Current Biology *16*, 2058–2065, October 24, 2006 DOI 10.1016/j.cub.2006.08.092

Report

Control of Compartment Size by an EGF Ligand from Neighboring Cells

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Summary

Insect bodies are subdivided into anterior (A) and posterior (P) compartments: cohesive fields of distinct cell lineage and cell affinity [1]. Like organs in many animal species, compartments can develop to normal sizes despite considerable variation in cell division [2, 3]. This implies that overall compartment dimensions are subject to genetic control, but the mechanisms are unknown. Here, studying *Drosophila*'s embryonic segments, I show that P compartment dimensions depend on epidermal growth factor receptor (EGFR) signaling. I suggest the primary activating ligand is Spitz, emanating from neighboring A compartment cells. Spi/EGFR activity stimulates P compartment cell enlargement and survival, but evidence is presented that Spitz is secreted in limited amounts, so that increasing the number of cells within the P compartment causes the per-cell Spitz level to drop. This leads to compensatory apoptosis and cell-size reductions that preserve compartment dimensions. Conversely, I propose that lowering P compartment cell numbers enhances per-cell Spitz availability; this increases cell survival and cell size, again safeguarding compartment size. The results argue that the gauging of P compartment size is due, at least in part, to cells surviving and growing according to Spi availability. These data offer mechanistic insight into how diffusible molecules control organ size.

Results and Discussion

How are the sizes of biological structures controlled? Cases abound in the literature of organs or whole animals reaching normal sizes despite variations in cell proliferation [4]. For example, tetraploid mouse fetuses have less than half the number of cells of diploid fetuses, but are 85% of their size [5]. Similarly, the vertebrate liver [6] and pancreas [7] can regenerate to a normal size after massive tissue loss; so too can the mammalian brain after cortical lesions [8]. Such observations have fueled the idea that to ensure developing organs reach the correct final size, tissue mass or organ dimensions per se are subject to genetic control. The mechanisms behind this kind of overall size regulation are a mystery; somehow, they must be able to transduce information about organ dimensions (or a correlated variable) into cellular responses, that is, a cell's decision to grow, divide, or die. This feedback makes it likely that organ size is regulated by extracellular signals. Compartments in the *Drosophila* wing provide a further example of dimension-sensing: Their size is unchanged across a 4–5-fold range of cell numbers, via compensatory adjustments of cell size [2, 3]. In this case, the likely size regulators are extracellular patterning molecules, namely ligands of the Decapentaplegic/TGF-β, Hedgehog, Wingless/Wnt, and epidermal growth factor (EGF) pathways. However, despite clearly influencing growth [9–15], how these molecules might function as part of a mechanism determining compartment dimensions—relatively independently of cell number—is unclear.

Herein, I describe a pattern-based mechanism of compartment-size control. Like the wing, each segment of the *Drosophila* epidermis comprises anterior (A) and posterior (P) compartments. I have focused on P compartments in an embryonic-ectoderm region that develops into the dorsal abdominal larval epidermis (Figures 1A–1D; see Supplemental Experimental Procedures in Supplemental Data available online). There are several advantages to studying these embryonic compartments. First, the epidermis has a well-described and easily tractable pattern of cell proliferation, in which most cells undergo 13 preblastoderm and three postblastoderm divisions, arresting finally in G1 of cycle 17 [16]. This small number of divisions brings a second benefit in that epidermal compartments contain a relatively small number of cells, making accurate quantification relatively easy. The third benefit is that the *Drosophila* embryonic epidermis boasts perhaps the best-understood patterning system of any animal (reviewed in [17]).

P Compartment Size Is Determined Independently of Cell Proliferation

By expressing cell-cycle regulatory genes specifically in the embryonic P compartment, I examined the effect of altered cell number on larval compartment size. Expression of *cyclin E* (*cycE*), the G1-S cell-cycle-checkpoint regulator [18], forced many P compartment cells into S phase and to divide (Figure 1E). Wild-type compartments contain 44 cells on average, but *cycE* increased this to 59 (Figure 1G). Despite containing more cells, larval compartment size was close to wild-type (Figure 1G). Conversely, expression of *p27Dacapo* (*dap*), the *Drosophila* Cyclin E/CDK2 inhibitor [19, 20], blocked proliferation (Figure 1F) and lowered cell number to 33 (Figure 1G). However, this failed to reduce compartment size (Figure 1G). In response to *cycE* and *dap*, cell size compensated for the altered cell number, leaving compartment dimensions relatively unaffected (Figure 1G). P compartment size is thus independent of cell number.

