Unsolved Mystery

How Are the Sizes of Cells, Organs, and Bodies Controlled?

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Why is an elephant bigger than a mouse? Why, luckily, are our arms precisely the same size? While developmental genetics over the past 20 years has provided us with fascinating insights into how segments form, limbs bud, and axons find their targets, we have made little progress towards answering these obvious questions in biology. Rather than attempting to provide the answers, we will try to frame the questions in a developmental context and highlight some approaches towards answering them. Somewhat artificially, we will consider separately the mechanisms of cell, organ, and body size control.

What Controls the Size of Eukaryotic Cells?

The size of a cell depends on intrinsic and extrinsic factors. For example, cell size can vary dramatically with cell type—some neurons or glia cells are up to 1,000 times larger than epithelial cells. Cell size is also influenced by the number of genome sets (ploidy). A haploid *Drosophila* epithelial cell is only about half the size of a diploid cell. A polyploid salivary gland cell, on the other hand, is more than 1,000 times larger than a diploid cell. Amongst the extrinsic factors, the availability of nutrients and temperature are well known for their effect on cell size. Starvation not only prolongs the cell doubling time in yeast and in *Drosophila* cells, it also reduces the size at which they divide.

Work from Zetterberg (Killander and Zetterberg 1965) in mammalian fibroblasts and subsequently from Nurse and Hartwell in yeast provided evidence for a cell size checkpoint (Nurse 1975; Johnston et al. 1977). In budding yeast, the protein Cln3p acts as a sizer. Cells only initiate the critical cell cycle step from G1 phase to S phase, when Cln3p has reached a certain threshold. The accumulation of Cln3p is, in turn, dependent on efficient translation of the *Cln3* mRNA, which is inefficiently translated until sufficient numbers of ribosomes have been generated (Polymenis and Schmidt 1997). In this way, the presence of an efficient translation machinery is a prerequisite for passing the cell size checkpoint. Indeed, in a whole-genome survey of mutants affecting cell size in budding yeast, many size mutants exhibited defects in ribosome biogenesis (Jorgensen et al. 2002).

Ribosome biogenesis also appears to be an important regulator of cell size in multicellular organisms. Phosphorylation of the ribosomal protein S6 by S6 kinase (S6K) results in the preferential translation of ribosomal proteins and thus in the replenishment of the protein synthesis machinery. *Drosophila* cells lacking functional S6K grow more slowly and are smaller than normal cells, possibly because of the earlier accumulation of a cell sizer analogous to Cln3p in yeast (Thomas 2000).

Unsolved Mysteries discuss a topic of biological importance that is poorly understood and in need of attention.

Abbreviations: *brk*, *brinker*; Dpp, Decapentaplegic; Hh, Hedgehog; IGF, insulin-like growth factor; *M*, *Minute*; S6K, S6 kinase; Shh, Sonic hedgehog; TGFβ, transforming growth factor β; Tkv, Thickveins

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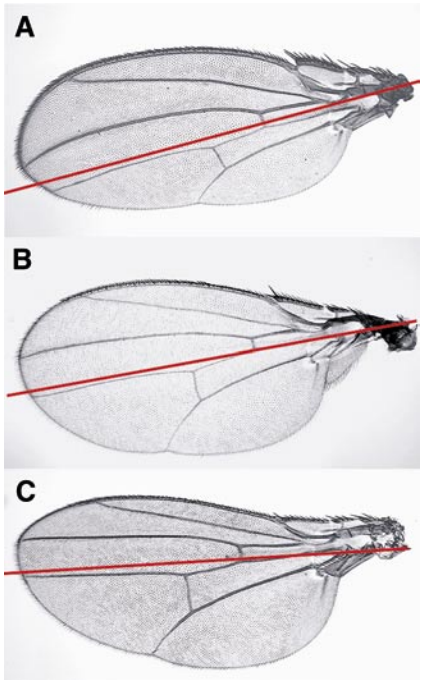
DOI: 10.1371/journal/pbio.0000086



DOI: 10.1371/journal/pbio.0000086.g001

Figure 1. The Insulin Signaling Activity Controls Organ Size in a Compartment-Specific Manner

Mosaic *Drosophila* wings with compartment-specific manipulations of dAkt function display striking size defects but normal patterning.

