Adhesion molecules in the stem cell niche – more than just staying in shape?

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Summary

The expression of adhesion molecules by stem cells within their niches is well described, but what is their function? A conventional view is that these adhesion molecules simply retain stem cells in the niche and thereby maintain its architecture and shape. Here, we review recent literature showing that this is but one of their roles, and that they have essential functions in all aspects of the stem cell-niche interaction – retention, division and exit. We also highlight from this literature evidence supporting a simple model whereby the regulation of centrosome positioning and spindle angle is regulated by both cadherins and integrins, and the differential activity of these two adhesion molecules enables the fundamental stem cell property of switching between asymmetrical and symmetrical divisions.

Key words: Cadherin, Integrin, Adherens junction, Basal lamina, Centrosome, Stem cell niche

Introduction

Stem cells are conventionally defined as slowly dividing cells that constantly replenish differentiated cells in adult tissues, although the term is also widely used to describe rapidly dividing cells in the embryo that give rise to the cells that are required for growth and tissue formation. Well-described examples of adult stem cells are the germline stem cells (GSCs) of the *Drosophila melanogaster* ovary or testes that produce new oocytes or spermatogonia, respectively, throughout life, and the stem cells of adult mammalian skin, gut and blood that replace differentiated cells that are lost through turnover in each of these tissues. Here, stem cells operate in a 'homeostatic' role to maintain the structure and function of adult tissues. Maintenance of the cell population required for this homeostasis appears to be an important function of the stem cell microenvironment – a specialised region termed a niche, which both maintains and protects the stem cells. Two broad classes of niche have been described (Morrison and Spradling, 2008) – epithelial niches, where stem cells are in direct contact with a basal lamina, and stromal niches, where stem cells contact another cell type that is in contact with basal lamina (Fig. 1A). In both cases, stem cells are also in contact with their own daughter cells. Therefore, the niche microenvironment contains various sources for the signals that regulate stem cell behaviour.

Despite studies showing high expression levels of adhesion molecules on stem cells in both the adult and the embryo, we still have a surprisingly poor understanding of the function of these molecules. Recent reviews have addressed this gap in our knowledge, focussing either on integrins or on each of the different niches (Ellis and Tanentzapf, 2010; Raymond et al., 2009). Here, at the risk of over-simplifying the biology of these complex structures, we attempt to tease out general mechanisms from prior studies by organising this Commentary around the basic aspects of stem cell behaviour within the niche – retention, division and exit. To achieve this, we focus on the two major classes of adhesion molecules – cadherins, which regulate cell-cell interactions, and integrins, which regulate cell-matrix interactions.

Retention of stem cells in the niche

The best examples of a function for adhesion molecules in retaining stem cells in the niche are based on studies of *Drosophila* gonads. In these stromal niches, the hub and cap cells of the testes and ovary, respectively, provide signals to maintain germline stem cells (Fuller and Spradling, 2007). Two distinct sets of adhesive interactions are therefore required: those that retain the hub (in the testes) or cap (in the ovary) cell on the basal lamina, and those that keep the stem cell in contact with the hub or cap cell. Integrins have been shown to have a necessary role in the former interaction. Loss of integrin function in the hub cell during morphogenesis results in its detachment from the basal lamina and mislocation into the gonad (Tanentzapf et al., 2007) (Fig. 1B). Importantly, the stem cell remains attached to the supporting hub cell throughout gametogenesis. In this stromal niche, therefore, integrins are required for the correct location of the support cell but not of the stem cell. Adhesion of the stem cell to the support cell, by contrast, appears to be mediated by cadherins. In the fly ovary, loss of E-cadherin from an individual stem cell leads to an inability of the stem cell to remain anchored in the niche (Song et al., 2002) (Fig. 1C). Moreover, mutant stem cells expressing high levels of E-cadherin displace normal stem cells expressing lower levels of E-cadherin from the niche (Jin et al., 2008). This suggests that cells 'compete' for niche space based on cadherin expression levels, providing a mechanism for the displacement of differentiated cells and a means to dislodge dysfunctional stem cells from the niche (Jin et al., 2008).

On the basis of the structure of epithelial niches, one would predict that integrin-mediated adhesion to the basal lamina is required for stem cell retention at these sites. This has been shown

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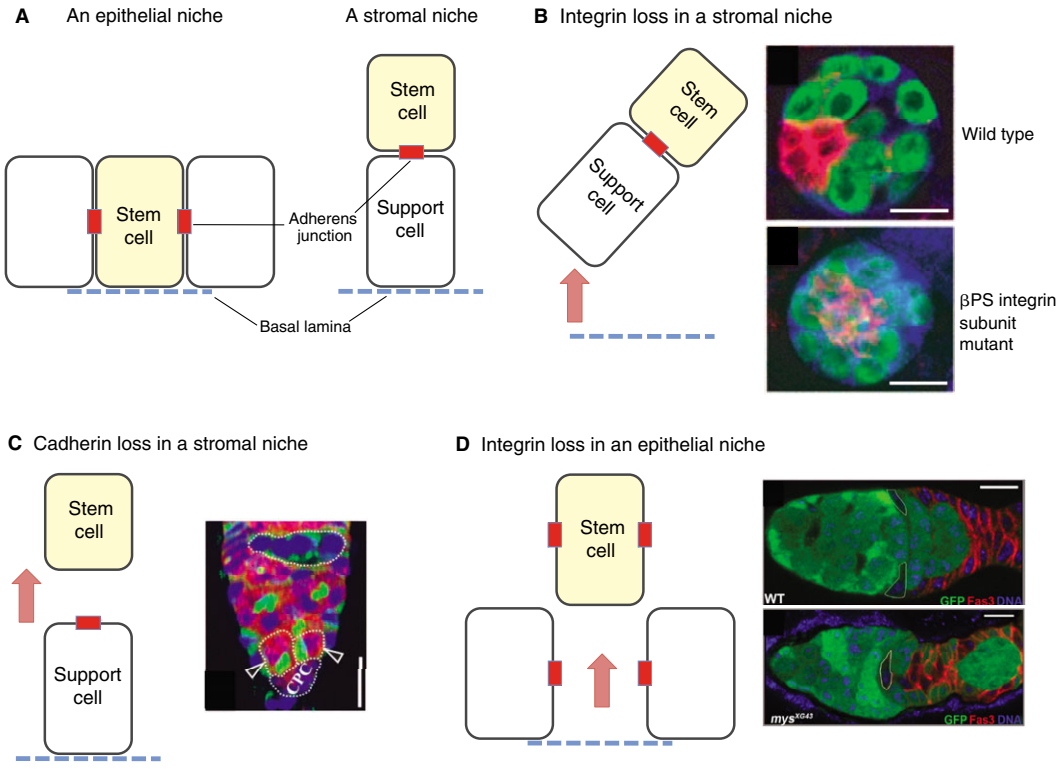