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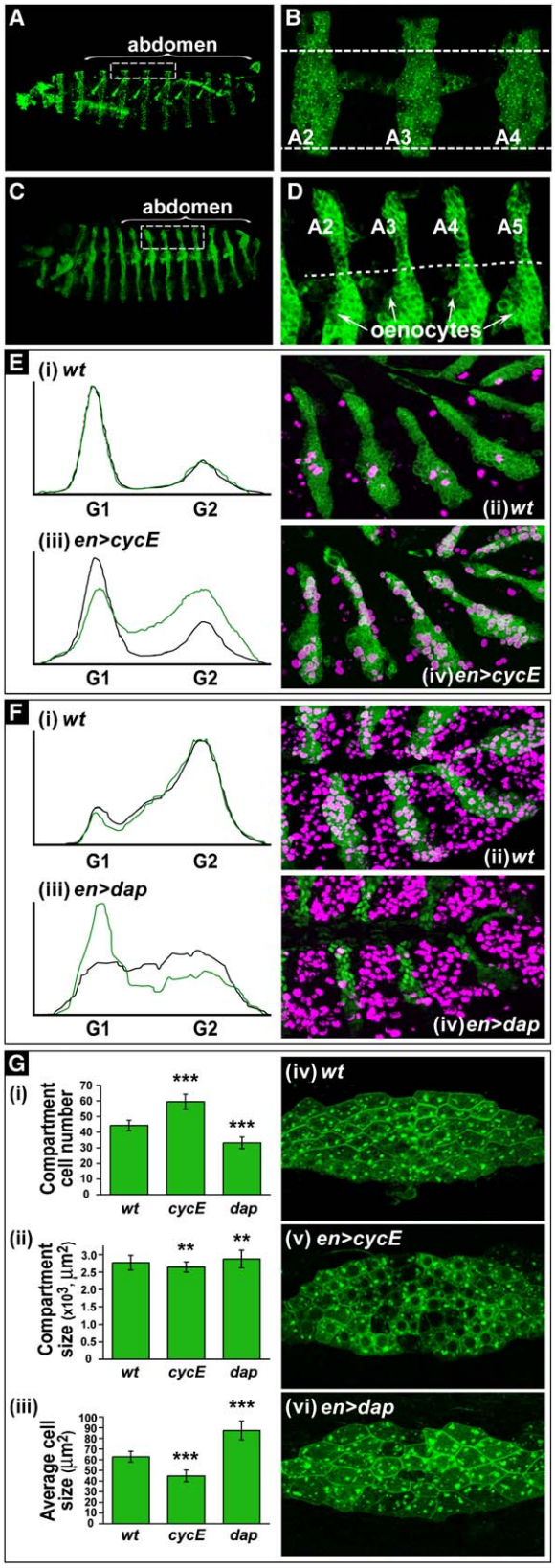

Figure 1. P Compartment Size is Independent of Cell Number

(A–D) Wild-type larvae and embryos expressing CD8-GFP (green) with *en-GAL4*.

(A) First-instar larva; box defines quantified regions of P compartments in abdominal segments A2–A4.

(B) Regions extend a defined width either side of dorsal midline.

(C) Stage-13 embryo; box defines quantified regions of abdominal segments A2–A5, from which analyzed larval regions derive.

(D) Regions lie dorsal to oenocytes.

(E_i), Flow cytometry of dissociated wild-type stage-12 embryos expressing cytoplasmic GFP with *en-GAL4*. En-positive (P compartment; green trace) and En-negative (black trace) cell populations are quiescent at this stage, with most cells in G1.

(E_{iii}) *cycE* expression forces many En-positive cells into S and G2 phases.

(E_{ii} and E_{iv}) Stage-12 embryos labeled for En (green) and mitosis marker PH3 (purple). (E_{ii}) Wild-type: Most epidermal cells are mitotically quiescent at stage 12. (E_{iv}) P compartment *cycE* expression induces additional mitoses.

(F_i) Stage-11 wild-type embryos show high proliferative activity in En-positive and En-negative populations.

(F_{iii}) *dap* causes P compartment cells to arrest prematurely in G1.

(F_{ii} and F_{iv}) Stage-11 embryos labeled for En (green) and PH3 (purple). (F_{ii}) Wild-type: Mitosis happens across the epidermis at this stage. (F_{iv}) *dap* expression inhibits this mitosis in the P compartment.

(G_i–G_{iii}) Effects of *cycE* and *dap* on first-instar larval compartments. In this and subsequent figures, mean values are shown, error bars are standard deviation (SD), and asterisks denote significances from t tests with wild-type compartments (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Number of compartments measured = 60 (*wt*), 35 (*en>cycE*), and 57 (*en>dap*). (G_i) *cycE* and *dap* alter compartment cell number, with little effect on compartment size (G_{ii}), because of cell-size compensation (G_{iii}). Slight overcompensation occurs: *dap* causes a 3.8% increase in compartment size, and *cycE* a 4.5% reduction. (G_{iv}–G_{vi}) First-instar larval P compartments expressing membrane-bound *CD8-GFP*, showing similarities in size despite cell-number differences: (G_{iv}), wild-type, (G_v), *en>cycE*, and (G_{vi}) *en>dap*.

