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The stem cell niche: lessons from the *Drosophila* testis

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Summary

In metazoans, tissue maintenance and regeneration depend on adult stem cells, which are characterized by their ability to self-renew and generate differentiating progeny in response to the needs of the tissues in which they reside. In the *Drosophila* testis, germline and somatic stem cells are housed together in a common niche, where they are regulated by local signals, epigenetic mechanisms and systemic factors. These stem cell populations in the *Drosophila* testis have the unique advantage of being easy to identify and manipulate, and hence much progress has been made in understanding how this niche operates. Here, we summarize recent work on stem cells in the adult *Drosophila* testis and discuss the remarkable ability of these stem cells to respond to change within the niche.

Key words: *Drosophila*, JAK-STAT, Niche, Stem cell, Testis

Introduction

Stem cells are undifferentiated cells with remarkable potential. When a stem cell divides, each daughter cell can either remain a stem cell (a process called self-renewal) or differentiate into a more specialized type of cell. Stem cells reside in specific microenvironments, called niches, which provide the molecular signals that maintain stem cells and regulate their division. In adult metazoans, the precise regulation of stem cells and their daughters is crucial for tissue maintenance and repair. Among the best-understood adult stem cell niches are those in the *Drosophila* testis and ovary, which house the germline stem cells (GSCs) that give rise to sperm or eggs. These anatomically simple niches contain stem cells that are easier to identify, image and manipulate than those in complex mammalian niches; therefore, they have become two of the best models for studying the biology of adult stem cells in vivo.

In this review, we focus on the stem cell niche of the adult *Drosophila* testis. This is not intended to be a comprehensive review but rather a sampling of recent findings, especially those that have shed light on previously unexplored topics or that have challenged our way of thinking about established topics. Comprehensive reviews of the *Drosophila* testis include those by Fuller (Fuller, 1993) and Davies and Fuller (Davies and Fuller, 2008). Recent reviews that focus on specific topics relevant to *Drosophila* testis stem cells include those on adhesion (Marthiens et al., 2010), asymmetric division (Yamashita et al., 2010), aging (Wang and Jones, 2010) and systemic regulation (Drummond-Barbosa, 2008; Jasper and Jones, 2010). For a comprehensive review of the *Drosophila* ovary stem cell niche, see Xie et al. (Xie et al., 2008); a recent review by Fuller and Spradling (Fuller and Spradling, 2007) compares and contrasts the testis and ovary stem cell niches.

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An overview of the *Drosophila* testis

Adult male *Drosophila* contain a pair of testes; each is a long blind-ended tube that is coiled around a seminal vesicle. The stem cell niche is located at the blind apical end of the testis. Here, GSCs divide asymmetrically to generate one cell that remains a stem cell and another, a gonialblast, that is displaced away from the niche and differentiates (Fig. 1). Each gonialblast is enveloped by two somatic cyst cells, which arise from cyst stem cells (CySCs) that also divide asymmetrically to self-renew and produce differentiating cyst cell daughters. A gonialblast progresses through four rounds of transit-amplifying divisions to produce a cluster of 16 spermatogonial cells; cytokinesis is incomplete in each division and the 16 cells remain connected by stable intercellular bridges called ring canals. These 16 spermatogonial cells progress through premeiotic S phase and then switch to a spermatocyte program of growth and gene expression; most of the gene products that are needed for the development of spermatocytes and spermatids are transcribed at this time (White-Cooper, 2010). GSCs, gonialblasts and spermatogonia are almost identical morphologically, but spermatocytes and spermatids undergo dramatic changes in both size and shape. The two cyst cells that envelop the gonialblast do not divide, but they continue to grow and encase the gonialblast and its progeny throughout spermatogenesis. At the end of spermatogenesis, the spermatids lose their interconnections and become surrounded by individual plasma membranes. Mature sperm are then released from the open end of the testis into the seminal vesicle, where they are stored until needed. Thus, the testis contains a gradient of developmental stages, from stem cells in the niche at the apical end to mature sperm at the basal end.

Morphology and development of the testis niche

Many stem cells, including those of the *Drosophila* testis, reside in stromal niches: the stem cells are anchored to specific stromal cells that regulate their division and differentiation (Spradling et al., 2008). At the apical tip of the testis, adjacent to the basement membrane, is a group of ~10-15 non-dividing stromal cells called the hub (Hardy et al., 1979) (Fig. 1). These hub cells are small and closely packed and they are arranged in a distinctive dome-shaped structure that protrudes into the testis. Surrounding the hub are GSCs; the number of GSCs can vary widely from one strain to another, but typically there are 6-9 GSCs per testis. GSCs are shaped like spheres but are flattened where they make broad contact with the hub. Each GSC is flanked by two CySCs; the number of CySCs per testis is therefore about twice the number of GSCs. CySCs also contact the hub, but their nuclei are located farther from the hub than those of the GSCs and they make small regions of contact with the hub via thin cytoplasmic extensions (Hardy et al., 1979). The CySCs and cyst cells completely encase their associated germ cells and isolate them from one another; thus, the only germ cells that contact each other are those that are connected by ring canals.

