Signalling by amino acid nutrients

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Abstract

It is clear that mTORC1 (mammalian target of rapamycin complex 1) is regulated by the presence of ambient amino acid nutrients. However, the mechanism by which amino acids regulate mTORC1 is still open to question, despite extensive efforts. Our recent work has revealed that PR61ε, a B56 family regulatory subunit of PP2A (protein phosphatase 2A), associates with and regulates the activity of MAP4K3 (mitogen-activated protein kinase kinase kinase kinase 3), a protein kinase regulated by amino acid sufficiency that acts upstream of mTORC1. In searching for a physiological process regulated by amino acids, we have demonstrated recently that arginine plays a role in the activation of LPS (lipopolysaccharide)-induced MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase]/ERK signalling in macrophages. PP2A similarly associates with the upstream regulator of MEK in this signalling pathway, TPL-2 (tumour progression locus-2), in response to arginine availability. Thus PP2A is a negative regulator of both MAP4K3 and TPL-2 in both mTORC1 and MEK/ERK signalling pathways.

Introduction

mTOR (mammalian target of rapamycin) is a conserved serine/threonine kinase that regulates cell growth and metabolism in response to environmental cues [1,2]. mTOR in mammalian cells exists in two distinct complexes, mTORC (mTOR complex) 1 and mTORC2. Unlike mTORC2, mTORC1 is regulated by extracellular nutrients including amino acids. In the absence of amino acids, mTORC1 becomes unresponsive to all agonists such as mitogens and hormones [3]. Moreover, although growth factors are thought to regulate GTP charging of the Ras-like GTPase Rheb, an upstream regulator of mTORC1, Rheb-GTP levels do not decrease in TSC (tuberous sclerosis complex) 1- or TSC2-null cells in the absence of amino acids, indicating that amino acid sufficiency utilizes a different mechanism to regulate mTORC1 [4,5]. Therefore extensive research efforts have centred on understanding how amino acid nutrients act on mTORC1. In the present paper, we discuss the latest developments in the mechanisms by which amino acid nutrients regulate signalling pathways.

PP2A$_{T61\varepsilon}$, a negative modulator of MAP4K3 (mitogen-activated protein kinase kinase kinase kinase 3), in the regulation of mTORC1 in response to amino acid sufficiency

We performed previously an RNA interference screen of *Drosophila* protein kinases required for signalling to dS6K

(*Drosophila* S6 kinase). This led us to identify MAP4K3 as a kinase that is required for maximal mTORC1-dependent S6K (S6 kinase)/4E-BP1 (eukaryotic initiation factor 4E-binding protein 1) phosphorylation and regulates cell growth [6]. Further studies identified a phosphorylation site at Ser^{170} in the kinase-activation loop of MAP4K3 by MS analysis [7]. The phosphorylation of Ser^{170} paralleled MAP4K3 activation and a mutation of Ser^{170} to alanine abolished MAP4K3 activity and blocked mTORC1-mediated phosphorylation of S6K1. Importantly, we showed that MAP4K3 Ser^{170} phosphorylation was rapidly inhibited by removal of all amino acids from the growth medium and was stimulated by their re-addition, but was relatively unaffected by stimulation of insulin. Finally, we presented evidence that the phosphorylation of MAP4K3 Ser^{170} was an autophosphorylation event catalysed by MAP4K3 itself.

Studies from two independent laboratories have shown that Rag GTPases are critical components in promoting amino-acid-induced mTORC1 activation [8,9]. The relationship between MAP4K3 and Rag proteins was investigated. We showed that suppression of Rag C and Rag D impaired the ability of MAP4K3 to activate mTORC1, indicating that activation of mTORC1 by MAP4K3 also requires Rag GTPase function [7]. Studies in *Drosophila* indicate that expression of Rag A-(Q61L) in a MAP4K3-knockdown mutant background is still able to induce tissue overgrowth [10]. This led to the suggestion that Rag proteins act genetically downstream of MAP4K3. However, our efforts to demonstrate a stable interaction between MAP4K3 and Rag C in mammalian cells or provide evidence that Rag A, B or C were substrates of MAP4K3 were unsuccessful [7]. Thus, at present, the exact relationship between MAP4K3 and Rag GTPases in the activation of mTORC1 remains unknown.

