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Novel insights into regulation of Target of Rapamycin Complex 2 by hyperosmotic stress.

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Target of Rapamycin (TOR) Complex 2 (TORC2) is a multi-subunit protein kinase associated with the plasma membrane that is an essential regulator of growth. Ample genetic and biochemical evidence indicates that, in *Saccharomyces cerevisiae*, TORC2 exerts its effects solely via direct phosphorylation and upregulation of the activity of the downstream protein kinase Ypk1 (and its paralog Ypk2). TORC2-Ypk1 signaling, in turn, regulates various aspects of the lipid composition and organization of the plasma membrane during normal cell growth. In addition, TORC2 activity, and thus Ypk1 function, is modulated by environmental insults that exert stress on the plasma membrane. Ypk1 action maintains plasma membrane homeostasis in multiple ways. For example, in response to sphingolipid depletion, TORC2 activates Ypk1, which then phosphorylates targets that stimulate sphingolipid synthesis, inhibit aminoglycero-phospholipid flipping, and block endocytosis of integral plasma membrane proteins. In contrast, in response to hyperosmotic shock, TORC2 stimulation of Ypk1 is rapidly lost, alleviating Ypk1-mediated inhibition of Gpd1-dependent glycerol production and preventing Ypk1-mediated opening of the aquaglyceroporin Fps1, thereby allowing cells to rapidly accumulate intracellular glycerol to counteract the increase in extracellular tonicity. How hypertonic conditions influence TORC2 is not well understood. We report here that the plasma membrane-localized osmosensor Sln1 is an upstream regulator of TORC2. Inactivation of Sln1, as occurs under hyperosmotic conditions, leads to loss of TORC2 phosphorylation of Ypk1. Upon Sln1 inactivation, the TORC2 subunit Avo2 is phosphorylated at its MAPK phosphoacceptor sites. This response requires two mitogen (messenger)-activated protein kinases (MAPKs), Hog1 and Slt2/Mpk1. Absence of Avo2 partially restores TORC2-mediated stimulation of Ypk1 under hyperosmotic conditions. These results suggest that the phosphorylation status of the Avo2 subunit of TORC2 serves as a rheostat for controlling TORC2 function.