Supplemental Data

Control of Compartment Size by an EGF Ligand from Neighboring Cells

Joseph Parker

Supplemental Experimental Procedures

Fly Stocks

Flies were maintained on a standard medium at low densities to prevent body size affecting egg size. For embryonic P compartment-specific overexpression, flies possessing the *engrailed-GAL4* driver recombined to *UAS-CD8-GFP* were crossed to flies carrying the following transgenes: *UAS-cyclin E* (C. Lehner), *UAS-dacapo* (I. Hariharan), *UAS-p35* (B. Hay), *UAS-dmyc* (L. Johnston), *UAS-arm.s10*, *UAS-EGFR** (*UAS-tor^{4021}-EGFR*), *UAS-ras** (*UAS-Ras85D.V12*), *UAS-spi^{s}* (*UAS-spi.s*), *UAS-EGFR^{dn}* (*UAS-Egfr.DN*), *UAS-ras^{dn}* (*UAS-Ras85D.N17*), and *UAS-aos* (*UAS-gil.H*). The lines *rho^{x81}*, *ru^{1} rho^{7m43}*, *Df(3L)XR38* (L. Johnston), *hid^{05014}*, *spi^{1}*, and *vn$^{\Delta P25}$* were also used. All stocks are described in Flybase (http://flybase.bio.indiana.edu/).

Immunochemistry, TUNEL Labeling, and Quantification

Embryos were fixed in 4% formaldehyde and incubated overnight at 4°C with primary antibodies rabbit anti-GFP (1:200; Molecular Probes), rabbit anti-PH3 (Phosphohistone H3, 1:250; Upstate Biotech), mouse anti-Engrailed (1:100; JP Vincent, NIMR), mouse anti-Wingless (1:100; DSHB), mouse anti-dMyc (undiluted; L. Johnston), and mouse anti-β-galactosidase (1:100; DSHB) and then for 2 hr at room temperature with Cyanine-conjugated secondaries (Jackson Immunoresearch). For TUNEL staining, antibody-labeled embryos were permeabilized at 70°C in sodium citrate and then subjected to a 3 hr TUNEL reaction at 37°C, with the Tetramethylrhodamine red In Situ Cell Death Detection Kit (Roche). Embryos were viewed with a Bio-Rad Radiance 2100 confocal microscope. For quantification of apoptosis, the numbers of TUNEL-positive nuclei in the region dorsal to the oenocytes of P compartments of abdominal segments 2–5 (A2–A5) were scored, during late embryonic stage 12 (>50% germ-band retraction) and stage 13—the window in which most embryonic apoptosis was observed to occur. The region of ectoderm studied differentiates directly into dorsal epidermis. The average number of dying cells per P compartment was calculated from the total number of compartments: all embryos and all compartments pooled. In *ru^{1} rho^{7m43}* embryos, readily distinguishable oenocytes were missing, so apoptotic cells were quantified in a region extending from the dorsal leading edge to approximately 1/3 the length of the dorso-ventral axis.

Larval Cuticle Preparations and Measurements

Unhatched first-instar larvae were dechorionated in 50% bleach, manually devitellinized, and rotated in 0.05% Triton-X in double-distilled water overnight in the dark until the cuticle had expanded sufficiently so that segmental grooves were flattened out. They were then fixed for 1 hr in 4% formaldehyde. Images of the dorsal epidermis of genotypes expressing CD8-GFP in P compartments were taken with the confocal microscope with a 40× objective. Images were input into Adobe Photoshop CS, and a template of fixed width (310 pixels) was placed along the dorsal midline. The template spans the region that differentiates from that analyzed in the embryo. A and P compartment sizes of abdominal segments A2–A4 were measured within this template region (A2–A5 did not fit into a single 40× image). CD8-GFP localizes to plasma membranes, enabling estimation of P compartment cell numbers; however, loss of GFP occurs after some time, so only preps in which marked cells and compartments were evident were measured. Cell size = compartment size/cell number. Data from A or P compartments of A2–A4 were pooled for each experiment.

