Int. J. Dev. Biol. 53: 1329-1339 (2009)
doi: 10.1387/ijdb.072431jp

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Recent advances in *Drosophila* stem cell biology

JOHN PEARSON*, LOURDES LÓPEZ-ONIEVA, PATRICIA ROJAS-RÍOS and ACAIMO GONZÁLEZ-REYES

Centro Andaluz de Biología del Desarrollo (CABD), CSIC / Universidad Pablo de Olavide, Sevilla, Spain

ABSTRACT Stem cells possess the unique properties of self-renewal and the ability to give rise to multiple types of differentiated tissue. The fruit fly *Drosophila melanogaster* retains several populations of stem cells during adulthood as well as transient populations of stem cells during development. Studies of these different populations of stem cells using the genetic tools available to *Drosophila* researchers have played an important role in understanding many conserved stem cell characteristics. This review aims highlight some of the recent contributions from this important model system to our understanding of the myriad of processes that interact to control stem cell biology.

KEY WORDS: *niche signaling, niche morphogenesis, somatic stem cell, germline stem cell, siRNA*

The essence of a stem cell

The self-renewal of stem cell populations is critical for animal development, growth, tissue homeostasis, damage repair and reproduction. Understanding what gives stem cells these unique characteristic is one of the most important aims in biology today. Researchers using a wide range of model systems including vertebrates, invertebrates and plants have made considerable progress in our knowledge of stem cell biology. While stem cell populations in different species and tissues are as divergent as the roles they perform, several common characteristics have also emerged (see reviews Nystul and Spradling, 2006, Wong *et al.*, 2005).

Niche regulation has been one of the key concepts to emerge in our understanding of stem cell self-renewal (See reviews e.g. Fuchs *et al.*, 2004, Lin, 2002, Nystul and Spradling, 2006, Ohlstein *et al.*, 2004, Spradling *et al.*, 2001). A stem cell niche has been defined as "a specific location where stem cells can reside for an indefinite period of time and produce progeny cells while self-renewing" (Ohlstein *et al.*, 2004). In the strictest sense this has meant a region which is stably maintained even in the absence of stem cells. While different classifications of stem cell niche organization have been proposed (Ohlstein *et al.*, 2004), they all involve the formation of a limited "permissive zone" for self-renewal. Those stem cells forced to leave this zone (e.g. by spatial constraints) lose the factors required for self-renewal and, typically, enter differentiation. Thus, stem cell niche regulation can be viewed as a homeostatic mechanism for controlling the proliferative potential of stem cells, yet affording the plasticity required to respond to changing conditions.

Formation and maintenance of stem cell microenvironments is often dependent on surrounding support or stromal cells and the secretion of extracellular factors. However, this traditional view has been challenged recently, especially after the description of new niches in *Drosophila* that lack a stable population of support cells.

This review sets out to provide an up-to-date guide to the latest trends in stem cell biology emerging from studies in *Drosophila.* We shall focus on organizational aspects of stem cell microenvironments — including the role of adhesion molecules and the interplay between stem cells and the surrounding stroma — the common properties of two well-defined germline niches, the contribution of small RNA molecules and chromatin remodeling factors to stem cell self-renewal, and the relationship between the control of centrosome dynamics and asymmetric stem cell division.

Drosophila stem cell populations – variations on the niche theme

Studies in *Drosophila* have resulted in the identification of several stem cell populations (See reviews e.g. Doe *et al.*, 1998,

Abbreviations used in this paper: BMP, bone morphogenetic protein; CPC, cyst progenitor cell; ESC, escort stem cells; FSC, follicle stem cell; GMC, ganglion mother cell; GSC, germline stem cell; fGSC, female germline stem cell; mGSC, male germline stem cell; RNSC, renal and nephric stem cell; HP, hematopoetic precursor; ISC, intestinal stem cell; JAK/STAT, janus kinase/signal transducer and activator of transcription; miRNA, microRNA; NB, neuroblast; PGC, primordial germ cell; piRNA, Piwi-interacting RNA; PSC, posterior signaling center; SGP, somatic gonadal precursor; TF, terminal filament.

***Address correspondence to:** John Pearson. Centro Andaluz de Biología del Desarrollo (CABD), CSIC / Universidad Pablo de Olavide, Carretera de Utrera Km. 1, 41013 Sevilla, Spain. Fax: +34-954-349-376. e-mail: jrobpea@upo.es - Lab webpage: http://www.upo.es/CABD

Final author-corrected PDF published online: 12 September 2008.

ISSN: Online 1696-3547, Print 0214-6282

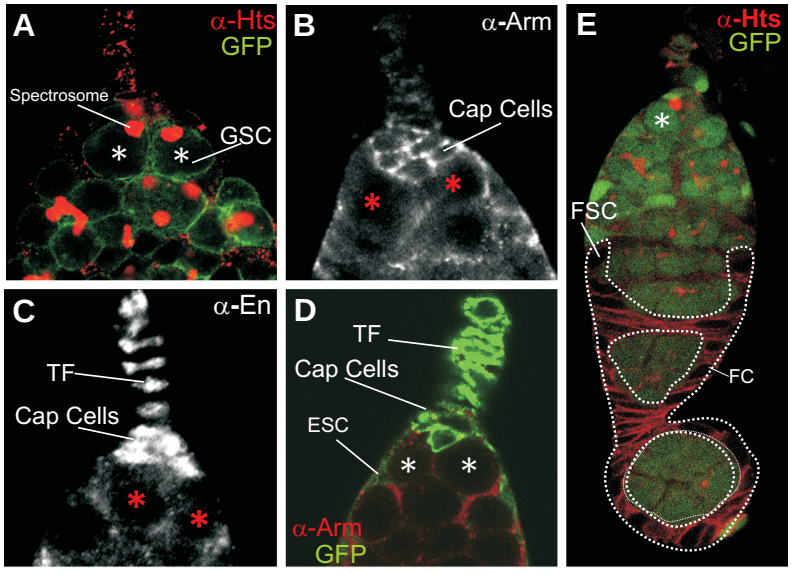

Fig. 1. Markers can be used to distinguish ovarian GSC niche cells. *Confocal images of the anterior tip of* Drosophila *germaria* **(A)** nanos-*Gal4, UAS-*Src:*GFP germarium double stained with anti-Hts (red) and anti-GFP (green). Germline cells are marked by* nanos*-driven expression of membrane-associated Src-GFP. GSCs (marked with an asterisk in each panel) can be distinguished by Hts-rich apical spectrosomes (apical, spherical organelles typical of GSCs). Other germline cells also contain Hts-rich structures which become branched during cyst divisions.* **(B)** *Germarium stained with anti-Armadillo (Arm) which is strongly accumulated in the stromal cap cells.* **(C)** *Germarium stained with anti-Engrailed (En) which accumulates at high levels in the nuclei of stromal cap and terminal filament (TF) cells.* **(D)** bab1-*Gal4, UAS-Src:GFP germarium double stained for anti-GFP and anti-Arm.* bab1-*Gal4 is specifically expressed in the stromal niche cells of the germaria (Bolívar* et al.*, 2006) included the TF, cap cells and escort stem cells (ESCs).* **(E)** *Flp-out recombination induced by* bab-*Gal4 UAS-Flipase (Bolívar* et al.*, 2006). Follicle stem cells (FSCs) can be identified by genetically dividing somatic cells, in this case by the loss of GFP; FSCs are usually the most anterior marked follicle cells (FCs) (Nystul and Spradling, 2007). Anterior is up in all figures.*

Fuller and Spradling, 2007, Kirilly and Xie, 2007, Lin, 2002). Investigation of these populations has proved pivotal in refining our conception of niche regulation of stem cell self-renewal.

Drosophila *germline stem cells – "classical" models of niche regulation*

Both male and female *Drosophila* retain germline stem cells (GSCs) during most of their adult life. Studies of GSC maintenance in *Drosophila* have been decisive in the establishment of the niche model of stem cell self-renewal (reviewed by Spradling *et al.*, 2001). The availability of markers allowing key germline and somatic cells to be unequivocally identified has been critical for the success of the *Drosophila* GSC niche models (see Fig. 1 as an example of the wealth of markers available and the cellular simplicity of the ovarian stem cell niche) (reviewed in Wong *et al.*, 2005). Although the *Drosophila* ovary and testis differ significantly in their organization, the arrangement of their respective GSC niches shares many architectural similarities (Fig. 2 A,B). In each ovarian niche, 2-3 female GSCs (fGSCs) keep contact with stromal cap cells, while in the testis approximately 8 male GSCs (mGSCs) are associated with stromal hub cells. The cap and hub cells are heavily implicated in forming their respective stem cell niches (reviewed in Kirilly and Xie, 2007, Wong *et al.*, 2005). This stereotyped organization and the powerful genetic tools of *Drosophila* have permitted a detailed analysis of the requirement for different cell types and signaling molecules in niche function. Such studies have played an important role in defining to what we will refer to as the "classical" stromal model of a stem cell niche. In this paradigm, summarized in Fig. 2C, the stromal cells define a stem cell-independent microenvironment primarily through their physical organization, cell-adhesion properties and expression of extracellular signals. Only cells capable of responding to the environment of a given niche, due to their intrinsic properties, are able to populate and self-renew. In fGSC and mGSC niches, this includes both actual GSCs as well as differentiating germ cells, which can be made to revert into functional stem cells (Brawley and Matunis, 2004, Kai and Spradling, 2004). The GSC niche model has proved a valuable basis for the study of stem cells in many species, with several stem cell populations appearing to be maintained in niches with similar properties (reviewed in Fuchs *et al.*, 2004).

