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Regulation of plasma membrane homeostasis: Dissecting TORC2 signaling

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Abstract

Cell survival requires preservation of plasma membrane (PM) integrity in response to environmental insults and changes in lipid metabolism. In eukaryotic cells, maintenance of PM homeostasis requires a conserved, PM-localized protein kinase complex, Target of Rapamycin (TOR) Complex 2 (TORC2). In budding yeast (*Saccharomyces cerevisiae*), the essential catalytic subunit of TORC2 is the TOR isoform Tor2 and the essential downstream effector of TORC2 is the AGC-family protein kinase Ypk1 (and its paralog Ypk2). Our prior work has shown that Ypk1 phosphorylates and thereby regulates proteins that stimulate the rate of sphingolipid production, control the amount of glycerol-3P available for glycerolipid biosynthesis, impede retrograde transport of sterols from the PM, and modulate the efficiency of clathrin-mediated endocytosis of integral PM proteins. More recently, we have confirmed that Muk1, a Rab5-specific guanine nucleotide exchange factor (GEF) we identified in a global screen for new Ypk1 targets, is a *bona fide* Ypk1 substrate whose GEF function is stimulated by Ypk1-mediated phosphorylation. Our studies further show that TORC2- and Ypk1-dependent activation of Muk1 provides a control circuit for positive (self-reinforcing) Rab5-mediated stimulation of TORC2 action. It has been presumed that three TORC2 subunits (Avo1, Slm1 and Slm2), which contain Pleckstrin Homology (PH) domains exhibiting specificity for phosphatidylinositol-4,5-*bis*phosphate (PtdIns4,5P2), tether TORC2 at the PM. To investigate assembly and PM localization of TORC2, we used auxin-inducible degradation and fluorescence microscopy of its constituent subunits to systematically examine the roles of these proteins and of PtdIns4,5P2 in TORC2 PM targeting. Contrary to prior assumptions, our findings pinpoint the essential subunit Avo3 (mammalian counterpart is RICTOR) as pivotal for TORC2 PM localization and assembly *in vivo*. However, how TORC2 activity is modulated in response to changes in the status of the cell envelope is unclear. In recent work, we found that TORC2 subunits Avo2 and Avo3 are direct targets of Slt2/Mpk1, the MAPK of the cell wall integrity pathway. Slt2-mediated phosphorylation of Avo2 at its MAPK phosphoacceptor sites inhibits TORC2-mediated activation of Ypk1. Deletion of Avo2 or expression of a phosphomimetic Avo2 allele rendered cells sensitive to two stresses (myriocin treatment and elevated exogenous acetic acid) that the cell requires Ypk1 activation by TORC2 to survive. Collectively, our studies have provided new insights about the assembly, localization, function and regulation of TORC2.

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