World: South America, R. S. Williams and J. G. Ferrigno, Eds., *U.S. Geol. Surv. Prof. Pap. 1386I* (1998), p. 148.
24. P. I. Moreno, T. V. Lowell, G. L. Jacobson, Jr., G. H. Denton, *Geogr. Ann.* **81A**, 285 (1999).
25. C. Singer, J. Shulmeister, B. McLea, *Science* **281**, 812 (1998).
26. T. Blunier *et al.*, *Geophys. Res. Lett.* **24**, 2683 (1997).
27. T. Blunier *et al.*, *Nature* **394**, 739 (1998).
28. E. J. Steig *et al.*, *Science* **282**, 92 (1998).
29. J. R. Petit *et al.*, *Nature* **399**, 429 (1999).
30. J. Jouzel *et al.*, *Clim. Dynam.* **11**, 151 (1995).
31. H. J. B. Birks, in *Climate and History: Studies in Past Climates and Their Impact on Man*, T. M. L. Wigley, M. J. Ingram, G. Farmer, Eds. (Cambridge Univ. Press, Cambridge, 1981), pp. 111–138.
32. J. Mangerud, S. T. Andersen, B. E. Berglund, J. J. Donner, *Boreas* **3**, 109 (1974).
33. M. Stuiver and P. J. Reimer, *Radiocarbon* **35**, 215 (1993).
34. H. Niemeyer R., J. Skarmeta M., R. Fuenzalida P., W. Espinosa N., *Technical Report 60–61* (Servicio Nacional de Geología y Minería, Santiago, Chile, 1984).
35. See *Science* Online for supplementary information, at www.sciencemag.org/feature/data/1053325.shl.
36. S. H. Lumley, thesis, University of Cambridge (1993).
37. J. Troels-Smith, *Danm. Geol. Unders. Raekke IV* **3(10)**, 73 (1955).
38. We thank Corporación Nacional Forestal for permission to work in San Rafael and Las Guaitecas National Parks, Raleigh International and their staff and venturers for logistical help, the Natural Environment Research Council (NERC) and the Leverhulme Trust for financial support, R. Switsur and NERC for radiocarbon age determinations, J. Szeicz for help with fieldwork, A. Ashworth for comments on the manuscript, and J. Temple-Smith for technical help. K.D.B. initiated the project, K.D.B. and S.G.H. devised the paper, S.G.H. and S.H.L. obtained the tephrochronological data, K.D.B., S.G.H., and S.H.L. carried out fieldwork, and S.H.L. did the pollen and sediment analyses (with additional sediment analyses by J. Temple-Smith).

20 June 2000; accepted 30 August 2000

A Niche Maintaining Germ Line Stem Cells in the *Drosophila* Ovary

Ting Xie* and Allan C. Spradling

Stromal cells are thought to generate specific regulatory microenviroments or "niches" that control stem cell behavior. Characterizing stem cell niches in vivo remains an important goal that has been difficult to achieve. The individual ovarioles of the *Drosophila* ovary each contain about two germ line stem cells that maintain oocyte production. Here we show that anterior ovariolar somatic cells comprising three cell types act as a germ line stem cell niche. Germ line stem cells lost by normal or induced differentiation are efficiently replaced, and the ability to repopulate the niche increases the functional lifetime of ovarioles in vivo. Our studies implicate one of the somatic cell types, the cap cells, as a key niche component.

Stem cells are defined by their ability to self-renew and to generate cell populations that differentiate to maintain adult tissues (*1–3*). Changes in stem cell behavior may contribute to aging and tumor formation, while stem cell populations will likely have therapeutic applications if their growth and differentiation can be controlled in vitro (*4–6*). However, their rarity and lack of distinctive characteristics make it difficult to study stem cells in their natural context within tissues. Stem cell behavior is thought to be controlled by neighboring stromal cells that create special microenvironments known as stem cell "niches" whose regulatory potential persists even when stem cells are absent. Until specific, individual niches can be identified and characterized, however, it will remain difficult to unravel their molecular regulatory mechanisms.

