Abstract

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The *Saccharomyces cerevisiae* protein kinases Rck1 and Rck2 (Radiation sensitivity Complementing Kinases) were originally cloned as suppressors of *Schizosaccharomyces pombe* checkpoint mutants (27, 28). In *Saccharomyces cerevisiae* we found that overexpression of either kinase has the ability to depress sporulation in *mec1* and *rad24* mutant backgrounds. Conversely, deletion of *RCK1* or *RCK2* accelerates the sporulation process.

In a parallel study, we found a strong constitutive interaction between the C-terminal portion of Rck2p and the MAPK Hog1p. This interaction allows the *in vitro* and *in vivo* phosphorylation of Rck2p by Hog1p upon hyperosmotic stress. Rck2p *in vivo* phosphorylation was mapped to Ser^{520}, and leads to a hyperactivation of its catalytic activity, measured by the autophosphorylation level of Rck2p. *RCK2* overexpression can suppress osmosensitivity of *hog1* and *pbs2* mutants, and *RCK2* deletion can suppress the growth arrest of a hyperactive HOG pathway. Together, these results allowed us to place Rck2p genetically and biochemically downstream of the MAPK Hog1p.

We also found that unlike previously stated in the literature, the HOG pathway can be activated by oxidative stress, as measured by Hog1p Tyr/Thr phosphorylation. This activation leads to Hog1 translocation into the nucleus and Rck2p phosphorylation, in an analogous however less pronounced way than observed upon hyperosmotic stress. Using the two-hybrid system, we identify a series of Rck1p/Rck2p interacting proteins with roles in oxidative stress resistance. Two of these putative interactions (Rck1p-Yap2p and Rck2p-Zrc1p), were verified by co-immunoprecipitation and genetic studies, strengthening the possibility of Yap2p and Zrc1p being relevant Rck1p/Rck2p *in vivo* targets.

Key words: *Saccharomyces* cerevisiae, *RCK1*, *RCK2*, MAPKAP kinases, meiosis, HOG pathway, oxidative stress

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