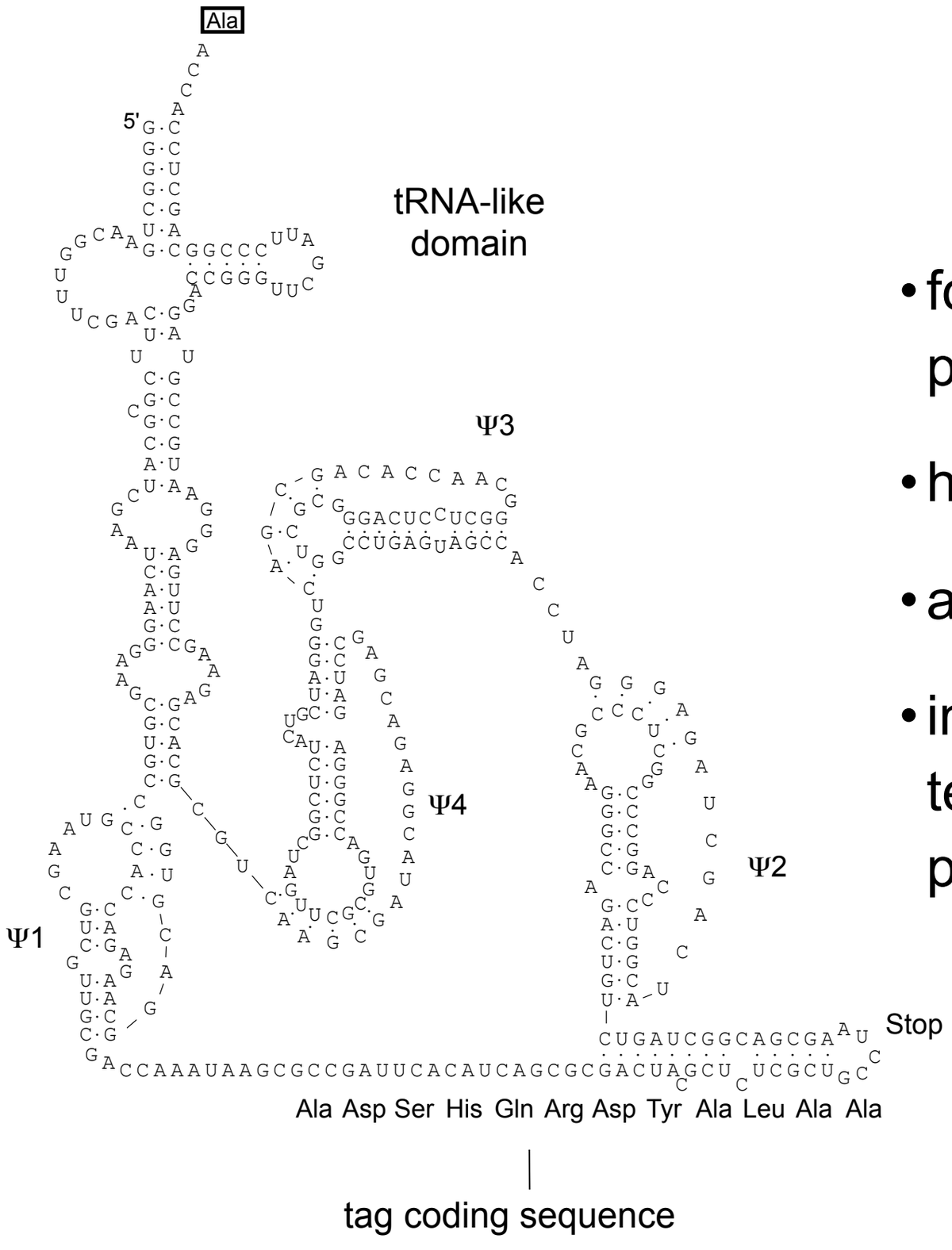
trans-translation: SsrA, SmpB, EF-Tu, RpsA, and PZA

Ken Keiler
Penn State University

tmRNA ribonucleoprotein complex



- found in every species of bacteria, some plastids & primitive mitochondria
- highly abundant (~10% rRNA level)
- also called SsrA (Small, stable RNA)
- in *E. coli,* 2-4% of translation initiations terminate by translating tmRNA (5 times per ribosome per cell cycle)

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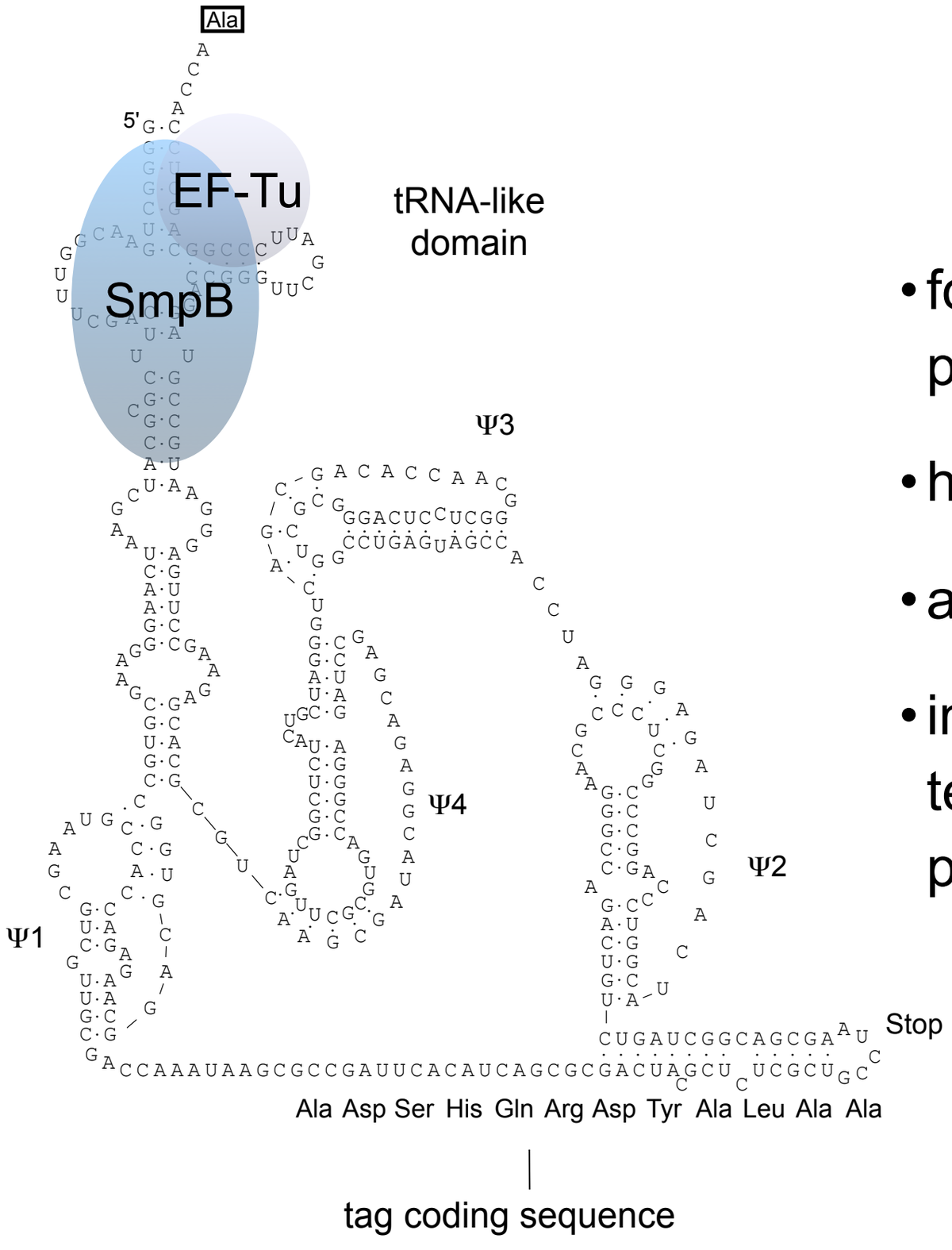

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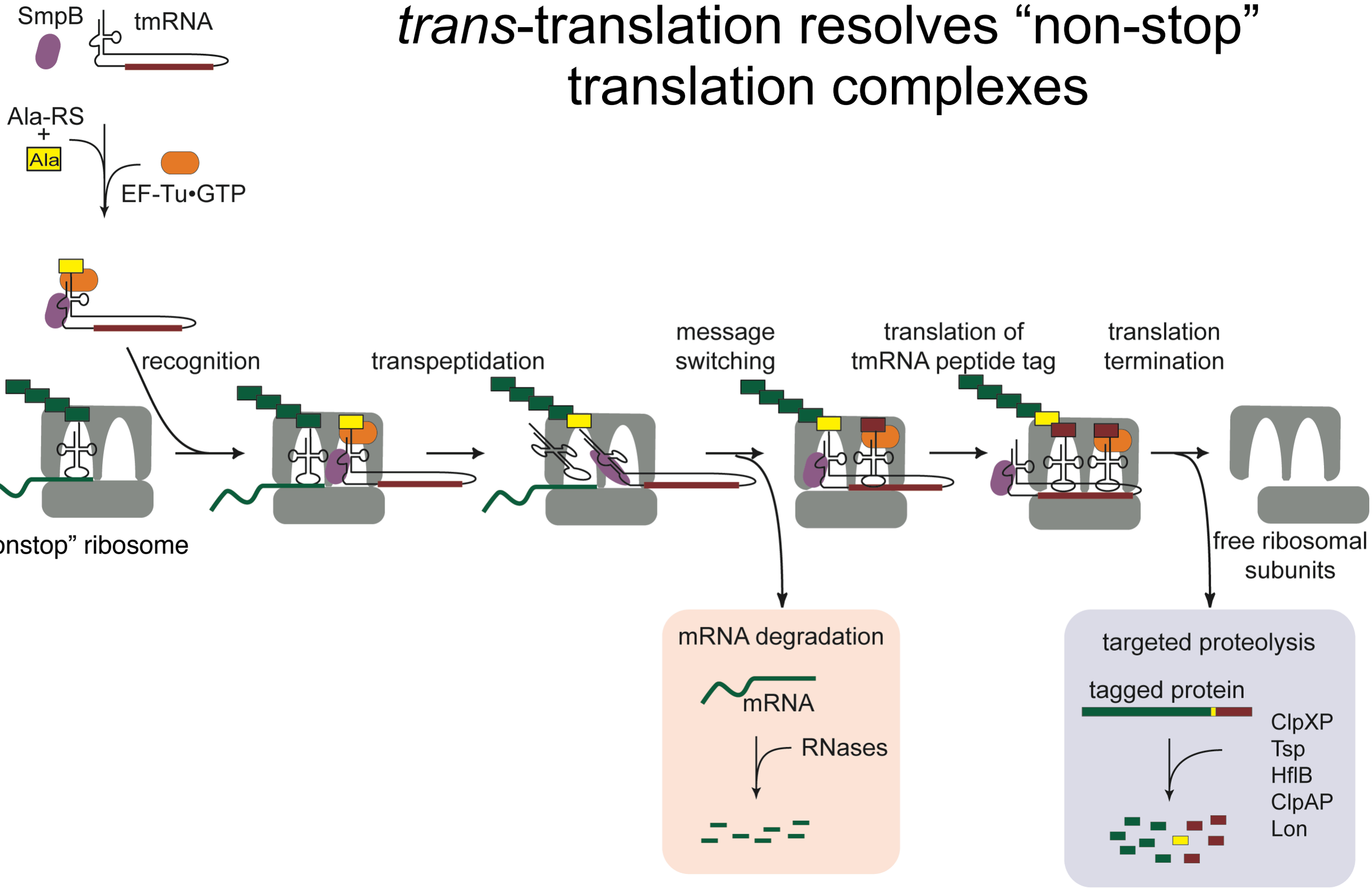
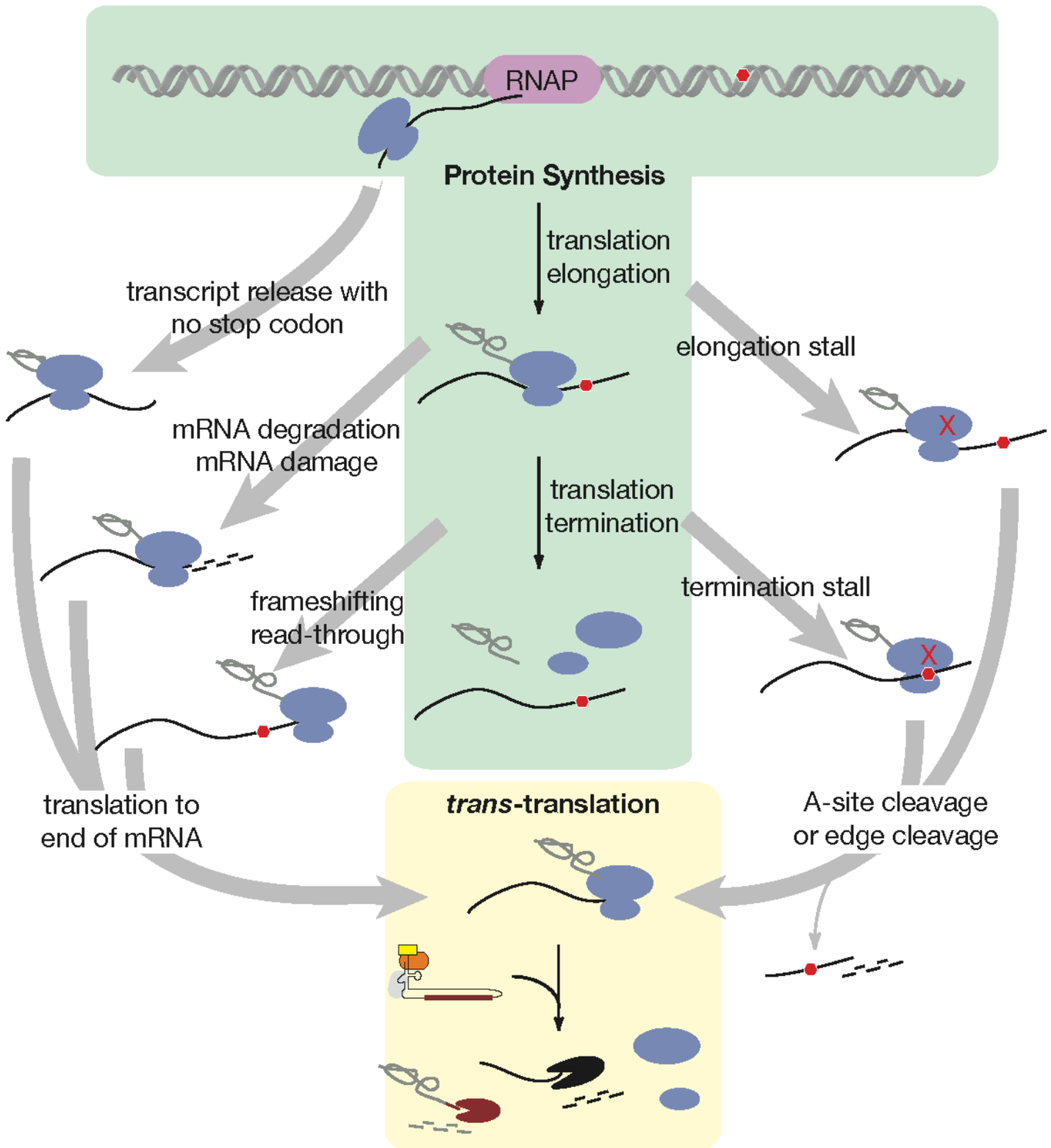
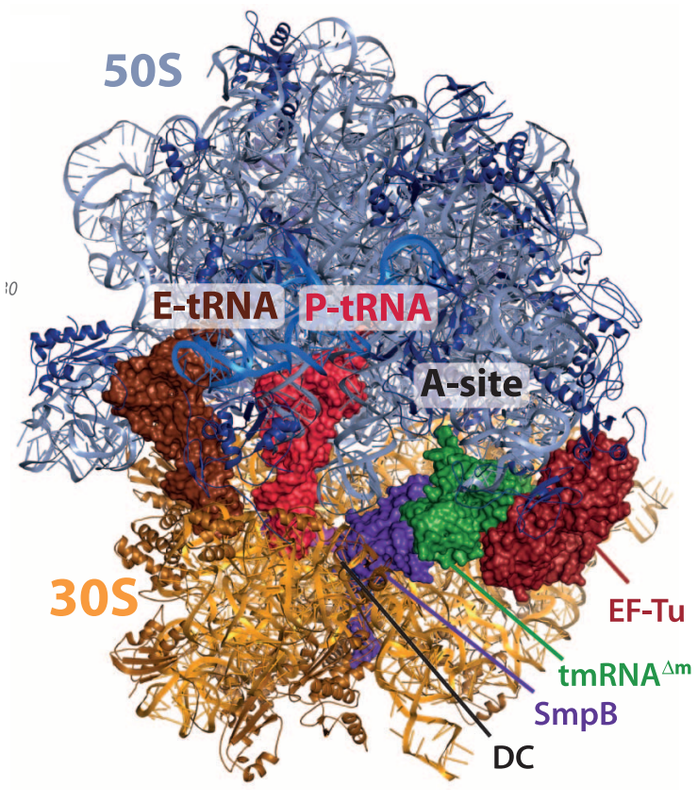
trans-translation resolves “non-stop” translation complexes

