GCN Sensitive Protein Translation in Yeast

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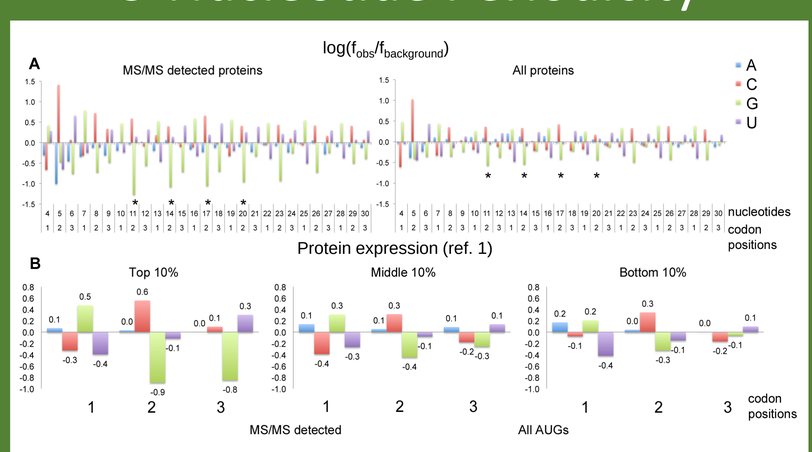
Abstract

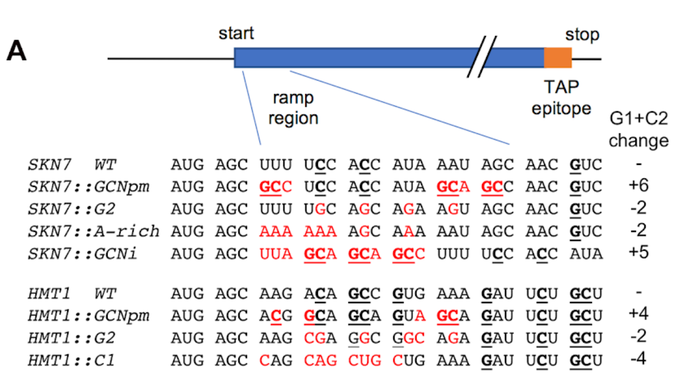
Our research is focused on elucidating the functional significance of a 'GCN' 3-nucleotide periodicity observed in protein coding open reading frames (ORFs). GCN is overrepresented in the initial codons of ORFs (the ramp region), particularly in highly expressed genes. The Weir lab has observed through Molecular Dynamics (MD) simulation that there is an interaction surface located in the mRNA entrance tunnel of the ribosome near the A site decoding center. This CAR interaction surface consists of 16S/18S rRNA C1054, A1196 (*E. coli* numbering), and yeast ribosomal protein Rps3 R146. Further investigation of this interaction surface revealed that there is an mRNA-ribosome interaction (through hydrogen bonding) between the CAR interface and GCN in the mRNA +1 codon which is 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. Previous wet lab experiments have shown that mutations that deviate from the GCN periodicity in the ramp region lead to changes in protein expression levels. To ensure the changes in protein expression were not a result of a change in mRNA abundance or protein stability, mRNA abundance and protein stability assays were carried out in parallel. We similarly made mutations in the mRNA in MD simulations of the ribosome to observe how the mRNA-ribosome interactions may change. Specifically, we observed that deviating from GCN led to decreased interactions between the CAR interface and the mRNA +1 codon. Indeed, A-rich and CGN 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.

