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Recent advances in Drosophila male germline stem cell biology

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The ability of stem cells to divide asymmetrically to produce both self-renewing and differentiating daughter cells sustains many adult tissues, but germline stem cells (GSCs) are unique among stem cells as they perpetuate the genome of the species. The cellular and molecular mechanisms regulating most mammalian stem cells in their endogenous local microenvironments, or niches, are quite challenging to study. However, studies of stem cell niches such as those found in the Drosophila gonads have proven very useful. In these tissues, GSCs are housed in a readily identifiable niche, and the ability to genetically manipulate these cells and their neighbors has uncovered several fundamental mechanisms that are relevant to stem cells more generally. Here, we summarize recent work on the regulation of GSCs in the Drosophila testis niche by intercellular signals, and on the intracellular mechanisms that cooperate with these signals to ensure the survival of the germline. This review focuses on GSCs within the adult Drosophila testis; somatic stem cells in this tissue are reviewed by Zoller and Schulz in this issue.[1] For a review of the testis niche as a whole, see de Cuevas and Matunis,[2] and for more comprehensive reviews of the Drosophila testis, refer to Fuller[3] and Davies and Fuller.[4]

The Morphology of the Drosophila Testis Germline Stem Cell Niche

Drosophila males have a pair of testes, which arise from the coalescence of somatic gonadal precursor cells and primordial germ cells during embryogenesis (reviewed in this issue by Whitworth et al.).[5] A morphologically distinct stem cell niche resides at the apical end of each adult testis, where GSCs adhere to a dome-shaped cluster of small, highly interdigitated quiescent somatic cells called the hub (**Fig. 1**).[6] Each hub contains about 10–15 hub cells; some adhere to the basement membrane at the testis apex, while others protrude into the lumen of the testis, where they serve as a docking site for adjacent stem cells. The number of GSCs varies among strains, but there are typically 6–9 GSCs per testis, and GSC number correlates with the number of hub cells.[7] GSCs undergo asymmetric divisions, typically generating one cell that remains attached to the hub and retains stem cell identity, and another cell, called a gonialblast, that is displaced from the hub (out of the niche) and begins to differentiate. As in the mammalian testis, germ cell differentiation depends on cues from multiple nearby somatic cells. In addition to the hub cells, GSCs intimately associate with somatic stem cells called cyst stem cells, or CySCs, which have a dual role in the niche. Approximately two CySCs flank each GSC, and they adhere to the hub via thin cytoplasmic extensions such that CySC nuclei are located slightly farther from the hub than GSC nuclei.[6,8] CySCs divide asymmetrically to generate squamous, quiescent somatic daughter cells called cyst cells, two of which envelop each gonialblast as it exits the niche.[8,9] CySCs also constitute an important signaling component of the GSC niche, as they produce signals that promote GSC self-renewal.[10,11]

After differentiating germ cells exit the niche, they undergo a steady progression of dramatic morphological and molecular changes that characterize the ten-day process of spermatogenesis.[6] Gonialblasts divide four times with incomplete cytokinesis to become clusters of 16 interconnected spermatogonia, which enter premeiotic S-phase shortly after their last mitotic division. As spermatocyte growth and spermatogenesis ensue, older spermatogonia and spermatocytes become displaced from the testis apex by newly generated spermatogonial cysts that arise from continual stem cell divisions in the niche. Thus, the testis contains a gradient of developmental stages from GSCs within the niche to differentiated sperm at the basal end.

Local and Systemic Signals Maintain Testis GSCs

Stem cells are generally thought to require local signals to promote their long-term maintenance within the niches where they reside, and studies of the Drosophila ovary and testis provided some of the first examples of how niches function at the cellular and molecular level.[12-14] While many tissues (for example, the mouse testis) do not contain morphologically distinct cells that constitute a candidate niche structure,[15] Drosophila gonads do, and these stromal cells were long suspected to be sources for niche signals.[16] The local and systemic signals known to act within the male GSC niche are summarized in **Figure 2**. The Bone morphogenetic protein (BMP) signaling pathway was the first signaling cassette identified as critical for the maintenance of ovarian GSCs, while the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway was the first found to regulate GSC and CySC maintenance in the testis.[12-14]

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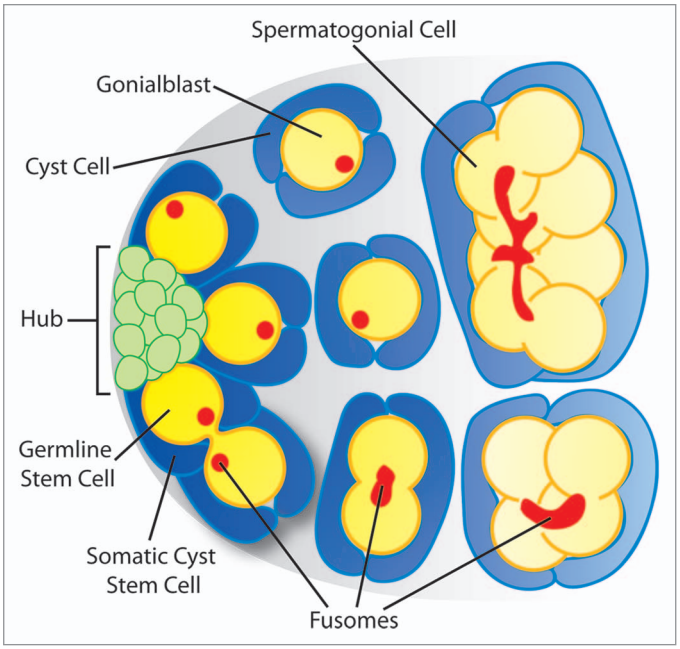

Figure 1. The Drosophila testis stem cell niche. Stromal hub cells (green) adhere to the apical tip of the testis. Surrounding the hub are germline stem cells (GSCs, yellow) and somatic cyst stem cells (CySCs, blue), which share the niche. GSCs and CySCs divide and produce daughter cells that remain 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 fusome (red), a germline-specific endoplasmic reticulum-like organelle, has a spherical shape in GSCs and gonialblast daughters and an elongated or branched shape in spermatogonia. (Adapted from ref. 2.)

