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Abstract

Growth factors and nutrients regulate the mechanistic target of rapamycin complex 1 (mTORC1) by different mechanisms. The players that link growth factors and mTORC1 activation have been known for several years and mouse models have validated its relevance for human physiology and disease. In contrast to the picture for growth factor signaling, the means by which nutrient availability leads to mTORC1 activation have remained elusive until recently, with the discovery of the Rag GTPases upstream of mTORC1. The Rag GTPases recruit mTORC1 to the outer lysosomal surface, where growth factor signaling and nutrient signaling converge on mTORC1 activation. A mouse model of constitutive RagA activity has revealed qualitative differences between growth factor- and nutrient– dependent regulation of mTORC1. Regulation of mTORC1 activity by the Rag GTPases in vivo is key for enduring early neonatal fasting, showing its importance for mammalian physiology.

Growth factors and nutrients

Since the establishment of a link between growth factors and mTORC1 activation via the interaction of Akt and the Tuberous sclerosis complex (TSC) more than a decade ago (1, 2), our understanding of the mechanism and players involved has increased substantially. In contrast, the picture of how nutrients activate mTORC1 is far less complete and we are just beginning to add pieces to a puzzle that remained virtually unknown until 2008, with the identification of the Rag GTPases as a direct link between amino acids and mTORC1 (3, 4).

Although both growth factors and nutrients culminate in the activation of mTORC1, the means by which each input does suggests cooperation in their ability to trigger mTORC1-based responses. Growth factor signaling drives kinase activation of mTORC1 through a process that starts at the plasma membrane with the transduction of a signal evoked by protein hormones like insulin, via tyrosine kinase receptors and activation of PI3K. PI3K, in turn, activates Akt, which phosphorylates and inhibits TSC (1, 2), a complex with GTPase activating protein (GAP) activity towards the Rheb GTPase (5-7), responsible for direct kinase activation of mTORC1. Nutrient signaling operates in a different manner: when the Rag GTPases where discovered, the originally puzzling observation that the activity of purified mTORC1 was not affected *in vitro* by the Rag GTPases lead to the realization that a different mechanism was responsible for Rag-dependent activation of mTORC1. Upon nutrient sufficiency, the Rag GTPases interact with and recruit mTORC1 to the outer lysosomal surface, where the Rag and Rheb GTPases reside (4, 8), allowing mTORC1

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kinase activation by the latter. This mechanistic insight explains why both nutrient and growth factor inputs cannot substitute each other and must simultaneously occur in a cell to achieve activation of mTORC1, and shows that these inputs cooperate to activate mTORC1. Logically, a cell that would trigger anabolism and increase its mass needs to engage cellular processes that are energetically expensive and regulated by mTORC1. Hence, such cooperation warrants that the cell will fully commit to it when 1) long-range growth factor signals are present and 2) local nutrient sensing by the Rag GTPases assures the availability of building blocks and energy.

There are, however, some aspects of Rheb and Rag –dependent regulation of mTORC1 that are similar. In particular, they both reside at the lysosomal surface, and they are both GTPases that change their nucleotide state upon physiological fluctuations of their upstream signals. In the case of Rheb, activation of TSC leads to Rheb loading with GDP, and conversely, TSC inactivation by Akt leads to Rheb loading with GTP, and activation of mTORC1. The four members of the Rag GTPase family behave conceptually differently to most GTPases, as they exist as obligate dimers, where RagA (or RagB) interacts with RagC (or RagD), and their nucleotide state is opposite. Nutrient availability leads to the loading of RagA/B with GTP and C/D with GDP, and its absence loads RagA/B with GDP and C/D with GTP. The role of RagA/B versus C/D is not identical, as single amino acid substitutions in RagA/B that mimic a constitutive loading with GTP, leads to constitutive recruitment (and activation) of mTORC1 regardless of the loading status of RagC/D.

