Drosophila Boi limits Hedgehog levels to suppress follicle stem cell proliferation

Tiffiney R. Hartman,[1] Daniel Zinshteyn,[1] Heather K. Schofield,[1] Emmanuelle Nicolas,[2] Ami Okada,[3] and Alana M. O'Reilly[1]

[1]Program in Cancer Biology and [2]Genomics Core Facility, Fox Chase Cancer Center, Philadelphia, PA 19111
[3]Department of Neurology, Stanford University School of Medicine, Stanford, CA 94305

Stem cells depend on signals from cells within their microenvironment, or niche, as well as factors secreted by distant cells to regulate their maintenance and function. Here we show that Boi, a Hedgehog (Hh)-binding protein, is a novel suppressor of proliferation of follicle stem cells (FSCs) in the *Drosophila* ovary. Hh is expressed in apical cells, distant from the FSC niche, and diffuses to reach FSCs, where it promotes FSC proliferation. We show that Boi is expressed in apical cells and exerts its suppressive effect on FSC proliferation by binding to and sequestering Hh on the apical cell surface, thereby inhibiting Hh diffusion. Our studies demonstrate that cells distant from the local niche can regulate stem cell function through ligand sequestration, a mechanism that likely is conserved in other epithelial tissues.

Introduction

Adult epithelial tissues depend on the presence of self-renewing stem cells for their long-term homeostasis. Signals from the immediate stem cell microenvironment, or niche, promote stem cell self-renewal, prevent differentiation, and control stem cell proliferation to produce the specialized daughter cells required for tissue homeostasis. Emerging data support the idea that niches are adapted for specific stem cell needs. Classical niches consist of differentiated cells that directly contact stem cells and direct their self-renewal and behavior (Morrison and Spradling, 2008). In contrast, other niches appear to lack a stable cellular component. Instead, stem cells generate some or all components necessary for their self-renewal and maintenance (O'Reilly et al., 2008; Sato et al., 2009). The *Drosophila* ovary houses both types of niche in a structure called a germarium. Germline stem cells (GSCs) adhere directly to postmitotic cells called terminal filament and cap cells (apical cells), which are located at the apical tip of the germarium (Fig. 1 A; Xie and Spradling, 2000). This adhesion-based mechanism promotes GSC retention in the niche and concomitantly maintains stem cell fate.

In contrast, follicle stem cells (FSCs) lack a permanent cellular niche, instead relying on transient cell–cell and cell–matrix adhesion to maintain their position (Song and Xie, 2002; Nystul and Spradling, 2007; O'Reilly et al., 2008). FSCs themselves produce the essential local niche component, Laminin A, which activates integrins on the FSC surface, thus promoting FSC anchoring and proliferation (O'Reilly et al., 2008). In addition, secreted signals produced by apical cells (Forbes et al., 1996a,b; Song and Xie, 2003; Kirilly et al., 2005) stimulate proliferation through canonical receptors expressed on the FSC surface, which is located 3–5 cell diameters to the posterior (Fig. 1 A; Margolis and Spradling, 1995).

In one well-characterized example, Hedgehog (Hh) is expressed and secreted by apical cells (Forbes et al., 1996a), and FSCs express its receptor, Patched (Ptc), and effector proteins Smoothened (Smo) and Cubitus Interruptus (Ci; the fly homologue of Gli; Forbes et al., 1996a,b; Zhang and Kalderon, 2000, 2001). Current genetic data support a model in which apical cell-derived Hh interacts with Ptc expressed by FSCs, relieving Ptc-mediated inhibition of Smo and activating Ci-mediated target gene expression. FSC proliferation rates are affected by mutation of *hh*, *ptc*, or *smo* (Forbes et al., 1996a; Zhang and Kalderon, 2001), which indicates an important role for this pathway in FSC proliferation control.

Correspondence to Alana M. O'Reilly: Alana.OReilly@fccc.edu

Abbreviations used in this paper: Ci, Cubitus Interruptus; ESC, escort stem cell; FSC, follicle stem cell; GSC, germline stem cell; Hh, Hedgehog; Ptc, Patched; Smo, Smoothened.

© 2010 Hartman et al. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/).

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 191 No. 5 943–952
www.jcb.org/cgi/doi/10.1083/jcb.201007142

THE JOURNAL OF CELL BIOLOGY

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

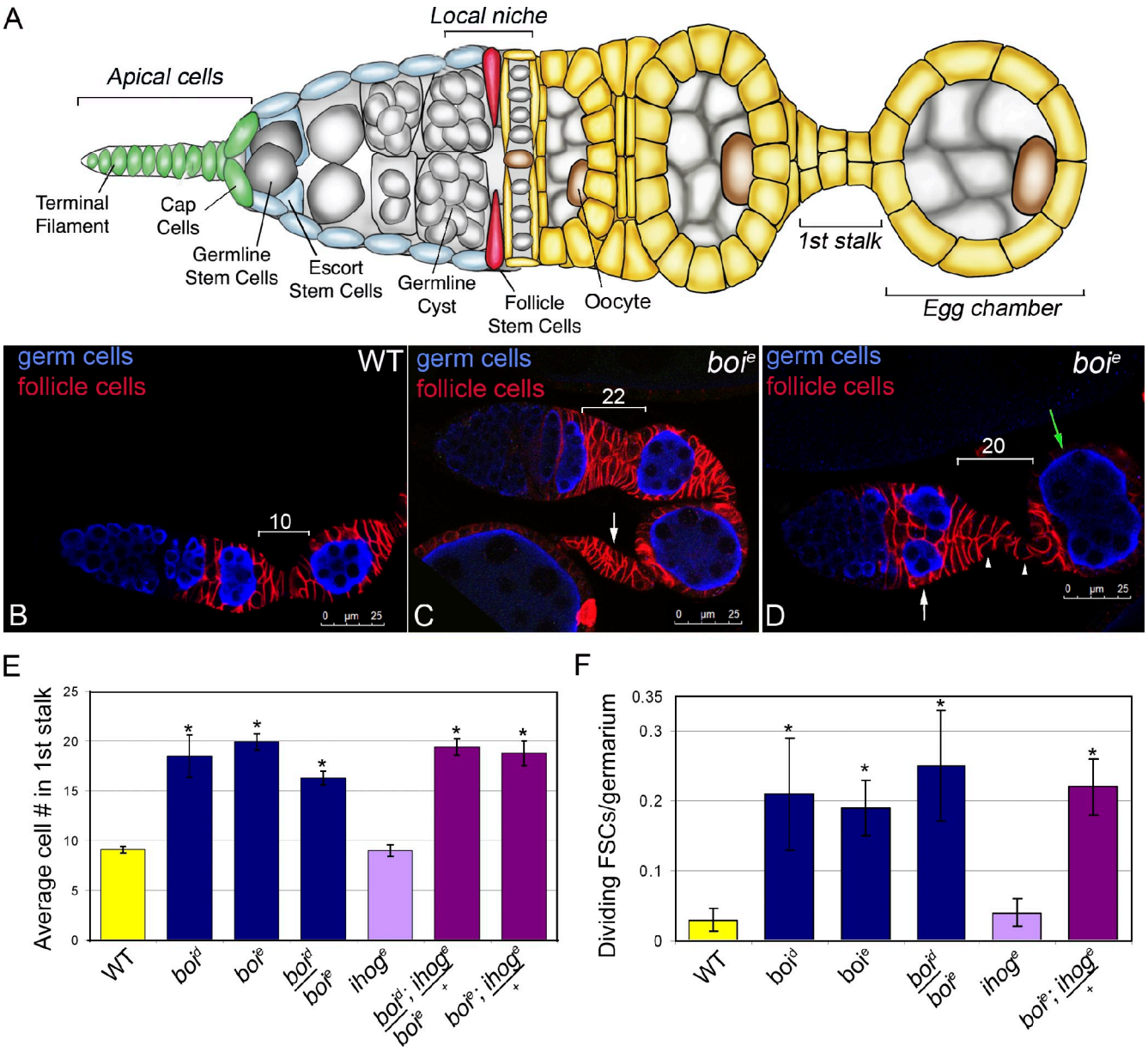

Figure 1. ***boi* controls FSC proliferation.** (A) Schematic of early oogenesis. GSCs (gray) and ESCs (light blue) contact a cellular niche composed of terminal filament and cap cells ("apical cells," green). FSCs (red) reside 3–5 cell diameters posterior to apical cells. These stem cells generate daughter cells that coordinate to produce follicles (egg chambers) composed of a 16-cell germline cyst (gray) surrounded by a single follicular epithelial layer (yellow). Egg chambers develop over 7 d to produce mature eggs. (B–D) Germaria in which germ cells (blue, anti-Vasa) and differentiating follicle cells (red, anti-Fas3) are labeled. Stalks between the germarium and the first budded egg chamber average 9 cells in wild type (WT; B) and 18 cells in boi^e mutants (C). Cell numbers for WT (B), and boi^e (C and D) are indicated (brackets). Stalks with excess follicle cells are observed at later stages (B, arrow). (D) *boi* mutants exhibit defects in cyst packaging (indicated by side-by-side cysts in a single plane (white arrow) or two cysts surrounded by a single epithelium (green arrow), and polarization defects (round cells, changes in Fas3 staining; arrowheads). (E) Mean cell number in the first stalk for each genotype is shown. *, significant differences relative to WT ($P < 0.00000006$). (F) Mean numbers of dividing FSCs ($PH3^+$) per germarium are shown. *, significant differences relative to WT ($P < 0.002$). Error bars represent SEM.

