Protein folding on the ribosome

Quyen Vu

Proteins are produced by macromolecular machines called ribosomes, which are found in living cells across all species, from bacteria to humans.
In this talk, we will discuss effects of the ribosome on a nascent protein (newly synthesized protein), specifically, how the ribosome exit tunnel modulates the ejection of the nascent protein and alters the co-translational protein folding pathways.

The ejection of the nascent protein occurs after its covalent bond with the transfer-RNA has been broken. We investigate this process using a combination of multiscale modeling, ribosome profiling, and gene ontology analyses. We find a greater than 1000-fold variation in ejection times. Nascent proteins enriched in negatively charged residues near their C-terminus eject the fastest, while nascent chains enriched in positively charged residues tend to eject much more slowly. More work is required to pull slowly ejecting proteins out of the exit tunnel than quickly ejecting proteins, according to all-atom simulations. An energetic decomposition reveals, for slowly ejecting proteins, that this is due to the strong attractive electrostatic interactions between the nascent chain and the negatively charged ribosomal-RNA lining the exit tunnel, and for quickly ejecting proteins, it is due to their repulsive electrostatic interactions with the exit tunnel. Ribosome profiling data from E. coli reveals that the presence of slowly ejecting sequences correlates with ribosomes spending more time at stop codons, indicating that the ejection process might delay ribosome recycling.

Interactions between the ribosome and nascent protein can destabilize folded domains in the ribosome exit tunnel's vestibule, the last 3 nm of the exit tunnel where tertiary folding can occur. Here, we test if a contribution to this destabilization is a weakening of hydrophobic association, the driving force for protein folding. Using all-atom molecular dynamics simulations, we calculate the potential-of-mean force between two methane molecules along the center line of the ribosome exit tunnel and in bulk solution. Associated methanes, we find, are half as stable in the ribosome's vestibule as compared to bulk solution, demonstrating that the hydrophobic effect is weakened by the presence of the ribosome. These findings mean that nascent proteins pass through a ribosome vestibule environment that can destabilize folded structures, which has the potential to influence co-translational protein folding pathways, energetics, and kinetics.

References:

(1) Nissley, D. A.; Vu, Q. V.; Trovato, F.; Ahmed, N.; Jiang, Y.; Li, M. S.; O'Brien, E. P. Electrostatic Interactions Govern Extreme Nascent Protein Ejection Times from Ribosomes and Can Delay Ribosome Recycling. *J. Am. Chem. Soc.* **2020**, *142* (13), 6103–6110. https://doi.org/10.1021/jacs.9b12264.

(2) Vu, Q. V; Jiang, Y.; Li, M. S.; O'Brien, E. The Driving Force for Co-Translational Protein Folding Is Weaker In the Ribosome Vestibule Due to Greater Water Ordering. *Chem. Sci.* **2021**. https://doi.org/10.1039/d1sc01008e.