the niche. Once recruited, the undifferentiated state of the PGCs would then be maintained by niche signals into adulthood as GSCs. The remainder of the PGCs, and the daughters of the newly formed GSC, do not receive the niche signals, assume the default fate, and differentiate.

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Fig. 1.1.

Primordial germ cell migration. Lateral view, dorsal up, anterior left (A–E). The germ-line is the first distinct cell lineage established in the *Drosophila* embryo when primordial germ cells (PGCs), or pole cells, cellularize at the posterior pole of the embryo (A). PGC fate is determined by their inheritance of polar granules, which are located in the posterior cytoplasm of the embryo. A polar granule component, Vasa, is used here to mark the germline (A–H). PGCs are carried dorsally and anteriorly as the germ band extends, and during gastrulation, the posterior midgut (PMG) invaginates and carries the PGCs into the PMG lumen (B and C). PGCs then migrate through the epithelium of the PMG into the interior of the embryo (D and E). PGCs migrate away from Wunen expression in the ventral PMG (grey in E') and toward

the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Hmgcr)-expressing mesoderm (black in **E'**) and thus move dorsally. Dorsal view, anterior left (**F–H**). PGCs migrate toward the Hmgcrexpressing mesoderm (black in **F'**) and split into two groups as they are repelled from the midline by Wunen expression in the central nervous system (CNS; gray in **F'**). Within the mesoderm, PGCs associate with the somatic gonadal precursors (SGPs) in parasegments 10–12 (**G**), before migrating anteriorly and coalescing to form embryonic gonads in parasegment 10 (**H**)

Fig. 1.2.

Female germline stem cell niche development. *hh-LacZ* is specifically expressed in the developing GSC niche in the anterior of the developing ovary (A–C). Niche development commences in early third larval instar with the identity of somatic terminal filament (TF) cells in the anterior of the gonad. Terminal filament cells intercalate and separate into clusters (A), then extend by cell shape changes and by cell recruitment during late third larval instar (B). The niche develops anterior to the primordial germ cell (PGC) population, which is located in the medial region of the ovary, and contacts a subset of PGCs, marked here with *Aub::GFP* (A', B'). Cap cells (CpC) are formed in pupal ovaries after TF formation is complete (C). GSC fate is restricted to anterior PGCs during the early pupal stage based on their juxtaposition to cap cells (D, early pupae; E, late pupae). Antibodies to α -Spectrin mark the cell membranes of TF cells, the spectrosomes of PGCs and GSCs (spec), and the fusomes of differentiating germline cells (fus). Anterior PGCs next to the developing niche maintain a single spectrosome

that becomes attached to the CpC–GSC contact site. The differentiation of germline cells further away from the niche is marked by the presence of branched fusomes (**D**, **E**)

Fig. 1.3.

The germline stem cell (GSC) niche of male third instar larvae. GSCs in the testis are maintained by a cluster of somatic hub cells that specifically express an *hh-lacZ* enhancer trap (A) and Fas3 protein (C). The position of the hub from A is noted in B (arrow). Antibodies to α -Spectrin mark GSC spectrosomes next to the hub and the fusomes of differentiating gonialblasts and dividing cysts (B). GSCs are arranged around the hub in a characteristic rosette pattern (C). The positions of GSCs from D are noted around the Fas3-expressing hub in C. Germline cells arranged around the hub (*) express an Aub::GFP (green fluorescent protein) fusion protein (D)

Table 1.1

Cell types in the male and female germline stem cell (GSC) niches

Cell type	Testis	Ovary
Primary GSC niche	Hub cells	Cap cells (CpC)
Primary niche–GSC pathway	Jak-Stat	Dpp-Bam
Differentiating GSC daughter	Gonialblast (GB)	Cystoblast (CB)
Somatic cells supporting differentiation of cysts	Somatic cyst cells (SCCs)	Inner germarial sheath (IGS) cells/escort cells (ECs)
Progenitor of somatic cyst cells	Somatic cyst progenitor stem (SCP) cells	Escort stem cells (ESCs)
Follicle cell progenitors (female specific)		Somatic stem cells (SSCs) or follicle stem cells (FSCs)

Table 1.2

Useful niche and germline stem cell (GSC) markers

LacZ P-element insertion lines	
<i>842-lacZ</i>	Hub cells, SSCs and cyst cells (87)
<i>1444-lacZ</i>	IGS cells (129)
<i>3914-lacZ</i>	Cap cells (weak: TF, IGS) and hub cells (weak: somatic cyst progenitors) (21)
<i>68-77-lacZ</i>	Gonadal mesoderm (37)
<i>bab-lacZ</i>	Somatic cells of the pupal niche (68)
<i>bluetail-lacZ</i>	Male-specific SGPs (86)
<i>esg-lacZ</i>	Male anterior SGPs and hub (88)
<i>hh-lacZ</i>	Hub cells and cap cells/TF (60)
<i>M5-4-lacZ</i>	Hub cells, GSCs and gonialblasts (130)
<i>ptc-lacZ</i>	Cap cells (weak: TF, IGS) (60)
<i>Stat-lacZ</i>	Cap and IGS cells (131)
<i>wingless-lacZ</i>	Cyst progenitor cells and cyst cells (17-en-40) (64)
Antibodies	
α -Spectrin	Spectrosome and fusome (70)
Bag of Marbles	Dividing cysts (106,119)
E-Cadherin	Cap cells (77), hub cells (83)
Eyes absent	Male-specific somatic gonadal precursors (msSGPs) (86)
Engrailed	Cap cells, terminal filament (64)
Nanos	Highest in GSCs and cystoblasts (female) (24)
pMad (phosphorylated Mothers against <i>Dpp</i>)	GSCs (weak in dividing cysts) (112,118)
<i>Vasa</i>	Germline (132)