Novel Drosophila *somatic stem cell niches: intestine and ovaries*

A completely novel *Drosophila* stem cell population has recently been described in publications from the Perrimon and Spradling laboratories. Using genetic lineage marking techniques, intestinal stem cell (ISC) populations were identified in the midgut of adult fruit flies (Micchelli and Perrimon, 2006, Ohlstein and Spradling, 2006) (Fig. 3C). ISCs remain attached to the basement membrane and also appear to retain some association with their daughter cells via Armadillo-rich junctions (Ohlstein and Spradling, 2006). However ISCs do not appear to associate with any other stromal cell types. These observations demonstrate the existence of self-renewing adult stem cell populations which are not defined by stromal cells. Interestingly, differentiating daughter cell fate is determined by differential Notch signaling (Ohlstein and Spradling, 2007). Thus, ISC self-renewal may depend primarily on intrinsic factors as is the case for the neural progenitor cells neuroblasts, whose asymmetric divisions also depend on asymmetric Notch activation (Micchelli and Perrimon, 2006, Ohlstein and Spradling, 2006, Ohlstein and Spradling, 2007, Yu *et al.*, 2006). The extent, to which the association of ISCs to the basement membrane and/or their own daughter cells might constitute a niche, remains to be determined. A second population of ISCs has recently been reported to localize to the anterior region of the hindgut and repond to Wingless and Hedgehog signaling (Takashima et al., 2008).

The existence of somatic stem cells in the ovary has been known for many years (Margolis and Spradling, 1995). There are at least two classes of somatic stem cells in the ovary, Follicle stem cells (FSCs) (previously known as Somatic Stem Cells) (Margolis and Spradling, 1995) and Escort Stem Cells (ESCs) (Decotto and Spradling, 2005) (See Fig. 2A). Each germarium retains two FSCs that generate the somatic cells which encapsulate the 16-cell germline cyst and play a fundamental role in defining the polarity of the developing oocyte (Reviewed in Poulton and Deng, 2007).

However, the lack of markers or easily stereotyped morphology has meant that the definition of FSC organization has been traditionally vague. Recently, the first detailed study of FSC morphology, cell division and migration has been published (Nystul and Spradling, 2007). The authors found that like ISCs, FSCs are associated with the basement membrane but lack stable contact with stromal cells. Nystul and Spradling also observed that FSC daughters are capable of displacing other FSCs within the same germarium, suggesting that we should regard the local microenvironment of each FSCs as a niche. Clearly, intrinsic factors expressed by FSCs (and/or their daughter cells) are likely to play an important role, perhaps not just for their asymmetric divisions but also in shaping their extracellular environment. Many questions remain open, such as which properties the FSC niche might possess to permit self-renewal and how this can be controlled in the absence of stromal cells. A number of signaling pathways required for FSC self-renewal have been identified (reviewed by Kirilly and Xie, 2007). Interestingly, many of the extracellular signaling molecules required for FSC maintenance are expressed by relatively distant cells, which participate also in the stromal component of the fGSC niche, indicating that specialized support cells do not necessarily have to contact their target stem cells to exert their influence in niche regulation. The emergence of these new and exciting models for *Drosophila* stem cell regulation have shown that we need to broaden our definition of what constitutes a stem cell niche to encompass regions whose unique properties permit limited stem cell self-renewal, even in the absence of defined stromal cells (Fig. 3).

Drosophila *neuroblasts – stem cells without a niche?*

During development, populations of stem cells have important roles in the generation of specific tissues and structures. In *Drosophila*, transient populations of neural stem (or progenitor) cells, known as neuroblasts (NBs), form during embryonic and larval stages to give rise to a range of sensory tissues (reviewed in Yu *et al.*, 2006). NBs are one of the best characterized models of asymmetric cell division (Fig. 3A). In the embryo approximately 60 NBs divide repeatedly to generate hundreds of differentiated neurons and glia cells. Typically NBs divide to produce two different daughter cells; the larger, apical daughter remains as a NB, while the smaller, basal cell becomes a ganglion mother cell (GMC) which undergoes further divisions prior to differentiation.

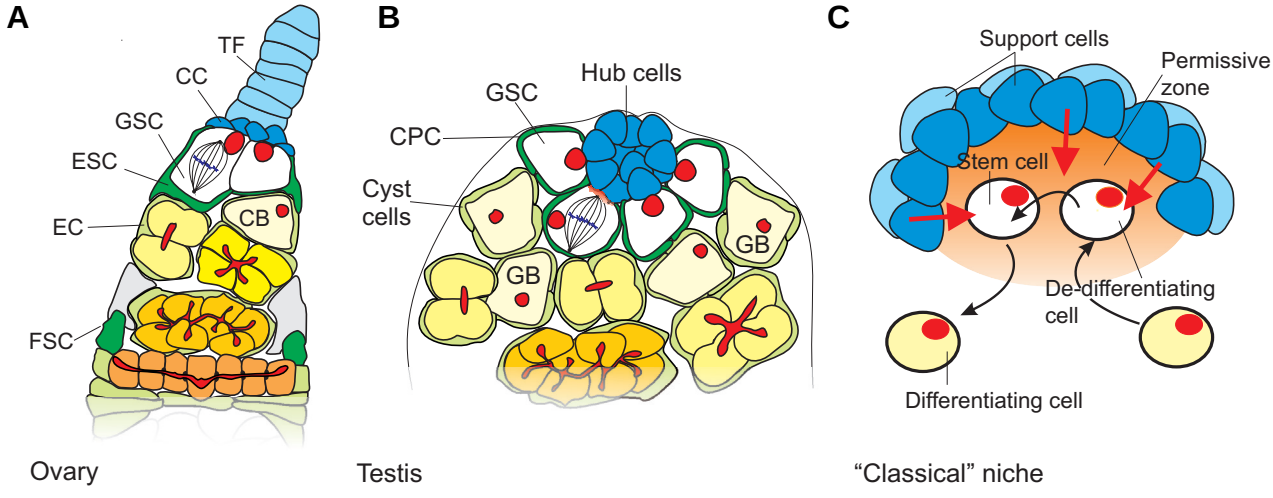

Fig. 2. Established *Drosophila* stem cell models. (A) *Schematic diagram of the* Drosophila *female GSC niche. The ovarian GSC niche is usually occupied by 2-3 fGSCs and is located near the anterior tip of each germarium, the region where egg chambers originate. fGSCs can be readily identified by the presence of an apical spherical spectrin-rich structure known as the spectrosome, and their position in direct contact with somatic cap cells (CC). CCs are located between fGSCs and the anterior terminal filament (TF). Escort stem cells (ESCs) are closely associated with the fGSCs and are also in contact with the cap cells. fGSC divisions are usually oriented along the anterior-posterior axis with one spindle pole seemingly organized by the spectrosome. A fGSC divides to regenerate itself and to produce a cystoblast (CB) which also inherits some spectrosome material. CBs associate with somatic escort cells (ECs), which are derived from ESCs, and accompany the germline during four mitotic divisions to form 16 cell-cysts (marked in yellow and orange). As cysts divide the spectrosome (now referred to as a fusome) becomes branched. Germline cysts become encapsulated by follicle cells (FC) to form egg chambers (not shown). FCs are derived from 2 Follicle stem cells (FSCs) located in the central region of the germarium. Their association with unknown inner germarium sheath cells (marked in grey) seems to be important for the FSC niche (Song and Xie, 2002).* **(B)** *Schematic diagram of the testicular stem cell niche. Each male GSC niche containing 7-9 GSCs associated with somatic hub cells near the anterior tip of the testis and can be identified by the same characteristic apical spectrosome as female GSCs. mGSCs are associated with cyst progenitor cells (CPCs), which associate with GSCs in pairs. CPCs, also known as Somatic stem cells, contribute to both hub and cyst cell lineages (Voog* et al., *2008). mGSCs divide to regenerate themselves and to produce differentiating gonialblasts (GBs), which like CBs retain a spherical fusome and associate with somatic cyst cells which are derived from CPCs. As in females, mGSC divisions are usually oriented along the anterior-posterior axis but the anterior spindle pole appears to associate which a cortical protein complex (marked in red) positioned apically where the mGSC contacts the hub cells rather than the spectrosome (Yamashita* et al., *2003). This association depends on both centrosome function and APC2 protein that accumulates at the apical region of the mGSC cortex.* **(C)** *"Classical" stromal model of a stem cell niche. Support, stromal cells define a permissive zone by regulating cell adhesion properties and extracellular signaling. Cells capable of responding to the environment due to their intrinsic properties (such as stem cell themselves and some de-differentiating cells) are able to populate and self-renew within the permissive zone. The exit from the niche influence zone often implies a certain degree of cell proliferation and differentiation.*

The association of NBs with epithelial cells appears to be important for the proper alignment of neuroblast polarity and/or cell division with respect to their neighboring cells, suggesting that extrinsic cues do play a role in NB divisions (Siegrist and Doe, 2006). However, in contrast to GSCs, intrinsic factors involving polarity, the mitotic apparatus and the distribution of fate determinants appear to be sufficient for NB self-renewal and GMC specification (Yu *et al.*, 2006). Thus, the self-renewal of NBs does not seem to be dependent on a niche. This difference might reflect the relatively transient nature of NBs progenitor cells. Nevertheless, *Drosophila* NBs have become established as an important reference in the field of stem cell biology with respect to the role of intrinsic factors and control of asymmetric cell division. Further recent findings will be discussed in more detail in the last section of this review.