Fig. 1. The essential features of the two basic types of niche, and the effects of adhesion molecule loss. (**A**) In an epithelial niche (left), the stem cell is in direct contact with the underlying basal lamina, whereas in a stromal niche (right) the stem cell contacts a support cell (e.g. the hub and cap cells in *Drosophila* testes and ovary, respectively) that contacts the basal lamina. Adherens junctions (AJs) anchor the stem cell to the support cell in a stromal niche and maintain contact with neighbours in an epithelial niche. (**B-D**) The effects on support and/or stem cells of integrin and cadherin loss of function: results are illustrated schematically (left) and experimentally (right). Pink arrows indicate the detachment of support and/or stem cells. (B) Integrin loss in a stromal niche. The hub cell becomes detached from the underlying basal lamina. Panels on right [reproduced with permission (Tanentzapf et al., 2007)] show hub cells labelled red and stem cells green. Note that, in the wild-type *Drosophila* testes, hub cells are attached to the basal lamina surrounding the gonad, whereas in a mutant of the βPS integrin subunit, they become mislocalised into the centre of the niche while remaining attached to the stem cells. (C) Loss of E-cadherin in the stem cells of *Drosophila* ovary leads to detachment from their supporting cap cells (indicated by pink arrow). In the experiment shown on the right [reproduced with permission (Song et al., 2002)], cells in which *Drosophila* E-cadherin has been lost as a result of genetic recombination are marked by loss of expression of *lacZ* (red). Note that two normal stem cells (arrowheads) remain in contact with the cap cell (CPC), whereas a clone of cells in which *Drosophila* E-cadherin function has been lost (top of panel, outlined by dotted line) has formed from a stem cell that has become detached from the niche. (D) Integrin loss in an epithelial niche. The stem cells become detached from the underlying basal lamina. Panels on right show this in a *Drosophila* ovary [reproduced with permission (O'Reilly et al., 2008)]. The follicle stem cells are normally found in contact with the basal lamina that surrounds the gonad (top panel); two wild-type follicle stem cells that have undergone genetic recombination are revealed by a loss of GFP staining (white cells). The use of this recombination strategy to generate both GFP loss and the myospheroid (*mys*) mutation of the integrin βPS subunit in these stem cells results in their mislocalisation into the centre of the ovary (bottom panel). Scale bars: 20 μm (B), 10 μm (C,D).

directly for follicle stem cells in the *Drosophila* ovary, where cells that lack the β-integrin subunit βPS were frequently mislocalised into the centre of the gonad, away from their normal location on the basal lamina at the edge (O'Reilly et al., 2008) (Fig. 1D). Interestingly, these stem cells also produce laminins, and laminins constitute one of the integrin ligands in the underlying basal lamina. This, therefore, provides an example of stem cells generating the signals required for their own maintenance within the niche.

In adult mammalian niches, the role for adhesion molecules in retaining the correct position of stem cells remains unproven. In the bone marrow niche of haematopoietic stem cells (HSCs), a population of HSCs is located adjacent to the endosteum, the cell layer on the bone surface (Kiel et al., 2005; Zhang et al., 2003). It has been proposed that the HSCs are anchored to a subpopulation of osteoblasts at this site by N-cadherin, with these osteoblasts providing a niche environment (Zhang et al., 2003) – a situation analogous to that in *Drosophila* stromal gonadal niches. However, studies that used the signalling lymphocyte activating molecule (SLAM) family of lymphocyte receptors as markers of HSCs revealed that only a minority of HSCs is found adjacent to the endosteum; by contrast, the majority was found to be associated with the sinusoidal endothelial cells within the bone (Kiel et al., 2005). Furthermore, mice with greatly reduced numbers of osteoblasts have no alterations in HSC numbers (Kiel et al., 2007), and the conditional deletion of N-cadherin from HSCs has no effect on haematopoiesis (Kiel et al., 2009). Together, these observations cast doubt on the concept of an N-cadherin-dependent osteoblastic niche as being required for haematopoiesis. With respect to integrins, it has been reported that epithelial stem cells in several tissues, including the skin and brain, express high levels of α6 and/or β1 integrins (which heterodimerise to generate a laminin receptor) (Hall et al., 2006; Jones and Watt, 1993). Equally, laminins that contact the stem cells are present in the underlying basal lamina or in finger-like extensions of extracellular matrix

extending from blood vessels termed fractones (Mercier et al., 2002). These latter structures are found in the subependymal zone (SEZ) niche of the adult central nervous system (CNS), which gives rise to new olfactory neurones and oligodendrocytes in rodents. Demonstration of an effect of integrin or laminin deletion on stem cell retention has been hampered by the lack of markers for these cells, preventing histological studies that might directly show displacement of stem cells. The recent demonstration that the expression of leucine-rich-repeat-containing G-protein-coupled receptor 5 (Lgr5) marks intestinal stem cells (Barker et al., 2007) might overcome this hurdle if further studies show that Lgr5 also marks other stem cell populations.

