Identifying target proteins of the CreB deubiquitination enzyme in the fungus *Aspergillus nidulans*

By

Niyom Kamlangdee

BSc. (Biology), MSc. (Biotechnology)
Prince of Songkla University, Thailand

A thesis submitted to the University of Adelaide in total fulfillment of the requirements for admission to the degree of Doctor of Philosophy.

Genetics Discipline
School of Molecular and Biomedical Sciences
Faculty of Sciences
The University of Adelaide

November 2007

Table of contents

Abstract

Carbon catabolite repression in *A. nidulans* is a regulatory system which allows the organism to utilize the most preferable carbon source by repressing the expression of genes encoding enzymes utilizing alternative carbon sources. A ubiquitination pathway was shown to be one of the key mechanisms which regulate carbon source utilization, when *creB* was found to encode a deubiquitinating enzyme. Strains containing mutations in *creB* show loss of repression for some metabolic pathways in carbon catabolite repressing conditions, and also grow very poorly on several sole carbon sources such as quinate and proline, suggesting CreB plays multiple roles in the cell.

This work describes the analysis of the interaction of CreB with CreA, and with PrnB and QutD. Various epitope-tagged versions of CreA were expressed in *A. nidulans*, and an internally located HA-epitope tag was found to allow detection of CreA using Western analysis. A diploid strain was constructed between strains containing HA-tagged CreA and FLAG-tagged CreB. When CreB was immunoprecipitated, HA-tagged CreA was also precipitated in the diploid, indicating that CreA and CreB are present in a complex *in vivo*. To determine whether CreA is a ubiquitinated protein, a version of CreA that was tagged with both an HA epitope and a His-tag was expressed in *A. nidulans*, and protein extracts were precipitated with an UbiQapture™-Q matrix. Western analysis was used to show that CreA was present in the precipitate. These findings suggest that CreA is a ubiquitinated protein, and a target of the CreB deubiquitination enzyme.

To determine whether the proline permease (PrnB) is a direct substrate of CreB, plasmids to express epitope-tagged versions of PrnB were constructed and introduced into the *prnB* mutant strain. No tagged protein could be detected by Western analysis, even when these constructs were over-expressed from the *gpdA* promoter. However, a construct to express an HA epitope tagged version of quinate permease (QutD) fully complemented the *qutD* mutant strain, and HA-tagged QutD could be easily detected in Western analysis when probed with the anti-HA monoclonal antibody. A diploid strain was made between a complementing transformant and a strain expressing a FLAG-tagged CreB construct. When QutDHA was immunoprecipitated, CreBFLAG was detected in the immunoprecipitate of the diploid. A proportion of QutDHA was also co-precipitated in the diploid when CreBFLAG was immunoprecipitated. Thus, CreB is

present in a complex with QutD *in vivo*. Further results showed that the concentration of QutD in the cell is lower in a *creB* null mutant background than in the wild-type background, indicating that deubiquitination is required to prevent protein turnover. Northern analysis of mRNA showed that the failure of *creB* mutant strains to grow on quinate medium was not due to a failure of transcriptional induction of *qutD*, as the amount of mRNA was not lower in a *creB1937* mutant background compared to the wild-type. Furthermore, experiments were undertaken that showed that QutD is a ubiquitinated protein. These findings suggest that quinate permease is regulated through deubiquitination involving the CreB deubiquitination protein in *A. nidulans*.

In addition to the candidate protein approach asking whether CreA is a substrate of CreB, a proteomics approach was also used to identify proteins that interact with CreA. However, no clear interacting proteins were identified using this approach.

Declaration

This work contains no material which has been accepted for the award of any other degrees 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 had been made in the text.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

I give consent to this copy of my thesis, when deposited in the University Library, being made available in all forms for loan and photocopying.

……..

Niyom Kamlangdee

22nd November, 2007

Data presented in this thesis has been presented and published as in the following:

Kamlangdee, N., Lockington, R. A., and Kelly, J. M. **2005**. Expression of the tagged PrnB and QutD proteins in *Aspergillus nidulans* to identify targets of the CreB deubiquitination protein. The ComBIO2005 Combined Conference. Adelaide Convention Center, Adelaide, South Australia, Australia. September 2005.

Kamlangdee, N., Lockington, R. A., and Kelly, J. M. **2006**. Transformational study of the tagged *prnB* and *qutD* genes in *Aspergillus nidulans* to identify targets of the CreB deubiquitinating enzyme. The 20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress. Kyoto International Conference Hall, Kyoto, Japan. June 2006.

Kamlangdee, N., Lockington, R. A., and Kelly, J. M. **2007**. The CreB deubiquitinating protein interacts with quinate permease (QutD) *in vivo* in *Aspergillus nidulans*. 28th Lorne Genome Conference. Erskine Mantra Resort, Lorne, Victoria, Australia. February 2007.

Kamlangdee, N., Lockington, R. A., and Kelly, J. M. **2007**. Roles of the CreB deubiquitination protein in carbon catabolite repression (CCR) in *Aspergillus nidulans*. Asian Mycology Congress 2007 and Xth International Maine and Freshwater Mycology Symposium. Royal Park Hotel, Penang, Malaysia, December 2007.

