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Identification of potential Gcn2 regulating proteins

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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.
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## Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AT</td>
<td>3-Amino-1, 2, 4-triazole</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium persulfate</td>
</tr>
<tr>
<td>A-site</td>
<td>Acceptor site</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>Co-IP</td>
<td>Co-immunoprecipitation</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>eEF1A</td>
<td>Eukaryotic translation elongation factor 1 A</td>
</tr>
<tr>
<td>eIF2</td>
<td>Eukaryotic initiation factor 2</td>
</tr>
<tr>
<td>eIF2α-P</td>
<td>Eukaryotic initiation factor 2 alpha phosphorylated</td>
</tr>
<tr>
<td>EtBr</td>
<td>Ethidium Bromide</td>
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<tr>
<td>GAAC</td>
<td>General amino acid control</td>
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<td>General control non-derepressible 4</td>
</tr>
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</tr>
<tr>
<td>kDa</td>
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</tr>
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<td>LiOAc</td>
<td>Lithium Acetate</td>
</tr>
<tr>
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<tr>
<td>Pgk1</td>
<td>Phosphoglycerate kinase 1</td>
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<tr>
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</tr>
<tr>
<td>PMSF</td>
<td>Phenylmethanesulfonyl fluoride</td>
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<tr>
<td>PVDF</td>
<td>Polyvinylidene fluoride</td>
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<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>SD</td>
<td>Synthetic dextrose</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SM</td>
<td>Sulfometuron methyl</td>
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