Defining the mutational and conformational space of prokaryotic ribosomes

Wenfa Ng

Citizen scientist, Singapore, Email: ngwenfa771@hotmail.com

Abstract

Ribosomes are macromolecular complexes important to protein translation, and thus, essential to life. Comprising an ensemble of ribosomal proteins and RNA molecules, ribosomes are conserved in structure and function across all domains of life, but recent structural studies have revealed differentiated structures of ribosomes from bacterial, archaea and eukaryotes. Additionally, unique ribosomal protein mass fingerprints have been found for individual species; thereby, indicating that ribosomes are differentiated in structure amongst different species. Given that structure defines function, differentiated function likely exists amongst ribosomes of different species, which could manifest as differences in translation efficiency that could impact on cell growth rate. But ribosomal proteins also hold phylogenetic significance in informing the evolutionary trajectory of each species. Such ribosomal proteins are thus not highly conserved and offers sufficient sequence space for the evolution of differentiated structure and function in different species. Using ribosomal proteins that hold phylogenetic significance as templates, this study sought to understand the mutational and conformational limits that define functional ribosomes. Specifically, ribosomal proteins in *Bacillus subtilis* that hold phylogenetic cues would be mutated through error-prone polymerase chain reaction to generate variants that are subsequently transformed into *Escherichia coli*. To help assess the functional properties of the heterologous ribosomal proteins, endogenous ribosomal protein genes would be inactivated by multiplex CRISPR interference. Since variants in ribosomal proteins would likely impact on ribosome function and translation efficiency, live/dead screening on LB agar would be effective as a preliminary screen for functional mutants. These mutants would subsequently be inoculated into liquid LB medium in 96 well plates to quantify relative growth rates between different strains harbouring different heterologous variants of ribosomal proteins. Plasmids containing different ribosomal protein mutants would be extracted from each functional strain and subjected to Sanger sequencing for determining the specific mutations involved. Collection of such mutations would provide a comprehensive mutational map that define the limits of ribosomal protein sequence space important to ribosome function. Furthermore, biochemical isolation of ribosomal proteins and their structural characterization by X-ray crystallography or cryo-electron microscopy would further illuminate the structural significance of each mutation on ribosome structure and function; thereby, elucidating the structural tolerance space for functional ribosomes. Overall, generating a diverse pool of mutant ribosomal proteins in viability assays followed by sequencing and structural characterization would help define the mutational and conformational limits of a functional and efficient ribosome.

Keywords: CRISPR interference, error-prone polymerase chain reaction, ribosomal proteins, ribosome, viability assay, *Bacillus subtilis*, *Escherichia coli*, sequence space, conformational space, mutational map,

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Conflicts of interest

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