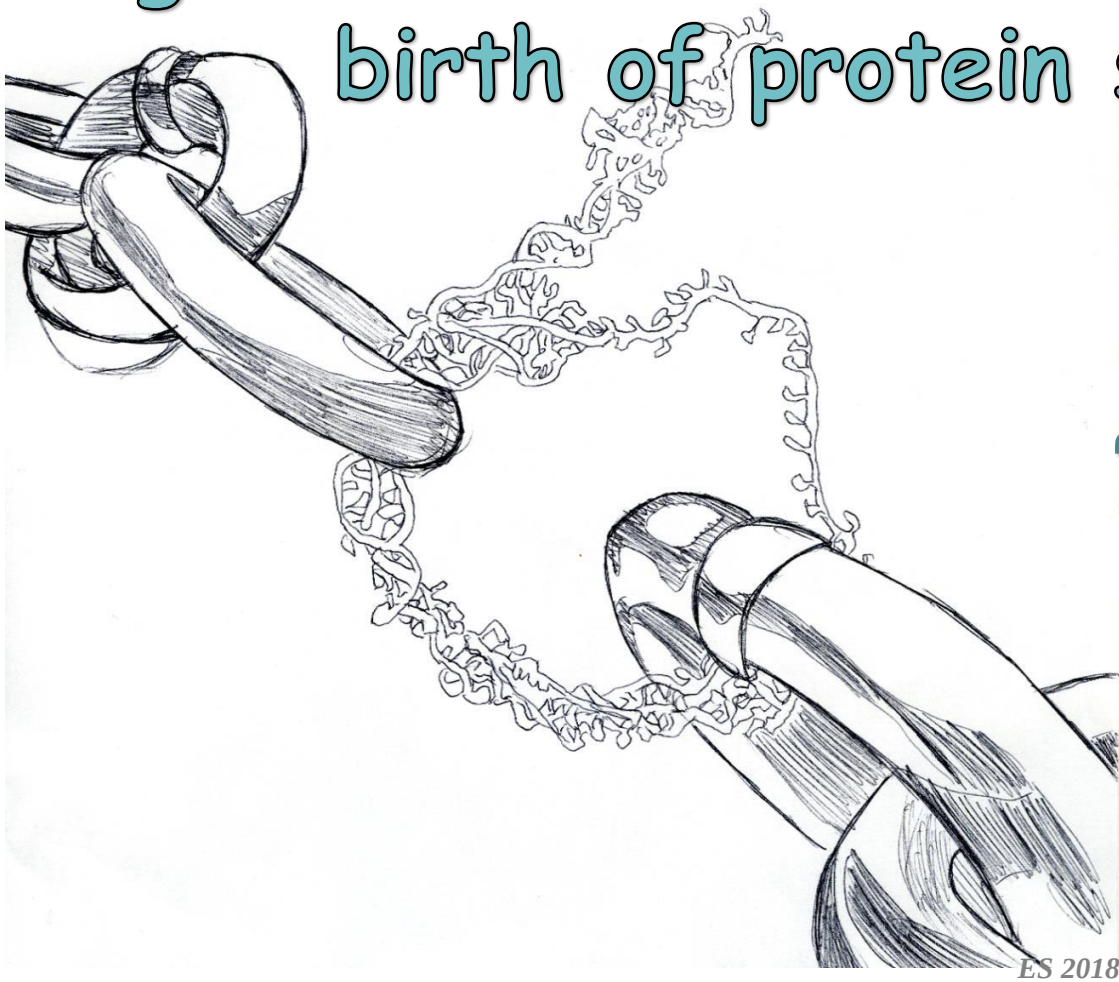
Origins of tmRNA: the missing link in the birth of protein synthesis?



"Evolution Symposium" Salzburg 2018

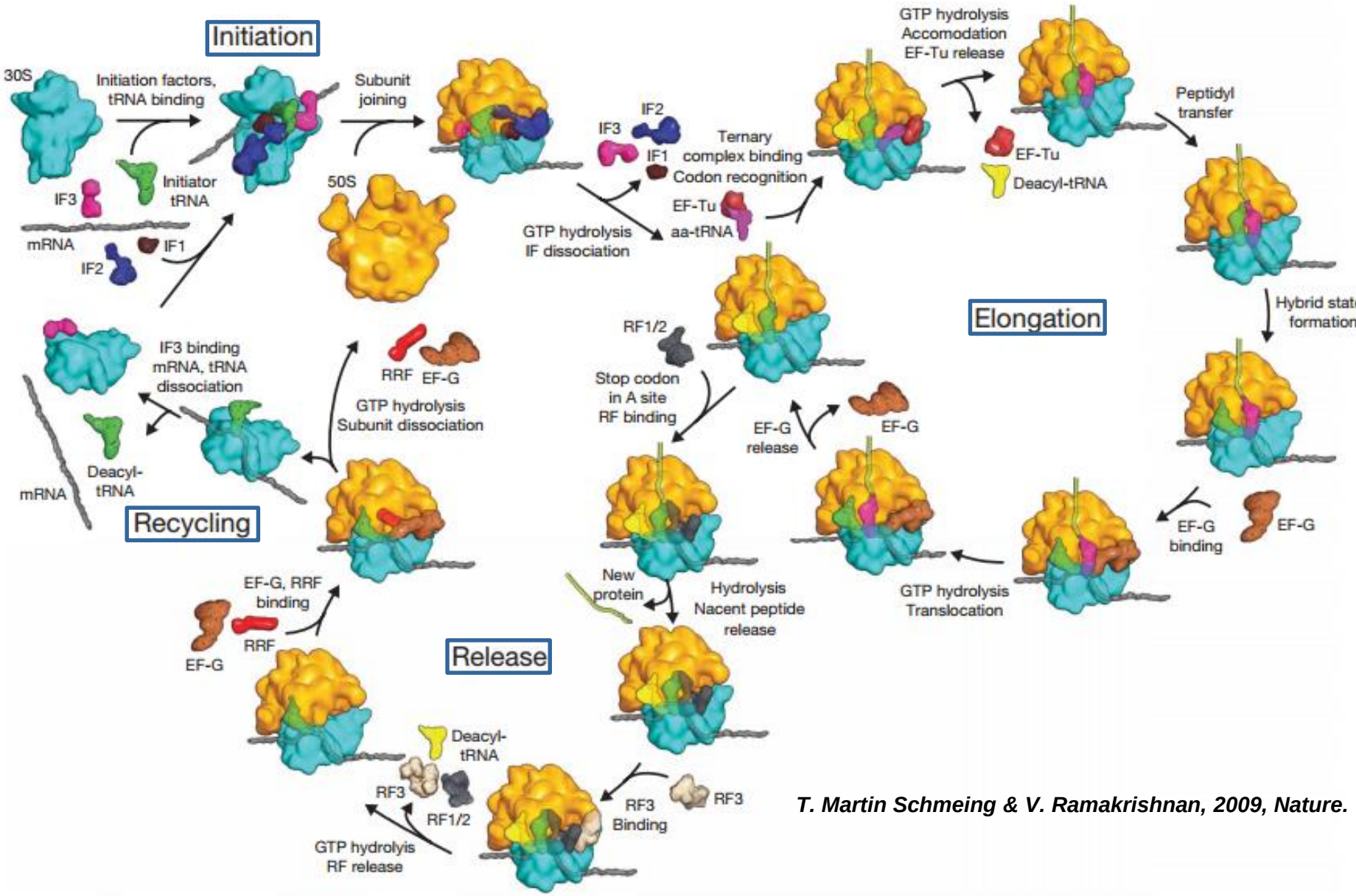
Reynald GILLET
IGDR - UMR CNRS 6290

igdr Institut Génétique et Développement de Rennes

UNIVERSITÉ DE RENNES 1

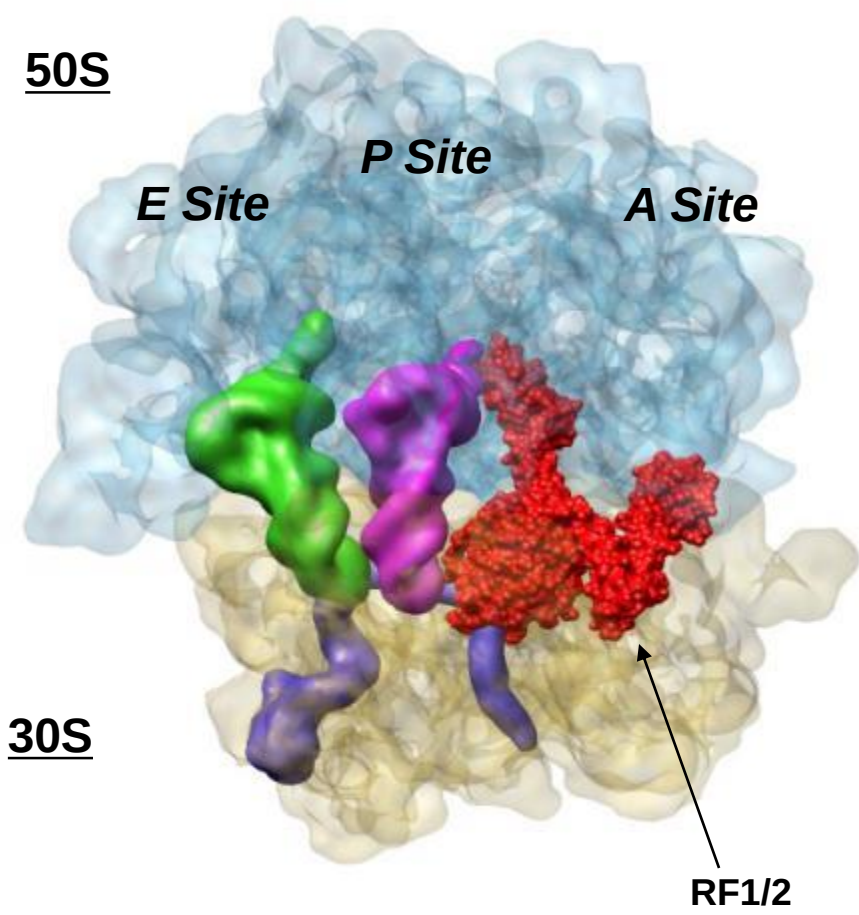
cnrs

Translation by the ribosome: 4 main steps



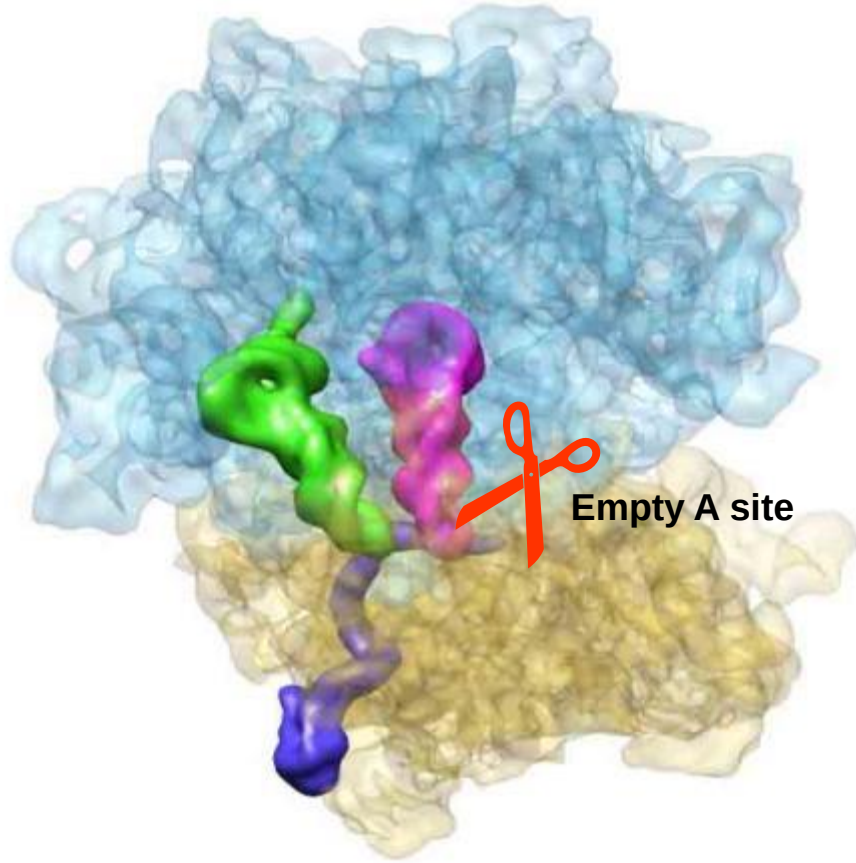
T. Martin Schmeing & V. Ramakrishnan, 2009, Nature.

What happens when termination goes wrong?



