

Mechanisms of size control

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The study of organ size control is a discipline of developmental biology that is largely unexplored. Although the size of an organ or organism depends largely on cell numbers and cell size, studies have found that the simple deregulation of cell proliferation or cell growth does not necessarily lead to changes in organ size. Recent genetic screens in *Drosophila* suggest that mutations that do affect organ size can be classified into three broad categories on the basis of their underlying effects: patterning, proliferation, and growth. Overall, experimental data suggest that organ size might be regulated by a 'total mass checkpoint' mechanism which functions to link the regulation of cell size and cell proliferation. The mechanisms of organ size control could also be critical targets for evolutionary events or disease processes such as tumorigenesis.

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Abbreviations

Dpp	Decapentaplegic
dTOR	<i>Drosophila</i> target of rapamycin
IGF	insulin receptor growth factor
IRS	insulin receptor substrate
TSC	tuberous sclerosis

Introduction

Why is a Great Dane never the size of a Dachshund? Why is the left kidney usually the same size as the right? The mechanisms that control the size of an organ, and thereby determine the size of the organism, are fundamental aspects of biology that remain largely unknown. Although one of the most dramatic differences between species are their individual and organ sizes, the individual members of the same species have remarkably characteristic body and organ sizes, suggesting that genetic mechanism likely play key roles in regulating organ size. Very little is known about the elements that contribute to size differences between species, but recent studies suggest that intrinsic mechanisms might be conserved in regulating organ size. In theory, the size of an organ is determined by a contribution of cell size, cell number, and intercellular space. Comparisons between species, however, suggest that organ size is largely determined by cell numbers and cell size [1]. For example, an elephant heart is larger than a mouse heart because of an increase in cell numbers [2], whereas a small wing of some Hawaiian species of *Drosophila* is as a result of decreased cell size [3].

Clearly, the growth of an organ and organism requires external nutrition and growth signals. Classic studies suggest that, in most cases, external signals are not rate-limiting factors for determining organ size and that final organ size depends largely on mechanisms intrinsic to the organ [4]. Although the molecular natures underlying these intrinsic mechanisms are largely unknown, genetic studies in *Drosophila* are starting to identify genes affecting the mechanisms of size control. The focus of this review is to discuss these intrinsic mechanisms of organ size control. (For additional discussions on organ size control, the reviews by Conlon and Raff [1] and Su and O'Farrell are recommended [5].)

Transplantation experiments and the existence of a size control mechanism

A first clue that organs contained an intrinsic mechanism to determine size was based on transplantation experiments. In mammalian studies, it was found that infant rat hearts or kidneys transplanted into adult rats grew at the same rate and attained the correct size [6,7]. Similar results were also found in transplantation experiments with fetal organs of the mouse. For example, a fetal mouse thymus gland transplanted into a developing adult mouse grew to the normal adult size [2].

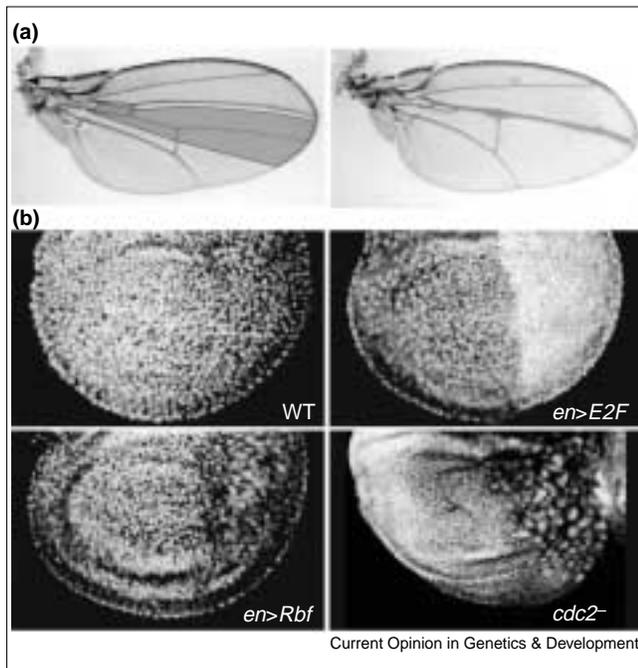
In experiments with salamanders, limbs or eyes exchanged from one smaller species of salamander to a larger species of salamander attained an organ size characteristic of the donor [8]. That is, the eyes or limbs originating from the smaller species were always smaller even when grafted onto the larger species.

