

A substrate localization model for the selective regulation of TORC1 downstream pathways

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ABSTRACT

Target of rapamycin complex 1 (TORC1) is a protein kinase complex conserved in eukaryotes that coordinates diverse cellular processes critical for cell growth to environmental conditions. Previous studies have shown that TORC1 is localized mainly in the lysosome/vacuoles, and its localization is important for signaling to downstream pathways. We recently demonstrated that signaling to Sch9, an S6K-related substrate of TORC1 in budding yeast, was selectively suppressed upon oxidative stress, which was mediated by the delocalization of phosphatidylinositol 3, 5-bisphosphate (PI[3,5]P₂) from vacuolar membranes following stress. We propose that TORC1 downstream pathways can be regulated separately via the modulation of organelle localization of a specific target protein.

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Target of rapamycin complex 1 (TORC1) is an evolutionarily conserved protein kinase complex in eukaryotes which, acting as a signaling hub, contributes to the coordination of cellular processes critical for cell growth, such as protein and lipid synthesis, or uptake and recycling of amino acids [1]. A variety of environmental cues affect TORC1 activity and thereby its downstream processes, including nutrient levels, growth factors, and cellular stresses [2]. Recent lines of evidence have shown the importance of TORC1 subcellular localization on endomembrane structures, such as lysosomes or vacuoles, in the regulation of its activity. In mammals, TORC1 is activated by localization on the cytoplasmic surfaces of lysosomes via the Ragulator-Rag complex, which senses amino acid availability [3].

The budding yeast *Saccharomyces cerevisiae* is a model organism suitable for the molecular dissection of TORC1 regulation. Rapamycin is a drug that selectively inhibits TORC1 activity, and screening for mutants showing different sensitivities to rapamycin enriched those affecting TORC1-dependent processes. Indeed, TOR1/2, the catalytic components of TORC1, were identified genetically in this organism [4]. In yeast, components of the Ego-Gtr (Ragulator-Rag) complex are largely conserved and localized to vacuoles, but TORC1 remains associated with the vacuolar membranes upon inactivation [5–7]. The close relationship between the

regulation of TORC1 activity and its lysosomal/vacuolar localization suggests that intracellular amino acid levels may be sensed somewhere near the lysosome/vacuole, because these organelles serve as a storage site for amino acids.

Genetic studies in yeast have identified roles for TORC1 in the regulation of multiple physiological functions through downstream signaling branches, each of which is involved in proteosynthesis, the stress response, nitrogen metabolism, uptake of nitrogen sources, and autophagy, among others. When yeast cells were subjected to stresses except for nutrient starvation, such as hyperosmolarity, oxidative stress, or heat shock, the downstream branches of yeast TORC1 do not always respond uniformly to changes in the environment [8,9]. These observations indicate that TORC1 downstream pathways can be regulated separately.

One possible mechanism for the selectivity of downstream signaling is the difference in inherent quality of each phosphorylation site as a TORC1 substrate [10]. In this ‘substrate quality’ model, the downstream effectors of TORC1 respond differentially by temporal and intensity alterations in TORC1 kinase activity according to changes in the levels of nutrients and growth factors. Such differential responses would serve to prioritize and/or coordinate the diverse functions of TORC1 in each situation. Although the difference in substrate quality to

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a certain kinase is useful for the hierarchical regulation of downstream functions, it may be difficult to explain the mechanism underlying reciprocal regulation. For example, the Ego complex is strictly required for normal activity of a branch involved in protein phosphatase 2A (PP2A) but is less critical for the Sch9 branch of TORC1 downstream signaling, whereas deletion of the HOPS complex severely reduces the activity of the Sch9 branch without affecting that of PP2A [9].

