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

A thesis submitted in partial fulfillment of the requirements for the degree Doctor of Philosophy in Biochemistry

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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 (kem1Δ, msn5Δ and sin3Δ), 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. Further the interactions were validated by reciprocal immunoprecipitation. Taken together, this study sheds light on novel Gcn1 binding proteins regulating Gcn2.
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The following abbreviations are used in addition to the chemical symbols from the periodic table of elements and the International System of Units (SI):

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3AT</td>
<td>3-Amino-1, 2, 4-triazole</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP Binding Cassette</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium PerSulphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine tri phosphate</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra acetic acid</td>
</tr>
<tr>
<td>Co-IP</td>
<td>Co-Immunoprecipitation</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra acetic Acid</td>
</tr>
<tr>
<td>eEF3</td>
<td>Eukaryotic Elongation Factor 3</td>
</tr>
<tr>
<td>eIF2</td>
<td>Eukaryotic Initiation Factor 2</td>
</tr>
<tr>
<td>eIF2α-P</td>
<td>Eukaryotic Initiation Factor 2 phosphorylated</td>
</tr>
<tr>
<td>alpha subunit</td>
<td>Guanine nucleotide exchange factor</td>
</tr>
<tr>
<td>eIF2B</td>
<td>Ethidium Bromide</td>
</tr>
<tr>
<td>GAAC</td>
<td>General Amino Acid Control</td>
</tr>
<tr>
<td>Gcn1</td>
<td>General control non-derepressible 1</td>
</tr>
<tr>
<td>Gcn2</td>
<td>General control non-derepressible 2</td>
</tr>
<tr>
<td>Gcn3</td>
<td>General control non-derepressible 3</td>
</tr>
<tr>
<td>Gcn4</td>
<td>General control non-derepressible 4</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>LB</td>
<td>Luria- Bertani</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
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</table>
OD  Optical Density
ORF  Open Reading Frame
p    Plasmid
PAGE Polyacrylamide Gel Electrophoresis
PEG  Polyethylene glycol
Pgk1 3-Phosphoglycerate kinase
PVDF Polyvinylidene 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- Tetramethylethlenediamine
Y2H  Yeast Two Hybrid
YPD  Yeast extract Peptone Dextrose
YPG  Yeast extract Peptone Glycerol