The *Drosophila* ovary is a tissue where stem cells can be studied at the cellular and molecular level in vivo (*7*, *8*). Near the beginning of each developing egg string (or ovariole) within the ovary reside about two germ line stem cells (GSCs) whose progeny differentiate into eggs within 8 days as they move at predictable rates along the ovariole. These stem cells are surrounded by three differentiated somatic cell types—terminal filament, cap, and inner sheath cells—that help make up an anatomically simple tubular structure known as the germarium (Fig. 1). GSCs are easily identified by size, location, and the shape of the fusome, an intracellular structure rich in membrane skeleton proteins. Stem cells usually contain a round fusome, but display a distinctive elongated fusome after division when they remain transiently connected with their daughter cell (*9*, *10*). Under appropriate conditions, GSCs divide about once per day and are randomly lost by differentiation with a half-life of 4 to 5 weeks (*11*, *12*). Recently, it was proposed that the somatic cells at the tip of the ovariole are organized into a niche that maintains and controls GSCs (*12*).

Ovariolar anatomy is consistent with the existence of a niche at the anterior tip. After stem cell division, the daughter that lies closer to the terminal filament and cap cells remains a stem cell, whereas the daughter that more closely adjoins the inner sheath cells differentiates into a cystoblast (Fig. 1). Anatomical asymmetry may ensure that equivalent stem cell daughters receive different fate-determining signals. GSCs require a signal mediated by Dpp, a homolog of human bone morphogenetic proteins 2 and 4, in order to remain as stem cells and to divide at a normal rate (*12*). Two other proteins needed to maintain GSCs, Piwi and Fs(1)Yb (Yb), act outside the germ line (*13*, *14*). However, a requirement for intercellular signals does not by itself indicate the presence of a niche. A true niche should function independently of resident stem cells and be able to reprogram newly introduced cells to become stem cells. Consequently, we investigated whether the microenvironment at the ovariolar tip can specify cells to become GSCs.

Ovarioles normally lose GSCs by differentiation, but the low rate of GSC loss and the possibility that rapid replacement quickly restores the original GSC configuration complicate observing such events. To study germaria with recently lost stem cells, we genetically marked and destabilized individual GSCs. We used FRT-mediated recombination (*12*) to generate mutant clones of *schnurri* (*shn*), a gene we postulated would reduce GSC lifetime by disrupting *dpp* signaling (*15*, *16*), under conditions where the mutant cells also lose an *armadillo-lacZ* marker (Fig. 2A). Because cystoblasts require 4 to 5 days to exit the germarium, the only remaining lacZ$^-$ cells 1 week after transiently activating the *hs-FLP* transgene by means of a heat shock will be clones consisting of *shn* mutant stem cells and their progeny of 4 to 5 days. With this marking system, marked *shn* GSCs that differentiate during the last 4.5 days can be recognized because lacZ$^-$ germ cells will remain in the germarium; moreover, the developmental age of the least mature such cell will indicate the elapsed time since GSC loss.

The results demonstrated that *shn* mutant stem cells are lost at an increased rate and are rapidly replaced by wild-type cells (*17*, *18*). Seventy-nine germaria were found that retained lacZ$^-$ germ cells, revealing that a *shn* stem cell had been recently lost (Fig. 2, B and C). In every case they contained two wild-type stem cells, indicating that the lost lacZ$^-$ stem cell had been replaced by a wild-type stem cell. Even when the stem cell was lost so recently

Department of Embryology, Howard Hughes Medical Institute, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, MD 21210, USA.

*Present address: Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA.
E-mail: tgx@stowers-institute.org; spradling@mail1.ciwemb.edu

that it remained a cystoblast, two lacZ$^+$ stem cells were present at the tip (Fig. 2D). These stem cells were connected by an elongated fusome, indicating that they were recently divided sister cells in early interphase (*4*); the fusome was oriented in an unusual manner, perpendicular to the anterior/posterior (a/p) axis (*10*). These observations suggest a specific model for GSC replacement (Fig. 2E). After one GSC is lost, its neighboring stem cell divides perpendicular to a/p axis, causing a daughter cell to occupy the environment recently vacated by the departed GSC. For this mechanism to work, the environment at the site of the lost GSC must be capable of programming the incoming cell to become a GSC rather than a cystoblast. Our observations indicate that it is capable of doing so, and hence that GSCs reside in a true stem cell niche.

