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Corresponding Author	Family Name	**You**
	Particle	
	Given Name	**Tao**
	Suffix	
	Division/Department	Computational Biology, Innovative Medicines
	Organization/University	AstraZeneca
	Street	Mereside, Alderley Park
	City	Macclesfield
	State	Cheshire
	Postcode	SK10 4TG
	Country	UK
	Phone	+44 (0)1625 514733
	Email	youtao80@gmail.com
Author	Family Name	**Coghill**
	Particle	
	Given Name	**George M.**
	Suffix	
	Division/Department	Institute for Complex System & Mathematical Biology
	Organization/University	University of Aberdeen
	Street	Foresterhill
	City	Aberdeen
	Postcode	AB25 2ZD
	Country	UK
	Phone	+44 (0)1224 273829
	Email	g.coghill@abdn.ac.uk
Author	Family Name	**Brown**
	Particle	
	Given Name	**Alistair J. P.**
	Suffix	
	Division/Department	Microbiology
	Organization/University	Institute of Medical Sciences, University of Aberdeen

Street	Foresterhill
City	Aberdeen
Postcode	AB25 2ZD
Country	UK
Phone	+44 (0)1224 555883
Fax	+44 (0)1224 555844
Email	al.brown@abdn.ac.uk

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Translational Control of GCN4

Tao You[1], George M. Coghill[2] and
Alistair J. P. Brown[3]
[1]Computational Biology, Innovative Medicines,
AstraZeneca, Macclesfield, Cheshire, UK
[2]Institute for Complex System & Mathematical
Biology, University of Aberdeen, Aberdeen, UK
[3]Microbiology, Institute of Medical Sciences,
University of Aberdeen, Aberdeen, UK

Synonyms

GCN control; *Gcn2*-dependent general translational control; uORF-mediated translational control in eukaryotes

Definition

In yeast, the *GCN4* (*General Control Nonderepressible 4*) gene mediates responses to environmental stress conditions including amino acid starvation. Its expression level is primarily controlled at the translational level. The *GCN4* mRNA has a long 5′-leader sequence of approximately 600-nucleotide that contains four upstream open reading frames (▶ uORFs). Under normal conditions, these uORFs impede the translation of the protein coding region via a mechanism that inhibits scanning of ▶ ternary complexes during the translation initiation pathway to the main *GCN4* coding region. Under various types of environmental stress, ternary complex levels are reduced, and the inhibition of *GCN4* mRNA translation by the uORFs is alleviated. Similar control mechanisms regulate the expression of other genes in yeast and are conserved in other eukaryotic organisms.

Characteristics

Gcn2-Dependent General Translational Control

In eukaryotic cells, the ternary complex (TC) (comprising eIF2, GTP, and initiator tRNA Met-$tRNA_i$) is required for ▶ translation initiation. The G-protein eIF2 has roughly tenfold higher affinity for GDP than GTP. The guanine nucleotide exchange factor eIF2B replaces this GDP for GTP only on the unphosphorylated form of eIF2 (Hinnebusch 2005).
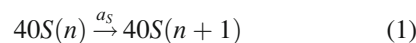
The yeast kinase Gcn2 (GCN2, PERK, PKR, and HRI in mammals) senses environmental stress (Fig. 1). Environmental cues such as amino acid starvation cause cytosolic accumulation of uncharged tRNA's, which consequently activate Gcn2. Its HisRS domain (histidyl-tRNA synthetase homologous) selectively binds uncharged tRNA's, and to activate the protein. Active Gcn2 phosphorylates eIF2 at the serine 51 residue in the eIF2α subunit, thereby enhancing its affinity for eIF2B. Phosphorylated eIF2 is a ▶ competitive inhibitor of unphosphorylated eIF2, and reduces eIF2B activity. This lowers the rate of formation of ternary complex, and impedes general mRNA translation (Hinnebusch 2005).

Stochastic modeling of translational control of *GCN4* mRNA

The 5′-leader sequence of *GCN4* mRNA contains four short uORFs (Fig. 2a). The translational control remains intact in the absence of uORF2 and uORF3,

