Germline stem cell niches

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Abstract

Germline stem cells (GSCs) can generate haploid gametes, sperms or oocyte, which are responsible for transmitting genetic information from generation to generation. Because GSCs can be easily identified and gene functions can be readily manipulated in *Drosophila* and *C. elegans*, their niches were among the first to be functionally and anatomically defined. Genetic and cell biological studies in these systems have first shown that stem cell function is controlled by extracellular cues from the niche, and intrinsic genetic programs within the stem cells. Important progress has also recently been made in localizing GSCs in the mouse testis. Here I will review recent progress and compare the differences and commonalities of GSC niches from different systems. Since the studies on GSC niches in *Drosophila* and *C. elegans* have provided guiding principles for initial identification of niches in other systems, I hope that this review will provide some stimulating thoughts about niche structures and functions of adult stem cells in somatic systems.

Stem cells are a group of undifferentiated cells having the dual ability to self-renew and differentiate into functional mature cells. Somatic stem cells play essential roles in organogenesis and tissue maintenance, while germline stem cells (GSCs) can only produce gametes for reproduction (Li and Xie, 2005). In most of invertebrates and low vertebrates, both male and female animals have long-term self-renewing GSCs. Although it remains controversial whether postnatal mammalian females also harbor GSCs (Eggan et al., 2006; Johnson et al., 2005; Johnson et al., 2004), all the mammalian males maintain GSCs in the testis to support spermatogenesis for a lifetime (Brinster, 2007). Since GSCs are responsible for passing on their genetic information from generation to generation, sustaining their self-renewal ability is of paramount importance to evolution and genetic continuity. Recently, mouse GSCs have been successfully cultured and expanded *in vitro*, and the cultured GSCs can be used successfully to repopulate germ cell-depleted testes and restore fertility (Kanatsu-Shinohara et al., 2005; Kubota et al., 2004). Surprisingly, the cultured

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GSCs from neonatal and adult mouse testes can produce embryonic stem cell-like cells, which have the capacity to differentiate into the cell types found in three different germ layers (Guan et al., 2006; Kanatsu-Shinohara et al., 2004). Therefore, the knowledge gained from studies on GSCs is important for applying human GSCs to treat infertility and degenerative diseases. In addition, because stem cells from different systems share many similarities such as self-renewal and the supporting niche, the knowledge of molecular mechanisms regulating GSCs is also important to understanding general stem cell regulation and cancer formation.

In 1978, Ray Schofield proposed the 'niche' hypothesis to describe the physiologically limited microenvironment that supports hematopoietic stem cells (HSCs; Schofield, 1978). While defining the stem cell niche in mammals has been difficult due to their complex anatomic structures, the stem cell niches in other genetic model systems, including *Drosophila* and *C. elegans*, were among the first to be defined. In 2000, the germarial tip adjacent to GSCs was defined as the niche to support GSCs in the *Drosophila* ovary (Xie and Spradling, 2000) while the hub, located at the apical end of the *Drosophila* testis, was found to serve this function in testis (Kiger et al., 2001; Tulina and Matunis, 2001). In *C. elegans*, a distal tip cell (DTC) located at the distal end of the gonad was found to function as the niche in supporting GSCs (Crittenden et al., 2002). Recently, significant progress regarding stem cells and their surrounding microenvironment has been made in different mammalian tissue types. Two types of niche, osteoblasts-based and vasculature-based, have been defined for supporting HSC self-renewal and regulating their proliferation (Calvi et al., 2003; Kiel et al., 2005; Sugiyama et al., 2006; Zhang et al., 2003). In the nervous system, the neural stem cell niche was localized to the base of the subventricular zone (SVZ) or subgranular zone (SGZ), and endothelial cells in the blood vessels were found to contribute to niche functions (Doetsch, 2003; Doetsch et al., 1999; Palmer et al., 1997; Shen et al., 2004). Also, epithelial stem cells in the skin were identified in the bulge area of hair follicles based on label-retention and transplantation (Cotsarelis et al., 1990; Tumbar et al., 2004). More recently, stem cells in the mouse intestine and colon have been localized to the bottom of the crypt (Barker et al., 2007). GSCs have also been localized close to blood vessels in the mouse testis, though its niche has not been defined (Yoshida et al., 2007). Generally speaking, in mammalian systems, niche location is defined largely based on its proximity to stem cells. The niche or stem cell regulatory microenvironment has been defined to include the cellular components and extracellular matrixes in proximity to stem cells, signals emanating within the support cells (Li and Xie, 2005). Therefore, it will be essential to determine how different cell types adjacent to stem cells in mammalian systems contribute to niche function and stem cell regulation. In this review, I will compare the differences and commonalities of the GSC niches in *C. elegans*, *Drosophila*, and mouse, and further discuss important future topics related to GSCs and their niche.

1. The anatomically simplest GSC niche in *C. elegans*

In the *C. elegans* hermaphrodite gonad, about 225 mitotic germ cells are located closest to the distal tip cell (DTC) at the distal end of the germ line tube; those proximal are in meiotic prophase (Hansen and Schedl, 2006; Kimble and Crittenden, 2007; see Figure 1A). Although GSCs likely reside in the mitotic region, which extends about 20 cell diameters along the gonadal axis from the DTC, it has not been definitively determined by lineage tracing if GSCs are only germ cells that are in direct contact with the DTC. The mitotic germ cells that are not in direct contact may represent transit amplifying or differentiating cells found in other systems (Hansen and Schedl, 2006; Kimble and Crittenden, 2007). Several pieces of experimental evidence support the idea that the DTC acts as a niche for GSCs. First, the somatic DTC was shown by laser ablation to be required for maintaining the germline mitotic region, indicating that the DTC supports germ cell proliferation and GSC maintenance (Kimble and White, 1981). Second, DTC relocation leads to a corresponding positional change for the mitotic region, and duplicated DTC cells support two pools of mitotically dividing germ cells, including GSCs (Kidd et al., 2005; Kipreos et al., 2000; Lam et al., 2006). Third, the signal from the DTC, Delta-like LAG-2, can directly activate Notch-like receptor GLP-1 in germ cells to maintain active Notch signaling, which is necessary and sufficient for maintaining GSCs and the mitotic zone of the gonad (Crittenden et al., 1994; Henderson et al., 1994;Fitzgerald and Greenwald, 1995). Therefore, the single DTC cell forms a functional niche sufficient for supporting GSC self-renewal and proliferation.