Drosophila *hematopoietic precursor cells – a novel progenitor cell niche*

Recent publications have now signaled the arrival of additional *Drosophila* stem cell populations as models with the potential to further enhance our understanding of stem cell self-renewal, stem cell niches, signaling pathways and asymmetric cell division.

Drosophila hemolymph cells are derived from hematopoietic precursor (HP) populations in embryonic and larval stages (reviewed in Crozatier and Meister, 2007; Martinez-Agosto et al,. 2008). Only recently has the development of molecular markers permitted the identification of specific regions of the lymph gland where larval hematopoiesis occurs and of a group of cells known as the posterior signaling center (PSC) thought to be involved in the regulation of HPs. Although it is unclear if *Drosophila* HP cells represent true stem cells, two recent studies by Mandal *et al.* and Krzeimer *et al.* have shown that the PSC cells are required for the establishment of an HP niche (Krzemien *et al.*, 2007, Mandal *et al.*, 2007) (Fig. 3B). As in the ovarian GSC niche, contact between 'support' PSC cells and HPs appears to be important for their maintenance (Krzemien *et al.*, 2007, Mandal *et al.*, 2007). Although specific markers can be used to distinguish PSC cells, the spatial relationship between the support and precursor/stem cells is less clearly defined than in the ovary. Nevertheless these findings suggest that the PSC cells may form the stromal component of a classical stem cell niche and suggest that the niche regulation of self-renewal may also play a role in some transiently-maintained progenitor cells.

Drosophila *renal and nephric stem cells – multipotent cells in the kidney*

It has recently been shown that the Malpighian tubules, the *Drosophila* renal organs, contain proliferating cells in the proximal

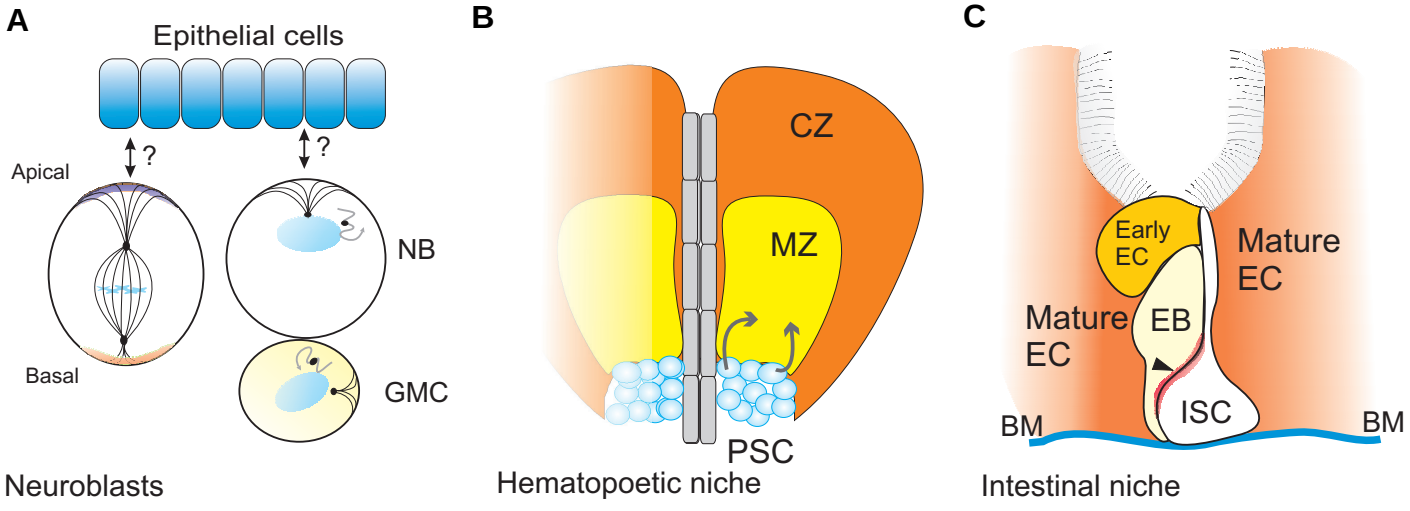

Fig. 3. Other *Drosophila* stem cell niches. (A) Drosophila *neuroblasts (NBs) originate from cells which delaminate from epithelia, but retain apical-basal polarity (reviewed in Yu* et al.*, 2006). NBs divide asymmetrically to form smaller ganglion mother cells (GMCs), which continue to divide a further two times prior to terminal differentiation. Correct positioning of the NB divisions appears to depend on maintaining contact with epithelial cells but the nature of this interaction is unknown. Recruitment of apical protein complexes (marked in purple), including the Par/Inscuteable complex, upon the entry into mitosis is required to organize the mitotic spindle and determine the basal positioning of fate determinant such as Numb and Prospero (marked in red). Recent findings suggest that NB centrosomes (black dots) divide during early interphase but that only one remains stably associated with apical astral microtubules, while the other moves rapidly around the cytoplasm (as indicated by the arrow) (Rebollo* et al.*, 2007).* **(B)** *Schematic diagram of the larval hematopoietic niche. Larval hematopoiesis occurs within the lymph gland. The medullary zone (MZ) contains hematopoietic precursor (HP) cells while the cortical zone (CZ) contains specialized blood cells. Posterior signaling center (PSC) cells are responsible for preventing premature differentiation of HP cells in the MZ. The PSC cells appear to act as stromal cells to form a hemopoetic niche and are required for activating the JAK/STAT pathway in HP cells. The Notch pathway is also required to maintain PSC identity (Crozatier and Meister, 2007).* **(C)** *Schematic diagram of the* Drosophila *intestinal stem cell niche. The* Drosophila *mid-gut contains intestinal stem cells (ISCs) able to produce both enterocytes (ECs) and the less frequent hormone producing enteroendocrine cells (not shown) (Micchelli and Perrimon, 2006, Ohlstein and Spradling, 2006). ISCs associate primarily with the basement membrane (BM) and appear not to associate with other stromal cells. However, ISCs do maintain contact with their daughter enteroblasts (EB), an intermediate stage before differentiation as either an EC or enteroendocrine cell. A strong accumulation of Armadillo (marked in red) between ISCs and EBs suggests that adherens junctions may mediate this association. It is suggested that this linkage may permit ISCs to move to different regions of the mid-gut (Ohlstein and Spradling, 2006).*

segment (Singh *et al.*, 2007). Using a linage tracing strategy, the authors demonstrated that a subpopulation of "small nuclear" cells in the proximal segment are multipotent stem cells, termed renal and nephric stem cells (RNSCs). RNSCs give rise to differentiated renalcysts in proximal segment and, remarkably, can also generate Type I and Type II cells, which are located in the upper tubule segments. Interestingly, Jak/Stat signaling seems to play a dual role regulating MT cell specification. Autocrine Jak/Stat signaling regulates RNSC self-renewal, while weaker Jak/Stat signaling appears to play a role in the differentiating RNSC daughter cells (renalblasts). RNSCs do not appear to associate with any particular cell type or reside in any recognizable niche. Autocrine Jak/Stat signaling potentially confers RNSCs with enough independence to self-renew in an environment without an identified stromal component. It remains to be seen whether the intrinsic properties of RNSCs are sufficient to resist the pull towards differentiation, or if extrinsic factors, such as receipt of unidentified signaling molecules or interaction with the basement membrane, are also required.

Stem cells as active participants in the niche environment

The classical view of a stem cell niche has been one where combinations of extrinsic factors interact with the intrinsic properties of stem cells to promote stem cell division and self-renewal. In this context, the stem cells are essentially passive with respect to the niche in which they survive (see Fig. 2C). This view is supported by observations suggesting that in *Drosophila* many somatic niche cell types of the ovary or testis are specified normally even in the absence of germline cells (Brookman *et al.*, 1992, Margolis and Spradling, 1995). However, recent evidence now suggests that stem cells are intimately involved in establishing and maintaining their own niche.

