Development of new peptide-based antimicrobial agents targeting *trans*-translation in bacteria

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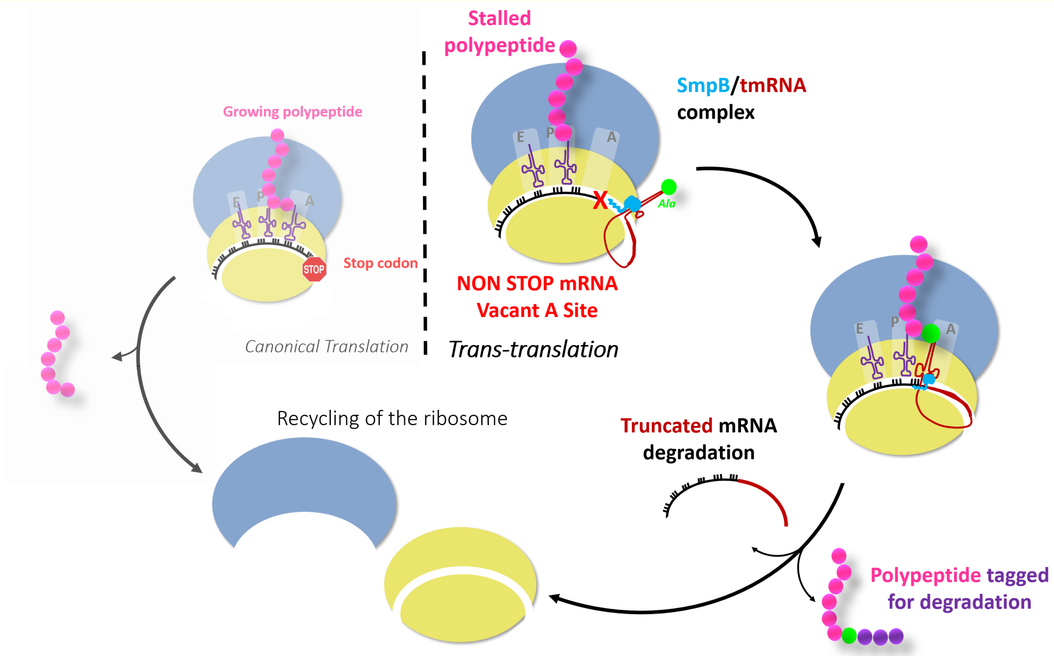
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Trans-translation is the main recue system in bacteria. It is required for bacteria stress tolerance, growth and virulence, and permits resuming stalled translation when errors occur, hence ensuring bacterial survival. In this bacteria specific process, a small protein called SmpB recognizes stalled ribosomes by inserting its helical C-terminal tail in the vacant ribosomal mRNA path and allows the correct positioning of its partner, transfer-messenger RNA. In the search for **alternative antibiotic strategies**, the **rational design of SmpB C-ter tail peptide decoy** would provide a **highly specific *trans*-translation inhibitor** with potentially **broad-spectrum antimicrobial activity** and **limited side-effects on host cells**.

1 - *Trans*-translation, an appealing target in the fight against antibioresistance[1]

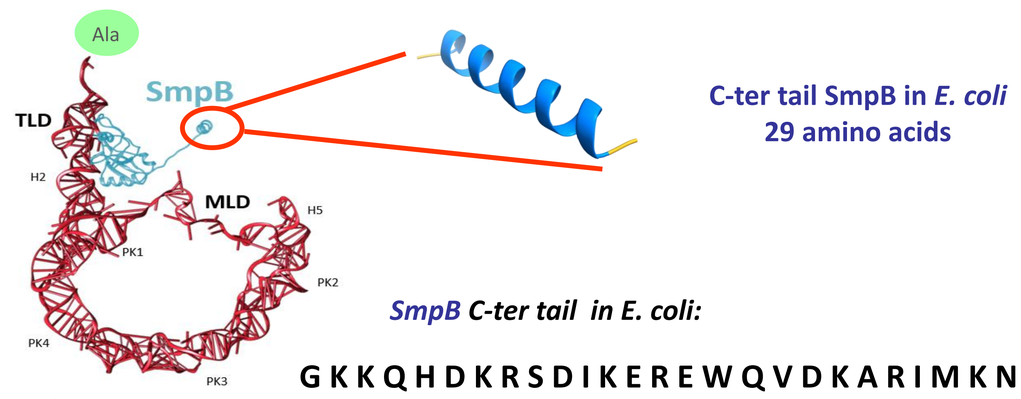
What is ***trans*-translation ?**

- Under stress, ribosome can get stalled during translation due to non-Stop mRNA
- SmpB/tmRNA complex identifies and initiates **rescue** of the stalled ribosome
- Release of the stalled ribosome and degradation of the faulty mRNA and incomplete peptide