Apoptosis is widely cited as a mechanism for removing unwanted cells from developing organs and tissues [21, 22]. I used TdT-mediated dUTP nick-end labeling (TUNEL) [23] to determine whether apoptosis responds to altered P compartment proliferation. Indeed, reducing the cell number by *dap* expression caused a drop in P compartment apoptosis (Figures 2A and 2C, Table S1). Conversely, increasing cell number by expressing *cycE* led to a large increase in TUNEL-positive nuclei (Figures 2A and 2B, Table S1). The proapoptotic genes *reaper* (*rpr*) and *head involution defective* (*hid*) (reviewed in [22]) mediate this apoptosis because expressing *cycE* with one copy of *Df(3L)XR38* (which removes *rpr*) lowered apoptosis by 59% (calculated across stage-12 and -13 embryos; Table S1), and with one copy of hid^{05014} by 65% (Table S1). Coexpressing the caspase inhibitor *p35* along with *cycE* blocked apoptosis (Table S1) and produced a compartment containing 74 cells (Figure 2D). This shows that half of the additional cells induced by expressing *cycE* alone normally die.

P Compartment Size Is Controlled by Pattern

Despite the simultaneous induction of proliferation and inhibition of apoptosis, P compartments expressing *cycE* and *p35* did not overgrow (Figures 2D and 2F). This was due to a more extreme reduction in average cell size than was witnessed on expression of *cycE* alone (Figure 2F). This result shows that the apoptotic removal of cells in response to *cycE* expression provides those cells that survive with more freedom to increase in size. This demonstrates the presence of cell growth (increase in individual cell mass) in this system. The amount a P compartment cell grows (presumably

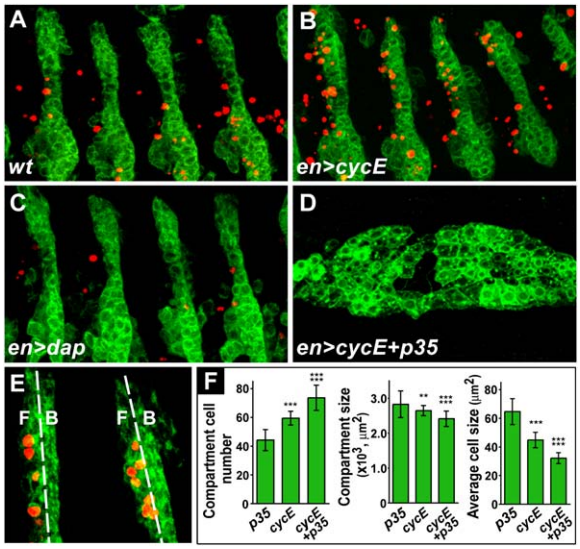

Figure 2. Compartment Cell Number Inversely Affects Cell Growth and Survival

(A–C) TUNEL-labeled (red) stage-13 embryos expressing CD8-GFP (green) with *en-GAL4*. (A) Wild-type P compartment cells apoptose relatively infrequently. (B) *cycE* increases P compartment apoptosis. (C) *dap* expression lowers P compartment apoptosis.

(D) First-instar larval compartment coexpressing *p35* and *cycE*. Such compartments contain 74 ± 9 cells.

(E) Close-up of stage-13 *en>cycE* P embryonic compartments. Dashed lines indicate division of compartments into front (anterior) and back (posterior) territories, used to calculate F:B. *cycE* induces apoptosis mostly in the front half of the P compartment.

(F) The effects of blocking cell death on cell number, cell size, and compartment size. *n* = 35 (*en>cycE*), 56 (*en>p35*), and 9 (*en>cycE+p35*). Asterisks denote significances from t tests; upper score denotes comparison with *en>cycE*, lower score with *en>p35*. Cells in *en>cycE* compartments are ~39% larger than those in *en>cycE+p35* compartments, indicating that cells modulate their growth in a manner inversely dependent on total compartment cell number. Overcompensation again occurs: *en>cycE+p35* compartments are 8.4% smaller than those expressing *cycE* alone.

via conversion of yolk into cytoplasm [24]) is thus inversely dependent on the number of cells in the compartment. Likewise, a cell's probability of survival depends inversely on cell number. Hence, a mechanism exists that restricts the number of cells within the compartment by limiting survival, and it sets the size of the compartment by limiting the growth of the individual cells that remain. The ability of the P compartment to maintain a normal size despite proliferative alterations suggests that P compartments act as unitary building blocks in the control of epidermis size. Their absolute dimensions are "measured" and constrained in a similar fashion to wing compartments [2, 3], indicating control by extracellular signals. These data also refute an alternative, more "passive" explanation for the cell-number independence of compartment size, in which the P compartment is allocated a certain mass of cytoplasm when compartment boundaries are determined, with any ensuing cell division simply cleaving this fixed volume into smaller cells.