Subsequent work has revealed that each pathway is important for GSC maintenance in both systems, with nuanced differences.[17]

JAK-STAT signaling promotes GSC maintenance in the Drosophila testis. In the testis, hub cells express a novel secreted cytokine called Unpaired (Upd, also called Outstretched), which activates the JAK-STAT signaling pathway in adjacent GSCs and CySCs. When stem cells divide, daughter cells that are displaced from the hub are thought to receive lower levels of hub-derived signals, such as Upd, and therefore differentiate. Pathway activation is required in both GSCs and CySCs for their maintenance. Conversely, misexpression of Upd throughout the testis causes ectopic GSCs and CySCs to accumulate outside the niche. These observations suggest that activation of STAT promotes the maintenance of stem cell identity directly within GSCs and CySCs.[13,14] As expected, ectopic activation of STAT in somatic cells outside the niche is sufficient to cause CySCs to self-renew outside the niche; ectopic GSCs are also found outside the niche in these testes. Activation of STAT in germline cells, however, is not sufficient to promote GSC self-renewal outside the niche.[10] Furthermore, GSCs lacking STAT lose the enrichment of E-cadherin that normally resides at the GSC-hub interface, and E-cadherin partially rescues the maintenance of STAT-depleted GSCs.[11] Together, these data support a model in which STAT activation in GSCs promotes their adhesion to the hub, while STAT activation in CySCs activates targets essential for their identity.[11]

The ability of CySCs to maintain GSCs is likely to be mediated by Zinc-finger homeodomain protein 1 (Zfh-1).[10] *zfh-1,* a target of JAK-STAT signaling, is expressed in CySCs and their immediate daughters and is required for CySC maintenance. Like STAT, when *zfh-1* is ectopically expressed in the cyst lineage outside the niche, it causes ectopic accumulation of both CySCs and GSCs outside the niche.[10] *zfh-1* is not expressed in GSCs indicating that STAT is able to regulate distinct targets in GSCs and CySCs. Another target of activated STAT, *chronologically inappropriate morphogenesis* (*chinmo*), is also required autonomously in CySCs but not GSCs for their self-renewal. Misexpression of *chinmo* in cyst cells also causes ectopic accumulation of GSCs and CySCs.[18] *ken-and-barbie* (*ken*) is another gene that is necessary and sufficient to promote CySC identity, but it acts independently of STAT.[19] Like *zfh-1* and *chinmo*, ectopic expression of *ken* in cyst cells leads to accumulation of CySCs and GSCs outside the niche. Interestingly, *zfh-1*, *chinmo* and *ken* are all known to act as transcriptional repressors, suggesting that transcriptional repression is important for the self-renewal of CySCs, and for the ability of CySCs to send self-renewal signals to the germline. STAT targets in GSCs await identification; adhesion molecules or their regulators are candidates,[11] but some targets may also play roles in the maintenance of GSC fate that are obscured by STAT's role in mediating adhesion to the hub. A stringent test for the requirement of CySCs in GSC renewal would be to genetically ablate all CySCs from wild type testes. Interestingly, the ligand hedgehog is expressed specifically within the hub, and transduction of hedgehog signaling is required in CySCs (but not GSCs) to promote their maintenance. Although loss of hedgehog signaling leads to a decrease is CySC maintenance, increased hedgehog signaling is not sufficient to promote ectopic accumulation of GSCs outside the niche as is the case with *zfh-1*, *chinmo* and *ken*.[20]

Testis GSCs are maintained by localized BMP signaling. The BMP signaling pathway also regulates GSC self-renewal in the Drosophila testis. Two BMP ligands, *decapentaplegic (dpp)* and *glass bottom boat (gbb)*, are expressed in the hub and CySCs; although the relative contribution of each source of BMPs to GSC maintenance is not known, it is clear that BMP pathway activation in GSCs represses transcription of the differentiation factor *bag-of-marbles (bam)*.[21-24] Although ectopic BMP pathway activation is sufficient to cause GSC self-renewal outside the niche in the ovary,[12] this is not the case in the testis.[21-23] Thus, BMPs are necessary but may not be sufficient to promote male GSC self-renewal. CySCs with ectopic STAT activation, however, are likely to mediate GSC self-renewal outside the niche by producing BMP ligands, and the level of BMP activation in these ectopic GSCs corresponds to the level of STAT activation in CySCs.[11] In this case, ectopic GSCs may receive more BMP signaling than normal, which could explain why they can be maintained outside the niche.

Learning more about how the BMP pathway functions in the testis niche is an important goal in the field. Toward this goal, the gene encoding the secreted protein Magu, which extracellularly

regulates BMP signaling in other instances,[25] was recently found to be necessary for male GSC maintenance.[26] *magu* is transcribed in hub cells, and the Magu protein accumulates in nearby cells and in the extracellular matrix. Since overexpression of a constitutively active BMP receptor rescues the *magu* GSC loss phenotype, Magu likely acts upstream of receptor binding.[26]

Another recent advance toward understanding BMP signaling in the testis niche is the development of a fluorescently-tagged reporter of BMP receptor activation.[27] This tool has revealed that BMP signaling is localized within GSCs to the GSC-hub cell interface and provides an interesting model whereby localized BMP receptor activation at adherens junctions may serve as a novel means for spatially limiting BMP signaling in this and perhaps other tissues.[27] Since GSCs depleted for STAT still maintain active BMP signaling,[11] future work regarding the requirements for adherens junctions during BMP signaling in GSCs, as well as an analysis of the behavior of this reporter in female GSCs, will be of interest.[28] It will also be interesting to know whether extracellular BMP signaling modulators such as Magu, or the heparin sulfate proteoglycans Dally and Dally-like, which also maintain GSCs, act cooperatively within the niche,[29,30] and whether any of these factors affect localized BMP receptor activation. The development of additional reporters to facilitate the spatial analysis of signaling pathways known to operate within the niche could also be very informative.