In *Drosophila*, adult organs are derived from the imaginal discs. Imaginal discs are specialized epithelial structures that give rise to the adult organs of the fly. Each disc begins as a cluster of 10–50 cells within the embryo, which then undergo massive proliferation during larval development to produce a mature disc of between 30,000–100,000 cells. When immature imaginal discs from young larvae were transplanted into adult hosts, they attained a size and shape characteristic of the mature discs. Furthermore, when full-grown imaginal discs were transplanted into adult hosts, they did not continue to grow even after extended periods [9]. These experiments suggest that organisms from invertebrates to vertebrates contain a mechanism to regulate organ size, and that this mechanism is intrinsic to the organ primordia.

Organ regeneration experiments suggest organ size is actively monitored

These transplantation experiments, although suggesting an intrinsic mechanism for organ growth, do not rule out a mechanism by which organ size is attained merely as a

Figure 1



Organ size control maintains a constant mass. (a) Mutation of *collier* can lead to deletion of the shaded region, but does not alter wing size [19]. (b) Organ size is also maintained when cell proliferation is increased (*en>E2F*), decreased (*en>Rbf*), or even stopped (*cdc2⁻*) [21,22]. Changes in proliferation instead lead to changes in cell size to maintain a constant organ mass.

result of a fixed number of cell divisions. That is, the cells of the immature rat heart or *Drosophila* wing disc might be programmed to divide only a finite number of times and thus result in a characteristic final size. Regeneration experiments, in which part of an organ is removed, suggest that organ size is instead actively monitored. The best known and characterized process of regeneration is the mammalian liver (reviewed in [10]): the rat liver can regenerate to its original size following removal of two thirds of its mass. Similar results are found in experiments with *Drosophila* imaginal discs [9,11]. When lesions were induced in the imaginal discs, pupation was delayed until the discs had obtained their wild-type size. Furthermore, the wing imaginal disc could fully regenerate even when half of the disc was removed [4].

Furthermore, mouse embryos exhibit an amazing ability to combat experimental changes in cell number during development. For example, if two morulae are aggregated to form a giant blastocyst, organ size is maintained by a decrease in cell proliferation during later development, with no significant increase in apoptosis [12]. Likewise, if cell numbers of the early embryo are decreased — either by chemical damage or physical removal — then there is a general elevation in proliferation during later development to compensate [13].

Pattern-formation-dependent and -independent mechanisms of size control

A key question is then how does an organ know how big to be or when a part of it is missing? The transplantation and regeneration experiments were first to suggest that organ size might be determined by a genetic program within each organ. If this program were to be disrupted, it is then predicted that organ size would be altered. Experiments in *Drosophila* indicated that mutations affecting the regulation of pattern formation could deregulate organ size. For example, mutations that affect wing imaginal disc patterning can dramatically alter wing size. In the wing imaginal disc, the Engrailed and Hedgehog proteins are normally expressed in the posterior compartment of the wing, and the Decapentaplegic protein (Dpp) is normally expressed as a stripe along the anterior/posterior border (see [14] for review). Mitotic clones that remove engrailed function in the posterior region of the wing disc can lead to duplicated wings [15] (Figure 2). Similarly, ectopic expression of either Hedgehog or Dpp in the wing disc can also lead to duplicated wing structures [16]. In addition, the loss of Dpp or Hedgehog leads to a failure of the wing organ to grow [17]. Thus, the overall size of an organ can be severely affected by the activity of patterning genes.

