ELSEVIER

Full text provided by www.sciencedirect.com
ScienceDirect

hvps34, an ancient player, enters a growing game: mTOR Complex1/S6K1 signaling

Takahiro Nobukuni, Sara C Kozma and George Thomas

Recent studies have shown that the nutrient input to the mTOR Complex1/S6K1 signaling pathway is mediated by class 3 PI3K or hVps34, the oldest member of the PI3K family. Moreover, studies to date would suggest that during the evolution of multicellular organisms this ancient branch of the pathway was merged with the growth-factor-hormone-controlled class 1 PI3K pathway at the level of mTOR Complex1 to control the development and growth of the organism. However, hVps34 also plays a role in the regulation of macroautophagy — the mechanism by which cells generate nutrients, such as amino acids, through the degradation of intracellular complexes, including mitochondria and ribosomes. These functions of hVps34 initially appear contradictory, since increased mTOR Complex1 activation is triggered by increased amino acid levels, while autophagy is triggered when cells are faced with amino acid deprivation.

Addresses
Genome Research Institute, University of Cincinnati, 2180 E Galbraith Rd, Cincinnati, OH 45237, USA

Corresponding author: Thomas, George (thomasg4@UC.edu)

Current Opinion in Cell Biology 2007, **19**:135–141

This review comes from a themed issue on
Cell regulation
Edited by Carl-Henrik Heldin and Peter ten Dijke

Available online 23rd February 2007

0955-0674/$ – see front matter
Published by Elsevier Ltd.

DOI 10.1016/j.ceb.2007.02.019

Introduction

It has been known for nearly a decade that the mammalian target of rapamycin (mTOR) Complex1 is exquisitely sensitive to nutrients. However, there has been little understanding of the underlying mechanisms which mediate this response, despite an increasing awareness of the importance of this pathway in metabolic syndromes such as diabetes, obesity and cancer. Here we review the recent finding that hVps34 mediates nutrient signaling to mTOR. We also discuss a potential framework to rationalize the distinct roles played by hVps34 in the control of growth versus autophagy.

