The genetic control of organ growth: insights from *Drosophila*

David Weinkove* and Sally J Leevers†

The genetic control of growth ensures that animals grow to reproducible sizes and that tumourous growth is rare. This year, the regulation of organ growth has been studied extensively in *Drosophila* imaginal discs, and a signalling pathway that regulates organ growth and size has been identified. Furthermore, the role of *Drosophila* homologues to human tumour suppressor genes and oncogenes in imaginal disc growth has been investigated.

Addresses
*The Netherlands Cancer Institute, Department of Molecular Biology, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands; e-mail: weinkove@nki.nl
†Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, UK and the Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK; e-mail: sallyl@ludwig.ucl.ac.uk

Current Opinion in Genetics & Development 2000, **10**:75–80

0959-437X/00/$ – see front matter

Abbreviations

DS6K	*Drosophila* p70 S6 kinase
IGF	insulin-like growth factor
INR	insulin/IGF receptor
IRS	insulin receptor substrate protein
PI3K	phosphoinositide 3-kinase
Rb	retinoblastoma protein

Introduction

Understanding how animal growth is controlled is a major goal of biological research for two reasons. First, from a medical perspective, it is important to understand what goes wrong in the numerous diseases that involve the loss of growth control. Second, from a wider biological viewpoint, it is important to understand how genes control animal size [1]. Natural selection has ensured that, for most species, individual animals and their constituent organs grow to highly reproducible and characteristic sizes. In addition, the observation that size can vary considerably between related species suggests that although size is very consistent within a species, it is also sensitive to small genetic differences between related species. At present, little is known about how genes control growth during development to generate animals of consistent sizes. Here, we discuss recent studies in *Drosophila* imaginal discs, which investigate how tumour suppressor genes and oncogenes influence growth and which identify a signalling pathway that regulates organ growth and size.

The *Drosophila* imaginal discs, which are larval epithelial organs that are re-organised to form adult epidermal structures such as wings and eyes during metamorphosis, provide an attractive system to study growth control for several reasons. First, in the space of a few days prior to metamorphosis, the discs undergo a dramatic increase in mass and, like mammalian epithelial organs, become patterned while they grow [2,3]. Second, also in common with many mammalian organs, disc growth and size are governed not only by external signals, but also by intrinsic mechanisms [4]. These mechanisms enable discs to grow to their normal size even when growth is perturbed. For example, organ size is unaffected by inducing apoptosis in small regions, by delaying larval development or by transplanting discs into animals at different stages of the life-cycle [5–8]. Third, many genes that have been implicated in the control of normal and/or tumourous growth in mammals have close homologues that can be studied in *Drosophila*. Fourth and finally, various techniques can be used to modulate gene activity during disc development and to monitor the consequences: for example, mitotic recombination can be used to generate clones of disc cells lacking a particular gene (the equivalent of the somatic loss-of-heterozygosity found in many tumours) [9]. In addition, genes can be over-expressed in mitotic clones [10], in specific regions of the disc [11] or throughout the disc during its development. The impact of these interventions can be assessed in the adult, or during development, by microscopic analysis of intact discs and by flow cytometry of dissociated disc cells.

