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REVIEW

Finding a niche: studies from the *Drosophila* ovary

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Abstract

Specialized microenvironments called niches help maintain stem cells in an undifferentiated and self-renewing state. The existence of niches has long been predicted from mammalian studies, but identifying stem cells in their native environments *in vivo* has remained a challenge in most vertebrates. Many of the mechanistic insights into how niches regulate stem cell maintenance have been obtained using invertebrate models such as *Drosophila*. Here, we focus on the *Drosophila* ovarian germline stem cell niche and review recent studies that have begun to reveal how intricate crosstalk between various signaling pathways regulates stem cell maintenance, how the extracellular matrix modulates the signaling output of the niche and how epigenetic programming influences cell development and function both inside and outside the niche to ensure proper tissue homeostasis. These insights will probably inform the study of mammalian niches and how their malfunction contributes to human disease.

Introduction

Stem cells are essential for tissue homeostasis, particularly in organs that exhibit high rates of cellular turnover such as the skin, intestine and hematopoietic system. Without the self-renewing capacity of stem cells, these tissues quickly cease to function properly, leading to various conditions including infertility, anemia and immunodeficiency. Overproliferation of stem cells is equally undesirable and can disrupt normal tissue homeostasis, possibly contributing to tumor formation and growth. Interestingly, cells within tumors often exhibit a hierarchy of malignant potential, giving rise to the notion that small populations of cancer stem cells may be responsible for propagating certain cancers [1,2]. Prospectively identifying these cells and determining how they differ from their normal stem cell counterparts will probably provide important insights into the origin and progression of malignancy.

The concept of the cellular niche represents one of the central paradigms in stem cell biology. First proposed by Schofield in 1978 [3], the niche hypothesis posits that specific locations or microenvironments within tissues prevent the maturation of resident stem cells. The niche model is consistent with many observations made in mammalian cell transplantation experiments, but difficulties in unequivocally identifying individual stem cells within their native environment prevented further testing of this hypothesis. Twenty years following Schofield's seminal publication, Xie and Spradling provided compelling experimental evidence that a cellular niche supports the maintenance of germline stem cells (GSCs) in the *Drosophila* adult ovary [4]. Shortly thereafter, similar findings were reported in the *Drosophila* testis [5,6]. Taken together, the study of the *Drosophila* ovary and testis has greatly enhanced our understanding of the basic principles that govern niche formation and function. Several recent publications have reviewed studies of stem cells within the testis [7,8]. Here we will focus on reviewing work describing the formation and regulation of the ovarian stem cell niche.

Organization of the adult *Drosophila* ovary

Drosophila females have two ovaries typically comprised of 16 to 21 tube-like structures called ovarioles [9]. Each ovariole contains six to eight sequentially developing egg chambers, each of which is initially assembled in a structure at the tip of the ovariole called the germarium (Figure 1). Two to three GSCs reside at the anterior tip of the germarium immediately adjacent to the niche, which includes a small cluster of five to seven cap cells attached to eight to 10 terminal filament cells. GSCs typically undergo asymmetric self-renewing divisions, producing one daughter stem cell that remains associated with the cap cell niche and a second daughter that is displaced away from the niche and as a result differentiates. This newly formed cystoblast undergoes four incomplete mitotic divisions to form an interconnected 16-cell cyst.

Escort cells, also called inner sheath cells or inner germarium sheath cells, line the anterior region of the germarium and send extensions between germline cysts during the earliest stages of their differentiation. Recent