The germline maintains a close association with somatic cells from earliest stages of gonadogenesis. *Drosophila* gonad formation begins as primordial germ cells (PGCs) complete a complex migration to come into contact with the somatic gonadal precursors (SGPs) (Starz-Gaiano and Lehmann, 2001). As the germline and the soma first contact each other, a subset of SGPs appear to emit long dynamic processes around each germline cell, which seem to be maintained during embryogenesis (Jenkins *et al.*, 2003). At later stages in ovarian development another population of somatic cells, termed «intermingled cells» appear to be associated with PGCs. This close association between the germline and subsets of somatic cells is maintained in adult gonads (Decotto and Spradling, 2005, Fuller, 1993). In the male GSC niche, cyst progenitor cells (CPCs) are implicated in GSC self-renewal (Leatherman and Dinardo, 2008) and the normal association between CPCs and GSCs requires activation of a classical EGFR-MAPK-Raf signaling cascade in the CPCs (Kiger *et al.*, 2000, Tran *et al.*, 2000). Recently, it has been shown that activation of the EGFR pathway in CPCs and cyst cells depends on an EGF ligand (encoded by *spi)* expressed in the germline. *spi* mutants lose the cytoplasmic extensions that envelop the germline, which causes a general disruption in the normal organization between germline and somatic cyst cells. Activation of the EGFR pathway in germline-associated cells appears to affect the local balance between antagonistic Rac and Rho GTPase activities which may control the formation of cytoplasmic processes (Sarkar *et al.*, 2007). Interestingly, enhancer trap evidence suggests that germline expression of *spi* may also be involved in interactions between PGCs and the intermingled cells of the developing ovary that control germ cell proliferation (Gilboa and Lehmann, 2006). These results point to an active role for GSCs and their precursors as contributors to their microenvironment. In this context, examination of the *in vivo* dynamics of cells in the hematopoietic niche revealed that the stromal PSC cells formed long cytoplasmic processes which extend into the niche (Mandal *et al.*, 2007), suggesting that the formation of cytoplasmic extensions by stromal cells may be a general feature of many stem cells niches.

The changing paradigm of niche morphogenesis and stem cell maintenance has been further strengthened by other recent discoveries. Although hub cells are specified (as judged by their expression of *upd*) in the absence of the germline, they are not organized properly (Le Bras and Van Doren, 2006). In fact, signaling from the germline is required to repress activation of the Sevenless kinase pathway in certain anterior somatic gonadal precursor cells in male embryos (Kitadate *et al.*, 2007). Loss of the Sevenless ligand (encoded by *bride of sevenless*) from the germline results in the ectopic specification of hub cells and a corresponding increase in the adult mGSC niche (Kitadate *et al.*, 2007). This suggests that the germline has a partial role in organizing the somatic cells of the male GSC niche.

Does this germline-soma relationship persist in the adult stem cell niche? In the ovary, recent publications have shown that continued Notch signaling from fGSCs is required for their own maintenance (Song *et al.*, 2007, Ward *et al.*, 2006). Interestingly, the requirement for Notch in promoting fGSC maintenance appears to act by contributing to the maintenance of cap cell identity and/or their expression of niche signals (Song *et al.*, 2007, Ward *et al.*, 2006). In the absence of Notch signaling from fGSCs, these key stromal cells are lost from the niche. Thus, far from being passive receivers of niche signaling, increasing evidence suggests that stem cells play critical roles in stem cell niche formation and maintenance.

The critical role of cell adhesion and the ECM in niche morphogenesis

How might the complex, stereotyped arrangements between stem cells and their surrounding support cells be established? Cell adhesion plays a critical role in tissue morphogenesis. Several recent studies in *Drosophila* have begun to uncover the molecular mechanisms by which cell adhesion drives the formation of stem cell niches. In fact, the balance of adhesive properties between cells can drive complex cell rearrangements, cell migration, the formation of structures such as epithelial sheets, as well as the sorting and separation of cell populations (McNeill, 2000). Cell adhesion proteins such as the homophilic adhesion protein *D*E-Cadherin play a critical role in the above processes. Stable adherens junctions are formed between Cadherin molecules on the surface of adjacent cells, linked to their respective cytoskeletons by α and β-Catenin (Halbleib and Nelson, 2006, McNeill, 2000). The presence of such junctions between cells is often indicated by an accumulation of these components on the contact surfaces of the cells involved. *D*E-Cadherin, β-Catenin and other adhesion molecules are concentrated between stromal cells and GSCs

(Jenkins *et al.*, 2003, Kiger *et al.*, 2000, Song *et al.*, 2002, Tazuke *et al.*, 2002), suggesting that they may be important for the stem cell niche. Indeed, the rapid loss of mutant fGSC clones when adherens junction-mediated cell adhesion is perturbed confirms that correct cell adhesion between GSCs and niche support cells is an important aspect of GSC maintenance (Gonzalez Reyes, 2003; Song *et al.,* 2002; Wang *et al.,* 2006). Indeed, recent results suggest than Cadherin-mediated niche-germline cell adhesion may be part of a competitive mechanism to ensure than differentiated or defective germ cells are displaced from the niche by fGSCs expressing higher levels of *D*E-Cadherin (Jin *et al.,* 2008). Conversely, declining expression of *D*E-Cadherin in stromal cells has also been associated with the age-related loss of stem cell maintenance and niche integrity in both male and female GSCs (Boyle *et al.,* 2007, Pan *et al.,* 2007) and Rab11-mediated trafficking of *D*E-Cadherin is required for GSC maintenance (Bogard *et al.,* 2007; but see Lighthouse *et al.,* 2008). Adherens junctions also appear to be necessary for maintaining other stem cell populations found within the ovary, such as the somatic FSCs (Song and Xie, 2002).

Recent studies into the formation of the male GSC niche have begun to shed light on the molecular mechanisms involved in controlling cell adhesion within a niche. Male GSC niche morphogenesis and PGC recruitment begin during embryonic development when a group of anterior somatic cells in the primitive male gonad start to accumulate many of the cell adhesion proteins typical of adult hub cells such as Fasciclin III, *D*E- and N- Cadherin (Le Bras and Van Doren, 2006). By the end of embryogenesis the presumptive hub cells become highly compacted and appear to organize PGCs into a rosette-like arrangement, characteristic of GSCs in the adult niche (Le Bras and Van Doren, 2006).

GTPase signaling seems to play a significant role in niche cell adhesion. Mutants for a Rap-GEF appear to be defective specifically for the recruitment of adherens junction components between mGSCs and hub cells even though levels of *D*E-Cadherin and β-Catenin between hub cells were normal and hub cell morphology was not affected. Consistent with a role of adherens junctions in niche formation/maintenance, GSCs were seen "drifting away" from hub cells during larval development in Rap-GEF mutants, resulting in male sterility. Although Rap GTPase activity seems to primarily affect the somatic hub cells, its loss could be compensated by over-expressing *D*E-Cadherin in mGSCs, demonstrating the importance of cell adhesion for niche function (Wang *et al.*, 2006).

The identification of putative stem cell niches such as ISCs and FSCs (Micchelli and Perrimon, 2006, Nystul and Spradling, 2007, Ohlstein and Spradling, 2006), which lack direct association with identified stromal cells, has highlighted the potential importance of adhesion to the basement membrane and indirect cell adhesion mediated via association with the Extracellular Matrix (ECM). Recent findings from Tanentzapt *et al.* appear to confirm this hypothesis (Tanentzapf *et al.*, 2007). During embryogenesis ECM components accumulate around the male gonad. By late embryogenesis hub cells adopt their anterior position and appear to make contact with the ECM. In the adult, ECM components appear enriched and convoluted where they contact the hub cells, indicating an enhanced association between the niche and the ECM (Hardy *et al.*, 1979). Tanentzapt *et al.* showed that loss of integrins resulted in a failure to currently organize the ECM in the embryo and properly position the hub cells. Moreover, reduced expression of Talin, an integrin-binding cytoskeletal linker, from somatic niche cells resulted in the frequent destabilization of the hub cells from the testis. Their findings suggest that Integrin-mediated organization of the ECM may play a key role in niche formation and maintenance. Interestingly many aspects of mGSC niche formation were unaffected by the loss of integrins (Tanentzapf *et al.*, 2007) suggesting that direct cell-cell adhesion and ECM-mediated adhesion may assume complimentary roles in forming a niche. A recent report has demonstrated that the ECM component Type IV collagens play an important role in defining the range of BMP signaling (see below) in the fGSC niche (Wang *et al.,* 2008), suggesting a role for the ECM in both niche morphology and regulation of niche signaling. Future work will need to address how the ECM is established and maintained in those niches that lack stable stromal cell associations.

Male and female germline stem cell niche signaling – different, but not so different

The two well characterized *Drosophila* GSC niches have revealed a wealth of information with respect to how different cell types within a niche can signal to each other. Although the signaling pathways involved in both the fGSC and mGSC niches are complex, genetic analysis has established individual signaling pathways which appear to act to define the stem cell self-renewal permissive zone (reviewed in Kirilly and Xie, 2007, Wong *et al.*, 2005). In the *Drosophila* ovary, fGSC maintenance is strictly dependent on their receipt of BMP ligands, Dpp (encoded by *decapentaplegic*) and Gbb (encoded by *glass-bottomed boat*), from somatic niche cells. Strong activation of the BMP pathway only occurs in those germline cells in contact with stromal cap cells, where it acts together with Otefin, a nuclear membrane protein, to repress the expression of bag-of-marbles (bam) (Jiang *et al.,* 2008), whose expression is sufficient to induce fGSC differentiation. In contrast, the mGSC niche is defined primarily by activation of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway. The stromal hub cells express one of the JAK/STAT ligands, unpaired (upd), which is received by the germline and results in phosphorylation and activation of the transcription factor STAT and expression of JAK/STAT target gene *Zfh1* (Leatherman and Dinardo, 2008). Ectopic activation of the JAK/STAT or BMP pathways in the male or female germline, respectively, results in the formation of GSC tumors.

