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The *AtMRS2* gene family from
Arabidopsis thaliana

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Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy
December 2004

Acknowledgements

Well it's been a long road getting here, but this is it.

If you are reading this book it's because I have finished my PhD. The people and events that we have to thank for me achieving this goal deserve as much credit as me in the end. So here is a selection, in chronological order, of those people and events.

Mum and Dad, and Troy, my brother. A great family. And also Fiona, Ryan and Logan, the other half of my family.

Gareth and Gavin my most steadfast childhood friends.

Mr Munroe, a primary school teacher. For annoying me to such an extent that I had to prove that he was wrong - that I was certainly amongst the smartest kids in the class.

Mr MacArthur, Ms Stuart and Ms O'Brian, the high school teachers that inspired me most. Mr Laver, who forced me to teach myself 7th form chemistry and Dr Colvine, who showed me physics was cool and also for annoying me in to proving that I was going to 'get somewhere', academically speaking, in the end.

Dan, Catherine and Vincent, Alexei, Jo S, Phil, Rachael, Dave, Caroline, Shelley, Jamie, Lee, Pete, Van, Jo H, Annemarie. Great friends.

Jeanette and Keith, the ever helpful and wise PMB lab technicians.

Richard Gardner. Supervisor, mentor. The guy most directly to blame for me walking around with an honorific.

And finally to Jo. My other half and great support crew.

Not exactly effusive but it's all you're getting. Thanks one and all.

Abstract

Magnesium (Mg^{2+}) is an essential mineral nutrient for plants and is the most abundant free divalent cation in plant cells. However, our knowledge of the role of this ion in the plant cell is limited, and the mechanisms of homeostasis and transport of the ion are almost completely unknown.

A. Tutone (this laboratory) identified an *Arabidopsis thaliana* gene by the complementation of a Mg^{2+} -uptake yeast mutant (CM66). This gene, referred to as *AtMRS2-11*, was expressed as cDNA from a strong yeast promoter and allowed the growth of the CM66 yeast strain on standard media. The conceptually translated AtMRS2-11 protein sequence was used in this study to identify nine additional proteins by sequence homology searches using the BLAST algorithm. The corresponding genes have been cloned from cDNA (*A. thaliana* ecotype Landsberg *erecta*) and sequenced. Protein sequence similarity suggests that the family forms a sub-section of the CorA super-family of Mg^{2+} transport proteins.

The mutant yeast used to identify the family initially was also used to show that two family members in addition to AtMRS2-11 were able to complement the Mg^{2+} -dependent growth phenotype. When fused to eGFP, these proteins showed a localisation consistent with some of the protein reaching the yeast cell membrane. The other members of the family were also fused to eGFP and showed a range of localisation patterns within the yeast cell. None of the three AtMRS2 proteins previously able to complement the yeast mutant phenotype did so when fused to eGFP.

RNA transcripts from the *AtMRS2* family were detected by RT-PCR in organ-scale preparations of total RNA from *A. thaliana*. Most family members were detected in all of the organs tested. Northern analysis of *AtMRS2-11* RNA transcript level showed that the gene was more highly expressed in leaf tissue, but was not affected by decreased levels of Mg^{2+} in the growth media. The levels of steady state *AtMRS2-11* mRNA transcript were elevated two-fold in the light during the diurnal cycle, but no change was detected during light-induced greening of etiolated seedlings. A stable transgenic *A. thaliana* line expressing the *gusA* gene from the promoter region of *AtMRS2-11* was used to localise the promoter's activity to cells containing chloroplasts. The expression appeared highest in younger cells.

The AtMRS2-11 protein was predicted to contain a chloroplast targeting peptide. Western analysis demonstrated that AtMRS2-11 was enriched in the total proteins of isolated chloroplasts as compared to extracts from whole plants. The AtMRS2-11:eGFP fusion protein was also detected in chloroplasts by fluorescence microscopy.

Flame atomic absorption spectroscopy was used in conjunction with isolated chloroplasts to try to determine the effects of the overaccumulation of the AtMRS2-11 protein in a transgenic *A. thaliana* plant line (constructed by A. Tutone). A rapid uptake or binding of Mg^{2+} was seen in chloroplasts isolated from both wild type and transgenic lines, but no differences were observed in either the rate of Mg^{2+} uptake/binding or the final Mg^{2+} content.

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Abbreviations

S.I. (Systeme Internationale) abbreviations for units and standard notations for chemical elements, nucleotides and amino acids are used in this thesis. Names of buffers and their abbreviations are given in Chapter 2, Table 2.1. Abbreviations of species and gene names are defined in the text. Other abbreviations used in the text are defined as below.

35S	cauliflower mosaic virus 35S promoter region
°C	degree celsius
β-gal	beta-galactosidase
ΔΨ	electric membrane potential
ΔpH	change in pH
AA	amino acid
AAS	atomic flame spectroscopy
ADP	adenosine diphosphate
AES	atomic emission spectroscopy
AMP	adenosine monophosphate
AP	alignment position
ATP	adenosine triphosphate
BAC	bacterial artificial chromosome
bp	base pair
C-terminus	carboxyl-terminus
CaMV	cauliflower mosaic virus
CIP	calf intestinal phosphatase
cDNA	complementary deoxyribonucleic acid
Co(III)Hex	cobalt (III) hexaamine
cryo-TEM	cryo-transmission electron microscopy
d	day
dCTP	2-deoxycytosine 5-triphosphate
DMDC	dimethyl-dicarbonate
DMF	dimethylformamide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleotide triphosphate
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
eGFP	enhanced green fluorescent protein
EGTA	ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N' tetraacetic acid

ER	endoplasmic reticulum
EST	expressed sequence tag
<i>g</i>	force of gravity at Earth's surface
GFP	green fluorescent protein
GST	glutathione-S-transferase
GUS	β -glucuronidase
h	hour
HEPES	N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
ICP	inductively coupled plasma
in prep.	in preparation
ITPG	isopropylthio- β -D-galactoside
kb	kilo base pair
kDa	kiloDalton
K_i	constant of inhibition
K_m	Michaelis constant
Kan ^R	kanamycin resistant
Kan ^S	kanamycin sensitive
KO	knockout
LB	left T-DNA border
MCS	multiple cloning site
min	minute
MOPS	3-[N-Morpholino]propanesulfonic acid
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MTS	methanethiosulphonate
N-terminus	amino-terminus
NMR	nuclear magnetic resonance
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PEG	polyethylene glycol
pers. comm.	personal communication
RACE	rapid amplification of genomic ends
RB	right T-DNA border
RNA	ribonucleic acid
RNase	ribonuclease
RNAi	RNA interference
RT-PCR	reverse transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
T-DNA	Transfer DNA

TM	transmembrane domain
T _m	melting temperature
Tris	Tris (hydroxymethyl)-aminomethane
U	Unit of enzyme activity
U.V.	ultra violet
V _{max}	maximum rate of uptake
v/v	volume/volume
WT	wild-type
w/v	weight/volume
X-gal	5-bromo-4-chloro-3-indoyl-β-thiogalactoside
X-gluc	5-bromo-4-chloro-3-indoyl-β-D-glucuronic acid
yr	year