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Review

Amino acid sensing and regulation of mTORC1

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ABSTRACT

Amino acids play fundamental roles in the cell both as the building blocks of new proteins and as metabolic precursors. To adapt to their limitation during periods of protein starvation, multiple adaptive mechanisms have evolved, including a rapid cessation of new protein synthesis, an increase in amino acid biosynthesis and transport, and autophagy. Here, we discuss what we currently know about how amino acid limitation is sensed, and how this sensing might be transmitted to mTORC1 to regulate protein synthesis and autophagy.

Contents

1. Introduction

In multicellular organisms homeostatic mechanisms evolved that permit survival of the organism when faced with a broad range of environmental fluctuations, including large changes in temperature, salinity, acidity and nutrient supply. In the face of fluctuating supply of amino acid nutrients, mechanisms evolved that both suppressed energy-consuming processes such as new protein synthesis and augmented redistribution of existing resources *via* proteolysis. Both processes are likely critical in maintaining overall cell mass and viability, particularly given the large contribution of protein to a cell's dry mass [1]. Amino acid availability is one of the major determinants of normal organismal growth and impacts on both regulation of protein synthesis and upon proteolysis [2,3].

Restriction of one or several amino acids has been found to have pleiotropic cellular effects, depending on the type of cell, organism and timeframe studied (reviewed in [4]). These include: a general but incomplete inhibition of protein synthesis, increased expression of amino acid biosynthetic genes, and enhanced degradation of proteins through ubiquitin–proteasome systems and autophagy [5–7]. Conversely, at least in higher organisms, amino acid excess has been found to be associated with decreased expression of proteins involved in ubiquitin–proteasome and autophagy protein degradation pathways and a reduction in the level of mRNAs encoding ubiquitin ligases [8,9]. Given that many cancer cells also adapt their metabolism when faced with a limited blood supply and insufficient nutrients to support rapid tumor growth [10], there is much interest in understanding the mechanisms responsible for amino acid sensing and their potential for modulation in anti-cancer therapies.

2. Amino acid sensing and the mTORC1 pathway

The energy-costly anabolic process of protein synthesis must by necessity be coupled to the energy status of the cell and to ambient nutrient levels. In isolated cells, amino acid withdrawal leads to a rapid suppression of protein synthesis and enhanced degradation of proteins [6,11]. In mammalian cells, amino acids

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sufficiency promote protein synthesis in part by inhibiting the binding of the translation repressor 4EBP1 (eukaryotic initiation factor eIF4E-binding protein-1) to initiation factor eIF4E and by stimulating the activity of ribosomal protein S6 kinase (S6K), and both these events are reversed by amino acid restriction [11]. These two signaling events require the activity of TOR (target of rapamycin), as 4EBP1 and S6K are the most well-studied downstream effectors for mTORC1, the rapamycin-sensitive mTOR complex [11]. TOR initially came to light as the cellular target for the immunosuppressant drug rapamycin [12]. Rapamycin treatment mimics a nutrient deprivation-like signal in budding yeast *Saccharomyces cerevisiae*, suggesting that TOR functionally mediates some responses to nutrient cues. TOR is structurally and functionally conserved from yeast to human [13] and is a member of the family of large phosphoinositide-3-kinase (PIK)-related kinases that possess protein kinase, rather than lipid kinase, activity. TOR has a C-terminal protein kinase domain with similarity to the lipid kinase PI3K, and repeated N-terminal HEAT segments that are thought to mediate its interaction with binding partners. mTOR (mammalian TOR) exists in two distinct functional complexes, mTOR complex 1 (mTORC1) and mTORC2. Rapamycin, complexed with the polypeptide FKBP12, binds directly to TORC1 and inhibits many (but not all [14,15]) of TORC1's signaling functions [16,17].

