How different is Venus from Mars? The genetics of germ-line stem cells in *Drosophila* females and males

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Development 131, 4895-4905
Published by The Company of Biologists 2004
doi:10.1242/dev.01373

Summary

In the fruit fly *Drosophila melanogaster*, both spermatogenesis and oogenesis rely on germ-line stem cells (GSCs). Intensive research has revealed many of the molecules and pathways that underlie GSC maintenance and differentiation in males and females. In this review, we discuss new studies that, some differences notwithstanding, highlight the similarities in the structural and molecular strategies used by the two sexes in GSC maintenance and differentiation. These include the tight control that somatic support cells exert on every aspect of GSC function and the similar molecular mechanisms for physical attachment, cell-cell signaling and gap-junction communication. Some common principles underlying GSC biology in the fly may be applied to stem cells in other organisms.

Introduction

The last few years have seen a surge in stem cell research, and our incentive to understand stem cell biology is only increased by the exciting promise of stem cell-based therapies. The definition of a stem cell is still under debate, but a general view is that stem cells are cells that have an unlimited (or an especially high) capacity for self-renewal, and that can produce at least one type of differentiated progeny. Accordingly, the two main questions that concern stem cell biology are how stem cells preserve their unique, undifferentiated identity through many rounds of divisions, and how their daughter cells choose and activate a differentiation program. Stem cell maintenance and differentiation is dependent on the microenvironment provided by surrounding cells, the 'niche' (Spradling et al., 2001; Watt and Hogan, 2000). Stem cells and niche cells must thus be regarded as a functional unit, and a better understanding of stem cell biology will be achieved by studying stem cells in vivo, within their natural surroundings.

The study of stem cells in many systems is hampered by several factors. In some cases, a set of markers to define stem cells and to distinguish them from their immediate daughter cells has not been found. In others, although the stem cells are defined, they constitute a very small percentage of the tissue, and are therefore hard to find and to study in their natural environment. Only lately have niches been identified for the important mammalian stem cells of the hematopoietic system and the epithelium (Calvi et al., 2003; Tumbar et al., 2004; Zhang et al., 2003). By contrast, the location of germ-line stem cells (GSCs) in both the male and female fruit fly, *Drosophila melanogaster*, is clearly defined and has long been studied. This, along with the power of genetic analysis, makes both spermatogenesis and oogenesis in fruit flies ideal systems in which to study stem cell maintenance and differentiation. The field of GSC biology in *Drosophila* has reached the stage where the analysis of the degree of similarity, and the nature of the differences, between males and females can allow us to make general conclusions about GSC biology. These conclusions may be applicable to other types of stem cells.

This review describes the GSC functional unit in male and female flies, and discusses some key issues that emerge when the mechanisms of GSC maintenance and differentiation in the two sexes are compared.

Basic topology of the stem cell niche

Stem cell function in vivo relies on their microenvironment. The cells and extracellular matrix that surround, support and direct stem cell function are termed the 'niche'. In *Drosophila*, as in other stem cell systems, the extracellular matrix that supports stem cells is ill defined and unstudied. We will therefore limit our discussion of the 'niche' only to the surrounding somatic cells that affect both male and female GSCs.

The principles of the architecture of the male and female GSC niche are quite similar (Fig. 1). The testis of the adult *Drosophila* male is shaped as a coiled tube, closed at the apical side and open to the seminal vesicle at the basal side. At the anterior tip (apical; Fig. 1B, Fig. 2B) is a group of somatic cells called the hub (Hardy et al., 1979). On average, nine GSC cells surround the hub, and are closely associated with it (Hardy et al., 1979; Yamashita et al., 2003). Each GSC is flanked by somatic stem cells (SSCs) (Lindsley and Tokuyasu, 1980). The division of a GSC is such that one daughter cell remains at the anterior, adjacent to the hub cell (Hardy et al., 1979). This cell remains a stem cell, while the other daughter cell, the gonialblast, which lies one-cell diameter away from the hub, begins to differentiate. Similarly, when SSCs divide, the daughter cells closer to the hub remain SSCs, while those away from the hub encapsulate the differentiating GSC daughter cell (Hardy et al., 1979; Lindsley and Tokuyasu, 1980). The subsequent differentiation of the GSC daughter cell is

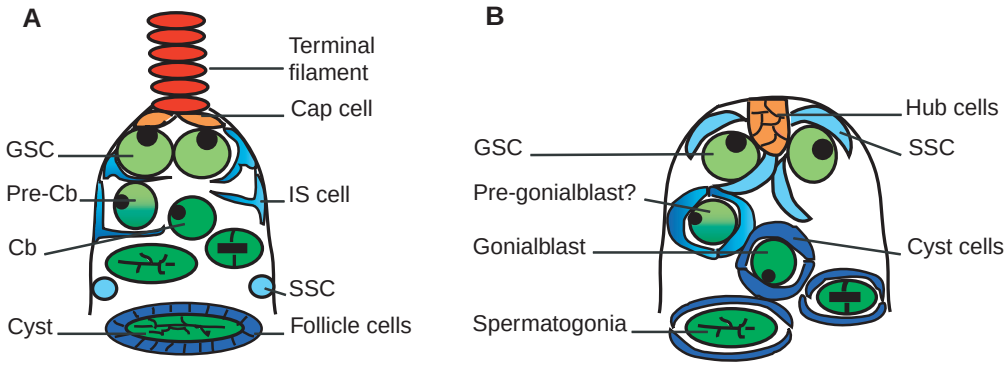

Fig. 1. Schematic of the anterior part of a female ovariole and male testis in *Drosophila melanogaster*. The description of the developmental stages of the germ cells is to the left of each structure and the somatic cells are to the right. Anterior is up. In the female ovariole (A) and male testis (B), germ-line stem cells (GSCs) are located at the anterior tip of the gonad. Upon division, the posterior daughter cell differentiates to a cystoblast (Cb, females) or a gonialblast (males). The differentiation may be gradual, as depicted by increasingly stronger shades of green. The daughter cell divides further and forms a cyst. GSCs and their immediate daughter cells harbor a spherical fusome (here marked in black). This organelle grows and extends into every cell of the cyst (black marking within the cyst). In females, the niche includes terminal filament (TF) and cap cells, which are located most anteriorly. Inner sheath (IS) cells may also be part of the niche and may perform similar functions to those of early somatic cyst cells in males. Somatic stem cells (SSCs) are located 'midway' down the germarium and they give rise to the follicle cells that envelope the cyst. In males, SSCs are attached to the hub, and their descendents (cyst cells) encapsulate the gonialblast. Color-coding is used to mark cells that have a similar function in males and females. Shades of red have been used for TF and cap cells as these two populations, although similar, are not identical in their gene expression profiles (Forbes et al., 1996a).