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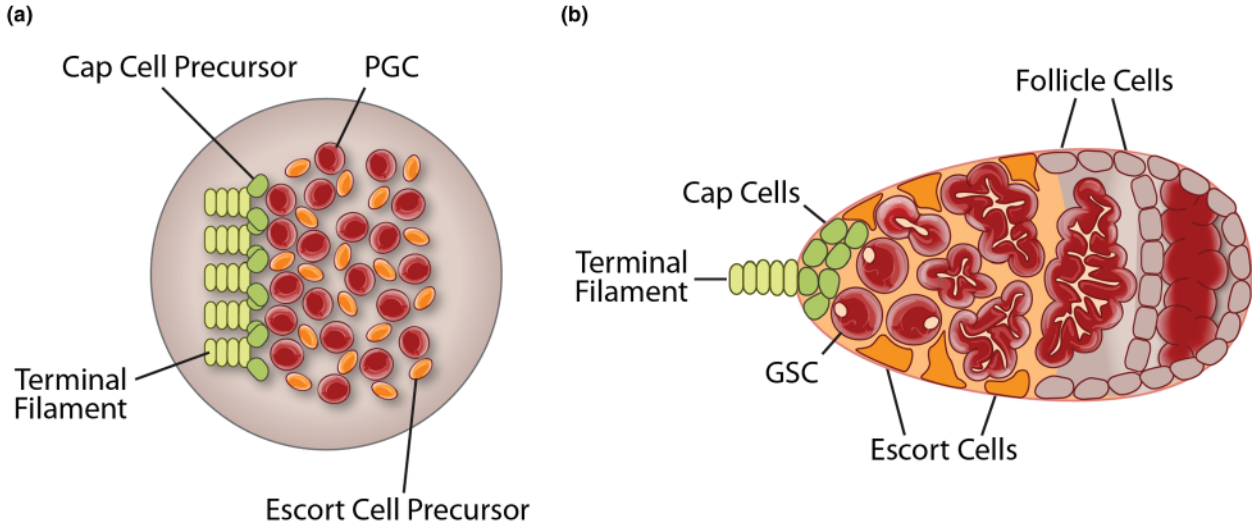

Figure 1. Organization of the developing female gonad and the adult germarium. (a) By the end of larval development, approximately 100 primordial germ cells (PGCs) (red) populate the gonad and associate with cap cell precursor (dark green) and escort cell precursor cells (orange). Terminal filament stacks (light green) begin to form and signal to adjacent somatic cells through the Delta–Notch pathway, inducing them to become cap cells. **(b)** The differentiation of adult germline cells (red) can be traced based on morphological changes in the fusome (beige), an endoplasmic reticulum-like organelle that appears round in the germline stem cells (GSCs) and becomes increasingly more branched as germline cysts develop [76]. Adult GSCs reside in a niche formed by the terminal filament (light green) and cap cells (dark green). Escort cells (orange) help to guide developing cysts as they pass through the germarium. Eventually a single layer of follicle cells (grey) surrounds the germline cysts and these enveloped cysts bud off the germarium to form an egg chamber.

live imaging experiments show that these escort cells help maturing germline cysts move posteriorly through the germarium [10]. Eventually progeny of two follicle stem cells envelop the 16-cell germline cyst, and together this cluster of cells buds off from the germarium to form an egg chamber.

Many of the aforementioned cell types can be identified at single-cell resolution based on the architecture of the germarium and through the use of morphological and molecular markers. The ability to distinguish individual cells within their native environment, coupled with the ability to genetically manipulate these cells, makes the *Drosophila* germarium a powerful platform with which to dissect the molecular mechanisms governing stem cell maintenance.

Bone morphogenetic protein signaling in the adult germline stem cell niche

Significant progress has been made in defining the signaling events that promote GSC self-renewal (Figure 2). One of the principle ligands required for GSC maintenance is Decapentaplegic (Dpp), a member of the bone morphogenetic protein (BMP) superfamily of signaling molecules [11]. Glass bottom boat (Gbb), a BMP5/6/7/8 homolog [12], also functions to support GSC maintenance [13]. Disruption of either *dpp* or *gbb* results in GSC loss, while overexpression of *dpp*, but not *gbb*, causes a GSC tumor phenotype. RT-PCR analysis of isolated cells suggests that several different subpopulations of somatic cells at the anterior tip of the germarium express *dpp* and *gbb* [13]. *In situ* hybridization also detects *dpp* transcripts within this region [4,14,15].