Similar conclusions were also reached when *Drosophila* mosaic screens were used to identify overgrowth mutants. In a mosaic screen, mitotic recombination is induced in animals that are heterozygous for a newly generated mutation. The result is a population of cells that are homozygous mutant for the new mutation in a heterozygous background. As a consequence, an organ that contains many mutant cells can be scored for an effect on size. A number of mutations from mosaic screens were found to affect patterning. For example, mutation of *slimb*, a negative regulator of *dpp* and *wingless*, can lead to the formation of duplicated organs [18] (Figure 2).

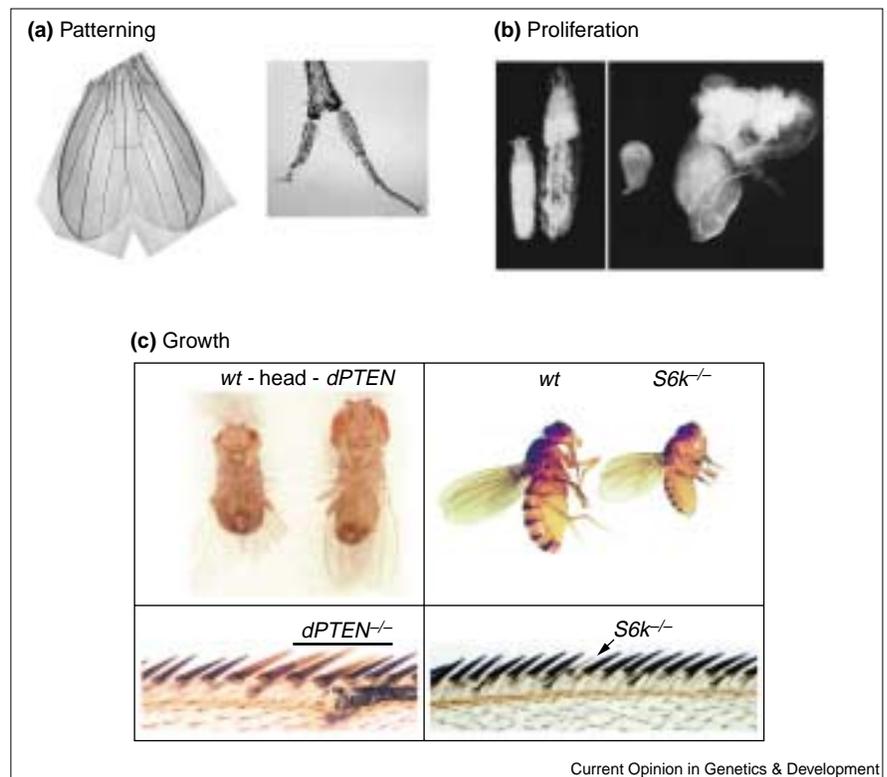
These data indicate that regulators of pattern formation must participate in defining organ size. However, other experimental data suggest patterning genes are not the sole players in monitoring organ size. For example, mutation of the *collier* transcription factor can lead to the loss of wing structures between the L3 and L4 interveins, and thereby disrupt the overall patterning of the wing. However, the overall size of the wing is unchanged, with the remaining structures filling in the missing space [19] (Figure 1). These data suggest that a mechanism separable from pattern formation most likely exists to regulate organ size.

Size-control mechanisms monitor total tissue mass instead of cell numbers

There are multiple experiments indicating that the mechanisms of size control can be distinguished from the simple regulation of cell proliferation. When cell proliferation was artificially increased in the wing imaginal disc by a ubiquitous pulse of *string/cdc25*, a phosphatase that promotes entry into mitosis, there was no final increase in cell number or a