dependent on its association with two somatic cyst cells (see below). The differentiation program of the gonialblast entails four rounds of mitotic division with incomplete cytokinesis, resulting in a 16-cell germ-line cyst. Both GSCs and gonialblasts harbor a spherical organelle called a spectrosome (or spherical fusome, Fig. 1A,B), which is composed of small vesicles and cytoskeletal proteins (Leon and McKearin, 1999; Lin et al., 1994; McKearin and Ohlstein, 1995; Roper and Brown, 2004). With each round of mitotic division, the fusome changes its shape, grows and branches, such that it penetrates each cell within the germ-line cyst. The fusome is instrumental in coordinating mitotic divisions in the cyst, and in oocyte determination in females. Following mitosis, all male germ-line cells enter the meiotic cell cycle. They then form a cohort of 64 interconnected spermatids, which differentiate further to form the mature sperm (Fuller, 1993).

The female ovary is composed of 16-20 units called ovarioles. At the anterior of each ovariole is the germarium, where new egg chambers are generated (King, 1970; Mahowald and Kambysellis, 1980; Spradling, 1993). At the anterior-most tip of the germarium is a group of six to ten disc-shaped somatic cells, the terminal filament (TF) cells, and located adjacent to these are the cap cells, which closely abut the GSCs (Forbes et al., 1996b; King, 1970) (Fig. 1A, Fig. 2A). A third group of somatic cells that occupy the niche and that may influence GSC maintenance and differentiation are the inner-sheath cells (IS), the cytoplasmic extensions of which protrude into the germarium, contacting GSCs, cystoblasts and germ-line cysts (Carpenter, 1975; King, 1970; Schulz et al., 2002). As in males, the division of a GSC results in one daughter cell, which lies in close apposition to the cap cells and remains a GSC, and another daughter cell, the differentiating cystoblast, which is located one-cell diameter away from the cap cells (Deng and Lin, 1997; King, 1970). The differentiation program of the cystoblast involves, as in males, four rounds of mitotic divisions that result in the formation of a 16-cell germ-line cyst. As in males, GSCs and their daughters contain a spherical fusome that branches and penetrates every cell in the germ-line cyst. However, unlike in males, only one cell within the germ-line cyst, the oocyte, completes the meiotic cell cycle. The other 15 cells become nurse cells (Spradling, 1993). The differentiation of the egg chamber requires the cooperation of somatic follicle cells. These originate from SSCs that are located 'midway' down the germarium (Margolis and Spradling, 1995). The SSC daughter cells contact the germ-line cyst only after the four mitotic rounds of division are completed and meiosis is underway.

Significant similarities exist between males and females in the asymmetric mode of GSC division, and in the close proximity of somatic cells to both GSCs and their differentiating daughters. The differentiation programs of male and female GSCs also exhibit marked similarities, although the different fate of one cell of the 16-cell cyst in females is a major difference between males and females. In the next part, we shall describe the molecular mechanisms that underlie GSC maintenance and differentiation, and discuss the similarities and differences between males and females at the molecular level (see also Table 1).

Molecular pathways of GSC maintenance

One of the most striking features of GSCs is their close association with and dependence on somatic support cells. The somatic niche is capable of retaining GSCs by a combination of extrinsic cues that include physical attachment and various signaling pathways. These signals are perceived by GSCs, which then employ a set of, as yet ill-defined, intrinsic stem-cell maintenance factors (Fig. 3).

Physical attachment to the niche

GSCs are attached to the niche through adherens junctions (see Fig. 3). Both hub and cap cells express high levels of DE-cadherin, a component of adherens junctions (Brower et al.,

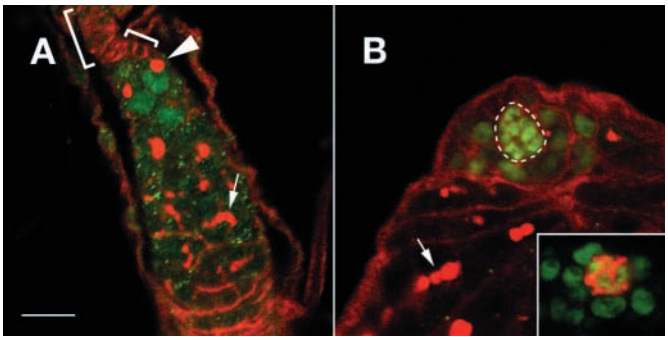

Fig. 2. The anterior part of a female ovariole and male testis in *Drosophila melanogaster*. Anterior is up. (A) An ovariole from a female *Drosophila* carrying a β-galactosidase (β-gal) enhancer trap in the *dad* gene, stained with antibodies against β-gal (green), and with the monoclonal antibody 1B1 (red), which stains the membrane cytoskeleton in somatic cells and the fusome within germ cells. The terminal filament and cap cells are at the anterior and are marked with brackets. GSCs are located just posterior to cap cells, and an arrowhead marks a spherical fusome in one GSC that is abutting cap cells. An arrow marks a branched fusome within a cyst in a more posterior location within the germarium. β-gal staining is strong in GSCs, but can also be observed in their immediate daughters and early cysts. This may be due to the produrence of the β-gal protein. (B) A testis from a male *Drosophila* carrying a β-gal enhancer trap in the *escargot* gene, stained with anti-β-gal (green) and 1B1 (red) antibodies. Hub cells express β-gal most strongly and are circled. Arranged around the hub are GSCs, which also express β-gal. Staining of β-gal in gonialblasts and early cysts may be due to the produrence of the protein. Posterior to the hub are the dividing cysts; a fusome in one cyst is marked by an arrow. (B inset) The same genotype as in B, stained with anti-β-gal (green) and with anti-Fasciclin III (red), which outlines the cells of the hub, showing its compact structure. Scale bar: 20 μm.