(A) Selective reduction of dAkt function in the posterior compartment by means of FLP-mediated mitotic recombination in posterior cells (using *engrailed–Gal4* to drive the expression of *UAS–Flp*) results in a small P compartment largely consisting of $dAkt^{3}$ mutant cells. The smaller compartment size is due to fewer and smaller cells.

(B) Wild-type wing for comparison.

(C) Expression of dAkt in posterior cells (*engrailed–Gal4 UAS–dAkt*) of wings with reduced dAkt function ($dAkt^{3}$) restores the size of the P compartment, whereas the A compartment remains small. The red lines mark the anterior–posterior compartment boundary. Note that similar results in the wing disc have been obtained by Teleman and Cohen (2000).

But is there a need for a cell size checkpoint in multicellular organisms? One would assume so, because otherwise cells would either become progressively smaller or larger. However, it has been suggested that this may be a problem for exponentially growing cells like yeast, but that mammalian cell growth is linear and, under these conditions, the need for a cell size checkpoint may be less stringent. Indeed, Conlon and Raff (2003) did not observe a cell size checkpoint in rat Schwann cells grown under different growth factor conditions (see also Grewal and Edgar 2003). Furthermore, the existence of cell size checkpoints may be cell-type dependent and stage specific. During *Drosophila* imaginal disc development, for example, cells are larger at the beginning of imaginal disc growth and become progressively smaller during later stages (Madhavan and Schneiderman 1977).

Until recently, more emphasis has been placed on understanding the genetic control of cell cycle progression than on the mechanisms regulating cell growth. This has often led to the use of cell proliferation and cell growth as synonymous terms. Analysis in *Drosophila* imaginal discs using cell clones either deficient in cell cycle progression or expressing cell cycle regulators that accelerate or slow down the cell cycle, however, have shown clearly that cell cycle progression alone is not sufficient to promote growth (Weigmann et al. 1997; Neufeld et al. 1998). In summary, cell size is altered by changing ploidy, by uncoupling cell cycle progression from cell growth, and by pathways regulating cell growth such as the insulin (see below) and S6K pathways. Of these three, only the modulation of cell growth has an effect on overall growth at the next level, the organ.

How Is the Size of Organs Controlled?

Changes in organ size are only partly due to changes in cell size. In *Drosophila*, the reduction in wing size in S6K mutant flies or in flies raised at higher temperature is caused by a reduction in cell size (Partridge et al. 1994; Montagne et al. 1999); in contrast, starvation or mutations in genes coding for insulin signaling components that mediate the starvation response affect body size and organ size by reducing cell size and cell number (Garofalo 2002). The effect of insulin pathway activity on growth is largely autonomous to cells and multicellular regions, called compartments. Specific reduction of dAkt function, an essential component of the insulin signaling pathway, in either the anterior or the posterior compartment of the wing imaginal disc results in a severe reduction of the respective compartment. Astonishingly, the small compartment is properly patterned and the size and patterning of the adjacent compartment remain untouched (Figure 1), demonstrating that the insulin pathway has a profound effect on the final size of an organ without interfering with the patterning mechanism.

