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Genetic control of size in *Drosophila*

Sean Oldham, Ruth Böhni, Hugo Stocker, Walter Brogiolo and Ernst Hafen*

Zoological Institute, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

During the past ten years, significant progress has been made in understanding the basic mechanisms of the development of multicellular organisms. Genetic analysis of the development of *Caenorhabditis elegans* and *Drosophila* has unearthed a fruitful number of genes involved in establishing the basic body plan, patterning of limbs, specification of cell fate and regulation of programmed cell death. The genes involved in these developmental processes have been conserved throughout evolution and homologous genes are involved in the patterning of insect and human limbs. Despite these important discoveries, we have learned astonishingly little about one of the most obvious distinctions between animals: their difference in body size. The mass of the smallest mammal, the bumble-bee bat, is 2 g while that of the largest mammal, the blue whale, is 150 t or 150 million grams. Remarkably, even though they are in the same class, body size can vary up to 75-million-fold. Furthermore, this body growth can be finite in the case of most vertebrates or it can occur continuously throughout life, as for trees, molluscs and large crustaceans. Currently, we know comparatively little about the genetic control of body size. In this article we will review recent evidence from vertebrates and particularly from *Drosophila* that implicates insulin/insulin-like growth factor-I and other growth pathways in the control of cell, organ and body size.

Keywords: insulin/IGF-I; Chico/IRS; PTEN; growth; nutritional sensor; *Drosophila*

1. FACTORS CONTROLLING BODY AND ORGAN SIZE

Body size is determined genetically. Within species, the genetic constitution of the individual, which controls the processes of growth, proliferation and apoptosis, dramatically controls body size (Conlon & Raff 1999). For instance, mice that lack growth hormone (GH), insulin-like growth factor-I (IGF-I), the IGF-I receptor (IGFR), or insulin receptor substrate (IRS-1) are dramatically reduced in size (Rimoin *et al.* 1966; Araki *et al.* 1994; Tamemoto *et al.* 1994; Efstratiadis 1998). Some mutations in the gene coding for the insulin receptor cause a form of growth retardation in humans known as leprechaunism (Taylor 1992). In addition to genetic determinants, environmental factors play a critical role in the control of body size. Nutrition is paramount in controlling organ and body size in all species. Limited caloric intake reduces growth in species as diverse as yeast and man.

Organ growth is regulated at many levels during development. First, the growth of organs is tightly coupled with the final body size (Stern & Emlen (1999), and references therein), and organ size is also influenced by the presence or absence of other tissues. For example, in butterflies, the size of the forewings is influenced by the presence of other competing tissues such as the posterior wing imaginal discs. If these discs are removed surgically during development, the forewing becomes larger (Nijhout & Emlen 1998). In mammals, organ size is differentially regulated in different organs. When foetal spleens are transplanted into a mouse embryo each will grow such that the sum of the spleen equals a spleen of normal size (Metcalf 1964). In contrast, when the same experiment is carried out with thymus, each thymus grows to the size of one thymus (Metcalf 1963). Second, growth is tightly coupled to pattern formation. Patterning processes must be coordinated with growth to orchestrate the final size and shape of the organ (Bryant & Simpson 1984). Induction of ectopic pattern elements like the duplications of chick limbs and *Drosophila* wings by ectopic expression of proteins of the Wingless or Hedgehog family is associated with additional growth (Riddle *et al.* 1993; Basler & Struhl 1994; Zecca *et al.* 1995). Third, alterations in cell–cell contacts can alter growth. Proteins associated with septate or adherens junctions also determine organ size by suppressing growth (Bryant *et al.* 1993; Bryant 1997). Mutations in *discs-large*, *expanded* and *lats* (also known as *warts*) result in tumorous outgrowths of imaginal discs (Woods & Bryant 1991; Boedigheimer & Laughon 1993; Xu *et al.* 1995). Fourth, apoptosis may also play a role in organ growth; blocking apoptosis in the compound eye of *Drosophila* results in the generation of additional cells (Hay *et al.* 1994). In the development of the nervous system, programmed cell death is an essential factor in determining the final number of neurons and support cells (Raff 1996). These few examples illustrate the multitude of extrinsic and intrinsic levels that operate in organ growth control.