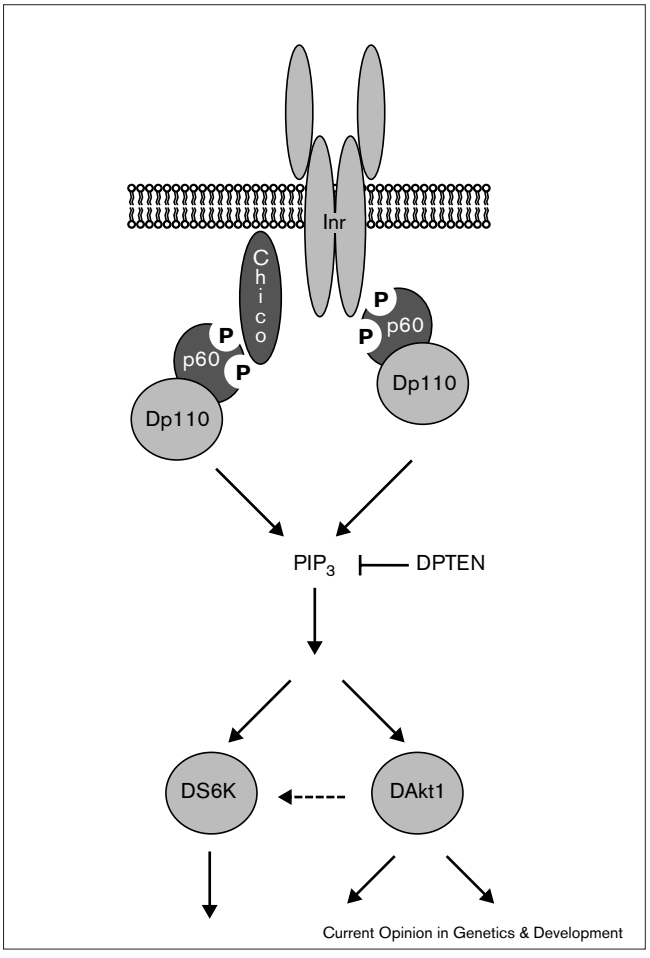
The role of the cell cycle

Animal and organ growth is generally accompanied by dramatic increases in cell number, not cell size. In addition, animals differ in size largely because of the number of cells that they contain, not the size of those cells [12]. Thus, growth has often been discussed in terms of the regulation of cell number — but unless cell division is accompanied by biosynthesis, organ growth will not occur. Consistent with this assertion, there is clear evidence that direct manipulation of the cell cycle in *Drosophila* imaginal discs does not alter organ growth [13–15]. In these experiments, the cell cycle was deregulated in one compartment of the wing imaginal disc by manipulating the activities of the cyclin-dependent kinase, Cdc2, the cell cycle transcription factors, E2F and DP, or the retinoblastoma protein, Rb. When division was slowed, the resulting compartments had fewer, bigger cells whereas when division was accelerated, the resulting compartments had more, smaller cells. In both cases, the compartments themselves were wild-type in size. These results demonstrate that the control of cell division is not the key mechanism through which growth is regulated. In addition, they suggest that whatever does control organ growth regulates and responds to organ size, rather than to cell number or cell size.

The role of protein synthesis

Although modulation of the cell cycle can affect the timing of cell division, growth ultimately requires biosynthesis. Thus, the regulation of biosynthesis could, in theory, be the

Figure 1



A signalling pathway controlling organ growth. Mutation of each of the molecules shown affects organ growth in *Drosophila*. At present, the evidence that these molecules form a signalling pathway comes almost exclusively from biochemical studies of mammalian homologues and genetic analyses of *C. elegans* homologues. In summary, activated Inr is predicted to phosphorylate itself and Chico on YXXM motifs, thereby generating docking sites for the SH2 domains of p60 [25••,27,28]. Thus, Dp110 is activated, phosphatidylinositol (3,4,5) trisphosphate (PIP_3) is generated, and the activities of the serine/threonine kinases DS6K and DAkt1 are increased. Like mammals and *C. elegans*, *Drosophila* possess a phosphoinositide-dependent kinase 1 (PDK1) homologue that probably mediates the activation of DS6K and DAkt1 by PIP_3 [49]. It is also likely that many of these molecules are activated by additional mechanisms and have additional targets.

mechanism through which organ size is regulated and tumour growth is prevented. Studies in *Drosophila* [16] suggest that although the level of general protein synthesis can influence the rate at which an organ grows, it does not necessarily affect final organ size. A class of mutations termed *Minutes* has been defined in *Drosophila* and several *Minute* genes have been cloned and shown to encode components of the ribosomal machinery. Heterozygous *Minute* organisms grow at a reduced rate. However, many of them achieve normal adult body sizes as a result of their developmental period being extended, and achieve normal cell sizes, as a result of their cell cycle time being increased [16,17]. In addition, although clones of wild-type imaginal disc cells induced in a heterozygous *Minute* organism outgrow their heterozygous *Minute* neighbours, cell size and final organ size are unaffected [17]. Similarly, mutation of the gene encoding the general translation initiation factor, EiF4A, delays larval development and slows disc growth, but does not affect cell size [18••]. In contrast to the effect of inhibiting global protein synthesis, recent results suggest that inhibiting the translation of a subset of mRNAs, through mutation of *Drosophila* p70 S6 kinase (*DS6K*), not only alters growth rates but also cell and organ size ([19••], see below).

A signalling pathway controlling growth?

