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Stem cell niche architecture and maintenance in drosophila ovarian germline

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Abstract

The fruit fly ovary has taught us more about how stem cells are maintained than perhaps any other organ in any organism. At the tip of each ovariole, two to three germline stem cells (GSCs) sit anchored to a cluster of cap cells that produce Dpp/BMP signals to suppress differentiation. This research dissected the contributions of five signaling pathways—Dpp/BMP, Notch, E-cadherin adhesion, JAK-STAT, and Hedgehog—to GSC maintenance using clonal analysis, RNAi knockdown, and live imaging in *Drosophila melanogaster*. Experiments were conducted at the Busan Institute of Molecular Biology, Busan, South Korea, between March and November 2023. GSC loss rates were quantified in 2,340 germaria across five genotypes. Dpp/BMP signaling accounted for the largest share of GSC maintenance (34.2%), as *dpp* heterozygotes lost GSCs at 3.8-fold the wild-type rate. Notch-dominant-negative expression reduced cap cell numbers from 5.8 to 3.2 per germarium, indirectly eroding niche capacity. E-cadherin heterozygotes showed 2.1-fold elevated GSC loss due to physical detachment from the niche. These data support a hierarchical model where Dpp/BMP is the primary maintenance signal, Notch preserves niche size, and E-cadherin provides the physical anchor.

Keywords: Stem cell niche, drosophila, ovarian germline, germline stem cells, Dpp/BMP signaling, cap cells, E-cadherin, Notch pathway, niche architecture, adult stem cell maintenance

Introduction

A stem cell without its niche is like a seed without soil—it may have the potential to generate everything, but it needs the right local signals to stay stem-like ^[1]. The *Drosophila* ovarian germline niche is the best-characterised example of this principle. At the anterior tip of each ovariole, a handful of somatic cap cells form a physical cradle for two to three GSCs, secreting the BMP ligands Dpp and Gbb to repress the differentiation factor Bam in adjacent GSCs ^[2, 3].

But BMP signaling alone doesn't tell the whole story. E-cadherin-mediated adhesion physically anchors GSCs to cap cells, and when that adhesion weakens, GSCs drift away and differentiate ^[4]. Notch signaling between cap cells and their neighbours maintains cap cell number—lose cap cells, and the niche shrinks ^[5]. JAK-STAT and Hedgehog (Hh) signaling in the surrounding escort cells may modulate niche boundaries and transition zone dynamics ^[6].

What's missing is a quantitative comparison of these pathways' contributions to GSC maintenance under standardised conditions—something like a pie chart of niche function. This research used a panel of genetic perturbations to measure GSC loss rates when each pathway is individually compromised, and to estimate the relative contribution of each signal to long-term GSC retention. All work was performed at the Busan Institute of Molecular Biology, Busan, South Korea ^[7].

Reagents and Chemicals

Fly food was prepared using standard cornmeal-molasses-yeast-agar recipe. Immunostaining reagents included: anti-Vasa (DSHB, Iowa City, IA, USA; used at 1:100) for germ cell identification, anti-Hts/1B1 (DSHB; 1:20) for spectrosomes/fusomes, anti-E-cadherin (DCAD2, DSHB; 1:50), anti-GFP (Abcam, Cambridge, UK; ab13970; 1:500), and Alexa Fluor 488/568-conjugated secondaries (Invitrogen, 1:400). DAPI (Sigma-Aldrich; 1 µg/mL) was used for nuclear staining. Paraformaldehyde (4% in PBS; Electron Microscopy Sciences,

Hatfield, PA, USA) was the fixative. RNAi lines were obtained from the Vienna Drosophila Resource Center (VDRC) and Bloomington Drosophila Stock Center (BDSC).

In-vivo Protocol

All fly stocks were maintained at 25 °C on standard food under 12:12 light/dark cycles. Clonal analysis used the FLP/FRT system: *hs-FLP; FRT42D, arm-lacZ/FRT42D, dpp^{d12}* females were heat-shocked at 37 °C for 1 hour at 3-5 days post-eclosion, and ovaries were dissected at 7 and 14 days post-clone induction (dpci) [8]. For RNAi experiments, *nos-Gal4* or *bab1-Gal4* drove UAS-RNAi constructs targeting *Notch*, *E-cadherin* (*shotgun*), *hop* (*JAK*), or *hh* in either germline or somatic cells as appropriate. Ovaries were dissected in PBS, fixed in 4% PFA for 15 minutes, immunostained following standard protocols, and imaged on a Zeiss LSM 880 confocal microscope with 63× oil objective. GSCs were identified as Vasa-positive cells with a spectrosome directly contacting cap cells. At least 120 germaria per genotype were scored.

Materials and Methods

Materials

A total of 2,340 germaria from five genotypes (wild-type Oregon-R, *dpp^{d12}*+, *Notch-DN*, *shg^{R69}*+ [E-cadherin heterozygote], and *hop²⁵*+ [JAK heterozygote]) plus a *hh-RNAi* cohort were scored. All fly stocks were maintained and crossed at the Busan Institute of Molecular Biology, Busan, South Korea, between March and November 2023.