The recent identification of additional Hh receptors (Lum et al., 2003a; Okada et al., 2006; Tenzen et al., 2006; Yao et al., 2006; Zhang et al., 2006; Williams et al., 2010; Yan et al., 2010) suggests that regulation of the FSC Hh response may be more complex. Ihog and its close homologue Boi bind Hh with high affinity (Yao et al., 2006; McLellan et al., 2008) and are good candidate proteins for Hh regulation in the germarium. Ihog and Boi function redundantly, acting as coreceptors for Ptc to promote cell-autonomous Hh responses (Yan et al., 2010; Zheng et al., 2010). The murine homologues of Ihog/Boi, called Cdo and Boc, also have been shown to act in Hh receiving cells to enhance signaling (Okada et al., 2006; Tenzen et al., 2006; Zhang et al., 2006). Although these experiments strongly indicate positive functions for Ihog/Boi and Cdo/Boc in Hh signaling during development, these receptors can limit Hh diffusion and negatively affect expression of Hh pathway reporters in some tissues (Tenzen et al., 2006; Yan et al., 2010). The dual function of Ihog/Boi in Hh regulation and the need for precise

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

Table I. **Quantification of FSC and follicle cell proliferation**

Genotype	Scoring average (SEM)[a]			P-value versus w^{1118}, *109-30*, or *babGal 4* wild-type control[b]			P-value versus boi^{e}, boi^{e}/*109-30*, or boi^{e}/*babGal 4* mutant controls[b]		
	Follicle cell number per germarium	Number of dividing follicle cells per germarium	Number of dividing FSCs per germarium	Follicle cell number per germarium	Number of dividing follicle cells per germarium	Number of dividing FSCs per germarium[c]	Follicle cell number per germarium	Number of dividing follicle cells per germarium	Number of dividing FSCs per germarium[c]
W^{1118}	38.5 (1.2)	0.55 (0.1)	0.03 (0.02)	NA	NA	NA	NA	NA	NA
boi^{e01708}	57.3 (2.7)	4.73 (0.5)	0.19 (0.04)	P ≤ 0.0000002	P ≤ 0.00000000002	P ≤ 0.0009	NA	NA	NA
boi^{d04917}	39.2 (2.5)	1.68 (0.4)	0.21 (0.08)	P ≤ 0.77	P ≤ 0.0006	P ≤ 0.001	P ≤ 0.00002	P ≤ 0.00006	P ≤ 0.81
boi^{d}/boi^{e}	37.2 (2.5)	1.88 (0.4)	0.25 (0.08)	P ≤ 0.62	P ≤ 0.00009	P ≤ 0.002	P ≤ 0.000004	P ≤ 0.0002	P ≤ 0.46
$ihog^{e02142}$	41.9 (2.2)	0.88 (0.3)	0.04 (0.02)	P ≤ 0.154	P ≤ 0.226	P ≤ 0.72	P ≤ 0.0002	P ≤ 0.0000006	P ≤ 0.007
boi^{e}; $ihog^{e/+}$	50.9 (2.1)	2.70 (0.4)	0.22 (0.04)	P ≤ 0.0000009	P ≤ 0.00000002	P ≤ 0.0001	P < 0.096	P < 0.005	P < 0.61
yw; smo^{RNAi}/*Cyo*	40.0 (2.0)	0.96 (0.3)	0.05 (0.03)	P ≤ 0.509	P ≤ 0.124	P ≤ 0.5	P ≤ 0.00002	P ≤ 0.0000008	P ≤ 0.02
boi^{e}; smo^{RNAi}/*Cyo*	43.6 (3.3)	2.63 (0.5)	0.21 (0.06)	P ≤ 0.09	P ≤ 0.000001	P ≤ 0.001	P ≤ 0.002	P ≤ 0.007	P < 0.8
Hh^{AC}/+	33.6 (1.6)	1.48 (0.4)	0.05 (0.02)	P ≤ 0.02	P ≤ 0.005	P ≤ 0.5	P ≤ 0.00000002	P ≤ 0.00002	P ≤ 0.005
boi^{e}; Hh^{AC}/+	31.8 (1.3)	1.50 (0.3)	0.08 (0.02)	P ≤ 0.0006	P ≤ 0.001	P ≤ 0.104	P ≤ 0.000000002	P ≤ 0.00002	P ≤ 0.016
smo^{3b}/+	35.3 (1.9)	1.40 (0.2)	0.07 (0.03)	P ≤ 0.15	P ≤ 0.001	P ≤ 0.2	P ≤ 0.0000002	P ≤ 0.000005	P ≤ 0.02
boi^{e}; smo^{3b}/+	39.8 (1.8)	1.56 (0.3)	0.07 (0.03)	P ≤ 0.55	P ≤ 0.001	P ≤ 0.309	P ≤ 0.00001	P ≤ 0.00002	P ≤ 0.038
boi^{d}/boi^{e}; smo^{3b}/+	39.3 (1.7)	0.84 (0.2)	0.04 (0.04)	P ≤ 0.706	P ≤ 0.144	P ≤ 0.729	P ≤ 0.000007	P ≤ 0.0000002	P ≤ 0.043
babGal4/+	45.4 (2.3)	1.12 (0.3)	0.04 (0.02)	NA	NA	NA	NA	NA	NA
boi^{e}; *babGal4/+*	50.8 (2.3)	3.56 (0.4)	0.24 (0.05)	P < 0.1	P < 0.000007	P < 0.0002	NA	NA	NA
hh^{RNAi}/*babGal4*	36.1 (1.7)	0.68 (0.2)	0.05 (0.03)	P ≤ 0.003	P ≤ 0.18	P ≤ 0.76	P ≤ 0.000006	P ≤ 0.0000001	P ≤ 0.0008
boi^{e}; hh^{RNAi}/ *babGal4*	42.1 (2.0)	1.92 (0.3)	0.09 (0.03)	P ≤ 0.298	P ≤ 0.045	P ≤ 0.152	P ≤ 0.006	P ≤ 0.002	P ≤ 0.02
boi^{e}; *UAS-Boi/ babGal4*	47.7 (2.0)	1.36 (0.3)	0.05 (0.03)	P ≤ 0.459	P ≤ 0.55	P ≤ 0.76	P ≤ 0.31	P ≤ 0.00009	P ≤ 0.0008
boi^{e}; *UAS-Boi-ΔFN1/bab-Gal4*	36.7 (1.8)	1.04 (0.3)	0.19 (0.04)	P ≤ 0.005	P ≤ 0.85	P ≤ 0.001	P ≤ 0.00001	P ≤ 0.00001	P ≤ 0.44
boi^{e}; *UAS-Boi-ΔFN2/bab-Gal4*	38.3 (1.5)	0.80 (0.3)	0.12 (0.05)	P ≤ 0.01	P ≤ 0.4	P ≤ 0.08	P ≤ 0.00003	P ≤ 0.000001	P ≤ 0.12
boi RNAi #4/ babGal4	38.6 (1.5)	0.96 (0.2)	0.11 (0.04)	P ≤ 0.02	P ≤ 0.631	P ≤ 0.152	P ≤ 0.00006	P ≤ 0.0000009	P ≤ 0.05
109-30/cyo	38.5 (2.5)	0.96 (0.3)	0.04 (0.02)	NA	NA	NA	NA	NA	NA
boi^{e}; *109-30/ Cyo*	43.7 (3.4)	2.56 (0.4)	0.20 (0.04)	P ≤ 0.219	P ≤ 0.001	P ≤ 0.007	NA	NA	NA
boi^{e}; smo^{RNAi}/ *109-30*	44.3 (2.2)	2.24 (0.4)	0.04 (0.02)	P ≤ 0.089	P ≤ 0.007	P ≤ 1.0	P ≤ 0.89	P ≤ 0.544	P ≤ 0.007
smo^{RNAi}/*109-30*	34.9 (1.4)	0.64 (0.2)	0.05 (0.04)	P ≤ 0.203	P ≤ 0.349	P ≤ 0.83	P ≤ 0.019	P ≤ 0.00006	P ≤ 0.023
Ci^{RNAi}/*109-30*	35.4 (1.5)	1.08 (0.3)	0.09 (0.03)	P ≤ 0.29	P ≤ 0.75	P ≤ 0.22	P ≤ 0.03	P ≤ 0.002	P < 0.05
boi^{e}; Ci^{RNAi}/ *109-30*	36.8 (1.8)	1.76 (0.4)	0.06 (0.02)	P ≤ 0.56	P ≤ 0.08	P ≤ 0.58	P ≤ 0.08	P ≤ 0.13	P < 0.003
boi^{RNAi} *#4/ 109-30*	35.1 (1.7)	0.28 (0.1)	0.01 (0.01)	P ≤ 0.26	P ≤ 0.023	P ≤ 0.34	P ≤ 0.03	P ≤ 0.0000007	P < 0.002

[a]Between 25 and 150 germaria from 7-d-old female flies were scored per genotype for each condition. Mean numbers are shown with SEM.
[b]A two-sample Student's *t* test was used for all statistical analysis. Significant differences were achieved at P ≤ 0.05.
[c]Statistical analysis for the number of dividing FSC per germarium compared to wild-type or boi^{e} mutant controls are shown in bold.

