Identification of Gcn1 binding proteins and characterization of their effect on Gcn2 function

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Abstract

All cells must have the ability to deal with a variety of environmental stresses. Failure to adapt and protect against adverse stress conditions can lead to cell death. One important stress that affects all cells is amino acid limitation. Amino acids are building blocks of proteins. Gcn2 is a protein kinase, activated under conditions of amino acid limitation and the active Gcn2 reduces the general protein synthesis and specifically increases the synthesis of a protein called Gcn4, a transcription factor of stress response genes.

Gcn2 is found in virtually all eukaryotes. In addition to the amino acid limitation it protects cells to a large array of stress conditions such as glucose and purine limitation, high salt, reactive oxygen species and UV irradiation. Interestingly, Gcn2 has been found to have acquired additional functions in higher eukaryotes such as cell cycle regulation, viral defense and memory formation. Not surprisingly, Gcn2 has been implicated in diseases and disorders such as abnormal feeding behaviour, cancer, Alzheimer's disease, impaired immune response, congestive heart failure, and susceptibility to viruses including HIV. Despite of its medical relevance, so far it is unknown how the cell ensures proper Gcn2 function.

Yeast studies have uncovered that for almost all Gcn2 functions Gcn2 must bind to its positive effector protein Gcn1. Gcn1 is proposed to be a scaffold protein, strongly suggesting that it serves as a platform for recruiting other proteins close to Gcn2 to fine-tune its activity. For this reason, in this study, we set out to comprehensively identify all proteins binding to Gcn1, i.e. generate the **Gcn1 interactome**, using a procedure that allowed us to also identify proteins that only weakly or transiently contact Gcn1 (a typical property of regulatory proteins). We have identified several potential Gcn1 binding proteins from published and *in house* data. Sixty six of these were further analyzed using the respective deletion strains. Ten of these deletion strains were unable to grow under amino acid starvation conditions. Five of these showed reduced eIF2α phosphorylation, strongly suggesting that they are positive effectors of Gcn2. Using plasmids from the Yeast Genome Tiling Collection, we were able to rescue the Gcn2 function of three deletion strains (*kem1*Δ, *msn5*Δ and *sin3*Δ), indicating that the defect was due to the deletion of the respective gene. In addition, some of these proteins were confirmed to reciprocally bind to Gcn1. Finally, we show that Kem1 partially facilitates activation of Gcn2 via Gcn1 and it may play a role as a positive regulator of Gcn2. Furhther the interactions were validated by reciprocal immunoprecipitation. Taken together, this study sheds light on novel Gcn1 binding proteins regulating Gcn2.

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Table of Contents

Table of Figures

Table of Tables

Abbreviations

The following abbreviations are used in addition to the chemical symbols from the periodic table of elements and the International System of Units (SI)

3AT	3-Amino-1, 2, 4-triaxole
ABC	ATP Binding Cassette
APS	Ammonium PerSulphate
ATP	Adenosine tri phosphate
BSA	Bovine Serum Albumin
DNA	Deoxyribonucleic acid
EDTA	Ehtylenediamine tetra acetic acid
Co-IP	Co-Immunoprecipitation
DMSO	Dimethylsulfoxide
EDTA	Ethylene Diamine Tetra acetic Acid
eEF3	Eukaryotic Elongation Factor 3
eIF2	Eukaryotic Initiation Factor 2
eIF2α-P	Eukaryotic Initiation Factor 2 phosphorylated alpha subunit
eIF2B	Guanine nucleotide exchange factor
EtBr	Ethidium Bromide
GAAC	General Amino Acid Control
Gcn1	General control non-derepressible 1
Gcn2	General control non-derepressible 2
Gcn3	General control non-derepressible 3
Gcn4	General control non-derepressible 4
HIV	Human immunodeficiency virus
kDa	Kilo Dalton
LB	Luria- Bertani
mRNA	Messenger ribonucleic acid
NaCl	Sodium Chloride
NaOH	Sodium hydroxide

OD	Optical Density
ORF	Open Reading Frame
p	Plasmid
PAGE	Polyacrylamide Gel Electrophoresis
PEG	Polyethylene glycol
Pgk1	3-Phosphoglycerate kinase
PVDF	Polyvinylidine Difluoride
RNase	Ribonuclease
rpm	Revolutions per minute
RT	Room Temperature
SD	Synthetic Dextrose
SDS	Sodium Dodecyl Sulphate
SM	Sulfometuron Methyl
SM^S	Sensitivity to sulfometuron methyl
ss	Single strand
Slg-	Slow growth
TAE	Tris-Acetate EDTA
TBS	Tris-Buffered Saline
TBS-T	TBS-Tween
TC	Tertiary Complex
TEMED	N, N, N, N- Tetramethylethylenediamine
Y2H	Yeast Two Hybrid
YPD	Yeast extract Peptone Dextrose
YPG	Yeast extract Peptone Glycerol