Nutrient sensing in the mTOR/S6K1 signalling pathway

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Abstract

Nutrient overload induces constitutive S6K1 (S6 kinase 1) activation, which leads to insulin resistance by suppressing insulin-induced class I PI3K (phosphoinositide 3-kinase) signalling [Um, Frigerio, Watanabe, Picard, Joaquin, Sticker, Fumagalli, Allegrini, Kozma, Auwerx and Thomas (2004) Nature 431, 200–205]. This finding gave rise to the question of the mechanism by which nutrients, such as AAs (amino acids), enter the mTOR (mammalian target of rapamycin)/S6K1 signalling pathway. Counter to the prevailing view, our recent studies have shown that the AA input into the mTOR/S6K1 signalling pathway is not mediated by the tumour suppressor TSC1 (tuberous sclerosis complex 1)/TSC2 or its target, the proto-oncogene Rheb (Ras homologue enriched in brain). Instead, we found that the AA input was mediated by class 3 PI3K, or hVps34 (human vacuolar protein sorting 34). In brief, ectopic expression of hVps34 drives S6K1 activation, but only in the presence of AAs, and this effect is blocked by small interfering RNAs directed against hVps34. Moreover, stimulation of cells with AAs increases hVps34 activity, as indicated by the production of PI3P (phosphatidylinositol 3-phosphate). PI3P mediates the recruitment of proteins containing FYVE (Fab1p, Yotb, Vac1p and Eea1) or PX (Phox homology) domains to endosomal membranes, with PI3P-rich micro-domains acting as signalling platforms. Additional evidence indicating hVps34 as the mediator of AA input to S6K1 came from experiments in which S6K1 activation was attenuated by ectopic expression of a cDNA containing two FYVE domains, which bind to PI3P, preventing binding of proteins containing either FYVE or PX domains [Nobukuni, Joaquin, Roccio, Dann, Kim, Gulati, Byfield, Backer, Natt, Bos, Zwartkruis and Thomas (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 14238–14243].

Introduction

The term ‘nutrient sensing’ has been defined and interpreted in different ways. In the narrowest sense, a nutrient sensor is a protein whose binding to a nutrient leads to a change in the function of that protein, which in some cases may trigger downstream activation of a signalling cascade. Nutrients such as AAs (amino acids), glucose and fatty acids have traditionally been considered as metabolic fuels for cell growth and development. But this view is undergoing a rapid change with an increased understanding of the mechanisms by which such nutrients can act as mediators of signal transduction pathways. These nutrient-mediated signalling cascades regulate specific aspects of fuel and energy metabolism, ultimately influencing cell growth, proliferation and survival.

Research performed in the past decade has shown that there are functionally distinct nutrient signalling pathways.

Substantial progress has been made in delineating the complex network of upstream events that regulate these pathways, and in identifying their downstream targets and functions. Many of these sensors and signalling pathways are conserved from yeast to mammals. All organisms appear to have the ability to sense nutrient availability on a moment-to-moment basis and to adjust flux through metabolic and signalling pathways accordingly. A major nutrient-signalling pathway emerging from such studies is the mTOR (mammalian target of rapamycin)/S6K1 (S6 kinase 1) signalling pathway, which is activated by specific nutrients [1,2] and is involved in the control of cell growth and proliferation.