The ability of the ovariole tip to act as a stem cell niche is likely to be biologically important. Females produce eggs for months, despite the 4- to 5-week half-life of an individual stem cell (*11*). To investigate whether stem cell replacement occurs normally, we measured the number of stem cells and somatic niche cells in aging females (Fig. 3). During the first 5 weeks of adult life the average number of GSCs per germarium declined from about 2.5 to 2.0 (Fig. 3A), significantly less than the 50% reduction expected in the absence of replacement ($P < 0.01$). Replacement stem cells must function efficiently because the rate of stem cell loss does not increase with age (*11*, *12*). Some of the ovarioles that did lose a stem cell started with three GSCs, because the number of such ovarioles declined over the same period.

One of the three somatic cell types, cap cells, interacted with stem cells in a manner that suggested they play a role in niche function. Over the 36-day period, the number of cap cells and GSCs remained closely correlated at about 2.5 cap cells per GSC (Fig. 3A). Moreover, GSCs were observed to always make special contacts with cap cells that characteristically align with the a/p axis of the ovariole. The GSC's fusome remains adjacent to the GSC/cap cell interface during most of the cell cycle. In contrast, the behavior of inner sheath cells and terminal filament cells did not correlate closely with GSCs. As germaria aged, terminal filament cells decreased in number from an average of 9.2 (3 days) to 5.0 (36 days) (Fig. 3A) and changed from a linear to a ball-like arrangement (Fig. 3, B to D) (*19*). Likewise, the relative number of inner germarium sheath (IGS) cells and GSCs varied (Fig. 3E). However, the number of IGS cells was closely correlated with the number of differentiating germ cells ($r = 0.88$). A functional connection between IGS cells and germ cell cysts has been previously suggested, because ovariole tips that develop without germ cells lack IGS cells (*11*).

To investigate the role of IGS cells in adult germaria we studied females carrying a *hs-bam* transgene, whose stem cells can be induced to differentiate (*20*). Over the course of several days after heat shock, GSCs were lost and all germ line cysts completed development and left the germarium. Such germaria also lost all IGS cells, further indicating that developing germ cells control IGS cell number (Fig. 3, F and G). In contrast, terminal filament and cap cells did not change in the absence of germ cells. Somatic cell divisions continued in their vicinity as in germaria that form in the absence of germ cells (*21*). Despite their presence near the GSC niche, these dividing somatic cells did not become GSCs.

Because the number of cap cells correlates closely with the number of GSCs, we investigated whether they might function by preferentially sending a *dpp* signal. Suitable antibodies to Dpp are unavailable, so we used whole-mount in situ hybridization to determine which cells at the ovariole tip express *dpp* mRNA. These experiments detected low levels of *dpp* mRNA in both cap cells and inner sheath cells, as well as higher levels in prefollicle cells farther posterior in the germarium. No *dpp* mRNA was seen in terminal filament cells or in any germ line cells, including GSCs (Fig. 4A). These results show that cap cells are one of several cell types located near the GSCs that express *dpp*. Moreover, it does not appear to be the absence of contact with a *dpp*-expressing cell that causes the posterior stem cell daughter to differentiate as a cystoblast.

Our studies suggest a working model for a GSC niche (Fig. 4B). We propose that cap cells are critical to the formation, maintenance, and regulation of the GSC niche. Cap cells and terminal filament cells form a characteristic structure with sufficient internal surface area to

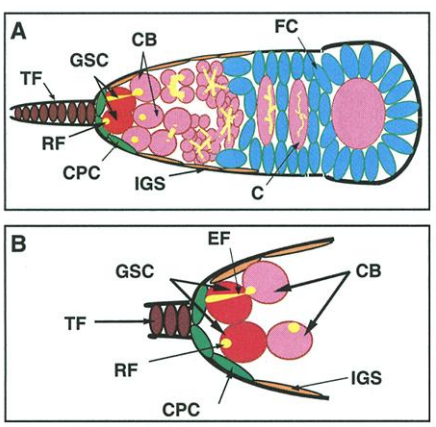

Fig. 1. Germarium structure and stem cells. (**A**) Diagram of a *Drosophila* germarium in cross section indicating germ line stem cells (GSCs, red), differentiating germ cells (pink), terminal filament cells (TF, brown), cap cells (CPC, green), inner germarium sheath cells (IGS, orange), somatic follicle cells (FC, blue), and fusomes (yellow). Fusome shape correlates with germ cell stage. (**B**) Asymmetric location of stem cell and cystoblast relative to somatic cells. C, germ line cyst; CB, cystoblast; EF, elongated stem cell fusome; RF, round stem cell fusome.

