# Signaling in stem cell niches: lessons from the *Drosophila* germline

**Yukiko M. Yamashita, Margaret T. Fuller and D. Leanne Jones*,‡**

Department of Development Biology, Stanford University School of Medicine, Stanford, CA 94305, USA

*Present address: Salk Institute for Biological Studies, Laboratory of Genetics, 10010 N. Torrey Pines Rd, La Jolla, CA 92037, USA

‡Author for correspondence (e-mail: ljones@salk.edu)

*Journal of Cell Science 118, 665-672 Published by The Company of Biologists 2005*
*doi:10.1242/jcs.01680*

## Summary

**Stem cells are cells that, upon division, can produce new stem cells as well as daughter cells that initiate differentiation along a specific lineage. Studies using the *Drosophila* germline as a model system have demonstrated that signaling from the stem cell niche plays a crucial role in controlling stem cell behavior. Surrounding support cells secrete growth factors that activate signaling within adjacent stem cells to specify stem cell self-renewal and block differentiation. In addition, cell-cell adhesion between stem cells and surrounding support cells is important for holding stem cells close to self-renewal signals. Furthermore, a combination of localized signaling and autonomously acting proteins might polarize stem cells in such a way as to ensure asymmetric stem cell divisions. Recent results describing stem cell niches in other adult stem cells, including hematopoietic and neural stem cells, have demonstrated that the features characteristic of stem cell niches in *Drosophila* gonads might be conserved.**

Key words: Germline, Stem cells, *Drosophila*, JAK-STAT, TGF-β, Cadherin

## Introduction

Stem cells are the building blocks of development and allow the maintenance and regeneration of tissues throughout the lifetime of an individual. The ability of stem cells to contribute to these processes depends on their ability to divide and generate both new stem cells (self-renewal) and specialized cell types (differentiation). In this way, adult stem cells provide a continuous supply of new cells to replace short-lived but highly differentiated cell types, such as blood, skin and sperm. Thus, adult stem cell populations are essential for both normal tissue homeostasis and repair of tissues after wounding or environmental insult.

The crucial decision between stem cell self-renewal and differentiation must be tightly controlled. If too many daughter cells differentiate, the stem cell population could be depleted. Alternatively, unchecked stem cell self-renewal could expand the number of proliferative, partially differentiated cells in which secondary mutations could arise, contributing to tumorigenesis. Understanding how the choice between stem cell self-renewal and the onset of differentiation is made might facilitate the expansion of adult stem cells in culture while maintaining essential stem cell characteristics – a crucial first step in the use of adult stem cells for tissue replacement and gene therapy.

To maintain a sufficient number and the correct ratio of stem cells and differentiating progeny, stem cells in many tissues have the potential to divide asymmetrically, giving rise to one daughter cell that retains stem cell characteristics and one that differentiates. Asymmetric cell division to generate daughter cells that assume different fates is used throughout development to generate and maintain hundreds of specialized cell types. Two classic mechanisms can ensure an asymmetric outcome following cell division. First, the two daughter cells can be placed in different microenvironments, which might then specify different cell fate choices through intercellular signaling. Alternatively, asymmetric partitioning of cell fate determinants in the mother cell can give rise to daughter cells that adopt different cell fates (Watt and Hogan, 2000).

Ultimately, stem cell number, division, self-renewal and differentiation are likely to be regulated by the integration of intrinsic factors with extrinsic cues provided by the surrounding microenvironment. Indeed, many stem cells lose the ability to self-renew when removed from their normal environment, which suggests that the stem cell niche plays a major role in controlling stem cell behavior. Here, we review the cellular mechanisms that function within the stem cell niche to influence stem cell behavior in *Drosophila*. In particular, we focus on how orientation within a niche can promote an asymmetric germline stem cell (GSC) division in the testis and the ovary.

## Portrait of a stem cell niche

Studies of the *Drosophila* germline have revealed several features of stem cell niches that are important for controlling stem cell behavior: (1) signals emanating from the niche regulate stem cell self-renewal, survival and maintenance; (2) cell-cell adhesion between stem cells and niche cells anchors stem cells within the niche and close to self-renewal signals; and (3) the physical organization of stem cells and niche cells can polarize the stem cells, providing spatial cues towards which stem cells can orient their mitotic spindles, which ensuries an asymmetric division (Xie and Spradling, 2000; Song, X. et al., 2002; Kiger et al., 2001; Tulina and Matunis, 2001) (reviewed by Jones and Fuller, 2004).

