

**Stony Brook University
The Graduate School**

Doctoral Defense Announcement

The role of SmpB protein in *trans*-translation

By

Thomas R Sundermeier

Abstract

Trans-translation is a quality control mechanism utilized by bacteria to cope with the consequences of translation of incomplete or damaged mRNAs. The two unique components of this system are tmRNA (transfer messenger RNA) and its protein partner SmpB (Small protein B). tmRNA is a unique RNA molecule that exhibits structural and functional similarity to both tRNA and mRNA. In the early stages of the *trans*-translation mechanism, SmpB and tmRNA must recognize stalled ribosomes and bind in the ribosomal A-site. I present results that argue for binding of a pre-formed SmpB•tmRNA•EF-Tu(GTP) quaternary complex to stalled ribosomes to initiate the *trans*-translation mechanism. SmpB protein possesses a C-terminal tail that lacks structure in solution. Through site directed mutagenesis and *in vivo* based functional assays, I've shown that deletion of the tail, or mutation of specific residues near the C-terminus abolishes SmpB's activity in supporting *trans*-translation. Interestingly, C-terminal tail mutants still retain their ability to support the two known functions of the protein, binding to tmRNA and promoting association of the SmpB•tmRNA complex with stalled ribosomes. Hence, I've identified a novel function of SmpB protein in *trans*-translation, a function that requires an intact C-terminal tail. I've assembled an *in vitro trans*-translation system in order to pinpoint the specific function of the SmpB C-terminal tail in the molecular mechanism of *trans*-translation. I've shown that the C-terminal tail is required to support transpeptidation onto tmRNA both *in vivo* and *in vitro*. Hence, the novel SmpB function performed by the tail occurs during the tRNA-like function of tmRNA. Utilizing the *in vitro trans*-translation system, I was able to demonstrate that, like canonical tRNAs, tmRNA requires EF-Tu-mediated GTP hydrolysis for peptide bond formation. However, the SmpB C-terminal tail does not appear to be involved in eliciting activation of the EF-Tu GTPase, as mutant SmpBs lacking the C-terminal tail were fully functional in supporting GTP hydrolysis activity *in vitro*. Overall, results presented in this thesis provide unique insight into the mechanism of the elegant translational quality control mechanism known as *trans*-translation.

Date: November 6, 2007

Time: 3:00PM

Program: MCB

Dissertation Advisor: Dr. A. Wali Karzai

Place: Life Sciences Bldg. Rm 038