BMP ligand produced at the anterior tip of the germarium transduces its effects through the type I receptors Thickveins and Saxophone and the type II receptor Punt. Genetic mosaic experiments show that these receptors function autonomously in GSCs and are necessary for their maintenance [11,16]. Activation of the receptor complex results in phosphorylation of Mothers Against Dpp (Mad), which then binds to its partner Medea [17] and translocates into the nucleus. Phosphorylated Mad and Medea associate with a specific silencer element in the promoter of the *bag of marbles* (*bam*) gene and repress its transcription [13,18,19]. Bam expression is both necessary and sufficient for germline differentiation [20-22]. Loss of *bam* results in germline tumors that contain undifferentiated cells that exist in a pre-cystoblast state, whereas misexpression of *bam* in GSCs results in their precocious differentiation.

BMP pathway activation also results in high levels of *Daughters against dpp* (*Dad*) expression in GSCs [13,23,24]. In GSC daughters displaced away from the cap cells, Dad expression decreases whereas *bam* transcription increases. Remarkably, this switch in *Dad* and *bam* expression occurs one cell diameter away from the cap cells. Several studies have begun to describe some of

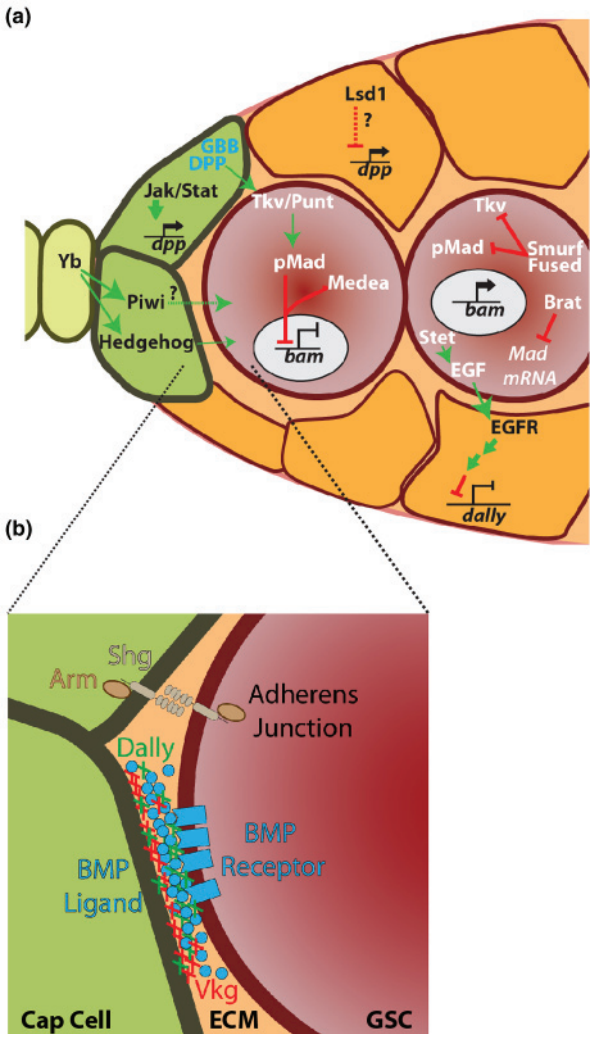

Figure 2. Signaling within the female germline stem cell niche. **(a)** Schematic illustrating that Decapentaplegic (Dpp) and Glass bottom boat (Gbb) produced in the anterior of the germarium binds to heterodimeric receptors on the surface of germline stem cells (GSCs). Activation of the receptor results in phosphorylation of Mad (pMad) which then partners with Medea and translocates into the nucleus, where it directly represses the transcription of *bag of marbles* (*bam*). This repression is relieved once a GSC daughter leaves the cap cell niche. Smurf, Fused, Brain tumor (Brat) and miR-184 all act to rapidly reduce bone morphogenetic protein (BMP) responsiveness within the cystoblast. Niche signaling is limited to the anterior of the germarium by Lsd1, which represses *dpp* expression outside the normal niche and by epidermal growth factor (Egf) signaling from the germline, which serves to limit *dally* expression in the escort cells. EGFR, epidermal growth factor receptor; JAK/STAT, Janus kinase/signal transducer and activator of transcription; pMad, phosphorylated Mothers Against Dpp; Tkv, Thickveins; YB, Female sterile (1) Yb. **(b)** Components of the extracellular matrix (ECM), including Viking (Vkg; red) and Division abnormally delayed (Dally; green) help to stabilize and limit BMP ligands (blue circles) within the anterior of the germarium. The adherens junction proteins Armadillo (Arm; brown) and Shotgun (Shg; grey) promote cell–cell adhesion between the cap cells (green) and GSCs (dark red).

