A Modified KESTREL Search Reveals a Basophilic Substrate Consensus for the Saccharomyces cerevisiae Npr1 Protein Kinase

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The Saccharomyces cerevisiae nitrogen permease reactivator Npr1 is a hyperphosphorylated protein that belongs to a family of Ser/Thr protein kinases dedicated to the regulation of plasma membrane transporters. Its activity is regulated by the Tor (target of rapamycin) signalling pathway. Inhibition of the Tor proteins by treating yeast cells with the immunosuppressant drug rapamycin promotes rapid dephosphorylation of Npr1. As an alternative to peptide arrays, the substrate requirement of Npr1 was probed with a peptide library that was generated by cleaving yeast cell extracts with CNBr and, after reverse-phase chromatography, the individual fractions were phosphorylated in vitro with recombinant Npr1. In this way, the ribosomal protein Rpl24a was found to be an excellent in vitro substrate for Npr1. Synthetic peptides tailored around the phosphorylation site of Rpl24a show that Npr1 is a Ser/Thr protein kinase with an absolute requirement for a basic residue at the P-3 position and a strong preference for basic P+1 residues, whereas proline at P+1 is strongly disfavoured. The results obtained with synthetic peptides suggest a (K/R)-X-X-S-(K/R) consensus sequence for Npr1. The availability of a consensus sequence allows a targeted search for physiologically relevant Npr1 substrates involved in the regulation of yeast amino acid permeases.

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