Molecular Analysis of Residues Essential for the Function of the Na+/H+ Antiporter of Fission Yeast, Schizosaccharomyces pombe.

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Abstract

Molecular analysis of residues essential for the function of the Na+/H+ antiporter of fission yeast, Schizosaccharomyces pombe. P. Dibrov, P. G. Young and L. Fliegel Department of Biochemistry, 347 Med. Sci. Bldg, University of Alberta, Edmonton, Alberta, Canada T6G 2H7; Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6. Using site-specific mutagenesis, we identified amino acid residues that are important for the function of sod2, the Na+/H+ antiporter of the fission yeast, Schizosaccharomyces pombe. All eight His residues of sod2 were mutated into Arg. Only the His367➔Arg substitution affected sod2 function. This mutation as well as the His367➔Ala mutation resulted in complete inability of mutant S. pombe to grow in the presence of LiCl. These mutants were unable to expel sodium from the cytoplasm in acidic (pH 4.0) medium. In more alkaline medium (pH 6.1) Na+-loaded S. pombe containing both mutant proteins failed to alkalinize the external Na+-free medium. When His367 was replaced by Asp, the sodium export was suppressed at acidic pH while the sodium-dependent proton influx at pH 6.1 was increased compared to wild type. To examine other amino acids important in cation transport we mutated three conserved aspartate residues within hydrophobic segments of sod2. S. pombe containing sod2 with the Asp241➔Asn and Asp266,267➔Asn mutations all showed greatly impaired growth in LiCl containing medium. In addition Na+-loaded S. pombe with the Asp➔Asn mutations could not exchange sodium ions for protons at pH 6.1. Sodium export of S. pombe at an external pH of 4.0 was almost completely abolished by the D266,267N mutation, however the D241N mutant protein retained almost normal Na+ efflux. Basing on the above experimental observations and on the sequence comparison, we suggest that His 367, Asp241 and Asp266,267 may be part of a common, conserved structural mechanism important in Na+/H+ exchange for both pro- and eukaryotic microorganisms.

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Presentation Number SAdibrov0630

Keywords: Na+/H+ antiport, fission yeast, mechanism, residues, sodium

Dibrov, P.; Young, P.G.; Fliegel, L.; (1998). Molecular Analysis of Residues Essential for the Function of the Na+/H+ Antiporter of Fission Yeast, Schizosaccharomyces pombe.. Presented at INABIS '98 - 5th Internet World Congress on Biomedical Sciences at McMaster University, Canada, Dec 7-

16th. Invited Symposium. Available at URL http://www.mcmaster.ca/inabis98/fliegel/dibrov0630/index.html