Canonical translation

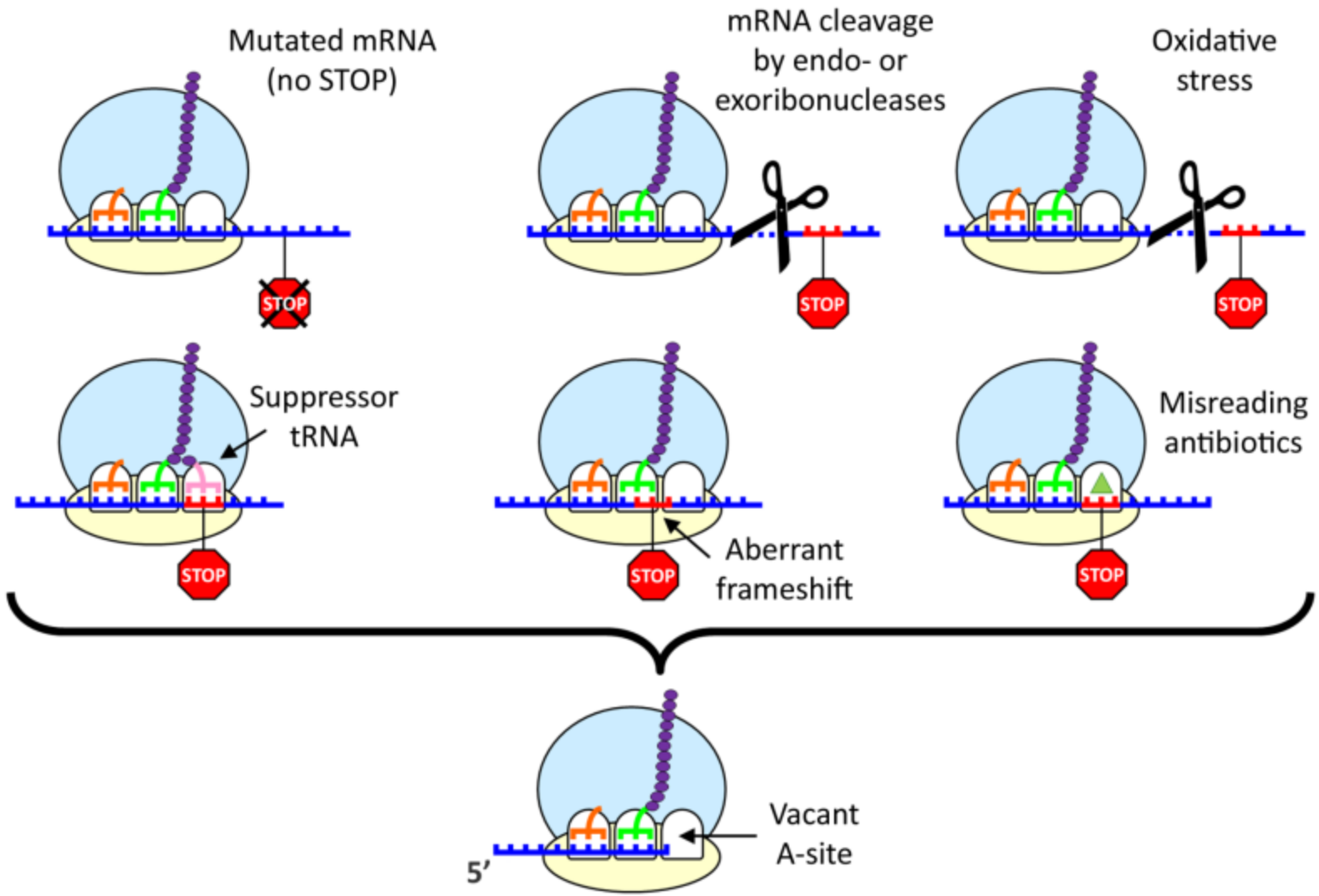
STOP = termination



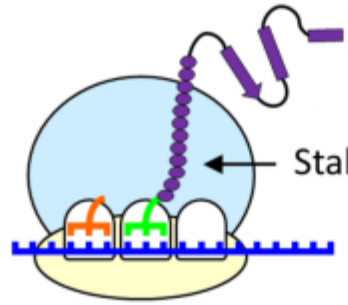
Non productive translation complex (NTC)

Stalled Ribosome
+
Incomplete Polypeptide
+
Problematic mRNA

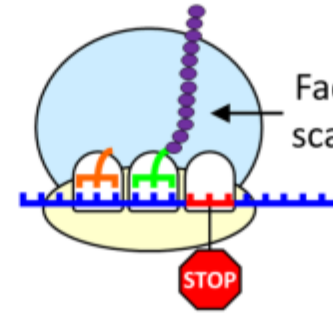
Non-stop


Mutated mRNA
(no STOP)
mRNA cleavage
by endo- or
exoribonucleases
Oxidative
stress
STOP
STOP
STOP
Suppressor
tRNA
Aberrant
frameshift
Misreading
antibiotics
STOP
STOP
STOP
Vacant
A-site
5’

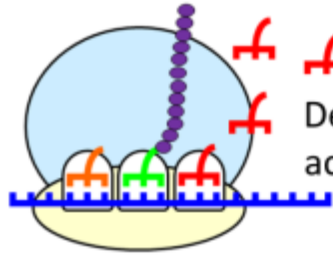
No-go



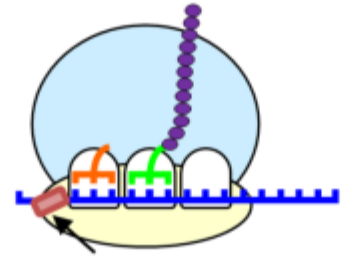
Stalling motif



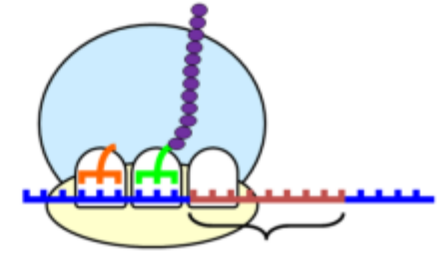
Factors scarcity (Starvation)



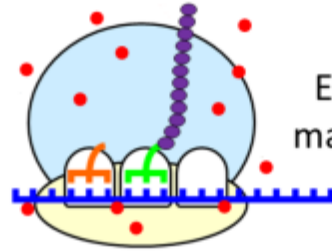
Deacyl-tRNAs accumulation



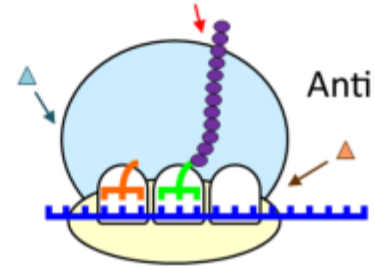
Shine-Dalgarno-like sequence



Rare codons



Excess of magnesium



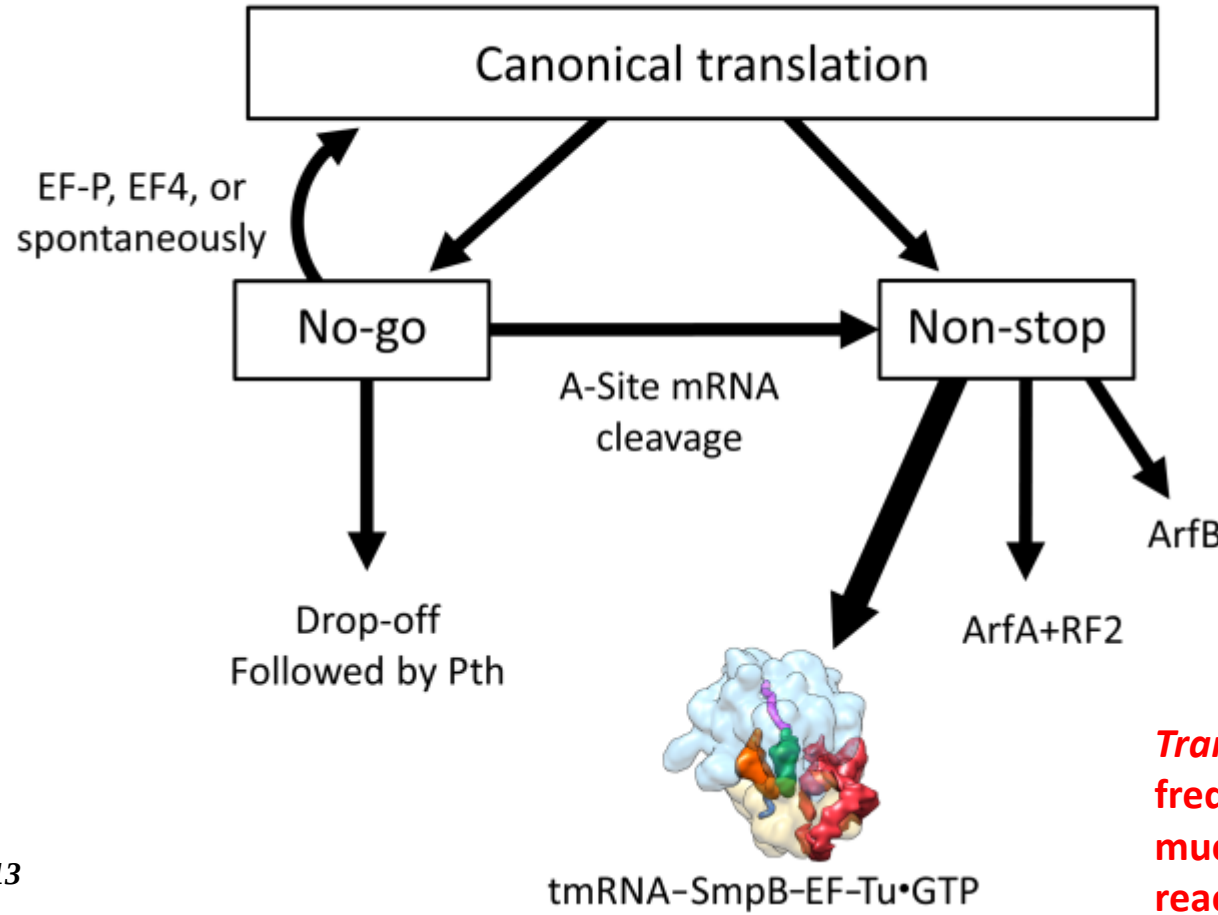
Antibiotics

How bacteria cope with such situations?

- **Quality control mechanisms of BACTERIAL translation :**

→ *the primary rescue system is trans-translation*, performed by tmRNA and SmpB

→ *Other systems: ArfA; ArfB; EFP; EF4; Peptidyl tRNA hydrolase*

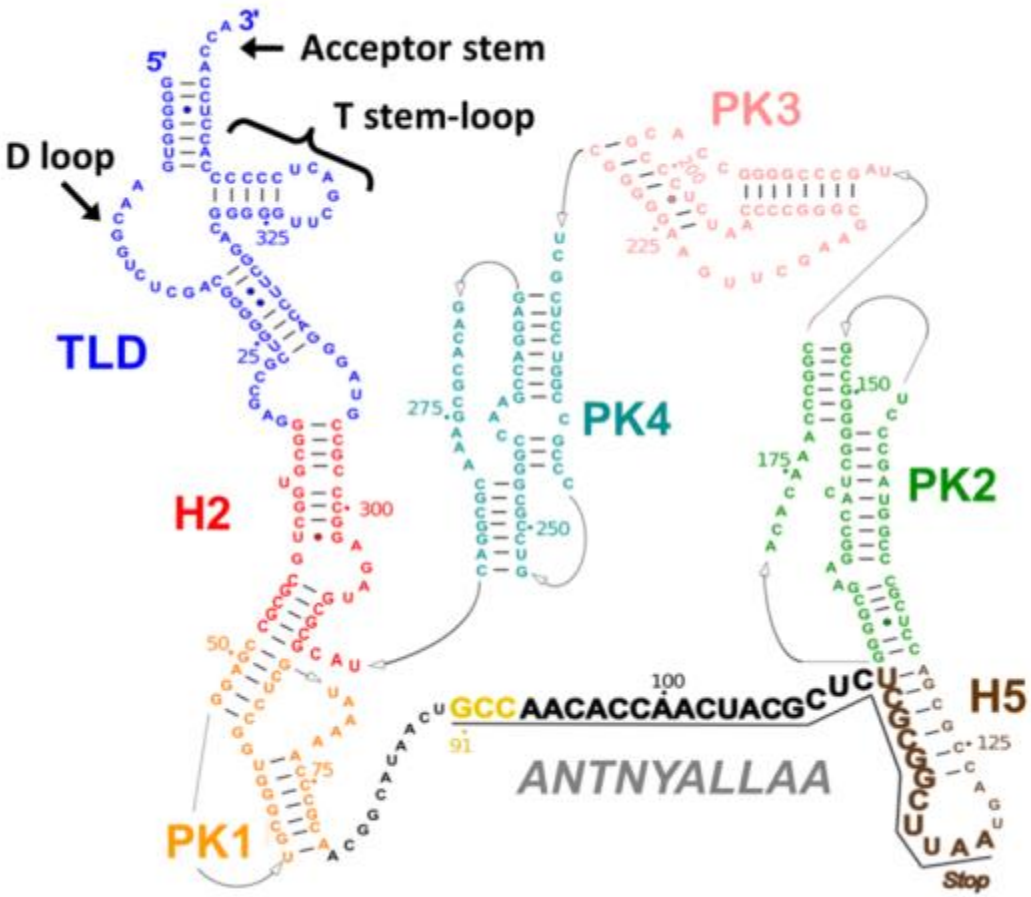


Giudice et Gillet, TIBS 2013

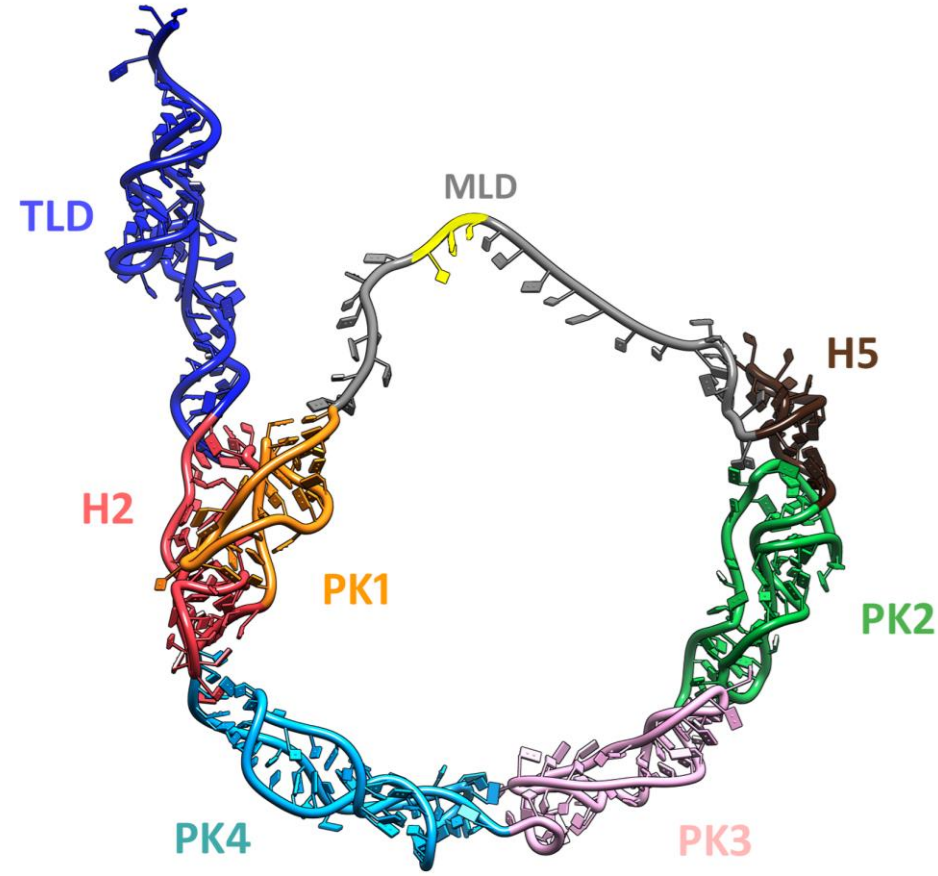
Trans*-translation occurs frequently, accounting for as much as 2 to 4% of translation reactions in *E. coli