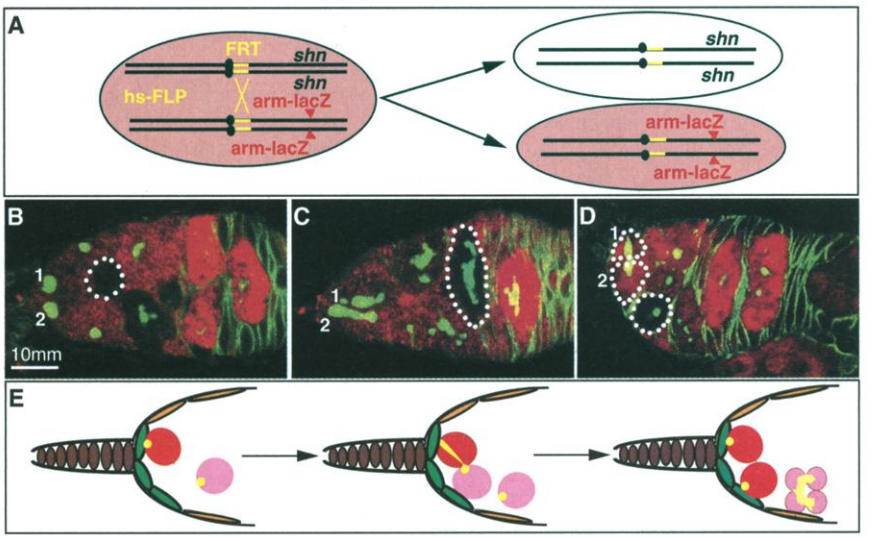

Fig. 2. A niche at the ovariolar tip can replace lost stem cells. (**A**) Generation of marked *shn* mutant GSC clones. All cells (ovals) express arm-lacZ marker (red), except *shn* mutant clones generated by FRT-mediated recombination as shown. (**B** to **D**). Germaria with a recently lost *shn* GSC, analyzed 7 days after a heat shock to induce recombination, display two GSCs (numbered), indicating replacement. For details of arm-lacZ marker (anti-lacZ, red), germ cell fusomes, and somatic cell membranes (anti-Hts, green), see (*18*). The lost stem cell has differentiated into a young 16-cell cyst (B and C, dotted ovals), but is still a cystoblast (large dotted circle) in (D), indicating a recent loss. A new cell (2) occupies the position of the lost GSC and is still connected to the remaining wild-type GSC (1) by an extended fusome. (**E**) An explanatory model for GSC replacement. A GSC differentiates and moves away from the cap cells (left). The other GSC divides perpendicular to the a/p axis (center). Both daughters become GSCs, whereas the lost GSC is now a four-cell cyst (right). Bar (B), 10 μm.

