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SHORT COMMUNICATION

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Genetic evidence for functional specificity of the yeast GCN2 kinase

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Abstract In yeast the GCN2 kinase mediates translational control of *GCN4* by phosphorylating the α subunit of eIF-2 in response to extracellular amino acid limitation. Although phosphorylation of eIF-2α has been shown to inhibit global protein synthesis, amino acid starvation results in a specific activation effect on *GCN4* mRNA translation. Under the same conditions, translation of other mRNAs appears only slightly affected. The mechanism responsible for the observed selectivity of the GCN2 kinase is not clear. Here, we present genetic evidence that suggests that locally restricted action of the GCN2 kinase facilitates *GCN4*-specific translational regulation.

Key words *GCN4* · eIF-2 · DAI kinase · tRNA · Translational regulation

Introduction

Formation of the 43S translation preinitiation complex appears to be the modulated step in most of the cases in which translational regulation of gene expression is employed in eukaryotes (reviewed by Merrick 1992). The initiation factor eIF-2 participates in the assembly of this complex by loading the 40S ribosomal subunit with the initiator $tRNA^{Met}$, permitting recognition of the initiation codon by the scanning ribosome (Kozak and Shatkin 1978; Donahue et al. 1988; Cigan et al. 1989; Kozak 1991). Three kinases have thus far been isolated that modulate the activity of eIF-2 and thereby determine the rate of translational initiation. In certain mammalian cell types, Double-stranded RNA Activated Inhibitor (DAI) and Heme-Regulated Inhibitor (HRI) kinases are the effectors of protein synthesis shutdown under conditions of viral infection and heme deprivation, respectively (Meurs et al. 1990; Chen et al. 1991). In yeast, GCN2 kinase regulates the synthesis of GCN4, a transcriptional activator responsible for the concerted activation of genes coding for amino acid and purine biosynthetic enzymes, under conditions of limited amino acid or purine availability in the culture medium (Driscoll et al. 1983; Jones and Fink 1985; Roussou et al. 1988; Wek et al. 1989; Hinnebusch 1990).

In addition to a centrally positioned kinase domain, the GCN2 kinase features a truncated kinase segment at the N-terminus, a histidyl-tRNA synthetase-like region near the C-terminus and a ribosome association domain at the C-terminal end (reviewed by Wek 1994). Based on this structure, an attractive model for regulation of GCN2 kinase activity has been formulated, initially involving monitoring of unchanged tRNAs that accumulate during amino acid starvation by the histidyl-tRNA synthetase-like domain and subsequent triggering of the adjacent kinase moiety (Wek et al. 1989). The consequent phosphorylation of the eIF-2α subunit at serine-51 attenuates initiation of polypeptide chains and derepresses *GCN4* translation by neutralizing the negative effects of four small open reading frames (ORFs) preceding the GCN4-coding ORF, thereby facilitating translation of GCN4 itself (Tzamarias and Thireos 1988; Dever et al. 1992).

Paradoxically however, the anticipated concomitant drop in general protein synthesis is not observed under conditions of continued starvation (Tzamarias et al. 1989; Wek 1994). To resolve this apparent discrepancy, it has been suggested that translation of the *GCN4* mRNA is particularly highly sensitive to a limited extent o eIF-2a phosphorylation which does not affect the majority of other mRNAs (Abastado et al. 1991).

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Alternatively, the specificity of the GCN2 effect could stem from phosphorylation of mainly those eIF-2α molecules engaged in translation of the *GCN4* RNA, as a result of specialized subcellular localization. A similar model has been proposed for the localized activity of the mammalian homologue, the DAI kinase (De Benedetti and Baglioni 1984; Edery et al. 1989). In this report, we present genetic evidence suggesting that localized GCN2 kinase action indeed accounts for the *GCN4*-specific effect on translation and implicate the *GCN4* mRNA leader sequence in establishing this specificity.

