An EGOcentric view of TORCI signaling

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The structurally and functionally conserved target of rapamycin complex 1 (TORC1) plays a pivotal role in the control of eukaryotic cell growth by promoting anabolic processes (such as protein synthesis) and inhibiting catabolic processes (such as autophagy) in response to diverse signals including hormones, growth factors, energy/ATP levels, and amino acids.^{1,2} The molecular pathways that couple hormones, growth factors, and energy levels to mammalian TORC1 (mTORC1) by and large converge on TSC2, a subunit of the heterodimeric TSC1/2 tuberous sclerosis complex. TSC1/2 functions as a GTPase activating protein (GAP) for the small GTP-binding protein Rheb (Ras-homolog enriched in brain) that directly interacts with and positively regulates mTORC1 in its GTP-bound state.³ Interestingly, TSC2, but not Rheb, appears to be dispensable for coupling amino acid signals to mTORC1, suggesting the existence of additional proteins that participate in amino acid regulation of mTORC1.4,5 In this context, two complementary studies have reported that the conserved Rag proteins, a family of four related small GTPases that associate into heterodimeric complexes between RagA/B and Rag C/D, mediate amino acid signaling to TORC1 in both Drosophila and mammalian cells.6,7 Accordingly, amino acids are thought, by a still unknown mechanism, to stabilize Rag heterodimers in an active conformation (i.e., with RagA/B and RagC/D in their GTP- and GDP-bound forms, respectively) to favor their binding to the TORC1 subunit raptor. Unlike Rheb, the Rag proteins do not directly stimulate mTORC1 kinase activity. However, in response to amino acids they may control mTORC1 function by driving the relocalization of mTORC1 from discrete locations in the cytoplasm to a Rheb-enriched, Rab-7-containing late endosomal and/or lysosomal compartment.⁷ Whether the Rag proteins, as implied by this model, co-localize with mTORC1 throughout this dynamic, amino-acid controlled process remains to be addressed.

Saccharomyces cerevisiae encodes single orthologs of metazoan RagA/B and RagC/D, Gtr1 and Gtr2, respectively. We have reported that Gtr1 and Gtr2 form, together with the fungal-specific Ego1 and Ego3 proteins, a complex (the EGO complex or EGOC) that localizes, like yeast TORC1, predominantly at late endosomal structures and to the periphery of the vacuole (yeast lysosome), irrespective of the presence or absence of amino acids such as leucine.^{8,9} Ego1 and Ego3 function in membrane anchoring of EGOC, and ego1 Δ , ego3 Δ and gtr2 Δ deletions were all identified in a selection for mutants that are unable to exit from a rapamycin-induced growth arrest.9 Similar to the Rag GTPases in higher eukaryotes, a Gtr1GTP/ Gtr2GDP heterodimer activates TORC1 to phosphorylate its downstream effector Sch9,8 which is closely related in structure and function to the mTORC1 target S6 kinase 1 (S6K1).¹⁰ Gtr1 physically interacts with the TORC1 subunits Kog1/raptor and Tco89 and these interactions are both sensitive to leucine starvation and strongly dependent on the nucleotide-binding status of Gtr1 suggesting that amino acids impinge on TORC1 via regulation of the nucleotide-binding status of Gtr1. Intriguingly, Vam6, which exerts a role in homotypic vacuolar fusion and vacuolar protein sorting within the HOPS complex by acting as GTP exchange factor (GEF) for the Rab-7 homolog Ypt7,11 binds to and regulates the nucleotide-binding status of Gtr1. Vam6 could therefore be potentially involved in coupling amino acid signals to EGOC and the HOPS complex.

While our studies in yeast confirm an evolutionarily conserved key role of Rag GTPases in mediating amino acid signaling to TORC1, they also raise a number of interesting issues. For instance, starvation for leucine strongly reduced TORC1 activity, but did not appreciably change the localization of either EGOC or the TORC1 subunits Tor1 and Tco89 in wildtype cells. These observations do not formally exclude a role of EGOC in ensuring proper localization of TORC1, but suggest that the Gtr proteins have at least an additional function in local activation of TORC1, which is unlikely to involve the TSC1/2-Rheb axis that is apparently not conserved in S. cerevisiae (N.P., unpublished).^{2,12} Notably, the observation that Gtr proteins, similar to Rag proteins,7 do not stimulate the kinase activity of TORC1 in vitro (Binda M, unpublished), is not necessarily at variance with such a model as it is quite possible that Gtr/Ragmediated in vitro activation of TORC1 may require additional, yet to be identified factors. Another interesting issue that remains to be addressed is whether the EGOC relays to TORC1 an amino acid signal that originates from the cytoplasm or, given its predominant localization at the vacuolar membrane, from the vacuolar compartment that represents a major intracellular nutrient reservoir in yeast. Studies centered on EGOC function in yeast are therefore likely to continue to contribute to our understanding of the evolutionarily conserved aspects of TORC1 regulation.

Acknowledgements

C.D.V. was supported by the Swiss National Science Foundation, the Leenaards Foundation, and the Canton of Fribourg. We thank Robbie Loewith for critically reading the manuscript.

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Previously published online: www.landesbioscience.com/journals/cc/article/10585

References

- 1. De Virgilio C, et al. Int J Biochem Cell Biol 2006; 38:1476-81.
- De Virgilio C, et al. Oncogene 2006; 25:6392-415. 2. 3. Sarbassov DD, et al. Curr Opin Cell Biol 2005; 17:596-603.
- 4. Roccio M, et al. Oncogene 2006; 25:657-64.
- 5. Smith EM, et al. J Biol Chem 2005; 280:18717-27.
- Kim E, et al. Nat Cell Biol 2008; 10:935-45.
 Sancak Y, et al. Science 2008; 320:1496-501.
- 8. Binda M, et al. Mol Cell 2009; 35:563-73.
- 9. Dubouloz F, et al. Mol Cell 2005; 19:15-26.
- 10. Urban J, et al. Mol Cell 2007; 26:663-74.
- 11. Wurmser AE, et al. J Cell Biol 2000; 151:551-62.
- 12. Soulard A, et al. Curr Opin Cell Biol 2009.

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