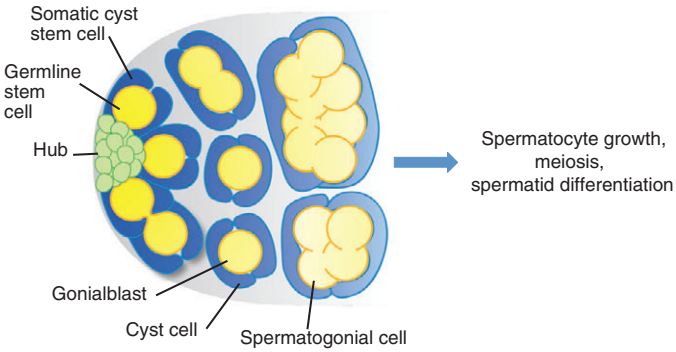

Fig. 1. The *Drosophila* testis stem cell niche. Stromal hub cells (green) adhere to the apical tip of the testis. Surrounding the hub are germline stem cells (GSCs, yellow) and somatic cyst stem cells (CySCs, blue), which share the niche. GSCs and CySCs divide and produce daughter cells that remain in the niche (self-renewal) or leave the niche and differentiate. GSCs give rise to spermatogonia (light yellow), which ultimately develop into sperm; CySCs give rise to cyst cells (light blue), which encase the developing spermatogonia.

The male gonad forms in mid-embryogenesis, when germ cells and somatic gonadal precursor cells (SGPs) coalesce to form a spherical gonad, and by the end of embryogenesis both hub cells and GSCs can be distinguished (Le Bras and Van Doren, 2006; Sheng et al., 2009b). Because the hub is not visible earlier, hub cell specification was thought to occur late in embryogenesis. However, recent work suggests that hub cells are specified much earlier in development, prior to gonad coalescence. Cells in the posterior midgut produce the ligand Delta, which activates the Notch signaling pathway in a subset of SGPs to specify hub cell fate (Okegbe and DiNardo, 2011). Epidermal growth factor receptor, which represses hub cell formation, is activated in posterior SGPs and restricts hub cell formation to the anterior of the gonad (Kitadate and Kobayashi, 2010). Hub cell specification also requires the gene *bowl*, which encodes a transcription factor (DiNardo et al., 2011). CySCs are also formed from SGPs, and the *lines* gene, which encodes an antagonist of Bowl, is required to prevent CySCs from expressing markers of hub cell fate (Hatini et al., 2005; DiNardo et al., 2011). Taken together, these studies suggest that CySCs and hub cells are derived from a common pool of precursor cells in the embryo, and that signaling through multiple pathways is required to specify the appropriate number of each cell type. The ability to follow its development at this level of detail makes the *Drosophila* male gonad one of the best models for understanding the process of niche formation.

Cellular mechanisms that regulate the *Drosophila* testis niche

Stem cell niches provide the local signals that maintain stem cell fate. When stem cells divide, daughters that remain in the niche continue to receive these signals and self-renew, whereas daughters that are displaced from the niche no longer receive these signals and differentiate. Therefore, to maintain tissue homeostasis, the cells that comprise the niche must be maintained, and the number of stem cell daughters that remain in the niche, as well as the number of those that differentiate, must be regulated. In the *Drosophila* testis niche, both GSCs and CySCs adhere to the hub, and their divisions are precisely oriented to balance self-renewal and differentiation.

Asymmetrically oriented GSC divisions rely on cell polarity

Testis GSCs, which are all mitotically active (Wallenfang et al., 2006), normally divide asymmetrically: one daughter cell stays in contact with the hub and retains the stem cell fate, whereas the other is displaced away from the niche and differentiates. This pattern of division results from the stereotypical orientation of centrosomes and spindles in GSCs (Hardy et al., 1979; Yamashita et al., 2003). During early interphase, GSCs contain a single centrosome located at the proximal end of the cell, next to the hub-GSC interface. Later in interphase, when the duplicated centrosomes separate, one centrosome remains anchored at the hub while the other moves to the distal end of the cell. Differential labeling of mother and daughter centrosomes in living testes has shown that the centrosome retained at the hub is the mother, whereas the daughter centrosome moves away (Yamashita et al., 2007). Both centrosomes keep their positions for the rest of the cell cycle; thus, in mitosis, the spindle is oriented perpendicular to the hub-GSC interface and the mother centrosome is retained in the daughter cell that remains at the hub. CySCs also divide asymmetrically but use a mechanism that is strikingly different from that used by GSCs (Cheng et al., 2011). The mitotic spindle in CySCs forms in a random orientation but then repositions in anaphase, when one spindle pole moves to the hub-CySC interface. Thus, as with GSCs, one daughter CySC remains attached to the hub while the other is displaced.

The mechanism controlling centrosome orientation in GSCs is intracellular and depends on polarity cues from the hub-GSC interface. Ultrastructural analysis of wild-type GSCs has shown that mother centrosomes are located near adherens junctions at the hub-GSC interface and are associated with a robust array of microtubules (Yamashita et al., 2007). Centrosomin, a centrosomal protein that tethers centrosomes to astral microtubules, and Adenomatous polyposis coli 2 (Apc2), which is thought to link astral microtubules to adherens junctions, are both required to anchor the mother centrosome to the hub-GSC interface; in GSCs lacking either protein, centrosomes are often misoriented, with neither located next to the hub (Yamashita et al., 2003; Inaba et al., 2010). Therefore, in wild-type GSCs it is likely that mother centrosomes are anchored by astral microtubules to adherens junctions at the hub-GSC interface. By contrast, new daughter centrosomes associate with very few microtubules, which might explain how they are able to move away from the hub. The polarity cue that positions the mother centrosome at the hub-GSC interface is likely to be the adhesion protein E-cadherin (Shotgun – FlyBase). E-cadherin is located exclusively at the hub-GSC interface, as is Apc2 (Yamashita et al., 2003; Inaba et al., 2010). However, when E-cadherin is expressed ectopically throughout the GSC cortex, Apc2 is also dispersed, and this dispersal of Apc2 results in a high frequency of misoriented centrosomes (Inaba et al., 2010). E-cadherin is therefore an important polarity cue for orienting centrosomes in GSCs.