A second indication of the kind of mechanism at work comes from the pattern of P compartment apoptosis. Across the antero-posterior (AP) axis of the wild-type compartment, the ratio of cells dying in the front half to those dying in the back half (herein termed Front:Back [F:B]) is 38 (Table S1). This anterior bias in apoptosis is especially clear to the eye in response to the extra cell proliferation induced by *cycE* expression in the P compartment (Figure 2E, Table S1). Whereas several studies have proposed that mitogen overexpression in *Drosophila* causes apoptosis [2, 25], in this instance at least, its cause is not mitogen expression per se, given that *cycE* is expressed—and hence induces mitosis—uniformly across the compartment (Figure 1E; F:B of mitotic cells in P compartments expressing *cycE* is 0.8, data not shown), yet cells die mostly at the front. This incongruence implies that apoptosis is not triggered by a cell-autonomous cue, but rather by a spatially patterned signal.

The EGFR Pathway Limits P Compartment Cell Survival

To identify this patterning signal, I expressed *cycE*, while simultaneously overactivating pathways that pattern the P compartment, and assayed apoptosis. Domains of patterning molecule expression flank the P compartment:

(1) To its anterior lies a stripe of *wingless* (*wg*) expression (Figure 3A). Wg, on binding to its receptor Frizzled, stimulates the nuclear accumulation of Armadillo (Arm) protein and activation of Wg target genes [26]. Simultaneously expressing *cycE* and overactivating Wg signaling (by coexpressing constitutively active $armadillo^{s10}$) had no effect on apoptosis at stage 12 and only a partial effect at stage 13 (Table S1), indicating that Wg is not the primary signal limiting cell survival.

(2) A row of cells posterior to the P compartment express *rhomboid* (*rho*) (Figure 3B) [27]. Intramembrane Rhomboid proteolytically cleaves golgi-localized, membrane-tethered EGF ligands, primarily Spitz (Spi) [28]. *spi* is expressed throughout the epidermis [29], but cleavage by Rhomboid is necessary for its release into the extracellular environment, where it binds the epidermal growth factor receptor (EGFR) and triggers the Ras/Raf/MAP-kinase pathway [30]. Unlike Wg, EGF-pathway overactivation with a constitutively active *EGFR* (*EGFR**) suppressed the apoptosis associated with *cycE* by 95% (Figure 3C, Table S1), causing compartment cell number to rise to approximately 76 (Figures 3I and 3K). Furthermore, when expressed alone, *EGFR** reduced apoptosis below wild-type levels (Table S1). *EGFR** had no apparent effect on mitosis, however, as indicated by near-wild-type compartment cell numbers (Figure 3K) and PH3 staining of post-stage-11 embryos (data not shown). Similar results were obtained with activated *ras* (*ras**; Figures 3H and 3K, Table S1) and also a secreted form of *spi* that does not require cleavage by Rho for extracellular release (spi^{s}; Table S1). Elevated EGF signaling thus appears to increase P compartment cell number not by inducing proliferation—as it does in some developmental circumstances in the fly [15, 31]—but purely by suppressing apoptosis—as it does in other situations [21, 32–34]. This makes Spi, acting through EGFR,