2. GROWTH AT THE CELLULAR LEVEL

Growth is associated with an increase in biomass through the stimulation of the biosynthesis of cellular components. Growth can occur in the absence of cell division by cell enlargement and by the deposition of extracellular matrix (accretion), but the most common

*Author for correspondence (hafen@zool.unizh.ch).

type of growth during development is coupled to cell division (Roush 1996; Neufeld & Edgar 1998; Polymenis & Schmidt 1999). When a cell divides, it will normally generate two daughter cells of half the size. These daughter cells then grow until they have reached the same size as the mother cell before they enter the next round of cell division. Therefore, growth—increase in biomass—is tightly coupled to cell-cycle progression. Given the importance of hyperplastic growth for tumour development, studies of growth control over the past years have primarily focused on the control of cell proliferation. It has been assumed that cell-cycle progression is an important regulator of growth. However, elegant experiments in *Drosophila* (Weigmann *et al*. 1997; Neufeld *et al*. 1998) have reminded us recently that the rate of growth regulates the rate of proliferation and not the contrary. Neufeld *et al*. induced genetically marked clones of cells expressing genes whose products promote (E2F and Dp) or slow down (Rb) cell-cycle progression. The overall growth was determined by measuring the area occupied by the cell clone in the imaginal disc after a fixed time. Acceleration of the cell cycle by overexpression of E2F and Dp promoted cell proliferation but not growth. The clone occupied the same area with more but smaller cells. Conversely, slowing down the cell cycle by expression of Rb produced fewer but larger cells without affecting overall growth. These results are consistent with classical experiments in yeast that demonstrated that growth rates determine the rate of proliferation and not vice versa (Nurse 1975; Johnston *et al*. 1977).

3. SOME OF THE BURNING QUESTIONS ON GROWTH CONTROL

One area concerns the regulation of cellular size. During normal growth, the size of the cells remains constant. Therefore, a cell must be able to determine when it has reached a certain size to initiate the next round of cell division. How do cells measure their size and what determines the critical size for cell-cycle progression? How is cell size regulated in response to extracellular cues? In yeast, the critical size when a cell undergoes cell division is dependent on the availability of nutrients. When yeast cells are placed on poor media, they divide at a smaller critical size (Johnston *et al*. 1977). Starved *Drosophila* larvae also develop into small flies that contain fewer and smaller cells than flies reared under non-starving conditions (Robertson 1959, 1963; Simpson 1979). At the level of the organ growth, how is the final size of an organ determined? What are the factors and signalling pathways that coordinate cell intrinsic and extrinsic growth of organs? There is a genetic programme that determines that a wing of a fly is larger than a haltere. Organ size determination appears not to be based on a cell-counting mechanism since organs of normal size can be formed by an increased number of genetically altered smaller haploid cells (Santamaria 1983). The last area concerns the control of body size. What are the evolutionary selective pressures that form body size? What are the cues that initiate and terminate growth in mammals to specify final body size? Clearly nutrition is a prerequisite, but other factors must dictate the production of GH, for example. Genetic dissection of growth control in genetically amenable organisms such as *Drosophila* may provide answers to some of these questions.