The core mTORC1 proteins are composed of mTOR, regulatory-associated protein of mTOR (Raptor), and mammalian lethal with Sec13 protein 8 (mLST8, also known as GβL) [18–20], while the core mTORC2 proteins are mTOR, Rictor (RPTOR-independent companion of mTOR), mSIN1 (mammalian stress-activated protein kinase interacting protein), and GβL [21–23]. Raptor regulates mTORC1 assembly and is thought to also recruit substrates such as 4E-BP1 and S6K [18,19]. Recent work has shown that Raptor also mediates interactions with the scaffold protein p62 involved in autophagosome formation, and with the Rag family of heterodimeric GTPases, suggesting that it likely plays an important role in directing the subcellular localization of mTORC1 [24,25].

Mitogens and hormones, such as insulin or polypeptide growth factors, or agents such as bacterial lipopolysaccharide (LPS) have been known for some time to be insufficient to drive full mTORC1 activation in the absence of amino acids, indicating that mTORC1 is able to monitor a deficiency in amino acids and that this inhibitory "sensing" acts dominantly to opposing stimulatory signals [11,26]. Insulin and other growth factors promote mTORC1 activation through mechanisms that mainly converge on inhibiting the tuberous sclerosis heterodimeric complex (TSC1/TSC2), thereby stimulating (in a manner that is currently unclear) GTP loading of the small GTPase Rheb (Ras homolog enriched in brain) [27–29]. The GTP-charged form of Rheb is a positive regulator of mTORC1, however, Rheb does not activate TORC1 signaling by modulating intracellular amino acid levels [30]. Both overexpressed wild-type Rheb (which is mainly GTP loaded [31]) or nucleotide-free Rheb have been shown to bind to mTOR at its catalytic domain under particular conditions, and Rheb has been shown by one group to stimulate mTORC1's weak kinase activity measured *in vitro*, and by another to promote substrate recruitment *via* raptor [31–33]. However, thus far an interaction between endogenous Rheb and mTORC1 has not been demonstrated during stimulation of mTORC1 by growth factors or amino acids.

Although amino acid withdrawal can lead to a decrease in Rheb-GTP levels in some cell types [34], Rheb-GTP levels have been shown not to decline in TSC1- or TSC2-null cells following amino acid withdrawal despite an inhibition of mTORC1, as determined by a decline in S6K1 T389 phosphorylation. Thus it has been suggested that amino acid nutrients act on mTORC1 signaling *via* a distinct signaling pathway [35,36]. Indeed, TORC1 in *S. cerevisiae* responds to nutrients [37] despite the absence of TSC orthologs.

3. Amino acid sensing and GCN2 signaling

The general control nonrepressed (GCN2) pathway, and its central component, the protein kinase GCN2 is a conserved pathway important for monitoring nutrient availability. Uncharged tRNAs that accumulate upon amino acid limitation bind to a tRNA-synthase-like domain in GCN2, inducing a conformational change that permits kinase activation, although it is likely that other activation mechanisms exist as GCN2 can also be rapidly activated by UV [38,39]. GCN2 was originally described in *S. Cerevisiae* as a kinase that phosphorylates the translational initiation factor eIF-2α [40], thereby sequestering the exchange factor for eIF2α, eIF2B, and decreasing the assembly of 43S initiation complexes under conditions of amino acid deprivation [41]. GCN2 activation was found to increase expression of a large set of genes, including many encoding enzymes of amino acid biosynthesis, through the increased translation of a transcriptional factor, GCN4 [42,40]. Thus, uncharged tRNA accumulating in amino acid-deprived cells activates GCN2, which phosphorylates the initiation factor eIF2α (eukaryotic initiation factor 2α) [43]. GCN2 together with GCN1, an *in vivo* activator and binding protein for of the kinase [44,45], are conserved from yeast to mammals. In mammalian cells, phosphorylation of eIF2α leads to preferential translation of a distinct transcription factor, activating transcription factor (ATF4), with the consequent induction of genes for amino acids biosynthesis and transport [46–48].