1981; Song et al., 2002; Yamashita et al., 2003). Likewise, GSCs were shown to accumulate wild-type or GFP-labeled DE-cadherin (in females and males, respectively) in the membrane that contacts niche cells (Song et al., 2002; Yamashita et al., 2003). Armadillo (β-catenin), a component of both adherens junctions and Wnt signaling, also co-localizes with DE-cadherin in males and females (Song et al., 2002; Yamashita et al., 2003). GSCs that lack DE-cadherin are 'lost' (i.e. they lose their position near somatic niche cells and commence differentiation). Thus, adherens junctions are functionally important for stem cell maintenance in both sexes (Song et al., 2002) (L. Jones and M. Fuller, personal communication). Using *armadillo* mutant alleles that specifically interfere with Wnt signaling, Song et al. showed that it is the participation of Armadillo in adherens junctions, rather than Wnt signaling, that is important for GSC maintenance (Song et al., 2002).

Physical attachment may be important for retaining stem cells in their niche in general. It has been shown that DE-cadherin is also important for maintaining SSCs in the *Drosophila* ovary (Song and Xie, 2002). Recently, asymmetric localization of both N-cadherin and β-catenin, in a subgroup of hematopoietic stem cells, to the membrane that contacts niche cells was demonstrated in mice (Zhang et al., 2003).

Adherens junctions may contribute to stem cell maintenance not only by providing physical attachment. These junctions may also regulate the Ras and Notch signaling pathways (Tepass et al., 2001), and thus may participate in the regulation of GSCs. Finally, adherens junctions may participate in orienting the plane of division in stem cells, as discussed below.

Major and minor signaling pathways

Besides physical attachment, signaling is also needed to maintain GSCs. Studies in both male and female flies suggest that the niche employs more than one signaling pathway to preserve GSCs. The various pathways can be divided into two classes, one major and the other minor. The first class has a major, instructive, effect on GSC maintenance and the other has a redundant, indirect or permissive effect. The major experimental difference is that upregulation of a major signaling pathway leads to an extensive accumulation of GSCs, whereas upregulation of a minor signaling pathway leads to only a minor accumulation of GSCs.

The Decapentaplegic (Dpp) pathway is a major signaling pathway for GSC maintenance. Overexpression of Dpp, the Bone Morphogenetic Protein 2/4 (BMP2/4) homolog in female flies, results in an extensive increase of single germ cells that resemble GSCs. Conversely, the GSC half-life and rate of division is reduced in GSCs deficient in signaling components of the Dpp pathway, such as *schnurri*, *thickveins* and *Medea* (Xie and Spradling, 1998; Xie and Spradling, 2000). Glass bottom boat (Gbb), another ligand of the Dpp family, also contributes to GSC maintenance, as GSCs are lost in *gbb* mutants. It is interesting to note, however, that unlike overexpression of Dpp, overexpression of Gbb does not lead to overproliferation of GSC-like cells (Song et al., 2004). *dpp* is expressed in cap, inner sheath and follicle cells, while *gbb* may be expressed in either inner sheath, early follicle cells, or both (Song et al., 2004; Xie and Spradling, 2000). These findings suggest that Dpp-like signals emanating from somatic cells are perceived directly by female GSCs, and control their maintenance and division rate. The differentiating progeny of GSCs actively repress Dpp signaling (Casanueva and Ferguson, 2004). This repression may be gradual, because although phosphorylated Mad, an indicator of an activated Dpp signaling pathway, is present at the highest levels in GSCs, it is present in decreasing amounts in cystoblasts and even in early cysts (Gilboa et al., 2003; Kai and Spradling, 2003; Song et al., 2004). The niche, then, may promote GSC identity by auxiliary mechanisms.

Several intriguing observations suggest that the JAK/STAT pathway may also play a minor role in the niche in maintaining female GSCs. First, the pathway components, the ligand Upd, its receptor, Domeless, and the transcriptional activator Stat92E, are present in cap cells (Silver and Montell, 2001) (R. Xi, J. McGregor and D. Harrison, personal communication) (Table 1). Second, overexpression of Upd causes a small increase in the number of single germ cells in the germarium. However, Stat92E protein cannot be detected in GSCs, and GSCs that lack the JAK kinase Hopscotch are maintained normally within the niche (R. Xi, J. McGregor and D. Harrison, personal communication). Thus, female GSCs can respond to the Upd signal, but this signaling pathway is not required in GSCs for their maintenance. It is possible that the increase in early germ cells is achieved indirectly, through modulation of the somatic niche by overexpressing Upd. Alternatively, Stat92E may be expressed at very low levels in GSCs, and the

pathway may function as a permissive or an auxiliary mechanism for GSC maintenance. If indeed the JAK/STAT pathway functions in such a manner, then stronger effects of the pathway on GSC maintenance may be revealed under conditions where the Dpp pathway is compromised.

The JAK/STAT pathway is a major signaling pathway required for stem cell maintenance in male flies. *upd* is expressed in hub cells, and its overexpression leads to an excess of germ cells with GSC characteristics (Kiger et al., 2001; Tulina and Matunis, 2001). Conversely, a viable

Table 1. Localization and function of genes that are expressed in GSCs or their niche

