Review Article

Keeping stem cells under control: New insights into the mechanisms that limit niche-stem cell signaling within the reproductive system†

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Abbreviations: BMP, bone morphogenetic protein; Dpp, Decapentaplegic; ECM, extracellular matrix; GDNF, glial cell-derived neurotrophic factor; GSC, germ line stem cells; MT-nanotubes, microtubule-based nanotubes; SSC, spermatogonial stem cell.

Quote: *Research on germ line stem cells has revealed remarkable complexity and precision in signaling mechanism regulating stem cell identity, differentiation, and asymmetric divisions.*

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SUMMARY

Adult stem cells reside in specialized microenvironments, called niches, that maintain stem cells in an undifferentiated and self-renewing state. Defining and understanding the mechanisms that restrict niche signaling exclusively to stem cells is crucial to determine how stem cells undergo self-renewal while their progeny, often located just one cell diameter away from the niche, differentiate. Despite extensive studies on the signaling pathways that operate within stem cells and their niches, how this segregation occurs remains elusive, Here we review recent progress on the characterization of niche-stem cell interactions, with a focus on emerging mechanisms that spatially restrict niche signaling.

INTRODUCTION

Cells communicate with their neighbors in the correct manner and at the right time to build and maintain functional tissues and organs. Only a handful of signaling pathways appear to mediate the majority of cell-to-cell communication within complex tissues. Although much has been learned about the molecular mechanics of these pathways, how signal transduction is spatially and temporally regulated in such a precise manner in vivo remains less well understood.

Adult tissue homeostasis depends on the correct spatio-temporal regulation of signaling between stem cells and their cellular neighbors. Improper signaling can lead to maladaptive increases or decreases in stem cell numbers, possibly resulting in cancer or tissue degeneration. Mechanisms that adjust stem cell signaling in the face of ever-changing conditions ensure the proper balance of stem cell self-renewal and differentiation needed for normal tissue function (reviewed in Morrison and Kimble 2006; Rando 2006). In this review, we highlight recent insights into the mechanisms that fine-tune stem cell signaling in vivo, with a particular focus on the reproductive system, as the underlying mechanisms involved in regulating stem cell-niche signaling in the ovary and testis are likely used in other stem cell systems as well.

STEM CELL NICHES AND SIGNALING

The "niche" hypothesis, first proposed by Schofield in 1978, posits that local environments determine whether or not stem cells remain in an undifferentiated state in vivo (Schofield 1978). Since this original publication, numerous cellular and non-cellular niches have been described in the literature (reviewed in Morrison and Spradling 2008; Wagers 2012; Scadden 2014). In "cellular niches", dedicated niche cells form specialized microenvironments that promote stem cell self-renewal and/or prevent stem cell differentiation. Niche cells influence stem cell behavior by producing various signaling molecules, such as Delta, Hedgehog, Bone morphologic proteins (BMPs), Wnt/Wingless, cytokines, chemokines, and other growth factors (reviewed in Li and Xie 2005; Morrison and Spradling 2008). In "non-cellular niches", extracellular molecules, such as extracellular matrix (ECM) proteins, provide essential signals that create the niche. The ECM can also concentrate self-renewing signaling molecules that originate from distant sources, thus creating a specialized microenvironment for stem cells. Variables beyond the niche itself can also influence stem cell behavior, division rates, and survival (reviewed in Li and Xie 2005; Morrison

and Spradling 2008). For example, pH, oxygen, ions, mechanical force, and electrical stimuli can all modulate stem cell activity, adding to the complexity in niche-mediated stem cell regulation (reviewed in Wagers 2012).

Significant progress has been made in understanding which niche signals foster stem cell self-renewal, yet a considerable lack of understanding remains regarding the mechanisms that prevent inappropriate delivery of self-renewing signals to stem cell progeny that have left the niche. Further insights into these mechanisms will have important implications for our understanding of tissue homeostasis and disease.

GERM LINE STEM CELL SYSTEMS IN INVERTEBRATE MODEL ORGANISMS

The germ line stem cells (GSCs) of *Caenorhabditis elegans* and *Drosophila melaongaster* have long served as useful models for studying stem cell niches. The simplicity and accessibility of worm and fly gonads, combined with the availability of robust and sophisticated genetic tools, have greatly accelerated the characterization of the in vivo cellular niches that help to maintain GSCs.

