Identification of novel proteins that potentially are in complex with Yih1 and that are required for promoting Gcn2 function

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The ability of organisms to respond to various stress conditions is important for life. Under amino acid starvation conditions the protein Gcn2 is activated and phosphorylates the translation initiation factor eIF2α. This leads to a downregulation of general protein synthesis and an upregulation of the synthesis of proteins that are involved in helping the cell overcome starvation, a process called General Amino Acid Control (GAAC). It is important that the GAAC is only switched on when necessary and for this Gcn2 needs to be regulated. For instance, the protein Gcn1 is needed for Gcn2 activation. Another protein, Yih1, inhibits Gcn2 activity by competing with Gcn2 for Gcn1 binding. However, the balance between Gcn2 activation by Gcn1 and Gcn2 inhibition by Yih1 is not well understood. Actin was already identified as a Yih1-binding protein and modelling exercises strongly suggests that additional proteins bind Yih1.

The aim of this project was to identify novel proteins that are in a complex with Yih1 (Yih1-binding proteins, YBP) and to then discover which are required for Gcn2 activation. For the first aim, YBP were ascertained from published large-scale protein-protein interactions studies and from data generated in-house.

19 different strains deleted for one putative YBP exhibited an impaired growth under starvation conditions. Of those, four deletion mutants showed a reduced Gcn2 activity. One protein was Spc72 which is involved in mitochondrial organisation. Another protein was Idh2, an enzyme of the citric acid cycle. The growth defect of strains deleted for *SPC72* or *IDH2* was complemented with a plasmid containing *SPC72* or *IDH2*, respectively, and other genes. This suggested their involvement in Gcn2 activation.

Elongation factor eEF1A was found as a putative YBP and as a co-precipitator of Yih1, supporting previous unpublished observations. eEF1A was found to bind Gcn2 in previous studies and this suggested that Yih1-eEF1A interaction may regulate Gcn2 activation.

Another putative YBP, the heat shock protein Hsc82, is needed for Gcn2 maturation. Strains deleted for *HSC82* showed an impaired growth under starvation conditions and this was reversed by deleting *YIH1*. This suggested that Yih1 may regulate Hsc82-Gcn2 interaction and thus Gcn2 activity.

This study was a step to further advance our understanding of Yih1-binding proteins and Gcn2 activity. In addition, this further emphasised the idea of Yih1 as an important regulator inside the cell.

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List of abbreviations

3AT	3-amino-1,2,4-triazole
APS	ammonium persulfate
AGC	automatic gain control
ATP	adenosine triphosphate
CID	collision induced dissociation
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
eIF2	elongation initiation factor 2
F-actin	filamentous actin
FTMS	Fourier transform mass spectrometry
GAAC	general amino acid control
G-actin	globular (monomeric) actin
Gcn	general control non-derepressible
GDP	guanosine diphosphate
GST	glutathione S-transferase
GTP	guanosine triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His_6	hexahistidine tag
HSP	heat shock protein
IMPACT	imprinted and ancient
IPTG	isopropyl β-D-1-thiogalactopyranoside
KCl	potassium chloride
kDa	kilodalton
LB	lysis broth
LC-MS/MS	liquid chromatography-tandem mass spectrometry
mRNA	messenger RNA
NaCl	sodium chloride
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PEG	polyethylene glycol
PIC	pre-initiation complex
PMSF	phenylmethylsulfonyl fluoride
PVDF	polyvinylidene difluoride
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RWD	RING finger, WD repeat, yeast DEAD-like helicase
SD	synthetic dextrose
SDS	sodium dodecyl sulfate

SM	sulfometuron methyl
SM^s	SM sensitivity
TAP	tandem affinity purification
TBS	Tris-buffered saline
TBS-T	TBS-Tween20
TOR	target of rapamycin
Tris	tris(hydroxymethyl)aminomethane
tRNA	transfer RNA
v/v	volume/volume
w/v	weight/volume
WCE	whole cell extract
YBP	Yih1-binding protein(s)
Yih1	Yeast Impact Homolog 1
YPD	yeast extract peptone dextrose
YPG	yeast extract peptone glycerol

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