3-Nucleotide Periodicity



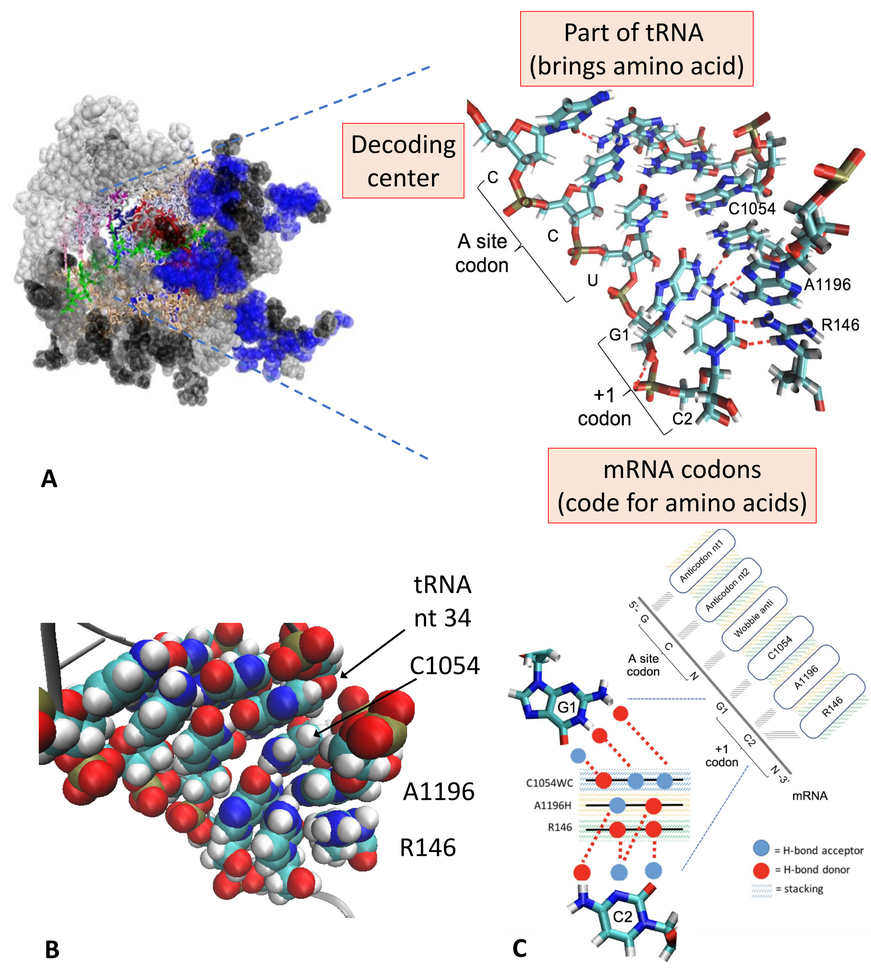
The mRNA sequences of yeast proteins were aligned at the start codon and the ratios of observed to expected frequencies of each nucleotide were measured. A 3-nucleotide periodicity, characterized by GCN, is particularly pronounced in the initial codons of ORFs of highly expressed proteins. (A) shows $\log(f_{obs}/f_{background})$ 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



