Characterisation of a Novel Family of Eukaryotic Ammonium Transport Proteins

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III. Abbreviations

3′	Three prime of nucleic acid sequence
5′	Five prime of nucleic acid sequence
~	Approximately
±	plus and minus
β	Beta
µM	Micromolar
ADP	Adenosine diphosphate
AGRF	Australian Genome Research Facility
AMF1	Ammonium Major Facilitator 1
AMP	Ampicillin
AMT	Ammonium Transporter
ATP	Adenosine triphosphate
bp	Base pairs
bHLH	basic Helix-Loop-Helix
BLAST	Basic Local Alignment Search Tool
BSA	Bovine Serum Albumin
CARB	Carbenicillin
CaMV 35S	Cauliflower Mosaic Virus Constitutive Promoter
cDNA	Complementary deoxyribonucleic acid
CDS	Coding DNA sequence
CFP	Cyan Fluorescent Protein
Ct	Threshold cycle
C-terminal	Carboxyl terminal

DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
cRNA	Capped RNA
EDTA	Ethylene Diamine Tetracetic Acid
EMS	Ethylene Methane Sulfonate
EMSA	Electromobility Shift Assay
g	Grams
Gal	Galactose
Gal1	Galactose inducible promoter 1
GFP	Green Fluorescent Protein
Glu	Glucose
GmSAT1	*Glycine max* Symbiotic Ammonium Transporter 1
h	Hour(s)
I/V	Current as a function of voltage
Kb	Kilo base(s)
kD	Kilo dalton(s)
LB	Luria broth
LiAc	Lithium acetate
M	Molar
MA	Methylammonium
MBS	Modified Barth's Solution
MEP	Methylammonium permease
MES	2- (N-Morpholino) ethanesulfonic acid, 4-morpholineethanesulfonic acid
MF	Major Facilitator
MFS	Major Facilitator Superfamily

Min	Minute(s)
mM	Millimolar
mRNA	messenger RNA
N	Nitrogen
NCBI	National Centre for Biotechnology Information
ng	Nanogram(s)
nl	Nanolitre(s)
nm	Nanometer(s)
N-terminal	Amine terminal
OD	Optical Density
P	Phosphate
PCR	Polymerase chain reaction
PEG	Polyethylene Glycol
PBS	Peribacteroid space
P_i	Inorganic phosphate
PAGE	Polyacrylamide Gel Electrophoresis
Pro	L-proline
PBS	Peribacteroid space
PEG	Polyethylene Glycol
PM	Plasma Membrane
Pro	L-proline
qPCR	quantitative PCR
Rh	Rhesus protein
RNA	Ribonucleic acid
RNase	Ribonuclease

RNAi	RNA Interference
s	Second(s)
SDS	Sodium Dodecyl Sulfate
SE	Standard Error
SM	Symbiosome Membrane
SPEC	Spectomycin
TAIR	The *Arabidopsis* Information Resource
TCA	Trichloroacetic acid
TEVC	Two-Electrode Voltage Clamp
TF	Transcription factor
Tris	Tris(hydroxymethyl)aminomethane
UTR	Untranslated region
v/v	volume/volume
w/v	weight/volume
YFP	Yellow Fluorescent Protein
YPD	Yeast extract peptone dextrose medium
YNB	Yeast Nitrogen Base

IV. Abstract

Species from the family *Leguminosae* are able to survive in nitrogen (N) limiting conditions via a symbiotic relationship with soil-borne N_2-fixing bacteria collectively known as *Rhizobium*. The symbiosis results in the development of the root nodule where invaded bacteria (bacteroids) reside within a plant derived membrane vesicle (symbiosome) located within the cytoplasm of infected nodule cortical cells. Bacterial nitrogenase activity converts atmospheric N_2 to ammonium (NH_4^+), which is delivered to the plant in exchange for photosynthetically derived carbon for bacterial consumption. The mechanism regulating the transfer of NH_4^+ to the plant across the symbiosome membrane is currently unknown. GmSAT1 (*Glycine max* Symbiotic Ammonium Transporter 1), a symbiosome membrane bound basic Helix-Loop-Helix (bHLH) transcription factor has previously been identified in soybean by its ability to enhance NH_4^+ and MA transport in the NH_4^+ transport deficient yeast strain 26972c (Kaiser et al., 1998). In this study, we have revisited microarray analysis of 26972c cells expressing *GmSAT1* to identify differentially regulated yeast genes with putative roles in NH_4^+/MA transport.

Central to this study is the identification of ScAMF1 (*Saccharomyces cerevisiae* Ammonium Major Facilitator 1), a previously uncharacterised major facilitator transport protein, which was upregulated 56.5-fold in response to GmSAT1 activity. ScAMF1 and GmAMF1;3, a representative AMF1 from soybean, were functionally analysed with respect to putative NH_4^+ transport using a combination of yeast and *Xenopus laevis* oocyte expression systems. Both AMF1 proteins enhanced ^{14}C-MA uptake and established a related sensitivity phenotype in 26972c and 31019b, an alternative NH_4^+ transport mutant strain. In the presence of low (1 mM) NH_4^+, *ScAMF1* overexpression partially rescued growth of 26972c but was unable to establish a similar phenotype in 31019b. The role of

ScAMF1 in NH_4^+ transport was less clear. However, this study reaffirmed endogenous high-affinity NH_4^+ transporters called MEPs (Methylammonium Permeases) play an important role in GmSAT1-mediated NH_4^+ complementation. Heterologous expression in *X. laevis* oocytes suggest that ScAMF1 and GmAMF1;3 behave as non-selective cation channels capable of low-affinity NH_4^+ transport, revealing NH_4^+ current activation by P_i or a product of P-metabolism and potential Ca^{2+}-gating. This study also provided a preliminary electrophysiological profile of the *Arabidopsis* AMF1 homologs with respect to NH_4^+ transport for future studies to explore in detail.

V. Declaration

I certify that 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Danielle Mazurkiewicz Date

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