The GSC maintenance in the adult *C. elegans* gonad is tightly controlled by the GLP-1/Notch signaling pathway (Kimble and Crittenden, 2007). The Notch-like receptor GLP-1 is expressed in the germ line and transduces the Delta-like LAG-2 signal from the DTC to promote mitotic divisions of GSCs and early mitotic cells (Austin and Kimble, 1987; Crittenden et al., 1994; Henderson et al., 1994; Fitzgerald and Greenwald, 1995). When *glp-1* temperature-sensitive mutants are shifted to a restrictive temperature, germ cells, including GSCs, leave the mitotic cell cycle and enter meiosis. In contrast, a *glp-1* gain-of-function mutation, resulting in constitutive Notch signaling, leads to formation of a germline tumor with all germ cells behaving like early mitotic germ cells (Berry et al., 1997;Fitzgerald and Greenwald,

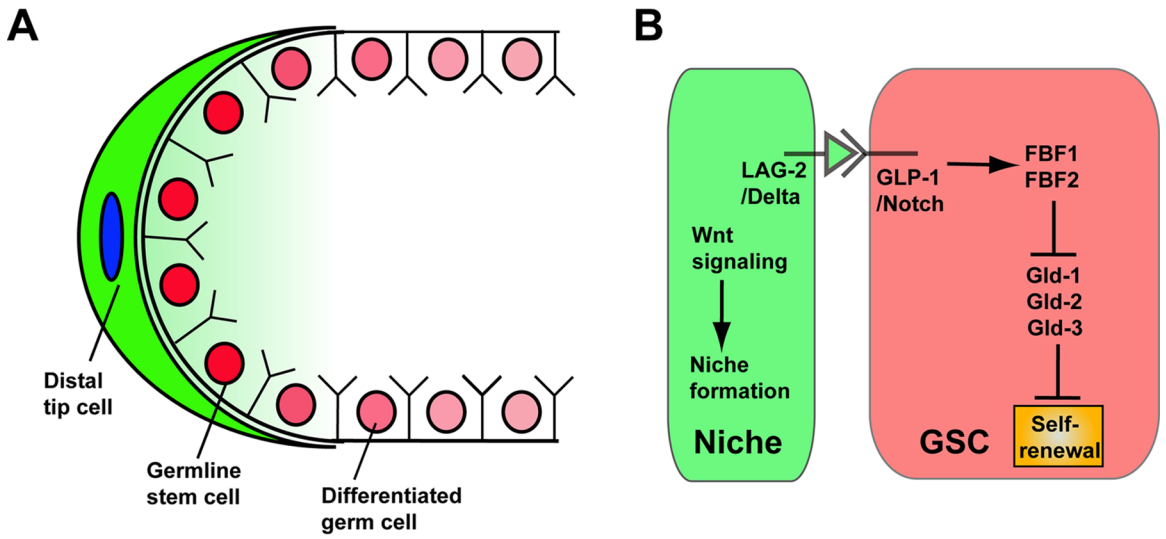

Figure 1. Structure and signaling mechanisms of the *C. elegans* GSC niche. (A). The Distal tip cell (DTC, green) functions as a niche to maintain GSCs (red, green shade representing the niche influence), allowing germ cells (pink) outside the influence of the niche to differentiate. **(B).** Wnt signaling controls the niche formation, while DTC-expressing LAG-2/Delta can activate Notch signaling, maintain functions of FBF1 and FBF2 and repress functions of differentiation-promoting GLD genes to control GSC self-renewal.

1995). Therefore, GLP1/Notch signaling is both necessary and sufficient for GSC maintenance and proliferation. The LAG-2/GLP-1 signaling pathway achieves its specificity for GSC regulation by the restricted expression of LAG-2 in DTC and of GLP-1 in GSCs and early mitotic germ cells (Crittenden et al., 1994; Henderson et al., 1994; Fitzgerald and Greenwald, 1995). GLP-1/Notch signaling can directly activate expression of *fbf-2* and *lip-1* in germ cells since the promoters of the two genes contain a binding site of the GLP-1/Notch pathway downstream transcription factor LAG-1 (Lamont et al., 2004; Lee et al., 2006). *fbf-2* and *lip-1* encode a Pumilio-like translational repressor and a MAPK phosphatase, respectively, which function in repressing intrinsic germ cell differentiation programs. Particularly, FBF-2 works with another Pumilio-like gene FBF-1 to repress expression of differentiation-promoting genes such as GLD-1, 2 and 3 (Crittenden et al., 2002). Therefore, the DTC-expressed LAG-2 activates GLP-1 signaling in GSCs to control their self-renewal and proliferation by repressing differentiation (see Figure 1B).

Much progress has also been made in understanding how the formation of the DTC cell is controlled (see Figure 1B). The DTC is generated during early larval development from the somatic gonadal progenitor cell through asymmetric division (Kimble and Hirsh, 1979). A Wnt pathway is necessary and sufficient for specification of the DTC fate (Kidd et al., 2005; Siegfried et al., 2004; Siegfried and Kimble, 2002). In the mutants defective for Wnt signaling, the DTC fails to form, while over-activation of Wnt signaling results in formation of extra DTCs. Consistently, a Wnt signaling pathway direct target, *ceh-22b*, encoding a conserved homeodomain transcription factor, is necessary and sufficient for DTC formation (Lam et al., 2006). In addition, the *daughterless* ortholog HLH-2 and a nuclear hormone receptor NHR-25 are also required for controlling the DTC fate (Asahina et al., 2006; Karp and Greenwald, 2004). HLH-2 acts positively to specify the DTC fate (Karp and Greenwald, 2004), while NHR-25 functions to negatively repress the DTC fate by antagonizing Wnt signaling (Asahina et al., 2006). In the future, it is important to figure out how different pathways or factors work synergistically to control the DTC fate.

In many stem cell systems, the stem cell division plane is always orientated in such way that only one of the newly generated stem cell daughters stays in the niche to self-renew and the other is positioned outside the niche to differentiate (Li and Xie, 2005). A recent elegant study shows that in the *C. elegans* germ line, the orientation of germ cell divisions can be perpendicular or parallel with regard to the distal-proximal axis (Crittenden et al., 2006). During larval development as well as in adults, some germ cells position both daughters in the same plane next to the DTC body, and others place one daughter next to the DTC body and the other daughter away from the DTC. In addition, the GSCs close to the DTC body and the mitotic germ cells away from the DTC have similar length in the cell cycle, indicating that all the mitotic germ cells including GSCs divide at a similar pace in *C. elegans*. These findings suggest that both daughters of stem cell divisions retain potential for self-renewal and differentiation and that the niche does not restrict stem cell proliferation.