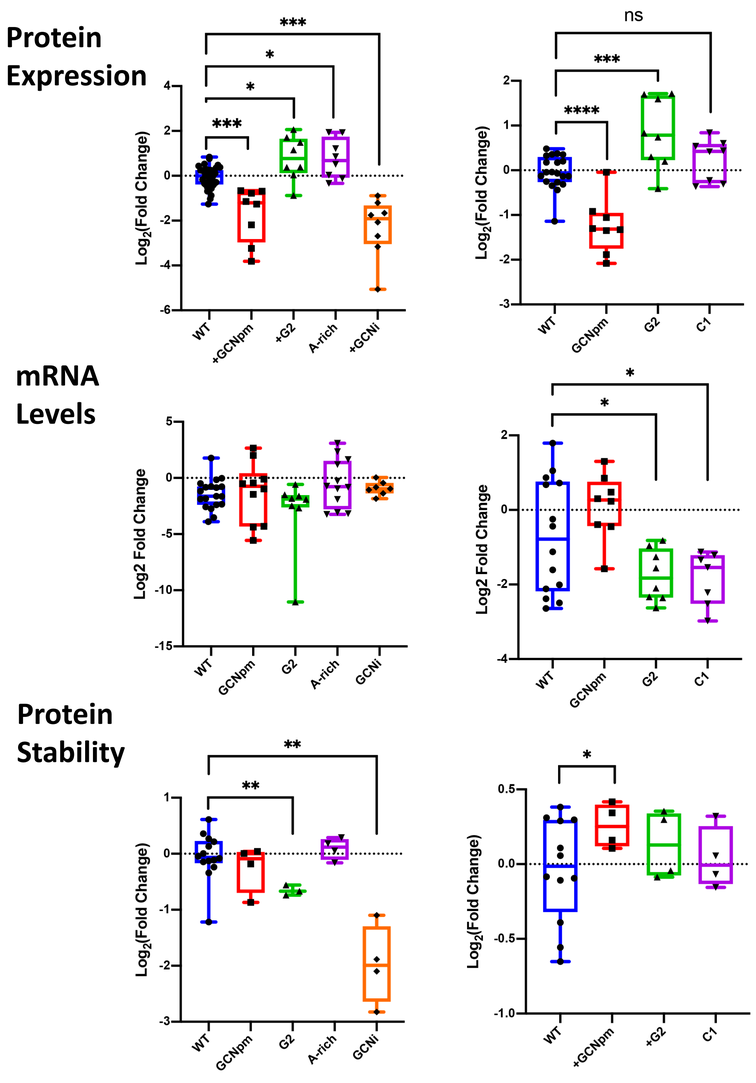
(A) Mutants of two candidate genes (*SKN7* and *HMT1*) were created to test our rRNA-mRNA base-pairing model by altering the GCN periodicity.

mRNA-CAR Interaction Surface



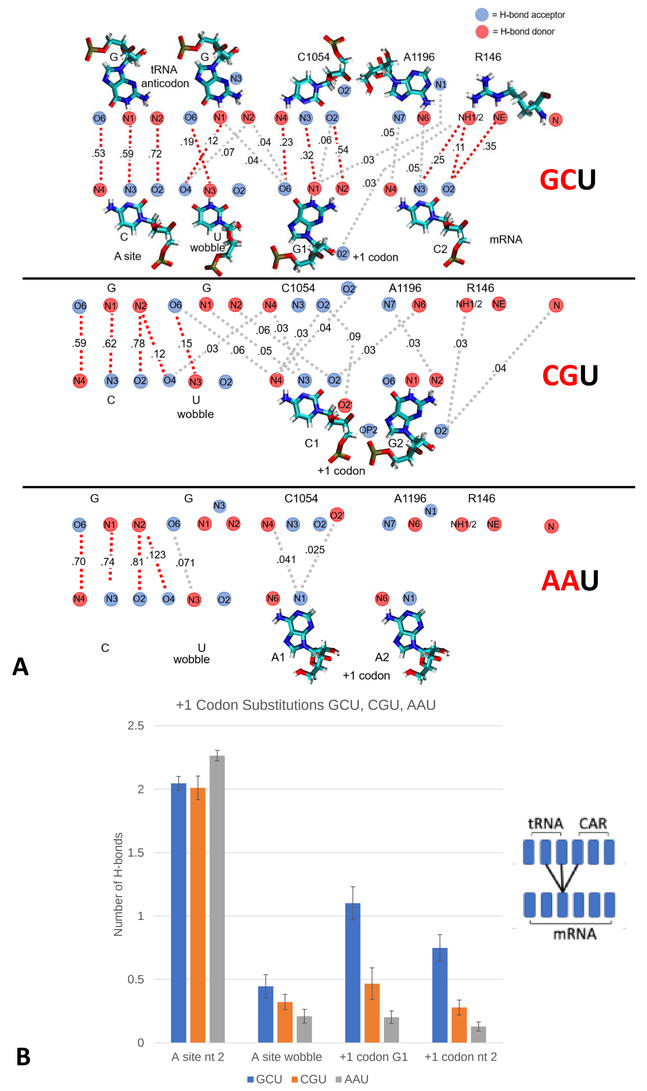
(A, B, C) Molecular Dynamics simulations show that 16S/18S rRNA bases C1054, A1196, and R146 (*E.coli* numbering) can form hydrogen bonds with the mRNA in the first two stages of ribosome translocation, potentially modulating protein expression. These rRNA residues can also pi-stack with one another as well as with tRNA nucleotide 34, forming the CAR-interaction surface.

