
rpmE2 and yidC, two novel mRNA targets of the multifaceted *Staphylococcus aureus* SprF1 antitoxin: Do these interactions contribute to SprF1-mediated antibiotic persistence?

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Résumé

Introduction

Bacteria can protect themselves from antibiotic treatment by becoming resistant or persistent. Persister cells are a subpopulation of transiently antibiotic-tolerant bacteria associated with antibiotic treatment failures and relapsing infections. Among others, type I toxin-antitoxin (TA) systems have been linked to persister cells formation. They are composed of a peptide toxin whose overexpression confers growth stasis or cell death, and of an RNA antitoxin that base-pairs with the toxin mRNA to inhibit its translation. In the *Staphylococcus aureus* SprG1/SprF1 type I TA system, SprF1 is a multifaceted antitoxin that, in addition to repress SprG1 expression and toxicity, can also bind ribosomes to inhibit global translation and promote persister cells formation (1). To better understand SprF1 regulatory implication in antibiotic persistence, we aim to identify its RNA direct targets using *in silico* analysis and MAPS (MS2-affinity purification coupled with RNA sequencing) experiments.

Material and methods

In silico SprF1 targets were identified using three softwares: RNApredator, IntaRNA and CopraRNA. In parallel, a MAPS experiment was carried out to identify *in vivo* SprF1 targets. To achieve this, an MS2 aptamer was fused at the 5' or 3'-end of SprF1 RNA. These chimeras were cloned in pRMC2 plasmid, allowing anhydrotetracycline (aTc) inducible expression, and transformed into *S. aureus* HG003 strain. After aTc induction, RNAs were extracted and purified. SprF1 targets were identified by RNA sequencing and differential expression analysis using the HTSeq/DESeq2 pipeline.

Results and conclusion

MAPS enabled to identify, besides SprG1, eleven novel SprF1 targets. These include mRNAs encoding the 50S ribosomal protein L31 (RpmE2) and the membrane protein insertase

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YidC. Interestingly, *yidC* is one of the five common mRNA targets found by the three *in silico* interaction predictions softwares. Based on our *in vivo* and *in vivo/in silico* investigations, these two candidates were selected. SprF1 interaction with *yidC* and *rpmE2* was more precisely defined *in silico* using IntaRNA and confirmed *in vitro* by gel retardation assays. Next, the functional link between SprF1, these two mRNA targets, and persister cells formation will be studied. Overall, this work could lead to the identification of novel therapeutic strategies to fight against recurrent *S. aureus* infections.

Mots-Clés: MAPS, Type I toxin, antitoxin system, SprF1 antitoxin, Antibiotic bacterial persistence, Staphylococcus aureus