Structural and functional studies of the *Staphylococcus aureus* ribosome hibernation

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Background: In bacteria, diminishing of protein synthesis is often accompanied with the formation of ribosomal dimers (or disomes). This process might be particularly important for pathogenic bacteria whose successful survival is a key factor for successful infection. Ribosome hibernation is driven by the binding of small stress-induced protein(s) to 70S ribosomes forming stable 100S ribosomal dimers. In contrast with *E. coli* where ribosome dimerization occurs in the presence of two proteins: hibernation promoting factor (HPF, 95 aa residues) and ribosome modulation factor (RMF, 55 aa residues) in *Staphylococcus aureus* only one protein (long version of HPF) is responsible for the formation of hibernating ribosome dimers, which in turn play a role in survival of this pathogen under unfavorable conditions.

Methods: By cryo-electron microscopy we obtained a high-resolution structure of the 100S ribosome dimer and by NMR spectroscopy and X-ray crystallography solved a NTD and CTD of SaHPF protein structures.

Results: Our recent cryo-EM studies demonstrated that integrity of 100S disomes is maintained primarily by interactions between C-terminal domains (CTD) of SaHPF with each other. N-terminal domain (NTD) of SaHPF binds to the 30S subunit at the P-site and A-site and may inhibit protein synthesis as

observed for shorter variants of HPF in other species. By NMR spectroscopy we have determined the solution structure of SaHPF-NTD and analyzed its binding with *S. aureus* 70S ribosome.

Conclusion: Based on NMR and crystal structures of SaHFP-CTD we predicted the key amino acids residues stabilizing dimer interface and made several single amino acid substitutions which allows us to abolish the ribosome hibernation. Obtained information about crucial for dimerization residues in long HPF may be used as a potential target for combating *S. aureus*.

Key Words: ribosome, translation, hibernation, NMR

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