Results

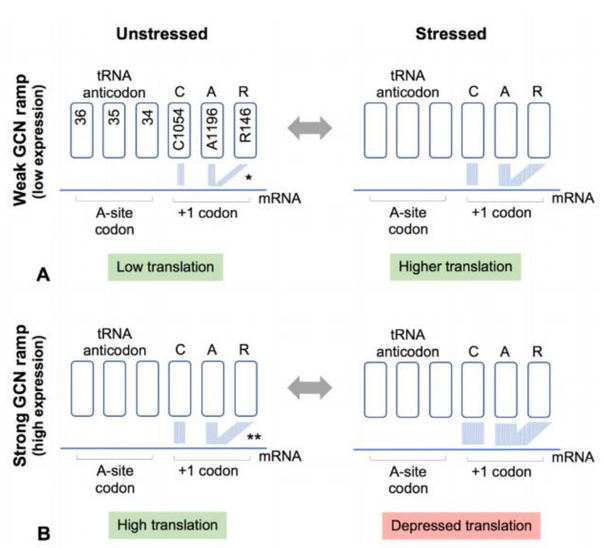


The protein expression of each mutant was measured using western blot analysis. Subsequent mRNA abundance and protein stability assays were performed for each mutant to ensure changes in protein expression level can be attributed to changes occurring at the translational level.

Results



Schematic and bar graph of average frequencies of H-bonds between CAR interaction surface and mRNA



Model of CAR-mRNA interaction surface and how translation may be modulated or tuned in response to different conditions and codon context

Conclusions and Future Directions

Conclusions:

- GCN periodicity downstream of translation start sites is enhanced in mRNAs with high protein expression levels
- The ribosome CAR interaction surface may transiently H-bond to the +1 codon about to enter the A site decoding center
- This hypothesized hydrogen bonding is strongest for GCN codons and could potentially modulate protein expression levels

Future Directions:

- Create tRNA nt 34 modifications to observe their effects on CAR interaction surface integrity and H-bonding
- Make additional +1 codon substitutions in MD simulations to gain more insight on effect of codon context on CAR-mRNA interactions.

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References: (1) Ghaemmaghami, S.; Huh, W. K.; Bower, K.; Howson, R. W.; Belle, A.; Dephoure, N.; O'Shea, E. K.; Weissman, J. S., Global analysis of protein expression in yeast. *Nature* **2003,** 425, (6959), 737-41.
(2) Abeyrathne, P.D.; Koh, C.S.; Grant, T.; Grigorieff, N.; Korostelev, A.A., Ensemble cryo-EM uncovers inchworm-like translocation of a viral IRES through the ribosome. *eLife* **5**, e14874 (2016).
(3) Scopino, K.; Williams, E.; Elsayed, A.; Barr, W.A.; Krizanc, D.; Thayer, K.M.; Weir, M.P. A Ribosome Interaction Surface Sensitive to mRNA GCN Periodicity. *Biomolecules* **2020**, *10*, 849.
(4) Barr, W.A.; Sheth, R.B.; Kwon, J.B.; Cho, J.W.; Glickman, J.W.; Hart, F.; Chatterji, O.K.; Scopino, K.; Voelkel-Meiman, K.; Krizanc D.; Thayer, K.M., Weir, M.P. GCN sensitive protein translation in yeast. *bioRxiv* **2020**, *05.01.072066.*