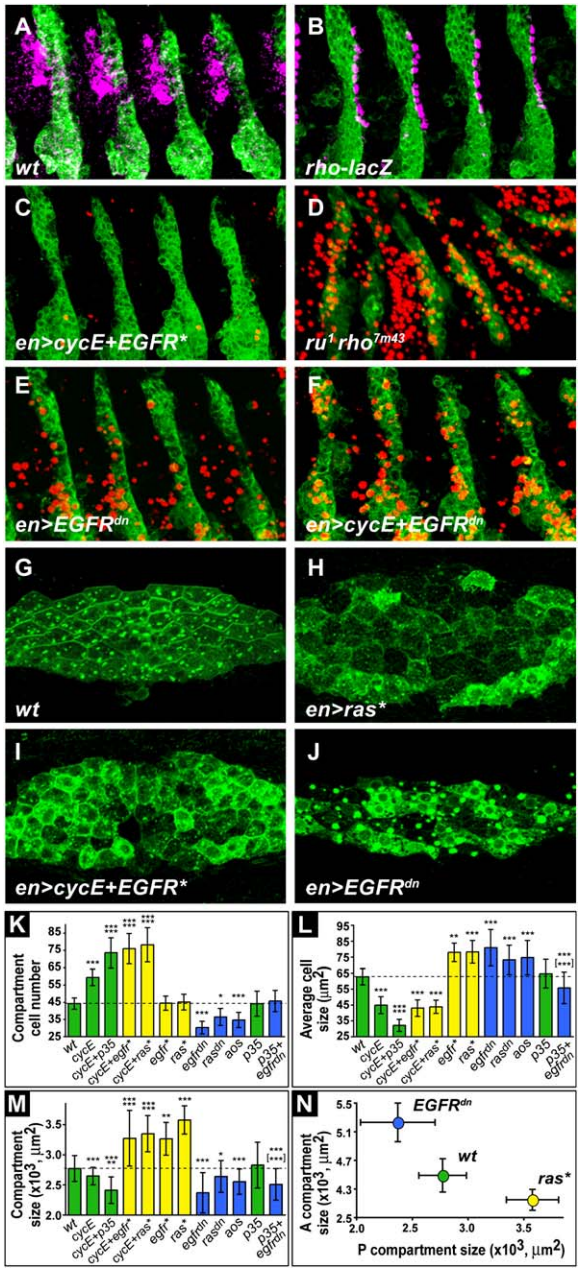

Figure 3. Spi/EGFR Activity Limits P Compartment Size

(A) *en>CD8-GFP* embryo. Cells anterior to P compartments express Wg (green, GFP; purple, Wg).

(B) *en>CD8-GFP rho-lacZ* embryo. Cells posterior to P compartments express *rho* (green, GFP; purple, β-galactosidase).

(C–F) Stage-13 (C, E, F) and stage-12 (D) TUNEL-labeled (red) embryos expressing CD8-GFP (green) with *en-GAL4*. (C) *EGFR** blocks *cycE*-induced P compartment apoptosis. (D) Stage-12 ru^1 rho^{7m43} embryo; removal of *rho* and *ru* leads to high levels of P compartment apoptosis (death is induced in A compartments also). (E) $EGFR^{dn}$ induces P compartment apoptosis. (F) Coexpression of *cycE* and $EGFR^{dn}$ increases cell death still further.

(G–J) CD8-GFP-labeled first-instar larval compartments. (G) Wild-type is shown. (H) *ras** causes compartment overgrowth. (I) *EGFR** and *cycE* coexpression increases cell number and overrides cell-size compensation, causing compartment overgrowth. (J) $EGFR^{dn}$ reduces compartment size.

(K–M) Effects of altered EGF signaling on first-instar larval P compartments; yellow bars indicate EGF overactivation, blue bars indicaet EGF inhibition, and green bars indicate the nonmanipulated EGF pathway. *n* = 60 (*wt*), 35 (*en>cycE*), 16 (*en>cycE+EGFR**), 23 (*en>cycE+ras**), 7 (*en>EGFR**), 32 (*en>ras**), 39 ($en>EGFR^{dn}$), 24 ($en>ras^{dn}$), 18 (*en>aos*), 56 (*en>p35*), and 36 ($en>p35+EGFR^{dn}$). Asterisks denote significances from t tests with wild-type compartments; where two scores are shown, unbracketed lower score denotes comparison with *en>cycE*, and bracketed lower score denotes comparison with *en>p35*. Dashed line indicates wild-type value. (K) EGF signaling controls P compartment cell number by promoting survival. (L) EGF signaling limits cell size. (M) EGF signaling limits P compartment size.

(N) P compartment EGF signaling inversely affects the size of the first-instar larval A compartment. Both $EGFR^{dn}$ and *ras** differ significantly from the wild-type at $p < 0.001$.

a strong candidate for the signal limiting P compartment cell survival. This conclusion also finds support from the location of the dying cells in wild-type animals, that is, at the front of the P compartment, furthest from Spi source (Table S1).

If Spi activity controls P compartment survival, then reductions in Spi activity should lead to P compartment apoptosis. I achieved this by two means. First, I assayed apoptosis in embryos completely devoid of Spi activity—namely, those mutant for both *rho* and *ru*, a *rho* paralog that functions redundantly in Spi cleavage [35]. In line with the hypothesis, starting at late embryonic stage 12, ru^1 rho^{7m43} embryos showed high levels of apoptosis throughout the P compartment (F:B = 1.1; Figure 3D, Table S1). Second, I partially blocked P compartment cells' ability to transduce Spitz by expressing dominant-negative *EGFR* ($EGFR^{dn}$). As predicted, $EGFR^{dn}$ expression caused many cells to apoptose, again beginning at late stage 12 (Figure 3E, Table S1), reducing cell number to approximately 30 cells (Figures 3J and 3K). Similar results were obtained by using dominant-negative *ras* (ras^{dn}; Figure 3K, Table S1).