In a recent study [9], we demonstrated that the change in substrate localization also contributes to the specific regulation of TORC1 downstream pathways (Fig. 1). Sch9 is a direct target of TORC1 [11], and signal transduction between TORC1 and Sch9 requires vacuolar localization of Sch9, which is mediated by the PI(3,5)P₂-binding C2 domain of Sch9 [12]. Following oxidative stress, the PI(3,5)P₂ content in vacuolar membranes is reduced, suppressing the Sch9 branch, despite the fact that other TORC1 downstream pathways are scarcely affected. Furthermore, the artificial tethering of Sch9 to vacuoles by other lipid-binding domains recovered TORC1-Sch9 signaling. Although incomplete rescue of Sch9 phosphorylation by tethering implies that TORC1 activity itself may be repressed to some extent, we concluded that TORC1 signaling to the Sch9 branch is regulated by the change in Sch9 localization upon oxidative stress (Fig. 2). Because inhibition of the Sch9 branch causes the activation of Rim15 and Sod2 [13,14], the physiological importance of the selective suppression of Sch9 under oxidative stress would be to confer resistance to this type of stress. Moreover, the change in distribution, but not the amount, of PI(3,5)P₂ underlies the regulation, suggesting that spatiotemporal alterations in the localization of inositol phospholipids contribute to the selective suppression of TORC1 downstream pathways.

Our results revealed that the phosphorylation of Sch9 by TORC1 exclusively requires the vacuolar localization of Sch9, which may be because Sch9 has less affinity to

TORC1 than the other TORC1 substrates (Fig. 2). Indeed, Atg13 is a direct target of TORC1 [15] but is not enriched in vacuoles [9], and its phosphorylation by TORC1 is less affected by vacuolar integrity [9]. The relocation of TORC1 and its associated proteins has been described upon other types of stresses; TORC1 is accumulated in stress granules after heat stress [16] or is sequestered to inhibited domains near vacuoles after glucose withdrawal [17]. In addition to these observations, our study demonstrated that TORC1 signaling is regulated by the relocation of a specific target protein, Sch9. To date, studies on TORC1 have not addressed substrate dynamics; future studies should focus on differences in the substrates to elucidate the regulation of TORC1 signaling *in vivo*.

The reversible phosphorylation of phosphatidylinositol is critical for the determination of organelle identity and regulates many biological processes [18,19]. Among the phosphatidylinositol phospholipids, PI(3,5)P₂ is less abundant in yeast, and the level of this phospholipid increases transiently in response to hyperosmotic stress [20]. We demonstrated that the delocalization of PI(3,5)P₂ from vacuoles following oxidative stress is primarily due to the loss of Fab1, the sole PI3P 5-kinase in yeast, from the vacuolar membrane [9]. Fab1 associates with vacuoles through its PI3P-binding FYVE domain, indicating that phosphatidylinositol dynamics within cells is altered or perturbed during the stress response. The mechanism remains unclear at present: the synthesis and/or transfer of specific lipid molecules may be regulated, actively or passively, by cellular stresses; or a physiological change in cells may induce the delocalization of a specific lipid according to its physicochemical nature [21].

Lastly, our results highlight the strategy used by cells to cope with a variety of stressors. Our finding that the signal from TORC1 to the Sch9 branch is notably more sensitive to stress signals due to the localization of Sch9

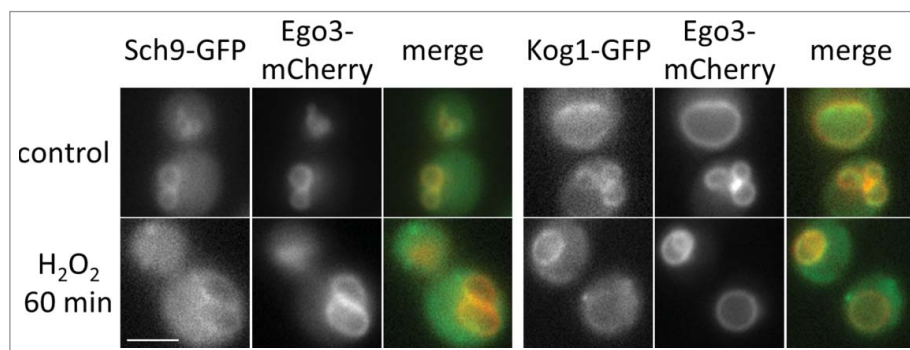


Figure 1. Representative images of cells expressing Sch9-GFP and Ego3-mCherry (left), or Kog1-GFP and Ego3-mCherry (right). Ego3 and Kog1 constitute the Ego complex and TORC1, respectively. Oxidative stress induces the delocalization of Sch9, but not the Ego complex or TORC1, from vacuoles.