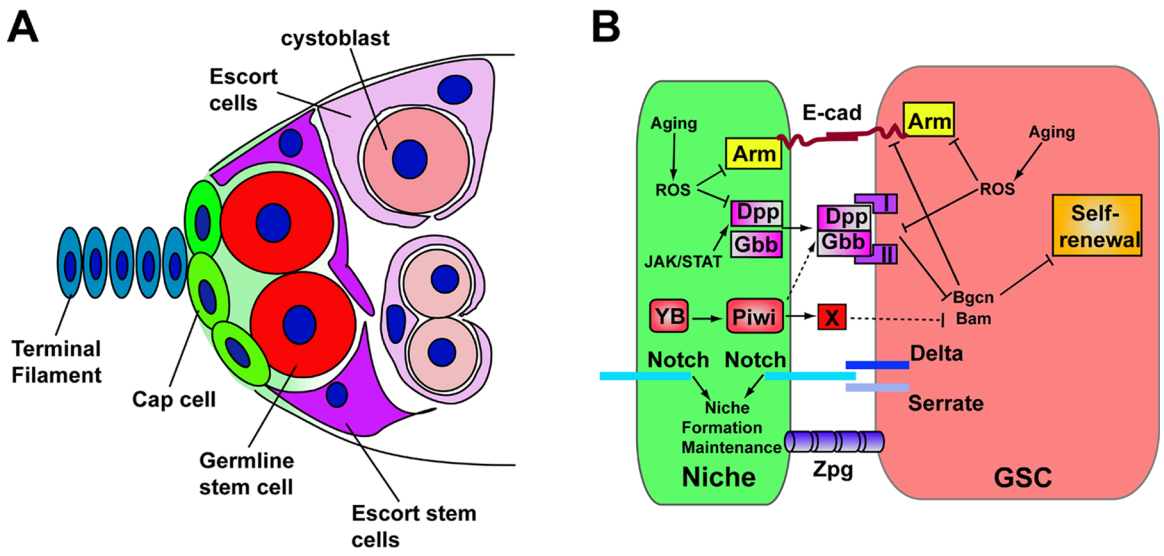

Figure 2. Structure and signaling mechanisms of the *Drosophila* ovarian GSC niche. (A). Cap cells (green) and escort stem cells (purple) function as a niche to maintain GSCs (red, green shade representing the niche influence), allowing germ cells (pink) outside the niche to differentiate. **(B).** Notch signaling controls the niche formation and maintenance, while aging affects BMP signaling activity and E-cadherin expression in niche cells. The Dpp/Gbb-mediated BMP signaling pathway and the Yb/Piwi-mediated unknown signaling pathway together repress expression of differentiation-promoting genes including *bam*, thereby maintaining GSC self-renewal. Intrinsic GSC aging affects E-cadherin expression and BMP reception. E-cadherin-mediated cell adhesion is required for GSC niche anchorage and competition for niche occupancy, while Zpg-containing gap junctions are required for GSC survival.

2. The first structurally and functionally defined GSC niche in the *Drosophila* ovary

In the structure called the germarium at the tip of the ovariole, an egg production unit of the *Drosophila* ovary, 2–3 GSCs, which contain an organelle known as the spectrosome, are surrounded by two types of somatic cells, cap cells and escort stem cells (ESCs; Kirilly and Xie, 2007; Lin, 2002; Xie and Spradling, 2001; see Figure 2A). Normally, a GSC divides to generate a self-renewing stem cell that stays in association with cap cells and a differentiating cystoblast that moves away from the cap cells and forms an interconnected 16-cell cyst through incomplete cytokinesis. Genetic and cell biological studies have demonstrated that cap cells and ESCs form the GSC niche (see Figure 2B). First, both GSC daughters remaining in contact with cap cells and ESCs following GSC division become GSCs, indicating that the direct contact with cap cells and ESCs is sufficient for GSC maintenance (Xie and Spradling, 2000). Second, the number of GSCs is closely related to the number of cap cells (Xie and Spradling, 2000). Third, the anchorage of GSCs to cap cells through E-cadherin-mediated cell adhesion is essential for GSC maintenance since loss of E-cadherin expression from GSCs results in their detachment from cap cells/ESCs, premature differentiation and loss (Song et al., 2002). Fourth, cap cells express genes that are known to be important for maintaining GSCs, such as *dpp*, *gbb*, *hh*, *piwi,* and *Yb* (Cox et al., 1998; Cox et al., 2000; King and Lin, 1999; King et al., 2001; Song et al., 2004; Xie and Spradling, 1998, 2000). Finally, ESCs also play an important role in maintaining GSCs since disruption of JAK-STAT signaling in ESCs also leads to rapid GSC loss (Decotto and Spradling, 2005). Therefore, cap cells and ESCs work together to form the GSC niche.

In the *Drosophila* ovary, niche structure and function are also relatively well understood in addition to the well-defined niche. Two BMP-like genes, *dpp* and *gbb*, are expressed in the somatic cells of the germarium including cap cells, and their signaling distance appears to be restricted to one cell diameter for maintaining active BMP signaling only in GSCs (Song et al., 2004). GSCs that are located in *dpp* and *ggb* mutant niche or are mutant for BMP downstream components (*punt*, *tkv*, *mad* and *Medea*) are lost rapidly due to differentiation, while *dpp* overexpression can completely block GSC differentiation and lead to formation of GSC-like tumors, indicating that BMP signaling is necessary and sufficient for controlling GSC self-renewal (Song et al., 2004; Xie and Spradling, 1998). *bam* is expressed in cystoblasts, and is necessary and sufficient for their differentiation since its mutation causes the accumulation of cystoblast-like cells and its forced expression in GSCs lead to their differentiation (McKearin and Ohlstein, 1995; Ohlstein and McKearin, 1997). BMP signaling was recently shown to control GSC self-renewal by directly repressing expression of *bam* (Chen and McKearin, 2003; Song et al., 2004). In differentiated cystoblasts, Bam expression then helps turn off residual BMP signaling to allow them to terminally differentiate (Casanueva and Ferguson, 2004). Interestingly, *dpp* overexpression can also revert differentiated mitotic cysts back into GSCs (Kai and Spradling,

2004). These findings lead to a simple model: BMP signals from the GSC niche directly repress differentiation, and thereby maintain GSC self-renewal (see Figure 2B).

In addition, the unknown signal regulated by Piwi/Yb in TFs and cap cells is also required for controlling ovarian GSC self-renewal (Cox et al., 1998; King and Lin, 1999). Yb, a novel protein, regulates expression of *piwi* and *hh* in TF/cap cells and controls GSC self-renewal; *piwi* encodes an Ago family protein involved in the biogenesis of small RNAs (King et al., 2001). Hh appears to play a redundant role with the Piwi-regulated niche signal in ensuring GSC self-renewal (King et al., 2001). Interestingly, the unknown signal regulated by Piwi in the niche is also involved in repressing *bam* expression and thereby maintaining GSC self-renewal since GSCs in *piwi* mutant niches upregulate *bam* transcription (Chen and McKearin, 2005; Szakmary et al., 2005). Because BMP downstream transcription factors Mad/Medea can directly bind to the *bam* promoter to repress its expression in GSCs, the BMP signal and the Piwi-regulated signal are likely intersected upstream of *bam* repression in controlling GSC self-renewal. It is possible that the Yb-Piwi genetic circuitry is involved in regulating BMP production in the niche. Recently, two independent studies have shown that JAK-STAT signaling in cap cells positively regulates *dpp* expression since in the absence of JAK-STAT signaling Dpp signaling activity in cap cells is downregulated (Lopez-Onieva et al., 2008; Wang et al., 2008). This regulation is likely direct since the *dpp* promoter is capable of responding to JAK-STAT signaling and harbors STAT binding elements. In addition, gap junctions, which are formed by a connexin-like protein Zpg (Zero population growth), are present in cytoplasmic membranes of GSCs and their differentiated progeny and are required for GSC survival and germ cell differentiation since loss of *zpg* function results in partial GSC loss due to cell death and accumulation of ill-differentiated germ cells (Giloa et al., 2003; Tazuke et al., 2002). Taken together, the niche communicates with GSCs through secreted growth factors and direct cell-cell contact in the *Drosophila* ovary (see Figure 2B).

