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Body building: regulation of shape and size by PI3K/TOR signaling during development

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Abstract

Growth of organisms and their constituent parts responds to both intrinsic and extrinsic cues during development: organisms of a given species generally grow at a predictable rate and to a specific body size, but individuals can modify this program during development in response to environmental conditions. Recent experiments, using gene knockouts and targeted overexpression, have revealed the central role of a signaling network controlled by the PI3K and TOR kinases in this regulation. These signaling molecules control growth by coordinately regulating a large number of cell biological processes. This review focuses on the cellular activities regulated by PI3K and TOR during development, and discusses how changes in different aspects of cellular metabolism may interact to regulate growth.

Keywords: Phosphatidylinositol 3-kinase; Target of rapamycin; Cell growth; Autophagy; Morphogenesis; Spindle orientation; *Drosophila*

1. Introduction

During development, a multitude of cellular processes are orchestrated to give rise to an organism of proper size, pattern and proportion. As these developmental programs unfold, in many cases they do so within an organism whose size is increasing exponentially. Growth of an organism thus affects nearly all aspects of its development. For example, morphogenetic patterning mechanisms that specify cell fate across a field of cells must account for the steadily increasing size of the field. Cellular movements such as migration or axonal pathfinding must also deal with a growing landscape, and must be coordinated with demands placed on the cytoskeleton by the mitotic apparatus of dividing cells. Given the evolutionary conservation of many developmental mechanisms, differences in body size across species also provide a challenge to these mechanisms. In addition, both the rate and extent of growth of many developing organisms are highly sensitive to environmental factors such as nutrition. Developmental programs must therefore be sufficiently robust and flexible to allow for changes in the size of a developing organ or organism, whether over developmental time, through evolution, or in response to external cues. Understanding how growth is controlled and how it is integrated with other cellular processes is of critical importance to developmental biology.

A variety of cellular activities contribute to growth during development, including nutrient uptake, macromolecular synthesis and degradation, cell division and cell death. In recent years a network of interacting signaling molecules has been identified which regulates these processes in response to growth factors and nutrients. These signaling pathways, governed by the class I phosphatidylinositol 3-kinase (PI3K) and the serine/threonine kinase Target of Rapamycin (TOR), have profound effects on growth at the cell, organ and organismal level. In this review, I primarily discuss the cell biological effects of PI3K and TOR signaling that in concert lead to cell growth. I first provide a brief description of these pathways, and summarize new insights into the connections between their components. Examples demonstrating the potent developmental effects of PI3K and TOR signaling on organismal size are reviewed. Finally, the role of spatially patterned cell growth and division in controlling organ morphogenesis is discussed.

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2. PI3K and TOR regulate interacting signaling pathways in response to growth factors and nutrients

The discovery of wortmannin and rapamycin as inhibitors of cell growth, and subsequent identification of their targets, PI3K and TOR, respectively, were critical steps leading to our present understanding of the signaling network depicted in Fig. 1. In response to insulin and other growth factors, class I PI3Ks promote the conversion of the membrane lipid phosphatidylinositol 4, 5-biphosphate (PIP2) into the second messenger phosphatidylinositol 3, 4, 5-triphosphate (PIP3). PIP3 can be reconverted to PIP2 by the lipid phosphatase activity of the tumor suppressor PTEN, which thus acts as a negative regulator of PI3K signaling. The current view is that increased levels of PIP3 cause translocation of the serine/threonine kinases Pdk1 and Akt to the cell membrane through interactions between PIP3 and the pleckstrin homology domains of these proteins. Pdk1 then promotes activation of both Akt and p70 S6 kinase (S6K) through phosphorylation of their activation loops. As discussed below, these kinases romote growth through a variety of mechanisms.

A potential role for PI3K in activating S6K was established through the use of wortmannin, which blocks S6K activation in response to growth factors (Han et al., 1995), as well as through studies showing that constitutively activated forms of PI3K can induce growth factor-independent phosphorylation of S6K (Weng et al., 1995a). However, the widely held view that PI3K mediates S6K activation has recently been challenged by genetic studies in *Drosophila*, which found that S6K activity is normal in animals carrying a null mutation in PI3K, and that PI3K is capable of promoting growth in S6K null animals (Radimerski et al., 2002a). A few earlier studies also argued against an essential role for mammalian PI3K in activating S6K: genetic inactivation of PI3K, either through dominant interfering mutations in the PI3K subunit p85, or by growth factor receptor mutations that prevent PI3K binding, was found to prevent activation of Akt but not S6K (Hara et al., 1995). Furthermore, the activity of Pdk1 toward S6K appears to be insensitive to PIP3 levels (Toker and Newton, 2000). In addition to its effects on PI3K, higher concentrations of wortmannin can also inhibit other kinases

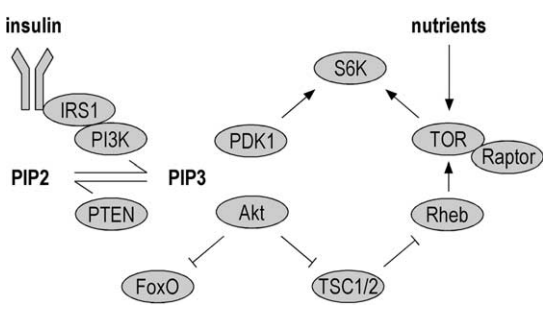

Fig. 1. Schematic representation of the PI3K/TOR pathway.

including TOR (Brunn et al., 1996), and thus studies relying on this drug need to be interpreted with some caution.

A stronger case can be made for activation of S6K through the serine-threonine kinase TOR. The phosphorylation and activity of S6K is thoroughly inhibited by rapamycin, and this effect can be overcome by specific point mutations in TOR which disrupt its ability to bind to rapamycin (Brown et al., 1995). The signaling function of TOR appears to be regulated in response to nutrient levels, particularly those of amino acids (Jacinto and Hall, 2003). How TOR activity responds to nutrient conditions is unclear, although several potential mechanisms have been proposed. Recent studies suggest that the intracellular rather than extracellular concentration of amino acids is important for TOR regulation (Christie et al., 2002). Amino acid alcohols, which cause uncharged tRNA pools to accumulate, have been shown to inhibit TOR signaling in some cell types (Iiboshi et al., 1999), suggesting that TOR (or an upstream regulator) may measure amino acid concentrations indirectly, by sensing levels of uncharged tRNA. Alternatively, nutrients may affect TOR activity indirectly through secondary effects on metabolism. For example, while cellular ATP levels are saturating for most protein kinases, TOR has been found to have a relatively high K_m for ATP (approaching that of intracellular ATP concentrations), which may allow TOR activity to respond to a cell's energy state (Dennis et al., 2001). Proteins of the TSC complex (TSC1 and TSC2) have also been identified as potential upstream negative regulators of TOR (McManus and Alessi, 2002), and may play a role in nutrient sensing. In cells lacking TSC1 or TSC2, phosphorylation of S6K is resistant to amino acid deprivation; conversely, increased levels of TSC1 and TSC2 prevent S6K phosphorylation even in the presence of abundant amino acids (Gao et al., 2002). Recent studies in *Drosophila* have identified the small GTPase Rheb as a link between TSC1/TSC2 and TOR (Saucedo et al., 2003; Stocker et al., 2003). Rheb, which is inhibited by the GTPase activating protein (GAP) activity of TSC2 (Zhang et al., 2003), appears to be a proximal activator of TOR, although its mechanism of action remains to be determined. Finally, TOR has recently been shown to form a nutrient-dependent complex with a WD40 and HEAT repeat-containing protein, raptor, which appears to have both positive and negative effects on TOR activity, perhaps depending on nutrient levels (Hara et al., 2002; Kim et al., 2002; Loewith et al., 2002).

What is the relationship between PI3K-mediated growth factor signaling on the one hand, and TOR-mediated signaling in response to nutritional cues on the other? Inhibition of TOR with rapamycin can potently block activation of S6K in response to growth factors, and thus TOR function is required for effective growth factor signaling (Jacinto and Hall, 2003). However, immunokinase assays have found that TOR activity increases only slightly in response to growth factors (Burnett et al., 1998; Scott et al., 1998). Furthermore, mutant forms of S6K have

been isolated that are resistant to rapamycin and amino acid withdrawal, but remain sensitive to wortmannin (Hara et al., 1998; Weng et al., 1995b). Thus TOR and PI3K appear to regulate essentially parallel signaling pathways which may ultimately converge on common targets. In this sense, TOR signaling can be thought of as a nutrient-dependent checkpoint on growth factor signaling.

The modest increase in TOR activity that does occur in response to growth factors was initially thought to be mediated through phosphorylation of TOR by Akt, which can directly phosphorylate TOR in vitro on sites whose phosphorylation is serum sensitive in vivo (Scott et al., 1998). However, mutation of these sites to alanine does not diminish signaling downstream of TOR (Sekulic et al., 2000); furthermore, these sites are not conserved in the fly, worm or plant TOR homologs. Recent experiments suggest that a link between PI3K and TOR may occur through Akt-mediated phosphorylation of TSC2, which was found to disrupt and inactivate the TSC1/TSC2 complex (Dan et al., 2002; Inoki et al., 2002; Potter et al., 2002). It will be important to determine whether phosphorylation by Akt is a critical factor regulating TSC function during normal development. Finally, the p85 regulatory subunit of PI3K was recently demonstrated to be capable of forming a ternary complex with TOR and S6K, and this was correlated with activation of S6K by growth factors (Gonzalez-Garcia et al., 2002).

An additional level of cross-talk between the PI3K and TOR pathways occurs through a negative feedback loop involving TOR-mediated inhibition of IRS1, an adapter protein required for PI3K activation by the insulin receptor. Activation of TOR results in phosphorylation and subsequent proteasomal degradation of IRS1, leading to reduced PI3K signaling (Haruta et al., 2000). TOR also regulates the subcellular distribution of IRS1 and PI3K, as inhibition of TOR can promote translocation of these proteins to low-density microsomes, thereby stimulating glucose uptake (Takano et al., 2001). Similarly, mutations in S6K have been found to increase the kinase activity of Akt (Radimerski et al., 2002a). These trans-pathway interactions are likely to play an important role in coordinating cell growth with other metabolic programs.

