GCN2, an old dog with new tricks

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Abstract
GCN2 was first described in budding yeast as a serine/threonine protein kinase involved in the response to amino acid starvation and this is its best characterized role to date. Recent work has revealed new and exciting roles for GCN2, which affect many aspects of cellular physiology in response to a number of stresses in addition to starvation. Furthermore, the GCN2 pathway has been implicated in diseases such as cancer and Alzheimer’s disease, and therefore elucidating the new roles of GCN2 seems ever more important.

Introduction
GCN2 was first described in budding yeast as a serine/threonine protein kinase involved in the response to amino acid starvation, hence the abbreviation GCN for general control non-repressed. This role is conserved from yeast to human cells and the extent of conservation is such that the human GCN2 can functionally replace the budding yeast GCN2 [1]. Recent work has revealed new roles for GCN2. The present review focuses on two aspects of GCN2 function: how it is activated and what the consequences of activation are. Finally, we shall discuss emerging evidence on how GCN2 function is relevant for human diseases.

Activation of GCN2
The mechanism of GCN2 activation in response to amino acid starvation has been much studied through the years. Under these conditions, activation of GCN2 requires accumulation of uncharged tRNAs, which bind a HisRS (histidyl-tRNA synthetase-like) domain in GCN2. This in turn leads to a conformational change and activates the kinase [2–4]. Consistently, activating mutations that mimic tRNA binding have been identified and GCN2 was shown to be associated with the translating ribosome [5], perfectly placed to interact with uncharged tRNAs.

More recently, it was shown in different model systems that GCN2 is activated by a number of other stresses, in addition to amino acid starvation. These include purine starvation, osmotic stress, UV irradiation, MMS (methyl methanesulfonate) treatment, oxidative stress (H2O2) and ER (endoplasmic reticulum) stress [6–11]. It is not immediately obvious for a number of these stresses how they might lead to the accumulation of uncharged tRNAs. It was shown that the HisRS domain is important for GCN2 activation not only upon amino acid starvation, but also upon starvation for purines [12], glucose limitation and growth on ethanol [13] as well as high-salt concentration [8,14]. It is not yet known whether H2O2, MMS, UV or ER stress-induced GCN2 activation also requires the HisRS domain. A trivial explanation for such a requirement would be that all treatments change the balance between charged and uncharged tRNAs, for example by impairing amino acid synthesis, or by inhibiting amino acid-tRNA synthetases or amino acid uptake. It would be interesting to know whether the uncharged tRNA mechanism is operative after all these stresses or whether novel and unknown mechanisms are at work.

Another interesting possibility is that GCN2 becomes covalently modified under stress conditions in a way that increases its affinity for uncharged tRNA or brings about the required conformation change without tRNA binding. This would allow for kinase activation even in the presence of the basal levels of uncharged tRNAs in amino-acid-replete cells. In budding yeast, phosphorylation of Ser577 inhibits GCN2 and the phosphorylation is removed in response to treatment with rapamycin, a known inhibitor of the TOR (target of rapamycin) pathway [15]. This observation has led to the model that TOR signalling inhibits GCN2 protein kinase activation in amino-acid-replete environmental conditions. However, this phosphorylation site is not conserved in fission yeast or in mammalian cells. Nonetheless, a recent study points to a link between TOR signalling and GCN2 activation also in fission yeast [16], suggesting that the pathway, if not the molecular details, might have been conserved. An alternative explanation for the apparent TOR-dependence of GCN2 activation in fission yeast might be that TOR is required to maintain efficient translation and thereby regulates the balance of charged and uncharged tRNAs. A similar reasoning might explain the recently reported role of Cpc2, the Schizosaccharomyces pombe homologue of RACK1 (receptor for activated C-kinase 1), in GCN2 activation in response to amino acid starvation [17]. RACK1 homologues are thought to act as scaffolding proteins to recruit key factors involved in cell signalling [18] and Cpc2 was shown to associate with the ribosomes [19]. Thus it is perfectly placed...
levels without translating them might be beneficial if the corresponding proteins [27–29]. Increasing mRNA upon oxidative stress do not show a concomitant increase in budding yeast, many mRNAs whose levels are increased either the extent of the increase or the identity of the mRNAs. Increased after exposure to stress are also translated at higher regulation. Although many mRNAs whose levels are increased after exposure to stress mainly depend on the MAPK (mitogen-activated protein kinase) pathways and no major requirement for Gcn2 in the regulation of the relevant transcription factors has been described. Consistently, there is no recognized GCN4 homologue, suggesting that translational regulation might play a major role as compared with transcriptional regulation. Interestingly, after UVC irradiation in G1-phase, the transcriptional response is not very pronounced, and among the few genes whose transcription is altered, as many as 15% affect the translational machinery [31].

