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22. By "one zone axis," we mean a low-index zone axis perpendicular to the stacking direction of tetrahedral close-packed sheets (e.g., cubic <011> and hexagonal <11$\bar{2}$0>).
23. The presence of 3C {111} twin boundaries in a SiC grain increases the number of unique <011> orientations. The presence of several 2H domains, orientationally related to different 3C {111} planes in a SiC intergrowth grain, increases the number of unique <11$\bar{2}$0> orientations.
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Germline Stem Cells Anchored by Adherens Junctions in the *Drosophila* Ovary Niches

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How stem cells are recruited to and maintained in their niches is crucial to understanding their regulation and use in regenerative medicine. Here, we demonstrate that DE-cadherin–mediated cell adhesion is required for anchoring germline stem cells (GSCs) in their niches in the *Drosophila* ovary. Two major components of this adhesion process, DE-cadherin and Armadillo/β-catenin, accumulate at high levels in the junctions between GSCs and cap cells, one of the niche components. Removal of these proteins from GSCs results in stem cell loss. Furthermore, DE-cadherin is required for recruiting GSCs to their niche. Our study demonstrates that anchorage of GSCs in their niche by DE-cadherin–mediated adhesion is important for stem cell maintenance and function.

Stem cells exist in many adult tissues and are responsible for generating differentiated cells that replace cells lost during an animal's lifetime (*1–3*). Understanding the molecular mechanisms controlling stem cell function in vivo is crucial to the future use of stem cells in regenerative medicine, as well as in understanding aging, tumor formation, and degenerative diseases (*4–6*). GSCs in the adult *Drosophila* ovary are an excellent system in which to study stem cells and niches in vivo at the cellular and molecular levels (*7*, *8*). At the tip of the germarium (Fig. 1A), located at the tip of each ovariole of an ovary, a niche exists for two or three GSCs whose progeny eventually develop into mature oocytes (*9–12*). GSCs can be reliably identified based on size, location, and the shape of their fusome. Stem cells contain fusomes (also known as spectrosomes) that are usually round but can be elongated in shape while transiently connected to their daughter cells after division (*13*, *14*). These stem cells directly contact cap cells and are close to two other somatic cell types: terminal filament cells and inner germarial sheath cells. Terminal filament cells and cap cells express the genes, *decapentaplegic* (*dpp*), *fs(1)Yb* (*Yb*), *piwi*, and *hedgehog* (*hh*), which are known to be important for GSC maintenance (*9–12*).

Cell adhesion mediated by DE-cadherin, a *Drosophila* classic cadherin encoded by the *shotgun* (*shg*), and Armadillo (Arm), the *Drosophila* β-catenin homolog, plays an important role between germ cells and follicle cells during oogenesis (*15–19*). To determine whether DE-cadherin–mediated cell adhesion is important for anchoring GSCs in their niches, we first examined the expression of DE-cadherin and Arm proteins in the germarium. The *hh-lacZ* line, in which the bacterial *lacZ* gene with a basal promoter is inserted into the *hh* gene, was used to identify cap cells and terminal filament cells (*20*). These germaria were also immunostained for Hu-li Tai Shao (Hts) and/or Vasa proteins, molecular markers for the fusomes and germline cells, respectively (*21*, *22*). DE-cadherin protein accumulated at high levels as a single band in the interfaces between cap cells and GSCs (Fig. 1B). With more detergent than used in the normal procedure (*23*), the DE-cadherin band separated into two bands just between cap cells and GSCs (Fig. 1C). DE-cadherin is a membrane-associated protein, which accumulates on the membranes of cap cells and GSCs. Arm and DE-cadherin colocalized in these sites (Fig. 1, D to F). Note that the distribution of these proteins was not uniform in the contact areas between GSCs and cap cells or between cap cells themselves. Instead, there were focal sites between the cells that were rich in DE-cadherin and Arm proteins (Fig. 1F), possibly representing adherens junctions. Consistent with this idea, typical adherens junctions were observed between cap cells and GSCs (Fig. 1G), suggesting that DE-cadherin–mediated cell adhesion between cap cells and GSCs in the form of adherens junctions may be involved in anchoring GSCs to cap cells.