Pathway	Function*		Gene/protein	Expression	
	Females	Males		Females	Males
TGF-β signaling pathway	GSC maintenance and rate of division	Cyst development (limiting mitosis/promoting meiosis) or gonialblast differentiation	*dpp/gbb*	*dpp*- Cap, IS cells, by ISH (Xie and Spradling, 2000). Not detected by enhancer trap (Forbes et al., 1996a). *gbb*- IS or early follicle cells or both, by RT-PCR (Song et al., 2004).	*dpp*- Not detected by enhancer trap (Matunis et al., 1997). No ISH data. *gbb*-SSCs, by ISH (Shivdasani and Ingham, 2003). Hub cells and SSCs/somatic cyst cells, by RT-PCR (Kawase et al., 2004).
	Possible later role in cyst development	Possible role in GSC survival/maintenance	*tkv*, *med*, *mad*, *pnt*	GSCs, inferred by genetic requirement for maintenance (Xie and Spradling, 1998).	GSCs, inferred by genetic requirement for maintenance (Kawase et al., 2004).
			Phosphoryl-ated Mad	Strong expression in GSCs. Weaker in dividing cysts, by Ab staining (Gilboa et al., 2003; Kai and Spradling, 2003).	GSCs, by Ab staining (Kawase et al., 2004).
			pnt, *shn*	GSCs, inferred by genetic requirement for maintenance (Xie and Spradling, 1998; Xie and Spradling, 2000).	Cyst cells, inferred by genetic requirement for germ line cyst differentiation (Matunis et al., 1997).
JAK/STAT signaling pathway	FC differentiation, BC migration	GSC, and possibly SSC maintenance	*upd*	Polar cells, possibly cap cells, by ISH (Silver and Montell, 2001).	Hub cells, by ISH (Kiger et al., 2001; Tulina and Matunis, 2001).
	Possible minor or indirect role in GSC maintenance		*stat92E*	Cap cells, by ISH (Silver and Montell, 2001).	GSCs, inferred by genetic requirement for maintenance (Kiger et al., 2001; Tulina and Matunis, 2001).
Piwi	GSC maintenance, rate of division	GSC maintenance	*piwi*	TF, by ISH (Cox et al., 1998).	Early germ cells, at anterior tip, by ISH (Schulz et al., 2002).
(PPD family protein)			Piwi	TF, Cap, IS, GSCs, dividing cysts, by Ab staining of a tagged *piwi* transgene (Cox et al., 2000).	
Yb (novel)	GSC maintenance, cyst differentiation, follicle cell patterning	Males fertile	*Yb*	TF, by ISH (King and Lin, 1999).	Expressed in males, by western blot (King and Lin, 1999).
Hh signaling pathway	Major function – SSC maintenance	No function in GSC maintenance	Hh	TF, Cap cells, by Ab staining (Forbes et al., 1996b). TF, Cap, IS cells, by enhancer trap (Forbes et al., 1996b).	Hub cells at third instar – enhancer trap (Forbes et al., 1996b).
			Ptc	Somatic and germ line cells throughout the ovary, by Ab staining (Forbes et al., 1996a).	Cyst cells – ptcGAL4 (Schulz et al., 2002).
Pum, Nos (transcriptional repression)	GSC maintenance	Not described	Pum	High levels in GSCs, lower in cystoblasts, by Ab staining (Forbes and Lehmann, 1998).	Not described
	Cyst differentiation		Nos	GSCs and their immediate daughters. Very high in a fraction of 16-cell cysts, by Ab staining (Gilboa and Lehmann, 2004; Wang and Lin, 2004).	Not described

mutation in the JAK kinase gene (*hopscotch*) results in GSC depletion, and GSCs that are mutant for *Stat92E* are not maintained in the niche and proceed to differentiate (Kiger et al., 2001; Tulina and Matunis, 2001). Thus Upd is a major stem cell maintenance factor for male GSCs. Upd may also control the maintenance of SSCs, as overexpressing Upd leads to an accumulation of somatic cells in testes (Kiger et al., 2001; Tulina and Matunis, 2001). It remains to be shown, however, whether this effect is direct, as the role of Stat92E in SSCs has not been tested. The function of the JAK/STAT pathway in

Table 1. *Continued*

Pathway	Function*		Gene/protein	Expression	
	Females	Males		Females	Males
Zpg (gap junction communication)	GSC differentiation and survival	GSC differentiation and survival	Zpg	Germ line cells from PGCs to late egg chambers, by Ab staining (Gilboa et al., 2003; Tazuke et al., 2002).	Germ line cells from PGCs to spermatocytes, by Ab staining (Tazuke et al., 2002).
Bam (novel)	Cystoblast differentiation	Cyst development (limiting mitosis/promoting meiosis) or gonialblast differentiation	BamC	Cystoblast and dividing cysts, by Ab staining (McKearin and Ohlstein, 1995).	Dividing cysts, by Ab staining and promoter construct (Brawley and Matunis, 2004; Gonczy et al., 1997; Kawase et al., 2004; Shivdasani and Ingham, 2003).
	Cyst division		BamF	GSCs to dividing cysts, by Ab staining (McKearin and Ohlstein, 1995).	Not described
EGF signaling pathway	FC differentiation, axis determination, BC migration	Differentiation of SSCs or their daughters	EGFR	Somatic cells from region 2b of the germarium onward, by Ab staining (Sapir et al., 1998).	Cyst cells, inferred genetically by requirement for germ line cyst differentiation (Kiger et al., 2000).
			raf	Follicle cells, inferred genetically by requirement for follicle cell differentiation (Brand and Perrimon, 1994).	Cyst cells, inferred genetically by requirement for germ line cyst differentiation (Tran et al., 2000).
			Activated MAPK	IS cells, by Ab staining (Schulz et al., 2002).	Hub cells, cyst cells, GSCs, spermatocytes, by Ab staining (Kiger et al., 2000; Schulz et al., 2002).
Stet	Early germ cell differentiation	Early germ cell differentiation	*stet*	Required genetically in early germ cells (Schulz et al., 2002). Germ cells in region 2B and 3 of germarium, by ISH (Schulz et al., 2002).	Required genetically in early germ cells (Schulz et al., 2002).
Notch signaling pathway	FC differentiation	Not described	N	Germ line region 1 of the germarium, low levels, by Ab staining (Xu et al., 1992). Somatic cells region 2 of the germarium onward, by Ab staining (Xu et al., 1992).	GSCs, gonialblasts, spermatogonia, by Ab staining (Kiger et al., 2000).
En (transcription factor)	Not described	Not described	En	TF, Cap cells, by Ab staining (Forbes et al., 1996a).	Not described
Wg (cell-cell signaling)	SSC maintenance	Not described	*wg*	Cap cells, by enhancer trap (Forbes et al., 1996a).	Cyst cells, by enhancer trap (Schulz et al., 2002).
Arm, E-cadherin (cell adhesion, cell-cell signaling)	GSC attachment to niche cells	GSC attachment to niche cells	Arm	High levels between GSCs and cap cells, by Ab staining (Song et al., 2002).	Hub cells, GSCs, by Ab staining (Yamashita et al., 2003).
			E-cadherin	High levels between GSCs and cap cells, by Ab staining (Song et al., 2002).	High levels between GSCs and hub cells, by Ab staining (Yamashita et al., 2003).

