UC RIVERSIDE

BCH 251/252 Seminar Series

Alexey Petrov, Associate Professor, Department of Biological Sciences, Auburn University

Seminar Title: "Non-Canonical Mechanisms of Translation"

Abstract: Non-canonical mechanisms of translation regulate protein expression, and yet they are poorly understood. We used single molecule fluorescence to reveal how leaderless mRNAs promote translation in bacteria and interrogate mechanism of ribosomal translocation in eukaryotes. In bacteria, the Shine-Dalgarno (SD) sequence of mRNA recruits the small ribosomal subunit by base pairing it with a complimentary anti-Shine-Dalgarno sequence in ribosomal RNA (rRNA). The strength of the SD-rRNA interactions and spacing between SD and the start codon define how well mRNA is translated, thus directly affecting protein abundance in bacterial cells. However, only a fraction of bacterial mRNAs uses this mechanism. Leaderless mRNAs lack a 5' UTR and SD sequence, and how these mRNAs are recruited to the ribosome and how translation is regulated is not well understood. Initiation may proceed through a pathway similar to the one for SD-containing mRNA, where mRNA is first recruited to the 30S subunit and is later followed by 50S subunit joining. Alternatively, it was proposed to occur via direct recruitment to the 70S ribosome. These are called "sequential" and "direct" pathways, respectively. However, neither pathway was directly demonstrated, and their molecular mechanisms are unknown. We showed that leaderless mRNAs can initiate translation via both pathways. We showed how pathway selection and initiation efficiency are regulated by Mg^{2+} concentration and initiation factors, with 70S ribosome concentration being a main determinant of the initiation pathway frequency. Under near physiological conditions ratio of sequential to direct pathways was 1:2 suggesting that both pathways might occur *in vivo*.

CRPV IRES is a unique mRNA, that begins translation from translocation reaction that must occur before first codon of mRNA is decoded. We observed that like canonical 80S-$tRNA_2$ pre-translocation ribosomes, 80S-IRES ribosomes spontaneously exchanged between non-rotated and semi-rotated conformations, but predominantly occupied a semi-rotated conformation. In the presence of eEF2, ribosomes underwent forward and reverse translocation. Both reactions were eEF2 concentration dependent, indicating that eEF2 promoted both forward and reverse translocation. The antifungal, sordarin, stabilizes eEF2 on the ribosome after GTP hydrolysis in an extended conformation. 80S-CrPV IRES-eEF2-sordarin complexes underwent multiple rounds of forward and reverse translocations per eEF2 binding event. In the presence of sordarin, neither GTP hydrolysis nor a phosphate release were required for IRES translocation. Together, these results suggest that in the presence of sordarin, eEF2 promotes the mid and late stages of CrPV IRES translocation by unlocking ribosomal movements, with mid and late stages of translocation being thermally driven. This directly confirms that ribosome is a Brownian molecular motor.

Tuesday, May 13, 2025 12:00 p.m. - 12:50 p.m. PST

In-Person: Genomics Auditorium 1102A

Host: Dr. Sean O'Leary