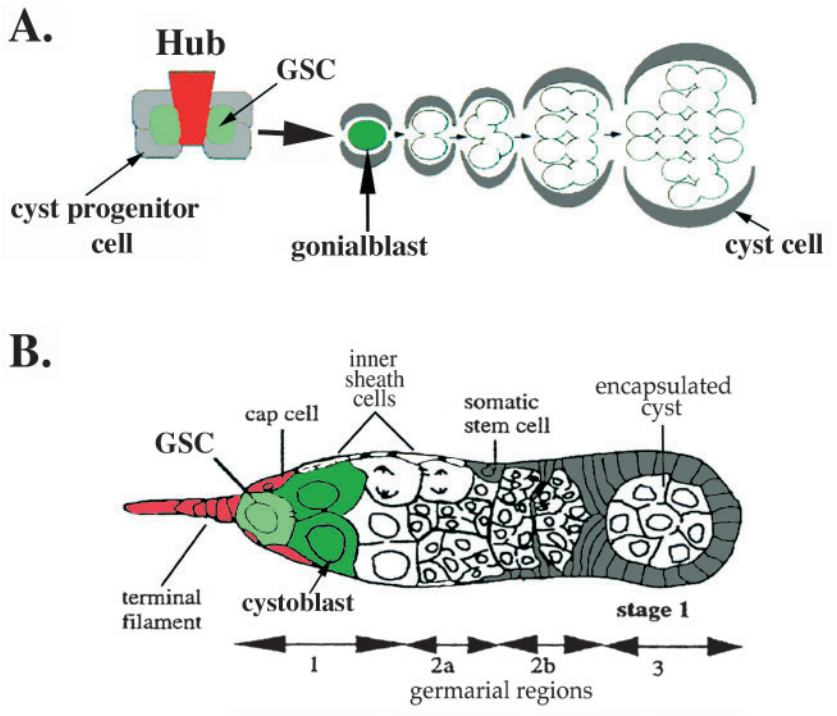


**Fig. 1.** GSC niches in the *Drosophila* ovary and testis. (A) Schematic of the early steps in *Drosophila* spermatogenesis. The GSCs (light green) surround and are in contact with a cluster of post-mitotic somatic cells known as the apical hub (red). The hub cells are a primary component of the male GSC niche. Each GSC is surrounded by two somatic stem cells known as the cyst progenitor cells (light gray). The GSC undergoes asymmetric cell division, giving rise to one daughter cell that will retain stem cell identity and one daughter cell, a gonialblast (dark green), which will undergo four rounds of cell division with incomplete cytokinesis to give rise to 16 spermatogonia. The gonialblast is surrounded by cyst cells (dark gray), which ensure spermatogonial differentiation. (B) Schematic of a *Drosophila* germarium, which houses the GSCs. Anterior is to the left and posterior is to the right. The terminal filament, cap and inner sheath cells (red) express molecules important for the maintenance and self-renewal of female GSCs and make up the stem cell niche. GSCs (light green) undergo asymmetric cell division, giving rise to one daughter cell that will retain stem cell identity and one daughter cell, a cystoblast, which will initiate differentiation (dark green). As these divisions are taking place, the more mature cysts are displaced towards the posterior of the germarium. Cyst encapsulation by the somatic stem cell derivatives (gray) occurs in region 2a/b. Mature encapsulated cysts budding off from the germarium make up region 3.

Stem cell niches are proposed to exist in several other adult tissues, including the hematopoietic system, neural tissues, skin and the gut epithelium. Recent results suggest that localized signaling between stem cells and niche cells, as well as cell adhesion between stem cells and niche cells, are conserved mechanisms that are important for maintaining stem cell identity and adequate stem cell numbers in animals (Calvi et al., 2003; Morris et al., 2004; Tumbar et al., 2004; Zhang et al., 2003) (reviewed by Fuchs et al., 2004). Likewise, the quiescent center in the root and the organizing center in the shoot apical meristem are considered to act as niches that maintain root and shoot stem cells in *Arabidopsis thaliana* (van den Berg et al., 1995; Sabatini et al., 2003; Aida et al., 2004) (reviewed by Baurle and Laux, 2003).

The *Drosophila* germline has emerged as a powerful model system in which to study how asymmetric stem cell division can be regulated by the surrounding microenvironment (reviewed by Jones and Fuller, 2004; Lin, 2002; Ohlstein et al., 2004). One advantage of *Drosophila* GSCs is that their asymmetric division normally always gives rise to one daughter cell that retains stem cell characteristics and one that commits to differentiation. In addition, the male and female *Drosophila* GSCs present an excellent opportunity to compare two different stem cell systems, particularly since they can be studied in their normal anatomical context.

## Extracellular signaling between stem cells and support cells

The *Drosophila* adult testis is a long, coiled tube filled with cells at all stages of spermatogenesis. In adult flies, approximately nine GSCs lie at the apical tip of the testis, forming a ring that closely surrounds a cluster of post-mitotic somatic cells called the hub (Fig. 1A). When a male GSC divides, it normally gives rise to one cell that will retain stem cell identity and one cell, called a gonialblast, that is displaced away from the hub and initiates differentiation (Fig. 1A). The gonialblast and its progeny undergo four rounds of transit-amplifying mitotic divisions, with incomplete cytokinesis, creating a cluster of 16 interconnected spermatogonia. In addition, each GSC is surrounded by a pair of somatic stem cells called the cyst progenitor cells (CPCs).

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is required for self-renewal of the male GSCs in *Drosophila* (Fig. 2A) (Kiger et al., 2001; Rawlings et al., 2004; Tulina and Matunis, 2001). Loss of function of either JAK, encoded by the *hopscotch* (*hop*) gene, or STAT, encoded by *Stat92E*, leads to rapid loss of stem cells and early germ cells (Kiger et al., 2001; Tulina and Matunis, 2001). The downstream targets of activated STAT that specify stem cell identity are currently unknown. The ligand that activates the *Drosophila* JAK/STAT pathway, Upd, is normally expressed in the 'apical hub cells'. Ectopic expression of Upd in early germ cells leads to a dramatic increase in the number of stem cells, as determined by markers available to distinguish stem cells from other early germ cells, with a concomitant decrease in the number of cells undergoing differentiation (Kiger et al., 2001; Tulina and Matunis, 2001).

Self-renewal signals from the hub appear to act over a short distance. The Upd ligand is glycosylated and is tightly associated with the extracellular matrix after secretion, which potentially limits its diffusion (Harrison et al., 1998). Furthermore, STAT activation (phosphorylation) is observed primarily in cells closest to the hub (E. Davis and M.T.F., unpublished observation). These observations suggest that only those cells that are adjacent to the hub receive the Upd signal and are capable of stem cell self-renewal (Fig. 2A). Consequently, hub cells have been proposed to contribute to the male GSC niche, instructing the adjacent germ cells to

maintain stem cell identity following division by providing a local source of the critical signal, Upd.

In the *Drosophila* ovary, the terminal filament, cap cells and inner germarial sheath cells constitute the GSC niche, expressing molecules that regulate critical aspects of GSC behavior (Fig. 1B) (reviewed by Jones, 2001; Lin, 2002). The vertebrate bone morphogenic protein (BMP) 2/4 homolog, *decapentaplegic* (*dpp*), plays a central role in the maintenance of GSCs in this tissue (Fig. 2B). Dpp binds and facilitates the association of type I and type II serine/threonine kinase receptors, allowing the type II receptor to phosphorylate and activate the type I receptor, which in turn phosphorylates the downstream activator Smad, Mothers against *dpp* (Mad) (reviewed by Moustakas, 2002). Mad facilitates nuclear translocation of *Medea* (*Med*), a transcriptional activator that stimulates Dpp target gene expression.

The cap cells and inner germarial sheath cells express Dpp, which activates Mad signaling in adjacent GSCs (Fig. 2B) (Kai and Spradling, 2003; Xie and Spradling, 1998; Xie and Spradling, 2000). Excessive Dpp signaling can block germ cell differentiation in the *Drosophila* ovary: ectopic expression of *dpp* produces enlarged germaria filled with cells resembling GSCs (Kai and Spradling, 2003; Xie and Spradling, 1998). By contrast, loss-of-function mutations in the type I receptor *saxophone* (*sax*) shorten the half-life of GSCs from one month to one week and slow the rate of GSC divisions. Clonal analysis revealed that the downstream signaling components *mad* and *Med* are required cell autonomously in the germline for maintaining the normal half-life of GSCs (Xie and Spradling, 1998).