In contrast to adult tissues, a clear example of the importance of adhesive interactions in retention within a niche is provided during development by neural stem cells (NSCs) in the mammalian forebrain. Despite the absence of stem-cell specific markers, NSCs can be recognised by their unique morphological features, as they display a radial morphology that spans the depth of the developing tissue. Throughout development, their short apical processes remain attached to the ventricular surface and their basal processes to the overlying pial surface (Fig. 2A), whereas their cell bodies are retained close to the ventricle in the so-called ventricular zone (VZ). Although different from an adult niche in that it is a transient structure, the VZ contains cells undergoing asymmetric self-renewing divisions, some of which become slowly dividing adult stem cells in the SEZ (Kriegstein and Alvarez-Buylla, 2009). The VZ, therefore, provides the signals required for stem cell maintenance and can appropriately be termed a niche. Here, adherens junctions (AJs) surround the very small apical membrane that is directly in contact with the ventricle and hold the apical processes in alignment at the ventricular surface. These N-cadherin-based junctions, therefore, preserve both lateral interactions between NSCs and the position of the cell body within the VZ (Junghans et al., 2005; Kadowaki et al., 2007) (Fig. 2B). Integrin-mediated extracellular matrix adhesion is partly responsible for attachment of the basal process to the overlying pial basal lamina (Haubst et al., 2006; Radakovits et al., 2009) (Fig. 2B). The remarkable observation that the thin basal process can be precisely split during division, thereby maintaining contact of both daughters with the basal lamina (Kosodo et al., 2008), strongly supports the idea that the basal lamina provides essential signals for NSC function. In keeping with this idea, detachment of stem cells following integrin loss was found to be associated with apoptosis (Radakovits et al., 2009). Interestingly, integrins also contribute to the attachment of apical processes, because integrin-blocking antibodies injected into the ventricle result in detachment of a subset of these processes, away from the ventricular surface (Lathia et al., 2007; Loulier et al., 2009). Together, these different experiments show that both cadherins and integrins retain stem cells in this embryonic niche.

Regulation of cell division

Asymmetric cell division is a fundamental property of stem cells that enables both self renewal and the generation of differentiated daughter cells. Elegant experiments in *Drosophila* and *C. elegans* have defined the three key steps in this process: (1) the establishment of polarity, (2) localisation of fate determinants to one or other pole of the cell and (3) subsequent regulation of the plane of cell cleavage upon division such that these fate determinants are asymmetrically distributed between the two daughter cells (Betschinger and Knoblich, 2004). One of the two thus retains stem cell characteristics while the other becomes a differentiated cell, constituting an asymmetric division. It is important to appreciate that asymmetric distribution of fate determinants is not the only way to maintain a self-renewing population, as the same result might be achieved when identical daughter cells are subsequently specified entirely by their position

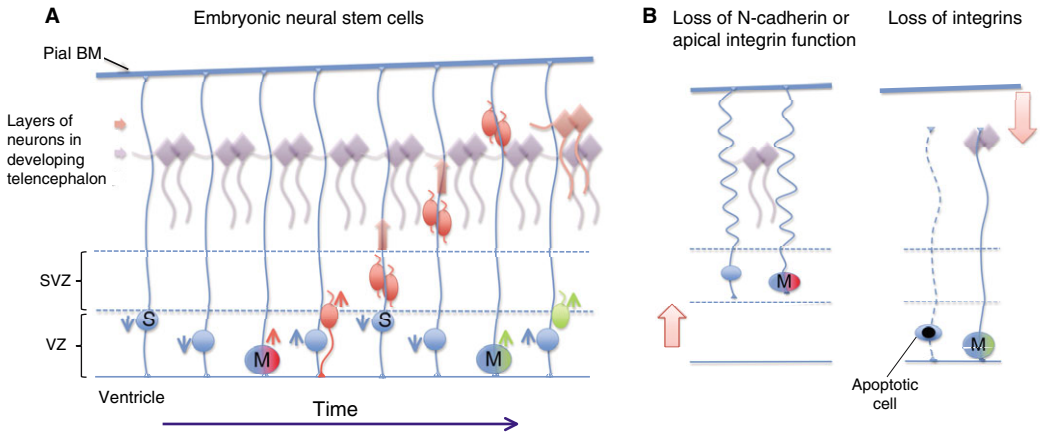

Fig. 2. The anatomy of embryonic NSCs in the mammalian telencephalon, and the effect of adhesion molecule loss. (**A**) Normal development, with stem cells in blue, spanning the depth of the developing telencephalon. A short apical process contacts the ventricular surface, whereas a longer basal process contacts the overlying pial basement membrane (Pial BM). The nucleus is retained in a region close to the ventricular surface, the ventricular zone (VZ). The cell undergoes mitosis (M) on the ventricular surface, after which the nucleus of the daughter that remains a stem cell moves away from the ventricular surface, undergoes S phase (S) and then drops down onto the ventricular surface to divide again (M). The daughter cell that will differentiate migrates out of the VZ into the underlying subventricular zone (SVZ) where it undergoes further division before migrating away along the basal process of the stem cell to find its correct location in the developing cortex. Successive waves of division generate the different neuronal types of the cortex. (**B**) Loss of N-cadherin expression from the apical adherens junctions results in detachment of the apical process (pink arrow) and mislocation of the mitotic NSCs into the SVZ. The injection of antibodies that block β1 integrin directly into the ventricle has the same effect on a small population of the NSCs. The effect of long-term loss of β1-integrin function caused by conditional deletion of the gene is shown on the right. The basal process becomes detached (pink arrow), leading to apoptosis of the NSC and/or mislocation of the neurons that normally migrate up the basal process.

and by external signals derived from cells outside the niche. Such a 'population' model has been proposed based on studies of the *C. elegans* germ line, and is thought to underlie the ability of stem cell numbers to expand during regeneration (Morrison and Kimble, 2006). More recently, specification by extrinsic signals has also been shown to operate in the mammalian hair follicle during cyclic hair growth (anagen phase) (Zhang et al., 2009). Although we recognise that genuinely asymmetric stem cell divisions might be the exception rather than the norm in many mammalian tissues, we focus in the next section on these asymmetric divisions, because it is here that putative roles for adhesion molecules are more clear.