tmRNA to the rescue

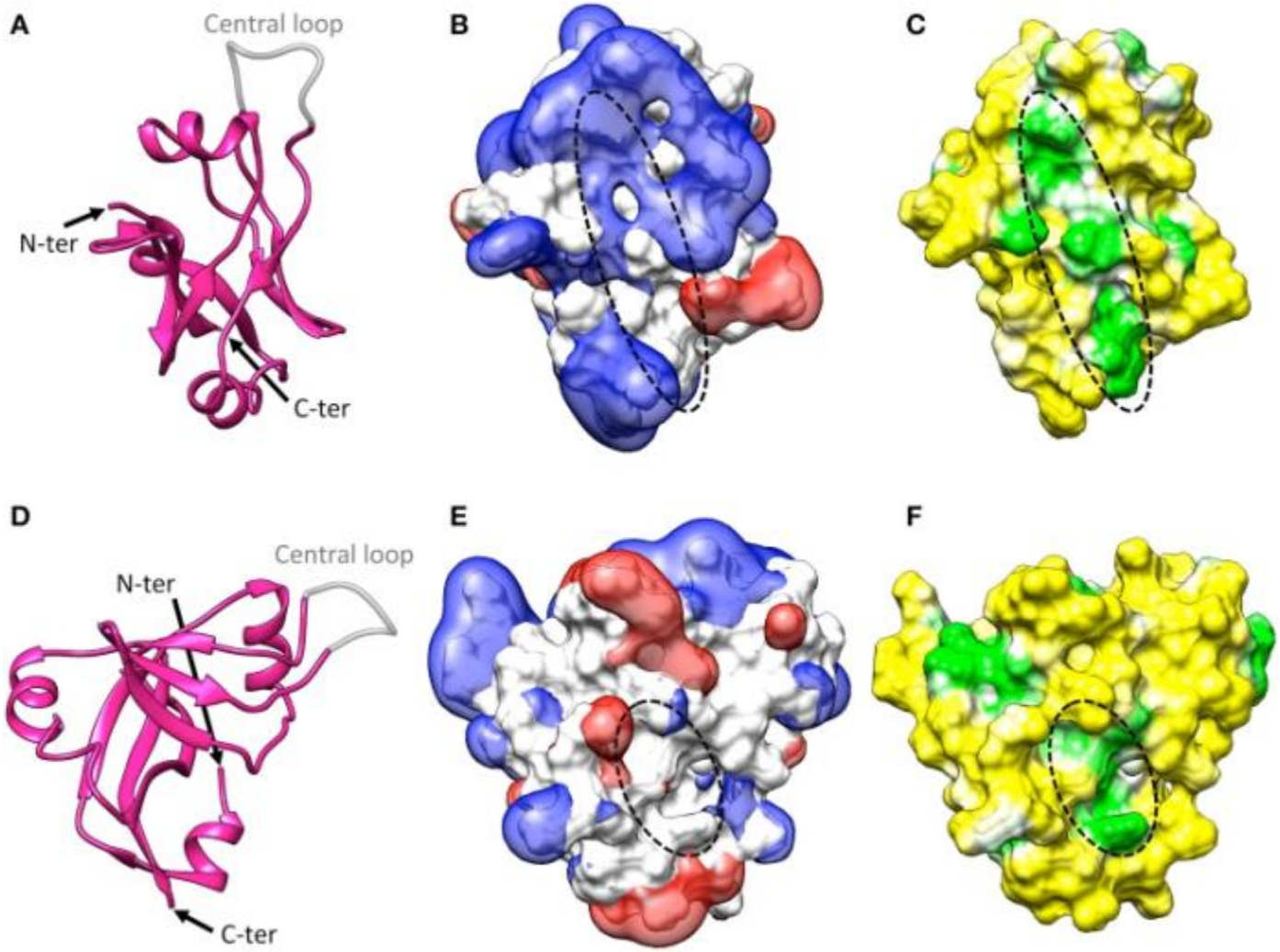
Diagram of the secondary structure of Thermus thermophilus tmRNA.



3D molecular model of tmRNA

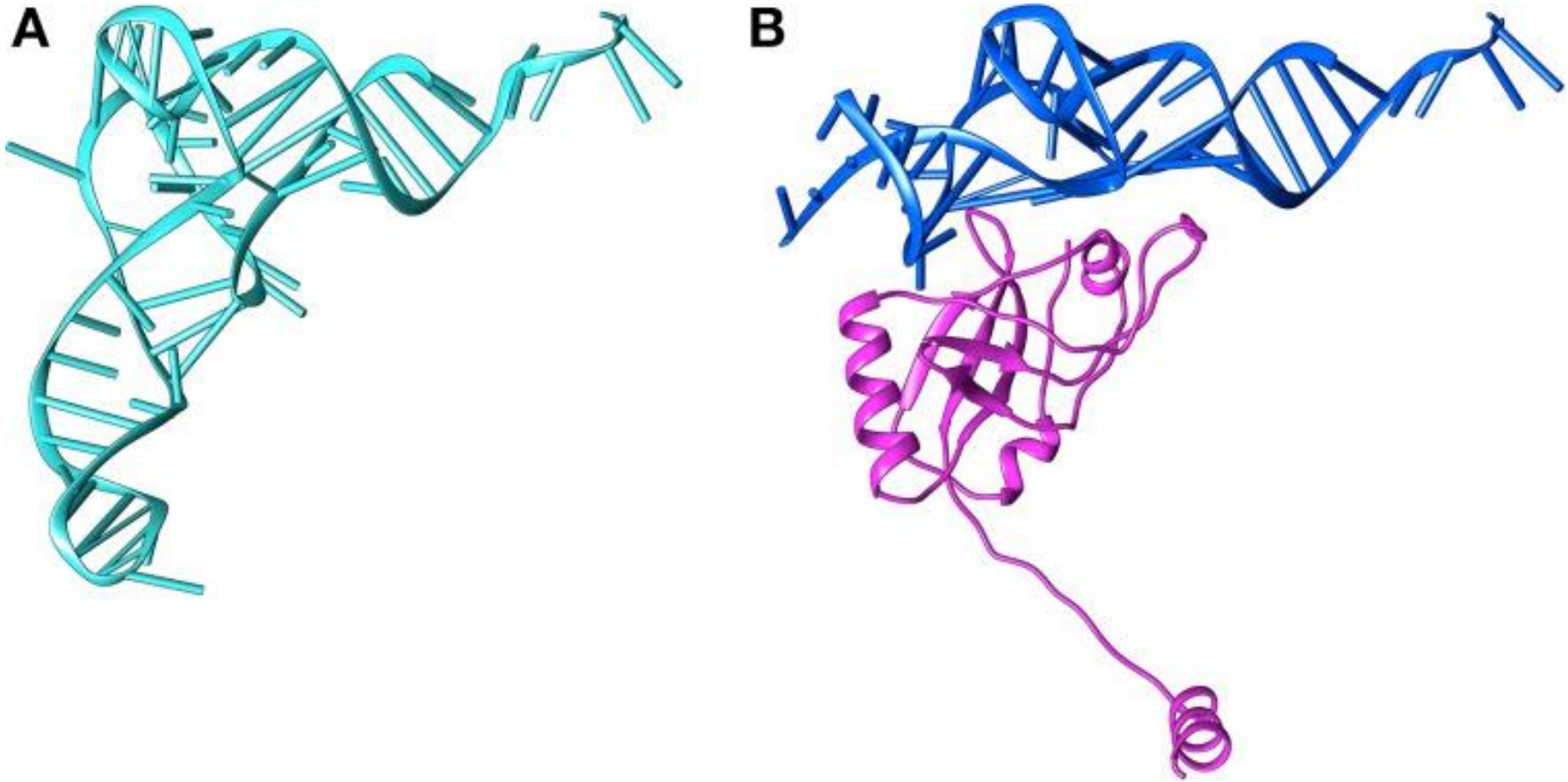


SmpB: the "handyman" of tmRNA



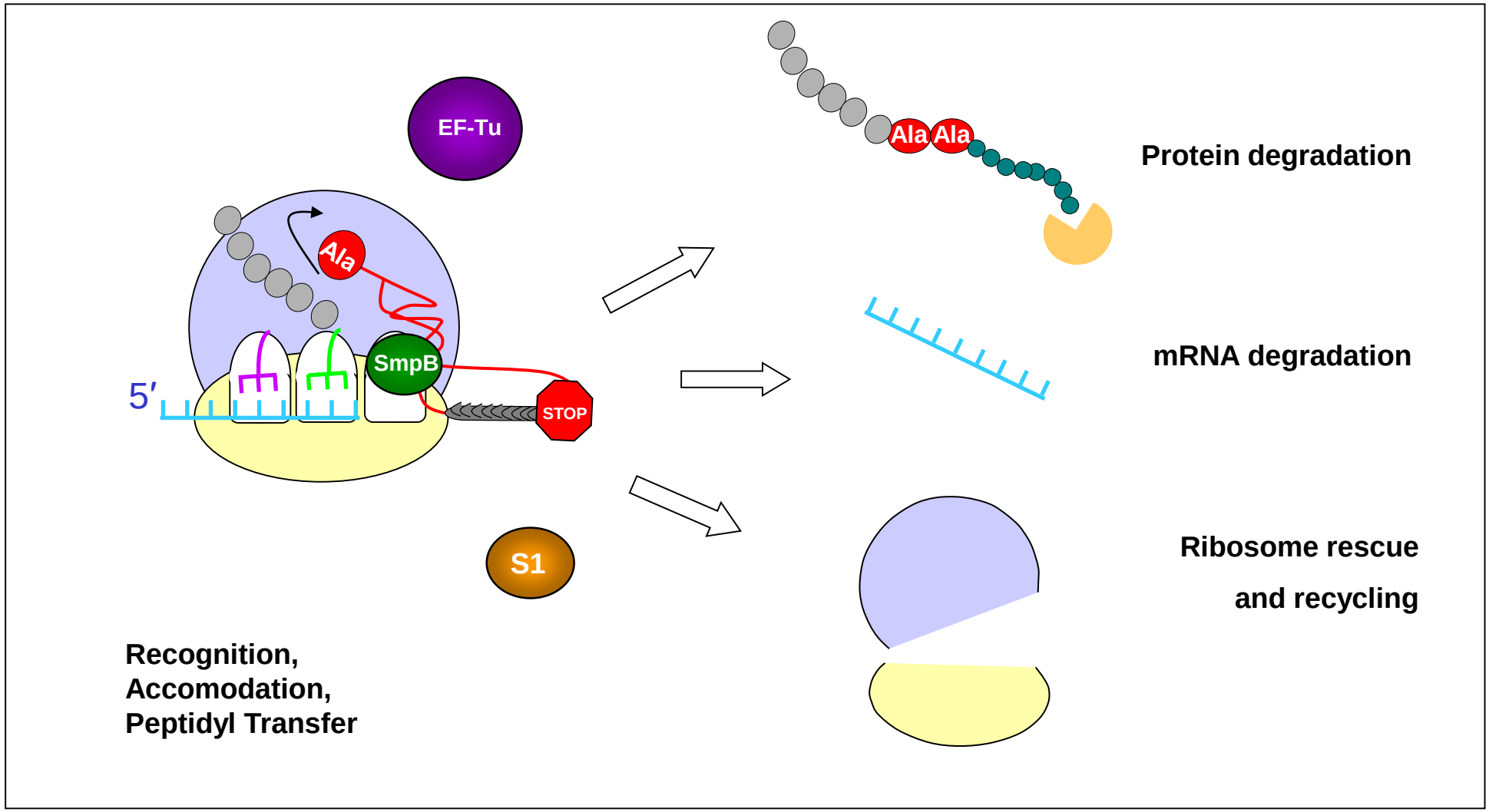
The N-terminal end, C-terminal end, and central loop are indicated. SmpB adopts an oligonucleotide-binding fold (OB fold) with a central β-barrel and three flanking α-helices. The C-terminal tail is unstructured in solution

tmRNA-SmpB primary interaction



Structural comparison between tRNA and the tRNA-like domain of tmRNA bound to SmpB. The TLD resembles the upper part of a tRNA, with SmpB replacing the tRNA anticodon stem-loop.