the intrinsic mechanisms responsible for this sharp gradient of BMP responsiveness. During *Drosophila* embryogenesis, the E3 ubiquitin ligase Smurf has been shown to oppose BMP signaling by targeting Mad for degradation [25]. Consistent with these observations, Smurf mutants also display greater Dpp responsiveness within the germline [23]. A recent study describes how Smurf partners with the serine/threonine kinase Fused to antagonize BMP signaling within cystoblasts and differentiating cysts by promoting the degradation of Thickveins [26]. In addition, the translational regulator Brain Tumor (Brat) acts as a germline differentiation factor and represses both *Mad* and *dMyc* [27]. Lastly, *mir-184* appears to regulate Saxophone levels within the cystoblast [16].

These findings suggest that multiple mechanisms ensure a very rapid downregulation of Dpp responsiveness in germline cells once they leave the niche. However, overexpression of *dpp* in somatic cells blocks germline differentiation [11,13], suggesting the existence of a Dpp signaling threshold above which pathway activation can overcome endogenous antagonists.

Building upon our understanding of how the Dpp–Thickveins–phosphorylated Mad–Bam pathway controls GSC maintenance, the field is beginning to delve more deeply into how the ovarian niche first forms, how Dpp signaling from the niche is modulated and how the niche responds to environmental cues. Addressing these fundamental questions will provide a framework with which to better understand niches across species.

Formation of the ovarian niche

GSCs arise from primordial germ cells (PGCs) that first form at the posterior pole of the embryo. Through a series of migratory events, these PGCs make their way towards the gonadal mesoderm and eventually coalesce with a subpopulation of surrounding somatic cells to form the embryonic gonad [28]. Initially, about seven to 13 PGCs are incorporated into each gonad [29]. This number expands to approximately 100 by the end of larval development. Cell–cell communication involving the epidermal growth factor (EGF) pathway helps to coordinate the expansion of the germline with the surrounding gonadal mesoderm [30].

Transformation of the primitive gonad into an adult ovary begins during late larval development, starting with the formation of terminal filaments [31] (Figure 1). These structures are composed of eight to 10 disc-shaped cells that demarcate individual ovarioles in the developing ovary. They arise from small clusters of cells that organize themselves into stacks. The actin-depolymerizing factor Cofilin/ADF, encoded by the *twinstar* gene, regulates the actin cytoskeletal rearrangements that drive the intercalation of presumptive terminal filament cells

[32]. Terminal filament formation occurs progressively, in a medial to lateral direction across the gonad [33]. The steroid hormone ecdysone or its metabolites probably govern the timing of these morphogenic events, as mutations in the *ecdysone receptor* or its binding partner *ultraspiracle* result in heterochronic defects and malformation of these structures [34].

