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Regulation of Stem Cells by Intersecting Gradients of Long-Range Niche Signals

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SUMMARY

We have used *Drosophila* ovarian follicle stem cells (FSCs) to study how stem cells are regulated by external signals and draw three main conclusions. First, the spatial definition of supportive niche positions for FSCs depends on gradients of Hh and JAK-STAT pathway ligands, which emanate from opposite, distant sites. FSC position may be further refined by a preference for low-level Wnt signaling. Second, hyperactivity of supportive signaling pathways can compensate for the absence of the otherwise essential adhesion molecule, DE-cadherin, suggesting a close regulatory connection between niche adhesion and niche signals. Third, FSC behavior is determined largely by summing the inputs of multiple signaling pathways of unequal potencies. Altogether, our findings indicate that a stem cell niche need not be defined by short-range signals and invariant cell contacts; rather, for FSCs, the intersection of gradients of long-range niche signals regulates the longevity, position, number, and competitive behavior of stem cells.

INTRODUCTION

Here we investigate *Drosophila* ovarian follicle stem cells (FSCs), which serve as a model for stem cells that support epithelia requiring continuous regeneration (Margolis and Spradling, 1995; Nystul and Spradling, 2007, 2010). Importantly, more than one FSC is present within each insulated developmental unit. This arrangement allows ready replacement of one FSC lineage by another and competition for supportive niche positions, with two major consequences. First, a healthy FSC population can be maintained beyond the lifetime of a single stem cell. Second, somatic mutations that enhance FSC duplication or niche affinity might allow that FSC and its descendants to outcompete neighboring FSCs and thus take over the tissue, as in early steps in cancer. Both phenomena are likely relevant to human epithelial stem cells.

FSCs and germline stem cells (GSCs) reside in the germarium (Figure 1A), which is the most anterior structure of each of the roughly 30 ovarioles of an adult female. These stem cells support continuous egg production throughout the lifetime of well-fed flies. FSCs are maintained in their niche by cadherin- and integrin-mediated adhesive interactions while producing daughters that escape the niche and surround passing germline cysts (O'Reilly et al., 2008; Song and Xie, 2002). Most FSC daughters form an epithelium around the germline cells of growing egg chambers and divide roughly eight times before differentiating into a variety of specialized follicle cell types. A minority of FSC daughters arrest cell division earlier and either form polar cells at the anterior and posterior poles of developing egg chambers or stalk cells that separate egg chambers (Dobens and Raftery, 2000; Margolis and Spradling, 1995; Nystul and Spradling, 2010).

GSCs in the *Drosophila* ovary have been studied more thoroughly than FSCs and have substantially molded popular perceptions of archetypal stem cell biology. Physical constraints limit the number of GSCs that can contact a small population of differentiated niche cells known as Cap cells (Figure 1A), which deliver a bone morphogenetic protein (BMP) signal locally to maintain GSCs in an undifferentiated state (Chen et al., 2011; Losick et al., 2011). FSC biology may be guided by quite different principles in several respects. First, FSC location is not simply defined as being adjacent to a single differentiated cell type (Nystul and Spradling, 2010). Second, FSCs respond to a far greater spectrum of extracellular signals than do GSCs (Kirilly et al., 2005; Song and Xie, 2003; Zhang and Kalderon, 2001). Third, FSC daughters only differentiate overtly after several rounds of proliferation.

FSCs are found at the beginning of germarial region 2b (Figure 1A), where germline cysts first span the whole width of the germarium as an elongated disc (Nystul and Spradling, 2007, 2010). Hedgehog (Hh) and Wingless (Wg) ligands, which are required for FSC maintenance, derive from distant terminal filament and Cap cells at the extreme anterior of the germarium (Figure 1A; Forbes et al., 1996b; Song and Xie, 2003; Zhang and Kalderon, 2001). FSC maintenance also requires activity of *Drosophila* epithelial (DE)-cadherin and integrin in the FSC (O'Reilly et al., 2008; Song and Xie, 2002). Escort cells are thought to be the critical FSC partners for homotypic cadherin-mediated interactions and are present throughout the germarium anterior to the FSCs (Decotto and Spradling, 2005; Song and Xie, 2002). A basement membrane lines the entire germarial wall and is seeded with a critical integrin ligand by the FSC lineage itself (O'Reilly et al., 2008). From knowledge of these factors alone we might expect the entire anterior half of the germarium to anchor and support FSCs with a preference for the most extreme