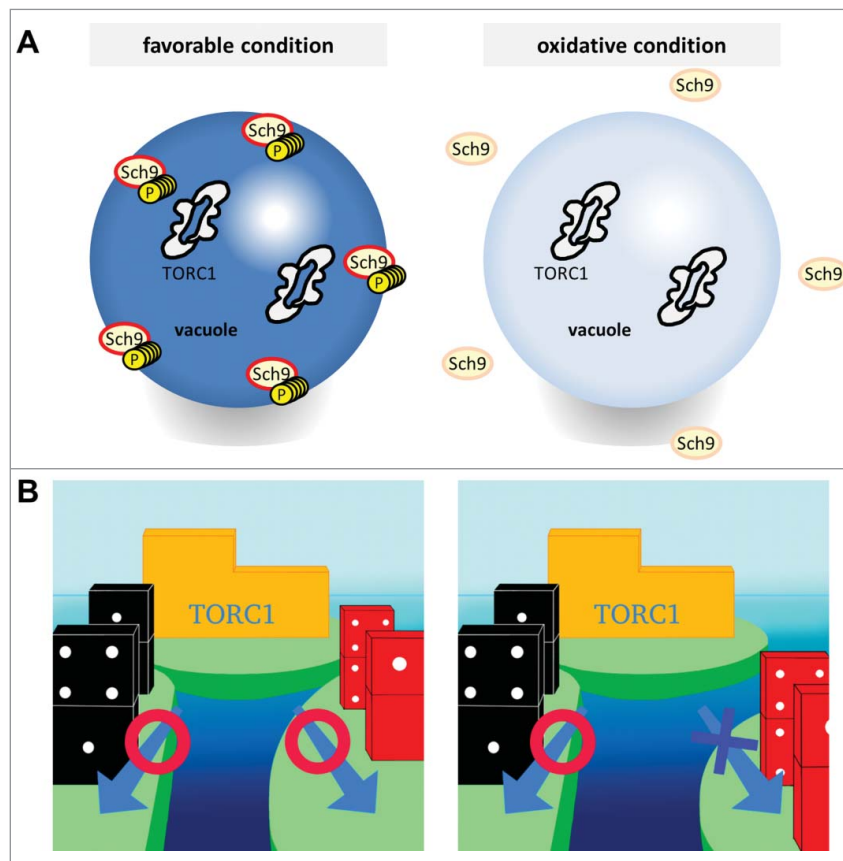


Figure 2. Localization-dependent signal transduction downstream of TORC1. (A) Subcellular localization of Sch9, a TORC1 substrate, before and after oxidative stress. (B) When TORC1, the first domino, is activated, the signal is transmitted to several downstream pathways like toppling dominoes (left). Suppression of a specific pathway can be achieved by moving the first domino of the line away from TORC1 (right).

suggests that localization in concert with TORC1 activity effect a more nuanced mode of regulation. In practice, this means that Sch9 can be blocked more easily than alternative pathways. In mammalian cells, the signal from TORC1 to the translational regulator S6K1, the functional homolog of Sch9, decreases during even mild amino acid starvation, but the TORC1 signal to ULK1, which modulates autophagy, declines only under conditions of severe starvation [10]. Therefore, it appears that cells employ a systematic approach in TORC1 signaling, dynamically prioritizing the regulation of TORC1-dependent branches in response to stress, with the inhibition of translation being the immediate top priority. The localization-dependent control of a TORC1 substrate in budding yeast may form one element of such a regulatory system.


Abbreviations

PI(3,5)P ₂	phosphatidylinositol 3, 5-bisphosphate
PI3P	phosphatidylinositol 3-phosphate
PP2A	protein phosphatase 2A.
TORC1	target of rapamycin complex 1

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

- [1] Soulard A, Cohen A, Hall MN. TOR signaling in invertebrates. *Curr Opin Cell Biol.* 2009;21(6):825–36. doi: 10.1016/j.ceb.2009.08.007.
- [2] Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell.* 2017;168(6):960–76. doi: 10.1016/j.cell.2017.02.004.
- [3] Sancak Y, Bar-Peled L, Zoncu R, et al. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell.* 2010;141(2):290–303. doi: 10.1016/j.cell.2010.02.024.
- [4] Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science.* 1991;253(5022):905–9.
- [5] Binda M, Péli-Gulli MP, Bonfils G, et al. The Vam6 GEF controls TORC1 by activating the EGO complex. *Mol Cell.* 2009;35(5):563–73. doi: 10.1016/j.molcel.2009.06.033.