An exciting advance in our understanding of growth regulation has been the demonstration that several molecules that are activated by insulin or insulin-like growth factors (IGFs) in mammals regulate disc growth and alter organ and organism size in *Drosophila* [20]. Biochemical analyses of the mammalian proteins and genetic studies in *Caenorhabditis elegans* suggest that these molecules form a signalling pathway (Figure 1). In mammals, this pathway has been implicated in the control of cell division and protein synthesis, as well as in the regulation of other processes that influence growth, including cell survival and glucose metabolism [21,22].

Initially, it was shown that flies with transheterozygous combinations of hypomorphic mutations in *Inr* (which encodes an insulin/IGF receptor) are smaller than wild type [23] and that over-expression of activated or dominant negative Dp110, a *Drosophila* phosphoinositide 3-kinase (PI3K) and target of insulin/IGF receptors, alters the size but not the shape or pattern of adult wings [24]. More recently, small flies containing small cells have been generated by null mutations in *chico*, which encodes an insulin receptor substrate protein (IRS) [25••], and in *DS6K*, a target of insulin/IGF and PI3K signalling in mammals [19••]. Interestingly, whereas *chico* flies contain fewer cells than wild-type flies, *DS6K* flies contain the wild-type number. This difference might arise for a number of reasons [20], one of which is that loss of *DS6K* can influence growth purely by reducing cell size and not cell number. S6 kinases phosphorylate the ribosomal protein S6 and thereby upregulate the translation of a subset of mRNAs containing 5′-terminal oligopyrimidine tracts [26]. Thus, in contrast to the presumed downregulation of general protein synthesis by mutation of the *Minutes*, altering the pattern of protein synthesis does seem to alter organ and cell size.

To establish whether this signalling pathway is required in the imaginal disc cells themselves, or is needed elsewhere to produce a systemic growth factor, mitotic clones lacking each gene were generated. Adult eye cells without *chico*, *DS6K*, or the genes encoding Dp110 or its adaptor p60 — which is predicted to mediate interaction of Dp110 with Chico and Inr [27,28] — are all smaller than the surrounding

Table 1

Control of organ growth.

Types of genes manipulated	Specific example	Effect on cell number	Effect on cell size	Effect on clone/comp size	Effect on final disc/organ size	References
Cell-cycle regulators	↓ Cdc2 activity	↓ (comp)	↑ (comp)	= (comp)*	= (disc)†	[13]
	↑ dE2F+dDP1activity	↑ (comp)	↓ (comp)	= (comp)	= (disc)	[14]
Protein synthesis regulators	Heterozygous null mutation of *Minutes*‡	↓ (clones)	= (clones)	↓ (clones)	= (disc, organ, fly#)	[14,16,39••]
Insulin/IGF/PI3K pathway	Homozygous null mutation in *DS6K*	↓ (clones)	↓ (clones)	↓ (clones)	↓ (disc, organ, fly§)	[19••]
	Homozygous null mutation in *chico*	↓ (clones)	↓ (clones)	↓ (clones)	↓ (disc, organ, fly**)	[25••]
	↓ Dp110 activity	↓ (clones)	↓ (clones)	↓ (clones, comp)	↓ (disc, organ)	[24,29••]
	↑ Dp110 activity	= (clones)††	↑ (clones)	↑ (clones, comp)	↑ (disc, organ)	[24,29••]
Others	Partial loss of function mutation in *dmyc*	↓ (clones)	↓ (clones)	↓ (clones)	↓ (organ, fly)	[32••]
	↑ dMyc activity	= (clones)	↑ (clones, comp)	↑ (clones), = (comp)‡‡	= (disc)	[32••]

A summary of the ways in which organ growth is affected by mutation or over-expression of some of the molecules discussed in this review. =, no change; comp, observations were made when activity was manipulated in one disc compartment; clones, observations were made when activity was manipulated in disc clones. For more details, refer to the original papers. *Although compartment size was sometimes reduced in these experiments, discs were observed in which cell size was increased, cell number was reduced and compartment size was maintained. †Although disc area was sometimes reduced in these experiments, discs were observed in which where cell size was increased, cell number was reduced and disc size was maintained. ‡Extrapolated from the phenotype of wild-type cells in a heterozygous *Minute* background. #Some heterozygous *Minutes* do have a reduced body size. §These adults contain small cells but the wild type number of cells. **These adults have cells that are reduced in size and number compared to wild type. ††Slight increases in cell number observed in adult wing after expression of Dp110 throughout wing development. ‡‡Although over-expression of dMyc in the posterior compartment increases cell size, posterior compartment size was not significantly altered, presumably because of compensatory