3. Regulation of developmental growth by PI3K and TOR signaling

Although much of our understanding of the signaling connections described above was gained from experiments in cell culture systems, the central role these pathways play in controlling growth during development has been revealed through genetic loss of function and targeted overexpression studies in model organisms. In *Drosophila*, dramatic reductions in organ and body size occur in response to inactivating mutations in insulin receptor, PI3K catalytic and regulatory subunits, IRS-1, Pdk1, Akt, Rheb, TOR or S6K (Bohni et al., 1999; Chen et al., 1996; Montagne et al., 1999; Oldham et al., 2000; Rintelen et al., 2001; Saucedo et al., 2003; Stocker et al., 2003; Verdu et al., 1999; Weinkove et al., 1999; Zhang et al., 2000), or upon overexpression of the negative regulators PTEN and TSC1/TSC2 (Gao and Pan, 2001; Potter et al., 2001; Tapon et al., 2001). Similarly, genetic disruption of Akt1, Pdk1 or S6K1 in the mouse reduces growth during development, both pre- and post-natally (Cho et al., 2001; Lawlor et al., 2002; Shima et al., 1998). These reductions in growth are associated with decreased cell size, and in some cases cell proliferation is reduced as well. Cell size is also decreased in mosaic clones of these mutants, indicating that their effects are cell autonomous. Conversely, increased growth is observed upon activation of signaling, through either overexpression of positive factors or loss of function mutations in negative signaling components. For example, transgenic mice with cardiac-specific expression of activated Akt1 or PI3K display an approximately two-fold enlargement of the heart, with a similar increase in myocyte size (Shioi et al., 2000, 2002). Together, these results argue that normal growth is achieved in response to proper levels of signaling through these pathways. In *Drosophila*, lethality due to loss of PTEN can be suppressed by a mutation in Akt that hinders PIP3 binding (Stocker et al., 2002); similarly, the lethal phenotypes of TSC1 and TSC2 mutants can be dominantly suppressed by heterozygous mutations in S6K or TOR (Radimerski et al., 2002b), again indicating that proper signal strength, rather than the level or activity of any particular signaling component, is essential for normal development. Interestingly, overexpression of TOR or the PI3K subunit P60/p85 causes phenotypes similar to loss of function mutations in these genes (Hennig and Neufeld, 2002; Weinkove et al., 1999), suggesting that signaling may be especially sensitive to the relative levels of these complex-forming proteins.

4. Cellular responses to growth-promoting signals

Having received cues instructing them to grow, how do individual cells alter their metabolism and physiology in response? Below I discuss the cellular events that collectively lead to changes in tissue growth in response to PI3K and TOR signaling (Table 1).

4.1. Translation

Because cell growth is so closely linked to rates of protein synthesis, efforts to address this question have focused on the mechanisms by which cells increase their rates of translation in response to growth factors or nutrients. Increased translation is one of the first responses to mitogen stimulation, and is required for transition through the ‘restriction point’ and entry into the cell cycle (Pardee, 1974). Much of the increase in translation capacity

Table 1
Growth-related cell biological processes regulated by PI3K/TOR signaling

Process	Effectors
Protein synthesis	p70 S6 kinase; 4E-BP1; RNA polymerase I; eIF2alpha; eIF2b; eEF2
Transcription	Transcription factors GLN3/GAT1, MSN2/MSN4, RTG1/RTG3, FoxO, STAT1/3; histone deacetylase Rpd3
Autophagy	APG1/APG13/APG17 complex; APG8
Nutrient import	Glucose transporter GLUT4; amino acid permeases TAT2, SAT2/ATA2, 4F2hc; LDL receptor; transferrin receptor CD71
Cell cycle progression	cyclin D; cyclin E; P27(Kip1); p130
Cell survival	FoxO, Fas, Bim, Bcl-2, Bcl-x, BAD, NF-KB, ASK-1

of a growth-stimulated cell stems from increases in ribosome biogenesis. In the early 1990s it was found that rapamycin selectively inhibits the translation of messages encoding components of the translation machinery, including ribosomal proteins and translation elongation factors. Translation of these messages, each of which contains a regulatory tract of oligo-pyrimidines at their immediate 5′ end (5′ TOP), was shown to correlate with the activity of p70S6K and the level of rpS6 phosphorylation (Jefferies et al., 1994; Terada et al., 1994). Further experiments with dominant-interfering and rapamycin-resistant mutants of p70S6K demonstrated a causal link between this kinase and 5′ TOP translation (Jefferies et al., 1997). Although these results did not directly implicate rpS6, previous studies had shown that 40S ribosomal subunits containing hyperphosphorylated rpS6 bound poly(U) more strongly than did unphosphorylated ribosomes (Gressner and van de Leur, 1980). Thus a model emerged in which growth factor binding stimulates ribosome biogenesis through a cascade of phosphorylations culminating in rpS6. Recently, however, it was found that 5′ TOP messages can be translated efficiently in the absence of detectable rpS6 phosphorylation in many cell types, including ES cells in which the S6K1 gene has been disrupted (Barth-Baus et al., 2002; Stolovich et al., 2002; Tang et al., 2001). In these experiments, 5′ TOP translation remained sensitive to PI3K inhibitors and amino acid withdrawal, suggesting that growth factor and nutrient signals may converge on a common target to control 5′ TOP translation, independently of rpS6. Furthermore, conditional genetic deletion of rpS6 was found to have no effect on cell growth in the livers of fed mice following starvation, despite preventing cell proliferation in a partial-hepatectomy assay (Volarevic et al., 2000). Thus, understanding the precise role of S6K, rpS6 and 5′ TOP translation in growth control awaits further experimentation, and should greatly benefit from merging biochemical and genetic approaches.

A second well-established link between these signaling pathways and the translational apparatus occurs through the translation initiation factor eIF4E. In some cell types eIF4E levels are rate limiting for translation, and its overexpression can cause cell transformation (Lazaris-Karatzas and Sonenberg, 1992). Regulation of eIF4E occurs in part through a family of inhibitory binding proteins, the 4E-BPs (reviewed in Sonenberg and Gingras, 1998). Under conditions favorable for growth, 4E-BPs are phosphorylated through rapamycin and wortmannin-sensitive pathways, and are thus unable to bind and inhibit eIF4E. Because these findings are based largely on in vitro experiments and overexpression in cell culture, it will be important to follow up these studies with in vivo genetic analyses. Although confounded by the presence of multiple family members in mammals, initial genetic characterization of 4E-BP homologs in other species suggests that these proteins may play a surprisingly minor role in regulating growth. For example, loss of function mutations in Thor and Eap1, 4E-BP homologs in flies and yeast, respectively, have no effect on growth under normal conditions, although Eap1 mutants are less sensitive than wild type to rapamycin (Bernal and Kimbrell, 2000; Cosentino et al., 2000; Miron et al., 2001). Furthermore, overexpression of Thor does not affect cell growth, but can suppress the overgrowth phenotypes resulting from PI3K or Akt overexpression. Thus 4E-BP activity may begin to influence growth at the upper and lower limits of PI3K and TOR signaling.

Other steps of the translation process are subject to regulation by PI3K and TOR as well. For example, it was recently shown that TOR controls the general amino acid control response in yeast by suppressing the activity of the eIF2alpha kinase GCN2 (Cherkasova and Hinnebusch, 2003; Kubota et al., 2003). In mammalian cells, the activities of translation factors eIF2B and eEF2 are responsive to both insulin and nutrient levels (Proud et al., 2001). Finally, TOR also regulates ribosome biogenesis in yeast and mammals by activating RNA polymerase I-dependent rRNA expression (Mahajan, 1994; Powers and Walter, 1999). Thus, PI3K/TOR signaling controls growth in part by regulating the synthesis and activity of the translation machinery at multiple points.

4.2. Transcription

PI3K and TOR-mediated signals also exert their growth effects at the transcriptional level. Akt controls gene expression in large part through negative regulation of the FoxO class of forkhead box transcription factors (FKHR, FKHRL1 and AFX). Akt has been shown to directly phosphorylate these factors on several sites, causing their retention in the cytoplasm (Kops and Burgering, 1999). Upon loss of Akt activity, FoxO factors activate expression of p27(Kip1) and the Rb-related p130 (Graff et al., 2000; Kops et al., 2002; Medema et al., 2000; Nakamura et al., 2000) and thus Akt activation may induce growth and proliferation in part by preventing expression of these negative regulators of growth. In addition, FoxO factors have recently been shown to down-regulate expression of

cyclin D (Schmidt et al., 2002). Interestingly, FoxO factors also have a positive role in cell cycle progression in some cell types, as mitotic exit and subsequent entry into G1 require transient PI3K/Akt down-regulation and FoxO activation (Alvarez et al., 2001).

Transcriptional profiling of cells treated with rapamycin or starved for various nutrients have revealed that TOR controls expression of broad groups of genes with roles in protein, lipid, and nucleic acid metabolism (Cardenas et al., 1999; Hardwick et al., 1999; Peng et al., 2002; Shamji et al., 2000). In yeast, TOR regulates expression of specific classes of starvation-response genes in part by cytoplasmic sequestration of transcription factors such as GLN3/GAT1 (regulated by nitrogen), MSN2/MSN4 (carbon) and RTG1/RTG3 (glutamine) (Beck et al., 1999; Komeili et al., 2000). Recently, TOR was also shown to promote expression of yeast ribosomal protein genes through the recruitment of histone acetylase complexes (Rohde and Cardenas, 2003). TOR also controls expression of a diverse spectrum of metabolism-related genes in metazoan cells. A recent genome-wide expression study found that loss of TOR activity in lymphocytes closely resembles the transcriptional effects of amino acid starvation (Peng et al., 2002). Although the mechanisms of transcriptional regulation by TOR are currently less well understood in higher eukaryotes than in yeast, TOR is required for phosphorylation and activation of the STAT1 and STAT3 transcription factors (Kristof et al., 2003; Yokogami et al., 2000). In addition, both TOR and PI3K have been shown to affect the expression and activity of the C/EBP family of transcriptional regulators, which control expression of a number of genes in response to nutrients (Entingh et al., 2001; Roesler, 2001).

4.3. Autophagy

Growth of cells and tissues during development is influenced not only by anabolic processes such as protein synthesis, but also by the rate and extent of catabolic, degradative processes. The bulk turnover of most long-lived cellular proteins occurs through the lysosomal pathway, by a process known as macroautophagy (reviewed in Kim and Klionsky, 2000). In this process, cytoplasm and organelles are non-selectively engulfed by a novel double membrane-bound vesicle, the autophagosome, which then fuses with the lysosomal and/or endosomal compartment. The resulting degradation products can be an important source of cellular nutrients, and indeed autophagy is strongly induced in response to nutrient deprivation in many cell types, and is required for survival during starvation in yeast.