In wild-type testes, GSCs with misoriented centrosomes are found occasionally, but misoriented spindles are almost never seen. What happens to GSCs with misoriented centrosomes? Time-lapse imaging of cultured live testes suggests that GSCs have a checkpoint mechanism for sensing and restoring centrosome orientation: GSCs with misoriented centrosomes do not divide, but instead pause until the correct orientation is restored and then continue dividing (Cheng et al., 2008). Thus, GSCs have robust mechanisms for ensuring that spindles are always oriented perpendicular to the hub, resulting in an asymmetric division. Surprisingly, GSCs are maintained and divide with correctly

oriented spindles even in the complete absence of centrosomes. In *Sas-4* mutants, which fail to replicate centrioles and therefore lack centrosomes, most GSCs still divide with spindles oriented perpendicular to the hub (Riparbelli and Callaini, 2011). This finding suggests that GSCs have a distinct 'back-up' mechanism for orienting spindles that enables them to divide asymmetrically in the absence of centrosomes. It is also possible that division orientation in GSCs without centrosomes is constrained simply by the shape of the cells within the tissue (Odde, 2011). *Sas-4* mutant CySCs have not been analyzed, so what happens to their division orientation in the absence of centrioles remains an open question. The analysis of CySC division orientation in agametic testes, where CySC shape is not constrained by neighboring GSCs, could also be informative.

As in the testis, centrioles in the *Drosophila* ovary are dispensable for GSC division and maintenance, but the mechanisms that orient divisions in wild-type GSCs may differ between the two sexes (Stevens et al., 2007). GSCs in the ovary divide asymmetrically, with spindles oriented perpendicular to the niche and with one pole of the spindle anchored near the niche (Deng and Lin, 1997). However, centrosomes in female GSCs do not remain anchored at the apical end of the cell during interphase, as they do in male GSCs. Instead, they appear to be randomly positioned until a mitotic spindle has formed (Stevens et al., 2007). Another difference is the role of the fusome, a germline-specific organelle, in orienting GSC divisions. During mitosis in female GSCs, the fusome is always located at the apical end of the cell, adjacent to the niche (Deng and Lin, 1997; de Cuevas and Spradling, 1998). The apical pole of the spindle is adjacent to the fusome, and the fusome is essential for spindle orientation; GSCs lacking Hu li tai shao, an essential component of the fusome, have misoriented spindles and no longer divide asymmetrically (Deng and Lin, 1997). In mitotic male GSCs, the apical spindle pole does not associate with the fusome (Sheng and Matunis, 2011), but the role of the fusome in spindle orientation has not been directly tested.

Adhesion molecules maintain cells in the niche

Two distinct types of adhesion molecules are known to be required in the testis niche: those that anchor the hub to the apical tip of the testis, and those that keep the stem cells attached to the hub. At the point at which the hub attaches to the testis wall, the extracellular matrix (ECM) is thick and convoluted and makes extensive connections with hub cells, suggesting that the hub is held in place by adhesion to the ECM (Hardy et al., 1979). Recent findings suggest that integrins are likely to mediate this interaction. When integrin function is removed from somatic cells in adult testes, hubs are mislocalized or lost, and testes with reduced integrin function have mislocalized hubs that no longer adhere to the ECM (Tanentzapf et al., 2007; Lee et al., 2008). Importantly, even in embryos completely lacking integrin function, mislocalized hubs are still surrounded by asymmetrically dividing stem cells, as in wild-type embryos. Thus, integrins anchor the hub to the ECM but are not required for attaching stem cells to the hub or for orienting their divisions.

Conversely, E-cadherin is not required for anchoring the hub to the ECM, but it is essential for maintaining stem cells at the hub (Voog et al., 2008). GSCs lacking E-cadherin are rapidly lost from the niche, suggesting that adherens junctions mediate GSC adhesion to the hub in addition to their role in orienting centrosomes and spindles. E-cadherin is also required for CySC maintenance (Voog et al., 2008), but whether it plays a role in polarizing CySC divisions is not known. As in the testis, E-cadherin in the *Drosophila* ovary is present at high levels in the junctions between stem cells and niche cells, and ovarian germline and ovarian somatic stem cells (called follicle stem cells, or FSCs) are both rapidly lost when E-cadherin is removed (Song and Xie, 2002; Song et al., 2002). Ovarian FSCs, however, also require integrin-mediated adhesion for their maintenance (O'Reilly et al., 2008). Integrins localize at high levels to the basal surface of follicle cells, and FSCs that lack integrins detach from the basal lamina and are lost from the niche, although more slowly than FSCs lacking E-cadherin. No changes in E-cadherin levels are evident in FSCs lacking integrins, which suggests that the two adhesion pathways act independently to anchor FSCs to their niche.

The hub is not a static structure

Hub cells in adult testes have not been found to divide or replicate their DNA, and the hub is usually described as a permanent structure (Hardy et al., 1979). It might not be a static structure, however. In young wild-type testes, the number of cells expressing hub cell markers does not change, but in agametic testes, in which all germ cells have been genetically ablated, the number of cells expressing hub cell markers increases significantly over time (Gönczy and DiNardo, 1996). No dividing hub cells are found even in these testes, which supports the idea that hub cells are postmitotic. Although markers for dividing cells do not label hub cells directly, marked cells are added to the hub periphery over time, suggesting that neighboring somatic cells can adopt a hub cell fate in agametic testes. In wild-type testes, similar experiments suggest that hub cells might turn over and be replaced by neighboring somatic cells, but these results are controversial. Marked cells can be incorporated into the hub as flies age, but the number of hubs that incorporate marked cells differs significantly between experiments (Voog et al., 2008; DiNardo et al., 2011). Therefore, although it is possible that CySCs can give rise to hub cells in wild-type testes, more experiments are needed to test this model. Whether this mechanism is sufficient to regenerate larger numbers of hub cells lost through damage is also unknown.