4. MUTATIONS AFFECTING GROWTH IN *DROSOPHILA*

In *Drosophila*, a number of mutations have been identified that affect growth at different levels. They can be divided into three different classes. The first class contains mutants that slow down overall growth but do not alter the final body size. The dominant *Minute* (*M*) mutations belong in this class. They cause a developmental delay and result in short, slender bristles (Lindsley & Zimm 1992). Many *M* genes have been shown to encode ribosomal proteins (Saeboe-Larssen *et al*. 1998). This suggests that *M* mutations slow down growth by reducing the efficiency of the translational machinery (Morata & Ripoll 1975). It is interesting to note that impairing translation by *M* mutations affects neither cell size nor body size. The second class contains mutations that affect the growth of individual organs. All the mutations known in this class affect organ size indirectly either by altering the organ identity like homeotic mutations, or by preventing differentiation and survival of specific cell types within an organ. The third class contains mutations that reduce overall body and organ size. One member of this class is *diminutive* (*dm*). The *dm* gene encodes the homologue of the c-*myc* proto-oncogene and has recently been shown to control growth rates and cell size (Gallant *et al*. 1996; Johnston *et al*. 1999). Another interesting member is the neurofibromatosis type tumour suppressor protein (NF1). Mutations in *NF1* result in flies that are reduced in size at all developmental stages (The *et al*. 1997). Lastly, the diffusible gas nitric oxide has been implicated in the negative control of proliferation (Kuzin *et al*. 1996). It is this third class of mutations that is most relevant for the understanding of the coordination of growth in *Drosophila*. In the following sections, we will summarize recent work on the role of the various signalling pathways that control growth without altering cell fate specification or pattern formation in *Drosophila*.

(a) *The insulin-signalling pathway*

(i) *Chico, the* Drosophila *IRS1–4 homologue controls cell size and overall body size*

In a search for mutations that result in a reduction of body size, we identified mutations in the gene coding for the homologue of the vertebrate IRSs 1–4 (Boehni *et al*. 1999). IRS proteins are multi-adaptor proteins containing an N-terminally located pleckstrin homology (PH) domain and a phosphotyrosine binding (PTB) domain as well as docking sites for SH2 domain-containing proteins including GRB2/DRK, which activate the Ras–mitogen-activated protein kinase (MAPK) pathway, and p85 adaptor that binds to the p110 phosphoinositide-3-kinase (PI3K) (White 1998). The activation of PI3K stimulates the synthesis of phosphatidylinositol-3,4,5 trisphosphate (PIP_3) (Whitman *et al*. 1988). The IRSs probably bind the activated insulin and IGFRs via their PTB domains and to PIP_3 in the plasma membrane via their PH domain (White 1998).

Flies homozygous for *chico* are approximately half the size of wild-type flies (figure 1). The reduction in overall

Figure 1. *chico* mutants are dramatically reduced in body size. *chico* homozygous mutant flies (−/−) are only about half the size of heterozygous siblings (+/−). Note that the reduction in size is proportional.

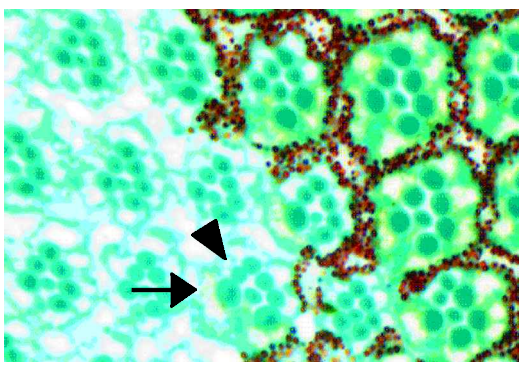

Figure 2. Autonomous cell size reduction in eye clones mutant for *chico*. Tangential section of the adult fly eye. Inspection of homozygous mutant tissue, marked by the absence of pigment, reveals that the cells are about half the size of normal cells. The presence of ommatidia consisting of heterozygous (arrow) and homozygous mutant (arrowhead) cells at the border of the clone indicates that *chico* function is autonomously required in each cell.

body size is due to a reduction in cell size and cell number. Since cell size and organ size are controlled by intrinsic and extrinsic factors, the loss of *chico* function may alter the production or secretion of humoral growth-promoting factors or the response of individual cells to these growth-promoting factors. The generation of *chico* mutant clones in a heterozygous background could distinguish between the different sites of action. Loss of *chico* function affects cell size in a cell-autonomous manner. This is most clearly seen in *chico* mutant clones in the eye,