Increasing evidence indicates that the differences between the mGSC and fGSC niches may be less than they originally appeared (reviewed in Fuller and Spradling, 2007). Analysis of loss-of-function conditions for JAK/STAT pathway components in the ovary shows that it is required for ovarian GSC maintenance (Decotto and Spradling, 2005, Lopez-Onieva *et al.*, 2008, Wang *et al.*, 2008). Moreover, the ectopic expression of upd-family ligands from somatic niche cells results in the formation of germ cell tumors, showing that the JAK/STAT pathway is sufficient to expand the fGSC niche in the ovary. However, in contrast to the testis, the JAK/STAT pathway is not required in the germline for fGSC maintenance (Decotto and Spradling, 2005, Lopez-Onieva *et al.*, 2008), meaning that it must mediate its effects indirectly. Significantly, the induction of ectopic germ cell tumors by *upd2* is

associated with increased *dpp* expression and activation of the BMP pathway in the germline (Lopez-Onieva *et al.*, 2008), suggesting that in the ovary the JAK/STAT pathway acts on the germline via the BMP pathway. Consistent with this model, loss of *hop* (which encodes the *Drosophila* Janus Kinase) function in the stromal cap cells is sufficient to promote GSC differentiation (Lopez-Onieva *et al.*, 2008, Wang *et al.*, 2008). In addition, the receipt of JAK/STAT pathway signals by the escort stem cells (ESCs) might also be required to co-ordinate somatic and germline line stem cell populations (Decotto and Spradling, 2005).

This interaction between BMP and JAK/STAT pathways in the fGSC niche raises the question of whether a similar interaction might occur in the testis. In fact, BMP signaling is required for the maintenance of mGSCs in the testis (Kawase *et al.*, 2004, Shivdasani and Ingham, 2003). Ectopic activation of the BMP pathway in the male germline results in a strong repression of *bam* expression, but in contrast to the fGSC niche, this is not sufficient to promote mGSC tumor formation (Kawase *et al.*, 2004, Shivdasani and Ingham, 2003). Interestingly, *upd*-induced mGSC tumors also fail to express *bam* (Tulina and Matunis, 2001). Given the fact that both JAK/STAT and BMP signals are received by the germline and that both pathways repress *bam* expression, it is possible that the JAK/STAT and the BMP pathways interact in germline cells. However, it has yet to be reported whether or not *upd*-induced tumors are associated with activation of the BMP pathway in mGSCs, leaving the possibility that the JAK/STAT pathway might act via a BMP independent mechanism. The inability to visualize BMP ligand gradients in *Drosophila* gonads means that their effects have to be inferred indirectly. However, the role of the BMP pathway and Bam in regulating the switch from amplifying cyst divisions to the initiation of spermatogenesis (Matunis *et al.*, 1997, Shivdasani and Ingham, 2003) suggests that BMP ligands may be more widely distributed in the testis than in the ovary, explaining why JAK/STAT, but not BMP signaling, is the limiting factor in defining the male GSC niche. Thus while clear differences exist in the role of signaling pathways in different stem cell niches, many aspects of the underlying mechanisms appear to be conserved.

Control of gene expression, small RNA molecules and chromatin remodeling as important intrinsic factors required for stem cell self-renewal

Stem cells possess intrinsic properties that enable them to divide many times, self-renew and retain the capacity to differentiate into several cell types. As discussed above, intrinsic properties may allow stem cells to contribute to the organization of their own niche microenvironment, yet their innate characteristics also impose a dependency on those factors produced by this environment. Where do these intrinsic properties come from? And how is the dependency on niche signaling maintained?

The role intrinsic factors play in stem cell maintenance has probably been best characterized in *Drosophila* GSCs. The germline is specified by the asymmetric positioning of particular factors (collectively known as the pole plasm), which are incorporated into the forming germ cells as they bud from the posterior pole of the embryo (reviewed in Okada, 1998). Pole plasm components, Nanos and Pumilio, are required for some of the characteristic features of *Drosophila* PGCs and GSCs such as the appearance of spherical spectrosomes during embryogenesis (Forbes and Lehmann, 1998, Lin and Spradling, 1997). Interestingly, both genes are also required for the maintenance of PGCs during development and later fGSCs in the adult ovary (Forbes and Lehmann, 1998, Gilboa and Lehmann, 2004, Wang and Lin, 2004). Thus, Nanos and Pumilio act as intrinsic factors required for activating and/or maintaining the special genetic programs involved in stem cell identity.

A plethora of recent papers have shown that post-transcriptional control of gene expression appears to be a conserved feature of many of the intrinsic factors involved in establishing and maintaining stem cell identity. Nanos and Pumilio are both translational repressors and appear to represent an ancient mechanism for germline specification found throughout the animal kingdom (Parisi and Lin, 2000).

Piwi is a conserved protein required for germline specification and fGSC self-renewal (Cox *et al.*, 1998, Lin and Spradling, 1997, Megosh *et al.*, 2006). Piwi is required in the female germline for fGSC maintenance and seems to play an important role in mediating *bam* silencing in response to niche signals (Chen and McKearin, 2005, Szakmary *et al.*, 2005). Piwi is the founding member of a novel family of proteins related to the Argonaute RNAi proteins. In *Drosophila*, the three members of the Piwi-family (Piwi, Aubergine and Ago3) mediate an RNAi-related mechanism involving the generation of novel classes of small RNA molecules named Piwi-interacting RNAs (piRNAs) (see reviews Lin, 2007, O'Donnell and Boeke, 2007). Piwi is thought to act to repress the amplification of selfish-genetic elements such as transposons in the germline of several species, at least in part by heterochromatic silencing of specific chromosomal regions. Thus, Piwi-family proteins appear to have evolved as both regulators of stem cell self-renewal and protectors against selfish DNA elements. Interestingly, the requirement for Piwi in fGSCs depends to a large extent on the expression of a single piRNA (3R-TAS1) (Smulders-Srinivasan and Lin, 2003, Yin and Lin, 2007). The expression of this piRNA requires a Piwi-dependent euchromatic remodeling of the heterochromatic region encoding the piRNA (Yin and Lin, 2007). Thus, Piwi may act to control the epigenetic state of GSCs through both activating and silencing specific chromatin regions. Future work will be needed to determine how 3R-TAS1 piRNA is involved in promoting fGSC maintenance and *bam* silencing.

Other RNAi related genes have been implicated in regulating GSC maintenance in *Drosophila*. The microRNA (miRNA) RNA interference pathway is a highly conserved mechanism involved in the regulation of several developmentally important processes (reviewed by Alvarez-Garcia and Miska, 2005). This mechanism is distinct from piRNA mediated-repression of transposons and in *Drosophila* specifically requires the activity of Dicer-1 (Dcr-1) and the miRNA pathway specific Argonaute protein, Ago1. In the ovary, Dcr-1 is required intrinsically within adult germline cells for the control of fGSC divisions possibly by controlling the expression of a cell cycle inhibitor encoded by *dacapo* (Hatfield *et al.*, 2005; Shcherbata *et al.,* 2007). In addition, Ago1, Dcr-1 and its partner Loqs (encoded by *loquacious*) are autonomously required for the long-term maintenance of fGSCs (Forstemann *et al.*, 2005, Jin and Xie, 2007, Park *et al.*, 2007, Yang *et al.*, 2007). Significantly, *dcr-1* is also required for the maintenance of ovarian FSCs (Jin and Xie, 2007) suggesting that miRNA-related mechanisms

may have a general role as innate factors in stem cell maintenance, as shown for the miRNA regulator Mei-P26 in fGSCs (Neumüller *et al.,* 2008).

Control of nuclear architecture has now emerged as an important intrinsic property of stem cells. A number of other factors involved in epigenetic control have also been identified as intrinsically required for stem cell maintenance. Dom and ISWI, ATP-dependent chromatin remodeling factors, are required for the maintenance of FSCs and fGSC respectively (Xi and Xie, 2005). In addition, Stonewall (Stwl), a DNA-associated protein involved in the chromatin-dependent general repression of gene expression, is required for the maintenance of fGSCs (Maines *et al.*, 2007).

In contrast to Piwi, the chromatin remodeling factor ISWI, Stwl and many of the components of the miRNA pathway appear to act independently of *bam* silencing to prevent fGSC differentiation (Jin and Xie, 2007, Maines *et al.*, 2007, Yang *et al.*, 2007). It is tempting to speculate that these pathways promote fGSC maintenance primarily by the repression of differentiation factors downstream of *bam*. However further work is required to definitively determine exactly how these complex intrinsic pathways of gene activation, transcriptional repression and chromatin silencing interact with each other and with extrinsic niche signaling in the ovary to regulate stem cell gene expression and maintenance.