Stem cell maintenance during homeostasis

To be maintained, stem cells must receive signals that prevent their differentiation and instruct them to self-renew. In stromal niches, in which stem cells are anchored to non-dividing stromal cells, the stromal cells are likely to be a source of these signals. In niches that contain more than one type of stem cell, one stem cell lineage might also receive signals from a different lineage. The *Drosophila* testis niche, which contains both GSCs and CySCs surrounding the hub, is an excellent system for analyzing the signaling pathways that maintain complex stem cell niches during tissue homeostasis.

Local signaling regulates stem cell adhesion and fate

The Janus kinase-Signal transducer and activator of transcription (JAK-STAT) signaling pathway was the first pathway found to regulate stem cell maintenance in the *Drosophila* testis (Kiger et al., 2001; Tulina and Matunis, 2001). The *Drosophila* JAK-STAT pathway is activated by a secreted ligand, Unpaired (Upd); binding of Upd to its receptor causes STAT to be activated and translocate to the nucleus, where it regulates the transcription of STAT-responsive genes (Hombria and Brown, 2002; Arbouzova and Zeidler, 2006). In the testis, Upd is expressed in hub cells and activates the JAK-STAT signaling pathway in adjacent stem cells (Fig. 2). When stem cells divide, daughter cells that remain in contact with the hub continue to receive the signal, whereas those

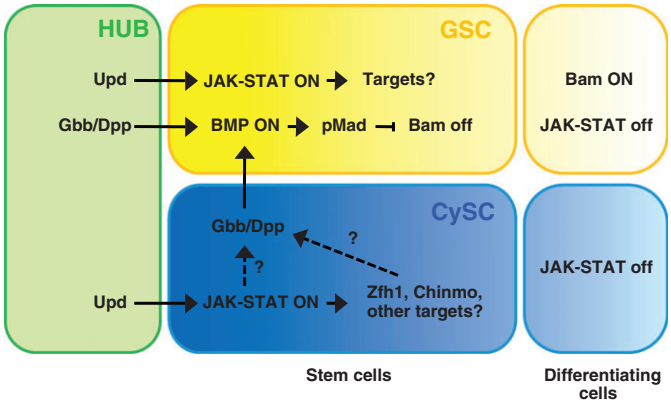

Fig. 2. Local signals maintain testis stem cells. Hub cells (green) secrete the ligand Upd, which activates JAK-STAT signaling in adjacent germline stem cells (GSCs) and somatic cyst stem cells (CySCs). In CySCs (blue), JAK-STAT activation is sufficient for CySC self-renewal. GSCs (yellow) are maintained by signals from both the hub and CySCs that independently regulate GSC self-renewal and adhesion to the hub. Two BMP ligands, Dpp and Gbb (produced by hub cells and CySCs), activate BMP signaling in GSCs, which in turn (via pMad) represses the differentiation factor Bam. By contrast, Bam is upregulated in differentiating daughters that are located further away from the hub. In CySCs, BMP ligands might be produced in response to activated STAT or one or more of its targets, as indicated by the dashed lines. Bam, Bag of marbles; BMP, Bone morphogenetic protein; Chinmo, Chronologically inappropriate morphogenesis; Dpp, Decapentaplegic; Gbb, Glass bottom boat; JAK, Janus kinase (Hopscotch – FlyBase), STAT, Signal transducer and activator of transcription (Stat92E – FlyBase); pMad, phosphorylated Mothers against dpp; Upd, Unpaired (Outstretched – FlyBase); Zfh1, Zinc-finger homeodomain protein 1.

that are displaced from the hub no longer receive enough signal to activate the JAK-STAT pathway at significant levels. JAK-STAT pathway activation is required intrinsically for the maintenance of both GSCs and CySCs: when STAT is depleted from all testis cells, both stem cell populations are completely lost, and individual GSCs or CySCs that lack STAT are also not maintained. Conversely, when Upd is misexpressed throughout the testis apex, both stem cell populations self-renew away from the hub. Based on these observations, JAK-STAT pathway activation was thought to be the critical event independently regulating the self-renewal of each population of stem cells. If this assumption were true, then one would expect that ectopic activation of STAT in germline or somatic cells would be sufficient to cause GSCs or CySCs to self-renew outside the niche. For germline cells, however, this assumption is not true. When STAT is activated in germline cells outside the niche, no self-renewing GSCs are found away from the hub (Leatherman and DiNardo, 2008). By contrast, activation of STAT in somatic cells outside the niche causes testes to fill with ectopic CySCs, and these testes are also filled with self-renewing GSCs interspersed among the CySCs. Thus, the activation of STAT in just the somatic cells is sufficient for the self-renewal of both CySCs and GSCs.

These findings suggest that STAT-activated CySCs produce a signal that promotes the self-renewal of adjacent GSCs. This signal may be mediated by Zinc-finger homeodomain protein 1 (Zfh1) (Leatherman and DiNardo, 2008). *zfh1* is a target of activated STAT and is normally expressed only in CySCs and their immediate daughters. Ectopic expression of *zfh1* in cyst cells outside the niche mimics the phenotype of ectopic STAT activation in these cells: testes fill with ectopic CySCs and GSCs that self-renew away from the hub. Another target of activated STAT, *chronologically inappropriate morphogenesis* (*chinmo*), is also expressed in CySCs and produces ectopic CySCs and GSCs when misexpressed in cyst cells (Flaherty et al., 2010). *zfh1* and *chinmo* are required for CySC self-renewal, but neither is required directly in GSCs for their maintenance (Leatherman and DiNardo, 2008; Flaherty et al., 2010). Thus, STAT may regulate stem cell maintenance through different downstream effectors in the two populations of stem cells.

