GCN Sensitive Protein Translation in Yeast

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Abstract

GCN codons are overrepresented in the initial codons of ORFs (the ramp region), particularly in highly expressed genes. Molecular Dynamics (MD) simulations have revealed an interaction surface in the mRNA entrance tunnel of ribosomes near the A site decoding center. This CAR interaction surface consists of 165/185 rRNA C1054, A1196 (E. coli numbering), and yeast ribosomal protein Rps3 A146. We observe H-bonding between the CAR interface and GCN in the mRNA +1 codon, the codon about to enter the A site. We hypothesize that this mRNA-ribosome interaction can lead to modulation in protein translation, and under different conditions (e.g. stress conditions) or sequence contexts, the mRNA-ribosome interactions can serve as a mode of regulation for protein translation. Our wet lab experiments have shown that mutations that deviate from the GCN periodicity in the ramp region lead to changes in protein expression levels. We similarly made mutations in the mRNA in MD simulations of the ribosome to observe how the mRNA-ribosome interactions may change. We observed that deviations from GCN led to decreased interactions between the CAR interface and the mRNA +1 codon. Indeed, A-rich and GCN codons show particularly weak CAR interactions. We hypothesize that the codon identity and the degree of conformance to the GCN periodicity of the codons in the ramp region determine the level of mRNA-CAR interaction and hence the level of protein expression.

Nucleotide Periodicity

The mRNA sequences of yeast proteins were aligned at the start codon and the ratios of observed to expected frequencies of each nucleotide were measured. A 3-nucleotide periodicity, characterized by GCN, is particularly pronounced in the initial codons of ORFs of highly expressed proteins. (A) shows log(fact/expected) for each nucleotide and highlights the GCN periodicity. (B) shows, in a separate set of experiments, the depression of the nucleotide G at codon positions of 2 and 3 especially in highly expressed proteins.

Ramp Mutants

(A) Mutants of two candidate genes (SKN7 and HMT2) were created to test our mRNA-mRNA base-pairing model by altering the GCN periodicity.

mRNA-CAR Interaction Surface

Part of tRNA (brings amino acid)

Results

Protein expression of mutants, measured in westerns, suggests that GCN enrichment depresses protein translation. mRNA abundance and protein stability assays were performed for each mutant to ensure that changes in protein expression level could be attributed to changes in translation.

Conclusions and Future Directions

Conclusions:

• GCN periodicity downstream of translation start sites is enhanced in mRNAs with high protein expression

• The ribosome CAR interaction surface H-bonds to the +1 codon about to enter the A site decoding center

• This CAR H-bonding is strongest for GCN codons and could potentially modulate protein expression levels

Future Directions:

• Create mRNA nt 34 modifications to observe their effects on CAR interaction surface integrity and H-bonding

• Make additional +1 codon substitutions in MD simulations to investigate codon context of CAR-mRNA interactions.

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References