Materials and methods

Yeast strains, manipulations and culture conditions

Yeast strains used in this study were all derivatives of S288C. The control strain wt is a *MAT***a**, leu*2-112*, *ura3*::*GCN4-LacZ* derivative, harboring an integrated *GCN4-lacZ* fusion reporter gene. This strain was constructed by transforming a *MAT***a**, *leu2-112* strain to 5-fluoro-orotic acid (5-FOA; Boeke et al. 1984) resistance with a 8.9-kb *Hin*dIII DNA fragment containing the *GCN4-LacZ* fusion (see below) inserted into the *Nco*I site of *URA3*. *gcn2Δ* carries a deletion in the *GCN2* gene (Roussou et al. 1988) and is otherwise isogenic to the wt strain. Transformations were carried out by the lithium acetate method (Ito et al. 1983). β-Galactosidase assays were performed as described (Tzamarias et al. 1986). *GCN4*-derepressing conditions were imposed by supplementing minimal medium (see below) with 10 mM 3-amino-1,2,4-triazole (3-AT), which stimulates histidine deprivation (Klopotowski and Wiater 1965). *GCN4* expression was assayed after allowing 6 h of growth in this medium at 30° C. Minimal medium contained yeast nitrogen base (Difco) and 2% glucose. All twenty amino acids were added when necessary.

Plasmids

Construction of Ycp50-*URA3-LacZ*, Ycp88-*DED1-LacZ*, Ycp50-*GCN4-LacZ*, Ycp50-Δ*ORFGCN4-LacZ*, and Yep24-*GCN2* has been described elsewhere (Rose and Botstein 1983; Hope and Struhl 1985; Tzamarias et al. 1986; Roussou et al. 1988, respectively). The *GCN4-LacZ* integration plasmid was constructed as follows. A 7.8-kb *Hin*dIII-*Sal*I blunt fragment from plasmid Ycp50-*GCN4-LacZ*, containing the *GCN4-LacZ* fusion (*GCN4* promoter plus 5′ UTR fused to the bacterial *LacZ* gene), was inserted into the *Nco*I site of plasmid pUC-*URA3* (generously provided by Despina Alexandraki). This plasmid harbors a 1.1-kb *Hin*dIII DNA fragment encompassing the *URA3* complementation unit, inserted into the *Hin*dIII site of pUC18. Insertion of *GCN4-LacZ* into the *Nco*I site disrupted the *URA3* ORF. The resulting plasmid was digested with *Hin*dIII prior to transformation, to facilitate efficient homologous recombination with the endogenous *URA3* locus. The 5′-UTR overexpressing plasmids Yep351-5′UTR(s)G and Yep351-5′UTR(as)G were constructed by initially inserting a blunt 0.4-kb *Sca*I-*Bst*EII DNA fragment from plasmid Ycp50-*GCN4-LacZ*, containing the 5′ UTR of *GCN4* up to the fourth upstream ORF into the *Hin*dIII site of vector pDB20, located between the *ADC1* promoter and transcription terminator (Fikes et al. 1990), either in the sense (s) or the anti-sense (as) orientation. A 2.5-kb *Bam*HI DNA fragment bearing the *ADC1* promoter and transcription terminator, together with the intervening *GCN4*-5′ UTR, was subsequently excised from each of the two parental plasmids and inserted into the *Bam*HI site of Yep351. Plasmid Yep351-5′UTR(s)A was constructed by inserting a 2-kb *Bam*HI DNA fragment, bearing the *ADC1* promoter and transcription terminator with the *ADC1* mRNA leader sequence from plasmid pDB20, into the *Bam*HI site of Yep351. Plasmid pDB20-Δ*R1GCN2* was constructed by inserting a 3.8-kb *Eco*RI-*Sal*I DNA fragment from plasmid Yep24-*GCN2* into the *Hin*dIII site of the vector pDB20. The in-frame ATG codon immediately beyond the *Eco*RI site in the *GCN2* gene was utilized for the translation of the truncated GCN2 derivative. No inhibitory out-of-frame initiation codons are present upstream this ATG in the transcript. Plasmid Yep24-Δ*KGCN2* was constructed by linearizing plasmid Yep24-*GCN2* at the unique *Kpn*I site between the kinase and tRNA synthetase domains, trimming with Mung Bean nuclease and religating. This treatment destroyed the *Kpn*I site by removing four nucleotides and generated a stop codon immediately after this site, resulting in production of a truncated GCN2 lacking the downstream tRNA synthetase-like domain.