* For reference to function see text.

Abbreviations: Ab, antibody; BamC, cytoplasmic Bam; BamF, fusomal Bam; BC, border cells; FC, follicle cells; GSC, germ line stem cell; IS, inner sheath cell; ISH, in-situ hybridization; PGC, primordial germ cell; SSC, somatic stem cell; TF, terminal filament.

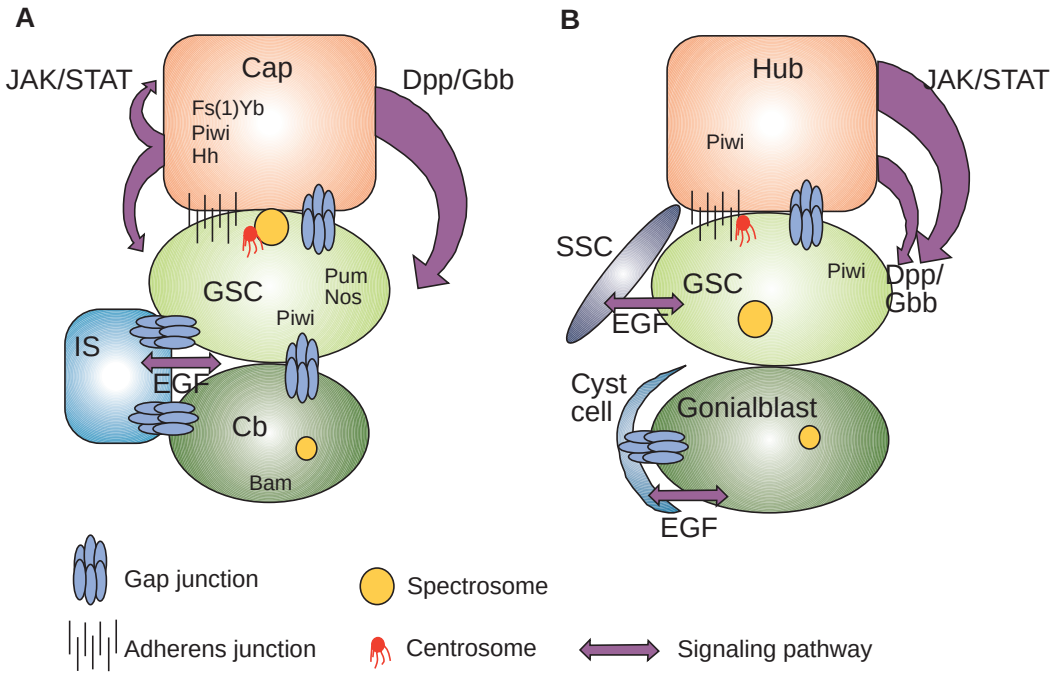

Fig. 3. Major participants in GSC maintenance and differentiation in (A) female and (B) male gonads. Germline stem cells (GSCs) and their differentiating daughter cells (Cb, cystoblast in females and gonialblast in males) are shown in shades of green. The somatic cells of the niche (cap cells in females and hub cells in males) are colored pink. Other somatic cells [inner sheath (IS) cells in females, and somatic stem cells (SSCs) and cyst cells in males) may affect either GSCs or their differentiating daughter cells, or both, and are shown in blue. Proteins and organelles that act within each cell type are noted. Major and minor signaling pathways emanating from the niche are marked with large and small arrows, respectively.

GSC maintenance in males closely resembles that of the Dpp pathway in females. In the female, Dpp also regulates the rate of GSC division. It would be interesting to know whether Upd has a similar role in the male.

Recent data suggest that the Dpp pathway also plays a role in male GSC maintenance or survival. Overexpression of Dpp or Gbb leads to a small increase in GSC-like cells. This may be due to a small increase in niche size rather than to a direct effect on GSCs (Kawase et al., 2004; Schulz et al., 2004; Shivdasani and Ingham, 2003). Gbb and Dpp are expressed in somatic cells that lie in proximity to GSCs, and eliminating some of the components of the Dpp signaling pathway from GSCs causes stem cell loss (Kawase et al., 2004; Shivdasani and Ingham, 2003). The relative potency of the two ligands is hard to compare as the effect of a complete loss of function of either ligand cannot be followed in the adult because of embryonic or larval lethality, and because they use the same signaling components within germ cells. A Gbb/Dpp heterodimer may also exist, conferring additional complexity to the system. The Dpp/Gbb pathway affects spermatogenesis at multiple steps, as it has also been shown to act in the germ line and in cyst cells during the differentiation of the cyst (Kawase et al., 2004; Matunis et al., 1997; Schulz et al., 2004; Shivdasani and Ingham, 2003).

It appears that flies use more than one signaling pathway to control GSCs. Males use the JAK/STAT pathway and also the Dpp pathway to maintain GSCs. Females use the Dpp pathway as a major signal for GSC maintenance, but they may also use the JAK/STAT pathway (perhaps indirectly); the functional importance of the two pathways has clearly shifted in the two sexes. The use of more than one signaling pathway perhaps provides robustness and flexibility to the system. In the somatic cells of the ovary, again, two signaling pathways (Hedgehog and Wingless) maintain SSCs (Forbes et al., 1996b; King et al., 2001; Song and Xie, 2003; Zhang and Kalderon, 2001). Further study should reveal how general this rule is in other stem cell systems.

Coordination in the niche

In males and females, the progeny of both GSCs and SSCs cooperate to form the gamete. Presumably, the division of the stem cells of both lineages needs to be coordinated such that there is no excess of either cell type. Coordination of GSCs and SSCs may be especially challenging in females, where, unlike in males, SSCs reside far from GSCs. The genes *fs(1)Yb* (*Yb*), *piwi* and *hedgehog* (*hh*) may have a role in this coordination.

