Structure
Previews

Amino Acid Signaling in High Definition

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In this issue of *Structure*, Zhang and colleagues present the structure of the Ego3 dimer, demonstrating that dimerization is an obligate prerequisite in amino acid-induced TORC1 activation.

The target of rapamycin (TOR) is a serine/threonine kinase that, in response to both internal and environmental stimuli, coordinates growth in addition to many other aspects of eukaryotic physiology. TOR kinases assemble into two distinct multiprotein complexes that are conserved from yeast to human (Durán and Hall, 2012). The activity of TOR complex 1 (TORC1), but not TORC2, is inhibited by the macrolide rapamycin, and, as a tool compound, rapamycin has greatly facilitated interrogation of the signaling events up- and downstream of TORC1.

TORC1 activity is acutely sensitive to nutritional cues, abiotic stress, and, in metazoans, circulating growth factors (Zoncu et al., 2011). Signals emanating from growth factors impinge upon the GTPase Rheb, which, in its GTP-bound state, activates mTORC1 (Figure 1). In contrast, the mechanisms by which abiotic stressors regulate TORC1/mammalian TORC1 (mTORC1) are not well defined, although it is appreciated that, in situations involving depletion of cellular energy, activation of the AMP-activated protein kinase results in diminished mTORC1 activity. Of the many nutritional cues upstream of TORC1, the best understood, in terms of signaling, is the branched chain amino acid leucine.

There is general concurrence now that amino acid levels are signaled to TORC1 via the EGO complex in yeast or the structurally orthologous (Kogan et al., 2010) **ragulator** complex in mammalian cells. However, precisely how amino acid abundance is signaled to the EGO (**ragulator**) complex and how this complex, in turn, stimulates TORC1 activity are both hotly debated. The EGO (**ragulator**) complex is composed of Ego1 (**p18/LAMTOR1**), Ego3 (**p14/LAMTOR2** + **MP1/LAMTOR3**), and the GTPases Gtr1 (**RagA** and **RagB**) and Gtr2 (**RagC** and **RagD**) (Binda et al., 2009; Sancak et al., 2010). The **ragulator** complex additionally contains **C7orf59/LAMTOR4** and **HBXIP/LAMTOR5** (Bar-Peled et al., 2012). The business end of the EGO (**ragulator**) complex is the pair of GTPases. Curiously, it is the $Gtr1^{GTP}$-$Gtr2^{GDP}$ conformation of the complex that is competent to activate TORC1, presumably through direct interaction with the Kog1 (**raptor**) subunit of TORC1 (Binda et al., 2009; Sancak et al., 2010). Presently, at least three mechanisms have been proposed for coupling amino acid levels, or, more specifically, leucine levels to the EGO (**ragulator**) complex (Figure 1). The Sabatini group (Bar-Peled et al., 2012) has proposed that amino acids within the lumen of the lysosome trigger a conformational switch in the transmembrane v-ATPase complex that, in turn, stimulates a guanosine-nucleotide exchange (GEF) activity, inherent to the **ragulator** complex, to load **RagA/B** with GTP. Thus activated, the ragulator complex serves to recruit mTORC1 to lysosomal membranes whereupon mTORC1 can be activated by Rheb. However, this does not explain a situation documented in yeast, where TORC1 is constitutively localized to the vacuolar membrane, and, thus, unlike the ragulator complex, the EGO complex appears not to control TORC1 activity via regulation of its localization. To reconcile these discrepancies, the Kim and De Virgilio groups (Han et al., 2012; Bonfils et al., 2012) have both proposed that cytoplasmic leucine levels are sensed by the leucyl-tRNA synthetase (LRS), albeit in significantly different ways. In one scenario, LRS was proposed to possess a GTPase activating (GAP) activity toward **RagD**, which is regulated in an amino acid-dependent fashion (Han et al., 2012). In the other scenario, leucine levels were suggested to regulate a conformational switch in the editing domain of LRS, with abundant leucine promoting interaction of the editing domain with Gtr1 and this association promoting the GTP-bound status of Gtr1, possibly by protecting GTP-loaded Gtr1 from an unknown GAP activity (Bonfils et al., 2012). Lastly, the Hall group has proposed that leucine, as an allosteric regulator of glutamate dehydrogenase, activates mTORC1 by promoting glutaminolysis (Durán et al., 2012). Glutamate dehydrogenase converts glutamate to α-ketoglutarate, which, in turn, is a putatively rate-limiting substrate for prolyl hydroxylases. Prolyl hydroxylase subsequently acts, in undefined ways, upstream of the ragulator complex.

In summary, the one thing that is clear from these disparate proposed mechanisms is that structural studies will have a substantial role to play in the unraveling of how amino acids signal to TORC1. In this issue of *Structure*, Zhang et al. (2012) report a high-resolution structure of Ego3. They find that Ego3 adopts an obligate dimeric conformation notable for its 12-stranded anti-parallel β sheet and a swapping of α helices from one monomer to the other. Importantly, this work confirms the observation made by Kogan et al. (2010) that Ego3 shares considerable structural similarity to the mammalian MP1, p14 heterodimer structure, despite the modest similarity of these proteins at the primary sequence level and despite the fact that Kogan et al. (2010) observed Ego3 as a tetramer, an apparent artifact caused by harsh crystallization conditions. Furthermore, mutational analyses done by Zhang et al. (2012) identify residues in Ego3 that may be involved in the binding to Gtr1 and -2.

