Synonymous codon usage determines sensitivity of gene expression to amino acid starvation

Arvind R. Subramaniam[1], Tao Pan[2], Philippe Cluzel[1]

Short Abstract — Synonymous codon usage in protein coding sequences affects gene expression. Current understanding of this effect is limited to rapidly growing cells in nutrient-rich media. Here, we experimentally demonstrate a novel role for synonymous codon usage in protein coding sequences – as a determinant of translational robustness in nutrient-limited environments. Guided by a recent model [3], we hypothesize that the sensitivity in translation rate to nutrient limitation is determined by a balance between the supply and demand for charged tRNA isoacceptors. Using fusions of arginine codon cassettes to the green fluorescent protein gene, we determined that the translation rate of arginine synonymous codons varied in sensitivity to arginine limitation. We could further modulate this sensitivity by increasing supply (intracellular arginine tRNA) or demand (intracellular mRNA) for arginine tRNA isoacceptors. We found that mutating robust synonymous codons to sensitive ones in arginine biosynthesis genes leads to a sizeable decrease in fitness during response to arginine starvation.

I. Background

THE degeneracy of the genetic code allows for a single protein to be encoded by a large number of DNA sequences. Multiple synonymous codons encode 18 of the 20 common amino acids. During normal growth conditions, synonymous codons read by abundant tRNA isoacceptors are typically translated faster [1] and more accurately [2] than those that are decoded by rare tRNA isoacceptors. However, it remains unknown how synonymous codon usage affects gene regulation in response to environmental changes. Recent studies [3, 4] indicate that tRNA isoacceptors vary in their charging levels for their cognate amino acid during starvation. Guided by these results, we hypothesized that synonymous codons that are read by tRNA isoacceptors with high charging levels during starvation, can confer robustness to the translation rate of the corresponding mRNA. In contrast, codons read by tRNA with low residual charging levels will make the translation of the corresponding mRNA sensitive to amino acid starvation.

[1]Center for Systems Biology, Department of Molecular and Cellular Biology, and School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138. Email: asubram@fas.harvard,edu

[2]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois 60637, USA. Email: taopan@uchicago.edu

II. Results

To measure the translation rates of the arginine synonymous codons in *E. coli*, we created six fusion constructs that each encoded five N-terminal arginine residues fused to the Green Fluorescent protein. For each construct, the N-terminal residues were encoded by a single arginine synonymous codon (AGA, AGG, CGA, CGC, CGG, or CGT). We cloned the fusion constructs downstream of a constitutive promoter on a very low copy plasmid. We worked with an *arg*$^-$ mutant that requires supplemented arginine for normal growth in a minimal medium. We induced partial arginine starvation by substituting a slowly hydrolyzed arginine analog, arginine methyl-ester, for the arginine.

By comparing the ratio of protein synthesis rates between arginine-limited and arginine-rich growth conditions, we identified which codons were robust (AGA, AGG and CGG), intermediate (CGT and CGC), and sensitive (CGA) to arginine limitation. The translation rate of robust codon-*gfp* constructs expressed from a high copy plasmid became sensitive to starvation. When we compensated for this high demand by simultaneously increasing the supply of the corresponding tRNA, the codon translation rates were robust to starvation again. To determine whether the robust codons confer a fitness advantage in natural genes, we mutated the robust codons to the sensitive codon in genes for enzymes in the arginine biosynthesis pathway (*carA* or *argA*). We compared the growth lag induced by arginine starvation between the wild-type and mutated strains. We found that the *carA* mutant had a growth lag that was twice as long as that of the wild-type strain. The *argA* mutant had the same growth lag as the wild-type strain.

III. Conclusions

This work demonstrates that in amino acid limited conditions, the translation rate of synonymous codons can be either robust or sensitive. We have shown that robust synonymous codons in an arginine biosynthesis gene confer a sizeable fitness advantage over sensitive codons during response to arginine starvation. Our results unveil a novel mechanism of post-transcriptional gene regulation that is based on the degeneracy of the genetic code.

References

[1] T. Tuller *et al.*, *Cell* **141**, 344 (2010).
[2] D. A. Drummond, C. O. Wilke, *Cell* **134**, 341 (2008).
[3] J. Elf *et al.*, *Science* **300**, 1718 (2003).
[4] K. A. Dittmar *et al.*, *EMBO Rep.* **6**, 151 (2005).