An all-in-one process: tagging; protein and mRNA degradation; ribosome rescue



Key points:

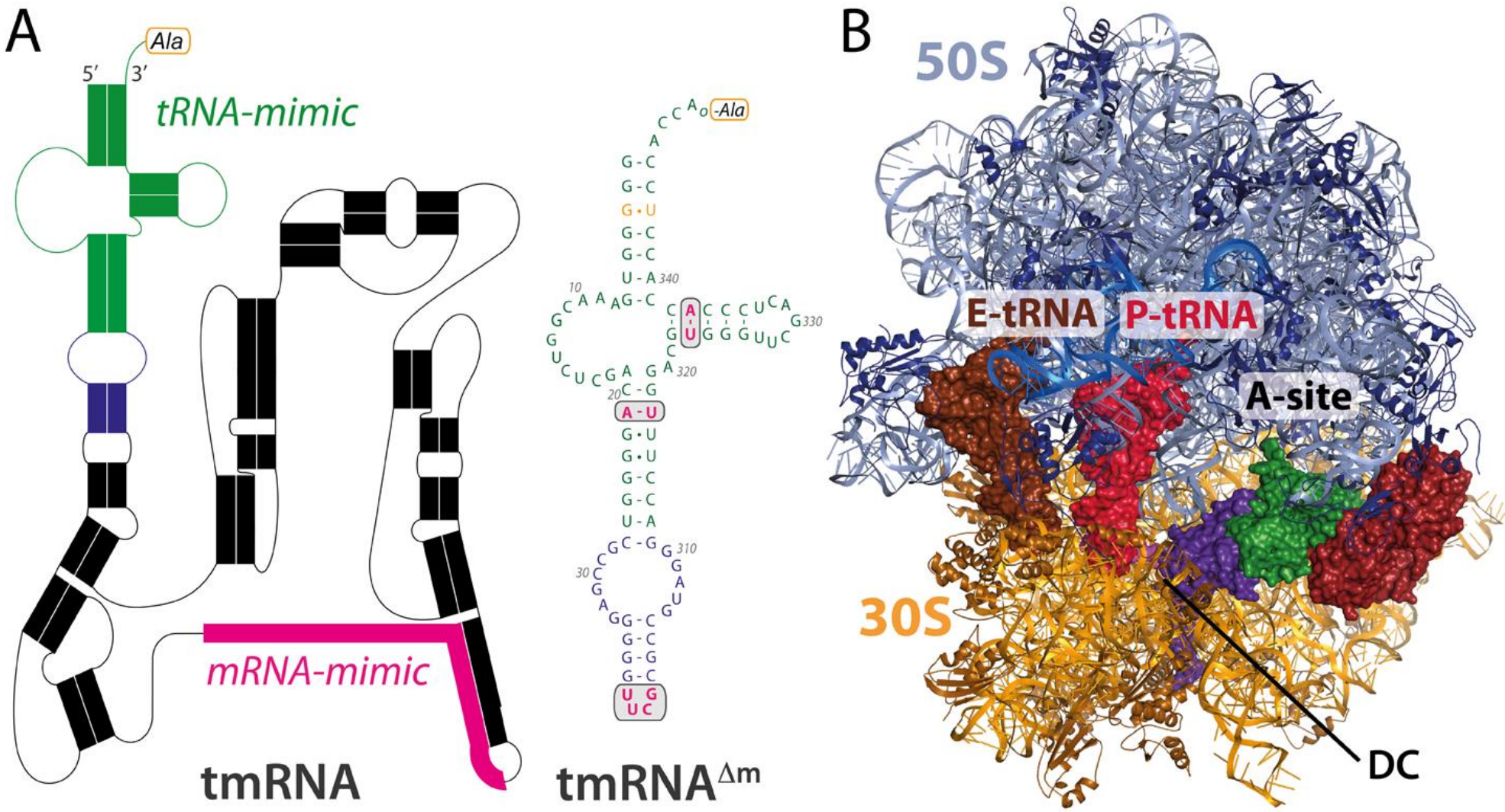
❶ **How tmRNA does recognize and bind stalled ribosomes without interfering with canonical translation?**

❷ **How can a large and complicated complex like tmRNA-SmpB make its way through the ribosome?**

❸ **How is the correct codon to restart translation from the ORF chosen?**

❹ **Where tmRNA comes from?**

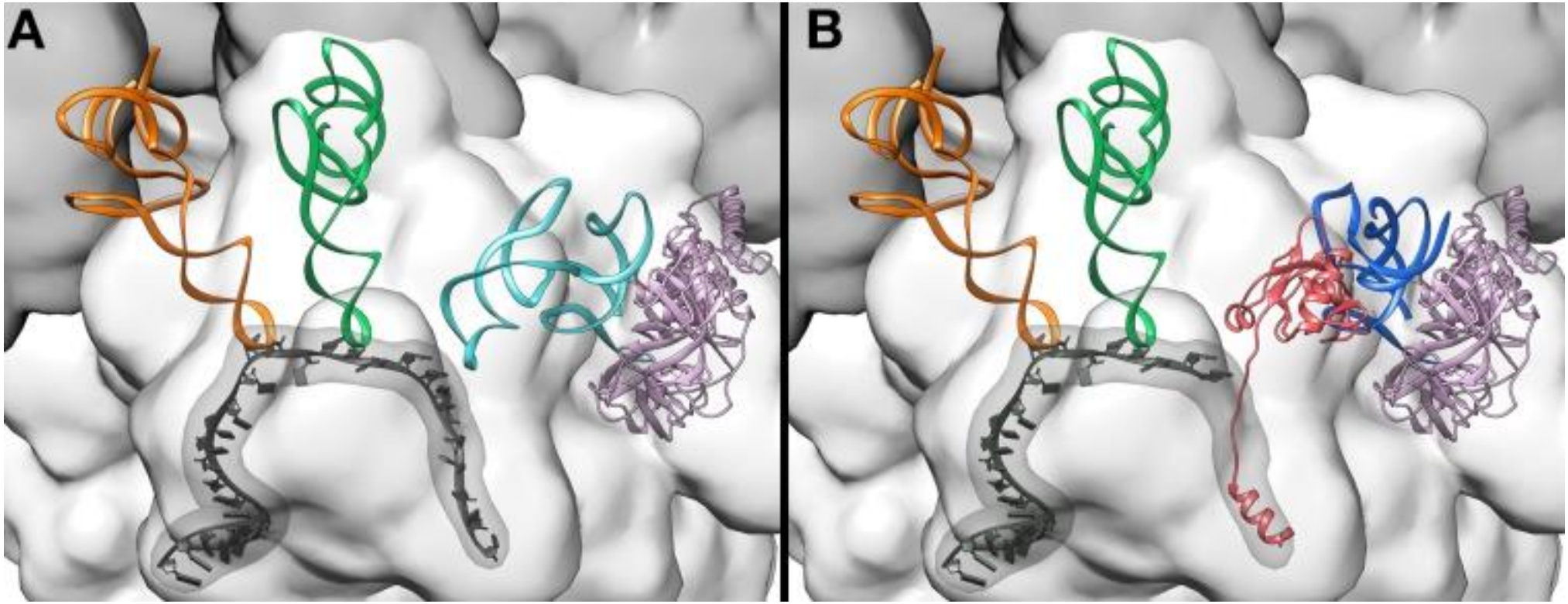
Crystal structure of a tmRNA fragment, SmpB and elongation factor Tu bound to the ribosome at 3.2 angstroms resolution.



Neubauer C, Gillet R, Kelley A, Ramakrishnan V, Science, 2012

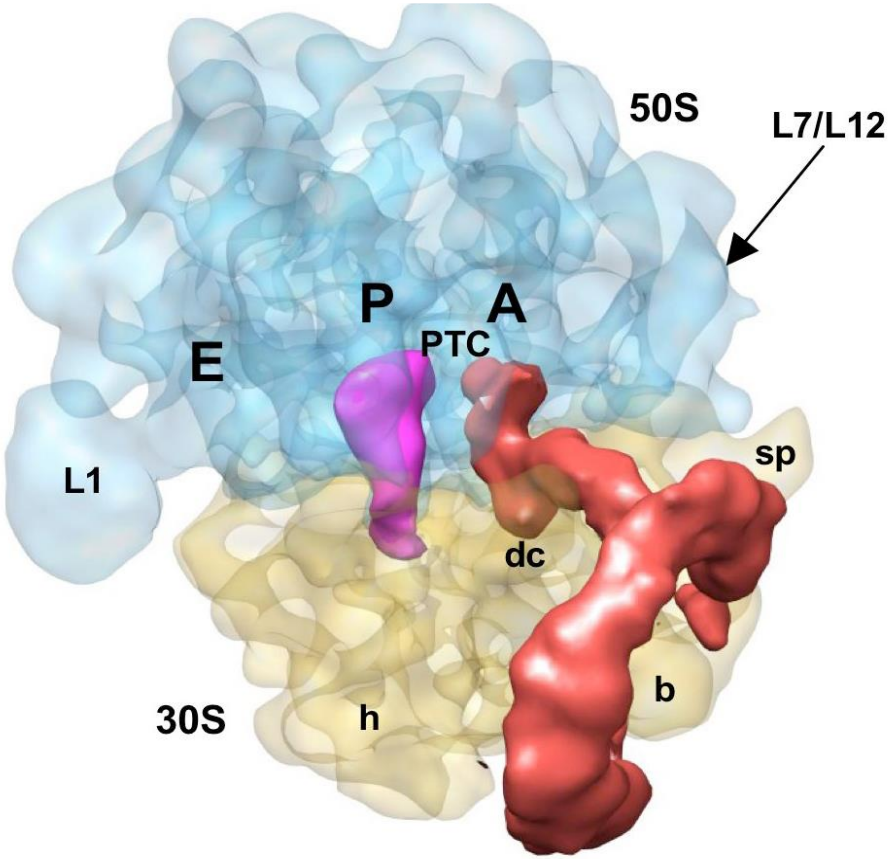
How tmRNA does recognize and bind stalled ribosomes without interfering with canonical translation?

The C-terminal tail of SmpB would clash with mRNA downstream of the A-site codon.

