

Thesis Abstract

Strategies and approaches for mechanistic understanding of bacterial ribosome assembly

Himanshu Sharma

Ribosomes are large ribonucleoprotein complexes that catalyze protein synthesis. Decades of efforts directed towards understanding ribosome structure and function have revealed crucial insights into the working of these complex molecular machines. In parallel, attempts to trace their biogenesis have uncovered an orchestrated interplay between synthesis and the hierarchical assembly of ribosomal components. However, due to the large size of the ribosomal RNA, its folding and the recruitment of ribosomal proteins are prone to a large kinetic barrier. In order to overcome these challenges and catalyze assembly, cells employ a gamut of non-ribosomal components called Ribosome Assembly Factors (RAFTs). However, we are yet to uncover the exact number and function of RAFTs that engage in ribosome assembly. Apart from its intrinsically complicated nature, efforts to understand assembly are also thwarted by a shortage of technologies to probe and monitor ribosome assembly in a scalable manner. In order to overcome this, we revisited bacterial ribosome assembly by devising novel strategies and approaches. At the outset, we describe the development of a strategy to identify RAFTs in a high-throughput manner using *in vivo* proximity-dependent biotinylation (BioID). In order to validate the BioID hits, we developed a reporter system that employs Bimolecular Fluorescence Complementation (BiFC) to capture assembly defects in a medium-throughput manner. Further, we have also attempted to characterize essential RAFTs using an *in vivo* targeted protein depletion strategy. Finally, the last part of this thesis attempts to understand how assembled ribosomes are quality controlled before initiating protein synthesis. Here, we establish the previously unknown ribosome quality control checkpoints in *E. coli* and show how these checkpoints are bypassed in cells with perturbed assembly.