and therefore for the sake of simplicity, we consider a construct that does not include these two uORFs (Fig. 2b). A ▶ 40S ribosomal subunit loads onto the 5′-end (i.e., beginning) of the mRNA in a closed conformation that is incompatible with ▶ scanning. Subsequently, it binds a ▶ TC, changes its conformation, and starts scanning toward the start codon (usually the 5′-proximal AUG). The 40S ribosomal subunit recognizes the ▶ start codon and initiates translation at uORF1 (Fig. 2b). Upon translational termination at uORF1, the ▶ 60S ribosomal subunit dissociates from the ribosome, and approximately 50% of 40S ribosomal subunits remain attached to the GCN4 mRNA and restarts scanning due to the properties of the mRNA sequence immediately 3′ to the uORF1 ▶ stop codon. When amino acids are replete, TC is abundant. Hence, there is a high probability that the scanning 40S ribosomal subunit will bind a TC before reaching uORF4 (Fig. 2c). As a result, most 40S ribosomal subunits then reinitiate at uORF4 and subsequently disassociate from the GCN4 mRNA due to the inhibitory nature of the sequence immediately 3′ to the uORF4 stop codon. Consequently, under replete conditions, the probability that a 40S ribosomal subunit will reach the protein coding region is low. Under conditions which activate Gcn2, ternary complex levels are reduced. As a result, the 40S ribosomal subunit is less likely to bind TC between uORF1 and uORF4, and the 40S ribosomal subunit is more likely to scan past uORF4. This elevates the chances of translating the main GCN4 coding region and increases GCN4 protein production (Fig. 2d). This regulation of GCN4 gene expression is known as ▶ translational control of GCN4 (Hinnebusch 2005).

This process can be modeled using the ▶ chemical master equation. Consider a 40S ribosomal subunit that scans downstream of uORF1 without steric hindrance. Suppose only two reactions are possible: a unidirectional scanning reaction whereby a 40S ribosomal subunit moves forward by one nucleotide with a ▶ propensity function of a_S s^{-1} (i.e., constant speed), and a second reaction in which a 40S ribosomal subunit binds a ternary complex with a propensity function of a_D s^{-1}.

$$40S(n) \xrightarrow{a_S} 40S(n + 1) \qquad (1)$$

$$40S(n) \xrightarrow{a_D} \qquad (2)$$

Denote the probability density function for a 40S ribosomal subunit at n nucleotides downstream of uORF1 at time t as $P(n, t)$. The chemical master equation states that during the infinitesimal time interval δt, the change in $P(n, t)$ is equal to the sum of the probability of a 40S ribosomal subunit moving into this site plus the probability that this site is originally occupied by a 40S ribosomal subunit which does not move during δt.

$$P(n, t + \delta t) = P(n - 1, t)a_S\delta t + P(n, t)(1 - a_S\delta t) \\ \times (1 - a_D\delta t) \qquad (3)$$

Using the boundary condition that a 40S ribosomal subunit initially starts from nucleotide 0, this equation can be solved to give:

$$P(n, t) = \frac{a_S^n \cdot t^n}{n!} exp[-(a_S - a_D)t]. \qquad (4)$$

Integrating $P(n, t)$ over t, the probability of the 40S ribosomal subunit being at nucleotide n is

$$P(n) = \int_{t=0}^{\infty} P(n, t)dt = \left(\frac{a_S}{a_S + a_D}\right)^n. \qquad (5)$$

Equation 5 reveals that $P(n)$ depends on the position n exponentially. Therefore, the probability that a 40S ribosomal subunit is at position n and has bound a ternary complex is $1 - \left(\frac{a_S}{a_S + a_D}\right)^n$. Consequently, the probability for a 40S ribosomal subunit to reinitiate at uORF4 is

$$P_{uORF4} = 1 - \left(\frac{a_S}{a_S + a_D}\right)^{n_1}. \qquad (6)$$

This decreases exponentially with the intercistronic distance between uORF1 and uORF4 n_1. This is supported by the experimental data in Fig. 3a (Grant et al. 1994).

The GCN4 protein coding region is translated when a 40S ribosomal subunit binds a ternary complex between uORF4 and the main GCN4 open reading frame. Hence, the probability of a 40S ribosomal subunit scanning downstream of uORF1 to reinitiate at GCN4 can be formulated as:

$$P_{GCN4} = \left(\frac{a_S}{a_S + a_D}\right)^{n_1}\left[1 - \left(\frac{a_S}{a_S + a_D}\right)^{n_2}\right]. \quad (7)$$