Recently, important progress in understanding how niche formation and maintenance are controlled has been made. In the early developing *Drosophila* female gonad of late third instar larval stage, newly formed TFs express Delta (Dl), a transmembrane ligand for Notch, which is expressed in all the somatic cells, including precursor cells for cap cells (Song et al., 2007). Expanded Notch activation causes the formation of more cap cells and bigger niches, which support more GSCs (Song et al., 2007; Ward et al., 2006), whereas compromising Notch signaling during niche formation decreases the cap cell number and niche size and consequently the GSC number (Song et al., 2007). Furthermore, the niches located away from their normal location can still sufficiently sustain GSC self-renewal by maintaining high local BMP signaling and repressing *bam* as in normal GSCs (Song et al., 2007). In the adult ovary, Delta and Serrate are expressed in GSCs and activate Notch signaling in cap cells (Ward et al., 2006), and loss of Notch function in adults results in rapid loss of the GSC niche, including cap cells and thus GSCs (Song et al., 2007), indicating that GSCs are also required for the maintenance of their niche. These findings demonstrate that Notch signaling is important for GSC niche formation and maintenance in the adult ovary.

Like many tissue types, the productivity of the *Drosophila* female ovary declines with age (Zhao et al., 2008b). Such decrease in the fecundity of old *Drosophila* females is likely in part attributed to the age-dependent decline of GSC number and proliferation activity (Pan et al., 2007; Zhao et al., 2008b). Interestingly, the number and the signaling activity of niche cells, namely cap cells, also decline with age, while artificially providing more BMP in old niche can significantly reduce age-dependent GSC loss (Pan et al., 2007; Zhao et al., 2008b). However, age-dependent increase in the degeneration of developing egg chambers also contribute to age-dependent decline in egg production, and therefore, increasing BMP signaling can transiently, but not permanently, improve egg production (Zhao et al., 2008b). In addition, E-cadherin accumulation in the stem cell-niche junction is reduced in aged ovaries in comparison with young ones, and E-cadherin overexpression in old GSCs can also slow down their age-dependent loss, indicating that the age-dependent decline in E-cadherin expression contributes to the age-related GSC loss (Pan et al., 2007). Reactive oxygen species (ROS)-induced cellular damage has long been proposed to contribute to cellular and organismal aging in *Drosophila* (Tower, 2000) Interestingly, overexpression of SOD (superoxide dismutase), which helps remove ROS in the cell, in either the niche or GSCs, can sufficiently prolong GSC lifespan, indicating that ROS-induced cellular damage causes niche aging and intrinsic aging, which collectively contribute to overall GSC aging (Pan et al., 2007). Taken together, age-dependent decline in niche function and intrinsic GSC function leads to GSC aging (see Figure 2B).

In the *Drosophila* ovary, each niche harbors two or three GSCs, but it remains unclear how stem cells in the same niche interact with one another. As mentioned earlier, *bam* and *bgcn* are required for cystoblast differentiation, and *bam* or *bgcn* mutant GSCs or cystoblasts continue to proliferate and fail to differentiate, behaving like cancer stem cells in mammals (McKearin and Ohlstein, 1995; Ohlstein et al., 2000). Interestingly, when it shares its niche with a wild-type GSC, a differentiation-defective *bam* or *bgcn* mutant GSC invades the niche space of neighboring wild-type GSC and

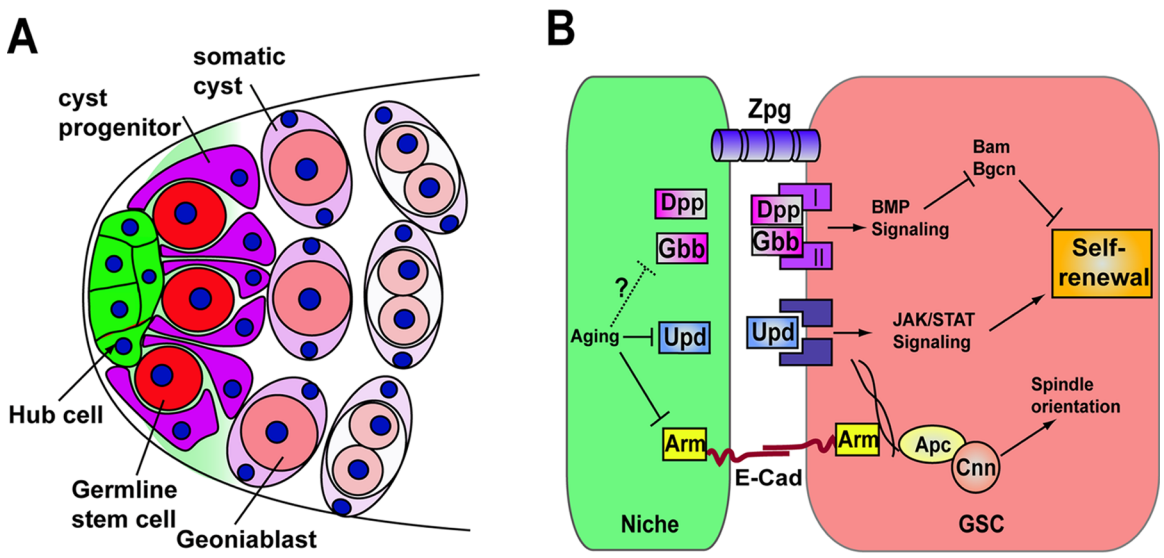

Figure 3. Structure and signaling mechanisms of the *Drosophila* testicular GSC niche. (A). Cap cells (green) and cyst progenitors (purple) function as a niche to maintain GSCs (red, green shade representing the niche influence), allowing germ cells (pink) outside the niche to differentiate. **(B).** Aging affects JAK-STAT signaling activity and E-cadherin expression. The Dpp/Gbb-mediated BMP signaling pathway represses expression of differentiation-promoting genes including *bam*, thereby maintaining GSC self-renewal, while the Upd-mediated JAK-STAT signaling pathway is necessary and sufficient for GSC self-renewal. E-cadherin-mediated cell adhesion is required for GSC anchorage and spindle orientation, while Zpg-containing gap junctions are required for GSC survival.