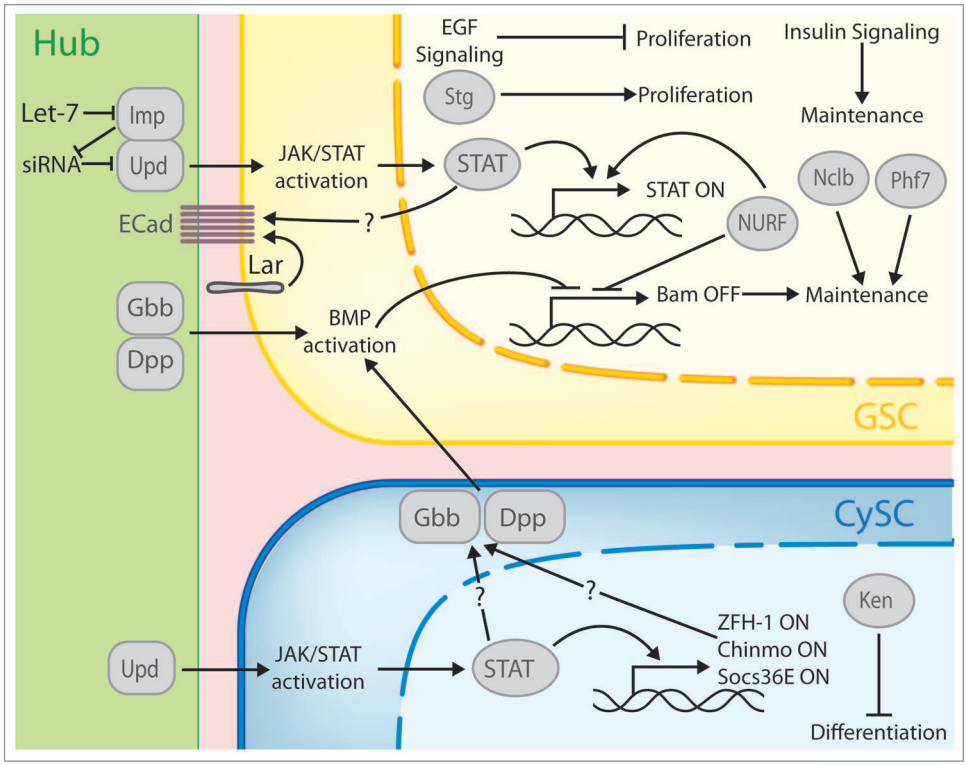

Figure 2. Local and systemic signals maintain testis stem cells. Hub cells (green) secrete the ligand Upd, which activates JAK-STAT signaling in adjacent germline stem cells (GSCs) and somatic cyst stem cells (CySCs). In CySCs (blue), JAK-STAT activation is sufficient for CySC self-renewal. GSCs (yellow) 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, leading to repression of the differentiation factor Bam. The epigenetic factors NURF, Nclb, and Phf7 are all required for maintenance of the GSCs. Insulin signaling also helps to maintain GSCs. Stg promotes proliferation in the GSCs while EGF signaling attenuates proliferation in the GSCs. In CySCs, BMP ligands might be produced in response to activated STAT or one or more of its targets, or in response to Ken. (Adapted from reference 87). Bam, Bag of marbles; BMP, Bone morphogenetic protein; Chinmo, chronologically inappropriate morphogenesis; Dpp, decapentaplegic; EGF, epidermal growth factor; Ken, ken and barbie; Lar, Leukocyte-antigen-related-like; NURF, nucleosome remodeling factor; Nclb, No child left behind; Phf7, PHD Finger Protein 7; Gbb, glass bottom boat; JAK, Janus kinase; Socs36E, Suppressor of cytokine signaling at 36E; STAT, signal transducer and activator of transcription; Stg, string; Upd, Unpaired; Zfh1, Zinc-finger homeodomain protein 1.

Nutrition and insulin signaling regulate GSC maintenance in the Drosophila testis. Stem cells respond to both local and systemic signals. Although more is known about the local signals that operate in the Drosophila testis, experiments regarding the effects of alterations in diet have revealed that nutritional status and insulin signaling impact GSC behavior in the testis, similar to the effects seen in the ovary (reviewed in reference 31). Male flies that are raised on a standard diet and then starved of protein show a significant decrease in GSC and CySC numbers in the testis, and the remaining GSCs fail to proliferate at normal rates.[32,33] Interestingly, this condition is reversible, and when starved flies are re-fed on a standard diet, missing stem cells are rapidly replaced. Constitutive insulin signaling can suppress the starvation-mediated loss of GSCs, and GSCs lacking the Drosophila Insulin receptor are not maintained, implicating the insulin signaling pathway both in GSC maintenance during homeostasis and in the response of GSCs to altered nutrition.[32-34] Since Insulin-like peptides are produced both in the brain and in the testis, local or systemic cues may be affecting GSC maintenance in males.[35,36] GSCs in the Drosophila ovary are also regulated by insulin signaling, and in this case, brain-derived insulin-like peptides play a major role.[37-40] The steroid hormone Ecdysone also regulates the proliferation and self-renewal of female GSCs, but it does so independently of insulin signaling.[41] Ecdysone is detected at very low levels in adult male flies,[42] but the role of ecdysone signaling in the testis niche is not known.

Epigenetic factors regulate Drosophila testis GSCs. Epigenetic control of gene expression through chromatin modifications has been shown in vitro to be critical for the maintenance and differentiation of numerous stem cell populations,[43,44] reviewed in reference 45. Cell signaling and chromatin structure

cooperatively regulate cell fate, but the mechanistic details are challenging to study in endogenous niches. The ATP-dependent chromatin remodeler NURF (nucleosome remodeling factor) complex, which uses the energy of ATP hydrolysis to alter histone-DNA contacts,[46] was the first epigenetic regulator found to be required for stem cell maintenance in the Drosophila testis.[47] In GSCs, the NURF complex promotes STAT expression while inhibiting the expression of Bam, preventing premature differentiation of GSCs from the niche. The NURF complex is also required for CySC maintenance in the testis, and for the maintenance of GSCs in the Drosophila ovary, where NURF functions together with the steroid hormone Ecdysone to promote GSC self-renewal and proliferation.[41] 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. Instead, each type of stem cell likely requires a unique combination of epigenetic regulators for its function. Recently, two additional chromatin-associated proteins have been shown to promote male GSC maintenance. The No child left behind (Nclb) protein is a member of a new family of conserved chromatin-associated proteins and is required within male but not female GSCs to promote their maintenance; male GSCs lacking *nclb* are rapidly lost from the niche and begin to differentiate but cannot complete spermatogenesis.[48] A third chromatin-associated protein, PHD Finger Protein 7 (Phf7), is also required for male GSC maintenance and germ cell differentiation but is not required in the female germline. Phf7 also has the striking ability to promote spermatogenesis in female germ cells when they are present in a male soma. Since Phf7 associates with chromatin and binds specifically to modified histones at sites of active transcription (dimethyl lysine 4 modified histone H3 tails or H3K4me2), Phf7 is proposed to be a conserved epigenetic "reader" that activates the male germline sexual identity.[49] Learning how such a widespread epigenetic mark could have a role in regulating sexual identity, and whether Phf7 and Nclb interact in this process, are interesting questions for the future.

Germline Stem Cell Polarity and Adhesion Within the Testis Niche

Stem cells are maintained by signals from the niche that promote their self-renewal and prevent differentiation. Daughter cells that remain in the niche continue to receive these signals and self-renew, while those that leave the niche stop receiving signals and differentiate. Therefore, the number of cells that remain in or leave the niche must be carefully regulated to maintain stem cell numbers. Since many different mechanisms function in the Drosophila testis to ensure tissue homeostasis, it is an excellent model for studying the different ways that stem cells are regulated in an endogenous niche.

Cell polarity stereotypically orients GSC divisions. Testis GSCs normally divide asymmetrically: one daughter cell stays in contact with the hub and retains stem cell identity, while the other is displaced from the hub and differentiates. An intracellular mechanism polarizes the GSC, ensuring that the mitotic spindle is always oriented perpendicularly with respect to the hub;[6,50] reviewed in reference 51. Early interphase GSCs contain a single centrosome located next to the hub-GSC interface, and differential labeling of mother and daughter centrosomes revealed that this is the older (mother) centrosome, which is retained in the GSC upon each division.[52] Mother centrosomes are thought to be anchored to the hub-GSC interface at adherens junctions, and several proteins, including the centrosomal protein Centrosomin and tumor suppressor Adenomatous Polyposis Coli 2 (Apc2), have been implicated in this process.[50,53] Despite the asymmetric segregation of centrosomes, parental DNA strands do not appear to be asymmetrically segregated.[54]

E-cadherin, a component of adherens junctions, also plays an important role in positioning the mother centrosome in GSCs. E-cadherin and Apc2 are highly enriched at the hub-GSC interface in wild type GSCs, but ectopic expression of E-cadherin throughout the GSC cortex causes mislocalization of Apc2 and results in a high frequency of misoriented centrosomes.[53] STAT-depleted GSCs, which have delocalized E-cadherin, also have misoriented centrosomes.[11] Interestingly, GSCs are maintained and the vast majority of GSCs divide with correctly oriented spindles even in DSas-4 mutants, which lack centrosomes,[55] indicating that GSCs have a centrosome-independent mechanism for orienting their spindles. A germline-specific endoplasmic reticulum-like organelle called the fusome was thought to act as a backup mechanism for orienting the male GSC mitotic spindle. In females, fusomes are always located at the GSC-niche interface, and the *hu li tai shao (hts)* gene, which encodes an essential component of the fusome, is required for spindle orientation in these cells.[56,57] In wild type male GSCs, however, fusomes are randomly localized, and in male GSCs lacking Hts, spindles are correctly oriented in young males, although spindle misorientation phenotypes do develop with age.[50,58,59] Interestingly, in DSas-4 mutant testes, fusomes are no longer randomly localized but instead are found at the GSC-hub interface.[59] Thus, the fusome is apparently dispensable for male GSC spindle orientation under normal conditions, but may play a role in this process if the centrosomes are absent. Removing both Hts and DSas-4 could be informative in clarifying the roles of the centrosome and the fusome in male GSC division orientation.