Could autophagy play a role in governing the rate of growth during development? A comparative study of adult and neonatal mouse livers found that rates of protein turnover, rather than protein synthesis, accounted for the greatest difference between growing and non-growing organs (Conde and Scornik, 1977). Conditions that stimulate tissue growth, such as partial hepatectomy, refeeding following starvation, and growth factor addition, have been shown to cause a reduction in the basal rate of autophagy (Muller et al., 1987; Pfeifer, 1979; Pfeifer and Bertling, 1977), whereas growth-suppressive signals such as contact inhibition and substrate detachment can induce autophagy (Otsuka and Moskowitz, 1978; Papadopoulos and Pfeifer, 1987). In addition, the tumor suppressor gene *Beclin 1* encodes the human homolog of Apg6p, one of a group of proteins required for autophagy in yeast (Liang et al., 1999). In general, rates of autophagy are higher in tumor-derived and transformed cells than in their normal counterparts (Ogier-Denis and Codogno, 2003). Finally, the rate of protein degradation has recently been shown to be a critical determinant of cell size (Conlon and Raff, 2003). Together, these observations suggest that modulation of autophagy rates may be an important strategy in regulating cell growth.

Interestingly, the insulin/PI3K/Akt and TOR/S6K signaling networks have been shown to play an important role in regulating autophagy, in addition to their better-known effects on protein synthesis (Blommaart et al., 1997b). Insulin has long been known to inhibit autophagic protein turnover in the liver (Pfeifer, 1978). More recently, it was found that autophagy is inhibited by class I PI3Ks (Petiot et al., 2000). Paradoxically, general PI3K inhibitors such as wortmannin, LY294002 and 3-methyladenine block autophagy, apparently through their effects on class III PI3Ks, whose activity is required for the membrane dynamics involved in autophagic vesicle trafficking (Blommaart et al., 1997a; Petiot et al., 2000; Seglen and Gordon, 1982). An inhibitory role for class I PI3Ks was further supported by the finding that autophagy is promoted by the lipid phosphatase PTEN, and is inhibited by activation of Akt (Arico et al., 2001). In addition, the role of TOR in regulating autophagy has been examined in number of cell types, and in all cases rapamycin was shown to be a potent inducer of autophagy (Blommaart et al., 1995; Eskelinen et al., 2002; Mordier et al., 2000; Noda and Ohsumi, 1998). In yeast, TOR regulates autophagy in part by controlling the phosphorylation and stability of a complex containing Apg1, Apg13 and Apg17 (Kamada et al., 2000), and by regulating the expression of Apg8 (Kirisako et al., 1999). Finally, the level of rpS6 phosphorylation has been shown to inversely correlate with rates of autophagy (Blommaart et al., 1995), although a causative role for S6K in this process has not been demonstrated.

Thus, protein synthesis and degradation are inversely regulated by the same signaling pathways. The endoplasmic reticulum is considered a possible source of membrane for autophagic vesicles (Dunn, 1990), suggesting a mechanism whereby occupation of the ER by the protein synthesis machinery may inhibit autophagy by physically preventing de novo autophagic vesicle formation (Blommaart et al., 1995). Regardless of mechanism, the intimate connection between these processes somewhat confounds efforts to

dissect the relative roles of anabolic and catabolic processes in mediating the growth effects of PI3K and TOR signaling. Characterization of autophagy-specific mutations should aid in this goal. For example, systematic inactivation of autophagy genes, followed by careful analysis of the effects on cellular growth rates under various nutrient and genetic conditions, could provide a rigorous test of the role of autophagy in regulating cell growth (Neufeld, 2003).

4.4. Nutrient import

One of the major anabolic activities of growth factor signaling is to promote uptake of a number of cellular nutrients, including amino acids and carbohydrates. Insulin-stimulated import of glucose has long been known to be achieved in part through recruitment of glucose transporters to the cell surface (Bryant et al., 2002). Accumulation of the glucose transporter GLUT4 at the plasma membrane occurs through translocation from a specialized pool of endosomally-derived vesicles (Aledo et al., 1997). This process is PI3K- and Akt-dependent (Cheatham et al., 1994; Tsakiridis et al., 1995), and is inhibited by PTEN (Nakashima et al., 2000). Although rapamycin does not acutely effect either rates of glucose import or GLUT4 translocation (Fingar et al., 1993), longer-term inactivation of TOR by rapamycin or amino acid depletion appears to reduce the localization of GLUT4 to the insulin-responsive vesicles (Bogan et al., 2001), suggesting a translationally or transcriptionally mediated effect. Little is known regarding the mechanism of GLUT4 vesicle fusion with the plasma membrane, although it was recently shown that dynein and the GTPase Rab5 are involved in GLUT4 translocation (Huang et al., 2001). In addition, the full level of insulin-stimulated glucose transport appears to require not only translocation of GLUT4 but also its activation, likely through a p38 MAPK-mediated process (Somwar et al., 2002). Finally, phosphorylation of GSK-3 by Akt promotes cellular retention of glucose through its conversion to glycogen (Cross et al., 1995).

Growth factors also stimulate uptake of amino acids, again through PI3K- and Akt-dependent signaling (Hajduch et al., 1998; McDowell et al., 1998; Tsakiridis et al., 1995). Although this process is less well understood, it was recently found that insulin induces translocation of the system A amino acid transporter SAT2/ATA2 from an endosomal-related vesicle pool to the plasma membrane, in a manner remarkably analogous to that of GLUT4 (Hyde et al., 2002). In yeast, TOR activity is required to prevent the ubiquitylation and degradation of the tryptophan transporter TAT2 (Beck et al., 1999; Schmidt et al., 1998). Finally, Akt and TOR were recently shown to promote cell surface expression of transporters for not only glucose and amino acids but also low-density lipoprotein and iron (Edinger and Thompson, 2002). Thus, these pathways exert their tremendous effects on tissue growth in part through widespread changes on the accumulation, assembly, and stability of biomolecules.

Can accumulation of nutrients and cellular building blocks be rate limiting for growth during normal development? Surgical experiments in insects have shown that different body parts do in fact compete with one another for nutrients. For example, ablation of developing hindwings in the butterfly results in disproportionate growth of forewings and other appendages; structures closest to site of ablation appear to receive the greatest growth stimulus (Emlen and Nijhout, 2000). In addition, stripe-wise activation of PI3K in *Drosophila* larvae causes a net reduction of PI3K activity in the interstripe regions, presumably due to disproportional accumulation of nutrients by the PI3K expressing cells (Britton et al., 2002). Interestingly, cells with a growth disadvantage have been shown to be eliminated by their faster growing neighbors in *Drosophila* imaginal discs, through a process that may involve competition for nutrients or growth factors (Simpson, 1979).

4.5. Cell division

Cell growth and division are normally coupled during most of development, such that cell size remains essentially constant. In yeast, the cell cycle responds to growth rates by linking accumulation of cyclins to mass increase (Polymenis and Schmidt, 1997). Surprisingly, stimulation of growth by PI3K and TOR signaling has little effect on cell division rates (Fig. 2). Thus, changes in animal or organ size

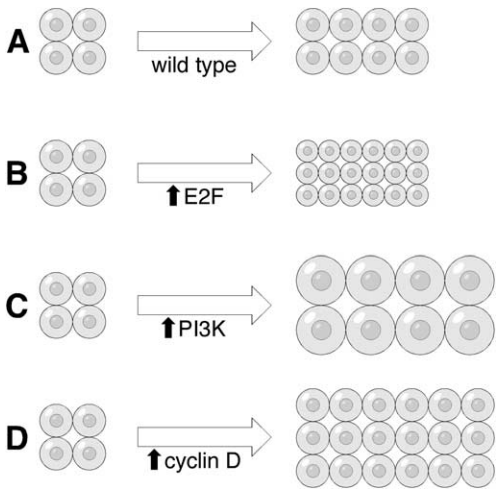

Fig. 2. Relationships between cell growth and division. In each panel, the group of cells on the left proliferate over a set amount of time to give rise to the cells on the right. In wild type controls (A), average cell size normally remains constant during proliferation. Overexpression of specific cell cycle regulators such as E2F (B) or cyclin E + Cdc25/string (not shown) causes acceleration of the cell division rate without increasing growth rate. Consequently, cell size is decreased. Activation of PI3K/TOR signaling by overexpression of PI3K (C), Akt or Rheb (not shown), increases the rate of cell growth but not division, resulting in cell hypertrophy. Some factors, such as cyclin D/CDK4 (D) or IGF-I, are able to stimulate both cell growth and cell division, and thus promote growth while maintaining normal cell size.

resulting from genetic alterations in these pathways are accompanied by corresponding changes in cell size, but in most cases cell numbers are similar to wild type tissues (Lawlor et al., 2002; Montagne et al., 1999; Shioi et al., 2000, 2002; Verdu et al., 1999; Weinkove et al., 1999). For example, examination of cells overexpressing PI3K, Akt or Rheb in the *Drosophila* wing imaginal disc found that these cells grew significantly faster than wild type, but their cell division rates were normal, leading to cell enlargement (Saucedo et al., 2003; Stocker et al., 2003; Verdu et al., 1999; Weinkove et al., 1999). By FACS analysis it was observed that these cells had a relatively shorter G1 phase of the cell cycle and an accelerated entry into S phase, but an increased proportion of cells accumulated in G2. Nearly identical effects were observed in cells overexpressing Myc or activated Ras, and in these cases it was shown that co-expression of the mitotic inducer String resulted in faster division rates and a return to normal cell size (Johnston et al., 1999; Prober and Edgar, 2000). Similarly, activation of PI3K in mammalian cells promotes S phase entry without increasing overall cell cycle rate (Klippel et al., 1998). Together, these results suggest a general mechanism in which cell growth regulates cell cycle progression at the G1/S boundary, whereas the decision to undergo mitosis is regulated independently, perhaps in response to patterning cues. Stimulation of both phase transitions is necessary to accelerate cell cycle rates.

PI3K and TOR signaling likely promote S phase entry in part by increasing the expression of proteins whose levels are limiting for the G1/S transition. Thus, cyclins D and E are down-regulated in response to TOR inactivation in mammalian and *Drosophila* cells, respectively, and forced expression of these proteins is sufficient to drive arrested cells into S phase (Hashemolhosseini et al., 1998; Muise-Helmericks et al., 1998; Nelsen et al., 2003; Zhang et al., 2000). In addition, negative cell cycle regulators such as P27 are induced in response to decreased PI3K and TOR signaling (Collado et al., 2000; Luo et al., 1996).

The ability of PI3K and TOR activation to promote cell growth but not cell cycle progression, leading to cell enlargement, is reminiscent of studies in which cell division rates were altered in the developing fly wing (Fig. 2). In these experiments, specific activation or inactivation of cell cycle regulators caused significant changes in cell doubling times and overall cell numbers, but had no effect on growth rates (Neufeld et al., 1998; Weigmann et al., 1997). As a result, increased cell division rates led to a greater number of cells occupying a given volume and mass, and hence a decrease in cell size. More recently it was found that growth and division rates of purified rat Schwann cells are differentially stimulated by different growth factors (Conlon et al., 2001). Together, these results demonstrate that cell growth and cell cycle progression can be regulated independently, and thus maintenance of cell size homeostasis is dependent upon coordinated activation of cell growth and division regulators. Uncoupling these processes may be a useful means of modulating cell size during development. Finally, it should be noted that some factors, such as cyclin D/CDK4, appear to promote cell growth and division in equal measures, and thus can stimulate cell proliferation while maintaining a normal cell size (Datar et al., 2000).