Asymmetric stem cell divisions – divergent control of centrosome dynamics in *Drosophila* stem cells

An important intrinsic property of many stem cells is the ability to divide asymmetrically. *Drosophila* NBs are the premier model for studying the intrinsic mechanisms of asymmetric cell division in *Drosophila* (reviewed by Wodarz, 2005, Yu *et al.*, 2006). Similar to *Drosophila* GSCs, larval NBs assemble their mitotic spindle in the orientation that they will eventually divide, and this orientation is determined by the polarity of the cell, which is retained following their delamination from the epithelium. NB polarity is governed by the apical Par/Inscuteable protein complex, which determines spindle orientation, spindle positioning and the basal localization of cell fate determinants such as Numb and Prospero (Wodarz, 2005, Yu *et al.*, 2006). The apical Par/Inscuteable complex appears to control spindle orientation via another apical complex, containing Pins, Gαi and Mushroom body defective (Mud) (Yu *et al.*, 2006). Recent results now suggest that this complex may act via the differential control of centrosome activation. Interphase NBs possess separated centrioles but only the apical centrosome is capable of acting as a stable microtubule organizing centre (MTOC) (Rebollo *et al.*, 2007, Rusan and Peifer, 2007). This contrasts with the behavior of the other centriole, which moves dramatically around the cell during interphase before eventually coming to rest opposite the apical centrosome at the start of mitosis (Rebollo *et al.*, 2007, Rusan and Peifer, 2007). Mutations affecting MTOC formation disrupt the fidelity of NB asymmetric divisions (Rusan and Peifer, 2007). Moreover loss of the apical Par/Inscuteable complex component Pins causes both centrioles to be destabilized and results in abnormal NB divisions affecting both spindle orientation and cell size (Rebollo *et al.*, 2007). Thus differential control of centrosomes seems to be a key intrinsic feature of asymmetric NB divisions.

Studies in the Fuller laboratory by Yamashita *et al.* on *Drosophila* testis have also suggested that differential regulation of the centrosomes is also a key factor in control of asymmetric mGSC divisions (reviewed by Yamashita and Fuller, 2008). *Drosophila* GSCs also possess polarity, but in contrast to NBs, it appears to be mediated by their contact with stromal cap or hub cells (Song *et al.*, 2002, Wang *et al.*, 2006, Yamashita *et al.*, 2003). In the male GSCs, as in larval NBs, the centrosome separates early in interphase, with one aster-associated centrosome remaining apical, close to the hub cells, while the other centriole migrates to the opposite side of the nucleus for most of interphase (Yamashita *et al.*, 2003). The position of the apical centrosome depends on Adenomatous Polyposis Coli (APC), which is recruited cortically to the region in contact with the hub cells, where *D*E-Cadherin and β-Catenin are also localized (Yamashita *et al.*, 2003). Mutations in APC2 or centrosome components result in a failure to properly orient GSC spindles. Interestingly, this increases the number of germ cells associated with the hub (Yamashita *et al.*, 2003), suggesting that in the male, the number of GSCs may be limited, in part, by controlling the orientation of their division. Recently, Yamashita *et al.* added to their earlier findings by showing that the mother centrosome is always maintained apically and inherited by each mGSC daughter (Yamashita *et al.*, 2007). Further investigation is required to determine if the mother centrosome retained by mGSCs has a role in determining GSC fate beyond controlling spindle orientation. In addition, it is unknown if a similar conservation of the mother centrosome occurs in NB divisions.

The similarity in the centrosome dynamics of mGSCs and NBs indicates that they might represent a conserved mechanism for asymmetric cell division, a suggestion supported by recent observations in fGSCs. During mitosis, the apical spindle pole of fGSCs appears to be associated with the apical spectrosome (Deng and Lin, 1997), an organelle characteristic of male and female GSCs (Fig. 1A). Mutations which disrupt the spectrosome also disturb spindle orientation and asymmetric division in ovarian GSCs (Deng and Lin, 1997, Lin and Spradling, 1997). However, a detailed study of centrosome dynamics in fGSC suggests that, in contrast to mGSCs, centrosome positioning in these cells is essentially random during interphase, even after centrosome separation (Stevens *et al.*, 2007). Even more surprisingly, fGSCs which lack centrosomes are capable of forming a mitotic spindle and orienting it normally along the anterior-posterior axis (Stevens *et al.*, 2007). Determination of the exact nature of centrosome dynamics and asymmetric divisions in male and female GSCs will require further investigation, particularly time-lapse studies of live GSC divisions. However, the current evidence suggests that control of centrosome dynamics may represent an important divergence between mGSC and fGSC asymmetric divisions. Such a divergence might be explained by differing spindle dynamics with respect to the spectrosome. In fGSCs, the mitotic spindle appears to associate with the spectrosome (Deng and Lin, 1997), whereas in mGSCs, the mitotic spindle appears to associate more closely with the cortex, with the spectrosome assuming a more basolateral position with respect to the hub (Yamashita *et al.*, 2003). It would be interesting to determine if fGSC spectrosomes possess a different composition from mGSC

spectrosomes which permits them to control spindle orientation independently of centrosomes. These latest findings in NBs and GSCs represent a major advance in understanding how intrinsic factors, in combination with internal or external polarity cues, can organize asymmetric stem cell divisions.

Concluding remarks

The last two years have seen major advances in our understanding of stem cell biology in both vertebrates and invertebrates. The RNAi-related "small RNA revolution" has had a major impact in developmental biology, and it looks like stem cell biology is no exception. Evidence suggesting a conserved role in stem cells for small-RNA containing complexes has now been seen in mammalian neurons (Shi *et al.*, 2007), and the GSCs of mouse, *C. elegans* and *Drosophila* (Alvarez-Garcia and Miska, 2005, Lin, 2007). Similarly, the control of epigenetic factors, especially chromatin structure, is emerging as an important mechanism for controlling the pluripotency of embryonic stem cells (Reik, 2007).

It remains to be seen the extent to which stem cells are themselves involved to promoting niche formation and/or signaling in other systems, as we have seen in *Drosophila*. In many stem cell models, the next challenge is to work out how the multiple signaling pathways, cell adhesion molecules and various intrinsic factors interact with each other to control self-renewal and differentiation. In this area, the arsenal of genetic tools at the disposal of *Drosophila* researchers will be of great value.

The dissimilarities between different populations of stem cells can sometimes be as striking as their similarities (Fuller and Spradling, 2007, Nystul and Spradling, 2006). Nevertheless, it can be difficult to compare the conservation of molecular mechanisms between different populations of stem cells because slight variations in the role of a given pathway can mask underlying similarity. In the case of *Drosophila* GSCs, there are clear differences between the stem cell regulation of males and females (Fuller and Spradling, 2007), yet recent discoveries have shown that the key factors involved in GSC self-renewal may be much more similar than was first thought.

The identification of additional stem cell populations in *Drosophila* is hugely significant. Each new stem cell population is likely to present distinctive features and regulatory mechanisms. These may represent special adaptations but they may also reflect conserved aspects of stem cell biology which are less easily observed in other systems. By comparing different stem cell populations within model organisms like *Drosophila*, *C. elegans* and vertebrates, we are much more likely to get a true understanding of how stem cell regulation has evolved and how it is conserved at the molecular level.

References

ALVAREZ-GARCIA, I. and MISKA, E.A. (2005). MicroRNA functions in animal development and human disease. *Development* 132: 4653-62.

BOGARD, N., LAN, L., XU, J. and COHEN, R.S. (2007). Rab11 maintains connections between germline stem cells and niche cells in the *Drosophila* ovary. *Development* 134: 3413-3418.

BOLÍVAR, J., PEARSON, J., LÓPEZ-ONIEVA, L. and GONZALEZ REYES, A. (2006). Genetic Dissection of a Stem Cell Niche: The Case of the Drosophila Ovary. *Developmental Dynamics* 235: 2969-2979.

BOYLE, M., WONG, C., ROCHA, M. and JONES, D.L. (2007). Decline in Self-Renewal Factors Contributes to Aging of the Stem Cell Niche in the *Drosophila* Testis. *Cell Stem Cell* 1: 470-478.

BRAWLEY, C. and MATUNIS, E. (2004). Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. *Science* 304: 1331-4.

BROOKMAN, J.J., TOOSY, A.T., SHASHIDHARA, L.S. and WHITE, R.A. (1992). The 412 retrotransposon and the development of gonadal mesoderm in Drosophila. *Development* 116: 1185-92.

COX, D.N., CHAO, A., BAKER, J., CHANG, L., QIAO, D. and LIN, H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes & Development* 12: 3715-3727.

CROZATIER, M. and MEISTER, M. (2007). Drosophila haematopoiesis. *Cell Microbiol* 9: 1117-26.

CHEN, D. and MCKEARIN, D. (2005). Gene Circuit Controlling a Stem Cell Niche. *Current Biology* 15: 179-184.