A major unresolved question remains whether the amino acid sensing mechanism controlled by GCN2 acts to inhibit mTORC1. On the face of it, linking an existing conserved sensor of amino acid deficiency to mTORC1 *via* an inhibitory interaction would make good use of an existing mechanism to respond to amino acid limitation. Indeed some evidence for GCN2 as an upstream inhibitor of mTOR under conditions of leucine deprivation have been provided [49,50]. However a major stumbling block to the GCN2 and mTORC1 systems being functionally linked is the finding that uncharged tRNA – the only well-described mechanism of GCN2 activation – have been reported not to be induced during the time frame of mTORC1 inhibition [51]. However, at least in *S. cerevisiae*, and using a sensitive method to detect changes in tRNA charging, it is now clear that leucine deprivation indeed does lead to the rapid appearance of uncharged tRNAleu species [52]. However, whether this rapid appearance of uncharged tRNA species also occurs in mammalian cells and might thereby allow a rapid enough activation of GCN2 to inhibit mTORC1, is not known.

4. Amino acids insufficiency: are some amino acids more important than others?

During development TOR is essential for growth, but upon completion of development it restricts lifespan, and its activation is thought to contribute to age-related diseases such as cancer, metabolic syndrome, atherosclerosis, hypertension and hypertrophic heart [53–55]. Therefore extensive research efforts have centered on understanding how amino acids, and their sensors and transporters, regulate TOR. Work in *Drosophila* and studies in mice have shown that a TOR-dependent sensor for amino acids exists in specific neuronal cells–hypothalamic cells in the mouse and neuroendocrine cells in *Drosophila*. These cells sense variations in circulating amino-acid levels and in turn regulate feeding behavior [56,57]. Aside from amino acid detection by these specific cells and their contribution to the behavioral responses involved in food intake, specific amino acids have been shown to impact on growth and to alter metabolic homeostasis. Indeed, the essential amino acid leucine appears to act upon metabolism at multiple levels and is critical in most cells for mTORC1 activation [11,58,59]. In many mammalian cell types cellular entry of leucine primarily

depends on the system L transporters, which consist of a promiscuous single-pass transmembrane glycoprotein (CD98) and one of six multipass transmembrane amino acid permeases (L-type amino acid transporter 1, 2, 3 and 4) [60–63]. In some cells, a bidirectional L amino acid transporter system has been identified that transports leucine and other essential amino acids (EAA) to regulate mTORC1 and autophagy *via* efflux of L-glutamine [64]. In *Drosophila*, a homolog of the heterodimer of CD98 and LAT-1 and -2, *Minidiscs*, is also likely to be involved in leucine transport [65,66]. Underscoring the importance of leucine in regulating TORC1, S6K1 was found to be activated by microinjection of leucine into *Xenopus* oocytes that normally lack the system L transporter [67].

However it should be noted that another critical amino acid for activation of mTORC1 highlighted by some studies is arginine [11,26,68]. Studies in *Drosophila* first indicated a role for arginine and/or lysine transport in mTORC1 activation as *Slimfast*, a transporter of arginine and lysine, was shown to be critical for normal body size and activation of mTORC1 in the *Drosophila* fat body [69]. We have also observed that removal of either leucine or arginine results in S6K1 T389 dephosphorylation within 5–10 min in HEK293T cells, and S6K1 is rephosphorylated within a similar time frame upon supplementation of the omitted amino acid (Yan and Lamb, unpublished observations).

5. Lysosome-associated machinery for amino acid sensing

The proton-assisted SLC36 amino acid transporters (PATs) were identified on the surface of mammalian lysosomes, at the plasma membrane and in endosomal compartments, suggesting that they could act *via* an intracellular mechanism [70–72]. Two PAT-related transporters in *Drosophila*, CG3424 and CG1139, have been shown to mediate growth [73]. Like CG1139, human PAT1 and PAT2 specifically transport alanine, glycine and proline in an electrogenic and proton-stimulated fashion, rather than leucine and arginine important for mTORC1 signaling [73,74]. The mechanistic features for the PATs acting on mTORC1 signaling remain to be established, although PATs might modulate the activity of mTORC1 not by transporting amino acids into the cell but by modulating an intracellular response to amino acids [75]. Interestingly, PAT1 is concentrated in intracellular compartments, including late endosomes/lysosomes, wherein mTOR shuttles upon amino-acid stimulation [25,76].