While the mechanisms that designate specific somatic cell fates across the larval gonad remain unclear, enhancer trap screens revealed a small number of genes that exhibit high levels of expression in the developing terminal filament [33]. One of these genes, *bric-a-brac* (*bab*), encodes a BTB/POZ domain transcription factor [33,35]. The expression of *bab* is first observed in the female gonad during late larval development and continues to mark terminal filament cells through adulthood. Disruption of *bab* results in terminal filament defects accompanied by severe morphological defects in the adult ovary, revealing that the overall organization of the adult ovary depends on proper terminal filament formation. A second transcription factor Engrailed also marks terminal filaments and is necessary for their development [36]. Identifying the transcriptional targets of Bab and Engrailed within the developing gonad remains important work for the future.

Cap cells, which help form the functional GSC niche in the adult ovary, are specified as the terminal filament formation nears completion. Cap cells can be distinguished based on a number of morphological and molecular markers. They form immediately adjacent to the posterior tips of the terminal filaments and express *bab*, *engrailed*, *hedgehog* and high levels of Lamin C [4,33,37,38], but are not incorporated into the growing terminal filament stack. Several studies have shown that the Notch pathway helps to promote cap cell formation [39,40]. Xie and colleagues showed that terminal filament cells express the Notch ligand Delta shortly after they begin to organize [39]. Delta activates Notch in adjacent somatic cells, inducing them to become cap cells. Overexpression of Delta or an activated form of Notch results in an accumulation of ectopic cap cells in the adult ovary. These extra cap cells are associated with ectopic GSCs, indicating that they act as functional niches. Heterozygous Notch mutant germaria carry a decreased number of cap cells, suggesting that Notch signaling is both necessary and sufficient for cap cell formation in the developing gonad. The expression of the *E(spl)m7-LacZ* Notch target reporter suggests that Notch signaling remains active in adult cap cells. Indeed, disruption of Notch specifically in adults leads to a decrease of cap cells within adult germaria over time and a subsequent reduction in the number of GSCs [39]. Overexpression of activated Notch in adult escort cells does not convert them into cap cells or result in ectopic niche formation, indicating that escort cell identity becomes set during pupal development. The basis for the stabilization of this cell fate remains uncharacterized.

Stem cell capture by the niche

Of the approximately 100 PGCs that populate each larval gonad, only a subset become GSCs while the rest differentiate to form germline cysts. The hallmarks of GSC selection become evident during the larval to pupal transition and involve a number of mechanisms. While germline cells of the larval gonad do not express *bam*, they differentiate in response to ectopic *bam* expression [41,42]. Moreover, all PGCs exhibit phosphorylated Mad expression prior to terminal filament formation, suggesting that BMP signaling blocks *bam* expression in larval gonads as it does in adults [41,42].

Upon terminal filament formation, PGCs begin to exhibit spatially restricted changes in gene expression. In the posterior of the gonad, away from the terminal filaments, germline cells begin to express *bam* and show morphological signs of cyst development, while germline cells immediately adjacent to the terminal filament and newly established cap cells remain undifferentiated and express markers of Dpp signal responsiveness [42]. These cells, which probably give rise to adult GSCs, can undergo clonal expansion, giving rise to daughter GSCs that inhabit the same adult germarium. These findings suggest a simple model wherein PGCs immediately adjacent to cap cells receive BMP signals, continue to repress *bam* transcription and thus become incorporated into the maturing cap cell niche.

Additional enhancer trap and cell transplantation experiments suggest there may be a bias in which PGCs associate with the nascent niche and ultimately become GSCs [43]. This mechanism appears flexible, however, as the same PGC can give rise to cells located both inside and outside the niche during its initial formation. How Dpp production and responsiveness become restricted during the transition from the larval/pupal gonad to the adult ovary and how PGCs home in on the newly formed niches remain unclear.

Modulation of adult niche signaling by the extracellular matrix

Recent work has begun to characterize how the extracellular matrix modulates BMP signaling in the adult ovarian niche. For example, type IV collagen – encoded by the *viking* gene – regulates the distribution of Dpp and helps foster interactions between BMP ligands and their receptors in the embryo [44]. Disruption of *viking* results in a modest GSC expansion phenotype, suggesting that this extracellular matrix component restricts the spread of Dpp, thereby creating a very localized source of ligand at the anterior tip of the germarium (Figure 2).

