Published in final edited form as:

Biochem Soc Trans. 2009 February ; 37(Pt 1): 289–290. doi:10.1042/BST0370289.

Rag Proteins Regulate Amino Acid-Induced mTORC1 Signaling

Yasemin Sancak^{1,2} and David M. Sabatini^{1,2,3}

¹Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Department of Biology, Nine Cambridge Center, Cambridge, MA 02142.

²MIT Center for Cancer Research, 77 Massachusetts Avenue, Cambridge, MA 02139.

³Broad Institute, Seven Cambridge Center, Cambridge, MA 02142.

Abstract

The serum and nutrient-sensitive protein kinase mTOR 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 rag family of GTPases as mediators of amino acid signaling to mTOR is an important step towards understanding this mechanism.

Keywords

mTOR; rag; amino acids; localization; rheb

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: mTORC1 and 2 (mTOR complex 1 and 2). mTOR and mLST8 are common members of both complexes; raptor and pras40 participate in mTORC1 specifically; and rictor, sin1 and protor are unique members of mTORC2 [1–13]. mTORC1 is downstream of both growth factor and nutrient signaling (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 (ras homolog enriched in brain) as a potent mTORC1 activator, and the finding that TSC1/2 (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 directly activates mTORC1 [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 over-expression can overcome amino acid-starvation induced mTORC1 inhibition, TSC2 null MEFs are sensitive to amino acid starvation [30]. This observation suggested that there are additional important players in the amino acid regulation of mTORC1. Earlier this year, the finding that rag proteins mediate amino acid signaling 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 (ragA, B, C and D) whereas yeast and fly have one ragA-like gene and one ragC-like gene.

We identified ragC by mass spectrometric analysis as a raptor-interacting protein. Kim *et al.* found that the rag proteins are important for amino acid-induced TORC1 activation in fly cells using an RNAi screen. Both groups showed that knocking down the rag proteins impairs amino acid signaling to TORC1, and that over-expression of constitutively GTP-bound ragA-like mutant makes TORC1 insensitive to amino acid deprivation. Moreover, Kim *et al.* – monitoring cell and organ size, as well as autophagy – observed that rag over-expression or deletion in flies directly parallels the effects of dTOR 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. Based on these observations, we proposed a model: upon amino acid stimulation, rag proteins initiate a localization change of mTORC1, taking it to rheb-containing vesicles, leading to its activation. This model explains (1) how over-expressed and thus mislocalized Rheb can overcome amino acid starvation, (2) 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 signaling can be targeted in disease are some of the exciting questions for which mTOR biologists will be seeking answers.

Abbreviations used

GTP	guanosine triphosphate
mTORC1	mammalian target of rapamycin complex 1
Rag	Ras-related GTPase
Rheb	Ras homolog enriched in brain
TSC	tuberous sclerosis complex
MEF	mouse embryonic fibroblast
RNAi	RNA interference

References

1. Frias M, et al. mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2. *Curr Biol.* 2006; 16(18):1865–1870. [PubMed: 16919458]
2. Hara K, et al. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell.* 2002; 110(2):177–189. [PubMed: 12150926]