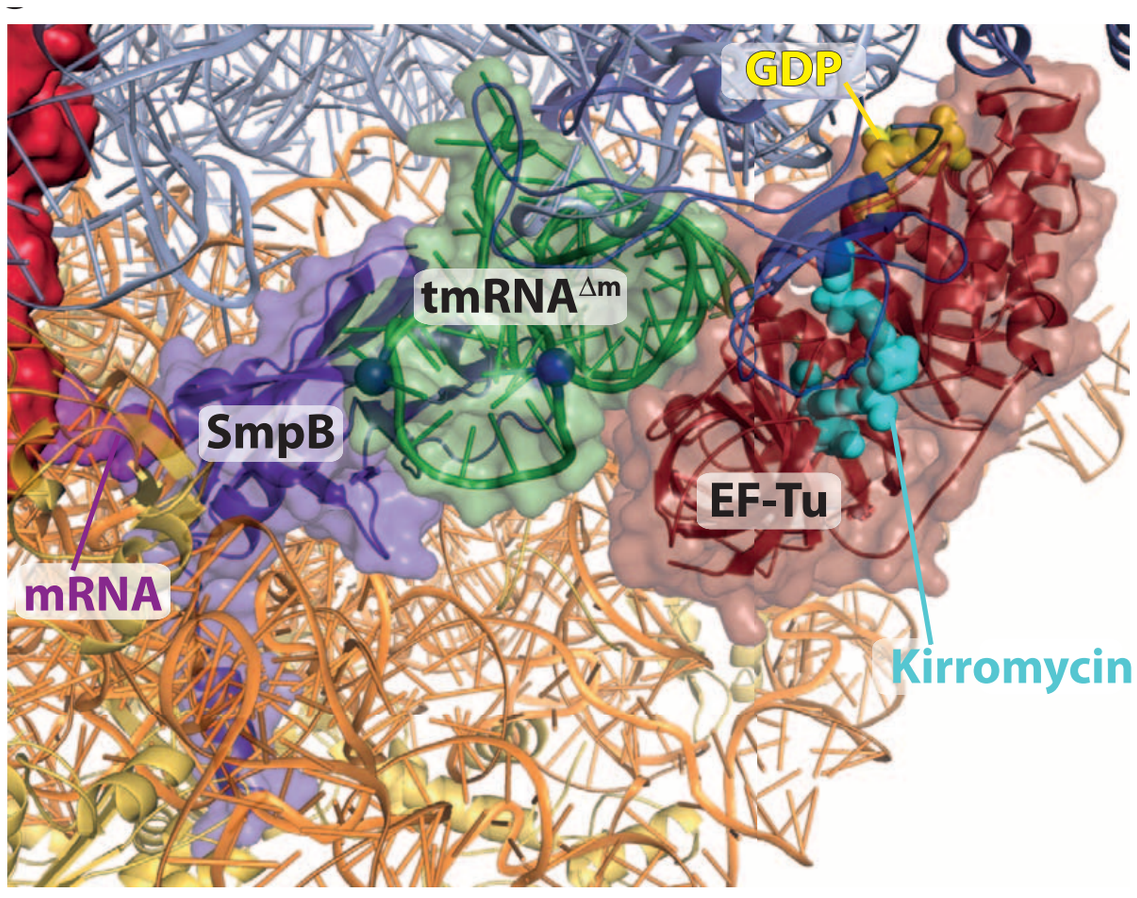


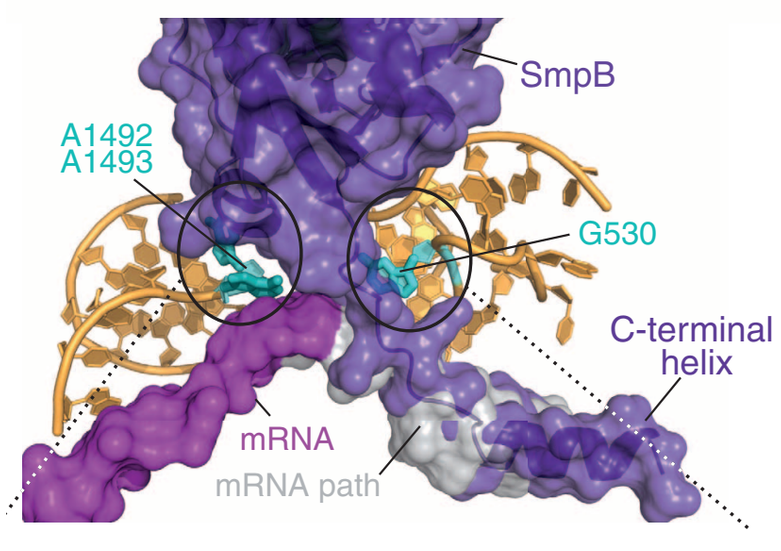
transcription and translation mistakes lead to *trans*-translation



EF-Tu binds the tRNA-like domain of tmRNA and SmpB mimics the missing mRNA







Decoding in the Absence of a Codon by tmRNA and SmpB in the Ribosome
Cajetan Neubauer, *et al.*
Science **335**, 1366 (2012);
DOI: 10.1126/science.1217039

the RpsA (S1) enigma

- RpsA from *E. coli* and *Thermus thermophilus* binds tmRNA, but not as well as mRNA
- RpsA is not required for *trans*-translation in vitro using *E. coli* or *T. thermophilus* components
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Could PZA act through RpsA and *trans*-translation in MTB?

- RpsA could be important for *trans*-translation in MTB
  - Mycobacterial RpsA has only 4 S1 repeats instead of 6
  - RpsA could be particularly important during latency
- RpsA-POA could inhibit *trans*-translation through a mechanism other than blocking RpsA-tmRNA binding

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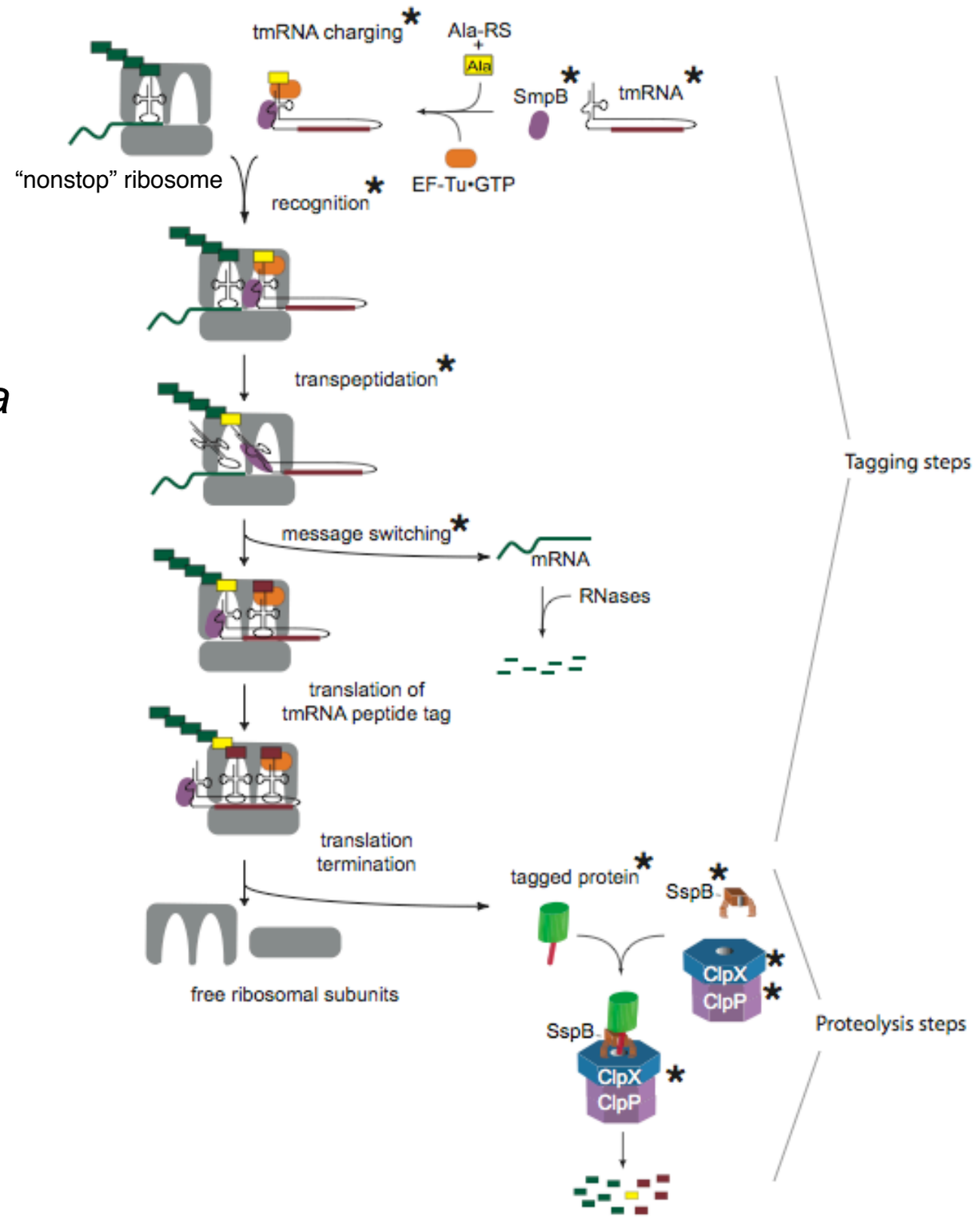
- need biochemical studies with mycobacterial components

