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Rag proteins regulate amino-acid-induced mTORC1 signalling

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Abstract

The serum- and nutrient-sensitive protein kinase mTOR (mammalian target of rapamycin) is a master regulator of cell growth and survival. The mechanisms through which nutrients regulate mTOR have been one of the major unanswered questions in the mTOR field. Identification of the Rag (Ras-related GTPase) family of GTPases as mediators of amino acid signalling to mTOR is an important step towards our understanding of this mechanism.

The mTOR (mammalian target of rapamycin) kinase is an evolutionarily conserved protein that regulates cell growth, survival and metabolism. mTOR participates in two biochemically and functionally distinct protein complexes: mTORC (mTOR complex) 1 and 2 [1]. mTOR and mLST8 (mammalian lethal with sec-13) are common members of both complexes; raptor (regulatory associated protein of mTOR) and PRAS40 (proline-rich Akt substrate of 40 kDa) participate in mTORC1 specifically; and rictor (rapamycin-insensitive companion of mTOR), sin1 and protor (protein observed with rictor) are unique members of mTORC2 [1–13]. mTORC1 is downstream of both growth factor and nutrient signalling (glucose and amino acids), and integrates the two so as to ensure cell growth only when conditions are ideal. Given the central role of mTORC1 in cell growth, it is not surprising that many upstream regulators of mTORC1 are involved in disease, and understanding how it is regulated is of great interest.

The identification of the small GTPase Rheb as a potent mTORC1 activator, and the finding that TSC (tuberous sclerosis complex) 1/2 is the GAP (GTPase-activating protein) for Rheb advanced our understanding of mTORC1 regulation significantly [14–20]. Both growth factors and cellular energy levels regulate TSC1/2 activity, which in turn modulates the level of Rheb-GTP, which binds to and activates mTORC1 directly [7,21–27]. In addition, two mTORC1 members, PRAS40 and raptor, are phosphorylated in response to mitogenic stimuli and cellular stress, leading to mTORC1 activation and inhibition respectively [6,7,28,29].

Although Rheb is necessary for amino-acid-induced mTORC1 activation and Rheb overexpression can overcome amino-acid-starvation-induced mTORC1 inhibition, TSC2-null MEFs (mouse embryonic fibroblasts) are sensitive to amino acid starvation [30]. This observation suggested that there are additional important players in the amino acid regulation of mTORC1. Recently, the finding that Rag (Ras-related GTPase) proteins mediate amino acid signalling to mTORC1 provided new insights into this problem [31,32].

The Rag proteins are a unique family of GTPases with a canonical N-terminal Ras-like GTPase domain and a unique C-terminal RagA conserved region. In mammals, there are four Rag genes (*Rraga*, *Rragb*, *Rragc* and *Rragd*), whereas yeast and fruitflies have one RagA-like gene and one RagC-like gene.

We identified RagC by MS mass spectrometric analysis as a raptor-interacting protein [32]. Kim et al. [31] found that the Rag proteins are important for amino-acid-induced TORC1 (target of rapamycin complex 1) activation in *Drosophila* cells using an RNAi (RNA interference) screen. Both groups showed that knocking down the Rag proteins impairs amino acid signalling to TORC1, and that overexpression of constitutively GTP-bound RagA-like mutant makes TORC1 insensitive to amino acid deprivation. Moreover, Kim et al. [31], monitoring cell and organ size, as well as autophagy, observed that Rag overexpression or deletion in *Drosophila* directly parallels the effects of dTOR (*Drosophila* target of rapamycin) activation or inhibition respectively.

Although we do not completely understand how Rag proteins activate mTORC1 in detail, the critical observation that amino acid stimulation induces a change in mTORC1 localization prompted us to hypothesize that Rag proteins may regulate mTORC1 localization. Supporting our hypothesis, when Rag proteins are knocked down, amino-acid-induced mTORC1 localization change is ablated. Similarly, when a constitutively GTP-bound RagB mutant is expressed, mTORC1 localization resembles the amino-acid-induced state, even in the absence of amino acids. We also showed that, after amino acid stimulation, mTORC1 moves to Rab7-containing vesicles, where its activator Rheb is thought to reside. On the basis of these observations, we proposed a model: upon amino acid stimulation, Rag proteins initiate a localization change of mTORC1, taking it to Rheb-containing

Key words: amino acid, localization, mammalian target of rapamycin (mTOR), Ras-related GTPase (Rag), Rheb.

Abbreviations used: MEF, mouse embryonic fibroblast; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; PRAS40, proline-rich Akt substrate of 40 kDa; Rag, Ras-related GTPase; raptor, regulatory associated protein of mTOR; rictor, rapamycin-insensitive companion of mTOR; TORC1, target of rapamycin complex 1; TSC, tuberous sclerosis complex.

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vesicles, leading to its activation. This model explains (i) how overexpressed and thus mislocalized Rheb can overcome amino acid starvation, and (ii) why TSC2-null MEFs are sensitive to amino acid starvation: even though Rheb is always in a GTP-bound state, mTORC1 is not in the same subcellular compartment as its activator when amino acids are not present.

The identification of Rag proteins as members of the mTORC1 pathway is an important first step towards deciphering the molecular events that signal nutrient availability to mTORC1 and evokes many interesting questions. How amino acid availability is sensed and communicated to the Rag proteins, how mTOR localization contributes to its activity, and whether Rag-related signalling can be targeted in disease are some of the exciting questions for which mTOR biologists will be seeking answers.

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