excretion of four nitrogen atoms as uric acid, six phosphoanhydride bonds are hydrolysed. However, PRPP is recycled. If we exclude the initial expenditure in making the primer molecule of PRPP, only five phosphoanhydride bonds are hydrolysed in the cycle, which is equivalent to 1.25 per nitrogen atom. This compares favourably with excretion of urea by mammals where two phosphoanhydride bonds are used for each nitrogen atom synthesised.

What about Glycine?

In considering the energy expended in the biosynthesis of uric acid it is easy to overlook that a molecule of glycine is incorporated. If this glycine was oxidised as fuel it would generate ATP as follows. Glycine can be metabolised to pyruvate and oxidised via the Krebs citric acid (TCA) cycle. The following NADH + H^+-dependent reactions – pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase – generate a total of four NADH + H^+ which, assuming a phosphorylation per oxygen atom (P/O) ratio of 2.5, yield 10 ATP. Succinyl-CoA synthetase produces one GTP (equivalent to ATP), and succinate dehydrogenase produces one $FADH_2$, yielding 1.5 ATP from oxidative phosphorylation. Thus a glycine molecule has the potential to produce 12.5 molecules of ATP.

Ammonotelic, Uricotelic, and Ureotelic Animals

Although all animals have the challenge of disposing of toxic ammonia from protein catabolism, there are three main nitrogenous waste products: ammonium ions, uric acid, and urea. For bony fish and larval amphibians, which inhabit an aquatic environment with unlimited water supply, ammonia is the principal excretory product. Approximately 400 ml of water is needed to excrete 1 g of ammonia. However, terrestrial animals such as insects, terrestrial reptiles, and birds can tolerate a dry environment and excrete uric acid, requiring only approximately 8 ml of water per g of nitrogen, whereas urea needs 40 ml of water.

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Figure 1. The Krebs Uric Acid Cycle for the Disposal of Nitrogenous Waste. Although other biochemists established the intermediates involved in purine metabolism as a linear process, it was Mapes and Krebs who organised the pathway as a metabolic cycle in which pyrophosphate acts in the manner of a catalyst, and 5-phosphoribosylpyrophosphate (PRPP) is the carrier on which the purine ring is assembled. The pathway, known here as the 'Krebs uric acid cycle', is published in their article cryptically entitled 'Rate-limiting factors in urate synthesis and gluconeogenesis in avian liver' [5]. Figure reproduced from [6] with permission from Wiley-Blackwell Publishing, License 4278631439239. Abbreviation: α-KG, α-ketoglutarate.

Spotlight

Translation Links Nutrient Availability with Inflammation

Karine Boulay,[1,2,3,*] Ivan Topisirovic,[3,4,*] and Frédérick A. Mallette[1,2,5,*]

Translation plays a crucial role in shaping the proteome during adaptation to various types of stress. A recent study by Gameiro and Struhl identified an inflammatory response which comprises coordination of transcriptional and translational programs, and which appears to be required for recovery from nutrient deprivation.

Protein synthesis consumes both amino acids and considerable amounts of energy. Consequently, translation relies on the availability of nutrients which provide building blocks for protein production and fuel the generation of energy. Cells adapt to nutrient limitation by attenuating global protein synthesis while enabling the translation of specific mRNAs involved in stress adaptation and recovery. The mechanistic/mammalian target of rapamycin (mTOR) [1] and the integrated stress response (ISR) [2] are two key pathways that adjust protein synthesis according to the nutrient availability.

The serine/threonine kinase mTOR integrates various stimuli through two different complexes, mTORC1 and mTORC2. mTORC1 is activated by nutrients and stimulates protein synthesis via phosphorylation of several translation regulators. For instance, mTORC1 phosphorylates and inactivates eIF4E-