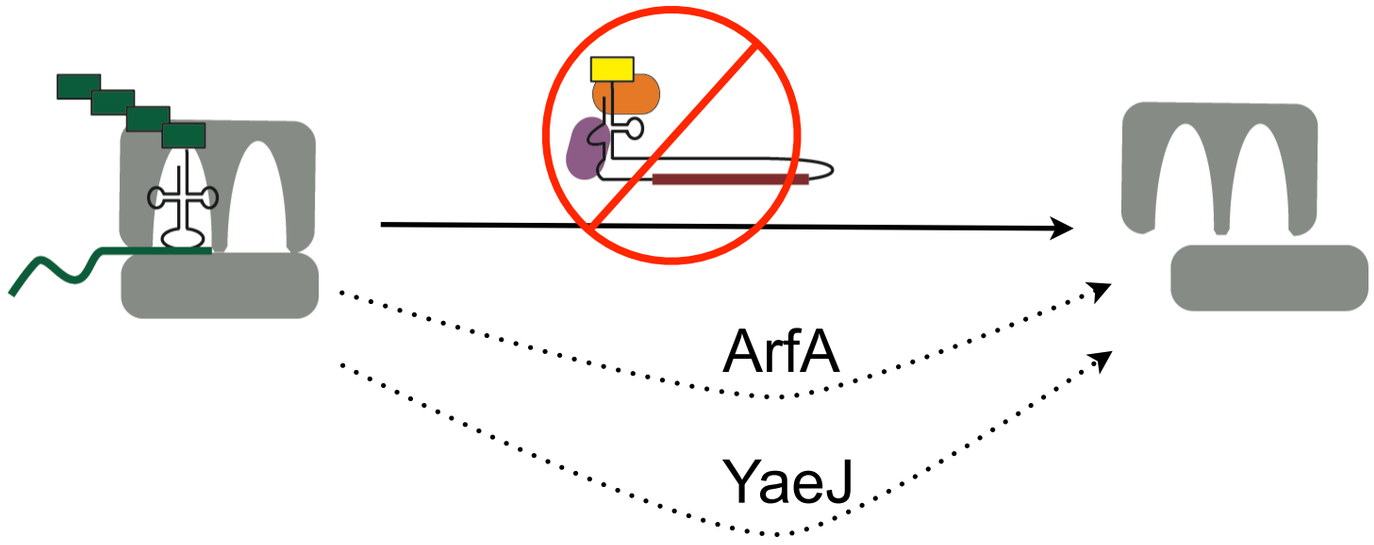
trans-translation is essential in many bacteria

Neisseria gonorrhoeae
Shigella flexneri
Haemophilus influenza
Helicobacter pylori
Mycoplasma genitalium and *M. pneumonia*
Bacillus anthracis
Staphylococcus aureus
Francisella tularensis

**Mycobacterium smegmatis*
Mycobacterium tuberculosis



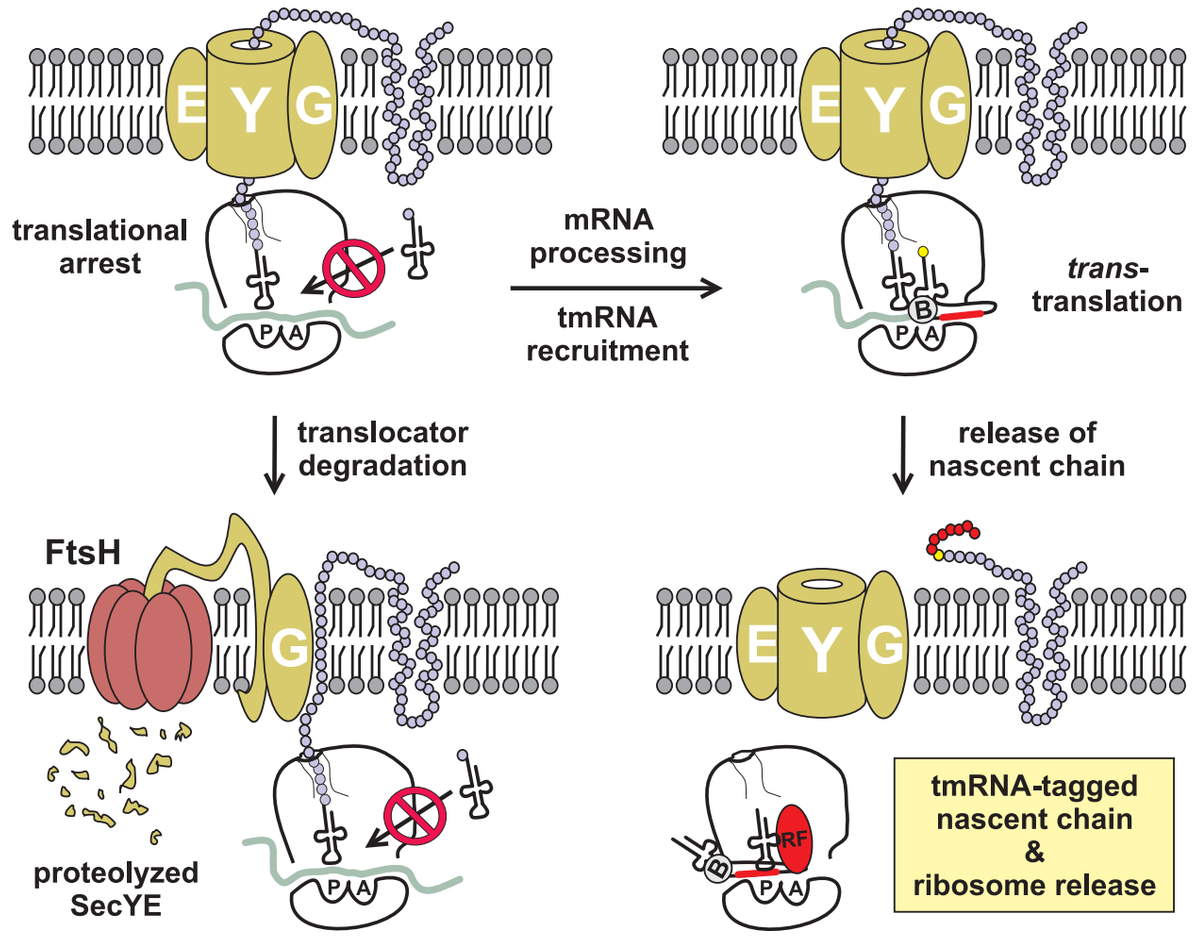
some species have backup systems for ribosome release



- in all cases studied, deletion of tmRNA and the backup system is lethal
- MTB and *M. smegmatis* do not have obvious homologs of ArfA or YaeJ

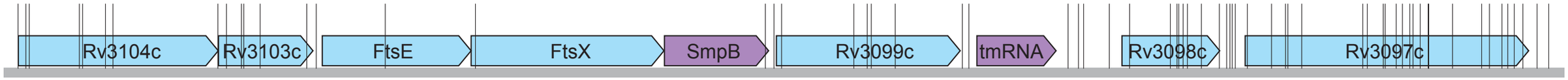
Why is loss of *trans*-translation detrimental?

1. loss of translation capacity
2. accumulation of incomplete proteins
3. particular problems with membrane proteins