Consistent with there being an EGFR activity gradient—high at the back of the compartment and low at the front—$EGFR^{dn}$ and ras^{dn} induced apoptosis mostly at the front (Figure 3E, Table S1); cells at the back are therefore particularly protected against the signaling reduction brought about by $EGFR^{dn}$ and ras^{dn}, presumably because they lie closest to the *rho*-expressing cells and hence experience higher concentrations of active Spi protein. However, the low F:B values of compartments expressing $EGFR^{dn}$ and ras^{dn} show that considerably more apoptosis is induced in the back half of the P compartment as compared to wild-type compartments (Table S1), indicating that cells across the entire compartment require Spi for survival. This demonstration of precise spatial control of P compartment cell survival by EGFR activity extends and clarifies a previous observation: that lowering EGF signaling across the ventral epidermis can increase apoptosis [32]. As a further test, I coexpressed $EGFR^{dn}$ with *cycE*. This approximately doubled the amount of apoptosis relative to expressing *cycE* alone (Figure 3F, Table S1). $EGFR^{dn}$ thus seems to augment the proposed *cycE*-induced reduction in per-cell ligand availability.

Spi Is the Activating Ligand

I note here that the *Drosophila* genome has three other EGF-ligand-encoding genes: *gurken* (*grk*), *keren* (*krn*), and *vein* (*vn*) [30]. It has been assumed thus far that *spi* is of relevance to this mechanism because of its

expression pattern and that of *rho*, as well as its established role in patterning the P compartment [32, 36, 37]. *grk* can be ruled out because it is transcribed only in the germline cells of the ovary, where it is required for oogenesis (reviewed in [38]). Likewise, *keren*, which encodes a structural homolog of Spi [39], is also unlikely to be the principal ligand: *keren*$^{-}$ flies are viable with no discernable phenotype (M. Freeman, personal communication), indicating that *keren* plays a negligible role in wild-type development. Several observations show that of the two other ligands, Vein and Spi, the latter is more important. First, the observation of high levels of P compartment apoptosis in *ru*$^{-}$ *rho*$^{-}$ embryos (Figure 3D, Table S1) implies that Spi is responsible, because Vn does not require Rho cleavage for extracellular release. Second, in *spi*^{1} homozygous embryos, apoptosis was induced throughout the P compartment (F:B = 1.6; Table S1), albeit at a relatively low level, possibly because *spi* is maternally contributed [29]. In contrast, *vn*$^{\Delta P25}$ embryos showed insignificant P compartment apoptosis and retained an anterior bias in cell death (F:B = 9.5; Table S1). Because *vn* is not maternally contributed [40], maternal *vn* transcripts cannot be responsible for P compartment cell survival in zygotic *vn*$^{-}$ embryos. Third, expression of *cycE* in *spi*^{1} heterozygous embryos increased cell death at stage 13 relative to *cycE* controls (Table S1). Fourth, cell death was induced, and compartment cell number reduced, by expressing *argos* (*aos*): a secreted protein that inhibits EGF signaling by directly binding to Spi [41, 42] (Figure 3K, Table S1). All of these observations point to Spi being the relevant ligand.

Spi/EGFR Limits P Compartment Cell Size and Stabilizes Compartment Dimensions

What may be regulating P compartment cell size, in such a way as to safeguard compartment dimensions? In imaginal discs, the EGF pathway is also implicated in the control of cell size [43]. Likewise, in epidermal P compartments, pathway overactivation with *EGFR** or *ras** increased cell size (Figures 3H and 3L), causing compartments to enlarge by 18% and 29%, respectively (Figures 3H and 3M). When coexpressed with *cycE*, *EGFR** and *ras** again produced compartment overgrowth (Figures 3I and 3M), although cells in these compartments were similarly sized to those produced by expressing *cycE* alone (Figure 3L). This apparently paradoxical result is explained by the fact that expressing *EGFR** or *ras** with *cycE* blocks cell death, causing cell numbers to rise higher than they do in response to *cycE* alone (Figure 3K). One may therefore expect cells in compartments coexpressing *cycE* with *EGFR** and *ras** to become smaller than they do in response to *cycE* expression, as happens when *cycE* is coexpressed with *p35* (Figure 3L, and compare Figure 3I with Figure 2D). That cells do not undergo a further size reduction indicates that *EGFR** and *ras** induce cell growth and compromise the P compartment's cell-size compensation mechanism, causing the entire compartment to expand. EGF-pathway overactivation thus increases P compartment size through cell enlargement and can also override the cell-size compensation that normally happens in response to manipulations of cell division. These results suggest that in addition to its role in limiting compartment cell number by apoptosis suppression, Spi/EGFR activity may also limit cell size. Hence, the activity of this pathway may account for the compensatory changes, both in survival and cell growth, that buffer compartment size from proliferative changes.