The *C. elegans* gonad represents perhaps one of the simplest examples of a cell-based stem cell niche: A distal tip cell, located at the tip of each gonad arm, extends a number of cellular projections that make contact with a small group of undifferentiated and mitotically active germ cells (Fig. 1A). Ablation of the distal tip cell causes germ cells at the tip of the gonad to exit mitosis and to initiate the meiotic program. Further work has shown that the distal tip cell prevents undifferentiated germ cells from entering meiosis via Notch signaling pathway (see below; also reviewed in Byrd and Kimble 2009; Kimble 2014).

Drosophila gonads house slightly more complex cellular niches. In male Drosophila, a cluster of hub cells located at the apical tip of each testis provides a niche for GSCs, whereas in females, a small group of 5-7 cap cells help form the female GSC niche in the ovary. Hub cells and cap cells both produce a number of ligands that are essential for GSC self-renewal. In males, hub cells produce Unpaired (Upd), a ligand in the Jak/Stat signaling pathway, and Decapentaplegic (Dpp) and Glass bottom boat (Gbb), ligands in the BMP pathway. BMP signaling also promotes GSC maintenance in the ovary (Michel et al. 2012; Amoyel et al. 2013; Luo et al. 2015). In both male and female Drosophila gonads, ectopic expression of niche ligands leads to expansion of GSC-like cells outside of the normal niche and/or delays the differentiation of GSC progeny,

demonstrating that niche-produced factors play a major role in stem cell fate determination (Xie and Spradling 1998; Kiger et al. 2001; Tulina and Matunis 2001).

THE MAMMALIAN SPERMATOGONIAL STEM CELL NICHE

Recent work has cast light on the complex nature of niche-stem cell interactions within the mammalian testis. Spermatogonia reside within the basal compartment of the seminiferous tubules, and are classified as A_{single}, A_{paired}, $A_{aligned}$, intermediate, and B-subtypes, based on morphological and molecular markers (Oatley and Brinster 2012; Chen and Liu 2015). Recent work using lineage tracing has shown that a PAX7-positive subset of the A_{single} population contains bona fide spermatogonial stem cells (SSCs) that are fast-cycling and have long-term self-renewal capacity (Aloisio et al. 2014). ID4 also marks a rare subset of A_{single} spermatogonia that are potentially enriched for stem cells (Chan et al. 2014; Sun et al. 2015). The relationship between PAX7-positive and ID4-positive A_{single} cells remains unknown; further quantitative analysis should help determine if and how these A_{single} cells parse as bona fide stem cells. Whether or not niche signaling directly influences the *Id4* and/or *Pax7* expression in neighboring germ cells, thus conferring SSC identity, represents a point of significant interest.

Glial cell-derived neutrophic factor (GDNF), a member of the Transforming growth factor beta superfamily of signaling molecules, and its receptor, GDNF-family receptor-α1 (GFRα1), comprise a core SSC self-renewal signaling pathway (Oatley and Brinster 2012; Chen and Liu 2015). GFRα1 is expressed in subsets of A_{single}, A_{paired}, and $A_{aligned}$ cells (Grasso et al. 2012), while Sertoli cells express GDNF (Meng et al. 2000). Interestingly, A_{paired} and $A_{aligned}$ cells have the ability to fragment into single cells under certain conditions, suggesting that these different cell types can dedifferentiate in response to niche signals (Nakagawa et al. 2010). Sertoli cells directly support the maintenance of SSCs, based on transplantation experiments (Oatley et al. 2011) and genetic evidence that *Gdnf* heterozygous mutants exhibit premature differentiation of SSCs (Meng et al. 2000) while decreases in *Gdnf* expression correlate with fewer functional SSCs during the course of aging (Ryu et al. 2006). Conversely, overexpression of *Gdnf* blocks germ cell differentiation, giving rise to an expansion of undifferentiated stem cell-like germ cells (Meng et al. 2000). These findings collectively point to Sertoli cell-produced GDNF as a critical factor in the maintenance of SSCs.