neither *ssrA* nor *smpB* can be deleted in *Mycobacteria*

1. no transposon hits from saturating mutagenesis in MTB

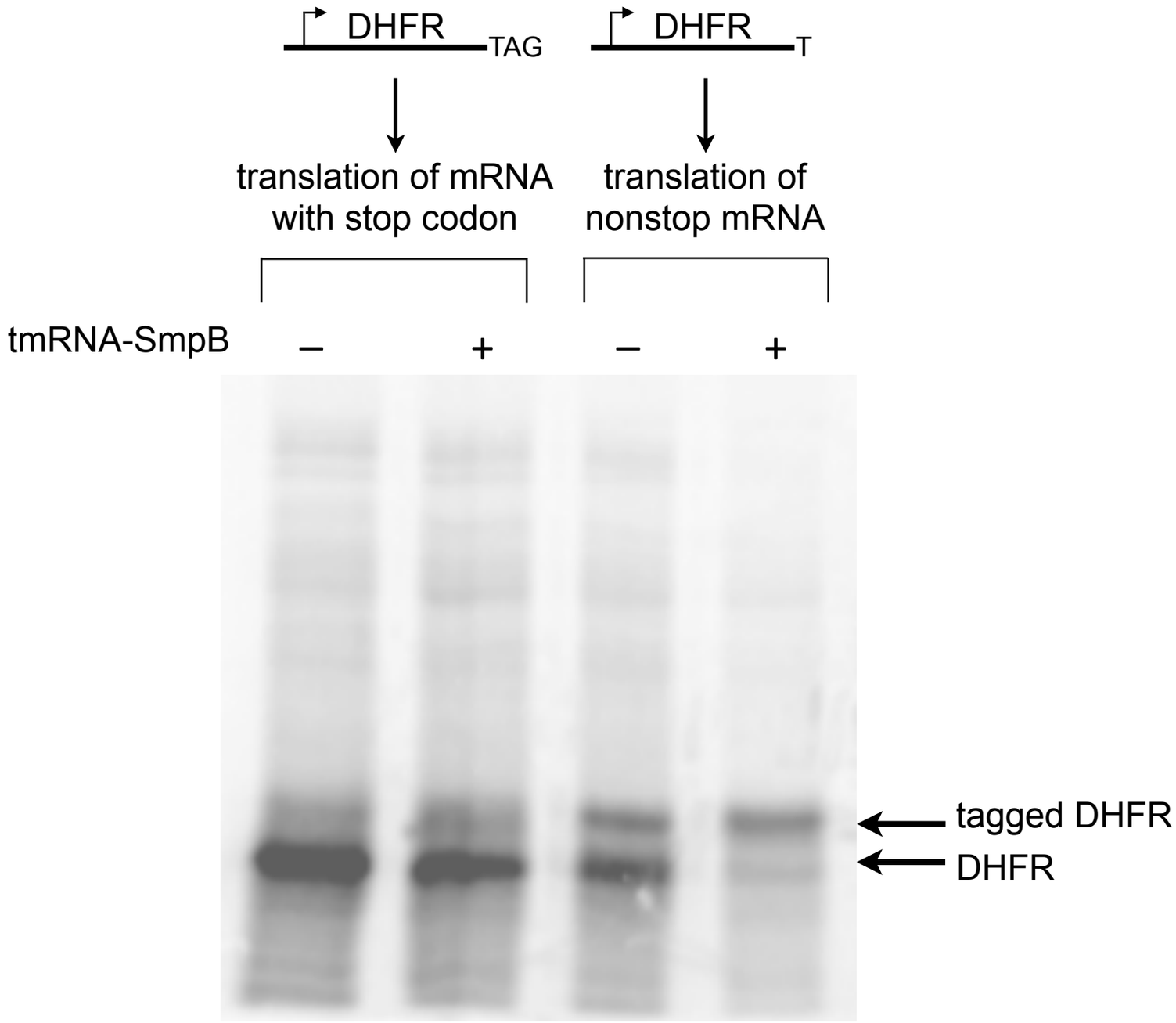


data from Griffin, et al. (2011) *PLOS Path.*

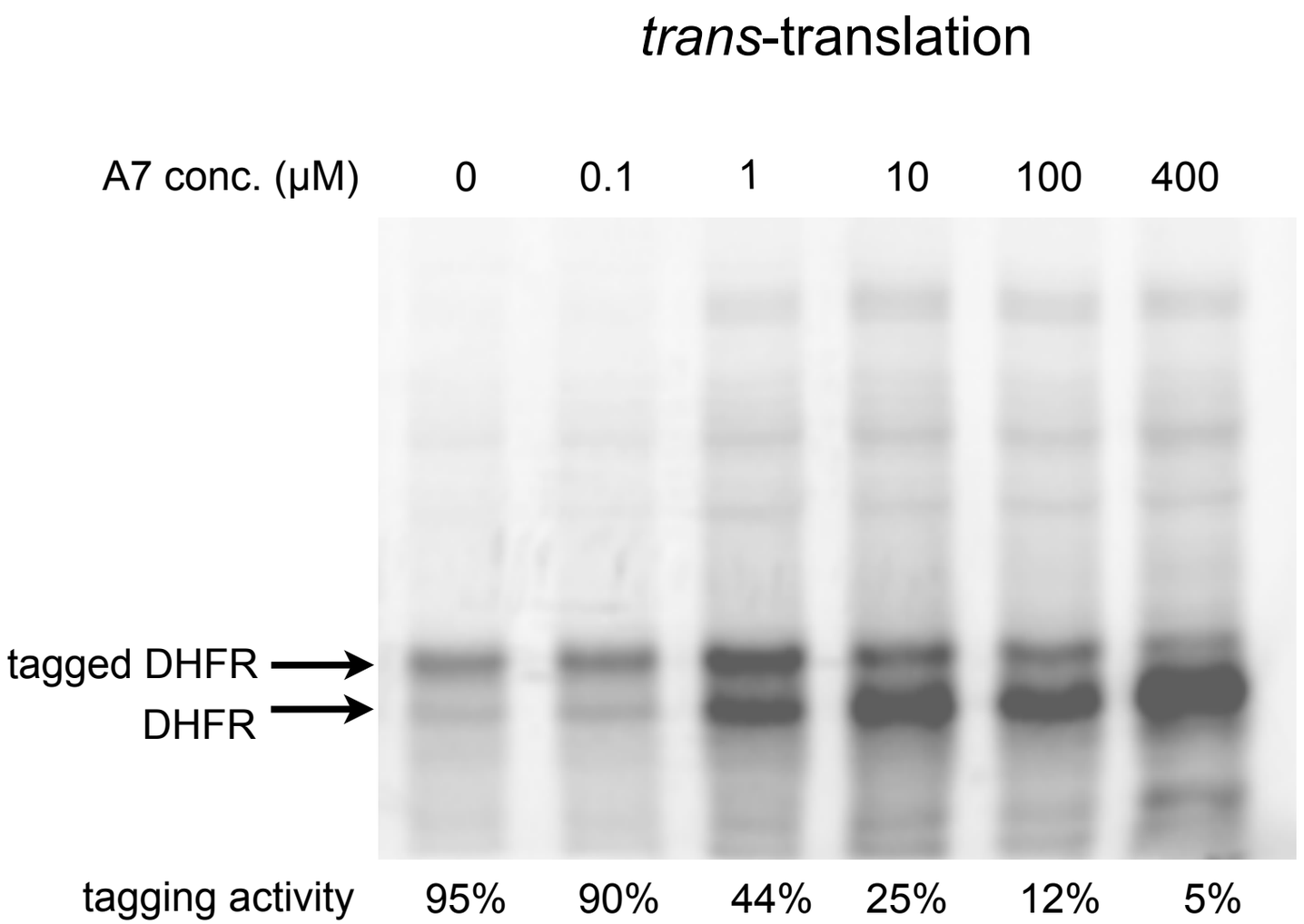
2. targeted genetic deletions cannot be isolated in MTB or *M. smegmatis*
   - depletion strains are under construction

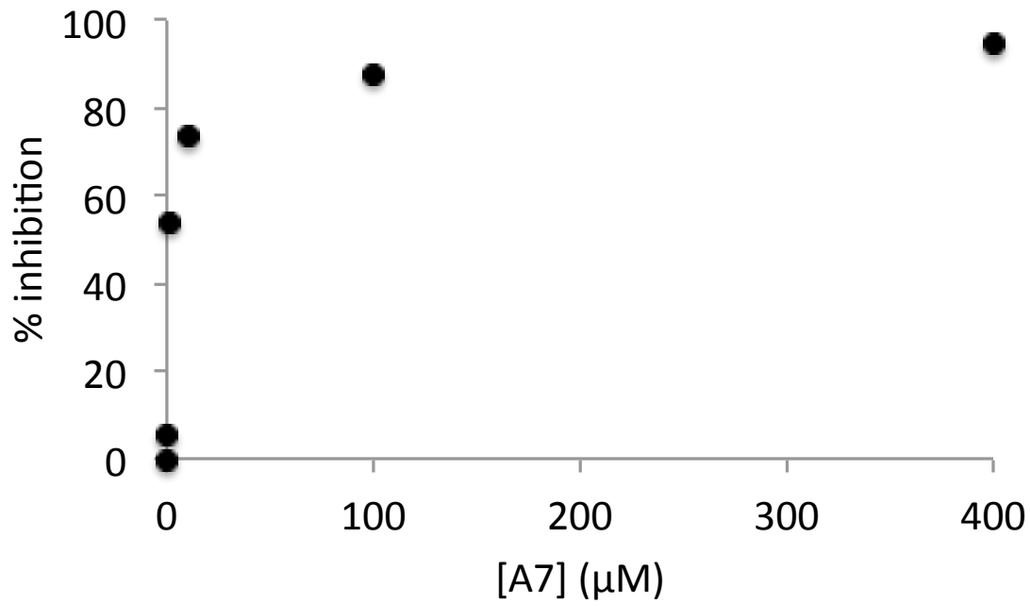
3. small molecule inhibitors of *trans*-translation kill MTB and *M. smegmatis*

in vitro *trans*-translation assay with *E. coli* extracts



A7 specifically inhibits *trans*-translation in vitro





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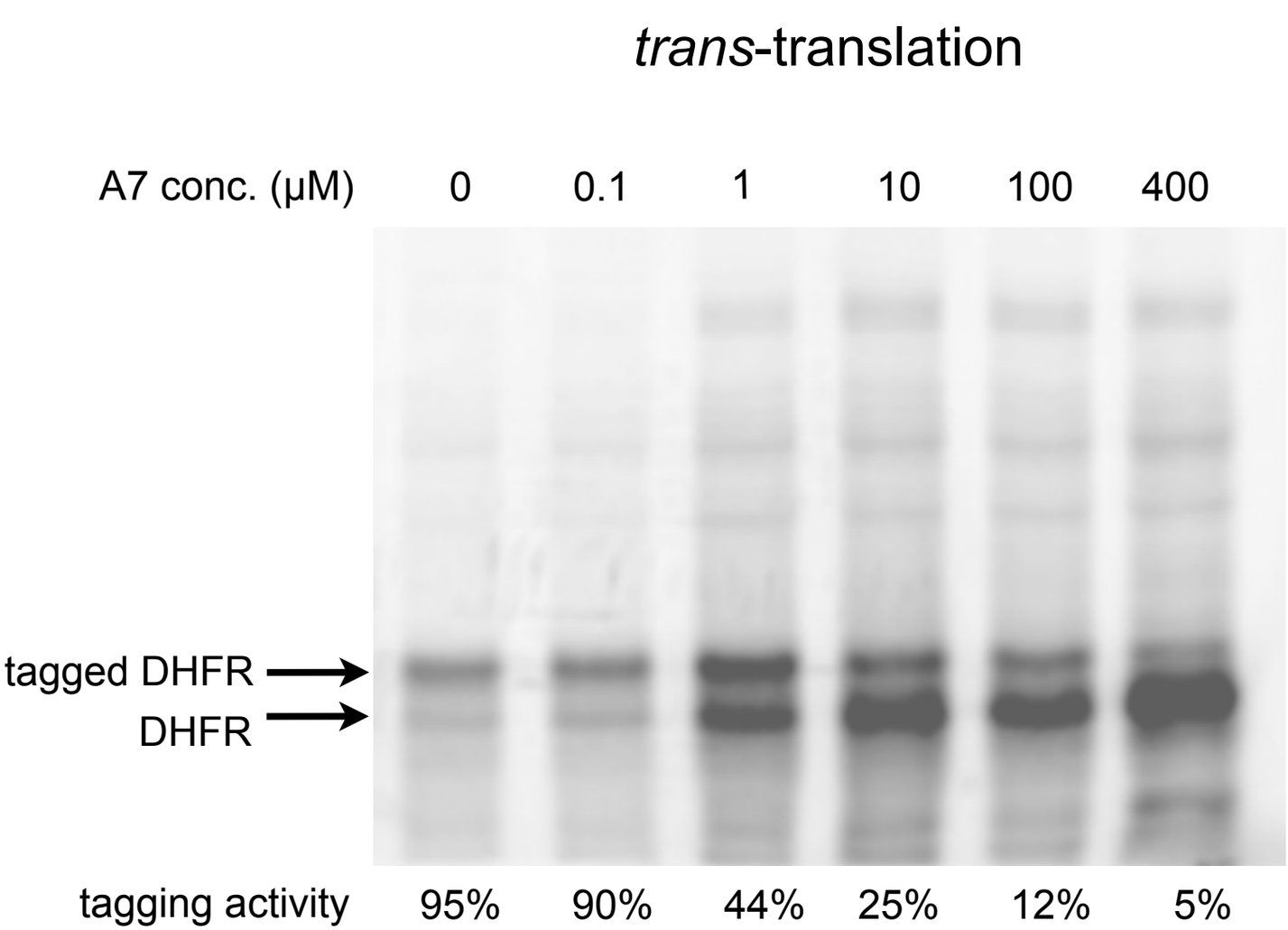