4.6. Cell death

The influence of cell survival on growth has been well established in a variety of developmental contexts. For example, genetic disruption of caspases can cause massive overgrowth of specific organs in the mouse (Kuida et al., 1998). In Hydra, starvation arrests growth but not cell proliferation; the excess cells are removed by apoptosis (Bosch and David, 1984). In contrast, in other developing tissues such as the wing imaginal disc of *Drosophila*, cell death is rarely observed and thus has little or no effect on growth.

Akt signaling plays a critical role in regulating cell death, through both transcriptional and non-transcriptional mechanisms. By inactivating FoxO transcription factors, Akt suppresses expression of the pro-apoptotic Fas ligand and the Bcl-2 family member Bim (Brunet et al., 1999; Stahl et al., 2002), and upregulates expression of apoptosis inhibitors such as Bcl-2 and Bcl-x (Chrysis et al., 2001; Leverrier et al., 1999), possibly through activation of NF-KB (Romashkova and Makarov, 1999). Akt can also directly phosphorylate and inhibit some components of the apoptotic machinery, such as caspase 9 and the Bcl-2 family member BAD (Cardone et al., 1998; Datta et al., 1997).

Inhibition of TOR signaling by rapamycin can result in cell death in some cases, although this effect appears to vary with cell type (Castedo et al., 2002). The degree to which rapamycin induces apoptosis appears to be influenced by the expression status of P53 and P21, the latter of which binds to and suppresses the apoptosis signal-regulating kinase 1 (ASK1) in response to rapamycin (Huang et al., 2003). In addition, the survival effects of TOR may be mediated in part through S6K-dependent phosphorylation and inactivation of BAD (Harada et al., 2001).

5. Connections between growth, cell division and morphogenesis

Growth of developing organs and appendages is often accompanied by significant changes in their shape. Thus, over time, some regions of a developing structure may grow and accumulate mass to a greater or lesser extent than other regions. In addition, in many instances growth is directed preferentially along one particular axis (anisotropic growth). A number of mechanisms can potentially contribute to these effects, including cell migration, cell survival and changes in cell shape. Below I consider the role of two cell proliferation-related mechanisms in controlling morphogenesis: spatial

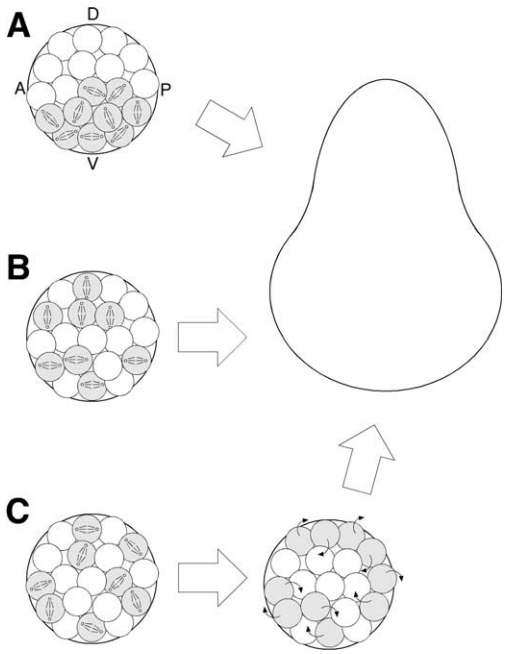

Fig. 3. Potential effects of cell growth and division on morphogenesis. In each example, an initially symmetrical primordium (left) gives rise to an asymmetric tissue enlarged in the ventral (V) region (right). (A) Preferential targeting of cell growth and proliferation to the ventral region of the primordium leads to an increase in ventral size. (B) In this mechanism, rates of cell growth and proliferation are uniform across the primordium, but spindle orientation is arranged such that growth is directed primarily along the anterior-posterior (A/P) axis ventrally, and along the dorsoventral (D/V) axis dorsally. (C) Uniform rates of cell growth and proliferation with random spindle orientation, followed by non-random cellular rearrangement and intercalation, can also lead to asymmetry.

differences in growth rates, and orientation of cell division axes (Fig. 3).

5.1. Control of morphogenesis through differential cell growth

To what extent is organ shape formation regulated by regional activation of growth-regulators such as PI3K or TOR? Several examples of morphogenesis resulting from spatial differences in growth rate can be found. In the vertebrate limb bud, FGF expression in the apical ectodermal ridge (AER) directs limb outgrowth by stimulating proliferation in a region of underlying mesenchymal cells known as the progress zone (Capdevila and Izpisua Belmonte, 2001). As limb outgrowth proceeds, maintenance of this zone at the distal edge of the limb causes growth to be primarily directed along the proximo-distal axis. Interestingly, the progress zone expresses high levels of IGF-1 and its receptor in response to AER signals, and ectopic IGF-1 is capable of directing limb outgrowth, suggesting localized PI3K activation may contribute to this growth (Dealy and Kosher, 1995). This topology is carried through into postembryonic limb development, as most growth occurs in the proliferation zone of chondrocytes localized at the proximal and distal epiphyses of long bones. A similar configuration is observed in the roots and shoots of developing plants, in which mitotic activity of the meristem is concentrated at the distal tips of these structures. The shape of the developing vertebrate neural tube is influenced by a more rapid rate of growth in its dorsal regions; this appears to be due to a gradient of Wnts secreted from the dorsal midline (Megason and McMahon, 2002). Finally, recent studies have suggested that elaboration of vertebrate digits may be due more to enhanced digit growth than to interdigit apoptosis as previously thought (Salas-Vidal et al., 2001).

Overexpression of PI3K in specific regions of the developing fly wing imaginal disc causes these regions to grow at a faster rate than normal, resulting in distortion of the normal shape of the wing disc (Weinkove et al., 1999). Interestingly, overexpression of other growth regulators such as dMyc does not cause similar distortions of the wing disc, despite the potent ability of dMyc to promote cell growth in this organ (Johnston et al., 1999). Similarly, imaginal discs that are mosaic for Minute mutations have regions with significant differences in growth rate, yet these discs also grow into normally shaped organs (Morata and Ripoll, 1975). Thus PI3K signaling would appear to be unique in its ability to alter or override the intrinsic growth compensatory mechanisms that normally regulate organ shape and size.

These examples demonstrate the potential for local differences in growth rates to regulate shape. However, analyses of loss of function mutant phenotypes argue against a significant role for PI3K/TOR mediated growth in such a mechanism. As described above, genetic disruption of various components of these signaling pathways, including null mutations in the sole S6K and IRS-1 genes in flies (Bohni et al., 1999; Montagne et al., 1999), causes marked reductions in body size; however, the resulting small mutant animals have a normal body shape and proportion. If normal morphogenesis required modulations of PI3K/TOR signaling, a uniformly low level of signaling would be expected to cause morphogenetic defects. Furthermore, using a pleckstrin homology domain-GFP fusion to measure in vivo PI3K activity, it was found that PI3K signaling is uniform across the *Drosophila* wing imaginal disc throughout development (Prober and Edgar, 2002). Together, these observations argue that modulation of these signals does not normally play an essential role in morphogenesis.

5.2. Role of spindle orientation in morphogenesis

Accurate positioning of the plane of cell division is critical for a number of developmental processes, such as segregation of morphogenic determinants and epithelial delamination of neuroblasts (Gonczy, 2002). In addition, by directing cells to specific regions of a tissue, spatial control of cell division can potentially have profound effects on morphogenesis.

Morphogenesis in plants is constrained by rigid cell walls which hinder cell movement, and thus it has traditionally been viewed that cell division orientation is critical to organ shape (Ashby, 1948). For example, leaf and petal cells preferentially divide either longitudinally or transversely to the long axis of these organs, and dynamic growth models have demonstrated that such oriented division and expansion could potentially drive morphogenesis (Rolland-Lagan et al., 2003). However, experimental disruption of cell division patterns has surprisingly little effect on plant morphogenesis. The *tangled-1* mutation of maize alters spindle orientation in the developing leaf, such that longitudinal divisions are replaced with aberrant, randomly oriented divisions (Smith et al., 1996). Surprisingly, leaf shape in this mutant is normal at all stages of development. Similarly, transgenic expression of dominant negative Cdc2 in Arabidopsis and tobacco plants was found to inhibit cell division but had no effect on morphogenesis (Hemerly et al., 1995). Even when cell division is completely arrested, leaf primordia can still initiate growth in the proper orientation (Haber, 1962). These results suggest that despite the close correlation between cell division patterns and morphogenesis in plants, these processes can be controlled independently.

In vertebrates, longitudinal growth of long bones occurs by endochondral ossification at the growth plate, a layer of cartilage between the metaphysis and the epiphysis. As they divide, chondrocytes in the proliferative zone of the growth plate align into clonally related 'isogenous groups, ' which are arranged in columns parallel to the long axis of the bone (Hunziker, 1994). Hypertrophy of these chondrocytes prior to their ossification is the major driving force of bone growth, and their stereotypical arrangement is thought to contribute to growth being directed along the longitudinal axis. However, recent experiments suggest that longitudinal arrangement of isogenous groups is not due to spindle orientation, but rather to post-mitotic realignment along this axis (Kimmel et al., 1998). Similarly, spindle orientation in the developing *Drosophila* wing has been shown to be random, yet marked clones of cells are found to align preferentially along the proximodistal axis of the wing, often forming long, continuous strings of cells (Milan et al., 1996a,b; Resino et al., 2002). As in the case of growth plate chondrocytes, wing cells appear to re-align themselves post-mitotically through a process of oriented cellular intercalation.

Although the cues used to guide these post-mitotic cell movements are not known, signals involving the planar cell polarity pathway are likely candidates. Grafting experiments suggest that cells in the resting zone of the growth plate may secrete a growth plate-orienting factor capable of directing the orientation of chondrocyte columns (Abad et al., 2002). Mutations in the *Drosophila nubbin* gene disrupt the proximodistal orientation of clones, and cause a reduction in growth along this axis (Resino et al., 2002). Interestingly, these cell movements are remarkably reminiscent of the convergent extension movements that drive elongation of the body axis during gastrulation (Keller, 2002). Recent studies have implicated the planar polarity signaling system in organizing these rearrangements (Mlodzik, 2002).

