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Review

Keeping the eIF2 alpha kinase Gcn2 in check

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ABSTRACT

The protein kinase Gcn2 is present in virtually all eukaryotes and is of increasing interest due to its involvement in a large array of crucial biological processes. Some of these are universally conserved from yeast to humans, such as coping with nutrient starvation and oxidative stress. In mammals, Gcn2 is important for *e.g.* long-term memory formation, feeding behaviour and immune system regulation. Gcn2 has been also implicated in diseases such as cancer and Alzheimer's disease. Studies on Gcn2 have been conducted most extensively in *Saccharomyces cerevisiae*, where the mechanism of its activation by amino acid starvation has been revealed in most detail. Uncharged tRNAs stimulate Gcn2 which subsequently phosphorylates its substrate, eIF2α, leading to reduced global protein synthesis and simultaneously to increased translation of specific mRNAs, *e.g.* those coding for Gcn4 in yeast and ATF4 in mammals. Both proteins are transcription factors that regulate the expression of a myriad of genes, thereby enabling the cell to initiate a survival response to the initial activating cue. Given that Gcn2 participates in many diverse processes, Gcn2 itself must be tightly controlled. Indeed, Gcn2 is regulated by a vast network of proteins and RNAs, the list of which is still growing. Deciphering molecular mechanisms underlying Gcn2 regulation by effectors and inhibitors is fundamental for understanding how the cell keeps Gcn2 in check ensuring normal organismal function, and how Gcn2-associated diseases may develop or may be treated. This review provides a critical evaluation of the current knowledge on mechanisms controlling Gcn2 activation or activity.

1. Introduction

The protein kinase Gcn2 was first found to be part of a signalling pathway that enables *Saccharomyces cerevisiae* to sense and overcome amino acid deprivation [1–3]. No matter whether the cell is starving for several or only one amino acid, this signalling pathway is activated, leading to the reprogramming of the cellular gene expression profile, which includes increased expression of genes that code for enzymes in various amino acid biosynthetic pathways. This regulatory module governed by Gcn2 was therefore called General Amino Acid Control (GAAC) in *S. cerevisiae*, or Cross Pathway Control (CPC) in the filamentous fungi *Neurospora* and *Aspergillus*. Mammals contain three kinases in addition to Gcn2 that phosphorylate the same substrate, the α subunit of eukaryotic translation initiation factor 2 (eIF2α), each responding to distinct stimuli: HRI, PKR and PERK(PEK) [1–3] (Fig. 1). As eIF2α is the common downstream target that integrates signalling from all eIF2α kinases, in mammals this pathway was termed the Integrated Stress Response (ISR) [4]. Gcn2 stands for "General control non-derepressible" 2, and in mammals it is also called EIF2AK4 (eukaryotic translation initiation factor 2 alpha kinase 4) [1–3]. The Gcn2 orthologue in *Neurospora* and *Aspergillus* is called Cpc3 and CpcC, respectively [5,6].

As all eIF2α protein kinases, Gcn2 exerts its function *via* phosphorylating a specific amino acid in eIF2α (Ser-51 in yeast and mammals) (Fig. 1) [1–3]. The molecular basis of Gcn2 function and the mechanisms underlying its activation have been predominantly studied in the yeast *S. cerevisiae* [1]. The immediate signal of amino acid starvation is uncharged tRNAs ($tRNA^{deacyl}$) accumulating in the cell. These are detected by Gcn2, leading to the stimulation of its protein kinase catalytic domain and subsequent phosphorylation of eIF2α [1]. eIF2 in a GTP-bound form is essential for initiating protein synthesis in that it delivers initiator methionyl tRNA (Met-$tRNA_i^{Met}$) to the ribosome. After completing translation initiation eIF2 is released in its GDP bound form, and it needs to be recycled to the GTP-bound form by its guanine nucleotide exchange factor (GEF) eIF2B. Phosphorylation of eIF2α by Gcn2 converts eIF2 to a competitive inhibitor of eIF2B, leading to reduced global protein synthesis and thus to reduced overall utilisation of amino acids (Fig. 1). Simultaneously, eIF2α phosphorylation evokes increased translation of specific mRNAs containing in their 5′ leader unique upstream open reading frames (see review [1,7,8]), such as those coding for Gcn4 in yeast and ATF4 in mammals (Fig. 1).

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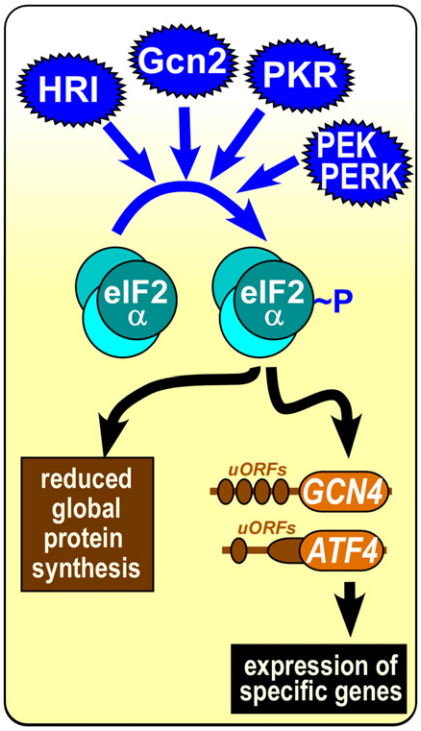

Fig. 1. In all eukaryotes, from yeast to mammals, phosphorylation of the α subunit of eIF2 is a major mechanism to adjust the cellular gene expression profile in response to specific cues. While global protein synthesis is reduced, eIF2α phosphorylation simultaneously leads to increased translation of mRNAs containing specific uORFs. These mRNAs code for transcription factors, *e.g.* Gcn4 in yeast and ATF4 in mammals, that regulate expression of a large array of genes. In all eukaryotes, the protein kinase phosphorylating eIF2α in response to amino acid starvation is Gcn2 (EIF2AK4; or Gcn2-like). Some eukaryotes contain additional eIF2α kinases, such as PKR, HRI and PEK/PERK in mammals. Heme regulated Inhibitor (called HRI or EIF2AK1) is activated by heme deprivation in erythroid cells; RNA-dependent protein kinase (PKR, EIF2AK2) is stimulated by double stranded RNAs accumulating during viral infections; and PKR-like endoplasmic reticulum kinase (PEK, PERK, EIF2AK3) is activated by the accumulation of misfolded proteins in the endoplasmic reticulum. Like Gcn2, these kinases have been found to respond to additional cues, and some overlap of function may occur [3,31].

These are transcriptional regulators that control the expression of hundreds of genes that promote the recovery of cells from the initial insult (*e.g.* [1–4,9–11]). For example, Gcn4/Atf4 induces expression of genes coding for key amino acid biosynthetic enzymes and amino acid transporters.

As in yeast, mouse Gcn2 is activated under low amino acid availability, and is required for adaptation to amino acid starvation [12–15]. Although mice lacking Gcn2 are viable, under amino acid deprivation they display aberrant protein production in the liver, and enhanced skeletal muscle loss, and *Gcn2*$^{-/-}$ mice starved after birth exhibit increased morbidity in response to amino acid deprivation [16]. Interestingly, feeding on diets devoid of essential amino acids results in Gcn2-dependent eIF2α phosphorylation in the anterior piriform cortex, and this is associated with the development of an aversive behaviour towards the amino-acid imbalanced food, the latter again being dependent on Gcn2 [17,18].

Notably, it is becoming evident that Gcn2 is implicated in many other biological processes that are seemingly unrelated to the maintenance of amino acid homeostasis (Table 1). Gcn2 has been associated with a range of normal physiological responses as well as with diseases or disorders (Table 1). For example, amino acid catabolising enzymes are used for signalling purposes, such as the Trp-specific Indoleamine-2,3-dioxygenase (IDO) [19]. IDO-expressing cells cause Trp depletion in the immediate cellular environment to trigger responses from neighbouring cells, *e.g.* suppressing T-cell proliferation in a manner that is dependent on Gcn2 function [20]. Gcn2 controls memory formation [21]. Cancer cells depend on Gcn2 for survival and proliferation [22]. Treatment of acute lymphoblastic leukemia with asparaginase (ASNase) is based on the depletion of serum asparagine, thereby depriving cancer cells of this amino acid. In mice, studies suggest that inhibition of Gcn2 may aid in enhancing the efficacy of ASNase and other anticancer drugs that involve deprivation of amino acids [23]. In plants (*Arabidopsis*), Gcn2 was found to respond to wounding, and it is essential for growth in stress conditions [24,25]. In *Caenorhabditis elegans*, hypertonic stress activates Gcn2, and the subsequent reduced protein synthesis is crucial for survival [26]. Also in *C. elegans*, Gcn2 affects the life span under dietary restricting conditions [27]. In the obligatory intracellular parasite *Toxoplasma gondii*, a Gcn2-like kinase promotes the survival of the parasite upon egress from the host cells [28]. Gcn2 may be involved in development, as knock-down of Gcn2 in *Drosophila* embryos inhibits larval development [29]. Differential expression of Gcn2 may also account for some of its biological effects. For example, several tumours have been identified with augmented levels of this kinase (total and active forms) relative to neighbouring normal cells [22]. Gcn2 is also present in high amounts in mouse oocytes [30]. Reports are constantly emerging on new biological roles for Gcn2. These have been the focus of several recent reviews (*e.g.* [3,31–37]), and examples for Gcn2 roles are summarised in Table 1.

Given that Gcn2 activation leads to a dramatic change in the cellular gene expression profile, tight regulation of Gcn2 activity is paramount to the cell and the organism. Several molecules have been reported that modulate Gcn2 function, and additional proteins have been found that again control Gcn2-regulatory proteins, and the list is still growing, suggesting that cells harbour a complex network that keeps Gcn2 in check (Fig. 2). Many of the Gcn2 regulators are highly conserved, from yeast to mammals. This is not surprising given that Gcn2 is implicated in many ubiquitous and fundamental biological functions. However, some Gcn2 regulators may have evolved to control Gcn2 in specific cells or under specific conditions. This review aims to give a comprehensive overview of the knowledge gained so far about molecules and molecular mechanisms regulating Gcn2 activity or activation.

2. Gcn2 domain composition and properties

The Gcn2 protein is composed of (from the N- to the C-terminus) an N-terminal RWD-domain (from its presence in RING finger proteins, WD-repeat-containing proteins, and yeast DEAD-like helicases), a pseudokinase domain with no enzymatic function, the eIF2α kinase catalytic domain, a domain with similarity to histidyl-tRNA synthetases (HisRS-like, is enzymatically inactive) that together with the C-terminus binds tRNAs^{deacyl}, and a C-terminal dimerisation and ribosome binding domain (CTD) [1] (Fig. 3). Adjacent to the RWD domain is a highly charged region [38]. Biochemical and genetic studies suggest that Gcn2 forms dimers or tetramers, and that dimerisation is mediated by a concerted action of the kinase domain, HisRS-like domain, and the CTD (Fig. 3) [39–41]. The latter is more critical for dimerisation *in vivo* [42]. Just recently, the crystal structures of yeast and mouse Gcn2 CTD dimers have been resolved, revealing structural similarities and differences, while their functions seem to be similar [253]. As found for yeast Gcn2, studies suggest that mouse Gcn2 function requires dimerisation of the CTD [42,253].

Gcn2 is held in its inactive state *via* several auto-inhibitory molecular interactions, ensuring that it remains in a latent state until exposed to an activating signal [40,41,43–45] (Fig. 3). Binding of tRNA^{deacyl} to the HisRS-like and CTD domain results in allosteric re-arrangements. This leads to Gcn2 auto-phosphorylation at specific amino acids in the activation loop of the protein kinase domain (Thr-882 and Thr-887 in *S. cerevisiae*, and the corresponding Thr-898 and Thr-903 in mouse Gcn2) [13,15,46,47], to then allow Gcn2 to efficiently phosphorylate its substrate, eIF2α [41,43,44,48,49] (Fig. 3).

Based on common denominators in the structure of the kinase domain of PKR, and the crystal structures of the kinase domain of inactive and constitutively active Gcn2, and on the phenotypes of several

Table 1
Gcn2 is involved in a large array of biological functions not directly related to overcoming nutrient starvation. Listed are examples of Gcn2 implications in organismal functions, diseases, disorders and pathogenicity.

	Organism and function	References
Human/mouse		
Metabolism	Regulates liver lipid metabolism	[222,223]
	Regulates liver gluconeogenesis	[224]
	Determines age-related macronutrient preference	[225]
	Increases insulin sensitivity in dietary restriction	[226]
Immune system	Promotes innate immunity	[227,228]
	Regulates T cell differentiation and proliferation	[20,203,229]
	Protects from autoimmune encephalomyelitis (disease model for multiple sclerosis)	[230]
	Promotes yellow fever vaccine efficacy	[228]
Neurobiology	Required for memory formation	[21]
	Regulates feeding behavior towards amino acid source	[17,18]
	Protects brain from lethal leukodystrophy in amino acid deficiency	[231]
	Contributes to neuronal dysfunction in Alzheimer's disease	[232]
Cancer	Promotes tumor angiogenesis and tumour growth	[22,233]
	Mitigates toxicity of asparaginase anti-cancer treatment	[23]
Viral infections	Host resistance to infection by RNA and DNA viruses	[96,184,186]
Other	Loss-of-function mutations cause familial pulmonary veno-occlusive disease	[234]
	Mediates dietary restriction induced protection from surgical stress	[235]
	Impairs adaptative responses to congestive heart failure	[236]
	Directs autophagy in response to different stress arrangements	[237–239]
	Contributes to redox homeostasis	[240]
	Protects renal cells from high urea concentrations	[241]
	Contributes to cell cycle arrest in response to hypoxia	[242]
Other organisms		
S. cerevisiae	Regulates life span	[243]
	Regulates a G_1/S cell cycle checkpoint in response to DNA damage	[244]
S. pombe	Regulates a G_1/S cell cycle checkpoint in response to UV irradiation	[245,246]
C. elegans	Regulates life span	[27]
D. discoideum	Regulates developmental programs	[144,247].
Drosophila	Confers susceptibility to bacterial infection	[248]
Arabidopsis	Confers resistance to herbicides	[249]
	Confers resistance to stresses, *e.g.*, cold shock, wounding	[24]
Parasites	Promotes extracellular viability of *Toxoplasma gondii*	[28]

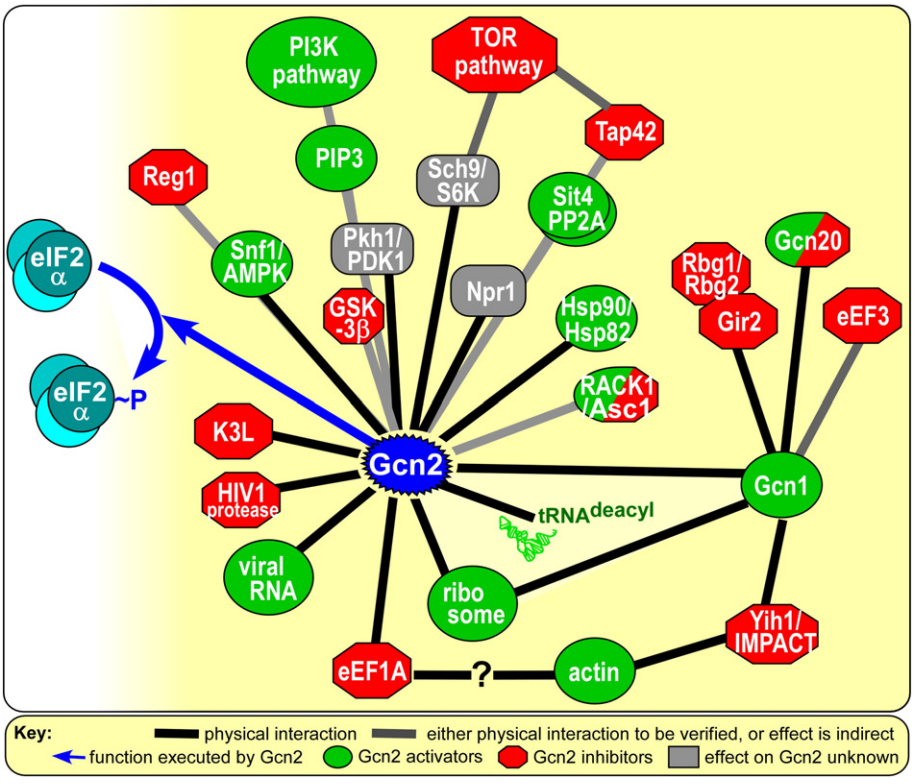

Fig. 2. Gcn2 activity is tightly controlled by a complex network of molecules that directly bind and regulate Gcn2, or that regulate Gcn2 indirectly by interacting with Gcn2-binding proteins, or that control Gcn2 *via* yet unknown mechanisms. For more see text.

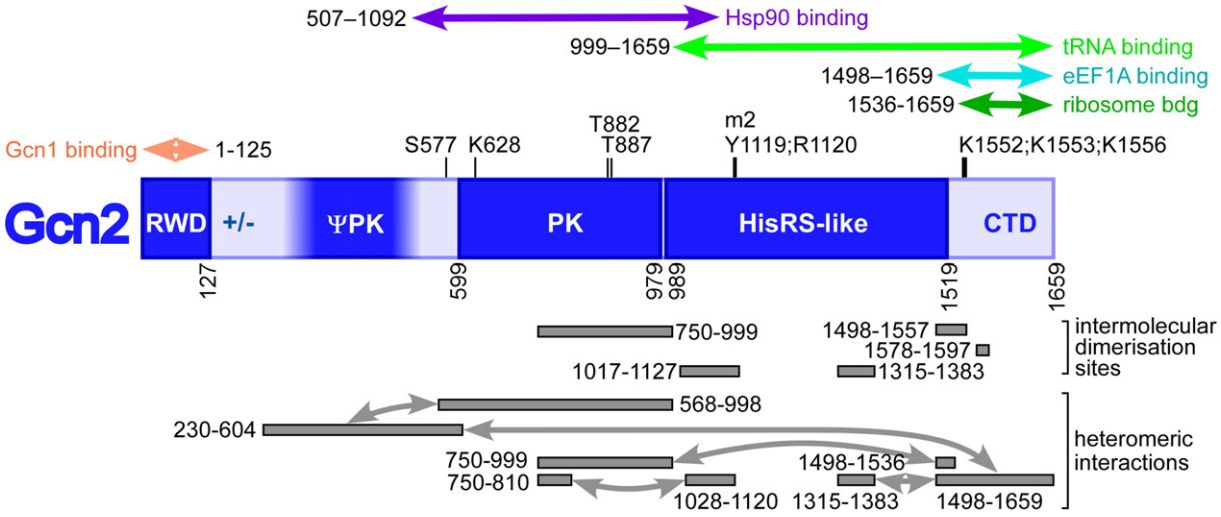

Fig. 3. Schematic representation of the domain arrangement in yeast Gcn2 (blue). From the N- to the C-terminus Gcn2 is composed of the RWD, pseudokinase (ΨPK), protein kinase (PK), HisRS-like, and C-terminal (CTD) domains. Next to the RWD domain is a highly charged region (+/−). Coloured double arrows above Gcn2 indicate areas that are sufficient for binding to Gcn1 [62], Hsp90 [205], tRNA [69], eEF1A [153] and ribosome [70]. Shown below Gcn2 are regions involved in intermolecular Gcn2 dimerisation, each region interacting with the identical region of a second Gcn2 molecule [41]. Areas involved in heteromeric interactions are indicated by grey double arrows [40,41]. Amino acid residues in Gcn2 with known function are shown, such as Ser-577 which reduces Gcn2 affinity to $tRNA^{deacyl}$ when phosphorylated by an unknown kinase [197]. Its dephosphorylation is regulated by the TOR pathway [88]. Lys-628 is crucial for the kinase catalytic activity [60]. Thr-882 and Thr-887 are auto-phosphorylation sites required for Gcn2 activation [46]. Tyr-1119 and Arg-1120 in the m2 motif of the HisRS-like domain are required or tRNA binding [67]. Lys-1552, Lys1553, and Lys-1556 are required for ribosome association and to some extent for $tRNA^{deacyl}$ binding [69,70]. The Gcn2 N-terminal 69 amino acids were uncovered around 1999, and any information on amino acid numbers taken from papers published before that year was adjusted accordingly.

mutants, it was found that in addition to auto-inhibitory interdomain interactions, mechanisms intrinsic to the kinase domain keep Gcn2 in its latent form. Within the kinase domain, the catalytic site is located in a cleft between the N- and C-terminal lobes that are connected by a hinge region. In the inactive state, hinge rigidity, a closed conformation of the lobes, distorted conformation of amino acid residues necessary for *e.g.* binding the ATP triphosphate moiety, and a flap located over the ATP binding pocket, prevent ATP and eIF2α binding and catalysis. Activation of the kinase is achieved by the re-arrangement of interactions between specific amino acid residues. Thereby, the hinge region loses its rigidity to allow inter-lobe flexibility and widening of the catalytic cleft, proper positioning of key residues, and removal of the 'gatekeeper' flap. ATP entering the catalytic site allows auto-phosphorylation of Gcn2 to then 'lock' it into its open active state. For more details on the proposed mechanism of Gcn2 autoinhibition and activation upon $tRNA^{deacyl}$ binding we refer to [41,43–45,48,49].

Upon kinase activation, substrate binding is allowed. The structure of eIF2α bound to PKR, and mutational and kinetic studies, revealed that phosphorylation of eIF2α is dependent on a conformational change of the region encompassing eIF2α Ser-51 which in the isolated protein is buried in a hydrophobic pocket [50,51]. A model is proposed in which upon binding to the kinase, an induced fit mechanism leads to the unfolding of the region carrying Ser-51, thereby exposing Ser-51 and allowing this phospho-acceptor to sufficiently project into the active site of the kinase. This mechanism ensures that Ser-51 in eIF2α is not phosphorylated by other kinases *in vivo* [50,51].

Substrate recognition by Gcn2, as well as by the other eIF2α kinases, is exquisitely dependent on residues far from the Ser-51 phosphorylation site, as indicated by extensive mutational studies, as well as by the structural determination of PKR-bound eIF2α [50,52]. Protein database searches revealed that only eIF2α (and its viral mimetics—Section 11) possesses these required amino acids to accommodate the appropriate folding for binding to eIF2α kinases [50,52]. It should be noted that, *in vitro*, PKR and HRI, and likely all eIF2α kinases, can phosphorylate other substrates, for example histone and an eIF2α peptide containing Ser-51, however with very low efficiency compared with intact eIF2α [53–55].

The pseudokinase domain is required *in vivo* [56] and *in vitro* [57] for Gcn2 kinase activity. This domain shows sequence similarity to multiple subdomains of eukaryotic Ser/Thr protein kinases but residues critical for enzyme function are lacking [57,58]. It was proposed that pseudokinase domains have a regulatory function by directly binding to the functional kinase domain [59]. The Gcn2 pseudokinase domain interacts with the kinase domain, raising the possibility that this interaction contributes to inhibiting the catalytic activity of the kinase domain under non-starvation conditions [40]. Furthermore, mutational studies suggest that the pseudokinase domain has a stimulatory role under amino acid starvation conditions [60].

Gcn2, through its RWD domain, must directly bind to its effector protein Gcn1 to sense amino acid starvation *in vivo* [61]. The minimal region in Gcn2 sufficient for Gcn1 binding encompasses amino acids 1–125 [62]. An independent study showed that Gcn1 binds to $Gcn2^{1–272}$ *in vitro*, but not to $Gcn2^{1–110}$ ($Gcn2^{1–110}$ lacks amino acid(s) proposed to contact Gcn1 [38,63,64]). Together with the fact that *in vivo* Gcn2 areas 10–109 and 110–235 are equally required for Gcn1 binding [38], this suggests that Gcn2 harbours Gcn1 binding activities upstream and downstream of amino acid 110.