Results and discussion

A non-functional, truncated GCN2 derivative lacking kinase activity reduces *GCN4* translational derepression, when overproduced in wild-type cells

Previous studies have demonstrated that GCN2 kinase is anchored to the ribosome via interactions facilitated by its extreme C-terminal domain (Ramirez et al. 1991; G. Thireos, unpublished observations). This suggests a localized action for GCN2 kinase. The specific impact of this kinase on *GCN4* expression further indicates a preference for ribosomes associated with *GCN4* mRNA. To investigate the mechanism responsible for such preference, we exploited the effects of overproduction of a truncated kinase-deprived GCN2 derivative (Δ*R1GCN2*), which has been shown to retain its subcellular localization properties intact (Ramirez et al. 1991).

As expected, this kinase-inactive derivative, when overexpressed (50-fold overexpression as assessed by Northern analysis; not shown) failed to complement a *gcn2* deletion, but, surprisingly, also blocked complete *GCN4* translational derepression in the wt strain and rendered the cells partially sensitive to amino acid starvation (Table 1; Δ*R1GCN2* vs. vector). In contrast, elevated (approximately 40-fold; Northern analysis not shown) production of intact GCN2 stimulated *GCN4* mRNA translation even in the absence of a starvation signal (Roussou et al. 1988; Table 1; *GCN2* vs. vector). An additional truncated GCN2 derivative (ΔKGCN2) lacking the histidyl-tRNA synthetase-like region, overproduced at the same level, failed to complement a *GCN2* lesion and did not result in any observable effect on the expression of *GCN4* (Table 1; Δ*KGCN2* vs. vector). No effect on the expression of two other genes, *DED1* and *URA3*, was observed under any of the above conditions (see Table 2; *GCN2*; Δ*R1GCN2*, Δ*KGCN2* vs. vector). In agreement with this observation, a *GCN4* derivative devoid of the upstream ORFs (Δ*ORFGCN4*) was insensitive to the effects of overexpression of the Δ*R1GCN2* derivatives (see Table 3; *GCN2*, Δ*R1GCN2*,

Table 1 Activation capacity of the general control of amino acid biosynthesis under conditions of steady overexpression of *GCN2* and *GCN4* 5′ UTR derivatives

Strain	wt				*gcn2Δ*			
Nutritional condition	R		D		R		D	
Overexpresed derivative								
Vector	+++	(4.1)	+++	(38.7)	+++	(2.7)	−	(2.6)
GCN2	++	(18.9)	++	(44.3)	++	(16.3)	++	(43.9)
Δ*R1GCN2*	+++	(3.5)	+	(14.7)	+++	(3.8)	−	(4.4)
Δ*KGCN2*	+++	(3.9)	+++	(36.4)	+++	(2.5)	−	(2.2)
5′UTR(s)G	+++	(3.7)	+	(12.1)	+++	(3.2)	−	(3.4)
5′UTR(as)G	+++	(3.2)	+++	(36.5)	+++	(2.9)	−	(3.1)
5′UTR(s)A	+++	(3.5)	+++	(37.3)	+++	(2.1)	−	(3.0)
5′UTR(s)G/*GCN2*	+++	(9.2)	+++	(38.1)	+++	(8.7)	+++	(34.3)
5′UTR(as)G/*GCN2*	++	(19.7)	++	(46.2)	++	(20.0)	++	(45.8)
5′UTR(s)A/*GCN2*	++	(18.3)	++	(41.9)	++	(24.7)	++	(48.4)
5′UTR(s)G/Δ*R1GCN2*	+++	(3.5)	++	(31.6)	+++	(3.7)	−	(4.0)
5′UTR(as)G/Δ*R1GCN2*	+++	(2.5)	+	(13.4)	+++	(4.2)	−	(4.3)
5′UTR(s)A/Δ*R1GCN2*	++	(2.3)	+	(11.3)	+++	(3.9)	−	(3.9)
5′UTR(s)G/Δ*KGCN2*	+++	(3.0)	+	(13.4)	+++	(3.3)	−	(3.9)
5′UTR(as)G/Δ*KGCN2*	+++	(3.9)	+++	(38.7)	+++	(2.1)	−	(3.6)
5′UTR(s)A/Δ*KGCN2*	+++	(3.7)	+++	(35.9)	+++	(2.6)	−	(3.3)