wild-type or heterozygous cells [19••,25••,29••]. Thus, these molecules function in the imaginal disc cells themselves, though they may have additional functions in other tissues. Similar results are seen in clones of cells lacking DAkt1, the *Drosophila* homologue of the downstream kinase Akt (J Verdu, MJ Birnbaum, personal communication), and flies with hypomorphic mutations in *DAkt1* are also small with small cells (H Stocker, E Hafen, personal communication). Importantly, as well as being required for normal growth, activation of this pathway is sufficient to increase growth. For example, when Dp110 is expressed in one compartment of the wing imaginal disc, the size of that compartment and the disc increases [29••]. This result is in direct contrast to the inability of cell-cycle manipulation to alter compartment and disc size (see above and [13,14]).

In mammals and *C. elegans*, PI3K signalling is antagonised directly by a lipid phosphatase encoded by the tumour suppressor gene, *PTEN* [30]. Consistent with these observations, mutation of *Drosophila* PTEN results in overgrown organs containing enlarged cells (D Goberdhan, C Wilson, personal communication). Thus, a pathway involving Inr, Chico, p60, Dp110, DAkt and DS6K, which is antagonised by PTEN, is likely to regulate disc growth and final organ size in *Drosophila*.

The molecules depicted in Figure 1 must also be regulated by other cellular components, forming a signalling network that regulates growth. One possibility is that, like mammalian Ras [31], *Drosophila* Ras interacts directly with PI3K, thereby enhancing its activation. Indeed, experiments in the wing disc have shown that over-expressing activated Ras increases clonal growth and cell size whereas over-expressing dominant negative Ras reduces clonal growth and cell size (D Prober, BA Edgar, personal communication). Another molecule that might interact with this pathway is the *Drosophila* homologue of the mammalian transcription factor and oncoprotein, Myc. It has recently been shown that *Drosophila* Myc (dMyc) also promotes clonal growth and increases imaginal disc cell size [32••]. Furthermore, *pitchoune*, a transcriptional target of dMyc also promotes growth. *pitchoune* encodes a protein homologous to RNA helicases, which are implicated in translation initiation and ribosomal RNA processing [33].

In Table 1, we have summarised the effects that some of the genes discussed here have on growth in the *Drosophila* imaginal disc.

The *Drosophila* tumour suppressor genes

Drosophila possess >50 tumour suppressor genes. The homozygous mutation of these genes induces over-growth in different tissues [34]. Several of the tumour suppressors that function in discs encode membrane-associated proteins and their mutation is postulated to induce over-growth by disrupting cell–cell contacts in the disc epithelia. In contrast, two recently identified *Drosophila* tumour suppressors that promote over-growth seem to

influence the cell cycle directly. Lats, also known as Warts, is a putative protein kinase that was first identified because of the dramatically enlarged outgrowths that form throughout the fly when both copies of *lats* are mutated [35,36]. The human and mouse homologues of *lats* have been cloned recently and there is evidence that their function is conserved — *lats* homozygous mice are highly susceptible to soft-tissue sarcomas that are often large and metastatic [37••]. Biochemical studies have shown that human LATS associates with and inhibits the activity of Cdc2 during early M phase [38••]. Thus, the mutation of *lats* is predicted to increase Cdc2 activity and to accelerate cell division. Consistent with this hypothesis, over-growth induced by mutation of *lats* in *Drosophila* is suppressed by loss of one copy of *cdc2* [38••]. At this stage, it is unclear how modulating the activity of Lats, in contrast to modulation of the cell cycle regulators discussed above, induces biosynthesis as well as proliferation, and hence results in dramatic over-growth.

Another tumour suppressor gene that influences the cell cycle and can also induce over-growth when mutated in *Drosophila* and mammals is *gigas*. Clones that are mutant for *gigas* contain enlarged cells that go through repeated rounds of endoreplication, and often cause enlargement of the discs [39••]. *Gigas* encodes a homologue of the tumour suppressor gene *TSC2*, and is one of two genes that result in tuberous sclerosis when one copy is mutated [40]. Patients with tuberous sclerosis suffer from benign tumours that arise from somatic loss of heterozygosity at either the *TSC1* or *TSC2* locus. Although the phenotype of *gigas* clones suggests that deregulation of the cell cycle can contribute to over-growth, it is likely that Gigas, which is predicted to encode an exchange factor for the small GTPase, Rap1, also affects other cellular processes.

