mTOR signalling

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AMPK knocks at the gate of GATOR

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Dai and colleagues show that activation of AMPK by glucose starvation leads to phosphorylation of GATOR2 and affects nutrient-dependent activation of mTORC1.

Our cells react with a repertoire of tightly regulated responses to fluctuations in the availability of nutrients and energy. Rates of build-up and breakdown of cellular biomass are tuned to adapt to abundance and scarcity. Among the critical players that couple nutrient and energy sufficiency to the execution of anabolic versus catabolic functions in the cell are the AMP-activated protein kinase (AMPK) and the mechanistic target of rapamycin complex 1 (mTORC1).

mTORC1 is a master switch of anabolic responses that drive cell growth (including protein, lipid and nucleotide synthesis), as well as an inhibitor of catabolic processes such as autophagy[1]. To ensure that onerous anabolic reactions occur only when nutrients and energy are plentiful, mTORC1 integrates cues from systemic nutrient availability (in the form of second messengers such as insulin and growth factors) with information on intracellular nutrient levels. Growth factor and nutrient signalling cues are funnelled independently to Rheb and Rag GTPases, respectively, which are located at the outer lysosomal surface[2]. In conditions of intracellular nutrient availability, the Rag GTPases promote the translocation of mTORC1 to the outer lysosomal surface, enabling activation of mTORC1 kinase via its interaction with Rheb downstream of growth factor signalling.

Several protein complexes act upstream of the Rag GTPases to relay information on the availability of different nutrients, including amino acids (such as leucine, arginine and methionine), glucose and lipids[3,4]. Amino acid signalling upstream of mTORC1 involves dedicated sensors that modulate the activity of two related multiprotein complexes called GATOR2 and GATOR1. GATOR2 represses the activity of GATOR1, which is itself a negative regulator of RAGA and RAGB. In addition to responding to changes in amino acid levels, GATOR2 and GATOR1 also signal glucose availability to the Rag GTPases and to mTORC1 via the detection of the intermediate metabolite dihydroxyacetone phosphate (DHAP) by an elusive sensor[5,6]. In addition, glucose starvation leads to an increase in the levels of AMP and ADP, which results in the activation of AMPK. Once activated, AMPK inhibits mTORC1 by at least two mechanisms. Active AMPK impairs growth factor-dependent activation of mTORC1 by directly phosphorylating the tuberous sclerosis complex, a negative regulator of RHEB[7]. In addition, AMPK directly phosphorylates the mTORC1 component RAPTOR[8], leading to an inhibitory interaction with 14-3-3 proteins.

In this issue of *Nature Metabolism*, Dai and colleagues show that AMPK controls GATOR2 upstream of mTORC1[9]. The authors identify AMPK-dependent phosphorylation of serine 155 in WDR24, a component of GATOR2, under conditions of glucose starvation, suppressing mTORC1 activation and partially impairing the ability of mTORC1 to become fully activated by other nutrients (Fig. 1).