Growth of yeast cells under *GCN4* repressing (R) and derepressing (D) nutritional conditions as a function of the genetic background. +++ indicates normal growth, − indicates no growth, + and ++ indicate different degrees of leaky growth. Plates were scored after incubation for 3 days at 30° C. Numbers *in parentheses* correspond to the levels of *GCN4* mRNA translation, measured indirectly by utilizing an integrated *GCN4-LacZ* reporter fusion. β-Galactosidase values are given in Miller units and represent the average of three independent experiments with less than 10% deviation

Table 2 Effects of overexpression of *GCN2* and *GCN4* 5′ UTR derivatives on two other genes, unrelated to the General Control of amino acid biosynthesis

Strain	wt				*gcn2Δ*			
Nutritional condition	R		D		R		D	
Overexpresed derivative								
Vector	95.6	(52.7)	91.6	(49.2)	96.6	(55.1)	88.5	(58.7)
GCN2	92.0	(47.2)	88.5	(45.7)	93.4	(52.0)	90.2	(50.1)
Δ*R1GCN2*	98.4	(59.1)	93.4	(51.7)	89.7	(59.6)	89.4	(56.3)
Δ*KGCN2*	96.1	(54.3)	92.0	(48.3)	95.1	(57.8)	91.9	(55.5)
5′UTR(s)G	94.6	(55.8)	89.6	(50.8)	97.9	(49.8)	94.4	(51.4)
5′UTR(as)G	100.2	(57.3)	92.1	(56.0)	99.0	(58.3)	96.1	(57.1)
5′UTR(s)A	97.3	(61.4)	87.9	(54.3)	104.1	(63.9)	92.3	(53.8)

Expression levels of *DED1* and *URA3* (shown in parentheses) under *GCN4* repressing (R) and derepressing (D) nutritional conditions are listed as a function of the genetic background. Levels of expression were measured indirectly by utilizing appropriate *LacZ* reporter fusions (see Plasmids section of Materials and methods). β-Galactosidase values are given in Miller units and represent the average of three independent experiments with less than 10% deviation

Δ*KGCN2* vs. vector). We conclude that the Δ*R1GCN2* mutant kinase derivative, when in present abundance, out-competes the endogenous GCN2 for ribosomal site occupation and/or titrates GCN2-activating molecules. The failure of the Δ*KGCN2* mutant to mimic this effect indicates that the tRNA synthetase-like domain is involved in mediating this phenomenon. Alternatively, trans-inhibition could also result from overproduction of the Δ*R1GCN2* deletion derivative via formation of non-functional, heteromeric complexes with the endogenous kinase (Diallinas and Thireos 1994). However, given the absence of observable effects on expression of the *GCN4* derivative lacking the 5′ UTR and the two unrelated reporters utilized (*DED1*, *URA3*), we consider

Table 3 A *GCN4* derivative devoid of upstream ORFs is insensitive to overexpression of Δ*R1GCN2* and 5′UTR(s)G

Strain	wt		*gcn2Δ*	
Nutritional condition	R	D	R	D
Overexpresed derivative				
Vector	155.3	147.8	164.1	151.7
GCN2	138.9	122.2	146.3	131.0
Δ*R1GCN2*	161.7	143.3	166.4	158.2
Δ*KGCN2*	157.5	139.1	156.3	149.4
5′UTR(s)G	147.5	146.2	158.1	144.6
5′UTR(as)G	154.7	145.3	163.0	152.4
5′UTR(s)A	161.1	160.0	159.2	152.4

Expression levels of an ORF-less *GCN4* derivative under *GCN4* repressing (R) and derepressing (D) nutritional conditions are listed as a function of the genetic background. Levels of expression were measured indirectly by utilizing a Δ*ORFGCN4-LacZ* reporter fusion (described in the Plasmids section of Materials and methods). *β*-Galactosidase values are given in Miller units and represent the average of three independent experiments with less than 10% deviation

this possibility unlikely. Rather, it appears that sequences in the 5′ UTR are required to establish altered *GCN4* expression under conditions of overproduction of a kinase-deficient GCN2 molecule.