6. Conclusions

Identification of PI3K and TOR as central mediators of growth has taken us a great stride toward understanding how size and shape are regulated during development. Mutations in the various components of these pathways will be extremely valuable experimental tools to address a number of remaining questions.

First, given the range of cell biological processes that respond to PI3K/TOR signaling, it will be important to learn how these processes are coordinated, and how they influence one another. For example, in addition to non-specifically promoting a general increase in mass, stimulation of protein synthesis can also affect specific cellular processes due to differences in translation efficiency of particular transcripts. Thus, cell cycle progression may be coordinated with growth rate through inefficient translation of cyclins and other cell cycle regulators, which ensures that these proteins accumulate only when growth is sufficient to warrant cell division (Polymenis and Schmidt, 1997). Paradoxically, stimulation of ribosome biogenesis may inhibit translation of such inefficient transcripts, at least initially, as the translational apparatus becomes targeted to the highly abundant 5′ TOP messages (Thomas, 2000). Changes in glucose transport might be expected to have indirect effects on TOR-mediated processes such as autophagy and ribosome biogenesis, by affecting ATP levels and thus TOR activity. Indeed, glucose appears to have a priming effect on the ability of amino acids to stimulate TOR (Proud, 2002). Mutations that block specific cellular responses to PI3K and TOR signals should be useful tools to study these connections.

Next, it is clear that TOR and PI3K do not signal along a simple linear pathway, but rather respond to multiple stimuli and activate multiple effectors. While our understanding of the links amongst known components of these pathways has advanced significantly in recent years, we lack a complete picture of the inputs and outputs. For example, the critical, physiologically relevant substrates of S6K remain to be determined. In addition to rpS6, recent studies have identified eEF2 kinase and BAD as S6K substrates (Harada et al., 2001; Wang et al., 2001). Identification of the full spectrum of S6K targets, perhaps through protein engineering-based methods (Shah et al., 1997), followed by genetic studies to determine relevance, should aid in this goal. Another critical gap in our knowledge concerns how TOR activity is regulated in response to nutrients. It remains unclear what metabolic factor(s) are measured, by what cellular component(s), how the signal is relayed to TOR,

and what aspect of TOR function is altered, be it kinase activity, substrate accessibility, association with regulatory factors, or cellular localization. Whatever the mechanism, nutrient-dependent differences in TOR activity appear to be lost upon cell disruption, as the in vitro kinase activity of TOR immunoprecipitated from extracts of starved or normal cells does not differ (Dennis et al., 2001). The identification of raptor, TSC1/2 and Rheb as regulators of TOR signaling provides new avenues to address this question. It will be interesting to learn whether and how these factors interact to regulate TOR function.

Finally, understanding the role of PI3K and TOR signaling during normal development will require investigations into how these pathways interact with the patterning systems that give form to bodies and organs. Analyses of mutant phenotypes suggest that these pathways are separable from and subservient to the global pattern regulators. Thus, mutations that block PI3K signaling reduce growth but do not abolish it, and do not affect patterning; likewise, despite their increased growth, cells in which PI3K signaling is activated are still able to exit the cell cycle and differentiate on schedule. These results suggest that responsiveness of cells to PI3K/TOR signaling may be regulated by an independent developmental mechanism. The ability of transplanted organ fragments to grow to a normal size further argues that signals intrinsic to a developing tissue can override external cues such as growth factors and nutrition. Together, the data suggest that PI3K and TOR signaling act to modify a program of growth which is established by a distinct patterning mechanism. Given the pervasive effects of these pathways on multiple cell biological processes, a challenge for the future will be to dissect how patterning and PI3K/TOR signals converge to regulate growth.

References

Abad, V., Meyers, J.L., Weise, M., Gafni, R.I., Barnes, K.M., Nilsson, O., Bacher, J.D., Baron, J., 2002. The role of the resting zone in growth plate chondrogenesis. Endocrinology 143, 1851–1857.

Aledo, J.C., Lavoie, L., Volchuk, A., Keller, S.R., Klip, A., Hundal, H.S., 1997. Identification and characterization of two distinct intracellular GLUT4 pools in rat skeletal muscle: evidence for an endosomal and an insulin-sensitive GLUT4 compartment. Biochem. J. 325, 727–732.

Alvarez, B., Martinez, A.C., Burgering, B.M., Carrera, A.C., 2001. Forkhead transcription factors contribute to execution of the mitotic programme in mammals. Nature 413, 744–747.

Arico, S., Petiot, A., Bauvy, C., Dubbelhuis, P.F., Meijer, A.J., Codogno, P., Ogier-Denis, E., 2001. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. J. Biol. Chem. 276, 35243–35246.

Ashby, E., 1948. Studies on the morphogenesis of leaves. I. An essay on leaf shape. New Phytol. 47, 153–176.

Barth-Baus, D., Stratton, C.A., Parrott, L., Myerson, H., Meyuhas, O., Templeton, D.J., Landreth, G.E., Hensold, J.O., 2002. S6 phosphorylation-independent pathways regulate translation of 5′-terminal oligopyrimidine tract-containing mRNAs in differentiating hematopoietic cells. Nucleic Acids Res. 30, 1919–1928.

Beck, T., Schmidt, A., Hall, M.N., 1999. Starvation induces vacuolar targeting and degradation of the tryptophan permease in yeast. J. Cell Biol. 146, 1227–1238.

Bernal, A., Kimbrell, D.A., 2000. *Drosophila* Thor participates in host immune defense and connects a translational regulator with innate immunity. Proc. Natl Acad. Sci. USA 97, 6019–6024.

Blommaart, E.F., Luiken, J.J., Blommaart, P.J., van Woerkom, G.M., Meijer, A.J., 1995. Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. J. Biol. Chem. 270, 2320–2326.

Blommaart, E.F., Krause, U., Schellens, J.P., Vreeling-Sindelarova, H., Meijer, A.J., 1997a. The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes. Eur. J. Biochem. 243, 240–246.

Blommaart, E.F., Luiken, J.J., Meijer, A.J., 1997b. Autophagic proteolysis: control and specificity. Histochem. J. 29, 365–385.

Bogan, J.S., McKee, A.E., Lodish, H.F., 2001. Insulin-responsive compartments containing GLUT4 in 3T3-L1 and CHO cells: regulation by amino acid concentrations. Mol. Cell. Biol. 21, 4785–4806.

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

Bosch, T.C., David, C.N., 1984. Growth regulation in Hydra: relationship between epithelial cell cycle length and growth rate. Dev. Biol. 104, 161–171.

Britton, J.S., Lockwood, W.K., Li, L., Cohen, S.M., Edgar, B.A., 2002. *Drosophila*'s insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. Dev. Cell 2, 239–249.

Brown, E.J., Beal, P.A., Keith, C.T., Chen, J., Shin, T.B., Schreiber, S.L., 1995. Control of p70 s6 kinase by kinase activity of FRAP in vivo. Nature 377, 441–446.

Brunet, A., Bonni, A., Zigmond, M.J., Lin, M.Z., Juo, P., Hu, L.S., Anderson, M.J., Arden, K.C., Blenis, J., Greenberg, M.E., 1999. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96, 857–868.

Brunn, G.J., Williams, J., Sabers, C., Wiederrecht, G., Lawrence, J.C. Jr., Abraham, R.T., 1996. Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. Eur. Mol. Biol. Org. J. 15, 5256–5267.

Bryant, N.J., Govers, R., James, D.E., 2002. Regulated transport of the glucose transporter GLUT4. Nat. Rev. Mol. Cell. Biol. 3, 267–277.

Burnett, P.E., Barrow, R.K., Cohen, N.A., Snyder, S.H., Sabatini, D.M., 1998. RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. Proc. Natl. Acad. Sci. USA 95, 1432–1437.

Capdevila, J., Izpisua Belmonte, J.C., 2001. Patterning mechanisms controlling vertebrate limb development. Annu. Rev. Cell Dev. Biol. 17, 87–132.

Cardenas, M.E., Cutler, N.S., Lorenz, M.C., Di Como, C.J., Heitman, J., 1999. The TOR signaling cascade regulates gene expression in response to nutrients. Genes Dev. 13, 3271–3279.

Cardone, M.H., Roy, N., Stennicke, H.R., Salvesen, G.S., Franke, T.F., Stanbridge, E., Frisch, S., Reed, J.C., 1998. Regulation of cell death protease caspase-9 by phosphorylation. Science 282, 1318–1321.

Castedo, M., Ferri, K.F., Kroemer, G., 2002. Mammalian target of rapamycin (mTOR): pro- and anti-apoptotic. Cell Death Differ. 9, 99–100.

Cheatham, B., Vlahos, C.J., Cheatham, L., Wang, L., Blenis, J., Kahn, C.R., 1994. Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. Mol. Cell. Biol. 14, 4902–4911.

Chen, C., Jack, J., Garofalo, R.S., 1996. The *Drosophila* insulin receptor is required for normal growth. Endocrinology 137, 846–856.

Cherkasova, V.A., Hinnebusch, A.G., 2003. Translational control by TOR and TAP42 through dephosphorylation of eIF2alpha kinase GCN2. Genes Dev. 17, 859–872.

Cho, H., Thorvaldsen, J.L., Chu, Q., Feng, F., Birnbaum, M.J., 2001. Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J. Biol. Chem. 276, 38349–38352.

Christie, G.R., Hajduch, E., Hundal, H.S., Proud, C.G., Taylor, P.M., 2002. Intracellular sensing of amino acids in *Xenopus laevis* oocytes stimulates p70 S6 kinase in a target of rapamycin-dependent manner. J. Biol. Chem. 277, 9952–9957.

Chrysis, D., Calikoglu, A.S., Ye, P., D'Ercole, A.J., 2001. Insulin-like growth factor-I overexpression attenuates cerebellar apoptosis by altering the expression of Bcl family proteins in a developmentally specific manner. J. Neurosci. 21, 1481–1489.

Collado, M., Medema, R.H., Garcia-Cao, I., Dubuisson, M.L., Barradas, M., Glassford, J., Rivas, C., Burgering, B.M., Serrano, M., Lam, E.W., 2000. Inhibition of the phosphoinositide 3-kinase pathway induces a senescence-like arrest mediated by p27Kip1. J. Biol. Chem. 275, 21960–21968.

Conde, R.D., Scornik, O.A., 1977. Faster synthesis and slower degradation of liver protein during developmental growth. Biochem. J. 166, 115–121.

Conlon, I., Raff, M., 2003. Differences in the way a mammalian cell and yeast cells coordinate cell growth and cell-cycle progression. J. Biol. 2, 7.

Conlon, I.J., Dunn, G.A., Mudge, A.W., Raff, M.C., 2001. Extracellular control of cell size. Nat. Cell. Biol. 3, 918–921.