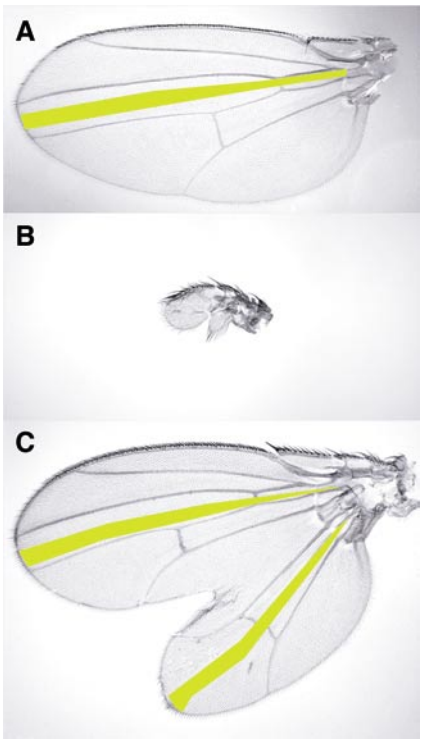
Recently, a novel signaling complex that restricts organ size by controlling both proliferation arrest and apoptosis has been discovered (Ryoo and Steller 2003). Mutations in either *hippo*, *salvador*, or *warts* result in a failure of cell cycle exit and in a protection from cell death, thus leading to massively overgrown organs. How an organ *knows* when it has reached its final size, however, is still mysterious and thus challenging.

It is clear that autonomous and nonautonomous factors control organ size, but their relative contribution varies depending on organ type and species. Multiple transplanted fetal thymus glands each grow to their normal size while multiple transplanted fetal spleens grow collectively to the size of one spleen (reviewed in Conlon and Raff 1999). In *Drosophila*, immature imaginal discs (larval structures that undergo metamorphosis and develop into structures such as legs, wings, and eyes in the adult) transplanted into a third instar larva do not undergo metamorphosis until they reach the final size (Bryant and Simpson 1984). But the size of insect appendages is not only controlled autonomously. Ablation of the hind wing discs in butterflies increases the size of the fore wings (Nijhout and Emlen 1998).

The Role of Cell Competition

Based on experiments in mammalian systems, it has been suggested that the competition for limiting growth or survival factors may be a general mechanism for organ size control (Conlon and Raff 1999). In *Drosophila*, cell competition is observed in imaginal discs. Slowly growing cells are eliminated when they are next to cells that grow at a normal rate (Simpson and Morata 1981). The slowly growing cells in these studies were heterozygous at one of several *Minute* (*M*) loci, some of which encode ribosomal proteins.

Recently, a link has been established



DOI: 10.1371/journal/pbio.0000086.g002

Figure 2. Changing the Patterning Mechansims during Wing Development Affects Growth
Compared with a wild-type wing (A), loss of Dpp function results in reduced growth and loss of pattern elements (B). Ectopic expression of Dpp in a clone of cells results in pattern duplications associated with massive extra growth (C). The region of Dpp expression in (A) and (C) is indicated by the green color. (Zecca et al. 1995; pictures courtesy of B. Müller and K. Basler.)

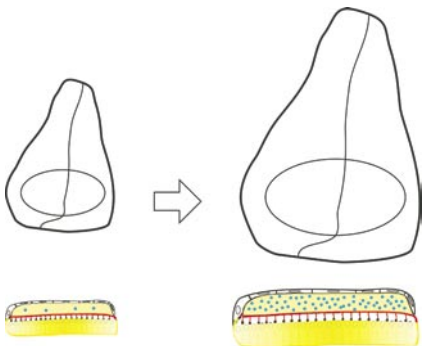
between cell competition and signaling by the secreted factor Decapentaplegic (Dpp) (Moreno et al. 2002). The elimination of slowly growing *M/+* cells is preceded by the upregulation of the gene *brinker* (*brk*), which triggers cell death. Expression of *brk* is downregulated by high Dpp levels. As in *M/+* cells, *brk* upregulation and cell elimination by apoptosis are also triggered in cells close to the Dpp source that are unresponsive to Dpp because they lack the Dpp receptor Thickveins (Tkv). Slowly growing cells may be outcompeted because they may be less efficient in internalizing Dpp via endocytosis and thus receive fewer survival signals. The problem with this simple model is that cells away from the anterior–posterior boundary—the site of Dpp production—possess high levels of Brk but do not die and grow at the same rate as cells close to the Dpp source. Indeed, *tkv* mutant clones also survive in these regions (Burke and Basler 1996). Therefore, *brk* levels do not correlate with the growth and survival potential of cells in all circumstances.