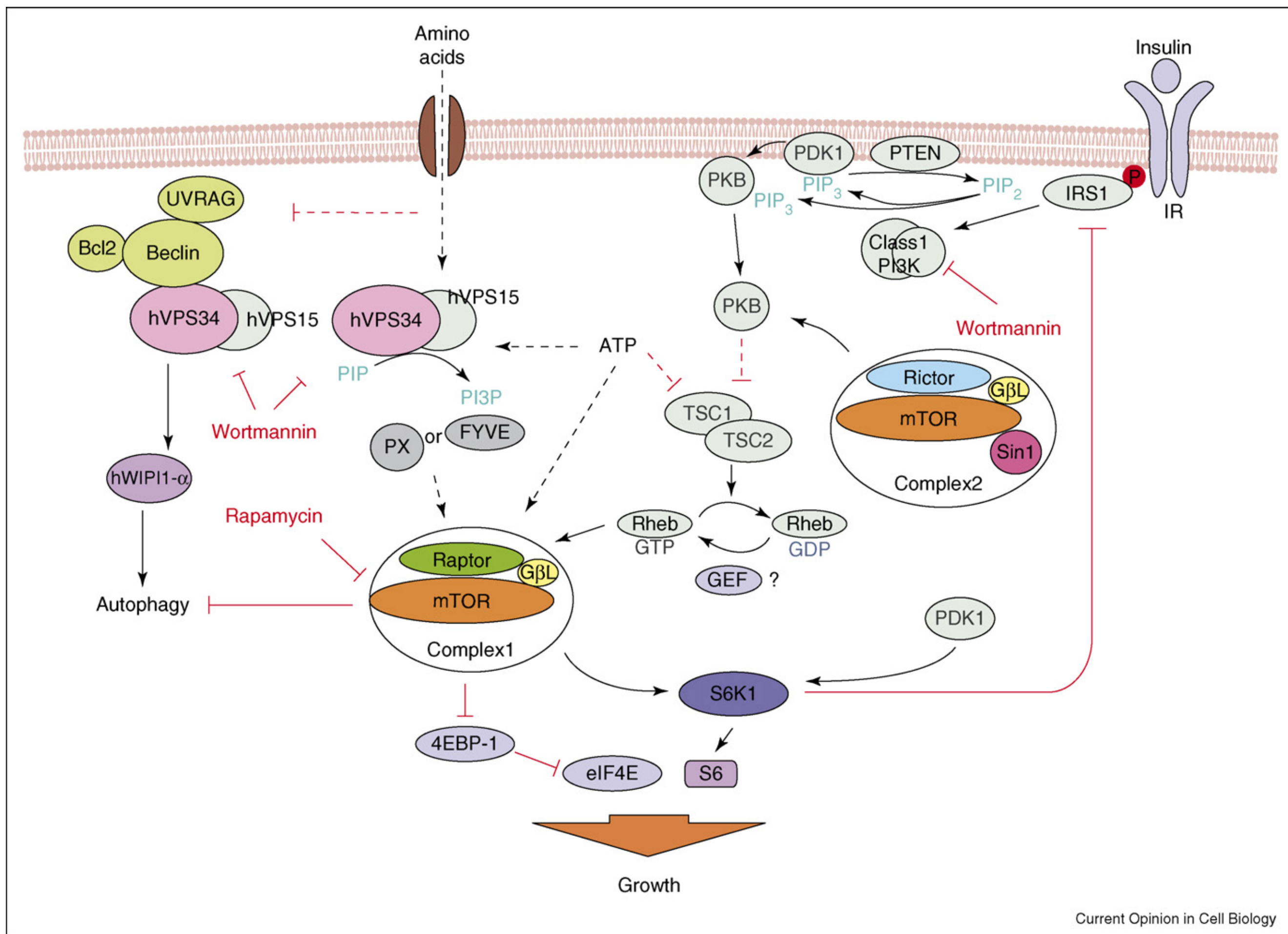
mTOR Complex1/S6K1

mTOR and its downstream effector S6 kinase 1 (S6K1), as well as initiation factor 4E binding protein (4E-BP1), have emerged as critical signaling components in the development, differentiation and growth of the organism [1], as well as in the maintenance of normal metabolic homeostasis [2,3]. In the case of S6K1, mTOR appears to influence these processes largely at the level of protein synthesis, through S6K1 phosphorylation of target proteins, such as ribosomal protein S6 [4], elongation factor 2 kinase [5], and initiation factor 4B [6] as well as raising the levels of initiation factor eIF4A, through the phosphorylation and degradation of its inhibitor, programmed cell death protein 4 [7]. Moreover, dysregulation of this pathway is associated with a number of pathological states, including obesity, insulin resistance and cancer [3,8,9]. Indeed, the rapamycin derivatives CCI779 (Wyeth-Ayerst) and RAD001 (Novartis) are being used in phase II and III clinical trials for the treatment of solid tumors [9].

In vivo, mTOR requires two associated proteins to signal to S6K1: raptor [10–12] and GβL [13]. This complex, termed mTOR Complex1 [3], interacts with downstream substrates through raptor, which recognizes substrates, such as S6K1, through their TOR signaling (TOS) motifs [14]. The rapamycins, when bound to FKBP-12, appear to interfere with raptor's ability to recruit substrates, such as S6K1, to mTOR, thus inhibiting their phosphorylation. Although GβL initially appeared to be required for a signaling-competent mTOR Complex1 [13], this view has recently been questioned [15]. Further studies have shown that mTOR also exists in a second complex, mTOR Complex2, which includes GβL, rictor and mSin1 [16–19]. mTOR Complex2 appears to be the major kinase involved in regulating the phosphorylation of S473 on protein kinase B (PKB/Akt), one of the two key residues required for the phosphorylation of specific substrates (see Figure 1). The rapamycins do not normally inhibit mTOR Complex2, although, under conditions of chronic exposure to the drug, they may do so, since they are able to bind nascent mTOR prior to its assembly into mature mTOR Complex2. Both mTOR complexes are activated by mitogens; however, only the mTOR Complex1/S6K1 signaling branch responds to nutrients, including amino acids and glucose [20]. Thus, to propagate downstream signals, mTOR Complex1/S6K1 must integrate growth factor and hormone signals with those of nutrients.

Earlier studies have demonstrated that growth factors and hormones, such as insulin, regulate mTOR Complex1/S6K1 activation via class 1 PI3K. Upon insulin stimulation, the insulin receptor autophosphorylates at specific tyrosine residues, leading to the creation of specific docking sites. One of these sites acts to recruit IRS1 and IRS2

Figure 1



Model of the mTOR Complex1/S6K1 signaling pathway (see text). Insulin and amino acids activate mTOR through distinct signaling cascades, with amino acids acting through hVps34. hVps34 also participates in the regulation of autophagy.

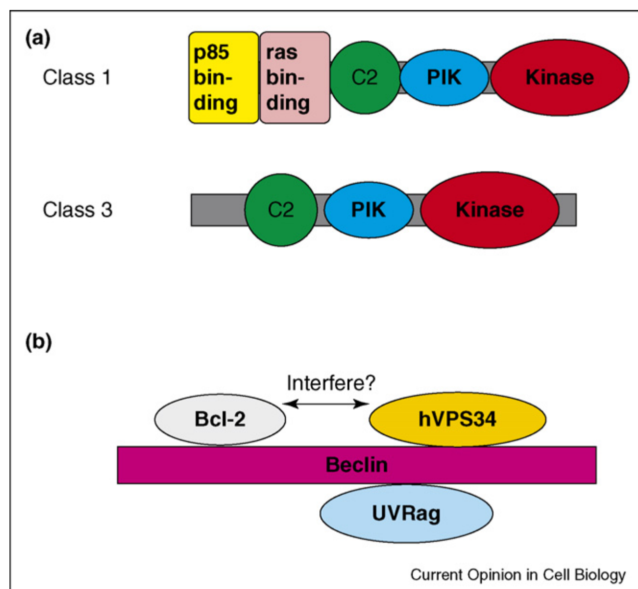
(see Figure 1), leading to their increased phosphorylation and to recruitment of adaptor molecules, such as the p85 regulatory subunit of class 1 PI3K [21]. In the case of class 1 PI3K, bringing the lipid kinase to the vicinity of the membrane leads to the transient production of the second messenger phosphatidylinositol (PtdIns (3,4,5)P_3). PtdIns (3,4,5)P_3 binds to the N-terminal plextrin-homology (PH) domain of PKB/Akt, recruiting PKB/Akt to the membrane, where it is phosphorylated by phosphoinositide-dependent protein kinase 1 (PDK1) [22,23] and mTOR Complex2 [19]. Activated PKB/Akt then acts to phosphorylate tuberous sclerosis complex (TSC) protein 2, which joins with TSC1 to make up a tumor suppressor complex associated with the autosomal-dominant genetic disorder, TSC [24]. Phosphorylation of TSC2 is thought to target TSC2 for destruction [25] or for 14-3-3 sequestration, where 14-3-3 proteins bind phosphorylated TSC2, thus keeping TSC2 in the cytosol and away from TSC1, which is always at the membrane [26]. In this way, the formation of the functional TSC1/TSC2 complex is inhibited [27], which may be a mechanism for inhibiting TSC2 tumor suppressor activity. In turn, TSC1/TSC2 functions as a GTPase that drives Ras homologue enriched in brain (Rheb) [28] into the inactive GDP-bound state [29–32]. Rheb directly interacts with mTOR Complex1, thus stimulating its ability to signal downstream to S6K1 [33]. The outcome of the insulin-induced response is elevated levels of protein synthesis and increased cell growth (see Figure 1).