Figure 2

The three classes of mutations that affect organ size. **(a)** Alterations in patterning genes can lead to duplications of organs. Mutant clones of *engrailed* can lead to a duplicated wing, and mutant clones of *slimb* can result in additional legs [18]. **(b)** Mutation of *lats* leads to an increase in proliferation, resulting in larvae and imaginal discs that are greatly enlarged [24]. **(c)** Mutations in the insulin pathway lead to changes in organ size by mainly affecting growth. Mutations of *dPTEN* lead to an increase in cell size and organ size. Mutant *dPTEN* bristles are larger than wild-type bristles. Mutation of *S6k* results in adult animals of smaller size [42]. An *S6k* mutant bristle is smaller than wild-type. Reprinted with permission from [42].



change in wing size [20]. Similar results were also found when cell cycling was affected in only one of the compartments of the wing [21]. When cell proliferation was increased in the posterior compartment by directed expression of *E2F*, there was an increase in cell number but also a decrease in cell size. As a result, the posterior compartment remained the same size (Figure 1). Conversely, slowing down the cell cycle by directed expression of the *Drosophila* homolog of the retinoblastoma family protein (Rbf) led to a decrease in cell number and an increase in cell size, but no change in compartment or wing size.

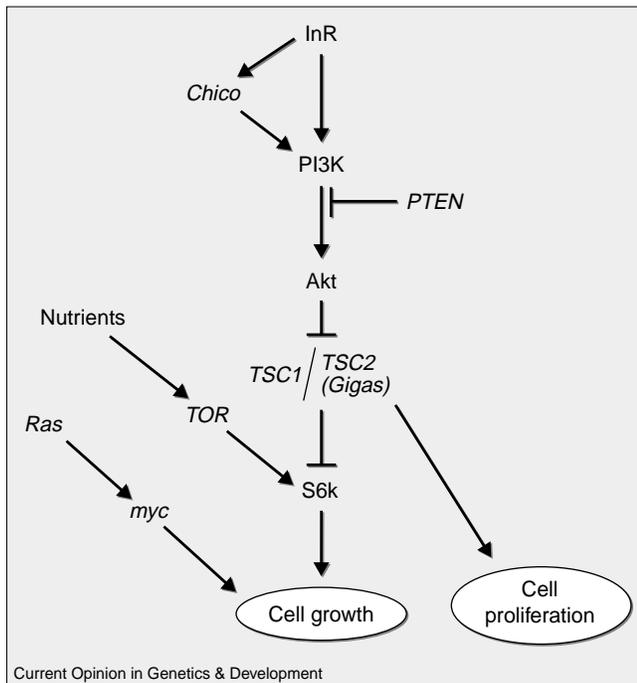
In a similar set of experiments, the function of Cdc2 kinase (which promotes entry into mitosis) was selectively removed from one of the wing compartments [22]. As a result, cell proliferation stopped, but cell growth continued. This led to a compartment that contained very few yet greatly enlarged cells and a normal organ size (Figure 1). These results suggest that altering cell proliferation does not necessarily lead to changes in organ size and that organ size is likely to be a function of organ mass, and not cell numbers.

This phenomenon is also supported by ploidy studies in newts and salamanders [23], which can vary from haploid to pentaploid, with an increase in cell size for every increase in ploidy. However, the organs from the adult tetraploid animal are the same size as the diploid animals, but instead contain half the number of cells. Thus, it

would appear that a major mechanism to regulate organ size would be a monitor of organ mass.

The preceding studies suggest that these known methods of regulating cell proliferation can be separated from organ size control. However, differences in organ size between species are most often a result of changes in cell number [2]. Thus, it is likely that organ size control does exert its effect through cell proliferation. It is possible that organ size control connects with the regulation of cell proliferation through a unique mechanism. If such a mechanism is disrupted, it is predicted that changes in cell numbers should now lead to changes in organ size. Interestingly, mutations such as *lats*, which fit such a prediction, were identified in a *Drosophila* mosaic screen [24]. Clones of the *lats* cells exhibit extensive overproliferation and develop into tumors, yet are still able to undergo some differentiation. Furthermore, homozygous *lats* mutant larvae are larger and contain imaginal discs that are dramatically increased in size as a result of an increase in cell numbers (Figure 2). The function of the *lats* genes are conserved from flies to humans as expression of human *LATS1* can rescue all fly mutant phenotypes [25]. Furthermore, genetic and biochemical studies indicate that one of the functions of *lats* is to regulate cell proliferation by inhibiting the activity of Cdc2 [25]. As discussed previously, although deregulation of Cdc2 activity at late imaginal development does not lead to changes in organ size, inactivation of Cdc2 early during development does decrease