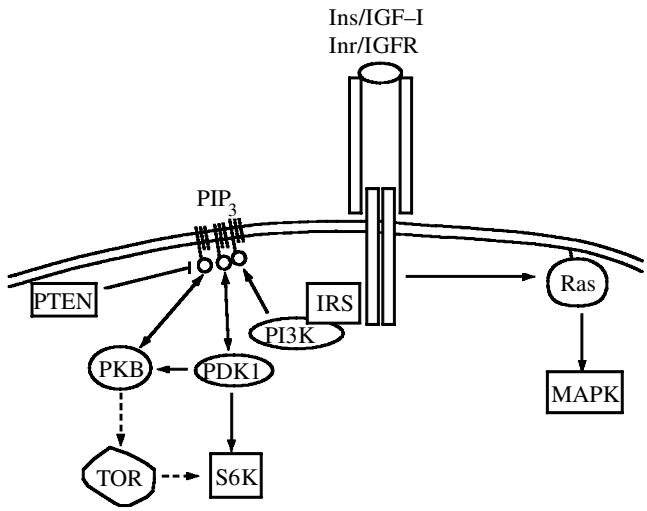

Figure 3. The *Drosophila* insulin/IGFR-signalling pathway. This pathway is conserved in its components and function with the vertebrate insulin/IGFR system. Depicted are components for which there is genetic evidence for growth regulation. Arrows indicate activation, dashed arrows indirect activation and double-headed arrows recruitment to the cell membrane. PTEN negatively regulates the pathway by decreasing PIP_3 levels. For abbreviations, see the main text.

where mutant photoreceptor cells (marked by the absence of pigment) are only half the size of adjacent cells that are heterozygous for *chico* (figure 2). Additionally, loss of Chico function in the entire eye and head produces flies with tiny heads relative to their normal-sized body (pinhead flies) capsule. Thus, Chico protein is required autonomously in cells and organs during development to transmit a growth-promoting signal.

(ii) *The insulin- and IGF-I-signalling pathway is conserved in* Drosophila *and is dedicated to growth control*

The homology between Chico and IRS1–4 suggests that other elements in the insulin signalling pathway may also control growth in a manner similar to Chico (figure 3). Indeed, mutations in the gene coding for the *Drosophila* homologue of the insulin/IGF-I receptor (Inr) affect overall growth (Rosen 1987; Fernandez *et al.* 1995; Chen *et al.* 1996). *C. elegans* possesses only one *Inr* gene in contrast to the two related insulin/IGFRs in vertebrates. The *Drosophila* Inr has almost equivalent sequence identity to the insulin/IGFRs in vertebrates and contains properties of both the vertebrate insulin receptor and IGFR: growth control as well as metabolic regulation. Although complete loss of Inr function causes embryonic lethality, partial loss-of-function mutations give rise to viable small flies with fewer and smaller cells much like *chico* mutant flies. In contrast to vertebrate insulin/IGFRs, *Drosophila* Inr contains a C-terminal extension with limited homology that can partially substitute for IRS function (Yenush *et al.* 1996). This extension is also conserved in the *C. elegans* homologue of the insulin receptor (Kimura *et al.* 1997). The difference between the lethal and viable phenotype of *Inr* and *chico* mutants, respectively, may be explained by the partially redundant function of the C-terminal extension of *Drosophila* Inr.