An alternative explanation for the observed parallels between the elimination of *tkv* mutant cells and *M/+* cells is that the juxtaposition of cells with different cell surface properties is the trigger for elimination. The upregulation of *brk* in *M/+* cells may trigger different surface properties (positional identities) in the same way as in *tkv* mutant cells. Thus, cell competition may be a cell-policing mechanism that eliminates cells that for various reasons do not fit into the community. Whether this mechanism of cell competition plays a major role in organ size control is still unclear.

How Are Pattern Formation and Growth Connected?

Organ size is coupled to pattern formation. Interfering with patterning mechanisms, for example, by implanting a bead soaked in the secreted factor Sonic hedgehog (Shh) into the anterior of the chick wing bud or by the ectopic expression of Hedgehog (Hh) or Dpp in the *Drosophila* wing, causes pattern duplications and concomitant growth. Conversely, partial loss-of-function mutations in *dpp* reduce wing size (Potter and Xu 2001) (Figure 2). In contrast to the effects caused by modulating insulin pathway activity, the stimulation of growth by Dpp appears to be tightly linked with pattern formation. How is patterning coupled to growth? This is one of the major unsolved questions in the field. It does not appear that the patterning morphogens like Dpp act by directly promoting growth since the cell division rates are the same in regions of high and low Dpp concentrations (Milan et al. 1996).

An attractive hypothesis put forward based on a previous model of regeneration postulates that the individual cells of an organ primordium measure the concentration gradients of specific signaling molecules, such as Dpp in the *Drosophila* wing disc and Shh in the vertebrate limb bud (Day and Lawrence 2000). In immature small primordia, the gradients are steep and cells continue to grow and divide. Since the source of the gradient stays approximately constant, its concentration gradient flattens as the tissue grows. When the difference in the morphogen concentration sensed by the two ends of the cells along the axis of the gradient falls below a certain threshold, the cells stop growing. Although this model could explain why cell growth and division are not concentrated around



DOI: 10.1371/journal/pbio.0000086.g003

Figure 3. Model for the Coordinated Control of Growth and Patterning in the *Drosophila* Wing Disc
A schematic representation of a growing (left) and a mature (right) wing disc is shown at the top. Corresponding cross-sections through the wing blade region are depicted below. The wing disc originates from the infolding of the embryonic ectoderm and consists of pseudostratified epithelial cells containing a basal–lateral side (yellow) and an apical side (red). The apical surface faces the disc lumen that is formed by the epithelium and the overlaying peripodial membrane (black), consisting of squamous epithelial cells. The morphogen and growth factor Dpp (yellow) is secreted basal–laterally by the Dpp-producing cells located anterior to the anterior–posterior compartment boundary (line through centre of wing disc). The Dpp concentration gradient from the anterior–posterior boundary to the periphery provides the anterior–posterior patterning cues. In addition, Dpp is also secreted apically into the disc lumen where is can diffuse freely. The model proposes that luminal Dpp acts as a growth-promoting factor stimulating disc growth in young discs. As the disc grows, a hypothetical growth inhibitor (blue dots) is also secreted apically and antagonizes the growth promoting activity of Dpp. Once the concentration of the inhibitor has reached a certain threshold, proliferation of wing imaginal disc cells ceases.

sources of morphogens, experimental evidence does not support it. Clones of cells expressing a constitutively active version of the Dpp receptor Tkv show increased growth when surrounded by cells of low Tkv activity. Furthermore, constant overexpression of activated Tkv in the entire disc also promotes growth, arguing against growth being induced by a differential of Tkv activity across the cell (Lecuit et al. 1996; Nellen et al. 1996).