Yb encodes a novel protein and is expressed in terminal filament cells. Mutations in *Yb* cause defects in the encapsulation of egg chambers by follicle cells (the descendents of SSCs), and in GSC maintenance (Johnson et al., 1995; King and Lin, 1999; King et al., 2001). Conversely, Yb overexpression induces a large excess of somatic cells. Overexpression of Yb also moderately increases the numbers of germ cells with stem cell character (King et al., 2001). As discussed below, given that *Yb* is required for the expression of *piwi* and *hh* in somatic cells of the niche, it may thus affect both germ line and soma (King et al., 2001).

Piwi belongs to the large PPD (containing PAZ and Piwi domains) family of proteins, which is found in diverse organisms, from plants to worms, flies and mammals (Cerutti et al., 2000; Cox et al., 1998; Schwarz and Zamore, 2002). PPD proteins, and Piwi amongst them, have been implicated in RNA-interference-mediated gene silencing (Aravin et al., 2001; Pal-Bhadra et al., 2002; Pal-Bhadra et al., 2004; Schwarz and Zamore, 2002). Piwi is expressed in terminal filament, cap and inner sheath cells, and is also expressed in the germ line, from GSCs to dividing cysts and egg chambers (Cox et al., 1998; Cox et al., 2000). GSCs are not maintained in females that are mutant for *piwi*, whereas overexpression of Piwi in the soma leads to increased numbers of germ cells with stem cell

character and to an elevated rate of GSC division (Cox et al., 2000; Lin and Spradling, 1997). In addition to affecting the maintenance and division rate of GSCs from the soma, Piwi acts within GSCs to control GSC division rate (Cox et al., 2000).

Hh is produced by terminal filament and cap cells, and affects the somatic stem cells, which are located several cell diameters away from its source of production. Decreased Hh signaling reduces the numbers of SSCs, whereas Hh overexpression induces extra somatic cells (Forbes et al., 1996b; King et al., 2001; Zhang and Kalderon, 2001). Interestingly, the overexpression of Hh rescues both the *Yb* and *piwi* mutant phenotypes (King et al., 2001). It is unclear how overexpression of Hh may affect GSC maintenance, because flies mutant for Hh or its receptor Patched do not show a marked GSC-maintenance phenotype. One possibility is that Hh affects GSC maintenance by affecting the niche.

The roles of Piwi and Yb in GSC maintenance are not well understood. A better understanding of the biochemical function and the targets of these genes may aid in elucidating their role in maintaining GSCs. Yb and Piwi may affect GSCs by influencing the fate determination of niche cells, or by controlling and coordinating additional signals that emanate from the niche. It is intriguing that, whereas Piwi has a role in male GSC maintenance, Yb males are fertile (King and Lin, 1999; Lin and Spradling, 1997). It is possible that Piwi is part of an ancient stem-cell maintaining mechanism, as suggested by the conserved role of other Piwi homologs, such as argonaute, ZWILLE and Piwi-Related-Gene (prg1,2) in stem cell biology (Cox et al., 1998). Yb, however, may be used only in females to coordinate GSC and SSC division, because, unlike males, these two cell populations do not lie adjacent to each other. There are currently no reports on the role of Hh in male GSC maintenance.

Acting from within – Nanos and Pumilio

Signals emanating from the niche must be translated into intrinsic factors that help maintain GSCs. Two such intrinsic stem cell factors are the RNA-binding proteins Nanos (Nos) and Pumilio (Pum), which are best known for their role in the translational repression of *hunchback* in the posterior of the embryo (Barker et al., 1992; Murata and Wharton, 1995; Wharton and Struhl, 1991; Zamore et al., 1997). *nos* and *pum* mutant female flies possess many empty ovarioles, a phenomenon that may be attributed to either defects in division or the development of germ cells prior to adulthood (Asaoka-Taguchi et al., 1999; Forbes and Lehmann, 1998; Lin and Spradling, 1997). Primordial germ cells (PGCs) in *nos* and *pum* mutant larvae begin to differentiate precociously, suggesting that both genes are required to repress the differentiation of PGCs (Gilboa and Lehmann, 2004; Wang and Lin, 2004). Consistently, Nos and Pum are expressed in GSCs, and are required to repress their differentiation (Forbes and Lehmann, 1998; Gilboa and Lehmann, 2004; Lin and Spradling, 1997; Wang and Lin, 2004). There have been no reports on the expression patterns of Nos and Pum, or on their possible roles in GSC maintenance in males.

RNA targets of Nos and Pum in GSCs have not been identified, and it is also unclear how Nos and Pum expression and function is regulated in the germ line. Dpp is unlikely to act through Nos and Pum, as the overexpression of Dpp can inhibit the precocious differentiation of PGCs that is observed in *nos* mutant gonads (Gilboa and Lehmann, 2004). Homologs of Nos and Pum have been described in many organisms, including the worm, frog and mouse (Mosquera et al., 1993; Nakahata et al., 2001; Subramaniam and Seydoux, 1999; Tsuda et al., 2003; White et al., 2001; Zamore et al., 1997; Zhang et al., 1997). Pum and Nos are part of the same functional complex in *Drosophila* embryos, and probably function in early germ-line development in *C. elegans* and in *Drosophila* GSC maintenance (Gilboa and Lehmann, 2004; Sonoda and Wharton, 1999; Subramaniam and Seydoux, 1999). As in flies, the *C. elegans* homologs of Pum, FBF-1 and FBF-2, function in GSC maintenance (Crittenden et al., 2002). However, *nos-3* has been shown to have an antagonistic function in promoting GSC differentiation in *C. elegans* (Hansen et al., 2004). Further research is required to determine whether other Nos or Pum proteins may function together in meiotic repression in the *C. elegans* germ line.

Molecular pathways of GSC differentiation

Signaling from the niche may not only promote GSC maintenance but also control GSC differentiation. In *Drosophila*, the niche directs the orientation of GSC division and represses the expression or function of genes that direct differentiation. Three molecular pathways have been shown to regulate the differentiation of the early male and female germ line: (1) a novel pathway that employs the gap junction protein Zero Population Growth (Zpg); (2) the major differentiation pathway, which is defined by the *bag of marbles* (*bam*) and *benign gonial cell neoplasm* (*bgcn*) genes; and (3) the Epidermal Growth Factor (EGF) pathway.