In wild type testes, although GSCs with misoriented centrosomes appear occasionally, misoriented spindles are almost never seen, suggesting that a checkpoint mechanism delays mitosis until proper centrosome orientation is achieved.[59,60] The serine/threonine kinase Par-1 is required for proper function of this centrosome orientation checkpoint, and it is thought to control cell cycle progression in GSCs with misoriented centrosomes by regulating the localization of cyclin A.[59] The centrosome orientation checkpoint may also delay GSC proliferation under poor nutrient conditions.[33] Perhaps nutrient sensing is a primary role for centrosome orientation, since mechanisms other than spindle orientation serve to increase the number of GSCs within the niche following nutrient deprivation, as discussed below.[32,58]

Adhesion of GSCs to their niche. Stem cells typically adhere to their niches, and this adhesion can be stable or transient. However, little is known about how stem cell-niche cell adhesion, which is often dynamic, is regulated within intact niches.

Work from the Drosophila testis is beginning to reveal more about this conserved aspect of stem cell biology. In addition to its role in polarizing GSCs and facilitating their stereotypically oriented divisions, the adherens junction component E-cadherin is also essential for the adhesion of both GSCs and CySCs to their niches in the Drosophila ovary and testis.[11,61-63]

Recently, the Leukocyte-antigen-related-like (Lar) receptor tyrosine phosphatase has been implicated in promoting GSC (but not CySC) maintenance through localized E-cadherin-based adherens junctions.[64] Lar localizes to the hub-GSC interface and is required cell-autonomously in GSCs for proper localization of Apc2 and E-cadherin at the hub-GSC interface and for the proper orientation of centrosomes. Ultrastructural analysis revealed abnormal adherens junctions between GSCs and hub cells in *Lar* mutant testes. Since STAT protein levels are normal in *Lar* mutant GSCs, and expression of E-cadherin in *Lar* mutant GSCs does not rescue GSC loss, Lar may function in parallel with JAK-STAT signaling to maintain attachment of GSCs to the hub. Interestingly, the heparin sulfate proteoglycan Dally-like (Dlp) is a ligand for Lar in the nervous system, and Dlp helps maintain GSCs in their undifferentiated state.[30] Identification of factors that interact with Lar to maintain GSC adhesion to the niche should provide additional insight into stem cell-niche cell adhesion.

Not only must stem cells adhere to the niche, but also the precise level of adhesion matters. Stem cells that do not adhere as well as neighboring cells can become displaced (or out-competed) from the niche. This is exemplified in testes lacking Suppressor of cytokine signaling at 36E (Socs36E) function, which encodes a negative regulator of the JAK-STAT pathway. In Socs36E mutant testes, STAT is upregulated in CySCs relative to neighboring GSCs, and this is accompanied by the upregulation of adhesive integrin receptors in CySCs.[65,66] As a result, over-adhesive CySCs outcompete GSCs for attachment to the hub. Loss of Socs36E does not cell-autonomously affect GSCs, suggesting that lineage-specific mechanisms independently regulate the adhesion of these two stem cell lineages within the niche.

Mechanisms for GSC Replacement in the Niche

In the Drosophila testis, individual GSCs have a half-life of about two weeks.[67] However, the number of GSCs per testis decreases only modestly in old flies,[7,67] indicating that mechanisms exist to replenish the GSC population. Stem cells that are lost from the niche could theoretically be replaced either by symmetric division of a remaining stem cell to generate two stem cell daughters, or by dedifferentiation, which is the process of a more differentiated cell reverting back to a stem cell fate. Both of these mechanisms have been shown to function in the Drosophila testis.

New GSCs can arise via "symmetric renewal" of existing GSCs. GSC divisions are nearly always oriented perpendicularly with respect to the hub. Instances where GSCs have been detected undergoing divisions with spindles oriented parallel to the hub are rare, and include *stat*-depleted GSCs self-renewing away from the hub[11] and GSCs with mutations that result in missing or misoriented centrosomes.[51,53,55] When a GSC divides with a misoriented spindle, the division could result in either an asymmetric or symmetric outcome, depending on whether or not both daughter cells remain attached to the hub. However, live imaging has revealed that a GSC dividing with a properly oriented spindle can also produce a symmetric outcome if the daughter cell that was initially displaced from the hub moves back toward the hub and regains hub contact and adhesion following mitosis.[58] Furthermore, in young, healthy testes as well as some mutant testes that have an increase number of misoriented divisions, there is little or no change in GSC number over time.[51,53,55,58] This indicates that there is a balance in the tissue between GSCs that divide to give two stem cell daughters and GSCs that are lost from the niche.

GSCs can also divide symmetrically in the Drosophila ovary to replace lost stem cells,[68] and stem cell replacement in the mouse testis is thought to include a symmetric renewal mechanism.[69] Together, these findings support the idea that GSCs behave in a highly dynamic manner within their niches, and that there is considerable plasticity in the lineage to ensure that these stem cells are maintained over time.

Differentiating spermatogonia can revert to GSCs. In addition to symmetric renewal, GSCs can be replaced in the testis niche by dedifferentiation of spermatogonial cysts. Spermatogonial dedifferentiation can be genetically induced by conditional loss of STAT or misexpression of the differentiation factor Bam within the testis.[70,71] In both cases, GSCs are initially lost by differentiation in response to signaling changes; after normal levels of signaling are restored to the tissue, the niche is replenished by new GSCs that arise from the reversion of differentiating spermatogonia back to stem cells. During the dedifferentiation process, the spermatogonia must not only reverse their fate but also physically separate into single cells and re-establish adhesion to the hub to form functioning GSCs.[58,60] Dedifferentiation occurs by a similar mechanism in the Drosophila ovary and has also been shown to occur in the mouse testis, underscoring its generality.[72-74] In the Drosophila testis, dedifferentiating germ cells are capable of migrating over a distance through remaining somatic cells to re-establish contact with the hub.[71] Although spermatogonial cysts that are not in contact with the hub are competent to initiate dedifferentiation, contact with CySCs may be required. Several observations support the requirement of CySCs for dedifferentiation. First, a greater percentage of Bam-manipulated testes, which maintain their CySCs, recover GSCs than do STAT depleted testes, which lose both GSCs and CySCs.[70,71] Second, in STAT depleted testes, GSCs are never recovered in testes that do not also recover CySCs.[70] Differentiating spermatogonia may require a signal from CySCs to dedifferentiate, or CySCs may play a role in breaking apart spermatogonial cysts. Alternatively, CySCs may not be required for dedifferentiation itself, but they may be necessary to maintain recovered GSCs in the niche.

