Translational regulation triggered by fungal pathogens: still a mystery

VS Alves*

Abstract

Introduction

Organisms are able to generate organised responses to environmental changes by controlling the level of messenger RNA translation. The ability to respond rapidly allows the mobilization of the host’s defences and coordination of innate response to different infections. A large number of microbial pathogens inhibit the translation of host cells. Bacterial and virus infections interfere with protein synthesis of the host and activating or suppressing the innate responses, thus manipulating the translational machinery. Mortality, morbidity and economic adverse effects associated with fungal infections along with the emergence of resistance to antifungal agents make it necessary to gain a better understanding of the pathogenesis and discovery of new agents for treatment of those infections. There is little knowledge regarding the role of translational regulation in fungus–host relationship. The present review aims to show how regulation in the context of fungal infections and coordination of innate responses, thus manipulating the translational machinery. Mortality, morbidity and economic adverse effects associated with fungal infections along with the emergence of resistance to antifungal agents make it necessary to gain a better understanding of the pathogenesis and discovery of new agents for treatment of those infections.

Introduction

The regulated translation of mRNAs in eukaryotes is a powerful tool of posttranscriptional control of gene expression. Translation has a fundamental role in cell growth and differentiation, development, learning, memory and synaptic plasticity. This process and its regulation have a relevant role in infections and host defence, allowing quick responses to contain pathogenic microorganisms. Regulation of these events occurs primarily at the steps of initiation and elongation. Figure 1 resumes the process.

Before mRNA decoding, the eukaryotic 40S ribosomal subunit forms a 43S pre-initiation complex composed of different eukaryotic translation initiation factors (eIFs) and initiator tRNA charged with methionine (Met-tRNAi—see Figure 1). This association involves eIF2, a heterotrimer that forms an active ternary complex (TC) containing GTP and aminoacylated initiator tRNA. In the presence of eIF4F complex, TC carries Met-tRNAi to 40S ribosome, a fundamental step to start protein synthesis at AUG codon or cognates. eIF4F binds to the cap structure present in the 5’-end of eukaryotic mRNAs. This interaction promotes correct positioning of ribosome relative to mRNA, thus facilitating the ATP-dependent movement of it that is named as ‘scanning’ and locates the translation initiation codon AUG. eIF4F is composed of a cap-binding protein (eIF4E), an RNA helicase (eIF4A) and a scavenger protein (eIF4G). eIF4E binds to the cap while eIF4G associates with eIF3 linked to a ribosome, allowing correct positioning of 43S complex at the mRNA 5’-end. Besides, eIF4E binds to PABP (poly(A)-binding protein) connecting 5’- and 3’-ends of mRNAs, resulting in mRNA circularization that is supposed to be important for a rapid turnover in protein synthesis.

Translation initiation is the limiting step of protein synthesis in most circumstances, and then the process is subject to elegant regulatory mechanisms. Initiation step is regulated by the availability of eIF4F. The binding of eIF4E to eIF4G is modulated by a serine/threonine kinase, TOR1, which responds to growth factors, nutrients, amino acids, oxygen and energy availability. In this context, TOR1 in a TORC1 complex, which controls eIF4E activity through phosphorylation of 4E-BPs (eIF4E-binding proteins). Hyperphosphorylated 4E-BPs interact with eIF4E, preventing the formation of eIF4F complex and thus suppressing the cap-dependent translation, while hypophosphorylation of these proteins by mTORC1 promotes the release of eIF4E-eIF4G binding, stimulating the cap-dependent translation.