Recently, elegant experiments have demonstrated that Dpp signaling supports stem cell maintenance by directly repressing transcription of the *bag of marbles* (*bam*) gene in female GSCs (Chen and McKearin, 2003a; Song et al., 2004). Bam is normally expressed in differentiating, early germ cells and is both necessary and sufficient for differentiation of female GSCs (Ohlstein and McKearin, 1997). Chen and McKearin mapped sequences upstream of *bam* that are important for regulating expression in differentiating germ cells (Chen and McKearin, 2003b). Subsequently, it was shown that a silencer element in the *bam* promoter is recognized and repressed directly by activated Mad and Medea complexes in GSCs (Chen and McKearin, 2003a; Song et al., 2004).

Casanueva and Ferguson extended these studies by demonstrating that redundant mechanisms might lead to the repression of Dpp signaling in differentiating germ cells (Casanueva and Ferguson, 2004). Overexpression of Bam suppresses the ‘tumorous ovary’ phenotype resulting from overexpression of an activated form of the Dpp receptor, Thick veins (Tkv), which suggests that Bam can repress Dpp signaling downstream of receptor activation. In addition, the authors suggest that the E3 ubiquitin ligase Smurf might target Mad for degradation, providing another mechanism for repression of Dpp signaling in cystoblasts and differentiating germ cells (Casanueva and Ferguson, 2004).

Transforming growth factor (TGF)-β signaling might also control Bam expression in the male germline, which indicates that some components of the differentiation program could be shared in male and female stem cell systems (Kawase et al., 2004; Schulz et al., 2004; Shivdasani and Ingham, 2003). However, it should be noted that, despite these similarities, the function of *bam* appears to be somewhat different in the male and female systems: in the female, *bam* expression is required for the initiation of differentiation of stem cell progeny, whereas in the male, *bam* expression is required to limit the number of transit-amplifying divisions that spermatogonia undergo before initiating terminal differentiation as a spermatocyte.

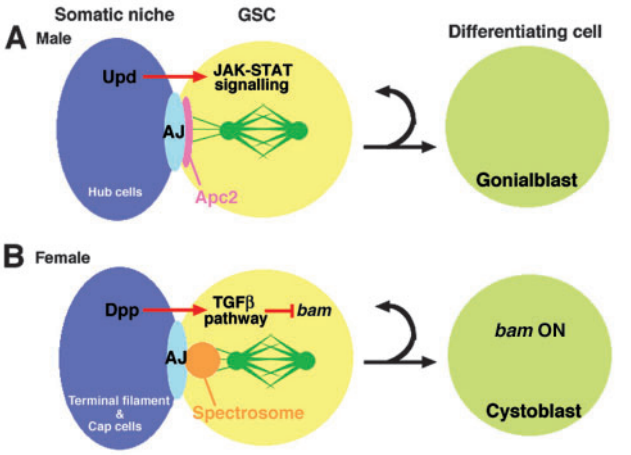


**Fig. 2.** Signaling from the stem cell niche regulates *Drosophila* GSC self-renewal and maintenance. GSCs (yellow) attach to niche cells (blue) through adherens junctions (AJ, grey). The mitotic spindle (green) is oriented orthogonally to the supporting niche cells, ensuring that one stem cell daughter remains within the niche, whereas the other daughter cell is displaced out of the niche and differentiates. (A) Secretion of the Upd ligand from hub cells (blue) activates JAK-STAT signaling in the male GSC. Mitotic spindles are anchored to the GSC cortex by Apc2 (pink), and GSC daughters initiate differentiation as gonialblasts (light green). (B) Secretion of Dpp from cap cells (blue) activates TGF-β signaling in female GSCs, which represses transcription of the differentiation factor *bag of marbles* (*bam*). Mitotic spindles are anchored to the GSC cortex by the spectrosome (orange), and GSC daughters initiate differentiation as cystoblasts (light green).

In addition to signals from the niche that specify stem cell identity, signals from surrounding cells can also direct differentiation of stem cell daughters. In the *Drosophila* male germline, interactions between germ cells and the somatic cyst cells appear to ensure that cells displaced away from the hub initiate differentiation. Activation of the epidermal growth factor receptor (EGFR) signaling pathway within the cyst cells is essential for preventing unlimited proliferation of GSCs and/or for promoting differentiation once germ cells leave the stem cell niche (Kiger et al., 2000; Matunis et al., 1997; Schulz et al., 2002; Tran et al., 2000). Loss of function of the EGFR or the downstream mediator Raf leads to the uncontrolled proliferation of early germ cells during the transit amplification stages (Kiger et al., 2000; Tran et al., 2000). Recent results suggest that a ligand secreted by early male germ cells might activate the EGFR signaling pathway in the somatic cyst cells (Schulz et al., 2002). The *Drosophila stet* gene encodes a homolog of Rhomboid, a transmembrane protease (Urban et al., 2001) that plays an essential role in EGFR signaling. Rhomboid proteolytically cleaves and activates the major EGFR ligand *Spitz* (*Spi*) in the signaling cell (reviewed by Klambt, 2002). In *stet*-mutant males, somatic cyst cells fail to

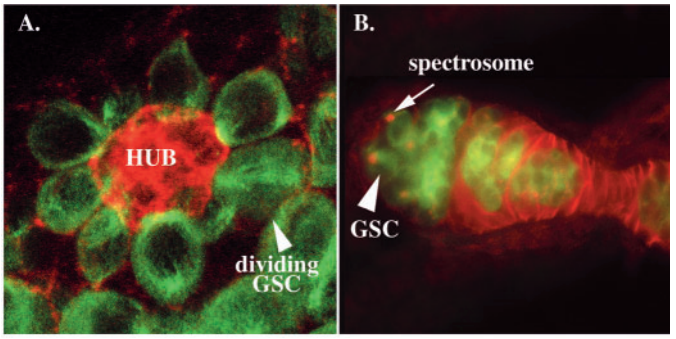


**Fig. 3.** Mitotic spindle orientation is regulated in *Drosophila* GSCs. (A) Male GSCs expressing a GFP-tagged α-tubulin were visualized using scanning laser confocal microscopy. *Drosophila* E-cadherin (red) localizes to the surfaces of hub cells and to the interface between hub cells and GSCs. The mitotic spindle visible in one GSC (arrow) is oriented orthogonally to the apical hub. (B) Immunofluorescence image of a *Drosophila* germarium. Germ cells are labeled with an antibody to the germ-cell-specific protein Vasa (green). Antibodies to the membrane protein α-spectrin (red) label the somatic cells within the germarium, as well as the vesiculated, cytoplasmic, ball-shaped structure known as the spectrosome in GSCs (arrow) and cystoblasts.

encapsulate germ cells properly, and early germ cells at mixed stages of differentiation accumulate, much as in EGFR-mutant males.