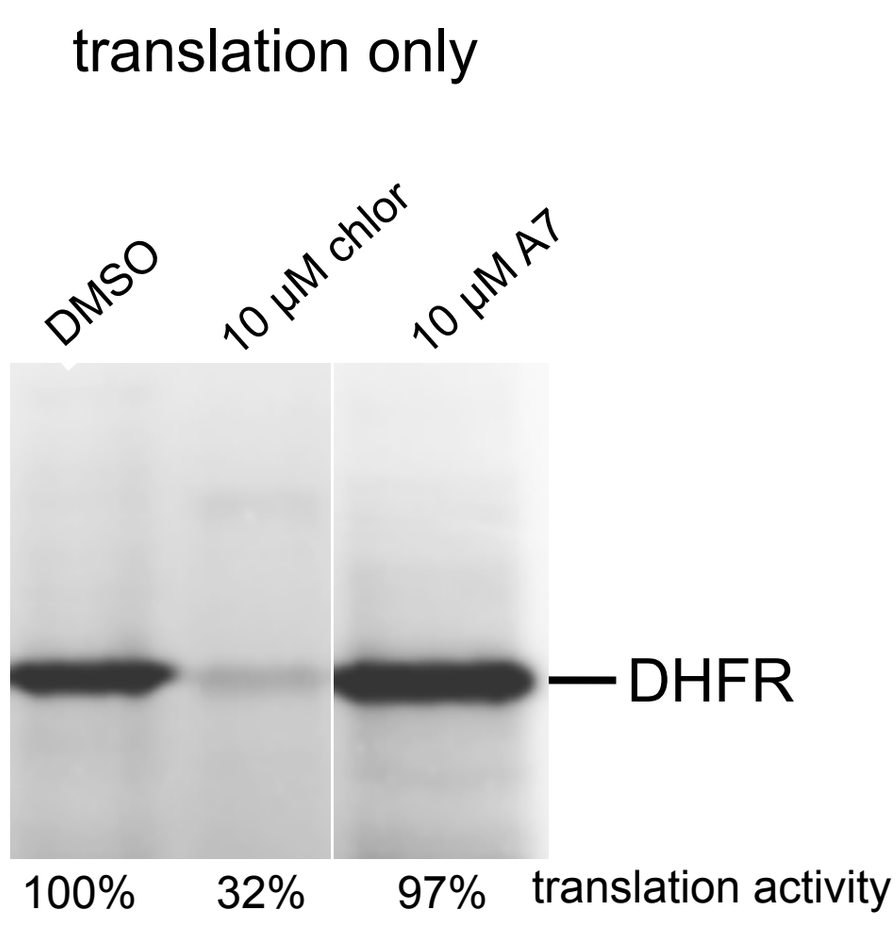

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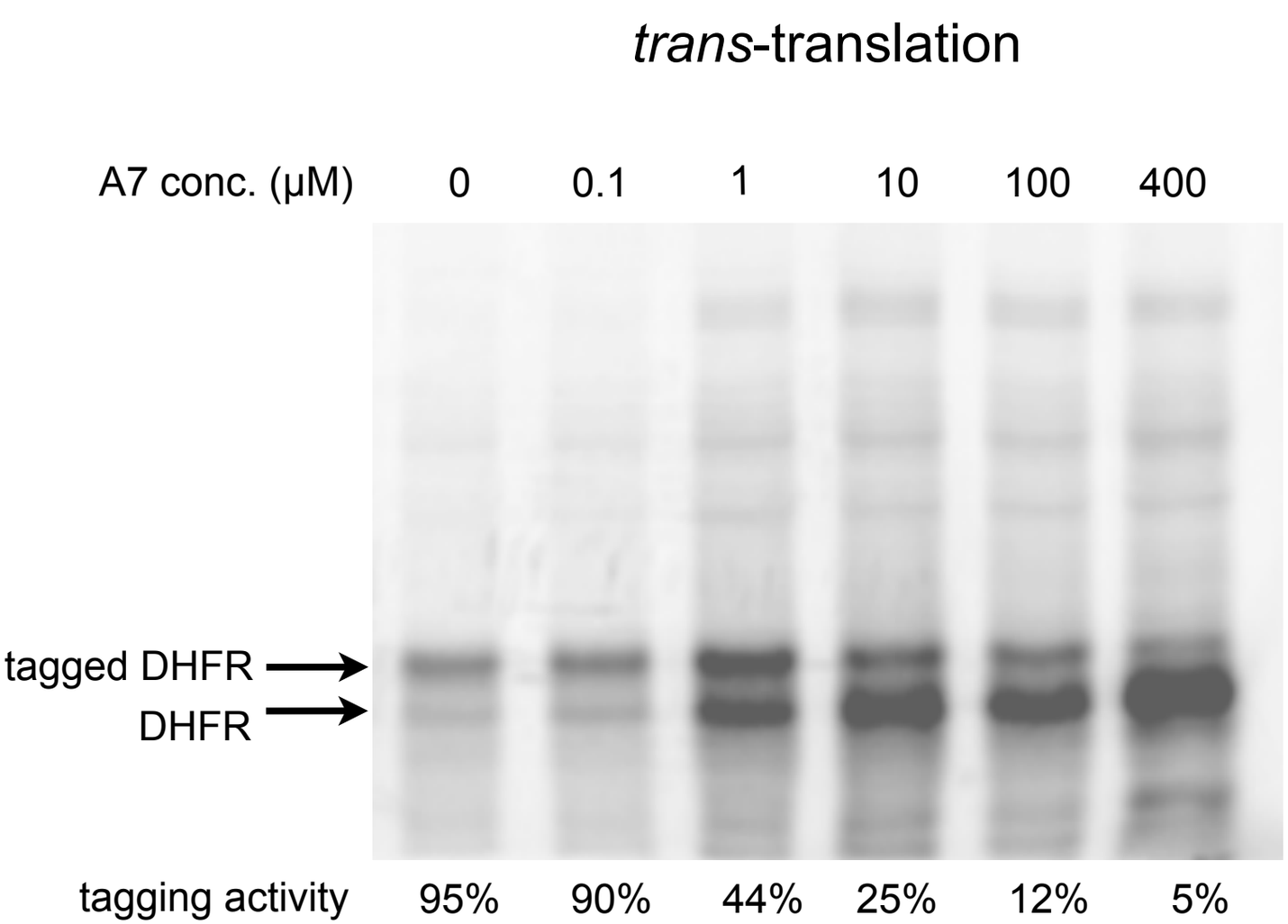

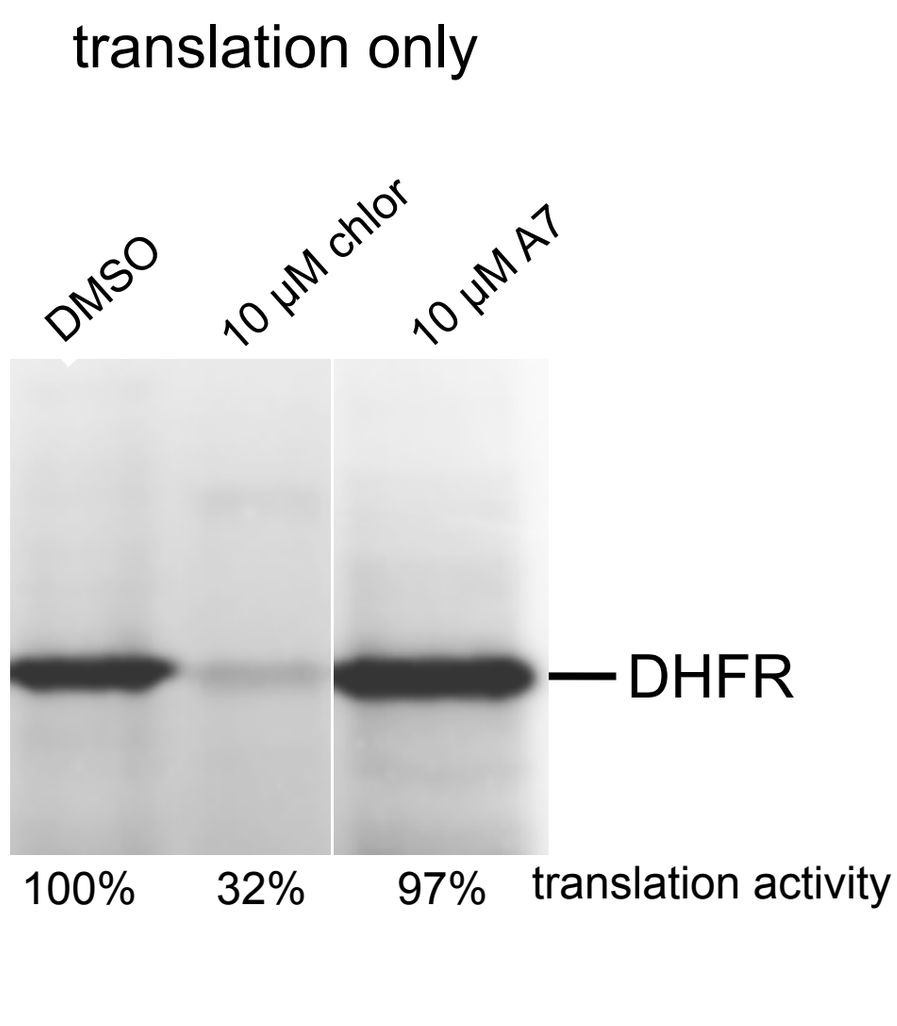

MIC of A7 against *M. tuberculosis H37Rv* = 12 μM (4 mg/ml)

M. smegmatis = 1.5 μM
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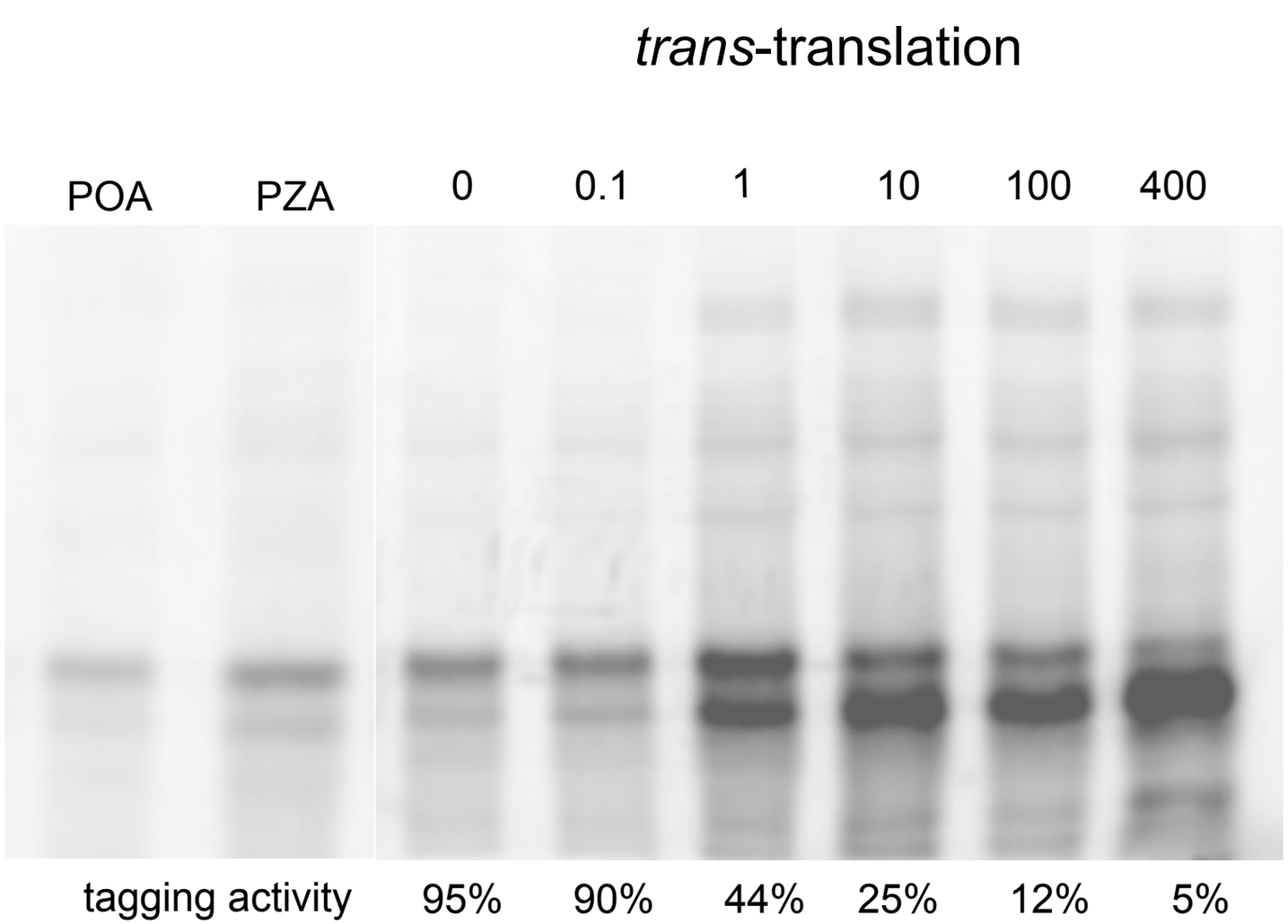

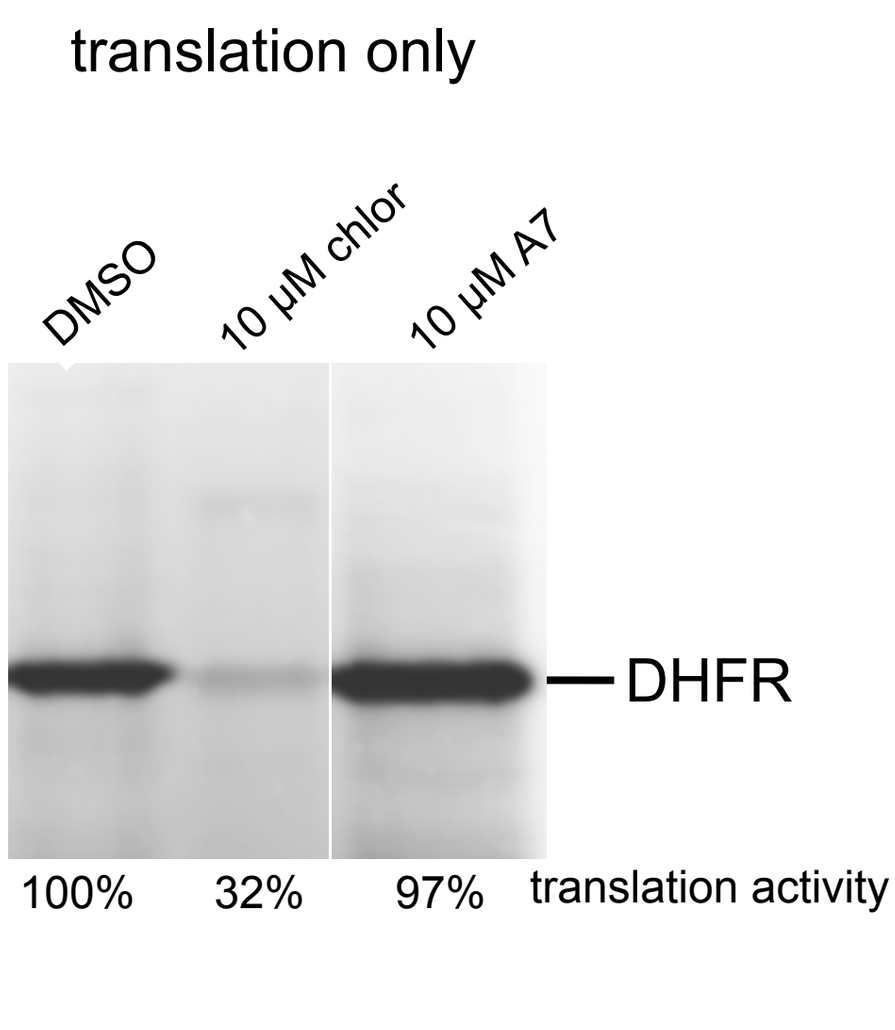

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If *trans*-translation is essential and PZA inhibits *trans*-translation through RpsA, why does PZA work so much better on latent cells?