The *division abnormally delayed* (*dally*) gene, a member of the glypican family of integral membrane heparin sulphate proteoglycans [45], also plays a critical role in regulating the distribution and stability of Dpp within the ovarian GSC niche. Dally, like other heparin sulphate proteoglycans, is a component of the extracellular matrix and covalently attaches to the plasma membrane by glycosylphosphatidylinositol linkage [45]. Heparin sulphate proteoglycans act as co-receptors for a variety of secreted proteins such as Wnts, Fibroblast Growth Factors, Transforming Growth Factor beta and Hedgehog [46]. In *Drosophila*, Dally promotes the stability and transport of Dpp [47]. Dally is expressed in the cap cells, and *dally* mutants display a GSC loss phenotype accompanied by reduced Dpp signaling and premature expression of Bam within the germline [48,49]. In contrast, *dally* overexpression in somatic cells outside the niche results in an expansion of GSC-like cells [14,48,49]. While these findings show that the extracellular matrix modulates Dpp signaling within germaria, further work will be needed to elucidate the mechanisms that coordinate the deposition of extracellular matrix components within the niche and control their functions.

Pathways that regulate niche signaling.

Several additional molecules function in the niche, either through or in parallel to Dpp signaling. The genes *female sterile (1) Yb* (*Yb*), *hedgehog* and *piwi* are expressed in somatic cells at the anterior tip of the germarium [37,50-53]. Loss of *Yb*, a large hydrophilic protein with limited homology to RNA helicases, disrupts the maintenance of both GSCs and follicle stem cells within the germarium [52,53]. Mutations in *piwi*, which encodes the founding member of a highly conserved family of proteins that function in various small RNA pathways, also cause a significant GSC loss phenotype. Overexpression of *piwi* within somatic cells of the germarium results in an expanded number of GSCs [50,51]. Hedgehog-mediated signaling primarily regulates follicle stem cells, but *hedgehog* mutants also exhibit a mild GSC loss phenotype [37,38,53]. *Yb* mutants exhibit reduced *hedgehog* and *piwi* expression in terminal filament and cap cells [53]. Further genetic evidence suggests that *Yb* regulates, through *piwi*-dependent and *hedgehog*-dependent mechanisms, parallel pathways that control GSC and follicle stem cell maintenance, respectively. *piwi* appears to regulate GSCs in a *dpp*-independent manner [53], suggesting that additional unidentified GSC maintenance signals emanate from the cap cells.

Recent work shows that components of the Janus kinase/signal transducer and activator of transcription (Jak/Stat) pathway promote Dpp production by cap cells [15,54,55]. Overexpression of the Jak/Stat ligands *unpaired* and *unpaired-2* in somatic cells results in GSC tumor formation, while mutations in pathway components cause a GSC loss phenotype [15,54,55]. Stat reporters show activation of the pathway in somatic cells at the anterior tip of the germarium, and clonal analysis reveals that pathway activation in cap cells is critical for GSC maintenance. Disruption of the Jak/Stat pathway does not affect terminal filament or cap cell formation and, unlike the Notch pathway, overactivation of the Jak/Stat pathway during development does not result in ectopic cap cells. Transcript analysis shows that the Jak/Stat pathway positively regulates *dpp* mRNA levels, providing a simple model for how this pathway promotes GSC self-renewal [15,55].