After AUG initiation codon recognition, eIF5 stimulates the hydrolysis of GTP bound to eIF2. In a reaction catalysed by eIF5B, the 40S ribosomal subunit binds to 60S and

*Corresponding author
Email: gouveia_va@yahoo.com; gouveiava@ufmg.br
Laboratório de Biologia Celular de Microrganismos, Departamento de Microbiologia – ICB, Universidade Federal de Minas Gerais, Brazil

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Viruses, which are completely dependent on the host translation machinery for replication, control the translation factors and its activity in eukaryotes.\(^5\)

Translation factors are highly sensitive to environmental changes, metabolic status and stresses as infections and can rapidly change gene expression limiting pathogen replication, acting like innate immunity effectors. \(\text{eIF2}\) is considered a guardian of the innate immunity. In this context, translation of cellular mRNAs can be inhibited by four kinases whose activity results in phosphorylation of a serine residue in the regulatory subunit of \(\text{eIF2} (\text{Ser}\,51)\) in response to metabolic and environmental stresses. PKR is a kinase activated primarily by double-stranded RNAs, and this is considered a pathogen-associated molecular pattern (PAMP) indicative of viral infection. Activated PKR inhibits protein synthesis in response to viral infection and induces IFN synthesis.\(^6\) Consequently, production of IFN by infected cells allows the accumulation of defence molecules in uninfected cells in order to restrict viral replication and dissemination.\(^7\)

When there are problems in protein folding on endoplasmic reticulum (ER), a membrane-associated kinase \(\text{PERK}\) is activated. After phosphorylation of \(\text{eIF2}\) by this kinase, there is a decrease in protein intake by ER, a process known as unfolded protein response (UPR).\(^8\) The lack of a haem group, arsenite-induced oxidative stress, heat shock and osmotic stresses induce \(\text{HRI}\) kinase, while starvation for amino acids and glucose and UV light activates \(\text{GCN2}\) kinase.\(^9,10\) Phosphorylated form of \(\text{eIF2} (\text{eIF2-P})\) has a great affinity for the recycling factor \(\text{eIF2B}\), and thus \(\text{eIF2-P}\) inhibits guanine exchange. \(\text{eIF2B}\) is a limiting factor in translation, and a higher concentration of \(\text{eIF2-P}\) has great impact in translation, resulting in a severe blocking of the process.\(^1\)

**Figure 1:** Simplified view of eukaryotic translation initiation. The first steps of translation initiation to form a ternary complex (TC) with \(\text{eIF2}\) are shown in the upper panel. This panel also shows the recycling of \(\text{eIF2}\) to its active form (\(\text{eIF2-GTP}\)). In the middle panel, 43S pre-initiation complex formation is shown. Ribosome ‘scanning’ to find initiation codon AUG, elongation and dissociation of translation factors including inactive \(\text{eIF2} (\text{eIF2-GDP})\) is shown in the last panel. In the upper panel, kinases that phosphorylate \(\text{eIF2}\) under stress conditions are indicated (\(\text{GCN2}\), \(\text{PERK}\), \(\text{PKR}\) and \(\text{HRI}\)).
The fundamental steps of the protein synthesis remained preserved in different forms of life along evolution; however in eukaryotes, the initiation step presented considerable complexity increase when compared to prokaryotes. Diversification has not reached the presence and activity of eIF2, nor their regulatory mechanisms; GCN2 is present in all eukaryotes; PERK is found only in metazoans; HRI is present in vertebrates, Diptera, Fungi and Echinoderms and PKR is found only in vertebrates.

Recently, it has been reported that infection with intracellular bacteria Listeria monocytogenes, Chlamydia trachomatis or Yersinia induces eIF2 phosphorylation. Cells unable to phosphorylate eIF2 showed a higher susceptibility to bacterial invasion, illustrating the role of eIF2-P on activation of NF-κB and proinflammatory cytokine production. Exposure of cells to LPS, a PAMP produced by gram-negative bacteria that activates TLR4, stimulates proinflammatory cytokines and antimicrobial peptides resulting in UPR. PERK, effector of UPR, phosphorylates eIF2, suppressing global translation and promoting translation of a set of specific mRNAs containing uORFs (upstream ORFs). Among these messages, translation of the mRNA of ATF4 transcription factor is induced, which in turn activates the transcriptional factors ATF-3 and CHOP. Temporary induction of CHOP has no deleterious effects and assists in cellular recovery, but if stress continues CHOP expression, it is dangerous and results in apoptosis. UPR activation also seems to be important to survival of Mycobacterium tuberculosis in macrophages.