Despite the clear niche-stem cell signaling relationship that exists between Sertoli-derived GDNF and GFR-α1-expressing spermatogonial cells, several fundamental questions

remain: (i) GDNF-GFR-α1 signaling likely occurs in a population of cells broader than bona fide SSCs (including $A_{aligned}$ populations). Does this indicate that the bona fide niche (e.g. potentially a subset of Sertoli cells) provides additional unidentified SSC-specifying signals? (ii) Sertoli cells are present throughout the seminiferous tubules, but the number and position of potential SSCs appears more limited. Does only a subset of Sertoli cells create and maintain niches? (iii) Sertoli cells are large cells that occupy space from the basement membrane to the lumen of the seminiferous tubules, and are thus intimately involved with and contact male germ cells at all stages – from SSCs to differentiating spermatids. How do Sertoli cells specify SSC identity while simultaneously encapsulating (and likely regulating) spermatid differentiation?

Evidence is building for the contribution of other signaling pathways to the SSC niche. Oatley et al. showed that Leydig cells and select peritubular myoid cells express colony-stimulating factor 1 (CSF1). An SSC-enriched Thy-positive population of germ cells expresses the receptor for CSF1, and recombinant CSF1 appears to enhance SSC self-renewal (Oatley et al., 2009). A more recent study showed that interstitial macrophages also express CSF1, in addition to enzymes involved in retinoic acid biosynthesis (DeFalco et al. 2015). The phenotypes resulting from the depletion of macrophages within the testis remain somewhat controversial, however, as early studies suggest that the loss of macrophages disrupt meiotic progression within germ cells whereas more recent findings indicate that ablation of macrophages results in reduced numbers of $A_{aligned}$ cells (Cohen et al. 1996; Cohen et al. 1997; Pollard et al. 1997; DeFalco et al. 2015).

Niche size is also flexible and regulated by other factors. For example, follicle-stimulating hormone and testosterone influence the activity of Sertoli cells (Oatley and Brinster 2012; Smith and Walker 2014). Other studies suggest that the basement membrane that lines seminiferous tubules and peritubular myoid cells may promote SSC maintenance; indeed, recent data implied that peritubular myoid cells express GDNF and can support SSC self-renewal in culture (Chen et al. 2014). The vasculature of the testis also appears to influence stem cell renewal as careful analysis using live cell imaging of mouse gonads showed that A_{single} cells tend to reside close to the vascular network, whereas their differentiating daughters move away from these regions and disperse through the basal compartment of the testis (Yoshida et al. 2007); however, a recent study on ID4-positive SSCs reported that this population of SSCs does not associate with the vasculature (Chan et al. 2014). Therefore, caution should be taken when considering vasculature as a possible niche component. A valve-like terminal segment of the seminiferous tubules may also support SSC maintenance in hamster testis, suggesting that niches come in different varieties

(Aiyama et al. 2015). Contributions of various cell types might explain why not all Sertoli cells can form and maintain the SSC niche: the combination of signals from Sertoli cells, Leydig cells, macrophages, and possibly additional somatic cells may be required to fully define the functional niche.

The active participation of somatic cells to maintenance of the SSC niche could be deceiving, given the possibility that intrinsic fate determinant(s) segregated during SSC divisions might conferring SSC identity to those that inherit the determinants. In this scenario, GDNF-expressing Sertoli cells may be the only population needed to provide SSC niche functionality as stem cell identity would be determined by cell-intrinsic fate determinants within the SSCs themselves.

The most challenging question is how a single population of Sertoli cells simultaneously regulate SSC and differentiating germ cells. Tight junctions form between Sertoli cells and germ cells, suggesting that germ cells are subjected to spatially segregated and distinct signaling events during each phase of spermatogenesis. Secretion from Sertoli cells may also be polarized (i.e. GDNF is only secreted toward the SSC area, whereas other factor(s) are secreted towards a different domain of the Sertoli cell surface). Alternatively, germ cells and/or Sertoli cells may extend distinct sets of nanotubes/cytonemes that mediate specific signaling (see "Protrusion-mediated access to ligand source"). Considering that Drosophila trachea air sac primordium extend distinct sets of cytonemes (fibroblast growth factor-specific cytonemes and Dpp-specific cytonenes) toward different target cells (Roy et al. 2014), the involvement of cytonemes in maintaining the testis SSC niche remain a possibility.

WHAT RESTRICTS NICHE SIGNALING?

