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Rho1 GTPase and PKC Ortholog Pck1 Are Upstream Activators of the Cell Integrity MAPK Pathway in Fission Yeast

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Abstract

In the fission yeast *Schizosaccharomyces pombe* the cell integrity pathway (CIP) orchestrates multiple biological processes like cell wall maintenance and ionic homeostasis by fine tuning activation of MAPK Pmk1 in response to various environmental conditions. The small GTPase Rho2 positively regulates the CIP through protein kinase C ortholog Pck2. However, Pmk1 retains some function in mutants lacking either Rho2 or Pck2, suggesting the existence of additional upstream regulatory elements to modulate its activity depending on the nature of the environmental stimulus. The essential GTPase Rho1 is a candidate to control the activity of the CIP by acting upstream of Pck2, whereas Pck1, a second PKC ortholog, appears to negatively regulate Pmk1 activity. However, the exact regulatory nature of these two proteins within the CIP has remained elusive. By exhaustive characterization of strains expressing a hypomorphic Rho1 allele (*rho1-596*) in different genetic backgrounds we show that both Rho1 and Pck1 are positive upstream regulatory members of the CIP in addition to Rho2 and Pck2. In this new model Rho1 and Rho2 control Pmk1 basal activity during vegetative growth mainly through Pck2. Notably, whereas Rho2-Pck2 elicit Pmk1 activation in response to most environmental stimuli, Rho1 drives Pmk1 activation through either Pck2 or Pck1 exclusively in response to cell wall damage. Our study reveals the intricate and complex functional architecture of the upstream elements participating in this signaling pathway as compared to similar routes from other simple eukaryotic organisms.

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Introduction

Studies on molecular clues involved in the regulation MAPK signaling pathways are essential to understand how eukaryotic cells are able to adapt and survive against suboptimal environmental conditions. The rod-shaped, fission yeast *Schizosaccharomyces pombe* is an excellent model organism to study mechanisms and cellular events linked to MAPK activation, given the significant functional homology between their regulatory circuits and those of higher cells [1,2]. The cell integrity pathway (CIP), one of the three MAPK pathways present in fission yeast, regulates multiple processes like cell wall construction and maintenance during stress, vacuole fusion, cytokinesis, morphogenesis, and ionic homeostasis through its central element, MAPK Pmk1 [3–8]. Pmk1 is ortholog to human ERK1/2 and associates *in vivo* with MAPKKK Mkh1and MAPKK Pek1 to form a ternary complex [9–12], becoming activated in response to multiple adverse conditions such as hyper- and hypo-osmotic stress, glucose withdrawal, cell wall damage, and oxidative stress induced by hydroperoxides or pro-oxidants [12]. Importantly, *S. pombe* mutants lacking Mkh1, Pek1, or Pmk1 display strong sensitivity to the above stresses [12], indicating that a functional MAPK module is required for cell adaptation and survival under such conditions.

Previous work demonstrated that Rho2 GTPase, one of the six Rho GTPases found in *S. pombe* proteome (Rho1 to Rho5, and Cdc42) which controls cell polarity and cell wall biosynthesis, is a positive regulator operating upstream of the CIP [13,14]. Rho2-dependent regulation of Pmk1 activity is mediated through Pck2, one of the two orthologs of protein kinase C (PKC) present in this organism [13,14]. On the contrary, Pck1, the second PKC ortholog, appears to negatively regulate the activity of the CIP by an unknown mechanism, since Pck1-less mutants display a moderate increase in basal Pmk1 phosphorylation [14]. Notably, simultaneous deletion of Pck1 and Pck2 is lethal, suggesting that both kinases share a functional role that is essential during fission yeast growth [15]. Importantly, we demonstrated that Pmk1 can still be activated in the absence of either Rho2 or Pck2, supporting the existence of a complex scenario where several routes involving various (known and unknown) elements regulate Pmk1 activation depending on the nature of the activating stimulus [14]. This model is in striking contrast to the situation in budding yeast *Saccharomyces cerevisiae*, where RHO1 GTPase and PKC1 (ortholog

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Author Contributions

Conceived and designed the experiments: PP JC. Performed the experiments: LSM TS AF MM RAV JV. Analyzed the data: LSM TS MG PP JC. Contributed reagents/materials/analysis tools: MG PP JC. Wrote the paper: JC.

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