3. Kim D, et al. betaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell*. 2003; 11(4):895–904. [PubMed: 12718876]
4. Kim DH, SD, 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(2):163–175. [PubMed: 12150925]
5. Sarbassov D, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol*. 2004; 14(14):1296–1302. [PubMed: 15268862]
6. Fonseca B, et al. PRAS40 is a target for mammalian target of rapamycin complex 1 and is required for signaling downstream of this complex. *J. Biol. Chem*. 2007; 282(34):24514–24524. [PubMed: 17604271]
7. Sancak Y, et al. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell*. 2007; 25(6):903–915. [PubMed: 17386266]
8. Wang L, Harris T, Lawrence JJ. Regulation of proline-rich Akt substrate of 40 kDa (PRAS40) function by mammalian target of rapamycin complex 1 (mTORC1)-mediated phosphorylation. *J. Biol. Chem*. 2008; 283(23):15619–15627. [PubMed: 18372248]
9. Wang L, et al. PRAS40 Regulates mTORC1 Kinase Activity by Functioning as a Direct Inhibitor of Substrate Binding*Formula. *J. Biol. Chem*. 2007; 282(27):20036–20044. [PubMed: 17510057]
10. Jacinto E, et al. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell*. 2006; 127(1):125–137. [PubMed: 16962653]
11. Woo S, et al. PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor beta expression and signaling. *J. Biol. Chem*. 2007; 282(35):25604–25612. [PubMed: 17599906]
12. Yang Q, et al. Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. *Genes Dev*. 2006; 20(20):2820–2832.
13. Pearce L, et al. Identification of Protor as a novel Rictor-binding component of mTOR complex-2. *Biochem J*. 2007; 405(3):513–522. [PubMed: 17461779]
14. Saucedo L, et al. Rheb promotes cell growth as a component of the insulin/TOR signalling network. *Nat Cell Bio*. 2003; 5(6):566–571. [PubMed: 12766776]
15. Stocker H, et al. Rheb is an essential regulator of S6K in controlling cell growth in *Drosophila*. *Nat Cell Bio*. 2003; 5(6):559–565. [PubMed: 12766775]
16. Inoki K, et al. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev*. 2003; 17(15):1829–1834. [PubMed: 12869586]
17. Tee A, et al. Tuberous sclerosis complex gene products, Tuberlin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol*. 2003; 13(15):1259–1268. [PubMed: 12906785]
18. Zhang Y, et al. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Bio*. 2003; 5(6):578–581. [PubMed: 12771962]
19. Gao X, PD. TSC1 and TSC2 tumor suppressors antagonize insulin signaling in cell growth. *Genes Dev*. 2001; 15(11):1383–1392. [PubMed: 11390358]
20. Potter C, Huang H, Xu T. *Drosophila* Tsc1 functions with Tsc2 to antagonize insulin signaling in regulating cell growth, cell proliferation, and organ size. *Cell*. 2001; 105(3):357–368. [PubMed: 11348592]
21. Inoki K, et al. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Bio*. 2002; 4(9):648–657. [PubMed: 12172553]
22. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. *Nat. Cell Biol*. 2002; 4(9):658–665. [PubMed: 12172554]
23. Manning B, et al. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberlin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell*. 2002; 10(1):151–162. [PubMed: 12150915]
24. Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J. 2005 Rheb binds and regulates the mTOR kinase. *Curr. Biol*. 702–713, Rheb binds and regulates the mTOR kinase. *Curr. Biol*. 2005; 15(8):702–713. [PubMed: 15854902]

25. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell*. 2003; 115(5):577–590. [PubMed: 14651849]
26. Lee D, et al. IKK beta suppression of TSC1 links inflammation and tumour angiogenesis via the mTOR pathway. *Cell*. 2007; 130(3):440–455. [PubMed: 17693255]
27. Ma L, et al. Phosphorylation and functional inactivation of TSC2 by Erk: implications for tuberous sclerosis and cancer pathogenesis. *Cell*. 2005; 121(2):179–193. [PubMed: 15851026]
28. Oshiro N, et al. The proline-rich Akt substrate of 40 kDa (PRAS40) is a physiological substrate of mammalian target of rapamycin complex 1. *J. Biol. Chem*. 2007; 282(28):20329–20339. [PubMed: 17517883]
29. Gwinn D, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*. 2008; 30(2):214–226. [PubMed: 18439900]
30. 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(19):18717–18727. [PubMed: 15772076]
31. Kim E, et al. Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol*. 2008:395–345. [PubMed: 18344988]
32. Sancak Y, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science*. 2008; 320(5882):1496–1501. [PubMed: 18497260]