Were it the case that Spi/EGFR activity regulates P compartment size, impeding the pathway should decrease compartment size. The severity of the *ru*$^{-}$ *rho*$^{-}$ cuticular phenotype precluded estimation of larval P compartment size, but partial inhibition of the pathway with *EGFR*^{dn} was sufficient to reduce compartment size by 15% (Figures 3J and 3M). Similar results were obtained with *ras*^{dn} (Figure 3M). Additionally, inhibiting Spi activity by expressing *aos* also reduced compartment size (Figure 3M). To study the effects of reduced EGFR-pathway activity on cell size, I expressed *EGFR*^{dn} while blocking apoptosis with *p35* and compared cell size in these compartments to those expressing *p35* alone, which have similar cell numbers (Figure 3K). Compartments coexpressing *EGFR*^{dn} with *p35* are smaller than those expressing *p35* (Figure 3M) because of a 14% reduction in cell size (Figure 3L). This result confirms that impeding EGF signaling decreases cell size; I conclude that in the P compartment, Spi/EGFR activity promotes both cell survival and cell growth but acts at a level that limits both processes and, as a consequence, determines compartment size.

Whether Spi/EGF signaling is the sole determinant of P compartment size remains to be seen. Inhibiting the pathway with *EGFR*^{dn}, *ras*^{dn}, or *aos* tends not to delete the larval P compartment, and one possible reason for this is that these constructs allow for a residual level of signaling. *EGFR*^{dn}, *ras*^{dn}, and *aos* cause apoptosis, but some cells survive, and these cells can also compensate for the reduction in cell number by becoming larger than normal (Figures 3J and 3L), albeit not large enough to rescue compartment size (Figure 3M). Perhaps the reduction in cell number provides remaining cells with higher-than-normal Spi levels. Increased Spi may override the effectiveness of *EGFR*^{dn}, *ras*^{dn}, and *aos*, causing the observed cell overgrowth. Alternatively, the effect of EGF signaling on size may be limited by the activity of additional forces that constrain growth either when compartment size is below a threshold size or when EGF-pathway activity below a threshold level. A drop in P compartment EGFR activity may be counterbalanced by reduced transcription of downstream targets of EGFR that encode pathway inhibitors, such as *aos* or *sprouty* (reviewed in [30]). Another explanation is that Wg promotes cell growth when EGFR activity is lowered; Wg is known to antagonize Spi activity across the P compartment's AP axis in the ventral epidermis [37, 44]. There also seems to be an upper limit to the effects of EGFR signaling on P compartment size: Compartments coexpressing *cycE* and *EGFR** or *ras** are overgrown relative to wild-type compartments, yet cell size is still much reduced (Figure 3L), despite the fact that these cells receive very high levels of signaling. It may be that the overgrowing compartment as a whole exhausts the limited resources available to it for growth.

How the pathway influences P compartment cell survival and cell size is also not clear, but extrapolation from the findings of other studies suggests that a direct mode of operation is likely. Ras/MAP-kinase signaling is

known to influence cell survival in a direct cell-autonomous fashion in the eye imaginal disc, by direct phosphorylation of Hid by MAP-kinase (thereby blocking Hid function [34]) and through suppression of *hid* and *rpr* transcription [33]. Likewise, EGF signaling may directly influence P compartment cell size: In imaginal discs, Ras activity increases the level of dMyc, a positive regulator of cell size [43], although in embryonic P compartments I detected no obvious changes in dMyc protein levels in response to EGFR overactivation (data not shown).

The Developmental Logic behind Compartment-Size Control

The mechanism by which Spi is used to measure and constrain P compartment size exemplifies one way in which diffusible molecules regulate organ size independently of cell division. The mode of Spi operation bears obvious parallels with how this same molecule controls the number of midline glial cells [21]; this "trophic" control system, in which cells can only survive and/or grow if they receive sufficient amounts of a limited signal, may be a common way in which EGFR/MAP-kinase signaling regulates tissue growth and cell numbers in developing organs. Because Spi activates EGFR [30], it is likely that the putative P compartment EGFR activity gradient is controlled directly by a Spi concentration gradient. With the assumption that a Spi gradient is responsible, a spatial model of how P compartment dimensions are controlled is shown in Figure 4. A key feature is that the level of Spi input to the P compartment should be unchanged by altered P compartment proliferation because its source is in the A compartment. Thus, the safeguarding of P compartment dimensions is made possible by having the signal that determines compartment size coming from a region unaffected by perturbations in proliferation—i.e., outside of the P compartment. For example, increasing the number of cells will not alter compartment size because the supply of ligand responsible for cell enlargement and survival is unchanged; hence, the excess cells either will die or will not be afforded the means to reach the normal size.