Studies in which the linkage between adhesion molecules and the astral microtubules that determine centrosome and spindle positioning have been perturbed show that both cadherins and integrins regulate the plane of cell division in stem cells and other cell types (Fig. 3). Deletion of the adenomatous polyposis coli protein (APC) that anchors astral microtubules to the E-cadherin-rich AJ between the hub cell and the stem cell in *Drosophila* testes leads to spindle misorientation with respect to the hub cell (Fig. 3A) (Yamashita et al., 2003). In *Drosophila* neuroepithelium, cell division is symmetric in epithelial cells but not in the neuroblasts that have lost contact with their epithelial neighbours after delamination from the epithelial layer. Here, division is now asymmetric and the plane of cleavage is perpendicular to the apico-basal axis, so that any putative fate determinants with an apical or basal location will be partitioned unequally. However, inhibition of the expression of APC – the molecule suspected to be the link between AJs and astral microtubules – in the epithelial cells switches their plane of cleavage from the normal vertical symmetric divisions (which generate two similar sized cells) to horizontal asymmetric divisions (which generate a smaller delaminating daughter cell) (Fig. 3B) (Lu et al., 2001). To explain this, the authors proposed an important model whereby two sets of cues compete to control the angle of cell division – a planar polarity cue supported by AJs, which is normally dominant, and an apico-basal polarity cue, which is only revealed after delamination when AJs are lost. In mammalian skin, where the switch from vertical to horizontal cleavage planes enables the formation of keratinocytes and a stratified skin, the deletion of cadherin-binding α-catenin also perturbs normal vertical symmetric divisions in the epidermal cells, but in this setting results in randomization of the angle of division rather than in a switch to horizontal asymmetric divisions (Lechler and Fuchs, 2005). A similar result was seen when β1 integrin was deleted in these cells (Lechler and Fuchs, 2005) and in another epithelial niche, the basal stem cells of the mammary gland (Taddei et al., 2008).

How do adhesion molecules control the angle of cell division? In the case of cadherins and AJs, the key interaction appears to be with the astral microtubules, which enables proper positioning of centrosomes. So, for example, in the stromal niche of germline stem cells in *Drosophila* testes, the ancestral centrosome is always localised adjacent to the support cell, and is tethered near the AJ that mediates adhesion between hub and stem cells (Yamashita et al., 2007). Consequently, the cleavage plane is parallel to the hub-cell–stem-cell junction, and one daughter always remains closer to the hub cell and associated self-renewing signals after cytokinesis (Fig. 4). Although a similar mechanism has not been formally demonstrated in an epithelial niche, the data on epithelial divisions discussed above are consistent with the centrosome-capture model involving AJs (Fig. 4). If one postulates that this cadherin-mediated centrosome capture is the 'default' mode of division, then the geometry of stromal and epithelial niches would lead to these default divisions being asymmetric in the former and symmetrical in the latter (Fig. 4). The question then becomes whether there are signals that enable the cell to switch from these default divisions to those that are symmetric (in stromal niches) and asymmetric (in epithelial niches)? There is, in fact, surprisingly little evidence that such changes do occur, either because continual asymmetric division is appropriate for gametogenesis as in the gonad, or because the population model whereby stem cells give rise to two identical daughter cells that are subsequently specified as described above suffices to regulate the supply of committed

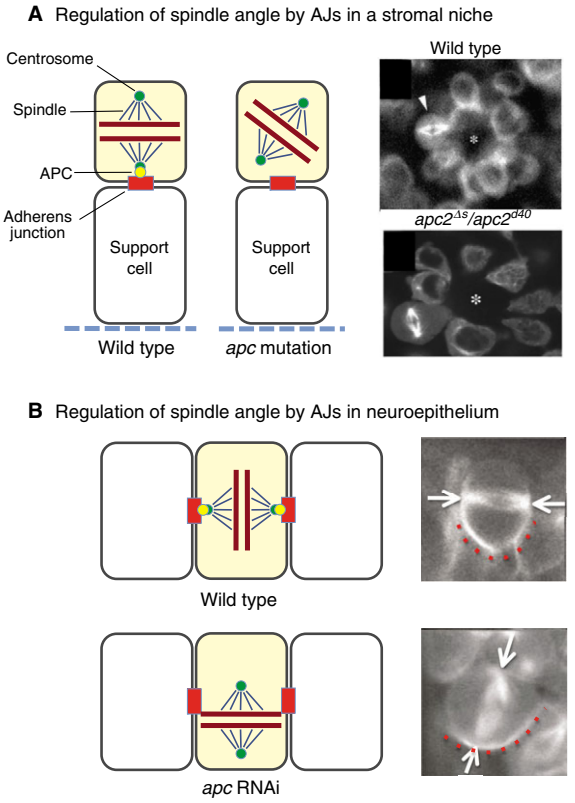

Fig. 3. AJs determine the positioning of the mitotic spindle by centrosome capture. Results are illustrated schematically (left) and experimentally (right). (**A**) In a stromal niche, the ancestral centrosome is held adjacent to the AJ. The effect of a mutation in one protein required for this capture, APC, is shown schematically and in an experiment in which stem cell division in *Drosophila* testes was examined [reproduced with permission (Yamashita et al., 2003)]. The hub cell, indicated by an asterisk, is surrounded by dividing stem cells. Note that, in the wild-type testes (top panel), the spindle angle is perpendicular to the hub cell (arrowhead), whereas in the lower panel showing stem cells in which APC has been mutated ($apc2^{\Delta s}/apc2^{d40}$), this precise orientation of the spindle has been lost and the angle is now nearly parallel to the hub cell surface. (**B**) Role of AJs in the neuroepithelium of *Drosophila*. The effect of decreased APC expression following RNAi is shown schematically (left panels) and in an experiment from Lu et al. [reproduced with permission (Lu et al., 2001)] (right panels). Upper panels show wild-type cells, with the centrosomes captured by the laterally located AJs, resulting in a horizontal spindle plane (white arrows). The basal pole of the cell in the micrograph is identified by the localisation of the protein partner of numb (PON) tagged with GFP (red dots). Following inhibition of APC expression (lower panels), the centrosome capture by the AJs appears to be lost, as the spindle reorientates along the apico-basal axis and now has a vertical orientation (white arrows).