The RWD domain of Gcn2 is related to the ubiquitin-conjugating enzymes (UBC) domain, forming the clade of Ubiquitin-conjugating enzyme/RWD-like domain (InterPro IPR016135, [65]), predicted to have a function in protein–protein interaction [66]. RWD domains including the Gcn2 RWD domain, however, lack the catalytic cysteine residue critical for ubiquitin-conjugating activity [66]. The structure of the GCN2 RWD domain has been solved by NMR [63]. The invariant motif YP*xxx*(*x*)P forms a triple β-turn that is unique to the RWD, UBC, and the Ubiquitin E2 variants (UEV). These residues maintain an internal hydrogen bond network shown to be essential for the structural conformation of this domain [63]). Discriminating the RWD domain from UEV and UBC is the helix α2, also found in Gcn2 [63]. Instead of this helix the UBC and UEV contain a long extended stretch of residues, which in UBC encompasses the catalytic Cys residue.

3. tRNAs

Northwestern assays showed that the Gcn2 HisRS-like domain interacts with $tRNAs^{deacyl}$ [67]. Class II synthetases, including histidyl-tRNA synthetases, contain a so-called motif 2 that is involved in binding the tRNA acceptor stem [68]. Residue substitutions in motif 2 of the HisRS-like domain (m2 mutation, Y1119L;R1120L) severely reduce

Gcn2 affinity to tRNAs *in vitro*, and the Gcn2-m2 mutant protein does not or hardly phosphorylate eIF2α *in vivo* or *in vitro*, providing the first evidence that tRNA binding to Gcn2 leads to Gcn2 activation [57,67]. Supporting the idea that increased pools of $tRNA^{deacyl}$ trigger Gcn2 activation *in vivo*, a temperature sensitive mutation in the HisRS gene, *hts1-1*, which reduces the levels of charged $tRNA^{His}$ under semipermissive conditions, leads to Gcn2 stimulation [67].

Gel shift experiments suggest that purified Gcn2 preferentially binds any $tRNA^{deacyl}$, rather than amino acylated tRNAs [69]. These studies also showed that for binding $tRNAs^{deacyl}$ with high affinity, both the HisRS and the CTD are necessary and sufficient [69]. Three highly conserved Lysine residues located in a predicted amphipathic α-helix in the CTD and known to be involved in ribosome binding (*K1552*, *K1553*, *K1556*; Section 5), were shown to be essential for $tRNA^{deacyl}$ binding, as well as for Gcn2 activation in response the amino acid starvation [69,70]. This suggests that Gcn2 contains a second tRNA binding site, located in the CTD, and/or that the CTD promotes dimerisation of the HisRS-like region which may be required for $tRNA^{deacyl}$ binding [41,69,71]. Supporting the latter, dimerisation of the N-terminal part of the HisRS-like domain is required for tRNA binding, while tRNA binding is not required for dimerisation [41]. Importantly, studies suggest that $tRNA^{deacyl}$ interacting with the bipartite binding module in Gcn2 neutralises the autoinhibitory interaction between the protein kinase domain and the CTD [41,69]. This would be in agreement with a model in which $tRNA^{deacyl}$ binding to Gcn2 contributes to its stimulation.

A sophisticated microarray-based approach for measuring genome-wide changes in tRNA charging in the cell provided *in vivo* support for the idea that any type of $tRNA^{deacyl}$ contributes to stimulating Gcn2 [72]. This method revealed that in *S. cerevisiae* the level of Gcn2 activation by amino acid starvation is directly correlated with the levels of cognate $tRNAs^{deacyl}$. Interestingly, in auxotrophic strains starved for an essential amino acid, in addition to the increase in cognate $tRNAs^{deacyl}$, other tRNAs become deacylated, even though the cellular levels of these non-starved amino acids do not decrease. Thus, mechanisms other than reduced amino acid availability can lead to deacylation of non-cognate tRNAs [72]. Contributors to this phenomenon may be the interconnection between synthesis and catabolism of certain amino acids, compartmentalisation of amino acids and/or tRNAs, tRNA synthetases influencing each other's activities, alteration in the activity of trans-editing proteins that can deacylate tRNAs, or the susceptibility of less abundant tRNAs to deacylation [72].

The mechanism of $tRNA^{deacyl}$-mediated Gcn2 activation is conserved in other organisms. For example, *Arabidopsis* Gcn2 was shown to bind $tRNAs^{deacyl}$ and to phosphorylate *Arabidopsis* eIF2α [73]. In mice, inhibition of tRNA aminoacylation by administering alcohol derivatives of amino acids directly into the anterior piriform cortex results in Gcn2-dependent increased eIF2α phosphorylation in the same brain area [18]. Halofuginone, a component of ancient Chinese medicine used to ameliorate inflammatory phenotypes, inhibits prolil-tRNA synthetase activity, and this correlates with Gcn2 activation [74,75]. Treatment of mammalian cells with borrelidin, a macrolide that inhibits the threonyl-tRNA synthetase, leads to Gcn2 activation [76].

Many other conditions that seem at first to be unrelated to amino acid starvation result in Gcn2 activation *in vivo*. However, for numerous of these stress arrangements there is evidence that the final signal does seem to be the accumulation of $tRNAs^{deacyl}$. These include starvation for nutrients other than amino acids. In yeast, glucose starvation triggers Gcn2 activation that is dependent on the m2 motif in the HisRS-like domain, indicating that Gcn2 must bind $tRNAs^{deacyl}$ for activation. It was suggested that usage of amino acids as alternative carbon source, and/or as secondary energy source, results in amino acid shortage. In line with this idea, a decrease in the cytoplasmic pool of amino acids was observed upon glucose starvation, consistent with the idea that accumulation of $tRNAs^{deacyl}$ stimulates Gcn2 [77]. Activation of yeast Gcn2 by purine starvation may be related to extensive modifications that occur in tRNAs [78]. For example, it has been shown that the post-transcriptional addition of a guanine residue to the 5′ end of $tRNA^{His}$ is required for its aminoacylation, and depletion of the corresponding modifying enzyme has been shown to activate GAAC in a Gcn2-dependent fashion [79]. Hence, the low availability of purines may increase the pool of non-chargable $tRNAs^{His}$ that stimulate Gcn2. Of course, other mechanisms cannot be excluded, such as reduced adenine availability leading to reduced ATP levels, that would impair amino acylation [78].

Stress regimens other than starvation also appear to involve $tRNA^{deacyl}$ for stimulating Gcn2. For example, acidic stress conditions in yeast results in accumulation of uncharged $tRNAs^{Leu}$ even though the intracellular Leu pool is not depleted [80]. It was suggested that this is due to the inhibition of the aminoacyl-tRNA synthetase. High salinity leads to a transient increase in $tRNAs^{deacyl}$ [72]. Activation of Gcn2 by boron treatment requires motif 2 in the HisRS-like domain, and boron inhibits charging of $tRNA^{Ser}$ and $tRNA^{Phe}$ by rabbit reticulocyte synthetases [81].

In mammals, UV-irradiation leads to Gcn2 activation [82]. Since no evidence was found for tRNA deacylation in UV-irradiated mammalian cells, the authors suggested that UV-light mediated crosslinking of tRNAs to Gcn2 may activate Gcn2. Studies by others suggest that Gcn2 activation by UV in mammalian cells may stem from the rapid consumption of Arg to produce nitric oxide, a process catalyzed by nitric oxide synthase (NOS) [83]. Interestingly, in mammals methionyl-tRNA synthetase (MRS) was reported to be a substrate of UV irradiation-stimulated Gcn2 [84]. Gcn2 appears to phosphorylate Ser-662 in MRS's tRNA binding domain. Those studies suggested that Ser-662 phosphorylation reduces $tRNA^{Met}$ binding to MRS, and as expected, UV irradiation decreases the levels of charged Met-$tRNA^{Met}$ *in vivo* and reduces global protein synthesis.

Intracellular events seemingly unrelated to tRNA charging appear to lead to Gcn2 activation. Methylglyoxal, an endogenous metabolite derived from glycolysis that in high concentrations is deleterious to the cell, activates Gcn2. No increase in the levels of uncharged $tRNA^{Met}$ was detected. It remains to be determined whether this compound increases the levels of other $tRNAs^{deacyl}$ [85–87]. Rapamycin, a drug that inhibits the kinase Tor, activates Gcn2 but no increase was observed in the two specific uncharged tRNAs studied ($tRNA^{His}$ and $tRNA^{Met}$) [87–89]. However, it is possible that the charging of other tRNAs may be affected. Supporting this idea, cells grown on the poor nitrogen source γ-aminobutyric acid, which deactivates the Tor signaling pathway, show increased levels of uncharged $tRNA^{Cys}$ and $tRNA^{Phe}$ [11]. Apart from that, rapamycin-mediated Tor inhibition increases the affinity of Gcn2 for $tRNA^{deacyl}$ [88] (see Section 12).

Other intracellular events may impair tRNA charging by affecting amino acid supply. For example, invasion of host cells by pathogenic bacteria (*Shigella*, *Salmonella*, *Listeria*) triggers Gcn2 activation, and this was associated with decreased levels of cellular amino acids due to membrane leakage [90,91]. In yeast, deletion of *GCN1* or *GCN2* renders cells sensitive to the tubulin depolymerizing agent benomyl, indicating that microtubule disassembly leads to Gcn2 activation [92]. Considering that tubulin is involved in various processes in the cell such as in intracellular transport [93], benomyl may affect, directly or indirectly, the intracellular localisation of amino acids, tRNAs, or tRNA synthetases, or the trafficking of amino acid transporters (especially considering that strains used in this experiment have amino acid auxotrophies). Thus, tubulin depolymerisation may impair the efficiency of tRNA charging.

Activation of Gcn2 by proteasome inhibition [94] appears to be due to the intracellular reduction of the free amino acid pool, in yeast and mammalian cells [95]. In *S. cerevisiae*, the genetic impairment of proteasome function by using the thermo-sensitive regulatory subunit Rpt6 (*cim3-1* mutant) results in decreased free pools of most amino acids, blocking protein synthesis and leading to lethality even in rich medium. Addition of all amino acids to the rich medium rescues protein synthesis

Table 2
Components required for Gcn2 activation in response to specific activating signals in *Saccharomyces cerevisiae*. For each cue is listed the phenotype conferred by Gcn2 (comparing the growth behaviour between a wild-type and *gcn2Δ* strain), whether Gcn2 activation requires Gcn2 dimerisation, Gcn2–ribosome interaction, the presence of Gcn1 or Gcn20, whether the cue leads to increased tRNAs^{deacyl} levels, whether the Gcn2 m2 sequence is required, and whether increased eIF2α phosphorylation (eIF2α-P) was observed. The activating cues are amino acid starvation, 8-azaadenine (purine starvation), Saline stress (excess NaCl), glucose starvation, H_2O_2 (oxidative stress), Acetic acid (acidic stress), Boron, Methyl Glyoxal, Rapamycin, Tunicamycin, Methyl methanesulfonate (DNA damage), Hydoxyurea, Heterologous gene expression, Benomyl (Microtubule depolymerisation), Methionine-S-sulfoximine (Gln/Nitrogen starvation). (—) No phenotype was found or reported.

Stress	Phenotype	Gcn2 dimerisation	Gcn2–ribosome binding	Gcn1 required	Gcn20 required	tRNAs^{deacyl} increased	Gcn2 m2 required	increased eIF2α-P	reference
Amino acid starvation	Resistance	Yes	Yes	Yes	Yes	Yes	Yes	Yes	See text
8-azaadenine	Resistance			Yes				Yes	[78]
				Yes	Yes		Yes	Yes	[77]
Excess NaCl	Sensitivity			Yes			Yes	Yes	[250]
		Yes		Yes	Yes		Yes	Yes	[42]
						Yes		Yes	[72]
Glucose starvation	–	Yes	Partially	Yes	Partially		Yes	Yes	[77]
H_2O_2	Resistance						Yes	Yes	[251]
Acetic acid	Resistance			Yes	Partially	Yes		Yes	[80]
Boron	Resistance					Yes	Yes	Yes	[81]
Methyl Glyoxal	Resistance			Yes				Yes	[86]
				Yes		No (tRNA^{Met})		Yes	[87]
Rapamycin	Sensitivity						Yes	Yes	[88]
				Yes		No (tRNA^{His})	Yes	Yes	[89]
		Yes		Yes	Yes		Yes	Yes	[42]
				After 30 min		No (tRNA^{Met})		Yes	[87]
Tunicamycin	–							Yes	[88]
Methyl methanesulfonate	–			Yes	Yes		Yes		[10]
								Yes	[88]
Hydoxyurea	–							Yes	[88]
Heterologous gene expr.	–			Yes	Yes		Yes	Yes	[252]
Benomyl	Resistance			Yes					[92]
Methionine-S-sulfoximine	Resistance							Yes	[165]
				Yes					[92]

and the viability of these *cim3-1* cells. In mammalian cells, pharmacological inhibition of proteasome activity by MG132 or Bortezomib causes decreased levels of free cysteine, asparagine and aspartate, and Gcn2 activation. Again, the addition of these amino acids to the medium reduces Gcn2 activation and rescues cells from death induced by MG-132. Thus, blocking proteasome function results in tRNAs^{deacyl} accumulation due to the lowered pool of cytoplasmic free amino acids, leading to Gcn2 activation [95].

Although for many stress conditions the levels of tRNAs^{deacyl} were not studied, the observations that the ensuing Gcn2 activity was dependent on the m2 sequence in the Gcn2 HisRS-like domain suggest that tRNA^{deacyl} is the direct activating signal (Table 2). However, one cannot exclude the possibility that Gcn2 requires co-activating ligands in addition to tRNA^{deacyl}, or that the m2 sequence is required for recognizing signals other than tRNA^{deacyl}, directly or indirectly. For example, some viral RNAs seem to promote Gcn2 activation in lieu of tRNAs^{deacyl}, as described in Section 11 [96].

In summary, for most situations that result in Gcn2 activation, the levels of tRNAs^{deacyl} are increased. Thus, any interference with the tRNA charging pathway should modulate Gcn2 activity. In this regard, it is interesting that mutations in the catalytic domain of several tRNA synthetases have been identified in human pathologies [97]. It is tempting to speculate that the severity of these diseases may be due in part to increased levels of ATF4 as a result of Gcn2 activation.

4. Ribosomes

Activation of Gcn2 *in vivo* requires its association with ribosomes as determined by studies in yeast [98]. Gcn2 is loosely associated with ribosomes rather than an integral component, and it appears to be in a dynamic equilibrium between its free and ribosome bound forms [98]. The majority of Gcn2 co-migrates with 80S ribosomes (monosomes) and with ribosomes actively engaged in translation (polysomes) in co-sedimentation assays using sucrose gradients, where Gcn2 was identified by its *in vitro* auto-phosphorylation activity (the kinase activity seemed to accurately measure the steady-state protein levels [70]). Co-migration assays using both native gel electrophoresis and sucrose gradients indicated that Gcn2 has strong affinity for the 60S subunit but not for 40S [98]. Under conditions that result in the accumulation of pre-initiation complexes (43S–48S), Gcn2 appears to also associate with these complexes, raising the possibility that this would facilitate a direct access to its substrate, eIF2α. Gcn2 kinase activity, its ability to bind tRNAs^{deacyl}, and the dimerisation activity in the CTD, are not necessary for Gcn2–ribosome association [42,70].

The Gcn2 CTD (amino acids 1536–1659) is sufficient and essential for binding to the 60S subunit and for polysome association, and necessary for overcoming amino acid starvation [56,70,98]. However, deletions of other parts in Gcn2 reduce Gcn2–ribosome co-migration to a small extent (N-terminus, portions of the HisRS-like domain), indicating that Gcn2 may have additional (weak) ribosome binding sites, or that Gcn2–ribosome association may be strengthened by other molecules that bind to ribosomes, *e.g.* Gcn1 [98].

Does Gcn2–ribosome interaction change upon sensing amino acid starvation? The three highly conserved Lys residues in the Gcn2 CTD (*K1552*, *K1553*, *K1556*) required for tRNA^{deacyl} binding are also essential for strong polysome and 60S association as determined by co-migration on sucrose gradients [69,70]. Under conditions that dissociate polysomes and monosomes into 40S and 60S subunits, however, the Lys residues are not absolutely required for Gcn2–60S association, but deletion of 20 amino acids encompassing these Lys residues nearly abolishes this interaction [69]. This raises the possibility that Gcn2 may utilise different subsets of binding determinants in the CTD for association either to translating ribosomes or to subunits. The observation that the Lys residues are required not only for ribosome-binding, but also for tRNA-binding, may indicate that these are mutually exclusive interactions [69,70]. If that is the case, then Gcn2 would dissociate off the ribosome upon tRNA^{deacyl} detection. Arguing against this scenario, other studies suggest that amino acid starvation does not significantly affect the steady-state ribosome-association of Gcn2, Gcn1 or Gcn20, or Gcn1–Gcn2 interaction [38,98,99]. Shedding light on this aspect is critical for

understanding Gcn2 activation and where it accesses its substrate, especially considering that the above assays are steady-state measurements.

The fact that ribosomal association is required for Gcn2 activation *in vivo* under amino acid deprivation supports the idea that Gcn2 does not simply monitor the cytoplasmic $tRNA^{deacyl}$ levels. Instead, it has been proposed first by Ramirez et al. [98] that a mechanism is in place that directs ribosomal A-site associated $tRNAs^{deacyl}$ to Gcn2 for their detection. This finds precedent in bacteria, where $tRNA^{deacyl}$ binding to the ribosomal A-site during amino acid starvation triggers the stringent response mediated by the RelA protein (see Section 7). Recently it was reported that acidic ribosomal proteins, in their non-ribosomal bound form, are required for Gcn2 activation under glucose starvation or high salinity stress (excess NaCl), but not amino acid starvation [100]. It remains to be verified whether these acidic proteins promote Gcn2 activation directly. Mapping the Gcn2-binding site on the ribosome will bring clues about the mechanism by which tRNAs are transferred to the Gcn2 HisRS-like domain to stimulate the adjacent kinase domain. Even though mouse Gcn2 appears to not associate with ribosomes as strongly as yeast Gcn2, this association may be sufficient for sensing the A-site associated $tRNA^{deacyl}$ [253].

5. Gcn1 and Gcn20

Gcn1 was the first protein found to promote Gcn2 function. A yeast strain deleted for the Gcn1 coding gene (*gcn1Δ* strain) is unable to activate Gcn2 upon amino acid starvation. Gcn2 kinase activity however, can be detected in the whole cell extract of a *gcn1Δ* strain, suggesting that Gcn1 is not required for the kinase activity *per se*, but for *in vivo* activation of Gcn2 in response to amino acid starvation [101]. Interestingly, mutations rendering Gcn2 constitutively active ($Gcn2^c$) still require Gcn1, with the exception of one $Gcn2^c$ allele that simultaneously does not require $tRNAs^{deacyl}$ for its constitutive activity [49,102]. All *in vivo* data in yeast support the idea that Gcn1 is absolutely required for Gcn2 to detect $tRNAs^{deacyl}$.

Gcn1 is a 2672 amino acids long protein and its middle portion (amino acids 1330–1641) has homology to the N-terminal HEAT repeat domain of fungal translation elongation factor 3 (eEF3) (Fig. 4) [101]. Some homology to eEF3 can be still detected beyond residue 1641. HEAT stands for the repeats first found in the proteins Huntingtin, eEF3, protein phosphatase 2 A, and Tor [103]. eEF3 is an ATPase that binds to ribosomes near the E-site, and studies suggest that its function is to promote the release of $tRNAs^{deacyl}$ from the E-site [104–106]. Except of the eEF3 similarity, Gcn1 has no significant homologies to any other protein [101].

In vitro and *in vivo* binding assays conducted by two independent research groups revealed that Gcn1 binds to the Gcn2 RWD domain [38,62], in particular the interaction is mediated by a region in Gcn1 encompassing amino acids 2052–2428 [61] or 2048–2383 [62]. Overexpression of the Gcn2 RWD domain or the $Gcn1^{2052–2428}$ fragment impaired cell growth under starvation conditions [38,61,62], and this was associated with reduced eIF2α phosphorylation [38,61], suggesting that Gcn1–Gcn2 interaction through these regions is required for Gcn2 activation. Compelling evidence for Gcn2 function depending on a *direct* Gcn1–Gcn2 interaction was provided by the amino acid substitution at Arg-2259 in Gcn1 by an alanine residue [61]. R2259A substitution in full length Gcn1 abolishes Gcn2 activation, and this can be reverted by overexpressing Gcn2. Arg-2259 is essential for Gcn2 binding *in vivo* and for direct Gcn2 binding *in vitro*, but not for binding to other known Gcn1 partners *in vivo*, such as Gcn20 or ribosomes [61]. Furthermore, the impairment of Gcn2 function *in vivo* by overexpression of $Gcn1^{2052–2428}$ is dependent on Arg-2259. Mutations in charged amino acids located in near proximity to Arg-2259 have no effect on Gcn2–Gcn1 interaction, *in vivo* or in *in vitro*. Additional Gcn1 mutations were found that impair Gcn1–Gcn2 interaction as judged by yeast 2-hybrid assays, all located C-terminal to R2259 (residues 2280–2370), and the F2291L substitution was shown to lead to reduced eIF2α phosphorylation [107]. The effect of this mutation on other Gcn1 functions has not been analysed yet, but most likely these are not affected.

Gcn1 may contain additional Gcn2 binding sites. For example, Gcn1 proteins lacking either one of its termini (residues 4–671 or 2476–2672) seem to have reduced Gcn2 affinity *in vivo* as judged in co-precipitation assays [61]. *In vivo*, Gcn2 activation is impaired if cells harbour one or the other Gcn1-truncation protein, and this can be rescued by Gcn2 overexpression, in contrast to a Gcn1 protein lacking residues 2052–2428. This suggests that 2052–2428 harbour the major Gcn2 binding determinant, while the others are minor as Gcn2 function can be rescued by driving the interaction with Gcn2 overexpression and thus *via* mass action [61]. It needs to be taken into consideration that the N-terminal deletion also affects Gcn1–ribosome interaction. However, a purified Gcn1 fragment encompassing the N-terminal 992 amino acids co-precipitates Gcn2 from whole cell extract derived from a *gcn1Δ* yeast strain. Conversely, apart from its RWD domain, Gcn2 may contain an additional Gcn1 binding site, because *in vitro* a GST-tagged Gcn2 fragment encompassing only the HisRS-like domain appears to co-precipitate more Gcn1 (and more of the Gcn1 binding protein Gcn20) than GST alone [38]. It remains to be determined whether these 'minor' interactions are direct or mediated by another molecule or the ribosome.

Gcn1 interacts with translating ribosomes through the region comprised of amino acids 1–2052, thus involving the majority of the Gcn1 protein [61] (Fig. 4). Mutations in physically distinct areas within this region, called M7 and M1, reduce Gcn1–ribosome association, but not Gcn1–Gcn20 interaction, and this is associated with impaired Gcn2 activation under amino acid starvation conditions, suggesting that Gcn1–ribosome interaction is required for promoting Gcn2 activation [108]. Area M1, constituting the amino acid sequence E*xx*WRTKR, is located in the eEF3-like region, while area M7, 12 basic residues in a 42 residue stretch, is N-terminal to the eEF3-like region (Fig. 4). The N-terminal HEAT repeat domain of eEF3 binds to the 40S subunit [105,109], and the similarity of Gcn1 with the eEF3 HEAT repeat domain raises the possibility that Gcn1 may also bind to the 40S subunit.

Interestingly, Gcn1 overexpression leads to a growth defect and increased Gcn1–ribosome interaction, implying that an essential cellular function is affected such as protein synthesis [61]. Gcn1 overexpression also leads to sensitivity to the drug paromomycin, while strains lacking Gcn1 are resistant to paromomycin as compared to the wild-type control. Together with the fact that the ribosome binding property of Gcn1 truncation proteins correlates with paromomycin sensitivity [61], this indicates that Gcn1 elicits paromomycin sensitivity while bound to the ribosome. As paromomycin is known to increase the error rate of translation by interfering with the anticodon recognition of the tRNA in the A-site [110], these observations suggest that Gcn1 binds close to the A-site or is able to affect A-site function, directly or indirectly.