Hh levels for normal oogenesis suggest that assessing the role of these novel Hh receptors in the ovary could further our understanding of stem cell regulation.

Results and discussion

Flies bearing homozygous or trans-heterozygous mutations in two loss-of-function *boi* mutant alleles exhibited excess follicle cells that accumulated between developing egg chambers (Figs. 1 and S1). Although stalks of follicle cells between egg chambers in wild-type flies contained nine follicle cells on average, *boi* mutant stalks had twice as many cells (Fig. 1, B–E). Additional defects associated with excessive follicle cell production, including improper egg chamber packaging, delayed differentiation, and follicle cell polarity defects were also observed (Fig. 1 D). *boi* mutant FSCs proliferated more frequently than wild-type FSCs (Fig. 1 F and Table I), which suggests that the increased numbers of follicle cells result, at least in part, from increased FSC proliferation in *boi* mutants. The number of dividing pre-follicle cells derived from *boi* mutant FSCs was also higher than wild type (Fig. S1), perhaps because of the inability of mutant cells to differentiate properly (Forbes et al., 1996a; Zhang and Kalderon, 2000; Bai and Montell, 2002). Although previous studies have shown that Boi and its close relative Ihog both function in wing imaginal disc and embryonic development (Yao et al., 2006; Zheng et al., 2010; Yan et al., 2010), Ihog does not appear to play a role in the control of FSC proliferation, as loss-of-function mutants exhibited wild-type FSC proliferation

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

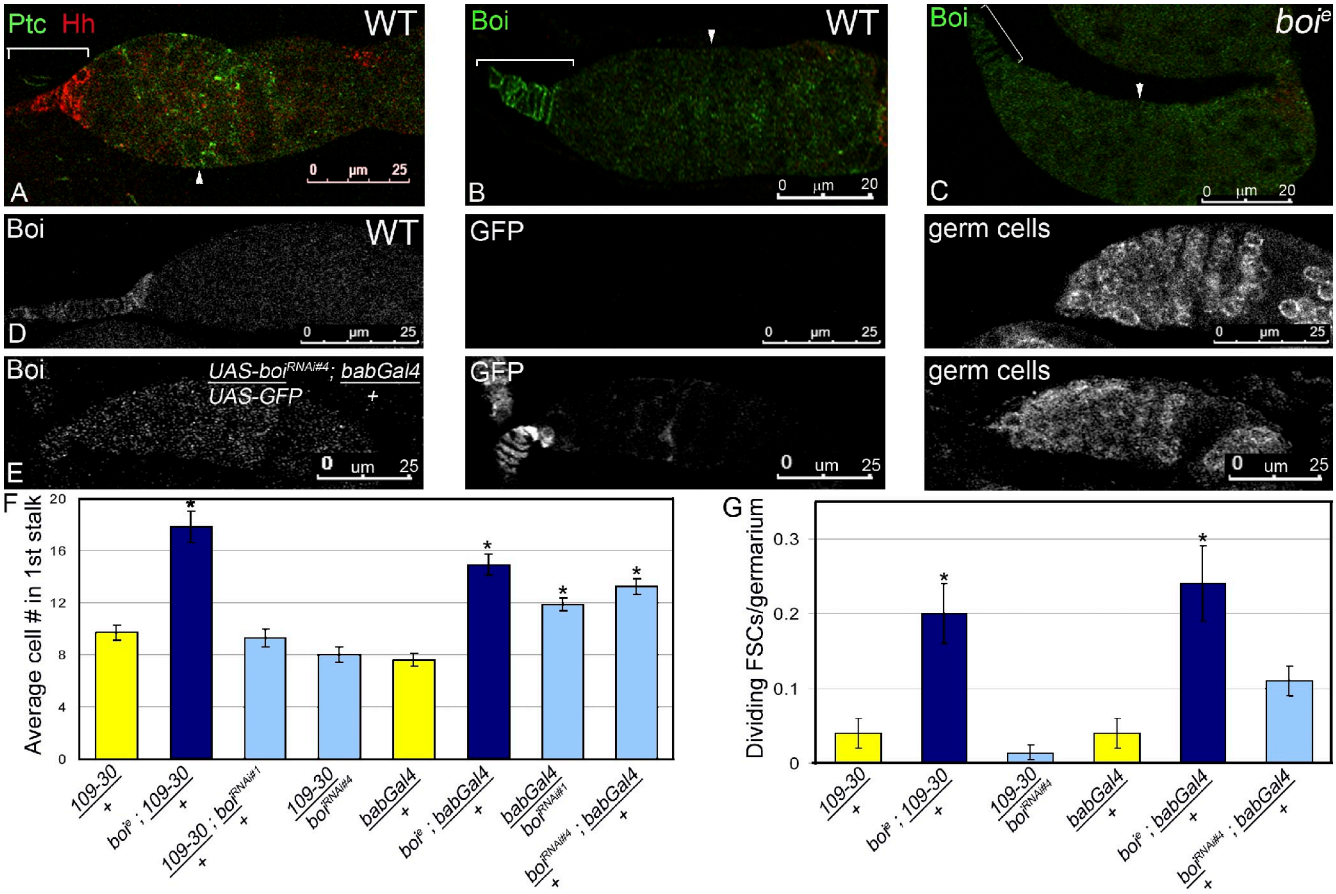

Figure 2. **Boi functions in apical cells to regulate FSC proliferation.** (A) Ptc expression (green) in wild-type (WT) germarium. Apical cells (red, anti-Hh) and FSC location (arrowhead) are indicated. (B and C) Boi (green) localizes predominantly to apical cells (brackets) in WT (B) but not *boi*^{e} mutant (C) germaria. FSCs are indicated (arrowheads). (D and E) *UAS-boi*^{RNAi} expression in apical cells reduces Boi levels. Boi (left), GFP (center), and germ cells (right, anti-Vasa) are labeled. WT germaria (D) express Boi but lack GFP. *UAS-boi*^{RNAi}*/UAS-GFP; babGal4/+* germaria (E) lose Boi and gain GFP in apical cells. (F) Mean cell number in the first stalk for each genotype is shown. Significant differences relative to control (*109-30/+* or *babGal4/+*) are indicated (*, $P < 0.000002$). (G) Mean numbers of dividing FSCs (PH3+) per germarium are shown. Significant differences relative to control (*109-30/+* or *babGal4/+*) are indicated (*, $P < 0.007$). Error bars represent SEM.

and follicle production (Fig. 1, E and F; and Table I). Moreover, the penetrance and severity of *boi* mutant defects were not affected by reducing *ihog* levels (*boi; ihog/+*; Fig. 1, E and F; Fig. S1; and Table I). These results suggest that *boi* plays a critical, nonredundant role in FSC proliferation control.

Boi is a known Hh receptor (Yao et al., 2006; McLellan et al., 2008), which suggests that direct Hh binding might contribute to Boi's role in suppressing FSC proliferation. Recent work in developing wing imaginal discs suggests that Boi functions as a coreceptor for Ptc, both stabilizing Ptc localization to the cell surface and promoting Hh pathway activation through Hh binding (Zheng et al., 2010). If a similar mechanism regulates FSC proliferation, Boi should be coexpressed together with Ptc in FSCs and be required cell autonomously within FSCs. Although low levels of Ptc were observed in most cells of the germarium including FSCs (Fig. 2 A; Forbes et al., 1996b), Boi localized predominantly to the surface of apical cells (Fig. 2, B and C). Moreover, reducing endogenous *boi* levels in apical cells by cell-specific *boi* RNAi expression (*boi*^{RNAi}*/+; babGal4/+*) led to follicle cell accumulation and FSC proliferation defects similar to those seen in *boi* homozygous mutants (Fig. 2, D–G; Fig. S2; and Table I). In contrast, expression of *boi* RNAi in FSCs and their progeny (*boi*^{RNAi}*/109-30 Gal4*) had no effect on follicle cell production or FSC proliferation (Fig. 2, F and G; and Table I). These data suggest that Boi functions in apical cells to suppress FSC proliferation rather than cell autonomously within FSCs.

