Stem cells, niches and cadherins: a view from *Drosophila*

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Summary

Stem cells are essential for the correct development and homeostasis of adult organisms, as well as having obvious potential therapeutic importance. Analysis of the biology of stem cells and their regulatory microenvironment in adult organs has, however, been hindered by the rarity of these cells in mature tissues and by the lack of positive markers for them. The ovary of the *Drosophila melanogaster* female is a stem cell niche in which such analyses can be performed. The stromal cells of the microenvironment act as a regulatory centre to control the proliferation and differentiation of the germline stem cells, using several signalling molecules, among them the protein DPP – a *Drosophila* homologue of the human bone morphogenetic proteins BMP2 and BMP4. Recent work shows that *D*E-cadherin-mediated adhesion is used for the initial recruitment and posterior anchoring of the germline-derived stem cells in their niche.

Key words: Stem cells, *D*E cadherin, germline, *Drosophila*

Introduction

Stem cells are of the utmost importance for the biology of developing and adult organisms, and have immense potential for therapeutic use in a variety of medical conditions (Daley, 2002). Adult stem cells allow patient-specific therapies to be carried out without the need for cloning to avoid immune rejection, and they provide an alternative to embryonic stem cells for use in regenerative medicine. Nonetheless, there are still fundamental questions that need to be answered before we fully understand the behaviour of stem cells in living tissues. For instance, the molecular parameters that define a stem cell are not known, nor are the mechanisms that control the rate of proliferation versus differentiation of stem cells (Marshak et al., 2001). Such questions are of major significance in stem cell research, because the characterisation and study of stem cells relies on our ability to identify and culture them. Moreover, in order to understand the mechanisms controlling the establishment and maintenance of stem cell types, we need to decipher the interactions between stem cells and the cellular microenvironments in which they are maintained – so-called stem cell niches (Morrison et al., 1997; Spradling et al., 2001).

Experimental model systems have been an invaluable tool for characterising stem cell regulation. Here, I review our current knowledge of a specific class of adult stem cell and its developmental niche: the germline stem cells present in the ovary of the *Drosophila* female. I focus on the signals that control stem cell function and on the cell biological mechanisms involved in the cellular organisation of the microenvironment.

The germarium: a simple niche for a few stem cells

Although stem cells are present in many mature tissues, they are rare and difficult to locate, which makes analysis of their stem cells properties and microenvironments hard. Some of the best-characterised niches are the hair follicles of mammalian skin, the endothelial gut crypts, the bone marrow in which mesenchymal and hematopoietic stem cells propagate and the mammalian and invertebrate testes (Fuchs and Segre, 2001; Morrison et al., 1997; Spradling et al., 2001). The *Drosophila* ovary constitutes another stem cell niche and is a simple, easy-to-manipulate structure for studies of adult stem cells and their interaction(s) with the surrounding stroma (Jones, 2001; Xie and Spradling, 2000). During its lifespan, the *Drosophila* female sustains a continuous production of eggs. This property relies upon the presence of two populations of stem cells in the ovary – germline stem cells (GSCs) and somatic follicle stem cells (King, 1970; Margolis and Spradling, 1995; Spradling et al., 1997). The coordinated activity of both stem cell types is responsible for homeostasis of the ovary, allowing the regular production of new egg chambers. Here, I concentrate on the origin and behaviour of the GSCs and their interaction with the surrounding tissue.

A *Drosophila* ovary is composed of a series of egg-producing tubes – ovarioles – each of which possesses, at its anterior tip, a conical structure called the germarium. Each germarium hosts on average two to three GSCs. These cells are located at the anterior end of the germarium, close to a group of specialized somatic cells termed cap cells (Forbes et al., 1996; Spradling et al., 1997). Two other somatic cell types are also part of the anterior end of the germarium, terminal filament cells and inner sheath cells. GSCs can be recognised because of their size, location and their possession of a cytoplasmic organelle termed the spectrosome or spherical fusome, which is located anteriorly in interphase cells, on the side of the GSC that contacts the cap cells (Fig. 1) (de Cuevas and Spradling, 1998; Lin et al., 1994).