Several lines of evidence indicate that the germline itself can signal back to the surrounding somatic cells to regulate their signaling output. As described above, the EGF pathway functions to regulate PGC and somatic cell numbers in the developing gonad [30]. This pathway also functions in adult germaria. Disruption of the *stem cell tumor* gene results in the cell-autonomous failure of germline differentiation in both male and females [56]. Stem cell tumor protein shares sequence similarity with Rhomboid and proteins within this class act to cleave transmembrane EGF proteins in the Golgi, thereby creating a diffusible ligand. EGF ligands produced by germline cells in turn activate the EGF receptor–RAS–RAF–MEK–mitogen-activated protein kinase pathway in the surrounding somatic cells of the germarium. This activation of the EGF pathway limits the number of GSCs in germaria by repressing *dally* expression in escort cells [14]. In contrast, disruption of EGF signaling causes an increase of *dally* expression outside the normal niche, presumably resulting in a broader distribution of stable Dpp [14]. In effect, this feedback loop ensures that differentiating germline cysts experience lower levels of BMP signaling.

Cell adhesion and cell competition in the adult niche

Drosophila E-cadherin promotes stem cell maintenance by anchoring the GSCs to the cap cells [57]. Encoded by the *shotgun* (*shg*) gene, E-cadherin is highly enriched at the adherens junctions between the cap cells and GSCs. Armadillo, a β-catenin homolog, also localizes to these sites. The *shotgun* and *armadillo* mutant GSCs quickly leave the anterior of the germarium [57]. The findings that *shotgun* and *armadillo* mutant PGCs within the developing gonad exhibit reduced interactions with newly formed cap cells [57] and the observation that E-cadherin contributes to the age-dependent decline of adult GSCs [58] highlight the importance of cell adhesion in promoting interactions between stem cells and their niches throughout life.

Several studies have shown that individual GSCs compete for space within niches [59,60]. Whether a

particular stem cell is more or less competitive often depends on expression levels of E-cadherin [59]. GSCs with relatively high levels of E-cadherin exhibit more competitiveness than neighboring cells and tend to have larger areas of contact with the cap cells. Bam, and its binding partner Benign gonial cell neoplasm [61], negatively regulate E-cadherin. The *bam* and *benign gonial cell neoplasm* mutant GSC clones express high levels of E-cadherin and outcompete the neighboring wild-type GSCs for the niche [59]. These results suggest that an important part of the GSC differentiation program may involve the rapid downregulation of genes involved in fostering cell–cell contacts between these stem cells and adjacent niche cells.

Insulin signaling influences the niche

Systemic factors that vary in response to diet and age play an important role in modulating niche output and stem cell responsiveness to niche signals. For example, insulin signaling contributes to the maintenance of the niche in adult ovaries. Activation of the insulin pathway through inhibition of FOXO by phosphatidylinositol 3-kinase activates Notch signaling in the cap cells [62]. *Drosophila insulin receptor* (*dinr*) mutants have a time-dependent cap cell loss phenotype, leading to a reduction of GSCs over time [63]. *dinr* mutants exhibit severely reduced Notch signaling, and expressing an activated form of Notch rescues the *dinr* mutant cap cell and GSC loss phenotypes. Moreover, insulin signaling influences E-cadherin levels at the junction between cap cells and GSCs as *dinr* mutant cap cells display decreased levels of E-cadherin, independent of Notch signaling. Steroid hormones also contribute to the formation and regulation of GSC maintenance [64,65], suggesting that multiple systemic inputs impinge upon the niche during development and in adulthood.

Programming inside and outside the niche

Several studies have begun to reveal how epigenetic programming regulates the function and identity of somatic cells within the niche. For example, mutations in the gene encoding the chromatin-associated protein Corto suppress the GSC loss phenotype exhibited by *piwi* mutants [66]. Disruption of *corto* also restores *hedgehog* expression in *Yb* mutant germaria. Corto protein interacts with both Polycomb and trithorax group proteins, suggesting that these chromatin-associated proteins may influence *Yb*, *piwi* and *hedgehog*-mediated regulation of the niche.