We recently revealed a new inhibitor of cell growth, PR61ε, a regulatory subunit for PP2A (protein phosphatase 2A), found in association with MAP4K3, by MS [11,12]. PP2A is

predominantly a heterotrimeric protein complex, consisting of a catalytic subunit (C), a scaffold subunit (A) and a regulatory subunit (B). Its substrate specificity, localization, activity and stability are regulated by a variety of regulatory subunit isoforms [13]. We showed that overexpression of one of these isoforms PR61ε inhibits both MAP4K3 Ser[170] phosphorylation and the ability of MAP4K3 to activate mTORC1/S6K1. In contrast, suppression of PR61ε was found to inhibit MAP4K3 Ser[170] dephosphorylation and impair mTORC1 inhibition following amino acid withdrawal. This finding therefore provides support for the idea that PP2A$_{T61ε}$ is a negative modulator of mTORC1 signalling via dephosphorylation and inhibition of MAP4K3 at Ser[170]. This view was strengthened by the finding that PR61ε suppression in HEK (human embryonic kidney)-293 cells caused an increase in cell size, whereas inhibition of MAP4K3 decreased cell size [6,7]. In accordance with this notion, a *Drosophila* MAP4K3-knockdown mutant displays low TOR (target of rapamycin) activity, reduced growth rate and small body size compared with wild-type animals [10].

PR61ε/B56ε has an alternative translation isoform, shared with a highly conserved B56 core domain, which is responsible for binding to the A–C heterodimer [14]. PR61ε/B56ε is mainly localized in the cytoplasm [15]. Although an important function of B56 subunits is to localize PP2A holoenzymes to specific intracellular compartments [16], we showed that the distribution between the soluble S100 and P100 fractions of MAP4K3 and PR61ε was unaffected by amino acid sufficiency [7]. Subsequent work established how PP2A$_{T61ε}$ and MAP4K3 are involved in the regulation of amino acid sufficiency upstream of mTORC1. We showed that PP2A$_{T61ε}$ preferentially interacts with MAP4K3 after amino acid withdrawal and leads to more rapid Ser[170] dephosphorylation and mTORC1-mediated dephosphorylation of S6K1. Since a truncation (MAP4K3 amino acids 1–431) exhibiting the least binding to PP2A$_{T61ε}$ is not inhibited by amino acid withdrawal, this appears to support the view that PP2A$_{T61ε}$ associates with MAP4K3 in an amino-acid-sensitive manner and is involved in amino acid signalling to mTORC1 via MAP4K3 [17].

PP2A, a negative modulator of TPL-2 (tumour progression locus-2) in arginine, facilitating MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase]/ERK signalling by LPS (lipopolysaccharide)

The susceptibility to infection diseases including HIV/AIDS, tuberculosis and malaria is augmented by protein malnutrition [18,19]. Likewise, nutrients are known to control several aspects of both innate and adaptive immunity [20]. Our contribution to this area was in discovering that a specific amino acid nutrient, arginine, is required for the innate immune response in macrophages responding to the bacterial surface component LPS, which activates

the Toll-like receptor pathway [11]. The stimulation of macrophages by LPS induces activation of MAPK cascades, including ERK1 and ERK2, resulting in production of pro-inflammatory cytokines such as TNFα (tumour necrosis factor α). We showed that arginine facilitated the activation of the ERK1/2 MAPKs by inhibiting the dephosphorylation of TPL-2, an upstream MEK kinase in macrophages. The results indicated that, when arginine was deficient, one or more undefined PP2A-type phosphatases associated with TPL-2, and induced dephosphorylation of regulatory phosphorylation sites in the kinase that normally promotes TPL-2 activity, thus inhibiting the activation of ERK1/2 by LPS. Arginine is a semi-essential amino acid that can be synthesized from citrulline, but is also required in the diet [21]. As such, it is well placed to play a role as an immune cell regulator, as, even during periods of malnutrition, it can be synthesized and potentially provide a minimal level of activation of innate immune cells during infection.

The pioneering studies by Avruch and colleagues first indicated the phosphorylation and activity of the best-studied mTORC1 targets S6K1 and 4E-BP1 are regulated by both arginine and leucine [3]. The identification of arginine also as a critical regulator of MEK/ERK signalling indicates that our current knowledge of amino acids as modulators of cell signalling pathways is at best fragmentary. How these amino acids are 'sensed' and relay their presence to mTORC1, MEK/ERK and, potentially, other signalling pathways in specific cell types remains an outstanding question for researchers interested in the role of nutrition in physiology.

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