When a GSC divides, one of the daughter cells always remains attached to the cap cells; the other ends up one cell diameter away from the cap cells. The cell contacting the cap cells stays on as a stem cell, whereas its sibling differentiates as a cystoblast and enters oogenesis (Lin and Spradling, 1997). This observation, together with an earlier report showing that laser ablation of terminal filament cells regulates stem cell division (Lin and Spradling, 1993), led to the hypothesis that the interaction between somatic cells and GSCs in the germarium is essential for the maintenance of the stem cell lineage and that the somatic cells surrounding the GSCs might form a niche in which GSCs are kept (Lin and Spradling, 1993; Xie and Spradling, 1998). Indeed, Xie and Spradling demonstrated that a signalling cascade involving both somatic and germline cells at the tip of the germarium maintains the stem cell lineage in the germline. The TGF-α family protein DPP, a homologue of the human bone morphogenetic proteins (BMPs) BMP2 and BMP4, is probably secreted by the somatic cells in the niche; the DPP receptor and the proteins downstream of it are needed in the GSCs. Upon removal of the receptor or genes encoding any of the downstream proteins in the germline, the GSCs are lost. By contrast, overexpression of *dpp* in the somatic cells of the germarium or hyperactivation of the pathway in the germline leads to supernumerary GSCs (Xie and Spradling, 1998). These results show that somatic cells of the germarium control the GSCs and thus form part of a stem cell regulatory microenvironment. Furthermore, when mosaic germaria containing both wild-type and germ cells that cannot transduce the DPP signal are produced, the mutant GSCs are efficiently replaced by wild-type germline cells that come to lie adjacent to cap cells and that behave like stem cells. This finding demonstrates that the somatic cells at the tip of the germarium constitute a niche for the GSCs (Xie and Spradling, 2000).

Taken together, the observations described above demonstrate that the differentiated somatic cells surrounding the GSCs in the germarium provide a molecular milieu able to regulate, induce and support the development of new stem