In keeping with the essential nature of Gcn1 for Gcn2 function, in yeast Gcn1 seems to be required in all stress arrangements that activate Gcn2 (Table 2). Gcn1 Arg-2259 found in *S. cerevisiae* to be specifically required for Gcn1–Gcn2 interaction and Gcn2 activation, and the amino acids immediately neighbouring this residue, are highly conserved, conforming to the consensus sequence ITGPLIR[bulky hydrophobic]$_2$G[negatively charged]RF [61,111]. The biological relevance of Gcn1 function in the activation of Gcn2 *in vivo* has already been discovered in other organisms.

In mammals, Gcn1 contains an amino acid equivalent to Arg-2259, with the surrounding sequences being evolutionary conserved [111]. In mammalian cells, Gcn1 complexes with Gcn2 as shown by co-immunoprecipitation experiments [92], and both proteins interact with translating ribosomes [112]. Furthermore, in mammalian cells, overexpression of a mammalian Gcn1 fragment equivalent to yeast $Gcn1^{2052–2428}$ impairs Gcn2 function under amino acid deprivation or proteasome inhibition (Section 4) [92]. Similarly, overexpression of IMPACT, a protein proposed to disrupt Gcn1–Gcn2 interaction (Section 9), impairs Gcn2 activation under amino acid deprivation, UV stress, glucose starvation, and proteasome inhibition [92].

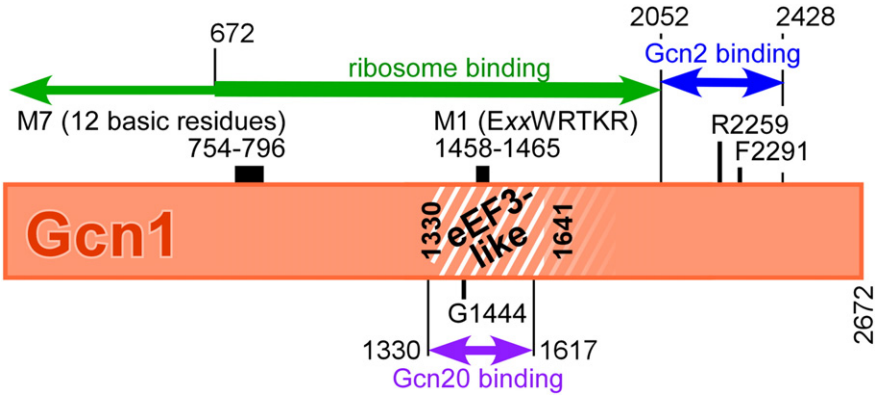

Fig. 4. Schematic representation of yeast Gcn1. The Gcn1 middle portion has homology to the eEF3 N-terminus (hatched) [101]. Areas indicated with double arrows are required for ribosome association [61], sufficient for Gcn20 binding [99], and required and sufficient for Gcn2 binding [61]. Amino acids are shown for which a biological function was discovered. 12 basic residues (dubbed area M7) and the sequence motif E*xx*WRTKR (dubbed area M1) are required for efficient ribosome binding [108]. Gly-1444 is required for Gcn20 binding [99]. Arg-2259 is specifically required for Gcn2 binding *in vitro* and *in vivo* [61]. Phe-2291 is required for Gcn2 binding *in vivo* [107]. For more see text.

In *C. elegans* hypertonic stress activates Gcn2 in a Gcn1-dependent manner [26]. Gcn1 is also involved in *C. elegans* morphogenesis. Loss-of-function mutations in Gcn1 or PERK, independently, suppress the defect of semaphorin mutants. Semaphorin-mediated signaling governs ray morphogenesis in the male tail, by reducing eIF2α phosphorylation [113]. Interestingly, however, knock-down of Gcn2 in semaphorin mutants leads to reduced eIF2α phosphorylation in the whole organism, but not in the ray precursor cells and this correlates with unaffected ray morphogenesis. It is possible that Gcn2 is not sufficiently knocked down in the ray precursor cells, or that Gcn1 has a Gcn2-independent role in this differentiation process.

Gcn1 is involved in embryogenesis in *Arabidopsis*, for which it was initially named after the Greek goddess of childbirth, ILITHYIA [114], and it is required for plant fertility and immunity against *Pseudomonas syringae* infections [115]. AtGcn1 has homology with eEF3 as found for scGcn1. Also, *At*Gcn1 contains an amino acid equivalent to *Sc*Arg-2259 (here Arg-2348), within the consensus sequence ITGPLIR[bulky hydrophobic]$_2$G[negatively charged]RF, which would support the idea that *Arabidopsis* Gcn1 interacts with Gcn2 [111]. The involvement of *At*Gcn2 in the above processes still remains to be verified.

Gcn1 may bind more proteins than those known so far, since it contains >20 HEAT repeats distributed throughout its length [103]. HEAT repeats are proposed to serve as interaction sites for other proteins and nucleic acids, suggesting that Gcn1 is a scaffold protein [103]. Despite its size and the presence of multiple HEAT repeats, large scale interaction studies did not consistently reveal any Gcn1-interactors, not even the known Gcn1-binding partners (Gcn2, Gcn20, Gir2, Yih1) (*e.g.* [116–119]). Thus, it is possible that protein–protein interactions with Gcn1 are too weak or transient to withstand the experimental procedures employed in these large scale studies.

Gcn1 forms a complex with Gcn20. Gcn20, or the Gcn1–Gcn20 interaction, promotes, but is not absolutely necessary for Gcn2 activation *in vivo* [99,120]. The Gcn20 C-terminus has homology to the C-terminus of eEF3 including the ATP binding cassettes (ABC) [120]. Both proteins belong to a subfamily of the ABC proteins that harbor twin ABC cassettes, but unlike other subfamilies they lack transmembrane domains [121].

The N-terminus of Gcn20 (residues 1–189) binds to the eEF3-like region in Gcn1 (amino acids 1330–1617), and this interaction is mediated by Gly-1444 in the eEF3-like region [99,120]. Gcn20 has an intrinsic ribosome binding activity, albeit weak, as detected by polysome co-sedimentation assays [99]. In the presence of ATP, the Gcn1–Gcn20 complex has a higher affinity for ribosomes than either protein alone, and this effect is predominantly mediated by the Gcn20 C-terminus, suggesting that Gcn20 promotes Gcn1/20–ribosome interaction [99]. Interestingly, in the absence of ATP, Gcn20 negatively regulates Gcn1–ribosome interaction [99]. Seemingly at odds with the co-sedimentation studies, the Gcn20 C-terminus is largely dispensable for promoting Gcn2 activation *in vivo* [99]. Thus, ATP mediated increased Gcn1–polysome interaction is dispensable for sensing amino acid starvation. It was proposed that the Gcn20 C-terminus modulates Gcn1–ribosome interaction under certain conditions in order to fine-tune Gcn2 activation.

A functional homologue of yeast Gcn20 has not yet been identified in humans, but considering the conservation of the Gcn2 regulatory module it is very likely that it does exist. The mammalian ABC-containing protein ABC50 described to participate in translation initiation, has some similarity with Gcn20 (20% identity and 30% similarity between their N-terminal region), but it cannot substitute for Gcn20 function in yeast [122]. However, as one cannot exclude the possibility of evolutionary divergence, it remains to be tested whether ABC50 interacts with mammalian Gcn1 [122].

In *Plasmodium falciparum* a homologue of Gcn20 was identified (PfGcn20), which complements a yeast *gcn20Δ* strain [123,124]. Interestingly, in certain developmental stages PfGcn20 is secreted into the infected erythrocyte [125]. It will be interesting to assess whether PfGcn20 participates in the regulation of Gcn2 in these parasites [126].

6. Working model for Gcn2 signal sensing

The evidence gathered thus far from work in *S. cerevisiae* has resulted in a working model of how Gcn2 detects tRNA^{deacyl} under amino acid starvation, as depicted in Fig. 5A [61,98,99]. Gcn1 and Gcn2 form a trimeric complex with the ribosome. Considering that Gcn20 associates with Gcn1, it is possible that Gcn20 is an integral part of this complex (Section 6), however, for simplicity, and as Gcn20 is necessary but not essential for Gcn2 activation, Gcn20 will not be further mentioned in this section. For eukaryotic ribosomes it was shown that tRNA^{deacyl} can enter the A-site in a codon-dependent manner *in vitro* [127]. When tRNAs^{deacyl} accumulate in the cell, the A-site of translating ribosomes may accommodate a cognate tRNA^{deacyl}, in particular when the cognate charged tRNA is scarce (Fig. 5A, step 1). This tRNA^{deacyl} is then transferred from the A-site to Gcn2 (Fig. 5A, step 2). The tRNA^{deacyl} entering the A-site prior to its detection by Gcn2 ensures that Gcn2 detects this starvation signal rather than tRNA^{deacyl} naturally occurring during the translational process. Gcn1 may function by delivering, or facilitating the entry of, tRNA^{deacyl} into the A-site, or by transferring tRNA^{deacyl} from the A-site to Gcn2. Alternatively, it is possible that Gcn1 is a scaffold protein that positions Gcn2 on the ribosome in such a way that tRNAs^{deacyl} can be transferred directly from the A-site to

Gcn2. In agreement with the idea that Gcn2 activation by tRNA^{deacyl} requires Gcn1 to deliver it to Gcn2, mutations were found in the Gcn2 kinase domain (R794G; F842L) that render it constitutively active *in vivo*, bypassing the requirement for ribosome as well as for tRNA^{deacyl} binding, and simultaneously bypassing the requirement for Gcn1 [49].

The hypothesis that Gcn2 is activated by an A-site bound cognate tRNA^{deacyl} is based largely on the bacterial RelA protein. RelA governs the 'stringent response' in which amino acid-starvation leads to the synthesis of guanosine tetra- and pentaphosphate ((p)ppGpp), an alarmone that mediates the regulation of a large number of adaptive responses [128–130]. In bacteria, *in vitro* studies suggest that the increase in tRNAs^{deacyl} as a result of amino acid starvation facilitates entry of tRNA^{deacyl} in the ribosomal A-site. *In vitro* data support a model in which cognate tRNAs^{deacyl} can enter the A-site of prokaryotic ribosomes in a weak but codon-dependent manner without the aid of other factors [131,132]. RelA binds to 70S ribosomes, and upon recognition of A-site-bound tRNA^{deacyl}, it converts ATP and (GTP)GDP into (p)ppGpp which simultaneously provides the energy for RelA to dissociate off the ribosome [133]. The enzymatic activity of RelA does not affect the amount of tRNA^{deacyl} bound to the A-site, suggesting that RelA does not remove tRNA^{deacyl} from the ribosome. After the cell has overcome starvation, the tRNA^{deacyl} may be chased out of the A-site by the cognate charged tRNA in complex with EF-Tu-GTP [133]. It has been proposed that RelA 'hops' from ribosome to ribosome to detect the presence of tRNAs^{deacyl} at A-sites, thereby the amount of synthesised (p)ppGpp reflects the amount of 'starved' ribosomes [133]. Recently, *in vivo* single-molecule imaging studies suggest that amino acid starvation causes the release of RelA from ribosomes as predicted from the *in vitro* data, and that RelA synthesises several (p)ppGpp molecules per 'hopping' event [134]. This raises the possibility that RelA remains active for some time after release from the ribosome [134]. This RelA 'hopping' model would be akin to the proposed idea that Gcn1/Gcn2 is in a dynamic equilibrium between free and ribosome-bound forms.

There is support for the idea that Gcn1 and Gcn2 form a trimeric complex with the ribosome to facilitate transfer of tRNA^{deacyl} from the A-site to Gcn2. First, the (major) binding domains in the Gcn1 and Gcn2 proteins required for Gcn1–Gcn2 interaction are physically distinct from the ribosome binding domains in either protein (Figs. 3, 4) [61,70,98]. Second, Gcn1 binding to the ribosome causes sensitivity to the A-site-binding drug paromomycin, suggesting that Gcn1 can access/affect the A-site directly or indirectly [61] (Section 6).

Curiously, Gcn1 and Gcn2 are far less abundant in the cell than ribosomes [135], as found for RelA (one RelA molecule per 200 ribosomes, [136]). Yeast cells grown in rich medium contain 7330 Gcn1 and only 279 Gcn2 molecules [135]. Taken into consideration results from polysome co-sedimentation profiles (cells grown in minimal medium) to gage the steady state distribution of the Gcn proteins [137], and assuming that the cellular amount of Gcn proteins does not change significantly depending on the growth medium, it is possible to reach an approximate estimate that 2430 Gcn1 and 74 Gcn2 molecules reside on the polysomes, and 2300/124 Gcn1/Gcn2 molecules reside on 80S and monosomes. As Gcn2 forms dimers (or even tetramers) [39–41], only 37 (or less) translating ribosomes would carry Gcn2. With the far higher abundance of ribosomes (200,000 in cells grown in rich medium [138]), the likelihood of Gcn1 and Gcn2 simultaneously contacting the same translating ribosome is extremely low. There are indications that Gcn1 and Gcn2 form a complex that 'hops' on and off the ribosome, thereby ensuring that both proteins reside on the same ribosome to allow tRNA^{deacyl} detection by Gcn2. For example, Gcn1 co-precipitates Gcn2 but not the integral 60S ribosomal protein Rpl39, and the ribosome binding activity of Gcn2 and Gcn1 are dispensable for Gcn1–Gcn2 interaction [38,61]. These data would also explain an observation that cells with 50% reduction in Gcn1 protein levels are still able to

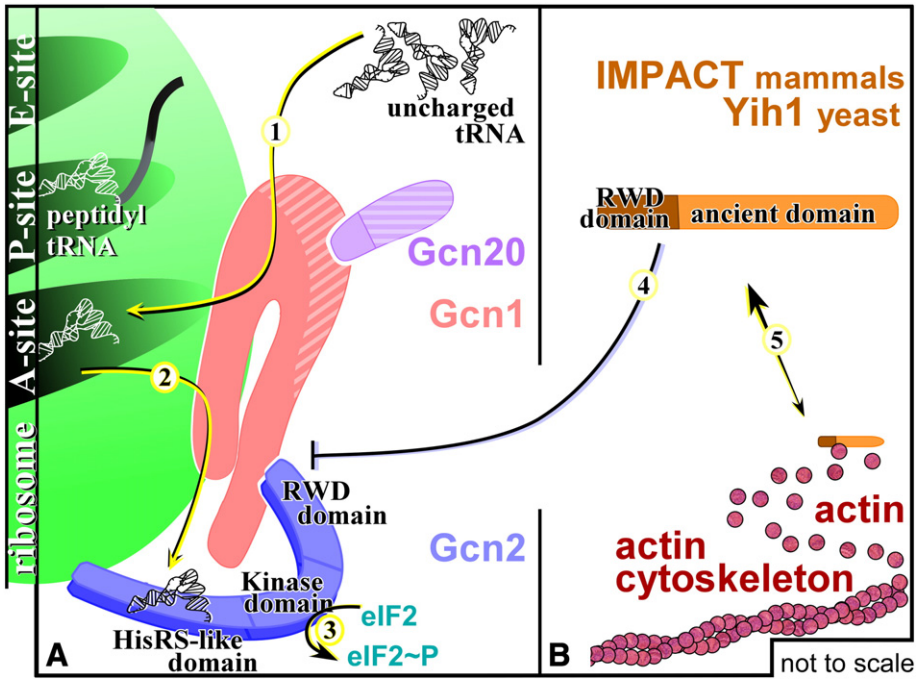

Fig. 5. (A) Working model for Gcn2 activation by uncharged tRNAs (tRNA^{deacyl}). [61,98,99] Gcn1 and Gcn2 bind to the ribosome, and contact each other involving the Gcn2 RWD domain. (1) Under amino acid starvation, or other stress conditions that lead to increased levels of tRNAs^{deacyl}, a cognate tRNA^{deacyl} enters the ribosomal A-site in a codon specific manner. (2) This tRNA is then transferred to the HisRS-like domain in Gcn2, (3) leading to the stimulation of the kinase domain, auto-phosphorylation, and phosphorylation of its substrate eIF2α. Gcn1 is directly involved in the transfer of the starvation signal to Gcn2. Areas in Gcn1 and Gcn20 that have homology to eEF3 are shaded. Because it is unknown where on the ribosome Gcn1 and Gcn2 bind, the ribosome is drawn in a simplified manner. (Figure adapted from [61]) (B) Working model for Gcn2 regulation by Yih1. [165] (4) The RWD domain of free Yih1 competes with Gcn2 for Gcn1 binding, thereby preventing transfer of the starvation signal to Gcn2 and impairing Gcn2 activation. (5) The Gcn2-inhibitory function of Yih1 is regulated. Studies suggest that Yih1 resides in the cell in an inactive heterodimeric complex with an actin monomer. So far the cue leading to Yih1 dissociating from actin is unknown.

respond to amino acid starvation similarly to a wild-type strain (Shanmugam & Sattlegger, unpublished and [139]).

The Gcn1–Gcn2 complex may randomly probe ribosomes for the presence of tRNAs^{deacyl} in the A-site. It is possible that the Gcn1–Gcn2 complex only recognises ribosomes which are in a certain stage in the elongation cycle, such as those that have accommodated a tRNA in the A-site but do not carry eEF3 [140] (see below). Under nutrient replete conditions, basal Gcn2 activity may be maintained by a rare event of tRNAs^{deacyl} occurring in the A-site, thereby ensuring basal Gcn4/ATF4 protein levels and basal expression levels of the Gcn4/ATF4 target genes [140] (see Section 8). Studies suggest that eEF3 triggers the release of tRNA^{deacyl} from the E-site during normal protein synthesis [104]. In analogy to this, Gcn1 may trigger the release of tRNA^{deacyl} as well, but from the A-site [101].

One approach to test the above model for Gcn2 signal sensing would require the development an *in vitro* assay using highly purified components. However, complicating the matter, studies hint towards Gcn2 becoming (partially) activated during cell breakage, possibly due to reduced tRNA charging in the cell extract, and/or due to the mixing of cell content and the concomitant exposure of Gcn2 to tRNA^{deacyl} or immature tRNAs that are normally not accessible to Gcn2 *in vivo* [57].

The model on starvation sensing by Gcn2 on the ribosome was built entirely on experimental evidence obtained in the yeast system. Although all the available information attests that tRNA^{deacyl} is the immediate signal for Gcn2 activation in all organisms investigated, the details of this model are still far from being understood relative to the participation of Gcn1 and the ribosomes in the activation of Gcn2, in yeast and other organisms. Mouse Gcn2 overexpressed in a *gcn2Δ* yeast strain is capable of phosphorylating eIF2α. It dampens cell growth even in replete medium, in a manner dependent on eIF2α Ser-51, possibly due eIF2α hyperphosphorylation. Curiously however, the growth defect is dependent on the m2 amino acids in Gcn2, but not on endogenous Gcn1. One possible reason for Gcn1 being dispensable may be the fact that the mammalian Gcn2 was overexpressed thereby artificially giving Gcn2 access to tRNA^{deacyl} [13].

Gcn2 variants have been described that lack the RWD domain required for Gcn1 binding. For example, in mouse, besides the bonafide Gcn2 that has homology to the entire length of yeast Gcn2 (Gcn2β isoform), two additional mRNAs were found encoding Gcn2 isoforms that differ in their N-terminus [13]. The γ form lacks part of the RWD domain (amino acids 1–86, equivalent to yeast Gcn2 residues 1–77) and harbours 8 amino acids unique to this isoform; and the α form lacks all RWD amino acids up to the pseudokinase domain (residues 1–197, equivalent to yeast Gcn2 residues 1–188). Although the RWD domain is truncated in the γ isoform, results from studies on the Yih1 RWD domain suggest that this truncated Gcn2 RWD domain may still be able to bind Gcn1 (see below) [64]. Thus only the α isoform completely lacks the Gcn1 interacting region. The Gcn2β mRNA is expressed in all examined organs (heart, brain, liver, lung, skeletal muscle, kidney, testis [12,13], and ovaries and oocytes [30]). The mRNAs of the other 2 isoforms are restricted to specific tissues (Gcn2γ in brain and testis; Gcn2α in brain, liver and testis [13]; no Gcn2α was detected in oocytes [30]). Given the expected size difference between the Gcn2α and the Gcn2β isoforms, Gcn2α should be detected in immunoblots of the mouse brain. However, no clear signal has been obtained with the expected size [141]. It is possible that Gcn2α is expressed in specific cells, which would not be sufficient for detection in extracts of whole brains or brain parts. Thus, an important issue that needs to be determined is whether the Gcn2α and Gcn2γ isoforms are expressed and functional, and to what conditions they respond to phosphorylate eIF2α.

RWD-less Gcn2 forms have also been found in other organisms. One of the two Gcn2-like kinases identified in the parasite *T. gondii* lacks the Gcn1-interacting RWD domain (TgIF2K-C). It is activated in intracellular parasites when glutamine is withdrawn from the host cell growth medium, and is required for parasite adaptation to imposed glutamine starvation on the host [142]. A close homolog in the malaria parasite *P. falciparum* (PfeIK1) also lacks the RWD domain [142] and is regulated by amino acid starvation [126]. In *Dictyostelium*, two of the three Gcn2-like kinases also lack the RWD domain [142–144]. It is possible that the Gcn2 isoforms lacking the RWD domain are activated by a mechanism that does not require Gcn1. In another scenario, the RWD-less Gcn2 may heterodimerise with and thereby regulate full-length Gcn2 *in vivo*. This important issue must be addressed in the future to further our understanding on the regulatory mechanisms impacting on Gcn2 activity in these other organisms or in mammalian cells that may express those alternative Gcn2 isoforms.

7. Translation elongation factors

Studies in *S. cerevisiae* suggest that the essential translation elongation factors eEF1A and eEF3 keep Gcn2 in its latent state under nutrient replete conditions [140,145]. eEF3 mediates the release of tRNA^{deacyl} from the E-site during each round of translation elongation, and this is coupled with eEF1A-mediated delivery of a cognate amino-acyl tRNA to the A-site [104,105,146]. eEF3 has two ATP binding cassettes and its ATPase activity is enhanced by ribosomes *in vitro* [146,147]. A homologue to eEF3 was not found in higher eukaryotes so far. In contrast to yeast ribosomes, mammalian ribosomes seem to have an intrinsic ATPase activity, and *in vitro* studies suggest that this may constitute the eEF3-equivalent function in these organisms [148,149]. Prokaryotes harbor a soluble ATPase, RbbA, that associates with ribosomes and may be the equivalent to eEF3 [150–152].

In vitro co-precipitation studies showed that eEF1A directly binds to the Gcn2 CTD, and *in vitro* kinase assays revealed that eEF1A inhibits Gcn2-mediated eIF2α phosphorylation but not Gcn2 autophosphorylation [153]. eEF1A may prevent Gcn2 from binding its substrate eIF2α [153]. Alternatively, considering that Gcn2 activation requires relief of autoinhibitory interactions and a rearrangement of the Gcn2 conformation, this raises the possibility that eEF1A may prevent the complete intramolecular rearrangement of Gcn2. This would allow ATP binding, but would not allow eIF2 to access the active site [153].

Gcn2–eEF1A interaction is reduced under starvation conditions *in vivo*, and this may be explained by the observation that tRNAs^{deacyl} dissociate eEF1A–Gcn2 interaction *in vitro*. As the Gcn2-CTD constitutes part of the tRNAs^{deacyl} binding site [69], one could envision that under amino acid starvation tRNAs^{deacyl} are channelled to Gcn2, dissociating eEF1A from Gcn2, thereby allowing Gcn2 activation [153].

eEF1A is also known to be implicated in a large array of other non-canonical functions that are seemingly unrelated to its function in protein synthesis [154,155]. For example, eEF1A regulates the actin cytoskeleton in eukaryotes by binding and bundling filamentous actin (F-actin) [155–157]. Conversely, actin regulates eEF1A [154,155]. For example, while bound to F-actin, eEF1A is unable to bind aminoacyl-tRNAs, suggesting that actin-binding drives eEF1A into its translation-inactive form. This raises the possibility that actin utilises eEF1A to also modulate Gcn2 activity.

Mammals harbor two eEF1A isoforms that function in protein synthesis but differ in their expression patterns. eEF1A2 is present during fetal development, and later-on is replaced by eEF1A1 in almost all tissues [158]. Increased abundance of eEF1A2 has been found in several tumours [158], and ectopic expression of this protein is tumorigenic [159]. It was reported that eEF1A1 can also be transforming as found for eEF1A2 [159]. Since Gcn2 activity has been shown to promote tumour growth [22], it will be relevant to address whether in cancer cells Gcn2 activity is modulated by some of the non-canonical functions of overexpressed eEF1A. For example, it is unknown whether free or actin-bound eEF1A inhibits Gcn2, or whether eEF1A1 and eEF1A2 differ in their affinity to Gcn2 or actin. Interestingly, eEF1A from metastatic cells has reduced F-actin affinity *in vitro* [160].

Overexpression of eEF3 leads to reduced eIF2α phosphorylation even under replete conditions [140]. Genetic studies suggest that this

is due to eEF3 inhibiting Gcn2 indirectly by preventing the formation of a functional Gcn1–ribosome interaction. Supporting this idea, Gcn2 activation is further impaired when eEF3 overexpression is combined with the Gcn1 M7A mutation known to have reduced ribosome affinity (amino acids in Gcn1 area M7 are substituted by alanine (chapter 5)) [140]. Furthermore, Gcn2 activation can be impaired by overexpressing eEF3 fragments that harbor only the ribosome binding activity, such as the eEF3 C-terminus (910–1044), or the eEF3 N-terminal HEAT domain (amino acids 100–367) which has homology to Gcn1 [140]. Together, these observations indicate that Gcn1 and eEF3 have common binding sites on the ribosome [140]. However, polysome co-sedimentation studies indicate that eEF3 overexpression does not reduce Gcn1–ribosome interaction, suggesting that eEF3 does not remove Gcn1 from the ribosome. It is possible though that the effect of eEF3 overexpression on the steady-state level of Gcn1–ribosome association may be too small for being detected by the employed assay. Since Gcn1 has a large ribosome binding region, eEF3 overexpression is likely to only affect a few of the Gcn1 ribosome contact points, in particular the ones that are crucial for Gcn1 function.

As a result of these observations, these elongation factors can be added to the current working model for Gcn2 activation (Fig. 6). During each round of translation elongation, eEF3 transiently binds to the ribosome to facilitate release of $tRNA^{deacyl}$ from the E-site. eEF3–ribosome binding would impair the formation of a functional Gcn1–ribosome complex. In addition, eEF1A bound to the Gcn2 CTD would prevent Gcn2 from phosphorylating its substrate eIF2α. Under conditions where the cognate charged tRNA is scarce, $tRNA^{deacyl}$ enters the A-site in a codon-dependent manner. Accommodation of A-site bound $tRNA^{deacyl}$ would not lead to the release of E-site bound $tRNA^{deacyl}$, thus eEF3 function would be redundant. The absence of eEF3 from the ribosome would allow Gcn1 to form a productive Gcn1–ribosome complex and to promote delivery of $tRNA^{deacyl}$ from the A-site to Gcn2. This $tRNA^{deacyl}$ would trigger eEF1A to dissociate from Gcn2 CTD, allowing full Gcn2 activation and eIF2α phosphorylation [140,145]. While the mechanism leading to eEF1A–Gcn2 dissociation under starvation conditions remains to be determined, current data suggest that $tRNAs^{deacyl}$ are a contributing factor [140]. The fact that eEF3 overexpression also reduces basal eIF2α phosphorylation levels suggests that a low amount of $tRNAs^{deacyl}$ occurs in the A-site even under replete conditions, leading to basal Gcn2 activity and basal eIF2α phosphorylation levels and thus basal levels of amino acid biosynthesis [140].

8. Yih1/IMPACT

In a search for imprinted genes in mice, a protein was uncovered that consisted of two domains [161]. Its C-terminal domain has sequence homology with proteins found in all kingdoms of life, for which the domain was called the ancient domain, and the mouse gene was named *Impact* for imprinted gene with ancient domain [161,162] (Fig. 7A). The N-terminal domain in IMPACT shares sequence similarity with the Gcn2 N-terminus, and also constitutes an RWD domain [62,64]. The gene encoding IMPACT is present in most eukaryotes [64,137,161,163,164].

The IMPACT protein in *S. cerevisiae* was called Yih1, for yeast Impact homologue [62]. Based on the discovery that the RWD domain of Gcn2 contacts its effector protein Gcn1 [61,62], it was proposed that IMPACT/Yih1 inhibits Gcn2 function by competing with Gcn2 for the interaction with Gcn1, mediated by its RWD region [62]. Supporting this idea, overexpression of the Yih1 RWD domain (amino acids 1–125) was shown to be sufficient for impairing cell growth under amino acid starvation conditions [62]. Evidence that Yih1 is a *de facto* negative regulator of Gcn2 by competing for Gcn1 binding was subsequently provided [64,165], by showing that: a) impaired growth under amino acid starvation observed with cells overexpressing Yih1 is reverted by increased Gcn2 levels; b) Yih1 overexpression reverts the growth defect associated with constitutively active Gcn2; c) *in vivo* overexpressed Yih1 co-precipitates Gcn1 but not Gcn2, reduces the cellular level of Gcn1–Gcn2 interaction, and leads to reduced phosphorylation of eIF2α; and d) purified $Gcn1^{2052–2428}$ binds to purified Yih1 *in vitro* and this interaction is dependent on the Gcn1 Arg-2259 residue, similarly to the Gcn1–Gcn2 interaction.

Several lines of evidence indicate that the function of IMPACT/Yih1 is evolutionarily conserved: a) purified yeast $Gcn1^{2052–2428}$ co-precipitates IMPACT from mouse brain extracts, in an Arg-2259 dependent manner [111]; b) endogenous IMPACT co-immunoprecipitates with Gcn1 in mouse brain extracts [111]; c) in mouse embryonic fibroblasts (MEFs), overexpressed IMPACT leads to reduced Gcn1–Gcn2 interaction [92]; d) IMPACT overexpressed in yeast inhibits Gcn2 in a variety of stress conditions that depend on Gcn1, as found for overexpressed Yih1 [92,111]; e) IMPACT overexpression in MEFs inhibits endogenous Gcn2 activation under amino acid or glucose starvation conditions, UV irradiation and proteasome inhibition, as determined by its auto-phosphorylation levels and the downstream response of eIF2α phosphorylation [92,111]; f) knock-down of endogenous IMPACT in undifferentiated neuronal-like N2a cells, which express high levels of IMPACT, resulted in stronger Gcn2 activation under leucine starvation conditions; [112]; g) in differentiated neuronal N2a cells, where IMPACT is even more abundant, knock-down experiments showed that endogenous IMPACT inhibits basal Gcn2 activity [112]; h) IMPACT does not inhibit PERK activation, as expected given that of the four mammalian eIF2α kinases, only Gcn2 contains an RWD domain [3,92].

Similarly to IMPACT-knock down in undifferentiated neuronal cells, deletion of Yih1 in yeast does not lead to a detectable increase in basal eIF2α phosphorylation [165]. To rule out the possibility that the Gcn2-inhibitory function of Yih1 is redundant with other proteins, all genes known to code for a protein with an N-terminal RWD-domain were deleted in the same yeast cell. Even then these cells did not show

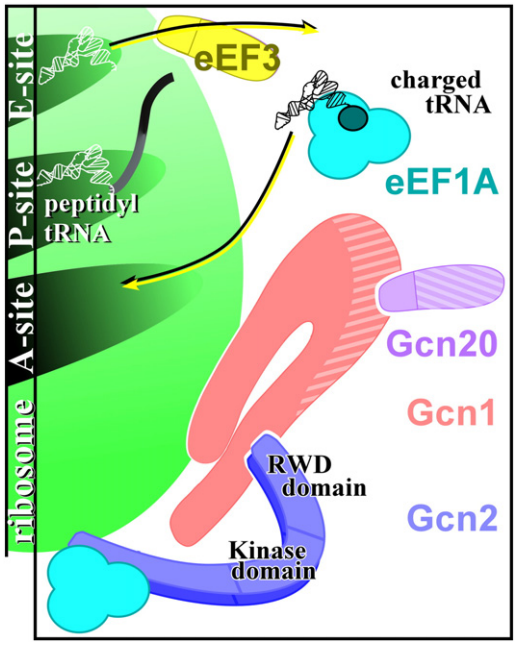

Fig. 6. Model for translation elongation factors keeping Gcn2 in its latent state. During protein synthesis eEF3 binding to the ribosome prevents the formation of a functional Gcn1–ribosome complex (see Fig. 5 for comparison), thereby preventing delivery of $tRNAs^{deacyl}$ to Gcn2 [140]. In addition, eEF1A bound to the Gcn2 CTD prevents Gcn2 from phosphorylating eIF2α [153]. It remains to be determined whether the eEF1A inhibiting Gcn2 is different to the one delivering aminoacyl-tRNA to the A-site, or whether this eEF1A is its GTP- or GDP-bound form. Areas in Gcn1 and Gcn20 with homology to eEF3 are hatched.

constitutive Gcn2 activity [165]. These findings seem to indicate that Yih1/IMPACT may be required under specific conditions, or in specific cells or cellular locations, where Gcn2-activity must be efficiently inhibited to allow for maximum rate of protein synthesis. This will be discussed later.

In vivo co-sedimentation and co-precipitation assays showed that endogenous, as well as overexpressed, Yih1 binds to translating ribosomes in a Gcn1-independent manner, and the same was found for IMPACT expressed in yeast [137]. Yih1 overexpression does not affect Gcn1–polyribosome or Gcn2–polyribosome interaction, indicating that Gcn1, Gcn2 and Yih1/IMPACT bind to the ribosome independently of each other [137]. The same may be true for the mammalian system since neuronal endogenous IMPACT also associates with polyribosomes [112]. It should be noticed that, both in yeast and in mammals, only a fraction of endogenous Yih1/IMPACT associates with polyribosomes [112,137]. Yih1/IMPACT may transiently associate with ribosomes or have additional functions unrelated to Gcn2 regulation.

Yih1/IMPACT–Gcn1 interaction must be somehow regulated, as otherwise Yih1/IMPACT would constitutively inhibit Gcn2 [165]. Supporting this idea studies in yeast uncovered that Yih1 function is regulated by actin. Yih1, expressed from its chromosomal locus and tagged with a flag epitope, forms a complex with monomeric actin (G-actin) in a 1:1 molar ratio, as determined by subjecting the Flag-immunoprecipitated material to separation on size exclusion chromatography or on velocity sedimentation on glycerol gradient [165]. IMPACT co-precipitates yeast actin *in vivo*, supporting the idea that IMPACT/Yih1–actin interaction is evolutionary conserved [137]. *In vivo* assays using *gcn1Δ* strains showed that the Yih1–actin interaction does not require Gcn1, while *in vitro* binding assays indicated that Yih1–Gcn1 interaction does not require actin [64,165]. These data support the idea that Yih1 may shuttle between its binding partners actin and Gcn1 (see below).

Indication that the connection of Yih1 and actin is relevant *in vivo* was obtained. Genetic reduction in actin levels, in heterozygous *ACT1*/*act1* diploid yeast strains, impairs the cell's ability to overcome amino acid starvation, and this in turn can be partially reverted by deleting *YIH1* [165]. The fact that in wild-type yeast cells endogenous Yih1 co-purified with actin but not with Gcn1 led to a model where Yih1 resides in the cell in an inactive Yih1–G-actin complex, and when released from actin, under certain conditions or in certain cellular locations, it then inhibits Gcn2 (Fig. 5B) [165]. Experimental support for this model would require showing that reduced actin levels increase Yih1–Gcn1 interaction at the expense of Gcn1–Gcn2 interaction, and that this is associated with reduced phosphorylation of eIF2α. However, this could not be observed so far, possibly because of the technicalities of the experimental procedure. Alternatively, Yih1 could be confined to inhibiting Gcn2 in certain regions of the cell where maximal protein synthesis is required. There are precedents for localised regulation of translation especially in neurons, but none has been described yet involving eIF2α phosphorylation [154,166,167]. The actin cytoskeleton provides a scaffold for components of the translational machinery [154], and its high rate of re-organisation in specific cell locations would allow for actin to mediate Yih1/IMPACT regulation of localised Gcn2 activity [64,137,165].

Interestingly, in neuronal N2a cells, IMPACT promotes neurite outgrowth induced by serum starvation. Conversely, Gcn2 inhibits spontaneous neurite outgrowth [112]. The involvement of IMPACT in induced N2a cell differentiation was, at least in part, mediated by its inhibition of Gcn2. Therefore, the possibility that IMPACT also functions in Gcn2-independent pathways is open to further studies. In primary hippocampal neuronal cultures, Gcn2 also negatively controls neuronal process extension [112]. Neurite outgrowth involves extensive actin remodelling, but it remains to be established whether actin modulates the ability of IMPACT to inhibit Gcn2. In addition, during the differentiation process, Gcn2 activity showed a marked decrease, as determined by its auto-phosphorylation levels in immunoblots, concomitant with the increase in the abundance of IMPACT. However, no detectable decrease in eIF2α phosphorylation was observed. This was probably due to the observed concurrent activation of PERK [112]. These observations may raise the possibility of a localised control of eIF2α phosphorylation by Gcn2, an event that cannot be detected by immunoblots of cell extracts, much in the same manner as Yih1 in the yeast model proposed above. These observations strengthen the necessity for further investigations, to address whether translation can be locally regulated by eIF2α phosphorylation mediated by Gcn2, and whether IMPACT regulates Gcn2 in subcellular locations.

In *Xenopus* oocytes, overexpression of IMPACT by microinjection leads to increased rates in gastrulation defects, indicating that its correct dosage is critical during this developmental process [163]. It remains to be verified whether this defect is due to Gcn2 inhibition.

More knowledge on the IMPACT/Yih1 domains may give a clue on how different interacting partners determine the function of this protein. Despite the low sequence conservation between the RWD domains of Yih1 and Gcn2, the RWD-motif of Yih1 (amino acids 1–114) was successfully modelled on the Gcn2 RWD structure [64]. Intramolecular contacts found for Gcn2 RWD seem to be preserved in Yih1, including the conserved YP*xxx*(*x*)P structural motif (Fig. 7A) [63,64].

The ancient domain is found throughout all kingdoms of life, suggesting that it is involved in a highly conserved and fundamental biological process [64,161,162]. Its function however, remains elusive. The *E. coli* YigZ protein is the prototype of an ancient domain, and its structure has been solved by crystallography [168]. Modelling of the Yih1 ancient domain (amino acids 125–258) suggests that it conforms well to the structure of YigZ [64]. Interestingly, three invariant sequence features found in both eukaryotes and prokaryotes (Fig. 7A) are present

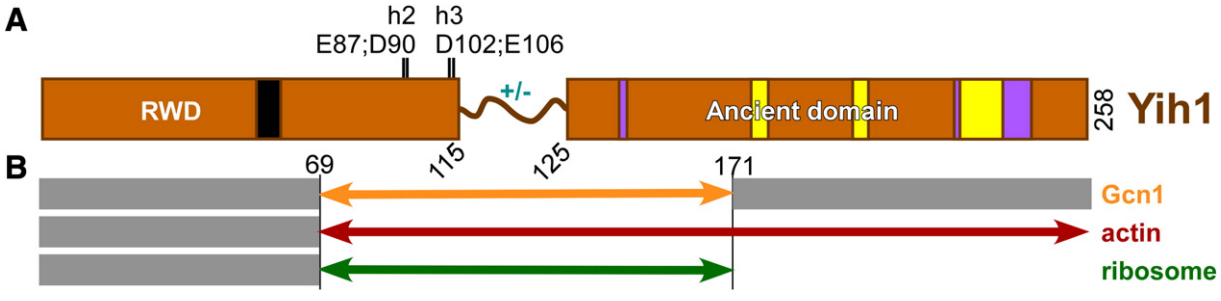

Fig. 7. (A) Overview of the Yih1 protein. In the RWD domain the location of motif *YPxxx*(*x*)*P* (amino acids 53–59) required for maintaining domain structure is indicated in black. Amino acids in RWD domain helix 2 (h2) are shown that when substituted by Ala increase interaction with Gcn1 and actin, while substitution of the indicated amino acids in helix3 (h3) impairs Gcn1 binding but not actin binding. The ancient domain harbours loop regions that form a conserved putative interaction surface for a yet-to-be discovered ligand (yellow and purple), and that harbours determinants characteristic to either eukaryotes or prokaryotes (purple). The linker region contains an abundance of charged amino acids, which is more evident in the proteins from higher eukaryotes (+/−) (B) Double arrows depict regions in Yih1 that are sufficient for binding the indicated proteins or ribosome. Regions in Yih1 identified to negatively impact on the respective binding properties are indicated by grey boxes.

in loop regions located on the same side of the molecule, suggesting that these motifs may be involved in binding a molecule that is evolutionarily conserved [64]. In addition, adjacent to these loop regions, and in a forth loop, sequences are found that are unique to either the prokaryotic or eukaryotic lineage [64].

The RWD and the ancient domains appear to be structurally largely independent of each other, and are connected by a linker region with low sequence conservation and longer in the vertebrate lineage [64]. This linker is rich in charged residues, similarly to what is found next to the RWD domain in Gcn2 [38]. The linker region seems to be unstructured, as indicated by biochemical studies, and prediction algorithms such as FoldIndex [64,169]. Areas of structural disorder and low sequence conservation are commonly associated with docking sites for different interactors in regulatory proteins [170]. Indeed, we found that the binding regions for Yih1-interacting proteins always encompass part of or the entire linker region, as shown in Fig. 7B, and discussed below.

In an effort to map binding regions in Yih1, *in vivo* co-precipitation studies were conducted using a set of GST-tagged Yih1 fragments. It was found that the interaction with Gcn1 is stronger with a Yih1 fragment comprising amino acids 68–171, encompassing the C-terminal part of the RWD domain and part of the highly charged linker region [64] (Fig. 7B). In Gcn2, partial deletion of the highly charged region adjacent to RWD (amino acids 184–237, Fig. 3) reduces Gcn1–Gcn2 interaction *in vivo*, suggesting that this region may also promote Gcn1–Gcn2 interaction [38]. Co-sedimentation assays revealed that the same Yih1$^{68–171}$ fragment harbours the main ribosome binding determinant(s) [64]. The actin binding site was mapped to a region encompassing part of the linker region and the ancient domain, amino acids 68–259 (Fig. 5B) [64].

Yih1 fragment 68–171 encompasses RWD helices h2 and h3 [64]. Within h3, Asp-102 and Glu-106, which are conserved in the Gcn2 RWD h3 helix (Glu-125, and Glu-136), are required for Gcn1 binding, but not for actin binding. As expected, Ala substitutions of these two residues impair the ability of overexpressed Yih1 to inhibit Gcn2. It will be interesting to verify whether the respective amino acids in Gcn2 RWD h3 also contact Gcn1 as suggested by [63]. Interestingly, Ala substitutions of Glu-87 and Asp-90 in h2 increase the ability of Yih1 to inhibit Gcn2, and this correlates with its stronger affinity to Gcn1, but also to actin. Together, these data strongly suggest different but overlapping determinants for actin and Gcn1 binding.

Binding properties seem to be subject to intra-molecular regulation (Fig. 7B). Whenever Yih1 fragments lack any of the termini (amino acids 1–67 or 172–258), Yih1–Gcn1 interaction is enhanced relative to that of full-length Yih1, suggesting that both termini negatively regulate Yih1–Gcn1 interaction. For actin and the polyribosome interaction, only the Yih1 N-terminus appears to have negative effects. Together this suggests, Yih1 function and regulation may be more complicated than anticipated [64]. It remains to be determined whether Yih1 exclusively binds actin, ribosomes and Gcn1, and whether one binding protein regulates the interaction of Yih1 with one or more of the other proteins.

These studies provided insights into how Yih1, and perhaps IMPACT as well, function in inhibiting Gcn2. The overlap in Gcn1- and actin-binding sites suggests that Yih1 binds either one or the other protein [64]. Curiously, the efficiency of a Yih1 fragment in interacting with Gcn1 does not necessarily dictate its ability to inhibit Gcn2 activation. In particular, Yih1$^{68–258}$ sequesters more Gcn1 than full length Yih1 but yet is less effective in impairing Gcn2 function [64]. This Yih1 fragment lacks helix h1, and the XP*xxx*(*x*)P motif, necessary to stabilise the conformation of h2 and h3 in the RWD, raising the possibility that this truncated RWD domain is unable to displace Gcn1 from preformed Gcn1–Gcn2 complexes. Thus, Yih1$^{68–258}$ may selectively bind Gcn1 molecules that are not associated with Gcn2 and not involved in Gcn2 regulation [64]. Supporting these ideas, not all Gcn1 appears to be engaged in contacting Gcn2, because a) there are 26 times more Gcn1 molecules than Gcn2 in the cell [135]; b) various polyribosome co-sedimentation assays indicate that only about 60% of Gcn1 resides on the ribosome [61,99,108,137]; and c) comprehensive protein–protein interaction studies found Gcn1 in several complexes lacking Gcn2 (*e.g.* [116–119]). In line with the idea that residues 1–67 are required for efficiently disrupting Gcn1–Gcn2 interaction, full-length Yih1 with the h2 amino acid substitutions also has increased affinity to Gcn1 and actin, except that it efficiently inhibits Gcn2 in contrast to Yih1$^{68–258}$ [64]. Considering that these h2 amino acids are predicted to be solvent exposed, their charge may restrict access to Gcn1, and the Ala substitutions would thus facilitate better access of Gcn1 to its binding site on h3, and better access of actin to its binding site. Possibly the increased actin affinity is counterbalanced by the increased Gcn1 affinity to still allow h2 mutated Yih1 to efficiently disrupt Gcn1–Gcn2 interaction. The above findings raise the possibility that Gcn1 and Yih1/IMPACT play additional roles in the cell apart from Gcn2 regulation. How Yih1/IMPACT is directed to the Gcn1/Gcn2 complex remains to be determined. One possible mechanism is the recruitment of Yih1/IMPACT to the ribosome where Gcn1 and Gcn2 are located as well [137].

While numerous large scale studies have shown that Yih1 expression does not change significantly (see http://spell.yeastgenome.org), IMPACT expression is highly regulated and may be relevant for adjusting Gcn2 function in specific tissues. The protein IMPACT is differentially expressed in specific organs and cells in adult rodents, being especially abundant in neurons in the central nervous system [111,141]. The hypothalamus is exceptionally rich in neurons with high IMPACT levels, particularly in the suprachiasmatic nucleus. Interestingly, the basal level of eIF2α phosphorylation in the hypothalamus is the lowest among the organs and brain parts analysed, suggesting that IMPACT keeps basal Gcn2-activity at very low levels [111]. The hypothalamus is in charge of maintaining body homeostasis such as controlling temperature and the balance of fluids and energy, and it is constantly adjusting the organism's metabolism and behaviour to its physiological needs. Along with this, protein synthesis must be maintained at constant levels, suggesting that high IMPACT abundance ensures low Gcn2 activity even under conditions in which Gcn2 would be activated in other brain regions [141].

Gcn2 function has been associated with synaptic plasticity and hippocampal memory [21,171]. *Gcn2*$^{-/-}$ mice have a lowered threshold for the induction of the late phase of long term potentiation (L-LTP). These mice show enhanced long term memory (LTM) in weak training programs that in the wild type mice do not result in LTM. On the other hand, *Gcn2*$^{-/-}$ are deficient in L-LTP and LTM induced by normal stimulation or strong training, respectively. Whether IMPACT participates in the modulation of Gcn2 activation in these paradigms remains to be determined [33]. Given that IMPACT expression can be regulated in neuronal cultures [112] (see above), it is possible that neuronal stimulation may as well regulate the levels of IMPACT, thus providing a means for the modulation of Gcn2 activity in L-LTP or LTM. In mouse, several lines of evidence suggest that Gcn2 function in the pyramidal neurons of the anterior piriform cortex is essential for developing aversive behaviour against food lacking essential amino acids [17,18,172]. It remains to be determined whether the same neurons that show increased eIF2α phosphorylation upon intake of this imbalanced food are devoid of IMPACT [141].

Together, these data indicate that IMPACT equips selected neurons with specialised functions such as down-regulating the Gcn2-dependent expression of ATF4, a protein proposed to play pivotal roles in neuronal functions [171,173]. Alternatively, or in addition to that, the highly abundant IMPACT in select neurons may function in other aspects of neuronal biology that may be independent of Gcn2, and this finds analogy with the data obtained for Yih1 in yeast [64] (see above).

IMPACT also provides the means for other cell types to control Gcn2. For example, human skin primary cells (fibroblasts and kerotinocytes) are more resistant than other cells (*e.g.* human T cells, and an immortalised human T cell line (Jurkat cells)) to co-

culturing with IDO-expressing cells [174,175]. IDO causes tryptophan depletion from the medium, leading to Gcn2 activation. Interestingly, human skin primary cells express high levels of IMPACT, and knock-down of IMPACT in the primary human skin fibroblasts increases their sensitivity to co-cultured IDO-expressing cells [175]. Conversely, overexpression of IMPACT in Jurkat cells, which have very little endogenous IMPACT, results in increased resistance under conditions of Trp depletion [175].

9. Gir2

Yeast Gir2 (Genetically Interacts with Ribosomal genes 2) is another protein with an N-terminal RWD domain that interacts with Gcn1 as determined by yeast-2-hybrid and pulldown assays. This interaction also requires Gcn1 Arg-2259, as found for Yih1/IMPACT and Gcn2 [61,111,165,176,177]. Overexpression of Gir2 inhibits Gcn2 function as judged by impaired growth under starvation conditions, and reduced eIF2α phosphorylation. This can be overcome by co-overexpressing Gcn2, suggesting that Gir2 downregulates Gcn2 by competing with Gcn2 for Gcn1 binding [176]. Deletion of Gir2 does not lead to increased Gcn2 activity, suggesting that Gir2 is not a general/continuous Gcn2-inhibitor as found for Yih1/IMPACT [165].

Unlike the RWD domains of other proteins studied so far, the Gir2 RWD is rich in acidic amino acids, particularly in an insert region unique to Gir2 (residues 85–101), which together with a large part of the C-terminus seems to be intrinsically unstructured [178,179]. It is possible that, in contrast to the other RWD domains, the Gir2 RWD region only folds into a structure homologous to the Gcn2 or Yih1/IMPACT RWD when complexed with a partner such as Gcn1. The insert in the Gir2 RWD domain also contains a PEST sequence (amino acids 62–102), known to target proteins for rapid degradation [179,180]. Supporting the idea of Gir2 being prone to degradation, purified Gir2 is highly sensitive to proteolysis [178].

The Gir2 C-terminus interacts with the small GTP-binding proteins Rbg1 and Rbg2 (RiBosome interacting Gtpase), but seems to mainly associate with Rbg2 [176,177]. While most of Rbg1 is ribosome-bound, only a small portion of Gir2 and Rbg2 is associated with translating ribosomes [176]. In contrast to Yih1/IMPACT, Gir2–ribosome association is partially dependent on Gcn1 [176]. Interestingly, Gir2–Rbg2 complex formation is enhanced by GTP, to some extent by GDP, but not by ATP [177]. Mutational studies showed that this is dependent on the GTP-binding domain in Rbg2, suggesting that Gir2–Rbg2 complex formation reflects the cellular level of GTP, which in turn indirectly reflects the cellular metabolic state. Guanine nucleotide enhanced interaction is not affected by Gcn1, indicating that this complex formation occurs independently of Gcn1 [177].

A biological relevance for the Gir2–Rbg interaction has been proposed recently. Gir2 was found to be required for maintaining cell doubling time, but only under amino acid starvation conditions, and mutational analyses indicated that this is mediated by the Gir2/Rbg2 or Gir2/Rbg1 complex [177]. Interestingly, Gir2–Rbg2 complex levels increase under amino acid starvation. These, as well as maintaining the doubling time under amino acid starvation, are dependent on the Rbg2 GTP binding activity [177]. Furthermore, amino acid starvation leads to increased binding of Rbg2–Gir2 to Gcn1 [177]. It was suggested that under amino acid starvation, Gir2–Rbg2 may sequester Gcn1 to blunt further Gcn2 activation [177]. It is tempting to speculate that the Rbg2–Gir2 complex adjusts the threshold level for Gcn2 activation to the energetic state of the cell. As all translation elongation factors (except of eEF3) consume GTP as an energy source, high GTP levels would signal availability of sufficient energy for proteins synthesis. High GTP levels would allow the Gir2–Rbg2 complex to dampen the Gcn2 response, thereby still allowing some general protein synthesis to occur while the cell engages mechanisms to overcome amino acid starvation [177].

Mammals contain a Gir2 orthologue called RWD domain-containing protein 1 (RWDD1, [181]) or developmentally regulated GTP-binding protein family regulatory protein 2 (DFRP2, [182]). As in yeast its main binding partner is the orthologue of Rbg2 called developmentally regulated GTP-binding protein 2 (DRG2) [182,183]. Evolutionary conservation of the Gir2–Rbg2 complex suggests its implication in a central biological process(es).

10. Viral proteins and RNA

Binding of viral RNAs to Gcn2 provides a mechanism for early cellular defence against a selection of viruses. *In vitro*, the Sindbis virus (SV) genomic RNA binds and activates Gcn2 in a manner that is dependent on the secondary structure of a bipartite sequence in the 5′ terminus of the SV genome [96]. This Gcn2 activation requires the m2 motif in the HisRS-like domain, suggesting that the viral RNA stimulates Gcn2 in the same manner as $tRNA^{deacyl}$ [96]. SV infection in MEFs leads to Gcn2 activation. This correlates with delayed synthesis of SV proteins and impaired viral replication, events not detected in $Gcn2^{-/-}$ MEFs. Furthermore, Gcn2 overexpression reduces SV replication and SV early protein synthesis [96]. In mammalian cells, Gcn2 overexpression also hampers the replication of other RNA viruses, such as the vesicular stomatitis virus (VSV) and Semliki forest virus (SFV). Compared to wild-type mice, $Gcn2^{-/-}$ mice are more susceptible to SV infection by intranasal administration, showing increased virus titers in the brain in the early days of infection [96].

In vitro transcribed HIV-1 genomic RNA also activates Gcn2 in a manner that is dependent on the m2 amino acids in the HisRS-like domain. Furthermore, reporter gene studies suggest that Gcn2 silencing leads to increased synthesis of HIV-1 proteins [184]. Gcn2 silencing in HeLa P4 cells relieves translational inhibition caused by HIV infection and results in increased viral infectivity [185]. HIV-1 in turn has developed counteracting mechanisms to inhibit Gcn2. At later times after infection, cells infected with HIV-1 show significant proteolytic degradation of Gcn2 which can be prevented by saquinavir, an inhibitor of the HIV-1 protease $HIV\text{-}1^{pro}$. *In vitro* assays confirm that the Gcn2 cleavage is catalysed by $HIV\text{-}1^{pro}$, cleaving Gcn2 C-terminal to the Tyr within the amino acid sequence Y[VI]ETVIP, thereby removing the N-terminal RWD and pseudokinase domains from Gcn2 and resulting into an N-terminally truncated Gcn2 with dramatically reduced kinase activity [184]. Curiously, the sequence of this protease site is present in mouse and human Gcn2 but not in yeast [184].

A recent study demonstrated that Gcn2 also protects against DNA virus infections. In a genetic screen, a mouse with a loss-of-function mutation in Gcn2 (*atchoum*) was identified that exhibits a modest increase in susceptibility to the double-stranded DNA virus mouse-cytomegalovirus (MCMV), and, contrary to wild type mice, fails to phosphorylate eIF2α upon viral infection, supporting the idea that Gcn2 contributes to viral defence against MCMV *in vivo* [186].

Some DNA viruses have evolved a mechanism to counteract PKR activation by ds-RNA that is formed during viral transcription/replication, which is the expression of eIF2α pseudosubstrates [187–189]. For example, vaccinia virus protein K3L has homology to the N-terminal part of eIF2α (eIF2α amino acids 1–88), but the amino acid equivalent to Ser51 is Lys, making K3L non-phosphorylatable. K3L, and similar proteins encoded by other viruses, function by competing with eIF2α for PKR binding. K3L-mediated inhibition of PKR antagonises the host′s main mechanism that prevents viral propagation by shutting down the translation machinery. K3L expression in yeast cells showed that K3L directly interacts with the Gcn2 protein kinase catalytic domain and impairs Gcn2 activation [190,191]. Even though the overexpression of Gcn2 in mammalian cells does not seem to hamper the replication of vaccinia virus, at least under these experimental conditions [96], the ability of vaccinia virus K3L to directly inhibit Gcn2 *in vivo* in mammalian cells has not yet been addressed experimentally.

11. Target of rapamycin

Apart from the GAAC, amino acid starvation is also sensed by the signalling pathway governing the protein kinase Tor (Target of rapamycin), specifically by the Tor containing protein complex 1 (TORC1). Active TORC1 promotes protein synthesis *e.g.*, by directly phosphorylating and activating the ribosomal protein S6 kinase (S6K in mammals and Sch9 in yeast), and additionally in mammals by phosphorylating and inhibiting the translational repressor eIF4E-binding protein 1 (4E-BP1) [192–196]. TORC1 is inactivated by several nutrient deprivation conditions as well as by the drug rapamycin. Hence, GAAC and Tor signalling pathways may co-ordinately prevent the translational machinery from using unnecessary amounts of vital resources under nutrient limiting conditions.

Cross-talks between GAAC and the TOR signalling pathway were found in *S. cerevisiae*. Tor inactivation promotes Gcn2 activation by a mechanism involving the dephosphorylation of Gcn2 Ser-577 [88,89]. Tor inactivity leads to the dephosphorylation of Tap42, thereby weakening its association with phosphatases [193]. It has been proposed that phosphatases (Sit4 and/or PP2A) freed from TAP42, mediate dephosphorylation of Gcn2 Ser-577 [88]. This mechanism of Gcn2 stimulation appears to require $tRNA^{deacyl}$, since the *gcn2-m2* mutation abolishes the activation [88,89]. S577A substitution increases Gcn2 affinity to $tRNA^{deacyl}$ *in vitro*, suggesting that Ser-577 dephosphorylation may be sufficient to allow Gcn2 to bind $tRNAs^{deacyl}$ *in vivo* to trigger its activation [197]. In fact, *in vivo* the S577A substitution leads to constitutively increased Gcn2 auto-phosphorylation at Thr882, and increased eIF2α phosphorylation [47,197].

Curiously, following 30 min rapamycin treatment, some eIF2α phosphorylation was observed in a *gcn1Δ* strain, while another group found that Gcn1 is absolutely required for Gcn2 activation 2 h after rapamycin treatment [87,89]. Supporting the idea that Gcn1 is relevant, growth assays show that rapamycin sensitivity is dependent on Gcn2 as well as Gcn1 [42]. In another study, after 30 min of rapamycin treatment, strains with a constitutively active TAP42 allele only show 30% reduction in Ser-577 phosphorylation levels as compared to the wild-type strain (80% reduction), and this level stays almost constant for up 4 h, while in the wild-type the phosphorylation level gradually reverts back to almost the original level [88]. At 30 min of rapamycin treatment, despite the large differences in Ser-577 phosphorylation between these strains, their eIF2α phosphorylation levels are increased to similar levels. These observations raise the possibility that a constitutive eIF2α phosphatase becomes transiently inhibited, or that an alternative pathway transiently activates Gcn2 that may not require Gcn1 [88].

In addition to the treatment of cells with rapamycin, the inhibition of Tor by shifting cells to a poor nitrogen source such as GABA (γ-aminobutyric acid) also activates Gcn2 through a mechanism involving the Sit4 phosphatase [11]. Shifting cells to GABA medium results in the accumulation of selected $tRNA^{deacyl}$, and GAAC response requires the Gcn2 m2 residues, pointing to $tRNAs^{deacyl}$ as the activating ligands for Gcn2 [11]. Curiously, shifting cells to GABA medium leads to a significant increase in $tRNAs^{deacyl}$ after 60 min but not by 15 min, and yet eIF2α phosphorylation was already at its maximum level at 15 min. It was suggested that $tRNAs^{deacyl}$ and Tor may contribute to Gcn2 activation at different time frames following exposure to GABA [11].

Other possible links by which the TOR pathway impinges on the GAAC pathway may be mediated *via* two other proteins that are each under the control of TOR and that have been found to be in complex with Gcn2, Sch9 and Npr1. Purified Gcn2, as well as the γ subunit of eIF2, co-precipiate Sch9 from yeast extract [198], but Sch9 does not phosphorylate Gcn2 Ser-577 [193]. *SCH9* deletion leads to constitutive eIF2α phosphorylation, while strains harbouring the rapamycin-insensitive $Sch9^{2D3E}$ (amino acid substitutions mimicking activated Sch9) are unable to increase eIF2α phosphorylation in response to rapamycin treatment [199]. The exact molecular mechanism by which Sch9 regulates eIF2α phosphorylation, and whether this is mediated *via* altering Gcn2 activity, remains to be determined.

The yeast protein kinase nitrogen permease reactivator/regulator 1 (Npr1) which controls amino acid permeases, is dephosphorylated and activated by rapamycin [195,200]. In an attempt to experimentally determine protein kinase interaction networks in yeast, followed by co-precipitation experiments, Npr1 was found to be in complex with Gcn2, raising the possibility that Npr1 is a novel Gcn2-regulator [201].

These findings support a model in which both signalling pathways act synergistically to promote cellular responses to changes in nutrient availability. The kinase(s) involved in Gcn2 down regulation by phosphorylating Ser-577, as well as the conditions leading to its(their) activation are yet to be determined. In mammals, evidences have not been reported yet that support a direct activation of Gcn2 by the inhibition of mammalian (mTOR). We cannot exclude the possibility that mammalian Gcn2 contains an amino acid equivalent to yeast Gcn2 Ser-577, though its identification is difficult given the low sequence conservation around Ser-577. Interestingly, studies in mammals raise the possibility that Gcn2 contributes to the regulation of the Tor pathway (*e.g.* [16, 202–204]; it remains to be determined whether the same is true in yeast.

12. Heat shock proteins

In yeast it appears that Gcn2 maturation and accumulation are reliant on the chaperone protein Hsp82, which alongside its isoform Hsc82, are orthologues of mammalian Hsp90. These proteins seem to be similar in function since Hsp90 can rescue the lethality of a *hsc82Δ*; *hsp82Δ* double deletion strain [205]. Mutations in Hsp82 were found to de-repress Gcn4 translation under amino acid replete conditions, and for at least one mutant (G313N) it was determined that this effect is dependent on Gcn2 [205]. This indicates that Hsp82 is required for maintaining Gcn2 in its repressed state.

Evidence that Hsp82 engages Gcn2 was obtained by showing that ectopically expressed Flag-tagged Hsp82 interacts with overexpressed GST–Gcn2. Using a strain expressing a tagged human Hsp90, it was shown that both the endogenous Gcn2 and an overexpressed GST–Gcn2 fusion protein interact with Hsp90 in co-immunoprecipitation assays. Gcn2–Hsp90 interaction appears to be mediated by a fragment encompassing mainly the Gcn2 kinase domain (residues 507–1092), and Gcn2 kinase activity is not necessary for this interaction [205]. Unfortunately, native Gcn2–Hsp82 interaction has not been reported so far [38]. Interestingly, the amount of *in vivo* Gcn2–Hsp90 interaction does not change when the cells are starved of amino acids (though data were not shown), nor does it change much when Hsp82 harbours a mutation (G313N) that results in Gcn2 activation [205]. It is possible that in these experiments the overexpression of Gcn2 may have masked subtle changes in Gcn2–Hsp90/82 interaction. Hsp82 mutations may weaken the Gcn2–Hsp90 interaction, still allowing Gcn2 maturation but promoting the release of functionally active Gcn2 even in the absence of any trigger, or promoting the release by the low levels of $tRNA^{deacyl}$ present under replete conditions [38]. The authors propose a model in which matured Gcn2 remains bound to Hsp82/90, and under amino acid starvation the binding of $tRNA^{deacyl}$ to Gcn2 releases it from Hsp82/90, to then allow for Gcn2 activation. Because of the known intrinsic activity of purified Gcn2, it was not possible to determine whether the binding of $tRNA^{deacyl}$ would trigger the dissociation of Hsp90 from Gcn2. Gcn2 synthesised in rabbit reticulocyte lysates in the presence of the Hsp90 inhibitor, geldanamycin, is inactive in autophosphorylation assays. In parallel, inhibition of Hsp90 also results in stronger interaction of Hsp90 with Gcn2. Together these data indicate that Hsp90 plays an essential role in Gcn2 maturation and for it to become an active kinase. Stronger inhibition of Hsp90 *in vitro* or inhibition of Hsp82 *in vivo* resulted in decreased amounts of Gcn2, indicating that this chaperone is required for stability of Gcn2 as well.

Hsp90 uses co-chaperones to regulate its ATPase activity and client binding [206]. Interestingly, yeast cells with mutated co-chaperone Cdc37 (p50 in mammals), or cells lacking co-chaperone Sba1 (p23) or Sti1/HOP (p60), are not capable of efficiently overcoming amino acid starvation. The possible mechanism of these co-chaperones in contributing to the maturation of Gcn2 remains to be studied.

Chaperones such as Hsp90 have been proposed to play a major role in promoting protein complex assembly in addition to folding [207], raising the possibility that Hsp90 may be involved in assembling complexes containing Gcn2 and its effector protein Gcn1, and possibly also the Gcn2-inhibitor eEF1A [153], to ensure that *de novo* synthesised Gcn2 does not become accidentally activated.

13. Snf1

The highly conserved AMP-activated serine/threonine protein kinase, AMPK in mammals or Snf1 in yeast, is part of a signalling pathway that ensures homeostasis of available chemical energy in the form of ATP [208,209]. It is activated when the AMP/ATP ratio increases, to then switch off energy-consuming anabolic pathways while turning on ATP-producing pathways. The so far best understood function of Snf1 is its activation under glucose starvation, or in presence of non-preferred carbon sources, in order to induce expression of genes that allow the utilisation of alternative carbon sources [210].

Snf1 was found to be also activated by amino acid starvation, and a physical and functional link between the Snf1/carbon signalling pathway and GAAC was uncovered [47]. In response to His starvation, Gcn2 auto-phosphorylation and eIF2α phosphorylation are increased in a manner that is (mostly) dependent on Snf1 kinase activity. This correlates with Snf1 phosphorylation at its Thr-210, suggesting that Snf1 becomes stimulated under His starvation, and this again is shown to be required for full Gcn2 activation. Snf1 and Gcn2 interact with each other *in vivo* as judged *via* co-immunoprecipitation experiments, however, no evidence was found for Snf1 directly phosphorylating Gcn2.

Reg1 is a negative regulator of Snf1 [210], and under amino acid starvation *REG1* deletion leads to increased phosphorylation of Gcn2 Thr-822 and eIF2α, in a manner that is dependent on Snf1. This indicates that under starvation, Reg1 dampens Snf1 stimulatory function on Gcn2. Under amino acid-replete conditions *reg1Δ* cells show decreased eIF2α phosphorylation but unaltered basal Gcn2 Thr-882 auto-phosphorylation levels, in a manner dependent on Snf1. Thus, it appears that in unstarved *reg1Δ* strains, the resultant increased activity of Snf1 somehow may stimulate an eIF2α phosphatase.

When cells are shifted from glucose to a non-preferred carbon source (galactose), Snf1 is required to maintain normal eIF2α phosphorylation levels, but this does not involve Gcn2 activation and instead involves regulation of eIF2α phosphatases [47]. Interestingly, in contrast to that, when cells starve for a carbon source the response mechanism appears to be different, since in this situation eIF2α phosphorylation is increased in a Gcn2-dependent manner (Section 4) [77].

14. RACK1/Asc1

Mammalian Receptor for Activated C-Kinase, Rack1, or its *S. cerevisiae* orthologue Asc1 (formerly called Cpc2), is a highly conserved protein consisting of 7 tryptophan-aspartate (WD) repeats. This protein has been shown to function as a scaffold for proteins in various signal transduction pathways, thereby allowing the localised orchestration of signalling events, and accordingly, it plays essential roles in regulating a wide array of biological processes [211].

Asc1 affects Gcn2 function in addition to events downstream of Gcn2. In *S. cerevisiae*, Asc1 contributes to the docking of translation initiation factors to the ribosome during general translation initiation, thereby blunting the inhibitory action of the uORFs in the *GCN4* mRNA leader [212]. Under amino acid starvation, *ASC1* deletion suppresses the growth defect of a *gcn2Δ* strain, and this is not due to simultaneous deletion of the snoRNA U24 located in the *ASC1* intron, again suggesting that Asc1 negatively regulates GAAC in *S. cerevisiae* [213]. In addition, *ASC1* deletion leads to increased eIF2α phosphorylation under replete conditions; although unlikely, it remains to be verified that this is not mediated by U24 [214].

Curiously, recent studies have revealed that the Rack1 homologue in *Schizosaccharomyces pombe*, Cpc2, actually promotes, rather than represses, the GAAC response [215]. Cpc2 is required for starvation-induced Gcn2 auto-phosphorylation, eIF2α phosphorylation and expression of amino acid biosynthesis genes [215]. It was confirmed that impaired Gcn2 activation is due to *CPC2* deletion and not due to lack of the *snoU24b* gene located in the *CPC2* intron [215]. Although Cpc2 appears to have an almost opposite effect in *S. pombe* as compared to *S. cerevisiae*, Gcn2 auto-phosphorylation in *cpc2* mutants can be restored by introducing Asc1 or mammalian RACK1 [215]. Thus, the apparent difference in regulation is not due to functional differences in the Cpc2 homologs themselves, but is more likely the result of differences in other components that regulate the GAAC system. Regulatory differences may be due to the fact that Gcn2 is the sole eIF2α kinase in *S. cerevisiae*, whereas Gcn2 is one of three eIF2α kinases in *S. pombe* [215]. It will be interesting to investigate how RACK1 controls Gcn2 in mammals.

Asc1 and RACK1 were shown to be a stochiometric ribosomal component [216]. *S. pombe* Gcn2 also contacts the ribosome as found for the *S. cerevisiae* counterpart [98,215], however, Cpc2 is not required for Gcn2 dimerisation or Gcn2–ribosome interaction, and genetic studies showed that ribosome-free Cpc2 retains its ability to regulate Gcn2 [215]. The authors suggest that Cpc2 modulates the activity of a Gcn2 regulatory protein, or Cpc2 may be involved in transmitting an activating signal to Gcn2 [215]. It is unknown whether Cpc2 and Gcn2 interact with each other, and a physical interaction between Asc1 and Gcn2 was not found [214], though one cannot exclude the possibility that it is too transient to be detected.

Clearly the role of Asc1/Cpc2/RACK1 in the regulation the GAAC response requires further investigation. Rack1 and its orthologues have also been shown to play significant roles in transporting proteins around the cell and ensuring sub-compartmental protein localisation, in addition to controlling the activity of proteins [211]. Thus, it is plausible that Asc1/Cpc2/RACK1 could spatially regulate protein interactions with or upon the ribosome and thus spatially regulate Gcn2.

15. PI3K pathway

The PI3K signalling pathway is involved in cell proliferation and has neuroprotective functions [217–219]. This pathway is stimulated by growth factors or insulin, and leads to the activation of phosphoinositide-3 kinases (PI3Ks) which phosphorylate inositol lipids in membranes to generate phosphoinositide 3,4,5-triphosphates (PIP3). This stimulates the phosphoinositide-dependent kinase 1 (PDK1) which then phosphorylates and activates Akt1. Akt1 is a kinase that in turn regulates many proteins, such as inhibiting glycogen synthase kinase 3β (GSK-3β), to then trigger further downstream events.

The yeast orthologue of mammalian PDK1, Pkh1, interacts with Gcn2 *in vitro* and *in vivo* [198]. Interestingly, in *in vitro* kinase assays Pkh1 phosphorylates Gcn2, even though Gcn2 does not contain a typical Pkh1 phosphorylation site [198]. However, in yeast, inactivation of all 3 orthologues of mammalian Pdk1 has no significant effect on the phosphorylation of eIF2α at Ser-51 or on *GCN4* translation in response to amino acid starvation [198]. This would suggest that either there is sufficient redundancy in the system so that another protein can substitute for the loss of Pkh1, or that it might regulate Gcn2 activity in response to conditions other than amino acid starvation.

Recently, it was described that Gcn2 activity is regulated by the PI3K/Akt/GSK-β pathway, both in neurons and in fibroblast cells [220]. Pharmacological inhibition of PI3K downregulates Gcn2 activity, as

determined by its reduced auto-phosphorylation, eIF2α phosphorylation, and ATF4 levels, while intracellular delivery of PIP3, or transient overexpression of the constitutively active PI3K p110*, have the opposite effect in that they lead to increased eIF2α phosphorylation and ATF4 expression. PI3K activation leads to the inhibitory phosphorylation of GSK-3β, and as expected, inhibiting GSK-3β results in increased levels of Gcn2 auto-phosphorylation, eIF2α phosphorylation and ATF4. Furthermore, these effects were shown to be specific since in *Gcn2*$^{-/-}$ cells, inhibition of GSK-3β did not affect eIF2α phosphorylation or ATF4 expression relative to control non-treated cells. Fibroblasts lacking each of the other three eIF2α kinases maintained the ATF4 response to either the PI3K inhibitor or to the GSK3 inhibitor, showing that the effect is directed to Gcn2. It is not known yet how GSK3β modulates Gcn2 activity.

It is also worth noting that eIF2α phosphorylation can activate the PI3K pathway [221]. Most likely this occurs indirectly as a result of the reduced translation of some protein(s) that normally represses PI3K signalling [221]. This raises the possibility of a feedback loop, mediating cross talks between the GAAC and PI3K pathways.

16. Conclusion

As highlighted throughout this review, there are still several important gaps that need to be filled on the mechanisms of how Gcn2 receives its activating signal. For example, it is still far from being understood how immediate players, such as Gcn1 and the ribosome, contribute to the exquisite *in vivo* responsiveness of this kinase. Many other molecules have been uncovered, and new molecules are constantly being added, that directly or indirectly modulate Gcn2 activation. It is likely that this regulatory circuitry is even more diverse in mammalian cells in order to provide further avenues for controlling Gcn2 function in response to the specific needs of different cell types. Indeed, large-scale studies suggest that many additional proteins may participate in the regulation of Gcn2, either by interacting directly with it, with Gcn1 or with the other proteins described here, or by stimulating Gcn2 *via* indirect mechanisms. New cross-talks between the GAAC and other central signalling pathways are being continuously unravelled (*e.g.* PI3K and Tor pathways). Understanding these links is paramount, as the last few years have seen an impressive accumulation of experimental evidence implicating Gcn2 in previously unforeseen physiological pathways and diseases. For example, Gcn2 has been implicated with cancer, and with the efficacy of anticancer drugs. Thus, it is fundamental to gain a detailed understanding of the function of each Gcn2 regulator, as well as of the comprehensive Gcn2-regulatory network, that continuously adjusts Gcn2 activity and activation. Simultaneously, this will allow the pinpointing of new drug targets for correcting and preventing Gcn2-associated diseases/disorders.

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References

[1] A.G. Hinnebusch, Translational regulation of GCN4 and the general amino acid control of yeast, Ann. Rev. Microbiol. 59 (2005) 407–450.
[2] C. Chaveroux, S. Lambert-Langlais, Y. Cherasse, J. Averous, L. Parry, V. Carraro, C. Jousse, A.C. Maurin, A. Bruhat, P. Fafournoux, Molecular mechanisms involved in the adaptation to amino acid limitation in mammals, Biochimie 92 (2010) 736–745.
[3] T.D. Baird, R.C. Wek, Eukaryotic initiation factor 2 phosphorylation and translational control in metabolism, Adv. Nutr. 3 (2012) 307–321.
[4] H.P. Harding, Y. Zhang, H. Zeng, I. Novoa, P.D. Lu, M. Calfon, N. Sadri, C. Yun, B. Popko, R. Paules, An integrated stress response regulates amino acid metabolism and resistance to oxidative stress, Mol. Cell 11 (2003) 619–633.
[5] E. Sattlegger, A.G. Hinnebusch, I.B. Barthelmess, cpc-3, the *Neurospora crassa* homologue of yeast GCN2, encodes a polypeptide with juxtaposed eIF2 alpha kinase and histidyl-tRNA synthetase-related domains required for general amino acid control, J. Biol. Chem. 273 (1998) 20404–20416.
[6] G.H. Braus, C. Sasse, S. Krappmann, Amino acid acquisition, cross-pathway control, and virulence in *Aspergillus*, Med. Mycol. 44 (2006) 91–94.
[7] P.D. Lu, H.P. Harding, D. Ron, Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response, J. Cell Biol. 167 (2004) 27–33.
[8] K.M. Vattem, R.C. Wek, Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 11269–11274.
[9] M.H. Jia, R.A. Larossa, J.M. Lee, A. Rafalski, E. Derose, G. Gonye, Z. Xue, Global expression profiling of yeast treated with an inhibitor of amino acid biosynthesis, sulfometuron methyl, Physiol. Genomics 3 (2000) 83–92.
[10] K. Natarajan, M.R. Meyer, B.M. Jackson, D. Slade, C. Roberts, A.G. Hinnebusch, M.J. Marton, Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast, Mol. Cell. Biol. 21 (2001) 4347–4368.
[11] K.A. Staschke, S. Dey, J.M. Zaborske, L.R. Palam, J.N. McClintick, T. Pan, H.J. Edenberg, R.C. Wek, Integration of general amino acid control and Target of Rapamycin (TOR) regulatory pathways in nitrogen assimilation in yeast, J. Biol. Chem. 285 (2010) 16893–16911.
[12] J.J. Berlanga, J. Santoyo, C.s. de Haro, Characterization of a mammalian homolog of the GCN2 eukaryotic initiation factor 2alpha kinase, Eur. J. Biochem. 265 (1999) 754–762.
[13] R. Sood, A.C. Porter, D. Olsen, D.R. Cavener, R.C. Wek, A mammalian homologue of GCN2 protein kinase important for translational control by phosphorylation of eukaryotic initiation factor-2alpha, Genetics 154 (2000) 787–801.
[14] P. Zhang, B.C. McGrath, J. Reinert, D.S. Olsen, L. Lei, S. Gill, S.A. Wek, K.M. Vattem, R.C. Wek, S.R. Kimball, L.S. Jefferson, D.R. Cavener, The GCN2 eIF2alpha kinase is required for adaptation to amino acid deprivation in mice, Mol. Cell. Biol. 22 (2002) 6681–6688.
[15] H.P. Harding, I. Novoa, Y. Zhang, H. Zeng, R. Wek, M. Schapira, D. Ron, Regulated translation initiation controls stress-induced gene expression in mammalian cells, Mol. Cell 6 (2000) 1099–1108.
[16] T.G. Anthony, B.J. McDaniel, R.L. Byerley, B.C. McGrath, D.D.R. Cavener, M.A. McNurlan, R.C. Wek, Preservation of liver protein synthesis during dietary leucine deprivation occurs at the expense of skeletal muscle mass in mice deleted for eIF2 kinase GCN2, J. Biol. Chem. 279 (2004) 36553–36561.
[17] A.C. Maurin, C. Jousse, J. Averous, L. Parry, A. Bruhat, Y. Cherasse, H.Q. Zeng, Y.H. Zhang, H.P. Harding, D. Ron, P. Fafournoux, The GCN2 kinase biases feeding behavior to maintain amino acid homeostasis in omnivores, Cell Metab. 1 (2005) 273–277.
[18] S.Z. Hao, J.W. Sharp, C.M. Ross-Inta, B.J. McDaniel, T.G. Anthony, R.C. Wek, D.R. Cavener, B.C. McGrath, J.B. Rudell, T.J. Koehnle, D.W. Gietzen, Uncharged tRNA and sensing of amino acid deficiency in mammalian piriform cortex, Science 307 (2005) 1776–1778.
[19] A.L. Mellor, D.H. Munn, IDO expression by dendritic cells: tolerance and tryptophan catabolism, Nat. Rev. Immunol. 4 (2004) 762–774.
[20] D.H. Munn, M.D. Sharma, B. Baban, H.P. Harding, Y.H. Zhang, D. Ron, A.L. Mellor, GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase, Immunity 22 (2005) 633–642.
[21] M. Costa-Mattioli, D. Gobert, H. Harding, B. Herdy, M. Azzi, M. Bruno, M. Bidinosti, C. Ben Mamou, E. Marcinkiewicz, M. Yoshida, H. Imataka, A.C. Cuello, N. Seidah, W. Sossin, J.C. Lacaille, D. Ron, K. Nader, N. Sonenberg, Translational control of hippocampal synaptic plasticity and memory by the eIF2 alpha kinase GCN2, Nature 436 (2005) 1166–1170.
[22] J. Ye, M. Kumanova, L. Hart, K. Sloane, H. Zhang, D. De Panis, E. Bobrovnikova-Marjon, J. Diehl, D. Ron, C. Koumenis, The GCN2–ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation, EMBO J. 29 (2010) 2082–2096.
[23] P. Bunpo, A. Dudley, J.K. Cundiff, D.R. Cavener, R.C. Wek, T.G. Anthony, GCN2 protein kinase is required to activate amino acid deprivation responses in mice treated with the anti-cancer agent l-asparaginase, J. Biol. Chem. 284 (2009) 32742–32749.
[24] S. Lageix, E. Lanet, M.N. Pouch-Pelissier, M.C. Espagnol, C. Robaglia, J.M. Deragon, T. Pelissier, Arabidopsis eIF2alpha kinase GCN2 is essential for growth in stress conditions and is activated by wounding, BMC Plant Biol. 8 (2008) (art. no. 134).
[25] Y. Zhang, J.R. Dickinson, M.J. Paul, N.G. Halford, Molecular cloning of an arabidopsis homologue of GCN2, a protein kinase involved in co-ordinated response to amino acid starvation, Planta 217 (2003) 668–675.
[26] E.C.H. Lee, K. Strange, GCN-2 dependent inhibition of protein synthesis activates osmosensitive gene transcription via WNK and Ste20 kinase signaling, Am. J. Physiol. Cell Physiol. 303 (2012) C1269–C1277.
[27] A. Rousakis, A. Vlassis, A. Vlanti, S. Patera, G. Thireos, P. Syntichaki, The general control nonderepressible-2 kinase mediates stress response and longevity induced by target of rapamycin inactivation in *Caenorhabditis elegans*, Aging Cell 12 (2013) 742–751.
[28] C. Konrad, R.C. Wek, W.J. Sullivan, A GCN2-like eukaryotic initiation factor 2 kinase increases the viability of extracellular *Toxoplasma gondii* parasites, Eukaryot. Cell 10 (2011) 1403–1412.

[29] E. Malzer, M. Szajewska-Skuta, L.E. Dalton, S.E. Thomas, N. Hu, H. Skaer, D.A. Lomas, D.C. Crowther, S.J. Marciniak, Coordinate regulation of eIF2alpha phosphorylation by PPP1R15 and GCN2 is required during *Drosophila* development, J. Cell Sci. 126 (2013) 1406–1415.

[30] V.S. Alves, F.L. Motta, M. Roffe, A. Delamano, J.B. Pesquero, B.A. Castilho, GCN2 activation and eIF2 alpha phosphorylation in the maturation of mouse oocytes, BBRC 378 (2009) 41–44.

[31] N. Donnelly, A.M. Gorman, S. Gupta, A. Samali, The eIF2alpha kinases: their structures and functions, Cell. Mol. Life Sci. (2013) 1–19.

[32] J.R. Murguía, R. Serrano, New functions of protein kinase Gcn2 in yeast and mammals, IUBMB Life 64 (2012) 971–974.

[33] M.A. Trinh, E. Klann, Translational control by eIF2alpha kinases in long-lasting synaptic plasticity and long-term memory, Neurobiol. Learn. Mem. 105 (2013) 93–99.

[34] M.N. Balasubramanian, E.A. Butterworth, M.S. Kilberg, Asparagine synthetase: regulation by cell stress and involvement in tumor biology, Am. J. Physiol. Endocrinol. Metab. 304 (2013) E789–E799.

[35] J. Gallinetti, E. Harputlugil, J.R. Mitchell, Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR, Biochem. J. 449 (2013) 1–10.

[36] T.M. Immanuel, D.R. Greenwood, R.M. MacDiarmid, A critical review of translation initiation factor eIF2α kinases in plants—regulating protein synthesis during stress, Funct. Plant Biol. 39 (2012) 717–735.

[37] T.G. Anthony, D.W. Gietzen, Detection of amino acid deprivation in the central nervous system, Curr. Opin. Clin. Nutr. Metab. Care 16 (2013) 96–101.

[38] M. Garcia-Barrio, J. Dong, S. Ufano, A.G. Hinnebusch, Association of GCN1–GCN20 regulatory complex with the N-terminus of eIF2alpha kinase GCN2 is required for GCN2 activation, EMBO J. 19 (2000) 1887–1899.

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

[40] H. Qiu, M.T. Garcia-Barrio, A.G. Hinnebusch, Dimerization by translation initiation factor 2 kinase GCN2 is mediated by interactions in the C-terminal ribosome-binding region and the protein kinase domain, Mol. Cell. Biol. 18 (1998) 2697–2711.

[41] H. Qiu, J. Dong, C. Hu, C.S. Francklyn, A.G. Hinnebusch, The tRNA-binding moiety in GCN2 contains a dimerization domain that interacts with the kinase domain and is required for tRNA binding and kinase activation, EMBO J. 20 (2001) 1425–1438.

[42] J. Narasimhan, K.A. Staschke, R.C. Wek, Dimerization is required for activation of eIF2 kinase Gcn2 in response to diverse environmental stress conditions, J. Biol. Chem. 279 (2004) 22820–22832.

[43] A.K. Padyana, H. Qiu, A. Roll-Mecak, A.G. Hinnebusch, S.K. Burley, Structural basis for autoinhibition and mutational activation of eukaryotic initiation factor 2alpha protein kinase GCN2, J. Biol. Chem. 280 (2005) 29289–29299.

[44] A. Garriz, H. Qiu, M. Dey, E.J. Seo, T.E. Dever, A.G. Hinnebusch, A network of hydrophobic residues impeding helix alphaC rotation maintains latency of kinase Gcn2, which phosphorylates the alpha subunit of translation initiation factor 2, Mol. Cell. Biol. 29 (2009) 1592–1607.

[45] M. Dey, C. Cao, F. Sicheri, T.E. Dever, Conserved intermolecular salt bridge required for activation of protein kinases PKR, GCN2, and PERK, J. Biol. Chem. 282 (2007) 6653–6660.

[46] P.R. Romano, M.T. Garcia-Barrio, X. Zhang, Q. Wang, D.R. Taylor, F. Zhang, C. Herring, M.B. Mathews, J. Qin, A.G. Hinnebusch, Autophosphorylation in the activation loop is required for full kinase activity in vivo of human and yeast eukaryotic initiation factor 2alpha kinases PKR and GCN2, Mol. Cell. Biol. 18 (1998) 2282–2297.

[47] V. Cherkasova, H. Qiu, A.G. Hinnebusch, Snf1 promotes phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2 by activating Gcn2 and inhibiting phosphatases Glc7 and Sit4, Mol. Cell. Biol. 30 (2010) 2862–2873.

[48] A.G. Hinnebusch, eIF2alpha kinases provide a new solution to the puzzle of substrate specificity, Nat. Struct. Mol. Biol. 12 (2005) 835–838.

[49] H. Qiu, C. Hu, J. Dong, A.G. Hinnebusch, Mutations that bypass tRNA binding activate the intrinsically defective kinase domain in GCN2, Genes Dev. 16 (2002) 1271–1280.

[50] A.C. Dar, T.E. Dever, F. Sicheri, Higher-order substrate recognition of eIF2alpha by the RNA-dependent protein kinase PKR, Cell 122 (2005) 887–900.

[51] M. Dey, A. Velyvis, J.J. Li, E. Chiu, D. Chiovitti, L.E. Kay, F. Sicheri, T.E. Dever, Requirement for kinase-induced conformational change in eukaryotic initiation factor 2alpha (eIF2alpha) restricts phosphorylation of Ser51, Proc. Natl. Acad. Sci. 108 (2011) 4316–4321.

[52] M. Dey, B. Trieselmann, E.G. Locke, J. Lu, C. Cao, A.C. Dar, T. Krishnamoorthy, J. Dong, F. Sicheri, T.E. Dever, PKR and GCN2 kinases and guanine nucleotide exchange factor eukaryotic translation initiation factor 2B (eIF2B) recognize overlapping surfaces on eIF2alpha, Mol. Cell. Biol. 25 (2005) 3063–3075.

[53] H. Mellor, C.G. Proud, A synthetic peptide substrate for initiation factor-2 kinases, Biochem. Biophys. Res. Commun. 178 (1991) 430–437.

[54] M. Dey, C. Cao, A.C. Dar, T. Tamura, K. Ozato, F. Sicheri, T.E. Dever, Mechanistic link between PKR dimerization, autophosphorylation, and eIF2alpha substrate recognition, Cell 122 (2005) 901–913.

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

[56] R.C. Wek, M. Ramirez, B.M. Jackson, A.G. Hinnebusch, Identification of positive-acting domains in GCN2 protein kinase required for translational activation of GCN4 expression, Mol. Cell. Biol. 10 (1990) 2820–2831.

[57] S. Zhu, A.Y. Sobolev, R.C. Wek, Histidyl-tRNA synthetase-related sequences in GCN2 protein kinase regulate in vitro phosphorylation of eIF-2, J. Biol. Chem. 271 (1996) 24989–24994.

[58] S.K. Hanks, A.M. Quinn, T. Hunter, The protein kinase family: conserved features and deduced phylogeny of the catalytic domains, Science 241 (1988) 42–52.

[59] J. Boudeau, D. Miranda-Saavedra, G.J. Barton, D.R. Alessi, Emerging roles of pseudokinases, Trends Cell Biol. 16 (2006) 443–452.

[60] R.C. Wek, B.M. Jackson, A.G. Hinnebusch, 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. 86 (1989) 4579–4583.

[61] E. Sattlegger, A.G. Hinnebusch, Separate domains in GCN1 for binding protein kinase GCN2 and ribosomes are required for GCN2 activation in amino acid-starved cells, EMBO J. 19 (2000) 6622–6633.

[62] H. Kubota, Y. Sakaki, T. Ito, GI domain-mediated association of the eukaryotic initiation factor 2a kinase GCN2 with its activator GCN1 is required for general amino acid control in budding yeast, J. Biol. Chem. 275 (2000) 20243–20246.

[63] N. Nameki, M. Yoneyama, S. Koshiba, N. Tochio, M. Inoue, E. Seki, T. Matsuda, Y. Tomo, T. Harada, K. Saito, N. Kobayashi, T. Yabuki, M. Aoki, E. Nunokawa, N. Matsuda, N. Sakagami, T. Terada, M. Shirouzu, M. Yoshida, H. Hirota, T. Osanai, A. Tanaka, T. Arakawa, P. Carninci, J. Kawai, Y. Hayashizaki, K. Kinoshita, P. Guntert, T. Kigawa, S. Yokoyama, Solution structure of the RWD domain of the mouse GCN2 protein, Protein Sci. 13 (2004) 2089–2100.

[64] E. Sattlegger, J.A.R.G. Barbosa, M.C.S. Moraes, R.M. Martins, A.G. Hinnebusch, B.A. Castilho, Gcn1 and actin binding to Yih1: implications for activation of the eIF2 kinase Gcn2, J. Biol. Chem. 286 (2011) 10341–10355.

[65] S. Hunter, P. Jones, A. Mitchell, R. Apweiler, T.K. Attwood, A. Bateman, T. Bernard, D. Binns, P. Bork, S. Burge, E. de Castro, P. Coggill, M. Corbett, U. Das, L. Daugherty, L. Duquenne, R.D. Finn, M. Fraser, J. Gough, D. Haft, N. Hulo, D. Kahn, E. Kelly, I. Letunic, D. Lonsdale, R. Lopez, M. Madera, J. Maslen, C. McAnulla, J. McDowall, C. McMenamin, H. Mi, P. Mutowo-Muellenet, N. Mulder, D. Natale, C. Orengo, S. Pesseat, M. Punta, A.F. Quinn, C. Rivoire, A. Sangrador-Vegas, J.D. Selengut, C.J.A. Sigrist, M. Scheremetjew, J. Tate, M. Thimmajanarthanan, P.D. Thomas, C.H. Wu, C. Yeats, S.-Y. Yong, InterPro in 2011: new developments in the family and domain prediction database, Nucleic Acids Res. 40 (2012) D306–D312.

[66] T. Doerks, R.R. Copley, J. Schultz, C.P. Ponting, P. Bork, Systematic identification of novel protein domain families associated with nuclear functions, Genome Res. 12 (2002) 47–56.

[67] S.A. Wek, S. Zhu, R.C. Wek, The histidyl-tRNA synthetase-related sequence in the eIF-2alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids, Mol. Cell. Biol. 15 (1995) 4497–4506.

[68] M. Ruff, S. Krishnaswamy, M. Boeglin, A. Poterszman, A. Mitschler, A. Podjarny, B. Rees, J.C. Thierry, D. Moras, Class II aminoacyl transfer RNA synthetases: crystal structure of yeast aspartyl-tRNA synthetase complexed with tRNA Asp, Science 252 (1991) 1682–1689.

[69] J. Dong, H. Qiu, M. Garcia-Barrio, J. Anderson, A.G. Hinnebusch, Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain, Mol. Cell 6 (2000) 269–279.

[70] S. Zhu, R.C. Wek, Ribosome-binding domain of eukaryotic initiation factor-2 kinase GCN2 facilitates translation control, J. Biol. Chem. 273 (1998) 1808–1814.

[71] J.G. Arnez, D.C. Harris, A. Mitschler, B. Rees, C.S. Francklyn, D. Moras, Crystal structure of histidyl-tRNA synthetase from *Escherichia coli* complexed with histidyl-adenylate, EMBO J. 14 (1995) 4143–4155.

[72] J.M. Zaborske, J. Narasimhan, L. Jiang, S.A. Wek, K.A. Dittmar, F. Freimoser, T. Pan, R. C. Wek, Genome-wide analysis of tRNA charging and activation of the eIF2 kinase Gcn2p, J. Biol. Chem. 284 (2009) 25254–25267.

[73] M.W. Li, W.K. Auyeung, H.M. Lam, The GCN2 homologue in *Arabidopsis thaliana* interacts with uncharged tRNA and uses *Arabidopsis* eIF2alpha molecules as direct substrates, Plant Biol. 15 (2013) 13–18.

[74] M.S. Sundrud, S.B. Koralov, M. Feuerer, D.P. Calado, A.E. Kozhaya, A. Rhule-Smith, R.E. Lefebvre, D. Unutmaz, R. Mazitschek, H. Waldner, M. Whitman, T. Keller, A. Rao, Halofuginone inhibits th17 cell differentiation by activating the amino acid starvation response, Science 324 (2009) 1334–1338.

[75] T.L. Keller, D. Zocco, M.S. Sundrud, M. Hendrick, M. Edenius, J. Yum, Y.J. Kim, H.K. Lee, J.F. Cortese, D.F. Wirth, J.D. Dignam, A. Rao, C.Y. Yeo, R. Mazitschek, M. Whitman, Halofuginone and other febrifugine derivatives inhibit prolyl-tRNA synthetase, Nat. Chem. Biol. 8 (2012) 311–317.

[76] D. Habibi, N. Ogloff, R.B. Jalili, A. Yost, A.P. Weng, A. Ghahary, C.J. Ong, Borrelidin, a small molecule nitrile-containing macrolide inhibitor of threonyl-tRNA synthetase, is a potent inducer of apoptosis in acute lymphoblastic leukemia, Investig. New Drugs 30 (2012) 1361–1370.

[77] R. Yang, S.A. Wek, R.C. Wek, Glucose limitation induces GCN4 translation by activation of Gcn2 protein kinase, Mol. Cell. Biol. 20 (2000) 2706–2717.

[78] R.J. Rolfes, A.G. Hinnebusch, Translation of the yeast transcriptional activator Gcn4 is stimulated by purine—implications for activation of the protein kinase Gcn2, Mol. Cell. Biol. 13 (1993) 5099–5111.

[79] W. Gu, R.L. Hurto, A.K. Hopper, E.J. Grayhack, E.M. Phizicky, Depletion of *Saccharomyces cerevisiae* tRNAHis guanylyltransferase Thg1p leads to uncharged tRNAHis with additional m5C, Mol. Cell. Biol. 25 (2005) 8191–8201.

[80] G. Hueso, R. Aparicio-Sanchis, C. Montesinos, S. Lorenz, J.R. Murguia, R. Serrano, A novel role for protein kinase Gcn2 in yeast tolerance to intracellular acid stress, Biochem. J. 441 (2012) 255–264.

[81] I. Uluisik, A. Kaya, D.E. Fomenko, H.C. Karakaya, B.A. Carlson, V.N. Gladyshev, A. Koc, Boron stress activates the general amino acid control mechanism and inhibits protein synthesis, PLoS One 6 (2011).

[82] J. Deng, H.P. Harding, B. Raught, A.C. Gingras, J.J. Berlanga, D. Scheuner, R.J. Kaufman, D. Ron, N. Sonenberg, Activation of gcn2 in UV-irradiated cells inhibits translation, Curr. Biol. 12 (2002) 1279–1286.

[83] W. Lu, C.F. Laszlo, Z. Miao, H. Chen, S. Wu, The role of nitric-oxide synthase in the regulation of UVB light-induced phosphorylation of the alpha subunit of eukaryotic initiation factor 2, J. Biol. Chem. 284 (2009) 24281–24288.

[84] N.H. Kwon, T. Kang, J.Y. Lee, H.H. Kim, H.R. Kim, J. Hong, Y.S. Oh, J.M. Han, M.J. Ku, S. Y. Lee, S. Kim, Dual role of methionyl-tRNA synthetase in the regulation of translation and tumor suppressor activity of aminoacyl-tRNA synthetase-interacting multifunctional protein-3, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 19635–19640.

[85] N. Rabbani, P.J. Thornalley, Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome, Amino Acids 42 (2012) 1133–1142.

[86] W. Nomura, K. Maeta, K. Kita, S. Izawa, Y. Inoue, Role of Gcn4 for adaptation to methylglyoxal in *Saccharomyces cerevisiae*: methylglyoxal attenuates protein synthesis through phosphorylation of eIF2alpha, Biochem. Biophys. Res. Commun. 376 (2008) 738–742.

[87] W. Nomura, K. Maeta, K. Kita, S. Izawa, Y. Inoue, Methylglyoxal activates Gcn2 to phosphorylate eIF2alpha independently of the TOR pathway in *Saccharomyces cerevisiae*, Appl. Microbiol. Biotechnol. 86 (2010) 1887–1894.

[88] V.A. Cherkasova, A.G. Hinnebusch, Translational control by TOR and TAP42 through dephosphorylation of eIF2alpha kinase GCN2, Genes Dev. 17 (2003) 859–872.

[89] H. Kubota, T. Obata, K. Ota, T. Sasaki, T. Ito, Rapamycin-induced translational derepression of GCN4 mRNA involves a novel mechanism for activation of the eIF2alpha kinase GCN2, J. Biol. Chem. 278 (2003) 20457–20460.

[90] I. Tattoli, M.T. Sorbara, D. Vuckovic, A. Ling, F. Soares, L.A.M. Carneiro, C. Yang, A. Emili, D.J. Philpott, S.E. Girardin, Amino acid starvation induced by invasive bacterial pathogens triggers an innate host defense program, Cell Host Microbe 11 (2012) 563–575.

[91] I. Tattoli, M.T. Sorbara, C. Yang, S.A. Tooze, D.J. Philpott, S.E. Girardin, Listeria phospholipases subvert host autophagic defenses by stalling pre-autophagosomal structures, EMBO J. 32 (2013) 3066–3078.

[92] T. Cambiaghi, C.M. Pereira, A. Delamano, R. Shanmugam, M. Bolech, R.C. Wek, E. Sattlegger, B.A. Castilho, Evolutionarily conserved IMPACT impairs various stress responses that require GCN1 for activating the eIF2 kinase GCN2, Biochem. Biophys. Res. Commun. 443 (2014) 592–597.

[93] R.H. Wade, On and around microtubules: an overview, Mol. Biotechnol. 43 (2009) 177–191.

[94] H.Y. Jiang, R.C. Wek, Phosphorylation of the alpha-Subunit of the Eukaryotic Initiation Factor-2 (eIF2) reduces protein synthesis and enhances apoptosis in response to proteasome inhibition, J. Biol. Chem. 280 (2005) 14189–14202.

[95] A. Suraweera, C. Munch, A. Hanssum, A. Bertolotti, Failure of amino acid homeostasis causes cell death following proteasome inhibition, Mol. Cell 48 (2012) 242–253.

[96] J.J. Berlanga, I. Ventoso, H.P. Harding, J. Deng, D. Ron, N. Sonenberg, L. Carrasco, C. de Haro, Antiviral effect of the mammalian translation initiation factor 2 alpha kinase GCN2 against RNA viruses, EMBO J. 25 (2006) 1730–1740.

[97] P. Yao, P.L. Fox, Aminoacyl-tRNA synthetases in medicine and disease, EMBO Mol. Med. 5 (2013) 332–343.

[98] M. Ramirez, R.C. Wek, A.G. Hinnebusch, Ribosome association of GCN2 protein kinase, a translational activator of the GCN4 gene of *Saccharomyces cerevisiae*, Mol. Cell. Biol. 11 (1991) 3027–3036.

[99] M.J. Marton, C.R. Vazquez De Aldana, H. Qiu, K. Chakraburtty, A.G. Hinnebusch, Evidence that GCN1 and GCN20, translational regulators of GCN4, function on elongating ribosomes in activation of eIF2alpha kinase GCN2, Mol. Cell. Biol. 17 (1997) 4474–4489.

[100] A. Jimenez-Diaz, M. Remacha, J.P. Ballesta, J.J. Berlanga, Phosphorylation of initiation factor eIF2 in response to stress conditions is mediated by acidic ribosomal P1/P2 proteins in *Saccharomyces cerevisiae*, PLoS One 8 (2013) e84219.

[101] M.J. Marton, D. Crouch, A.G. Hinnebusch, GCN1, a translational activator of GCN4 in *Saccharomyces cerevisiae*, is required for phosphorylation of eukaryotic translation initiation factor 2 by protein kinase GCN2, Mol. Cell. Biol. 13 (1993) 3541–3556.

[102] M. Ramirez, R.C. Wek, C.R. Vasquez de Aldana, B.M. Jackson, B. Freeman, A.G. Hinnebusch, Mutations activating the yeast eIF-2alpha kinase GCN2: isolation of alleles altering the domain related to histidyl-tRNA synthetases, Mol. Cell. Biol. 12 (1992) 5801–5815.

[103] M.A. Andrade, C. Petosa, S.I. O'Donoghue, C.W. Muller, P. Bork, Comparison of ARM and HEAT protein repeats, J. Mol. Biol. 309 (2001) 1–18.

[104] F.J. Trianaalonso, K. Chakraburtty, K.H. Nierhaus, The elongation-factor-3 unique in higher fungi and essential for protein-biosythesis is an E-site factor, J. Biol. Chem. 270 (1995) 20473–20478.

[105] C.B. Andersen, T. Becker, M. Blau, M. Anand, M. Halic, B. Balar, T. Mielke, T. Boesen, J.S. Pedersen, C.M. Spahn, Structure of eEF3 and the mechanism of transfer RNA release from the E-site, Nature 443 (2006) 663–668.

[106] K. Chakraburtty, Functional interaction of yeast elongation factor 3 with yeast ribosomes, Int. J. Biochem. Cell Biol. 31 (1999) 163–173.

[107] H. Kubota, K. Ota, Y. Sakaki, T. Ito, Budding yeast GCN1 binds the GI domain to activate the eIF2alpha kinase GCN2, J. Biol. Chem. 276 (2001) 17591–17596.

[108] E. Sattlegger, A.G. Hinnebusch, Polyribosome binding by GCN1 is required for full activation of eIF2a kinase GCN2 during amino acid starvation, J. Biol. Chem. 280 (2005) 16514–16521.

[109] R.R. Gontarek, H. Li, K. Nurse, C.D. Prescott, The N terminus of eukaryotic translation elongation factor 3 interacts with 18 S rRNA and 80 S ribosomes, J. Biol. Chem. 273 (1998) 10249–10252.

[110] J.M. Ogle, A.P. Carter, V. Ramakrishnan, Insights into the decoding mechanism from recent ribosome structures, Trends Biochem. Sci. 28 (2003) 259–266.

[111] C.M. Pereira, E. Sattlegger, H.Y. Jiang, B.M. Longo, C.B. Jaqueta, A.G. Hinnebusch, R.C. Wek, L. Mello, B.A. Castilho, IMPACT, a protein preferentially expressed in the mouse brain, binds GCN1 and inhibits GCN2 activation, J. Biol. Chem. 280 (2005) 28316–28323.

[112] M. Roffe, G.N.M. Hajj, H.F. Azevedo, V.S. Alves, B.A. Castilho, IMPACT is a developmentally regulated protein in neurons that opposes the eIF2a kinase GCN2 in the modulation of neurite outgrowth, J. Biol. Chem. 288 (2013) 10860–10869.

[113] A. Nukazuka, H. Fujisawa, T. Inada, Y. Oda, S. Takagi, Semaphorin controls epidermal morphogenesis by stimulating mRNA translation via eIF2alpha in *Caenorhabditis elegans*, Genes Dev. 22 (2008) 1025–1036.

[114] J. Monaghan, X. Li, The HEAT repeat protein ILITYHIA is required for plant immunity, Plant Cell Physiol. 51 (2010) 742–753.

[115] A.J. Johnston, P. Meier, J. Gheyselinck, S.E.J. Wuest, M. Federer, E. Schlagenhauf, J.D. Becker, U. Grossniklaus, Genetic subtraction profiling identifies genes essential for *Arabidopsis* reproduction and reveals interaction between the female gametophyte and the maternal sporophyte, Genome Biol. 8 (2007).

[116] A.C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J.M. Rick, A.M. Michon, C.M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M.A. Heurtier, R.R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, Functional organization of the yeast proteome by systematic analysis of protein complexes, Nature 415 (2002) 141–147.

[117] P. Uetz, L. Giot, G. Cagney, T.A. Mansfield, R.S. Judson, J.R. Knight, D. Lockshon, V. Narayan, M. Srinivasan, P. Pochart, A. Qureshi-Emili, Y. Li, B. Godwin, D. Conover, T. Kalbfleisch, G. Vijayadamodar, M.J. Yang, M. Johnston, S. Fields, J.M. Rothberg, A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*, Nature 403 (2000) 623–627.

[118] Y. Ho, A. Gruhler, A. Heilbut, G.D. Bader, L. Moore, S.L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutilier, L. Yang, C. Wolting, I. Donaldson, S. Schandorff, J. Shewnarane, M. Vo, J. Taggart, M. Goudreault, B. Muskat, C. Alfarano, D. Dewar, Z. Lin, K. Michalickova, A.R. Willems, H. Sassi, P.A. Nielsen, K.J. Rasmussen, J.R. Andersen, L.E. Johansen, L.H. Hansen, H. Jespersen, A. Podtelejnikov, E. Nielsen, J. Crawford, V. Poulsen, B.D. Sørensen, J. Matthiesen, R.C. Hendrickson, F. Gleeson, T. Pawson, M.F. Moran, D. Durocher, M. Mann, C.W. Hogue, D. Figeys, M. Tyers, Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry, Nature 415 (2002) 180–183.

[119] N.J. Krogan, G. Cagney, H.Y. Yu, G.Q. Zhong, X.H. Guo, A. Ignatchenko, J. Li, S.Y. Pu, N. Datta, A.P. Tikuisis, T. Punna, J.M. Peregrin-Alvarez, M. Shales, X. Zhang, M. Davey, M.D. Robinson, A. Paccanaro, J.E. Bray, A. Sheung, B. Beattie, D.P. Richards, V. Canadien, A. Lalev, F. Mena, P. Wong, A. Starostine, M.M. Canete, J. Vlasblom, S. Wu, C. Orsi, S.R. Collins, S. Chandran, R. Haw, J.J. Rilstone, K. Gandi, N.J. Thompson, G. Musso, P. St Onge, S. Ghanny, M.H.Y. Lam, G. Butland, A.M. Altaf-Ui, S. Kanaya, A. Shilatifard, E. O'Shea, J.S. Weissman, C.J. Ingles, T.R. Hughes, J. Parkinson, M. Gerstein, S.J. Wodak, A. Emili, J.F. Greenblatt, Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*, Nature 440 (2006) 637–643.

[120] C.R.V. deAldana, M.J. Marton, A.G. Hinnebusch, Gcn20, a novel ATP binding cassette protein, and GCN1 reside in a complex that mediates activation of the eIF-2-alpha kinase GCN2 in aminoacid-starved cells, EMBO J. 14 (1995) 3184–3199.

[121] I.D. Kerr, Sequence analysis of twin ATP binding cassette proteins involved in translational control, antibiotic resistance, and ribonuclease L inhibition, Biochem. Biophys. Res. Commun. 315 (2004) 166–173.

[122] J.K. Tyzack, X. Wang, G.J. Belsham, C.G. Proud, ABC50 interacts with eukaryotic initiation factor 2 and associates with the ribosome in an ATP-dependent manner, J. Biol. Chem. 275 (2000) 34131–34139.

[123] Z. Bozdech, U. Delling, S.K. Volkman, A.F. Cowman, E. Schurr, Cloning and sequence analysis of a novel member of the ATP-binding cassette (ABC) protein gene family from *Plasmodium falciparum*, Mol. Biochem. Parasitol. 81 (1996) 41–51.

[124] Z. Bozdech, E. Schurr, Protein transport in the host cell cytoplasm and ATP-binding cassette proteins in *Plasmodium falciparum*-infected erythrocytes, Novartis Found. Symp. 226 (1999) 231–245.

[125] Z. Bozdech, J. VanWye, K. Haldar, E. Schurr, The human malaria parasite *Plasmodium falciparum* exports the ATP-binding cassette protein PFGCN20 to membrane structures in the host red blood cell, Mol. Biochem. Parasitol. 97 (1998) 81–95.

[126] C. Fennell, S. Babbitt, I. Russo, J. Wilkes, L. Ranford-Cartwright, D.E. Goldberg, C. Doerig, PfeIK1, a eukaryotic initiation factor 2 alpha kinase of the human malaria parasite *Plasmodium falciparum*, regulates stress-response to amino-acid starvation, Malar. J. 8 (2009) (art. no. 99).

[127] M.J. Murchie, D.P. Leader, Coden-specific interaction of uncharged transfer-RNA with eukaryotic ribosomes, Biochim. Biophys. Acta 520 (1978) 233–236.

[128] M. Cashel, D. Gentry, V. Hernandez, D. Vinella, *Escherichia coli* and *Salmonella*: cellular and molecular biology, *Escherichia coli* and *Salmonella*, Cell. Mol. Biol. 1 (1996) 1458–1496.

[129] E. Goldman, H. Jakubowski, Uncharged tRNA, protein synthesis, and the bacterial stringent response, Mol. Microbiol. 4 (1990) 2035–2040.

[130] K. Potrykus, M. Cashel, (p) ppGpp: still magical?*, Annu. Rev. Microbiol. 62 (2008) 35–51.

[131] W.A. Haseltine, R. Block, Synthesis of guanosine tetra- and pentaphosphate requires the presence of a codon-specific, uncharged transfer ribonucleic acid in the acceptor site of ribosomes, Proc. Natl. Acad. Sci. 70 (1973) 1564–1568.

[132] S. Schilling-Bartetzko, F. Franceschi, H. Sternbach, K.H. Nierhaus, Apparent association constants of tRNAs for the ribosomal A, P, and E sites, J. Biol. Chem. 267 (1992) 4693–4702.

[133] T.M. Wendrich, G. Blaha, D.N. Wilson, M.A. Marahiel, K.H. Nierhaus, Dissection of the mechanism for the stringent factor RelA, Mol. Cell 10 (2002) 779–788.

[134] B.P. English, V. Hauryliuk, A. Sanamrad, S. Tankov, N.H. Dekker, J. Elf, Single-molecule investigations of the stringent response machinery in living bacterial cells, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) E365–E373.