Figure 3



Schematic representation of the insulin-signaling pathway. The insulin-signaling pathway regulates both cellular growth and cell proliferation. All components of the insulin pathway can also alter organ size. As S6k does not affect cell numbers, this suggests a branchpoint above S6k in this process. Arrows and bars represent genetic relationships, and do not imply a direct interaction.

organ size [22]. The detailed mechanism through which *lats* decouples cell proliferation from organ size control, however, is not well understood.

The role of cell growth in organ size control

As organ size appears to monitor tissue mass, the possibility is raised that changes in cellular growth might affect the regulation of organ size. Studies in *Drosophila*, however, suggest that the effect of cellular growth on organ size is complex: some changes in cellular growth do not change organ size, whereas other alterations in cellular growth do change organ size.

Recent studies in *Drosophila* have indicated that the *myc* and *Ras* genes can affect cell growth [26••,27]. For example, when *myc* and *Ras* were overexpressed in clones of the second instar wing disc and examined at the third instar stage, there was an increase in cell size but not an increase in cell numbers. Interestingly, the function of *myc* to promote cellular growth appears to be conserved, as overexpression of *myc* in human B-cells also leads to increases in cell size without increases in cell proliferation [28]. When *myc* was overexpressed in one compartment of the wing, however, the size of the wing was not changed. Thus, alterations in cellular growth in these examples was not sufficient to alter organ size.

However, a number of studies in *Drosophila* have provided strong evidence that changes in cellular growth in certain

instances can lead to changes in organ size. In fact, some of the overgrowth mutants that deregulate organ size in mosaic animals occur as a result of cellular growth deregulation. For example, organs that contain large percentages of *Drosophila PTEN* (*dPTEN*) mutant cells are larger than wild type (Figure 2) [29–31], a result of a 2–3-fold autonomous increase in size of the *dPTEN* mutant cells. Furthermore, overexpression of *dPTEN* leads to a cell-autonomous decrease in cell size and organ size. For example, overexpression of *dPTEN* in the posterior compartment of the wing leads to a decrease in both cell and compartment size [30]. Thus, in contrast to *myc*, alterations of *dPTEN* function might decouple an organ size control mechanism and now allow changes in cell mass to drive changes in organ size.

Interestingly, PTEN functions to negatively regulate insulin signaling in invertebrate and mammalian systems (see [32] for review). Indeed, altering the activities of any component of the insulin pathway can affect both cell and organ size in *Drosophila* (see [33] for review and Figure 3). For example, overexpression of the positive components of the insulin pathway — such as InR, PI3K, and Akt/PKB — lead to increases in cell and organ size. Furthermore, loss of function mutations in *Inr*, *Akt*, *chico*, and *S6k* lead to decreases in cell and organ size. With the exception of S6k, the known components of the insulin pathway also affect cell proliferation. However, the effects of insulin signaling on organ size are contributed mainly by its effects on cellular growth. A well characterized example of this is the *Drosophila* tuberous sclerosis (TSC) genes (CJ Potter *et al.*, unpublished data; see also ‘Update’), which have been found recently to be components of the insulin-signaling pathway (CJ Potter *et al.*, unpublished data). For example, large mutant clones of *TSC1* in the eye lead to a dramatic increase in eye size. An increase in cell size contributes 95% to this increase in eye size, whereas increases in cell number contribute only 5% (CJ Potter *et al.*, unpublished data).