If STAT activation in GSCs does not mediate their self-renewal, then what does it do? Testes that are globally depleted of *stat* lose both populations of stem cells, but CySCs can be rescued by restoring *stat* expression in somatic cells (Leatherman and DiNardo, 2008). Surprisingly, despite the fact that they make no functional STAT, GSCs are also rescued in these testes (Leatherman and DiNardo, 2010). The *stat*-depleted GSCs no longer contact the hub, however; instead, they contact the layer of *stat*-expressing CySCs that now surrounds the hub. Despite this atypical arrangement, the *stat*-depleted GSCs continue to self-renew and produce robust quantities of differentiating progeny. These results suggest that STAT activation in GSCs mediates adhesion to the hub rather than self-renewal. Consistent with this idea, in testes that are globally depleted of *stat*, GSCs show defects in adhesion and severe disruption of the hub-GSC interface before other signs of differentiation; even in GSCs that are still adjacent to the hub, E-cadherin is delocalized, centrosomes are misoriented and divisions are no longer oriented perpendicular to the hub. In light of these findings, GSCs might be lost in *stat*-depleted testes because CySCs are lost first, not because GSCs require STAT intrinsically for self-renewal. Moreover, in testes that are otherwise wild-type, *stat*-null GSCs might be lost because they lose their attachment to the hub and are replaced by wild-type GSCs, which are better able to adhere to the hub. Removing all CySCs from wild-type testes could be informative.

Another candidate pathway for regulating GSC self-renewal is the Bone morphogenetic protein (BMP) signaling pathway. In *Drosophila*, when BMP ligands bind to their receptors, Mothers against dpp (Mad) is phosphorylated and translocates to the nucleus, where it affects the transcription of target genes (Raftery and Sutherland, 1999; Affolter and Basler, 2007). Two BMP ligands, Decapentaplegic (Dpp) and Glass bottom boat (Gbb), are expressed in the hub and CySCs and activate signaling in the GSCs (Shivdasani and Ingham, 2003; Kawase et al., 2004; Schulz et al., 2004). As in the *Drosophila* ovary, BMP pathway activation is required in GSCs to repress transcription of the differentiation factor *bag of marbles* (*bam*). In both ovaries and testes, GSCs that lack downstream BMP pathway components upregulate *bam*, differentiate prematurely, and are consequently lost from the niche (Chen and McKearin, 2003; Kawase et al., 2004; Song et al., 2004). Intriguingly, in the testis, BMP signaling is activated not only in wild-type GSCs, but also in ectopic GSCs outside the niche; BMP signaling is also activated in *stat*-depleted GSCs and is required for their rescue by *stat*-expressing CySCs, as discussed above (Leatherman and DiNardo, 2010). Thus, BMP ligands might be one of the signals produced by STAT-activated CySCs that promote self-renewal in adjacent GSCs, but they cannot be the only signal required for GSC self-renewal. In the ovary, ectopic BMP pathway activation is sufficient for GSC self-renewal outside the niche (Xie and Spradling, 1998). In the testis, however, no extra GSCs are found either in or outside the niche when BMP signaling

is upregulated (Shivdasani and Ingham, 2003; Kawase et al., 2004; Schulz et al., 2004). Perhaps another signaling pathway is required; it is also possible that STAT plays a role in the maintenance of GSC fate that is obscured by its role in mediating adhesion to the hub. Activating the BMP and JAK-STAT pathways together in germ cells outside the niche might provide an answer; perhaps, in this case, GSCs would be able to self-renew away from both the hub and CySCs.

Stem cells are regulated by microRNAs

MicroRNAs regulate protein translation by silencing or degrading specific mRNAs. In *Drosophila*, mature microRNAs are generated from precursors by the Dicer-1 RNase and double-stranded RNA-binding protein Loquacious (Lee et al., 2004; Förstemann et al., 2005; Saito et al., 2005). In the ovary, the microRNA pathway is required in both germline and somatic stem cells to regulate their division and self-renewal, suggesting that ovarian stem cell maintenance depends on microRNA-mediated gene regulation (Hatfield et al., 2005; Jin and Xie, 2007; Park et al., 2007; Yu et al., 2009). The requirement for microRNA pathway components in the testis has not been determined, but one specific microRNA, miR-7, could play a role in GSC maintenance. miR-7 targets the 3′UTR of *bam* mRNA and represses its expression (Pek et al., 2009). *miR-7* expression is repressed by Maelstrom (Mael), which is required for differentiation of the GSC lineage. In *mael* mutants, GSCs are unaffected, but spermatogonia fail to upregulate Bam, differentiate abnormally and often revert to GSCs (Pek et al., 2009). These results suggest that microRNAs promote stem cell maintenance by repressing specific differentiation factors, such as Bam, and that microRNAs are themselves tightly regulated in stem cell lineages.

