Control of growth and organ size in *Drosophila*

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Summary

Transplantation experiments have shown that developing metazoan organs carry intrinsic information about their size and shape. Organ and body size are also sensitive to extrinsic cues provided by the environment, such as the availability of nutrients. The genetic and molecular pathways that contribute to animal size and shape are numerous, yet how they cooperate to control growth is mysterious. The recent identification and characterization of several mutations affecting growth in *Drosophila melanogaster* promises to provide insights. Many of these mutations affect the extrinsic control of animal size; others affect the organ-intrinsic control of pattern and size. In this review, we summarize the characteristics of some of these mutations and their roles in growth and size control. In addition, we speculate about possible connections between the extrinsic and intrinsic pathways controlling growth and pattern.
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Introduction

The mechanisms that determine the shape of organs have long interested developmental biologists. This interest has led to the identification of "patterning" signal transduction pathways, such as those controlled by the secreted proteins Wingless/Wnt, Dpp/BMP/TGF-β, and Hedgehog. Until recently, however, little attention has been directed at the mechanisms that guarantee the correct size of an organ. This review focuses on recent advances in our understanding of the mechanisms that control growth and organ size in *Drosophila*.

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Abbreviations: BHLHZ, basic, helix-loop-helix, leucine zipper; PDZ, PSD95-Dlg-ZO1; MAGUK, membrane-associated guanylate kinase; LRR, leucine-rich repeat; RNAi, double-stranded RNA-interference.

We will describe genes that are involved in two different aspects of growth. The first class controls the synthesis of proteins and other metabolic processes: these genes affect both the rate of growth and the final size of the organ. Many of these genes respond to environmental cues such as nutrient availability and temperature, which we will refer to as "extrinsic" signals. The second class of genes determines the identity, pattern, structure, and final size of imaginal discs, the organs that give rise to the appendages of the adult fly; the effects of these genes on growth rates are less clear. We discuss disc intrinsic signals including Wingless and Dpp, as well as the regulation derived from communication between neighboring cells within the disc epithelium.

Growth is an increase in mass over time, and the term can describe mass increases of individual cells ("cell growth") as well as tissues, organs, or entire animals ("growth"). Growth is normally accompanied by an increase in cell number. We limit our discussion in this review to the regulation of cell growth, its coordination with the cell cycle, and the resulting overall organ and animal growth. Not discussed here are processes such as cell death that also contribute to determining the final size of an organ.[1]

During the growth of most organs, rates of cell growth and cell division are coordinated so that cell size does not change much over time. The mechanisms that match rates of cell growth with cell division are mysterious, and are only recently being addressed in metazoans. In the developing *Drosophila* wing disc, growth can be uncoupled from cell division. For example, when cell division is slowed or blocked, cells continue to accumulate mass (cell growth), and hence increase in size. Conversely, when cell division rates are accelerated by overexpression of specific cell-cycle regulators, cell growth rates are unaffected and the cells divide at a smaller size.[2] These observations demonstrate that, in metazoans, as in the unicellular yeast,[3,4] cell division rates do not drive growth. It is unclear whether the converse is true and cell growth is sufficient to drive cell division.[2,5]

What determines the rate of cellular growth? Clearly, the identity of a particular organ, the position of a cell within this organ and its interactions with neighboring cells all play important roles in determining growth rates. Although different models have been put forward to explain this local, organ-intrinsic control of growth, its molecular basis has remained elusive.[6–8] In addition to local control, cells also experience

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more global signals that control their growth rates.[7] Temperature and nutrient availability, for example, strongly influence growth rates and can affect the final size of many animals.

Much of the recent work on growth has been carried out using the wing and eye imaginal disc cells of *Drosophila* larvae. Disc cells are subject to similar cell-cycle and growth regulatory controls as mammalian cells, and all of the genes discussed in this review have mammalian homologs. The disc is a simple epithelium made up of a single sheet of cells, which takes on characteristic folds as it grows in size. During larval development wing discs grow from about 50 to 50,000 cells in 4 days, and this growth is tightly linked to the acquisition of cell fates. Below, we discuss several genes that apparently cooperate to control the growth of imaginal discs.

Control of growth rate and organ size

Insulin receptor signaling

The synthesis of new proteins is at the heart of animal growth during development. In flies, mutations in components of the translation machinery have long been known to reduce the rate of growth. Prominent examples are mutations in genes encoding ribosomal proteins (collectively known as *Minute* mutations) and ribosomal RNA (e.g., the *miniature* and the *bobbed* mutations; reviewed in Ref. 9). Additional genes involved in protein synthesis have been identified through a recent genetic screen for growth-defective mutants, including the translation factor eIF4A.[10] Hence, the protein translation apparatus constitutes a critical target for the pathways that control growth rate. Amongst these, the insulin receptor (Inr) signaling pathway has emerged in recent years as the major regulator of growth in *Drosophila*. Whereas vertebrates contain two related receptors (insulin receptor and insulin-like growth factor receptor-1; reviewed in Ref. 11), *Drosophila* has only one insulin-receptor (dInr).

Upon binding to a ligand, dInr activates an evolutionarily conserved signal transduction cascade. Intracellular transducers of this cascade include the adaptor protein "insulin-receptor substrate" (IRS/chico), the lipid kinase dp110, and the protein serine/threonine kinases PDK1 and Dakt (for a detailed discussion of the molecular functions of these proteins and of their interactions the reader is referred to the article by Kozma & Thomas [this issue of BioEssays]). Together with the pathway acting through dTOR and the ribosomal protein S6 kinase (dS6K), the dInr pathway controls the activity of the protein translation machinery. Mutations in any of the components listed above result in a reduction of the cellular and organismal growth rate. Similarly, overexpression of inhibitory components of the dInr pathway (the lipid phosphatase dPTEN and the inhibitor of the cap binding protein, 4E-BP) slows growth rates.

Signaling via Inr and IGF-1R in vertebrates is initiated by the binding of insulin and two insulin-like growth factors (IGF-1 and IGF-2), respectively. Until recently, no protein corresponding to IGFs or insulin was known in *Drosophila*. By searching the *Drosophila* genome database, Brogiolo and colleagues identified seven genes they named *D*rosophila insulin-like peptide (DILP) genes.[12] Based on the predicted amino acid sequences and protein domain structures, the DILPs are more closely related to vertebrate insulin than to IGF-1 or IGF-2. So far, no direct binding to dInr has been demonstrated for any of the DILPs; however, one appears to play an important role in growth control. Ubiquitous expression of DILP2 under the control of the hsp70 promoter leads to a significant increase in body mass.[12] Furthermore, DILP2 shows a strong genetic interaction with both dInr and Dakt:[12] the embryonic lethality caused by ubiquitous overexpression of DILP2 is suppressed by either heterozygosity for dInr, or by heteroallelic combinations of Dakt. Conversely, the increase in growth caused by overexpression of dInr in the developing eye is dominantly suppressed by a deficiency that removes DILP2. Although this deficiency also uncovers DILP1, DILP3, DILP4 and DILP5 as well as other genes, the importance of DILP2 for this suppression is demonstrated by the observation that reintroduction of DILP2 into this genetic background reverses the suppressive effect of the deficiency.

Taken together, these data suggest that the DILPs constitute physiological ligands for dInr. A hormonal function for at least some of the DILPs is further suggested by their pattern of expression during late larval stages. DILP2, DILP3 and DILP5 were found to be expressed in two (bilaterally symmetrical) groups of 7 cells in the brain hemispheres. These cells project into the ring gland, the compound endocrine gland of *Drosophila*,[13] from which the DILPs could be secreted into the hemolymph. A more detailed characterization of these cells and of the DILP translation products will be required to further validate this hypothesis. An equally important challenge will be the identification of the signals that regulate DILP levels. The observation that mutations in several dInr-pathway components phenocopy the effects of starvation supports the idea that nutritional status plays an important role in the control of dInr signaling and, by inference, DILP levels (e.g., Refs. 12,14,15). In this context, it is also intriguing that the levels of bombyxins, the insulins of the silk moth *Bombyx mori*, are controlled by blood glucose levels.[16] With the molecular identification of the DILPs, these upstream signals can now be experimentally addressed.

In addition to the DILPs, five factors have been isolated that are able to cooperate with human insulin in promoting the proliferation of cultured *Drosophila* S2 cells.[17] These factors were termed imaginal-disc derived growth factors (IDGFs) for their tissue of origin. IDGFs are related in primary amino acid sequence to chitinases, enzymes that hydrolyze chitin polymers. Since they do not contain a certain conserved amino acid in the active site, however, IDGFs are likely to be enzymatically inactive.[17] IDGFs are expressed in several

tissues in addition to imaginal discs, most notably the fat body, which was previously shown to emit a diffusible growth signal in response to nutrient status.[18] It is tempting to speculate that the IDGFs might take part in nutritional signaling. Future work is needed to address this possibility and establish the molecular details of an interaction with dInr signaling in vivo.

Other genes controlling growth

In addition to components of the basic translation machinery and the dInr pathway, several other genes, previously thought to act as regulators of the cell cycle, have recently been shown to control growth.

dMyc. *dmyc* encodes the single *Drosophila* homolog of the Myc family of proto-oncogenes.[19] These proteins are transcription factors of the BHLHZ family (basic, helix-loop-helix, leucine zipper), and they have long been known to promote cell-cycle progression and apoptosis, as well as cellular transformation.[20] Recent studies in *Drosophila* point to an additional role for Myc in the control of cellular and organismal growth. While a null mutation in *dmyc* is lethal, viable hypomorphic mutants have been isolated that show phenotypes similar to those described for dInr pathway components, including a reduction in cell size and number, resulting in smaller adult flies.[21] Conversely, ectopic expression of dMyc promotes growth, and exponentially proliferating imaginal disc cells overexpressing dMyc reach nearly twice the size of control cells. At the same time, dMyc expression also shortens G_1 phase of the cell cycle by inducing Cyclin E, the limiting regulator of the G_1/S transition in discs.[21,22] Interestingly, the overall doubling time of these cells is unchanged due to a compensatory lengthening of G_2.[21] Co-expression of dMyc with the protein phosphatase String (Stg)/Cdc25, rate limitor for entry into mitosis,[2] readjusted the cell-cycle profile and prevented the lengthening of G_2 phase, but not the increase in growth.[21] These experiments demonstrated that dMyc's role in the fly is primarily as regulator of cell growth, and that dMyc's effects on cell-cycle progression may be secondary to its acceleration of the cellular growth rate. Subsequent studies in vertebrate systems indicated that c-Myc also increases cellular growth rates.[23,24]

A molecular explanation for the effect of dMyc on growth is likely to require the characterization of its transcriptional targets.[20] So far, only one such target has been found in *Drosophila*, *pitchoune* (*pit*, Ref. 25). *pit* codes for a nucleolar DEAD-box RNA helicase, and it has been suggested that Pit is involved in ribosome biogenesis.[25] *pit* mutant flies arrest growth during larval development, although they survive for extended periods of time, indicating that Pit might be an important mediator of growth downstream of dMyc. In contrast, more than hundred Myc targets have been identified in vertebrates (see for example Refs. 20,26,27). Many of these correspond to components of the translation machinery, e.g., eIF4E and genes coding for ribosomal proteins (e.g. Refs. 20,26,27).

Ras. While signaling through Ras plays an important role in many different contexts during development (reviewed in Ref. 28), recently a direct role for Ras in controlling cellular growth was also suggested.[22] Overexpression of activated Ras (dRasV12) in clones of proliferating cells resulted in similar phenotypes as overexpression of dMyc. Indeed, dMyc protein (but not mRNA) levels were elevated in these clones, suggesting that the growth effect of Ras might be mediated by post-transcriptional regulation of dMyc.[22] A possible mechanism is suggested by recent reports that, in vertebrate tissue culture cells, Ras induces phosphorylation of c-Myc on serine 62 which in turn increases the stability of c-Myc protein by several-fold.[29,30]

Cyclin D/cdk4. Complexes between the regulatory subunit Cyclin D and the kinase Cdk4 (or Cdk6) are important cell-cycle regulators in vertebrates, and also involved in regulating growth. In the absence of this complex, cells are impaired in their progression through the cell cycle. Mice lacking either D-type cyclin or Cdk4 are viable but reduced in size. Biochemical and molecular studies have focused on pRB, a tumor suppressor and negative regulator of the cell cycle, as the major regulatory target of the Cyclin D/Cdk4 complex.[31]

Homologs for all these genes have been found in *Drosophila*. In contrast to vertebrates, however, flies contain only one D-type cyclin[32] and one associated kinase subunit (called Cdk4, Ref. 33). While no mutations have been described yet for *cyclin D*, a null mutation in *cdk4* was recently characterized.[34] Similar to mice, flies lacking Cdk4 are viable but reduced in size, due to a decrease in cell number. These defects can be partly rescued by reducing the dose of *rbf* (a *Drosophila* RB homolog; Ref. 35). Conversely, over-expression of Cyclin D together with Cdk4 coordinately accelerates the rates of cell-cycle progression and cell growth, resulting in more cells that retain their normal size.[36] When Cyclin D and Cdk4 were co-expressed in postmitotic cells in the eye disc, cell size was significantly increased.[36] Together, these experiments suggest that Cyclin D/Cdk4 complexes are true growth promoters: they stimulate both cellular growth and cell-cycle progression coordinately. In this respect, they differ from factors, such as dMyc, components of the dInr pathway and activated Ras, that promote growth without altering cell-cycle rates when overexpressed.

TSC1 and TSC2. Vertebrate TSC1 and TSC2 were initially identified as the two loci most frequently mutated in heritable forms of "tuberous sclerosis complex" (TSC), a disease characterized by tumorous growths called hamartomas.[37,38] The *Drosophila* homologs of the proteins encoded by these two genes, TSC1 and TSC2 (also known as Gigas), were identified by virtue of their sequence and their mutant

phenotype.[39–41] Proliferating and differentiating cells lacking either TSC1 or TSC2 are substantially larger than control cells, and progression through the cell cycle is significantly accelerated (in particular passage through G_1 phase). Lack of TSC1 in post-mitotic cells of the eye disc induces ectopic S-phases and mitoses, presumably caused by an increase in the levels of Cyclin E and Cyclin A.[40,41] Co-overexpression of TSC1 and TSC2 strongly inhibits growth and extends G_1 and, to a lesser extent, G_2 phase of the cell cycle. These results demonstrate a clear role for TSC1 and TSC2 in the regulation of growth and cell-cycle progression.

How do TSC1 and TSC2 fit in with other pathways controlling growth, in particular dInr signaling? Genetic epistasis experiments carried out by Potter, Tapon, and their colleagues[40,41] place TSC1/2 either in the dInr pathway between Dakt and dS6K, or in a parallel pathway that converges with dInr signaling to control growth (possibly at the level of dS6K). While the molecular mechanism for such an interaction between TSC1/2 and the dInr pathway is currently unknown, both human and *Drosophila* TSC2 contain putative phosphorylation sites for Akt (PG, unpublished observation; Ref. 42). It will be interesting to determine whether TSC2 is phosphorylated on this site in vivo, and what the consequences of this phosphorylation might be. In addition, TSC1/2 also interact genetically with *dmyc*, *cyclin D/cdk4*, and *ras*.[41] Together, these results provide an intriguing basis for additional experiments to address the molecular function of TSC1/2 in the control of growth.

Disc intrinsic control of growth and size: pattern organizers and cell–cell communication

While the dInr pathway controls how signals from outside the animal and outside the disc influence growth, the coordination of cell proliferation within imaginal discs is mediated by local signals that control cell–cell interactions.[43] Although all adult wings look essentially the same, cell lineages in discs are not fixed, and patterns of cell proliferation during wing development vary from disc to disc. Small clusters of cells within the disc, unrelated by clonal origin, progress through the cell cycle together, so that at any given time several neighboring cells will be in S phase, or in G_2.[44] Although they progress through S phase together, they may not all go through G_2 at the same rate: some cells will drop out of the cluster, while other nearby cells will enter it. This sort of disc-intrinsic lineage plasticity must be controlled via communication between neighboring cells, but the actual mechanisms at work are unclear. Below, we discuss different modes of disc-intrinsic growth regulation.

The influence of pattern organizers on growth

The organization of pattern in imaginal discs requires the activity of the signaling pathways activated by Wg, Dpp, Hedgehog, Notch, and EGF. Genetic analysis has demonstrated that most of these pathways influence growth, yet surprisingly little is known about how they do this. We restrict our discussion here to Wg and Dpp, since they are the primary pattern organizers of the wing itself. Wg and Dpp are expressed in two perpendicular stripes of cells in wing imaginal discs (Fig. 1). Both molecules act as secreted morphogens that form perpendicular, intersecting gradients across the disc. These gradients are critical for the instruction of cells about their location within the disc and the fate that each acquires. Thus, cells close to Wg or Dpp receive different information than cells that are farther away.[45–48]

Genetic manipulation of the Wg and Dpp pathways has revealed a tight link between their role in disc patterning and their role in its growth. In the wing disc, Wg and Dpp are both required for cells to form the wing pouch, the portion of the wing disc that forms the adult wing blade. Mutations in either pathway prevent cell proliferation within this region.[49,50] In mosaic wing discs, cells within the wing pouch that are mutant for the Dpp receptor, *tkv*, do not proliferate, although their neighboring, phenotypically wild-type cells proliferate normally.[50] When a Minute mutation is used to impair protein synthesis and growth rate specifically in the surrounding cells, however, *tkv* mutant cells are able to proliferate and form a clone. This behavior of *tkv* mutant cells indicates that they are unable to compete with wild-type cells for growth and survival factors (a phenomenon known as "cell competition"). Other mutants known to suffer from cell competition are defective in their growth pathways (such as components of the dInr pathway), suggesting that one role of Dpp signaling is to promote cellular growth.

Cells in the wing pouch with mutations that eliminate Wg signaling are unable to proliferate even when given a growth advantage.[49] However, if their death is prevented by expression of the Baculovirus caspase inhibitor, P35,[51] cells unable to transduce the Wg signal can proliferate, and in fact do so at a faster rate than wild-type cells (LJ, unpublished). This observation implies that Wg is necessary for cell survival, but not to promote cell growth and proliferation. Instead, overactivation of Wg signaling in the wing pouch leads to a slowing of cell division and growth (LJ, unpublished; Ref. 52), suggesting that, under normal circumstances, Wg activity is required to constrain growth rates.[52]

Activation of the Wg or Dpp pathways in cells that do not normally receive high levels of the signals can cause tremendous overgrowth.[53–56] Cells in these overgrowths proliferate faster than in the wild type. The overgrowth also delays development, which is probably due to a reprogramming of cell identities. Interestingly, the cells retain their normal size even though they are dividing at an increased rate (LJ & C. Martin-Castellanos, unpublished). Thus, even during aberrant overgrowth, rates of cell growth and cell division are tightly coupled.

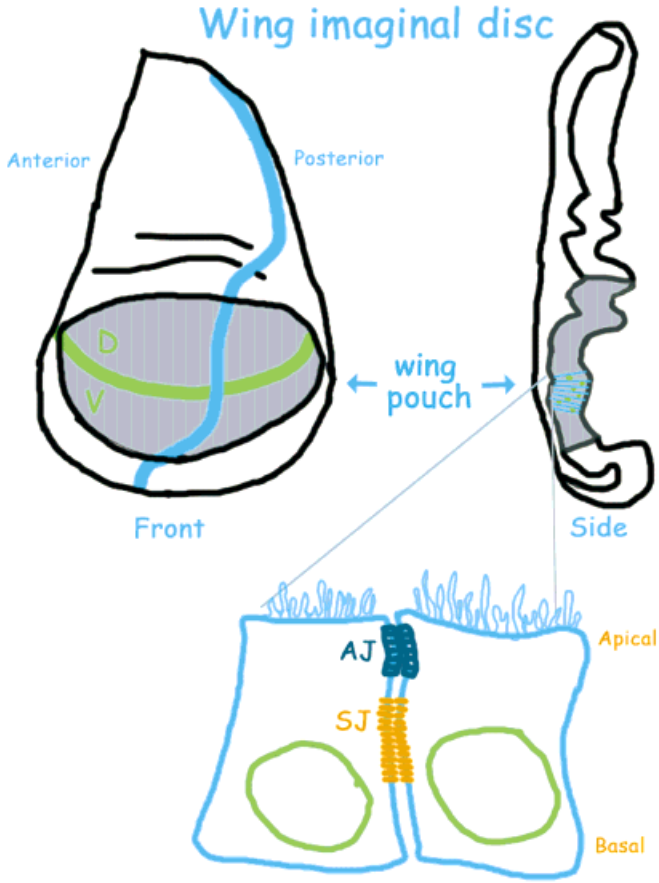

Figure 1. Scheme of a wing imaginal disc. Top: front (left) and side (right) views of the wing disc. The center of the disc (grey) is the wing pouch, which gives rise to the blade of the adult fly. Imaginal discs are divided into developmental compartments, shown here by the blue line (separating anterior cells from posterior cells), which also corresponds to the zone of Dpp expression, and the green line (separating dorsal from ventral cells), where Wingless is expressed. The disc is made up of a single sheet of columnar epithelial cells, seen in the side view, and takes on a series of characteristic folds as it grows in size. Bottom: structure of two cells, showing their apical–basal polarity. AJ, adherens junction;. SJ, septate junction; Green circles, cell nuclei.

Cell–cell communication and growth control

Imaginal discs are able to coordinate events between neighboring cells (such as a partial synchronization of cell-cycle phase) in part because the disc epithelium is a single layer of cells with defined apical and basal sides. This polarized architecture allows cells to share information by connecting neighboring cells with organized multiprotein structures such as adherens junctions, septate junctions, and gap junctions. The junctions provide the tools for anchoring the actin cytoskeleton of one cell to another, coordinating signaling between neighboring cells, and for clustering receptors and their ligands into signaling foci within the plasma membrane.[57] Connections between neighboring cells are critical for the maintenance of a functional epithelium such as the disc, but also need to be flexible to accommodate cell division, and folding of the disc as it grows.

Several *Drosophila* genes are classified as tumor suppressors since they cause overgrowth of certain tissues when mutated. In many of the mutants, imaginal discs are hugely overgrown with disorganized folding patterns (for a comprehensive list of these genes, see Ref. 58). Many of the tumor suppressor and the related overgrowth genes encode components of adherens and septate junctions (Fig. 1), thus linking control of growth with the structural integrity of the cell. Below, we discuss new information about a few of these genes, along with some possible links between their gene products and the machinery that controls cell proliferation.

Neoplastic mutants are required for epithelial integrity

Tumor suppressor mutants have been divided into two groups based on their overgrowth characteristics.[59] In the "neoplastic" class, cells lose their apical–basal polarity and the imaginal discs form a disorganized mass. In the "hyperplastic" class, epithelial cells retain their polarized structure and continue to grow as a single layer of cells, but discs dramatically overgrow and generate many additional folds.

Research within the last few years has revealed that three of the neoplastic tumor suppressor genes function together to establish and maintain the architecture of an epithelial cell. *lethal giant larvae (lgl)*, *discs large (dlg)*, and *scribbled (scrib)*

each encode components of the basal-laterally located septate junctions and, as a large complex, they are required to organize the apical–basal axis of epithelial cells. Individually, *lgl, dlg*, and *scrib* mutants each show characteristics of human neoplasms: loss of epithelial integrity, low adhesiveness, hyperproliferation and invasiveness (reviewed in Ref. 58).

The proteins encoded by these three tumor suppressor genes are large and structurally complex. Lgl (also called p127) is a large cytoplasmic protein that concentrates at the basal-lateral plasma membrane near septate junctions, and associates with the actin cytoskeleton. Dlg, enriched in septate junctions and in neuromuscular junctions, contains several protein–protein interaction domains: PDZ (*P*SD95-*D*lg-*Z*O1) and SH3 domains, a HOOK domain required for plasma membrane localization, and a guanylate kinase domain, which can bind GMP but appears not to function as a guanylate kinase.[60] Dlg is a member of the MAGUK (membrane associated guanylate kinase) family of proteins, which bind to a variety of cellular proteins involved in cell signaling, and serve as scaffolds for signaling complexes within diverse cell types.[61,62] Scrib, another sizeable protein, has leucine-rich repeats (LRR) and PDZ domains of the type found in Dlg; like Dlg, Scrib is concentrated in septate junctions.[63]

Lgl, Dlg and Scrib thus are all located at the basal-lateral plasma membrane, and depend on each other for this position: in mutants of any one of the three, the localization of the other two proteins is disrupted.[64] Furthermore, all three proteins are required for the formation of the apically located adherens junctions. Interestingly, this activity occurs from a distance. Through their multiple interaction domains, Lgl, Dlg and Scrib appear to assemble the apical–basal axis by forming a platform on which proteins are positioned at appropriate sites in the plasma membrane. In the absence of Lgl, Dlg or Scrib, the adherens junctions as well as the epithelial character of the cell is lost. The importance of junctional integrity in growth control is strikingly demonstrated in *scrib* mutants, where discs are severely disorganized and contain about five times more cells than normal.[63]

Dlg and Lgl interact with proteins that control the cell cycle

Just how the apical–basal polarity of epithelial cells exerts an effect on growth is not obvious. However, recent work in several different systems has provided links between Dlg and other tumor suppressors, and with cell-cycle regulation. The PDZ domains of both Dlg and Scrib are predicted to bind the tripeptide sequence S/TXV present in carboxy-terminal regions of many cellular proteins, including the tumor suppressors adenomatous polyposis coli (APC) and PTEN, and Protein 4.1 family members.[64–66] The human Dlg homolog, hDlg, co-localizes with APC in basal-lateral plasma membranes of both neuronal and colon epithelial cells, and through its second PDZ domain binds APC in vitro.[65] This latter interaction is potentially revealing because in mammalian cell culture APC negatively regulates progression through G_1 into S phase of the cell cycle, possibly by inhibiting the activity of Cyclin E/Cdk2.[67] Moreover, over-expression of hDlg in NIH 3T3 cells blocks the G_1–S phase transition, whereas mutants of APC that lack the hDlg-binding motif fail to do so.[68]

Does Dlg associate with APC to regulate cell division in flies? When overexpressed in wing discs, Dlg blocks cell proliferation and causes tissue loss,[60] although whether APC is involved is not clear. *Drosophila* has two APC genes, dAPC and E-APC (for epithelial-enriched APC, also known as dAPC2) but neither contains the tripeptide Dlg-binding motif.[69,70] Nevertheless, E-APC was recently identified as a component of adherens junctions of epithelial cells of the brain, and shown to be required for symmetrical division of those cells.[71] In epithelial cells, the orientation of the mitotic spindle is independent of apical–basal cell polarity and cells divide along a planar axis. In contrast, neuroblasts divide asymmetrically because their spindles are tightly linked to asymmetrically located proteins such as Numb and Prospero. Blocking E-APC function in the brain epithelial cells with RNAi resulted in connection of the mitotic spindle with the apical–basal axis, causing asymmetric cell division.[71] Thus, although as yet there is no direct link between Dlg and E-APC in control of growth, E-APC is required for an important aspect of epithelial cell division.

Recently, *scrib* and *dlg* mutants were identified as dominant suppressors of a hypomorphic *cyclin E* mutant phenotype. In this mutant, eyes take on a rough appearance due to fewer S phases during development.[72] Having the dosage of *scrib* or *dig* increases the number of S phase cells in the cyclin E mutant eye discs to nearly normal. (H. Richardson, personal communication). Although preliminary, these observations suggest that both Scrib and Dlg control cell proliferation via an interaction with Cyclin E. Since Scrib contains PDZ domains that recognize S/TXV, it is possible that like Dlg, Scrib interacts with APC. Together, these observations raise the possibility that APC, Dlg and Scrib function together in a complex to negatively regulate Gl/S transition, and provide a rudimentary mechanism for how all three function as tumor suppressors.

Another link between epithelial cell structure and cell division comes from the demonstration that Lgl also binds to NAP 1, a protein that, in *Drosophila*, is linked to cytoskeletal and mitotic spindle dynamics.[73] NAP 1 is cytoplasmic and associated with the cytoskeleton during interphase, but at mitosis becomes nuclear and binds to mitotic spindles.[73] In yeast, NAP 1 binds the mitotic cyclin, Cyclin B and, in the absence of NAP 1, cells cannot carry out Cyclin B-induced tasks at mitosis.[74,75] Thus, Lgl may have a role in cytoskeletal alterations prior to mitosis, and with assembly of the mitotic spindle. Whether Nap 1 interacts with mitotic cyclins in

Drosophila, and whether it plays a role in control of cell proliferation by Lgl remains to be determined.

Mutations in adhesion proteins cause hyperplastic overgrowth

Within the hyperplastic class of overgrowth mutants, proteins involved in cell–cell adhesion form the largest group. This group includes the cadherins, catenins, and the Protein 4.1 family and, with the exception of the 4.1 family, the proteins are located primarily in adherens junctions. The phenotypes of mutations in adhesion proteins differ from those of the neoplastic class in that epithelial cell integrity is generally maintained even though the discs overgrow.

Protein 4.1 family: Merlin/NF2 and expanded. Merlin/NF2 (Mer) and *expanded* (*ex*) encode members of Protein 4.1 family that co-localize in apical cell membranes.[76] These proteins organize the membrane actin network by linking transmembrane proteins to the cytoskeleton. *Ex* is mislocalized in Dlg mutants, suggesting that Dlg is required to anchor it in septate junctions.[76] Mutations in *Mer* and *Ex* cause excessive disc growth, and *Mer/ex* double mutants produce wings 30% larger than wild type.[76]

Cadherins and catenins. The transmembrane cadherins and their cytoplasmic associates, catenins, form the core of adherens junctions. Catenins link the cadherins with the actin microfilament network. β-catenin, known as Armadillo (Arm) in *Drosophila*, binds to the cytoplasmic tail of E-cadherin in adherens junctions.[57] α-catenin binds to Arm, and provides a bridge between the complex and the actin cytoskeleton. Although in *Drosophila* no α-catenin mutants that cause overgrowth exist, in mice, targeted loss of α-catenin in skin causes hyperproliferation and disordered proliferation of epidermal cells.[77] Arm/β-catenin is also a major effector of the Wingless/Wnt signal transduction pathway. How much Arm accumulates in the cell is under extremely tight regulation. Any non-membrane-associated Arm is bound by a multi-protein complex that includes Axin and APC, which targets Arm for degradation. Wingless signaling blocks this process, and allows Arm to accumulate in the cytoplasm and transduce a nuclear signal in collaboration with the DNA-binding protein, TCF. Activating mutations of mammalian β-catenin are highly oncogenic, and implicated in several types of cancer.[78]

Mutations in E-cadherin are frequently associated with invasive tumors in mice and humans; however, mutants of the closest *Drosophila* homolog, DE-cadherin, do not exhibit overgrowth phenotypes. However, other members of the cadherin superfamily have been implicated in growth control. The *fat (ft)* and *fat-like* mutants cause massively overgrown discs with convoluted folds that caricature the normal folding patterns of the disc. In *ft* mosaic wings, mutant clones contain many densely packed, small cells.[79] These mutant clones protrude out of the epithelium and produce massive extra folds, although the expression pattern of Wingless (Wg) and Decapentaplegic (Dpp) is not altered.[79] Another cadherin family member, *dachsous (ds)*, shows overgrowth phenotypes only when in *trans* to other overgrowth mutants, or when mutant cells are given a growth advantage.[79] Genetic interactions between *ft* and *ds* suggest that they may physically interact, although most cadherins engage in homophilic interactions.[80] The cytoplasmic regions of Ft and Ds share homology with the catenin-binding domain of vertebrate cadherin, suggesting that they could bind β-catenin/Armadillo.[80] In support of this idea, *ds* and *ft* mutations show genetic interactions with overexpressed Arm/β-catenin.[81]

Connections between extrinsic and intrinsic modes of growth control

In this review, we have discussed recent literature describing genes involved in growth control in *Drosophila*. Many of these genes act in distinct pathways: cell–cell communication, control of cell identity, and regulation of protein synthesis. Some receive input from the environment and other extrinsic cues, and others from more local, disc-intrinsic signals. Yet all of these genes and the pathways in which they function participate in the developmental control of organ size and shape. How do all of these pathways intersect? We would like to end this review with some speculations addressing this question.

Cell–cell communication and the cell cycle

Both the "neoplastic" and "hyperplastic" tumor suppressor mutants cause massive disc overgrowth, yet their gene products have different roles in cellular architecture and intercellular communication. Do both classes of genes use common mechanisms to regulate growth? Among these mutants, there are no reported cases of hypertrophy, or growth in the absence of cell division. In addition, cell proliferation rates have not been examined in detail in many mutants. *ex* mutant disc cells divide faster than normal,[82] however, and *ex* and *ft* mutations give rise to outgrowths with smaller cells than normal.[79,82] Thus in these two mutants at least, cell division is stimulated at a faster rate than the cells can grow. The genetic and biochemical interactions between Dlg, Scrib, APC, and Cyclin E provide some clues as to how the cell-cycle control machinery might be a target of regulation, but how these mutants also stimulate cell growth remains a mystery.

Why is the integrity of cellular junctional complexes so highly correlated with control of cell proliferation? One appealing explanation is that the junctions are required to localize foci of signaling molecules that are essential for growth regulation in each cell. Loss of such signaling centers can have drastic developmental outcomes. In the nematode, *C. elegans*, the EGF receptor, LET-23 is mislocalized in *lin-2*, *lin-7* or *lin-01* mutants and, as a consequence, signaling is

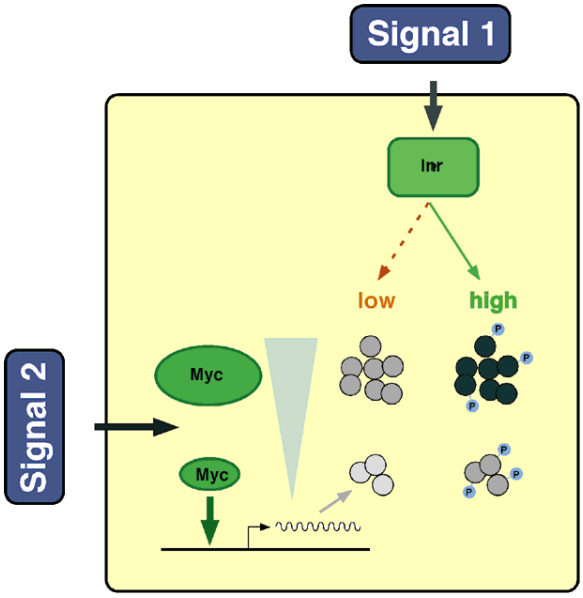

Figure 2. Model for cooperation between two growth-promoting pathways. Activation of Myc stimulates the transcription of genes coding for components of the translation machinery (indicated by grey circles); this induction may be graded depending on the levels of Myc activity. Upon activation, the Inr pathway post-translationally activates the protein translation apparatus (symbolized by a black "P" in a blue circle). Maximum stimulation of growth requires high levels of Myc and Inr activity, whereas intermediate growth may be induced by combinations of "high Myc" with "low Inr" and by "low Myc" with "high Inr".

impaired and vulval development is disrupted.[83] The Lin-2, Lin-7, and Lin-10 proteins form a ternary complex that binds EGFR/LET-23 through the PDZ domain of Lin 7, and localizes it to the basal-lateral plasma membrane of epithelial cells.[83] In *Drosophila*, EGFR is also found in a complex with Dlg and Scrib.[84] In addition, many *Drosophila* overgrowth mutants delay developmental timing to allow for the extra growth, rather than accelerate proliferation rates.[85] Thus, a critical role of intact cell junctions may be to allow cells to respond appropriately to developmental signals.

How are growth and cell division coordinated locally?

Experiments in yeast and *Drosophila* have led to the hypothesis that growth can drive cell-cycle progression.[2,4,86] However, this notion has been challenged by recent studies of growth promoters. Increasing levels of dMyc or components of dInr signaling substantially stimulates cell growth, but is not sufficient to drive cell division.[21,22,87] Although the potential for artifact always exists in over-expression experiments, a more likely explanation is that cell-cycle progression is controlled via two inputs: one at the G_1-to-S transition by growth regulators (such as dMyc and the dInr pathway) to increase Cyclin E levels, and the second at the G_2-to-M transition, by developmental signals.[21,52,88,89] In mature wing discs, where many of these experiments were carried out, passage through G_2 phase is rate limiting, and cells eventually arrest in G_2 presumably because Stg becomes scarce.[2,21,90] In contrast, in younger discs, cells are cycling very rapidly, and cell-cycle profiles indicate that cells spend more time in G_1 than in G2.[2] Conceivably, passage through G_1, controlled by input from growth signals, is limiting for cell-cycle progression at this stage. Consistent with this idea, in immature wing discs both *dmyc* and *stg/cdc25* mRNAs are expressed at high levels fairly uniformly (LJ, unpublished). It has been proposed that growth in imaginal discs is controlled locally through modulation of *dmyc* and *stg/cdc25* transcription by developmental signals such as Wg.[21] Both *dmyc* and *stg/cdc25* are under Wg control in wing discs: as part of a developmental cell-cycle arrest in the wing disc, expression of both *dmyc* and *stg/cdc25* are lost in a Wg-activity-dependent fashion.[21,52] Although *stg/cdc25* expression is repressed by the activity of proneural transcription factors induced by Wg, whether Wg regulation of *dmyc* is direct or indirect remains to be determined. In vertebrates, however, Wg signaling directly activates the transcription of a *c-myc* reporter transgene.[91]

How do the intrinsic and the extrinsic signals intersect?

A major gap in our knowledge concerns how both extrinsic and intrinsic inputs to growth are linked during development. However, an interesting possibility for collaboration exists between the dInr pathway and dMyc. Some of the proteins targeted at the post-translational level by Inr have been identified as transcriptional targets of mammalian c-Myc, including S6, other ribosomal proteins and the translation initiation factor eIF-4E.[26,27] Although our knowledge of dMyc targets is currently minimal, the vertebrate data suggest a scenario whereby activation of dMyc will increase the number of available substrates for the dInr pathway. In addition, the

rather moderate transactivation potential of c-Myc suggests that Myc might not act as a binary on/off-switch, but instead provide a graded response to sensitize a cell for the action of a second signal from dInr (Fig. 2). Situations may also exist where a second signal, for example from the Inr pathway, already acts at intermediate levels. In this case, activation of Myc may be sufficient to trigger cell growth and division (Fig. 2).

Drosophila has been a pioneering, genetic model system for many years, and once again leads the way in studies of growth control during animal development. We have described recent data regarding the cloning and characterization of several genes involved in growth regulation, and have put forward thoughts about how they might be linked to control organ and animal size during development. The future holds much promise for elucidating the control of growth at the genetic and molecular level.

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