The next steps

The experiments described in this review identify a signalling pathway that regulates organ growth, and provide new insights into how tumour formation is suppressed in *Drosophila*. One possibility that has yet to be addressed is whether signalling via the molecules shown in Figure 1 is enhanced when organ over-growth is induced through the mutation of *lats*, *gigas* and other tumour suppressors. Is the insulin/IGF/PI3K pathway activated during over-growth and is its activation necessary for over-growth? In addition, it will be interesting to determine whether signalling through this pathway participates in the intrinsic mechanisms that maintain organ size. For example, is the pathway upregulated when the growing organ is damaged and compensatory growth is induced?

Although a signalling pathway that can modify organ growth and size has been identified, we know little about the upstream regulators and downstream targets of this pathway. What controls the level and duration of signalling via this pathway during development, what are its targets, and how do they influence growth? Clearly, the regulation of protein synthesis is a probable target. However, DAkt1 homologues in mammals phosphorylate many substrates and influence various cellular processes [21], raising the possibility that this pathway has multiple effectors that enable it to influence organ growth.

Another important issue is how growth and pattern formation are coordinated during development. Perturbation of signalling via the insulin/IGF/PI3K pathway affects organ size without altering organ shape or pattern. In contrast, both imaginal disc growth and patterning are affected by modulating the activity of the ligands Notch, Wingless or Depentaplegic (a TGF-β homologue) [41–43]. It will be interesting to investigate whether insulin/IGF/PI3K signalling is required for Wingless, Decapentaplegic and Notch to direct growth as well as pattern formation. One possibility is that Wingless, Decapentaplegic and Notch synergise with insulin/IGF homologues during disc development [43]. Synergy between insulin and TGF-β signalling has been observed in mammalian cells [44] and in *C. elegans*, insulin and TGF-β signalling act together to control the decision to form dauer larvae [45]. In addition, a family of secreted factors termed 'imaginal disc growth factors', which synergise with insulin to stimulate the proliferation of cultured imaginal disc cells has recently been identified [46••]. Thus, disc growth and patterning might be coordinated through the synergistic activation of the insulin/IGF/PI3K pathway by a number of secreted molecules.

In addition, it is important that the hypotheses arising from these studies in *Drosophila* are tested in other animals. Mutation of a mouse homologue of *DS6K* [47] and of other molecules on the insulin/IGF/PI3K has been reported to reduce body size in mice [48]. As has been the case in *Drosophila*, the use of techniques to modulate gene activity in particular groups of cells during organogenesis should provide insights into the control of organ growth in mammals.

Conclusions

Studies in *Drosophila* have demonstrated that organ size is not controlled solely by regulating the cell cycle or by regulating general protein synthesis. However, a signalling pathway that can control organ growth and size, and that can alter both cell size and cell number, has been identified. This pathway may also participate in intrinsic mechanisms that normally maintain organ size and that prevent tumour formation.

Note added in proof

The works referred to as J Verdu, MJ Birnbaum, personal communication, and D Goberdhan, C Wilson, personal communication, have now been published as [50,51], respectively. (Furthermore, another paper on *Drosophila* PTEN has been published [52].)

Acknowledgements

We thank Carmen Coelho, Ian Conlon, Jennifer Rohn and Bart Vanhaesebroeck for helpful advice on this manuscript. D Weinkove is supported by the Human Frontiers Science Program Organization, SJ Leevers is supported by the Royal Society and the Ludwig Institute for Cancer Research.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Conlon I, Raff M: **Size control in animal development.** *Cell* 1999, **96**:235-244.

2. Madhavan MM, Scheiderman HA: **Histological analysis of the dynamics of growth of the imaginal discs and histoblast nests during larval development of *Drosophila melanogaster*.** *Wilhelm Roux's Arch* 1977, **183**:269-305.

3. Cohen S. **Imaginal disc development.** In *The Development of Drosophila melanogaster*. Edited by Bate M, Martinez-Arias A. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1993:747-842.

4. Bryant PJ, Simpson P: **Intrinsic and extrinsic control of growth in developing organs.** *Q Rev Biol* 1984, **59**:387-415.

5. Milan M, Campuzano S, Garcia-Bellido A: **Developmental parameters of cell death in the wing disc of *Drosophila*.** *Proc Natl Acad Sci USA* 1997, **94**:5691-5696.