In wild-type germaria, Hh accumulates on the surface of the apical cells where it is produced rather than in the FSC niche (Fig. 3 A; Forbes et al., 1996a). Moreover, ectopic Hh expression strongly promotes FSC proliferation (Forbes et al., 1996a,b) which suggests that Hh release from apical cells may be a limiting event for FSC proliferation control. If this is the case, then Boi binding to Hh in apical cells may control Hh levels available to FSCs. Consistent with this model, a dramatic redistribution of Hh from apical cells to the extracellular space of the local FSC niche occurred in the absence of *boi*, without changes in Hh transcriptional activation (Fig. 3 B and Fig. S3). Expression of wild-type Boi in apical cells rescued both Hh localization and the *boi* mutant FSC hyperproliferation phenotypes (*boi*; *UAS-boi/+*; *babGal4/+*; Fig. 3, C, F, and G; and Table I). In contrast, a mutant form of Boi lacking the Hh-binding domain

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

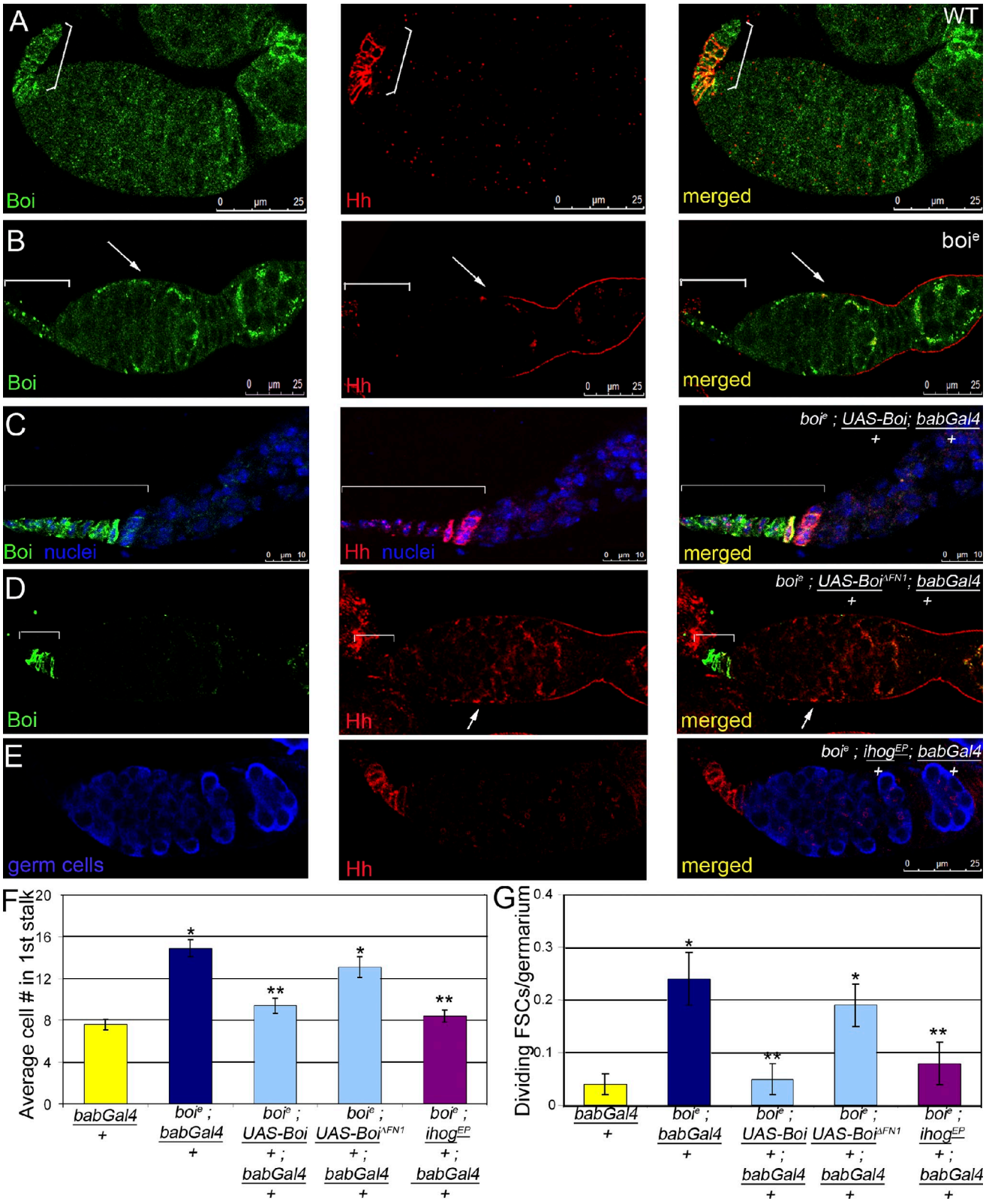

Figure 3. **Boi sequesters Hh on the surface of apical cells.** (A and B) Wild-type (WT; A) or *boi*^{e} mutant (B) germaria showing Boi (green) and Hh (red) expression in apical cells (brackets). Redistributed Hh is indicated (B, arrows). (C) Rescue of Hh (red) and Boi (green) localization by wild-type *boi* expression in apical cells (bracket, *boi*^{e}*; UAS-boi/+; babGal4/+*). Nuclei are labeled (Draq5, blue). (D) Boi$^{\Delta FN1}$ (green) lacks the Hh-binding domain and fails to rescue Hh (red) localization (brackets, *boi*^{e}*; UAS-boi*$^{\Delta FN1}$*/+; babGal4/+*). Hh accumulates near FSCs (arrows) as in *boi* mutants. (E) Ihog expression (*boi*^{e}*; ihog*^{EP}*/+; babGal4/+*) rescues Hh localization (red). Germ cells are labeled (anti-Vasa, blue). (F) Mean cell number in the first stalk for each genotype is shown. *, significant differences relative to control (*babGal4/+*, $P < 0.00002$). **, significant differences relative to *boi*^{e}*; babGal4/+* ($P < 0.00002$). (G) Mean numbers of dividing FSCs (PH3+) per germarium are shown. *, significant differences relative to control (*babGal4/+*, $P < 0.001$). **, significant differences relative to *boi*^{e}*; babGal4/+*, ($P < 0.02$). Error bars represent SEM.