Cosentino, G.P., Schmelzle, T., Haghighat, A., Helliwell, S.B., Hall, M.N., Sonenberg, N., 2000. Eap1p, a novel eukaryotic translation initiation factor 4E-associated protein in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 20, 4604–4613.

Cross, D.A., Alessi, D.R., Cohen, P., Andjelkovich, M., Hemmings, B.A., 1995. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378, 785–789.

Dan, H.C., Sun, M., Yang, L., Feldman, R.I., Sui, X.M., Ou, C.C., Nellist, M., Yeung, R.S., Halley, D.J., Nicosia, S.V., Pledger, W.J., Cheng, J.Q., 2002. Phosphatidylinositol 3-kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by phosphorylation of tuberin. J. Biol. Chem. 277, 35364–35370.

Datar, S.A., Jacobs, H.W., de la Cruz, A.F., Lehner, C.F., Edgar, B.A., 2000. The *Drosophila* cyclin D-Cdk4 complex promotes cellular growth. Eur. Mol. Biol. Org. J. 19, 4543–4554.

Datta, S.R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y., Greenberg, M.E., 1997. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91, 231–241.

Dealy, C.N., Kosher, R.A., 1995. Studies on insulin-like growth factor-I and insulin in chick limb morphogenesis. Dev. Dyn. 202, 67–79.

Dennis, P.B., Jaeschke, A., Saitoh, M., Fowler, B., Kozma, S.C., Thomas, G., 2001. Mammalian TOR a homeostatic ATP sensor. Science 294, 1102–1105.

Dunn, W.A. Jr., 1990. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. J. Cell Biol. 110, 1923–1933.

Edinger, A.L., Thompson, C.B., 2002. Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. Mol. Biol. Cell 13, 2276–2288.

Emlen, D.J., Nijhout, H.F., 2000. The development and evolution of exaggerated morphologies in insects. Annu. Rev. Entomol. 45, 661–708.

Entingh, A.J., Law, B.K., Moses, H.L., 2001. Induction of the C/EBP homologous protein (CHOP) by amino acid deprivation requires insulin-like growth factor I, phosphatidylinositol 3-kinase, and mammalian target of rapamycin signaling. Endocrinology 142, 221–228.

Eskelinen, E.L., Prescott, A.R., Cooper, J., Brachmann, S.M., Wang, L., Tang, X., Backer, J.M., Lucocq, J.M., 2002. Inhibition of autophagy in mitotic animal cells. Traffic 3, 878–893.

Fingar, D.C., Hausdorff, S.F., Blenis, J., Birnbaum, M.J., 1993. Dissociation of pp70 ribosomal protein S6 kinase from insulin-stimulated glucose transport in 3T3-L1 adipocytes. J. Biol. Chem. 268, 3005–3008.

Gao, X., Pan, D., 2001. TSC1 and TSC2 tumor suppressors antagonize insulin signaling in cell growth. Genes Dev. 15, 1383–1392.

Gao, X., Zhang, Y., Arrazola, P., Hino, O., Kobayashi, T., Yeung, R.S., Ru, B., Pan, D., 2002. Tsc tumour suppressor proteins antagonize amino-acid-TOR signalling. Nat. Cell Biol. 4, 699–704.

Gonczy, P., 2002. Mechanisms of spindle positioning: focus on flies and worms. Trends Cell Biol. 12, 332–339.

Gonzalez-Garcia, A., Garrido, E., Hernandez, C., Alvarez, B., Jimenez, C., Cantrell, D.A., Pullen, N., Carrera, A.C., 2002. A new role for the p85-phosphatidylinositol 3-kinase regulatory subunit linking FRAP to p70 S6 kinase activation. J. Biol. Chem. 277, 1500–1508.

Graff, J.R., Konicek, B.W., McNulty, A.M., Wang, Z., Houck, K., Allen, S., Paul, J.D., Hbaiu, A., Goode, R.G., Sandusky, G.E., Vessella, R.L., Neubauer, B.L., 2000. Increased AKT activity contributes to prostate cancer progression by dramatically accelerating prostate tumor growth and diminishing p27Kip1 expression. J. Biol. Chem. 275, 24500–24505.

Gressner, A.M., van de Leur, E., 1980. Interaction of synthetic polynucleotides with small rat liver ribosomal subunits possessing low and highly phosphorylated protein S6. Biochim. Biophys. Acta 608, 459–468.

Haber, A.H., 1962. Nonessentiality of concurrent cell divisions for degree of polarization of leaf growth. I. Studies with radiation-induced mitotic inhibition. Am. J. Bot. 49, 583–589.

Hajduch, E., Alessi, D.R., Hemmings, B.A., Hundal, H.S., 1998. Constitutive activation of protein kinase B alpha by membrane targeting promotes glucose and system A amino acid transport, protein synthesis, and inactivation of glycogen synthase kinase 3 in L6 muscle cells. Diabetes 47, 1006–1013.

Han, J.W., Pearson, R.B., Dennis, P.B., Thomas, G., 2000. Rapamycin, wortmannin, and the methylxanthine SQ20006 inactivate p70s6k by inducing dephosphorylation of the same subset of sites. J. Biol. Chem. 270, 21396–21403.

Hara, K., Maruki, Y., Long, X., Yoshino, K., Oshiro, N., Hidayat, S., Tokunaga, C., Avruch, J., Yonezawa, K., 2002. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell 110, 177–189.

Hara, K., Yonezawa, K., Sakaue, H., Kotani, K., Kotani, K., Kojima, A., Waterfield, M.D., Kasuga, M., 1995. Normal activation of p70 S6 kinase by insulin in cells overexpressing dominant negative 85kD subunit of phosphoinositide 3-kinase. Biochem. Biophys. Res. Commun. 208, 735–741.

Hara, K., Yonezawa, K., Weng, Q.P., Kozlowski, M.T., Belham, C., Avruch, J., 1998. Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. J. Biol. Chem. 273, 14484–14494.

Harada, H., Andersen, J.S., Mann, M., Terada, N., Korsmeyer, S.J., 2001. p70S6 kinase signals cell survival as well as growth, inactivating the pro-apoptotic molecule BAD. Proc. Natl. Acad. Sci. USA 98, 9666–9670.

Hardwick, J.S., Kuruvilla, F.G., Tong, J.K., Shamji, A.F., Schreiber, S.L., 1999. Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins. Proc. Natl. Acad. Sci. USA 96, 14866–14870.

Haruta, T., Uno, T., Kawahara, J., Takano, A., Egawa, K., Sharma, P.M., Olefsky, J.M., Kobayashi, M., 2000. A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. Mol. Endocrinol. 14, 783–794.

Hashemolhosseini, S., Nagamine, Y., Morley, S.J., Desrivieres, S., Mercep, L., Ferrari, S., 1998. Rapamycin inhibition of the G1 to S transition is mediated by effects on cyclin D1 mRNA and protein stability. J. Biol. Chem. 273, 14424–14429.

Hemerly, A., Engler Jde, A., Bergounioux, C., Van Montagu, M., Engler, G., Inze, D., Ferreira, P., 1995. Dominant negative mutants of the Cdc2

kinase uncouple cell division from iterative plant development. Eur. Mol. Biol. Org. J. 14, 3925–3936.

Hennig, K.M., Neufeld, T.P., 2002. Inhibition of cellular growth and proliferation by dTOR overexpression in *Drosophila*. Genesis 34, 107–110.

Huang, J., Imamura, T., Olefsky, J.M., 2001. Insulin can regulate GLUT4 internalization by signaling to Rab5 and the motor protein dynein. Proc. Natl Acad. Sci. USA 98, 13084–13089.

Huang, S., Shu, L., Dilling, M.B., Easton, J., Harwood, F.C., Ichijo, H., Houghton, P.J., 2003. Sustained activation of the JNK cascade and rapamycin-induced apoptosis are suppressed by p53/p21(Cip1). Mol. Cell 11, 1491–1501.

Hunziker, E.B., 1994. Mechanism of longitudinal bone growth and its regulation by growth plate chondrocytes. Microsc. Res. Tech. 28, 505–519.

Hyde, R., Peyrollier, K., Hundal, H.S., 2002. Insulin promotes the cell surface recruitment of the SAT2/ATA2 system A amino acid transporter from an endosomal compartment in skeletal muscle cells. J. Biol. Chem. 277, 13628–13634.

Iiboshi, Y., Papst, P.J., Kawasome, H., Hosoi, H., Abraham, R.T., Houghton, P.J., Terada, N., 1999. Amino acid-dependent control of p70(s6k). Involvement of tRNA aminoacylation in the regulation. J. Biol. Chem. 274, 1092–1099.

Inoki, K., Li, Y., Zhu, T., Wu, J., Guan, K.L., 2002. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat. Cell Biol. 4, 648–657.

Jacinto, E., Hall, M.N., 2003. Tor signalling in bugs, brain and brawn. Nat. Rev. Mol. Cell. Biol. 4, 117–126.

Jefferies, H.B., Fumagalli, S., Dennis, P.B., Reinhard, C., Pearson, R.B., Thomas, G., 1997. Rapamycin suppresses 5′TOP mRNA translation through inhibition of p70s6k. Eur. Mol. Biol. Org. J. 16, 3693–3704.

Jefferies, H.B., Reinhard, C., Kozma, S.C., Thomas, G., 1994. Rapamycin selectively represses translation of the 'polypyrimidine tract' mRNA family. Proc. Natl Acad. Sci. USA 91, 4441–4445.

Johnston, L.A., Prober, D.A., Edgar, B.A., Eisenman, R.N., Gallant, P., 1999. *Drosophila* myc regulates cellular growth during development. Cell 98, 779–790.

Kamada, Y., Funakoshi, T., Shintani, T., Nagano, K., Ohsumi, M., Ohsumi, Y., 2000. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. J. Cell Biol. 150, 1507–1513.

Keller, R., 2002. Shaping the vertebrate body plan by polarized embryonic cell movements. Science 298, 1950–1954.

Kim, D.H., Sarbassov, D.D., Ali, S.M., King, J.E., Latek, R.R., Erdjument-Bromage, H., Tempst, P., Sabatini, D.M., 2002. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 110, 163–175.

Kim, J., Klionsky, D.J., 2000. Autophagy, cytoplasm-to-vacuole targeting pathway, and pexophagy in yeast and mammalian cells. Annu. Rev. Biochem. 69, 303–342.

Kimmel, C.B., Miller, C.T., Kruze, G., Ullmann, B., BreMiller, R.A., Larison, K.D., Snyder, H.C., 1998. The shaping of pharyngeal cartilages during early development of the zebrafish. Dev. Biol. 203, 245–263.