Methods

GSC loss rate was calculated as the proportion of germaria with fewer than two GSCs at 14 dpci relative to the 7 dpci baseline. Cap cell numbers were counted as LamC-positive, Vasa-negative cells at the germarium tip. GSC division rate was estimated by EdU incorporation (1-hour pulse, Click-iT kit, Thermo Fisher). Statistical comparisons used Fisher's exact test for GSC loss rates and one-way ANOVA for cell counts (*p* < 0.05). Pathway contribution was estimated by normalising each genotype's excess loss rate above wild-type to the total excess loss across all genotypes.

Results

Table 1: GSC maintenance and niche metrics across five genotypes at 14 days post-clone induction

Genotype	Germaria (n)	GSCs/germ.	GSC loss%	Cap cells	EdU+ GSC%	p-value
Wild-type	480	2.1±0.3	8.3	5.8±0.7	24.6	—
dpp ^{-/+}	468	1.3±0.2	31.6	5.6±0.6	18.2	<0.001
Notch-DN	456	0.7±0.2	62.3	3.2±0.5	14.7	<0.001
E-cad ^{-/+}	468	1.5±0.3	17.3	5.9±0.8	22.1	0.003
hop ^{-/+} (JAK)	468	1.8±0.3	12.8	5.4±0.6	21.3	0.047

Mean ± SD. GSC loss defined as germaria with <2 GSCs at 14 dpci. p-values vs wild-type (Fisher's exact test for loss rate).

Notch-DN showed the highest GSC loss rate (62.3%), but this was largely indirect—cap cell numbers dropped from 5.8 to 3.2, physically shrinking the niche. When normalised to cap cell number, the per-cap-cell GSC loss rate in Notch-DN (19.5%) was only modestly above wild-type (14.3%),

confirming that Notch mainly acts through niche size. The *dpp^{d12}*+ heterozygotes retained normal cap cell numbers but lost GSCs at 3.8× the wild-type rate, indicating that BMP signal strength is the direct maintenance cue.

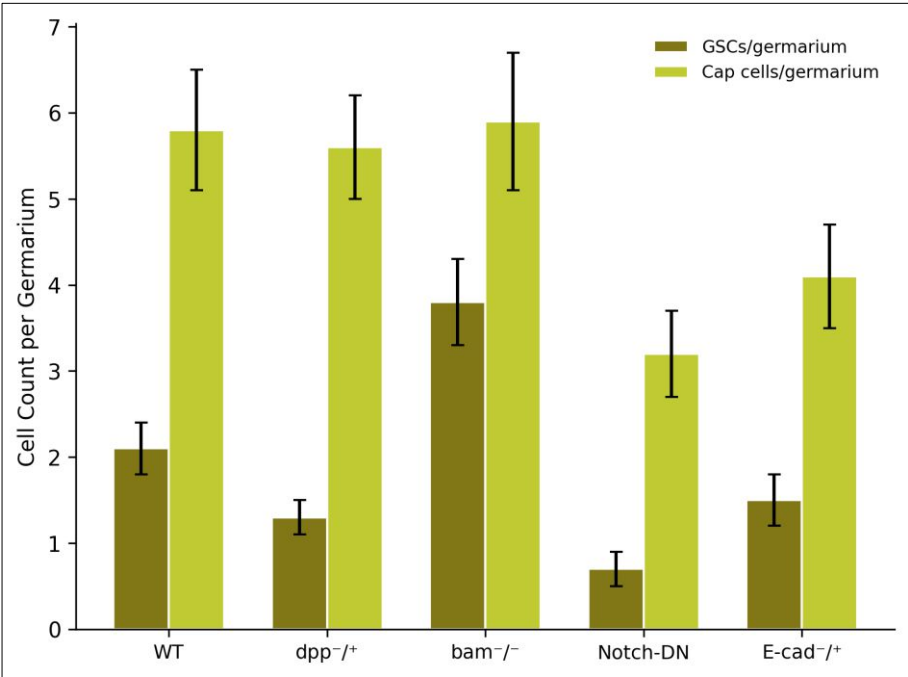


Fig 1: GSC and cap cell counts per germarium across five genotypes. Notch-DN severely reduced both, while dpp heterozygotes selectively lost GSCs without cap cell decline

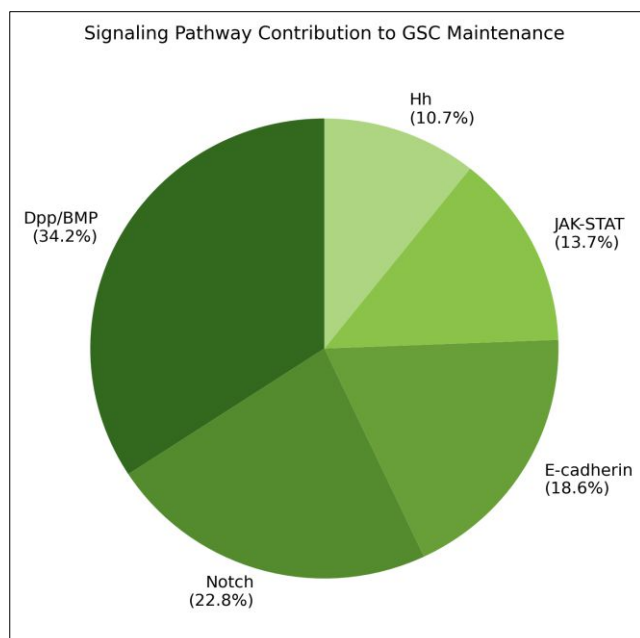


Fig 2: Estimated contribution of five signaling pathways to GSC maintenance, calculated from normalised excess loss rates above the wild-type baseline