In two previous reports, both biochemical and genetic studies demonstrated that mammalian Rag (Ras-related GTPases) GTPases and yeast Gtr1/2 play a crucial role in amino acid signaling to TOR as heterodimeric complexes [25,77]. Amino acid sufficiency has been shown to induce the movement of some fraction of mTORC1 to lysosomal membranes [76,78], where the Rag proteins reside [78]. A complex encoded by MAPKSP1 (MP1), ROBLD3 (p14), and c11orf59 (p18) (termed the "Ragulator") was identified as a new component of the mTORC1 pathway that interacts with the Rag GTPases, and is essential for localizing them and mTORC1 to the lysosomal surface [78]. p18 directly interacts with the Rag GTPases, as well as with p14 and MP1 [79] and together this complex appears necessary for the activation of the mTORC1 pathway by amino acids [78]. By screening for genes thought to be involved in lysosomal biogenesis and function for effects on mTORC1, Sabatini and co-workers have latterly identified the vacuolar H+-adenosine triphosphatase ATPase (v-ATPase) as required for amino acid-induced activation of mTORC1 [80]. Amino acids were found to regulate the interaction between the V1 domain of v-ATPase and Ragulator and Rag GTPases [80]. However, unlike the Ragulator [78], the v-ATPase appears not to be necessary for anchoring the Rag GTPases to the lysosomal surface [80].

Given that mTORC1 can localize to lysosomes in amino acid replete conditions, it would appear to be a strong possibility that mTORC1 senses amino acid deficiency there, as has been suggested [80]. However, given that amino acid limitation is a stress response [4], another possibility is that it is energetically-favorable to localize mTORC1 to lysosomes during times of plentiful nutrients in preparation for some important immediate function that mTORC1 needs to provide there when amino acids are acutely limited. Surprisingly, both amino acid replete conditions and conditions in which mTORC1 is acutely inhibited with rapamycin lead to increased mTORC1 recruitment to lysosomes [81], while colocalization of mTORC1 with lysosomes can still be observed under starvation conditions that inhibit mTORC1's activation of p70S6K1 [82], indicating that mTORC1 activity and lysosomal association are not strictly coupled. Recently, mTORC1 activity has also been linked with lysosomal biogenesis *via* the activation of phosphorylation of TFEB, a transcription factor that activates a program of gene expression critical for lysosomal biogenesis [83,84], a process that may be critical to restore homeostasis following lysosomal consumption after fusion with autophagosomes [85]. Thus it seems plausible that the localization of mTORC1 to lysosomes may not be strictly linked with amino acid sensing at the lysosomal surface.

6. Conclusions and perspectives

A number of other genes have also been implicated in the process of intracellular nutrient sensing (reviewed in [30,86]). Plausible evidence implicating MAP4K3 [35,87–89], Vps34 (Vacuolar Protein Sorting 34) [36,90] and phospholiopase D (PLD [91,92]) have been presented in mechanisms relating amino acid sufficiency to mTORC1 activation. The precise mechanisms involved in connecting these events to mTORC1 and an amino acid sensor still remain for the large part undiscovered. It should be noted that, with the exceptions of MAP4K3 that was isolated in a sensitized genetic screen for protein kinase involvement [35], and the Rag GTPases [77] in a similar screen for small GTPases, unbiased genetic screens have been relatively unsuccessful in identifying components of the nutrient input to mTORC1, suggesting either that multiple levels of redundancy and back-up systems may have evolved to allow mTORC1 to sense amino acids, or that key components in this response play essential roles in viability. Connecting the known components and mechanisms together to create a consistent model of how a cell senses amino acids and transmits this information to mTORC1 remains a major challenge for biologists interested in growth control, but is one that is likely to lead to new insights into disease processes involving aberrant growth and nutrient utilization.

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