Many signaling pathways contribute to the function of GSC systems; however, the mechanisms that restrict niche signaling to foster the appropriate expansion of stem cells needed for tissue homeostasis under different environmental conditions remains poorly understood. Recent work using simple model systems may provide important clues that inform the types of mechanisms utilized to limit signaling in different contexts. Below, we describe several biological processes that can modulate the range of the niche signaling within model systems.

Tissue geometry

Tissue architecture, and more specifically the exact spatial positioning of cells relative to one another, can dictate cell fate. GSCs in model systems directly adhere to their niche cells (Fig. 1): Drosophila GSCs, for example, typically align their spindles perpendicularly toward the hub or cap cells, placing one daughter cell in direct contact with the niche while displacing the other daughter away from the niche (Yamashita et al. 2003). Such positioning ensures an asymmetric outcome of to the division – i.e. self-renewal and differentiation (Fig. 1B). The close proximity of GSCs to their differentiating daughter cells, versus their distinct fates, indicate that the effective range of niche signaling is tightly restricted. Considering that many niche ligands act over a long range (~100 μm) in other contexts – such as Dpp in developing imaginal discs – mechanisms that limit the effective range of these ligands within the niche to 1 cell diameter (~7μm) must be in place.

Juxtacrine or contact-dependent signaling

By its very nature, contact-dependent or "juxtacrine" signaling allows for highly selective cell-to-cell communication. The Notch pathway (reviewed by Kopan and Ilagan 2009) represents one of the best-studied examples of juxtacrine signaling: Notch and its ligands are transmembrane proteins, so activation of this signaling pathway occurs only when the communicating cells are in direct contact with one another. These molecules are not released into the extracellular space, further minimizing the possibility of ectopic signaling.

The Notch pathway functions in a number of stem cell niches (Liu et al. 2010). Within the *C. elegans* gonad, Notch signaling keeps GSCs in an undifferentiated state by repressing three pathways: gld-1, gld-2, and a third meiotic entry pathway that remains poorly understood (Kadyk and Kimble 1998; Eckmann et al. 2004; Hansen et al. 2004; Fox et al. 2011). The distal tip cell expresses the Notch ligand LAG-2 while the germ line expresses the receptor GLP-1. Notch pathway activation within germ cells induces the transcription of a number of target genes whose products act in concert with additional factors to repress germ cell entry into meiosis (Brenner and Schedl 2016). Thus, by using the Notch pathway, the *C. elegans* distal tip cells directly and precisely regulate the size of the GSC population (Byrd and Kimble 2009; Kimble 2014). Notch signaling also controls GSC numbers in Drosophila gonads – but the Notch pathway does not mediate direct communication between niche cells and GSCs, instead acting during the formation of the niche itself. In the developing Drosophila ovary, limited cell-to-cell communication is essential for the formation of properly sized niches. Expression of the Notch

ligand Delta by developing terminal filament cells normally induces pathway activation in immediately adjacent somatic cells, specifying them to become cap cells (Song et al. 2007), whereas ectopic activation of the Notch pathway in more cells within the developing gonad leads to the formation of ectopic niches and the inappropriate expansion of the GSC population in adults (Ward et al. 2006; Song et al. 2007). A similar scenario occurs during male gonad development, wherein the Notch pathway regulates the differentiation of somatic gonadal precursors, the precursors of hub cells. Notch signaling within somatic gonadal precursors act with the epidermal growth factor (EGF) pathway of primordial germ cells to determine appropriate niche size (Kitadate and Kobayashi 2010).

Notch is not the only example of a juxtacrine signal, as several ligands once thought to function as secreted factors were later found to act in a juxtacrine manner. For example, cytokines and growth factors (e.g. Transforming growth factor alpha [TGF-α], c-KIT, and Amphiregulin) can act in a juxtacrine manner in specific contexts (reviewed in Singh and Harris 2005): Pro-TGF-α, tethered to the plasma membrane of a mouse bone marrow stromal cell, binds to EGFR on an adjacent hematopoietic progenitor cell (Anklesaria et al. 1990). A second example is Steel factor, the ligand for c-KIT, exists in a secreted and membrane-bound form. Bone marrow niche cells lacking membrane-bound Steel factor failed to maintain hematopoetic stem cells (Barker 1997; Ding et al. 2012). In all cases, juxtacrine signaling guarantees that cell-cell communication will be spatially restricted to those neighbors that immediately contact one another, thus making them ideal participants in the type of spatially-limited signaling observed in most stem cells niches.