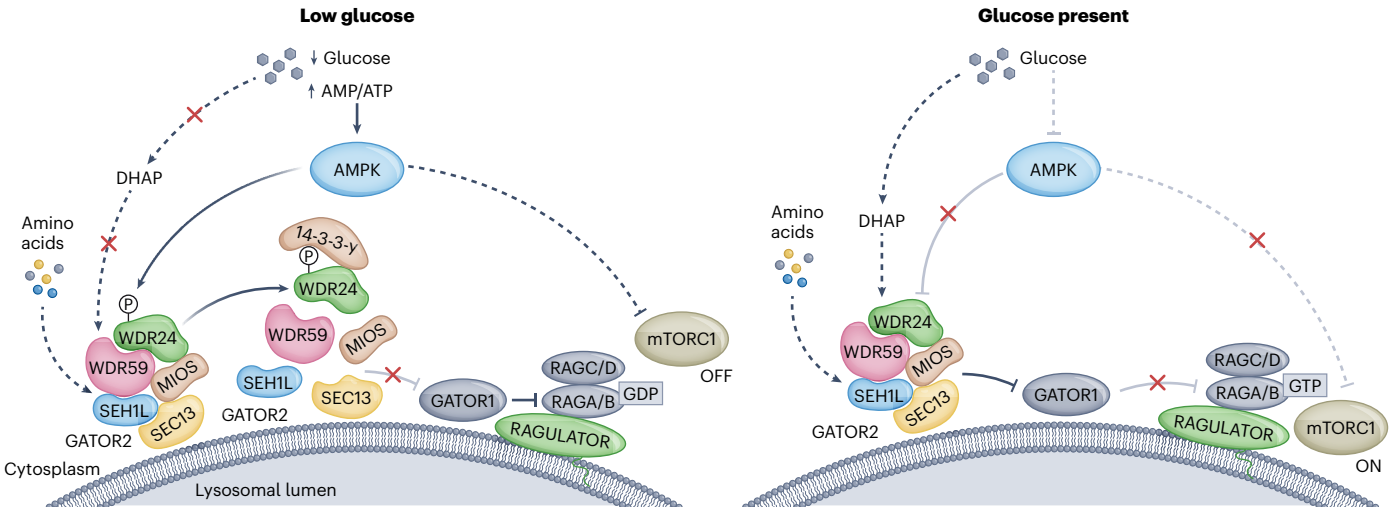

Fig. 1 | GATOR2 is phosphorylated by AMPK in response to glucose starvation, suppressing mTORC1 activation. mTORC1 is tightly regulated by changes in the levels of intracellular nutrients. Different cellular nutrients, such as amino acids and glucose, via the glycolytic intermediate metabolite DHAP, signal through GATOR2 and GATOR1 multiprotein complexes, ultimately leading to the activation of the Rag GTPases, which promote the translocation of mTORC1 to the outer lysosomal surface, an essential step for its activation. Under conditions of glucose starvation (left), an increase in the AMP:ATP ratio switches on AMPK. Activated AMPK phosphorylates WDR24, a component of GATOR2, and creates a binding site for 14-3-3γ protein, promoting the sequestration of WDR24 and compromising the integrity of the whole GATOR2 complex, thereby restricting the activation of mTORC1. Under conditions of glucose sufficiency (right), AMPK remains inactive, thereby releasing its inhibitory effects towards GATOR2 and mTORC1. Moreover, glucose signals to GATOR2 through DHAP via an unknown sensor, leading to the repression of GATOR1 and activation of the Rag GTPases. GDP, guanosine diphosphate; GTP, guanosine triphosphate.

Extensive biochemical data generated in this work show that both GATOR1 and GATOR2 are necessary for regulating mTORC1 activity in response to glucose by controlling its lysosomal localization, as previously described. Interestingly, the authors find that AMPK activation also suppresses the recruitment of mTORC1 to the outer lysosomal surface and thus propose a potential functional relationship between AMPK activity and GATOR1/2 complexes to link glucose levels and the regulation of mTORC1. They find that AMPKα1 interacts with WDR24, WDR59 and Mios, which are components of GATOR2. Although these interactions are not glucose sensitive, they are largely compromised in *WDR24* knock-out cells, pointing to WDR24 as the critical factor of the AMPK–GATOR2 interaction. Moreover, they identify the evolutionarily conserved serine 155 in WDR24, located within an AMPK consensus motif, and use elegant and robust biochemical assays to show that this serine is phosphorylated by AMPK under conditions of glucose deprivation and that this phosphorylation event is critical for the ability of AMPK to control GATOR2.

