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Report

Slit/Robo Signaling Regulates Cell Fate Decisions in the Intestinal Stem Cell Lineage of *Drosophila*

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SUMMARY

In order to maintain tissue homeostasis, cell fate decisions within stem cell lineages have to respond to the needs of the tissue. This coordination of lineage choices with regenerative demand remains poorly characterized. Here, we identify a signal from enteroendocrine cells (EEs) that controls lineage specification in the *Drosophila* intestine. We find that EEs secrete Slit, a ligand for the Robo2 receptor in intestinal stem cells (ISCs) that limits ISC commitment to the endocrine lineage, establishing negative feedback control of EE regeneration. Furthermore, we show that this lineage decision is made within ISCs and requires induction of the transcription factor Prospero in ISCs. Our work identifies a function for the conserved Slit/Robo pathway in the regulation of adult stem cells, establishing negative feedback control of ISC lineage specification as a critical strategy to preserve tissue homeostasis. Our results further amend the current understanding of cell fate commitment within the *Drosophila* ISC lineage.

INTRODUCTION

Whereas determinants of lineage specification in several somatic stem cell lineages of vertebrate model systems have been identified (Beck and Blanpain, 2012; Rock and Hogan, 2011; Yeung et al., 2011), little is known about how tissue needs are monitored and information about specific missing cell types is relayed to stem cells. The *Drosophila* posterior midgut has emerged as a powerful genetically tractable system for the characterization of stem cell function and the control of epithelial homeostasis, serving as an ideal model for the identification of such signaling interactions (Biteau et al., 2011; Casali and Batlle, 2009; Jiang and Edgar, 2012; Wang and Hou, 2010). Intestinal stem cells (ISCs) can regenerate all cell types of the intestinal epithelium, producing, through asymmetric and symmetric divisions, precursor cells (such as enteroblasts [EBs]) that differentiate into either enterocytes (ECs) or enteroendocrine cells (EEs) (de Navascués et al., 2012; Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006, 2007).

Homeostasis of the intestinal epithelium is maintained both by cell-autonomous control of proliferation and differentiation in the ISC lineage as well as by cell-cell interactions. One example is the induction of ISC proliferation by damaged ECs, a mechanism that allows regenerating new ECs as needed (Amcheslavsky et al., 2009; Buchon et al., 2009; Chatterjee and Ip, 2009; Cronin et al., 2009; Jiang et al., 2009). So far, it remained unclear if EEs have a similar ability to control the regeneration of their own lineage. The balance between EC and EE differentiation is influenced by Notch signaling. High expression of the Delta ligand in ISCs activates Notch in EBs, promoting EC differentiation. Low-Delta-expressing ISCs, on the other hand, promote the differentiation of their daughter cells into EEs (Ohlstein and Spradling, 2007). However, the signals that control ISC cell fate decisions or regulate the level of Delta expression in ISCs have not been identified to date.

Here, we report the identification of Slit/Robo2 signaling as a critical regulator of the balance between the EE and EC lineages. We show that the Slit ligand is expressed in EEs, establishing a retrograde signal that controls cell fate decisions in ISCs. Our results suggest that Robo2 regulates lineage specification by inhibiting the expression of the transcription factor Prospero in ISCs prior to cell division, acting upstream of the establishment of differential Notch signaling.

RESULTS AND DISCUSSION

Slit/Robo Signaling between Enteroendocrine and Progenitor Cells in the Posterior Midgut

In a screen for new signaling molecules involved in the regulation of tissue homeostasis in the posterior midgut, we identified the secreted ligand Slit as a factor specifically expressed in EEs. Using a LacZ-expressing reporter line inserted in the *slit* locus ($Slit^{PZ05248}$), we found that the Slit promoter is active in a subset of cells in the intestinal epithelium (Figure S1A) and that these cells represent Prospero-positive EEs (Figure 1A), but not small esg-positive ISCs/EBs or polyploid ECs (Figure 1B). Immunocytochemistry confirmed that high levels of Slit protein are present in the cytoplasm of Prospero-positive and esg-negative diploid EEs (Figures 1C, 1D, and S1B). Interestingly, the Slit protein can also be detected on escargot-positive ISCs and EBs (Figures 1D and S1C), suggesting that this secreted molecule diffuses from EEs to these progenitors.