Limit the amount of ligand production and/or secretion

Several mechanisms may control the range of niche signaling involving secreted ligands. For example, simply modulating ligand production at the level of transcription or translation influences the range of a local signaling gradient. The availability of niche ligands can also be regulated by the secretion rate, given that exocytosis itself is a highly regulated process, both in terms of the amount and the subcellular location of the molecules targeted for secretion. Indeed, polarized exocytosis plays an important role in most eukaryotic cells (He and Guo 2009), involving the multi-protein exocyst complex. The exocyst resides at sites of active exocytosis and mediates the targeting and tethering of post-Golgi vesicles to the plasma membrane prior to membrane fusion. Michel et. al. showed that BMP secretion and E-cadherin membrane targeting

require exocytosis and recycling endosomes in the Drosophilia male GSC niche, specifically observing the colocalization of E-Cadherin and BMP ligand at adherens junctions within the niche cell membrane (Michel et al. 2011). Whether or not adherens junctions are functionally required for BMP ligand secretion is unclear. Nevertheless, this study suggests that specific mechanisms regulate the secretion of ligands, which allows for the precise control of signal availability to stem cells within the niche.

Modulation of ligand diffusion outside the ligand-producing cells

Regulating how a ligand diffuses through a tissue is another method to modulate the range of signaling. The ECM can influence how far a ligand travels from its source, either by retarding its diffusion or by functioning as reservoirs through direct ligand binding, which limits local availability (reviewed in Hynes 2009). For example, fibronectin, vitronectin, collagens, and proteoglycans are known to bind BMPs and growth factors such as fibroblast growth factor and hepatocyte growth factor, thereby influencing the solubility and activity of these ligands. The remodeling activity of enzymes, such as matrix metalloproteinases, on the ECM permit the release of factors as necessary (Hynes 2009). For example, the Drosophila heparan sulfate proteoglycan protein Dally is essential for concentrating Dpp molecules on the surface of cells in wing discs (Akiyama et al. 2008). Dally is also specifically expressed in female GSC niche cells to ensure a high level of BMP signaling, and thus promotes GSC identity (Guo and Wang 2009). This heparan sulfate proteoglycan is thought to function as an activating co-receptor that enhances the specificity between ligand-producing and -receiving cells. Strikingly, ectopic expression of Dally in the Drosophila ovary expands the number of undifferentiated germ line stem cells, suggesting that Dally influences the range of niche signaling (Hayashi et al. 2009). In the male Drosophila GSC niche, the secreted ECM protein Magu/Pentagone (Pent) is specifically expressed in hub cells and modulates Dpp activation exclusively in the GSC population (Zheng et al. 2011).

ECM proteins do not always restrict ligand availability; they can also increase the distance over which signals act. Type IV collagens bind to Dpp and regulate BMP signaling in both the Drosophila embryo and ovary (Akiyama et al. 2008; Guo and Wang 2009). Interaction between Dpp and type IV collagen appears to promote long-range gradient formation in the embryo, whereas it restricts the range of BMP pathway activation in the ovary through sequestration of Dpp.

Protrusion-mediated access to ligand source

Our recent discovery of microtubule based-nanotubes (MT-nanotubes) identifies another mechanism that influences which cells can respond to niche signals (Fig. 2A) (Inaba et al. 2015). MT-nanotubes are microtubule-based protrusions that extend from GSCs into the hub cell area. Similar to other thin protrusions reported to date, such as cytonemes and tunneling nanotubes, MT-nanotubes are sensitive to fixation, explaining why they have escaped detection in previous studies.