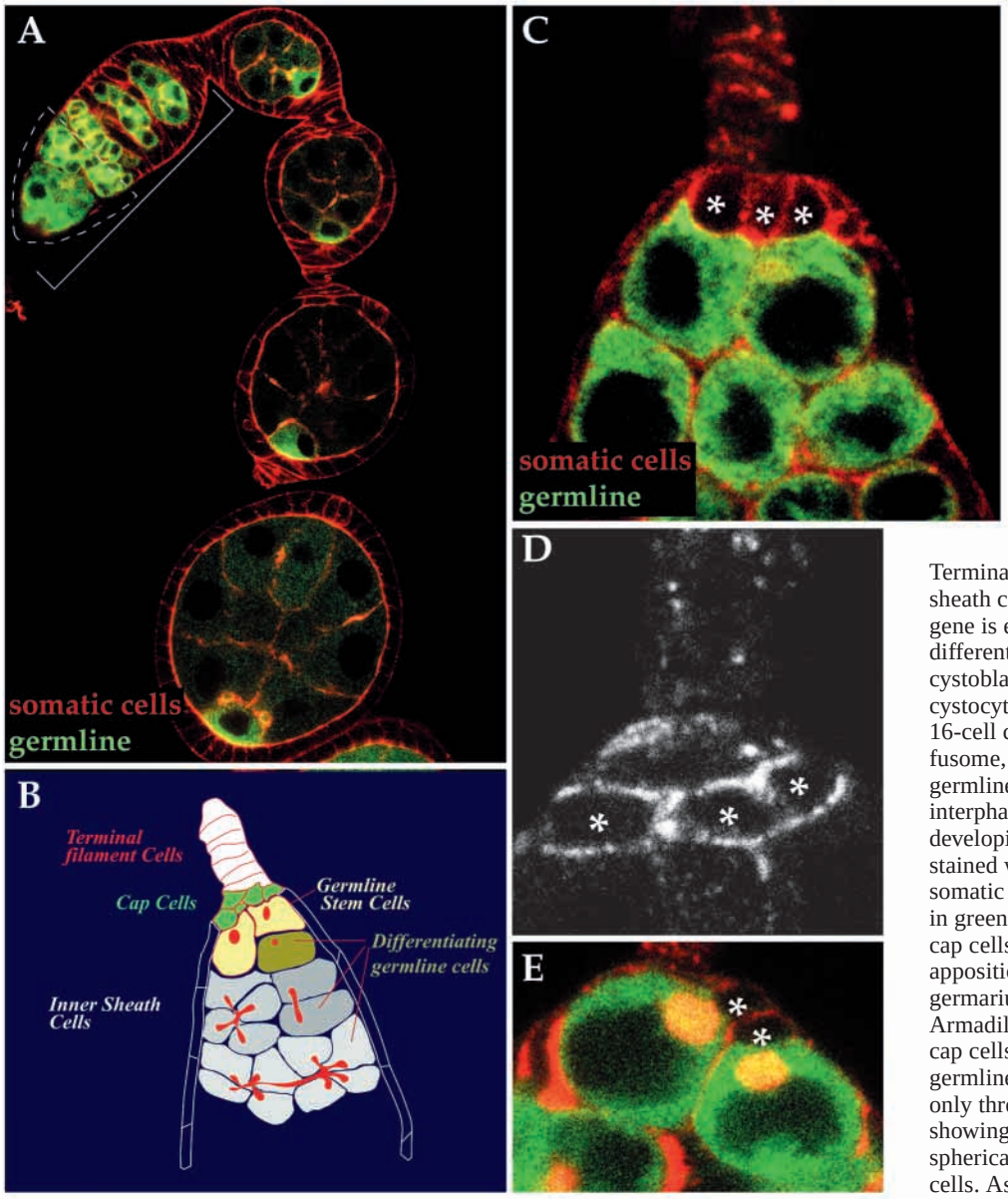

Fig. 1. The germarium, a niche for germline stem cells. (A) A wild-type ovariole showing the germarium (anterior tip; bracket) and a string of developing egg chambers, all of which derive from the activity of stem cells in the germarium. The oocyte is the cell located at the posterior of the cysts that accumulates most of the green staining. The dashed line labels the anterior half of the germarium hosting the germline stem cells represented in B and pictured in C and E. Red: F-actin staining to show the shape and arrangement of somatic and germline cells. (B) The scheme represents different cell types present in a wild-type germarium. Terminal filament cells, cap cells and inner sheath cells are of somatic origin (the *dpp* gene is expressed in the cap cells); the differentiating germline cells are the cystoblast (daughter of a stem cell) and the cystocytes, which form part of 2-, 4-, 8- and 16-cell cysts. The organelle in red is the fusome, which acquires a spherical shape in germline stem cells and in cystoblasts in interphase, and a branched appearance in developing cysts. (C) Wild-type germarium stained with F-actin to visualise the shape of somatic cells (red); germline cells are stained in green. The large cells in contact with the cap cells are germline stem cells. Note the apposition of both cell types. (D) Wild-type germarium showing the localisation of the Armadillo protein in the junctions between cap cells, and between the cap cells and the germline stem cells. This focal plane depicts only three cap cells. (E) Wild-type germarium showing the apical localisation of the spherical fusome present in germline stem cells. Asterisks label cap cells.

cells. Furthermore, terminal filament cells and cap cells seem to play an essential role in the niche. First, the number of cap cells per GSC is maintained in adult germaria [2.5 cap cells/GSC on average (Xie and Spradling, 2000)]. Second, cap cells express *dpp* mRNA, implicating them as the source of the DPP that regulates GSC proliferation and differentiation (Xie and Spradling, 2000). Third, other genes known to be required for GSC survival in the ovary, such as *fs (1) Yb*, *piwi* and, to a lesser extent, *hedgehog*, are expressed in the terminal filament and cap cells (Bhat, 1999; Cox et al., 1998; Cox et al., 2000; Forbes et al., 1996; King and Lin, 1999; King et al., 2001; Lin and Spradling, 1997; Parisi and Lin, 1999; Zhang and Kalderon, 2001). Although other somatic cells in the germarium express *dpp* mRNA, the spatial organisation of terminal filament cells, cap cells and GSCs makes the terminal filament and cap cells the best candidates for a signalling centre essential for the proper function of the niche. Decisive proof that these specialised somatic cells indeed regulate the GSC microenvironment awaits experiments in which they are genetically manipulated so that they cannot produce functional DPP or other candidate factor(s) that may play a part in controlling the behaviour of GSCs.

