Identification of ribosomal proteins that are necessary for fully activating the protein kinase Gcn2

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Abstract

The environment in which cells grow often changes rapidly and in order to survive, cells need to adjust their metabolic pathway to these changes. Vitally important for all organisms is the constant availability of amino acids as they are building blocks for proteins. Proteins are essential molecules involved in most biological processes in a cell. Yeast and mammals overcome amino acid limitation by switching on a signalling pathway named General Amino Acid Control (**GAAC**), which triggers a decrease in general protein synthesis by inhibiting translation initiation while upregulating the transcription of stress-response genes.

For sensing starvation in yeast, the GAAC requires the kinase Gcn2 and its effector protein Gcn1. Gcn2 phosphorylates the α-subunit of the eukaryotic initiation factor 2 (**eIF2α**), which ultimately induces the selective expression of stress-response genes, leading to the *de novo* synthesis of all amino acids. In order to recognize the deacylated tRNA as an immediate signal for starvation, Gcn1 and Gcn2 need to be in direct contact and associated with the translating ribosome. The current model for sensing starvation by Gcn2 suggests that deacylated tRNA enters the ribosomal A-site and Gcn1 concomitantly transfers the starvation signal to Gcn2. However, the molecular details of this process are still unclear. Deletion analysis of *GCN1,* suggested that Gcn1 has multiple contact points with the ribosome. We therefore aim to uncover ribosomal proteins that are required to fully activate Gcn2 in order to better understand the starvation recognition process. The fact that Gcn1 has many ribosomal contact points implies that the deletion of one contact point will not remove Gcn1 from the ribosome and therefore maintains Gcn2 activation. This allows us to identify Gcn1-ribosome interaction points which are not only required to position Gcn1on the ribosome but also facilitate in Gcn1 mediated Gcn2 activation per se.

Genetic studies conducted in this thesis reveal that ribosomal proteins rps18, rps26, rps28, rpl21 and rpl34 are necessary for full Gcn2 activation. The deletion of their genes resulted in an impaired growth on starvation media and in a reduction in eIF2α phosphorylation. With these results we are able to create a first map of Gcn1 contact points of the ribosome that are necessary to promote Gcn2 activation. Two ribosomal

proteins that are necessary for fully activated Gcn2 are located on the large ribosomal subunit. Three others are located on the ribosomal head region of the small ribosomal subunit in proximity to the A-site region. Considering that Gcn1 is a large protein, our results support the idea that Gcn1 has multiple contact points with the ribosome and that some important contact points for Gcn2 activation are located near the ribosomal A-site.

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Abbreviations

In addition to the Système international d' unites (SI), the following abbreviations are used:

ABC	ATP-binding cassette
ALS	**A**ceto**l**acetate **S**ynthase
A-site	**A**cceptor-site
ATF4	**A**ctivating **T**ranscription **F**actor **4**
BSA	**B**ovine **S**erum **A**lbumin
c	**C**oncentration
Co-IP	**Co**-**I**mmuno**p**recipitation
cryo-EM	**Cryo**-**E**lectron **M**icroscopy
DMSO	Dimethylsulfoxide
EDTA	**E**thylene **D**iamine **T**etra acetic **A**cid
eEF3	**E**ukaryotic **E**longation **F**actor 3
eIF2	**E**ukaryotic **I**nitiation **F**actor 2
eIF2α-P	**E**ukaryotic **I**nitiation **F**actor 2 phosphorylated alpha subunit
eIF2B	Guanine nucleotide exchange factor
EtBr	**Et**hidium **Br**omide
E-site	**E**xit-site
GAAC	**G**eneral **A**mino **A**cid **C**ontrol
Gcn1	**G**eneral **c**ontrol **n**on-derepressible **1**
Gcn2	**G**eneral **c**ontrol **n**on-derepressible **2**
Gcn4	**G**eneral **c**ontrol **n**on-derepressible **4**
His	Histidin
HisRS	Histidyl-tRNA synthetase
kDa	Kilo Dalton
LB	**L**uria- **B**ertani
Met-tRNA$_i$	Methionyl initiator tRNA
mRNA	Messenger ribonucleic acid
NaCl	Sodium Cloride
NaOH	Sodium hydroxide
OD	**O**ptical **D**ensity
ORF	**O**pen **R**eading **F**rame

p	**P**lasmid
PAGE	**P**olyacryl**a**mide **G**el **E**lectrophoresis
PEG	**P**oly**e**thylene **g**lycol
Pgk1	3-Phosphoglycerate kinase
P-site	Peptidyl donor site
PVDF	**P**oly**v**inylidine **Dif**luoride
RNase	Ribonuclease
Rp(s/l)	**R**ibosomal **p**rotein (small/large)
rpm	**R**evolutions **p**er **m**inute
RT	**R**oom **T**emperature
SC	**S**ynthetic **C**omplete
SD	**S**ynthetic Dropout
SDS	**S**odium **D**odecyl **S**ulphate
SM	**S**ulfometuron **M**ethyl
SM^S	Sensitivity to sulfometuron methyl
Slg^-	**Sl**ow **g**rowth
TAE	**T**ris-**A**cetate **E**DTA
TBS	**T**ris-**B**uffered **S**aline
TBS-T	TBS-Tween
TC	**T**ertiary **C**omplex
TEMED	N,N,N, N- Tetramethylethylenediamine
WCE	**W**hole **C**ell **E**xtract
Y2H	**Y**east **Two H**ybrid
YPD	**Y**east extract **P**eptone **D**extrose
YPG	**Y**east extract **P**eptone **G**lycerol