The mechanism by which nutrients, such as amino acids and glucose, signal to mTOR Complex1/S6K1 is largely unknown. The importance of understanding this phenomenon has been underscored by two recent observations: first, that this branch of the pathway appears to play a critical role in insulin resistance, through a negative feedback loop from mTOR Complex1/S6K1 to IRS1 [3] (see Figure 1), and second, that in the environment of a solid tumor, where nutrients may be scarce, upregulation of this branch of the pathway may facilitate tumor growth [20]. Recent studies have indicated that the amino acid

response is, in part, mediated by a novel signaling pathway that is triggered by class 3 PI3K, also known as human vacuolar protein sorting 34 (hVps34) [34•,35••] (see Figure 2). hVps34 differs from class 1 PI3K in its substrate specificity and salt requirement in *in vitro* assays, in its binding partners and in its primary structure. *In vitro*, class 1 PI3K is more active with Mg^{2+} than with Mn^{2+}, whereas hVps34 exhibits the opposite preference. Moreover, hVps34 uses PtdIns (phosphoinositide) as a substrate to produce PtdIns(3)P_1, whereas class 1 is more active toward PtdIns(4, 5)P_2 to produce PtdIns(3,4,5)P_3. The catalytic subunits of both class 1 PI3K and hVps34 harbor a C2 domain, PIK domain and lipid kinase domain, though class 1 also has a p85-binding and a ras-binding domain. In addition, both lipid kinases form heterodimers with unique regulatory subunits: the adapter protein p85 for class 1 PI3K, and S/T protein kinase p150 for hVps34.

The role of amino acids in mTOR Complex1/S6K1 signaling was initially demonstrated in studies on macroautophagy, a cellular process in which portions of cytoplasm are sequestered within double-membrane vesicles known as autophagosomes before being delivered to lysosomes for degradation and recycling of cellular components [36]. Earlier studies showed that inhibition of macroautophagy by the addition of amino acids was directly paralleled by increased S6 phosphorylation and that this response was suppressed by rapamycin [37]. Subsequently, it was shown that amino-acid-induced S6K1 activation was dependent on mTOR Complex1 [38–40]. Moreover, a number of studies have shown that essential amino acids, especially the branched-chain amino acids (BCAAs), are critical in potentiating mTOR Complex1/S6K1 signaling [41]. However, it is clear that non-essential amino acids can also affect mTOR Complex1/S6K1 signaling [38,39], consistent with their role in driving early embryonic growth [42], a process in which mTOR Complex1 has been implicated [43].

Figure 2



Class 3 PI3Kinase is structurally distinct from Class 1 PI3Kinase and forms a unique complex with other signaling components. **(a)** Comparison between class 1 and 3 PI3Kinases. **(b)** Beclin complex containing hVPS34.

The yeast orthologue of hVps34 was first identified in a screen for genes involved in vacuolar protein localization and processing [44]. Further genetic [45,46] and biochemical [47,48] studies revealed that hVps34 resides in a membrane-associated complex with a serine/threonine kinase, hVps15, whose activity is essential for hVps34 function [45]. The sequence of hVps34 contains a C2 Ca^{2+}-binding motif [49] at its N-terminus, which was recently identified as the domain by which hVps34 interacts with beclin, the human orthologue of the autophagic gene Atg6 [50]. The C2 Ca^{2+}-binding motif of hVps34 is followed by a conserved PIK accessory domain, first described in yeast PI4K [51], and a highly conserved lipid kinase domain located at the C-terminus [47]. hVps15 (p150) contains a consensus sequence for the addition of myristic acid at its N-terminus, which is thought to target the hVps34/hVps15 complex to membranes, followed by a conserved serine/threonine catalytic kinase domain, a single HEAT repeat, and multiple WD40 repeats. Moreover, Rabs have been shown to bind to the HEAT and WD40 protein-interaction domains of hVps15, which may direct the hVps34/hVps15 complex to distinct intracellular compartments (see below). At these sites of recruitment, PtdIns(3)P_1 is produced, which in turn acts to recruit proteins containing FYVE or PX domains [52]. Many of these FYVE- and PX-domain-containing proteins are thought to be Rab effectors [53].