gradually pushes the latter out of the niche (Jin et al., 2008). Although BMP signaling can directly repress *bam* expression in GSCs, this repression is not complete, leaving low levels of *bam* expressions in wild-type GSCs. The reason why a *bam* or *bgcn* mutant GSC can outcompete a wild-type GSC in the same niche is that the ability of *bam* and *bgcn* to negatively regulate E-cadherin makes the mutant GSC have more E-cadherin than the wild-type one. In addition, the GSC that expresses more E-cadherin and is otherwise wild type can gradually push its neighboring GSC having less E-cadherin out of the niche. These findings can well explain why *bam* or *bgcn* mutant GSCs are more competitive than wild-type ones, and may also help explain how cancer stem cells can outcompete normal stem cells for niche occupancy (Jin et al., 2008). The stem cell competition mechanism also helps explain how the niche expels differentiated stem cells and maintains normal stem cells. If a differentiated GSC upregulates *bam* expression and consequently downregulates E-cadherine expression, it is quickly pushed out of the niche by its neighboring stem cell and is then replaced with a normal GSC. Therefore, stem cell competition may serve as a quality control mechanism to ensure that accidentally differentiated stem cells are rapidly removed from the niche and replaced by functional ones (Jin et al., 2008).

3. The structurally and mechanistically well-studied GSC niche in the *Drosophila* testis

In the apical tip of the *Drosophila* testis, 7–10 GSCs and 14–20 cyst progenitor cells are directly attached to hub cells, and are responsible for producing differentiated germ cells and somatic cyst cells that wrap around differentiated germ cells and support their development, respectively (Fuller, 1993; Gonczy and DiNardo, 1996; Hardy et al., 1979; Kiger and Fuller, 2001; Lindsley and Tokuyasu, 1980; Yamashita et al., 2003; see Figure 3A). As in the *Drosophila* ovary, GSCs and their immediate daughters, gonialblasts, contain a spectrosome, while the further differentiated germ cell clusters contain a branched fusome (Hime et al., 1996). A male GSC divides asymmetrically to generate one self-renewing stem cell that remains in contact with the hub and one differentiating gonialblast that is positioned away from the hub (Hardy et al., 1979; Lindsley and Tokuyasu, 1980; Yamashita et al., 2003). As a GSC divides to produce a gonialblast, neighboring cyst progenitors also divide to generate two cyst cells that encase the gonialblast (Gonczy and DiNardo, 1996; Hardy et al., 1979). Also as in the *Drosophila* ovary, adjacent somatic cells, hub cells and possibly cyst progenitors, form a niche for GSCs in the *Drosophila* testis (see Figure 3B).

As expected, niche cells produce essential signals for promoting GSC self-renewal and proliferation in the *Drosophila* testis. The hub generates signals including Unpaired (Upd) and BMP to control GSC self-renewal (Brawley and Matunis, 2004; Kawase et al., 2004; Kiger and Fuller, 2001; Shivdasani and Ingham, 2003; Tulina and Matunis, 2001). Upd from the hub activates the JAK-STAT pathway in GSCs; in the absence of JAK-STAT signaling, GSCs differentiate and are lost prematurely, while *upd* overexpression is sufficient to repress germ cell differentiation and

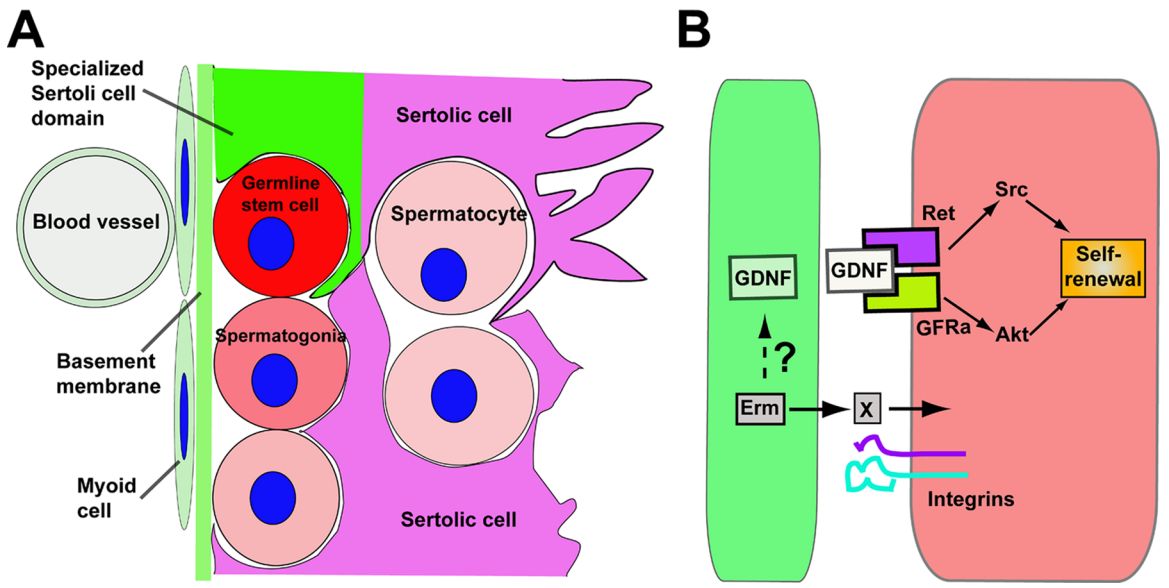

Figure 4. Structure and signaling mechanisms of the putative mouse testicular GSC niche. **(A).** Sertoli cells (green-specialized domain contacting and supporting GSCs), blood vessel and perhaps other cellular and non-cellular components including myoid cells and basal membrane function as a niche to maintain GSCs (red), allowing germ cells (pink) outside the influence of the niche to differentiate. **(B).** Sertoli cell-produced GDNF can activate Src and Akt signaling in GSCs, thereby maintaining their self-renewal, while integrins rich in GSCs may help anchor GSCs to the basal membrane. Erm expressed in Sertoli cells can help maintain GSCs by regulating the GDNF signaling pathway or an unknown signal pathway.

