Supporting Online Material for

Multipotent Drosophila Intestinal Stem Cells Specify Daughter Cell Fates by Differential Notch Signaling

Benjamin Ohlstein and Allan Spradling*

*To whom correspondence should be addressed. E-mail: spradling@ciwemb.edu

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Materials and Methods

Drosophila stocks

Information on Drosophila genes and stocks is available from Flybase (http://flybase.bio.indiana.edu). A y w stock was used as wild type. All Drosophila strains were cultured with daily changes of wet yeast paste at 21-23 °C. Flies expressing the Notch activation reporter Gbe SuHlacZ-4 have been described previously (S1).

Clonal analysis

GFP-marked ISC clones were generated as previously described (S2). Mutants were analyzed using a positive marking (MARCM) system (S3). Samples of flies were analyzed at various times after heat-shock by staining dissected guts with antibody combinations as described in the text.

Immunostaining and fluorescence microscopy

Guts were prepared for immunostaining by immediately fixing in 100 mM glutamic acid, 25 mM KCl, 20 mM MgSO4, 4 mM Sodium Phosphate, 1 mM MgCl2, 4% EM-grade formaldehyde (TedPella) for 30 min. Subsequent rinses, washes and incubations with primary and secondary antibodies were done in 1X PBS, 0.5% BSA, 0.3% TritonX-100. Midguts stained for Delta were prepared and processed as described above except that individual midguts were dissected and placed into separate wells of a 48-well tissue culture plate containing 0.2 ml of fixative for 30 min. Subsequent steps were carried in the same wells using 0.2 ml of the appropriate solutions. The following anti-sera were used: anti-Delta (1:100), anti-Prospero (1:100), anti-Armadillo (1:20), and anti-Notch-intracellular (1:100) (Developmental Studies Hybridoma Bank); anti-mouse or anti- rabbit phosphohistone H3 (1:2000)(Cellsignal.com), rabbit anti-GFP (1:5000) (Torrey Pines), rat anti-alpha tubulin (1:50) (immunologicalsdirect.com), mouse anti-gamma tubulin (1:500) (Sigma), rabbit anti-Anillin (1:1000) a gift of Chris Field, rabbit polyclonal anti-
β Gal (pre-absorbed against fixed midguts and used at 1: 3000) (Cappel), mouse monoclonal anti-β Gal (1: 400) (Promega). Secondary antibodies were used at 1:2000 and are as follows: goat anti-mouse and goat anti-rabbit IgG conjugated to either of Alexa 488 or Alexa 568 (Molecular Probes). Also goat anti-mouse IgG conjugated to Alexa 633 (Molecular Probes) (highly absorbed) was used in combination with goat anti-rat conjugated to Rhodamine (Jackson Labs)(minimal cross reactive). DAPI (Sigma) was used at 1 µg/ml. Images were taken by a Zeiss Axioimager Z1 equipped with an Apotome system. Three-dimensional reconstructions were generated using Zeiss software.

**Calculation of mitotic angles**

ISCs undergoing mitosis were identified by positive staining of the mitotic marker phospho-histone H3 (PH3) and the centrosome marker gamma-tubulin. Images of metaphase and anaphase chromosomes were taken by a Zeiss Axioimager Z1 equipped with an Apoptome system and three-dimensional reconstructions were rotated 90 degrees. The angle of division was determined by the orientation of the two centrosomes relative to the plane of the slide. Under our mounting conditions, controls showed that this yielded the same answer as calculating the angle relative to the basement membrane marker Viking.

**Analysis of Notch signaling**

The following chromosomes were used to generate MARCM clones. FRT82B Δelta<sup>rev10</sup>, FRT82B Serrate<sup>Rx106</sup>, and FRT40A Su(H)<sup>047</sup> (kind gifts of H. Ruhoula-Baker); FRT19A Notch<sup>55e11</sup> (a kind gift of Ken Irvine), FRT82B neuralized<sup>11</sup> (a kind gift of Steve DiNardo), UAS-Nact<sup>1768</sup> (a kind gift of Ilaria Rebay).

**SOM References**


**Figure S1. Models of ISC multipotency and daughter specification.**

Model of ISC multipotency, showing that ISCs cycle between states where Dl is high and low in response to postulated signals from surrounding tissue cells. Asymmetric Delta-Notch signaling programs enterocyte differentiation when Delta levels and Notch signaling is high, and enteroendocrine differentiation when Delta levels and Notch signaling is low. **B.** Diagrammatic representation of a stromal cell niche such as the niche regulating Drosophila female germline stem cells. Niche stromal cells (blue) form junctions (black boxes) with the stem cell (yellow), and produce a local signal (red arrow). The signal maintains the stem cell, while its loss programs daughter cell fate (pink). **C.** Diagrammatic representation of the ISC niche. The stem cell (yellow) resides adjacent to the basement membrane (ECM, black line), and sends a signal of variable strength (red arrow) that programs the fate of its daughter cell (pink).