Navigating upstream

The discovery of the Rags also constituted a handle that allowed further interrogation of how nutrients activate mTORC1. The Rag GTPases, which have no lipid modification to allow them be membrane-bound, are tethered to the lysosomal surface by constitutive interaction with the multiprotein complex Ragulator (9) that is anchored to the lysosomal surface and is necessary and sufficient to recruit the Rag proteins. In addition to its role as scaffold for Rag-mTORC1 interaction, the Ragulator complex also exerts a regulatory role, as it works as a guanine nucleotide exchange factor (GEF) for the Rag GTPases (10). A complete picture of how fluctuations in nutrients affect the loading of the Rag GTPases is far from complete, but we have identified the vacuolar H^+-ATPase (v-ATPase) as a key regulator upstream of the Rag GTPases. The v-ATPase, in addition to its prime function of maintaining the pH gradient between the cytoplasm and the lysosome at the expense of ATP, engages into interactions with Rag-Ragulator that are sensitive to nutrient levels. Furthermore, the activity of the v-ATPase is critical for recruitment and activation of mTORC1 (11). The identity of the direct nutrient sensor and how it is connected to mTORC1 recruitment by the Rag GTPases is still unknown, but at least part of this sensing seems to occur in a lysosomal inside-out manner that involves the v-ATPase.

mTORC1 regulation in a physiological setting

Tuberous sclerosis is the prime example of a human syndrome driven by constitutive mTORC1 activity. It is caused by germline mutations in TSC1 or TSC2 and causes benign and malignant tumors, cysts, seizures, mental retardation, and other symptoms (12,13). Proteus and Proteus-like syndromes, neurofibromatosis, von Hippel Landau, are also caused by genetic alterations in regulators of mTORC1 activity that operate in the growth-factor branch (14). Furthermore, sporadic mutations in these genes are also key players in tumorigenesis. Nutrient-dependent regulation of mTORC1 is also associated with a human syndrome caused by a germline mutation in one of the Ragulator proteins (15), implicating both main axes upstream of mTORC1 are at the core of pathogenesis of human disease.

Although cultured cells have taught us valuable aspects of mTORC1 biology, in order to model human disease, experimental mouse-driven approaches are required, and some efforts to manipulate mTORC1 activity in mice have been pursued. Loss of function of mTORC1 key components leads to embryonic lethality (16-18), which precludes deeper insight *in vivo*, so conditional deletions followed those studies (reviewed in (19)). Because most human syndromes of deregulated mTORC1 consist of increased activity, gain-of-function mouse strains may prove invaluable as tools for understanding and intervening human disease. As for human syndromes, the prime mouse model of hyperactive mTORC1 is loss of TSC1 or TSC2 (20-22). TSC-deficient mice die at E9.5-11.5 with hyperactive mTORC1, severe anomalies, and cells derived from them undergo senescence. Conditional deletion of TSC function in adult mice has recapitulated most characteristics of tuberous sclerosis, including neuronal defects and cancer, as well as its response to the mTORC1 inhibitor rapamycin.