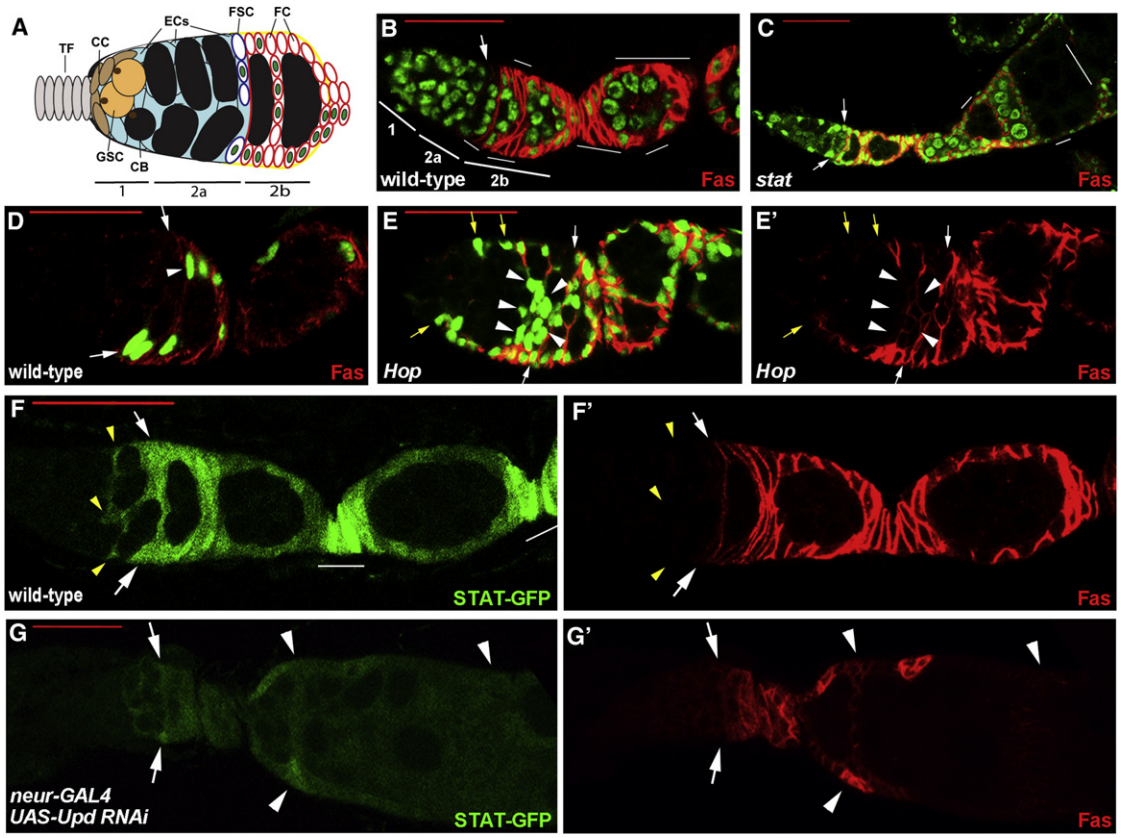

Figure 1. FSC Responses to JAK-STAT Pathway Activity

(A) Germarium cartoon showing differentiated Terminal Filament (TF) and Cap Cells (CC), which maintain Germline Stem Cells (GSCs) and express Hh and Wg strongly. Cystoblasts (CB) and subsequent, 2, 4, 8, and 16 cell germline cysts are in black, surrounded by somatic (blue) Escort Cells (ECs). A typical single marked Follicle Stem Cell (FSC) lineage is indicated by green nuclei, including FSC daughters that move laterally (up-down) or to the posterior (right). Most FSC daughters continue to proliferate as prefollicle cells (FC) that express Fas3 (denoted by red outlines).

(B and C) FSC clones negatively marked by the loss of GFP, showing (B) a wild-type FSC (arrow) and its derivatives (no green GFP; thin white lines) and (C) a *Dstat92E* mutant FSC clone where the mutant FSC has been lost and replaced by GFP-positive FSCs (arrows), while some GFP-negative FSC derivatives are still detected in later egg chambers (white lines). FSCs (arrows) are the most anterior (left) cells in their lineage, contact the germarial walls and do not express the surface protein Fas3 (red). In (C) there are some "fused" egg chambers, as expected from the requirement for JAK-STAT pathway activity to specify stalk cells.

(D and E) FSC clones positively marked by GFP (green) and stained with antibody to Fas3 (red) for (D) a wild-type FSC and (E) an FSC expressing excess Hop (E′ shows Fas3 staining only). Excess JAK-STAT signaling produced extra marked cells anterior to normal FSC positions (white arrows). The most anterior ectopic GFP-positive cells did not express Fas3 (yellow arrows) but Fas3 was expressed in most ectopic anterior cells, whether lining the germarial walls (no arrows) or at internal positions (arrowheads).

(F and G) JAK-STAT pathway activity reported by *10xSTAT-GFP* (green) in ovarioles costained for (F′ and G′) Fas3 (red). (F) In wild-type ovarioles the JAK-STAT pathway reporter was active in FSCs (arrows), their posterior derivatives (no arrows) and in posterior Escort Cells (yellow arrowheads). (G) In ovarioles where *UAS-upd RNAi* was driven by *neur-GAL4* in polar cells JAK-STAT reporter activity was greatly reduced in the entire germarium, including FSCs (arrows) and in egg chambers, which were sometimes fused (arrowheads) with no intervening stalks.

Red scale bars represent 25 μm. See also Figure S1.

anterior positions close to Hh and Wg sources. Hence, a key question is whether additional signals or localized adhesive interactions define FSC position more precisely or control the number of FSCs supported in a germarium. Here we show that the JAK-STAT pathway is also a key regulator of FSCs, and we examine how multiple signaling pathways collaborate to regulate FSC position and competitive behavior.

RESULTS

JAK-STAT Pathway Activity Is Critical for FSC Function

JAK-STAT pathway activity is required directly in both germline and somatic "cyst" stem cells in the *Drosophila* testis for each type of stem cell to be maintained in its normal niche position (de Cuevas and Matunis, 2011). In *Drosophila* ovaries JAK-STAT signaling regulates GSC function indirectly by activating expression of a key GSC factor (Decapentaplegic; Dpp) in GSC-niche cells (Cap cells) (Losick et al., 2011). JAK-STAT requirements for FSC function have not been reported. The JAK-STAT pathway in *Drosophila* involves three Unpaired (Upd) family ligands, a receptor (Domeless), a single Janus Kinase, Hopscotch (Hop), and a single STAT (DStat92E), which is converted to a nuclear transcriptional activator by ligand-induced JAK phosphorylation (Arbouzova and Zeidler, 2006). We tested autonomous requirements for JAK and STAT components by creating mutant FSC clones marked by the loss of green