DECOTTO, E. and SPRADLING, A. (2005). The Drosophila Ovarian and Testis Stem Cell Niches: Similar Somatic Cells and Signals. *Developmental Cell* 9: 501-510.

DENG, W. and LIN, H. (1997). Spectrosomes and fusomes anchor mitotic spindles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in Drosophila. *Developmental Biology* 189: 79-94.

DOE, C.Q., FUERSTENBERG, S. and PENG, C.Y. (1998). Neural stem cells: from fly to vertebrates. *J Neurobiol* 36: 111-27.

FORBES, A. and LEHMANN, R. (1998). Nanos and Pumilio have critical roles in the development and function of Drosophila germline stem cells. *Development* 125: 679-90.

FORSTEMANN, K., TOMARI, Y., DU, T., VAGIN, V.V., DENLI, A.M., BRATU, D.P., KLATTENHOFF, C., THEURKAUF, W.E. and ZAMORE, P.D. (2005). Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. *PLoS Biol* 3: e236.

FUCHS, E., TUMBAR, T. and GUASCH, G. (2004). Socializing with the neighbors: stem cells and their niche. *Cell* 116: 769-78.

FULLER, M.T. (1993). Spermatogenesis. In *The Development of Drosophila melanogaster*, vol. 1 (ed. BATE, M. and MARTINEZ-ARIAS, A.). Cold Spring Harbor Laboratory Press, New York, pp.71-147.

FULLER, M.T. and SPRADLING, A.C. (2007). Male and female Drosophila germline stem cells: two versions of immortality. *Science* 316: 402-4.

GILBOA, L. and LEHMANN, R. (2004). Repression of Primordial Germ Cell DIfferentiation Parallels Germ Line Stem Cell Maintenance. *Current Biology* 14: 981-986.

GILBOA, L. and LEHMANN, R. (2006). Soma-germline interactions coordinate homeostasis and growth in the Drosophila gonad. *Nature* 443: 97-100.

GONZALEZ REYES, A. (2003). Stem cells, niches and cadherins: a view from Drosophila. *Journal of Cell Science* 116: 949-54.

HALBLEIB, J.M. and NELSON, W.J. (2006). Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes & Development* 20: 3199–3214.

HARDY, R.W., TOKUYASU, K.T., LINDSLEY, D.L. and GARAVITO, M. (1979). The germinal proliferation center in the testis of Drosophila melanogaster. *J Ultrastruct Res* 69: 180-90.

HATFIELD, S.D., SHCHERBATA, H.R., FISHER, K.A., NAKAHARA, K., CARTHEW, R.W. and RUOHOLA-BAKER, H. (2005). Stem cell division is regulated by the microRNA pathway. *Nature* 435: 974-978.

JENKINS, A.B., MCCAFFERY, J.M. and VAN DOREN, M. (2003). Drosophila E-cadherin is essential for proper germ cell-soma interaction during gonad morphogenesis. *Development* 130: 4417-26.

JIANG, X., XIA, L., CHEN, D., YANG, Y., HUANG, H., YANH, L., ZHAO, Q., SHEN, L., WANG and CHEN, D. (2008). Otefin, a Nuclear Membrane Protein, Determines the Fate of Germline Stem Cells in *Drosophila* via Interaction with Smad Complexes. *Developmental Cell* 14: 494-506.

JIN, Z. and XIE, T. (2007). Dcr-1 maintains Drosophila ovarian stem cells. *Curr Biol* 17: 539-44.

JIN, Z., KIRILLY, D., WENG, C., KAWASE, E., SONG, X., SMITH, S., SCHWARTZ, J. and XIE, T. (2008). Differentiation-Defective Stem Cells Outcomplete Normal Stem Cells for Niche Occupancy in the *Drosophila* Ovary. *Cell Stem Cell* 2: 39-49.

KAI, T. and SPRADLING, A. (2004). Differentiating germ cells can revert into functional stem cells in *Drosophila melanogaster* ovaries. *Nature* 428: 564-569.

KAWASE, E., WONG, M.D., DING, B.C. and XIE, T. (2004). Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the Drosophila testis. *Development* 131: 1365-75.

KIGER, A.A., WHITE-COOPER, H. and FULLER, M.T. (2000). Somatic support cells restrict germline stem cell self-renewal and promote differentiation. *Nature* 407: 750-4.

KIRILLY, D. and XIE, T. (2007). The Drosophila ovary: an active stem cell community. *Cell Res* 17: 15-25.

KITADATE, Y., SHIGENOBU, S., ARITA, K. and KOBAYASHI, S. (2007). Boss/Sev Signaling from Germline to Soma Restricts Germline-Stem-Cell-Niche Formation in the Anterior Region of Drosophila Male Gonads. *Dev Cell* 13: 151-159.

KRZEMIEN, J., DUBOIS, L., MAKKI, R., MEISTER, M., VINCENT, A. and CROZATIER, M. (2007). Control of blood cell homeostasis in Drosophila larvae by the posterior signalling centre. *Nature* 446: 325-8.

LEATHERMAN, J.L. and DINARDO, S. (2008). Zfh-1 controls somatic stem cell self-renewal in the *Drosophila* Testis and nonautonomously influences germline stem cell self-renewal. *Cell Stem Cell* 3: 44-54.

LE BRAS, S. and VAN DOREN, M. (2006). Development of the male germline stem cell niche in Drosophila. *Dev Biol* 294: 92-103.

LIGHTHOUSE, D.V., BUSZACAK, M. and SPRADLING, A.C. (2008) New components of the Drosophila fusome suggest it plays novel roles in signaling and transport. *Developmental Biology* 317: 59-71.

LIN, H. (2002). The Stem-Cell Niche Theory: Lessons from flies. *Nature Reviews - Genetics* 3: 931-940.

LIN, H. (2007). piRNAs in the germ line. *Science* 316: 397.

LIN, H. and SPRADLING, A. (1997). A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the Drosophila ovary. *Development (Cambridge, England)* 124: 2463-2476.

LOPEZ-ONIEVA, L., FERNANDEZ-MINAN, A. and GONZALEZ-REYES, A. (2008). Jak/Stat signalling in niche support cells regulates dpp transcription to control germline stem cell maintenance in the Drosophila ovary. *Development* 135: 533-40.

MAINES, J.Z., PARK, J.K., WILLIAMS, M. and MCKEARIN, D.M. (2007). Stonewalling Drosophila stem cell differentiation by epigenetic controls. *Development* 134: 1471-9.

MANDAL, L., MARTINEZ-AGOSTO, J.A., EVANS, C.J., HARTENSTEIN, V. and BANERJEE, U. (2007). A Hedgehog- and Antennapedia-dependent niche maintains Drosophila haematopoietic precursors. *Nature* 446: 320-4.

MARGOLIS, J. and SPRADLING, A. (1995). Identification and behavior of epithelial stem cells in the Drosophila ovary. *Development (Cambridge, England)* 121: 3797-807.

MARTINEZ-AGOSTO, J.A., MIKKOLA, H.K.A., HARTENSTEIN, V and BANERJEE, U. (2008) The hematopoietic stem cell and its niche: a comparative view. *Genes and Development* 21:3044-3060.

MATUNIS, E., TRAN, J., GONCZY, P., CALDWELL, K. and DINARDO, S. (1997). punt and schnurri regulate a somatically derived signal that restricts proliferation of committed progenitors in the germline. *Development* 124: 4383-91.

MCNEILL, H. (2000). Sticking together and sorting things out: adhesion as a force in development. *Nat Rev Genet* 1: 100-8.

MEGOSH, H.B., COX, D.N., CAMPBELL, C. and LIN, H. (2006). The role of PIWI and the miRNA machinery in Drosophila germline determination. *Curr Biol* 16: 1884-94.

MICCHELLI, C.A. and PERRIMON, N. (2006). Evidence that stem cells reside in the adult Drosophila midgut epithelium. *Nature* 439: 475-9.

NEUMÜLLER, R.A., BETSCHINGER, J., FISCHER, A., BUSHATI, N., POERNBACHER, I., MECHTLER, K., COHEN, S. and KNOBLICH, J.A. (2008) Mei-P26 regulates microRNAs and cell growth in the *Drosophila* ovarian stem cell lineage. *Nature* 454: 241-245.

NYSTUL, T.G. and SPRADLING, A. (2007). An Epithelial Niche in the *Drosophila* Ovary Undergoes Long-Range Stem Cell Replacement. *Cell Stem Cell* 1: 277-285.

NYSTUL, T.G. and SPRADLING, A.C. (2006). Breaking out of the mold: diversity within adult stem cells and their niches. *Curr Opin Genet Dev* 16: 463-8.

O'DONNELL, K.A. and BOEKE, J.D. (2007). Mighty Piwis defend the germline against genome intruders. *Cell* 129: 37-44.

OHLSTEIN, B., KAI, T., DECOTTO, E. and SPRADLING, A. (2004). The stem cell niche: theme and variations. *Current Opinion in Cell Biology* 16: 693-699.

OHLSTEIN, B. and SPRADLING, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. *Nature* 439: 470-4.