6. Haynie JL, Bryant PJ: **The effects of X-rays on the proliferation dynamics in the imaginal wing disc of *Drosophila melanogaster*.** *Wilhelm Roux's Arch* 1977, **183**:85-100.

7. Simpson P, Berreur P, Berreur-Bonnenfant J: **The initiation of pupariation in *Drosophila*: dependence on growth of the imaginal discs.** *J Embryol Exp Morphol* 1980, **57**:155-165.

8. Garcia-Bellido A: **Larvalentwicklung Transplantierter Organe von *Drosophila melanogaster* im Adultmilieu.** *J Insect Physiol* 1965, **11**:1071-1078. [Title translation: Transplantation of developing larval organs into an adult environment.]

9. Xu T, Rubin GM: **Analysis of genetic mosaics in developing and adult *Drosophila* tissues.** *Development* 1993, **117**:1223-1237.

10. Pignoni F, Zipursky SL: **Induction of *Drosophila* eye development by decapentaplegic.** *Development* 1997, **124**:271-278.

11. Brand AH, Perrimon N: **Targeted gene expression as a means of altering cell fates and generating dominant phenotypes.** *Development* 1993, **118**:401-415.

12. Raff MC: **Size control: the regulation of cell numbers in animal development.** *Cell* 1996, **86**:173-175.

13. Weigmann K, Cohen SM, Lehner CF: **Cell cycle progression, growth and patterning in imaginal discs despite inhibition of cell division after inactivation of *Drosophila* Cdc2 kinase.** *Development* 1997, **124**:3555-3563.

14. Neufeld TP, de la Cruz AF, Johnston LA, Edgar BA: **Coordination of growth and cell division in the *Drosophila* wing.** *Cell* 1998, **93**:1183-1193.

15. Su TT, O'Farrell PH: **Size control: cell proliferation does not equal growth.** *Curr Biol* 1998, **8**:R687-R689.

16. Lindsley DL, Zimm GG: *The Genome of* Drosophila melanogaster. New York: Academic Press; 1992.

17. Morata G, Ripoll P: **Minutes: mutants of *Drosophila* autonomously affecting cell division rate.** *Dev Biol* 1975, **42**:211-221.

18. •• Galloni M, Edgar BA: **Cell-autonomous and non-autonomous growth-defective mutants of *Drosophila melanogaster*.** *Development* 1999, **126**:2365-2375.

This paper describes a genetic screen to identify genes required for normal larval growth. Several genes were identified, including an allelic series for the gene encoding the translation initiation factor EiF4A. Clones of cells mutant for EiF4A and several of the other genes described contain reduced numbers of cells but these are wild-type in size. Thus, this study shows that there are genes, which may be involved in protein synthesis, that are needed for growth but do not affect cell size.

19. •• Montagne J, Stewart MJ, Stocker H, Hafen E, Kozma SC, Thomas G: ***Drosophila* S6 kinase: a regulator of cell size.** *Science* 1999, **285**:2126-2129.

This paper describes how mutation of the gene encoding *Drosophila* S6 kinase (*DS6K*) in larvae results in smaller flies with smaller cells. These flies, however, have the same number of cells as wild-type flies because, although the mutant cells divide more slowly, development is delayed, and the normal cell number is reached. Organ growth may, therefore, be regulated by S6 kinase through effects on cell size and not number.

20. Leevers SJ: **All creatures great and small.** *Science* 1999, **285**:2082-2083.

21. Coffer PJ, Jin J, Woodgett JR: **Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation.** *Biochem J* 1998, **335**:1-13.

22. Shepherd PR, Withers DJ, Siddle K: **Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling.** *Biochem J* 1998, **333**:471-490.

23. Chen C, Jack J, Garofalo RS: **The *Drosophila* insulin receptor is required for normal growth.** *Endocrinology* 1996, **137**:846-856.

24. Leevers SJ, Weinkove D, MacDougall LK, Hafen E, Waterfield MD: **The *Drosophila* phosphoinositide 3-kinase Dp110 promotes cell growth.** *EMBO J* 1996, **15**:6584-6594.

25. •• Bohni R, Riesgo-Escovar J, Oldham S, Brogiolo W, Stocker H, Andruss BF, Beckingham K, Hafen E: **Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1-4.** *Cell* 1999, **97**:865-875.