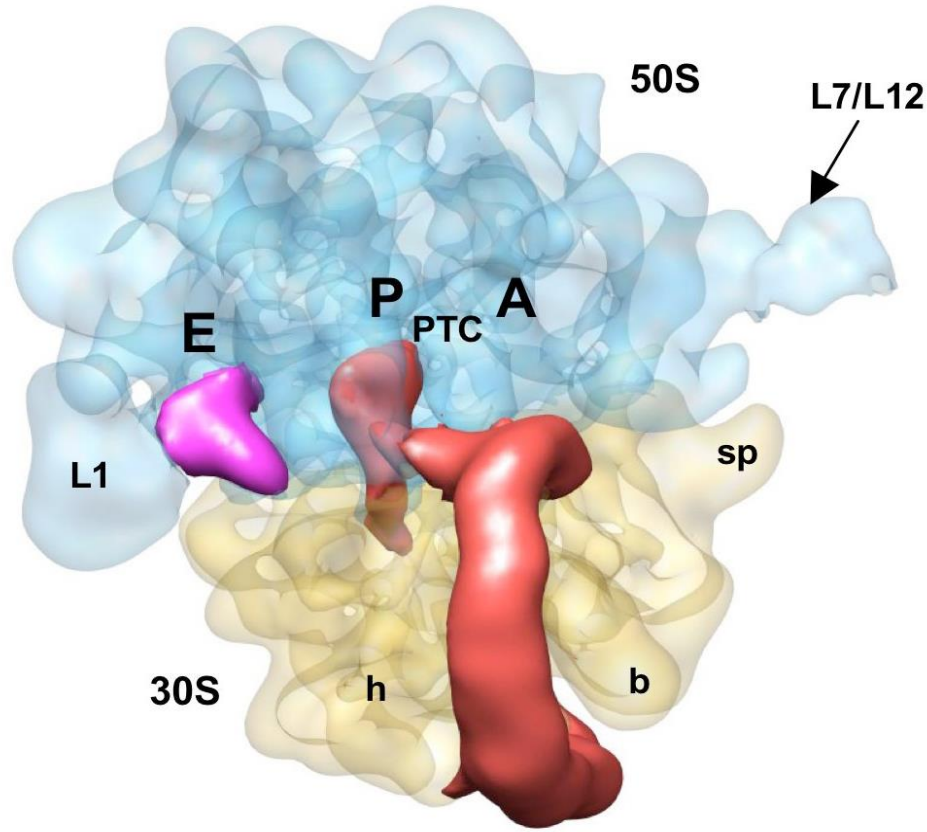


tmRNA-SmpB: moving forward into the ribosome





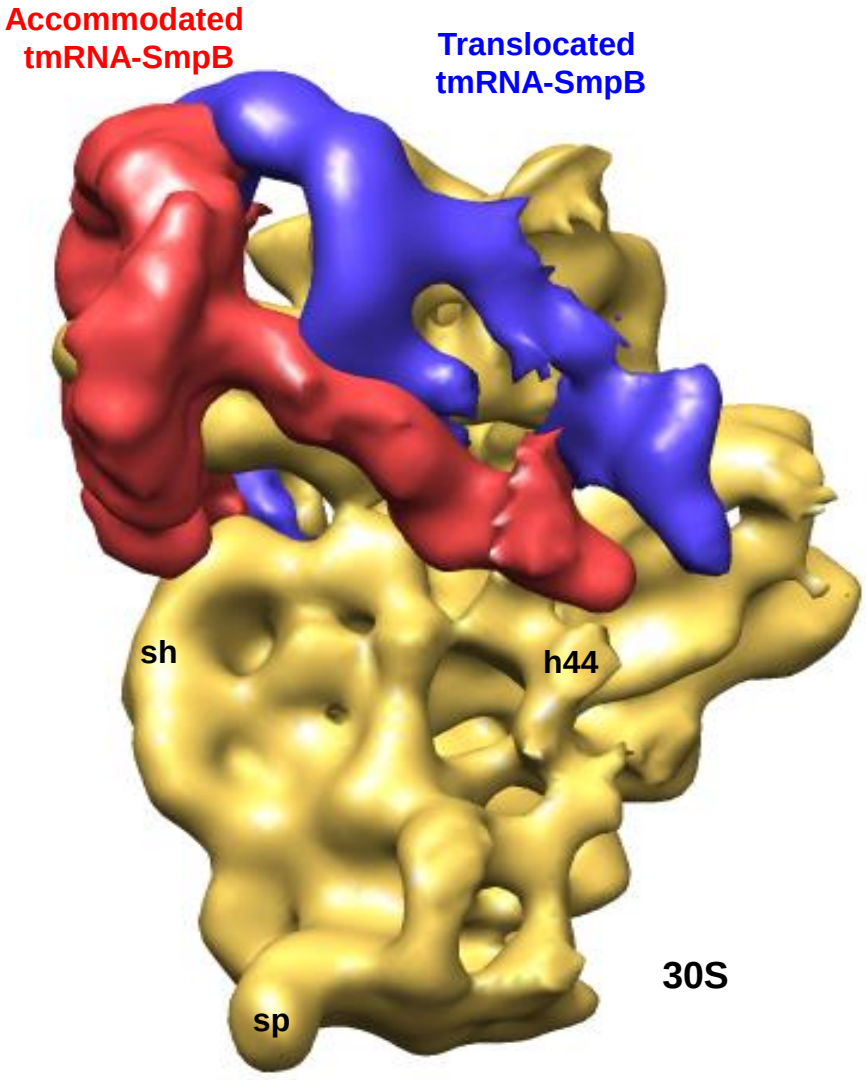
Accomodated state

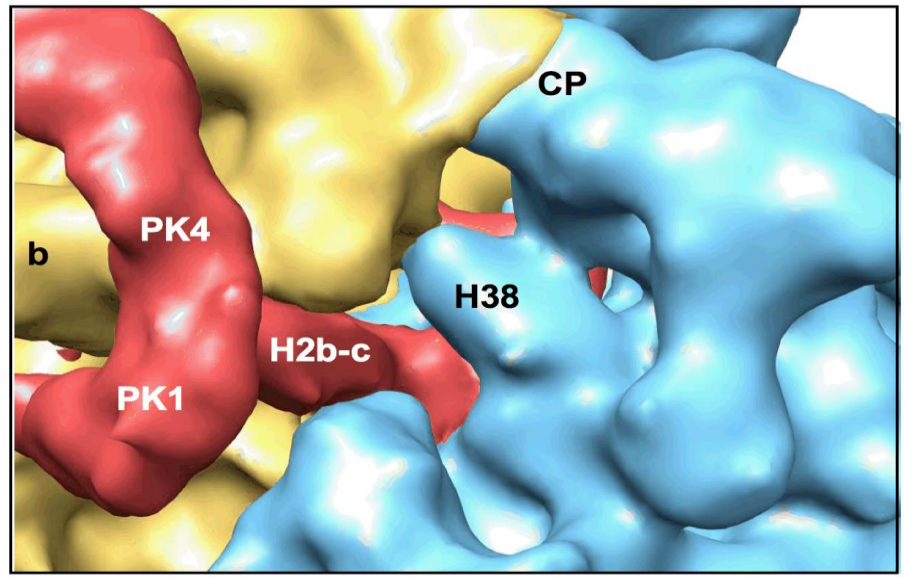


Translocated state

Weis et al.,
EMBO J 2010

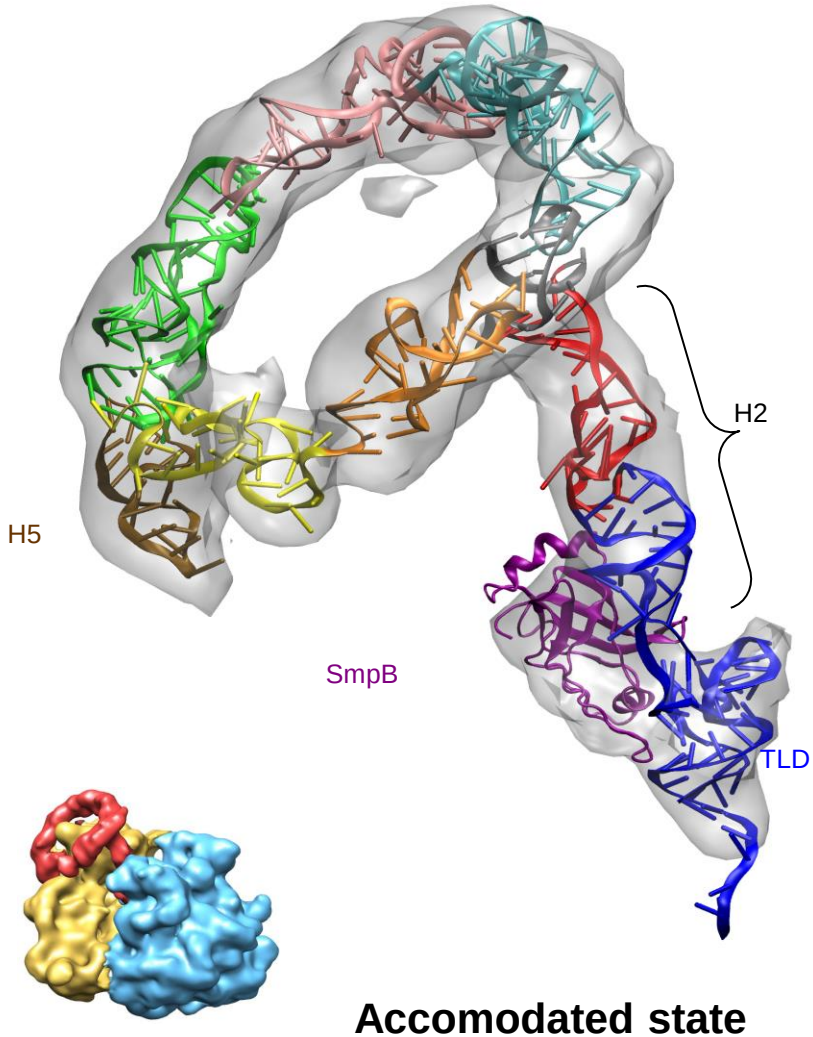
How tmRNA-SmpB makes its way through the ribosome ?



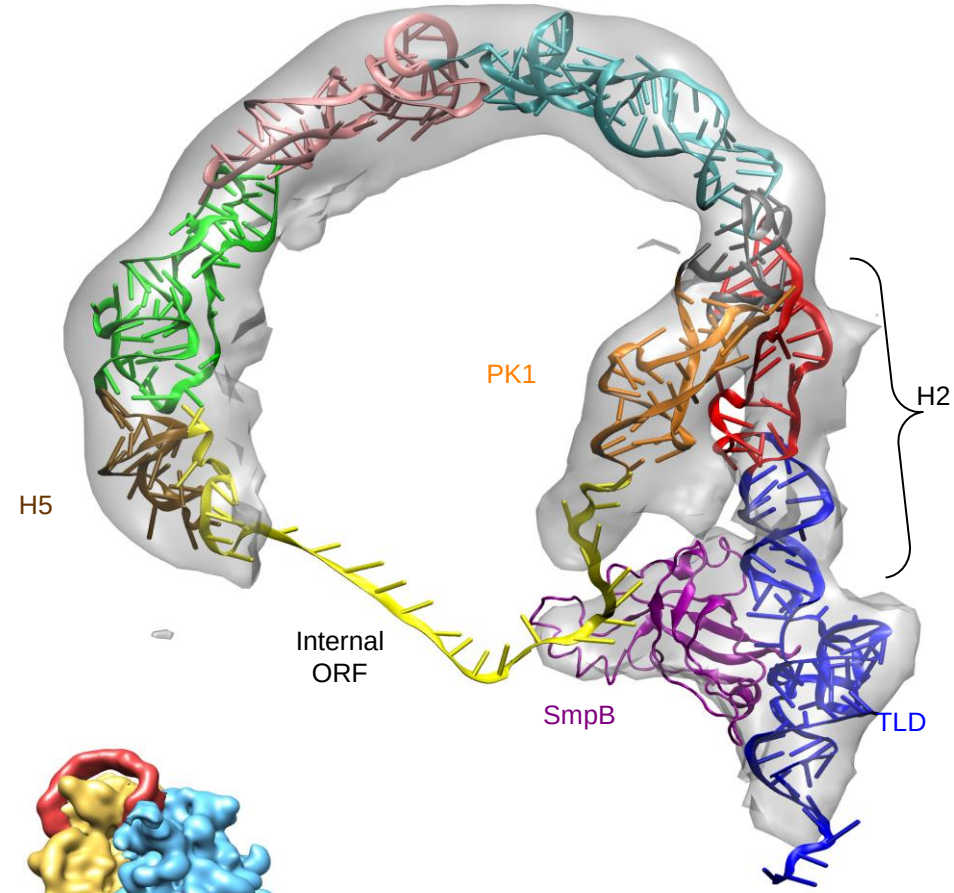


A-site finger opening

SmpB is instrumental when switching the templates

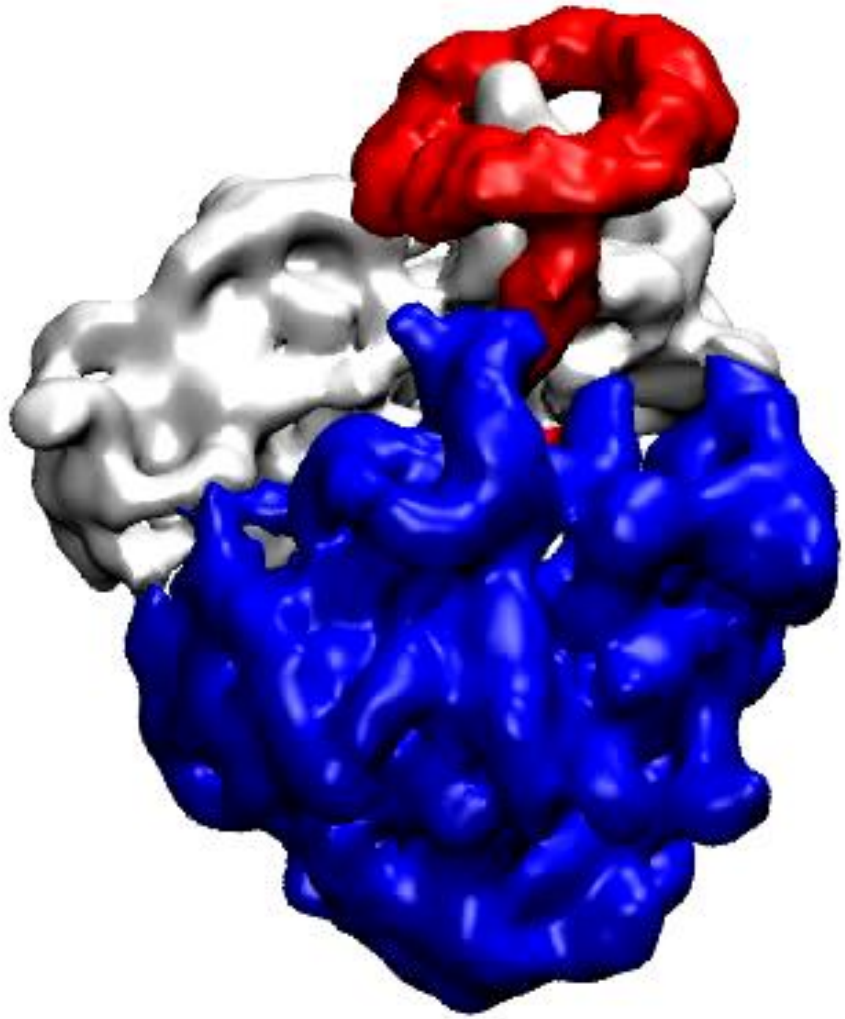


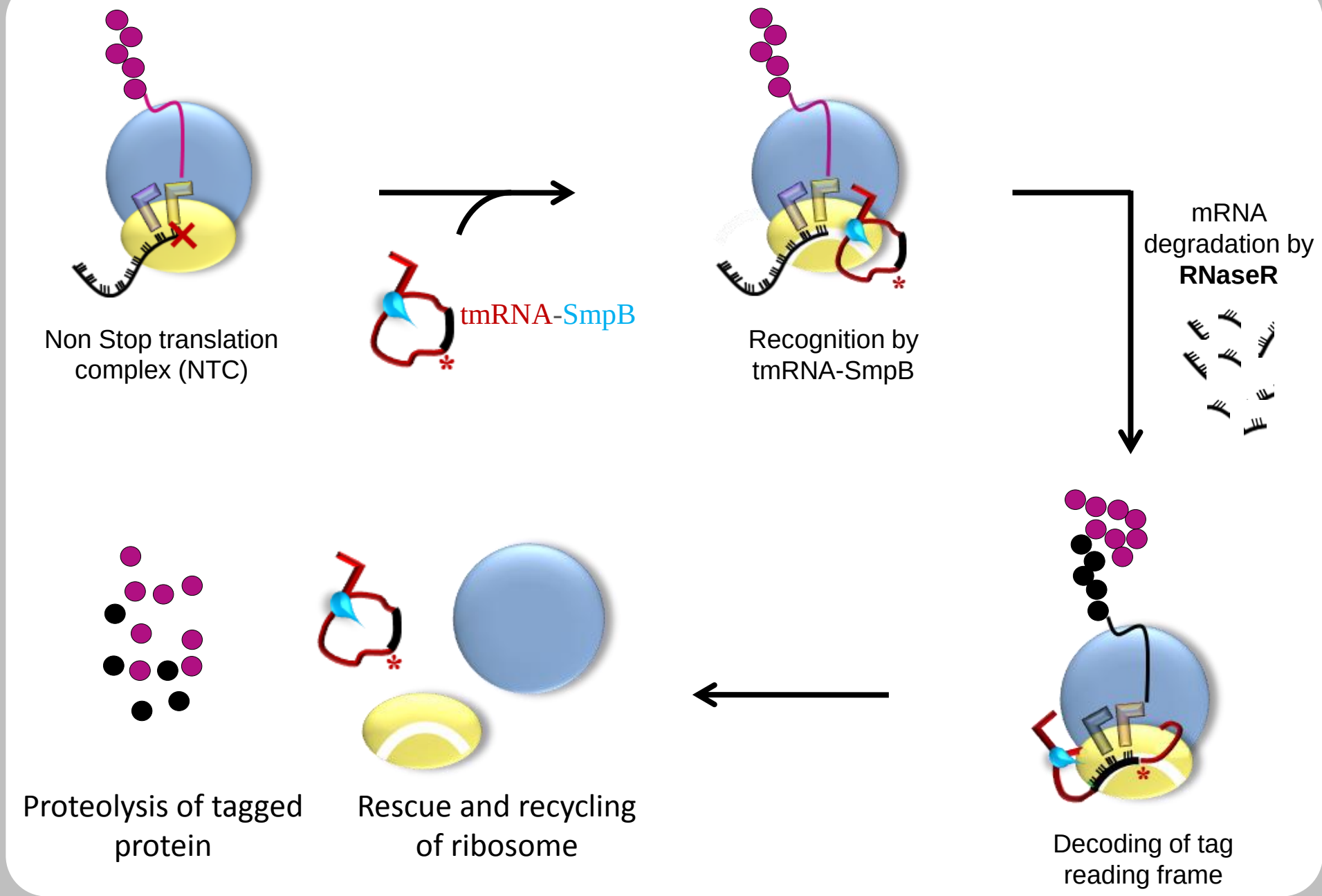
Accomodated state



Translocated state

How is the correct codon to restart translation from the ORF chosen ?