Trans-translation is an **appealing target** because:

- It is absent in eukaryotes
- It is necessary for bacterial **survival**
- It is required for the **virulence** of some species
- When deletion is not lethal (due to backup systems), it induces **hypersensitive** phenotypes



C-ter tail SmpB in *E. coli*
29 amino acids

***SmpB* C-ter tail in *E. coli*:**

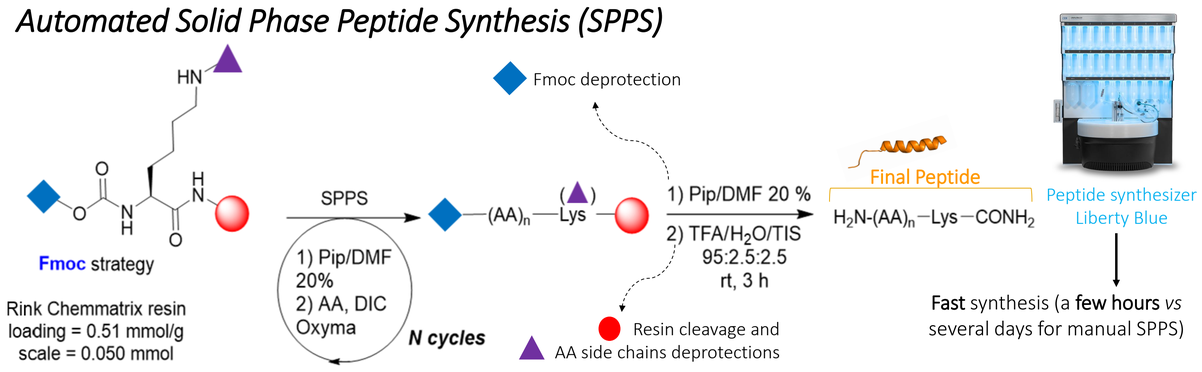
G K K Q H D K R S D I K E R E W Q V D K A R I M K N A H R

Goal:

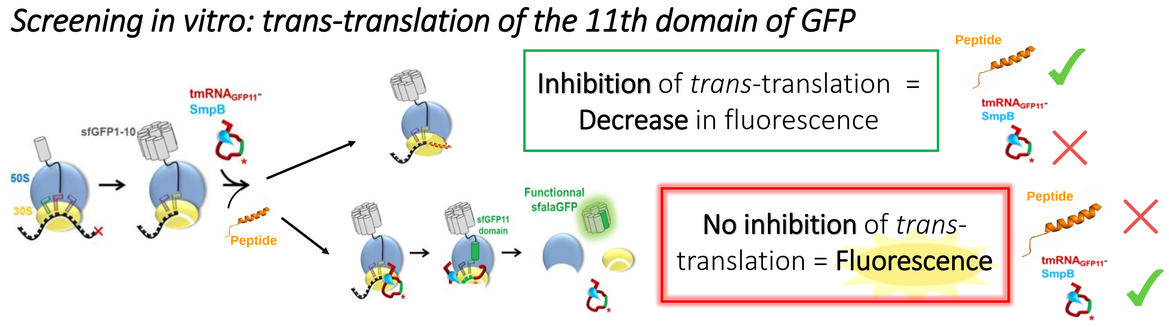
Design of the shortest **mimetic** of the SmpB C-ter tail that **selectively** inhibits *trans*-translation

2 - Synthesis and *in vitro* screening[3] of the peptides

Automated Solid Phase Peptide Synthesis (SPPS)



Screening in vitro: trans-translation of the 11th domain of GFP



In vitro experiments are implemented to determine the **inhibition capacity** of a designed peptide over the ***trans*-translation** using GFP fluorescence[3].

3 - Results on the mimic SmpB_29 (*E. coli*)

The 29 amino acids peptide corresponding to the C-ter tail of the protein SmpB (SmpB_29) was tested with the *in vitro* system.