Piwi and small piwi-interacting RNAs (piRNAs) play an essential role in programming chromatin within the germarium and in defending the germline against unwanted transposable element activity [67-70]. Recent results show that piRNA production is intimately linked with heterochromatin formation [70]. Functional analysis of *eggless*, a histone methyltransferase that acts to modify lysine 9 on histone H3 (H3K9), shows that this histone modification enzyme is needed for normal germline differentiation [70,71]. Loss of *eggless* results in sterility marked by the accumulation of undifferentiated germ cells, a reduction of piRNA production and a subsequent increase in transposable element levels. Interestingly, Eggless activity is required in both germ cells and in the surrounding escort cells. Vreteno, a tudor domain-containing protein involved in piRNA production, is also required in both the germline and surrounding somatic cells [72]. Exploring the links between germline and somatic piRNA-mediated chromatin silencing and how they relate to the function of the niche will be important work for the future.

Loss of another chromatin-associated protein, the histone demethylase Lsd1, results in the formation of GSC tumors [73-75]. Lsd1 acts in a cell nonautonomous manner, and cell-specific knockdown experiments show that Lsd1 functions in escort cells to repress the expression of niche-specific signals [74]. Undifferentiated germ cells in Lsd1 mutants exhibit increased Dpp signaling, and reducing *dpp* levels within escort cells suppresses the Lsd1 phenotype. The loss of Lsd1 during development results in the misexpression of cap cell markers in escort cells. While lineage tracing needs to be performed to determine whether cap cells and escort cells have a common precursor, the finding that escort cells can potentially express cap cell markers and *vice versa* suggests that these two cell populations may have similar developmental potential within the developing gonad [13,39,42,74]. Furthermore, these findings suggest that certain factors play a crucial role in limiting the size of the cap cell niche.

Lsd1 also functions to repress *dpp* expression in adult escort cells independent of any changes in cell fate [74]. Whether Lsd1 directly targets the *dpp* gene or some upstream regulator remains unknown. Lsd1 expression is ubiquitous within the germarium, and overexpression of an Lsd1 transgene in cap cells does not result in a stem cell loss phenotype (SE and MB, unpublished data). Lsd1 activity may therefore be spatially limited in some way or this histone demethylase could be recruited to specific sites by other proteins that have more cell-specific expression patterns. The characterization of Lsd1 function in escort cells reveals that the active repression of niche-specific signals outside the normal microenvironment may be essential for proper tissue homeostasis in certain contexts.

Conclusions

Over the past decade, the study of *Drosophila* GSCs has yielded a wealth of information about the fundamental principles that govern cellular niches, and the

characterization of *in vivo* mammalian niches will certainly benefit from these lessons. Mammalian niches will probably share common features with *Drosophila* GSC niches, but perhaps they will also share common markers as well. Aside from these cross-species comparisons, many basic questions about niche biology remain. How does the signaling output of the niche change in response to environmental cues or to aging? How does metabolism affect the size of the niche? How do stromal cells inside and outside the niche interact with one another? How are niche cells specified and how is their fate stabilized? Do niche cells perform functions aside from producing localized signaling molecules? We can anticipate that the continued study of model stem cell systems will lead to a deeper understanding of the formation and function of niches across tissues and across species, improved tissue engineering, advances in regenerative medicine and insights into how perturbations in microenvironments contribute to human disease.

This article is part of a review series on *Stem cell niche*. Other articles in the series can be found online at http://stemcellres.com/series/stemcellniche

Abbreviations

Bab, Bric-a-brac; Bam, Bag of marbles; BMP, bone morphogenetic protein; Dad, Daughters against dpp; Dally, Division abnormally delayed; Dinr, Drosophila insulin receptor; Dpp, Decapentaplegic; EGF, epidermal growth factor; Gbb, Glass bottom boat; GSC, germline stem cell; JAK/STAT, Janus kinase/signal transducer and activator of transcription; Mad, Mothers Against Dpp; PGC, primordial germ cell; piRNA, piwi-interacting RNA; RT-PCR, reverse transcriptase-polymerase chain reaction; YB, Female sterile (1) Yb.

Competing interests

The authors declare that they have no competing interests.

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