Drosocin-like peptides exhibit highly diverse antimicrobial activity and inhibit translation at two different stages

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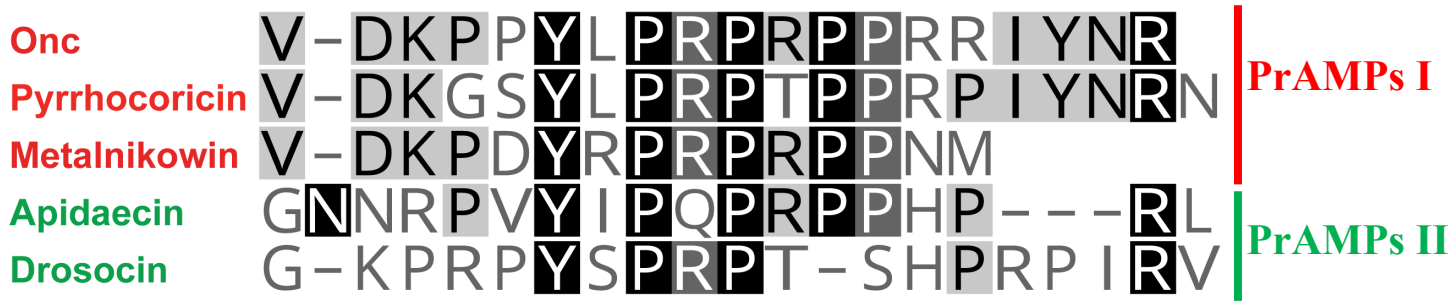
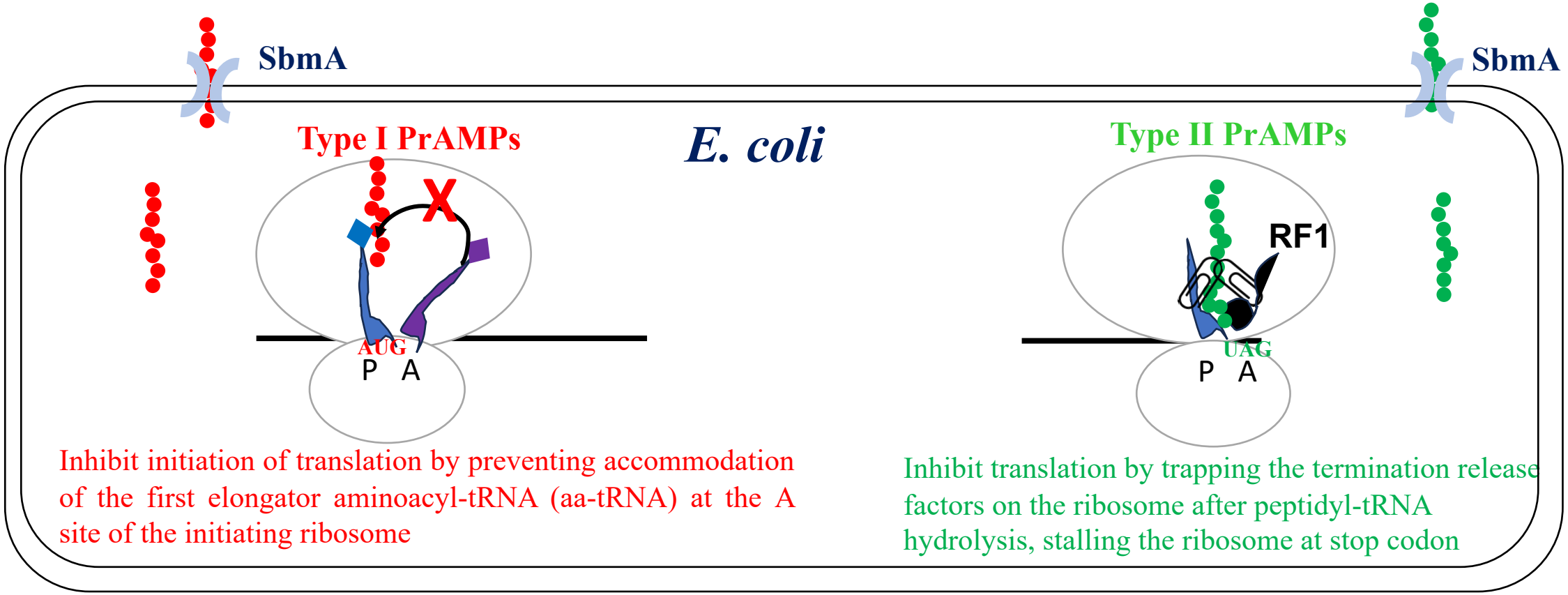