hVps34 and mTOR

The link between hVps34 and mTOR Complex1 signaling emerged independently in studies designed to elucidate the mechanism by which nutrient input, particularly of amino acids and glucose, leads to increased S6K1 T389 phosphorylation as a readout of mTOR Complex1 activation [34•,35••]. Initially it was presumed that amino acids affected mTOR Complex1/S6K1 by acting through the generic class 1 PI3K branch of this pathway [35••]. In the absence of TSC1/TSC2, although mTOR Complex1/S6K1 activation was elevated and refractive to stimulation by mitogens [54–56], such as insulin, it was still regulated by amino acids [35••]. However, this was not the case for Rheb, as siRNA knock-down of Rheb protein levels blocked both the insulin and amino acid input to S6K1 [35••]. Nonetheless, withdrawal of amino acids, which triggers S6K1 inactivation, had no effect on elevated Rheb–GTP levels in TSC2-deficient cells, leading to the hypothesis that endogenous, in contrast to overexpressed, Rheb–GTP [29] is necessary, but not sufficient, to drive S6K1 activation in the absence of amino acids.

The reason for the difference between the amino acid requirements associated with endogenous and overexpressed Rheb is not entirely understood. However, our unpublished results suggest that, normally, amino acids are necessary to induce a change in Rheb localization or a conformational change in mTOR Complex1 that allows active Rheb (or an immediate target effector of Rheb other than mTOR) to trigger mTOR substrate phosphorylation. However, it appears that an overwhelming amount of active Rheb can bypass this activation mechanism and signal to mTOR without amino acid stimulation. These findings suggested that the amino acid input to mTOR Complex1/S6K1 acts on a pathway parallel to the TSC1/2–Rheb axis. As earlier studies demonstrated that wortmannin, a class 1 PI3K inhibitor, blocked amino acid-induced S6K1 activation and that amino acids did not induce PKB activation [38,40], this suggested that a novel wortmannin-sensitive signaling component was responsible for mediating the amino acid input to the mTOR Complex1/S6K1. These observations led to the identification of hVps34 as the novel wortmannin-sensitive target by which these responses were mediated [34•,35••]. In brief, it was shown that ectopic expression of hVps34 drives S6K1 activation in the presence, but not in the absence, of amino acids, and that this effect is blocked by siRNAs [34•,35••] or neutralizing antibodies [34•] directed against hVps34. Moreover, stimulation of cells with amino acids appears to increase hVps34 activity, as measured by the production of PtdIns(3)P_1. Consistent with hVps34 mediating the amino acid input to S6K1, this response is attenuated by expression of a cDNA whose protein product contains two FYVE domains and binds to PtdIns(3)P_1, blocking the binding of FYVE- or PX-domain-containing proteins and preventing S6K1 activation [34•,35••]. Interestingly, hVps34 is the only PI3K found in yeast. Indeed, it may be that, during the evolution of metazoans and the rise of systemic hormonal regulation, the insulin–class 1 PI3K signaling pathway was merged with the primordial amino-acid-driven class 3 PI3K signaling pathway so that both pathways act through the mTOR Complex1 to control the growth and development of the organism (see Figure 1).

The mechanism by which PtdIns(3)P_1 signals to mTOR Complex1/S6K1 is not known; however, the data above suggest that it requires the increased production of PtdIns(3)P_1. One possibility is that hVps34, through PtdIns(3)P_1, acts to increase the intracellular concentrations of amino acids, either by enhancing their uptake from the external milieu or by increasing protein degradation through macro- or microautophagy. To test this possibility, the concentration of individual amino acids was measured in HeLa cells in which hVps34 protein levels were depleted by siRNA treatment. The amino acid content did not differ between cells treated with hVps34 siRNA and those treated with a control siRNA (T Nobukuni and G Thomas, unpublished). This indicates that hVps34 stimulation of mTOR Complex1/S6K1 signaling is not mediated through increased levels of amino acids, but instead acts as a component of the pathway downstream of amino acids. Given the role of PtdIns(3)P_1 in recruiting proteins containing either FYVE or PX domains, it is highly possible that hVps34 is involved in forming a signaling complex that includes mTOR Complex1 and potentially S6K1 (see below). It should also be noted that glucose depletion has been reported to inhibit hVps34 activity, suggesting that amino acids or glucose are converging through a common mechanism to activate mTOR Complex1/S6K1 signaling [34•]. Although earlier it was argued that glucose signals to mTOR Complex1/S6K1 through the activation of AMPK kinase and the phosphorylation of TSC2 [57], this model has been called into question by two recent publications. These studies showed that REDD1, which negatively regulates mTOR activity in response to energy deprivation, does not require AMPK to mediate this response [58], and reagents that affect AMPK activity affected a downstream target of mTOR, eEF2 kinase, in the absence of TSC2 [59]. It should also be noted that energy deprivation does not have an immediate impact on intracellular amino acid levels, nor does amino acid deprivation affect ATP levels [60]. Key questions, then, are the identity of the mechanism leading to hVps34 activation, and whether this mechanism is shared by amino acids, glucose and other nutrients.

