# PROTEIN SYNTHESIS IN EUKARYOTES

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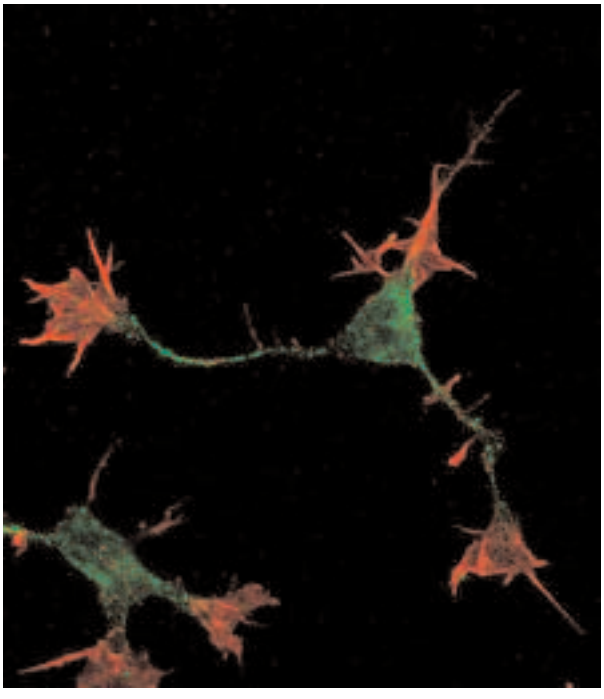

*Distribution of IMPACT in neurons. Primary culture of hippocampal neurons (1 day* in vitro*) stained with antibody anti-IMPACT (*green*) and rhodamine-phalloidin to label F-actin (*red*). IMPACT is found in the cell body and in neurites. Filamentous actin accumulates in growth cones, the leading tip of a neurite*

Protein synthesis in eukaryotes is regulated mainly through the activities of the initiation factors eIF2 and eIF4F. eIF2 is a heterotrimer, with the a subunit playing a regulatory role. Its phosphorylation by specific kinases results in inhibition of translation. The B subunit mediates interactions with other translation initiation components, and stabilizes the binding of GTP to the eIF2 complex; the y subunit, due to its similarity to EF-Tu, might bind GTP and the initiator tRNA. GCN2, the only eIF2a kinase found in the yeast *S. cerevisiae*, is activated by the binding of uncharged tRNA's. In mammals, besides GCN2, three other eIF2a kinases are known: PKR, HRI and PERK, activated by dsRNA, lack of hemin and endoplasmic reticulum stress, respectively. eIF4F, also a heterotrimeric factor, is responsible for the coupling of the 43S complex to the 5' end of mRNA ' s through the direct binding of its eIF4E subunit to the cap. eIF4E can be sequestered away from this interaction through its binding to 4E-BP, an interaction that is regulated by the phosphorylation of 4E-BP by mTOR. The activity of these factors, besides controlling general protein synthesis, can lead to the differential translation of rnRNA's, thus representing mechanisms for adjusting the synthesis of specific proteins to the immediate needs of cells, independently of transcriptional regulation. This project will address the function and regulation of eIF2 and eIF4E in *S. cerevisiae*, in trypanosomatids and mammals, focusing on the following specific aspects:

1. Functional study of the beta subunit of eIF2 in yeast – analysis of the association of eIF2B with eIF2y, and determination of the function of the C2C2 region of eIF2B.

2. GCN2-dependent translational regulation in yeast – determination of the function of proteins encoded by ORF's YDR152W and YLR419W, which share conserved sequences with the N-terminus of GCN2.

3. Translational regulation in trypanosomatids – studies on the phosphorylation of eIF2a, and characterization of eIF4E.

4. Translational regulation in the mammalian brain – mechanism of translation regulation in the experimental model of epilepsy; determination of the expression of GCN1 and GCN2 in brain; study of the function of the protein Impact, which shares conserved sequences with the N-terminus of GCN2.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Translational control mediated by phosphorylation of eI F2 plays a pivotal role in cell biology by inducing a downstream response aimed at cellular recovery from stresses. In mammals, four eIF2α kinases are found, GCN2, PKR, PERK and HRI, activated by different stress conditions. In the absence of this response, or when exacerbated, cell death ensues. Abnormally high levels of eIF2α(P) are found in several neuropathologies. In this project, we have shown that also in epilepsy there is a large increase in eIF2α(P) levels, caused by activation of PKR, leading to a drastic shut off of translation in the brain. On the other hand, this pathway is required for normal physiological responses in animals. GCN2 manages starvation for nutrients and determines feeding behavior and memory. During this project, we described a novel protein, called IMPACT, that functions as an inhibitor of GCN2. IMPACT was found predominantly in neurons, being extremely abundant in the hypothalamus. Our results suggest that under physiological stresses IMPACT functions to maintain constant protein synthesis required for neuronal signaling in brain areas that control homeostasis.

Translational control by eIF2 phosphorylation is also a means by which microorganisms adapt to changing environmental conditions. Trypanosomatid parasites, such as *T. cruzi*, *T. brucei* and *Leishmania*, encounter in their life cycles different environments in the mammalian host and insect vector. We have in this project addressed the eIF2 pathway in this group of parasites. We have characterized their unusual eIF2α subunit, and a unique trans-membrane kinase localized in the flagellar pocket in *T. brucei*, the only region of these cells with direct communication with the environment. These findings suggest that this novel eIF2 kinase is involved in cross-talks between host and parasite.

## MAIN PUBLICATIONS


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