IDENTIFICATION AND FUNCTIONAL CHARACTERISATION OF A CLASS ON NON-SELECTIVE CATION CHANNELS IN *SACCHAROMYCES CEREVISIAE*

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Abstract:

Na^+ accumulation is a significant component in salt toxicity in plants. Although many proteins that facilitate Na^+ flux have been identified in plants, investigations aimed at identifying the initial mechanism of Na^+ entry into plants have failed. The catalysis of rapid, high capacity flux of Na^+ across plant cell membranes, currently through unknown means, is of particular importance. Observations of Na^+ flux across cellular membranes, using techniques such as electrophysiology and radiotracer flux, suggests this is a protein-based catalysis (Demidchik, 2002a; Demidchik and Tester, 2002c) (Essah et al., 2003). Based on these data, a class of protein described as voltage insensitive non-selective cation channel(s) (viNSCCs) are considered a good candidate for this Na^+ flux. This is due in part to characteristics they possess. These characteristics include catalysis of high capacity / low affinity cation flux, being relatively non-selective to the point where Na^+, K^+, NH_4^+ and MA^+ flux with similar properties and this flux being sensitive to elevated Ca^{2+} and changes in pH.

A screen using the toxic ammonium analogue methylammonium (MA^+) was developed using *Saccharomyces cerevisiae* strains that have minimal high affinity ammonium uptake capability. An *in silico* screen was developed and a number of candidate genes were identified as being possible viNSCCs. Preliminary selection of these was then conducted using the developed *S. cerevisiae* screen. Two genes, belonging to the same protein family, were selected based upon these results.

Analysis of these proteins using radiotracer flux in *S. cerevisiae* and electrophysiological examination using *Xenopus laevis* oocytes revealed these

proteins catalyse the non-selective flux of mono-valent cations following unsaturable kinetics, indicative of a low affinity transport system. Further analysis revealed this cation flux is sensitive to external Ca^{2+}. These properties strongly indicate these proteins form voltage insensitive non-selective cation channels in their native system.

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Table of Figures

List of Abbreviations used:

AMT: ammonium / methylammonium transporter

cAMP: cyclic adenosine monophosphate

cDNA: complimentary deoxyribonucleic acid

cGMP: cyclic guanosine monophosphate

CNGC: cyclic nucleotide gated channel

cRNA: complimentary ribonucleic acid

GLR: glutamate-like receptor

GPCR: G protein coupled receptor

HKT: A transporter family originally thought to catalyse H^+ and K^+ flux

iPCR: inverse polymerase chain reaction

MA: methylammonium

MEP: A transporter family originally known as methylammonium permeases

MES: 2-(*N*-morpholino)ethanesulfonic acid

NSCC: non-selective cation channel

ORF: open reading frame

PCR: polymerase chain reaction

TMD: trans-membrane domain

Tris: tris(hydroxymethyl)aminomethane

viNSCC: voltage insensitive non-selective cation channel

WU-BLAST: basic local alignment search tool maintained by Washington University