Unraveling the dynamics of protein-protein interactions in the Gcn2 signal transduction pathway

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Abstract

Eukaryotic cells regulate protein synthesis (translation) for a rapid response to various types of stress, and this involves several protein-protein interactions (PPIs) and protein phosphorylation. Phosphorylation of eukaryotic initiation factor-2 α (eIF2α) is a common regulatory mechanism to adjust protein synthesis in response to various stimuli. Gcn2 (General Control Non-derepressible) is an eIF2α kinase that is conserved from yeast to mammals, that is activated in response to amino acid starvation. Gcn2 activation leads to a reduction in global protein synthesis and simultaneous augmented translation of GCN4, a transcriptional activator of genes that are necessary to overcome stress. This cascade of events that allows cells in stress adaptation constitutes the General Amino Acid control (GAAC) pathway in yeast.

Gcn2 activity is controlled by a large array of proteins that directly or indirectly regulate Gcn2. Gcn2 has to bind another protein called Gcn1, in order to be activated in response to amino acid starvation. Yih1 (Yeast IMPACT homolog 1) in yeast and its counterpart IMPACT (IMPrinted and AnCienT) in mammals are homologous proteins that indirectly regulate Gcn2. Yih1/IMPACT inhibit Gcn2 by competing for Gcn1 binding. Yih1 associates with Actin, and studies so far have suggested that Yih1 only inhibits Gcn2 when it dissociates from Actin**.** The focus of this thesis work was to shed more light on those interactions relevant for Gcn2 regulation.

Firstly, we have identified that the Yih1 mediated interactions occur at distinct cellular locations within the cell, supporting the idea that spatially restricted cellular interactions controlled Gcn2 function. Using *in vitro* studies we have identified the regions on eEF1A that are involved in Gcn2 and Yih1 binding. The distinct binding sites for both proteins on eEF1A led to further investigations on how the dynamics of these interactions involving eEF1A might affect Gcn2 function. Together with unpublished observations by E Sattlegger and B Castilho, a function for the Yih1 ancient domain in interacting with eEF1A has been identified. Finally, the mechanisms by which Actin might control Gcn2 function were studied. In this regard, we have identified the Yih1-Actin interaction as one of the key PPIs involved in the crosstalk between the cytoskeleton and Gcn2 regulation. Together, the findings presented in this thesis, support the hypothesis that Gcn2 activity is spatiotemporally controlled by dynamic PPIs that occur at specific time at particular locations.

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Abbreviations

In addition to the chemical symbols from the periodic table of elements and the système international d'unités (SI), the following abbreviations are used:

3AT	3 amino 2, 4 triazole
$3AT^S$	sensitive to 3AT
A-site	acceptor-site
APS	ammonium persulphate
ATP	adenosine triphosphate
BiFC	Bimolecular fluorescence complementation
BSA	Bovine Serum Albumin
CIP	calf intestinal phosphatase
CTD	C-terminal domain
DAPI	4',6-diamidino-2-phenylindole
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DTT	dithiothreitol
E-site	exit-site
EDTA	ethylenediamine tetra acetic acid
eEF1A	eukaryotic elongation factor 1A
eEF1A-I	eEF1A domain I
eEF1A-II	eEF1A domain II
eEF1A-III	eEF1A domain III
eEF1A-I+II	eEF1A domains I and II
eEF1A-IImut	eEF1A with mutations in domain II
eEF3	eukaryotic elongation factor 3
EF2	elongation factor 2
eIF2	eukaryotic initiation factor 2
FITC	Fluorescein isothiocyanate
GAAC	general amino acid control
Gcd	general control derepressed
Gcn	general control non derepressible
GDP	guanosine diphosphate
GFP	green fluorescent protein
Gir2	genetically interacts with ribosomal genes 2
GTP	guanosine triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonicacid
HisRS	histidyl-tRNA synthetase

IMPACT	Imprinted and Ancient
IPTG	isopropyl-β-D-thiogalactopyranoside
LB	Luria-Bertani
OD	optical density
ORF	open reading frame
P-site	peptidyl donor site
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
PMSF	phenylmethanesulphonyl fluoride
PVDF	polyvinylidine difluoride
RNA	ribonucleic acid
RNase	ribonuclease
RPM	revolutions per minute
RWD	RING finger proteins, WD-repeat-containing proteins, yeast DEAD-like helicases
SD	synthetic defined
SDS PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SDS	sodium dodecyl sulfate
SM	sulfometuron methyl
SM^S	sensitive to SM
TBS	tris-buffered saline
TEMED	N,N,N',N'-Tetramethylethylenediamine
tRNA	transfer RNA
UV	Ultraviolet
VC	C terminal fragment of Venus fluorescent protein
VN	N terminal fragment of Venus fluorescent protein
WCE	whole cell extract
$xleu^S$	Sensitive to excess leucine
YFP	Yellow fluorescent protein
Yih1	Yeast IMPACT Homolog 1
YPD	yeast peptone dextrose
YPG	yeast peptone glycerol

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