The origin of the germarial niche for germline stem cells

The precursors of the GSCs in the *Drosophila* embryo are the pole cells, a group of cells set aside at the start of embryogenesis that constitute the first distinct cell lineage established in the embryo. Pole cells, which are equivalent to primordial germ cells of vertebrate embryos, are initially located at the posterior pole of the blastoderm embryo. At the beginning of gastrulation, they move dorsally and are carried inside the embryo by posterior midgut invagination. Pole cells next migrate across the midgut to contact the embryonic mesoderm and subsequently divide into two groups, which interact with the gonadal mesoderm to form the primitive gonads (Starz-Gaiano and Lehmann, 2000). Each female embryonic gonad hosts 12 pole cells on average. During embryogenesis and the larval stages, these cells proliferate to make 80-110 pole cells per larval ovary. At the end of larval development, pole cells stop proliferating and the first signs of oogenic differentiation are observed (King, 1970; Lin and Spradling, 1997). Concomitantly, pole cells become refractory to hybrid dysgenesis at the larval-pupal transition, which suggests that larval and pupal germline cells possess different properties (Bhat and Schedl, 1997).

The somatic cells of the gonad also proliferate during the larval stages, remaining relatively undifferentiated. At the end of the larval phase of development, they begin to differentiate, ultimately subdividing the gonad into 17-20 ovarioles. At this stage, the terminal filament cells and a group of cells that, at least on morphological grounds, look like cap cells, form the anterior structure of the germarium (Chen et al., 2001; Godt and Laski, 1995) (Fig. 2). In close contact with these presumptive cap cells are two or three germline cells that might be the precursors of adult GSCs. These germline-derived cells behave like the stem cells in mature ovaries: they possess a spherical fusome located apically, near the boundary with the cap cells; and they divide so that one of the daughter cells always remains in contact with the cap cells (J. Bolívar and A.G.-R., unpublished observations) (Fig. 2). Considering that germ cells become refractory to hybrid dysgenesis at the larval-pupal transition, and that this coincides with the differentiation of the terminal filament and presumably cap cells, it is tempting

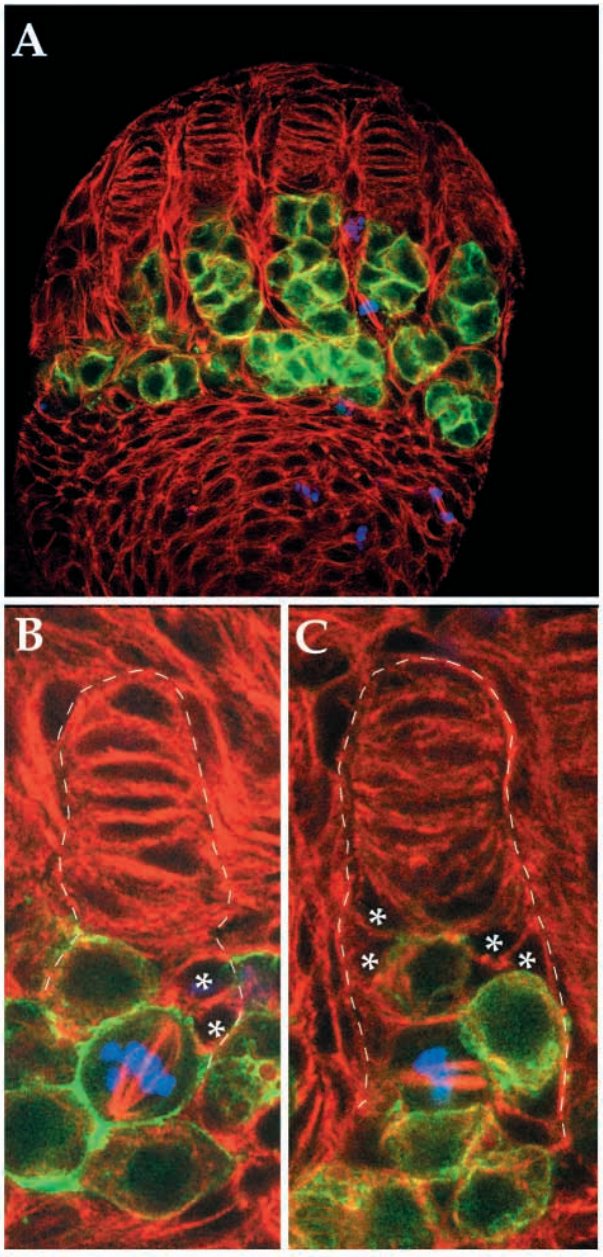

Fig. 2. The generation of a niche during gonadal development. (A) An early pupal gonad (0-2 hours after puparium formation) showing the disposition of terminal filament cells and cap cells, and germline cells. This focal plane depicts five ovarioles. (B,C) Magnification of ovarioles from 0-2 hour pupal gonads. (B) Germline cell in metaphase. The mitotic spindle is oriented so that one of the daughter cells will remain in contact with the cap cells. (C) Cystoblast dividing; the mitotic spindle of these differentiating cells is oriented randomly with respect to the cap cells. In this particular case it is perpendicular to the anterior-posterior axis of the ovariole. Red: tubulin staining to show cell shapes and mitotic spindles; green: membrane-bound GFP driven by a germline specific promoter to label germline cells; blue: phospho-histone H3 to label dividing cells.