Although the signals that regulate dedifferentiation are not yet understood, the JAK-STAT signaling pathway is thought to be involved in the process.[71] Activation of JAK-STAT signaling is normally restricted to GSCs and GSC-gonialblast pairs; however, in testes undergoing dedifferentiation following Bam manipulation, STAT levels increase in some four to 16-cell spermatogonia

near the hub. Moreover, spermatogonia expressing a JAK-STAT inhibitor are not able to repopulate the niche as efficiently as uninhibited spermatogonia. The ability of dedifferentiating spermatogonia to re-establish contact with the hub may depend on STAT and on other adhesion-regulating pathways including the Lar receptor.[64,71]

Aging and Stress-Related Changes to GSCs and Their Niche

Aging is accompanied by intrinsic and extrinsic changes that alter the behavior of stem cells and compromise their ability to maintain tissues. In mammals, many stem cells are thought to enter or exit quiescent states depending on the needs of the tissue.[75] Because the GSCs in the testis niche are constantly turning over, mechanisms must be in place to replace lost stem cells and ensure that old male flies can maintain their fertility. Dedifferentiation of spermatogonia occurs in wild type testes over the life of the fly, and as flies age, GSCs resulting from dedifferentiation events accumulate in greater numbers in the niche.[60] Additionally, under conditions where STAT function is conditionally removed and then restored, dedifferentiation has been shown to occur with comparable efficiency in both young and old flies, indicating that an aging niche is capable of supporting robust dedifferentiation.[76] Exposure to X-irradiation triggers a dramatic increase in dedifferentiation, but starving and refeeding flies, which leads to loss and then regaining of GSCs, does not trigger dedifferentiation.[32,33,60] In this case, symmetric renewals serve as a mechanism to replace lost GSCs.[32,58] Thus, the relative contributions of symmetric renewal and dedifferentiation in maintaining tissue homeostasis in aging or damaged testes remains to be clarified, and the development of additional tools for studying stem cell replacement could be helpful in this regard.

Interestingly, GSCs that arise from dedifferentiation have a much higher frequency of misoriented centrosomes than other GSCs in the niche.[60] This indicates that dedifferentiating GSCs cannot reliably orient their centrosomes as they re-enter the niche. By the time these GSCs divide, however, they do not have an increased frequency of misoriented spindles compared with other GSCs, indicating that, like wild type GSCs, GSCs that arise from dedifferentiation do not divide until their centrosomes are oriented correctly. In aging testes, although the frequency of GSCs with misoriented centrosomes increases dramatically, it is not yet clear if the rate of dedifferentiation increases with age, or if GSCs that arise from dedifferentiation continue to have a centrosome misorientation defect in subsequent divisions. Regardless of the underlying mechanism, centrosome misorientation and other changes to the niche, discussed below, are thought to contribute to the decline in stem cell division rate that is seen in old flies.[7,60,67,77]

In Drosophila males, although all GSCs remain mitotically active, their rate of division declines with age.[67,77] Recently, a mechanism that can alter GSC mitotic activity has been described. Epidermal growth factor (EGF) signaling, which promotes gonialblast differentiation and enclosure of germ cells by somatic cyst cells in the testis, also affects the frequency of GSC divisions.[77-81] Decreased EGF signaling causes adult GSCs to divide more often, and this defect can be reversed by restoring EGF signaling in GSCs.[81] This regulation of GSC division frequency occurs in adult testes but not in larval testes, suggesting that additional factors may counteract EGF-mediated cell cycle repression in larvae. GSC division rate is also quite sensitive to environmental factors such as temperature,[81] nutrition[32] and even exposure to carbon dioxide,[82] suggesting that its regulation is likely to be complex. A novel environmental means of increasing GSC division frequency, at least in *Drosophila mauritiana* females, is infection of their niche cells with the intracellular parasite Wolbachia. Wolbachia also targets the hub cells in *D. mauritiana* males, but the phenotypic consequences for male GSCs are not apparent.[83]

An age-related decline in the expression of core cell cycle components in GSCs also contributes to a decline in their function. The Cdc25 homolog String, which is a phosphatase essential for activating cyclin-dependent kinases and promoting the cell cycle,[84] is required in both GSCs and CySCs for their proliferation and maintenance.[82] In aging testes, levels of String decrease in GSCs and correlate with a decline in GSC proliferation; restoring expression of String in the germline reverses the age-associated decline in GSC function. In contrast, String expression levels do not decline with age in CySCs, and the number of dividing CySCs per testis also remains unchanged over time, although the number of CySCs per testis does decrease. These results suggest that String is an important regulator of testis stem cell function during both homeostasis and aging.[82]

Aging causes not only a decline in GSC proliferation, but also a decrease in the number of GSCs per testis.[7,67] This loss of GSCs correlates with an age-related decline in the levels of *upd* in hub cells, and it can be suppressed by constitutive expression of *upd* in the hub.[7] IGF-II mRNA binding protein (Imp), which is a member of a conserved family of regulatory RNA-binding proteins, was recently shown to play a key role in regulating *upd* levels during aging.[85] Imp binds to *upd* mRNA and protects it from degradation caused by short interfering RNAs (siRNAs). However, *Imp* is itself targeted by the *let-7* microRNA (miRNA), which is expressed at higher levels in the hub cells of aging males. Constitutive expression of an *Imp* construct that is resistant to *let-7*-mediated degradation is sufficient to suppress not only the decline in *upd* levels but also the loss of GSCs in older males. These data suggest that the testis stem cell niche is regulated in older males by both the siRNA pathway, which targets *upd*, and the miRNA pathway, which targets *Imp*, leaving *upd* susceptible to siRNA-mediated degradation. As Imp is expressed in germ cells as well as hub cells, it may also play an intrinsic role in regulating GSC function during aging.[86]

Conclusion

Recent work on the Drosophila testis stem cell niche has added to the general understanding of how stem cells are regulated in vivo in both young and aging animals. Perhaps because of their unique capacity to generate gametes, GSCs are highly plastic and can be replaced by multiple mechanisms that enable them to resist damage accruing with age or poor environmental conditions. Future

work 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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References

