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# Translation of Master Regulator GCN4 Is Resistant to eIF4E Mediated Inhibition of Cap Recognition in Saccharomyces cerevisiae

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# Translation of Master Regulator $GCN_4$ Is Resistant to $eIF_4E$ Mediated Inhibition of Cap Recognition in *Saccharomyces cerevisiae*

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In Eukaryotes, mRNAs possess a 5' 7-methylguanosine cap, which stabilizes the mRNA and recruits the ribosome to initiate translation. However, not all genes require the cap to be translated. There are several mechanisms by which mRNAs can be translated without cap recognition. Cap-independent translation in eukaryotic systems has not been comprehensively characterized but is implicated in cellular functions such as stress response, apoptosis, and cell cycle control. Studies of cap-independent translation in eukaryotes have the possibility to unearth key conserved regulatory mechanisms in gene expression. We used ribosome profiling to map ribosome protected RNA fragments, providing a comprehensive view of transcripts that are actively translated. We used a *Saccharomyces cerevisiae* strain with a conditional mutant for eIF4E, the eukaryotic translation initiation factor responsible for cap recognition and binding, to halt cap-dependent translation under non-permissive conditions. In this way, we are able to locate genes which can be translated in a cap-independent manner. Our findings indicate that master regulator GCN4 can be translated cap-independently and was an interesting gene with significantly increased translational efficiency. GCN4 is a key transcription factor that activates over 50 genes in amino acid biosynthetic pathways in response to amino acid starvation—we saw a corresponding increase in mRNA from amino-acid biosynthetic genes in the GCN4 regulon. GCN4 is a well-known model for translational regulation and is activated by inhibition of eIF2α by phosphorylation. When eIF2α is phosphorylated, it inhibits ternary complex formation, allowing the ribosome to scan through upstream open reading frames to reach the true start codon. Via western blotting we found that our observed increase in translational efficiency was independent of eIF2α-P. Our investigation further underscores the importance of GCN4's role in the stress response and complicates the accepted model of translational regulation. This work yields novel understanding of stress-mediated translational control in eukaryotes.