Modeling constitutive RagA activity

Given the recent discovery of genes involved in nutrient-dependent regulation of mTORC1, mouse models are just underway. In a recent report, we generated a mouse strain with constitutive RagA activity (23). We introduced a single nucleotide substitution in the endogenous RagA gene, which renders a Q to L change in amino acid 66 in RagA protein sequence. This point-mutant form of RagA protein is constitutively bound to GTP ($RagA^{GTP}$) and allowed us generate mice with constitutive nutrient-dependent activation of mTORC1 in every cell. We first obtained MEFs either $RagA^{+/+}$, or that harbored one copy of the $RagA^{GTP}$ allele ($RagA^{GTP/+}$), and analyzed mTORC1 activity in those cells. To our surprise, in spite of normal expression of the mutant allele, regulation of mTORC1 activity in $RagA^{GTP/+}$ cells was normal, suggesting that the presence of the wild-type allele was somehow compensating for the effect of the $RagA^{GTP}$ allele. This turned to be true also in mouse tissues. When we intercrossed $RagA^{GTP/+}$ mice, $RagA^{GTP/GTP}$ embryos at E13.5, in spite of having complete insensitivity to amino acid deprivation in culture, were macroscopically indistinguishable from $RagA^{+/+}$ embryos. This result is in sharp contrast to $TSC1^{-/-}$ or $TSC2^{-/-}$ embryos, which succumb at ~E10.5 with serious anomalies, and cells derived from them undergo rapid p53-dependent senescence in culture, to a point where culturing these cells is almost impossible. $RagA^{GTP/GTP}$ cells proliferated in culture with similar kinetics to those of $RagA^{+/+}$ cells. Moreover, $RagA^{GTP/GTP}$ embryos developed normally and were born with mendelian ratios and with minimal phenotypic defects. The different outcomes of constitutive growth factor dependent activation (death at E10.5 and premature senescence) and nutrient dependent activation of mTORC1 (normal embryonic development) may underlie different causes. One possibility is that oscillations of growth factors during embryonic development must be accurately detected and cells in the embryo must properly respond to them, whereas oscillations in nutrients do not occur. Provided that trans-placental supply of nutrients is supposed to be steady during embryonic development, this possibility seems reasonable. In addition, there may be an intrinsic difference in the extent to which growth factors and nutrients can activate mTORC1. Indeed, even though mTORC1 activity in $RagA^{GTP/GTP}$ mice and cells was insensitive to amino acid withdrawal, maximal activity was comparable to that of $RagA^{+/+}$ cells in the presence of amino acids and growth factors. This contrasts with mTORC1 activity in TSC-deficient cells, which show a several-fold increase in maximal mTORC1 activity. This difference may constitute an alternative explanation for the substantial difference between TSC-deficiency and RagA constitutive activity in the developing embryo. Whether this is related to the intrinsic nature of each type of stimulus (regulating recruitment versus kinase activation) is not clear, but raises the question of how much TSC-deficient mice and cells, great models for tuberous sclerosis syndrome, mimic physiological states associated with deregulation in mTORC1 activity, as nutrient overload, early stages of type 2 diabetes and aging.

Nutrient crisis and death of RagA$^{GTP/GTP}$ neonates

In spite of being barely distinguishable from wild-type littermates, RagA$^{GTP/GTP}$ mice succumb during the first day of life. As expected, interruption of maternal supply of nutrients at birth lead to a profound drop in circulating glucose and amino acids in mice of all genotypes, but mTORC1 activity was reduced only in wild-type neonates, and not in RagA$^{GTP/GTP}$ littermates. This is consistent with the critical role of mTORC1 in regulating fasting/feeding responses (24). Upon prolonged fasting in isolation following C-section, RagA$^{GTP/GTP}$ neonates were unable to recover from this reduction in circulating nutrients, and succumbed to fatal hypoglycaemia within ~15 hours. In contrast, RagA$^{+/+}$ mice lived ~24 hours and endured their fasting state by mobilizing internal energetic sources, reflected by their recovery from the initial hypoglycaemia. Glycogen is a critical source of energy after birth, but RagA$^{GTP/GTP}$ mice showed no alterations in glycogen levels or impaired consumption after birth. Gluconeogenesis is also critical, but again, RagA$^{GTP/GTP}$ neonates were able to execute gluconeogenesis when gluconeogenic substrates were present. Because mice are born without significant amounts of fat, they rely on amino acids as substrates for gluconeogenesis. Hence, we hypothesized that the fatal hypoglycaemia was secondary to a reduction in circulating amino acids to be used as substrates for gluconeogenesis. Importantly, free amino acids are the main product of autophagy, which is triggered immediately after birth, and autophagy-deficient mice share characteristics observed in RagA$^{GTP/GTP}$ neonates, including early lethality and reduced circulating amino acids (25, 26). We tested whether RagA$^{GTP/GTP}$ neonates had impairment in the induction of autophagy after birth, and indeed, they showed a striking defect in their ability to induce autophagy. As a consequence, circulating amino acids were reduced, and the reduction in gluconeogenic amino acids failed to fuel a significant amount of gluconeogenesis, leading to a hypoglycaemic state that became fatal as soon as glycogen reserves were exhausted. This argues that nutrient signaling upstream of mTORC1 is the key regulator of autophagy in mammals, a surprising finding provided the multiple regulators of autophagy described in cultured cells.