1. Zoller R, Schulz C. The Drosophila cyst stem cell lineage: Partners behind the scenes? Spermatogenesis 2012; 3:1-13. http://dx.doi.org/10.4161/spmg.21380.
2. de Cuevas M, Matunis EL. The stem cell niche: lessons from the Drosophila testis. Development 2011; 138:2861-9; PMID:21693509; http://dx.doi.org/10.1242/dev.056242.
3. Fuller MT. Spermatogenesis. In: Bate M, Martinez Arias A, eds. The Development of Drosphila melanogaster. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1993:71-147.
4. Davies EL, Fuller MT. Regulation of self-renewal and differentiation in adult stem cell lineages: lessons from the Drosophila male germ line. Cold Spring Harb Symp Quant Biol 2008; 73:137-45; PMID:19329574; http://dx.doi.org/10.1101/sqb.2008.73.063.
5. Whitworth, C, Jimenez, E and Van Doren, M. Development of sexual dimorphism in the Drosophila testis. Spermatogenesis 2012; 3:1-8; http://dx.doi.org/10.4161/spmg.21780.
6. Hardy RW, Tokuyasu KT, Lindsley DL, Garavito M. The germinal proliferation center in the testis of Drosophila melanogaster. J Ultrastruct Res 1979; 69:180-90; PMID:114676; http://dx.doi.org/10.1016/S0022-5320(79)90108-4.
7. Boyle M, Wong C, Rocha M, Jones DL. Decline in self-renewal factors contributes to aging of the stem cell niche in the Drosophila testis. Cell Stem Cell 2007; 1:470-8; PMID:18371382; http://dx.doi.org/10.1016/j.stem.2007.08.002.
8. Cheng J, Tiyaboonchai A, Yamashita YM, Hunt AJ. Asymmetric division of cyst stem cells in Drosophila testis is ensured by anaphase spindle repositioning. Development 2011; 138:831-7; PMID:21303845; http://dx.doi.org/10.1242/dev.057901.
9. Gönczy P, DiNardo S. The germ line regulates somatic cyst cell proliferation and fate during Drosophila spermatogenesis. Development 1996; 122:2437-47; PMID:8756289.
10. Leatherman JL, DiNardo S. Zfh-1 controls somatic stem cell self-renewal in the Drosophila testis and nonautonomously influences germline stem cell self-renewal. Cell Stem Cell 2008; 3:44-54; PMID:18593558; http://dx.doi.org/10.1016/j.stem.2008.05.001.
11. Leatherman JL, DiNardo S. Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in Drosophila testes. Nat Cell Biol 2010; 12:806-11; PMID:20622868; http://dx.doi.org/10.1038/ncb2086.
12. Xie T, Spradling AC. decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell 1998; 94:251-60; PMID:9695953; http://dx.doi.org/10.1016/S0092-8674(00)81424-5.
13. Tulina N, Matunis E. Control of stem cell self-renewal in Drosophila spermatogenesis by JAK-STAT signaling. Science 2001; 294:2546-9; PMID:11752575; http://dx.doi.org/10.1126/science.1066700.
14. Kiger AA, Jones DL, Schulz C, Rogers MB, Fuller MT. Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. Science 2001; 294:2542-5; PMID:11752574; http://dx.doi.org/10.1126/science.1066707.
15. Yoshida S. Elucidating the identity and behavior of spermatogenic stem cells in the mouse testis. Reproduction 2012; PMID:22733803; http://dx.doi.org/10.1530/REP-11-0320.
16. Spradling AC, Nystul T, Lighthouse D, Morris L, Fox D, Cox R, et al. Stem cells and their niches: integrated units that maintain Drosophila tissues. Cold Spring Harb Symp Quant Biol 2008; 73:49-57; PMID:19022764; http://dx.doi.org/10.1101/sqb.2008.73.023.
17. Losick VP, Morris LX, Fox DT, Spradling A. Drosophila stem cell niches: a decade of discovery suggests a unified view of stem cell regulation. Dev Cell 2011; 21:159-71; PMID:21763616; http://dx.doi.org/10.1016/j.devcel.2011.06.018.
18. Flaherty MS, Salis P, Evans CJ, Ekas LA, Marouf A, Zavadil J, et al. chinmo is a functional effector of the JAK/STAT pathway that regulates eye development, tumor formation, and stem cell self-renewal in Drosophila. Dev Cell 2010; 18:556-68; PMID:20412771; http://dx.doi.org/10.1016/j.devcel.2010.02.006.
19. Issigonis M, Matunis E. The Drosophila BCL6 homolog ken and barbie promotes somatic stem cell self-renewal in the testis niche. Dev Biol 2012; 368:181-92; PMID:22580161; http://dx.doi.org/10.1016/j.ydbio.2012.04.034.
20. Michel M, Kupinski AP, Raabe I, Bökel C. Hh signalling is essential for somatic stem cell maintenance in the Drosophila testis niche. Development 2012; 139:2663-9; PMID:22745310; http://dx.doi.org/10.1242/dev.075242.
21. Shivdasani AA, Ingham PW. Regulation of stem cell maintenance and transit amplifying cell proliferation by tgf-beta signaling in Drosophila spermatogenesis. Curr Biol 2003; 13:2065-72; PMID:14653996; http://dx.doi.org/10.1016/j.cub.2003.10.063.
22. Kawase E, Wong MD, Ding BC, Xie T. Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the Drosophila testis. Development 2004; 131:1365-75; PMID:14973292; http://dx.doi.org/10.1242/dev.01025.
23. Schulz C, Kiger AA, Tazuke SI, Yamashita YM, Pantalena-Filho LC, Jones DL, et al. A misexpression screen reveals effects of bag-of-marbles and TGF beta class signaling on the Drosophila male germline stem cell lineage. Genetics 2004; 167:707-23; PMID:15238523; http://dx.doi.org/10.1534/genetics.103.023184.
24. Chen D, McKearin D. Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. Curr Biol 2003; 13:1786-91; PMID:14561403; http://dx.doi.org/10.1016/j.cub.2003.09.033.
25. Vuilleumier R, Springhorn A, Patterson L, Koidl S, Hammerschmidt M, Affolter M, et al. Control of Dpp morphogen signalling by a secreted feedback regulator. Nat Cell Biol 2010; 12:611-7; PMID:20453847; http://dx.doi.org/10.1038/ncb2064.
26. Zheng Q, Wang Y, Vargas E, DiNardo S. magu is required for germline stem cell self-renewal through BMP signaling in the Drosophila testis. Dev Biol 2011; 357:202-10; PMID:21723859; http://dx.doi.org/10.1016/j.ydbio.2011.06.022.
27. Michel M, Raabe I, Kupinski AP, Pérez-Palencia R, Bökel C. Local BMP receptor activation at adherens junctions in the Drosophila germline stem cell niche. Nat Commun 2011; 2:415; PMID:21811244; http://dx.doi.org/10.1038/ncomms1426.
28. Ma X, Xie T. Stem cells: keeping BMP signaling local. Curr Biol 2011; 21:R809-11; PMID:21996502; http://dx.doi.org/10.1016/j.cub.2011.08.051.
29. Guo Z, Wang Z. The glypican Dally is required in the niche for the maintenance of germline stem cells and short-range BMP signaling in the Drosophila ovary. Development 2009; 136:3627-35; PMID:19793889; http://dx.doi.org/10.1242/dev.036939.
30. Hayashi Y, Kobayashi S, Nakato H. Drosophila glypicans regulate the germline stem cell niche. J Cell Biol 2009; 187:473-80; PMID:19948496; http://dx.doi.org/10.1083/jcb.200904118.
31. Drummond-Barbosa D. Stem cells, their niches and the systemic environment: an aging network. Genetics 2008; 180:1787-97; PMID:19087970; http://dx.doi.org/10.1534/genetics.108.098244.
32. McLeod CJ, Wang L, Wong C, Jones DL. Stem cell dynamics in response to nutrient availability. Curr Biol 2010; 20:2100-5; PMID:21055942; http://dx.doi.org/10.1016/j.cub.2010.10.038.
33. Roth TM, Chiang CY, Inaba M, Yuan H, Salzmann V, Roth CE, et al. Centrosome misorientation mediates slowing of the cell cycle under limited nutrient conditions in Drosophila male germline stem cells. Mol Biol Cell 2012; 23:1524-32; PMID:22357619; http://dx.doi.org/10.1091/mbc.E11-12-0999.
34. Ueishi S, Shimizu H, H Inoue Y. Male germline stem cell division and spermatocyte growth require insulin signaling in Drosophila. Cell Struct Funct 2009; 34:61-9; PMID:19384053; http://dx.doi.org/10.1247/csf.08042.
35. Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E. An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. Curr Biol 2001; 11:213-21; PMID:11250149; http://dx.doi.org/10.1016/S0960-9822(01)00068-9.
36. Wang L, McLeod CJ, Jones DL. Regulation of adult stem cell behavior by nutrient signaling. Cell Cycle 2011; 10:2628-34; PMID:21814033; http://dx.doi.org/10.4161/cc.10.16.17059.
37. Drummond-Barbosa D, Spradling AC. Stem cells and their progeny respond to nutritional changes during Drosophila oogenesis. Dev Biol 2001; 231:265-78; PMID:11180967; http://dx.doi.org/10.1006/dbio.2000.0135.
38. LaFever L, Drummond-Barbosa D. Direct control of germline stem cell division and cyst growth by neural insulin in Drosophila. Science 2005; 309:1071-3; PMID:16099985; http://dx.doi.org/10.1126/science.1111410.
39. Hsu HJ, Drummond-Barbosa D. Insulin levels control female germline stem cell maintenance via the niche in Drosophila. Proc Natl Acad Sci U S A 2009; 106:1117-21; PMID:19136634; http://dx.doi.org/10.1073/pnas.0809144106.
40. Hsu HJ, Drummond-Barbosa D. Insulin signals control the competence of the Drosophila female germline stem cell niche to respond to Notch ligands. Dev Biol 2011; 350:290-300; PMID:21145317; http://dx.doi.org/10.1016/j.ydbio.2010.11.032.