In *Drosophila*, three Roundabout receptors (Robo1, Robo2/leak, and Robo3) have been shown to transduce the Slit signal

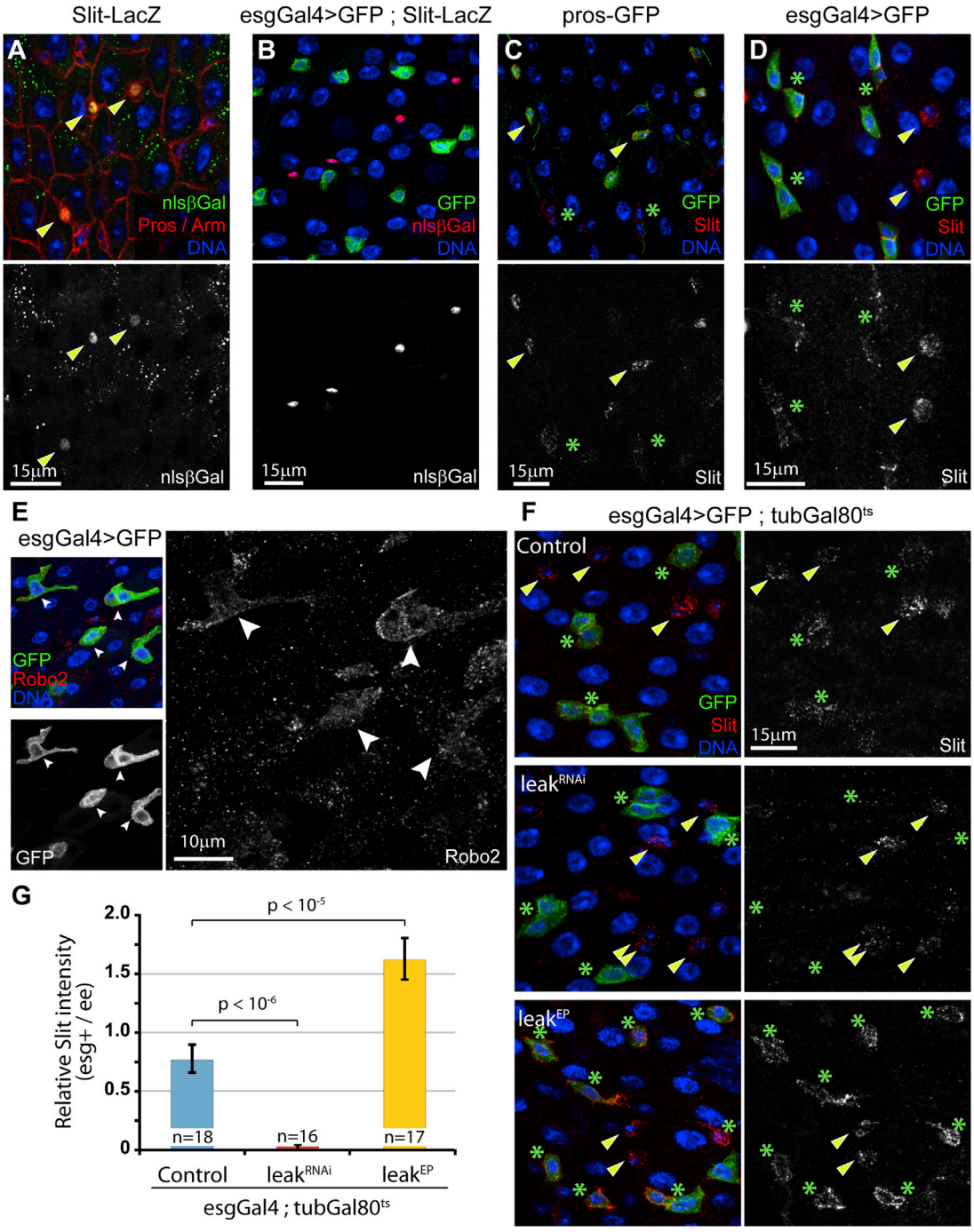

Figure 1. Slit/Robo2 Signaling between Enteroendocrine and Stem/Progenitor Cells in the Posterior Midgut

(A and B) The Slit promoter is active in EEs, as shown by the detection of β-galactosidase in Prospero-positive cells, using the Slit^{PZ05248}-LacZ reporter line (A; arrowheads), and inactive in escargot-positive progenitors and polyploid enterocytes (B).

(C and D) The Slit protein is detected in the cytoplasm of Prospero-GFP-positive cells (C; arrowheads; see Figure S2B for the characterization of the pros-GFP reporter) and at the periphery of escargot-positive cells (D; asterisks).

(E) The Robo2 receptor is expressed in esg-positive cells, as shown by immunocytochemistry using a Robo2-specific antibody.

(F) Knockdown of lea/Robo2 in esg-positive cells is sufficient to abolish the accumulation of Slit at the surface of these cells (asterisks) without affecting Slit expression in EEs (arrowheads). Overexpressing Robo2, using the lea^{EP} line, increases the signal at the periphery of ISCs.

(G) Quantification of Slit immunostaining intensity in esg-positive ISCs/EBs compared to esg-negative diploid EEs in similar conditions as Figure 1F.

n represents the number of pairs of diploid cells (one esg-positive and one esg-negative) that were analyzed. p value from two-tailed Student's t test. Values are presented as average ± SEM. See also Figure S1.