http://researchspace.auckland.ac.nz

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage. http://researchspace.auckland.ac.nz/feedback

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.

Note : Masters Theses

The digital copy of a masters thesis is as submitted for examination and contains no corrections. The print copy, usually available in the University Library, may contain alterations requested by the supervisor.

Mutagenesis of the Yeast *ALR1* Mg Transport Gene

Jong-min Lee

Plant Science Research Group, School of Biological Sciences

University of Auckland

Thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy
February 2006

Acknowledgments

First of all, I would like to express my infinite gratitude to Professor Richard Gardner. Your guidance and support made the completion of this thesis a most worthwhile experience. I thank for your patience and understanding which encouraged me to finish my study successfully in a second language. There are not enough words to express how much I appreciate everything you have done.

I would also like to express my appreciation to Dr. Jo Putterill who kept an eye on the progress of my work and always was available when I needed her advice. My thanks also go to all the PMBies for the support, friendship and advice - especially Dr. Revel Drummond, Keith Richards, Jeannette Keeling, Shelley Robison and Soon Lee who provided wonderful proof-reading for my thesis.

I am very grateful to the Korean government and University of Auckland for the scholarship they provided and the contestable fund which supported me financially and enabled me to complete my study.

I would also like to acknowledge Shaoshan Chen. Without your daily encouragement, I would not have completed this study successfully. Thanks for your care and inspiration during my studies. My appreciation also goes to my dear friends, Marcus Henning, John Lambert, Jung-hyun Rho, Woo-hyun Ko, Jin-gyung Lee, Ivy Yao, Sunday Tsoi and Horace Xu who encouraged me and shared the knowledge and experience.

Finally, I am grateful to my family. To my father and mother, thank you very much for inspiring me to pursue my goal and encouraging me to do my best. To my sister Eun-jung Lee and her husband, thank you very much for taking care of mother and father in Korea so that I could concentrate fully on my study in New Zealand.

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 Ser^{729} and Ile^{746} 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, Ile^{746} 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
E. coli	*Escherichia coli*
EDTA	ethylenediamine tetraacetic acid
EMS	ethyl methyl sulfonate
g	times the force of gravity
G418	geneticin
GFP	green fluorescent protein
GTP	guanosine triphosphate
h	hour(s)
HA	haemaglutinin
HIV	human immunodeficiency virus
K	potassium
kb	kilo base pairs
L, mL, µL	litre, millilitre, microlitre
LPM	low phosphate, low pH, low magnesium medium
M, mM, µM	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
^{o}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
S. cerevisiae	*Saccharomyces cerevisiae*
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

Table of Contents