FACS Analysis

Between 100 and 300 appropriately staged embryos were partially Dounce homogenized in Trypsin-EDTA (Sigma) for initial fragmentation and left for 1 hr in Trypsin-EDTA and Hoechst (Molecular Probes). The resulting cell suspension was analyzed in a MoFlow flow cytometer (Cytomation) with an ultraviolet lamp and a 488 nm laser. Flow-cytometry data were analyzed with FCSPress 1.3a and 1.4.

Table S1. Apoptosis in the Posterior Compartment

Genotype	TUNEL-Positive Nuclei (mean ± SE)				
(*en>CD8-GFP*) +	Stage 12	*n*	Stage 13	*n*	F:B
- *(wild type)*	0.6 ± 1.5	16	0.7 ± 0.8	40	38
UAS-cycE	2.8 ± 2.9 ***	48	3.7 ± 3.1 ***	80	11
UAS-dap	0.0 ± 0.2 *	40	0.2 ± 0.5 ***	40	3
UAS-cycE + UAS-p35	0.0 ± 0.0 (***)	40	0.2 ± 0.5 (***)	40	∞
UAS-cycE; hid^{05014}/+	0.6 ± 0.9 (***)	48	1.7 ± 2.0 (***)	68	10
UAS-cycE; Df(3L)XR38/+	1.2 ± 1.8 (**)	40	1.4 ± 1.5 (**)	36	5.1
UAS-cycE + UAS-arm^{s10}	2.4 ± 2.6 (NS)	40	2.1 ± 2.4 (**)	36	8.5
*UAS-cycE + UAS-EGFR**	0.2 ± 0.5 (***)	40	0.1 ± 0.4 (***)	40	5.5
*UAS-cycE + UAS-ras**	0.6 ± 1.2 (***)	44	0.2 ± 0.5 (***)	40	0.7
UAS-cycE + UAS-spis	0.6 ± 1.3 (***)	40	1.0 ± 1.0 (***)	40	1.6
*UAS-EGFR**	0.0 ± 0.2 *	36	0.1 ± 0.3 ***	40	0.3
*UAS-ras**	0.1 ± 0.3 NS	40	0.1 ± 0.3 ***	40	8
UAS-spi^{s}	0.1 ± 0.5 NS	24	0.5 ± 1.0 NS	40	1
UAS-p35	0.1 ± 0.3 *	40	0.2 ± 0.5 ***	40	2
UAS-EGFR^{dn}	3.7 ± 2.8 ***	48	2.6 ± 2.6 **	80	4
UAS-ras^{dn}	2.6 ± 2.8 **	48	1.4 ± 3.3 *	48	2.8
UAS-cycE + UAS-EGFR^{dn}	7.3 ± 4.6 (***)	56	7.0 ± 4.3 (***)	64	2.5
UAS-aos	2.0 ± 1.9 ***	40	1.5 ± 1.4 ***	56	7.1
UAS-cycE; spi^{1}/+	2.9 ± 3.3 (NS)	80	4.4 ± 3.0 (*)	72	6.8
ru^{1} rho^{7m43}	6.8 ± 5.2 ***	44	-		1.1
spi^{1}/+	0.9 ± 1.1 NS	40	0.8 ± 1.6 NS	40	7.5
spi^{1}	1.9 ± 1.9 *	44	1.2 ± 1.6 NS	32	1.6
vn$^{\Delta P25}$	0.8 ± 1.3 NS	20	0.6 ± 0.9 NS	56	9.5

Values are mean and standard deviation (SD) of apoptotic cells in P compartments of fixed, TUNEL-labeled embryos, and so are relative, not absolute, measures. n = number of compartments. Asterisks denote significance scores from t tests with wild-type compartments (unbracketed) or with *en>CD8-GFP+cycE* (bracketed). NS denotes not significant. All genotypes possess *en-GAL4 UAS-CD8-GFP* except if underlined. F:B denotes the ratio of cells dying in the front half of the P compartment to those dying in the back half, calculated across both stages. F:B decreases in response to reduced EGF signaling (due to induction of apoptosis in the back half) or large reductions in apoptosis (due to elevated EGF signaling or reduced proliferation, for example). Stage-13 *ru^{1} rho^{7m43}* embryos were not examined because staging became unreliable after stage 12. No dying cells were observed in the back half of *en>cycE+p35* compartments.