Kamlangdee, N., Lockington, R. A., and Kelly, J. M. **2007**. Ubiquitinated CreA is a target and interacts with the CreB deubiquitinating protein *in vivo* in *Aspergillus nidulans*. International Conference on Cellular & Molecular Bioengineering 2007, Nanyang Technological University, Singapore. December 2007.

Acknowledgements

I would like to thank Thai Government for providing me the Ph.D. Scholarship to study at the University of Adelaide. Most importantly, I wish to acknowledge the guidance I have received from my proficient supervisor, Dr. Joan M. Kelly. I am very fortunate to have my comprehensive training experience with her, and Joan has been ever generous with her time and patient with my labours. Joan is not only a professional supervisor but she is always the great "*Teacher*" in my mind. I am grateful for her assistance in this thesis preparation. Her sagacious comments and positive approach have sustained my enthusiasm through all the tasks.

I would like to acknowledge Professor Dr. Jeremy N. Timmis, co-supervisor, for his support and constructive advice, and for putting up with me during these years.

Thank you to Dr. Robin Lockington, who has an enthusiasm and with a huge of knowledge in the *Aspergillus* experiences. You always give me with the excellent answers in all of my questions, and thank again for reading the thesis manuscript.

Thank to my fellow Ph.D. students, Jai Denton, Preeti Roy and Adrian Hunter. It had been the great to have all of you in this lab. Thank you for all of your helps, supports and for making me a lot of fun. My time here was shares with Damien Ferragamo, Jianliang Liang and Vivian Georgakopoulos as well as another adjoining laboratory. Hopefully all of you will successful with a real fungi world on the Globe.

I would like to thank School of Molecular and Biomedical Science (SMBS), and Faculty of Health Sciences (FHS) for granting me the Postgraduate traveling fellowship to presenting parts of this work both in National and International conferences. I also thank for people in SMBS for all of their help when I was having any problems.

Finally, I would like to acknowledge all of supports and assistances of my family in Thailand. They never come here but I always know their concerns through their gentle words. I am appreciative of the encouragement I have received from my parents.

List of Figures

Chapter 6

List of Tables

List of Abbreviations

ANM	*Aspergillus* nitrogen free media
A	Adenine
ANGIS	Australian National Genomic Information Service
[α^{-32}P]dATP	alpha-labelled deoxyadenosine triphosphate
BLAST	basic local alignment search tool
bp, kb	base pairs, kilobase pairs
BSA	bovine serum albumin
C	cytosine
^{0}C	degree Celsius
cDNA	deoxyribonucleic acid complementary to ribonucleic acid
CCR	carbon catabolite repression
C-terminal	carboxy-terminal
Da, kDa	Dalton(s), kiloDaltons
dATP	2'-deoxyadenosine-5'-triphosphate
dCTP	2'-deoxycytosine-5'-triphosphate
dGTP	2'-deoxyguanosine-5'-triphosphate
dTTP	2'-deoxythymidine-5'-triphosphate
DNA	deoxyribonucleic acid
dNTPs	2'-deoxynucleotide-5'-triphosphates
DTT	dithiothreitol
EDTA	ethylenediaminetetraaminoacetic acid
G	guanine
g, mg, µg, ng	gram(s), milligram(s), microgram(s), nanogram(s)
GFP	green fluorescent protein
His	histidine
IPTG	isopropylthio-β-D-galactosidase (or 1-isopropyl-β-D-1-thiogalactopyranoside
KOP	potassium orthophosphate
L, ml, µl	litre(s), millilitre(s), microlitre(s)
M, mM	mole per litre, millimole per litre
mCi	microCurie
min, hr	minute(s), hour(s)
mRNA	messenger ribonucleic acid
NAD	*β*-nicotinamide adenine dinucleotide
NCBI	National Center of Biotechnology Information
N-terminal	amino-terminal
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
®	registered
RNA	ribonucleic acid
rpm	revolutions per minute
RSP	reverse sequencing primer
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS polyacrylamide gel electrophoresis
SSC	saline sodium citrate buffer
SSPE	saline sodium phosphate-EDTA buffer

STC	Sorbital-TrisHCL-$CaCl_2$ buffer
™	Trademark
T	thymidine
TAE	Tris-acetate EDTA
Taq	*Thermus aquaticus*
TE	Tris-EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	Tris[hydroxymethyl] aminomethane
U	unite(s) of enzyme
UAS	Upstream activation sequence
Ub	ubiquitin
X-gal	5'-bromo-4-chloro-3-idoyl-β-D-galactopyranoside
(%) v/v	percent volume per volume
(%) w/v	percent weight per volume

Chemical Formula

$CaCl_2$	calcium chloride
CH_3COONa	sodium acetate
$CHCl_3$	chloroform
HCl	hydrochloric acid
KCl	potassium acetate
KH_2PO_4	potassium dihydrogen phosphate (monopotassium phosphate)
$MgSO_4$	Magnesium sulfate
MOPS	3-(N-Morpholino)propanesulfonic acid
NaCl	sodium chloride
NaH_2PO_4	monosodium phosphate
Na_2HPO_4	sisodium hydrogen phosphate
$Na_3C_6H_5O_7.2H_2O$	sodium citrate dihydrate
NaOH	sodium hydroxide