Three-dimensional reconstitution of confocal stacks revealed that the MT-nanotubes invaginate into, but do not breach the membranes of, hub cells. Double plasma membranes from both cells appeared to wrap around the core microtubule bundle extending from the GSC. Tkv receptors expressed by GSCs translocate to the tips of MT-nanotubes, where they interacts with Dpp ligand expressed by hub cells (Fig. 2A-B). Dpp ligand fused to mCherry expressed within hub cells exhibits a punctate pattern within homotypic hub cell junctions, likely marking sites where MT-nanotubes foster efficient signal reception (Fig. 2B) (Inaba et al. 2015). Perturbation of MT-nanotubes compromises activation of Dpp signaling within GSCs, leading to loss of the GSCs, thus indicating that MT-nanotubes promote signal reception (Inaba et al. 2015). Similar to the cytonemes, whose formation and/or stabilization requires ligand-receptor interactions (Roy et al. 2014), MT-nanotube formation and/or maintenance depends on interactions between Dpp and Tkv as well as intraflagellar transport proteins. Taken together, these data suggest that GSCs sense niche-produced ligands and extend/stabilize MT-nanotubes towards the source of these signals. MT-nanotube formation, in turn, allows GSCs around the hub to experience the signaling needed for their self-renewal. By contrast, MT-nanotubes do not promote stem cell self-renewal via Jak/Stat signaling between hub cells and GSCs (__). This specificity of MT-nanotubes for BMP signaling suggests that stem cells employ multiple mechanisms to receive signals from the niche.

MT-nanotube-mediated signaling represents one of the first examples in which cells utilize cellular protrusions to foster short-range signaling, thereby promoting efficient signal transduction in response to a limited amount of ligand produced by a local source, such as niche cells. Other examples of protrusion-mediated niche-stem cell regulation follow: Cap cells use short filopodia (cytonemes) to transport Hedgehog protein to escort cells (Rojas-Ríos et al. 2012). Co-cultured osteoblast and human hematopoietic progenitor cells form long distance cytoplasmic connections (tunneling/membrane nanotubes) that mediate trafficking of SARA endosomes between cells to regulate SMAD signaling (Gillette et al. 2009), which presents an intriguing

model wherein cytoplasmic contents may be directly transported between niche cells and stem cells. Whether or not tunneling/membrane nanotubes foster in vivo niche-stem cell interactions is not clear.

Ligand diffusion versus contact-dependent/protrusion-mediated signaling?

Integration of protrusion-mediated signaling and ECM-mediated diffusion may serve to fine-tune the delivery of niche ligands to stem cells. For example, the ECM may increase the local concentration of ligands specifically around protrusions, making the protrusion-mediated restriction of ligand delivery even tighter. Whether both mechanisms function together in the same niche or these mechanisms play distinct roles in the regulation of signaling remains an open question.

Many niches rely on signaling molecules that are presumably secreted into the extracellular space. A potential benefit of using diffusible ligands within niches includes the ability to adaptively adjust stem cell numbers in response to physiological change. Namely, if niche signaling solely depended on juxtacrine signaling, re-establishment of the stem cell population after stem cell loss would be difficult. By also employing diffusible ligands, niches can influence cell fate at a distance, potentially allowing for the dedifferentiation of distal cells – which has been observed in a number of systems, including the Drosophila ovary and testis (Brawley and Matunis 2004; Kai and Spradling 2004). When properly controlled, diffusible ligands allow for adaptable stem cell regeneration. Thus, ECM-mediated control of ligand diffusion/concentration may complement protrusion-dependent restriction of the niche signaling to maintain long-term tissue homeostasis.

Intrinsic factors that mediate the ability of a cell to respond to a signal

Intrinsic factors within individual cells also help to sharpen the boundary defining which cells experience signal transduction in response to ligands and which do not. In the differentiating daughters of female GSCs (Xia et al. 2010) and male GSCs (Chang et al. 2013), BMP signaling is actively repressed by the HECT-domain ubiquitin E3 ligase SMAD ubiquitination regulatory factor (Smurf), which targets Tkv for degradation. *smurf*-mutant ovaries exhibit an expansion of GSC-like cells outside of the niche (Xia et al. 2010), indicating that the degradation of Tkv promotes female germ cell differentiation. Likewise, *smurf*-mutant testes show increased/expanded Mad phosphorylation and more GSCs that exhibit transit-amplifying cell

divisions (Chang et al. 2013). These results indicate that prompt inactivation of Dpp signaling is essential for the timely differentiation of germ cells. Yet how the degradation activity is differently regulated between stem cells and their differentiating daughters remains unclear in both cases, so additional mechanism(s) for decoding a cells' location within the tissue must exist.

