Investigating the role of the small GTPase Rhb1 in the TORC1 pathway in the budding yeast

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TORC1 (target of rapamycin complex 1) is a protein kinase complex that controls growth and proliferation by integrating multiple signals including hormones and nutrients such as amino acids. In higher eukaryotes, mTORC1 is regulated by its recruitment to the lysosomal membrane, a process that is mediated by the heterodimeric small Rag GTPases under amino-acid rich conditions. mTORC1 is also regulated by growth factor signaling via the small GTPase Rheb (Ras homolog enriched in brain). When it is GTP-bound, Rheb activates TORC1 allosterically at lysosomal membranes. Loss of Rheb causes altered TORC1 signaling, impaired development, and embryonic death in mice, which underscores its importance in mammals.

Some yeast species like *Schizosaccharomyces pombe* and *Candida albicans*, which lack growth factor signaling pathways, also express a Rheb-orthologous Rhb1 protein that is essential and that critically controls TORC1 activity. Curiously, however, loss of Rhb1 appears to have little impact on the viability and TORC1 activity in the budding yeast *Saccharomyces cerevisiae*. The only reported phenotype of *S. cerevisiae rhb1*Δ cells is their pronounced sensitivity to the toxic arginine analog canavanine, which is presumably due to an enhanced expression of the Can1 arginine permease at the plasma-membrane.

In this study, we aimed to uncover a potential link between Rhb1 and TORC1 in budding yeast. While our initial experiments confirmed the previously reported canavanine sensitivity of *rhb1*Δ mutant cells, they did not readily support a role of Rhb1 in TORC1 control, as the sensitivity of *rhb1*Δ cells to low levels of the TORC1 inhibitor rapamycin was comparable to the one of wild-type cells. Further corroborating this finding, loss of Rhb1 did also not alter the TORC1 kinase activity towards a known *bona fide* downstream effector at the vacuole (*i.e.* Sch9). Nevertheless, we also considered the possibility that Rhb1 may control a sub-population of TORC1 to locally phosphorylate a defined set of effectors. This hypothesis was fostered by our discovery that Rhb1 not only localized in the cytosol, but was also associated with foci that we colocalized with Golgi and early endosomal markers. Interestingly, our SILAC-based phosphoproteome analyses pinpointed various residues in Golgi-resident and early endosomal proteins, which were differentially phosphorylated in WT and *rhb1*Δ cells. This suggests that Rhb1 may perhaps control TORC1 at the Golgi or at early endosomes to regulate the phosphorylation state of a specific set of target proteins. To further support this idea, we have also set out to probe the Rhb1 interactome using a biotinylation-based proximity-labeling method. We will discuss our current progress and show how it delineates future studies in this context.

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