Control of orientation of GSC division

In stem cell systems that use a strategy of asymmetric cell division to determine the fate of the daughter cells (such as male and female GSCs), controlling the plane of division of the stem cell is of great importance. In both male and female gonads, GSC division occurs such that the anterior GSC daughter cell, which lies close to the cap cells or the hub, remains a GSC, while the posterior daughter, which is removed from these somatic cells, begins to differentiate. It has been shown in males, that changes in the plane of division result in more GSCs, because both division products remain close to the hub (Yamashita et al., 2003).

In male GSCs, one spindle pole always associates with the GSC-hub interface. This spindle orientation depends on Centrosomin (Cnn), and on the Adenomatous Polyposis Coli tumor suppressor (APC) protein homologs. Interestingly, although Cnn and APC1 localize to centrosomes, APC2 is enriched at the interface between GSCs and hub cells (Yamashita et al., 2003). APC family members were shown to localize to actin-rich regions in the membrane of epithelial cells, with which mitotic spindles associate. APC proteins also bind to β-catenin, a component of adherens junctions (Bienz, 2002). APC2 could thus provide a link between astral microtubules and the adherens junctions at the cell cortex. The adherens junctions may in turn provide not only physical anchorage, but also a way to ensure that GSC division results in two cells destined for different fates.

In female GSCs, the spectrosome is asymmetrically localized, and abuts the cap cells in late interphase and throughout mitosis

(de Cuevas and Spradling, 1998). This is in contrast to males, where the spectrosome has no specific location (Yamashita et al., 2003). During mitosis, one spindle pole co-localizes with the spectrosome; abolishing the spectrosome causes the randomization of this spindle orientation in female GSCs (Deng and Lin, 1997). The fusome associates with microtubules not only in GSCs, but also throughout cyst development (Grieder et al., 2000). This association is important for setting one cell in the cyst (the oocyte) aside, a process that does not take place in males. It remains unknown whether the molecules that anchor the spectrosome to the cell cortex abutting cap cells, and those that anchor the mitotic spindles to the spectrosome, bear any resemblance to those that function in orienting the spindles in male GSCs.

Gap junctions and GSC differentiation

Intercellular communications are important not only for GSC maintenance, but also for their differentiation. This is exemplified in *Drosophila* by a requirement for gap junctions in the earliest steps of GSC differentiation. Females mutant for the gap junction protein Zpg have a unique phenotype: only a few germ cells that morphologically resemble GSCs are located at the anterior tip of the gonad, suggesting that *zpg* is necessary for early germ cell differentiation (Gilboa et al., 2003; Tazuke et al., 2002). Indeed, lack of Zpg causes death of the differentiating GSC daughter cell, thus Zpg may be necessary for their survival (Gilboa et al., 2003). Zpg may also be necessary for the process of differentiation itself, as indicated by the fact that GSCs in flies mutant for both *pum* and *zpg* remain undifferentiated at the niche; although GSCs that are mutant for *pum* alone are not maintained at the niche (Gilboa et al., 2003).

In males, *zpg* mutant germ cells differentiate further than in *zpg* mutant females (Tazuke et al., 2002). In *zpg* mutant females, most germ cells are single cells that carry a spectrosome. However, in *zpg* mutant males, more clusters of partially differentiating germ cells are observed (Tazuke et al., 2002). Because *zpg* acts within germ cells, these different mutant phenotypes might reflect inherent differences in the differentiation program of male and female germ cells.

Gap junctions can be observed in GSCs of wild-type ovaries. These connect germ-line cells (either between GSCs, or between GSCs and cystoblasts), or connect somatic and germ-line cells (GSCs and inner sheath cells, GSCs and cap cells) (Tazuke et al., 2002). However, it remains unclear which of the observed gap junctions contains Zpg, and what signal is transmitted through these gap junctions. The *zpg* mutant phenotype is very different from other mutations that disrupt GSC differentiation, such as mutations in the genes *bam* or *bgcn*. This suggests that separate pathways regulate germ cell differentiation. Although the disruption of some of these pathways leads to the accumulation of GSC-like cells, the disruption of others may lead to germ cell death.

Bam and Bgcn – major differentiation factors

Our best insights into how the niche preserves GSCs have arisen from our understanding of how Dpp/Gbb signaling represses the transcription of the important differentiation factor Bam. *bam* and *bgcn* have very similar phenotypes and interact genetically with each other. We will therefore focus on the one studied in more detail – *bam*.

bam mutant ovaries are filled with cells that have stem cell characteristics. Accordingly, the *bam* mutant phenotype has been described as a ‘stem cell tumor’, and it has been proposed that Bam controls the differentiation of the stem cell into a cystoblast (McKearin and Ohlstein, 1995). Consistent with this hypothesis, the overexpression of Bam in female GSCs leads to their differentiation, indicating that Bam is both necessary and sufficient for GSC differentiation (Ohlstein and McKearin, 1997). The central role of Bam in female GSC differentiation is emphasized by the fact that Bam expression is repressed by the major, GSC-maintaining, Dpp pathway. It has recently been shown that *bam* transcription may be directly silenced in GSCs by the Dpp pathway, as the *Drosophila* Smads, Medea and Mad, bind to the *bam* promoter (Chen and McKearin, 2003; Song et al., 2004).

