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In *vivo* expression of H69-targeting peptides in bacteria

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Identifying novel drug targets within the bacterial ribosome is an important approach to overcome the well-known problem of antibiotic resistance. The specific region of the ribosome under investigation in this study is helix 69 (H69) of 23S ribosomal RNA. Considering the variety of functions of H69 in protein biosynthesis, as well as differences between the bacterial and human H69 sequences, this RNA is an attractive antibacterial drug target. In a previous study, short peptides that specifically bind to H69 were isolated by using phage-display libraries. In a second approach, modified variants of the selected parent peptide sequence were synthesized to identify more tight binders. The main objective of the current study was to express these H69-targeting peptides in *E. coli* to investigate the inhibitory effects on protein synthesis. H69-targeting peptides were expressed *in vivo* as Green Fluorescence Protein-fusion proteins and their activities were monitored through cellular fluorescence levels. The seven-mer peptide sequence was cloned behind the Tobacco Etch Virus (TEV) protease recognition sequence. Expression of TEV protease from another plasmid in the same cell cleaves the TEV-recognition peptide sequence, and the peptide is then exposed at the N-terminus of GFP. The construct was prepared in which a his tag was placed at the C- terminus of the GFP gene such that the peptide could be purified for further experiments. In order to further characterize selected peptides as potential drug leads it is necessary to determine their activity outside the context of the fusion protein. Therefore, currently we are using a different plasmid system which can be used to express short peptides as free peptides in bacteria cells. Expression of two different peptides was shown to have an inhibitory effect on bacterial cell growth. These findings will be helpful for future antimicrobial drug development.

Keywords: Ribosome, helix 69, Peptides, Antibiotic resistance