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

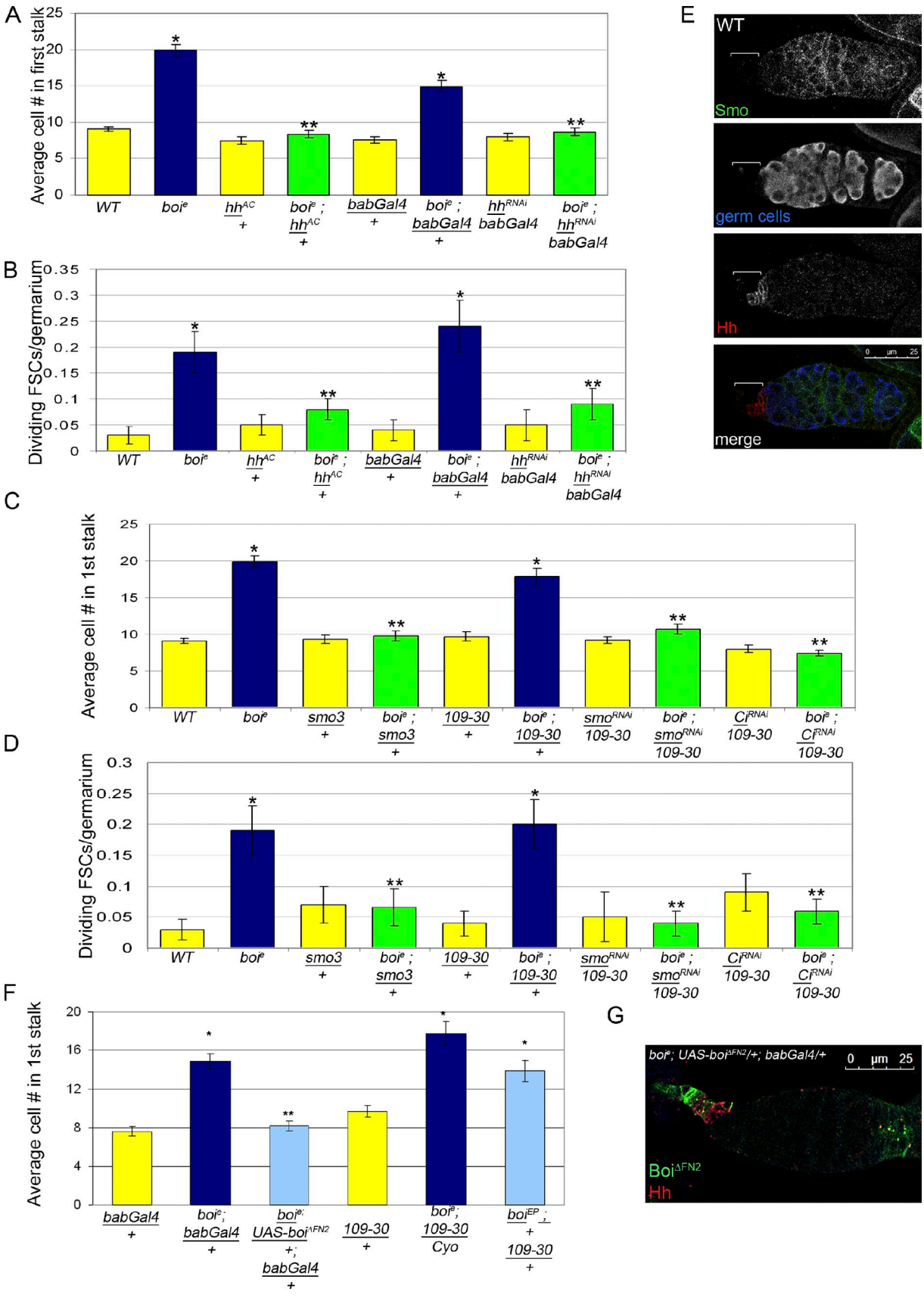

Figure 4. **Boi acts through the canonical Hh pathway.** (A and B) Reducing Hh expression in all cells (hh^{AC}/+) or in apical cells (hh^{RNAi}/*bab-Gal4*) suppresses *boi* mutant defects. (A) The mean cell number in the first stalk for each genotype is shown. *, significant differences relative to control (wild-type [WT] or *bab-Gal4/+*, P < 0.00000006). **, significant differences relative to boi^e or boi^e; *babGal4/+* (P < 0.000001). (B) Mean numbers of dividing

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

(Yao et al., 2006; McLellan et al., 2008) failed to rescue both Hh localization and the *boi* mutant proliferation defects (Fig. 3, D, F, and G; and Table I), which suggests that direct binding of Hh to Boi mediates its function. Apical cell expression of Ihog, which also binds Hh directly (Yao et al., 2006; McLellan et al., 2008), rescued *boi* mutant defects (*boi*; $ihog^{EP}$/+; *babGal4/+*; Fig. 3, E–G; and Table I); this further supports the model that retention of Hh on the apical cell surface is critical for FSC proliferation control.

To confirm that the FSC hyperproliferation observed in *boi* mutants is caused by increased Hh signaling, we reduced the levels of (a) Hh ligand produced by apical cells or (b) Hh effector proteins in FSCs in *boi* mutant germaria. Consistent with the model, the FSC hyperproliferation and follicle cell accumulation defects observed in *boi* mutants were restored to wild-type levels by reducing Hh levels either in all cells of the germarium (*boi*; hh^{AC}/+) or just in apical cells (*boi*; hh^{RNAi}*/bab-Gal4*; Fig. 4, A and B; and Table I). Moreover, genetic (*boi*; smo^{3}/+) or RNAi-mediated reduction (*boi*; smo^{RNAi}*/109-30 Gal4* or *boi*; *109-30 Gal4/+*; Ci^{RNAi}/+) of Smo or Ci levels in *boi* mutants also suppressed *boi* mutant phenotypes (Fig. 4, C and D; Table I). Finally, the levels of activated Ci in FSCs and their derivatives were higher in *boi* mutants than in wild-type germaria (Fig. S3). Collectively, these data support the model that Boi binds and sequesters Hh on apical cells, limiting the levels of ligand available for receipt by FSCs and thus controlling their proliferation.

In most documented cases, Boi and its homologues increase the local Hh concentration in cells that also express the Hh effector proteins Ptc, Smo, and Ci/Gli, promoting Hh signaling cell autonomously (Okada et al., 2006; Tenzen et al., 2006; Yao et al., 2006; Zhang et al., 2006, 2010). Hh binds to apical cells in a Boi-dependent manner (Fig. 3), but the expression patterns of the downstream effectors Smo and Ci have not been documented. Strikingly, we found that Smo and the full-length, activated form of Ci were expressed in most cells of the germarium including FSCs, but were excluded from Boi-expressing apical cells (Figs. 4 E and S3). This suggests that Boi suppresses Hh signaling in cells lacking expression of its downstream effectors. Consistent with this idea, *boi* mutant defects were rescued by a form of Boi that lacks the Ptc-binding domain (boi^{e}; *UAS-*$boi^{\Delta FN2}$ /+; *babGal4/+*; Fig. 4, F and G; Yao et al., 2006; McLellan et al., 2008), demonstrating that Boi-mediated suppression of FSC proliferation is Ptc independent. Finally, ectopic expression of Boi in FSCs and their progeny (boi^{EP}*/+;109-30 Gal4/+*), the Hh target cells in this system, led to the accumulation of 1.5-fold excess follicle cells between stalks (Fig. 4 F). Thus, Hh signaling was enhanced in cells coexpressing Boi and Smo/Ci, which is consistent with observations in other fly tissues (Yao et al., 2006; Yan et al., 2010; Zheng et al., 2010). Together, these observations support the idea that the role of Boi in Hh signaling depends on the presence or absence of Hh pathway effectors in Boi-expressing cells.

Here we demonstrate that the primary function of Boi in FSC proliferation control is to limit the access of Hh ligand to FSCs. Similar nonautonomous inhibition of Sonic Hedgehog (Shh) signaling has been observed upon overexpression of the Boi/Ihog homologues Cdo or Boc in the developing chick neural tube (Tenzen et al., 2006), which suggests that Hh sequestration may be a conserved function for Boi family members. However, roles for Boi or its homologues in nonautonomous control of stem cell factors have not been demonstrated previously. The requirement for precise control of Hh levels for proliferation control in prostate and neural stem cells (Lai et al., 2003; Machold et al., 2003; Karhadkar et al., 2004) suggests the possibility that this mechanism may be conserved in those tissues.

Although classical niches are composed of differentiated cells that provide all information necessary to direct stem cell fate (Xie and Spradling, 2000), FSCs rely on factors, including Hh, produced in cells residing at a distance from their immediate niche (Forbes et al., 1996a; Zhang and Kalderon, 2000; Zhang and Kalderon, 2001; Song and Xie, 2003; Kirilly et al., 2005). The benefit of this architecture for egg production is unclear. In other tissues, complex mechanisms control Hh transcription, secretion, cleavage, time of exposure, and range of diffusion in order to concentrate Hh signal on the appropriate receiving cells (Dahmann and Basler, 2000; Varjosalo and Taipale, 2008). In some cases, such as during neural tube development in mammals, the fate of several neural cells is determined by their position along a Shh concentration gradient (Ingham and Placzek, 2006). In other cases, long-range Hh diffusion is blocked by strong expression of Ptc in cells close to the signal source, promoting short-range, high-level Hh stimulation (Chen and Struhl, 1996).

For FSC regulation, Hh likely acts as a long-range signal that diffuses in a gradient from the apical cells where it is produced (Forbes et al., 1996a,b; Zhang and Kalderon, 2000, 2001). Perhaps an unidentified component of the FSC niche helps concentrate the ligand at the FSC surface, promoting proliferation. Hh accumulation in the extracellular space near FSCs in *boi* mutants (Fig. 3) supports this idea. Moreover, the FSC-autonomous requirement for *ptc*, *smo*, and *Ci* is consistent with direct, long-range stimulation of proliferation by Hh (Forbes et al., 1996a,b; Zhang and Kalderon, 2000, 2001).