## The role of cell-cell and/or cell-extracellular matrix (ECM) adhesion

Cell adhesion between niche cells and stem cells has also been shown to be required for stem cell maintenance. Clusters of adherens junctions are present between female GSCs and cap cells (Song, X. et al., 2002) and between male GSCs and the adjacent hub cells (T. Mahowald and M.T.F., unpublished). Immunofluorescence analysis revealed that the *Drosophila* E-cadherin homolog Shotgun (Shg) and β-catenin homolog Armadillo (Arm) are highly concentrated at the interface between GSCs and cap cells in females (Song, X. et al., 2002) and between GSCs and hub cells in males (Yamashita et al., 2003). Presumably, cell adhesion mediated by adherens junctions is required for holding stem cells within the niche, close to maintenance signals and away from differentiation cues (Fig. 2) (Gonzalez-Reyes, 2003; Song, X. et al., 2002).

In addition to adherens junctions, intercellular communication through gap junctions appears to play an important role in the survival of early germ cells in *Drosophila.* Animals that have a null mutation in the *zero population growth* (*zpg*) gene, which encodes the germline-specific gap junction protein Innexin 4, are viable but sterile. Gonads from these animals are populated with a few early germ cells, but lack later stages (Gilboa et al., 2003; Tazuke et al., 2002). Zpg localizes to the surface of spermatogonia in males and probably mediates interactions between germ cells and surrounding somatic cyst cells, which are required for differentiation. In females, Zpg localizes to the interface between niche cells and GSCs, as well as to the surface of more-mature germ cells (Gilboa et al., 2003; Tazuke et al., 2002).

## Stem cell polarity within the niche

Theoretically, the limited availability of essential signals that specify stem cell identity and the physical space within the niche could be sufficient for regulating stem cell number. However, some stem cell systems employ elaborate mechanisms to ensure that one daughter cell resulting from a stem cell division remains within the niche, whereas the other is placed outside the niche. This means that the two daughter cells of the stem cell division do not compete for limited space and maintenance signals.

In *Drosophila*, mitotic spindles in both female and male GSCs are oriented orthogonally to the support cells that secrete signals promoting stem cell identity (Fig. 2, Fig. 3A) (Deng and Lin, 1997; Yamashita et al., 2003). This precise orientation of the mitotic spindle within the stem cell might force the displacement of one of the daughter cells outside the stem cell niche. Even though the spindle is oriented to ensure asymmetric GSC divisions in both the female and male gonad, the mechanisms that ensure this might be different in the two systems. In female GSCs, one of the spindle poles is anchored to the apical side of the GSC by a subcellular organelle called the spectrosome (Fig. 2B, Fig. 3B) (Deng and Lin, 1997). By contrast, in male GSCs, the spectrosome is not consistently oriented towards the hub. Instead, stereotyped movement of the centrosome during interphase correlates with, and appears to establish, orientation of the mitotic spindle (Yamashita et al., 2003).

## Male GSC polarity

Polarization of male GSCs with respect to the hub is maintained throughout the cell cycle. In early interphase, the single centrosome is located between the nucleus and the region of cortex where the GSC touches the hub. When the centrosome duplicates, one daughter centrosome remains adjacent to the hub whereas the other migrates to the opposite side of the GSC nucleus to set up an oriented spindle.

The integral centrosomal protein Centrosomin (Cnn) is required for normal centrosome positioning and spindle orientation in GSCs, which indicates that centrosomes and/or astral microtubules have roles in maintaining GSC polarity (Yamashita et al., 2003). The average number of stem cells around the hub is larger in *cnn* mutant males, although the hub diameter remains the same. This suggests that misoriented GSC spindles can lead to symmetric divisions that produce two daughter GSCs (Yamashita et al., 2003). Thus, spindle orientation plays a crucial role in regulating stem cell behavior.

Since Cnn is required for anchoring astral microtubules to the centrosome, astral microtubules might link the centrosome and cell cortex to orient the spindle. A specialized region of the GSC cortex that contains adherens junctions might provide a polarity cue or anchor for astral microtubules emanating from the centrosome. Interestingly, a homolog of the adenomatous polyposis coli (APC) tumor suppressor protein, *Drosophila* Apc2, localizes to the interface between the hub and GSC, where it colocalizes with the E-cadherin Shg and Armadillo (β-catenin) (Yamashita et al., 2003). APC proteins interact both with β-catenin and microtubules. Therefore, Apc2 is a good candidate for a protein that links the centrosome and cell cortex by anchoring astral microtubules. Analysis of loss-of-function mutants suggested that Apc2 does indeed play a role in

centrosome and spindle orientation in the male GSC. GSCs from *apc2* mutant males have mis-oriented centrosomes and spindles similar to those observed in *cnn* mutants. Another APC homolog, *Drosophila* Apc1, localizes to the spindle pole during late G2-phase prophase and is also required for proper centrosome and spindle orientation.

The observations discussed above support a model in which astral microtubules emanating from the centrosome are captured by a protein complex containing Apc2, which is localized to the interface between the GSC cortex and the hub (Yamashita et al., 2003). Thus, asymmetric male GSC divisions appear to be controlled by a combination of extrinsic factor(s) that specify stem cell identity and an intrinsic cellular machinery that acts at the centrosome and cell cortex to orient the cell division plane with respect to the signaling microenvironment.

## Female GSC polarity

The spectrosome is a cytoplasmic structure in GSCs that is composed largely of membrane skeletal proteins such as α- and β-spectrin, the adducin-like protein Hu-li tai shao (Hts), and ankyrin (de Cuevas et al., 1996; Lin et al., 1994). As germ cells initiate mitotic amplification divisions, this structure becomes highly branched and is called the fusome. In female GSCs, the spectrosome is consistently located at the apical GSC cortex adjacent to cap cells during interphase and most stages throughout mitosis (Fig. 2B, Fig. 3B) (Deng and Lin, 1997). In addition, it is associated with the mitotic spindle pole located adjacent to cap cells from interphase through late anaphase (Deng and Lin, 1997). Loss of the spectrosome, owing to mutations in *hts*, results in randomization of mitotic spindle orientation, suggesting that the spectrosome in female GSCs plays a role in anchoring and/or orienting the mitotic spindle perpendicular to the cap cells (Deng and Lin, 1997). Although we do not known for sure whether female GSCs are oriented with respect to the cap cells as early as interphase, this is likely to be the case if orientation is dependent on spectrosome positioning.