In the system :

- $C_{estimated\ ribosome}$ = 1,5 µM
- C_{SmpB} = 2,5 µM
- C_{tmRNA} = 1,25 µM

Ratio protein SmpB/peptide:

- 100x (250 µM)
- 50x (125 µM)
- 10x (25 µM)
- 1x (2,5 µM)

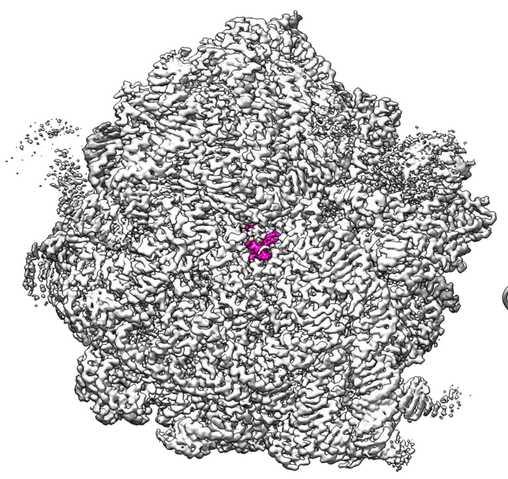
The peptide SmpB_29 showed great efficiency toward the inhibition of *trans*-translation : **still 88% of inhibition of *trans*-translation at 10x (25 µM)**

Comparatively, **only 20% of inhibition of translation at 10x (25 µM)**

A **peptide** mimicking the C-ter tail of SmpB can **selectively** inhibit *trans*-translation over translation at low concentration, and **in a competitive manner** in regard with the SmpB/tmRNA complex.

4 - Cryo-electron microscopy of SmpB_29 bound to a stalled ribosome

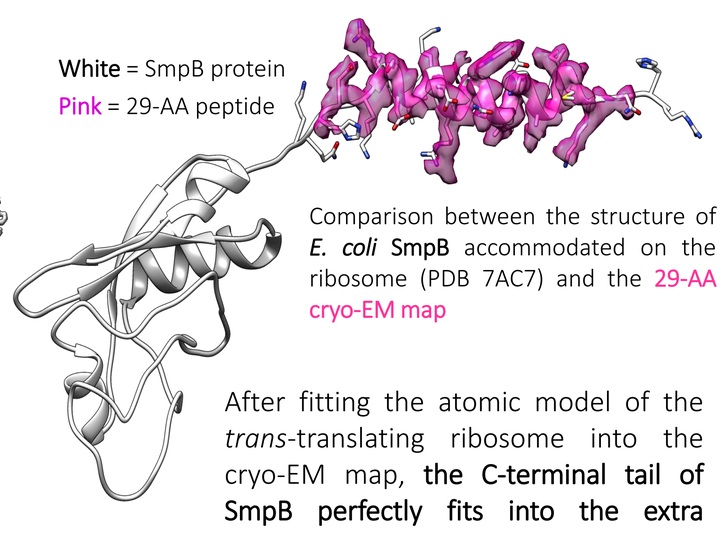
To precisely confirm the target of the peptide SmpB_29, we obtained a Cryo-EM density map of the peptide into a stalled *E. coli* ribosome.



Resolution : **1.84Å**

Extra density corresponding to the peptide

Huge excess of peptide (SmpB_29 / ribosome = 250)



White = SmpB protein

Pink = 29-AA peptide

Comparison between the structure of ***E. coli* SmpB** accommodated on the ribosome (PDB 7AC7) and the 29-AA cryo-EM map

After fitting the atomic model of the *trans*-translating ribosome into the cryo-EM map, **the C-terminal tail of SmpB perfectly fits into the extra density**

The 29-AA peptide occupies the **same binding site** as SmpB C-ter tail, and can therefore **compete** with the *trans*-translation machinery

5 - Conclusion and perspectives

- **Selective inhibition** of *trans*-translation by a **decoy peptide** is possible
- Inhibition of *trans*-translation without inhibiting translation is possible even
- Truncated versions showed inhibition too, allowing the design of a **shorter peptide**

→*Perspectives:*

- **Strengthen the interactions** with the ribosome and improve the **metabolic stability: chemical modifications** on strategic positions of the peptide chain identified through *in silico* studies
- ***In vivo* activity** of the "hit" peptide to be determined: introduction of a **cell-penetrating peptide** (CPP)
- Extrapolation of the *E. coli* results on other pathogenic bacteria (**ESKAPEE**)

References

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3. Thépaut, M.; Campos-Silva, R.; Renard, E.; Barloy-Hubler, F.; Ennifar, E.; Boujard, D.; Gillet, R. Safe and Easy in Vitro Evaluation of TmRNA-SmpB-Mediated Trans-Translation from ESKAPE Pathogenic Bacteria. *RNA* **2021**, *27* (11), 1390–1399. https://doi.org/10.1261/rna.078773.121.