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# Insulin signals control the competence of the *Drosophila* female germline stem cell niche to respond to Notch ligands

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ABSTRACT

Adult stem cells reside in specialized microenvironments, or niches, that are essential for their function *in vivo*. Stem cells are physically attached to the niche, which provides secreted factors that promote their self-renewal and proliferation. Despite intense research on the role of the niche in regulating stem cell function, much less is known about how the niche itself is controlled. We previously showed that insulin signals directly stimulate germline stem cell (GSC) division and indirectly promote GSC maintenance via the niche in *Drosophila*. Insulin-like peptides are required for maintenance of cap cells (a major component of the niche) via modulation of Notch signaling, and they also control attachment of GSCs to cap cells and E-cadherin levels at the cap cell–GSC junction. Here, we further dissect the molecular and cellular mechanisms underlying these processes. We show that insulin and Notch ligands directly stimulate cap cells to maintain their numbers and indirectly promote GSC maintenance. We also report that insulin signaling, via phosphoinositide 3-kinase and FOXO, intrinsically controls the competence of cap cells to respond to Notch ligands and thereby be maintained. Contrary to a previous report, we also find that Notch ligands originated in GSCs are not required either for Notch activation in the GSC niche, or for cap cell or GSC maintenance. Instead, the niche itself produces ligands that activate Notch signaling within cap cells, promoting stability of the GSC niche. Finally, insulin signals control cap cell–GSC attachment independently of their role in Notch signaling. These results are potentially relevant to many systems in which Notch signaling modulates stem cells and demonstrate that complex interactions between local and systemic signals are required for proper stem cell niche function.


## Introduction

The microenvironment (niche) where stem cells reside provides physical contact and local signals to retain and modulate stem cells. Systemic factors that vary with physiological changes also influence stem cells either directly or by altering the niche (Drummond-Barbosa, 2008). It is largely unknown, however, how systemic factors interact with local signals to maintain the niche.

The *Drosophila* female germline stem cell (GSC) niche, located in the anterior germarium of each ovariole, is well described. The GSC niche is composed of cap cells, terminal filament cells, and escort cells (Kirilly and Xie, 2007). Cap cells are major cellular components of the niche, as they are directly attached to GSCs through E-cadherin (Song et al., 2002) (Fig. 1A). GSCs self-renew and produce cystoblasts that divide to form 16-cell cysts (Kirilly and Xie, 2007). One cell becomes the oocyte, the others become nurse cells, and follicle cells surround the cyst to generate a developing egg chamber (Spradling, 1993).

GSC number correlates with cap cell number (Hsu and Drummond-Barbosa, 2009; Xie and Spradling, 2000), which in turn is regulated by Notch signaling (Song et al., 2007; Ward et al., 2006). The Notch receptor and its ligands are transmembrane proteins, thus requiring cell–cell contact for signaling. *Drosophila* has one Notch receptor (encoded by *N*) and two ligands, Delta and Serrate (encoded by *Dl* and *Ser*, respectively), and full ligand activity requires the E3 ubiquitin ligase Neuralized (encoded by *neur*) in signal-producing cells (Fiuza and Arias, 2007). Ligand stimulation induces proteolytic cleavage of Notch and translocation of its intracellular domain into the nucleus, where it regulates gene expression (Fiuza and Arias, 2007). Notch inactivation leads to cap cell and GSC loss (Song et al., 2007), whereas overexpression of Delta in the germline or of actived Notch in somatic cells of the germarium has the opposite effect (Song et al., 2007; Ward et al., 2006). A report that GSCs mutant for *neur*, *Dl*, and *Dl Ser* are lost from the niche led to the model that Notch ligands produced in GSCs signal to cap cells to maintain their own niche (Ward et al., 2006). It remained experimentally untested, however, whether Notch ligands produced

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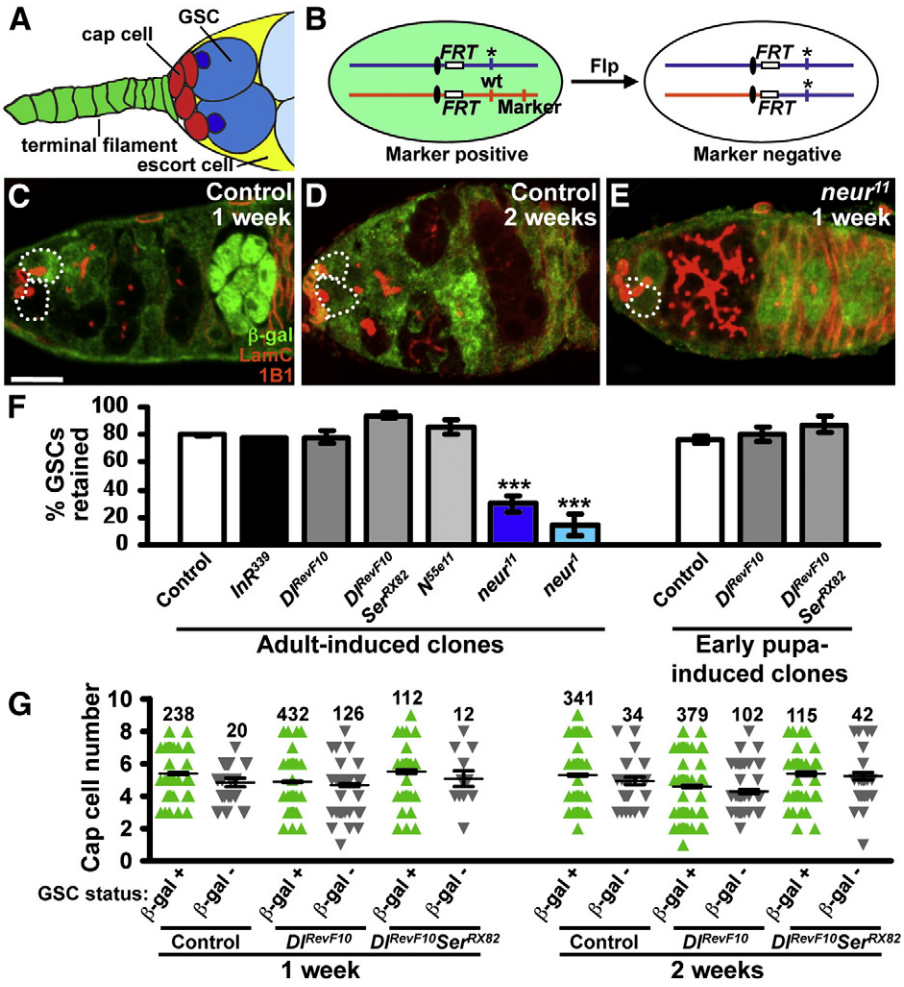