This model can be extended to include additional factors that might also be required for translation initiation (You et al. 2007, 2011). The spatial hindrance should also be taken into account when crowding effects are significant (You et al. 2011).
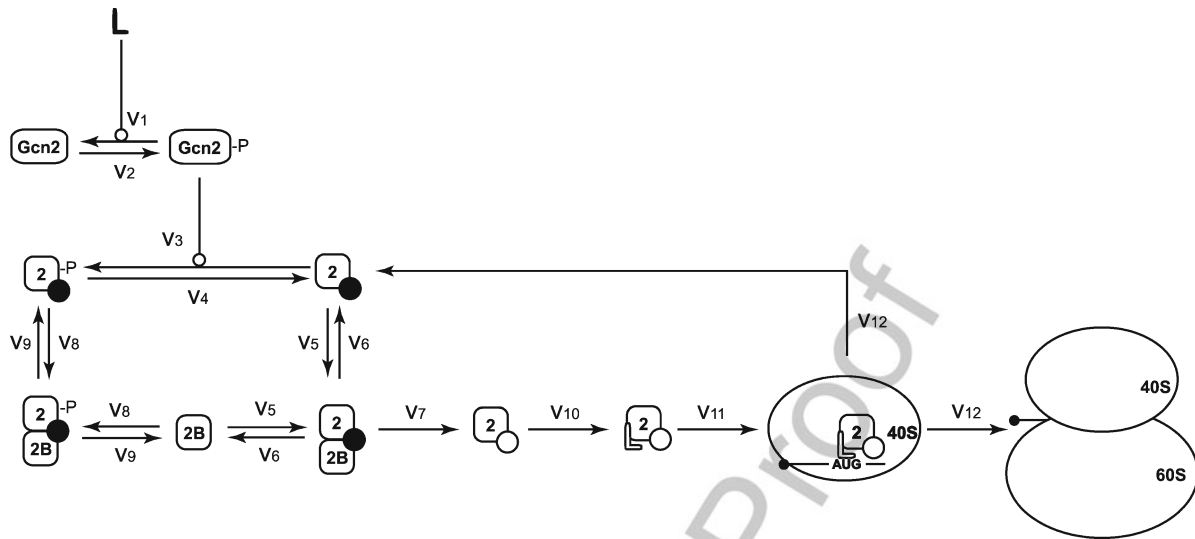
Cross-References

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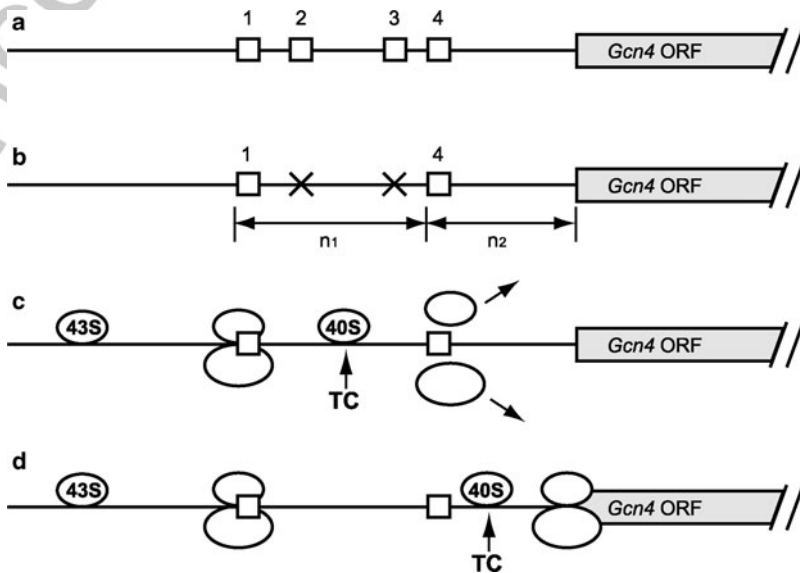
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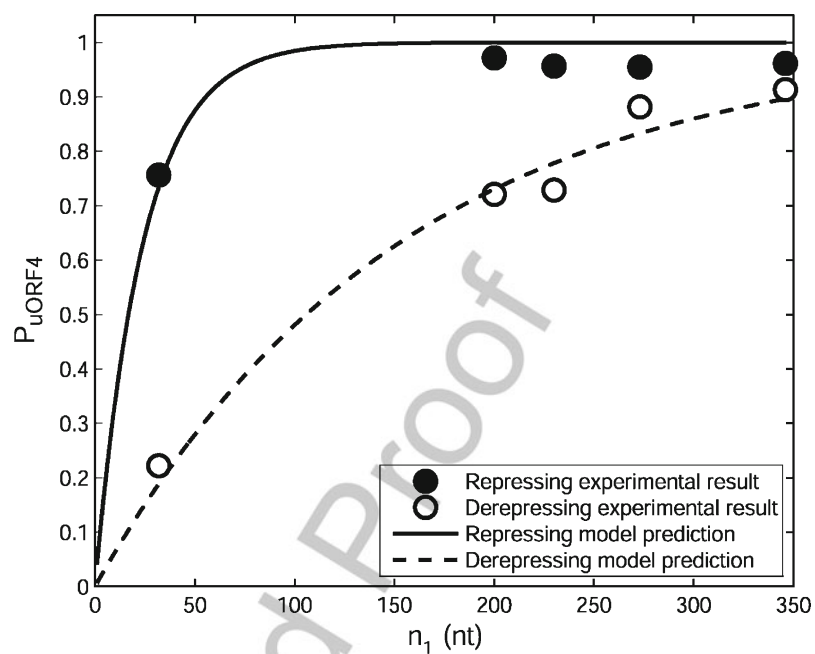
Translational Control of *GCN4*, Fig. 1   Schematic diagram of *Gcn*2-dependent general translational control

Translational Control of *GCN4*, Fig. 2   Schematic diagram of *GCN4* mRNA structure

Translational Control of
***GCN4*, Fig. 3**   Probability of
translation reinitiation at
uORF4 as a function of n_1