Structural and mechanistic insights into the FusB family of fusidic acid resistance proteins

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Introduction

A major focus in the O'Neill laboratory is on understanding the mechanisms by which bacteria resist the effects of the antibiotics that are used to treat the infections they cause. The antibiotic fusidic acid inhibits bacterial protein synthesis in the bacterial pathogen *Staphylococcus aureus* by blocking release of the translocase, elongation factor G (EF-G), from the ribosome. Resistance to fusidic acid is most commonly mediated by proteins of the FusB family, which bind to elongation factor G and protect it from the inhibitory effect of fusidic acid. The mechanism by which the FusB-type proteins interact with EF-G to mediate resistance to fusidic acid resistance is not fully understood, and recent efforts have therefore focused on gaining a detailed understanding of this protein-protein interaction (PPI).

Mapping of the binding interface between FusB and EF-G by mutagenesis

Previous studies using NMR chemical shift mapping have established that it is the C-terminal domain of FusB-type proteins that is responsible for mediating the interaction with EF-G. To more precisely delineate the EF-G binding site within this region, alanine-scanning mutagenesis was employed to systematically substitute conserved, surface exposed residues of FusB. Of 18 alanine substitution mutations generated, four resulted in FusB proteins that were no longer capable of binding to EF-G. The four residues identified lie in close proximity to each other in the three dimensional structure of the FusB protein, and reside within a region that matches previous *in silico* predictions for the EF-G-binding site on FusB.

Mapping the FusB binding site by NMR

We have previously shown that the C-terminal half of the EF-G protein (a protein fragment referred to here as EF-G_{C3}) contains the binding site for the FusB-type proteins. NMR backbone assignment of EF-G_{C3} has recently been undertaken in both the FusB-bound and free forms, and work is underway to use NMR chemical shift changes to identify the binding site for FusB on EF-G_{C3}. Preliminary docking studies show that the interaction site of FusB on EF-G is similar, but not identical to, that previously predicted *in silico*.

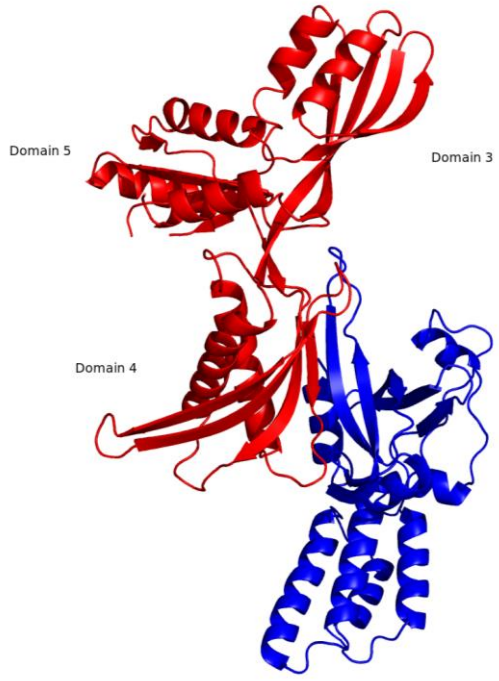

Figure 1: proposed model of the interaction between EF-G and FusB from initial docking of the crystal structures of the two proteins (PDB IDs 2XEX and 4ADN, respectively). Domains 3-5 of EF-G are shown in red, and FusB is shown in blue.

Our current model for the interaction between FusB and EF-G is shown in Figure 1; additional NMR and mutagenesis experiments are currently underway to further refine our model of this PPI, and thereby facilitate a better understanding of this unusual antibiotic resistance mechanism.

Publications

Cox, G., Thompson, G., Jenkins, H., Peske, F., Savelsbergh, A., Rodnina, M., Wintermeyer, W., Homans, S., Edwards, T. & O'Neill, A. Ribosome clearance by FusB-type proteins mediates resistance to the antibiotic fusidic acid. (2012) *Proc. Natl. Acad. Sci. U S A* **109:** 2102-7.

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