To investigate whether DE-cadherin–mediated cell adhesion is essential for maintaining GSCs in their niche, we used FLP-mediated recombination of the FLP recombination target sequences (FRTs) (*24*, *25*) to produce marked GSCs that lack functional copies of either *shg* or *arm* (*23*). The marked GSCs were identified by their loss of expression of *lacZ* (*9*). To investigate whether the removal of DE-cadherin from GSCs disrupts its accumulation in the contact sites, we used the deletion allele of *shg*, shg^{R69}, to remove DE-cadherin proteins from GSCs in the adult ovary (*15*). With this allele, DE-cadherin accumulation diminished in the interfaces between GSCs and cap cells within 1 week (Fig. 2, A and B). Partial depletion was observed in some cases, which may be due to the persistence of the protein (Fig. 2A). In the germaria containing shg^{R69} GSCs, the accumulation of DE-cadherin was maintained between wild-type GSCs and cap cells.

To further determine the importance of DE-cadherin–mediated adhesion in maintaining GSCs in their niche, we used two *shg* alleles: the deletion allele (shg^{R69}) and a weak allele (shg^{10469}) (*15*). Consistent with the prediction that the adhesion is important for anchoring GSCs, mutant shg^{R69} GSCs were lost very quickly (with a half-life of 0.8 weeks), causing marked shg^{R69} GSCs to occur at a much lower frequency in germaria during the first week after

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clone induction (10.0%, n = 251), compared with the control (32.1%, n = 109) and shg^{10469} (26.8%, n = 149) germaria. During the first 2 weeks, germaria carrying one or a few shg^{R69} mutant cysts without marked GSCs were observed (Fig. 3, A and B), suggesting recently lost mutant GSCs. Additionally, more than 95% of marked shg^{R69} GSCs observed during the first week were lost within 2 weeks (n = 360). The weak allele, shg^{10469}, had no dramatic effect on GSC maintenance, with 60% of marked GSCs from the first week being maintained for two more weeks (n = 252) (Fig. 3A). As a control, 70% of marked wild-type GSCs observed 1 week after clone induction were maintained in their niche for two more weeks (n = 221) (Fig. 3, A and C). Our mutant clonal analysis demonstrates that DE-cadherin is essential for anchoring GSCs in their niches.

Because *arm* is involved in *wingless* (*wg*) signaling and DE-cadherin–mediated adhesion, two classes of *arm* mutants were used to differentiate the role of *arm* in GSC adhesion. The arm^2 and arm^8 alleles were used for their ability to specifically block *wg* signaling with no obvious effects on cell adhesion, whereas arm^4 was chosen for its defects in *wg* signaling and cell adhesion (*26*). Approximately 60% of marked GSCs mutant for arm^2 and arm^8 observed during the first week after induction were maintained for two more weeks, suggesting that disrupting *wg* signaling does not dramatically affect GSC maintenance (Fig. 3, A and D). Consistently, marked GSCs mutant for *dishevelled* and *shaggy* (two essential downstream components in *wg* signaling) were maintained normally in their niche (*27*). In contrast, arm^4 severely affected GSC maintenance (Fig. 3A). A lower percentage of arm^4 mutant GSC clones were observed during the first week (1.5%, n = 200) after clone induction, in comparison with those for arm^2 (9.4%, n = 159) and arm^8 (6.8%, n = 219). Furthermore, more than 80% of marked arm^4 GSCs were lost 3 weeks after clone induction (Fig. 3, A and E). These results indicate that *arm* is required for anchoring GSCs in their niches through DE-cadherin–mediated adhesion. We further show that DE-cadherin–mediated adhesion does not play an important role in directly regulating stem cell identity and division (supporting online text).