The mechanism by which the insulin-signaling pathway can deregulate organ size is not fully understood. Phosphorylation of the small ribosomal S6 subunit by S6k enhances the ability of the ribosome to transcribe mRNAs that contain 5′ terminal oligopyrimidine tracts [34]. These tracts are found mostly in ribosome proteins and rarely in other types of RNA, suggesting that S6k activation leads to a general increase in protein synthesis and that this is the predicted reason for the increase in cell growth. However, mutations in ribosome proteins (Minutes) that decrease cell growth only lead to a developmental delay, but final organ size is not changed. Therefore, inactivation of S6k might somehow decouple cellular growth from organ size control.

Interestingly, flies that are raised under poor growth conditions phenocopy mutations in the insulin-signaling pathway, raising the possibility that there might be a genetic connection. Recently, a mosaic screen to identify genes that affect growth and organ size isolated mutations in *Drosophila target of rapamycin* (*dTOR*) [35••,36••].

Similar to components of the insulin pathway, *dTOR* mutants exhibit a decrease in cell and organ size; however, *dTOR* mutants also exhibited additional phenotypes, such as lipid vesicle aggregation and decreased nucleolar size that more closely resembled the phenotypes caused by poor growth conditions. These observations fit remarkably well with previous research that implicated TOR in S6k activation as well as a sensor for amino acid concentrations (reviewed in [37]). In fact, overexpression of *dS6k* could rescue *dTOR* lethality [35**]. Taken together, these studies suggest that *dTOR* might be a major sensor for nutrient conditions in flies and that it exerts its effects on cell growth and organ size by converging on the insulin-signaling pathway.

The functions of insulin signaling in the regulation of organ growth and size also appear to be conserved in mammalian systems as well. For example, in the mouse, mutation of insulin receptor (*InR* and *IGF1 receptor*), insulin receptor growth factors (*IGF1* and *IGFII*), insulin receptor substrates (*IRS-1* and *IRS-2*) or *S6k* lead to smaller organs and smaller animals (reviewed in [38]). Similarly, expression of an activated *PI3K* in the mouse heart can lead to an increase in heart organ size due to an increase in cell size [39**]. Likewise, expression of a dominant negative *PI3K* in the heart leads to a decrease in cell and organ size. Organ size in humans might also be regulated by insulin signaling. For example, babies from diabetic mothers exhibit increases in organ size, most likely as a result of an increase in insulin production [4]. Conversely, germline mutations of insulin receptor can lead to a disorder known as leprechaunism, which is characterized by a decrease in organ size and growth.

Model for a mechanism of size control

Current data have not yet led to a coherent hypothesis of how an organ determines its final size. Genetic manipulations in *Drosophila* that alter organ size, however, provide a starting point to develop working models for further discussion. It is now becoming clear that organ size most likely depends on total mass and not cell number [5]. It is possible that there exists a checkpoint for measuring organ size that depends on the total mass of the tissue (Figure 4). Such a 'total mass checkpoint' may function to insure a constant total mass by linking the regulation of cell number to cell size. In this model, cell size and cell number are affected by growth and proliferation signals, respectively. When cell numbers are increased abnormally, the total mass checkpoint functions to inhibit cell growth. Similarly, when cellular growth is increased abnormally, then cell proliferation is inhibited. Consistent with this model, overexpression of *E2F* stimulates abnormal cell proliferation, for which the total mass checkpoint mechanism compensates by inhibiting cellular growth. As a result, an increase in cell number leads to a decrease in cell size but not a change in total mass or organ size. When cell proliferation is decreased by overexpression of *Rbf*, the total mass checkpoint leads to decreased inhibition of cell growth, resulting in an increase

in cell size but no change in total mass. Similarly, when growth signals are increased by overexpression of *myc*, the total mass checkpoint causes an increase in the inhibition of cell proliferation. The outcome is an increase in cell size, but a decrease in cell proliferation.