Epigenetic mechanisms regulate stem cell maintenance

Epigenetic mechanisms modulate chromatin structure without changing the underlying genomic DNA sequence. Both cell signaling and chromatin structure can participate in regulating cell fate, but how these two regulatory mechanisms are coordinated in endogenous niches remains largely unknown. Nucleosomes, the fundamental units of chromatin, contain DNA and histones and are regulated by two main classes of chromatin-remodeling enzymes: those that catalyze covalent modifications of histone proteins, and those that use the energy of ATP hydrolysis to alter histone-DNA contacts (Becker and Horz, 2002). In *Drosophila*, at least nine different ATP-dependent chromatin remodelers are currently known, which can act as repressors or activators of transcription by regulating chromatin structure (Bouazoune and Brehm, 2006). One of these remodelers, the nucleosome remodeling factor (NURF) complex, is essential for stem cell maintenance in the *Drosophila* testis (Cherry and Matunis, 2010). In GSCs, the NURF complex promotes the expression of STAT and inhibits the expression of Bam, thereby maintaining GSCs and preventing them from differentiating prematurely in the niche. The NURF complex is also required for the maintenance of CySCs. By contrast, it is not required in differentiating daughter cells, as spermatogonia and cyst cells differentiate normally without a functional NURF complex. In the *Drosophila* ovary, the steroid hormone ecdysone functions together with the NURF complex to promote GSC self-renewal and proliferation, suggesting that stem cells can be modulated systemically by steroid hormones that act directly on their intrinsic chromatin remodeling machinery (Ables and Drummond-Barbosa, 2010). It is tempting to speculate that similar mechanisms regulate stem cells in the *Drosophila* testis, although the role of ecdysone signaling in testis GSCs is not yet known.

Although the NURF complex may play a conserved role in stem cell maintenance in both males and females, this role is not a general property of all chromatin remodelers. ISWI, the ATPase subunit of the NURF complex, is a component of two other chromatin remodelers in *Drosophila*, neither of which is required for GSC or CySC maintenance (Cherry and Matunis, 2010). Mi-2, the core ATPase for another family of chromatin remodelers, is also dispensable for GSC maintenance in the testis. In the ovary, ISWI is required for the maintenance of GSCs but not FSCs; conversely, Domino, the ATPase subunit of the INO80 family of chromatin remodelers, promotes self-renewal of FSCs but is not required in GSCs (Xi and Xie, 2005). Taken together, these results suggest that each type of stem cell requires a unique constellation of epigenetic regulators and that additional changes in epigenetic regulation are needed as cells leave the niche and differentiate.

Other types of epigenetic mechanisms are likely to regulate *Drosophila* testis stem cells. In mammals, chromatin in embryonic and adult stem cells is thought to be maintained in a unique ‘poised’ status: genes that will be needed for subsequent differentiation are kept silent but poised for expression as differentiation ensues (Boyer et al., 2006; Guenther et al., 2007). These genes are labeled bivalently by opposing active and repressive histone modifications and although they recruit the transcription initiator RNA polymerase II (Pol II), it remains in a stalled position. Genome-wide analyses in the *Drosophila* testis, however, suggest that most differentiation-associated genes in stem cells contain only repressive histone marks or no mark; they contain no active marks or stalled Pol II (Gan et al., 2010). This distinct chromatin signature, which might reflect species or cell-type specificity, is an exciting topic for future studies.

Stem cell maintenance following aging or damage

To maintain tissue homeostasis throughout the lifetime of an organism, stem cells must be regulated in response to diverse physiological or pathological conditions. Stem cell niches must have mechanisms not only for regulating stem cells, but also for replacing stem cells lost through aging or damage to the tissue in which they reside. The *Drosophila* testis stem cell niche has the remarkable ability to regenerate GSCs even in testes that have lost all GSCs.

Differentiating cells can revert to stem cells

In the *Drosophila* testis, the half-life of individual GSCs is ~2 weeks (Wallenfang et al., 2006). If lost GSCs were not replaced, testes in flies that are several weeks old would contain far fewer GSCs than testes in young flies, whereas in fact the number of GSCs per testis is only slightly reduced in old flies (Wallenfang et al., 2006; Boyle et al., 2007). Therefore, wild-type flies must have mechanisms for maintaining the GSC population by replacing lost GSCs (Fig. 3). Theoretically, lost stem cells can be replaced either by dedifferentiation, which is the reversion of a differentiating cell to a stem cell, or by symmetric self-renewal of a remaining stem cell to generate two daughter stem cells. Direct evidence for both of these mechanisms has been found in testes from flies that have been genetically manipulated to induce a high rate of GSC loss, as well as in wild-type testes. Although rarely employed during homeostasis, both mechanisms are likely to maintain GSC numbers in testes that are aging or recovering from damage.

Rapid loss of GSCs from the testis niche can be induced genetically by removal of the stem cell maintenance factor STAT or by ectopic expression of the differentiation factor Bam (Brawley

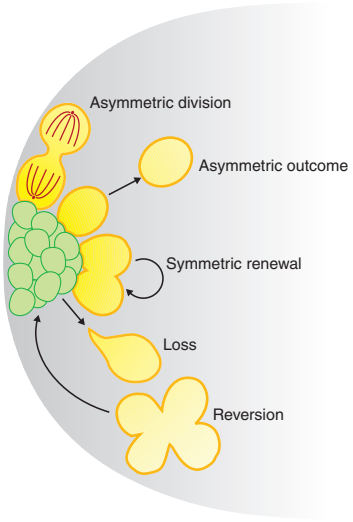

Fig. 3. Multiple mechanisms maintain the testis GSC population. Testis GSCs (yellow) divide asymmetrically, with spindles (red) oriented perpendicular to the hub (green). Asymmetric division can result in an asymmetric outcome (giving rise to one self-renewing GSC and one differentiating daughter) or in symmetric renewal (the displaced daughter returns to the hub). GSCs can also arise from reversion of spermatogonia or they can be lost from the niche. These various mechanisms are regulated by local and systemic factors to maintain the GSC population during homeostasis and following perturbations to the niche.

and Matunis, 2004; Sheng et al., 2009a). In both cases, GSCs are lost via differentiation, but they can be restored via dedifferentiation when conditions in the niche are returned to normal. These studies suggest that gonialblasts and spermatogonia are capable of reverting to GSCs. In addition to reversing their fate, interconnected spermatogonia must also close their ring canals and separate into single cells to form functional GSCs (Cheng et al., 2008) (Sheng and Matunis, 2011). Dedifferentiation occurs by a similar mechanism in the *Drosophila* ovary (Kai and Spradling, 2004) and also in the mouse testis, where clusters of interconnected spermatogonia fragment into single cells during GSC regeneration (Barroca et al., 2009; Nakagawa et al., 2010).