Non Stop translation complex (NTC)
tmRNA-SmpB
Recognition by tmRNA-SmpB
mRNA degradation by **RNaseR**
Decoding of tag reading frame
Rescue and recycling of ribosome
Proteolysis of tagged protein

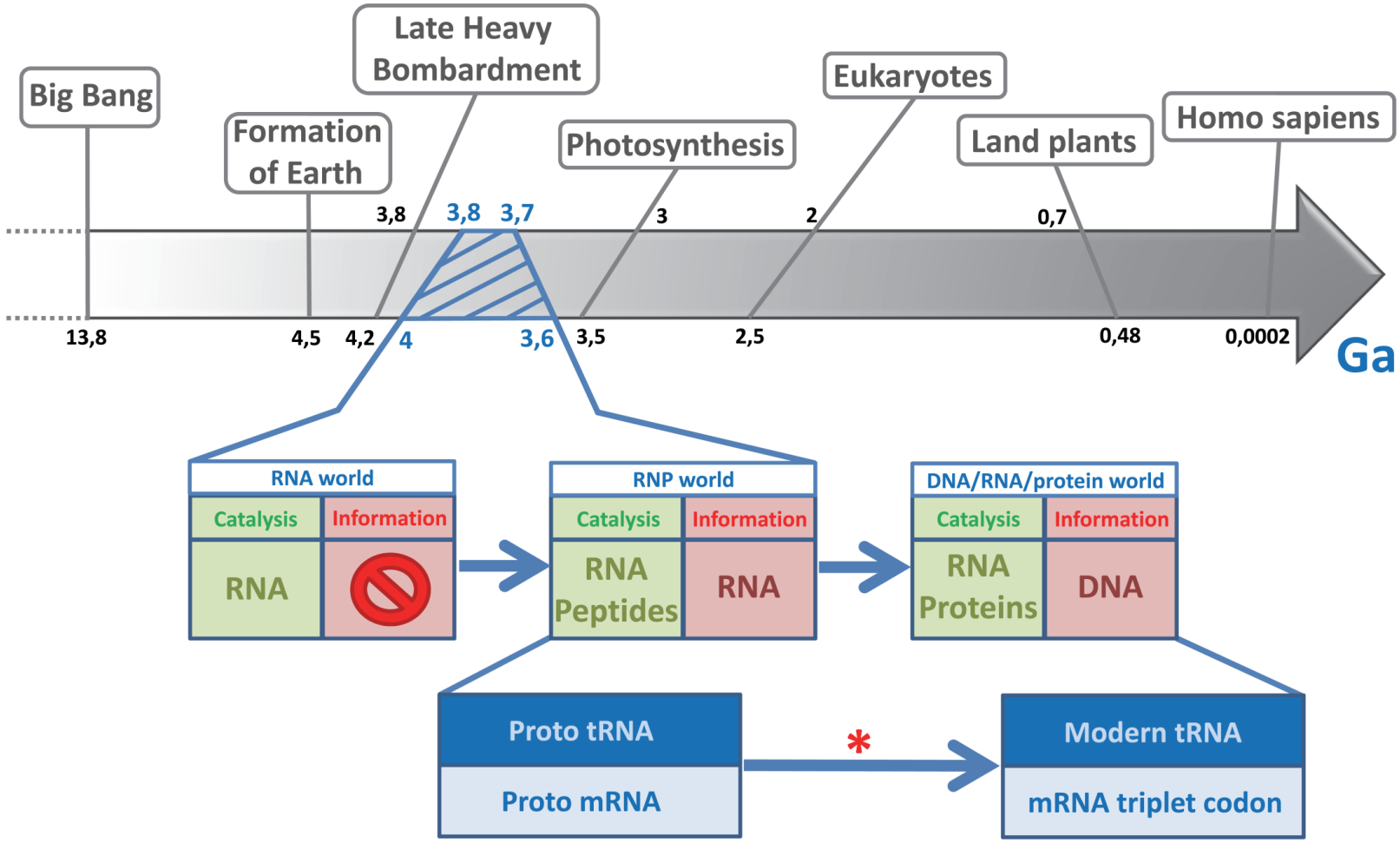
trans-translation is a perfect target for the development of new antibiotics

- **No *trans*-translation in eukaryotes**
- **Essential to the survival of many pathogenic bacteria**
  Staphylococcus aureus, Mycobacterium tuberculosis, Neisseria gonorrhoeae, Helicobacter pylori…
- **Required for the virulence of some species**
  Bacillus anthracis, Yersinia pestis or Francisella tularensis
- **When deletion is not lethal, it induces hypersensitive phenotypes in many bacterial species**

tmRNA Origins?

Looking for the scars of an ancient (RNA) world into the modern (DNA-protein) world

Timeline of early events

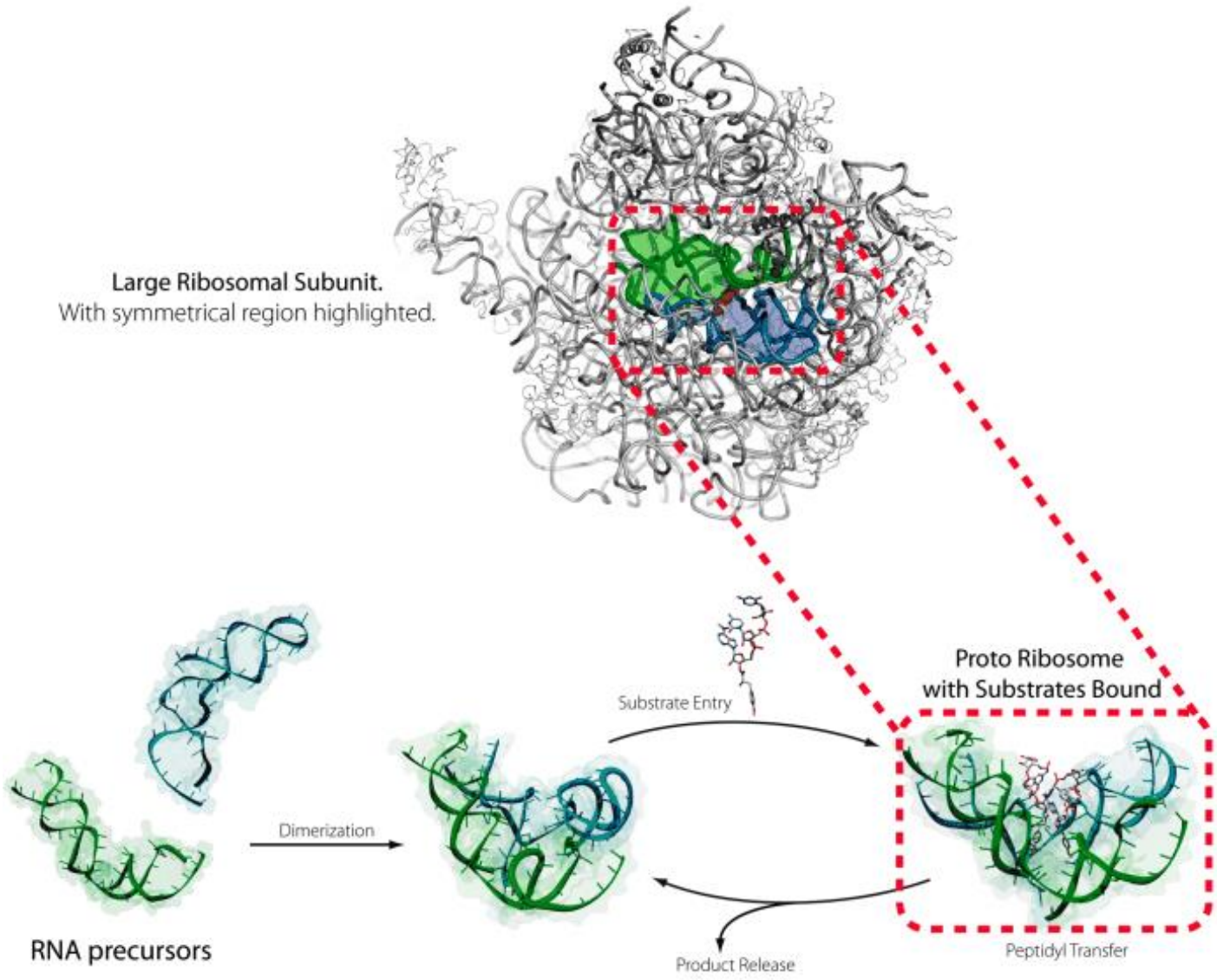


RNA was central in assuring both genetic continuity and catalysis

Macé and Gillet, NAR 2016

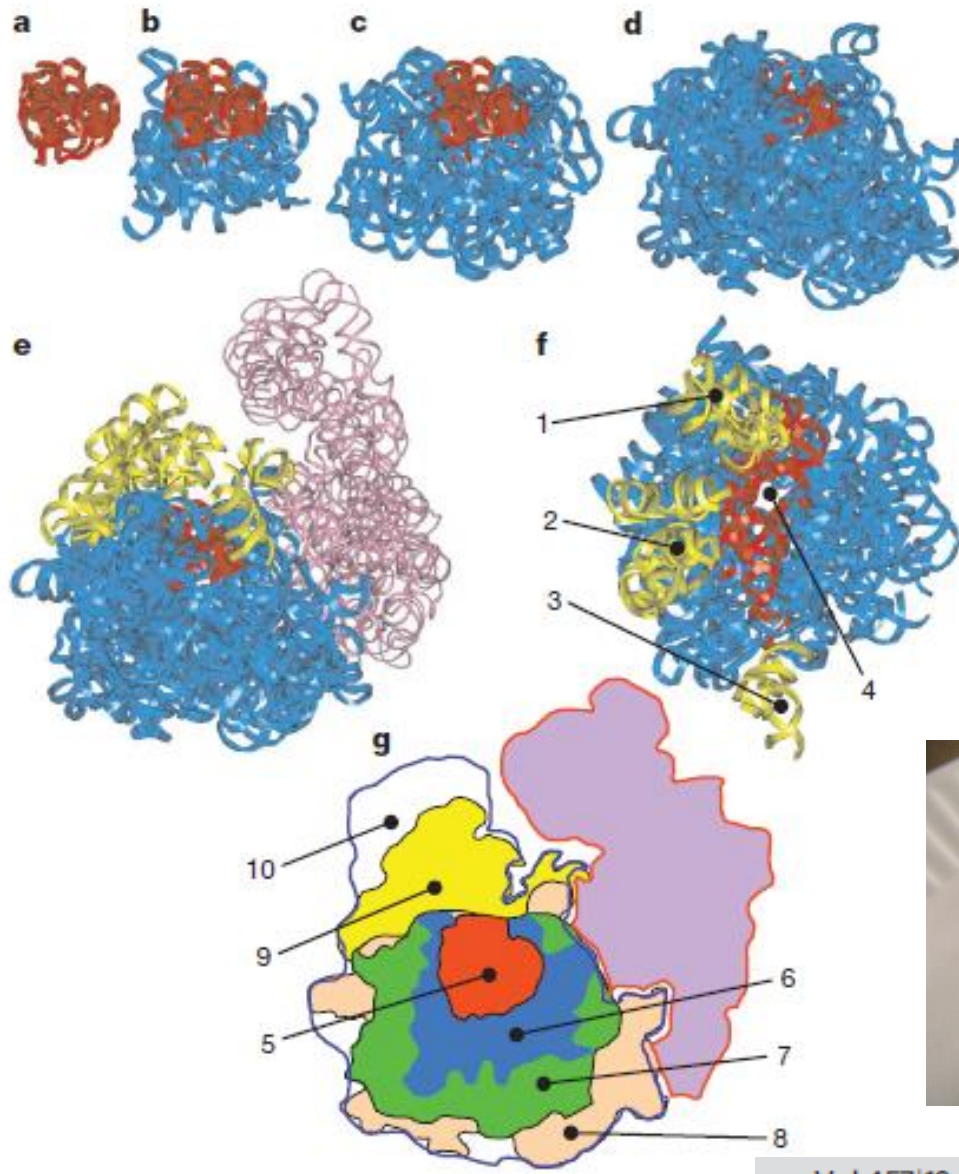
Proto-Ribosome:

an ancient nano-machine dedicated to the binding of amino-acids



Israel Journal of Chemistry
Volume 50, Issue 1, pages 29-35, 10 JUN 2010

Evolution of the ribosome:



a=0 new element

b= 8

c = 20

d= 50

e = 59

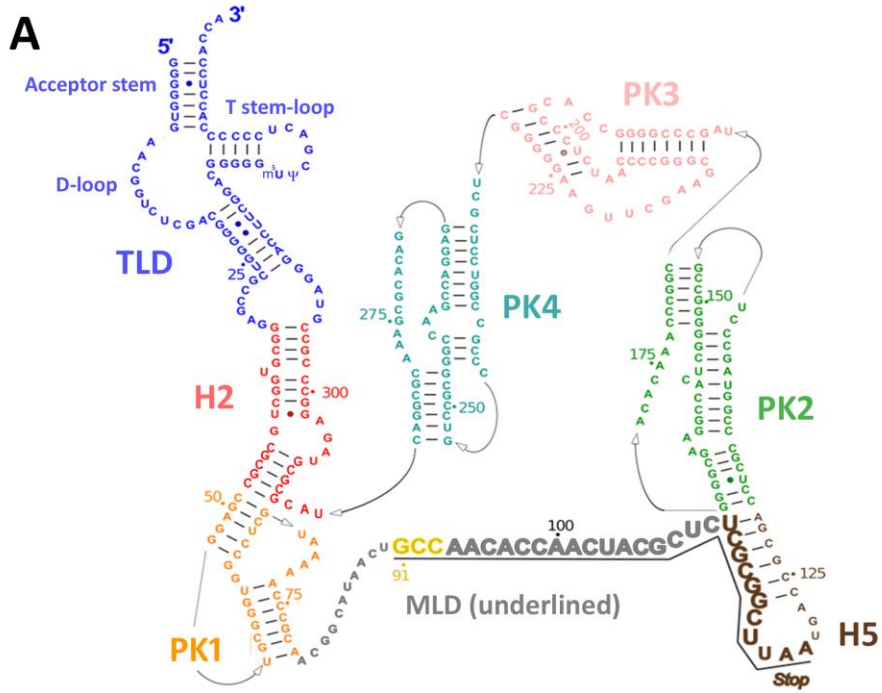
Vol 457|19 February 2009|doi:10.1038/nature07749