[135] S. Ghaemmaghami, W.K. Huh, K. Bower, R.W. Howson, A. Belle, N. Dephoure, E.K. O'Shea, J.S. Weissman, Global analysis of protein expression in yeast, Nature 425 (2003) 737–741.
[136] F.S. Pedersen, N.O. Kjeldgaard, Analysis of the relA gene product of *Escherichia coli*, Eur. J. Biochem. 76 (1977) 91–97.
[137] T. Waller, S.J. Lee, E. Sattlegger, Evidence that Yih1 resides in a complex with ribosomes, FEBS J. 279 (2012) 1761–1776.
[138] J.R. Warner, The economics of ribosome biosynthesis in yeast, Trends Biochem. Sci. 24 (1999) 437–440.
[139] A.G. Cridge, J. Visweswaraiah, R. Ramesh, E. Sattlegger, Semi-quantitative colony immunoassay for determining and optimizing protein expression in *Saccharomyces cerevisiae* and *Escherichia coli*, Anal. Biochem. 447 (2013) 82–89.
[140] J. Visweswaraiah, S.J. Lee, A.G. Hinnebusch, E. Sattlegger, Overexpression of eukaryotic translation Elongation Factor 3 (eEF3) impairs GCN2 activation, J. Biol. Chem. 287 (2012) 37757–37768.
[141] S. Bittencourt, C.M. Pereira, M. Avedissian, A. Delamano, L.E. Mello, B.A. Castilho, Distribution of the protein IMPACT, an inhibitor of GCN2, in the mouse, rat, and marmoset brain, J. Comp. Neurol. 507 (2008) 1811–1830.
[142] C. Konrad, R.C. Wek, W.J. Sullivan Jr., GCN2-like eIF2alpha kinase manages the amino acid starvation response in *Toxoplasma gondii*, Int. J. Parasitol. 44 (2014) 139–146.
[143] R. Fang, Y. Xiong, C.K. Singleton, IfkA, a presumptive eIF2alpha kinase of Dictyostelium, is required for proper timing of aggregation and regulation of mound size, BMC Dev. Biol. 3 (2003) 1–19 (art. no. 1).
[144] M. Rai, Y.H. Xiong, C.K. Singleton, Disruption of the ifkA and ifkB genes results in altered cell adhesion, morphological defects and a propensity to form pre-stalk O cells during development of Dictyostelium, Differentiation 74 (2006) 583–595.
[145] J. Visweswaraiah, S. Lageix, B.A. Castilho, L. Izotova, T.G. Kinzy, A.G. Hinnebusch, E. Sattlegger, Evidence that eukaryotic translation elongation factor 1A (eEF1A) binds the Gcn2 C-terminus and inhibits Gcn2 activity, J. Biol. Chem. 286 (2011) 36568–36579.
[146] T.E. Dever, R. Green, The elongation, termination, and recycling phases of translation in eukaryotes, Cold Spring Harb. Perspect. Biol. 4 (2012).
[147] B. Dasmahapatra, K. Chakraburtty, Protein synthesis in yeast. I. Purification and properties of elongation factor 3 from *Saccharomyces cerevisiae*, J. Biol. Chem. 256 (1981) 9999–10004.
[148] A.V. El'skaya, G.V. Ovcharenko, S.S. Palchevskii, Z.M. Petrushenko, F.J. Triana-Alonso, K.H. Nierhaus, Three tRNA binding sites in rabbit liver ribosomes and role of the intrinsic ATPase in 80S ribosomes from higher eukaryotes, Biochemistry 36 (1997) 10492–10497.
[149] M.V. Rodnina, A.I. Serebryanik, G.V. Ovcharenko, A.V. El'skaya, ATPase strongly bound to higher eukaryotic ribosomes, Eur. J. Biochem. 225 (1994) 305–310.
[150] M.C. Kiel, H. Aoki, M.C. Ganoza, Identification of a ribosomal ATPase in *Escherichia coli* cells, Biochimie 81 (1999) 1097–1108.
[151] M.C. Kiel, M.C. Ganoza, Functional interactions of an *Escherichia coli* ribosomal ATPase, Eur. J. Biochem. 268 (2001) 278–286.
[152] J. Xu, M.C. Kiel, A. Golshani, J.G. Chosay, H. Aoki, M.C. Ganoza, Molecular localization of a ribosome-dependent ATPase on *Escherichia coli* ribosomes, Nucleic Acids Res. 34 (2006) 1158–1165.
[153] J. Visweswaraiah, S. Lageix, B.A. Castilho, L. Izotova, T.G. Kinzy, A.G. Hinnebusch, E. Sattlegger, Evidence that eukaryotic translation elongation factor 1A (eEF1A) binds the Gcn2 C-terminus and inhibits Gcn2 activity, J. Biol. Chem. 286 (2011) 36568–36579.
[154] S. Kim, P.A. Coulombe, Emerging role for the cytoskeleton as an organizer and regulator of translation, Nat. Rev. Mol. Cell Biol. 11 (2010) 75–81.
[155] M.K. Mateyak, T.G. Kinzy, eEF1A: thinking outside the ribosome, J. Biol. Chem. 285 (2010) 21209–21213.
[156] S.R. Gross, T.G. Kinzy, Translation elongation factor 1A is essential for regulation of the actin cytoskeleton and cell morphology, Nat. Struct. Mol. Biol. 12 (2005) 772–778.
[157] A.N. Sasikumar, W.B. Perez, T.G. Kinzy, The many roles of the eukaryotic elongation factor 1 complex, Wiley Interdiscip. Rev. RNA 3 (2012) 543–555.
[158] M.H. Lee, Y.J. Surh, eEF1A2 as a putative oncogene, Ann. N. Y. Acad. Sci. 1171 (2009) 87–93.
[159] S. Thornton, N. Anand, D. Purcell, J. Lee, Not just for housekeeping: protein initiation and elongation factors in cell growth and tumorigenesis, J. Mol. Med. 81 (2003) 536–548.
[160] B.T. Edmonds, J. Wyckoff, Y.G. Yeung, Y. Wang, E.R. Stanley, J. Jones, J. Segall, J. Condeelis, Elongation factor-1 alpha is an overexpressed actin binding protein in metastatic rat mammary adenocarcinoma, J. Cell Sci. 109 (1996) 2705–2714.
[161] Y. Hagiwara, M. Hirai, K. Nishiyama, I. Kanazawa, T. Ueda, Y. Sakaki, T. Ito, Screening for imprinted genes by allelic message display: identification of a paternally expressed gene *Impact* on mouse chromosome 18, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 9249–9254.
[162] E.V. Koonin, P. Bork, C. Sander, Yeast chromosome III: new gene functions, EMBO J. 13 (1994) 493–503.
[163] Y. Yamada, Y. Hagiwara, K. Shiokawa, Y. Sakaki, T. Ito, Spatiotemporal, allelic, and enforced expression of Ximpact, the Xenopus homolog of mouse imprinted gene Impact, Biochem. Biophys. Res. Commun. 256 (1999) 162–169.
[164] K. Okamura, Y. Sakaki, T. Ito, Comparative genomics approach toward critical determinants for the imprinting of an evolutionarily conserved gene Impact, Biochem. Biophys. Res. Commun. 329 (2005) 824–830.
[165] E. Sattlegger, M.J. Swanson, E.A. Ashcraft, J.L. Jennings, R.A. Fekete, A.J. Link, A.G. Hinnebusch, YIH1 is an actin-binding protein that inhibits protein kinase GCN2 and impairs general amino acid control when overexpressed, J. Biol. Chem. 279 (2004) 29952–29962.
[166] T. Shigeoka, B. Lu, C.E. Holt, RNA-based mechanisms underlying axon guidance, J. Cell Biol. 202 (2013) 991–999.
[167] E.T.E. Klann, Biochemical mechanisms for translational regulation in synaptic plasticity, Nature Reviews Neuroscience, vol. 5, Nature Publishing Group, 2004. 931–942.
[168] F. Park, K. Gajiwala, G. Eroshkina, E. Furlong, D. He, Y. Batiyenko, R. Romero, J. Christopher, J. Badger, J. Hendle, J. Lin, T. Peat, S. Buchanan, Crystal structure of YIGZ, a conserved hypothetical protein from *Escherichia coli* K12 with a novel fold, Proteins Struct. Funct. Bioinform. 55 (2004) 775–777.
[169] V.N. Uversky, J.R. Gillespie, A.L. Fink, Why are "natively unfolded" proteins unstructured under physiologic conditions? Proteins Struct. Funct. Bioinform. 41 (2000) 415–427.
[170] A. Tantos, K.H. Han, P. Tompa, Intrinsic disorder in cell signaling and gene transcription, Mol. Cell. Endocrinol. 348 (2012) 457–465.
[171] M. Costa-Mattioli, W.S. Sossin, E. Klann, N. Sonenberg, Translational control of long-lasting synaptic plasticity and memory, Neuron 61 (2009) 10–26.
[172] D.W. Gietzen, C.M. Ross, S.Z. Hao, J.W. Sharp, Phosphorylation of eIF2 alpha is involved in the signaling of indispensable amino acid deficiency in the anterior piriform cortex of the brain in rats, Experimental Biology 2003 Meeting, San Diego, Ca, 2003, pp. 717–723.
[173] A. Chen, I.A. Muzzio, G. Malleret, D. Bartsch, M. Verbitsky, P. Pavlidis, A.L. Yonan, S. Vronskaya, M.B. Grody, I. Cepeda, T.C. Gilliam, E.R. Kandel, Inducible enhancement of memory storage and synaptic plasticity in transgenic mice expressing an inhibitor of ATF4 (CREB-2) and C/EBP proteins, Neuron 39 (2003) 655–669.
[174] R.B. Jalili, F. Forouzandeh, A. Moeenrezakhanlou, G.R. Rayat, R.V. Rajotte, H. Uludag, A. Ghahary, Mouse pancreatic islets are resistant to indoleamine 2,3 dioxygenase-induced general control nonderepressible-2 kinase stress pathway and maintain normal viability and function, Am. J. Pathol. 174 (2009) 196–205.
[175] D. Habibi, R.B. Jalili, F. Forouzandeh, C.J. Ong, A. Ghahary, High expression of IMPACT protein promotes resistance to indoleamine 2,3-dioxygenase-induced cell death, J. Cell. Physiol. 225 (2010) 196–205.
[176] P. Wout, E. Sattlegger, S.M. Sullivan, J.R. Maddock, *Saccharomyces cerevisiae* Rbg1 protein and its binding partner Gir2 interact on polyribosomes with Gcn1, Eukaryot. Cell 8 (2009) 1061–1071.
[177] K. Ishikawa, K. Ito, J.I. Inoue, K. Semba, Cell growth control by stable Rbg2/Gir2 complex formation under amino acid starvation, Genes Cells 18 (2013) 859.
[178] V.S. Alves, B.A. Castilho, Gir2 is an intrinsically unstructured protein that is present in Saccharomyces cerevisiae as a group of heterogeneously electrophoretic migrating forms, Biochem. Biophys. Res. Commun. 332 (2005) 450–455.
[179] V.S. Alves, D.C. Pimenta, E. Sattlegger, B.A. Castilho, Biophysical characterization of Gir2, a highly acidic protein of *Saccharomyces cerevisiae* with anomalous electrophoretic behavior, Biochem. Biophys. Res. Commun. 314 (2004) 229–234.
[180] M. Rechsteiner, S.W. Rogers, PEST sequences and regulation by proteolysis, Trends Biochem. Sci. 21 (1996) 267–271.
[181] N. Kang, D. Chen, L. Wang, L. Duan, S.R. Liu, L. Tang, Q.F. Liu, L.X. Cui, W. He, Rwdd1, a thymus aging related molecule, is a new member of the intrinsically unstructured protein family, Cell. Mol. Immunol. 5 (2008) 333–339.
[182] K. Ishikawa, S. Azuma, S. Ikawa, K. Semba, J.I. Inoue, Identification of DRG family regulatory proteins (DFRPs): specific regulation of DRG1 and DRG2, Genes Cells 10 (2005) 139–150.
[183] K. Ishikawa, T. Akiyama, K. Ito, K. Semba, J.I. Inoue, Independent stabilizations of polysomal Drg1/Dfrp1 complex and non-polysomal Drg2/Dfrp2 complex in mammalian cells, Biochem. Biophys. Res. Commun. 390 (2009) 552–556.
[184] J. del Pino, J.L. Jimenez, I. Ventoso, A. Castello, M.A. Munoz-Fernandez, C. de Haro, J.J. Berlanga, GCN2 has inhibitory effect on human immunodeficiency virus-1 protein synthesis and is cleaved upon viral infection, PLoS One 7 (2012) e47272.
[185] O. Cosnefroy, A. Jaspart, C. Calmels, V. Parissi, H. Fleury, M. Ventura, S. Reigadas, M. L. Andreola, Activation of GCN2 upon HIV-1 infection and inhibition of translation, Cell. Mol. Life Sci. (2013) 1–11.
[186] S. Won, C. Eidenschenk, C.N. Arnold, O.M. Siggs, L. Sun, K. Brandl, T.M. Mullen, G.R. Nemerow, E.M.Y. Moresco, B. Beutler, Increased susceptibility to DNA virus infection in mice with a GCN2 mutation, J. Virol. 86 (2012) 1802–1808.
[187] E. Beattie, J. Tartaglia, E. Paoletti, Vaccinia virus-encoded eIF-2alpha homolog abrogates the antiviral effect of interferon, Virology 183 (1991) 419–422.
[188] M. Kawagishi-Kobayashi, C. Cao, J. Lu, K. Ozato, T.E. Dever, Pseudosubstrate inhibition of protein kinase PKR by swine pox virus C8L gene product, Virology 276 (2000) 424–434.
[189] S. Rothenburg, V.G. Chinchar, T.E. Dever, Characterization of a ranavirus inhibitor of the antiviral protein kinase PKR, BMC Microbiol. 11 (2011) 56.
[190] W. Qian, S. Zhu, A.Y. Sobolev, R.C. Wek, Expression of vaccinia virus K3L protein in yeast inhibits eukaryotic initiation factor-2 kinase GCN2 and the general amino acid control pathway, J. Biol. Chem. 271 (1996) 13202–13207.
[191] M. KawagishiKobayashi, J.B. Silverman, T.L. Ung, T.E. Dever, Regulation of the protein kinase PKR by the vaccinia virus pseudosubstrate inhibitor K3L is dependent on residues conserved between the K3L protein and the PKR substrate eIF2 alpha, Mol. Cell. Biol. 17 (1997) 4146–4158.
[192] C.G. Proud, Control of the translational machinery by amino acids, Am. J. Clin. Nutr. 99 (2014) 231S–236S.
[193] R. Loewith, M.N. Hall, Target of rapamycin (TOR) in nutrient signaling and growth control, Genetics 189 (2011) 1177–1201.
[194] S. Sengupta, T.R. Peterson, D.M. Sabatini, Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress, Mol. Cell 40 (2010) 310–322.
[195] E. Jacinto, M.N. Hall, TOR signalling in bugs, brain and brawn, Nat. Rev. Mol. Cell Biol. 4 (2003) 117–126.
[196] G. Thomas, M.N. Hall, TOR signalling and control of cell growth, Curr. Opin. Cell Biol. 9 (1997) 782–787.
[197] M. Garcia-Barrio, J. Dong, V.A. Cherkasova, X. Zhang, F. Zhang, S. Ufano, R. Lai, J. Qin, A. G. Hinnebusch, Serine 577 Is phosphorylated and negatively affects the tRNA binding and eIF2alpha kinase activities of GCN2, J. Biol. Chem. 277 (2002) 30675–30683.

[198] M. Kimpe, K. Voordeckers, J.M. Thevelein, G. Van Zeebroeck, Pkh1 interacts with and phosphorylates components of the yeast Gcn2/eIF2alpha system, Biochem. Biophys. Res. Commun. 419 (2012) 89–94.

[199] J.r. Urban, A. Soulard, A. Huber, S. Lippman, D. Mukhopadhyay, O. Deloche, V. Wanke, D. Anrather, G. Ammerer, H. Riezman, Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*, Mol. Cell 26 (2007) 663–674.

[200] A. Schmidt, T. Beck, A. Koller, J. Kunz, M.N. Hall, The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease, EMBO J. 17 (1998) 6924–6931.

[201] A. Breitkreutz, H. Choi, J.R. Sharom, L. Boucher, V. Neduva, B. Larsen, Z.Y. Lin, B.J. Breitkreutz, C. Stark, G. Liu, J. Ahn, D. Dewar-Darch, T. Reguly, X. Tang, R. Almeida, Z.S. Qin, T. Pawson, A.C. Gingras, A.I. Nesvizhskii, M. Tyers, A global protein kinase and phosphatase interaction network in yeast, Science 328 (2010) 1043–1046.

[202] Y. Iiboshi, P.J. Papst, H. Kawasome, H. Hosoi, R.T. Abraham, P.J. Houghton, N. Terada, Amino acid-dependent control of p70s6k: involvement of tRNA aminoacylation in the regulation, J. Biol. Chem. 274 (1999) 1092–1099.

[203] P. Bunpo, J.K. Cundiff, R.B. Reinert, R.C. Wek, C.J. Aldrich, T.G. Anthony, The eIF2 kinase GCN2 is essential for the murine immune system to adapt to amino acid deprivation by asparaginase, J. Nutr. 140 (2010) 2020–2027.

[204] M. Cully, A. Genevet, P. Warne, C. Treins, T. Liu, J. Bastien, B. Baum, N. Tapon, S.J. Leevers, J. Downward, A role for p38 stress-activated protein kinase in regulation of cell growth via TORC1, Mol. Cell. Biol. 30 (2010) 481–495.

[205] O. Donze, D. Picard, Hsp90 binds and regulates the ligand-inducible alpha subunit of eukaryotic translation initiation factor kinase Gcn2, Mol. Cell. Biol. 19 (1999) 8422–8432.

[206] A. Rohl, J. Rohrberg, J. Buchner, The chaperone Hsp90: changing partners for demanding clients, Trends Biochem. Sci. 38 (2013) 253–262.

[207] T. Makhnevych, W.A. Houry, The role of Hsp90 in protein complex assembly, Biochim. Biophys. Acta Mol. Cell Res. 1823 (2012) 674–682.

[208] D.G. Hardie, AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy, Nat. Rev. Mol. Cell Biol. 8 (2007) 774–785.

[209] D.G. Hardie, D. Carling, M. Carlson, The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu. Rev. Biochem. 67 (1998) 821–855.

[210] K. Hedbacker, M. Carlson, SNF1/AMPK pathways in yeast, Front. Biosci. 13 (2008) 2408–2420.

[211] D.R. Adams, D. Ron, P.A. Kiely, RACK1, A multifaceted scaffolding protein: structure and function, Cell Commun. Signal. 9 (2011).

[212] T. Kouba, E. Rutkai, M. Karaskova, L.S. Valasek, The eIF3c/NIP1 PCI domain interacts with RNA and RACK1/ASC1 and promotes assembly of translation preinitiation complexes, Nucleic Acids Res. 40 (2012) 2683–2699.

[213] B. Hoffmann, H.U. Mosch, E. Sattlegger, I.B. Barthelmess, A. Hinnebusch, G.H. Braus, The WD protein Cpc2p is required for repression of Gcn4 protein activity in yeast in the absence of amino-acid starvation, Mol. Microbiol. 31 (1999) 807–822.

[214] O. Valerius, M. Kleinschmidt, N. Rachfall, F. Schulze, S.L. Marin, M. Hoppert, K. Streckfuss-Bomeke, C. Fischer, G.H. Braus, The *Saccharomyces* homolog of mammalian RACK1, Cpc2/Asc1p, is required for FLO11-dependent adhesive growth and dimorphism, Mol. Cell. Proteomics 6 (2007) 1968–1979.

[215] Y. Tarumoto, J. Kanoh, F. Ishikawa, Receptor for activated C-kinase (RACK1) homolog Cpc2 facilitates the general amino acid control response through Gcn2 kinase in fission yeast, J. Biol. Chem. 288 (2013) 19260–19268.

[216] V.R. Gerbasi, C.M. Weaver, S. Hill, D.B. Friedman, A.J. Link, Yeast Asc1p and mammalian RACK1 are functionally orthologous core 40S ribosomal proteins that repress gene expression, Mol. Cell. Biol. 24 (2004) 8276–8287.

[217] B. Vanhaesebroeck, L. Stephens, P. Hawkins, PI3K signalling: the path to discovery and understanding, Nat. Rev. Mol. Cell Biol. 13 (2012) 195–203.

[218] X. Sun, H. Yao, R.M. Douglas, X.Q. Gu, J. Wang, G.G. Haddad, Insulin/PI3K signaling protects dentate neurons from oxygen-glucose deprivation in organotypic slice cultures, J. Neurochem. 112 (2010) 377–388.

[219] A. Quesada, B.Y. Lee, P.E. Micevych, PI3 kinase/Akt activation mediates estrogen and IGF-1 nigral DA neuronal neuroprotection against a unilateral rat model of Parkinson's disease, Dev. Neurobiol. 68 (2008) 632–644.

[220] J. Lewerenz, P. Baxter, R. Kassubek, P. Albrecht, J. Van Liefferinge, M.A. Westhoff, M.E. Halatsch, P.J. Meakin, G. Karpel-Massler, J.D. Hayes, E. Aronica, I. Smolders, A. Ludolph, A. Methner, A. Massie, G.E. Hardingham, P. Maher, Phosphoinositide 3-kinases upregulate system xc- via eIF2alpha and ATF4—a pathway active in glioblastomas and epilepsy, Antioxid. Redox Signal. (2014) (in press).

[221] S. Kazemi, Z. Mounir, D. Baltzis, J.F. Raven, S. Wang, J.L. Krishnamoorthy, O. Pluquet, J. Pelletier, A.E. Koromilas, A novel function of eIF2alpha kinases as inducers of the phosphoinositide-3 kinase signaling pathway, Mol. Biol. Cell 18 (2007) 3635–3644.

[222] F.F. Guo, D.R. Cavener, The GCN2 eIF2 alpha kinase regulates fatty-acid homeostasis in the liver during deprivation of an essential amino acid, Cell Metab. 5 (2007) 103–114.

[223] X. Xu, J. Hu, B.C. McGrath, D.R. Cavener, GCN2 in the brain programs PPARgamma2 and triglyceride storage in the liver during perinatal development in response to maternal dietary fat, PLoS One 8 (2013).

[224] X. Xu, J. Hu, B.C. McGrath, D.R. Cavener, GCN2 regulates the CCAAT enhancer binding protein beta and hepatic gluconeogenesis, Am. J. Physiol. Endocrinol. Metab. 305 (2013) E1007–E1017.

[225] A.C. Maurin, C. Chaveroux, S. Lambert-Langlais, V. Carraro, C. Jousse, A. Bruhat, J. Averous, L. Parry, D. Ron, J. Alliot, P. Fafournoux, The amino acid sensor GCN2 biases macronutrient selection during aging, Eur. J. Nutr. 51 (2012) 119–126.

[226] F. Xiao, Z. Huang, H. Li, J. Yu, C. Wang, S. Chen, Q. Meng, Y. Cheng, X. Gao, J. Li, Y. Liu, F. Guo, Leucine deprivation increases hepatic insulin sensitivity via GCN2/mTOR/S6K1 and AMPK pathways, Diabetes 60 (2011) 746–756.

[227] H. Liu, L. Huang, J. Bradley, K. Liu, K. Bardhan, D. Ron, A.L. Mellor, D.H. Munn, T.L. McGaha, GCN2-dependent metabolic stress is essential for endotoxemic cytokine induction and pathology, Mol. Cell. Biol. 34 (2014) 428–438.

[228] R. Ravindran, N. Khan, H.I. Nakaya, S. Li, J. Loebbermann, M.S. Maddur, Y. Park, D.P. Jones, P. Chappert, J. Davoust, D.S. Weiss, H.W. Virgin, D. Ron, B. Pulendran, Vaccine activation of the nutrient sensor GCN2 in dendritic cells enhances antigen presentation, Science (2013) 1–8.

[229] F. Fallarino, U. Grohmann, S. You, B.C. McGrath, D.R. Cavener, C. Vacca, C. Orabona, R. Bianchi, M.L. Belladonna, C. Volpi, M.C. Fioretti, P. Puccetti, Tryptophan catabolism generates autoimmune-preventive regulatory T cells, International Symposium on Transplant Immunology—New Ways of Tolerance, Murcia, SPAIN, 2006, pp. 58–60.

[230] H. Orsini, L. Araujo, J. Maricato, B. Castilho, M. Mariano, A. Basso, General control nonrepressed 2 (GCN2) kinase participation in the remission phase of experimental autoimmune encephalomyelitis (EAE) in mice, Front. Immunol. Conference Abstract: 15th International Congress of Immunology (ICI), vol. 546, 2013, http://dx.doi.org/10.3389/conf.fimmu.

[231] P. She, P. Bunpo, J.K. Cundiff, R.C. Wek, R.A. Harris, T.G. Anthony, General control nonderepressible 2 (GCN2) kinase protects oligodendrocytes and white matter during branched-chain amino acid deficiency in mice, J. Biol. Chem. 288 (2013) 31250–31260.

[232] T. Ma, M.A. Trinh, A.J. Wexler, C. Bourbon, E. Gatti, P. Pierre, D.R. Cavener, E. Klann, Suppression of eIF2alpha kinases alleviates Alzheimer's disease-related plasticity and memory deficits, Nat. Neurosci. 16 (2013) 1299–1305.

[233] Y. Wang, Y. Ning, G.N. Alam, B.M. Jankowski, Z. Dong, J.E. Nor, P.J. Polverini, Amino acid deprivation promotes tumor angiogenesis through the GCN2/ATF4 pathway, Neoplasia (United States) 15 (2013) 989–997.

[234] M. Eyries, D. Montani, B. Girerd, C. Perret, A. Leroy, C. Lonjou, N. Chelghoum, F. Coulet, D. Bonnet, P. Dorfmuller, E. Fadel, O. Sitbon, G. Simonneau, D.A. Tregouet, M. Humbert, F. Soubrier, EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension, Nat. Genet. 46 (2014) 65–69.

[235] W. Peng, L. Robertson, J. Gallinetti, P. Mejia, S. Vose, A. Charlip, T. Chu, J.R. Mitchell, Surgical stress resistance induced by single amino acid deprivation requires Gcn2 in mice, Sci. Transl. Med. 4 (2012) 118ra111.

[236] Z. Lu, X. Xu, J. Fassett, D. Kwak, X. Liu, X. Hu, H. Wang, H. Guo, D. Xu, S. Yan, E.O. McFalls, F. Lu, R.J. Bache, Y. Chen, Loss of the eukaryotic initiation factor 2alpha kinase general control nonderepressible 2 protects mice from pressure overload-induced congestive heart failure without affecting ventricular hypertrophy, Hypertension 63 (2014) 128–135.

[237] W. B'Chir, A.C. Maurin, V. Carraro, J. Averous, C. Jousse, Y. Muranishi, L. Parry, G. Stepien, P. Fafournoux, A. Bruhat, The eIF2alpha/ATF4 pathway is essential for stress-induced autophagy gene expression, Nucleic Acids Res. 41 (2013) 7683–7699.

[238] K. Ohta, A. Mizuno, M. Ueda, S. Li, Y. Suzuki, Y. Hida, Y. Hayakawa-Yano, M. Itoh, E. Ohta, M. Kobori, T. Nakagawa, Autophagy impairment stimulates PS1 expression and gamma-secretase activity, Autophagy 6 (2010) 345–352.

[239] S. Fougeray, I. Mami, G. Bertho, P. Beaune, E. Thervet, N. Pallet, Tryptophan depletion and the kinase GCN2 mediate IFN-gamma-induced autophagy, J. Immunol. 189 (2012) 2954–2964.

[240] C. Chaveroux, S. Lambert-Langlais, L. Parry, V. Carraro, C. Jousse, A.C. Maurin, A. Bruhat, G. Marceau, V. Sapin, J. Averous, P. Fafournoux, Identification of GCN2 as new redox regulator for oxidative stress prevention in vivo, Biochem. Biophys. Res. Commun. 415 (2011) 120–124.

[241] Q. Cai, H.L. Brooks, Phosphorylation of eIF2alpha via the general control kinase, GCN2, modulates the ability of renal medullary cells to survive high urea stress, Am. J. Physiol. Ren. Physiol. 301 (2011) F1202–F1207.

[242] Y. Liu, C. Laszlo, W. Liu, X. Chen, S.C. Evans, S. Wu, Regulation of G1 arrest and apoptosis in hypoxia by PERK and GCN2-mediated eIF2alpha phosphorylation, Neoplasia 12 (2010) 61–68.

[243] M. Molin, J. Yang, S. Hanzen, M.B. Toledano, J. Labarre, T. Nystrom, Life span extension and H2O2 resistance elicited by caloric restriction require the peroxiredoxin Tsa1 in *Saccharomyces cerevisiae*, Mol. Cell 43 (2011) 823–833.

[244] M. Menacho-Marquez, J. Perez-Valle, J. Arino, J. Gadea, J.R. Murguia, Gcn2p regulates a G1/S cell cycle checkpoint in response to DNA damage, Cell Cycle 6 (2007) 2302–2305.

[245] T. Tvegard, H. Soltani, H.C. Skjolberg, M. Krohn, E.A. Nilssen, S.E. Kearsey, B. Grallert, E. Boye, A novel checkpoint mechanism regulating the G1/S transition, Genes Dev. 21 (2007) 649–654.

[246] C.A. Boe, M. Krohn, G.E. Rodland, C. Capiaghi, O. Maillard, F. Thoma, E. Boye, B. Grallert, Induction of a G 1-S checkpoint in fission yeast, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 9911–9916.

[247] R.L. Bowman, Y.H. Xiong, J.H. Kirsten, C.K. Singleton, eIF2 Alpha kinases control chalone production in *Dictyostelium discoideum*, Eukaryot. Cell 10 (2011) 494–501.

[248] S. Chakrabarti, P. Liehl, N. Buchon, B. Lemaitre, Infection-induced host translational blockage inhibits immune responses and epithelial renewal in the *Drosophila* gut, Cell Host Microbe 12 (2012) 60–70.

[249] Y. Zhang, Y. Wang, K. Kanyuka, M.A.J. Parry, S.J. Powers, N.G. Halford, GCN2-dependent phosphorylation of eukaryotic translation initiation factor-2alpha in *Arabidopsis*, J. Exp. Bot. 59 (2008) 3131–3141.

[250] A. Goossens, T.E. Dever, A. Pascual-Ahuir, R. Serrano, The protein kinase Gcn2p mediates sodium toxicity in yeast, J. Biol. Chem. 276 (2001) 30753–30760.

[251] C. Mascarenhas, L.C. Edwards-Ingram, L. Zeef, D. Shenton, M.P. Ashe, C.M. Grant, Gcn4 is required for the response to peroxide stress in the yeast *Saccharomyces cerevisiae*, Mol. Biol. Cell 19 (2008) 2995–3007.

[252] L. Steffensen, P.A. Pedersen, Heterologous expression of membrane and soluble proteins derepresses GCN4 mRNA translation in the yeast *Saccharomyces cerevisiae*, Eukaryot. Cell 5 (2006) 248–261.

[253] H. He, I. Singh, S.A. Wek, S. Dey, T.D. Baird, R.C. Wek, M.M. Georgiadis, Crystal structures of GCN2 C-terminal domains suggest regulatory differences in yeast and mammals, J. Biol. Chem. (2006), http://dx.doi.org/10.1074/jbc.M114.560789 (in press).