Rag GTPases beyond amino acids

Considering that the Rag GTPases regulate mTORC1 activation by amino acids, the fact that RagA$^{GTP/GTP}$ neonates had high mTORC1 activity in spite of a significant reduction in amino acid levels was not surprising. What was unexpected was that mTORC1 activity in RagA$^{GTP/GTP}$ neonates was also insensitive to profound hypoglycaemia. This led us to consider that the Rag GTPases could also regulate mTORC1 by glucose levels. Indeed, RagA$^{GTP/GTP}$ cells in culture were resistant to amino acid deprivation, glucose deprivation or deprivation of both glucose and amino acids. Furthermore, deprivation of glucose, as that of amino acids, led to dispersion of mTORC1 in the cytoplasm in wild-type cells, but mTORC1 was constitutively recruited to the lysosomal surface, regardless of amino acid or glucose levels in RagA$^{GTP/GTP}$ cells. Glucose, as shown previously for amino acids, regulated the physical interactions of the v-ATPase with the Ragulator complex, implicating the same machinery in sensing amino acids and glucose. Whether the lysosomal nutrient sensing machinery detects both nutrients independently, or on the contrary, it detects the levels of a common intermediate, awaits further research, but conceptually, this finding implies that the Rag GTPases behave as global nutrient regulators of mTORC1, conveying global information about the nutritional status of the cell. Several nodes seem to converge into the Rag GTPases, as anti-hyperglycaemic drugs biguanides also regulate Rag function (27), as well as alpha-ketoglutarate (28).

Concluding remarks

The discovery of the proteins involved in mTORC1 regulation by nutrients has opened a new area of research, still in its infancy. The identification of the sensor (or sensors), the mechanism of convergence of the lysosomal inside-out sensing versus a potential cytoplasmic sensing, the existence of additional roles of the Rag GTPases upstream of mTORC1 and why they show this unique dimeric nature for GTPases, are all outstanding questions awaiting answers. Provided the numerous human syndromes associated with PI3K-mTORC1 genetic defects, it will be no surprise to find the involvement Rag-mTORC1 pathway in more of these. In addition, deregulation in nutrient sensing, as occurring in $RagA^{GTP/GTP}$ neonates, will almost certainly prove relevant for human diseases as cancer, neurodegeneration, diabetes and aging. Additional mouse models of deregulated nutrient sensing will certainly teach us additional aspects of physiology that are critically regulated by this pathway, which may be similar or different to those where growth factors are involved.

References

1. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nature cell biology. 2002; 4:648–657.
2. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. Mol Cell. 2002; 10:151–162. [PubMed: 12150915]
3. Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL. Regulation of TORC1 by Rag GTPases in nutrient response. Nat Cell Biol. 2008; 10:935–945. [PubMed: 18604198]
4. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science. 2008; 320:1496–1501. [PubMed: 18497260]
5. Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Roccio M, Stocker H, Kozma SC, Hafen E, Bos JL, Thomas G. Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. Mol Cell. 2003; 11:1457–1466. [PubMed: 12820960]
6. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev. 2003; 17:1829–1834. [PubMed: 12869586]
7. Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. Nat Cell Biol. 2003; 5:578–581. [PubMed: 12771962]
8. Takahashi K, Nakagawa M, Young SG, Yamanaka S. Differential membrane localization of ERas and Rheb, two Ras-related proteins involved in the phosphatidylinositol 3-kinase/mTOR pathway. J Biol Chem. 2005; 280:32768–32774. [PubMed: 16046393]
9. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell. 2010; 141:290–303. [PubMed: 20381137]
10. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. Cell. 2012; 150:1196–1208. [PubMed: 22980980]
11. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H-ATPase. Science. 2011; 334:678–683. [PubMed: 22053050]
12. van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, Lindhout D, van den Ouweland A, Halley D, Young J, Burley M, Jeremiah S, Woodward K, Nahmias J, Fox M, Ekong R, Osborne J, Wolfe J, Povey S, Snell RG, Cheadle JP, Jones AC, Tachataki M, Ravine D, Sampson JR, Reeve MP, Richardson P, Wilmer F, Munro C, Hawkins TL, Sepp T, Ali JB, Ward S, Green AJ, Yates JR, Kwiatkowska J, Henske EP, Short MP, Haines JH, Jozwiak S, Kwiatkowski DJ. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. Science. 1997; 277:805–808. [PubMed: 9242607]