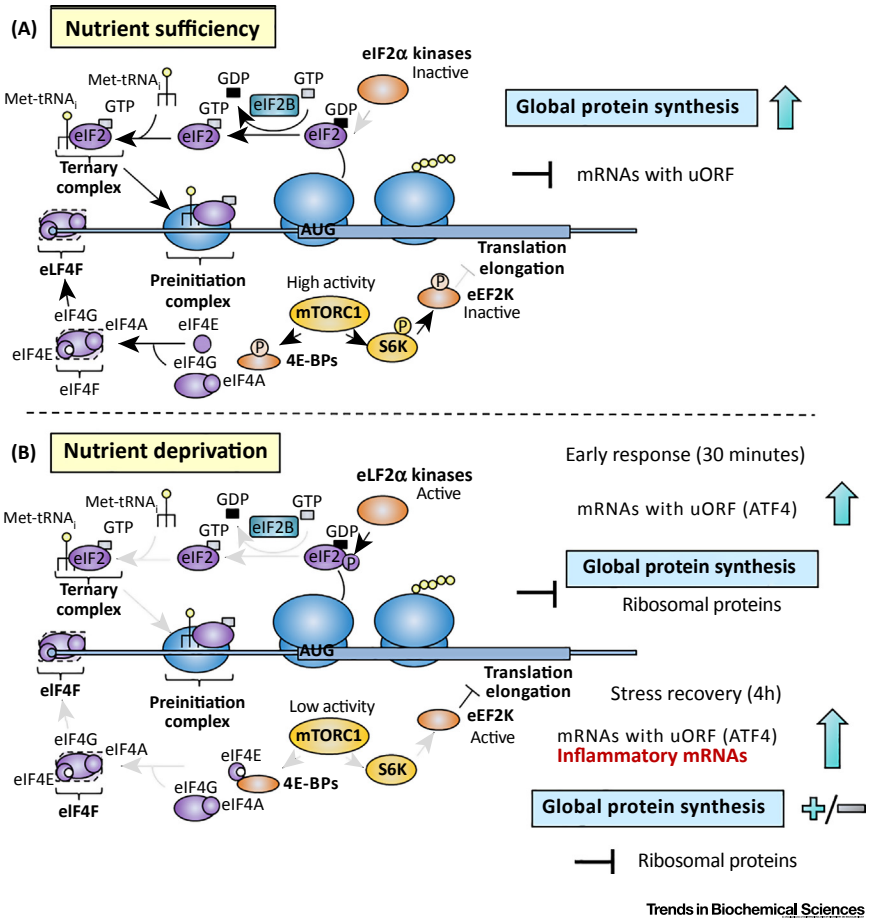

Figure 1. Translational Regulation by Nutrient Availability. The mechanistic/mammalian target of rapamycin complex 1 (mTORC1) and eIF2α kinases are important sensors that coordinate translational activity with nutrient levels. (A) Nutrients stimulate mTORC1, which phosphorylates (P) eIF4E-binding proteins (4E-BPs), triggering their dissociation from the translation initiation factor eIF4E and allowing assembly of the eIF4F complex. eIF4F binds to the mRNA cap and facilitates the recruitment of the preinitiation complex (PIC) that scans the mRNA towards the start codon (AUG). Moreover, mTORC1 promotes translation elongation via a signaling cascade involving activation of S6 kinase (S6K) and downstream inactivation of eEF2 kinase (eEF2K). Efficient translation initiation also relies on guanidine exchange factor eIF2B activity for recycling of the ternary complex (TC) formed by eIF2, GTP, and a methionyl-initiator transfer RNA (Met-tRNA$_i$). The TC assembles with the small ribosomal subunit to form the PIC and, upon Met-tRNA$_i$ delivery to the initiation codon, eIF2-GDP is released. Overall, in the presence of nutrients, global protein synthesis rates are increased while mRNAs harboring inhibitory upstream open reading frames (uORFs) are poorly translated. (B) Nutrient deprivation strongly reduces mTORC1 activity, thus attenuating global protein synthesis and decreasing the translation of mRNAs encoding ribosomal proteins. Nutrient depletion also activates the integrated stress response (ISR) whereby eIF2α kinases inhibit TC recycling through phosphorylation of eIF2. Limited TC levels further suppress general protein synthesis, but favor the translation of mRNAs containing uORFs, such as *ATF4*. Cells recovering from metabolic stress selectively translate inflammatory mRNAs and restore global protein synthesis to different extents, depending on the limiting nutrient.

binding proteins (4E-BPs), thus promoting translation initiation factor eIF4F assembly. In addition, mTORC1 stimulates translation elongation through the mTORC1/S6 kinase (S6K)/eukaryotic elongation factor 2 kinase (eEF2K) axis [1] (Figure 1). Although mTORC1 stimulates global translation, some mRNAs are particularly sensitive to mTORC1 inhibition, including mRNAs encoding components of the translation apparatus, proliferation- and survival-promoting factors, and proteins with mitochondrial functions [3].

