Identification of potential Gcn2 regulating proteins

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in Genetics

at Massey University, Albany, New Zealand

Hayley Dawn Prescott

2016

Abstract

The viability of any organism relies greatly on their ability to adapt their metabolic processes in response to environmental stimuli. Proteins are essential for almost every intracellular biological process. Proteins are composed of amino acid precursors and the levels of amino acids within the cell available for protein synthesis requires careful monitoring. Amino acid conservation needs to be initiated in response to declining availability. Under conditions of amino acid deprivation, cessation of non-essential protein synthesis and activation of amino acid biosynthetic pathways is initiated. This regulatory mechanism is referred to as the General Amino Acid Control (GAAC) pathway.

General control non-derepressible 2 (Gcn2) is a fundamental constituent of the GAAC response pathway. Gcn2 senses a decline in amino acid availability and initiates the stress response by phosphorylating the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α). The phosphorylation of eIF2α triggers a sequence of events resulting in increased translation of the transcriptional activator Gcn4, which subsequently induces selective expression of genes necessary for *de novo* amino acid synthesis.

The activation and activity of Gcn2 is moderated by inhibitory and facilitative protein interactions. Published large scale purification studies identified many novel Gcn2 binding partners, some of which may function in Gcn2 regulation. However known Gcn2 regulators were absent from these datasets indicating that they were incomplete.

This work aimed to identify and screen potential Gcn2 binding partners for those that regulate Gcn2 activity. Analysis identified 135 proteins that were potentially in complex with Gcn2. Of those, Sse1, Chs5, Ncl1, Tir4 and Npr1 were subsequently identified as potential Gcn2 regulators.

For the purpose of comprehensively identifying novel Gcn2 binding proteins, a protocol was successfully optimised to enable Gcn2 affinity purification under conditions that would be specifically conducive for the maintenance of bonds between Gcn2 and its interaction partners. In this method, Gcn2 was overexpressed in cells to drive weak and/or transient interactions, and the usage of formaldehyde to crosslink interactions and further stabilise them was explored. This method will enable the future compilation of a comprehensive Gcn2 interactome.

Acknowledgements

In this space I would like to acknowledge those people who have contributed to the content of this thesis.

I am extremely grateful to my supervisor Evelyn Sattlegger. I would like to thank you for your support, advice and encouragement during the course of this research. You have been an incredible mentor to me.

I would like to thank my laboratory colleagues and friends Rashmi Ramesh and Kayleigh Evans who have always been on hand to offer advice and encouragement, and for proof reading my thesis for me.

I would also very grateful to Renuka Shanmugam for her incredibly valuable technical advice and encouragement.

Last of all I would like to thank my family, who have supported me wholeheartedly throughout the course of my studies.

Table of contents

List of Figures

List of Tables

Abbreviations

AT	3-Amino-1, 2, 4-triazole
APS	Ammonium persulfate
A-site	Acceptor site
BSA	Bovine serum albumin
Co-IP	Co-immunoprecipitation
EDTA	Ethylenediaminetetraacetic acid
eEF1A	Eukaryotic translation elongation factor 1 A
eIF2	Eukaryotic initiation factor 2
eIF2α-P	Eukaryotic initiation factor 2 alpha phosphorylated
EtBr	Ethidium Bromide
GAAC	General amino acid control
Gcn1	General control non-derepressible 1
Gcn2	General control non-derepressible 2
Gcn4	General control non-derepressible 4
HCl	Hydrochloric acid
kDa	Kilo Dalton
LiOAc	Lithium Acetate
mRNA	Messenger ribonucleic acid
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
ORF	Open reading frame
PEG	Polyethylene glycol
Pgk1	Phosphoglycerate kinase 1

PMSF	Phenylmethanesulfonyl fluoride
PVDF	Polyvinylidene fluoride
rpm	Revolutions per minute
SD	Synthetic dextrose
SDS	Sodium dodecyl sulfate
SM	Sulfometuron methyl
TBS	Tris Buffered Saline
TBST	Tris buffered Saline Tween
TC	Ternary complex
YPD	Yeast peptone dextrose
YPG	Yeast peptone glycerol