Characterization Of GmSAT1 And Related Proteins From Legume Nodules

Submitted by

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This thesis is submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy

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August, 2012

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I. Abstract

Nitrogen is an essential nutrient for plant growth and is normally obtained from the soil medium. Legumes are a unique group of plants that acquired the ability to form a symbiosis with nitrogen-fixing bacteria, called rhizobia, enabling growth in nitrogen-poor soils. *GmSAT1,* a predicted bHLH transcription factor from soybean, is essential for nitrogen fixation; however the role of this protein remains elusive (Kaiser et al., 1998; Loughlin, 2007).

In this work, a further functional characterization of GmSAT1 was undertaken. Using the promoters of known upregulated genes in yeast upon expression of *GmSAT1*, it was found that purified GmSAT1 directly interacts with DNA. Further, GFP-fusion analysis in onion epidermal cells, found that GmSAT1 localizes to the nucleus, as well as peripheral vesicles, demonstrating that GmSAT1 is a likely a transcription factor. Residues from both the N- and C-termini required for GmSAT1 activity were also identified by exchanging domains with GmSAT2, a protein that arose during the relatively recent whole-genome duplication in soybean.

Recently, GmSAT1 was shown to be essential for proper nodule development in soybean (Loughlin, 2007). Therefore, a DNA microarray analysis was conducted to identify transcripts that are differentially expressed after silencing of *GmSAT1* by RNA interference (RNAi) in soybean nodules. Of the ninety-five genes downregulated, twelve were associated with the circadian clock, potentially explaining the *GmSAT1* RNAi phenotype. Investigations were also initiated in the model legume *Medicago truncatula* to identify and characterize *GmSAT1* orthologues. Two genes, *MtSAT1* and *MtSAT2* were cloned and analyzed. *MtSAT1* and *MtSAT2* are expressed in roots and the inner cortex of nodules, similar to *GmSAT1*. By *in planta* GFP-fusion analysis, both MtSAT1 and MtSAT2 were found to associate with vesicles and the nucleus. Insertional mutations in either gene alone did not render a phenotype, however downregulating both genes by RNAi disrupted nodule formation.

Using the sequence of a newly discovered ammonium channel protein from yeast (ScAMF1), which is activated by GmSAT1, a novel subfamily of major facilitator transporter proteins (MFSs) from plants was identified. Interestingly, members of the

MFS gene family are found linked with *GmSAT1* loci in soybean, as well as *M. truncatula* and many sequenced dicots. *GmMFS1.3,* a representative from soybean, was cloned and characterized. *GmMFS1.3* expression was localized to the root stele and the inner cortex of nodules. Further, expression of GmMFS1.3 in yeast induced the uptake of methylammonium. Interestingly, *GmMFS1.5* was found to be downregulated in *GmSAT1* RNAi nodules. The link between GmSAT1 and the MFS transporters *in planta* will be the focus of future experiments.

A novel receptor-like kinase protein was also characterized from soybean nodules. GmCaMK1 was identified in a protein interaction screen using conserved calmodulin as bait. The calmodulin-binding domain overlaps the GmCaMK1 kinase subdomain XI, however it was found that GmCaMK1 is able to auto-phosphorylate independent of calmodulin. Therefore, calmodulin binding may influence the interaction of GmCaMK1 with its phosphorylation targets. Taken together, these studies have enriched our knowledge of nitrogen fixation, a critically important component of agricultural practice.

II. 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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III. Acknowledgements

I would like to acknowledge those people that have contributed to my research. First, I must thank my supervisor, Dr. Brent Kaiser, for supporting my project and allowing me to explore new ideas and possibilities. Brent was always available for discussions on new data and enthusiastic about the potential implications of our discoveries. I am also grateful for the support I received to conduct research overseas and attend conferences in some great locations.

I am also indebted to those who helped me conduct my research. I would like to thank Thomas DeFalco and Dr. Wayne Snedden for the opportunity to collaborate on the GmCamK1 manuscript. It was a great boost early in my PhD to be involved in a project that was leading to an immediate publication. I would also like to recognize Sergey Ivanov, Elena Fedorova, Eric Limpens, and Dr. Ton Bisseling from Wageningen University for help with *Medicago* experiments. I must also acknowledge Manijeh Mohammadidehcheshmeh and Danielle Mazurkiewicz for help with soybean transformations and yeast uptake experiments, respectively.

I would like to thank my wife, Louise Gillis, who lovingly supported me throughout my PhD. She had to cope with my irregular schedule and frequent weekend disappearances to the lab, not to mention living through the ups and downs of my results. She even had to move to The Netherlands for four months with me for research.

Finally, I must also acknowledge the generous support I received through scholarships from the Natural Sciences and Engineering Research Council of Canada (NSERC), The University of Adelaide, the Australian Government.

Cheers!

III. Abbreviations

AA	Amino Acid
bHLH	Basic Helix-Loop-Helix
BLAST	Basic Local Alignment Search Tool
cDNA	Complementary DNA
CaMV 35S	Cauliflower Mosaic Virus Constitutive Promoter
DNA	Deoxyribonucleic Acid
EMSA	Electromobility Shift Assay
ER	Endoplasmic Reticulum
EST	Expressed Sequence Tag
GFP	Green fluorescent protein
GUS	ß-Glucuronidase
kB	Kilobase
kD	Kilodalton
LB	Luria Broth
MA	Methylammonium
OD	Optical Density
PBM	Peribacteroid Membrane
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
RACE	Rapid Amplification of cDNA Ends
RNA	Ribonucleic Acid
RNAi	RNA Interference
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	SDS Polyacrylamide Gel Electrophoresis
TBST	Tris Buffered Saline (with Tween 20)
TMD	Transmembrane Domain
Tris	Tris(hydroxymethyl)aminomethane
UTR	Untranslated Region
v/v	Volume/Volume
w/v	Weight/Volume
X-gluc	5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid
YNB	Yeast Nitrogen Base