Translation initiation rates also depend on the availability of the ternary complex (TC), which is composed of the translation initiation factor eIF2, a methionyl-initiator transfer RNA (Met-tRNA$_i$), and GTP. The TC is bound to the small ribosomal subunit together with other initiation factors to form the 43S preinitiation complex that scans the mRNA until it encounters a start codon in a favorable context. Upon Met-tRNA$_i$:start codon base-pairing, eIF2-associated GTP is hydrolyzed to GDP, triggering the release of eIF2-GDP from the small ribosomal subunit. To be recycled for another round of translation initiation, eIF2-GDP relies on guanidine exchange factor (GEF) eIF2B for GTP reloading [4]. This is attenuated via the ISR, wherein phosphorylation of the α subunit of eIF2 (eIF2α) inhibits eIF2B GEF activity [2] (Figure 1).

The eIF2α protein can be phosphorylated by four different kinases. General control nonderepressible 2 (GCN2) is stimulated by amino acid deprivation, whereas protein kinase R (PKR), PKR-like endoplasmic reticulum (ER) kinase (PERK), and heme-regulated inhibitor kinase (HRI) are induced by viral infection, ER stress, and heme deficiency, respectively. While eIF2α phosphorylation strongly reduces global protein synthesis, it specifically enhances the translation of mRNAs harboring inhibitory upstream open reading frames (uORFs). Activating transcription factor 4 (*ATF4*) mRNA displays such characteristics, and its encoded protein plays an important role in modulating the transcriptome under stress. Under prolonged ISR, protein synthesis levels partially recover, whereby the translational machinery is reprogrammed to allow translation of the stress-induced transcriptome [5].

Owing to insufficient vascularization, cancer cells in solid tumors are constantly subjected to cycles of nutrient deprivation. To sustain proliferation and survival under these conditions, cancer cells are thought to undergo profound metabolic reprogramming [6]. Nonetheless, how adaptation and recovery from nutrient depletion may affect translation in transformed versus non-transformed cells remained underexplored. Gameiro and Struhl addressed this question by using a breast epithelial cell line transformed with Src [7]. Measurements of methionine analog incorporation revealed different levels of protein synthesis reduction in response to short-term withdrawal of either glucose or diverse amino acids that are known to support cell proliferation. Analyses performed at later timepoints further indicated that cells eventually restored protein synthesis in the absence of some nutrients, but recovered poorly when deprived of branched-chain amino acids or glutamine. Interestingly, both transformed cells and their non-transformed counterparts were comparably affected by specific nutrient limitation, although transformed cells displayed an increased sensitivity to glutamine depletion.

To identify mRNAs that are differentially regulated upon nutrient deprivation, the authors carried out ribosome profiling. This technique provides ribosome positioning on transcripts at single-nucleotide resolution, from which changes in translational efficacy are inferred [8]. As expected, nutrient removal diminished mTOR activity and triggered ISR, which reduced the translation of mRNAs coding for ribosomal proteins and translation factors, while stimulating the translation of transcripts harboring uORFs including *ATF4*. Similar investigations performed on cells deprived for longer periods uncovered selective induction of genes with functions in inflammation, both at the transcriptional and the translational levels (Figure 1). For instance, nutrient depletion enhanced the translation of mRNAs encoding regulators of the inflammatory response, including interleukins 6 and 8 [7]. Although similar transcriptional stimulation has been described for glutamine-restricted cells [9], the findings of Gameiro and Struhl provide insights into the generality of inflammatory gene upregulation upon limitation of several different nutrients while highlighting the contribution of translation. Considering that the stability of multiple inflammatory mRNAs is also tightly controlled [10], it may be pertinent to evaluate how different layers of regulation of gene expression are coordinated in the context of nutrient deprivation to finely tune levels of factors implicated in inflammatory responses.

The inflammatory response observed when nutrients are scarce does not appear to stem from reduced mTOR signaling because acute mTOR inhibition did not affect translation of inflammatory mRNAs. Instead, ISR inhibitor (ISRIB) lowered cytokine expression in nutrient-deprived cells, thus suggesting that this response is mostly mediated via eIF2α phosphorylation. Intriguingly, most of the inflammatory mRNAs that were selectively translated under conditions where global protein synthesis is attenuated were devoid of uORFs, suggesting alternative translational activation mechanisms under nutrient deprivation [7]. The authors noticed that cells subjected to acute ER stress also show increased translation of inflammation-related mRNAs, and activation of the ISR pathway may generally induce inflammatory genes, although this remains to be tested.