- [6] Péli-Gulli MP, Sardu A, Panchaud N, et al. Amino Acids Stimulate TORC1 through Lst4-Lst7, a GTPase-Activating Protein Complex for the Rag Family GTPase Gtr2. *Cell Rep.* **2015**;13(1):1–7. doi: 10.1016/j.celrep.2015.08.059.
- [7] Kira S, Kumano Y, Ukai H, et al. Dynamic relocation of the TORC1-Gtr1/2-Ego1/2/3 complex is regulated by Gtr1 and Gtr2. *Mol Biol Cell.* **2016**;27(2):382–96. doi: 10.1091/mbc.E15-07-0470.
- [8] Hughes Hallett JE, Luo X, Capaldi AP. State transitions in the TORC1 signaling pathway and information processing in *Saccharomyces cerevisiae*. *Genetics.* **2014**;198(2):773–86. doi: 10.1534/genetics.114.168369.
- [9] Takeda E, Jin N, Itakura E, et al. Vacuole-mediated selective regulation of TORC1-Sch9 signaling following oxidative stress. *Mol Biol Cell.* **2018**;29(4):510–22. doi: 10.1091/mbc.E17-09-0553.
- [10] Kang SA, Pacold ME, Cervantes CL, et al. mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin. *Science.* **2013**;341(6144):1236566. doi: 10.1126/science.1236566.
- [11] Urban J, Soulard A, Huber A, et al. Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol Cell.* **2007**;26(5):663–74. doi: 10.1016/j.molcel.2007.04.020.
- [12] Jin N, Mao K, Jin Y, et al. Roles for PI(3,5)P2 in nutrient sensing through TORC1. *Mol Biol Cell.* **2014**;25(7):1171–85. doi: 10.1091/mbc.E14-01-0021.
- [13] Fabrizio P, Liou LL, Moy VN, et al. SOD2 functions downstream of Sch9 to extend longevity in yeast. *Genetics.* **2003**;163(1):35–46.
- [14] Wei M, Fabrizio P, Hu J, et al. Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. *PLoS Genet.* **2008**;4(1):e13. doi: 10.1371/journal.pgen.0040013.
- [15] Kamada Y, Yoshino K, Kondo C, et al. Tor directly controls the Atg1 kinase complex to regulate autophagy. *Mol Cell Biol.* **2010**;30(4):1049–58. doi: 10.1128/MCB.01344-09.
- [16] Takahara T, Maeda T. Transient sequestration of TORC1 into stress granules during heat stress. *Mol Cell.* **2012**;47(2):242–52. doi: 10.1016/j.molcel.2012.05.019.
- [17] Prouteau M, Desfosses A, Sieben C, et al. TORC1 organized in inhibited domains (TOROIDS) regulate TORC1 activity. *Nature.* **2017**;550(7675):265–9. doi: 10.1038/nature24021.
- [18] Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. *Nature.* **2006**;443(7112):651–7. doi: 10.1038/nature05185.
- [19] Strahl T, Thorner J. Synthesis and function of membrane phosphoinositides in budding yeast, *Saccharomyces cerevisiae*. *Biochim Biophys Acta.* **2007**;1771(3):353–404. doi: 10.1016/j.bbaliip.2007.01.015.
- [20] Bonangelino CJ, Nau JJ, Duex JE, et al. Osmotic stress-induced increase of phosphatidylinositol 3,5-bisphosphate requires Vac14p, an activator of the lipid kinase Fab1p. *J Cell Biol.* **2002**;156(6):1015–28. doi: 10.1083/jcb.200201002.
- [21] Rayermann SP, Rayermann GE, Cornell CE, et al. Hallmarks of reversible separation of living, unperturbed cell membranes into two liquid phases. *Biophys J.* **2017**;113(11):2425–32. doi: 10.1016/j.bpj.2017.09.029.