It is possible that the intrinsic cellular machinery responsible for establishing polarity and orienting the division plane is conserved in *Drosophila* male and female GSCs. Although the available data suggest that female GSCs rely on spectrosome position for appropriate orientation of the mitotic spindle, there is evidence that centrosomes are closely associated with the fusome in female early germ cells, and these may be associated with the spectrosome in GSCs (Bolivar et al., 2001; Lin et al., 1994). Therefore, centrosome positioning might also be involved in orienting the mitotic spindle in female GSCs; analysis of mitotic spindle orientation in *cnn* mutant female GSCs would address this question.

Adherens junctions between cap cells and female GSCs (Song, X. et al., 2002) might provide a polarity cue or anchor for spectrosomes within GSCs. It would be interesting to examine whether adherens junctions are required to position the spectrosome and thereby orient the mitotic spindle and, if so, what the mechanical link is between adherens junctions and the spectrosome.

## Regulation of differentiation within the niche

Recent studies have demonstrated that *Drosophila* germ cells that have initiated differentiation are capable of reassuming stem cell properties. Ectopic expression of the differentiation factor *bam* leads to premature differentiation of female GSCs (Ohlstein and McKearin, 1997). Therefore, by overexpressing *bam* in the larval ovary using a heat shock regime, Kai and Spradling were able to force the differentiation of GSCs, causing depletion of the stem cell pool and accumulation of differentiating cysts of germ cells (Kai and Spradling, 2004). Once *bam* expression returned to wild-type levels, germ cell cysts composed of 4-8 cystocytes broke down, giving rise to female GSCs that repopulated the otherwise empty stem cell niches in developing larval ovaries (Kai and Spradling, 2004).

Brawley and Matunis reported that a similar phenomenon occurs in the male germline, where 4-cell and 8-cell spermatogonial cysts break down to form individual cells that re-initiate self-renewing divisions (Brawley and Matunis, 2004). Using a temperature-sensitive allele of *Stat92E*, the authors demonstrated significant loss of GSCs at the restrictive temperature and an accumulation of differentiating germ cells adjacent to the hub. Upon a shift back to the permissive temperature, ring canals adjoining interconnected spermatogonia disintegrated, giving rise to single cells that began to divide individually as GSCs.

These results suggest that partially differentiated stem cell progeny undergoing transit-amplifying divisions might be able to reassume stem cell identity given the appropriate environment. Such de-differentiation probably requires an empty stem cell niche as well as re-initiation of a stem cell gene expression program and rearrangement of subcellular structures characteristic of differentiating cells.

## Asymmetry regulated by intrinsic cell fate determinants

Cells can divide asymmetrically by unequal segregation of cell fate determinants upon cell division. The intrinsic mechanisms that control asymmetric division may first require that cells polarize by segregating fate determinants unequally within the cell. Subsequently, mitotic spindles become oriented such that the two daughter cells inherit different contents. The *Drosophila* neuroblast provides one of the most well characterized examples of this phenomenon. Neuroblasts, which serve as neuronal stem cells in *Drosophila*, divide asymmetrically to generate one neuroblast and a ganglion mother cell (GMC) that divides once more to generate fully differentiated neurons. In the dividing neuroblast, determinants such as Numb and Prospero (Pros) are localized to one side of the cell, the basal side, and reorientation of the mitotic spindle ensures that when the cell divides, only the GMC inherits these two proteins (Fig. 4). Although no intrinsic determinants have been identified in *Drosophila* female and male GSCs, it is reasonable to postulate that intrinsic cell fate determinants may act to direct asymmetric cell divisions in concert with extrinsic signals from the niche. Asymmetrically localized cell adhesion molecules, such as Shg, Arm, and APC2 could be examples (Song, X. et al., 2002; Yamashita et al., 2003).

## Mammalian stem cell niches

*Drosophila* male and female germline systems are incredibly

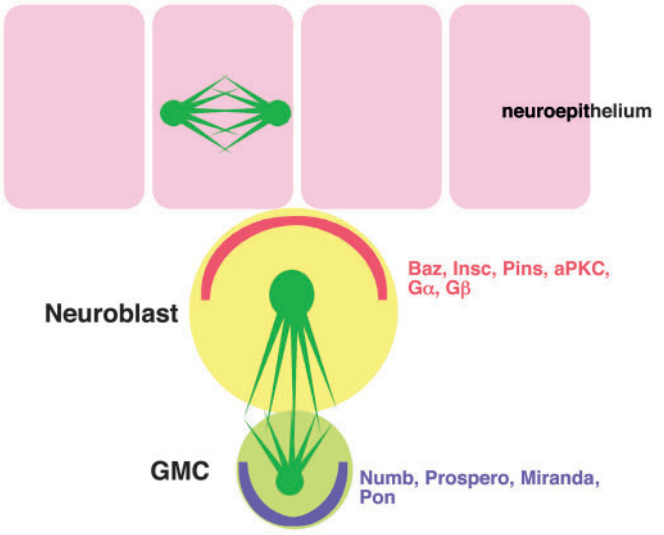


**Fig. 4.** Asymmetric division of *Drosophila* neuroblasts is regulated by intrinsic cell fate determinants. A *Drosophila* neuroblast divides asymmetrically, generating another neuroblast and a smaller ganglion mother cell (GMC) that will produce mature neurons. Cell fate determinants such as Numb and Prospero (blue crescent) are segregated only into the GMC. Spindle orientation is determined by the apical complex (red crescent), containing proteins such as Bazooka (Baz)/Par-3 and Inscuteable (Insc). Spindle orientation in the neuroblast is established by the programmed rotation of the spindle during mitosis so that it becomes perpendicular to the neuroepithelium, from which the neuroblast is derived. Pins, partner of Inscuteable; aPKC, a typical protein kinase C; Pon, partner of Numb.

powerful model systems for studies of how stem cell behavior is controlled by the surrounding microenvironment. Such studies have provided a paradigm for the characterization and analysis of stem cell niches in other systems, defining support cells that constitute the niche, signals that control stem cell self-renewal and proliferation, and cell adhesion molecules that might be important for holding stem cells within the niche. Two examples are discussed below.

## The hematopoietic stem cell niche

Characterization of the hematopoietic stem cell (HSC) niche and the signaling molecules that influence HSC maintenance and self-renewal is in its initial stages. HSCs reside along the endosteal surface of the bone, whereas differentiating hematopoietic cells migrate towards the center of the bone marrow cavity. Recently, the bone-forming osteoblasts have been proposed to be a major cellular component of the HSC niche because increases in the number of osteoblasts lead to a concomitant increase in the number of long-term HSCs (LT-HSCs) (Calvi et al., 2003; Zhang et al., 2003). Calvi et al. demonstrated that these osteoblasts secrete an elevated level of the Notch ligand Jagged 1, raising the possibility that activation of the Notch signal transduction pathway in HSCs supports HSC proliferation (Calvi et al., 2003). In addition, studies have shown that signaling through the canonical Wnt pathway can direct HSC self-renewal in vitro and in vivo (Reya et al., 2003; Willert et al., 2003). The Wnt family of proteins are secreted growth factors that bind to members of the Frizzled (Fz) family of cell-surface receptors. The β-catenin molecule serves as a positive regulator of the pathway by mediating transcription in cooperation with members of the Lef/TCF transcription factor family (reviewed by Nelson and Nusse, 2004).

Currently, there are no data to suggest that osteoblasts secrete a potential Wnt ligand that could provide self-renewal signals to dividing HSCs; however, this is clearly a possibility and should be investigated further. Interestingly, Reya et al. demonstrated that the level of *Notch 1* transcription increases 2.5-fold upon stimulation of HSCs by activated/stabilized β-catenin (Reya et al., 2003). Therefore, crosstalk between Notch and Wnt signaling pathways could be used to sustain long-term and short-term HSC proliferation within the niche.

The cell-cell and cell-ECM adhesion molecules that are involved in anchoring HSCs within the bone marrow have not yet been identified. Interestingly, Zhang et al. suggested that the cell adhesion molecule N-cadherin, which is expressed by the spindle-shaped N-cadherin$^{+}$ CD45$^{-}$ osteoblasts, might be responsible for holding HSCs within the niche and close to self-renewal and survival signals (Zhang et al., 2003). Expression of N-cadherin within HSCs has not been examined, however. The mobility of HSCs indicates that adhesion between HSCs and niche cells could be highly regulated. HSCs are detectable in the peripheral blood, spleen and liver, which suggests that they can migrate out of the niche (Wright et al., 2001). The mechanisms regulating homing and recruitment of HSCs to the niche after transplantation have not been clearly elucidated, although cellular adhesion molecules and chemokine receptors are probably involved (Wagers et al., 2002).

To date, there is no information regarding whether HSCs have orientated divisions with respect to the surrounding osteoblasts. Nevertheless, it will be interesting to examine whether the mitotic spindle is oriented within the HSCs towards the N-cadherin patch between HSCs and osteoblasts.

## The neural stem cell niche

Neural stem cells (NSCs) that have the capacity to self-renew and give rise to precursors that differentiate both into neurons and glia can be cultured from the subventricular zone (SVZ) of the lateral ventricle and the hippocampus (Gage, 2000; Pevny and Rao, 2003). When cultured in vitro in the presence of fibroblast growth factor (FGF)-2 and EGF, cells from these tissues can give rise to free-floating, spherical clusters called neurospheres that contain mixed populations of stem cells and precursor cells (Gage, 2000; Reynolds and Weiss, 1992; Uchida et al., 2000).

Astrocytes from both the SVZ and hippocampus provide neurogenic signals to progenitor cells, which suggests that astrocytes might serve a crucial role in regulating NSC fate within the niche (Lim and Alvarez-Buylla, 1999; Song, H. et al., 2002a; Song, H. J. et al., 2002b). In addition, astrocytes might possess progenitor cell activity: SVZ astrocytes from adult rodent and human brains have been reported to proliferate in vivo and give rise to multipotent, self-renewing neurospheres in culture (Doetsch et al., 1999; Sanai et al., 2004).

Neurogenesis occurs close to blood vessels, and Shen et al. recently demonstrated that endothelial cells secrete soluble factors capable of promoting the proliferation of NSCs and the

maintenance of neurogenic potential (Shen et al., 2004). Therefore, endothelial cells could function in vivo as niche cells that maintain NSC populations; however, the growth factors provided by endothelial cells that promote NSC self-renewal have not been identified. Wurmser et al. showed that NSCs can differentiate into endothelial cells (Wurmser et al., 2004), raising the intriguing possibility that NSCs have the ability to create their own stem cell niche in vivo.

Although candidates for growth factors that are secreted from niche cells and support the growth of NSCs in vivo have not yet been identified, there is evidence that FGF-2 and EGF can promote NSC and progenitor cell proliferation in vivo, as well as in vitro. These data suggest that FGF-2 and/or EGF could act within the niche to control stem cell self-renewal and the initiation of differentiation (Kuhn et al., 1997; Taupin et al., 2000; Wagner et al., 1999).

## Concluding remarks

*Drosophila* stem cell systems have proven to be incredibly powerful models for studying the mechanisms by which stem cell behavior is controlled by the surrounding microenvironment and intrinsic cell fate determinants. These studies have provided a paradigm for the characterization and analysis of stem cells in other systems, including the identification of support cells that constitute the niche, signals that control stem cell self-renewal and proliferation, and cell adhesion molecules that are important for holding stem cells within the niche (reviewed by Fuchs et al., 2004; Jones and Fuller, 2004).

A general picture of how the stem cell niche controls stem cell number and maintains the correct balance between self-renewal and differentiation is beginning to emerge from work on *Drosophila* male and female GSCs. This process involves complex crosstalk between intercellular and intracellular mechanisms. First, the size or number of stem cell niches defines the correct number of stem cells by sending short-range signal(s) for self-renewal or maintenance to the neighboring stem cells. The signal transduction pathways that are involved in stem cell maintenance do not appear to be conserved between the male and female GSC systems. Consequently, it is likely that the same signaling pathway will not prove to be sufficient to maintain stem cell identity in all systems. Second, cell-cell adhesion between supporting niche cells and stem cells enables stem cells to remain tightly associated with the niche. Cell-cell adhesion between GSCs and niche cells is required for stem cell maintenance, both physically maintaining stem cells within the niche and ensuring that GSCs are held close to self-renewal signals emanating from the microenvironment. Third, stem cells are polarized with respect to the niche. Either localized signals from the niche or the geometry of the cell adhesion junctions between stem cells and the niche could provide polarity cues to orient stem cells towards the niche. Stem cells polarized through contact with the niche can orient their mitotic spindles to ensure an asymmetric outcome of stem cell divisions by reliably placing one daughter cell firmly within the niche, where it can both maintain its attachment and come under the influence of the self-renewal signal(s), while placing the other daughter cell outside the niche. These three mechanisms – attachment to, orientation towards, and signaling from a supporting niche – might also play important roles in regulating stem cell number, self-renewal and differentiation in tissues maintained by stem cell populations in other organisms.