The consequences of Gcn2 activation

Translational regulation

The best described and perhaps the most important consequence of Gcn2 activation is the phosphorylation of the translation initiation factor eIF2α, which is thought to lead to an inhibition of general translation. It should be noted that the extent of reduction in general translation as a direct consequence of eIF2α phosphorylation has not been studied after exposure to many of the stresses that induce eIF2α phosphorylation. The best studied consequence of eIF2α phosphorylation after amino acid starvation is selective translational up-regulation of the GCN4 transcription factor in budding yeast, resulting in transcriptional induction of nearly all genes encoding amino acid biosynthetic enzymes [24]. In human cells a similar pathway has been described, including translational up-regulation of ATF4 (activating transcription factor 4) [25]. Thus Gcn2 activation leads to a pronounced and characteristic transcriptional response, which targets genes required to deal with starvation.

Further work has suggested that the most important effect of eIF2α phosphorylation is a widespread reprogramming of translation which is uncoupled from transcriptional regulation. Although many mRNAs whose levels are increased after exposure to stress are also translated at higher levels [26], the correlation is not perfect when considering either the extent of the increase or the identity of the mRNAs. For example, after amino acid starvation or oxidative stress in budding yeast, many mRNAs whose levels are increased upon oxidative stress do not show a concomitant increase in the corresponding proteins [27–29]. Increasing mRNA levels without translating them might be beneficial if the corresponding proteins are required for recovery from the stress at a later stage. A similar trend was observed in human cells exposed to hypoxic conditions: the abundance of some mRNAs increase at the same time as they are subject to a general translational repression, whereas other mRNAs are less abundant than under normal conditions, but their translation is induced [30]. These observations imply that translation is extensively regulated to ensure the cells’ survival when exposed to stress, but the mechanisms responsible for this regulation are largely unknown. We propose that fission yeast is a good model organism to study the regulation of selective translation, including the effects of Gcn2. In fission yeast, the transcriptional response to stress mainly depends on the MAPK (mitogen-activated protein kinase) pathways and no major requirement for Gcn2 in the regulation of the relevant transcription factors has been described. Consistently, there is no recognized GCN4 homologue, suggesting that translational regulation might play a major role as compared with transcriptional regulation. Interestingly, after UVC irradiation in G1-phase, the transcriptional response is not very pronounced, and among the few genes whose transcription is altered, as many as 15% affect the translational machinery [31].

Gcn2 and the cell cycle

Several reports have shown that Gcn2 affects cell cycle progression. Formation of the pre-replication complex, an obligatory step in preparation for DNA replication, is delayed in a Gcn2-dependent manner in fission yeast in response to UVC, MMS or H2O2 [9,11]. Similarly, a Gcn2-dependent checkpoint delays entry into S-phase in MMS-treated budding yeast cells [32]. Gcn2 activation and eIF2α phosphorylation are important for the nitrogen starvation-induced cell cycle arrest in fission yeast [33]. G1 arrest after ER stress in human cells depends on eIF2α phosphorylation by PERK [PKR (double-stranded-RNA-dependent protein kinase)-like ER kinase] and Gcn2 [7]. It is unclear whether the cell-cycle effects are mediated through general downregulation of translation, translational regulation of selected proteins or by phosphorylating substrates other than eIF2α. In response to ER stress, a single translationally regulated key factor, cyclinD1, was shown to be responsible for the observed eIF2α phosphorylation-dependent cell-cycle effect [7,34]. Furthermore, cells expressing non-phosphorylatable eIF2α have phenotypes similar to a gcn2 mutant in the UVC-induced G1/S-phase checkpoint [9] or the nitrogen-starvation-induced G1-phase arrest [33], suggesting that the cell-cycle effect is largely due to eIF2α phosphorylation and thereby selective translation of key regulators. However, several observations point to the possibility that Gcn2 and the other eIF2α kinases have additional substrates. First, both in fission yeast and in mammalian cells, there are several eIF2α kinases that are activated in response to different stresses. For example, in fission yeast Gcn2 is activated in response to oxidative stress, UV, MMS and amino acid starvation, Hri2 is activated by oxidative stress, and Hr1 is activated as cells enter stationary phase [33]. In human...
cells, amino acid starvation and UV activate GCN2, and ER stress activates PERK and GCN2, whereas iron deficiency activates HRI and viral infection activates PKR. All of these kinases phosphorylate eIF2α and yet elicit different responses. This leads us to speculate that each of the kinases have major additional targets that determine the nature of the response. Secondly, in fission yeast, Gcn2 is required for the acceleration of mitotic entry upon nutritional shift to poorer growth conditions, but cells expressing non-phosphorylatable eIF2α accelerate mitotic entry as wild-type cells do [35], again suggesting the existence of additional Gcn2 substrate(s). Thirdly, an additional substrate of Gcn2 has recently been identified in mammalian cells. Upon UV irradiation Gcn2 phosphorylates the MRS (methionyl-tRNA synthetase), thereby inhibiting its binding to tRNA^Met, contributing to a down-regulation of general translation, in a mechanism additive to eIF2α phosphorylation [36]. Furthermore, the identification of this substrate reveals a potential link between Gcn2 activation and cell cycle progression. MRS resides in a complex that also includes other amino-acyl-tRNA synthetases and a tumour suppressor, p18/AIMP3 (aminocyl-tRNA synthetase-interacting multifunctional protein 3). MRS phosphorylation leads to the release of p18/AIMP3, which translocates to the nucleus [36]. Interestingly, p18/AIMP3 was previously reported to up-regulate p53 through interactions with ATR/ATM [37]. It remains to be seen to what extent the much-studied ATR- and p53-dependent checkpoint responses are controlled by Gcn2 in human cells and whether the Gcn2/MRS/AIMP pathway might be required for the Gcn2-dependent G_1/S-phase checkpoint in fission and budding yeast.

In summary, it appears that, in addition to their well-characterized role in starvation responses, Gcn2 and eIF2α phosphorylation have unexplored roles in other stress responses and cell-cycle regulation.

**Gcn2 and eIF2α phosphorylation in disease**

Considering the emerging roles of Gcn2, eIF2α phosphorylation and selective translation in stress responses, it is not that surprising that an increasing number of reports emphasize the importance of Gcn2 function in human diseases. Gcn2 has been implicated in diseases affecting many people, such as cancer and Alzheimer’s disease, and therefore elucidating the new roles of Gcn2 seems ever more important.

Rapidly growing tumour cells are surrounded by a microenvironment containing low levels of oxygen and nutrients. Tumour cells can adapt to this stressful environment by inducing angiogenesis and altering metabolic strategies, thus ensuring survival and continued proliferation. The GCN2/eIF2α/ATF4 pathway was found to be critical for maintaining metabolic homeostasis in tumour cells [38]. In that work, Ye et al. [38] showed that knockdown of ATF4 in human fibrosarcoma or colorectal tumour cells leads to lowered survival owing to decreased proliferation and increased apoptosis. Interestingly, these effects were reversed by the addition of non-essential amino acids, in particular asparagine. Asparagine is formed from aspartic acid in a reaction catalysed by ASNS (asparagine synthetase), the gene for which is a well-characterized target of ATF4. These results suggest that the GCN2 pathway supports tumour survival largely through ensuring sufficient ASNS and thereby asparagine levels.

Interestingly, clinical treatments of childhood acute lymphoblastic leukaemia employ asparaginase for depletion of blood asparagine. However, in many cases, drug resistance develops that correlates with up-regulation of ASNS [39]. It remains to be seen whether drug resistance in these cases is due to activation of the Gcn2 pathway.

A hallmark of cancer cells is a metabolic reprogramming involving a reduction in oxidative phosphorylation and an increase in glycolysis, even under aerobic conditions; the Warburg effect [40]. It is now thought that aerobic glycolysis is beneficial to cancer cells because it allows them to re-tune the control of biosynthetic pathways that use intermediates derived from glucose metabolism to generate amino acids, lipids and nucleotides [41]. Recent work has provided evidence that the Gcn2 pathway is important for the Warburg effect. A key enzyme in the switch from oxidative phosphorylation to aerobic glycolysis is pyruvate kinase, which catalyses the last reaction of glycolysis. Of the several isoforms of pyruvate kinase, cancer cells selectively express the less active isoform, PKM2 (pyruvate kinase muscle 2), which is thought to allow accumulation of TCA (tricarboxylic acid) cycle intermediates and reroutes these into biosynthetic pathways. Interestingly, modulation of PKM2 activity requires the GCN2/ATF4 pathway through up-regulation of the enzymes involved in the biosynthesis of serine [42], which in turn is an allosteric activator of PKM2 [43]. Indeed, PKM2 provides a proliferative advantage to the cells during serine starvation as opposed to other isoforms, suggesting that tumour cells use serine-dependent regulation of PKM2 and GCN2 to modulate the flux of glycolytic intermediates to support cell proliferation [42].

Another example of the importance of Gcn2 function for the Warburg effect is through the regulation of mitochondrial H^+-ATP synthase. A reduction in oxidative phosphorylation involves down-regulation of this enzyme, which is exerted at the level of translational regulation [44–46]. Selective translational regulation of H^+-ATP synthase requires the Gcn2 pathway [47]. Thus Gcn2 activation is required for metabolic reprogramming in cancer cells through selective translational regulation of key proteins involved in the regulation of glycolysis and mitochondrial function.

Gcn2 has also been linked to neuronal function. First, an inhibitor of Gcn2, IMPACT, is up-regulated during neuron development, promoting protein synthesis and neurogenesis, and opposing GCN2 [48,49]. Another study showed the importance for Gcn2 in the regulation of nitric oxide (NO) levels [50]. NO is a diffusible neuronal second messenger that can be synthesized in the nervous system by three distinct enzymes, including iNOS [inducible NOS (NO synthase)]. L-Arginine is the only endogenous nitrogen-containing substrate of NOS, and therefore the activity of iNOS and the availability of arginine govern
the production of NO during nervous system development as well as in diseases including multiple sclerosis, Huntington’s disease, Alzheimer’s disease, Parkinson’s disease and stroke. Intracellular arginine regulates iNOS expression through translational control pathways involving GCN2 and eIF2α phosphorylation [50].

In summary, Gcn2 activation and eIF2α phosphorylation have far-reaching consequences for cellular physiology in response to a number of stresses. Our current understanding of Gcn2 function and regulation largely stems from studies on starvation responses in budding yeast, but Gcn2 obviously has many unidentified tricks up its sleeve. The Gcn2 pathway is emerging as an attractive target for therapy in diseases such as cancer and possibly Alzheimer’s disease, and we need to understand more about its functions and its involvement in the regulation of selective translation in order to reveal its full potential for clinical applications.

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