In vertebrates, activation of the insulin/IGFRs triggers a wide variety of intracellular signalling pathways including the Ras–MAPK pathway and the PI3K pathway (Avruch 1998). In *Drosophila*, the PI3K pathway is essential for growth regulation. The Chico protein contains two docking sites for the p60 adaptor subunit of Dpl10 PI3K and amino-acid substitutions at these sites inactivate Chico function (S. Oldham and E. Hafen, unpublished data). Mutations in *p60* and *p110 PI3K* reduce the growth rate and cell size in a way similar to *chico* (Leevers *et al.* 1996; Weinkove *et al.* 1999). In vertebrates, increased PIP_3 levels by PI3K trigger the translocation of protein kinase B (PKB) to the membrane by binding of the PH domain of PKB to PIP_3. Indeed, partial loss of PKB function also causes a reduction in body and cell size (Verdu *et al.* 1999; Stocker *et al.* 2000)

(iii) Drosophila *S6 kinase (S6K): a target of insulin signalling*

One of the targets of the PI3K/PKB pathway is p70S6K (Chou & Blenis 1995; Dufner & Thomas 1999). In response to PI3K and PKB activation, p70S6K phosphorylates the ribosomal protein S6 (Pullen & Thomas 1997). This phosphorylation event permits the preferential translation of an mRNA population with a specific 5′-end (5′TOP) (Brown & Schreiber 1996; Jeffries *et al.* 1997). Most 5′TOP mRNAs code for ribosomal proteins and are essential components for ribosome biogenesis and protein synthesis (Meyuhas *et al.* 1996). Mutations in *Drosophila S6K* exhibit a phenotype similar to its putative upstream activators, namely delay in development and a reduction in body size (Montagne *et al.* 1999). In contrast to *chico* mutant flies, however, the reduction in body size appears to be exclusively due to a reduction in cell size and not cell number. Therefore, *dS6K* and *chico* affect cell and organ size to a different degree. Consistent with this notion is the fact that development lasts two days longer in *dS6K* mutants than in *chico* mutants. The link between dS6K and PI3K is likely to involve the *Drosophila* TOR (target of rapamycin) homologue. In mammalian cells, activation of dS6K requires PI3K and TOR activity, while in yeast the TORs have been shown to control cytoskeletal organization (TOR2), ribosome biogenesis, and nutrient-dependent changes in amino-acid permeability (Thomas & Hall 1997; Lawrence & Abraham 1997; Heitman *et al.* 1991; Schmidt *et al.* 1998; Powers & Walter 1999). Indeed, mutations in *dTOR* have growth defects similar to those of other components like *dPDK1* and *dPKB* in the Inr pathway (F. Rintelen, S. Oldham, H. Stocker and E. Hafen, unpublished data).

(iv) *PTEN: an important negative regulator of growth*

The Inr/PI3K pathway is an essential positive regulator of growth in *Drosophila* and must be under stringent control to ensure that appropriate growth happens at the correct place and time. Various levels for negative regulation of the vertebrate insulin and IGFRs have been shown: the tyrosine phosphatases PTP1B and LAR can inhibit insulin receptor function (Goldstein *et al.* 1998) and the insulin and IGFRs undergo ligand-induced internalization and downregulation (Lammers *et al.* 1989). IRS has multiple serine residues whose phosphorylation has been suggested to mediate dampening of the insulin signal (Sun *et al.* 1992; Hotamisligil *et al.* 1996; Peraldi *et al.* 1996). The lipid product of PI3K can be subject to regulation as well. PTEN is a lipid phosphatase with specificity for PIP_3 and is a negative regulator of Inr signalling in *Drosophila* (Huang *et al.* 1999; Maehama & Dixon 1999). Loss of PTEN (phosphate and tensin homologue at chromosome 10) function in marked clones in *Drosophila* results in the generation of bigger cells and increased proliferation (Goberdhan *et al.* 1999; Huang *et al.* 1999). Loss of PTEN in the entire head of *Drosophila* creates flies with giant heads relative to their normal bodies, which demonstrates that PTEN normally regulates organ size in a negative fashion (S. Oldham and E. Hafen, unpublished data).