In *Drosophila*, testes that contain only spermatocytes are not able to regain GSCs (Brawley and Matunis, 2004), which suggests that spermatocytes, unlike gonialblasts and spermatogonia, cannot dedifferentiate into GSCs, most likely because their chromatin has transitioned irreversibly towards a terminally differentiated state. Spermatogonia do not have to contact the hub to revert but can move back to the hub from a distance, displacing the somatic cells that surround the hub as they move (Sheng et al., 2009a). Although hub contact is not required, reversion might depend on contact with CySCs. In support of this idea, nearly all testes are able to recover GSCs following manipulation of Bam, which triggers loss of GSCs only; however, far fewer recover after STAT manipulation, which depletes both GSCs and CySCs (Brawley and Matunis, 2004; Sheng et al., 2009a). Furthermore, after STAT manipulation, GSCs repopulate the niche only in testes that also regain CySCs. CySCs could produce a regulatory signal that promotes spermatogonial dedifferentiation, or they could play a physical role in breaking apart interconnected spermatogonia. It is also possible that CySCs are required not for the process of dedifferentiation, but for maintenance of the repopulating GSCs.

The mechanisms that regulate dedifferentiation are poorly understood, but it is likely that the JAK-STAT signaling pathway is required (Sheng et al., 2009a). This pathway is normally inactive outside the niche; however, in testes undergoing dedifferentiation after manipulation of Bam, STAT is upregulated in some spermatogonia near the hub. Moreover, spermatogonia that express an inhibitor of JAK-STAT signaling do not repopulate the niche as efficiently as uninhibited spermatogonia. Therefore, JAK-STAT pathway activation could be required for dedifferentiating cells to re-establish contact with the hub or to transition to a GSC fate. Although the precise mechanism is unclear, these results suggest that dedifferentiation is a regulated process that depends on local signals from the niche and not on chance encounters between differentiated cells and the hub.

Dedifferentiation has been characterized not only in genetically manipulated testes, but also in wild-type testes. Although dedifferentiating spermatogonia are rarely seen in healthy testes from young flies, the frequency of dedifferentiation increases dramatically in flies that are old or recovering from exposure to X-irradiation (Cheng et al., 2008). Dedifferentiation is therefore an important mechanism for maintaining tissue homeostasis in aging or damaged testes. Interestingly, a much higher frequency of misoriented centrosomes is found in GSCs that arise from dedifferentiation than in constitutive GSCs, suggesting that centrosomes do not orient towards the hub as dedifferentiating GSCs re-enter the niche. However, the frequency of misoriented spindles is not much higher in GSCs that arise from dedifferentiation than in constitutive GSCs. This suggests that both dedifferentiating and constitutive GSCs wait until their centrosomes are correctly oriented before entering mitosis. Since the frequency of dedifferentiation increases as flies age, centrosome misorientation in dedifferentiating GSCs could contribute to the decline in the stem cell division rate that is seen in old flies (Wallenfang et al., 2006; Boyle et al., 2007; Cheng et al., 2008).

Stem cells can be replenished via multiple mechanisms

In unperturbed wild-type testes, most GSC divisions are stereotypically oriented and result in an asymmetric outcome: mitotic spindles are oriented perpendicular to the hub, and only one daughter cell stays attached to the hub and self-renews. Symmetric divisions, with spindles oriented parallel to the hub, are almost never seen, except in *stat*-depleted GSCs that self-renew away from the hub (Leatherman and DiNardo, 2010) or in mutant GSCs with missing or misoriented centrosomes (Yamashita et al., 2003; Inaba et al., 2010; Riparbelli and Callaini, 2011), in which misoriented spindles are more frequent. GSCs dividing with misoriented spindles could potentially result in an asymmetric or symmetric outcome, depending on how many daughter cells remain attached to the hub and self-renew. A GSC dividing with properly oriented spindles can also produce a symmetric outcome if the daughter cell that was initially displaced from the hub moves back to the hub and regains hub contact after mitosis (Sheng and Matunis, 2011). However, in unperturbed young testes – and even in mutant testes with many misoriented divisions – the number of GSCs generally remains constant or changes only modestly (Yamashita et al., 2003; Inaba et al., 2010; Riparbelli and Callaini, 2011) (Sheng and Matunis, 2011). Therefore, during homeostasis, divisions that give rise to two GSC daughters are likely to be balanced by loss of GSCs from the hub. In aging or damaged testes, however, symmetric renewals could be a means of replacing lost GSCs.

Symmetric renewals arising from misoriented spindles can also replace lost GSCs in the *Drosophila* ovary (Xie and Spradling, 2000). These findings suggest that lost GSCs can be replaced by multiple mechanisms in both sexes, and the frequency of replacement is likely to be regulated in response to aging, damage or other changes in the GSC content of the niche. In the mouse testis, stem cells are also lost and replaced stochastically on a time-scale of weeks, and stem cell loss is likely to be balanced by both symmetric renewal and dedifferentiation (Klein et al., 2010). Together, these findings support the idea that the GSC pool is both highly dynamic and carefully regulated by homeostatic mechanisms.