Overexpression of the *GCN4* 5′ UTR interferes with translation of endogenous *GCN4* mRNA

Dissection of the effects of eIF-2α phosphorylation on initiation of polypeptide chain synthesis has revealed several instances where these effects are restricted to specific mRNAs, both in vitro and in vivo (De Benedetti and Baglioni 1984; Kaufman and Murtha 1987; Edery et al. 1989; Kaufman et al. 1989). In the case of the DAI kinase, cis determinants on certain mRNAs, such as RNA secondary structures, may provide signals that enable the kinase to phosphorylate eIF-2α in the vicinity of the message and thereby ensure specificity of translational repression (Edery et al. 1989). It has been demonstrated that GCN2 regulates re-initiation by ribosomes scanning the *GCN4* 5′ untranslated region (UTR), in a manner that is dependent on the arrangement of four small ORFs located in the UTR (Tzamarias and Thireos 1988). This observation implicates the 5′ UTR in establishing the specificity for *GCN4* exhibited by GCN2.

To test this hypothesis, we monitored the translation of the endogenous *GCN4* mRNA in the presence of excess amounts (50-fold, as verified by Northern analysis; data not shown) its 5′ UTR, both in the sense and antisense orientations. A severe impact on *GCN4* translational derepression was only observed upon overproduction of the 5′ UTR RNA in the sense (5′UTR(s)G) orientation. Additionally, 50-fold overproduction of the *ADC1* 5′ UTR (5′UTR(s)A) did not interfere with *GCN4* translation. The effects resembled those associated with the kinase-deficient Δ*R1GCN2* derivative: initiation of translational derepression was manifested as inability to fully overcome starvation (Table 1; 5′UTR(s)G vs. 5′UTR(as)G, 5′UTR(s)A and vector). 5′ UTR-dependent sequestration of regulatory molecules required to establish a successful response to amino acid deprivation could account for this observation. Since no interference with the expression of other genes was evident (*DED1*, *URA3*, Table 2; 5′UTR(s)G vs. 5′UTR(as)G, 5′UTR(s)A and vector), such regulatory molecules should be specifically related to *GCN4* expression. Furthermore, none of the UTRs altered the translation of *GCN4* mRNA lacking the regulatory upstream ORFs, suggesting that molecules involved in the neutralization of the negative effects of these ORFs are affected (Table 3; 5′UTR(s)G vs. 5′UTR(as)G, 5′UTR(s)A and vector).

Co-overexpression of the truncated *GCN2* derivative quenches the effect of the *GCN4* 5′ UTR

Increased amounts of the sequestered molecule(s) should alleviate the consequences of *GCN4* 5′ UTR overexpression. We tested whether GCN2 kinase is such a molecule by examination of strains that overproduce GCN2 kinase and *GCN4* 5′ UTR simultaneously. Overexpression of both the *GCN2* gene and the *GCN4* 5′ UTR was monitored by Northern analysis (not shown). In these strains, endogenous *GCN4* expression is restored, indicating a functional association between GCN2 and *GCN4* 5′ UTR (Table 1; 5′UTR(s)G/*GCN2* vs. 5′UTR(as)G/*GCN2*, 5′UTR(s)A/*GCN2* and vector). Alternatively, the 5′ UTR-induced phenotype may not be manifest in *GCN2*-overexpressing strains where *GCN4* is constitutively derepressed (Roussou et al. 1988). To discriminate between these possibilities, we utilized the Δ*R1GCN2* and Δ*KGCN2* derivatives, which do not impose similar constitutive activation of *GCN4* when overexpressed. Co-overexpression of Δ*R1GCN2* and the *GCN4* 5′ UTR resulted in mutual cancellation of their negative effects on translation of the endogenous *GCN4* message (Table 1; 5′UTR(s)G/Δ*R1GCN2* vs. 5′UTR(as)G/Δ*R1GCN2*, 5′UTR(s)A/Δ*R1GCN2* and vector). In contrast, overproduction of the Δ*KGCN2*-derived protein, could not reverse the effects of overexpression of the *GCN4* 5′ UTR (Table 1; 5′UTR(s)G/Δ*KGCN2* vs. 5′UTR(as)G/ Δ*KGCN2*, 5′UTR(s)A/Δ*KGCN2* and vector). Northern analysis was employed to verify that expression levels of the *GCN2* derivatives and 5′ UTRs was maintained during the experiments (not shown).