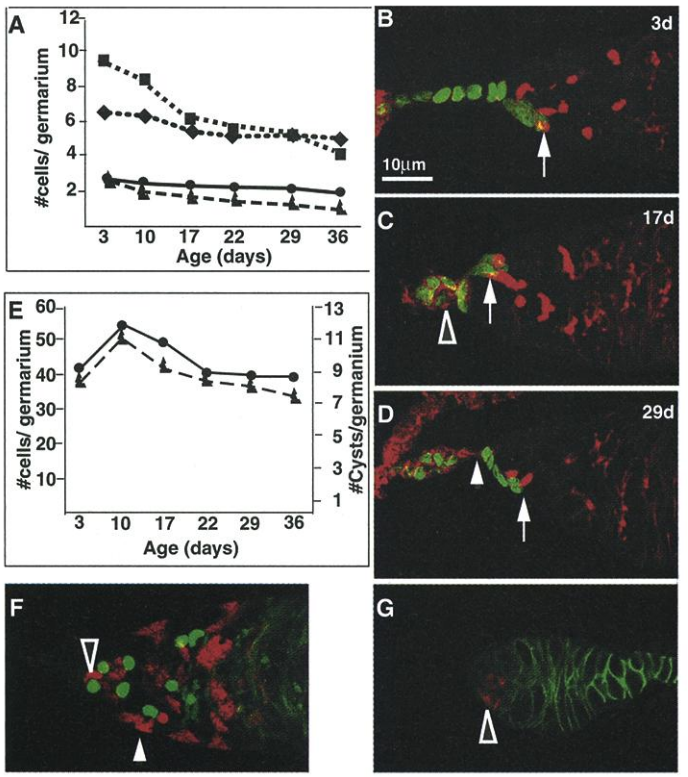

Fig. 3. Changes in niche-associated cells during aging. (**A**) The average numbers per germarium of various types are shown as a function of age. GSCs (●), GSCs expected on the basis of a half-life of 4.6 weeks (▲), cap cells (◆), and terminal filament cells (■). (**B** to **D**) Cap cells stably associate with GSCs through special junctions (arrows) located near the fusome. Terminal filament organization changes from an ordered stack (B) to a ball-like structure (C, open arrowhead) to a disordered clump attached to cap cells by a thin cellular process (D, arrowhead). Anti–laminC staining (green) labels terminal filament and cap cells; anti–α-Spectrin or anti-Hts staining (red) labels fusomes. (**E**) The average number of IGS cells per germarium (●) and germ cell cysts (▲) as a function of age. (**F** and **G**) Germ cells maintain inner sheath cells. Germaria from *hs-bam* females heterozygous for PZ1444 enhancer trap (*7*) before (F) or 72 hours after (G) a 1-hour heat shock. Loss of the germ line in (G) is associated with loss of virtually all IGS cells (arrowhead). Cap cells [open arrowheads in (F) and (G)] remain unchanged. Anti-lacZ (red) labels IGS cells, whereas anti-Hts (green) labels fusomes. See (*18*). Bar (B), 10 μm.

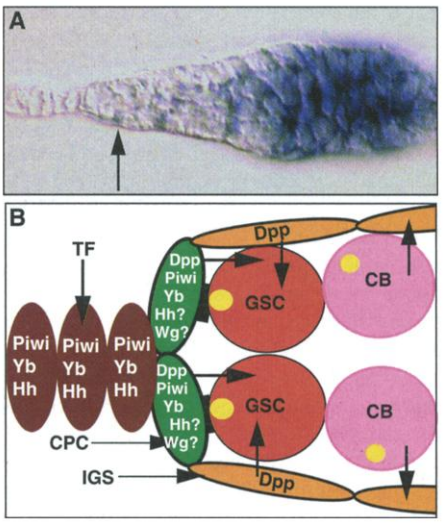

Fig. 4. Model of the stem cell niche and its relation to *dpp* expression. (**A**) A germarium hybridized in situ with an antisense probe specific for dpp mRNA. A *dpp* sense probe did not show any labeling under these conditions (*29*). Cap cells and inner sheath cells (arrow) are labeled in an apparently uniform manner. Strong *dpp* expression is observed in developing follicle cells, but none can be detected in terminal filament or germ line cells. (**B**) A model of niche structure and function. The postulated junction between cap cells and the GSCs is shown as a black box. Genes that are known or thought to be involved in niche function are indicated within the cells where they are expressed, and arrows show potential intercellular signals. Other abbreviations are as in Fig. 1.

contact two or three GSCs. A special cell-cell junction is likely to form between GSCs and cap cells to explain their intimate juxtaposition throughout adult life. Such a junction likely holds a GSC at the anterior and prevents it from moving away from the ovariolar tip where it might receive differentiation cues. An intercellular signal, possibly *dpp*, would be needed to maintain this junction and control the rate of GSC division, but need not be spatially graded. Additional signals appear to be involved in niche function as well. Terminal filament cells and/or cap cells express *hedgehog*, *wingless*, and *armadillo*, although roles for these signaling molecules in regulating GSCs remain unclear (*19*, *22*). *Yb* and *piwi* function outside the germ line in maintaining GSCs. The combined action of these genes ensures that precisely one of the GSC daughters loses cap cell contacts and differentiates.