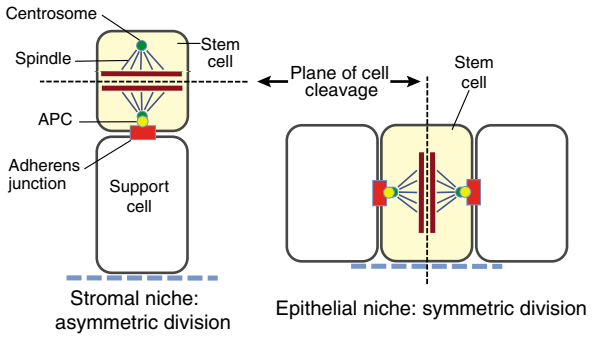

Fig. 4. Predicted 'default' division patterns when centrosome position is instructed by AJs. The capture of the centrosomes by the AJs prior to division locks the spindle plane perpendicular to the support cell or parallel to the plane of the epithelium. The consequence is asymmetric divisions in a stromal niche and symmetric divisions in an epithelial niche.

progeny from the niche. However, one clear example of switching from symmetric to asymmetric division has been provided by studies of embryonic NSCs: here, evidence hints that integrin signalling might be required to re-orient the divisions. Elegant work from Huttner and colleagues has shown that very small changes in the axis of division – that either split (with a vertical cleavage plane) or partition (with an oblique cleavage plane) the apical membrane and associated fate determinants between the daughter cells – are sufficient to switch between symmetric and asymmetric divisions (Kosodo et al., 2004) (Fig. 5). As would be expected, asymmetric divisions with oblique cleavage planes that generate neuronal progenitor cells are more frequent during the phase of neurogenesis. Transient inhibition of integrin activity in the VZ was sufficient in some regions of the telencephalon to alter the axis of division, as evidenced by the disappearance of cells with oblique cleavage planes (Loulier et al., 2009) (Fig. 5). Although differential effects of integrin inhibition on the retention in the VZ of distinct populations of NSCs that divide either vertically or obliquely cannot be excluded, a speculative model to explain these data is that the symmetric divisions result from cadherin-mediated centrosome capture, whereas integrin signalling is one of the components required to overcome the cadherin-mediated capture and enable an (oblique) asymmetric division (Fig. 6). When integrin signalling is blocked, these cells revert back to vertical symmetric divisions (Fig. 6).

Data from other developmental and cell biology studies support this model by showing that integrins can regulate spindle angles. Follicular epithelial cells of the *Drosophila* ovary that lack the integrin βPS subunit show altered spindle orientation without any changes in the localization of polarity markers; this phenotype is also seen when integrin signalling is blocked without perturbing

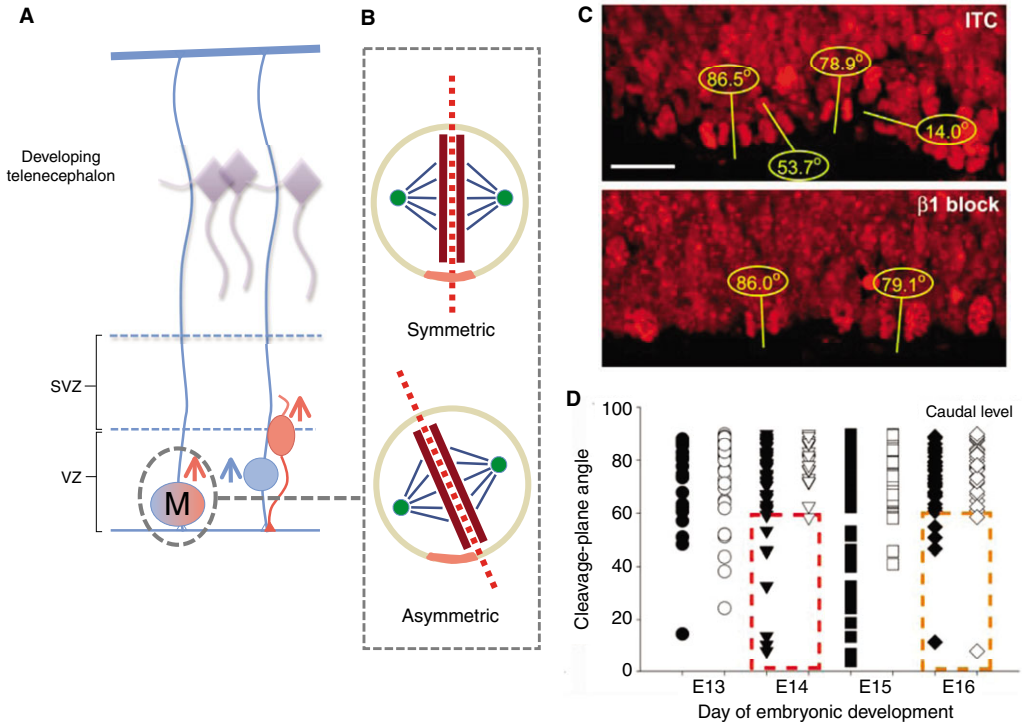

Fig. 5. Transient inhibition of β1 integrin changes division angle in embryonic NSCs. In the developing telencephalon, the fate of the daughter cells of embryonic NSC division is related to the angle of division. (**A**) Mitosis of the NSC occurs at the ventricular surface, as explained in Fig. 2. (**B**) Symmetric divisions are vertical and split the apical membrane (and its putative fate determinants), whereas asymmetric divisions are oblique and partition the apical membrane to one or other daughter cell. (**C**) Effect of the injection of antibodies that specifically block β1 integrin (bottom micrograph) during embryonic development in mice compared with control injections (top micrograph) [reproduced with permission (Loulier et al., 2009)]. Following control injections, a small number of oblique divisions (angle less than 60°) are seen. When β1 integrin is blocked, only vertical divisions can be seen in the experimental embryo. (**D**) The graph shows the results of repetitions of the experiment shown in C; filled symbols represent control injections and open symbols represent β1 integrin block. Each symbol represents the angle of division relative to the ventricular surface at the angles shown on the *y* axis. Following injection of the β1-integrin-function-blocking antibodies, there is a reduction in the number of oblique divisions (highlighted by dashed red and orange boxes). Scale bar, 15 µm.

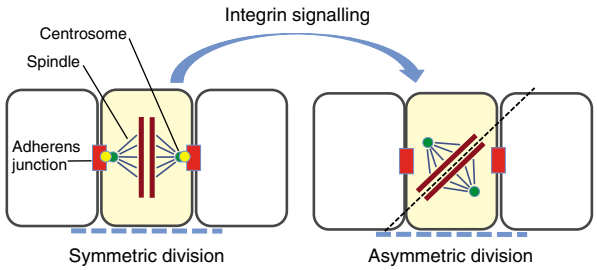

Fig. 6. A speculative model for how integrin signalling in stem cells enables asymmetric divisions by overriding AJ capture. As a result, the spindle plane in a dividing NSC is no longer parallel to the plane of the neuroepithelium and the cleavage plane becomes oblique. See main text for further discussion.

adhesion through the expression of mutant forms of focal adhesion kinase (FAK) or integrins (Fernández-Miñán et al., 2007). Perturbation of integrin signalling by expression of cytoplasmic-domain mutants in cell culture leads to abnormalities of microtubule assembly and cytokinesis, a phenotype that is rescued by activation of the integrin signalling pathway (Reverte et al., 2006). Mitotic spindles orient parallel to extracellular substrates (when viewed in the *z* axis), an effect that is inhibited by blocking either the function of integrins or the formation of astral microtubules (Toyoshima and Nishida, 2007). It has been shown that integrins can reorient microtubules by regulating the accumulation of phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] and recruitment of dynactin-dynein complexes that pull the spindle into place (Toyoshima et al., 2007), and/or by the inhibition of glycogen synthase kinase 3β (GSK3β) that normally inhibits the interaction between APC and microtubules (LaFlamme et al., 2008). As integrins are expressed immediately basal to (but distinct from) the AJ in embryonic NSCs (Lathia et al., 2007; Loulier et al., 2009), their asymmetric activation could rotate the spindle away from the adherens junctions to the oblique plane observed. Such a model would also explain why loss of integrin function from *Drosophila* testes stem cells had no effect on the angle of division relative to the hub cell (Tanentzapf et al., 2007), because these cells are dividing in a cadherin-dependent 'default' mode. However, this model does not explain the randomization of division angle in mammary stem cells or epidermal cells observed when β1 integrins were deleted using a conditional knockout strategy (Lechler and Fuchs, 2005; Taddei et al., 2008), as it predicts that these would revert to a vertical division plane. A problem here is that conditional deletion of the integrin (in contrast to short-term inhibition using antibodies, as in the embryonic NSC experiments) might have secondary effects on the adhesion of cells to the underlying basal lamina in all of these epithelial niches, and/or on the localization of key intracellular and extracellular spatial cues, making interpretation of division angles difficult. Thus, the polarity marker Discs Large (Dlg) becomes mislocalised following integrin deletion in the epidermal cells (Lechler and Fuchs, 2005), and there are abnormalities in spindle angle in wild-type *Drosophila* follicular epithelial cells located adjacent to cells carrying integrin mutations (Fernández-Miñán et al., 2007). These findings indicate that there are other extracellular cues that are localised by integrins and that are responsible for instructing spindle angle during division. The identification and specific inhibition of molecules that link integrins and centrosomes, but do not perturb adhesion or other signalling pathways, and/or the use of blocking antibodies in other niches will be required to test this speculative model in NSCs. Thereafter, further experiments will help to establish whether this role of integrins is context-dependent or whether it also operates in other stem cell types.

Exit from the niche

Given that the role of a niche is to provide a source of cells for turnover or repair, it follows that newborn cells fated to differentiate must exit from the niche after their birth. We have already described how levels of adhesion molecules determine which stem cells are retained in the niche, and evidence suggests that differences in the expression of adhesion molecules also instruct which cells exit the niche. The clearest example of this is provided by studies of HSCs, in which deletion of *Myc* was shown to lead to an accumulation of stem cells in the bone marrow, a decrease in the number of differentiated cells of all haematopoietic lineages and development of anaemia (Wilson et al., 2004). In this study, the HSCs in the bone marrow showed upregulated expression of both N-cadherin and several integrin subunits, but were still able to differentiate normally in vitro. This leads to the hypothesis that the apparent failure to differentiate in vivo reflects an inability of the cells to leave the niche, where they are retained owing to the overexpression of adhesion molecules. Thus, the defects occur because niche signals prevent stem-cell differentiation, rather than because there is any intrinsic defect in the differentiation process. In support of this, overexpression of Myc in HSCs leads to premature differentiation in vivo, as would be expected if downregulation of adhesion molecules led to accelerated exit from the niche. Moreover, normally differentiated cells that have exited the niche have higher levels of Myc than the HSCs remaining in the niche (Wilson et al., 2004). Together, these results suggest a model whereby Myc regulates the expression level of adhesion molecules and the exit from the niche of the daughter cells from HSC divisions.

Levels of Myc might also control exit from the epidermal niche. The local activation of Myc by expression of a tamoxifen-activated form of Myc, or by deletion of *Rac1* (which negatively regulates Myc activity) leads to depletion of epidermal stem cells owing to their premature entry into transit-amplifying and differentiated cell compartments (Benitah et al., 2005). As in the bone marrow, Myc activation is associated with downregulation of integrin expression. Direct evidence for premature niche exit and subsequent depletion is provided by the temporary increase in the number of proliferating transit-amplifying cells observed following Myc activation, after which numbers decline to levels below those found in normal skin. Interestingly, and in contrast to the work on HSCs, cadherin expression levels were not altered as a result of Myc activation in epidermal stem cells (Benitah et al., 2005). If the effect of Myc being explored in these studies is physiological, this might reflect a difference in the structure of the two niches. The epidermal niche is epithelial: here, the stem cells are in contact with the underlying basal lamina. At least part of the bone marrow niche, by contrast, has features more in keeping with a stromal niche, where stem cells interact with osteoblasts, and N-cadherin has been suggested to have a role in this interaction (Zhang et al., 2003). The significance of this is controversial, as discussed above, but a role for cell-cell contact in stem cell retention within the bone marrow niche but not the epidermal niche would generate different requirements for cell-cell adhesion molecules in the two environments.