thus causing the accumulation of GSC-like cells, indicating that JAK-STAT signaling promotes their self-renewal by preventing differentiation (Kiger et al., 2001; Tulina and Matunis, 2001). In addition, JAK-STAT signaling can also reprogram early differentiated germ cell cysts back into GSCs (Brawley and Matunis, 2004). As in the ovary, the somatic cells surrounding GSCs, hub cells and somatic cyst cells, express *gbb* at high levels and *dpp* at much lower levels, and BMP signaling activity is primarily restricted to GSCs (Kawase et al., 2004). Consequently, BMP downstream components are essential for controlling testicular GSCs since GSCs that are located in *gbb* mutant niches or are mutant for BMP downstream components (*tkv*, *punt*, *mad* and *Medea*) are lost prematurely (Kawase et al., 2004; Shivdasani and Ingham, 2003). Overactivation of BMP signaling is not sufficient to repress GSC differentiation in the *Drosophila* testis, which is in contrast with its necessary and sufficient role in controlling GSC self-renewal in the *Drosophila* ovary (Kawase et al., 2004; Schulz et al., 2004). As in *Drosophila* ovarian GSCs, BMP signaling also maintains GSC self-renewal by repressing *bam* expression in the *Drosophila* testis since *bam* is upregulated in GSCs defective for BMP signaling and forced *bam* expression sufficiently causes GSC differentiation (Kawase et al., 2004). However, there is a significant difference between BMP signaling and JAK-STAT signaling in the control of male GSC maintenance; BMP signaling plays a permissive role, while JAK-STAT signaling has an instructive role. It remains to be determined in the future how BMP and JAK-STAT signaling pathways are integrated in GSCs. In addition, Zpg-containing gap junctions formed between germ cells and surrounding somatic cells are required for germ cell differentiation since adult mutant *zpg* testes contain a small number of germ cells resembling GSCs or early spermatogonia (Tazuke et al., 2002).

In the *Drosophila* testis, asymmetric stem cell division through the control of spindle orientation is an important mechanism to ensure two daughters generated by a GSC division adopt different cell fates, namely a self-renewing stem cell and a differentiating gonialblast (Yamashita et al., 2003). Cnn and APC1, which are centrosomal components in GSCs, direct orientation of the spindle perpendicular to the hub. APC2 (an APC-like) and E-cadherin are concentrated around the junction between GSCs and hub cells, where a centrosome is normally anchored (Yamashita et al., 2003). This anchored centrosome appears to be very special since it is only inherited by the stem cell and has more tubulin bundles associated with it, but its biological significance remains unclear (Yamashita et al., 2007). Furthermore, APC2 is also important for controlling GSC spindle orientation perpendicular to the hub, suggesting that the apically anchored centrosome may play an important role in orientating the GSC spindle. These findings indicate that spindle orientation controlled by adherens junction-associated *APC*2 and centrosome components is important for maintaining stem cell identity in conjunction with the niche (Yamashita et al., 2003; Yamashita et al., 2007; see Figure 3B). For the time being, it is not clear whether this spindle orientation is independent of niche signaling or a consequence of asymmetric signaling from the niche.

Similar to GSCs in the *Drosophila* ovary, GSC number and/or activity undergo an ageing-related decline in the *Drosophila* testis (Boyle et al., 2007; Wallenfang et al., 2006; see Figure 3B). The division rate of GSCs slows significantly during aging, and this slowing correlates with a reduction in the number of somatic hub cells that contribute to the stem cell niche (Wallenfang et al., 2006). Interestingly, long-lived *methuselah* mutant males do not exhibit age-dependent decline in GSC division rate (Wallenfang et al., 2006). Hub cells in testes of older males display reduced expression of E-cadherin and *upd* transcription, which correlates with an overall decrease in stem cell number in each niche (Boyle et al., 2007). Conversely, forced expression of *upd* within niche cells prolongs GSC lifespan in older males, but does not increase production of mature sperms. Consistent with GSC aging in the *Drosophila* female (Pan et al., 2007), age-related changes within stem cell niches contribute to age-dependent decline in stem cell number and spermatogenesis (Boyle et al., 2007; Wallenfang et al., 2006).

4. The complex GSC niche yet to be defined structurally and functionally in the mouse testis

Stem cell transplantation, simple tubular structure and genetics make the mouse testis an attractive model for studying GSCs and their niche (Brinster, 2007). The male GSC in the postnatal mouse testis, also known as the spermatogonial stem cell (SSC), resides on the basal membrane in the periphery of the seminiferous tubule, and divides to generate two A_{single} (A_s) spermatogonia (see Figure 4A). As spermatogonia can either self-renew or differentiate and divide to form interconnected A_{pair}, A_{align4} and A_{align8} spermatogonia. A_{align} spermatogonia further produce interconnected differentiated A1 to A4 spermatogonia that are then capable of maturing into intermediate and type B spermatogonia (de Rooij and Russell, 2000). GSCs, A_s spermatogonia, A_{pair} spermatogoina, A_{align} spermatogonia, A1 to A4 spermatogonia and B spermatogonia represent premeoitic germ cells and are localized on the surface of the seminiferous tubules, while meiotic germ cells enter the lumen of the tubule. GSCs in the normal testis can be isolated as a population of MHC-$1^-$$\alpha$v-integrin$^-$ C-kit$^-$Sca-1^-CD34^-CD24^+ α6integrin$^+$ Thy-1^+ using a combination of fluorescence-activated cell sorting (FACS) and spermatogonial transplantation, and there are about 18,000–19,000 GSCs in a young adult testis based on transplantation results (Kubota et al., 2003; Ryu et al., 2006). Recently, a combined strategy of lineage tracing and transplantation has estimated that the total GSC in the mouse testis is about 2000 and has also shown that a GSC is responsible for producing differentiated germ cells that occupy the about 1.4 mm long segment of the seminiferous tubule (Nakagawa et al., 2007). The inconsistency in the GSC number in the mouse testis could be attributed to different methodologies. In light of recent experimental evidence that differentiated mitotic germline cysts can be reverted back to GSCs in the presence of niche signals in the *Drosophila* ovary and testis (Brawley and Matunis, 2004; Kai and Spradling, 2004), the transplantation assay could potentially overestimate the GSC number in the mouse testis due to the contribution of transplanted early spermatogonia to the formation of stem cell colonies. Transplantation and genetic studies suggest that the niche is critical to GSC maintenance in the mouse testis (Brinster and Zimmermann, 1994; Chen et al., 2005; Meng et al., 2000). However, the cellular components of the niche have not been functionally identified. In the mouse testis, the seminiferous tubules form the convoluted loops, between which the interstitium contains the vasculature network, Leydig cells, and macrophages, while the seminiferous tubule contains myoid cells on the surface, which are responsible for producing the basal membrane, and Sertoli cells inside the tubule, which intimately interact with and support germ cells of different developmental stages (Russell et al., 1990; Figure 4A). It is conceivable that Sertoli cells, myoid cells, along with blood vessels and Leydig cells**,** could constitute a functional niche for stem cells.