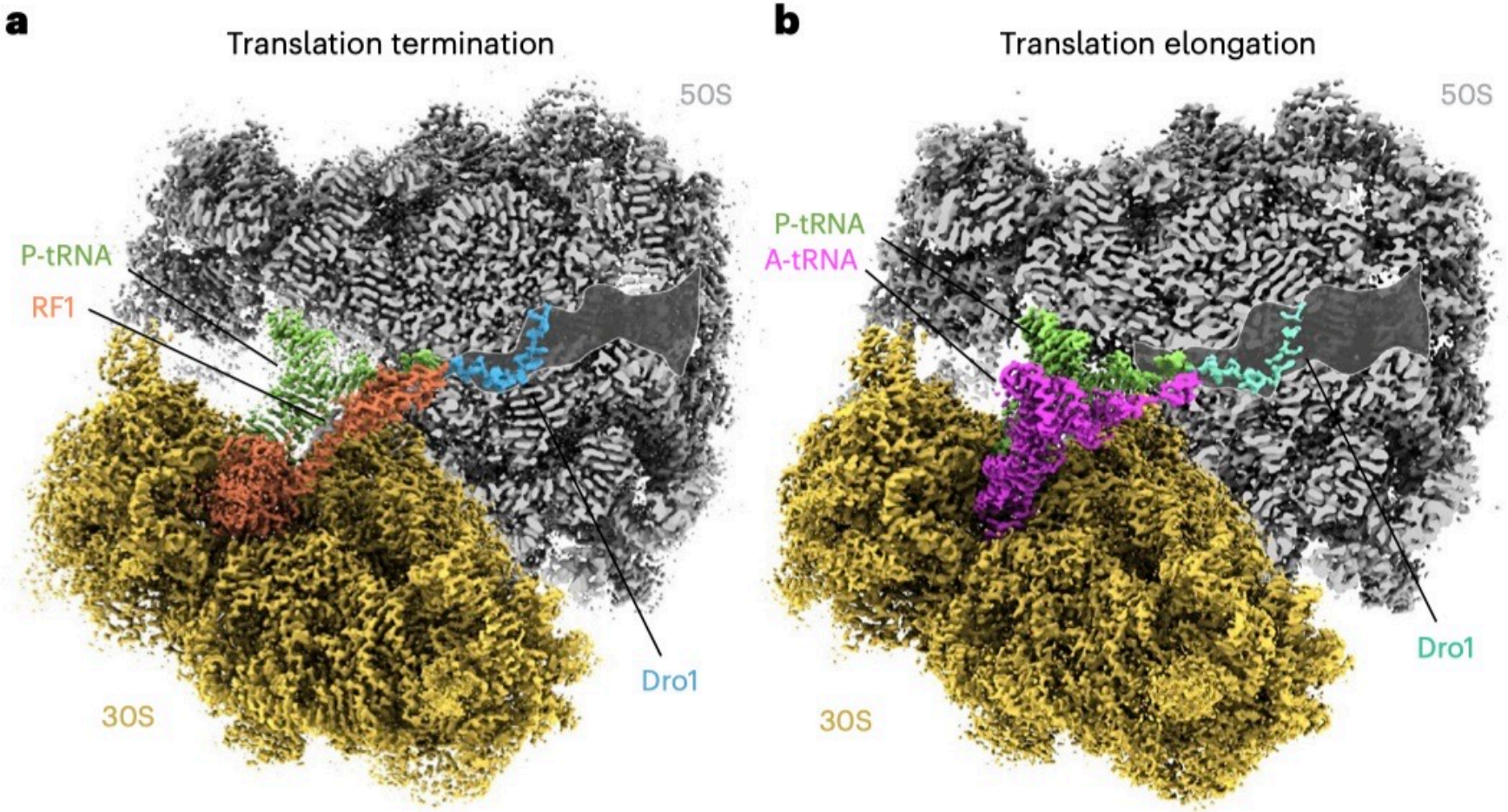
Proline-rich antimicrobial peptides (PrAMPs) are short peptides naturally synthesized by arthropods and mammals. According to their ability to inhibit translation, PrAMPs are classified into two subgroups: PrAMPs I and PrAMPs II