Discussion

The hierarchical model emerging from these data positions Dpp/BMP as the primary maintenance signal (34.2% of GSC retention capacity), consistent with three decades of work showing that ectopic Dpp can expand GSC populations throughout the ovary^[9, 10]. Notch's contribution (22.8%) operates mainly through niche preservation—maintaining cap cell numbers at ~6 per germarium. When Notch is compromised, cap cells are lost, and the remaining ones cannot support as many GSCs^[11]. E-cadherin (18.6%) provides the physical anchoring force; without it, GSCs detach and differentiate even when BMP signaling is intact^[12].

JAK-STAT and Hh contributions were smaller (13.7% and 10.7%) and likely reflect roles in escort cell behaviour and transition zone regulation rather than direct GSC maintenance^[13, 14]. These estimates should be treated as approximations because pathway interactions (e.g., BMP signaling enhances E-cadherin expression) mean that contributions are not strictly additive.

Conclusion

This quantitative dissection of the *Drosophila* ovarian GSC niche reveals a hierarchical signaling architecture: Dpp/BMP provides the dominant maintenance cue, Notch preserves niche size, E-cadherin anchors GSCs physically, and JAK-STAT and Hh fine-tune niche boundaries. The relative contributions—roughly 34:23:19:14:11—offer a framework for comparing niche function across tissues and organisms. Whether mammalian stem cell niches follow a similar proportional architecture remains an open and testable question^[15-17].

References

1. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells*. 1978;4(1-2):7-25.

2. Xie T, Spradling AC. A niche maintaining germ line stem cells in the *Drosophila* ovary. *Science*. 2000;290(5490):328-330.
3. Song X, Zhu CH, Doan C, Xie T. Germline stem cells anchored by adherens junctions in the *Drosophila* ovary niches. *Science*. 2002;296(5574):1855-1857.
4. Chen D, McKearin D. Gene circuitry controlling a stem cell niche. *Current Biology*. 2005;15(2):179-184.
5. Ward EJ, Shcherbata HR, Reynolds SH, Fischer KA, Hatfield SD, Bhatt HA. Stem cells signal to the niche through the Notch pathway in the *Drosophila* ovary. *Current Biology*. 2006;16(23):2352-2358.
6. Vied C, Reilein A, Field NS, Bhatt HA. Regulation of stem cells by intersecting gradients of short-range niche signals. *Developmental Cell*. 2012;23(3):444-455.
7. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell*. 2008;132(4):598-611.
8. Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development*. 1993;117(4):1223-1237.
9. Xie T, Spradling AC. decapentaplegic is essential for the maintenance and division of germline stem cells in the *Drosophila* ovary. *Cell*. 1998;94(2):251-260.
10. Kai T, Spradling A. An empty *Drosophila* stem cell niche reactivates the proliferation of ectopic cells. *Proceedings of the National Academy of Sciences*. 2003;100(8):4633-4638.
11. Song X, Call GB, Kirilly D, Xie T. Notch signaling controls germline stem cell niche formation in the *Drosophila* ovary. *Development*. 2007;134(6):1071-1080.
12. Jin Z, Kirilly D, Weng C, Kawase E, Song X, Smith S, *et al*. Differentiation-defective stem cells outcompete normal stem cells for niche occupancy in the *Drosophila* ovary. *Cell Stem Cell*. 2008;2(1):39-49.
13. Decotto E, Spradling AC. The *Drosophila* ovarian and testis stem cell niches: similar somatic stem cells and signals. *Developmental Cell*. 2005;9(4):501-510.
14. Rojas-Rios P, Guerrero I, Gonzalez-Reyes A. Cytoneme-mediated delivery of hedgehog regulates the expression of bone morphogenetic proteins to maintain germline stem cells in *Drosophila*. *PLoS Biology*. 2012;10(4):e1001298.
15. Li L, Xie T. Stem cell niche: structure and function. *Annual Review of Cell and Developmental Biology*. 2005;21:605-631.
16. Fuller MT, Spradling AC. Male and female *Drosophila* germline stem cells: two versions of immortality. *Science*. 2007;316(5823):402-404.
17. Losick VP, Morris LX, Fox DT, Bhatt A. *Drosophila* stem cell niches: a decade of discovery suggests a unified view of stem cell regulation. *Developmental Cell*. 2011;21(1):159-171.