We thank E. Davies, A. Wurmser and two anonymous reviewers for suggestions and a critical reading of the manuscript. Y.M.Y. was supported by JSPS and is currently supported by the Jose Carreras International Leukemia Foundation; D.L.J. was a Lilly Fellow of the Life Sciences Research Foundation. This was supported in part by a grant to M.T.F. (NIH grant#P01 DK53074, subproject #4).

## References

**Aida, M., Beis, D., Heidstra, R., Willemsen, V., Blilou, I., Galinha, C., Nussaume, L., Noh, Y. S., Amasino, R. and Scheres, B.** (2004). The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. *Cell* **119**, 109-120.

**Baurle, I. and Laux, T.** (2003). Apical meristems: the plant's fountain of youth. *BioEssays* **25**, 961-970.

**Bolivar, J., Huynh, J. R., Lopez-Schier, H., Gonzalez, C., St Johnston, D. and Gonzalez-Reyes, A.** (2001). Centrosome migration into the *Drosophila* oocyte is independent of BicD and egl, and of the organisation of the microtubule cytoskeleton. *Development* **128**, 1889-1897.

**Brawley, C. and Matunis, E.** (2004). Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. *Science* **304**, 1331-1334.

**Calvi, L. M., Adams, G. B., Weibrecht, K. W., Weber, J. M., Olson, D. P., Knight, M. C., Martin, R. P., Schipani, E., Divieti, P., Bringhurst, F. R. et al.** (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841-846.

**Casanueva, M. O. and Ferguson, E. L.** (2004). Germline stem cell number in the *Drosophila* ovary is regulated by redundant mechanisms that control Dpp signaling. *Development* **131**, 1881-1890.

**Chen, D. and McKearin, D.** (2003a). Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. *Curr. Biol.* **13**, 1786-1791.

**Chen, D. and McKearin, D. M.** (2003b). A discrete transcriptional silencer in the bam gene determines asymmetric division of the *Drosophila* germline stem cell. *Development* **130**, 1159-1170.

**de Cuevas, M., Lee, J. K. and Spradling, A. C.** (1996). alpha-spectrin is required for germline cell division and differentiation in the *Drosophila* ovary. *Development* **122**, 3959-3968.

**Deng, W. and Lin, H.** (1997). Spectrosomes and fusomes anchor mitotic spindles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in *Drosophila*. *Dev. Biol.* **189**, 79-94.

**Doetsch, F., Caille, I., Lim, D. A., Garcia-Verdugo, J. M. and Alvarez-Buylla, A.** (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703-716.

**Fuchs, E., Tumbar, T. and Guasch, G.** (2004). Socializing with the neighbors: stem cells and their niche. *Cell* **116**, 769-778.

**Gage, F. H.** (2000). Mammalian neural stem cells. *Science* **287**, 1433-1438.

**Gilboa, L., Forbes, A., Tazuke, S. I., Fuller, M. T. and Lehmann, R.** (2003). Germ line stem cell differentiation in *Drosophila* requires gap junctions and proceeds via an intermediate state. *Development* **130**, 6625-6634.

**Gonzalez-Reyes, A.** (2003). Stem cells, niches and cadherins: a view from *Drosophila*. *J. Cell Sci.* **116**, 949-954.

**Harrison, D. A., McCoon, P. E., Binari, R., Gilman, M. and Perrimon, N.** (1998). *Drosophila* unpaired encodes a secreted protein that activates the JAK signaling pathway. *Genes Dev.* **12**, 3252-3263.

**Jones, D. L. and Fuller, M. T.** (2004). Stem cell niches. In *Handbook of Stem Cells*, Vol. 1: *Adult and Fetal Stem Cells* (ed. R. Lanza), pp. 59-72. San Diego, CA: Academic Press.

**Jones, L.** (2001). Stem cells: so what's in a niche? *Curr. Biol.* **11**, R484-486.

**Kai, T. and Spradling, A.** (2003). An empty *Drosophila* stem cell niche reactivates the proliferation of ectopic cells. *Proc. Natl. Acad. Sci. USA* **100**, 4633-4638.

**Kai, T. and Spradling, A.** (2004). Differentiating germ cells can revert into functional stem cells in *Drosophila melanogaster* ovaries. *Nature* **428**, 564-569.

**Kawase, E., Wong, M. D., Ding, B. C. and Xie, T.** (2004). Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the *Drosophila* testis. *Development* **131**, 1365-1375.

**Kiger, A. A., White-Cooper, H. and Fuller, M. T.** (2000). Somatic support

cells restrict germline stem cell self-renewal and promote differentiation. *Nature* **407**, 750-754.

**Kiger, A. A., Jones, D. L., Schulz, C., Rogers, M. B. and Fuller, M. T.** (2001). Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. *Science* **294**, 2542-2545.

**Klambt, C.** (2002). EGF receptor signalling: roles of star and rhomboid revealed. *Curr. Biol.* **12**, R21-23.

**Kuhn, H. G., Winkler, J., Kempermann, G., Thal, L. J. and Gage, F. H.** (1997). Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J. Neurosci.* **17**, 5820-5829.

**Lim, D. A. and Alvarez-Buylla, A.** (1999). Interaction between astrocytes and adult subventricular zone precursors stimulates neurogenesis. *Proc. Natl. Acad. Sci. USA* **96**, 7526-7531.

**Lin, H.** (2002). The stem-cell niche theory: lessons from flies. *Nat. Rev. Genet.* **3**, 931-940.

**Lin, H., Yue, L. and Spradling, A. C.** (1994). The *Drosophila* fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. *Development* **120**, 947-956.

**Matunis, E., Tran, J., Gonczy, P., Caldwell, K. and DiNardo, S.** (1997). punt and schnurri regulate a somatically derived signal that restricts proliferation of committed progenitors in the germline. *Development* **124**, 4383-4391.

**Morris, R. J., Liu, Y., Marles, L., Yang, Z., Trempus, C., Li, S., Lin, J. S., Sawicki, J. A. and Cotsarelis, G.** (2004). Capturing and profiling adult hair follicle stem cells. *Nat. Biotechnol.* **22**, 411-417.

**Moustakas, A.** (2002). Smad signalling network. *J. Cell Sci.* **115**, 3355-3356.

**Nelson, W. J. and Nusse, R.** (2004). Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* **303**, 1483-1487.

**Ohlstein, B. and McKearin, D.** (1997). Ectopic expression of the *Drosophila* Bam protein eliminates oogenic germline stem cells. *Development* **124**, 3651-3662.

**Ohlstein, B., Kai, T., Decotto, E. and Spradling, A.** (2004). The stem cell niche: theme and variations. *Curr. Opin. Cell Biol.* **16**, 693-699.