Autophagy and endosome signaling

hVps34 is known to play a critical role in autophagy, and has been shown to directly interact with the autophagic gene beclin. Beclin was first identified as a Bcl-2-interacting protein in a yeast two-hybrid screen [61]. Subsequently it has been shown to be a haploinsufficient tumor suppressor gene [62,63], and it has been demonstrated that overexpression of Bcl-2 disrupts the hVps34–beclin interaction [64•]. Moreover, it is hypothesized that, in a nutrient-replete setting, Bcl-2 inhibits autophagy by sequestering beclin away from hVps34 [64•]. However, this latter view has been challenged recently, in that Bcl-2 and hVps34 are argued to bind within the same complex through two distinct domains in beclin, along with a third protein, UVRAG, which is also a haploinsufficient tumor suppressor gene [65••]. How then does hVps34 trigger autophagy? Recent studies have led to the identification of a WD40-repeat-containing protein, hWIPI-1α (WIPI49) (see Figure 1), the human orthologue of the yeast autophagy gene Atg18, which, following amino acid deprivation, co-localizes with the autophagosomal marker LC3 at punctate cytoplasmic structures in cells. Moreover, structural analysis data suggest that hWIPI-1α contains an evolutionarily conserved phospholipid-binding domain [66•]. In yeast, this domain has been shown to bind PtdIns(3)P_1 and to be involved in the formation of the pre-autophagosome structures (PASs) [67], which act as a donor compartments for the biogenesis of autophagosomes [68]. Given the

common role of WD40-repeat-containing proteins in coordinating multiprotein complex assemblies, with the repeating units serving as a rigid scaffold for protein interactions [69], it has been hypothesized that hWIPI-1α may also act as a scaffold for building the autophagosome [66•].

The suggestion that hVps34 is a positive effector in both autophagy and mTOR Complex1/S6K1 signaling appears incongruent because autophagy is thought to be under the negative control of mTOR [36]. A potential explanation is that hVps34 may be operating in specific cellular compartments. As indicated above, hVps34 potentially plays a distinct role in the recruitment of hWIPI-Iα during the generation of the autophagosome. However, it is known that the hVps34/hVp15 complex is also recruited to early endosomes by Rab5 [70] through the direct interaction of Rab5 with the carboxyl tail of hVp15 [71]. In this way, Rab5 is more readily able to recruit its downstream FYVE-domain effector early-endosomal autoantigen 1 (EEA1) to early endosomes, where it is required for endosome fusion [72]. The primary roles of hVps34 in endosomes are in growth-factor-receptor sorting and the formation of intralumenal endosomal vesicles; it does not appear to play a role in targeting cargo to the late endosome/lysosome [73]. Interestingly, early endosomes have been implicated in signaling [74]. The concept of endosome signaling emanates from studies showing that, following ligand addition, activated epidermal growth factor receptors were found on early endosomes with their downstream signaling effectors. Similarly, nerve growth factor was found with its activated TrkA receptor and phospholipase γ1 (PLC-γ1) in endocytic organelles, which were capable of signaling to downstream effectors such as MAPK [74]. Relevant to this concept, we have found that mTOR Complex1 co-immunoprecipitates with hVps34 (P Gulati and G Thomas, unpublished), and others have reported that Rheb, through its carboxyl CaaX box, associates with the Golgi [75]. These findings raise the possibility that mTOR Complex1 may signal to downstream effectors through such vesicles.

Future perspectives

Given the ability of mTOR Complex1 to suppress autophagy, and the importance of both endosome trafficking and autophagy to the malignant phenotype, it will be critical to identify the mechanisms by which these two pathways interact at the intracellular level. Currently, hVps34 and hVps15 are the only known components of the pathway linking nutrient input to mTOR Complex1 activation. Thus, identifying the upstream and downstream elements of this pathway will be critical to deciphering the mechanism of the cellular response to nutrients. To find the downstream targets of hVps34, it will be important to find the PtdIns $(2)P_1$-binding protein responsible for the activation of the pathway, especially since it seems that hVps34's ability to produce PtdIns $(3)P_1$ is critical for activation of the pathway. It is also of importance to find the binding partners of hVps34/hVps15 to identify the upstream regulator of the pathway.