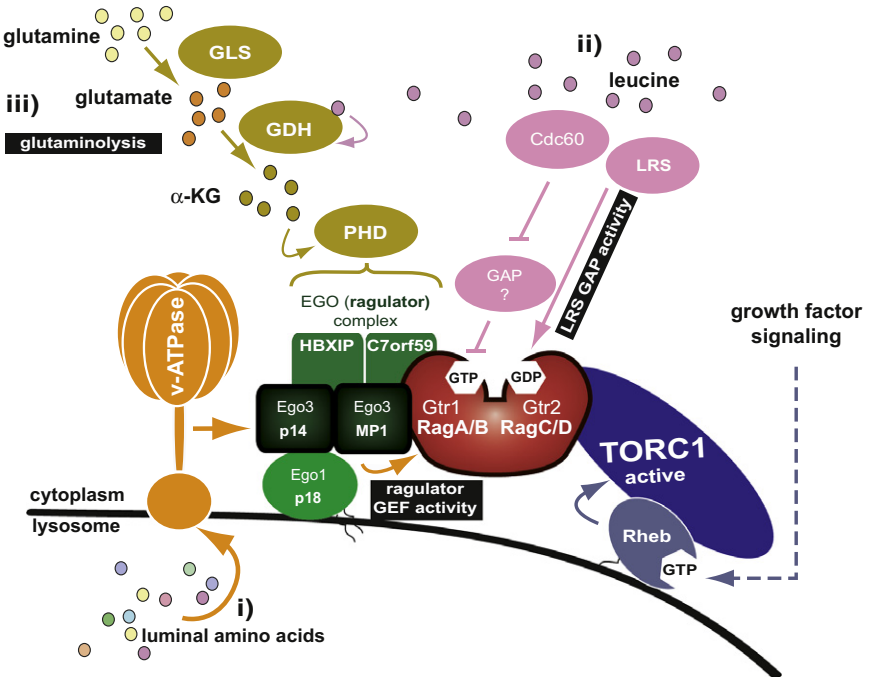

Figure 1. Amino Acid-Induced Activation of TORC1
Three mechanisms have been proposed to signal amino acid levels to TORC1 as detailed in the main text: (i) luminal amino acids stimulate GTP loading of **Rag** proteins through conformational regulation of the v-ATPase, which itself stimulates the GEF activity of the **ragulator** complex; (ii) leucine levels are sensed by leucyl-tRNA synthetases (**LRS**, aka Cdc60 in yeast), which regulate the guanine nucleotide loading status of Gtr1 or **RagD**; and (iii) glutamine and leucine levels are integrated through glutaminase (GLS) and glutamate dehydrogenase (GDH) during glutaminolysis. The product of these two enzymes, α-ketoglutarate (αKG), is a substrate for an unidentified prolyl hydroxylase (PHD), which acts upstream of the **ragulator** complex. Names of mammalian **ragulator** proteins are written in bold. EGO (**ragulator**) complex components drawn with a 3D effect indicate that high resolution structures are available for these proteins.

Although the swapping of α helices seems to be unique to Ego3, the overall structure and particularly the extended β sheet are similar to the Roadblock/LC7 (RLC7) protein superfamily. Intriguingly, several other EGO (**ragulator**) complex components including the above-mentioned **MP1** and **p14**, the C-termini of Gtr1 and Gtr2, and the recently described **C7orf59** and **HBXIP** proteins also contain Roadblock domains. Indeed, as one would predict, dimerization of Gtr1 and Gtr2 is mediated by their C-terminal Roadblock domains (Gong et al., 2011). It remains to be seen if Roadblock domain proteins also function upstream of TORC2.

It is still early in the atomic resolution of TORC1 signaling. Structures of **C7orf59**, **HBXIP**, and Ego1 (**p18**) are missing. Next, it will be important to obtain structures of the entire EGO and/or **ragulator** complexes, ideally in active and inactive conformations. Toward this end, the structure of a $Gtr1^{GTP}/Gtr2^{GDP}$ complex has recently been solved (Jeong et al., 2012) and comparison to the $Gtr1^{GMPPNP}/Gtr2^{GMPPNP}$ structure (Gong et al., 2011) suggests mechanisms by which nucleotide binding regulates the interaction with TORC1. Structural interrogations of the v-ATPase have been ongoing, and it will be fascinating to determine how it interacts functionally with the **ragulator** complex. Last, but certainly not least, the high resolution structure of TORC1 itself is eagerly awaited by all in the TOR field.

Why TOR? Beyond its fundamental role in eukaryote biology, the mTORC1 signaling pathway is clinically validated as a drug target in cancer and will likely soon be targeted to ameliorate symptoms associated with metabolic syndrome. Identification of the signaling events upstream of mTORC1 thus represents new nodes that can potentially be targeted for therapeutic gain, and atomic resolution of these signaling nodes will help guide the synthesis of these targeted therapeutics.

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