(v) *Insulin signalling is required for growth but not survival during imaginal disc development*

The IGF-I growth factor is a potent survival factor in tissue culture cells. IGF-I promotes survival of some neuronal cell types through the activation of PI3K and PKB and subsequent inactivation of Bad (Yang *et al.* 1995; Datta *et al.* 1997). Is the slower growth and the reduction in cell number of *chico* mutant flies caused by increased apoptosis of imaginal disc cells during development? This does not seem to be the case, since no evidence for increased cell death is observed in *chico* mutant tissues and the expression of the apoptosis inhibitor p35 does not rescue the reduced cell number phenotype (Boehni *et al.* 1999). Similarly, cells lacking PI3K function do not show a detectable increase in programmed cell death (Weinkove *et al.* 1999). Thus, in imaginal discs cell survival does not require Chico/PI3K signalling but is probably promoted by other factors. Possible survival signals may come from the epidermal growth factor receptor (EGFR) pathway or the extracellular matrix (Bergmann *et al.* 1998; Ilic *et al.* 1998; Kurada & White 1998).

Although complete loss of PKB, PI3K, and insulin/IGFR function does not absolutely block growth in mutant clones of cells, loss of function mutations in *PKB* (in germ line clones) and *Inr* cause embryonic lethality and an increase in cell death has been reported in *PKB* mutant embryos (Staveley *et al.* 1998). The role of the insulin-signalling pathway during embryogenesis appears to be different from its role in a growing epithelium. During embryogenesis, there is little cell growth and cell division occurs without cell enlargement. Consequently, cells become progressively smaller. Whether the increased apoptosis observed in PKB mutant embryos is a direct consequence of the loss of PKB survival signalling or a more indirect effect of abnormal specification or proliferation remains to be tested.

(b) *Coordination of growth control pathways in* Drosophila

(i) Drosophila *Myc controls growth*

Recent genetic analysis of the *Drosophila* homologue of c-*myc* demonstrated that it also controls growth without any apparent effect on the apoptotic rate. Flies mutant for partial loss-of-function mutations in *dMyc* are delayed in development, reduced in size and possess small, slender bristles (Gallant *et al.* 1996; Johnston *et al.* 1999). The reduction in body size is caused by a reduction in the number and the size of individual cells. Although the *dMyc* phenotypes are similar to those of *chico*, there are a

few notable differences. Loss of *dMyc* function disproportionately reduces bristle size, a phenotype characteristic of the *Minute* mutations that affect ribosome biogenesis. Indeed one of the conserved target genes of dMyc in *Drosophila* is called *pitchoune* and encodes an RNA helicase that may be involved in some aspects of ribosome function (Zaffran *et al*. 1998). Overexpression of dMyc in the posterior wing compartment increases cell size but does not alter organ size. Overexpression of p110 PI3K, however, increases cell size and the size of the same compartment in the wing (Weinkove *et al*. 1999). dMyc expression is tightly regulated during development and responds to patterning signals such as Wingless (Johnston *et al*. 1999). Myc may, therefore, be a critical component in integrating patterning and growth information. The insulin-signalling pathway, on the other hand, may regulate growth in response to environmental factors such as the availability of nutrients. Thus, it appears that dMyc and the insulin-signalling pathway control overlapping subsets of growth functions.

(ii) *The Ras pathway controls growth in* Drosophila

In addition to its better understood roles in cell fate specification and survival, the EGFR–Ras–MAPK pathway also is involved in growth control (Diaz-Benjumea & Garcia-Bellido 1990; Diaz-Benjumea & Hafen 1994; Karim & Rubin 1998). Loss-of-function clones of surviving cells are reduced in size in the adult wing. Whether this level of control occurs during proliferation or during differentiation, needs to be examined.