This paper describes the identification the gene encoding Chico, a *Drosophila* insulin receptor substrate protein. Complete mutation of both copies of *chico* results in small but viable flies with reduced cell size and cell number. Mitotic clones of *chico* contain cells that are smaller than their heterozygous and wild-type neighbours. This paper provides further evidence for the role of an IGF/PI3K pathway in imaginal disc growth control.

26. Jefferies HB, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G: **Rapamycin suppresses 5′TOP mRNA translation through inhibition of p70s6k.** *EMBO J* 1997, **16**:3693-3704.

27. Weinkove D, Leevers SJ, MacDougall LK, Waterfield MD: **p60 is an adaptor for the *Drosophila* phosphoinositide 3-kinase, Dp110.** *J Biol Chem* 1997, **272**:14606-14610.

28. Fernandez R, Tabarini D, Azpiazu N, Frasch M, Schlessinger J: **The *Drosophila* insulin receptor homolog: a gene essential for embryonic development encodes two receptor isoforms with different signaling potential.** *EMBO J* 1995, **14**:3373-3384.

29. •• Weinkove D, Neufeld T, Twardzik T, Waterfield M, Leevers S: **Regulation of imaginal disc cell size, cell number and organ size by *Drosophila* class I_A phosphoinositide 3-kinase and its adaptor.** *Curr Biol* 1999, **9**:1019-1029.

This paper shows that *Dp110* and *p60* are essential genes and that their mutation, in mitotic clones, reduces cell size and cell number. Inhibiting Dp110 activity reduces the cell size in developing wing imaginal discs, whereas over-expression of Dp110 increases wing disc cell size, and causes cells to accumulate in G_2. Over-expression of Dp110 in the posterior compartment of wing imaginal discs increases the size of that compartment and of the disc as a whole. Thus, activation of Dp110 is both necessary and sufficient for imaginal disc growth.

30. Maehama T, Dixon JE: **PTEN: a tumour suppressor that functions as a phospholipid phosphatase.** *Trends Cell Biol* 1999, **9**:125-128.

31. Rodriguez-Viciana P, Warne PH, Vanhaesebroeck B, Waterfield MD, Downward J: **Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation.** *EMBO J* 1996, **15**:2442-2451.

32. •• Johnston LA, Prober DA, Edgar BA, Eisenman RN, Gallant P: ***Drosophila* Myc regulates cellular growth during development.** *Cell* 1999, **98**:779-790.

The experiments described here investigate how dMyc regulates growth. Loss of dMyc is shown to reduce growth and cell size, whereas over-expression increases growth and cell size. In addition, dMyc is shown to promote G_1/S progression but not G_2/M. Finally, Wingless is shown to negatively regulate Myc expression in a specific region of the developing wing disc.

33. Zaffran S, Chartier A, Gallant P, Astier M, Arquier N, Doherty D, Gratecos D, Semeriva M: **A *Drosophila* RNA helicase gene, *pitchoune*, is required for cell growth and proliferation and is a potential target of d-Myc.** *Development* 1998, **125**:3571-3584.

34. Bryant PJ, Watson KL, Justice RW, Woods DF: **Tumor suppressor genes encoding proteins required for cell interactions and signal transduction in *Drosophila*.** *Development* (Suppl.) 1993:239-249.

35. Xu T, Wang W, Zhang S, Stewart RA, Yu W: **Identifying tumor suppressors in genetic mosaics: the *Drosophila lats* gene encodes a putative protein kinase.** *Development* 1995, **121**:1053-1063.

36. Justice RW, Zilian O, Woods DF, Noll M, Bryant PJ: **The *Drosophila* tumor suppressor gene *warts* encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation.** *Genes Dev* 1995, **9**:534-546.

37. •• St John MA, Tao W, Fei X, Fukumoto R, Carcangiu ML, Brownstein DG, Parlow AF, McGrath J, Xu T: **Mice deficient of Lats1 develop soft-tissue sarcomas, ovarian tumours and pituitary dysfunction.** *Nat Genet* 1999, **21**:182-186.
The authors describe the phenotype of *Lats1* knock-out mice. Mutation of *Lats* has multiple effects, including an increased frequency of soft-tissue sarcomas, ovarian stromal cell tumours and high sensitivity to carcinogens. In flies, all *lats* mutant tissues over-grow, whereas, in mice, only restricted *Lats1* mutant tissues develop tumours. This paper, however, clearly demonstrates that a tumour suppressor identified in *Drosophila* has a tumour suppressor function in mammals.