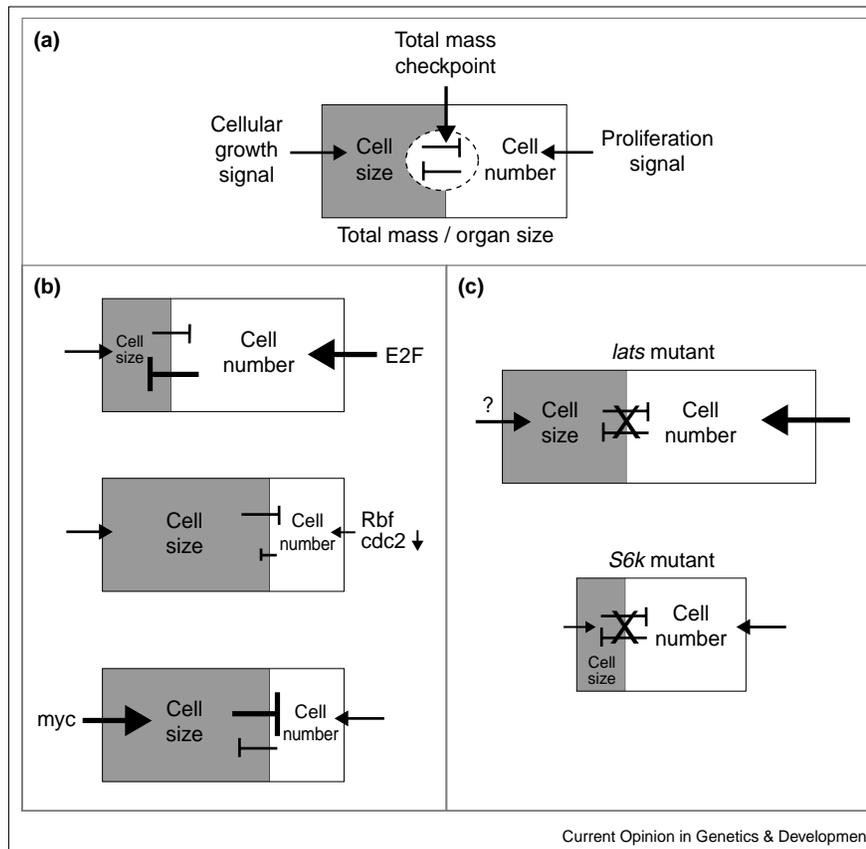
If the total mass checkpoint does not function properly, however, then organ size can be changed. This might be the situation for the *lats* mutant animals. In this case, we suggest that the overproliferation signal no longer leads to an inhibition on cell growth, and thus results in an increase in cell number and organ size. The *S6k* mutant could also decouple the total mass checkpoint such that the decrease in growth signals are no longer compensated by an increase in cell numbers. The result is a smaller animal with smaller cells. This model indeed raises some intriguing predictions. For example, as the *S6k* mutant no longer has a total mass checkpoint, overexpression of *E2F* in these flies should now lead to an increase in organ size because of an increase in cell numbers.

The molecular mechanisms by which an organ senses its size or total mass is not clear. It is not even known whether such a mechanism depends on direct contact between neighboring cells. For example, T-cells in mammals are tightly regulated such that constant numbers are maintained. Perhaps a mechanism similar to bacterium quorum sensing might function to regulate organ size for dispersed or solid organs.

The total mass for an organ may be specified by the genes that regulate pattern formation, such that alterations of these genes lead to a resetting of organ size. Thus, it is understandable that alterations of patterning genes, such as *slimb* and *dpp*, lead to changes in total tissue mass. Furthermore, enlarged imaginal discs caused by some mutations, such as the *Drosophila lethal giant larva* tumor suppressor, are most likely caused by alterations in patterning genes, as Dpp expression is altered in these discs [40]. After the initial specification by the patterning genes, the mechanisms that monitor organ size or total mass most likely function to coordinate cellular growth and proliferation. Mutations that disrupt such size-control mechanisms are likely to deregulate organ size yet not affect pattern formation. For example, it was found that increasing the size of a wing compartment by overexpression of *PI3K* led to an increase in the Dpp-responsive region but not the pattern of Dpp expression [41**]. Overall, these observations are consistent with hypothetical size control mechanisms that function downstream of patterning processes to coordinate cell proliferation and cellular growth in order to insure the correct size and total mass of the organ.