Hh also may act as a short-range morphogen in the ovary. Ptc and Smo are expressed in most cells of the germarium,

FSCs ($PH3^{+}$) per germarium are shown for genotypes indicated. *, significant differences relative to control (WT or *bab-Gal4/+*, $P < 0.0002$). **, significant differences relative to boi^{e} or boi^{e}*; bab-Gal4/+* ($P < 0.02$). (C and D) Reducing Smo or Ci expression in all cells (smo^{3}/+) or in FSCs and their progeny (*smo*^{RNAi}*/109-30-Gal4, or* Ci^{RNAi}*/109-30-Gal4*) suppresses *boi* mutant defects. (C) Mean cell number in the first stalk for each genotype is shown. *, significant differences relative to control (WT or *109-30/+*, $P < 0.0000009$). **, significant differences relative to boi^{e} or boi^{e}*;109-30/+* ($P < 0.00001$). (D) Mean numbers of dividing FSCs per germarium ($PH3^{+}$) are shown. *, significant differences relative to control (WT or *109-30/+*, $P < 0.007$). **, significant differences relative to boi^{e} or boi^{e}*;109-30/+* ($P < 0.04$). (E) Wild-type germaria immunostained with antibodies against Smo (green), Vasa (germ cells, blue), and Hh (red). Brackets indicate apical cells. (F) Mean cell number in the first stalk for each genotype is shown. *, significant differences relative to control (*babGal4/+* or *109-30/+*, $P < 0.004$). **, significant differences relative to boi^{e}*; babGal4/+*, $P < 0.0000002$). (G) Expression of $Boi^{\Delta FN2}$ (green) in *boi* mutant apical cells (boi^{e}*; UAS-*$boi^{\Delta FN2}$*/+; babGal4/+*) rescues Hh localization (red). Error bars represent SEM.

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

including GSCs and escort stem cells (ESCs) lying immediately adjacent to apical cells (Fig. 4; Forbes et al., 1996a,b). *ptc-lacZ* and *Ci-lacZ* reporters are strongly activated in ESCs and their progeny (Forbes et al., 1996b; Vied and Kalderon, 2009). Moreover, Hh expression in apical cells apparently coordinates FSC and GSC cell division via an undefined mechanism (King et al., 2001). Perhaps Boi binds Hh within apical cells to increase its local concentration for short-range action, in a manner similar to that observed for the Decapentaplegic (Dpp) receptor Dally in GSC proliferation control (Guo and Wang, 2009; Hayashi et al., 2009). In this case, Hh might activate downstream signaling in ESCs, initiating a signaling relay system that affects GSC and FSC proliferation indirectly. Further work is needed to determine whether long-range, short-range, or both signaling mechanisms regulate stem cell proliferation in the ovary.

The role of nonadjacent growth factor producing cells in limiting stem cell proliferation signals may be a general mechanism in epithelial tissues. In the murine hair follicle and intestine, where stem cells can be identified in situ, underlying mesenchymal cells produce factors that are critical for stem cell self-renewal and function (Rendl et al., 2005; Schmidt-Ullrich and Paus, 2005; Kosinski et al., 2007; Li et al., 2007; McLin et al., 2009). Transcriptional analysis indicates that these same cells express receptors that can limit the release or range of the growth factor signals they produce (Rendl et al., 2005; Kosinski et al., 2007; Li et al., 2007). Although current data support critical functions for the growth factors in stem cell maintenance (Blanpain et al., 2007; Blanpain and Fuchs, 2009; McLin et al., 2009), the roles of their membrane-bound inhibitors is less clear. Based on our observations, we propose that inhibitory receptors sequester ligand on the surface of the cells that produce them to limit the levels of signal available to the stem cell niche. If this model is correct, then the cellular source of secreted stem cell regulators that reside outside of the classical stem cell niche may be identified by the presence of membrane-bound receptors with high levels of surface-localized ligand. Understanding how these cells regulate stem cell function will be critical for treating stem cell–based diseases and for future development of effective stem cell replacement therapies.

Materials and methods

Fly strains

boi^{d04197} (*boi^{d}*), *boi^{e01708}* (*boi^{e}*), and *$ihog^{e02142}$* (*$ihog^{e}$*) were generated by Exelixis and maintained by the Harvard stock center. *boi^{d}* and *boi^{e}* are loss-of-function alleles expressing 8% and 0.2% of wild-type, full-length *boi* transcript, respectively, in the ovary (Fig. S1). *$ihog^{e}$* has been described previously (Yao et al., 2006; Zheng et al., 2010). In the ovary, 10% of full-length wild-type *ihog* transcript is expressed in *$ihog^{e}$* mutants (Fig. S1). *smo^{3}* (Chen and Struhl, 1998) and *hh^{AC}* (Lee et al., 1992) are null alleles of *smo* and *hh*, respectively. RNAi directed against *boi* (*$boi^{GD14474}$* [*boi^{RNAi} #1*] or *boi^{GD60}* [*boi^{RNAi}#4*]), *smo* (*P{UAS-smo^{RNAi}}2 P{UAS-smo^{RNAi}}*), *Ci* (*P{TRiP.JF01715}attP2*), or *hh* (*P{TRiP.JF01804}attP2*) was expressed either in apical cells using *babGal4* (*P{GawB}bab1{Pgal4-2}*) or in FSCs and their progeny using *109-30-Gal4* (*P{GawB}109-30*) (Fig. S2). Boi was expressed in FSCs and their progeny by generating female flies of the genotype *boi^{EP}/109-30* (*P{EPgy2}$boi^{EY09847}$/+; 109-30-Gal4/+*). Ihog was expressed in apical cells by generating female flies of the genotype *$ihog^{EP}$* (*P{EP}$ihog^{G13202}$)/+; bab-Gal4/+*.

Transgenic fly lines

pUASt-boi (*UAS-boi*) was generated by cloning the full-length *boi* transcript *boi-RB* from pOT2-SD07678 (GenBank/EMBL/DDBJ accession No. AY061833, *Drosophila* Genomics Resource Center) into *pUASt*. *pUASt-$boi^{\Delta FN1}$* was created by site-directed excision of bases corresponding to amino acids 456–598 of Boi. *pUASt-$boi^{\Delta FN2}$* was created by site-directed excision of bases corresponding to amino acids 604–701 of Boi. *pUASt-boi*, *pUASt-$boi^{\Delta FN1}$*, and *pUASt-$boi^{\Delta FN2}$* were created using the Gateway *Drosophila* cloning system (Carnegie Institution of Washington). Transgenic flies were generated by Best-Gene, Inc. *pUASt-boi*, *pUASt-$boi^{\Delta FN1}$*, and *pUASt-$boi^{\Delta FN2}$* map to the second chromosome.

Generation of anti-Boi antibodies

Polyclonal anti-Boi antibodies were developed from the injection of a GST fusion protein of amino acids 936–1013 (D isoform) in the cytoplasmic domain of all Boi isoforms into Sprague Dawley rats. Epitope identification was aided by the bioinformatics tools *Antigenic* (Kolaskar and Tongaonkar, 1990), *Phobius* (Käll et al., 2007), *Disopred* (Ward et al., 2004), and *Swiss EMBL* (Schwede et al., 2003). The construct was generated by amplifying the *boi* fragment using genomic PCR (5′-CATGGATCCCATCAGAATGGCCTTCACCAC-3′ and 5′-GATATCTCGAGCAGGGATGGTATCCTGGTC-3′) and cloning it into pGEX4T-2.

Immunofluorescence

Fly ovaries were dissected and fixed as described previously (O'Reilly et al., 2008). Wild-type and mutant ovaries were compared directly by dissecting, fixing, and immunostaining with premixed primary and secondary antibodies at the same time. For anti-Boi immunostaining, ovaries were fixed in 2% formaldehyde on ice. For nuclear staining, fixed ovaries were incubated for 15 min with Draq5 (Cell Signaling Technology). After staining, ovaries were mounted in Vectashield medium (Vector Laboratories). Primary antibodies were 1:50 rat anti-Boi, 1:2,000 rabbit anti-Vasa (Hay et al., 1990); 1:100 rabbit anti-Hh (Calvados; provided by P. Therond, Institute of Developmental Biology and Cancer, Université Nice, Sophia Antipolis, Parc Valrose, France; Gallet et al., 2003), 1:100 goat anti-Hh (Santa Cruz Biotechnology, Inc.), 1:25 mouse anti-Fas3 (Developmental Studies Hybridoma Bank [DSHB]; Patel et al., 1987); 1:1,000 rabbit anti–phospho-histone H3 (Millipore); 1:100 mouse anti-Ptc (DSHB; Capdevila et al., 1994); and 1:100 mouse anti-Smo (DSHB; Lum et al., 2003b). Secondary antibodies used were FITC, Cy3, and Cy5 conjugated to species-specific secondary antibodies (Jackson ImmunoResearch Laboratories, Inc.). Samples were mounted in Vectashield mounting medium. Images were collected at room temperature (~22°C) using 40× (1.25 NA) or 63× (1.4 NA) oil immersion lenses (Leica) on an upright microscope (DM 5000; Leica) coupled to a confocal laser scanner (TCS SP5; Leica). LAS AF SP5 software (Leica) was used for data acquisition. Images representing individual channels of single confocal slices from the center of each germarium were exported as TIFF files, and images were converted to figures using Photoshop software (Adobe). For analysis of Boi expression in wild-type or mutant tissue, ideal settings were determined for immunostaining wild-type tissue, and the level of signal in *boi* mutants was compared. To ensure that residual Boi expression was not observed in mutant tissue, the images presented were taken at higher gain than the wild-type tissue, resulting in slightly higher background levels than observed in the wild type.