38. •• Tao W, Zhang S, Turenchalk GS, Stewart RA, St John MA, Chen W, Xu T: **Human homologue of the *Drosophila melanogaster lats* tumour suppressor modulates CDC2 activity.** *Nat Genet* 1999, **21**:177-181.
This paper describes the identification and biochemical characterisation of the human homologue of Lats, LATS1. LATS1 is phosphorylated and associates with CDC2 in early mitosis, thereby inhibiting CDC2 activity. Consistent with these observations, mutation of one copy of *Drosophila* cdc2 suppresses imaginal disc overgrowth induced by loss of *lats*. Thus cell-cycle control may be an important mechanism by which Lats suppresses tumour growth.

39. •• Ito N, Rubin GM: ***gigas*, a *Drosophila* homolog of tuberous sclerosis gene product-2, regulates the cell cycle.** *Cell* 1999, **96**:529-539.
This paper shows that cells mutant for *gigas*, the *Drosophila* homologue of the tuberous sclerosis complex gene-2 (TSC2), are larger than surrounding heterozygous and wild-type cells. The *gigas* mutant cells replicate their DNA without division, which presumably enables them to increase in size. Although greatly increased in size, *gigas* mutant cells can still differentiate. When large *gigas* mutant clones were generated, the imaginal discs became enlarged, though this effect was accompanied by an extension of the larval period. The *Drosophila* homologue of the TSC1 gene is also identified.

40. Young J, Povey S: **The genetic basis of tuberous sclerosis.** *Mol Med Today* 1998, **4**:313-319.

41. Dominguez M, de Celis JF: **A dorsal/ventral boundary established by Notch controls growth and polarity in the *Drosophila* eye.** *Nature* 1998, **396**:276-278.

42. Burke R, Basler K: **Dpp receptors are autonomously required for cell proliferation in the entire developing *Drosophila* wing.** *Development* 1996, **122**:2261-2269.

43. Serrano N, O'Farrell PH: **Limb morphogenesis: connections between patterning and growth.** *Curr Biol* 1997, **7**:R186-R195.

44. Krymskaya VP, Hoffman R, Eszterhas A, Ciocca V, Panettieri RA Jr: **TGF-beta 1 modulates EGF-stimulated phosphatidylinositol 3-kinase activity in human airway smooth muscle cells.** *Am J Physiol* 1997, **273**:L1220-L1227.

45. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G: **The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*.** *Nature* 1997, **389**:994-999.

46. •• Kawamura K, Shibata T, Saget O, Peel D, Bryant PJ: **A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disc.** *Development* 1999, **126**:211-219.
This study identifies a family of secreted factors termed imaginal disc growth factors (IDGFs) that stimulate the proliferation, polarisation and motility of cultured *Drosophila* imaginal disc cells. The IDGFs are homologous to chitinases, but have an amino acid substitution predicted to abolish catalytic activity. Importantly, recombinant IDGFs synergise with insulin to stimulate the proliferation of a wing imaginal disc derived cell line.

47. Shima H, Pende M, Chen Y, Fumagalli S, Thomas G, Kozma SC: **Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase.** *EMBO J* 1998, **17**:6649-6659.

48. Lamothe B, Baudry A, Desbois P, Lamotte L, Bucchini D, De Meyts P, Joshi RL: **Genetic engineering in mice: impact on insulin signalling and action.** *Biochem J* 1998, **335**:193-204.

49. Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, Norman DG, Gaffney P, Reese CB, MacDougall CN, Harbison D *et al.*: **3-Phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the *Drosophila* DSTPK61 kinase.** *Curr Biol* 1997, **7**:776-789.

50. Verdu J, Buratovich MA, Wilder EL, Birnbaum MJ: **Cell-autonomous regulation of cell and organ growth in *Drosophila* by Akt/PKB.** *Nat Cell Biol* 1999, **1**:500-506.

51. Goberdhan DCI, Paricio N, Goodman EC, Mlodzik M, Wilson C: ***Drosophila* tumor suppressor PTEN controls cell size and number by antagonizing the Chico/PI3-kinase signaling pathway.** *Genes Dev* 1999, **13**:3244-3258.

52. Huang H, Potter CJ, Tao W, Li D, Brogiolo W, Hafen E, Sun H, Xu T: **PTEN affects cell size, cell proliferation and apoptosis during Drosophila eye development.** *Development* 1999, **126**:5365-5372.