**Fig. 1.** Notch ligands from GSCs do not control cap cell number or GSC maintenance. (A) *Drosophila* female GSC niche. The GSC niche comprises terminal filament (green), cap cells (red), and a subset of escort cells (yellow). The morphology and position of the fusome (dark blue), a membranous structure present in early germ cells, allow the identification of GSCs (medium blue) versus their progeny (light blue). (B) *FLP/FRT* system. In females carrying a wild-type allele (wt) linked to a marker gene *in trans* to a mutant allele (*), FLP-mediated recombination between *FRT* sites during mitotic division generates a homozygous mutant cell recognized by the absence of marker expression. (C–E) Mosaic germaria labeled with β-gal (green, wild-type cells), 1B1 (red, fusomes), and LamC (red, cap cell nuclear envelopes). GSCs are outlined. Scale bar, 10 μm. One-week-old control mosaic germarium (C) shows a β-gal-negative GSC and its progeny. Two-week-old mosaic germarium (D) shows β-gal-negative progeny, but the β-gal-negative GSC has been lost. One-week-old *neur*$^{11}$ mosaic germarium (E) shows a large β-gal-negative cyst displaying an abnormal, highly branched fusome, and the β-gal-negative GSC is absent. (F) Percentage of mosaic germaria retaining β-gal-negative GSCs at 2 weeks after clone induction (for adult-induced clones) or eclosion (for early-pupa-induced clones) relative to 1-week time point. (G) Cap cell number in individual mosaic germaria carrying all β-gal-positive (β-gal +) or -negative (β-gal−) GSCs 1 or 2 weeks after clone induction. The number of analyzed germaria is shown above each bar. Error bars, mean ± SEM, ***$P<0.001$.

in GSCs control cap cell number, or whether Notch activation is cell autonomously (i.e., intrinsically) required for cap cell maintenance.

Insulin signaling ties diet to function of the GSC niche, at least in part via modulation of Notch signaling (Hsu and Drummond-Barbosa, 2009). The evolutionarily conserved insulin/insulin-like growth factor (IGF) pathway controls processes linked to nutrient sensing (Goberdhan and Wilson, 2003; Hafen, 2004). Insulin-like peptides activate the *Drosophila* insulin receptor (encoded by *InR*), leading to phosphorylation of the insulin receptor substrate (encoded by *chico*). Subsequent phosphoinositide 3-kinase (PI3K) stimulation leads to cytoplasmic retention of the transcriptional factor FOXO, thus preventing target gene activation (Oldham and Hafen, 2003). We previously showed that systemic insulin-like peptides promote both the maintenance of cap cells, via positive regulation of Notch signaling, and cap cell–GSC attachment, likely via E-cadherin (Hsu and Drummond-Barbosa, 2009). It remained unclear, however, how insulin and Notch signaling interact to control niche size, or if Notch modulates E-cadherin.

Here, we show that the insulin pathway and Notch signaling are both intrinsically required to maintain cap cell numbers. Notch ligands produced in GSCs are not required to activate Notch or to maintain cap cells or GSCs. Instead, ligands are produced within the niche itself to stimulate Notch signaling in cap cells. Further, our results demonstrate that insulin-like peptides, acting via PI3K and FOXO, directly control the competence of cap cells to respond to Notch ligands. Finally, Notch does not control cap cell–GSC attachment, indicating that this is a Notch-independent role of insulin signaling. These results connect systemic factors to the competence of niche cells to receive local signals and highlight the complexity of systemic effects on the function of niches and their stem cells.

## Materials and methods

### *Drosophila strains and culture*

*Drosophila* stocks were maintained at 22–25 °C on standard medium. *yw* is a wild-type control. Null *InR*$^{339}$, *Dl*$^{RevF10}$, *Ser*$^{RX82}$, *neur*$^{1}$, *neur*$^{11}$, *chico*$^{1}$, *foxo*$^{21}$, and *foxo*$^{25}$ alleles; hypomorphic *N*$^{55e11}$ and *InR*$^{E19}$ alleles; and the temperature-sensitive *N*$^{ts2}$ allele have been described (Hsu et al., 2008; Shellenbarger and Mohler, 1975; Wang et al., 2007; Ward et al., 2006). *Dl-lacZ*, *Ser-lacZ*, *E(spl)m7-lacZ*, *c587-Gal4*, *bab1-Gal4*, *UAS-Dp110*, and *UAS-Dp110*$^{CAAX}$ have been described (Bachmann and Knust, 1998; Grossniklaus et al., 1989; Bolivar et al., 2006; Hsu and