OHLSTEIN, B. and SPRADLING, A. (2007). Multipotent Drosophila intestinal stem cells specify daughter cell fates by differential notch signaling. *Science* 315: 988-92.

OKADA, M. (1998). Germline cell formation in Drosophila embryogenesis. *Genes Genet Syst* 73: 1-8.

PAN, L., CHEN, S., WENG, C., CALL, G., ZHU, D., TANG, H., ZHANG, N. and XIE, T. (2007). Stem Cell Aging Is Controlled Both Intrinsically and Extrinsically in the *Drosophila* Ovary. *Cell Stem Cell* 1: 458-469.

PARISI, M. and LIN, H. (2000). Translational repression: a duet of Nanos and Pumilio. *Curr Biol* 10: R81-3.

PARK, J.K., LIU, X., STRAUSS, T.J., MCKEARIN, D.M. and LIU, Q. (2007). The miRNA pathway intrinsically controls self-renewal of Drosophila germline stem cells. *Curr Biol* 17: 533-8.

POULTON, J.S. and DENG, W.M. (2007). Cell-cell communication and axis specification in the Drosophila oocyte. *Dev Biol* 311: 1-10.

REBOLLO, E., SAMPAIO, P., JANUSCHKE, J., LLAMAZARES, S., VARMARK, H. and GONZALEZ, C. (2007). Functionally unequal centrosomes drive spindle orientation in asymmetrically dividing Drosophila neural stem cells. *Dev Cell* 12: 467-74.

REIK, W. (2007). Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* 447: 425-32.

RUSAN, N.M. and PEIFER, M. (2007). A role for a novel centrosome cycle in asymmetric cell division. *J Cell Biol* 177: 13-20.

SARKAR, A., PARIKH, N., HEARN, S.A., FULLER, M.T., TAZUKE, S.I. and SCHULZ, C. (2007). Antagonistic Roles of Rac and Rho in Organizing the Germ Cell Microenvironment. *Current Biology* 17: 1253-1258.

SHCHERBATA, H.R., WARD, E.J., FISCHER, K.A., YU, J.Y., REYOLDS, S.H., CHEN, C.H., XU, P., HAY, B.A. and RUOHOLA-BAKER, H. (2007). Stage-specific differences in the requirements for germline stem cell maintenance in the Drosophila ovary. *Cell Stem Cell* 13: 698-709.

SHIVDASANI, A.A. and INGHAM, P. (2003). Regulation of Stem Cell Maintenance and Transit Amplifying Cell Proliferation by TGF-β Signaling in *Drosophila* Spermatogenesis. *Current Biology* 13: 2065-2072.

SINGH, S.R., LIU, W. and HOU, S.X. (2007). The adult Drosophila malpighian tubules are maintained by multipotent stem cells. *Cell Stem Cell* 1: 191-203.

SHI, Y., SUN, G., ZHAO, C. and STEWART, R. (2007). Neural stem cell self-renewal. *Crit Rev Oncol Hematol*.

SIEGRIST, S.E. and DOE, C.Q. (2006). Extrinsic cues orient the cell division axis in Drosophila embryonic neuroblasts. *Development* 133: 529-36.

SMULDERS-SRINIVASAN, T.K. and LIN, H. (2003). Screens for *piwi* Suppressors in Drosophila Identify Dosage-Dependent Regulators of Germline Stem Cell Division. *Genetics* 165: 1971-1991.

SONG, S. and XIE, T. (2002). DE-cadherin-mediated cell adhesion is essential for maintaining somatic stem cells in the *Drosophila* ovary. *Proc. Natl. Acad. Sci. USA* 99: 14813-14818.

SONG, X., CALL, G.B., KIRILLY, D. and XIE, T. (2007). Notch signaling controls germline stem cell niche formation in the *Drosophila* ovary. *Development (Cambridge, England)* 134: 1071-1080.

SONG, X., ZHU, C.-H., DOAN, C. and XIE, T. (2002). Germline Stem Cells Anchored by Adherens Junctions in *Drosophilia* Ovary Niches. *Science* 296: 1855-1857.

SPRADLING, A., DRUMMOND-BARBOSA, D. and KAI, T. (2001). Stem cells find their niche. *Nature* 414: 98-104.

STARZ-GAIANO, M. and LEHMANN, R. (2001). Moving towards the next generation. *Mech Dev* 105: 5-18.

STEVENS, N.R., RAPOSO, A.A., BASTO, R., ST JOHNSTON, D. and RAFF, J.W. (2007). From stem cell to embryo without centrioles. *Curr Biol* 17: 1498-503.

SZAKMARY, A., COX, D.N., WANG, Z. and LIN, H. (2005). Regulatory Relation-

ships among *piwi*, *pumilio*, and *bag-of-marbles* in *Drosophila* Germline Stem Cell Self-Renewal and Differentiation. *Current Biology* 15: 171-178.

TAKASHIMA, S., MKRTCHYAN, M., YOUNOSSI-HARTENSTEIN, A., MERRIAM, J. and HARTENSTEIN, V. (2008). The behaviour of Drosophila adult hindgut stem cells is controlled by Wnt and Hh signalling. *Nature* 454:651-655.

TANENTZAPF, G., DEVENPORT, D., GODT, D. and BROWN, N.H. (2007). Integrin-dependent anchoring of a stem-cell niche. *Nat Cell Biol* 9: 1413-8.

TAZUKE, S.I., SCHULZ, C., GILBOA, L., FOGARTY, M., MAHOWALD, A.P., GUICHET, A., EPHRUSSI, A., WOOD, C.G., LEHMANN, R. and FULLER, M.T. (2002). A germline-specific gap junction protein required for survival of differentiating early germ cells. *Development* 129: 2529-39.

TRAN, J., BRENNER, T.J. and DINARDO, S. (2000). Somatic control over the germline stem cell lineage during Drosophila spermatogenesis. *Nature* 407: 754-7.

TULINA, N. and MATUNIS, E. (2001). Control of Stem Cell Self-Renewal in Drosophila Spermatogenesis by JAK-STAT Signaling. *Science* 294: 2546-2549.

VOOG, J., D'ALTERIO, C. and JONES, D.L. (2008). Multipotent somatic stem cells contribute to the stem cell niche in the *Drosophila* testis. *Nature*. 454: 1132-1136.

WANG, L., LI, Z. and CAI, Y. (2008). The JAK/STAT pathway positively regulates DPP signaling in the Drosophila germline stem cell niche. *J Cell Biol* 180: 721-8.

WANG, Z. and LIN, H. (2004). Nanos Maintains Germline Stem Cell Self-Renewal by Preventing Differentiation. *Science* 303: 2016-2019.

WANG, H., SINGH, S.R., ZHENG, Z., OH, S.W., CHEN, X., EDWARDS, K. and HOU, S.X. (2006). Rap-GEF signaling controls stem cell anchoring to their niche through regulating DE-cadherin-mediated cell adhesion in the Drosophila testis. *Dev Cell* 10: 117-26.

WANG, X., HARRIS, R.E., BAYSTON, L.J. and ASHE, H.L. (2008). Type IV collagens regulate BMP signalling in Drosophila. *Nature.* doi:10.1038/nature07214.

WARD, E.J., SHCHERBATA, H.R., REYNOLDS, S.H., FISCHER, K.A., HATFIELD, S.D. and RUOHOLA, H. (2006). Stem Cells Signal to the Niche through the Notch Pathway in the Drosophila Ovary. *Current Biology* 16: 2352-2358.

WODARZ, A. (2005). Molecular control of cell polarity and asymmetric cell division in *Drosophila* neuroblasts. *Current Opinion in Cell Biology* 17: 475-481.

WONG, M.D., JIN, Z. and XIE, T. (2005). Molecular Mechanisms of Germline Stem Cell Regulation. *Annu. Rev. Genet.* 39: 173-95.

XI, R. and XIE, T. (2005). Stem cell self-renewal controlled by chromatin remodeling factors. *Science* 310: 1487-9.

YAMASHITA, Y.M. and FULLER, M.T. (2008). Asymmetric centrosome behavior and the mechanisms of stem cell division. *Journal of Cell Biology* 180: 261-266.

YAMASHITA, Y.M., JONES, D.L. and FULLER, M.T. (2003). Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. *Science* 301: 1547-50.

YAMASHITA, Y.M., MAHOWALD, A.P., PERLIN, J.R. and FULLER, M.T. (2007). Asymmetric inheritance of mother versus daughter centrosome in stem cell division. *Science* 315: 518-21.

YANG, L., CHEN, D., DUAN, R., XIA, L., WANG, J., QURASHI, A. and JIN, P. (2007). Argonaute 1 regulates the fate of germline stem cells in Drosophila. *Development* 134: 4265-72.

YIN, H. and LIN, H. (2007). An epigenetic activation role of Piwi and a Piwi-associated piRNA in Drosophila melanogaster. *Nature* 450: 304-8.

YU, F., KUO, C.T. and JAN, Y.N. (2006). Drosophila neuroblast asymmetric cell division: recent advances and implications for stem cell biology. *Neuron* 51: 13-20.