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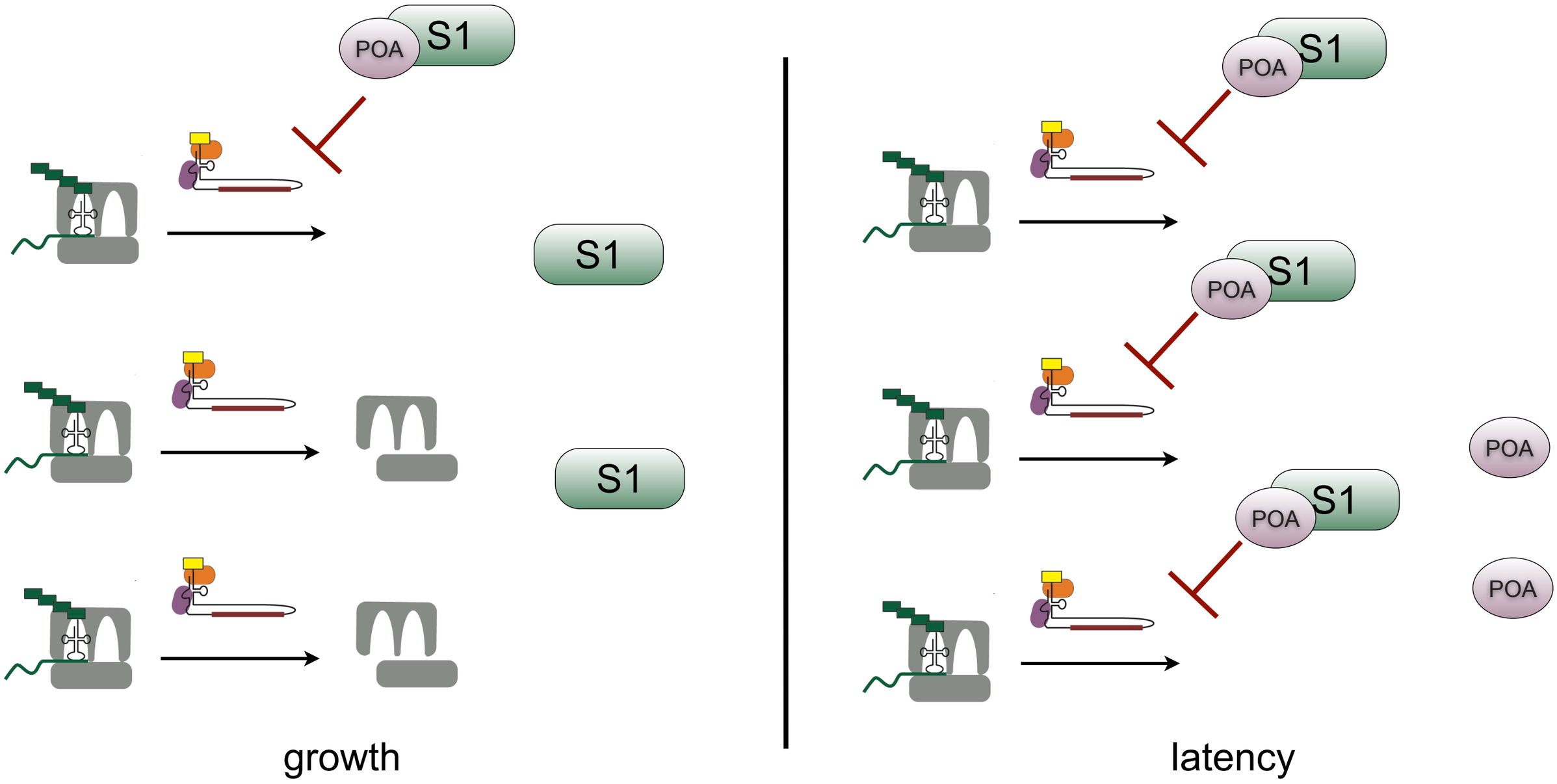

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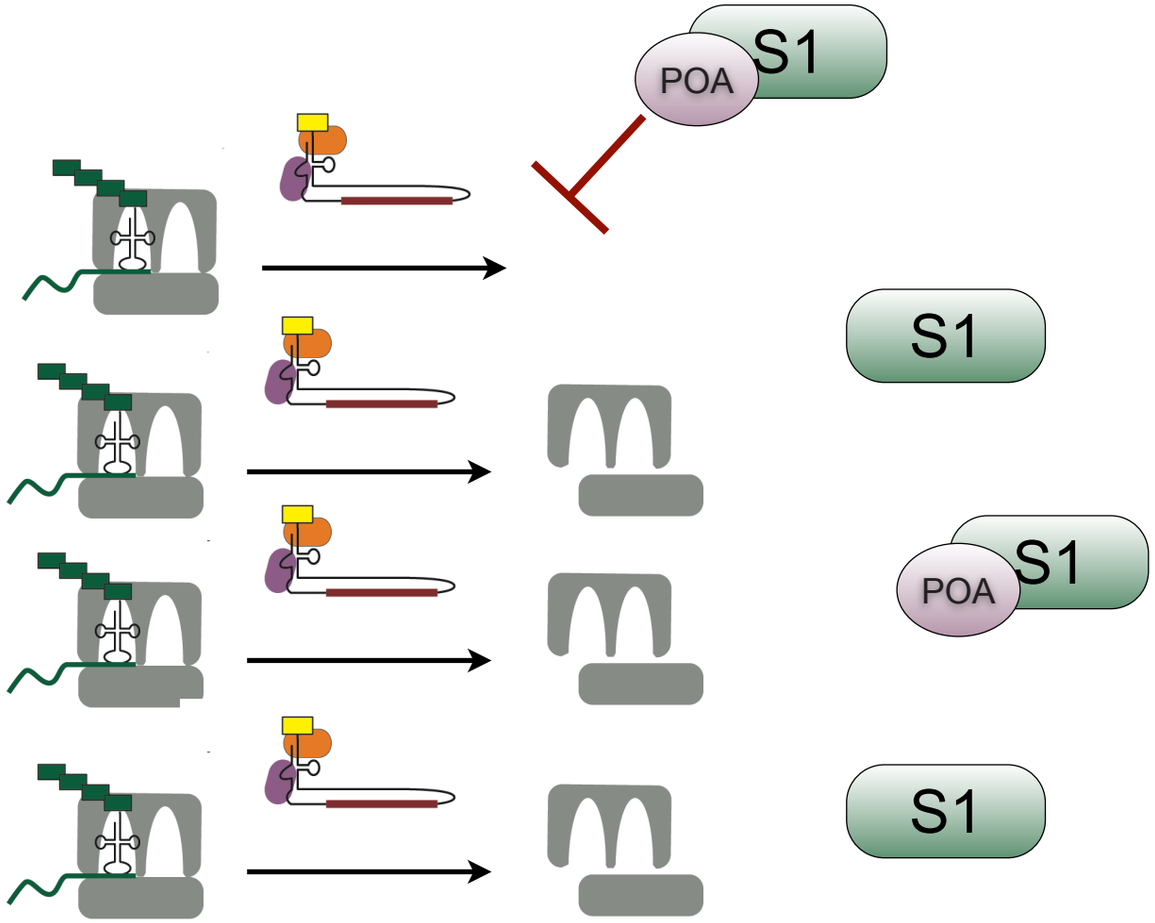
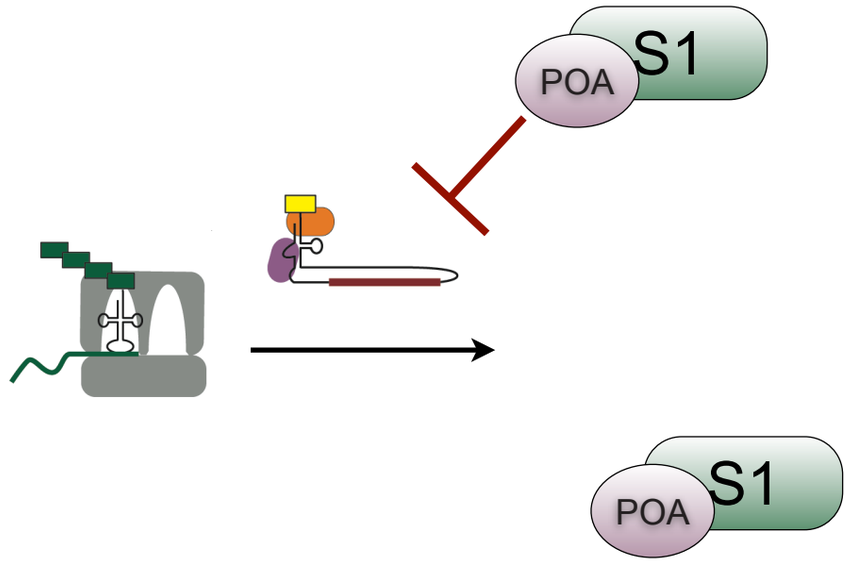


growth



latency

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- if *trans*-translation is the target of PZA, other *trans*-translation inhibitors should be as effective as PZA against latent cells

- likewise, drugs that act like PZA might inhibit *trans*-translation
  - ➡ test in vitro
  - ➡ test in vivo with reporters for *trans*-translation activity

conclusions and suggestions

- loss of *trans*-translation in MTB is either lethal or has a major growth defect

  ➡ do other *trans*-translation inhibitors act like PZA on latent cells?

  ➡ do molecules that act like PZA inhibit *trans*-translation?

- RpsA is not required for *trans*-translation in other species

  ➡ test the role of RpsA on *trans*-translation in vitro using MTB components

  ➡ test whether RpsA-POA can interfere with *trans*-translation in vitro using MTB components

Nitya Ramadoss
John Alumasa
Lin Cheng

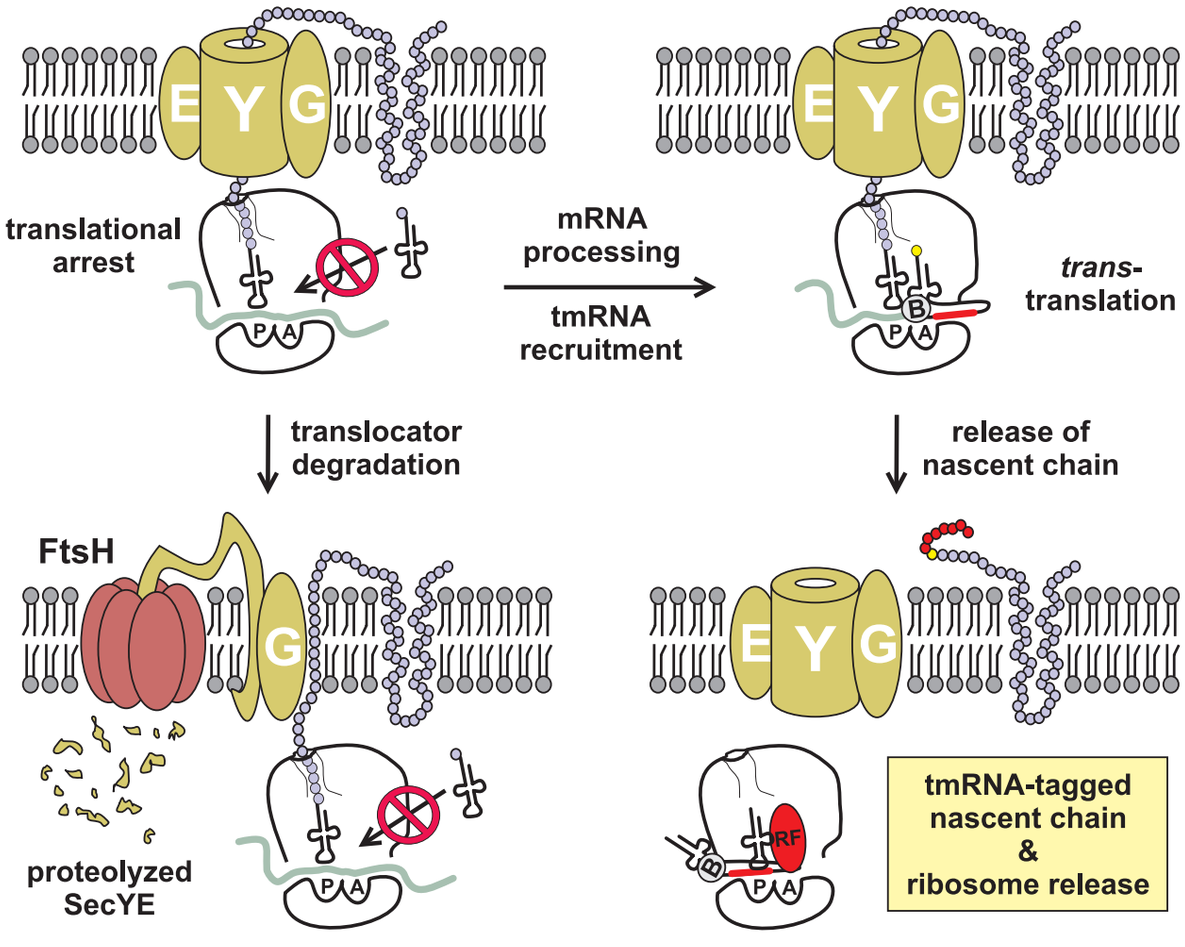
Jeff Cox (UCSF)
Paolo Manzanillo
Lynn Connolly
Trisha Lundrigan