AAs and the mTOR signalling pathway

The first indication of AAs acting as signal transducers in the control of metabolic pathways came from studies on the regulation of macroautophagy in hepatocytes [3]. In response to AA deprivation, autophagy is up-regulated to produce AAs that are inhibitory for autophagy and are needed for survival of the cell [4]. Later it was shown that the addition of AAs to the absence of insulin or other growth factors resulted in an acute, rapid stimulation of the phosphorylation of two mTOR substrates, ribosomal protein S6K1 [5–7] and initiation factor 4E-BP1 (4E-binding protein 1) [6,7]. Since the stimulation of S6 phosphorylation by AAs could be completely prevented by rapamycin, it was concluded that mTOR-dependent activation of S6K1 must be responsible...
for AA-stimulated S6 phosphorylation, a downstream target of S6K1 [8,9]. The mechanism(s) responsible for the AA-induced mTOR phosphorylation of S6K1 and 4E-BP1 and their synergism with that of insulin has been the subject of intense study. Contrary to earlier models, the studies carried out in our laboratory using either cells depleted of TSC1 (tuberous sclerosis complex 1) or TSC2 as well as TSC1- or TSC2-null cells suggested that nutrients do not act through TSC1/2. Rather, recent results support a model in which nutrients act on mTOR signalling, independently and downstream of TSC1/2 [10,11]. Moreover, GTP levels in the Rheb (G-protein homologue enriched in brain), a small GTPase, do not change in TSC1- or TSC2-null cells following AA withdrawal, although S6K1 is inactivated [10,12]. The failure of AA deprivation to alter levels of Rheb-GTP, and their exclusive activation of S6K1, and not of PKB (protein kinase B) [6,10], suggested that AAs transduce signals to mTOR via a pathway that operates in parallel to the TSC1/2–Rheb signalling axis [10]. Studies performed in our and other laboratories had shown that wortmannin, a potent class I PI3K (phosphoinositide 3-kinase) inhibitor, blocks AA-mediated S6K1 activation via mTOR signalling [6,13]. To analyse the role of class I PI3K in AA signalling to mTOR, we depleted cells of class I PI3K using a previously described siRNA (small interfering RNA) [10] and found that AAs were still capable of activating S6K1. However, under these same conditions we found that insulin-induced stimulation of S6K1 or PKB was largely suppressed. These observations imply that AA stimulation of S6K1 via mTOR is not transduced through class I PI3K or PKB [10], but rather it involves a class I PI3K-independent wortmannin-sensitive AA input to mTOR. Further studies revealed the identification of the wortmannin-sensitive target as class 3 PI3K, or hVps34 (human vacuolar protein sorting 34). This was demonstrated in experiments in which siRNA knockdown of hVps34 blocked AA- and insulin-induced S6K1 activation, but had no effect on PKB activation [10,14]. In addition, stimulation of cells with AAs led to an increase in the concomitant production of PI3P (phosphatidylinositol 3-phosphate), the product of hVps34 [10,14]. This response was not altered in TSC2-null cells, consistent with the state of S6K1 activation in these cells [10]. Using a monoclonal antibody that specifically recognizes PI3P, we employed confocal microscopy to show that PI3P levels decreased in cells after AA deprivation, whereas re-addition of AAs to AA-starved cells led to increased PI3P levels, confirming the earlier observed regulation of hVps34 activity by AAs. It is known that PI3P mediates the recruitment of proteins containing FYVE (Fab1p, YOTB, Vac1p and EEA1) or PI3P-targeting PX (Phox homology) domains to endosomal membranes [15], with PI3P-rich micro-domains having been shown to act as signalling platforms [16–18]. Consistent with a role for PI3P, the product of hVps34 kinase, in AA-induced S6K1 activation, this response is blocked by the overexpression of a PI3P-interacting mutant construct encoding two FYVE domains [10]. Moreover, Byfield et al. [14] have shown that inputs such as energy and osmotic stress also appear to regulate hVps34 activity in a manner similar to that of AAs, suggesting a possible role of hVps34 as a common nutrient sensor for mTOR activation leading to S6K1 stimulation (Figure 1). Previously, it was shown that mTOR exists in a complex with Raptor (regulatory-associated protein of mTOR) [19] and GβL (G-protein β-subunit-like protein) [20], termed mTOR Complex 1 [22]. Moreover, nutrients such as AAs activate S6K1 by modulating mTOR Complex 1 activity through the regulation of dynamic interaction between Raptor and mTOR, which is sensitive to the mTOR inhibitor, rapamycin [20]. Interestingly, insulin-induced PKB activation is mediated by the rapamycin-insensitive mTOR Complex 2, a complex comprising Rictor (rapamycin-insensitive companion of mTOR), rather than Raptor, GβL and mTOR [22]. Thus both mTOR complexes play a role in S6K1 activation through nutrients and insulin (Figure 1).

An important function for hVps34 is to control macroautophagy. However, under conditions of AA withdrawal, macroautophagy is thought to be under the negative control of mTOR and the positive control of hVps34 [23]. This seeming contradiction could be explained by previous findings of Kihara et al. [24]. Their results suggest the existence of multiple cellular hVps34 complexes of distinct identities. Thus some hVps34-containing complexes may be involved in the control of autophagy, whereas others may be implicated in the regulation of endocytic trafficking and protein sorting. Recent reports suggest that hVps34 complexes that contain Beclin-1 [25] and UVRAG (UV irradiation resistance-associated gene) [26] act specifically as modulators of autophagy, while hVps34 complexes without tumour

Figure 1 | A model depicting the interplay of signals between the signalling molecules involved in the nutrients (AAs) and growth factor (insulin) signalling pathways

Broken arrows indicate steps where mechanism(s) are not yet known.
mTORC1/2, mTOR Complex 1/2; PKD1, phosphoinositide-dependent kinase 1; PI, phosphatidylinositol; PI3P, phosphatidylinositol 3-phosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate.
suppressors beclin-1 and UVRAG are important for protein-
trafficking events in the cell.

An important unifying concept emerging from our current
understanding of nutrient-signalling pathways is that there
must be cross-talk among these pathways and to other meta-
bolic pathways operating in the cell [27]. Overall, these sig-
nalling cascades may be involved in forming a larger metabolic
regulatory network that co-ordinates cellular metabolism
and regulates energy homoeostasis in the cell and/or whole
organism. Further unravelling of these nutrient-mediated
signalling cascades will help us to better understand how
nutrients control intermediary metabolism, and to concep-
tualize how nutrient excess, growth factor signalling defects,
or both, might play a role in the pathogenesis of various
diseases such as diabetes, obesity and cancer.

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Received 23 October 2006