**Pevny, L. and Rao, M. S.** (2003). The stem-cell menagerie. *Trends Neurosci.* **26**, 351-359.

**Rawlings, J. S., Rosler, K. M. and Harrison, D. A.** (2004). The JAK/STAT signaling pathway. *J. Cell Sci.* **117**, 1281-1283.

**Reya, T., Duncan, A. W., Ailles, L., Domen, J., Scherer, D. C., Willert, K., Hintz, L., Nusse, R. and Weissman, I. L.** (2003). A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* **423**, 409-414.

**Reynolds, B. A. and Weiss, S.** (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**, 1707-1710.

**Sabatini, S., Heidstra, R., Wildwater, M. and Scheres, B.** (2003). SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. *Genes Dev.* **17**, 354-358.

**Sanai, N., Tramontin, A. D., Quinones-Hinojosa, A., Barbaro, N. M., Gupta, N., Kunwar, S., Lawton, M. T., McDermott, M. W., Parsa, A. T., Manuel-Garcia Verdugo, J. et al.** (2004). Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* **427**, 740-744.

**Schulz, C., Wood, C. G., Jones, D. L., Tazuke, S. I. and Fuller, M. T.** (2002). Signaling from germ cells mediated by the rhomboid homolog stet organizes encapsulation by somatic support cells. *Development* **129**, 4523-4534.

**Schulz, C., Kiger, A. A., Tazuke, S. I., Yamashita, Y. M., Pantalena-Filho, L. C., Jones, D. L., Wood, C. G. and Fuller, M. T.** (2004). A misexpression screen reveals effects of bag-of-marbles and TGFbeta class signaling on the *Drosophila* male germ-line stem cell lineage. *Genetics* **167**, 707-723.

**Shen, Q., Goderie, S. K., Jin, L., Karanth, N., Sun, Y., Abramova, N., Vincent, P., Pumiglia, K. and Temple, S.** (2004). Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338-1340.

**Shivdasani, A. A. and Ingham, P. W.** (2003). Regulation of stem cell maintenance and transit amplifying cell proliferation by TGF-beta signaling in *Drosophila* spermatogenesis. *Curr. Biol.* **13**, 2065-2072.

**Song, H., Stevens, C. F. and Gage, F. H.** (2002a). Astroglia induce neurogenesis from adult neural stem cells. *Nature* **417**, 39-44.

**Song, H. J., Stevens, C. F. and Gage, F. H.** (2002b). Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat. Neurosci.* **5**, 438-445.

**Song, X., Zhu, C. H., Doan, C. and Xie, T.** (2002). Germline stem cells anchored by adherens junctions in the *Drosophila* ovary niches. *Science* **296**, 1855-1857.

**Song, X., Wong, M. D., Kawase, E., Xi, R., Ding, B. C., McCarthy, J. J. and Xie, T.** (2004). Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the *Drosophila* ovary. *Development* **131**, 1353-1364.

**Taupin, P., Ray, J., Fischer, W. H., Suhr, S. T., Hakansson, K., Grubb, A. and Gage, F. H.** (2000). FGF-2-responsive neural stem cell proliferation requires CCg, a novel autocrine/paracrine cofactor. *Neuron* **28**, 385-397.

**Tazuke, S. I., Schulz, C., Gilboa, L., Fogarty, M., Mahowald, A. P., Guichet, A., Ephrussi, A., Wood, C. G., Lehmann, R. and Fuller, M. T.** (2002). A germline-specific gap junction protein required for survival of differentiating early germ cells. *Development* **129**, 2529-2539.

**Tran, J., Brenner, T. J. and DiNardo, S.** (2000). Somatic control over the germline stem cell lineage during *Drosophila* spermatogenesis. *Nature* **407**, 754-757.

**Tulina, N. and Matunis, E.** (2001). Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signaling. *Science* **294**, 2546-2549.

**Tumbar, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W. E., Rendl, M. and Fuchs, E.** (2004). Defining the epithelial stem cell niche in skin. *Science* **303**, 359-363.

**Uchida, N., Buck, D. W., He, D., Reitsma, M. J., Masek, M., Phan, T. V., Tsukamoto, A. S., Gage, F. H. and Weissman, I. L.** (2000). Direct isolation of human central nervous system stem cells. *Proc. Natl. Acad. Sci. USA* **97**, 14720-14725.

**Urban, S., Lee, J. R. and Freeman, M.** (2001). *Drosophila* rhomboid-1 defines a family of putative intramembrane serine proteases. *Cell* **107**, 173-182.

**van den Berg, C., Willemsen, V., Hage, W., Weisbeek, P. and Scheres, B.** (1995). Cell fate in the *Arabidopsis* root meristem determined by directional signalling. *Nature* **378**, 62-65.

**Wagers, A. J., Allsopp, R. C. and Weissman, I. L.** (2002). Changes in integrin expression are associated with altered homing properties of Lin(–/lo)Thy1.1(lo)Sca-1(+)c-kit(+) hematopoietic stem cells following mobilization by cyclophosphamide/granulocyte colony- stimulating factor. *Exp. Hematol.* **30**, 176-185.

**Wagner, J. P., Black, I. B. and DiCicco-Bloom, E.** (1999). Stimulation of neonatal and adult brain neurogenesis by subcutaneous injection of basic fibroblast growth factor. *J. Neurosci.* **19**, 6006-6016.

**Watt, F. M. and Hogan, B. L.** (2000). Out of Eden: stem cells and their niches. *Science* **287**, 1427-1430.

**Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T., Yates, J. R., III and Nusse, R.** (2003). Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* **423**, 448-452.

**Wright, D. E., Wagers, A. J., Gulati, A. P., Johnson, F. L. and Weissman, I. L.** (2001). Physiological migration of hematopoietic stem and progenitor cells. *Science* **294**, 1933-1936.

**Wurmser, A. E., Nakashima, K., Summers, R. G., Toni, N., D'Amour, K. A., Lie, D. C. and Gage, F. H.** (2004). Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. *Nature* **430**, 350-356.

**Xie, T. and Spradling, A. C.** (1998). decapentaplegic is essential for the maintenance and division of germline stem cells in the *Drosophila* ovary. *Cell* **94**, 251-260.

**Xie, T. and Spradling, A. C.** (2000). A niche maintaining germ line stem cells in the *Drosophila* ovary. *Science* **290**, 328-330.

**Yamashita, Y., Jones, D. L. and Fuller, M. T.** (2003). Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. *Science* **301**, 1547-1550.

**Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W. G., Ross, J., Haug, J., Johnson, T., Feng, J. Q. et al.** (2003). Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* **425**, 836-841.