Kirisako, T., Baba, M., Ishihara, N., Miyazawa, K., Ohsumi, M., Yoshimori, T., Noda, T., Ohsumi, Y., 1999. Formation process of autophagosome is traced with Apg8/Aut7p in yeast. J. Cell Biol. 147, 435–446.

Klippel, A., Escobedo, M.A., Wachowicz, M.S., Apell, G., Brown, T.W., Giedlin, M.A., Kavanaugh, W.M., Williams, L.T., 1998. Activation of phosphatidylinositol 3-kinase is sufficient for cell cycle entry and promotes cellular changes characteristic of oncogenic transformation. Mol. Cell. Biol. 18, 5699–5711.

Komeili, A., Wedaman, K.P., O'Shea, E.K., Powers, T., 2000. Mechanism of metabolic control. Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. J. Cell Biol. 151, 863–878.

Kops, G.J., Burgering, B.M., 1999. Forkhead transcription factors: new insights into protein kinase B (c-akt) signaling. J. Mol. Med. 77, 656–665.

Kops, G.J., Medema, R.H., Glassford, J., Essers, M.A., Dijkers, P.F., Coffer, P.J., Lam, E.W., Burgering, B.M., 2002. Control of cell cycle exit and entry by protein kinase B-regulated forkhead transcription factors. Mol. Cell. Biol. 22, 2025–2036.

Kristof, A.S., Marks-Konczalik, J., Billings, E., Moss, J., 2003. Stimulation of STAT1-dependent gene transcription by lipopolysaccharide and interferon-gamma is regulated by mammalian target of rapamycin. J. Biol. Chem..

Kubota, H., Obata, T., Ota, K., Sasaki, T., Ito, T., 2003. Rapamycin-induced translational derepression of GCN4 mRNA involves a novel mechanism for activation of the eIF2[alpha] Kinase GCN2. J. Biol. Chem. 278, 20457–20460.

Kuida, K., Haydar, T.F., Kuan, C.Y., Gu, Y., Taya, C., Karasuyama, H., Su, M.S., Rakic, P., Flavell, R.A., 1998. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 94, 325–337.

Lawlor, M.A., Mora, A., Ashby, P.R., Williams, M.R., Murray-Tait, V., Malone, L., Prescott, A.R., Lucocq, J.M., Alessi, D.R., 2002. Essential role of PDK1 in regulating cell size and development in mice. Eur. Mol. Biol. Org. J. 21, 3728–3738.

Lazaris-Karatzas, A., Sonenberg, N., 1992. The mRNA 5′ cap-binding protein, eIF-4E, cooperates with v-myc or E1A in the transformation of primary rodent fibroblasts. Mol. Cell. Biol. 12, 1234–1238.

Leverrier, Y., Thomas, J., Mathieu, A.L., Low, W., Blanquier, B., Marvel, J., 1999. Role of PI3-kinase in Bcl-X induction and apoptosis inhibition mediated by IL-3 or IGF-1 in Baf-3 cells. Cell Death Differ. 6, 290–296.

Liang, X.H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., Levine, B., 1999. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 402, 672–676.

Loewith, R., Jacinto, E., Wullschleger, S., Lorberg, A., Crespo, J.L., Bonenfant, D., Oppliger, W., Jenoe, P., Hall, M.N., 2002. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol. Cell 10, 457–468.

Luo, Y., Marx, S.O., Kiyokawa, H., Koff, A., Massague, J., Marks, A.R., 1996. Rapamycin resistance tied to defective regulation of p27Kip1. Mol. Cell. Biol. 16, 6744–6751.

Mahajan, P.B., 1994. Modulation of transcription of rRNA genes by rapamycin. Int. J. Immunopharmacol. 16, 711–721.

McDowell, H.E., Eyers, P.A., Hundal, H.S., 1998. Regulation of System A amino acid transport in L6 rat skeletal muscle cells by insulin, chemical and hyperthermic stress. Federation Eur. Biochem. Soc. Lett 441, 15–19.

McManus, E.J., Alessi, D.R., 2002. TSC1-TSC2: a complex tale of PKB-mediated S6K regulation. Nat. Cell Biol. 4, E214–E216.

Medema, R.H., Kops, G.J., Bos, J.L., Burgering, B.M., 2000. AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. Nature 404, 782–787.

Megason, S.G., McMahon, A.P., 2002. A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. Development 129, 2087–2098.

Milan, M., Campuzano, S., Garcia-Bellido, A., 1996a. Cell cycling and patterned cell proliferation in the *Drosophila* wing during metamorphosis. Proc. Natl. Acad. Sci. USA 93, 11687–11692.

Milan, M., Campuzano, S., Garcia-Bellido, A., 1996b. Cell cycling and patterned cell proliferation in the wing primordium of *Drosophila*. Proc. Natl. Acad. Sci. USA 93, 640–645.

Miron, M., Verdu, J., Lachance, P.E., Birnbaum, M.J., Lasko, P.F., Sonenberg, N., 2001. The translational inhibitor 4E-BP is an effector of PI(3)K/Akt signalling and cell growth in *Drosophila*. Nat. Cell Biol. 3, 596–601.

Mlodzik, M., 2002. Planar cell polarization: do the same mechanisms regulate *Drosophila* tissue polarity and vertebrate gastrulation? Trends Genet. 18, 564–571.

Montagne, J., Stewart, M.J., Stocker, H., Hafen, E., Kozma, S.C., Thomas, G., 1999. *Drosophila* S6 kinase: a regulator of cell size. Science 285, 2126–2129.

Morata, G., Ripoll, P., 1975. Minutes: mutants of drosophila autonomously affecting cell division rate. Dev. Biol. 42, 211–221.

Mordier, S., Deval, C., Bechet, D., Tassa, A., Ferrara, M., 2000. Leucine limitation induces autophagy and activation of lysosome-dependent proteolysis in C2C12 myotubes through a mammalian target of rapamycin-independent signaling pathway. J. Biol. Chem. 275, 29900–29906.

Muise-Helmericks, R.C., Grimes, H.L., Bellacosa, A., Malstrom, S.E., Tsichlis, P.N., Rosen, N., 1998. Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/Akt-dependent pathway. J. Biol. Chem. 273, 29864–29872.

Muller, J., Pfeifer, U., Dammrich, J., 1987. Inhibited autophagic degradation during ACTH-stimulated growth of rat adrenal zona fasciculata. Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. 52, 429–441.

Nakamura, N., Ramaswamy, S., Vazquez, F., Signoretti, S., Loda, M., Sellers, W.R., 2000. Forkhead transcription factors are critical effectors of cell death and cell cycle arrest downstream of PTEN. Mol. Cell. Biol. 20, 8969–8982.

Nakashima, N., Sharma, P.M., Imamura, T., Bookstein, R., Olefsky, J.M., 2000. The tumor suppressor PTEN negatively regulates insulin signaling in 3T3-L1 adipocytes. J. Biol. Chem. 275, 12889–12895.

Nelsen, C.J., Rickehim, D.G., Tucker, M.M., McKenzie, T.J., Hansen, L.K., Pestell, R.G., Albrecht, J.H., 2003. Amino acids regulate hepatocyte proliferation through modulation of cyclin D1 expression. J. Biol. Chem..

Neufeld, T.P., 2003. Role of autophagy in developmental cell growth and death: insights from *Drosophila*. In: Klionsky, D.J., (Ed.), Autophagy, RG Landes Co., Georgetown, in press.

Neufeld, T.P., de la Cruz, A.F., Johnston, L.A., Edgar, B.A., 1998. Coordination of growth and cell division in the *Drosophila* wing. Cell 93, 1183–1193.

Noda, T., Ohsumi, Y., 1998. Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. J. Biol. Chem. 273, 3963–3966.

Ogier-Denis, E., Codogno, P., 2003. Autophagy: a barrier or an adaptive response to cancer. Biochim. Biophys. Acta 1603, 113–128.

Oldham, S., Montagne, J., Radimerski, T., Thomas, G., Hafen, E., 2000. Genetic and biochemical characterization of dTOR, the *Drosophila* homolog of the target of rapamycin. Genes Dev. 14, 2689–2694.

Otsuka, H., Moskowitz, M., 1978. Differences in the rates of protein degradation in untransformed and transformed cell lines. Exp. Cell Res. 112, 127–135.

Papadopoulos, T., Pfeifer, U., 1987. Protein turnover and cellular autophagy in growing and growth-inhibited 3T3 cells. Exp. Cell Res. 171, 110–121.

Pardee, A.B., 1974. A restriction point for control of normal animal cell proliferation. Proc. Natl. Acad. Sci. USA 71, 1286–1290.

Peng, T., Golub, T.R., Sabatini, D.M., 2002. The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. Mol. Cell. Biol. 22, 5575–5584.

Petiot, A., Ogier-Denis, E., Blommaart, E.F., Meijer, A.J., Codogno, P., 2000. Distinct classes of phosphatidylinositol 3′-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. J. Biol. Chem. 275, 992–998.

Pfeifer, U., 1978. Inhibition by insulin of the formation of autophagic vacuoles in rat liver. A morphometric approach to the kinetics of intracellular degradation by autophagy. J. Cell Biol. 78, 152–167.

Pfeifer, U., 1979. Inhibited autophagic degradation of cytoplasm during compensatory growth of liver cells after partial hepatectomy. Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. 30, 313–333.

Pfeifer, U., Bertling, J., 1977. A morphometric study of the inhibition of autophagic degradation during restorative growth of liver cells in rats re-fed after starvation. Virchows Arch. B Cell Pathol. 24, 109–120.

Polymenis, M., Schmidt, E.V., 1997. Coupling of cell division to cell growth by translational control of the G1 cyclin CLN3 in yeast. Genes Dev. 11, 2522–2531.

Potter, C.J., Huang, H., Xu, T., 2001. *Drosophila* Tsc1 functions with Tsc2 to antagonize insulin signaling in regulating cell growth, cell proliferation, and organ size. Cell 105, 357–368.

Potter, C.J., Pedraza, L.G., Xu, T., 2002. Akt regulates growth by directly phosphorylating Tsc2. Nat. Cell Biol. 4, 658–665.

Powers, T., Walter, P., 1999. Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in *Saccharomyces cerevisiae*. Mol. Biol. Cell 10, 987–1000.

Prober, D.A., Edgar, B.A., 2000. Ras1 promotes cellular growth in the *Drosophila* wing. Cell 100, 435–446.

Prober, D.A., Edgar, B.A., 2002. Interactions between Ras1, dMyc, and dPI3K signaling in the developing *Drosophila* wing. Genes Dev. 16, 2286–2299.

Proud, C.G., 2002. Regulation of mammalian translation factors by nutrients. Eur. J. Biochem. 269, 5338–5349.