To determine the relationship between DE-cadherin and the development of GSCs, we examined the expression of DE-cadherin in the ovaries of late third-instar larvae. At this stage, 8 to 10 terminal filament cells were fully developed, and cap cells were beginning to form (*27*), suggesting that niche development was still occurring. In all ovarioles, DE-cadherin was present between terminal filament cells (Fig. 4, A to C); however, DE-cadherin expression between cap cells and GSCs was variable, and its relative abundance appeared to depend on the location of future GSCs' fusomes. When the fusomes of future GSCs were not localized to anterior contact sites, DE-cadherin expression was either absent (Fig. 4A) or punctate (Fig. 4B). Conversely, in ovarioles in which fusomes of future GSCs were localized to the anterior sites where GSCs contact cap cells, DE-cadherin accumulated at high levels (Fig. 4C). We hypothesize that after cap cell differentiation, DE-cadherin initially helps to keep cap cells and GSCs in juxtaposition. We surmise that permanent contact sites are established where DE-cadherin and Arm accumulate in high levels and form adherens junctions.

To determine whether DE-cadherin–mediated cell adhesion is required for recruiting GSCs to their niches, we used the FLP-mediated FRT recombination technique to mark wild-type, mutant shg^{10469} and shg^{R69} primordial germ cells (PGCs) just before the late third-instar stage (*9*). We then determined the efficiency of these marked PGCs to be recruited to their niches in 1- to 2-day-old females. Only a fraction of PGCs are incorporated into niches and become GSCs; however, if DE-cadherin–mediated cell adhesion is important for initial interactions between cap cells and GSCs, we expect that marked wild-type and mutant *shg* PGCs would

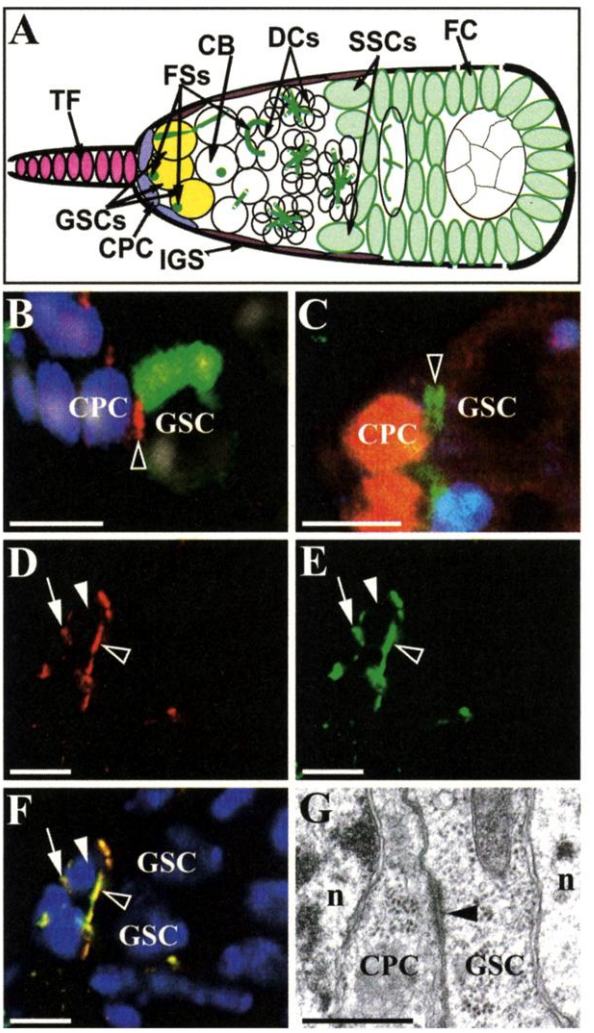


Fig. 1. DE-cadherin and Arm in the interface between GSCs and cap cells. (**A**) A cross-sectional diagram of a *Drosophila* germarium. (**B**) A confocal section of the tip of a *hh-lacZ* germarium labeled for DE-cadherin (red), Hts (green), LacZ (blue), and nuclei (gray). DE-cadherin has accumulated as a single band (open arrowhead) located at the interface between a cap cell and a GSC indicated by an elongated fusome. (**C**) The tip of a *hh-lacZ* germarium labeled for DE-cadherin (green), Hts (blue), LacZ (bright red), and Vasa (dark red) in the presence of more detergent than normally used. Two DE-cadherin–positive bands (open arrowhead) are apparent between a cap cell and a GSC. (**D** to **F**) The tip of a wild-type germarium labeled for DE-cadherin [(D), red], Arm [(E), green)], and nuclei [(F), blue], respectively. The bright yellow band in (F) indicates colocalization, or overlap, of DE-cadherin and Arm. (**G**) An electron micrograph showing the presence of adherens junctions (arrowhead) between a cap cell and a GSC (n, nuclei). CB, cystoblast; CPC, cap cell; DCs, developing cysts; FC, follicle cell; FS, fusome; GSCs, germline stem cells; IGS, inner germarial sheath cell; SSCs, somatic stem cells; TF, terminal filament cell. Scale bars (B to F) represent 5 μm. Scale bar in (G) represents 0.3 μm.