TB Alliance
Anna Upton
Takushi Kaneko

Pete Schultz (GNF)
Achim Brinker
Ingo Engels
Yu Wang

NIH/NIGMS

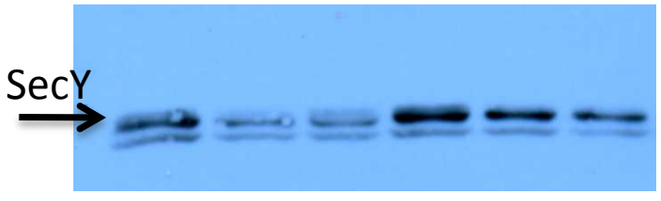
A7 causes rapid turnover of SecY



A7 treated

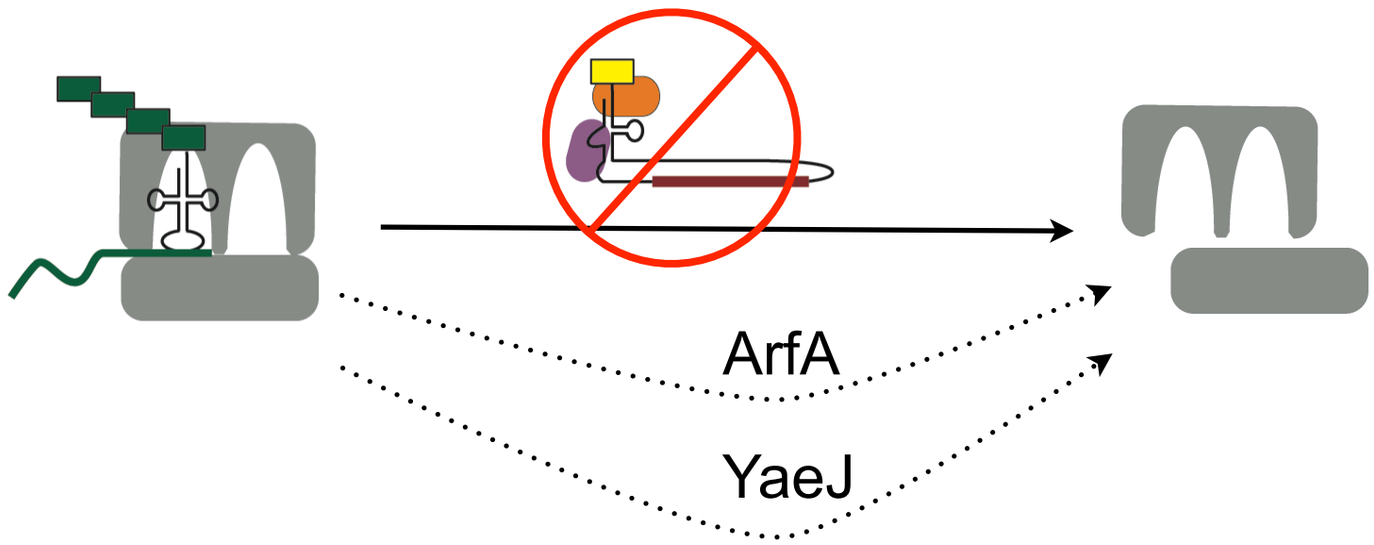
CAM treated

0’ 30’ 60’ 0’ 30’ 60’



Holly Cardoso and Tom Silhavy

when there is a backup system, inactivation of *trans*-translation typically causes defects in resuming proliferation



Proliferation after stationary phase and stasis induced by T/A system toxins: ***E. coli***

Proliferation after macrophage invasion: ***Yersinia pestis, Y. pseudotuberculosis***
Salmonella enterica*, *S. typhimurium

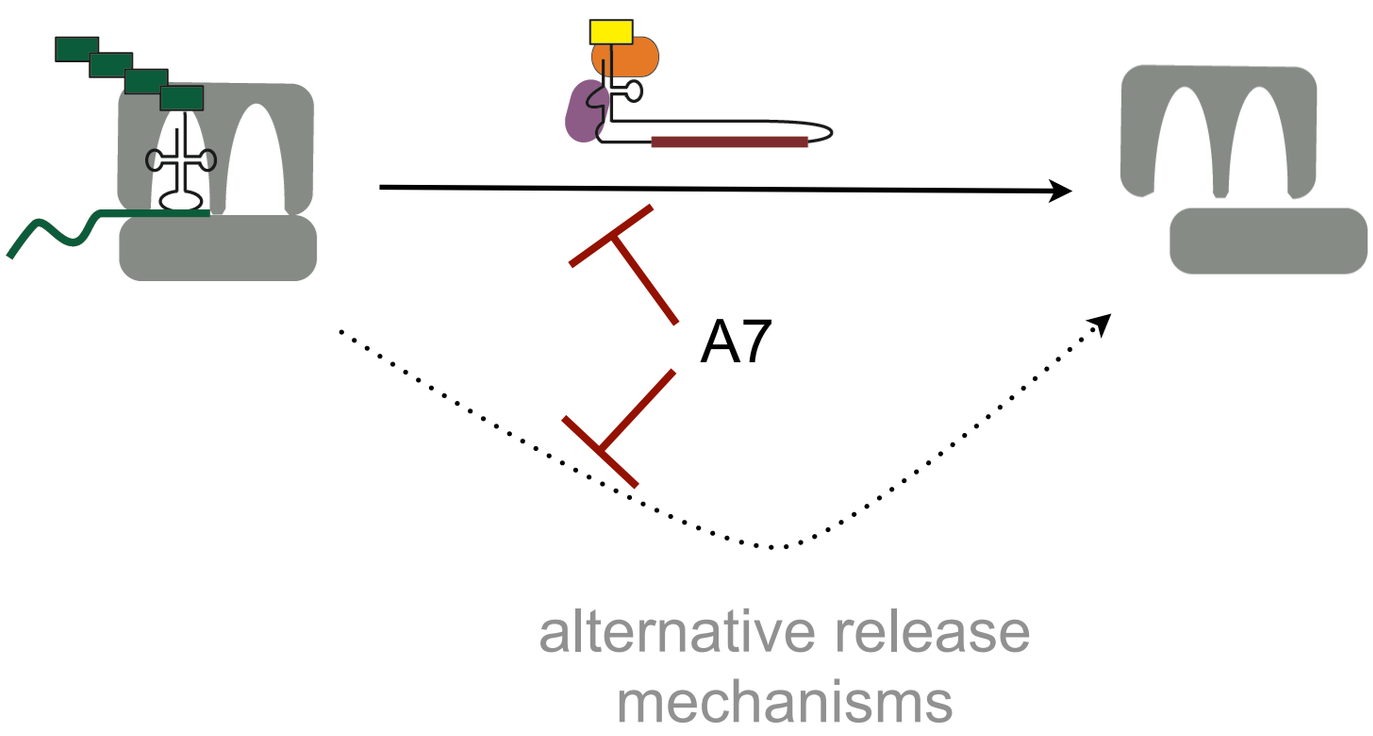
Proliferation after root cell invasion: ***Bradyrhizobium japonicum***

Proliferation after dispersal state: ***Caulobacter crescentus***

Other general phenotypes: increased sensitivity to oxidative stress and antibiotics

neither *ssrA* nor *smpB* can be deleted in *Mycobacteria*, but...

- severe growth defects can prevent recovery of Tn insertion mutants and genetic deletions (depletion strains are required to confirm)
- A7 acts upstream of alternative release mechanisms



conclusions and speculations

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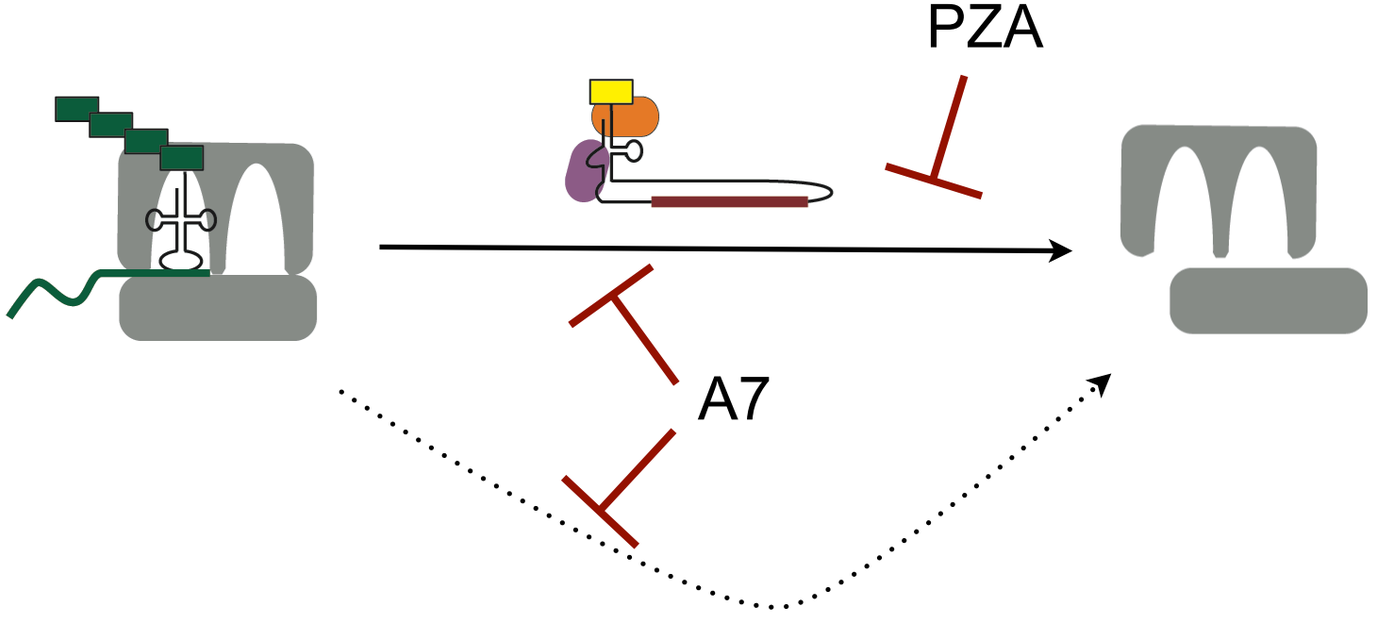
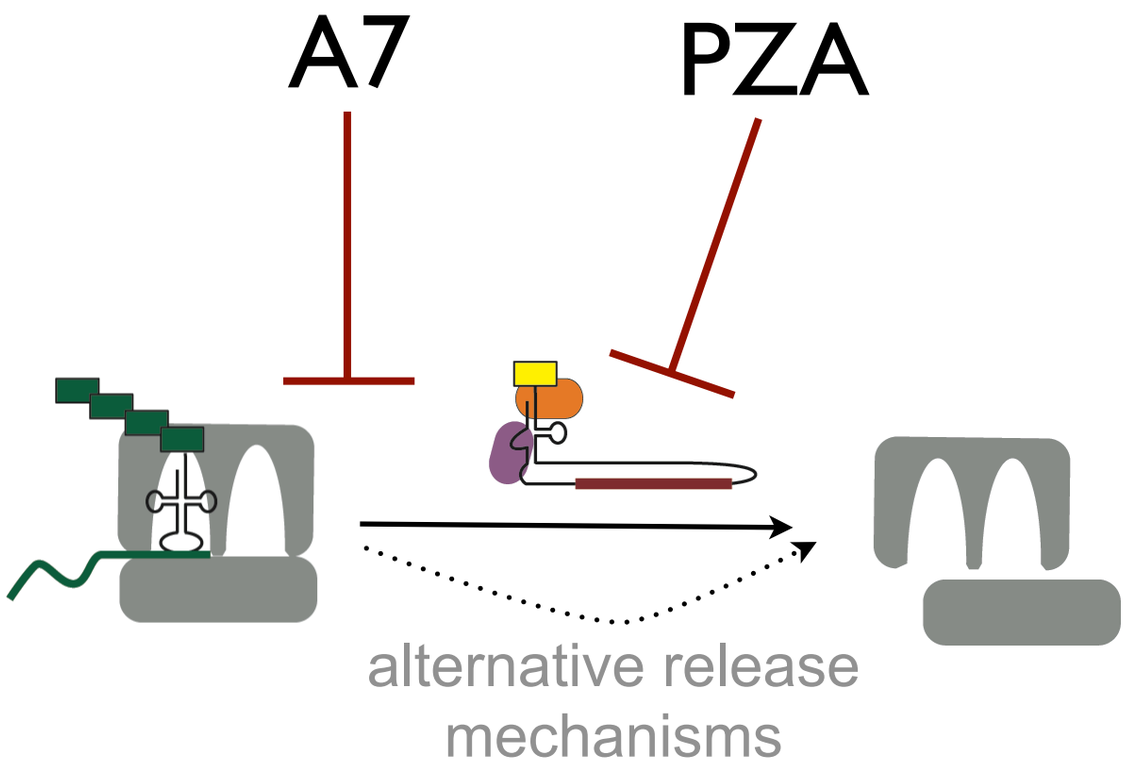
➡If MTB has a backup system for *trans*-translation, PZA may still act through *trans*-translation by preventing a return to proliferation.