Our experiments show that a small group of stromal cells located at the tip of the *Drosophila* ovariole acts as a stem cell niche. Stem cells in many different tissues and organisms may be regulated in a similar manner. In the *Drosophila* testis, five to seven stem cells are anchored on terminally differentiated somatic hub cells, suggesting that both the ovary and testis could use similar strategies to regulate their stem cells (*23*). In *Caenorhabditis elegans*, distal tip cells have been directly implicated in the maintenance of the GSC population (*24*). In the *Arabidopsis* shoot meristem, an organizing center located nearby is required to maintain meristem stem cells (*25*). The reported plasticity of some mammalian stem cells may result from the existence of niches that can reprogram stem cell identity (*26*–*28*). Our studies provide a basis for detailed comparisons between the structure and regulatory properties of niches supporting different stem cells and will assist efforts to elucidate the molecular signals that control stem cell division and differentiation.

References and Notes

1. C. S. Potten and M. Loeffler, *Development* **110**, 1001(1990).
2. S. J. Morrison, N. M. Shah, D. J. Anderson, *Cell* **88**, 287 (1997).
3. F. M. Watt and B. L. M. Hogan, *Science* **287**, 1427 (2000).
4. M. J. Shamblott *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13726 (1998).
5. O. Brustle *et al.*, *Science* **285**, 754 (1999).
6. I. L. Weissman, *Science* **287**, 1442 (2000).
7. A. Spradling *et al.*, *Cold Spring Harbor Sym. Quant. Biol.* **62**, 25 (1997).
8. H. Lin, *Curr. Opin. Cell Biol.* **10**, 687 (1998).
9. M. de Cuevas and A. Spradling, *Development* **125** 2781 (1998).
10. W. Deng and H. Lin, *Dev. Biol.* **189**, 79 (1997).
11. J. Margolis and A. Spradling, *Development* **121**, 3797 (1995).
12. T. Xie and A. Spradling, *Cell* **94**, 251 (1998).
13. D. Cox, A. Chao, H. Lin, *Development* **127**, 503 (2000).
14. F. J. King and H. Lin, *Development* **126**, 1833 (1999)
15. K. Arora *et al.*, *Cell* **81**, 781 (1995).
16. N. C. Grieder *et al.*, *Cell* **81**, 791 (1995).
17. The half-life of shn^{04738} GSCs was measured to be 2.3 weeks as described (*12*). Using *mothers against dpp*, *Medea*, *thick veins*, or *punt* to destabilize stem cells yielded similar results (*18*). Clone induction and stem cell loss were independent of the number of stem cells initially present in a germarium. Thus, at least half the mutant stem cells were present in germaria containing only one other stem cell.
18. Supplementary data are available at *Science* Online at www.sciencemag.org/feature/data/1052793.shl.
19. A. J. Forbes, H. Lin, P.W. Ingham, A. C. Spradling, *Development* **122**, 1125 (1996).
20. B. Ohlstein and D. McKearin, *Development* **124**, 3651 (1997).
21. C. J. Fielding, *J. Embryol. Exp. Morphol.* **17**, 375 (1967).
22. A. J. Forbes, A. C. Spradling, P. W. Ingham, H. Lin, *Development* **122**, 3283 (1996).
23. M. Fuller, in *Development of* Drosophila melanogaster (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1993), p. 71.
24. J. Kimble and P. Simpson, *Annu. Rev. Cell Dev. Biol.* **13**, 333 (1997).
25. K. F. X. Mayer *et al.*, *Cell* **95**, 805 (1998)
26. P. H. Jones, *BioEssays* **19**, 683 (1997)
27. G. Ferrari *et al.*, *Science* **279**, 1528 (1998)
28. C. R. R. Bjornson *et al.*, *Science* **283**, 534 (1999).
29. T. Xie and A. C. Spradling, data not shown.

30. We thank S. Cohen, R. Dubreuil, D. McKearin, M. Sanders, and the Bloomington stock center for antibodies and fly stocks, and members of the Spradling and Xie laboratory for discussions.

2 June 2000; accepted 22 August 2000