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Mutagenesis of the Yeast *ALR1* Mg Transport Gene

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Abstract

Magnesium is an essential element and the most abundant divalent cation in eukaryotic and prokaryotic cells, but its transport is not well understood. Mutagenesis was used to study the function of the *ALR1* (aluminium resistance) gene, which encodes the major Mg^{2+} uptake system in *Saccharomyces cerevisiae*.

Random PCR mutagenesis was undertaken of the C-terminal part of *ALR1* that is homologous to the bacterial CorA magnesium transport family. The mutants with the most severe phenotype all had amino acid changes in a small region of Alr1 containing the putative transmembrane (TM) domains. Eighteen single amino acid mutants in this critical region were classified into three categories: no, low and moderate activity. One 'no activity' mutation, M762L, affected the GMN motif that is a characteristic of the CorA super-family genes. Two other conservative mutations that reduced or inactivated uptake led me to identify Ser⁷²⁹ and Ile⁷⁴⁶ as critical amino acid residues in Alr1. High expression of inactive mutants inhibited the capability of the wild-type Alr1 protein to transport magnesium, consistent with the idea that Alr1 may form homo-oligomers. The results confirm the classification of *ALR1* as a member of the CorA family of magnesium transport genes

Random mutagenesis was also undertaken of the critical region of Alr1 containing the TM domains, in order to find important residues for Al^{3+} toxicity. Two types of Al -tolerant mutants were obtained: one with increased sensitivity to Co^{2+} and a second with no change in sensitivity to Co^{2+} ions. The former class was shown to have an increased rate of Mg^{2+} uptake, consistent with the hypothesis that Al^{3+} toxicity results from Mg^{2+} deficiency via inhibition of Alr1 activity. The latter class of mutants was shown to have normal rates of Mg^{2+} uptake but with a reduced sensitivity to inhibition by Al^{3+} ions. The three individual mutants in the latter class were combined in all combinations and the results indicated that their Al^{3+} tolerance was likely to be additive and that the mutants operate independently. The most tolerant mutant in this class, I746L, involved a conservative change (alteration of the relative location of methyl groups on the amino acid side chain), to a residue that is located within a TM and that was shown above to be critical for Mg^{2+} uptake. Therefore, Ile⁷⁴⁶ plays a very important role in both Mg^{2+}

uptake and Al^{3+} tolerance in Alr1. These results indicate that Al^{3+} may inhibit Mg^{2+} uptake by directly competing for binding sites within the pore of the Alr1 protein.

Truncation of N-terminal extension of Alr1 showed that the N-terminal 239 amino acids and the C-terminal 53 amino acids are not essential for magnesium uptake. They might be serving some other functions such as protein regulation.

In conclusion, these mutagenesis results firmly establish *ALR1* as a magnesium transport gene belonging to the CorA super-family and provide direct experimental support for the hypothesis that Al^{3+} toxicity in yeast occurs by direct inhibition of Mg^{2+} uptake via the Alr1 protein.

Abbreviations

%	percentage
35S	cauliflower mosaic virus 35S promoter
aa	amino acid
AAS	atomic absorption spectroscopy
Al	aluminium
ARS	autonomous replication sequence
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
bp	base pair(s)
Ca	calcium
CEN	centromere
Co	cobalt
C-terminus	carboxyl-terminus
DNA	deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylenediamine tetraacetic acid
EMS	ethyl methyl sulfonate
<i>g</i>	times the force of gravity
G418	geneticin
GFP	green fluorescent protein
GTP	guanosine triphosphate
h	hour(s)
HA	haemagglutinin
HIV	human immunodeficiency virus
K	potassium
kb	kilo base pairs
L, mL, μ L	litre, millilitre, microlitre
LPM	low phosphate, low pH, low magnesium medium
M, mM, μ M	moles per litre, millimoles per litre, micromoles per litre
Mg	magnesium
min	minute(s)

Mn	manganese
mRNA	messenger RNA
Ni	nickel
nm	nanometers
NMR	Nuclear magnetic resonance
N-terminus	amino-terminus
°C	degrees Celsius
OD	optical density
P	phosphate
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pers. comm.	personal communication
Pol	polymerase
RNA	ribonucleic acid
rpm	revolutions per minute
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
SC	synthetic complete medium
SC4	SC media containing 4 mM Mg ²⁺
SCM	SC media containing 250 mM Mg ²⁺
Sr	strontium
TBE	tris borate EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
tRNA	transfer RNA
UPR	unfolded protein response
v	volume
w	weight
YPD	yeast extract, peptone, dextrose rich medium
YPDM	YPD containing 250 mM Mg ²⁺
Zn	zinc
Δ	deletion mutant

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