Another interesting question raised by these findings is the link between differentiation of the daughter cells that exit the

niche and their downregulated expression of adhesion molecules. Is the loss of adhesion an instructive first step in a sequential process in which the cell is then exposed to differentiation factors that determine its fate – i.e. does the decrease in adhesion molecule expression enable a subsequent differentiation? Alternatively, does the decrease in adhesion molecule expression and the generation of a differentiated fate occur in a single coordinated step? As noted above, either model can enable the maintenance of self-renewing populations of stem cells, and a sequential process might provide a greater degree of flexibility as to the choice of fates appropriate for a tissue at a given time. Further work examining the kinetics with which decreased adhesion molecule expression and differentiation occurs is required to test this model.

A further nuance in the sequential mechanisms regulating niche exit is that these steps might need to be timed to enable transient but instructive cell-cell interactions between the parent stem cell and the daughter cell after cytokinesis is complete. In some cases, such as in *Drosophila* intestinal stem cells, these interactions instruct daughter cell fate. *Drosophila* E-cadherin-mediated maintenance of the contact between the stem cell and its progeny after division prolongs Notch signalling in the daughter cell (Maeda et al., 2008), and this activity regulates its differentiation (Ohlstein and Spradling, 2007). Knockdown of *Drosophila* E-cadherin expression by using RNAi reduced Notch signalling in daughter cells and increased the number of enteroendocrine cells that are normally specified by reduced levels of Notch activity (Maeda et al., 2008). A prolonged interaction between stem cells and daughter cells is also seen in the developing mammalian CNS, where the apical processes of embryonic NSCs and daughter cells in the VZ retain contact with the ventricular surface (and therefore their relationship with one another) for several hours after division. Here, instructive signalling between the cells during this time might contribute to maintenance of the NSC pool. One outcome of such signalling is the activation of the Notch pathway, which contributes to maintaining the stem cell population. Current hypotheses propose that Notch ligands are expressed cyclically in all neuroepithelial cells [driven by oscillating levels of hairy and enhancer of split 1 (Hes1)], thereby maintaining them all as stem cells through the consequent transient activation of Notch (Kageyama et al., 2008; Shimojo et al., 2008). Later during development, it is hypothesised that the expression of Notch ligands is sustained on differentiated precursor cells, keeping the parent radial glial cell in the stem cell state (Yoon et al., 2008). Interestingly, the Notch ligand delta-like 1 (Dll1) is known to be associated with cadherins via the scaffolding protein MAGI1 (Mizuhara et al., 2005). It will be interesting to determine whether this segregates the molecule to the apical processes, thereby facilitating these signalling interactions.

In those cell divisions in which the prolonged interaction between the parent stem cell and the daughter cell has a necessary role in determining which cell becomes a committed precursor, it follows that both daughters must retain the necessary cell-cell adhesion molecules. This raises an interesting question with respect to embryonic NSCs. Here, the change in cleavage plane that generates an asymmetric division is proposed to segregate fate determinants, such as Par3, Par6 and atypical protein kinase C (aPKC), that are associated with the AJ (Götz and Huttner, 2005). Therefore, it would be predicted that these junctions segregate to one or other daughter cell. As their role is to retain the apical process at the ventricular surface, how then do both apical processes retain attachment to the ventricular surface after asymmetric division, as is known to occur from live imaging studies (Miyata et al., 2004)? Careful imaging of the AJ during symmetric and asymmetric divisions answers this question. The AJ is comprised of three microdomains (Fig. 7A): cadherins and zona occludens 1 (ZO-1) are located in basal domains, and the Par complex proteins are located in the apical domain. Symmetric divisions split the apical membrane equally but asymmetric divisions always partition the AJ between the adhesive basal domains and the fate-determining apical domain (Marthiens and ffrench-Constant, 2009) (Fig. 7B). So, both daughters always inherit significant levels of cadherins and ZO-1 [explaining the apical process retention for which N-cadherin is required (Kadowaki et al., 2007)] but only one inherits the fate determinants. These data also explain the apparent contradiction between a model for generating asymmetry on the

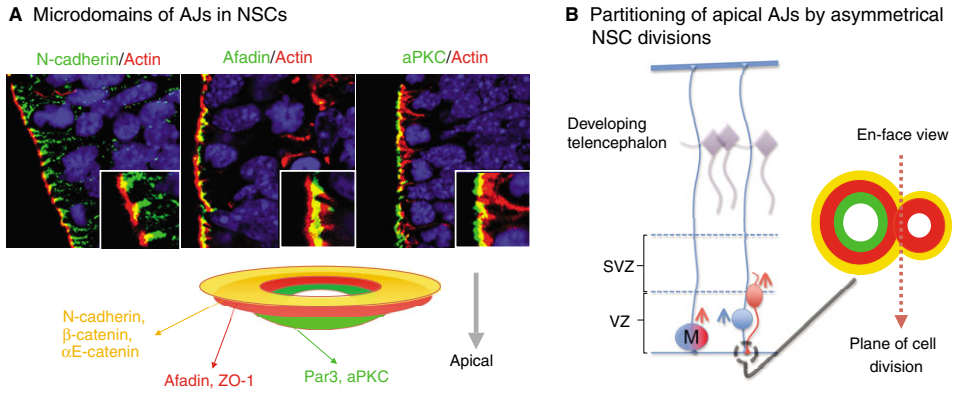

Fig. 7. Architecture and behaviour during division of the AJ in embryonic NSCs. (**A**) Confocal microscopy reveals that the AJ contains three microdomains, shown experimentally and schematically [reproduced with permission (Marthiens and ffrench-Constant, 2009)]. Using the ring of actin staining as a position of reference, it can be seen that N-cadherin is located basal to this actin ring and that other junctional adhesive complexes (as revealed by their cytoplasmic partners afadin and ZO-1) are in an intermediate position, whereas Par-complex proteins, including aPKC, are located at the most apical position. (**B**) Schematic of the split of AJ during division. The microdomains are precisely partitioned such that adhesion molecules (shown in orange and red) are inherited by both daughter cells, whereas fate determinants (Par3, aPKC; shown in green) are inherited by only one daughter cell. This enables asymmetric fate-determining divisions in which both daughter cells retain contact with the ventricular surface.

basis of the regulation of cleavage plane and en face live-imaging studies of the ventricular surface showing that the adhesion molecule ZO-1 is always inherited by both daughters (Konno et al., 2008) (Fig. 7).