Coordinating the regulation of multiple stem cell types in one niche

Many niches contain multiple stem cell types that are housed together in a single niche and produce a balanced mix of differentiated cell types. In these niches, the number of each type of stem cell must be regulated to ensure that no one type overtakes the niche. Moreover, the self-renewal and differentiation of all stem cell types must be coordinated to produce an appropriate ratio of differentiated cell types. Populations of stem cells can be regulated not just by local signals from within the niche, but also by systemic signals from outside the tissue. The *Drosophila* testis niche contains two stem cell types (i.e. GSCs and CySCs), the behavior of which is coordinately regulated by both local and systemic signals to produce the precise ratio of germline and somatic cells required for the development of sperm.

Competition between stem cells is mediated by local signaling

In the *Drosophila* testis, GSCs and CySCs are both anchored via an E-cadherin-based mechanism to a fixed population of hub cells. Each GSC is flanked by approximately two CySCs, which results in a ratio of ~2:1 CySCs to GSCs around the hub. *Suppressor of cytokine signaling at 36E* (*Socs36E*) plays a role in maintaining this ratio. SOCS proteins are highly conserved antagonists of JAK-STAT signaling that dampen signaling by binding to and inhibiting JAKs or their associated receptors, or by targeting the JAK-receptor complex for proteasomal degradation (Croker et al., 2008). In *Socs36E* mutant testes, the ratio of CySCs to GSCs is disrupted; CySCs overtake the niche and displace most of the GSCs (Issigonis et al., 2009; Singh et al., 2010). No changes in E-cadherin levels are detectable in either cell type, but integrin levels are elevated in *Socs36E* mutant CySCs (Issigonis et al., 2009). Furthermore, CySCs with enhanced integrin-mediated adhesion are able to displace GSCs even in testes that are otherwise wild-type. Thus, although integrin is not essential for anchoring stem cells to the hub, elevated levels of integrin in CySCs are sufficient for CySCs to outcompete GSCs. In wild-type testes, *Socs36E* is expressed in CySCs and is thought to dampen JAK-STAT signaling in these cells. Therefore, competition between GSCs and CySCs may be mediated by differentially regulating JAK-STAT signaling levels in the two different stem cell types. These results illustrate how one signaling pathway can be modulated in two different stem cell types to maintain both types in a single niche. Although JAK-STAT pathway activation is required in both CySCs and GSCs, it is likely that the lower levels of signaling in CySCs are sufficient to maintain CySCs in the niche while preventing them from outcompeting the GSCs.

Competition can also occur between neighboring stem cells of the same type (Johnston, 2009). In the *Drosophila* testis, *Socs36E* mutant CySCs can outcompete not just GSCs, but also neighboring wild-type CySCs; testes that contain a small number of *Socs36E* mutant CySCs, but are otherwise wild-type, gain mutant CySCs and lose wild-type CySCs over time (Issigonis et al., 2009). In the *Drosophila* ovary, in which two to three GSCs are anchored to the niche via E-cadherin-based adhesion, different levels of E-cadherin can induce competition between neighboring GSCs; GSCs that express more E-cadherin displace neighboring GSCs that express less E-cadherin (Jin et al., 2008). *bam* mutant GSCs, which express higher than normal levels of E-cadherin, are also able to displace neighboring wild-type GSCs (Jin et al., 2008), as are GSCs with higher levels of Dpp signaling, which promotes GSC self-renewal (Rhiner et al., 2009). Competition between neighboring GSCs might also occur in the testis, but this has not yet been reported.

Taken together, these results suggest that neighboring stem cells actively compete with one another for space around the niche. This type of competition is especially evident in stromal niches in which stem cells are anchored to a fixed population of stromal cells. Moreover, regulating adhesion between stem cells and their niches is an important mechanism for regulating competition between different stem cell populations.

Stem cells respond to changes in nutrition

Stem cells are regulated not just by local signals from within their niches, but also by external signals from outside the tissue. One example that has been especially well studied in *Drosophila* is the ability of stem cells to respond to changes in nutrient availability. When males are raised on a standard diet and then switched to a diet lacking protein, the numbers of GSCs and CySCs per testis are reduced and GSC proliferation rates decline (McLeod et al., 2010). Remarkably, these effects are completely reversible; when starved flies are shifted back to a standard diet, the lost stem cells are rapidly replaced. The mechanism by which they are replaced (symmetric renewal, dedifferentiation, or a combination of the two) is not yet clear.

The ability of stem cells to sense changes in diet is likely to be mediated by insulin signaling. Insulin-like peptides are produced in the brain and signal through the *Drosophila* insulin receptor (InR) (Brogiolo et al., 2001). In the testis, *InR* mutant GSCs are not maintained, and GSC division rates decline in testes with reduced levels of insulin signaling (Ueishi et al., 2009; McLeod et al., 2010). Moreover, constitutive insulin signaling can suppress the loss of GSCs in response to starvation. GSCs in the *Drosophila* ovary are also regulated directly by insulin signaling and respond to starvation with decreased proliferation rates (Drummond-Barbosa and Spradling, 2001; LaFever and Drummond-Barbosa, 2005). Follicle cells also respond to changes in diet but are not affected directly by loss of InR, suggesting that diet regulates FSCs only indirectly, in response to a secondary signal from the germline. Whether testis CySCs are controlled directly by insulin signaling or whether their response to starvation is coordinated by secondary signals from GSCs remains to be seen.

Conclusion

Recent work on the *Drosophila* testis has greatly improved our understanding of how this stem cell niche is regulated during homeostasis and how it responds to perturbation. The picture that has emerged is of a system that is far more resilient and plastic than previously appreciated. Future studies will continue to reveal the complex regulatory networks that control stem cell maintenance, to determine how these networks sense and

respond to change within or outside the niche, and to extend our understanding of stem cell regeneration. As many features of the *Drosophila* testis stem cell niche are likely to be conserved, these studies have broad implications for mammalian stem cell biology and stem cell-based medicine.

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Competing interests statement
The authors declare no competing financial interests.

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