13. Consortium., E. C. T. S. Identification and characterization of the tuberous sclerosis gene on chromosome 16. Cell. 1993; 75:1305–1315. [PubMed: 8269512]

14. Efeyan A, Zoncu R, Sabatini DM. Amino acids and mTORC1: from lysosomes to disease. Trends Mol Med. 2012; 18:524–533. [PubMed: 22749019]

15. Bohn G, Allroth A, Brandes G, Thiel J, Glocker E, Schaffer AA, Rathinam C, Taub N, Teis D, Zeidler C, Dewey RA, Geffers R, Buer J, Huber LA, Welte K, Grimbacher B, Klein C. A novel human primary immunodeficiency syndrome caused by deficiency of the endosomal adaptor protein p14. Nat Med. 2007; 13:38–45. [PubMed: 17195838]

16. Gangloff YG, Mueller M, Dann SG, Svoboda P, Sticker M, Spetz JF, Um SH, Brown EJ, Cereghini S, Thomas G, Kozma SC. Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development. Mol Cell Biol. 2004; 24:9508–9516. [PubMed: 15485918]

17. Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, Sabatini DM. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. Dev Cell. 2006; 11:859–871. [PubMed: 17141160]

18. Murakami M, Ichisaka T, Maeda M, Oshiro N, Hara K, Edenhofer F, Kiyama H, Yonezawa K, Yamanaka S. mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Molecular and cellular biology. 2004; 24:6710–6718. [PubMed: 15254238]

19. Polak P, Hall MN. mTOR and the control of whole body metabolism. Current opinion in cell biology. 2009; 21:209–218. [PubMed: 19261457]

20. Kwiatkowski DJ, Zhang H, Bandura JL, Heiberger KM, Glogauer M, el-Hashemite N, Onda H. A mouse model of TSC1 reveals sex-dependent lethality from liver hemangiomas, and up-regulation of p70S6 kinase activity in Tsc1 null cells. Hum Mol Genet. 2002; 11:525–534. [PubMed: 11875047]

21. Onda H, Lueck A, Marks PW, Warren HB, Kwiatkowski DJ. Tsc2(+/-) mice develop tumors in multiple sites that express gelsolin and are influenced by genetic background. The Journal of clinical investigation. 1999; 104:687–695. [PubMed: 10491404]

22. Zhang H, Cicchetti G, Onda H, Koon HB, Asrican K, Bajraszewski N, Vazquez F, Carpenter CL, Kwiatkowski DJ. Loss of Tsc1/Tsc2 activates mTOR and disrupts PI3K-Akt signaling through downregulation of PDGFR. J Clin Invest. 2003; 112:1223–1233. [PubMed: 14561707]

23. Efeyan A, Zoncu R, Chang S, Gumper I, Snitkin H, Wolfson RL, Kirak O, Sabatini DD, Sabatini DM. Regulation of mTORC1 by the Rag GTPases is necessary for neonatal autophagy and survival. Nature. 2013; 493:679–683. [PubMed: 23263183]

24. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol. 2010; 12:21–35. [PubMed: 21157483]

25. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, Kominami E, Tanaka K, Chiba T. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J Cell Biol. 2005; 169:425–434. [PubMed: 15866887]

26. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N. The role of autophagy during the early neonatal starvation period. Nature. 2004; 432:1032–1036. [PubMed: 15525940]

27. Kalender A, Selvaraj A, Kim SY, Gulati P, Brule S, Viollet B, Kemp BE, Bardeesy N, Dennis P, Schlager JJ, Marette A, Kozma SC, Thomas G. Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. Cell Metab. 2010; 11:390–401. [PubMed: 20444419]

28. Duran RV, Oppliger W, Robitaille AM, Heiserich L, Skendaj R, Gottlieb E, Hall MN. Glutaminolysis activates Rag-mTORC1 signaling. Molecular cell. 2012; 47:349–358. [PubMed: 22749528]