CONCLUDING REMARKS

Our understanding of potential regulatory mechanisms that control communication between niche cells and stem cells has greatly improved by studying model organisms. Research on GSCs has revealed remarkable complexity and precision in signaling mechanism regulating stem cell identity, differentiation, and asymmetric divisions. At the same time, these studies have raised more interesting questions: How are multiple mechanisms that control cell-cell signaling integrated into a single asymmetric event? Which event happens first? How flexible is the system? Does the effective range of signaling change to adapt to developmental and physiological changes? Are these mechanisms mutually dependent or do they provide redundancy to protect against the failure of one another? What ultimately happens when the spatial specificity of signaling is disturbed? These and other interesting questions about niche regulation await future study.

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Figure legends

Figure 1. Short range niche signaling in *C. elegans* and *D. melaongaster* gonadal niches

A: In *C. elegans,* one distal tip cell forms the niche for germ line stem cells located at the distal end. This distal tip cell extends long projections that contact stem cells. **B**: Asymmetric fate determination of Drosophila GSCs largely depends on the differential placement of two stem cell daughters to distinct locations: cells within the niche self-renew whereas cells outside the niche differentiate. The niche cell cluster (hub cells in males, terminal filament and cap cells in females) provides signals for stem cell self-renewal to the juxtaposed stem cells, but not other daughter cells that are displaced 1 cell diameter away from the niche cells (gonialblast in males, cystoblast in females).

Figure 2. MT-nanotube mediated niche-stem cell signaling

A: Model for MT-nanotube-mediated signaling. Dpp induces MT-nanotube formation, and receptor–ligand interaction occurs at the surface of MT-nanotubes, leading to signaling activation in GSCs. **B**: Dpp-mCherry (red) expressed in hub cells together with GFP-α–tubulin (green, hub cell coltex), via the hub-specific unpaired (Upd) promoter. Dpp-mCherry forms punctae along the hub cell coltex (arrowheads). The entire hub area is encircled by a white broken line. GSCs are attached to hub from surrounding area (not visible here). Scale bar, 10 μm.

