Characterisation of PpMDHARs and PpENA1 from the moss, *Physcomitrella patens*

by

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ABSTRACT

Identifying a genetic basis for the tolerance to salinity exhibited by the resilient moss, *Physcomitrella patens,* could provide valuable information for use in the selection or modification of salinity tolerance in crop plants. The overall aim of the work described in this thesis was to identify, express and functionally characterise the protein products of two putative salinity tolerance genes from *Physcomitrella*, namely *PpMdhar* and *PpENA1*. The characterisation of *PpMdhar* and *PpENA1* represents a two-pronged approach into investigating the salinity tolerance of *Physcomitrella* at the biochemical and transport level, respectively. The enzymes encoded by *PpMdhars,* monodehydroascorbate reductases (MDHARs), are central to the ascorbate-glutathione cycle, and recycle monodehydroascorbate molecules into the antioxidant, ascorbate. Hence, MDHARs play a part in maintaining the capacity of plant cells to remove toxic reactive oxygen species. Given that the production of reactive oxygen species is greatly increased in plants under salt stress, and that *Physcomitrella* is tolerant of high salt, MDHAR enzymes were expressed to determine whether they exhibit increased enzymic activity when compared with MDHARs from higher plants. The protein encoded by *PpENA1* is Na^+ transporting ATPase, which actively transports toxic Na^+ ions across the cell membranes, and thereby minimizes the level of Na^+ that accumulates in the cytoplasm. Thus, in contrast to the mechanism by which MDHARs may help *Physcomitrella* deal with the secondary effects of high salt, the PpENA1 protein could enable the moss to actively exclude Na^+ ions, and thereby avoid cellular toxicity.

A link between salinity and the transcription of *PpMdhar* and *PpENA1* is reported here, and the function of each gene is investigated. A comprehensive characterisation of the enzymic action of expressed PpMDHAR enzymes is described, demonstrating that the biochemical mechanisms used by *Physcomitrella* in dealing with salt-induced reactive oxygen species are likely to be conserved with vascular plants. The physiological effects of the expression of PpENA1 are investigated *via* complementation experiments in yeast, and the membrane location of the protein is determined. The Na^+ binding-sites of PpENA1 are predicted using homology modelling and amino acid residues crucial for Na^+ transport are tested experimentally *via* site-directed mutagenesis. Finally, the introduction of a new, functional Na^+ binding-site into an inactivated form of the PpENA1 protein demonstrates that a degree of control is possible over the Na^+ binding-sites in PpENA1.

STATEMENT OF AUTHORSHIP

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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ABBREVIATIONS

A	Absorbance
ABA	Abscisic acid
APX	Ascorbate peroxidase
ATP	Adenosine triphosphate
BLAST	Basic local alignment search tool
bp	Base pairs
CAT	Catalase
cDNA	Complementary DNA
CMB	Chloromercuribenzoate
d	Days
Da	Daltons
DHAR	Dehydroascorbate reductase
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra-acetic acid
ER	Endoplasmic reticulum
EST	Expressed sequence tag
FAD	Flavin adenine dinucleotide
g	Gram
g	Units of centrifugal force
GR	Glutathione reductase
h	Hour
HA	Haemagluttin
HPLC	High-performance liquid chromatography
HRP	Horseradish peroxidase
IMAC	Immobilised metal affinity chromatography
IPTG	Isopropylthiogalactoside
kb	Kilobase
LB	Luria-Bertani
M	Molar
MCS	Multiple cloning site
MDHA	Monodehydroascorbate reductase
MDHAR	MDHA reductase
min	Minutes
mRNA	Messenger RNA
NADH	Nicotinamide adenine dinucleotide
Ni-NTA	Nickel-nitrilotriacetic acid
OD	Optical density
PBS	Phosphate buffered saline
PMSF	Phenylmethylsulphonyl fluoride
PCR	Polymerase chain reaction
Q-PCR	Quantitative PCR
PEG	Polyethylene glycol
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Room temperature (22°C)
SC	Synthetic complete
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
sec	Seconds
SOD	Superoxide dismutase
TBS	Tris buffered saline
TEA	Triethylamine
TEMED	Tetramethylethylene-diamine
Tris	Tris[hydroxymethyl] amino methane
tRNA	Transfer RNA
U	Units
UTR	Untranslated region
UV	Ultraviolet
v/v	Volume for volume
w/v	Weight for volume
YPD	Yeast peptone dextrose