Published studies support the notion that Sertoli cells are one of the cellular components of the GSC niche in the mouse testis (see Figure 4B). Transplantation of GSCs and Sertoli cells into infertile mice with defective Sertoli cells has shown that Sertoli cells can indeed support GSC maintenance, proliferation**,** and production of differentiated germ cells (Ogawa et al., 2000; Shinohara et al., 2000; Shinohara et al., 2003). In addition, Sertoli cells express a glial derived neurotrophic factor (GDNF), a member of the TGF-β superfamily, which is necessary and sufficient for GSC self-renewal *in vivo* (Meng et al., 2000). In the heterozygous *Gdnf* mutant testis, GSCs differentiate and are lost prematurely, while *Gdnf* overexpression in the mouse testis can sufficiently suppress GSC differentiation and cause accumulation of stem cell-like undifferentiated germ cells. Recently, it has been also shown that GDNF is essential for GSC proliferation and expansion *in vitro* (Kanatsu-Shinohara et al., 2004; Kubota et al., 2004). Furthermore, it has been shown *in vitro* that GDNF can directly act on GSCs themselves for controlling self-renewal and proliferation through activating Src and Akt signaling activities (Lee et al., 2007; Oatley et al., 2007). Finally, Ets related molecule (ERM) is expressed exclusively within Sertoli cells in the testis and is required for GSC self-renewal (Chen et al., 2005). Targeted disruption of ERM causes a premature loss of GSCs without affecting normal spermatogenic differentiation, resulting in a Sertoli-cell-only syndrome. However, the signal regulated by Erm in Sertoli cells remains to be identified. Therefore, Sertoli cells are either a part of the GSC niche in the mouse testis or indirectly contribute to the function of the GSC niche. If Sertoli cells are a part of the GSC niche, it remains unclear how they exert distinct regulations

on GSCs and their differentiated progeny since Sertoli cells are present throughout the periphery of the seminiferous tubule and interact with both GSCs and differentiated germ cells.

A recent study shows that the vasculature surrounding the seminiferous tubule contributes to niche function or the regulation of niche function (Yoshida et al., 2007). Using time-lapse imaging of GFP–labeled undifferentiated spermatogonia and three-dimensional reconstitution, GSCs are biased toward the location close to the vascular network in intact testes, while differentiating A_{pair} and A_{align} spermatogonia move away from the vascular network and spread throughout the basal compartment of the seminiferous epithelium. Following alteration of the vasculature, GSCs are relocalized close to the newly positioned vascular network. These findings raise an interesting possibility that the GSC niche in the mouse testis is established near the vasculature (Yoshida et al., 2007). Interestingly, neural stem cells (NSCs) in the subventracular zone are also localized close to the blood vessel, and endothelial cells can help support NSC self-renewal and proliferation *in vitro* (Zhao et al., 2008a), while HSCs have recently been shown to be localized near the vasculature in the bone marrow, and the reticular cells on the vasculature express secreted CXCL12, a chemokine that directly acts on HSCs and promotes their maintenance (Kiel et al., 2005; Sugiyama et al., 2006). These studies indicate that the proximity to the blood vessel is critical for the stem cell maintenance. However, it remains unclear whether endothelial cells themselves, the cells associated with the blood vessel and/or factors supplied by the blood flow contribute to GSC maintenance. Thus, it is critical to reveal the source and the identity of the factors that regulate GSC maintenance in the mouse testis.

In the hematopoietic system, HSCs have been shown to be controlled by the factors produced by osteoblasts and blood vessel-associated cells (Calvi et al., 2003; Kiel et al., 2005; Sugiyama et al., 2006; Zhang et al., 2003). It is also conceivable that in the mammalian testis, Leydig cells and macrophages in the interstitium, endothelial cells and peritubular cells in the blood vessel, and myoid cells and Sertoli cells in the seminiferous tubule could be involved in the regulation of GSC behavior, contributing directly or indirectly to the function of the GSC niche. The factors produced by the cells that are not in direct contact with GSCs have to diffuse a distance in order to act directly on GSCs, while the other factors produced by Sertoli cells that contact GSCs can also be delivered through cell-cell contact. Some contributions to the niche may be direct, whereas others may act indirectly through intermediate cells. For example, the factors produced by the cells in the interstitium and the blood vessel can act on Sertoli cells and myoid cells, which produce the secondary signals that directly act on GSCs. Therefore, the key for defining the GSC niche in the future lies in identifying essential signals for GSC self-renewal and their physiological origins by conditionally removing these factors from specific cell types in the mouse testis. This strategy will also help elucidate the relative contributions of different cell types in the seminiferous tubule, the blood vessel, and the interstitium to GSC maintenance in the mouse testis.

Although the GSC niche has not been definitively defined, a great deal has been learned about how the niche function changes with age using stem cell transplantation. By using the GSC transplantation assay, it has been determined that there is a 39-fold increase in male GSCs during development from birth to adult in the mouse, though GSCs from the different developmental stages proliferate at similar rates (Shinohara et al., 2001). Surprisingly, the niche in the immature mouse pup testis supports colonization and proliferation of transplanted GSCs much better than the one in adult testis. Thus, GSCs and their niche in the mouse testis undergo dramatic changes in the postnatal testis (Shinohara et al., 2001). Testes in aging mammals undergo a variety of age-related changes, such as reduction of size, lower sperm output, an increase in abnormal forms of sperm, and endocrine malfunctions. Between 12 and 24 months of age, male mice show a declining level of fertility associated with decreased testis weight, level of spermatogenesis, and bilateral testis atrophy (Ryu et al., 2006; Zhang et al., 2006). In the atrophic testes, numbers and proliferation potential of GSCs decrease in an age-dependent manner, indicating that GSCs undergo the aging process. Shortening of the telomere length, which is thought to be caused by the declined telomerase activity, has been associated with cellular aging in different tissue types. Surprisingly, the telomerase activity in GSCs and their differentiated progeny is unaffected by aging and remains high in aged GSCs (Riou et al., 2005). It has been hypothesized that the spermatogenic defects are due to loss and dysfunction of GSCs as well as deterioration of their niche. Transplantation of GSCs from young fertile male donors into atrophic testes of old recipients of different ages shows a host age-dependent decline in the restoration of spermatogenesis (Zhang et al., 2006; Ryu et al., 2006). However, it is controversial about the outcome for GSCs of different ages transplanted into GSC-depleted young testes: Zhang et al. (2006) reported a decline in colony number and length when GSCs from 2-year-old atrophied testes were transplanted into young GSC-depleted recipients; while Ryu et al. (2006) showed no decline in colony number and length. This inconsistency could be due to the experimental variations or different genetic backgrounds of the mouse strains. Taken together, these findings demonstrate that both GSCs and their niche in the mouse testis undergo the aging process. In the future, it will be important to reveal the underlying molecular mechanisms for the age-dependent functional deterioration of GSCs and their niche.