Proud, C.G., Wang, X., Patel, J.V., Campbell, L.E., Kleijn, M., Li, W., Browne, G.J., 2001. Interplay between insulin and nutrients in the regulation of translation factors. Biochem. Soc. Trans. 29, 541–547.

Radimerski, T., Montagne, J., Rintelen, F., Stocker, H., van der Kaay, J., Downes, C.P., Hafen, E., Thomas, G., 2002a. dS6K-regulated cell growth is dPKB/dPI(3)K-independent, but requires dPDK1. Nat. Cell Biol. 4, 251–255.

Radimerski, T., Montagne, J., Hemmings-Mieszczak, M., Thomas, G., 2002b. Lethality of *Drosophila* lacking TSC tumor suppressor function rescued by reducing dS6K signaling. Genes Dev. 16, 2627–2632.

Resino, J., Salama-Cohen, P., Garcia-Bellido, A., 2002. Determining the role of patterned cell proliferation in the shape and size of the *Drosophila* wing. Proc. Natl. Acad. Sci. USA 99, 7502–7507.

Rintelen, F., Stocker, H., Thomas, G., Hafen, E., 2001. PDK1 regulates growth through Akt and S6K in *Drosophila*. Proc. Natl. Acad. Sci. USA 98, 15020–15025.

Roesler, W.J., 2001. The role of C/EBP in nutrient and hormonal regulation of gene expression. Annu. Rev. Nutr. 21, 141–165.

Rohde, J.R., Cardenas, M.E., 2003. The tor pathway regulates gene expression by linking nutrient sensing to histone acetylation. Mol. Cell. Biol. 23, 629–635.

Rolland-Lagan, A.G., Bangham, J.A., Coen, E., 2003. Growth dynamics underlying petal shape and asymmetry. Nature 422, 161–163.

Romashkova, J.A., Makarov, S.S., 1999. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. Nature 401, 86–90.

Salas-Vidal, E., Valencia, C., Covarrubias, L., 2001. Differential tissue growth and patterns of cell death in mouse limb autopod morphogenesis. Dev. Dyn. 220, 295–306.

Saucedo, L.J., Gao, X., Chiarelli, D.A., Li, L., Pan, D., Edgar, B.A., 2003. Rheb promotes cell growth as a component of the insulin/TOR signalling network. Nat. Cell Biol. 5, 566–571.

Schmidt, A., Beck, T., Koller, A., Kunz, J., Hall, M.N., 1998. The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease. Eur. Mol. Biol. Org. J. 17, 6924–6931.

Schmidt, M., Fernandez de Mattos, S., van der Horst, A., Klompmaker, R., Kops, G.J., Lam, E.W., Burgering, B.M., Medema, R.H., 2002. Cell cycle inhibition by FoxO forkhead transcription factors involves downregulation of cyclin D. Mol. Cell. Biol. 22, 7842–7852.

Scott, P.H., Brunn, G.J., Kohn, A.D., Roth, R.A., Lawrence, J.C. Jr, 1998. Evidence of insulin-stimulated phosphorylation and activation of the mammalian target of rapamycin mediated by a protein kinase B signaling pathway. Proc. Natl. Acad. Sci. USA 95, 7772–7777.

Seglen, P.O., Gordon, P.B., 1982. 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. Proc. Natl. Acad. Sci. USA 79, 1889–1892.

Sekulic, A., Hudson, C.C., Homme, J.L., Yin, P., Otterness, D.M., Karnitz, L.M., Abraham, R.T., 2000. A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of

rapamycin in mitogen-stimulated and transformed cells. Cancer Res. 60, 3504–3513.

Shah, K., Liu, Y., Deirmengian, C., Shokat, K.M., 1997. Engineering unnatural nucleotide specificity for Rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. Proc. Natl. Acad. Sci. USA 94, 3565–3570.

Shamji, A.F., Kuruvilla, F.G., Schreiber, S.L., 2000. Partitioning the transcriptional program induced by rapamycin among the effectors of the Tor proteins. Curr. Biol. 10, 1574–1581.

Shima, H., Pende, M., Chen, Y., Fumagalli, S., Thomas, G., Kozma, S.C., 1998. Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. Eur. Mol. Biol. Org. J. 17, 6649–6659.

Shioi, T., Kang, P.M., Douglas, P.S., Hampe, J., Yballe, C.M., Lawitts, J., Cantley, L.C., Izumo, S., 2000. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. Eur. Mol. Biol. Org. J. 19, 2537–2548.

Shioi, T., McMullen, J.R., Kang, P.M., Douglas, P.S., Obata, T., Franke, T.F., Cantley, L.C., Izumo, S., 2002. Akt/protein kinase B promotes organ growth in transgenic mice. Mol. Cell. Biol. 22, 2799–2809.

Simpson, P., 1979. Parameters of cell competition in the compartments of the wing disc of *Drosophila*. Dev. Biol. 69, 182–193.

Smith, L.G., Hake, S., Sylvester, A.W., 1996. The tangled-1 mutation alters cell division orientations throughout maize leaf development without altering leaf shape. Development 122, 481–489.

Somwar, R., Koterski, S., Sweeney, G., Sciotti, R., Djuric, S., Berg, C., Trevillyan, J., Scherer, P.E., Rondinone, C.M., Klip, A., 2002. A dominant-negative p38 MAPK mutant and novel selective inhibitors of p38 MAPK reduce insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting GLUT4 translocation. J. Biol. Chem. 277, 50386–50395.

Sonenberg, N., Gingras, A.C., 1998. The mRNA 5′ cap-binding protein eIF4E and control of cell growth. Curr. Opin. Cell Biol. 10, 268–275.

Stahl, M., Dijkers, P.F., Kops, G.J., Lens, S.M., Coffer, P.J., Burgering, B.M., Medema, R.H., 2002. The forkhead transcription factor FoxO regulates transcription of p27Kip1 and Bim in response to IL-2. J. Immunol. 168, 5024–5031.

Stocker, H., Andjelkovic, M., Oldham, S., Laffargue, M., Wymann, M.P., Hemmings, B.A., Hafen, E., 2002. Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. Science 295, 2088–2091.

Stocker, H., Radimerski, T., Schindelholz, B., Wittwer, F., Belawat, P., Daram, P., Breuer, S., Thomas, G., Hafen, E., 2003. Rheb is an essential regulator of S6K in controlling cell growth in *Drosophila*. Nat. Cell Biol. 5, 559–566.

Stolovich, M., Tang, H., Hornstein, E., Levy, G., Cohen, R., Bae, S.S., Birnbaum, M.J., Meyuhas, O., 2002. Transduction of growth or mitogenic signals into translational activation of TOP mRNAs is fully reliant on the phosphatidylinositol 3-kinase-mediated pathway but requires neither S6K1 nor rpS6 phosphorylation. Mol. Cell. Biol. 22, 8101–8113.

Takano, A., Usui, I., Haruta, T., Kawahara, J., Uno, T., Iwata, M., Kobayashi, M., 2001. Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. Mol. Cell. Biol. 21, 5050–5062.

Tang, H., Hornstein, E., Stolovich, M., Levy, G., Livingstone, M., Templeton, D., Avruch, J., Meyuhas, O., 2001. Amino acid-induced translation of TOP mRNAs is fully dependent on phosphatidylinositol 3-kinase-mediated signaling, is partially inhibited by rapamycin, and is independent of S6K1 and rpS6 phosphorylation. Mol. Cell. Biol. 21, 8671–8683.

Tapon, N., Ito, N., Dickson, B.J., Treisman, J.E., Hariharan, I.K., 2001. The *Drosophila* tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. Cell 105, 345–355.

Terada, N., Patel, H.R., Takase, K., Kohno, K., Nairn, A.C., Gelfand, E.W., 1994. Rapamycin selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins. Proc. Natl. Acad. Sci. USA 91, 11477–11481.

Thomas, G., 2000. An encore for ribosome biogenesis in the control of cell proliferation. Nat. Cell Biol. 2, E71–E72.

Toker, A., Newton, A.C., 2000. Cellular signaling: pivoting around PDK-1. Cell 103, 185–188.

Tsakiridis, T., McDowell, H.E., Walker, T., Downes, C.P., Hundal, H.S., Vranic, M., Klip, A., 1995. Multiple roles of phosphatidylinositol 3-kinase in regulation of glucose transport, amino acid transport, and glucose transporters in L6 skeletal muscle cells. Endocrinology 136, 4315–4322.

Verdu, J., Buratovich, M.A., Wilder, E.L., Birnbaum, M.J., 1999. Cell-autonomous regulation of cell and organ growth in *Drosophila* by Akt/PKB. Nat. Cell Biol. 1, 500–506.

Volarevic, S., Stewart, M.J., Ledermann, B., Zilberman, F., Terracciano, L., Montini, E., Grompe, M., Kozma, S.C., Thomas, G., 2000. Proliferation, but not growth, blocked by conditional deletion of 40S ribosomal protein S6. Science 288, 2045–2047.

Wang, X., Li, W., Williams, M., Terada, N., Alessi, D.R., Proud, C.G., 2001. Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. Eur. Mol. Biol. Org. J. 20, 4370–4379.

Weigmann, K., Cohen, S.M., Lehner, C.F., 1997. Cell cycle progression, growth and patterning in imaginal discs despite inhibition of cell division after inactivation of *Drosophila* Cdc2 kinase. Development 124, 3555–3563.

Weinkove, D., Neufeld, T.P., Twardzik, T., Waterfield, M.D., Leevers, S.J., 1999. Regulation of imaginal disc cell size, cell number and organ size by *Drosophila* class I(A) phosphoinositide 3-kinase and its adaptor. Curr. Biol. 9, 1019–1029.

Weng, Q.P., Andrabi, K., Klippel, A., Kozlowski, M.T., Williams, L.T., Avruch, J., 1995a. Phosphatidylinositol 3-kinase signals activation of p70 S6 kinase in situ through site-specific p70 phosphorylation. Proc. Natl. Acad. Sci. USA 92, 5744–5748.

Weng, Q.P., Andrabi, K., Kozlowski, M.T., Grove, J.R., Avruch, J., 1995b. Multiple independent inputs are required for activation of the p70 S6 kinase. Mol. Cell. Biol. 15, 2333–2340.

Yokogami, K., Wakisaka, S., Avruch, J., Reeves, S.A., 2000. Serine phosphorylation and maximal activation of STAT3 during CNTF signaling is mediated by the rapamycin target mTOR. Curr. Biol. 10, 47–50.

Zhang, H., Stallock, J.P., Ng, J.C., Reinhard, C., Neufeld, T.P., 2000. Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. Genes Dev. 14, 2712–2724.

Zhang, Y., Gao, X., Saucedo, L.J., Ru, B., Edgar, B.A., Pan, D., 2003. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. Nat. Cell Biol. 5, 578–581.