In addition to exit out of the niche by cells fated for differentiation, another aspect of stem cell behaviour illustrated by HSCs is movement between niches, or 'homing'. This is evidenced by the presence of circulating HSCs, and might contribute to the replenishment of the various niches when rapid generation of committed cells is required. These circulating cells require adhesion molecules to stick to the sides of blood vessels and then migrate through them into their new niche (Lapidot et al., 2005). The mechanism by which this occurs is highly similar to that of immune cells exiting through blood-vessel walls to enter sites of inflammation, and involves selectins (which initiate adhesion) and β1 integrins (which tether and promote migration through the vessel wall). This potentially common mechanism of HSCs and immune cells is intriguing, given the increasing evidence that HSCs and other stem cells can have immunomodulatory functions that might underlie some of the beneficial effects apparent in different tissues following stem cell transplantation. Homing also occurs following injection of spermatogonial stem cells into the testes of mice: these transplanted cells establish themselves in appropriate locations and function as stem cells (Kanatsu-Shinohara et al., 2008). However, the significance of homing is uncertain outside the haematopoietic system, as there is little evidence that transfer between niches is an important part of normal stem cell behaviour.

Other roles, other molecules?

Two other facets of stem cell behaviour that are regulated during development and in the adult are stem cell maintenance (the ability to undergo self-renewing divisions, also referred to as 'stemness') and the rate of proliferation. Several experiments suggest that adhesion molecules regulate stemness, although none are conclusive because it is difficult to exclude non cell-autonomous effects. As conditional E-cadherin knockout mice show reduced NSC numbers with age, E-cadherin might promote NSC self renewal; however, studies of neurospheres reveal that this decrease probably results from altered interactions with other cells that normally signal to the stem cells (Karpowicz et al., 2009). Laminins might also contribute to stemness because laminin 511 promotes ES cell maintenance in vitro (Domogatskaya et al., 2008). Here, integrins are the obvious candidates because laminin receptors and integrin signalling in cell culture have been shown to promote ES cell self renewal (Hayashi et al., 2007). Mouse ES cells cultured in 3D matrices engineered to activate α5β1, αVβ5, α6β1 and α9β1 integrins showed sustained expression of stemness genes equivalent to the levels seen when the cells were plated on feeder layers of embryonic fibroblasts (conventionally used to maintain ES cells) (Lee et al., 2010). However, there is no clear evidence that integrins promote stemness, even though they are highly expressed by many stem cells. Ablation of β1 integrin in NSCs does not reduce their ability to self renew, as measured by neurosphere formation (Leone et al., 2005), and does not alter the morphology of the VZ (where, as discussed above, NSC bodies are found) in the embryonic CNS (Graus-Porta et al., 2001). Whereas deletion of β1 integrin in basal mammary stem cells does reduce self-renewing capacity, as measured by serial transplantation (Taddei et al., 2008), this might reflect a secondary effect following loss of adhesion to the underlying basal lamina. Examination of other laminin receptors, such as dystroglycan, syndecans and lutheran is, therefore, required to establish the signalling pathways by which laminins promote self-renewal behaviour in stem cells.

Integrins regulate stem cell proliferation because conditional deletion of β1 integrin from the mouse intestinal epithelium results in increased proliferation of intestinal stem cells without any loss of adhesion. Thus, integrin signalling per se regulates stem cell proliferation independently of the role of integrins in anchoring the stem cell to the basal lamina (Jones et al., 2006). These proliferative defects result from impaired sonic hedgehog (Shh) expression and signalling, because β1-integrin signalling normally stimulates Shh transcription that is dependent on the winged-helix transcription factor HNF3β (Jones et al., 2006). Here, laminins are the most likely extracellular matrix ligand for these integrins, but regulation of proliferation might also be an important function of other extracellular matrix molecules, such as tenascin-C and chondroitin, or dermatan sulfate proteoglycans that are expressed in niches such as the SEZ of the adult CNS (Akita et al., 2008; Kazanis et al., 2007). One way these other extracellular matrix molecules might exert their effects is by direct or indirect amplification of growth-factor signalling. Both laminins and tenascin-C amplify platelet-derived-growth-factor (PDGF) signalling in oligodendrocyte precursor cells by integrin-mediated mechanisms (Baron et al., 2002; Garcion et al., 2001), whereas proteoglycans present in fractones can sequester fibroblast growth factors (FGFs), thereby increasing the local availability of the growth factors (Kerever et al., 2007). However, in vivo studies have yet to confirm a role for the matrix in stem cell regulation; for example, tenascin-C-knockout mice showed no abnormalities of SEZ regeneration, which is a stem-cell-dependent process (Kazanis et al., 2007). As mentioned above, a significant problem in these type of studies has been the lack of stem cell markers, making it difficult to distinguish effects on transit amplifying precursors and the stem cells themselves. Knowledge of these markers, and the reagents to label them, will lead to rapid progress in this area.

Concluding remarks

This Commentary has focussed on the normal (homeostatic) niche in which dividing stem cells generate cells required for normal tissue function (as in the gonads) or turnover (as in many adult tissues such as skin and brain). Stem cells in the niche also have essential roles in regeneration, giving rise to additional cells required for repair, and it will be important to determine whether the signalling mechanisms that regulate homeostasis also control the increased rate of division that underlies many repair processes. Another important question is what the role of adhesion molecules is in niche dysfunction. In the *Drosophila* testes, aging is associated with a decrease in the production of spermatogonia. There is an associated decrease in E-cadherin expression within the hub, prompting the hypothesis that loss of stem cells (which are normally attached to the hub cell by the cadherin) from the niche contribute to aging (Boyle et al., 2007). In addition, aging is associated with an increase in the number of divisions in which the centrosome is not tightly associated with the AJ, which would also be expected to decrease stem cell number in the niche. This centrosome abnormality precedes the reduction in *Drosophila* E-cadherin (Cheng et al., 2008). Such evidence suggests that aging results from dysfunction in niche regulation at multiple levels, providing insights into a fascinating area for future research of great relevance to human health.

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