5. Commonalities and differences in different GSC niches

Based on earlier descriptions of structures and functions of the GSC niches in *Drosophila* ovary and testis, in the *C. elegans* gonad, as well as in the mammalian testis, the common features, structures, and functions of the stem cell niche can be summarized as follows: First, though their overall structures are variable, GSC niches are composed of a group of specialized cells in a special location for controlling stem cell maintenance and proliferation such as cap cells for ovarian GSCs and Hub cells for testicular GSCs in *Drosophila*, DTC for *C. elegans* GSCs, and vasculature and Sertoli cells for mouse testicular GSCs. Second, the niche functions as a physical anchor for stem cells using the same or different adhesion molecules. E-cadherin-mediated cell adhesion and Delta-Notch interaction are required for anchoring GSCs in *Drosophila* ovary and testis (Song et al., 2007; Song et al., 2002; Ward et al., 2006). The LAG-2/GLP-1 interaction may be required for anchoring GSCs in addition to regulating their self-renewal (Crittenden et al., 1994; Henderson et al., 1994). Integrins may be needed to anchor GSCs to the basal membrane in the mouse testis since they are rich in GSCs (Shinohara et al., 1999). Third, the niche generates extrinsic factors that function in short range to control stem cell fate by directly repressing expression of differentiation-promoting genes. For example, the DTC-expressed LAG-2 activate Notch signaling in adjacent GSCs to control their self-renewal by repressing functions of differentiation-promoting *GLD* genes in *C. elegans* (Kimble and Crittenden, 2007); the BMP signal maintains GSC self-renewal by repressing expression of differentiation-promoting genes such as *bam* in *Drosophila* ovary and testis (Chen and McKearin, 2003; Kawase et al., 2004; Song et al., 2004). In the *Drosophila* testis, JAK-STAT signaling maintains GSCs by preventing differentiation, but its targets in GSCs have not been identified (Kiger et al., 2001; Tulina and Matunis, 2001). Fourth, GSC niches in invertebrates and mice exhibit asymmetry such that one daughter of a GSC division is maintained in the niche for self-renewal, while the other daughter cell leaves the niche and differentiates (Li and Xie, 2005; Morrison and Spradling, 2008). A unifying idea is that GSCs generally produce daughters with equivalent potential and their subsequent location relative to the niche determines their fate. Finally, the aged niche in *Drosophila* and mouse contributes to GSC aging (Boyle et al., 2007; Pan et al., 2007; Ryu et al., 2006). In *Drosophila* ovary and testis, the aged niche reduces its ability to produce the self-renewing signal and niche anchorage protein E-cadherin (Boyle et al., 2007; Pan et al., 2007).

As one would have expected for different sexes and organisms, GSC niches also show many differences. First, the pathways controlling niche formation are quite different in different organisms and between sexes of the same organism. For example, Notch signaling is required in *Drosophila* females for GSC niche formation and maintenance (Song et al., 2007; Ward et al., 2006), while the Wnt signaling pathway is required in *C. elegans* for GSC niche specification and maintenance (Lam et al., 2006). Second, the physical structures of different GSC niches and the GSC number per niche are quite different. The GSC niche is composed of a single DTC cell supporting at least more than 5 GSCs in *C. elegans* (Crittenden et al., 2006), of 5–7 cap cells supporting 2–3 GSCs in the *Drosophila* ovary (Xie and Spradling, 2000), and of about 13 hub cells supporting 8–10 GSCs (Gonczy and DiNardo, 1996). Somatic cyst progenitor cells in the *Drosophila* testis and escort stem cells in the *Drosophila* ovary may also contribute to GSC niche function or a part of the GSC niche (Decotto and Spradling, 2005; Kiger et al., 2000; Tran et al., 2000). In the mouse testis, the blood vessel, Sertoli, the basal membrane and even myoid cells may work together to form the GSC niche, but their contribution to niche function needs to be experimentally demonstrated (Chen et al., 2005; Meng et al., 2000; Yoshida et al., 2007). Finally, different signaling pathways are needed for different GSCs. *Drosophila* ovarian GSCs require the BMP signal and the Yb/Piwi-regulated signal (Cox et al., 2000; King et al., 2001; Song et al., 2004; Xie and Spradling, 1998), while *Drosophila* testicular GSCs need the BMP signal and the JAK-STAT signal (Kawase et al., 2004; Kiger et al., 2001; Shivdasani and Ingham, 2003; Tulina and Matunis, 2001). In contrast, *C. elegans* GSCs only need the Delta-Notch signal (Crittenden et al., 1994; Henderson et al., 1994). GDNF and FGF are required for successful expansion of mouse testicular GSCs in vitro (Kanatsu-Shinohara et al., 2004; Kubota et al., 2004), and GDNF has been demonstrated to be required for GSC maintenance *in vivo* (Meng et al., 2000). Therefore, GSC niches share many commonalities in structures and functions but also show many differences.

6. Conclusions and future directions

Like other adult stem cells, GSCs are regulated by coordinated actions of environmental signals and intrinsic programs (Li and Xie, 2005; Morrison and Spradling, 2008). Using a combination of genetic, molecular and cell biological approaches, important signaling pathways from the GSC niches in *C. elegans*, *Drosophila*, and mouse have been identified for their ability to maintain and regulate GSC self-renewal. Cellular components of GSC niches in *Drosophila* and *C. elegans* have been well defined *in vivo*, and the nature of these components for the GSC niche in the mouse testis has started to be revealed. Although GSC niches in different organisms and sexes have different structures and produce different combinations of signals, the signals themselves all appear to work in short range, approximately one cell diameter, to control stem cell self-renewal by repressing differentiation. All the GSC niches

maintain self-renewal only of stem cells within, but allow daughters outside to differentiate. Therefore, these studies on GSC niches in different organisms have provided insight into fundamental principles about the niche structure and function. As it has done, the knowledge gained from these GSC studies will continue to provide stimulating thoughts and guidelines for defining the stem cell niche and studying its function in other systems. Although we have learned a great deal from studying GSCs in different systems, many important and urgent questions still await to be addressed.

In *C. elegans* and *Drosophila*, genetic screening will continue to be an efficient method to uncover other molecular components of the GSC niche and intrinsic factors within GSCs, which could be used to answer how the production of niche signals is regulated in young and old animals and how the niche signals are interpreted in GSCs in conjunction with intrinsic pathways. Two recent studies in *Drosophila* show that the committed but not terminally differentiated daughter cells are able to revert to stem cells when given access to a niche (Brawley and Matunis, 2004; Kai and Spradling, 2004). It will be important to investigate how differentiated germ cells can be reverted back to GSCs at the molecular level, and the information gained from such studies will help reprogram differentiated tissue cells back to stem cells. In the mammalian testis, it remains the most important task to define the cellular components and signals of the GSC niche using systemically genetic and molecular analyses of signaling and adhesion molecules expressed in nearby somatic cells. In addition, a comparison of niche-and stem cell-specific gene profiles in different systems will provide important clues about the niche signals and intrinsic factors critical for GSC functions. Further dissection of the cellular and molecular components of GSC niches in different organisms will shed more light on the mechanisms regulating GSC self-renewal and maintenance and provide important insights into identification and functional studies of stem cell niches in other somatic systems. Finally, understanding the interaction between stem cells and their natural partners will substantially benefit the therapeutic approach to treating human infertility and degenerative diseases.

7. References

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