to speculate that it is the apposition of cap cells and pole cells that pushes the latter into acquiring a stem cell fate. In this model, pole cells proliferate throughout larval stages to make the pool of germline cells present in third instar larvae; once the terminal filament and cap cells of the gonad differentiate and are organised into a niche, they induce the germline cells in contact with the latter to adopt a stem cell fate. The newly formed GSCs will then initiate asymmetric divisions to sustain oogenesis.

The recruitment and anchoring of germline stem cells to their niche is cadherin dependent

Given the significance of the microenvironment for regulation of stem cell homeostasis, it is important to understand the mechanisms responsible for maintaining the architecture of stem cell niches. For some time now, we have known that adhesion molecules are important for the stroma–stem-cell interactions, and recent work has demonstrated that cadherin-mediated adhesion is central to such interactions. The cadherins are a superfamily of Ca^{2+}-dependent cell-cell adhesion molecules originally classified as single-pass transmembrane glycoproteins responsible for adherens junctions (Takeichi, 1995). 'Classical' cadherins are a subgroup of this superfamily present throughout metazoa characterised by their interactions with the actin cytoskeleton through cytoplasmic catenins (Angst et al., 2001; Steinberg and McNutt, 1999; Tepass et al., 2000). They are involved in a variety of cell biological processes that require the establishment of adhesive connections between cells, including neural development, maintenance of apical-basal polarity in epithelial cells, cell migration, cell sorting, establishment of tissue boundaries and tissue rearrangements during morphogenesis (Dumstrei et al., 2002; Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998; Inoue et al., 2001; Le Borgne et al., 2002; Martinek and Gaul, 1997; Murase and Schuman, 1999; Niewiadomska et al., 1999; Oda et al., 1997; Price et al., 2002; Tanaka-Matakatsu et al., 1996; Tepass et al., 2002; Tepass et al., 1996; Uemura, 1998; Uemura et al., 1996). Classical cadherins are also implicated in cell signalling and control of cell proliferation and differentiation (Knudsen et al., 1998; Willert and Nusse, 1998).

The generation of different structures during morphogenesis relies upon the ability of cells to sort themselves into distinct cell populations. The physical segregation of kindred cell types depends on differences in the relative strengths of cell adhesions and non-directed cell motility (García-Bellido, 1975; Tepass et al., 2002), as formulated by Steinberg in his 'differential adhesion' hypothesis (Steinberg, 1963). Steinberg and Takeichi initially demonstrated the crucial role of cadherins in cell sorting by in vitro experiments in which they cultured pellets of cells expressing different amounts of P-cadherin together. In these experiments, the different cell populations eventually segregated from each other to form a sphere in which the cells that expressed higher levels of P-cadherin were concentrated in the center, whereas cells that expressed lower levels of P-cadherin formed a layer around this core, a 'sphere-within-a-sphere' (Steinberg and Takeichi, 1994). In vivo experiments using oogenesis in *Drosophila* as an experimental system provided further evidence that classical cadherins mediate cell sorting (Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998). In a wild-type egg chamber, a layer of follicle cells surrounds the germline cells, the oocyte and the nurse cells. Early in oogenesis, the oocyte comes to lie posterior to the nurse cells; this arrangement is maintained during the rest of oogenesis and is essential for proper polarisation of the embryo (Fig. 1) (van Eeden and St Johnston, 1999). Cadherin-mediated cell sorting and adhesion play an active role in the posterior positioning of the oocyte. The oocyte possesses higher levels of *D*E-cadherin than the nurse cells; conveniently, the follicle cells at the poles of the egg chamber also possess higher levels of *D*E-cadherin than the rest of the follicle cells. As in Steinberg and Takeichi's experiment, the oocyte recognises the increased levels of *D*E-cadherin in the follicle cells at the posterior and adheres to them, thus achieving its posterior positioning (Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998).