How then does normal graded Tkv activity produce homogenous growth? One possible solution to this problem comes from the observation that Dpp in the *Drosophila* wing is secreted basal–laterally as well as apically (Figure 3). While Dpp secreted on the basal–lateral side in the epithelium has been detected in a concentration gradient (Teleman and Cohen 2000), Dpp secreted on the apical side accumulates in the disc lumen formed by the disc epithelium proper and the peripodial membrane, whose cells also secrete Dpp (Gibson et al. 2002). It is tempting to speculate that Dpp in the lumen functions as a general growth-promoting factor, while Dpp secreted in a graded fashion from the basal–lateral side induces pattern formation. A growth-promoting function has been suggested for the luminally produced Dpp (Gibson et al. 2002). This model implies that Dpp received on the apical side of the cell triggers a different cellular response (growth, survival, or both) than Dpp received on the basal–lateral side (patterning) and would probably require an unequal distribution of Dpp receptors or signaling components along the apical–basal axis of the cell.

At present, the most attractive hypothesis for how intrinsic control of organ size is achieved postulates that a secreted growth-promoting factor accumulates in the organ primordium and that its function is counteracted by an inhibitor accumulating with a delay (Nijhout 2003). Once the inhibitor reaches a certain threshold and/or the growth factor is consumed, organ growth stops (Figure 3). Although hypothetical, activator and inhibitor models have been postulated for many patterning processes. Dpp and related transforming growth factor β (TGFβ) molecules provide a particularly well-established case. TGFβ agonists and antagonists are involved in patterning the dorsal–ventral axis in the *Drosophila* embryo and the left–right asymmetry in the vertebrate embryos (Capdevila and Belmonte 1999). Further genetic and biochemical experiments are needed to identify the components involved in intrinsic organ growth control.

Which Growth Promoting Pathways Are Regulated by Secreted Factors with Patterning Functions?

Although little is known about the connection between patterning factors and growth pathways, a few potential links have been described. For example, in the *Drosophila* eye imaginal disc, Hh regulates growth directly by controlling the expression of cyclin E, a promoter of the G1/S transition, and by cyclin D, a promoter of cell growth (Duman-Scheel et al. 2002). Whether this is a general mechanism by which Hh controls cell growth and cell division is unclear, however, since in the wing disc at least, the effect of Hh appears to be mediated by Dpp. Comprehensive surveys of target genes regulated by these patterning factors in the specific developing tissues using microarray technology may provide further insight into how they control cell growth directly or indirectly.

How Is Body Size Controlled?

Can the question of body size regulation be reduced to simply summing up the mechanisms that regulate the size of individual organs? In contrast to organ size control that involves local cell interactions, locally produced growth factors as well as systemic growth factors, overall body size is controlled primarily by systemic factors. Vertebrate body size is controlled by growth hormone and the subordinate insulin-like growth factors (IGFs) (Butler and Roith 2001). In invertebrates, growth and body size are also regulated by the insulin/IGF system in response to nutrients. In addition, final body size in insects is determined by the number of molting cycles, and these are under the control of the steroid hormone ecdysone and the sesquiterpenoid juvenile hormone (Nijhout 2003). Nevertheless, changing ecdysone or insulin-like peptide levels in invertebrates or overproducing growth hormone in vertebrates can increase body size only within a certain range. It is obviously not possible to turn a mouse into the size of an elephant, although the recent identification of fossils of *Phoberomys pattersoni* indicates that rodents were once a great deal larger than they are today (Sanchez-Villagra et al. 2003). In addition to the hormonal control of body size, there are intrinsic genetic constraints to organ and body size. Understanding the mechanism underlying these constraints will be another challenge for the future.

Conclusions

In contrast to the control of cell fate, segment number, or patterning, which is largely determined by genetic regulatory mechanisms, the control of size is influenced by genetic, hormonal, and environmental inputs. Understanding this phenomenon requires a combination of developmental genetic, physiological, and evolutionary approaches. Given the significant interest that has been generated in growth control, it should not be long before some of these old mysteries in biology are explained. This will not only reward us with a better understanding of this important aspect of developmental biology, but it will also provide better insight into human diseases, such as cancer, that are associated with a misregulation of cellular growth. ■

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