The physiological relevance of induction of an inflammatory response in cells coping with metabolic stress remains to be established. Because the secretome of glutamine-deprived cells stimulates migration, it is plausible that cytokine production may trigger the movement of cells towards areas with more nutrients [7], which requires *in vivo* evaluation. In conclusion, given the multiple roles of inflammation in tumor development [11], Gameiro and Struhl shed light on an unsuspected translational adaptation to stress that may be important to consider when evaluating the benefits of therapies targeting cancer cell nutrient dependencies.

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Spotlight

Insights into Proteasome Conformation Dynamics and Intersubunit Communication

Grazia R. Tundo,[1] Diego Sbardella,[1] and Massimo Coletta[1,*]

A recently published paper applies cryo-electron microscopy (EM) studies and biochemical/genetic approaches for the elucidation of the mechanisms linking nucleotide binding by ATPases, proteasome conformation dynamics, and gate opening of the 20S core particle. These insights potentially represent a milestone in our understanding of the structural dynamics of the 26S proteasome.

The dynamics through which the 19S regulatory particle couples its substrate-processing activity with the opening of the 20S core particle gate during the 26S proteasome catalytic cycle is the focus of investigation by experts in the field of proteasome structural biology.

The 'rigid' structure of the 20S (comprising four stacked rings, two outer α and two inner β, each of seven repeated subunits, named α_{1-7} and β_{1-7}), which houses the catalytic activity, renders this assembly suitable for crystallographic resolution. Thus, it is well known that the narrow central pore through which substrates are pulled for catalysis is gated by the N-terminal tails of the seven α-subunits: furthermore, this gate-keeping mechanism displays a significant degree of conservation across evolution.

However, this extensive knowledge is in contrast to the persistent difficulty in determining the structure of the 19S (and, thus, of the 26S proteasome), which can be subdivided into two modules, the lid (involved with substrate recognition) and the base, mainly constituted by ATPases (Rpt1–6; forming a pore loop laying above the α-ring of the 20S and involved in substrate unfolding and translocation), which have highly dynamic properties that render crystallographic analyses problematic. Therefore, the mechanism by which ATPases carry out the ATP-dependent unfolding and translocation of polyubiquitinated substrates into the 20S, and the associated remodeling of the α-ring configuration, can only be unveiled by using alternative methodological approaches to traditional X-ray crystallography. Thus, cryo-EM coupled with integrative modeling has emerged as a fascinating and potent tool to investigate these unresolved structures and mechanisms.

In their recent paper [1], Eisele *et al*. applied cryo-EM and biochemical/genetic approaches to the yeast 26S proteasome to cast light on the mechanisms linking nucleotide binding by ATPases, proteasome conformation dynamics, and gate opening of the 20S core particle. Their findings potentially represent a milestone in our understanding of the structural dynamics of the 26S proteasome.

The study is the last in a series that has provided detailed structural insights into the different configurations of the 19S in the presence of ATP (and ATP analogs or substrates) throughout the catalytic cycle of the 26S. The existence of these configurations appears to be conserved among eukaryotic proteasomes, and the 19S modules clearly arrange into spatially and chronologically defined configurations that are energetically favored to accomplish substrate unfolding and translocation, and 20S gate opening.

In Archaea, the access of substrates to the 20S is regulated by the PAN-ATPase complex (the homolog of the 19S eukaryotic regulatory particle), which contains a conserved C-terminal hydrophobic-tyrosine-X (HbYX) motif that inserts into the α-pocket, thus triggering gate opening [2,3]. However, the engagement of the subunits of yeast 19S that have these motifs (the ATPase Rpt2, Rpt3, and Rpt5 subunits) is not sufficient to stimulate the gate opening of the yeast 20S [4]. The study by Eisele *et al*. provides a convincing allosteric model in which the docking of the C-terminal tails of Rpt1 and Rpt6 into the α4–α5 and α2–α3 pockets, respectively, of the 20S induces the structural rearrangement necessary for gate opening (Figure 1). It has long been debated whether the recruitment of ATPases is stochastic or spatially