Organisation of the germline stem cell niche in the *Drosophila* ovary constitutes another use of the adhesive properties of classical cadherins during ovary development (Song et al., 2002). Germline cells come into contact with the specialised somatic cells that form the tip of the germarium during late larval development. The juxtaposition of germline cells with future cap cells seems to initiate the establishment of adherens junctions that are maintained throughout pupal development and adult life. Indeed, germline cells lacking *D*E-cadherin are incorporated at a lower frequency in the adult germarium than are their wild-type siblings. This result demonstrates that cadherin-mediated adhesion, probably in concert with other factors, is necessary to recruit stem cells to their developmental microenvironment. In addition, *D*E-cadherin and the *Drosophila* homologue of β-catenin, Armadillo, are concentrated at the interface between cap cells and GSCs in the adult ovary, which supports a role for this adhesion system in anchoring GSCs to their niche (Fig. 1D). The fact that in mosaic germaria containing both wild-type and cadherin-mutant GSCs the mutant cells leave the niche, differentiate and are replaced by their wild-type neighbours is also consistent with this idea (Song et al., 2002). However, a lack of *D*E-cadherin or Armadillo does not seem to compromise the identity or behaviour of stem cells, since the mutant stem cells persist for at least three weeks after removal of *D*E-cadherin or Armadillo (Song et al., 2002). Altogether, these results indicate that adhesion of GSCs to the somatic cap cells is mediated mainly by *D*E-cadherin. This adhesion system is thus responsible for the recruitment and anchoring of GSCs to their niche. However, it is not required for GSC development as stem cells: as long as these cells remain in the appropriate environment, even in the absence of *D*E-cadherin or Armadillo proteins, they retain the self-renewal capacity and asymmetric division typical of stem cells. These observations argue against a role for cadherin-mediated signalling in GSC development.

Conclusions

The identification of the molecular mechanisms that are responsible for organising a stem cell niche is the initial step in understanding the biology of stem cells in vivo. Here, I have attempted to describe the evidence emerging from the analysis of the regulatory microenvironment of adult stem cells in *Drosophila*. The simplicity of the system, and its susceptibility to genetic manipulation, has helped enormously in elucidating

some of the genes and molecules involved in establishing and maintaining adult stem cells. The stem cells present in this particular niche are germline cells and have therefore a highly restricted developmental potential, because they ultimately give rise to a single differentiated cell type, the gamete. However, at least some aspects of the stroma–stem-cell interactions in the *Drosophila* germarium appear to be conserved in other stem cell niches.

The success of stem cell transplantations depends on the ability of these cells to interact with stromal cells and to re-populate the empty niches, a property of stem cells known as homing. Perhaps the best-studied experimental model in this regard is the hematopoietic system, where stem cells originating in the hematopoietic organs migrate between and populate different niches during development and adult life (Orkin, 2001). Under experimental conditions and upon transplantation, infused stem cells can re-populate the bone marrow in two phases: first, they can engraft the bone marrow stroma transiently and sustain hematopoiesis for up to four weeks; subsequently, they interact with the stromal cells and occupy the empty niches to provide long-term protection (Cumano and Godin, 2001; Whetton and Graham, 1999). Despite its importance, the mechanisms regulating stem cell homing are not fully understood. Signalling molecules such as chemokines and growth factors are known to be involved, and local secretion of proteases by stromal cells and the concomitant release of stem-cell cytokinesis is an essential step contributing to the mobilisation of stem cells (Heissig et al., 2002; Whetton and Graham, 1999). Cell adhesion molecules have also been proposed to mediate the interaction between stem cells and stromal cells in vitro. For instance, epithelial cadherin is present in human bone marrow stroma and $CD34^+$ bone marrow stem cells, which suggests that, like the situation in the *Drosophila* ovary, this molecule is involved in stem cell adhesion in vertebrates (Turel and Rao, 1998). Other adhesion molecules important in this context include mucin-like molecules, such as CD164, and integrins (Teixido et al., 1992; Zannettino et al., 1998). It might be interesting to test these candidates in an experimental in vivo system such as the *Drosophila* ovary. In the future, advances in our understanding of the signalling pathways used by stromal cap cells to influence the behaviour of germline stem cells should help broaden our comprehension of the biology of other stem cell types and shed light on the development of therapeutic applications for stem cell research.

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