(c) *The insulin-signalling pathway: an evolutionarily conserved nutritional sensing pathway?*

The similarity between flies reared under limiting food conditions and flies carrying viable mutations in components of the insulin-signalling pathway is striking. In each case, development is delayed and body size is reduced owing to fewer and smaller cells. In yeast, poor nutrition also reduces the critical cell size when cells undergo cell division. In these cells, TOR controls growth in response to changing nutrient conditions (Beck & Hall 1999; Cardenas *et al*. 1999; Hardwick *et al*. 1999). In *C. elegans*, the insulin-signalling and transforming growth factor-β (TGF-β)-signalling pathways coordinately control alternative developmental programmes in response to nutrient conditions (Estevez *et al*. 1993; Kenyon *et al*. 1993; Gottlieb & Ruvkun 1994; Ren *et al*. 1996). In this immature dauer stage, nematodes can endure periods of low food availability largely due to the accumulation of fat (Riddle 1988; Kimura *et al*. 1997). A similar increase in lipid stores has been described in *chico* mutant flies and IRS-2 knock-out mice (Boehni *et al*. 1999; D. Burks and M. White, unpublished data).

Although most members of the TGF-β family in *Drosophila* are involved in pattern formation during development, mutations in *baboon*, the *Drosophila* homologue of the type I activin receptor, cause a reduction of size without any overt patterning defects (Brummel *et al*. 1999). It is interesting to note that mutations in TGF-β pathway homologues in *C. elegans* result in reduced body size similar to mutations in the insulin-signalling pathway in *Drosophila* (Krishna *et al*. 1999; Morita *et al*. 1999; Suzuki *et al*. 1999). From this correlative evidence,

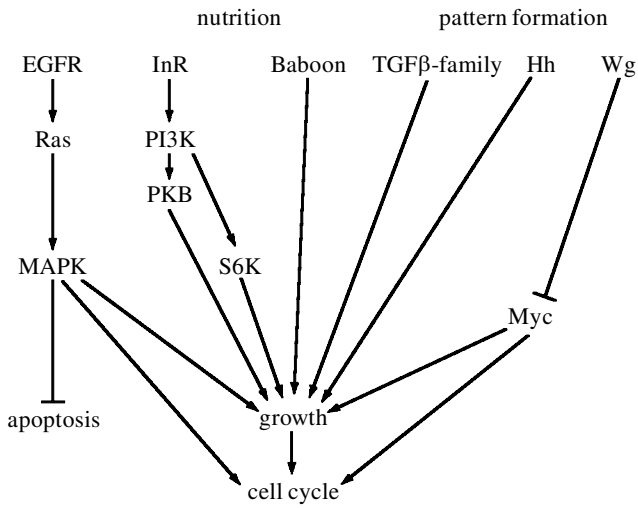

Figure 4. Schematic representation of multiple pathways affecting growth. Nutritional and patterning cues must be coordinated to regulate growth and cell cycle. For abbreviations, see the main text.

it appears that IGF-I/insulin and TGF-β signalling may act as a general nutritional sensing system to regulate final body size. Confirmation of this hypothesis will depend on the genetic characterization of the insulin-like and TGF-β ligands whose expression levels may change in response to different nutrient conditions. Although in *C. elegans* and in *Drosophila* there are multiple genes coding for insulin-like and TGF-β peptides, no mutations have yet been identified in these genes in *Drosophila* (Kutty *et al*. 1998; W. Brogiolo and E. Hafen, unpublished data). Another class of growth-promoting factors are the imaginal disc growth factors (Kawamura *et al*. 1999). They are secreted by the fat body into the haemolymph and can stimulate proliferation in imaginal discs. They are potentially a link between nutritional sensing of amino acids in the fat body and growth of imaginal discs (Zinke *et al*. 1999; Britton & Edgar 1998). Their synergy with insulin to promote imaginal disc growth supports the idea that multiple signalling pathways are required for coordinated growth.

5. SUMMARY

In summary, the similarity of the phenotypes caused by the loss of components in the insulin/IGF-I-signalling pathway in invertebrates and vertebrates strongly suggests a conserved role of this pathway in the control of overall growth during development. The intricate coordination of the various growth- and pattern-formation pathways requires a complex and probably partially redundant interplay of multiple signalling systems (figure 4). This is evident from the fact that cells lacking any one of the components in the insulin pathway are still able to grow and differentiate albeit at a severely reduced rate. Determining how the signals from these pathways are integrated is a major challenge for the future.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES