Discovering links between elongation factors and general amino acid control in *Saccharomyces cerevisiae*

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Abstract

Continuous protein synthesis is essential for life; hence, a steady supply of amino acids must be maintained. In order to respond appropriately to amino acid shortages, cells need to constantly monitor their availability. Cells have a signal transduction pathway, called the general amino acid control (GAAC), for sensing and ameliorating amino acid shortages. Since the sensing occurs on translating ribosomes, the objective of this study was to investigate links between translation elongation and the general amino acid control in *S. cerevisiae.* In all eukaryotes, Gcn2 and its effector Gcn1 are responsible for monitoring amino acid availability. Active protein synthesis requires eukaryotic translation elongation factors (eEFs) to associate with translating ribosomes. This study focussed on two eEFs, eEF3 and eEF1A, and their potential role in GAAC.

Gcn1 has homology to eEF3, which suggests that both proteins utilise overlapping binding sites on the ribosome. Supporting this idea, it was found that over-expression of eEF3 caused sensitivity to amino acid analogues (AAA^S), suppressed the growth defect associated with constitutively active Gcn2, and impaired Gcn2 function. The C-terminal domain in eEF3 was found to be responsible for affecting Gcn2 function. Over-expression of this domain was sufficient for ribosome binding and for causing AAA^S. These findings suggest that eEF3 influences Gcn1 negatively.

For signal perception, Gcn1 and Gcn2 need to access the ribosomal A-site where eEF1A is functional. This suggests a link exists between eEF1A and GAAC. This link was confirmed by the discovery that eEF1A interacts with Gcn2 *in vivo*. The Gcn2 C-terminal domain was sufficient to precipitate eEF1A, independent of ribosomes, other yeast proteins and RNA. The interaction was lost under amino acid starvation conditions, suggesting that eEF1A is a negative regulator of Gcn2 activation under replete conditions.

This study reveals a link between translation elongation and GAAC. As eEF3 and eEF1A are known to interact with each other it is proposed here that they act in concert to inhibit Gcn1 and Gcn2 under replete conditions, hence suggesting a novel mechanism of Gcn2 regulation.

~ श्रद्धा सबुरी ~

(Faith & Perseverance)

- Sai Baba

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No woman is an island – adapted from John Donne

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Abbreviations

In addition to the chemical symbols from the periodic table of elements and the *système international d'unités* (SI), the following abbreviations are used:

3AT	3 amino triazole
aa	amino acid
aa-tRNA	amino acylated tRNA
ABC	ATP-binding cassette
APS	ammonium persulphate
A-site	acceptor-site
ATP	adenosine triphosphate
bp	base pair
BSA	Bovine Serum Albumin
CIA	chloroform: iso-amyl alcohol
CIP	calf intestinal phosphatase
CTD	C-terminal domain
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DTT	dithiothreitol
EDTA	ethylenediamine tetra acetic acid
eEF1A	eukaryotic elongation factor 1A
eEF3	eukaryotic elongation factor 3
EF2	elongation factor 2
eIF2	eukaryotic initiation factor 2
E-site	exit-site
GAAC	general amino acid control
Gcn	general control non derepressible
GDP	guanosine diphosphate
GTP	guanosine triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His-RS	histidyl-tRNA synthetase
IPTG	isopropyl-β-D-thiogalactopyranoside
LB	luria-bertani
Ni-NTA	nickel-nitrilo triacetic acid
OD	optical density
ORF	open reading frame
p	plasmid
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PEG	polyethylene glycol
PMSF	phenylmethanesulphonyl fluoride
PRS	post ribosomal supernatent
P-site	peptidyl donor site
PVDF	polyvinylidine difluoride
rpm	revolutions per minute
RNase	ribonuclease
RWD	RING finger proteins, WD-repeat-containing proteins, yeast DEAD-like helicases
SD	synthetic dropout
SDS	sodium dodecyl sulfate

SM	sulfometuron methyl
$tRNA_i^{Met}$	Methionyl-tRNA
Tris	tris(hydroxymethyl)aminomethane
TAE	tris-acetate EDTA
TBS	tris-buffered saline
TBS-T	TBS-Tween
TE	Tris EDTA
TEMED	N,N,N',N'-Tetramethylethylenediamine
TOR	target of rapamycin
v/v	volume/ volume
WCE	whole cell extract
w/v	weight/ volume