Acknowledgments

We thank M Daston for editing and members of Kozma/Thomas laboratory for discussions and insights concerning this review. GT is supported by a U01 grant of the Mouse Models of Human Cancer Consortium (CA-84292-06) and an R01 grant (DK-73802-01) from the NIH. SK is supported by a R01 grant (CA-120475-01) from the NIH.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Wullschleger S, Loewith R, Hall MN: **TOR signaling in growth and metabolism**. *Cell* 2006, **124**:471-484.
2. Kahn BB, Myers MG Jr: **mTOR tells the brain that the body is hungry**. *Nat Med* 2006, **12**:615-617.
3. Um SH, D'Alessio D, Thomas G: **Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1**. *Cell Metab* 2006, **3**:393-402.
4. Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, Dor Y, Zisman P, Meyuhas O: **Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis**. *Genes Dev* 2005, **19**:2199-2211.
5. Wang X, Li W, Williams M, Terada N, Alessi DR, Proud CG: **Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase**. *Embo J* 2001, **20**:4370-4379.
6. Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, Mayeur GL, Polakiewicz RD, Sonenberg N, Hershey JW: **Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases**. *Embo J* 2004, **23**:1761-1769.
7. Dorrello NV, Peschiaroli A, Guardavaccaro D, Colburn NH, Sherman NE, Pagano M: **S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth**. *Science* 2006, **314**:467-471.
8. Krebs M: **Amino acid-dependent modulation of glucose metabolism in humans**. *Eur J Clin Invest* 2005, **35**:351-354.
9. Faivre S, Kroemer G, Raymond E: **Current development of mTOR inhibitors as anticancer agents**. *Nat Rev Drug Discov* 2006, **5**:671-688.
10. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM: **mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery**. *Cell* 2002, **110**:163-175.
11. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K: **Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action**. *Cell* 2002, **110**:177-189.
12. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN: **Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control**. *Mol Cell* 2002, **10**:457-468.
13. Kim DH, Sarbassov dos D, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, Tempst P, Sabatini DM: **GβL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR**. *Mol Cell* 2003, **11**:895-904.
14. Schalm SS, Fingar DC, Sabatini DM, Blenis J: **TOS motif-mediated raptor binding regulates 4E-BP1 multisite phosphorylation and function**. *Curr Biol* 2003, **13**:797-806.
15. Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, Sabatini DM: **Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals**

that mTORC2 is required for signaling to Akt-FOXO and PKCα, but not S6K1. *Dev Cell* 2006, **11**:859-871.

16. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN: **Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive**. *Nat Cell Biol* 2004, **6**:1122-1128.

17. Yang J, Wong RK, Park M, Wu J, Cook JR, York DA, Deng S, Markmann J, Naji A, Wolf BA *et al.*: **Leucine regulation of glucokinase and ATP synthase sensitizes glucose-induced insulin secretion in pancreatic β-cells**. *Diabetes* 2006, **55**:193-201.

18. Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA, Sabatini DM: **mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s**. *Curr Biol* 2006, **16**:1865-1870.

19. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM: **Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex**. *Science* 2005, **307**:1098-1101.

20. Dann SG, Thomas G: **The amino acid sensitive TOR pathway from yeast to mammals**. *FEBS Lett* 2006, **580**:2821-2829.

21. Fruman DA, Meyers RE, Cantley LC: **Phosphoinositide kinases**. *Annu Rev Biochem* 1998, **67**:481-507.

22. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P: **Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha**. *Current Biology* 1997, **7**:261-269.

23. Stokoe D, Stephens LR, Copeland T, Gaffney PR, Reese CB, Painter GF, Holmes AB, McCormick F, Hawkins PT: **Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B**. *Science* 1997, **277**:567-570.

24. Montagne J, Radimerski T, Thomas G: **Insulin signaling: lessons from the drosophila tuberous sclerosis complex, a tumor suppressor**. *Sci STKE* 2001, **2001**:E36.

25. Nellist M, Goedbloed MA, de Winter C, Verhaaf B, Jankie A, Reuser AJ, van den Ouweland AM, van der Sluijs P, Halley DJ: **Identification and characterization of the interaction between tuberin and 14-3-3ζ**. *J Biol Chem* 2002, **277**:39417-39424.

26. Li Y, Inoki K, Yeung R, Guan KL: **Regulation of TSC2 by 14-3-3 binding**. *J Biol Chem* 2002, **277**:44593-44596.

27. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC: **Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway**. *Mol Cell* 2002, **10**:151-162.

28. Yamagata K, Sanders LK, Kaufmann WE, Yee W, Barnes CA, Nathans D, Worley PF: **rheb, a growth factor- and synaptic activity-regulated gene, encodes a novel Ras-related protein**. *J Biol Chem* 1994, **269**:16333-16339.

29. Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Roccio M, Stocker H, Kozma SC, Hafen E, Bos JL, Thomas G: **Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2**. *Mol Cell* 2003, **11**:1457-1466.

30. Saucedo LJ, Gao X, Chiarelli DA, Li L, Pan D, Edgar BA: **Rheb promotes cell growth as a component of the insulin/TOR signalling network**. *Nat Cell Biol* 2003, **5**:566-571.

31. Stocker H, Radimerski T, Schindelholz B, Wittwer F, Belawat P, Daram P, Breuer S, Thomas G, Hafen E: **Rheb is an essential regulator of S6K in controlling cell growth in *Drosophila***. *Nat Cell Biol* 2003, **5**:559-565.

32. Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D: **Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins**. *Nat Cell Biol* 2003, **5**:578-581.

33. Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J: **Rheb binds and regulates the mTOR kinase**. *Curr Biol* 2005, **15**:702-713.

34. • Byfield MP, Murray JT, Backer JM: **hVps34 is a nutrient-regulated lipid kinase required for activation of p70 s6 kinase**. *J Biol Chem* 2005, **280**:33076-33082.

This paper shows that hVps34 is a critical component of the nutrient signaling apparatus leading to S6K1 activation. Amino acids, which activate S6K1, positively affect hVps34 activity, as measured in an *in vitro* kinase assay. In contrast, negative effectors of mTOR signaling, including the mitochondrial poison, oligomycin, and AMP kinase inhibit hVps34 activity, arguing that both nutrients and energy mediate mTOR signaling through hVPS34.