These results support the idea of localization of GCN2 function, as has been postulated for the homologous mammalian DAI kinase, and further suggest

a regulatory interaction with *GCN4* mRNA. The inability of the GCN2 derivative lacking the histidyl-tRNA synthetase-like region to quench the effects of 5′ UTR overexpression points to that region as a candidate interaction-facilitating domain. It is possible that this interaction is maintained in the context of the ribosome rather than being a direct physical association. This notion is supported by the fact that this region of GCN2 partially overlaps with the ribosome association domain on the protein. Furthermore, mutations in this domain constitutively activate the kinase and result in broader spectrum of effects on general protein synthesis. Some of these mutations disrupt the subcellular localization of GCN2 (Ramirez et al. 1991). This disruption of localization could relax the specificity of GCN2 for *GCN4* and consequently allow this kinase to affect translation more broadly. Features of the *GCN4* 5′ UTR could serve as identity flags to guide such an interaction (Miller and Hinnebusch 1989). Indeed, substitution of part of the *GCN4* leader with heterologous ORFs leads to loss of some aspects of intrinsic *GCN4* translational regulation (Williams et al. 1988).

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References

Abastado JP, Miller PF, Hinnebusch AG (1991) A quantitative model for translational control of the *GCN4* gene of *Saccharomyces cerevisiae*. New Biologist 3:511–524

Boeke J, LaCroute F, Fink GR (1984) A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoroorotic acid resistance. Mol Gen Genet 197: 345–346

Chen JJ, Throop MS, Gehrke L, Kuo I, Pal JK, Broodsky M, London IM (1991) Cloning of the cDNA of the heme-regulated eukaryotic initiation factor 2α (eIF-2α) kinase of rabbit reticulocytes: homology to yeast GCN2 protein kinase and human double-stranded-RNA-dependent eIF-2α kinase. Proc Natl Acad Sci USA 88:7729–7733

Cigan AM, Pabich EK, Feng L, Donahue TF (1989) Yeast translation initiation suppressor *sui2* encodes the α subunit of eukaryotic initiation factor 2 and shares sequence identity with the human α subunit. Proc Natl Acad Sci USA 86:2784–2788

De Benedetti A, Baglioni C (1984) Inhibition of mRNA binding to ribosomes by localized activation of dsRNA-dependent protein kinase. Nature 311:79–81

Dever TE, Feng L, Wek RC, Cigan AM, Donahue TD, Hinnebusch AG (1992) Phosphorylation of the initiation factor 2α by protein kinase GCN2 mediates gene-specific translational control of *GCN4* in yeast. Cell 68:585–596

Diallinas G, Thireos G (1994) Genetic and biochemical evidence for yeast GCN2 protein kinase polymerization. Gene 143:21–27

Donahue TF, Cigan AM, Pabich EK, Valavicius BC (1988) Mutations at a Zn(II) finger motif in the yeast IF-2 gene alter ribosomal start-site selection during the scanning process. Cell 54:621–632

Driscoll Penn M, Galgoci B, Greer H (1983) Identification of *AAS* genes and their regulatory role in general control of amino acid biosynthesis in yeast. Proc Natl Acad Sci USA 80:2704–2710

Edery I, Petryshyn R, Sonenberg N (1989) Activation of double-stranded RNA-dependent kinase (dsl) by the TAR region of HIV-1 mRNA: a novel translational control mechanism. Cell 56:303–312