The eIF4F complex and its regulators also control innate immunity through multiple independent signalling pathways that act synergistically, regulating different components of the innate response of host cells. In this response, there is a TLR signalling pathway involvement, which, in addition to regulating translation in response to metabolic and physiological status, controls production of type I IFN. Changes in protein synthesis indicate the presence of potentially dangerous microorganisms in hosts and allow distinction between self and pathogens. Using the nematode Caenorhabditis elegans as model, which usually feeds nonpathogenic Escherichia coli, it was shown that Pseudomonas aeruginosa ingestion causes intestinal infection involving the same virulence factors employed during infection of mammals. The virulence of P. aeruginosa is partly due to the exotoxin (Tox), which ribosylates eEF2 elongation factor, inhibiting translation. This inhibition activates cellular zip-2/arg-1, which induces the production of defence factors, and thus inhibition of global translation act as sensors for the presence of the pathogen. Zip2 has several uORFs, and these messages are selectively translated when eIF2 is phosphorylated. This selectivity is a conserved mechanism similar to activation of Gcn4 in S. cerevisiae and ATF4 in Mus musculus via GCN2. In macrophages infected with Legionella pneumophila, there is a decrease of global translation. In this circumstance, glycosyltransferases lgt are translated modifying eEF1A and suppressing translation. Modification of eEF1A results in a powerful innate response that allows distinction between pathogenic and non-pathogenic bacteria. Cytoplasmic membrane damage induced by Shigella or Salmonella decreases intracellular concentration of amino acids, activating GCN2. This activation is accompanied by an integrated stress response in mammals that induces translation of ATF4 and culminates with the expression of stress responsive genes.

Translation inhibition is not restricted to bacterial infections, occurring in response to protozoa and viral infections. While suppression of protein synthesis in bacteria-infected cells enables translation of specific cellular mRNAs, important for protecting the host, severe inhibition of translation facilitates viral replication. During viral infection, translation of host mRNA is reduced and viral mRNAs are translated using cellular eIFs and ribosomes, which restricts translation of inflammatory cytokines, HLA and other necessary molecules for antigen presentation or antiviral proteins.

There is little information regarding how yeast and filamentous fungi infections can affect translational machinery. Murine macrophages exposed to Candida albicans exhibit a decrease in eIF3 concentration and appearance of a modified form of eEF2, apparently phosphorylated. The 4E-BP1 transcript is induced after Drosophila infection with C. albicans and 4E-BP1 deficient flies are 50% more susceptible to infection than controls. Gene silencing of TOR pathway components in Drosophila has shown that some host proteins are important factors for intracellular growth of Cryptococcus neoformans. This fungus is detected by the host via Dectin-1, a receptor for PAMP, important in induction of innate responses, and thus dectin-1 deficient mice are more susceptible to infection. Researchers believe that pathogenic fungi, like bacteria, can be detected by host cells using mechanisms of translation inhibition. Larger studies in this subject are necessary, including experimental validation in different infection models.

Conclusion

In the last few years, incidence of fungal diseases has dramatically increased especially in immunodepressed individuals, representing a serious health problem since these diseases are associated with high mortality. There is a growing list of reports describing antifungal resistance in clinical isolates. Considering these facts, it is necessary for a better understanding of the
pathophysiology of fungal infections that may reveal new therapeutic targets. My group is interested in the mechanisms of translation initiation regulation triggered by Cryptococcus gattii in mouse model that can reveal how the host restricts fungal dissemination and how fungus can subvert translational machinery and can reveal new targets to antifungal development.

Abbreviations list
eIF, eukaryotic translation initiation factor; ER, endoplasmic reticulum; GEF, guanine exchange factor; PABP, poly(A)-binding protein; PAMP, pathogen-associated molecular pattern; TC, ternary complex; UPR, unfolded protein response

References