35. •• Nobukuni T, Joaquin M, Roccio M, Dann SG, Kim SY, Gulati P, Byfield MP, Backer JM, Natt F, Bos JL *et al.*: **Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase**. *Proc Natl Acad Sci U S A* 2005, **102**:14238-14243.

This study uses genetic, biochemical, and pharmacologic approaches to demonstrate that amino-acid-mediated mTOR activation is independent of the generic class 1 signaling pathway. Instead it is demonstrated that amino acids mediate mTOR signaling through class 3 PI3K, hVps34.

36. Klionsky DJ, Emr SD: **Autophagy as a regulated pathway of cellular degradation**. *Science* 2000, **290**:1717-1721.

37. Blommaart EF, Luiken JJ, Blommaart PJ, van Woerkom GM, Meijer AJ: **Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes**. *J Biol Chem* 1995, **270**:2320-2326.

38. Hara K, Yonezawa K, Weng QP, Kozlowski MT, Belham C, Avruch J: **Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism**. *J Biol Chem* 1998, **273**:14484-14494.

39. Xu G, Kwon G, Marshall CA, Lin TA, Lawrence JC Jr, McDaniel ML: **Branched-chain amino acids are essential in the regulation of PHAS-I and p70 S6 kinase by pancreatic β-cells. A possible role in protein translation and mitogenic signaling**. *J Biol Chem* 1998, **273**:28178-28184.

40. Iiboshi Y, Papst PJ, Kawasome H, Hosoi H, Abraham RT, Houghton PJ, Terada N: **Amino acid-dependent control of p70(s6k). Involvement of tRNA aminoacylation in the regulation**. *J Biol Chem* 1999, **274**:1092-1099.

41. Kimball SR, Jefferson LS: **Molecular mechanisms through which amino acids mediate signaling through the mammalian target of rapamycin**. *Curr Opin Clin Nutr Metab Care* 2004, **7**:39-44.

42. Lee ES, Fukui Y: **Synergistic effect of alanine and glycine on bovine embryos cultured in a chemically defined medium and amino acid uptake by vitro-produced bovine morulae and blastocysts**. *Biol Reprod* 1996, **55**:1383-1389.

43. Gangloff YG, Mueller M, Dann SG, Svoboda P, Sticker M, Spetz JF, Um SH, Brown EJ, Cereghini S, Thomas G *et al.*: **Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development**. *Mol Cell Biol* 2004, **24**:9508-9516.

44. Herman PK, Emr SD: **Characterization of VPS34, a gene required for vacuolar protein sorting and vacuole segregation in *Saccharomyces cerevisiae***. *Mol Cell Biol* 1990, **10**:6742-6754.

45. Stack JH, Herman PK, Schu PV, Emr SD: **A membrane-associated complex containing the Vps15 protein kinase and the Vps34 PI 3-kinase is essential for protein sorting to the yeast lysosome-like vacuole**. *Embo J* 1993, **12**:2195-2204.

46. Schu PV, Takegawa K, Fry MJ, Stack JH, Waterfield MD, Emr SD: **Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting**. *Science* 1993, **260**:88-91.

47. Volinia S, Dhand R, Vanhaesebroeck B, MacDougall LK, Stein R, Zvelebil MJ, Domin J, Panaretou C, Waterfield MD: **A human phosphatidylinositol 3-kinase complex related to the yeast Vps34p-Vps15p protein sorting system**. *Embo J* 1995, **14**:3339-3348.

48. Panaretou C, Domin J, Cockcroft S, Waterfield MD: **Characterization of p150, an adaptor protein for the human phosphatidylinositol (PtdIns) 3-kinase. Substrate presentation by phosphatidylinositol transfer protein to the p150. Ptdins 3-kinase complex**. *J Biol Chem* 1997, **272**:2477-2485.

49. Nishizuka Y: **The molecular heterogeneity of protein kinase C and its implications for cellular regulation**. *Nature* 1988, **334**:661-665.

50. Kihara A, Kabeya Y, Ohsumi Y, Yoshimori T: **Beclin-phosphatidylinositol 3-kinase complex functions at the trans-Golgi network**. *EMBO Rep* 2001, **2**:330-335.

51. Flanagan CA, Schnieders EA, Emerick AW, Kunisawa R, Admon A, Thorner J: **Phosphatidylinositol 4-kinase: gene structure and requirement for yeast cell viability**. *Science* 1993, **262**:1444-1448.

52. Lemmon MA: **Phosphoinositide recognition domains**. *Traffic* 2003, **4**:201-213.

53. Grosshans BL, Ortiz D, Novick P: **Rabs and their effectors: achieving specificity in membrane traffic**. *Proc Natl Acad Sci U S A* 2006, **103**:11821-11827.

54. Inoki K, Li Y, Zhu T, Wu J, Guan KL: **TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signaling**. *Nat Cell Biol* 2002, **4**:648-657.

55. Jaeschke A, Hartkamp J, Saitoh M, Roworth W, Nobukuni T, Hodges A, Sampson J, Thomas G, Lamb R: **Tuberous sclerosis complex tumor suppressor-mediated S6 kinase inhibition by phosphatidylinositide-3-OH kinase is mTOR independent**. *J Cell Biol* 2002, **159**:217-224.