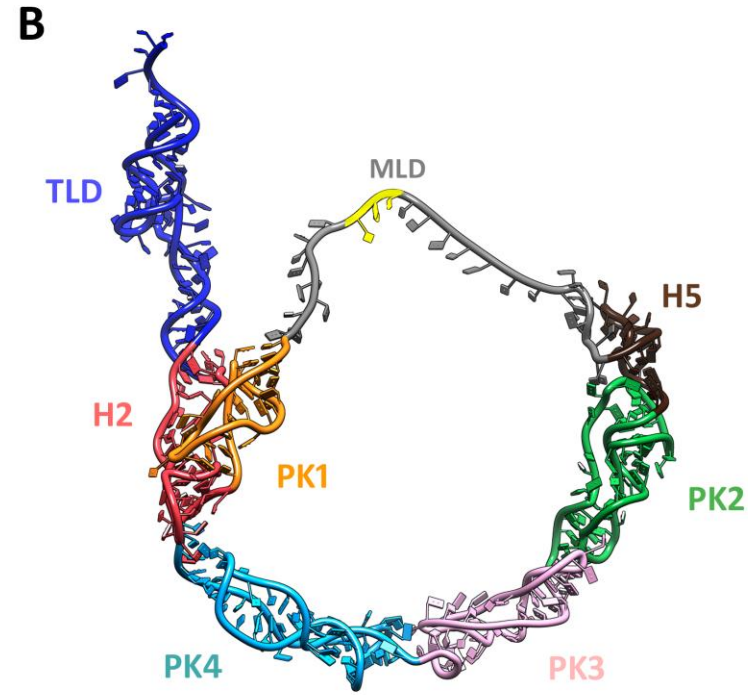
The ribosome must have evolved with:

The genetic code / mRNAs

tRNAs

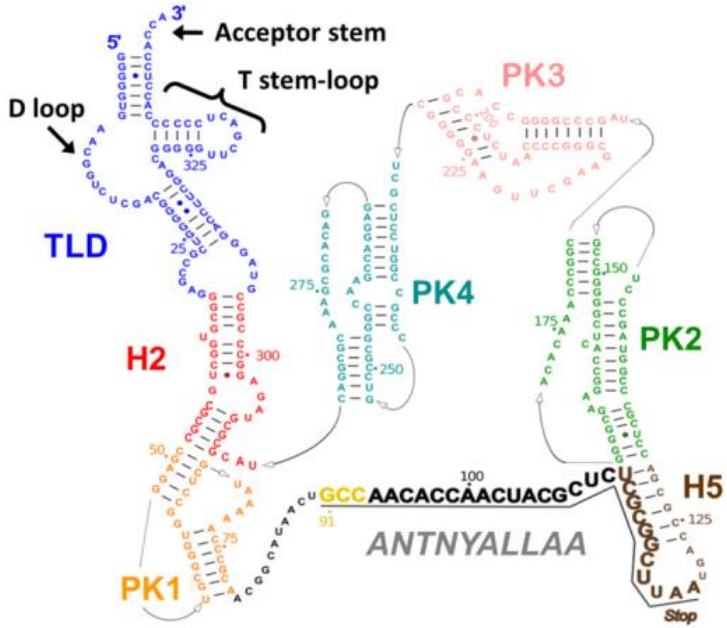
Two essential functions carried out by tmRNA

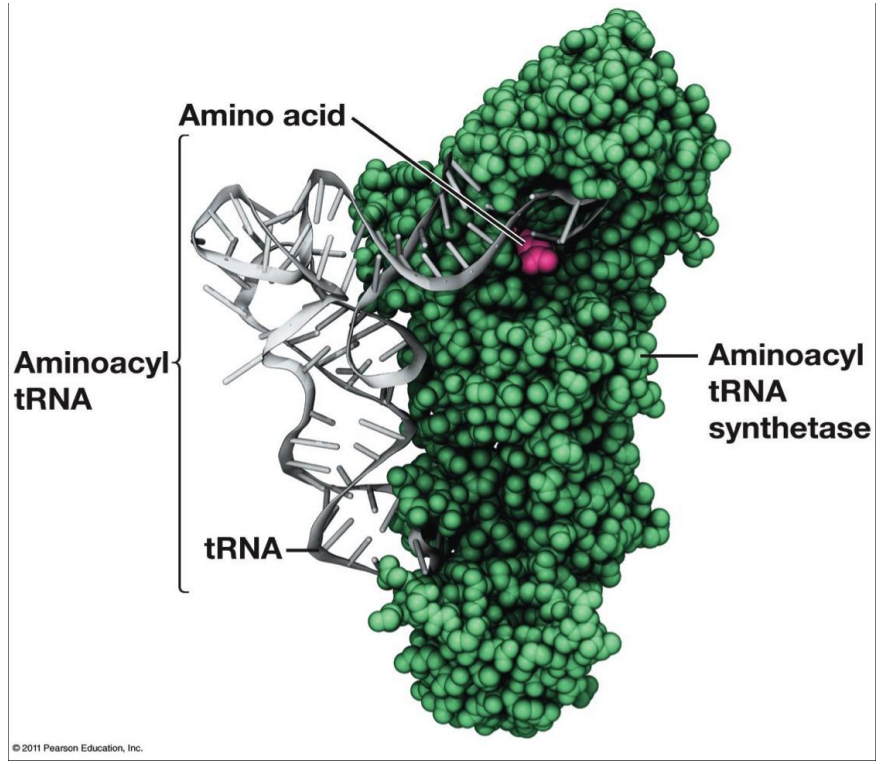


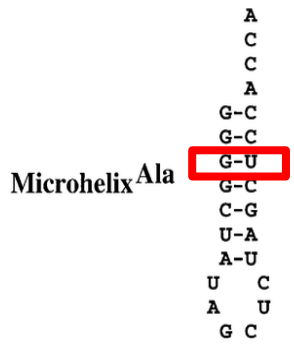


I- Scars into the tRNA like domain

1) G:U recognition by AlaRS





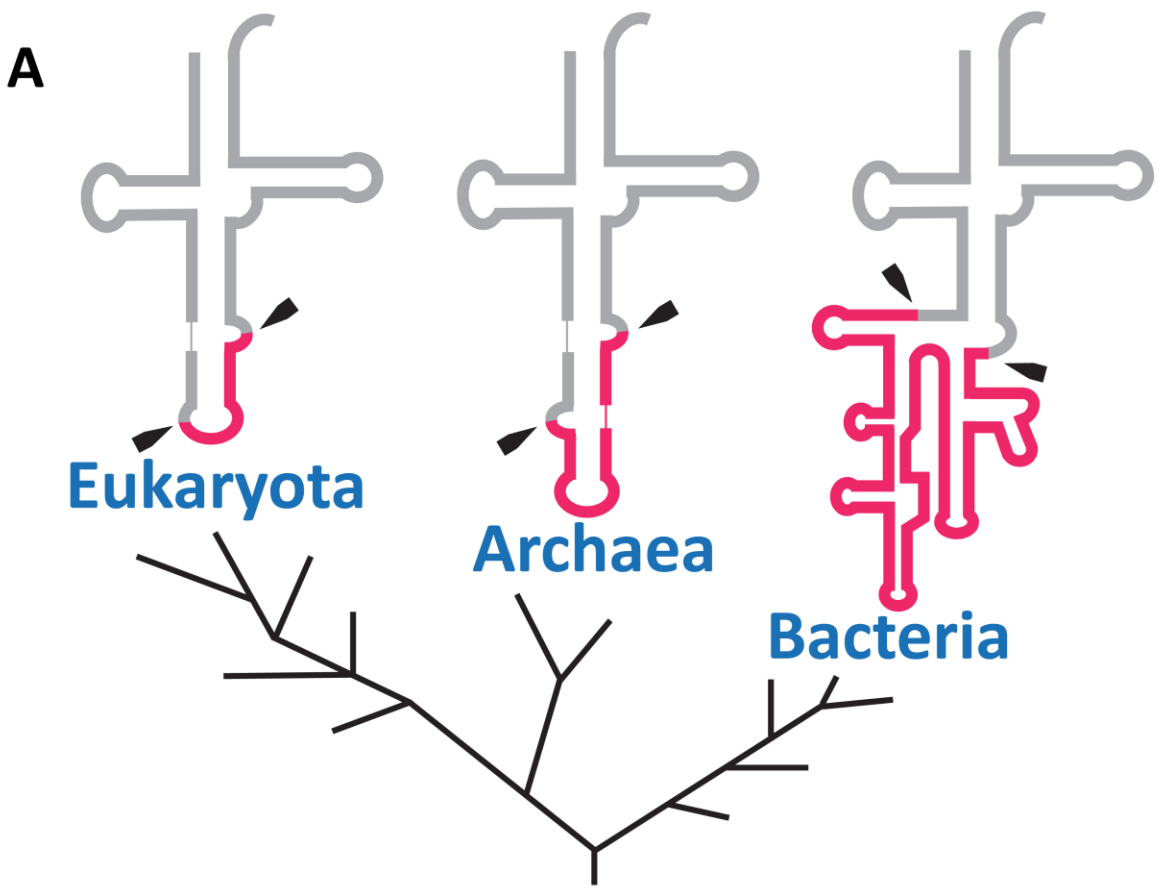


Reflects the ancient recognition of RNA minihelices by the first enzymes

P Schimmel

I- Scars into the tRNA like domain

2) Position and secondary structure similarities between the tRNA introns and tmRNA pseudoknots

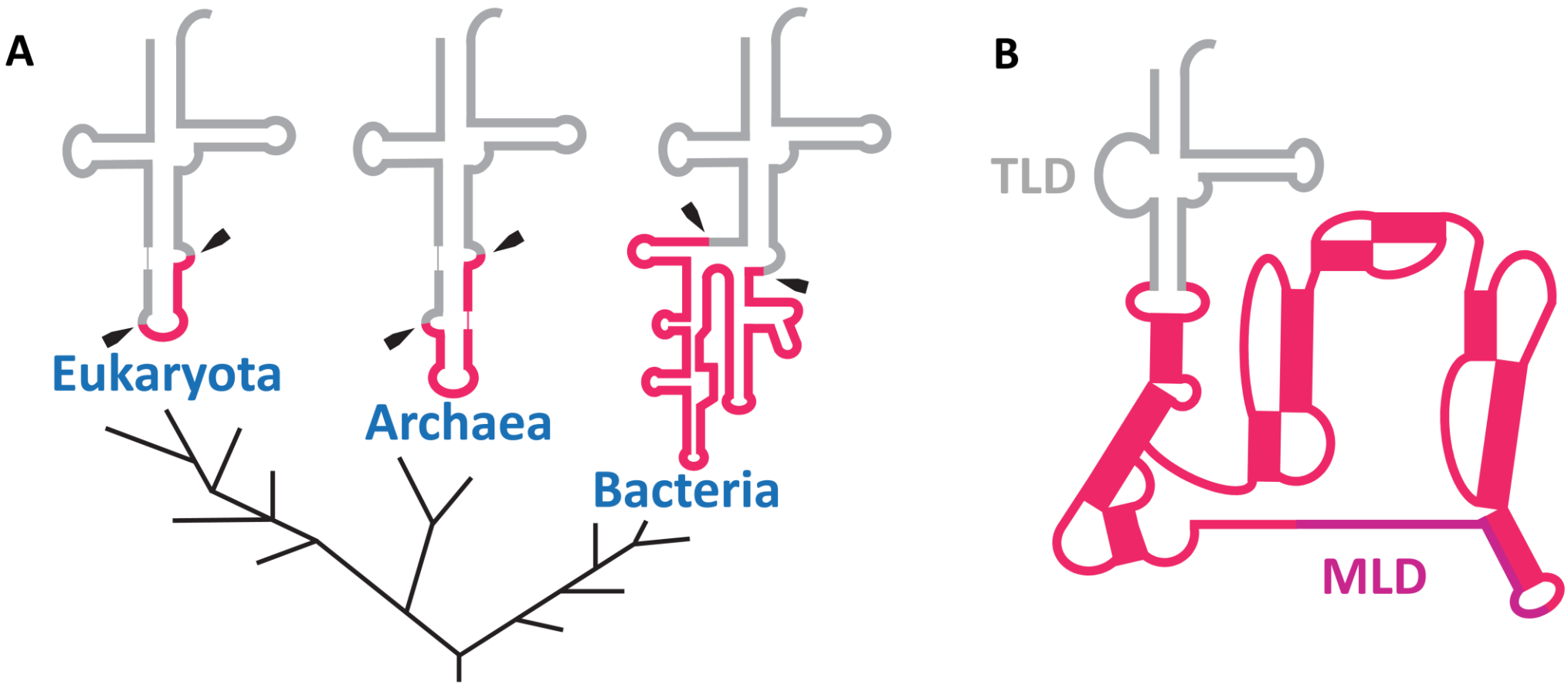


In the three domains of life, some rare tRNA still undergo splicing of non-coding sequences (introns) located within the anticodon stem-loop

A: The tRNA intron in the three major kingdoms (Bacteria, Archaea and Eukaryota), adapted from Akio Kanai. Intron clipping sites are indicated with black arrows.