Experiments with cells expressing either a serine-to-alanine (phospho-deficient) mutant or a serine-to-aspartic acid (phosphomimetic) mutant in amino acid position 155 show that the phosphorylation of WDR24 at serine 155 suppresses mTORC1 activation under conditions of glucose deprivation. Moreover, this suppression is strictly dependent on the activity of the Rag GTPases, establishing a connection between the energy sensor AMPK and the Rag GTPase–nutrient sensing pathway. Although AMPK does not regulate mTORC1 signalling in response to amino acids, under glucose starvation this identified AMPK–GATOR2 connection prevents full activation of mTORC1 by amino acids. Thus, this work establishes a hierarchical role of AMPK in the ability of mTORC1 to respond to amino acid sufficiency under glucose deprivation and, thus, to energetic stress. But what is the consequence of phosphorylation of serine 155 on WDR24 in GATOR2? This phosphorylation induces the binding of WDR24 to 14-3-3γ and compromises the integrity of the whole GATOR2 complex. Additional studies will help understand how the structural changes caused by WDR24 phosphorylation affect the interactions and conformation of the other subunits and, ultimately, the integrity of the GATOR2 multiprotein complex (Fig. 1).

This AMPK–GATOR2 axis seems to be of relevance in mammalian organs, as seen in the phenotype of mice that the authors knocked-in to express phospho-mimetic WDR24 S155D and phospho-deficient S155A mutant variants, mirroring constitutive and impaired phosphorylation of S155, respectively. *Wdr24*$^{S155D/S115D}$ mice suffer mid-embryonic lethality and have greatly decreased body size, accompanied by reduced mTORC1 activity. These phenotypes are reminiscent of those of *Raga* (also known as Rraga)-deficient mice[10,11], supporting the relevance of WDR24 serine 155 phosphorylation in the control of mTORC1. By contrast, homozygous expression of the phospho-deficient S155A variant does not cause obvious developmental defects: *Wdr24*$^{S155A/S155A}$ mice are born with expected Mendelian ratios and present no gross defects or abnormalities. Strikingly, tissues derived from *Wdr24*$^{S155A/S155A}$ mice show increased mTORC1 activity after fasting when compared with *Wdr24*$^{+/+}$ counterparts. Consistent with disruption of the AMPK–GATOR2 axis, mouse embryonic fibroblasts derived from *Wdr24*$^{S155A/S155A}$ mice in culture show a partial resistance to glucose − but not amino acid − starvation, as revealed by phosphorylation status of different mTORC1 targets and also by the lysosomal localization of mTORC1, which is constitutively present in the outer lysosomal surface, even under conditions of glucose starvation.

These findings provide strong support for the relevance of the AMPK–GATOR2 axis in mammalian physiology and also raise further questions. The relative resistance of mTORC1 to fasting in tissues from *Wdr24*$^{S155A/S155A}$ mutant mice partially resembles that of other models with constitutive nutrient signalling, such as mice expressing the constitutively active *Raga*^{Q66L} variant and mice lacking the amino acid sensors SESTRIN1–3[6,12,13]. However, unlike *Raga*^{Q66L} and *Sesn1/2/3* knock-out mice, *Wdr24*$^{S155A/S155A}$ mice survive the neonatal starvation period, which is consistent with the partial effect of the AMPK–GATOR2 axis in the control of mTORC1 and highlights the existence of additional, convergent cues upstream of the Rag GTPases. *Wdr24*^{S155A} knock-in mice are born normally and do not present gross abnormalities, but to what extent are these animals able to engage metabolic responses to fasting? Do they present alterations in systemic metabolism? Future characterization of *Wdr24*$^{S155A/S155A}$ mice will probably reveal in which particular tissue or cell this molecular axis is most prominent. Another layer of interest for future research would be to understand the integration of the AMPK and amino acid-related cues on GATOR signalling in pathophysiological settings in which the availability of glucose and amino acids are dissimilar. These include the control of metabolism by mTORC1 in feeding-to-fasting transitions, during chronic and pathologic hyperglycaemia and in the abnormal nutrient fluctuations that occur within the tumour microenvironment.

Our current knowledge spans a multitude of cues that link the sensing of different nutrient species to the activation of mTORC1. With the identification of this AMPK–GATOR2 connection, this work writes an important chapter on a much less understood phenomenon: how all these signalling cues quantitatively and qualitatively integrate for the execution of coherent metabolic responses to fluctuations in nutrients and energy levels, and how these convergent inputs upstream of mTORC1 may determine the pathophysiological control of metabolism.

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Competing interests

The authors declare no competing interests.