41. Ables ET, Drummond-Barbosa D. The steroid hormone ecdysone functions with intrinsic chromatin remodeling factors to control female germline stem cells in Drosophila. Cell Stem Cell 2010; 7:581-92; PMID:21040900; http://dx.doi.org/10.1016/j.stem.2010.10.001.
42. Parisi MJ, Gupta V, Sturgill D, Warren JT, Jallon JM, Malone JH, et al. Germline-dependent gene expression in distant non-gonadal somatic tissues of Drosophila. BMC Genomics 2010; 11:346; PMID:20515475; http://dx.doi.org/10.1186/1471-2164-11-346.
43. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 2006; 125:315-26; PMID:16630819; http://dx.doi.org/10.1016/j.cell.2006.02.041.
44. Gaspar-Maia A, Alajem A, Polesso F, Sridharan R, Mason MJ, Heidersbach A, et al. Chd1 regulates open chromatin and pluripotency of embryonic stem cells. Nature 2009; 460:863-8; PMID:19587682.
45. Meshorer E, Misteli T. Chromatin in pluripotent embryonic stem cells and differentiation. Nat Rev Mol Cell Biol 2006; 7:540-6; PMID:16723974; http://dx.doi.org/10.1038/nrm1938.
46. Becker PB, Hörz W. ATP-dependent nucleosome remodeling. Annu Rev Biochem 2002; 71:247-73; PMID:12045097; http://dx.doi.org/10.1146/annurev.biochem.71.110601.135400.
47. Cherry CM, Matunis EL. Epigenetic regulation of stem cell maintenance in the Drosophila testis via the nucleosome-remodeling factor NURF. Cell Stem Cell 2010; 6:557-67; PMID:20569693; http://dx.doi.org/10.1016/j.stem.2010.04.018.
48. Casper AL, Baxter K, Van Doren M. no child left behind encodes a novel chromatin factor required for germline stem cell maintenance in males but not females. Development 2011; 138:3357-66; PMID:21752937; http://dx.doi.org/10.1242/dev.067942.
49. Yang SY, Baxter EM, Van Doren M. Phf7 controls male sex determination in the Drosophila germline. Dev Cell 2012; 22:1041-51; PMID:22595675; http://dx.doi.org/10.1016/j.devcel.2012.04.013.
50. Yamashita YM, Jones DL, Fuller MT. Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. Science 2003; 301:1547-50; PMID:12970569; http://dx.doi.org/10.1126/science.1087795.
51. Pereira G, Yamashita YM. Fly meets yeast: checking the correct orientation of cell division. Trends Cell Biol 2011; 21:526-33; PMID:21705221; http://dx.doi.org/10.1016/j.tcb.2011.05.004.
52. Yamashita YM, Mahowald AP, Perlin JR, Fuller MT. Asymmetric inheritance of mother versus daughter centrosome in stem cell division. Science 2007; 315:518-21; PMID:17255513; http://dx.doi.org/10.1126/science.1134910.
53. Inaba M, Yuan H, Salzmann V, Fuller MT, Yamashita YM. E-cadherin is required for centrosome and spindle orientation in Drosophila male germline stem cells. PLoS One 2010; 5:e12473; PMID:20824213; http://dx.doi.org/10.1371/journal.pone.0012473.
54. Yadlapalli S, Cheng J, Yamashita YM. Drosophila male germline stem cells do not asymmetrically segregate chromosome strands. J Cell Sci 2011; 124:933-9; PMID:21325028; http://dx.doi.org/10.1242/jcs.079798.
55. Riparbelli MG, Callaini G. Male gametogenesis without centrioles. Dev Biol 2011; 349:427-39; PMID:20974123; http://dx.doi.org/10.1016/j.ydbio.2010.10.021.
56. Lin H, Yue L, Spradling AC. The Drosophila fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. Development 1994; 120:947-56; PMID:7600970.
57. Deng W, Lin H. 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 1997; 189:79-94; PMID:9281339; http://dx.doi.org/10.1006/dbio.1997.8669.
58. Sheng XR, Matunis E. Live imaging of the Drosophila spermatogonial stem cell niche reveals novel mechanisms regulating germline stem cell output. Development 2011; 138:3367-76; PMID:21752931; http://dx.doi.org/10.1242/dev.065797.
59. Yuan H, Chiang CY, Cheng J, Salzmann V, Yamashita YM. Regulation of cyclin A localization downstream of Par-1 function is critical for the centrosome orientation checkpoint in Drosophila male germline stem cells. Dev Biol 2012; 361:57-67; PMID:22024320; http://dx.doi.org/10.1016/j.ydbio.2011.10.010.
60. Cheng J, Türkel N, Hemati N, Fuller MT, Hunt AJ, Yamashita YM. Centrosome misorientation reduces stem cell division during ageing. Nature 2008; 456:599-604; PMID:18923395; http://dx.doi.org/10.1038/nature07386.
61. Song X, Zhu CH, Doan C, Xie T. Germline stem cells anchored by adherens junctions in the Drosophila ovary niches. Science 2002; 296:1855-7; PMID:12052957; http://dx.doi.org/10.1126/science.1069871.
62. Wang H, Singh SR, Zheng Z, Oh SW, Chen X, Edwards K, et al. Rap-GEF signaling controls stem cell anchoring to their niche through regulating DE-cadherin-mediated cell adhesion in the Drosophila testis. Dev Cell 2006; 10:117-26; PMID:16399083; http://dx.doi.org/10.1016/j.devcel.2005.11.004.
63. Voog J, D'Alterio C, Jones DL. Multipotent somatic stem cells contribute to the stem cell niche in the Drosophila testis. Nature 2008; 454:1132-6; PMID:18641633; http://dx.doi.org/10.1038/nature07173.
64. Srinivasan S, Mahowald AP, Fuller MT. The receptor tyrosine phosphatase Lar regulates adhesion between Drosophila male germline stem cells and the niche. Development 2012; 139:1381-90; PMID:22378638; http://dx.doi.org/10.1242/dev.070052.