This dependence of compartment size on a signal emanating from cells in a neighboring compartment contrasts with the idea of compartments acting autonomously in the control of size [1, 45]. Instead, it raises the possibility that growth of neighboring compartments is more integrated. A system of interactions between neighboring compartments makes intuitive sense for the maintenance of body proportions, the communication between compartments offering a greater level of security to prevent structures growing too much or too little. Perhaps a signal from the P compartment determines A compartment size; consistent with this kind of scenario, manipulations that expand the P compartment lead to size reductions in the A compartment, whereas reductions in P compartment size result in enlarged A compartments (Figure 3N). The only molecule known to travel from the P to the A compartment is Hedgehog (Hh; [46]), which is needed for cell survival in the dorsal epidermis [47]. Hh may therefore influence A compartment size, and its secretion might respond to changes in P compartment size or EGFR activity. In the wing, Decapentaplegic (Dpp: a long-range patterning molecule that promotes wing growth [9–11]) emanates from A compartment cells. It may be that wing P compartment size is unaltered by changes in cell number [2, 3] because these changes have little impact on Dpp output. Analogous logic of growth-promoting ligands from external sources nonautonomously determining dimensions may be found elsewhere. For example, liver regeneration depends on serotonin from platelets [48], and the observation of competition between growing wings discs of butterflies, and eyes and fighting horns of beetles [49], indicates similar nonautonomous regulation.

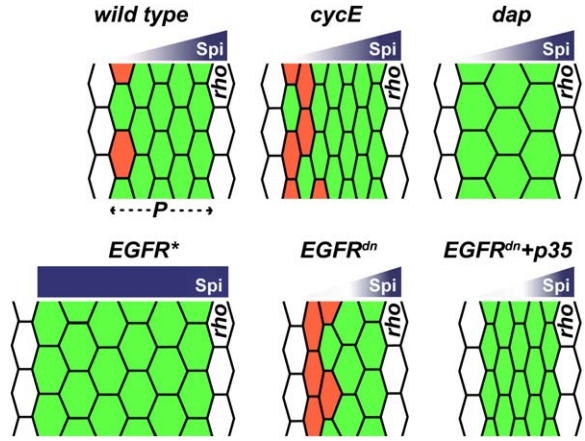

Figure 4. A Model of P Compartment-Size Control

Top row: After establishment of P compartment boundaries, growth of the P compartment becomes dependent on Spi. Spi is secreted at a fixed level from *rho*-expressing cells in the neighboring A compartment, setting a limit for P compartment expansion. Spi promotes P compartment cell survival and cell growth (increase in individual cell mass); cells failing to see sufficient Spi levels apoptose (red). Spi output remains unchanged by altered P compartment proliferation. *cycE*-induced overproliferation reduces per-cell Spi availability: Cell size decreases and more cells die farthest from Spi source. *dap*-induced underproliferation is assumed to increase per-cell Spi availability: Cells survive and increase in size.

Bottom row: Altering EGFR activity changes cells' perception of Spi. *EGFR** provide excess signaling: Cells survive and grow large, causing compartment overgrowth. $EGFR^{dn}$ reduces compartment size by apoptosis and by a failure of surviving cells to fully compensate; surviving cells increase in size perhaps because of elevated per-cell Spi levels. Coexpression of $EGFR^{dn}$*+p35* reduces cell size and compartment size.

Supplemental Data

Supplemental Data include Experimental Procedures and one table and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/20/2058/DC1/.

Acknowledgments

I am extremely grateful for the wonderful support I received from Peter Lawrence throughout the course of this study. For their helpful advice J.P. Vincent and Damon Page also receive my thanks, as do Richard Grenfell for assistance with FACS and Simon Bullock, Matthew Freeman, Martin Kerr, Peter Lawrence, G. Angus McQuibban, Matthew Piper, Leonie Quinn, and two anonymous reviewers for critically evaluating the manuscript. Matthew Freeman, Iswar Hariharan, Bruce Hay, Laura Johnston, Christian Lehner, and J.P. Vincent kindly provided fly stocks and antibodies. This work was supported by a Medical Research Council Studentship, and revision was assisted by funds from NIH-HD42770 (L. Johnston).

Received: December 20, 2005
Revised: August 24, 2006
Accepted: August 24, 2006
Published: October 23, 2006

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