bam transcript is the same in males and females, and cytoplasmic Bam can be observed in the dividing cyst in both sexes (Table 1). However, while BamC can be detected in the cystoblast in females, it cannot be detected in its male counterpart – the gonialblast. Furthermore, the phenotype of *bam*- and *bgcn*-mutant testes is not identical to that of mutant ovaries. In males, *bgcn* and *bam* mutant cysts contain many more than 16 germ cells, which divide in unison and are connected to at least one neighbor, and often to more (Gonczy et al., 1997). Marker analysis of these germ cells has shown them to have a mixed character of GSCs, gonialblasts, and primary and secondary spermatogonia (Gonczy et al., 1997). The difference in *bam* phenotypes between males and females can be interpreted in two ways. First, Bam may be needed for the differentiation of GSCs in both sexes. The ability of *bam* mutant male germ cells to differentiate further than their female counterparts may indicate a fundamental difference between the differentiation process in males and females that may be connected with the sexual identity of GSCs. It is interesting that, like *bam* mutant male cells, *stet* (see below) and *zpg* mutant germ cells in males progress further in the differentiation pathway than their female counterparts. The second interpretation of the *bam* phenotype is that in males the primary role of *bam* is to limit the mitotic proliferation of the cyst or to promote the meiotic cell cycle (Gonczy et al., 1997). A proliferative role for Bam was also suggested in females, based on the observation that mutations in *bam* enhance the tumorous phenotype of the meiotic gene *mei-P26*, and suppress an additional round of germ cell division in *encore* mutants and in flies overexpressing Cyclin A (Hawkins and Thorpe, 1996; Lilly et al., 2000; Page et al., 2000). It is notable, however, that although Bam may limit mitotic divisions in males, its role in cyst division in females appears to be the opposite – the facilitation of mitotic divisions. A better understanding of the molecular function of Bam is needed to understand how this molecule may regulate GSC differentiation and division in the two sexes.

As in females, the overexpression of Bam in males causes GSC loss (Kawase et al., 2004; Shivdasani and Ingham, 2003). Some of that loss, however, may be attributed to the death of either GSCs or their differentiated daughters (Schulz et al., 2004). The level of Bam overexpression in GSCs may determine whether they differentiate or die. Recent reports suggest that, like in females, activation of the Dpp pathway in male germ-line cysts represses Bam expression (Kawase et al., 2004; Schulz et al., 2004; Shivdasani and Ingham, 2003). Thus,

the control of Bam expression may be similar in males and females.

Somatic control of GSC differentiation – the EGF pathway

In both sexes, the differentiation of the GSC daughter cell depends on their tight association with somatic cells in the niche, and the EGFR pathway plays a crucial role in establishing these connections. In male flies that are mutant for the EGFR signaling component Raf or a temperature-sensitive allele of the EGF receptor ($EGFR^{ts}$), many germ cells have some GSC characteristics (Kiger et al., 2000; Tran et al., 2000). The accumulation of germ cells with partial GSC characteristics has also been observed in *stet* mutants (Schulz et al., 2002). Stet is a homolog of Rhomboid, which is needed for the cleavage and activation of Spitz, an EGFR ligand (Lee et al., 2001; Urban et al., 2001). Cell-autonomy experiments have shown that Stet function is required in the germ line, whereas the EGFR pathway needs to be activated in the soma to promote the association of GSC daughters with somatic cyst cells (Kiger et al., 2000; Schulz et al., 2002; Tran et al., 2000). Ovarioles of *stet* mutant females accumulate GSC-like cells, similarly to *stet* mutant males (Schulz et al., 2002). However, a function for EGFR or its known ligands in female GSC differentiation has not been reported.

Abrogation of the EGF signaling components in male cyst cells, or mutations in *stet* in both male and females, disrupt the normal connections that exist between the germ line and somatic cells (somatic cyst cells in males and inner sheath cells in females) (Kiger et al., 2000; Schulz et al., 2002; Tran et al., 2000). Thus, EGFR pathway activation may directly promote the association of somatic cells with early germ cells in males and females. This close association, in turn, may be important for the transmission of reciprocal signals, generated in the somatic cells, that are necessary to control the early steps of GSC differentiation. In males, signaling occurs between the somatic cyst cells and the germ line, whereas, in the female, the signal may be transmitted between the inner sheath and the germ cells, suggesting that inner sheath cells may perform a similar role to that of the somatic cyst cells (Schulz et al., 2002).

This interplay between soma and germ line in the differentiation of GSCs bears a morphological resemblance to the tight association that exists between germ cells and Sertoli cells, and germ cells and Granulosa cells, in the mammalian testes and ovaries, respectively. Future studies should determine whether this morphological resemblance is reflected at the molecular level.

Conclusion

According to prevailing beliefs, the stem cell stage is unique in the life cycle of the germ cell, and GSCs should be distinguished from both their predecessors (PGCs) and their successor (the developing cyst). Recent findings suggest that the GSC may not be as distinct as we used to think. First, under certain conditions in both males and females, a differentiating cyst can revert and form GSCs (Brawley and Matunis, 2004; Kai and Spradling, 2004), thus blurring the divide between a GSC and a cyst. Second, GSC tumor cells can be transplanted back to the embryo and be re-established as GSCs (Niki and Mahowald, 2003), suggesting that GSC-like cells have the capacity to behave like PGCs. Indeed, many of the genes that are required for GSC maintenance, such as *dpp*, *nos* and *pum*, are also required to repress differentiation in PGCs (Gilboa and Lehmann, 2004; Wang and Lin, 2004). All this suggests that the somatic cells surrounding the germ cell greatly influence its developmental state.

In both males and females, GSCs and their differentiating daughters contact each other and also two types of somatic cells (Fig. 1). Intensive research in the *Drosophila* GSC field has shown that this tight surrounding is mirrored by a myriad of molecular cross talk (Fig. 3). Adherens molecules, gap junctions and several signaling pathways are all employed in a complex network whose outcome is a balance between GSC preservation and differentiation. There is still much to learn about this process. Other signals emanating from the niche may be over-shadowed by the major signaling pathways, making them hard to find by genetic screens. How those signals integrate in GSCs is also a mystery. What other genes do these signals target? Even the function of the known molecules within GSCs that are responsible for GSC maintenance and differentiation, Pum, Nos, Piwi, Bam and Bgcn, is still unclear. The combined study of GSCs in male and female flies will surely answer some of these questions, and bring us closer to understanding the stem cell unit.

We wish to thank those members of the fly community who contributed unpublished data so generously; in particular Doug Harrison, Minx Fuller, Leanne Jones, Ting Xie and their lab members. We also thank Minx Fuller, all members of the Lehmann lab, and especially Jason Morris and Caryn Navarro, for their thorough reading of and useful comments on this manuscript.

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