

**Stony Brook University  
The Graduate School**

Doctoral Defense Announcement

**Abstract**

Identification and Characterization of Lon Protease as a Component of  
Bacterial *trans*-Translation

By

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The *trans*-translation pathway of bacteria serves to counteract problems associated with interrupted protein synthesis. During *trans*-translation, a trapped ribosomal complex that is unable to continue or terminate is properly released, the associated mRNA is rapidly degraded, and the incomplete peptide is tagged for targeted proteolysis. Ribosomal rescue helps maintain the pool of functional ribosomes, degradation of the ribosome-stalling mRNA prevents the recurrence of interrupted translation, and degradation of the incomplete peptide limits the cellular burden of abnormal proteins. The bifunctional transfer-messenger RNA (tmRNA) and its dedicated cofactor SmpB protein are essential components of *trans*-translation. Genetic screens were designed to identify novel components of *trans*-translation and further characterize this highly conserved pathway. 18,929 *Escherichia coli* mutants generated by transposon mutagenesis were screened for a specific bacteriophage phenotype associated with cells defective in tmRNA and SmpB, producing 148 primary candidates. Colony PCR analyses of the segment of *E. coli* genomic DNA containing the genes encoding tmRNA and SmpB suggested that two of the primary candidates contained transposon in this region. To remove mutants that were generally resistant to bacteriophage infection, the primary candidates were screened for sensitivity to a bacteriophage whose development is independent of tmRNA and SmpB function. The secondary bacteriophage screening of the primary candidates left 16 secondary candidates, which were mapped to determine transposon integration sites. Assessment of *trans*-translation function in the secondary candidates using an endogenous protein tagging assay revealed that each of the three candidates with transposon integrated in the gene encoding Lon protease accumulated excessive levels of the tagged proteins produced during *trans*-translation. Two reporter assays were optimized specifically for the study of tagged protein turnover *in vivo* and confirmed that cells defective in Lon protease are unable to efficiently dispose of tagged peptides compared to wild-type cells. *In vitro* proteolysis experiments using highly purified components showed that Lon preferentially degrades tagged proteins compared to untagged control proteins, thus complementing *in vivo* experiments. This dissertation discusses the use of genetic screens for the investigation of *trans*-translation and the experimental course used for the characterization a strong screen candidate.

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**Dissertation Advisor:** Dr. A. Wali Karzai