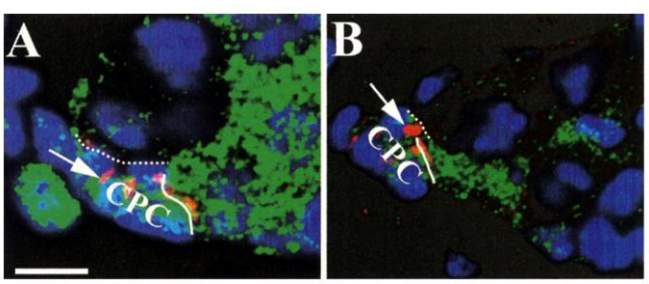
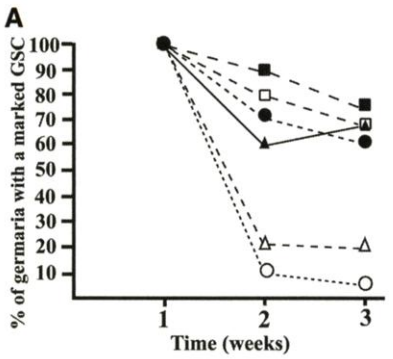


Fig. 2. Diminished accumulation of DE-cadherin in the junctions between GSCs and cap cells after removal of a functional *shg* gene from GSCs. (**A** and **B**) The tips of 1-week-old germaria labeled for LacZ (green), DE-cadherin (red), and nuclei (blue). Both panels illustrate the interface (dotted line) between mutant shg^{R69} GSCs and cap cells, as well as the interface (solid line) between wild-type GSCs and cap cells. Arrows indicate the normal accumulation of DE-cadherin between cap cells themselves. Two micrographs are shown at the same scale. Scale bar in (A) represents 6.7 μm.



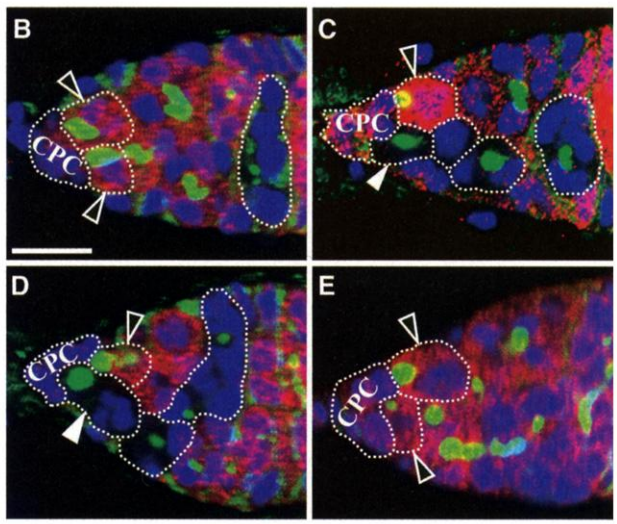


Fig. 3. DE-cadherin and Arm anchoring GSCs in their niches. (**A**) The percentage of germaria carrying marked wild-type or mutant GSCs is shown as a function of time. Wild type (filled squares), shg^{10469} (solid circles), shg^{R69} (open circles), arm^{2} (open squares), arm^{4} (open triangles), and arm^{8} (filled triangles). Because individual experiments started with different percentages of germaria carrying marked GSCs, we normalized them to 100% for comparison during the first week. (**B** to **E**) Germaria labeled for LacZ (red), Hts (green), and nuclei (blue). All four panels indicate marked GSCs (outlined with open arrowheads), unmarked GSCs (outlined with solid arrowheads), cap cells (outlined and marked as CPC), and marked cysts (outlined only). (B) A germarium carrying two wild-type GSCs and a mutant cyst generated by a lost mutant shg^{R69} GSC 2 weeks after clone induction. (C) A 3-week-old marked wild-type GSC clone. (D) A 3-week-old arm^{2} GSC clone. (E) A germarium carrying two unmarked GSCs with a marked arm^{4} GSC differentiating into a cyst that exits the germarium 2 weeks after clone induction. All the germaria are shown at the same scale. Scale bar in (B) represents 10 μm.