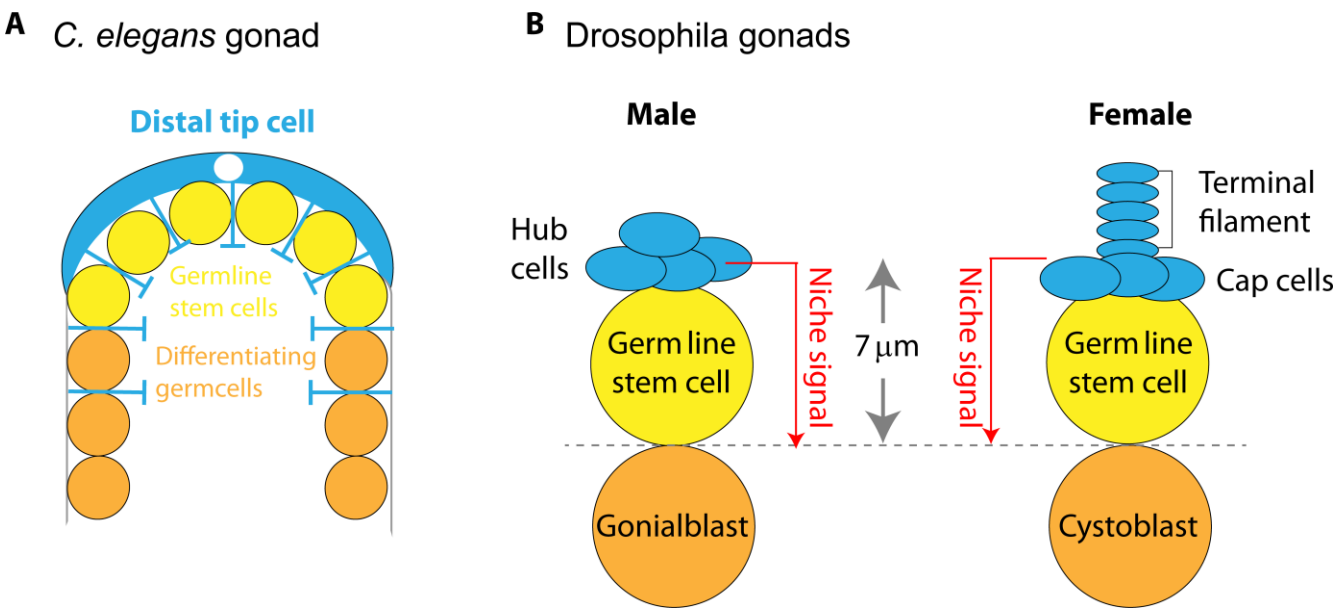
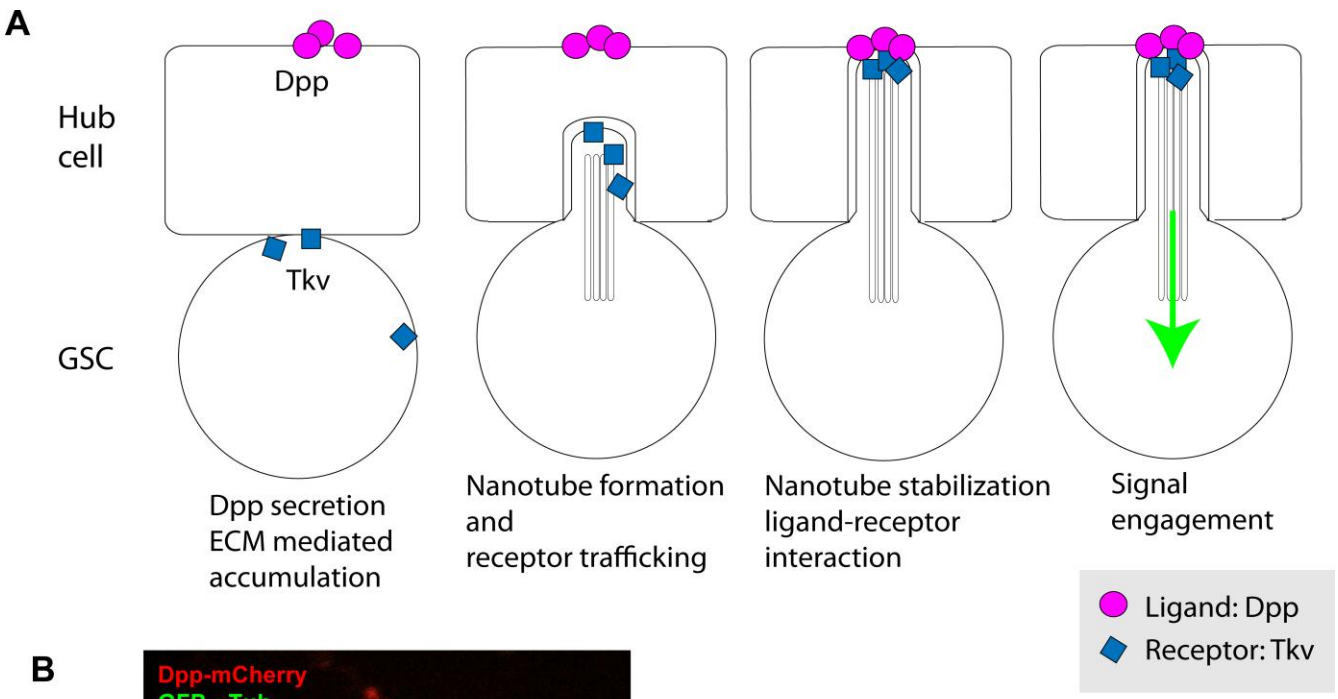


Figure 1



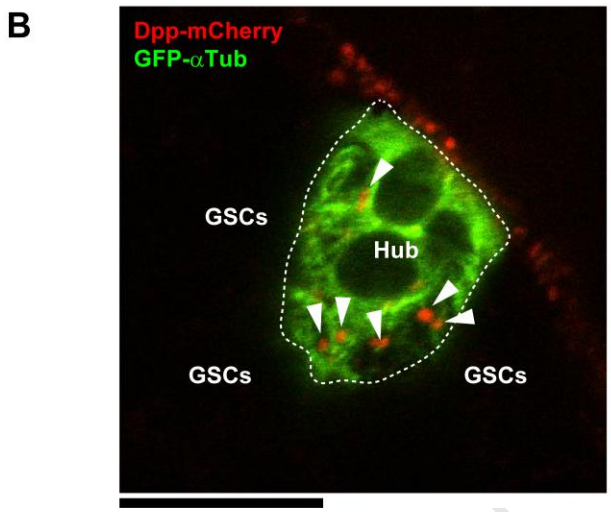


Figure 2