

## ABSTRACT

### INVESTIGATING THE ROLE OF THE ESSENTIAL GTPASE RBGA IN THE ASSEMBLY OF THE LARGE RIBOSOMAL SUBUNIT IN BACILLUS SUBTILIS

By

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Ribosomes are large ribonucleoprotein complexes required for synthesis of proteins and are a major target of several types of antibiotics. Ribosome assembly defects have been linked to several diseases, most notably Diamond-Blackfan anemia and Schwachman-Diamond syndrome. Although ribosome assembly has been studied extensively through in vitro reconstitution analysis, relatively little is known about the steps involved in vivo. Assembly factors such as GTPases are a crucial universal requirement for ribosome assembly yet their precise role has remained elusive. This thesis focuses on characterization of RbgA (ribosome biogenesis GTPase A), an essential GTPase in *Bacillus subtilis*, and its role in the assembly of the large ribosomal subunit. RbgA is widely distributed evolutionarily and its homologs have been functionally implicated in ribosome assembly in yeast (Lsg1p, Nog2p and Nug1p) as well as mammalian cells (Mtg1). Analysis of the ribosome assembly intermediate isolated from RbgA-depleted cells indicated that the GTPase plays a crucial role at a late stage in maturation of the 50S subunit and in recruitment of r-proteins L16, L27 and L36 during the assembly process. To elucidate the role of RbgA in 50S assembly, first, we performed extensive biochemical analysis to determine the kinetic parameters of RbgA and its interaction with the 50S subunit and ribosomal intermediates (Chapter 2). These studies revealed that RbgA requires K<sup>+</sup> ion for optimal GTPase activity and this activity is enhanced ~60 fold only in the presence of mature 50S subunit, and laid the groundwork for developing a model for the role of RbgA in the 50S assembly process. Next, we

generated a library of loss-of-function mutant RbgA proteins through site-directed mutagenesis and performed kinetic analyses and biochemical characterization to delineate the critical functional sites of the enzyme (Chapter 3). To analyze the mutations in vivo, we engineered *B. subtilis* strains to express mutated RbgA protein(s) and analyzed the ribosomal intermediates formed in the cells. These studies identified a potential catalytic residue of RbgA and provided insight into its catalytic mechanism when complexed with its ribosomal substrate. Further, we identified and characterized an RNA-binding domain required for RbgA function. Lastly, we designed a genetic suppressor screen, and isolated, and characterized six independent extragenic suppressor mutations that partially alleviated the growth defect in the RbgA-defective *B. subtilis* strain (Chapter 4). These studies revealed a novel in vivo ribosomal intermediate and determined a functional interaction between RbgA and r-protein L6. Together, these studies provide a model for the molecular mechanism of RbgA interaction with the 50S ribosomal subunit and the role of the enzyme in the assembly process.