RNA isolation and quantitative RT-PCR

For RNA isolation, whole ovaries were dissected from female flies. Ovaries were lysed with a dounce homogenizer and RNA isolated with an RNeasy kit (QIAGEN). RNA concentrations were determined with a spectrophotometer (NanoDrop; Thermo Fisher Scientific). For each sample, two reverse-transcription reactions were performed with 100 and 20 ng of input RNA. 5′ nuclease assays using TaqMan chemistry were run on a sequence detection system (7900 HT; Applied Biosystems). Ct (cycle threshold) values were converted to quantities (in arbitrary units) using standard curve (five points, fivefold dilutions) established with a calibrator sample. Quantitative real-time RT-PCR results were normalized to RPlI140 mRNA levels. For each sample, the two values of relative quantity (from two PCR assays) were averaged, and a representative sample from three independent biological assays is shown. Full-length Boi oligos: forward, 5′-TGGATTTGGATAGAGGATTTAGCTG-3′; and reverse, 5′-GCTGTCTTGCTGTCTCTTTCCA-3′. Full-length Ihog oligos: forward, 5′-AAAACCAGCACCACAGAGGAG-3′; and reverse, 5′-ACTCATATTGAATGTCTCGTTATGACTG-3′.

Protein isolation and Western blot

For protein isolation, whole ovaries were dissected from female flies, and ovaries were lysed using a dounce homogenizer and RIPA buffer (50 mM Tris, pH 8.0, 0.1% SDS, 1% Triton X-100, 150 mM NaCl, 1% deoxycholic acid, 2 mM PMSF, and protease inhibitors [Sigma-Aldrich]). Proteins were run on an 8% SDS-PAGE gel, then transferred to an Immobilon-P (Millipore) membrane. Membranes were probed with anti-Boi antibodies and β-tubulin (EMD).

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

Statistics

To determine the number of follicle cells in the first stalk, 25–150 germaria were scored for follicle cell number (not touching a germ cell). Follicle cell number was compared with controls (w^{1118}, *109-30/Cyo*, or *babGal4/+*) or boi^e mutants (boi^e/boi^e, boi^e/boi^e*;109-30/Cyo*, or boi^e/boi^e*; babGal4/+*). Statistical differences were determined using the Wilcoxon two-sample test, with significance achieved at $P \leq 0.05$. To determine the number of dividing follicle cells and FSCs per germarium, 75–150 germaria were analyzed for the presence of a phospho-histone-H3 positive ($PH3^+$) FSC. FSCs were identified by their location at the border of germarial regions 2A and 2B, low-level expression of Fas3 ($Fas3^{lo}$), a marker for prefollicle cells, and the presence of a triangular nucleus, a feature that distinguishes FSCs from their daughter cells and neighboring escort cells (Nystul and Spradling, 2007). The number of $PH3^+$ dividing FSCs was compared with controls (w^{1118}, *109-30/Cyo*, or *babGal4/+*) or boi^e mutants (boi^e/boi^e, boi^e/boi^e*;109-30/Cyo*, or boi^e/boi^e*; babGal4/+*). To measure dividing prefollicle cells, the total number of prefollicle cells that express high levels of Fas3 was determined by counting nuclei stained with Draq5. The number of $PH3^+$ prefollicle cells in the same germaria was counted and a ratio of $PH3^+$/total was determined. Statistical differences were determined using the Student's *t* test for two samples, with significance achieved at $P \leq 0.05$.

Online supplemental material

Fig. S1 shows that insertion of transposable elements in *boi* intron 2 significantly reduces expression of *boi* mRNA. Fig. S2 shows expression of UAS transgenes in apical cells or FSCs and their progeny. Fig. S3 shows normal Hh transcription and increased Ci activity in *boi* mutants. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201007142/DC1.

We thank J. Valvardi, J. Hittle, S. Litwin, M. Andrake, and K. Trush for technical assistance; D. Connolly, M. Murphy, J. Peterson, T. Rando, F. Roegiers, and A. Tulin for critical comments; P. Therond for the anti-Hh Calvados antibody, and the resource centers at Bloomington, Harvard, Vienna, Drosophila Genomics Resource Center, and Developmental Studies Hybridoma Bank.

This study was supported by grants from Fox Chase Cancer Center (to D. Zinshteyn), Pennsylvania Department of Health (Health Research Formula Funds to A. O'Reilly), Commonwealth of Pennsylvania Ben Franklin Technology Development Authority (KISK C000026964 to A. O'Reilly), American Cancer Society (IRG-92-027-16 to A. O'Reilly), and National Institutes of Health (CA06927 [Fox Chase Cancer Center], DK076443-01 to T.R. Hartman, and NS053775 to A. Okada).

Submitted: 26 June 2010
Accepted: 26 October 2010

References

Bai, J., and D. Montell. 2002. Eyes absent, a key repressor of polar cell fate during *Drosophila* oogenesis. *Development.* 129:5377–5388. doi:10.1242/dev.00115

Blanpain, C., and E. Fuchs. 2009. Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat. Rev. Mol. Cell Biol.* 10:207–217. doi:10.1038/nrm2636

Blanpain, C., V. Horsley, and E. Fuchs. 2007. Epithelial stem cells: turning over new leaves. *Cell.* 128:445–458. doi:10.1016/j.cell.2007.01.014

Capdevila, J., M.P. Estrada, E. Sánchez-Herrero, and I. Guerrero. 1994. The *Drosophila* segment polarity gene patched interacts with decapentaplegic in wing development. *EMBO J.* 13:71–82.

Chen, Y., and G. Struhl. 1996. Dual roles for patched in sequestering and transducing Hedgehog. *Cell.* 87:553–563. doi:10.1016/S0092-8674(00)81374-4

Chen, Y., and G. Struhl. 1998. In vivo evidence that Patched and Smoothened constitute distinct binding and transducing components of a Hedgehog receptor complex. *Development.* 125:4943–4948.

Dahmann, C., and K. Basler. 2000. Opposing transcriptional outputs of Hedgehog signaling and engrailed control compartmental cell sorting at the *Drosophila* A/P boundary. *Cell.* 100:411–422. doi:10.1016/S0092-8674(00)80677-7

Forbes, A.J., H. Lin, P.W. Ingham, and A.C. Spradling. 1996a. hedgehog is required for the proliferation and specification of ovarian somatic cells prior to egg chamber formation in *Drosophila. Development.* 122:1125–1135.

Forbes, A.J., A.C. Spradling, P.W. Ingham, and H. Lin. 1996b. The role of segment polarity genes during early oogenesis in *Drosophila. Development.* 122:3283–3294.

Gallet, A., R. Rodriguez, L. Ruel, and P.P. Therond. 2003. Cholesterol modification of hedgehog is required for trafficking and movement, revealing an asymmetric cellular response to hedgehog. *Dev. Cell.* 4:191–204. doi:10.1016/S1534-5807(03)00031-5

Guo, Z., and Z. Wang. 2009. The glypican Dally is required in the niche for the maintenance of germline stem cells and short-range BMP signaling in the *Drosophila* ovary. *Development.* 136:3627–3635. doi:10.1242/dev.036939

Hay, B., L.Y. Jan, and Y.N. Jan. 1990. Localization of vasa, a component of *Drosophila* polar granules, in maternal-effect mutants that alter embryonic anteroposterior polarity. *Development.* 109:425–433.