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

57. Inoki K, Zhu T, Guan KL: **TSC2 mediates cellular energy response to control cell growth and survival**. *Cell* 2003, **115**:577-590.

58. Sofer A, Lei K, Johannessen CM, Ellisen LW: **Regulation of mTOR and cell growth in response to energy stress by REDD1**. *Mol Cell Biol* 2005, **25**:5834-5845.

59. Smith EM, Finn SG, Tee AR, Browne GJ, Proud CG: **The tuberous sclerosis protein TSC2 is not required for the regulation of the mammalian target of rapamycin by amino acids and certain cellular stresses**. *J Biol Chem* 2005, **280**:18717-18727.

60. Dennis PB, Jaeschke A, Saitoh M, Fowler B, Kozma SC, Thomas G: **Mammalian TOR: a homeostatic ATP sensor**. *Science* 2001, **294**:1102-1105.

61. Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, Herman B, Levine B: **Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein**. *J Virol* 1998, **72**:8586-8596.

62. Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y *et al.*: **Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene**. *J Clin Invest* 2003, **112**:1809-1820.

63. Yue Z, Jin S, Yang C, Levine AJ, Heintz N: **Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor**. *Proc Natl Acad Sci U S A* 2003, **100**:15077-15082.

64. • Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B: **Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy**. *Cell* 2005, **122**:927-939.

These authors show that Bcl-2 inhibits beclin-1-dependent autophagy in yeast and mammalian cells, but not beclin 1 binding to defective mutants of Bcl-2. They also show that transgenic expression of Bcl-2 in heart inhibits cardiac muscle autophagy. Finally, beclin 1 mutants defective in Bcl-2 binding promote higher levels of autophagy than wild-type beclin 1.

65. •• Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, Jung JU: **Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG**. *Nat Cell Biol* 2006, **8**:688-699.

This paper reports the identification of a novel coiled-coil UV irradiation resistance-associated protein (UVRAG) as a component of a Bcl-2–Beclin–hVps34 complex, required for autophagy. UVRAG is monoallelically mutated at increased frequency in human colon cancers. The UVRAG-mediated activation of the Bcl-2–Beclin–hVps34 complex promotes autophagy and suppresses the proliferation of human colon cancer cells. Thus UVRAG appears to be a tumor suppressor candidate.

66. • Proikas-Cezanne T, Waddell S, Gaugel A, Frickey T, Lupas A, Nordheim A: **WIPI-1α (WIPI49), a member of the novel 7-bladed WIPI protein family, is aberrantly expressed in human cancer and is linked to starvation-induced autophagy**. *Oncogene* 2004, **23**:9314-9325.

WD-repeat proteins are β-propeller platforms that are involved in the assembly of multiprotein complexes. WIPI-1α is a member of a novel WD-repeat protein family, which plays a positive role in autophagy in yeast and *C. elegans*. The data presented in this study suggest that human WIPI proteins share an evolutionarily conserved function in autophagy, playing a critical role in generating the autophagosome. Moreover, WIPI genes were found to be aberrantly expressed in a variety of matched tumor tissues, suggesting that they may be compromised in human cancers.

67. Krick R, Tolstrup J, Appelles A, Henke S, Thumm M: **The relevance of the phosphatidylinositolphosphat-binding motif FRRGT of Atg18 and Atg21 for the Cvt pathway and autophagy**. *FEBS Lett* 2006, **580**:4632-4638.

68. Suzuki K, Kirisako T, Kamada Y, Mizushima N, Noda T, Ohsumi Y: **The pre-autophagosomal structure organized by concerted functions of APG genes is essential for autophagosome formation**. *Embo J* 2001, **20**:5971-5981.

69. Saxena K, Gaitatzes C, Walsh MT, Eck M, Neer EJ, Smith TF: **Analysis of the physical properties and molecular modeling of Sec13: A WD repeat protein involved in vesicular traffic**. *Biochemistry* 1996, **35**:15215-15221.

70. Christoforidis S, Miaczynska M, Ashman K, Wilm M, Zhao L, Yip SC, Waterfield MD, Backer JM, Zerial M: **Phosphatidylinositol-3-OH kinases are Rab5 effectors**. *Nat Cell Biol* 1999, **1**:249-252.

71. Murray JT, Panaretou C, Stenmark H, Miaczynska M, Backer JM: **Role of Rab5 in the recruitment of hVps34/p150 to the early endosome**. *Traffic* 2002, **3**:416-427.

72. Simonsen A, Lippe R, Christoforidis S, Gaullier JM, Brech A, Callaghan J, Toh BH, Murphy C, Zerial M, Stenmark H: **EEA1 links PI(3)K function to Rab5 regulation of endosome fusion**. *Nature* 1998, **394**:494-498.

73. Gruenberg J, Stenmark H: **The biogenesis of multivesicular endosomes**. *Nat Rev Mol Cell Biol* 2004, **5**:317-323.

74. Miaczynska M, Pelkmans L, Zerial M: **Not just a sink: endosomes in control of signal transduction**. *Curr Opin Cell Biol* 2004, **16**:400-406.

75. Buerger C, DeVries B, Stambolic V: **Localization of Rheb to the endomembrane is critical for its signaling function**. *Biochem Biophys Res Commun* 2006, **344**:869-880.