Subject of Internship: Mutational robustness of 23S rRNA in *Escherichia coli*

Laboratory: Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)

PI: Kentaro Miyazaki, Ph.D., miyazaki-kentaro@aist.go.jp; +81 11-857-8489

Supervisor: Miyuki Tsukuda (Ph.D. student), Mitsuharu Sato (Ph.D. student)

Abstract of internship subject:

The bacterial ribosome consists of 3 rRNA molecules and 57 proteins and plays a crucial role in translating mRNA-encoded information into proteins. Because of the ribosome's structural and mechanistic complexity, it is believed that each ribosomal component coevolves to maintain its function. Unlike 5S rRNA, 16S and 23S rRNAs appear to lack mutational robustness, because they form the structural core of the ribosome. However, using *Escherichia coli* Δ7 (null mutant of operons) as a host, we have recently shown that an active hybrid ribosome whose 16S rRNA has been specifically substituted with that from non-*E. coli* bacteria can be reconstituted *in vivo*. To investigate the mutational robustness of 16S rRNA and the structural basis for its functionality, we used a metagenomic approach to screen for 16S rRNA genes that complement the growth of *E. coli* Δ7. Various functional genes were obtained from the Gammaproteobacteria and Betaproteobacteria lineages. Despite the large sequence diversity (80.9-99.0% identity with *E. coli* 16S rRNA) of the functional 16S rRNA molecules, the doubling times (DTs) of each mutant increased only modestly with decreasing sequence identity (average increase in DT, 4.6 s per mutation). The 3D structure of the 30S ribosome showed that at least 40.7% (628/1,542) of the nucleotides were variable, even at ribosomal protein-binding sites, provided that the secondary structures were properly conserved. Our results clearly demonstrate that 16S rRNA functionality largely depends on the secondary structure but not on the sequence itself. The aim of this internship program is to apply the same genetic selection system to unveil the mutational robustness of 23S rRNA.

Keywords: ribosome; 16S rRNA; 23S rRNA; metagenomics; screening; *Escherichia coli*

References:

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