Hayashi, Y., S. Kobayashi, and H. Nakato. 2009. *Drosophila* glypicans regulate the germline stem cell niche. *J. Cell Biol.* 187:473–480. doi:10.1083/jcb.200904118

Ingham, P.W., and M. Placzek. 2006. Orchestrating ontogenesis: variations on a theme by sonic hedgehog. *Nat. Rev. Genet.* 7:841–850. doi:10.1038/nrg1969

Käll, L., A. Krogh, and E.L. Sonnhammer. 2007. Advantages of combined transmembrane topology and signal peptide prediction--the Phobius web server. *Nucleic Acids Res.* 35:W429–W432. doi:10.1093/nar/gkm256

Karhadkar, S.S., G.S. Bova, N. Abdallah, S. Dhara, D. Gardner, A. Maitra, J.T. Isaacs, D.M. Berman, and P.A. Beachy. 2004. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. *Nature.* 431:707–712. doi:10.1038/nature02962

King, F.J., A. Szakmary, D.N. Cox, and H. Lin. 2001. Yb modulates the divisions of both germline and somatic stem cells through piwi- and hh-mediated mechanisms in the *Drosophila* ovary. *Mol. Cell.* 7:497–508. doi:10.1016/S1097-2765(01)00197-6

Kirilly, D., E.P. Spana, N. Perrimon, R.W. Padgett, and T. Xie. 2005. BMP signaling is required for controlling somatic stem cell self-renewal in the *Drosophila* ovary. *Dev. Cell.* 9:651–662. doi:10.1016/j.devcel.2005.09.013

Kolaskar, A.S., and P.C. Tongaonkar. 1990. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett.* 276:172–174. doi:10.1016/0014-5793(90)80535-Q

Kosinski, C., V.S. Li, A.S. Chan, J. Zhang, C. Ho, W.Y. Tsui, T.L. Chan, R.C. Mifflin, D.W. Powell, S.T. Yuen, et al. 2007. Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors. *Proc. Natl. Acad. Sci. USA.* 104:15418–15423. doi:10.1073/pnas.0707210104

Lai, K., B.K. Kaspar, F.H. Gage, and D.V. Schaffer. 2003. Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo. *Nat. Neurosci.* 6:21–27. doi:10.1038/nn983

Lee, J.J., D.P. von Kessler, S. Parks, and P.A. Beachy. 1992. Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene hedgehog. *Cell.* 71:33–50. doi:10.1016/0092-8674(92)90264-D

Li, X., B.B. Madison, W. Zacharias, A. Kolterud, D. States, and D.L. Gumucio. 2007. Deconvoluting the intestine: molecular evidence for a major role of the mesenchyme in the modulation of signaling cross talk. *Physiol. Genomics.* 29:290–301. doi:10.1152/physiolgenomics.00269.2006

Lum, L., S. Yao, B. Mozer, A. Rovescalli, D. Von Kessler, M. Nirenberg, and P.A. Beachy. 2003a. Identification of Hedgehog pathway components by RNAi in *Drosophila* cultured cells. *Science.* 299:2039–2045. doi:10.1126/science.1081403

Lum, L., C. Zhang, S. Oh, R.K. Mann, D.P. von Kessler, J. Taipale, F. Weis-Garcia, R. Gong, B. Wang, and P.A. Beachy. 2003b. Hedgehog signal transduction via Smoothened association with a cytoplasmic complex scaffolded by the atypical kinesin, Costal-2. *Mol. Cell.* 12:1261–1274. doi:10.1016/S1097-2765(03)00426-X

Machold, R., S. Hayashi, M. Rutlin, M.D. Muzumdar, S. Nery, J.G. Corbin, A. Gritli-Linde, T. Dellovade, J.A. Porter, L.L. Rubin, et al. 2003. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. *Neuron.* 39:937–950. doi:10.1016/S0896-6273(03)00561-0

Margolis, J., and A. Spradling. 1995. Identification and behavior of epithelial stem cells in the *Drosophila* ovary. *Development.* 121:3797–3807.

McLellan, J.S., X. Zheng, G. Hauk, R. Ghirlando, P.A. Beachy, and D.J. Leahy. 2008. The mode of Hedgehog binding to Ihog homologues is not conserved across different phyla. *Nature.* 455:979–983. doi:10.1038/nature07358

McLin, V.A., S.J. Henning, and M. Jamrich. 2009. The role of the visceral mesoderm in the development of the gastrointestinal tract. *Gastroenterology.* 136:2074–2091. doi:10.1053/j.gastro.2009.03.001

Morrison, S.J., and A.C. Spradling. 2008. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell.* 132:598–611. doi:10.1016/j.cell.2008.01.038

Nystul, T., and A. Spradling. 2007. An epithelial niche in the *Drosophila* ovary undergoes long-range stem cell replacement. *Cell Stem Cell.* 1:277–285. doi:10.1016/j.stem.2007.07.009

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025

Okada, A., F. Charron, S. Morin, D.S. Shin, K. Wong, P.J. Fabre, M. Tessier-Lavigne, and S.K. McConnell. 2006. Boc is a receptor for sonic hedgehog in the guidance of commissural axons. *Nature.* 444:369–373. doi:10.1038/nature05246

O'Reilly, A.M., H.H. Lee, and M.A. Simon. 2008. Integrins control the positioning and proliferation of follicle stem cells in the *Drosophila* ovary. *J. Cell Biol.* 182:801–815. doi:10.1083/jcb.200710141

Patel, N.H., P.M. Snow, and C.S. Goodman. 1987. Characterization and cloning of fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. *Cell.* 48:975–988. doi:10.1016/0092-8674(87)90706-9

Rendl, M., L. Lewis, and E. Fuchs. 2005. Molecular dissection of mesenchymal-epithelial interactions in the hair follicle. *PLoS Biol.* 3:e331. doi:10.1371/journal.pbio.0030331

Sato, T., R.G. Vries, H.J. Snippert, M. van de Wetering, N. Barker, D.E. Stange, J.H. van Es, A. Abo, P. Kujala, P.J. Peters, and H. Clevers. 2009. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* 459:262–265. doi:10.1038/nature07935

Schmidt-Ullrich, R., and R. Paus. 2005. Molecular principles of hair follicle induction and morphogenesis. *Bioessays.* 27:247–261. doi:10.1002/bies.20184

Schwede, T., J. Kopp, N. Guex, and M.C. Peitsch. 2003. SWISS-MODEL: An automated protein homology-modeling server. *Nucleic Acids Res.* 31:3381–3385. doi:10.1093/nar/gkg520

Song, X., and T. Xie. 2002. DE-cadherin-mediated cell adhesion is essential for maintaining somatic stem cells in the *Drosophila* ovary. *Proc. Natl. Acad. Sci. USA.* 99:14813–14818. doi:10.1073/pnas.232389399

Song, X., and T. Xie. 2003. Wingless signaling regulates the maintenance of ovarian somatic stem cells in *Drosophila*. *Development.* 130:3259–3268. doi:10.1242/dev.00524

Tenzen, T., B.L. Allen, F. Cole, J.S. Kang, R.S. Krauss, and A.P. McMahon. 2006. The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice. *Dev. Cell.* 10:647–656. doi:10.1016/j.devcel.2006.04.004

Varjosalo, M., and J. Taipale. 2008. Hedgehog: functions and mechanisms. *Genes Dev.* 22:2454–2472. doi:10.1101/gad.1693608

Vied, C., and D. Kalderon. 2009. Hedgehog-stimulated stem cells depend on non-canonical activity of the Notch co-activator Mastermind. *Development.* 136:2177–2186. doi:10.1242/dev.035329

Ward, J.J., J.S. Sodhi, L.J. McGuffin, B.F. Buxton, and D.T. Jones. 2004. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *J. Mol. Biol.* 337:635–645. doi:10.1016/j.jmb.2004.02.002

Williams, E.H., W.N. Pappano, A.M. Saunders, M.S. Kim, D.J. Leahy, and P.A. Beachy. 2010. Dally-like core protein and its mammalian homologues mediate stimulatory and inhibitory effects on Hedgehog signal response. *Proc. Natl. Acad. Sci. USA.* 107:5869–5874. doi:10.1073/pnas.1001777107

Xie, T., and A.C. Spradling. 2000. A niche maintaining germ line stem cells in the *Drosophila* ovary. *Science.* 290:328–330. doi:10.1126/science.290.5490.328

Yan, D., Y. Wu, Y. Yang, T.Y. Belenkaya, X. Tang, and X. Lin. 2010. The cell-surface proteins Dally-like and Ihog differentially regulate Hedgehog signaling strength and range during development. *Development.* 137:2033–2044. doi:10.1242/dev.045740

Yao, S., L. Lum, and P. Beachy. 2006. The ihog cell-surface proteins bind Hedgehog and mediate pathway activation. *Cell.* 125:343–357. doi:10.1016/j.cell.2006.02.040

Zhang, Y., and D. Kalderon. 2000. Regulation of cell proliferation and patterning in *Drosophila* oogenesis by Hedgehog signaling. *Development.* 127:2165–2176.

Zhang, Y., and D. Kalderon. 2001. Hedgehog acts as a somatic stem cell factor in the *Drosophila* ovary. *Nature.* 410:599–604. doi:10.1038/35069099

Zhang, W., J.S. Kang, F. Cole, M.J. Yi, and R.S. Krauss. 2006. Cdo functions at multiple points in the Sonic Hedgehog pathway, and Cdo-deficient mice accurately model human holoprosencephaly. *Dev. Cell.* 10:657–665. doi:10.1016/j.devcel.2006.04.005

Zheng, X., R.K. Mann, N. Sever, and P.A. Beachy. 2010. Genetic and biochemical definition of the Hedgehog receptor. *Genes Dev.* 24:57–71. doi:10.1101/gad.1870310

Downloaded from http://rupress.org/jcb/article-pdf/191/5/943/1570793/jcb_201007142.pdf by guest on 02 December 2025