The total mass checkpoint could also be a major target in evolution for altering the size of an organism. For example, if the total mass checkpoint were increased, then a slight increase in proliferation (i.e. one extra round of cell division) could lead to a dramatic increase in organ and

Figure 4



Model for a total mass checkpoint in the regulation of organ size. (a) The total mass checkpoint functions to insure a constant total mass by linking cell numbers and cell size. (b) Abnormal changes in cell growth (*myc* overexpression) or cell proliferation (*E2F* overexpression) lead to a proportional inhibition on cell numbers or cell size. (c) We suggest that the total mass checkpoint is not functioning in *lats* and *S6k* animals such that changes in signaling now lead to changes in organ size. (See main text for details.)

animal size. This might also explain why changes in organ and animal size are most often associated with changes in cell number.

Size-control mechanisms and tumorigenesis

The study of tumor development has focused on the regulation of cell proliferation; however, tumorigenesis is a process of increased total tissue mass. Thus, in addition to alterations in cell proliferation, it is possible that deregulation of size-control mechanisms might also be essential in the process of tumor development. This idea is supported by the observation that many components of the insulin-signaling pathway are altered in tumorigenesis. For example, *PTEN* is one of the most frequently mutated tumor suppressor genes in a wide variety of human tumors [32]. The *TSC* genes also function as tumor suppressors in humans. In addition, activation of insulin receptor, PI3K, and Akt have also been linked to tumorigenesis [32]. Furthermore, the mammalian *lats* homolog, *Lats1*, also behaves as a tumour suppressor in mice. Therefore, the studies of size-control mechanisms are important for our understanding of cancer biology and for developing potential therapeutic agents.

Conclusions

The mechanisms that control organ size are beginning to be discovered. It is likely that a mechanism of size

control measures total tissue mass, and coordinates cell proliferation and cell growth to insure the correct organ size. At present, the molecules responsible for such organ-size regulation are unknown but further genetic experiments in *Drosophila* and mammalian systems could prove fruitful in their identification. Such molecules might provide important insights into the processes of evolution, developmental biology, and disease.

Update

During the preparation of this article, a description and re-examination of TSC mutants [43**] has been accepted for publication (see also [44]).

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In this study, *Drosophila* mutant for *TSC1* are described, and mutations of *Drosophila TSC2* are re-examined. It is found that mutations of *TSC1* and *TSC2* behave similarly and both lead to increases in cell size and cell numbers, but not changes in ploidy. Alterations in cell proliferation are most likely caused by affecting G₁. In fact, genetic experiments suggested interactions between *TSC1/TSC2* and G₁ cell-cycle components.
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Now in press

The work referred to in the text as CJ Potter *et al.*, unpublished data, is now in press:

45. Potter CJ, Huang H, Xu T: ***Drosophila* Tsc1 functions with Tsc2/gigas to antagonize insulin signaling in the regulation of cell growth, cell proliferation, and organ size.** *Cell* 2001, in press.
In this study, a *Drosophila TSC1* mutation is described. Mutation of *TSC1* results in an increase in cell size in both proliferating and differentiated cells, with a 2–3 fold increase in cell size by the adult stage. Flow cytometry analysis of mutant cells shows that this increase in cell size is not due to changes in ploidy. Organs that contain large *TSC1* mutant clones are also increased in size. It is found that *Drosophila TSC1* binds directly to *Drosophila TSC2*, and that co-overexpression of both genes is required to affect cell size, numbers, and organ size, suggesting that *TSC1* and *TSC2* form a functional unit. Genetic epistasis experiments also suggest that *TSC1/TSC2* function as components of the insulin signaling pathway to regulate cell size.