Drosocin (Dro), produced by fruit fly *Drosophila melanogaster*, is a translation termination inhibitor although it can bind to both translation termination and early elongation complex

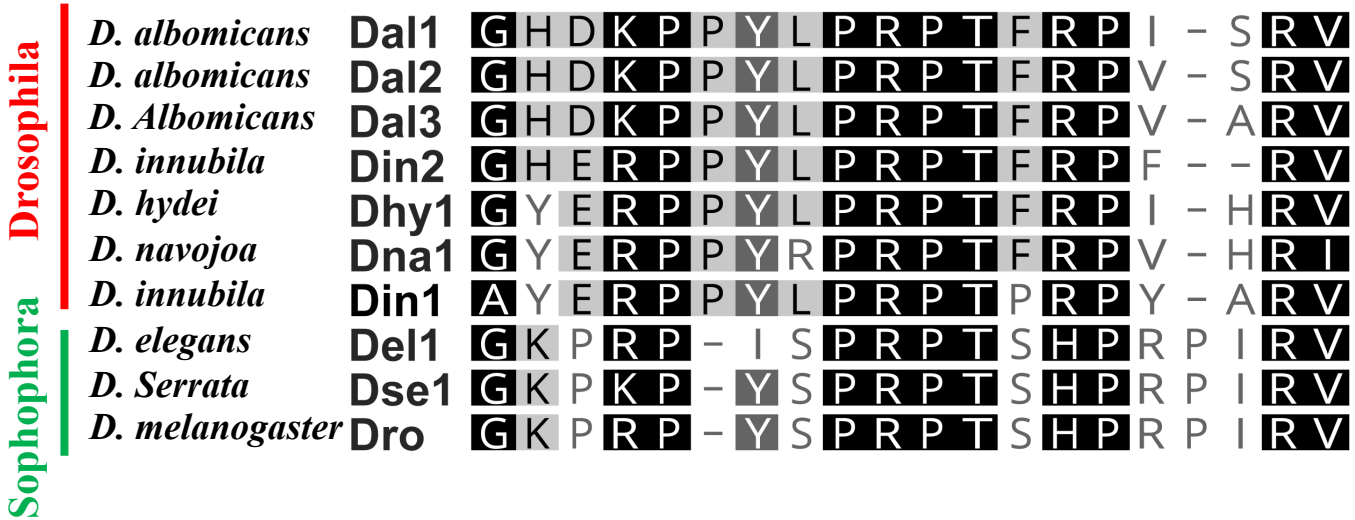


Thr11 (underlined) is O-glycosylated with N-acetylgalactosamine and galactose in natural Drosocin

Koller, et al. Nat Chem Biol 19, 1072–1081 (2023)
Mangano, et al. Nat Chem Biol 19, 1082–1090 (2023)

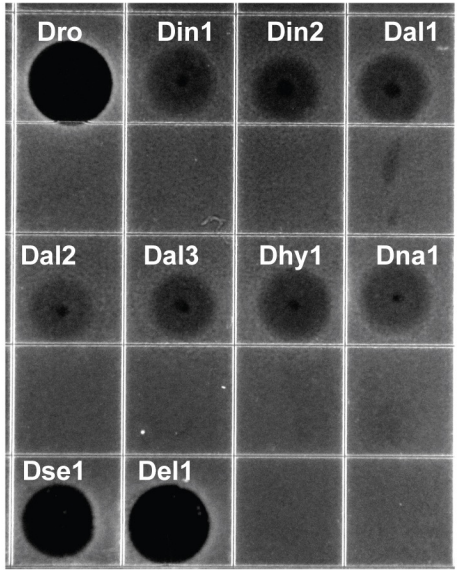
What is the extent of diversity among Dro-like peptides that share the same mode of action as Dro?

Dro-like peptides were identified through bioinformatic analysis from a wide range of fly species. Dro homologs within the subgenus *Drosophila* are distinct from those within the subgenus *Sophophora*. A total of nine Dro-like were chemically synthesized in a non-glycosylated form.

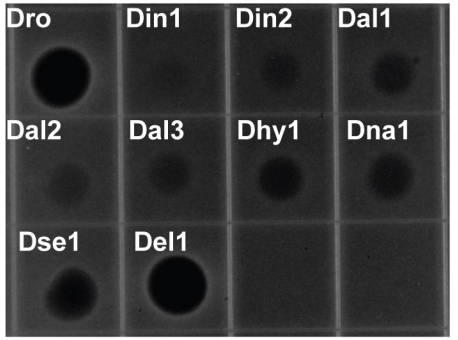


Two of the-Dro-like PrAMPs (besides the already characterized Dro from *D. melanogaster*) were able to kill the *E. coli* and *K. pneumoniae*, others displayed weak antimicrobial activity.