Fikes J, Becker D, Winston F, Guarente L (1990) Striking conservation of TFIID between *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Nature 346:291–294

Hinnebusch AG (1990) Transcriptional and translational regulation of gene expression in the general control of amino acid biosynthesis in *Saccharomyces cerevisiae*. Prog Nucleic Acid Res Mol Biol 38:195–240

Hope I, Struhl K (1985) GCN4 protein synthesized in vitro binds *HIS3* regulatory sequences: implications for the general control of amino acid biosynthesis in yeast. Cell 43:177–188

Ito H, Fukuda Y, Murata K, Kimura A (1983) Transformation of intact yeast cells treated with alkali cations. J Bacteriol 153:163–168

Jones EW, Fink GR (1985) Regulation of amino acid and nucleotide biosynthesis in yeast. Microbiol Rev 5:181–293

Kaufman RJ, Murtha P (1987) Translational control mediated by eukaryotic initiation factor-2 is restricted to specific mRNAs in transfected cells. Mol Cell Biol 7:1568–1571

Kaufman RJ, Davies MV, Pathak VK, Hershey JWB (1989) The phosphorylation state of eukaryotic initiation factor 2 alters translational efficiency of specific mRNAs. Mol Cell Biol 9:946–958

Klopotowski T, Wiater A (1965) Synergism of aminotriazole and phosphate on the inhibition of yeast imidazoleglycerol-phosphate dehydratase. Arch Biochem Biophys 112:562–566

Kozak M (1991) Structural features in eukaryotic mRNAs that modulate the initiation of translation. J Biol Chem 266: 19867–19870

Kozak M, Shatkin AJ (1978) Migration of 40S ribosomal subunits on messenger RNA in the presence of edeine J Biol Chem 253:6568–6577

Merrick WC (1992) Mechanism and regulation of eukaryotic protein synthesis. Microbiol Rev 56:291–315

Meurs E, Chong K, Galabru J, Shaun N, Thomas B, Kerr IM, Williams BRG, Hovanessian AG (1993) Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. Cell 62:379–390

Miller PF, Hinnebusch AG (1989) Sequences that surround the stop codons of upstream open reading frames in *GCN4* mRNA determine their distinct functions in translational control. Genes Dev 3:1217–1225

Ramirez M, Wek RC, Hinnebusch AG (1991) Ribosome association of GCN2 protein kinase, a translational activator of the *GCN4* gene of *Saccharomyces cerevisiae*. Mol Cell Biol 11:3027–3036

Rose M, Botstein D (1983) Structure and function of the yeast *URA3* gene. Differentially regulated expression of hybrid β-galactosidase from overlapping coding sequences in yeast. J Mol Biol 170:883–904

Roussou I, Thireos G, Hauge BM (1988) Transcriptional-translational regulatory circuit in *Saccharomyces cerevisiae* which involves the GCN4 transcriptional activator and the GCN2 protein kinase. Mol Cell Biol 8:2132–2139

Tzamarias D, Thireos G (1988) Evidence that the GCN2 protein kinase regulates reinitiation by yeast ribosomes. EMBO J 7: 3547–3551

Tzamarias D, Alexandraki D, Thireos G (1986) Multiple cis-acting elements modulate the translational efficiency of *GCN4* mRNA in yeast. Proc Natl Acad Sci USA 83:4849–4853

Tzamarias D, Roussou I, Thireos G (1989) Coupling of *GCN4* mRNA translational activation with decreased rates of polypeptide chain initiation. Cell 57:947–954

Wek RC (1994) eIF-2 kinases: regulators of general and gene specific translational initiation. Trends Biochem Sci 227:491–496

Wek RC, Jackson BM, Hinnebusch AG (1989) Juxtaposition of domains homologous to protein kinases and histidyl-tRNA synthetases in GCN2 protein suggests a mechanism for coupling *GCN4* expression to amino acid availability. Proc Natl Acad Sci USA 86:4579–4583

Williams NP, Mueller PP, Hinnebusch AG (1988) The positive regulatory function of the 5′-proximal open reading frames in *GCN4* mRNA can be mimicked by heterologous, short coding sequences. Mol Cell Biol 8:3827–3836