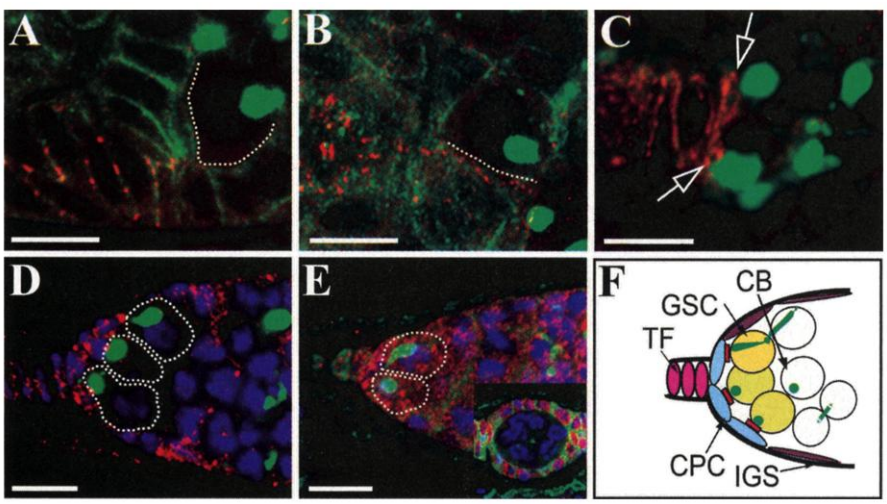


Fig. 4. DE-cadherin–mediated cell adhesion recruiting GSCs to niches during niche formation. (**A** to **C**) Ovaries of late third-instar larvae labeled for DE-cadherin (red) and Hts (green). (A) Ovarioles show no expression of DE-cadherin on juxtaposing membranes (outlined) between GSCs and cap cells; (B) shows punctate expression. (C) An ovariole showing high DE-cadherin expression on the membranes (arrows) between GSCs and cap cells (an overlay of multiple confocal sections). (**D** and **E**) Germaria from 1- to 2-day-old females labeled for LacZ (red), Hts (green), and nuclei (blue). (D) A germarium carrying three marked wild-type GSC clones (outlined). (E) A germarium carrying two wild-type unmarked GSCs (outlined) with mutant shg^{R69}germline cells in the same ovariole. The insert shows a mutant stage 2 chamber in the same ovariole. (**F**) The current model for anchorage of GSCs in their niche. Red bars between GSCs and cap cells indicate the presence of adherens junctions. All bars represent 10 μm.

have different efficiencies of incorporation into niches. Marked wild-type PGCs (control) were incorporated into 17.3% of niches (n = 294) (Fig. 4D), a percentage similar to that of the marked shg^{10469} mutant PGCs (13.1% incorporation, n = 236). However, PGCs homozygous for the deletion allele shg^{R69} were only incorporated into 0.4% of niches (n = 252), with 41.2% of the ovarioles carrying one or more mutant germline cysts (Fig. 4E), suggesting that DE-cadherin–independent mechanisms exist. Therefore, disrupting the expression of DE-cadherin in PGCs reduced their ability to be recruited to their niches and from becoming GSCs.

Our studies provide a working model to explain how GSCs are recruited to and anchored in their niches (Fig. 4F). DE-cadherin proteins accumulate on the contacting membranes between cap cells and GSCs from the beginning of niche establishment. This is important for recruiting GSCs to the niche. DE-cadherin and Arm form adherens junctions that are essential for holding GSCs in their niche and for maintaining their stem cell identity. Similarly, cadherin-related adhesion molecules may also help recruit and anchor stem cells in their niches in other organisms, including humans.

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Fig. S1

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