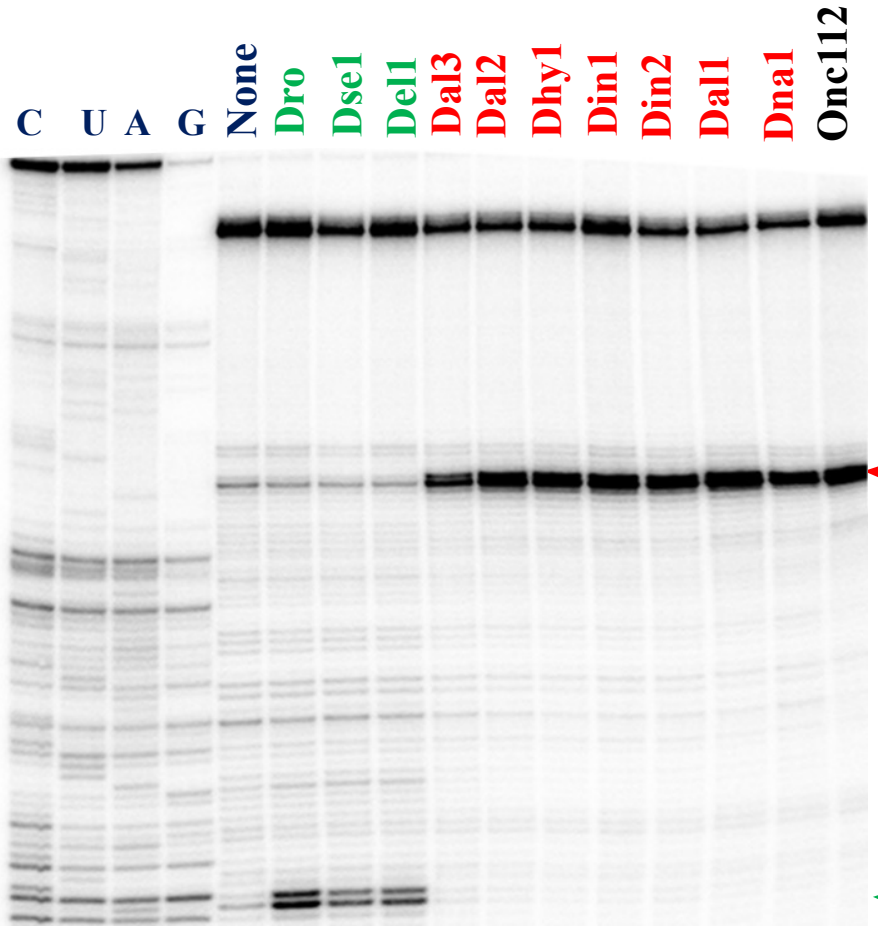
Escherichia coli BL21

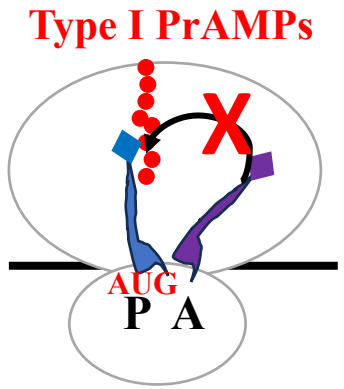


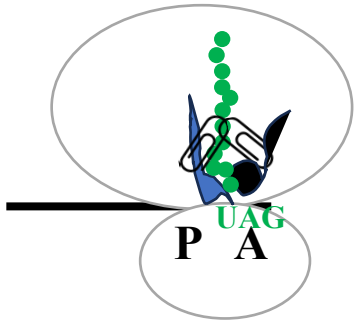
Klebsiella pneumoniae AR0112



Dro-like peptides inhibit translation at different stages

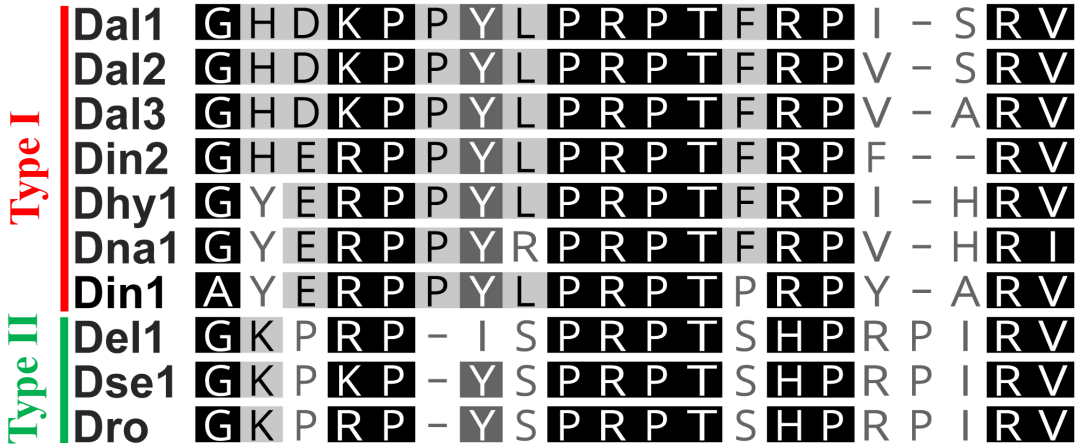






The toeprinting assay relies on reverse transcription to pinpoint the specific positions of ribosome stalling is induced by the inhibitors on a reporter mRNA

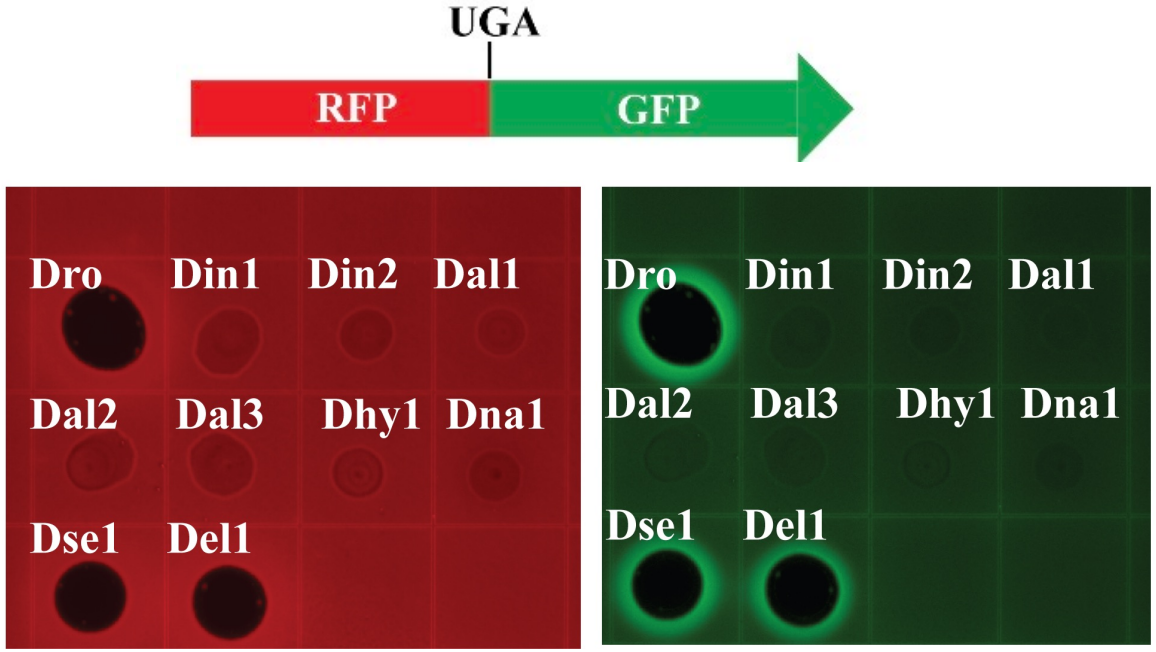
Some Dro-like peptides arrested ribosomes at start codons, resembling the behavior of type I PrAMPs.



Dro-like peptides with superior antimicrobial activity arrested ribosomes at stop codons, resembling the behavior of type II PrAMPs.

Type II Dro -like PrAMPs induce stop codon readthrough

In agreement with toe-printing assay, only **Dro, Dse1 and Del1** promote stop codon readthrough, resembling the behavior of type II PrAMPs.



Drops of peptide solutions were placed on agar plates containing a lawn of cells transformed with a reporter plasmid, in which an in frame fused RFP-GFP coding sequences are separated by a UGA stop codon. The green halo of GFP expression reveals stop codon, readthrough while expression of RFP serves as an internal control.

Conclusion

- Two out of the nine new Dro-like peptides demonstrate antibacterial activity, inducing stop codon readthrough and arresting ribosomes at stop codons
- Dro-like peptides whose mode of action resembles type I PrAMPs were less active in inhibiting growth of the tested strains, possibly reflecting poor peptide uptake
- The outcomes of our study underscore the complexity of predicting MOA solely based on similarity of PrAMP sequences

Acknowledgement

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