65. Issigonis M, Tulina N, de Cuevas M, Brawley C, Sandler L, Matunis E. JAK-STAT signal inhibition regulates competition in the Drosophila testis stem cell niche. Science 2009; 326:153-6; PMID:19797664; http://dx.doi.org/10.1126/science.1176817.
66. Singh SR, Zheng Z, Wang H, Oh SW, Chen X, Hou SX. Competitiveness for the niche and mutual dependence of the germline and somatic stem cells in the Drosophila testis are regulated by the JAK/STAT signaling. J Cell Physiol 2010; 223:500-10; PMID:20143337.
67. Wallenfang MR, Nayak R, DiNardo S. Dynamics of the male germline stem cell population during aging of Drosophila melanogaster. Aging Cell 2006; 5:297-304; PMID:16800845; http://dx.doi.org/10.1111/j.1474-9726.2006.00221.x.
68. Xie T, Spradling AC. A niche maintaining germ line stem cells in the Drosophila ovary. Science 2000; 290:328-30; PMID:11030649; http://dx.doi.org/10.1126/science.290.5490.328.
69. Klein AM, Nakagawa T, Ichikawa R, Yoshida S, Simons BD. Mouse germ line stem cells undergo rapid and stochastic turnover. Cell Stem Cell 2010; 7:214-24; PMID:20682447; http://dx.doi.org/10.1016/j.stem.2010.05.017.
70. Brawley C, Matunis E. Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. Science 2004; 304:1331-4; PMID:15143218; http://dx.doi.org/10.1126/science.1097676.
71. Sheng XR, Brawley CM, Matunis EL. Dedifferentiating spermatogonia outcompete somatic stem cells for niche occupancy in the Drosophila testis. Cell Stem Cell 2009; 5:191-203; PMID:19664993; http://dx.doi.org/10.1016/j.stem.2009.05.024.
72. Ohlstein B, Kai T, Decotto E, Spradling A. The stem cell niche: theme and variations. Curr Opin Cell Biol 2004; 16:693-9; PMID:15530783; http://dx.doi.org/10.1016/j.ceb.2004.09.003.
73. Barroca V, Lassalle B, Coureuil M, Louis JP, Le Page F, Testart J, et al. Mouse differentiating spermatogonia can generate germinal stem cells in vivo. Nat Cell Biol 2009; 11:190-6; PMID:19098901; http://dx.doi.org/10.1038/ncb1826.
74. Nakagawa T, Sharma M, Nabeshima Y, Braun RE, Yoshida S. Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. Science 2010; 328:62-7; PMID:20299552; http://dx.doi.org/10.1126/science.1182868.
75. Li L, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. Science 2010; 327:542-5; PMID:20110496; http://dx.doi.org/10.1126/science.1180794.
76. Wong C, Jones DL. Efficiency of spermatogonial dedifferentiation during aging. PLoS One 2012; 7:e33635; PMID:22442704; http://dx.doi.org/10.1371/journal.pone.0033635.
77. Tran J, Brenner TJ, DiNardo S. Somatic control over the germline stem cell lineage during Drosophila spermatogenesis. Nature 2000; 407:754-7; PMID:11048723; http://dx.doi.org/10.1038/35037613.
78. Kiger AA, White-Cooper H, Fuller MT. Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature 2000; 407:750-4; PMID:11048722; http://dx.doi.org/10.1038/35037606.
79. Schulz C, Wood CG, Jones DL, Tazuke SI, Fuller MT. Signaling from germ cells mediated by the rhomboid homolog stet organizes encapsulation by somatic support cells. Development 2002; 129:4523-34; PMID:12223409.
80. Sarkar A, Parikh N, Hearn SA, Fuller MT, Tazuke SI, Schulz C. Antagonistic roles of Rac and Rho in organizing the germ cell microenvironment. Curr Biol 2007; 17:1253-8; PMID:17629483; http://dx.doi.org/10.1016/j.cub.2007.06.048.
81. Parrott BB, Hudson A, Brady R, Schulz C. Control of germline stem cell division frequency--a novel, developmentally regulated role for epidermal growth factor signaling. PLoS One 2012; 7:e36460; PMID:22586473; http://dx.doi.org/10.1371/journal.pone.0036460.
82. Inaba M, Yuan H, Yamashita YM. String (Cdc25) regulates stem cell maintenance, proliferation and aging in Drosophila testis. Development 2011; 138:5079-86; PMID:22031544; http://dx.doi.org/10.1242/dev.072579.
83. Fast EM, Toomey ME, Panaram K, Desjardins D, Kolaczyk ED, Frydman HM. Wolbachia enhance Drosophila stem cell proliferation and target the germline stem cell niche. Science 2011; 334:990-2; PMID:22021671; http://dx.doi.org/10.1126/science.1209609.
84. Edgar BA, O'Farrell PH. Genetic control of cell division patterns in the Drosophila embryo. Cell 1989; 57:177-87; PMID:2702688; http://dx.doi.org/10.1016/0092-8674(89)90183-9.
85. Toledano H, D'Alterio C, Czech B, Levine E, Jones DL. The let-7-Imp axis regulates ageing of the Drosophila testis stem-cell niche. Nature 2012; 485:605-10; PMID:22660319; http://dx.doi.org/10.1038/nature11061.
86. Picard D, Miller S, Hawkins CE, Bouffet E, Rogers HA, Chan TS, et al. Markers of survival and metastatic potential in childhood CNS primitive neuro-ectodermal brain tumours: an integrative genomic analysis. Lancet Oncol 2012; 13:838-48; PMID:22691720; http://dx.doi.org/10.1016/S1470-2045(12)70257-7.
87. Issigonis M, Matunis E. SnapShot: stem cell niches of the Drosophila testis and ovary. Cell 2011; 145:994-e2, e2; PMID:21663800; http://dx.doi.org/10.1016/j.cell.2011.05.037.