Distribution of proteins involved in carbon catabolite repression in *Aspergillus nidulans*

By

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Abstract

Carbon catabolite repression (CCR) is a mechanism by which micro-organisms preferentially utilize more easily metabolizable carbon sources in comparison to less easily metabolizable carbon sources. It prevents the organisms from unnecessary expenditure of energy and enables them to exploit the nutrients in appropriate manner. It represents a complex system of gene regulation.

The main aim of this study was to study the intracellular localization of proteins involved in CCR including CreA, CreB, CreC and CreD in *A. nidulans* in repressing and derepressing conditions. The major regulatory protein involved in CCR in *A. nidulans* is CreA. It is a DNA-binding repressor, but very little is known about the molecular events that allow CreA function to result in appropriate regulation in response to carbon source. To determine the amount and localization of CreA in different carbon sources, strains were made over-expressing GFP and HA tagged CreA. Western analysis showed that high levels of full length CreA can be present in cells that show normal responses to carbon catabolite repression, whether they are grown in repressing or derepressing media. Hence the amount of CreA is similar in both the conditions and thus degradation of CreA is not a key step in carbon catabolite repression. Fluorescence microscopy studies have shown that CreA is in the nucleus under repressing and derepressing carbon conditions and this is not affected by the absence of CreB or CreD, the other important proteins in *A. nidulans*. Thus mere localization of CreA in nucleus is not sufficient to cause carbon catabolite repression and there is some modification process involved for CreA to act as a repressor protein in CCR.

CreB is a deubiquitinating protein and CreC is a protein containing five WD 40 repeats, a putative nuclear localization signal (NLS) and a proline rich region and both the proteins are present in the cell in a complex. CreB was localized using strains that over-expresses GFP tagged CreB and fluorescence microscopy. CreB is present mainly in the cytoplasm in both repressing and derepressing conditions. Moreover, intracellular localization of CreB is unaffected by the presence or absence of CreD. However, the amount of CreB was higher in a *creD+* background as compared to a *creD34* mutant background, implying that the presence of CreD affects the amount of CreB in the cell.

CreC was localized by using strain that over-expresses YFP tagged CreC and it is also present mainly in the cytoplasm.

CreD contains arrestin domains and PY motifs and is highly similar to the Rod1p and Rog3p from *S. cerevisiae*. CreD is proposed to be involved in ubiquitination process in CCR in *A. nidulans*. Localization studies have shown that CreD is present throughout the cell in a punctate pattern with more in the cytoplasm than in the nucleus. CreB and CreD co-localize in some regions of the cell whereas in other regions either CreB or CreD is present.

Declaration

This thesis has 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 references has made in the text.

I give consent to this copy of my thesis, when deposited in The University of Adelaide library, being available for loan and photocopying.

Signed:

Data presented in this thesis have been published in the following article:

Roy, P., Lockington, R.A., and Kelly, J.M. (2008). CreA-mediated repression in *Aspergillus nidulans* does not require transcriptional auto-regulation, regulated intracellular localisation or degradation of CreA. Fungal Genetics & Biology 45, 657-670 (Appendix 2).

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alcA	Alcohol dehydrogenase A
[α-32 P] dATP	Alpha-labelled deoxyadenosine triphosphate
bp, kb	Basepair, kilobase(s)
BSA	Bovine serum albumin
CCR	Carbon catabolite repression
CM	Complete medium
dATP	2'-Deoxyadenosine triphosphate
dCTP	2'-Deoxycytosine triphosphate
dGTP	2'-Deoxyguanosine triphosphate
dTTP	2'-Deoxythymidine triphosphate
DIC	Differential interference contrast
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
DUB	Deubiquitinating enzyme
EDTA	Ethylene diaminetetraacetic acid
EGTA	Ethylene glycol–bis (2-aminoethylether) N, N, N', N' -tetra acetic acid
GFP	Green fluorescent protein
GPD	Glyceraldehyde-6-phosphate dehyrogenase
gpdA	Glyceraldehyde-6-phosphate dehyrogenase
ng, µg, mg, gm	nanogram(s), microgram (s), milligram(s), Gram(s)
HA	Haemagglutinin
HCHO	Formaldehyde
HCl	Hydrochloric acid
KCl	Potassium Chloride
KDa	Kilodalton(s)
KH_2PO_4	Monosodium potassium phosphate
$MgSO_4$	Magnesium sulphate
µl, ml	Microlitre(s), millilitre(s),
MM	Minimal medium
mM, M	Millimolar, Molar
µm	Micrometre
Na_2HPO_4	Disodium hydrogen orthophosphate
NaH_2PO_4	Sodium dihydrogen phosphate
NaOH	Sodium hydroxide
NaCl	Sodium Chloride
NLS	Nuclear localization sequence/signal
PAGE	Polyacrylamide gel electrophoresis

PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PIPES	Piperazine -N, N'-bis [2-ethanesulfonic acid]
PVDF	Polyvinyiedene Fluoride
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT	Room temperature
ribo	Riboflavine
SDS	Sodium dodecyl sulphate
SSC	Saline sodium citrate
TAE	Tris-acetate EDTA
TBS	Tris buffered saline
TGN	Trans-Golgi Network
Tris	Tris[hydroxymethyl] amino methane
UBP	Ubiquitin processing protease(s)
UCH	Ubiquitin carboxy-terminal hydrolase(s)
UV	Ultraviolet
V	Volt(s)
YFP	Yellow fluorescent protein
% (v/v)	Percent volume per volume
% (w/v)	Percent weight per volume
°C	Degree Celsius