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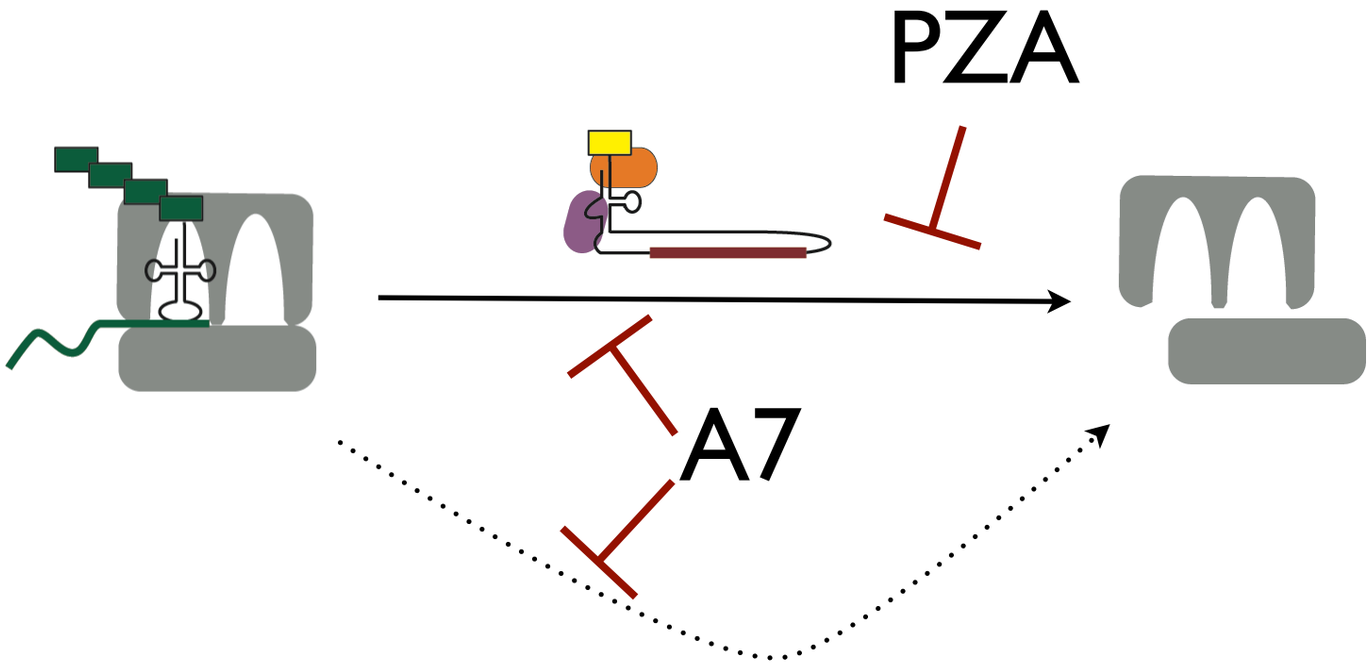
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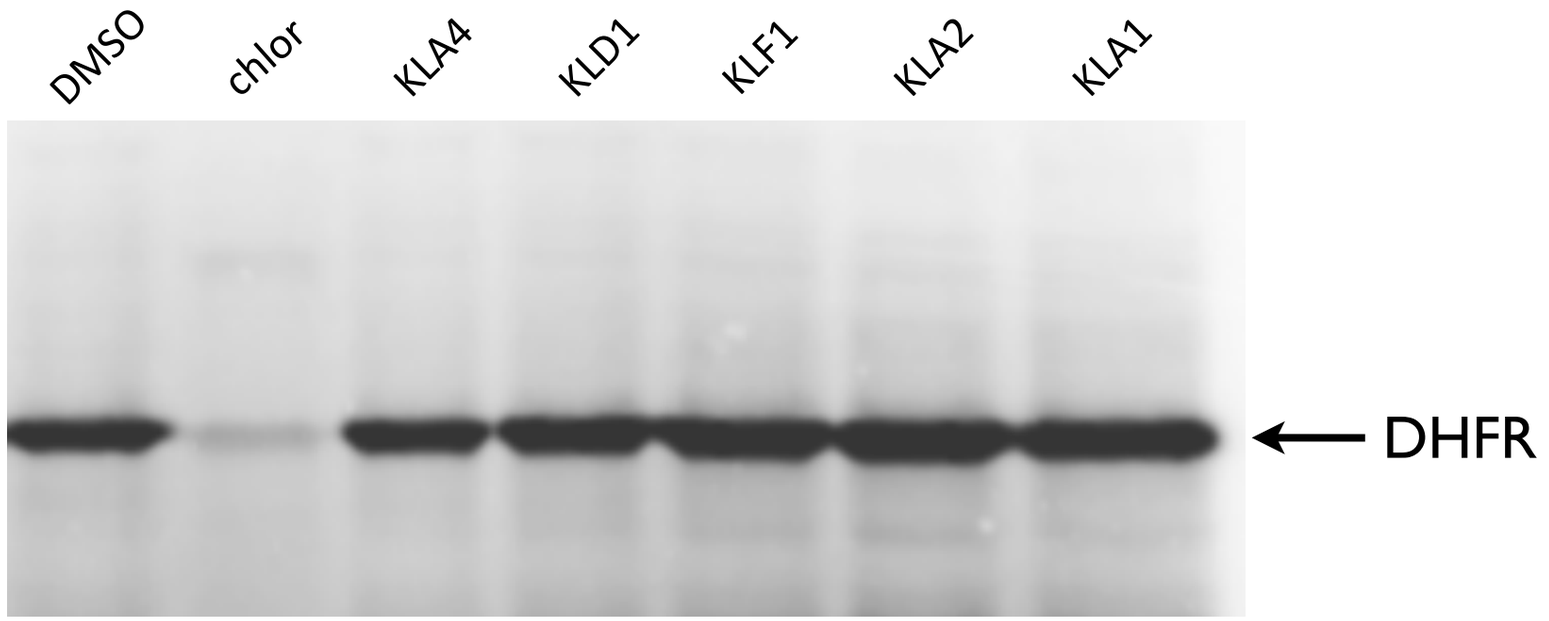
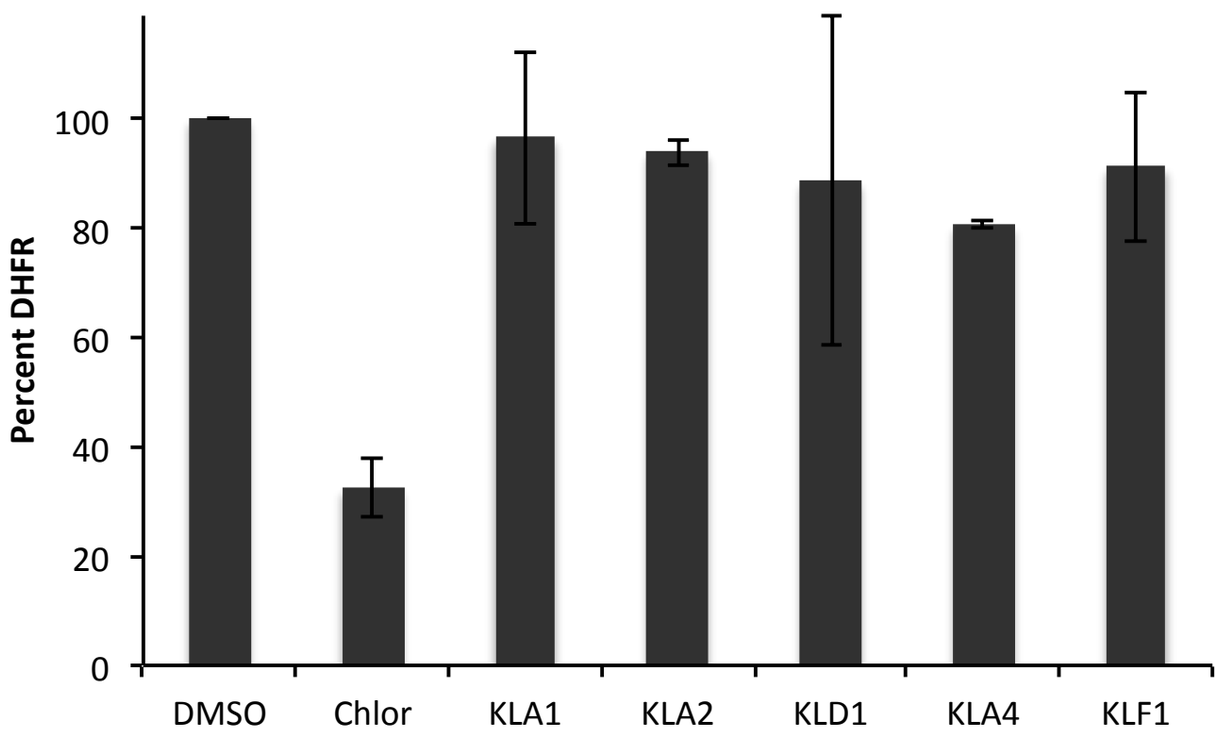
in vitro translation is not inhibited

representative gel





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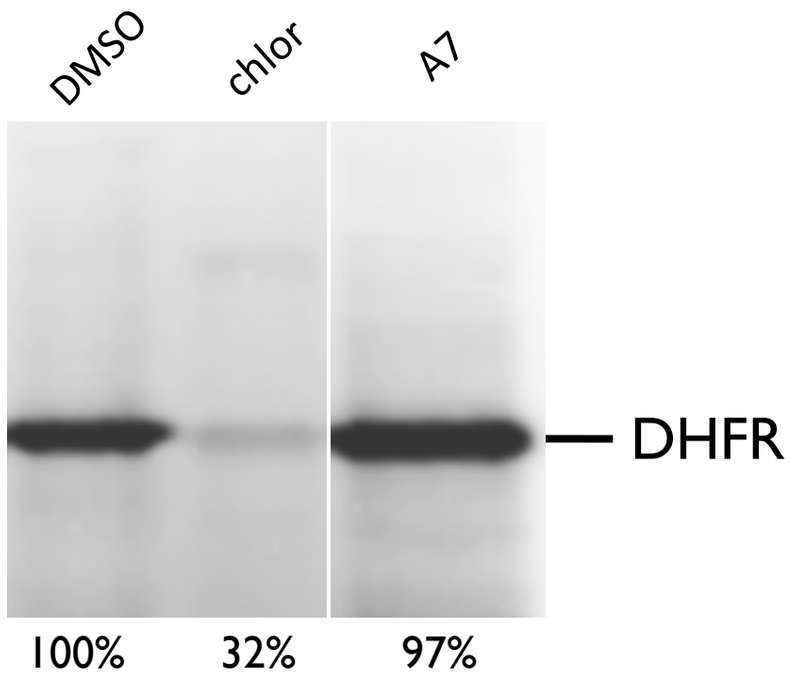

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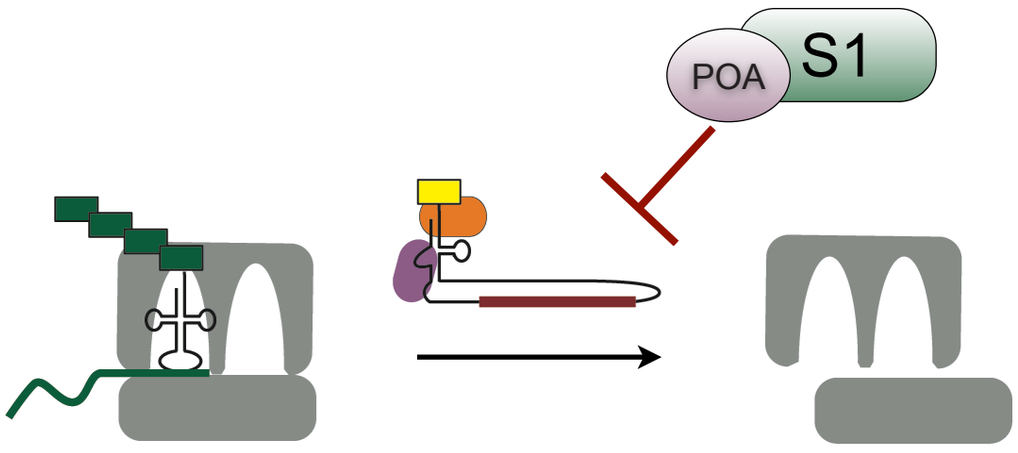

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