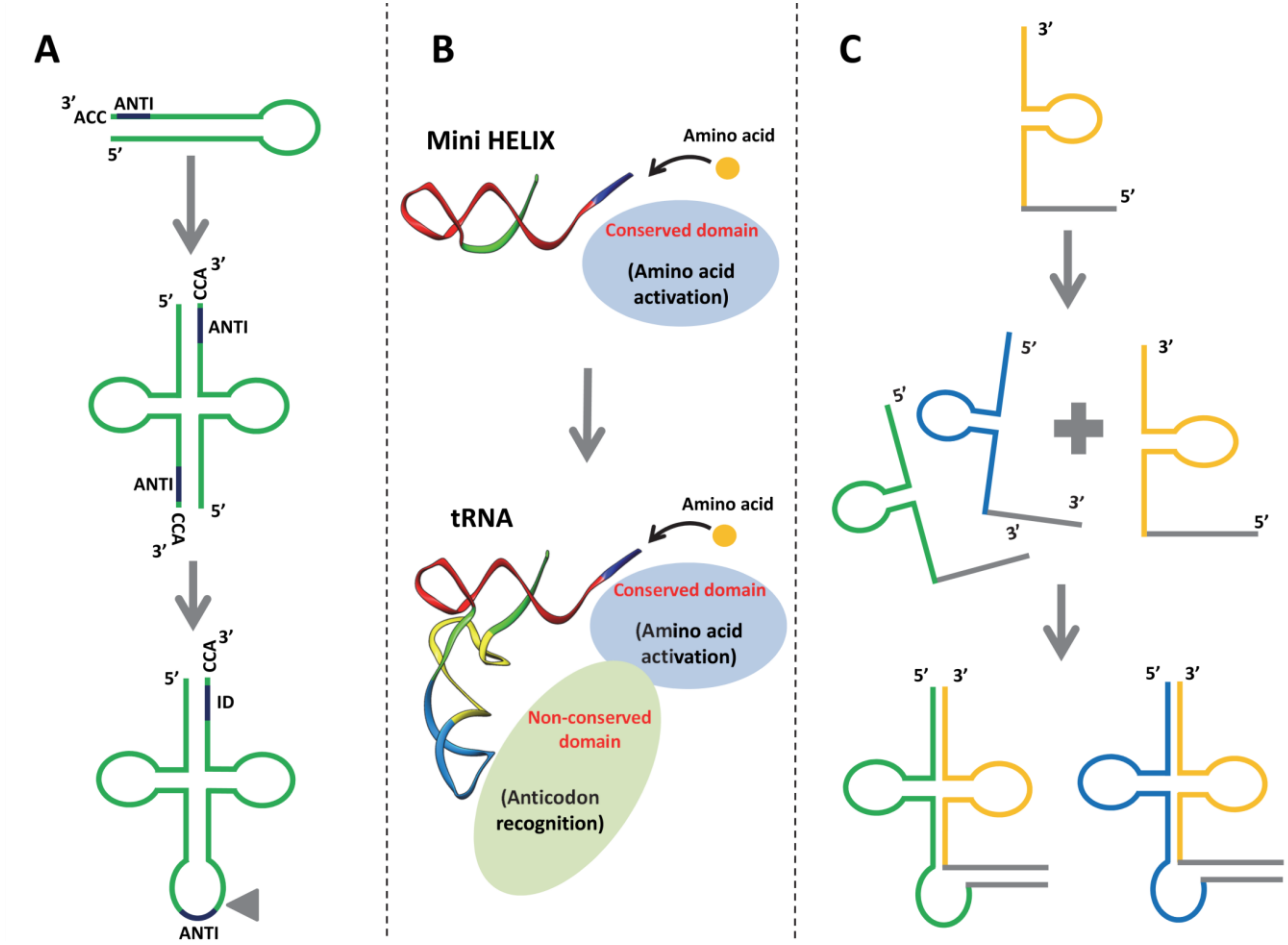
Scars into the tRNA like domain

2) Position and secondary structure similarities between the tRNA introns and tmRNA pseudoknots



A: The tRNA intron in the three major kingdoms (Bacteria, Archaea and Eukaryota), adapted from Akio Kanai Intron clipping sites are indicated with black arrows. B: Secondary structure of tmRNA. Note the similar positioning of the tRNA introns in the three domains of life and in the other tmRNA domains

Different models explaining the origins of tRNA



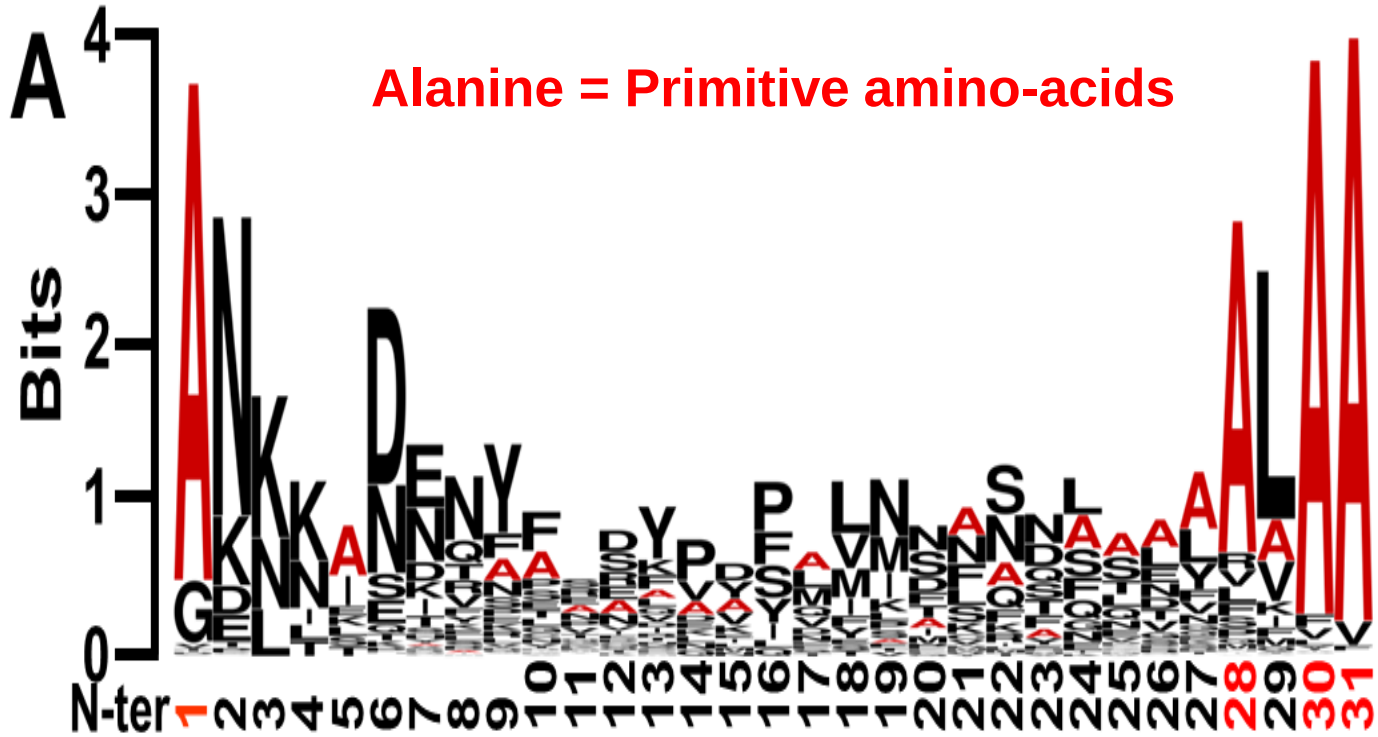
A: tRNA may originate from the dimerization of two hairpin structures. The triangle represents the position where the intron is found in tRNA genes (66).

B: tRNA may originate from the late fusion between two RNA minihelices.

C: tRNA may originate from the fusion of split genes of non-contiguous tRNAs.

II- Scars into the mRNA like domain

1) Alanines at the crossroads of tmRNA-based aminoacylation and tagging events

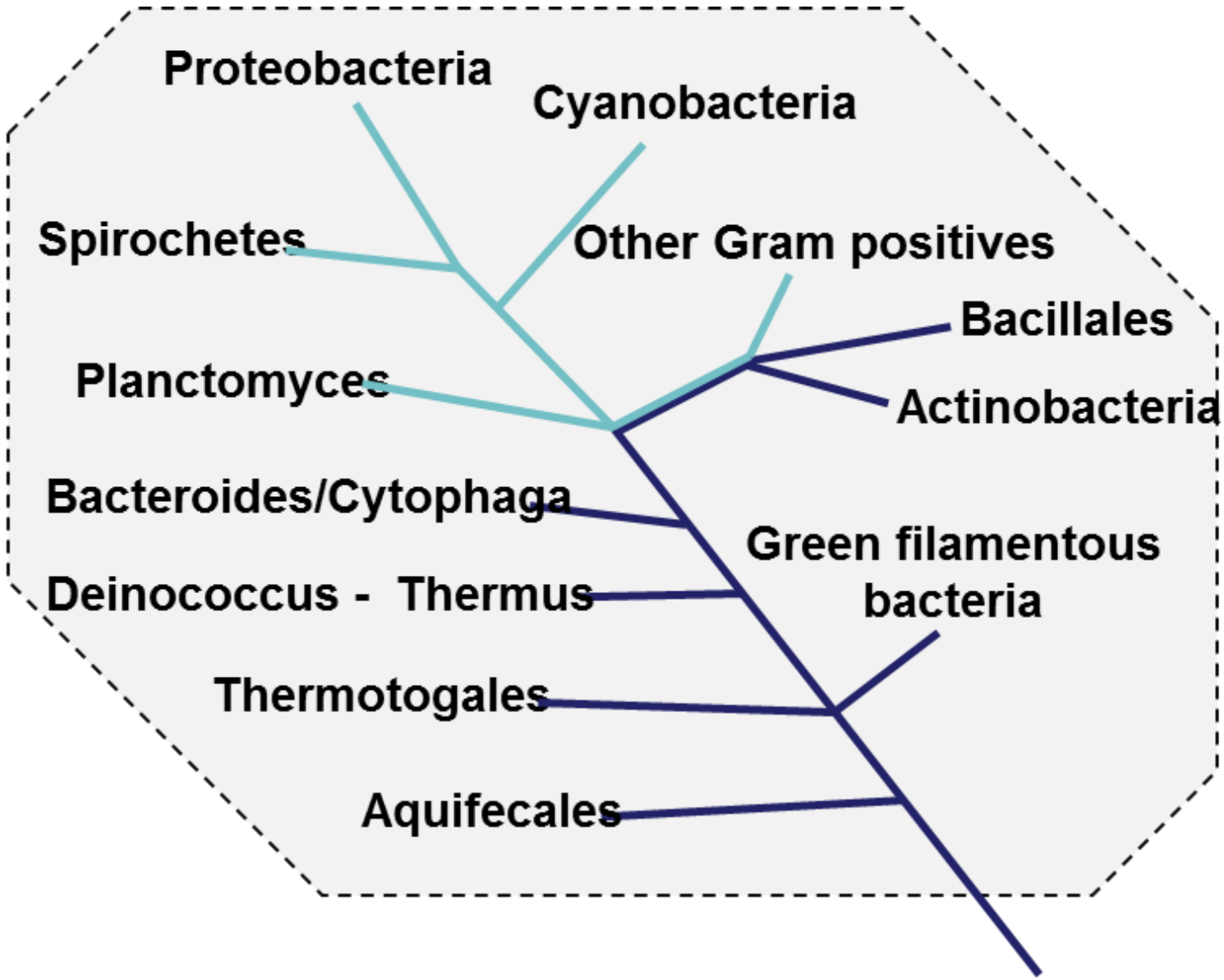


Sequence of the consensus sequence and diversity of the amino acids in the MLD

Analysis of the first MLD codon in different bacteria phyla

	ANCESTRAL CODON			OTHER CODON		
PHYLUM	Alanine % GCC	Glycine % GGC	TOTAL % GNC	Alanine % GCA	OTHER OTHER	TOTAL % GNN
Aquifecales	100 (4)	0 (0)	100 (4)	0 (0)	0 (0)	0 (0)
Thermotogales	100 (4)	0 (0)	100 (4)	0 (0)	0 (0)	0 (0)
Deinococcus - Thermus	50 (2)	50 (2)	100 (4)	0 (0)	0 (0)	0 (0)
Green filamentous bacteria	33 (1)	67 (2)	100 (3)	0 (0)	0 (0)	0 (0)
Bacillales	0 (0)	92 (36)	92 (36)	0 (0)	8 (3)	8 (3)
Actinobacteria (HIGH G+C)	90 (14)	0 (0)	90 (14)	5 (1)	5 (1)	10 (2)
Bacteroides/Cytophaga	73 (8)	0 (0)	73 (8)	18 (2)	9 (1)	27 (3)
Cyanobacteria	0 (0)	0 (0)	0 (0)	15 (4)	85 (22)	100 (26)
Planctomyces	0 (0)	0 (0)	0 (0)	50 (1)	50 (1)	100 (2)
Other Gram Positive	5 (4)	0 (0)	5 (4)	64 (49)	30 (23)	95 (72)
Proteobacteria	23 (64)	1 (2)	24 (66)	71 (195)	5 (14)	76 (209)
Spirochetes	40 (2)	0 (0)	40 (2)	40 (2)	20 (1)	60 (3)
All bacteria	23 (108)	9 (42)	32 (150)	54 (254)	14 (66)	68 (320)
Plastids	0 (0)	0 (0)	0 (0)	59 (13)	41 (9)	100 (22)
Bacteriophages	0 (0)	0 (0)	0 (0)	50 (1)	50 (1)	100 (2)

Variations in the first MLD codon in the different bacteria phyla



The dark blue line indicates ancestral codons for the first codon of the MLD, and the light blue line indicates other codons.

II- Scars into the mRNA like domain

2) First mRNAs on earth might have been peptidated.

This is obviously the case of tmRNA.

Co-evolution of tRNAs and mRNAs favored the evolution of the genetic code

III- Phylogenetic Scars

No trans-translation in eukaryotes and archea

(very few exceptions: plastomes of some primitive algae; some rare diatoms (Stramenopila) that acquired genes from marine bacteria; Jakobids (carrying uniquely bacterial-lie mitochondrial genomes)

Provocative model representing the different possibilities for the origins of tmRNA.

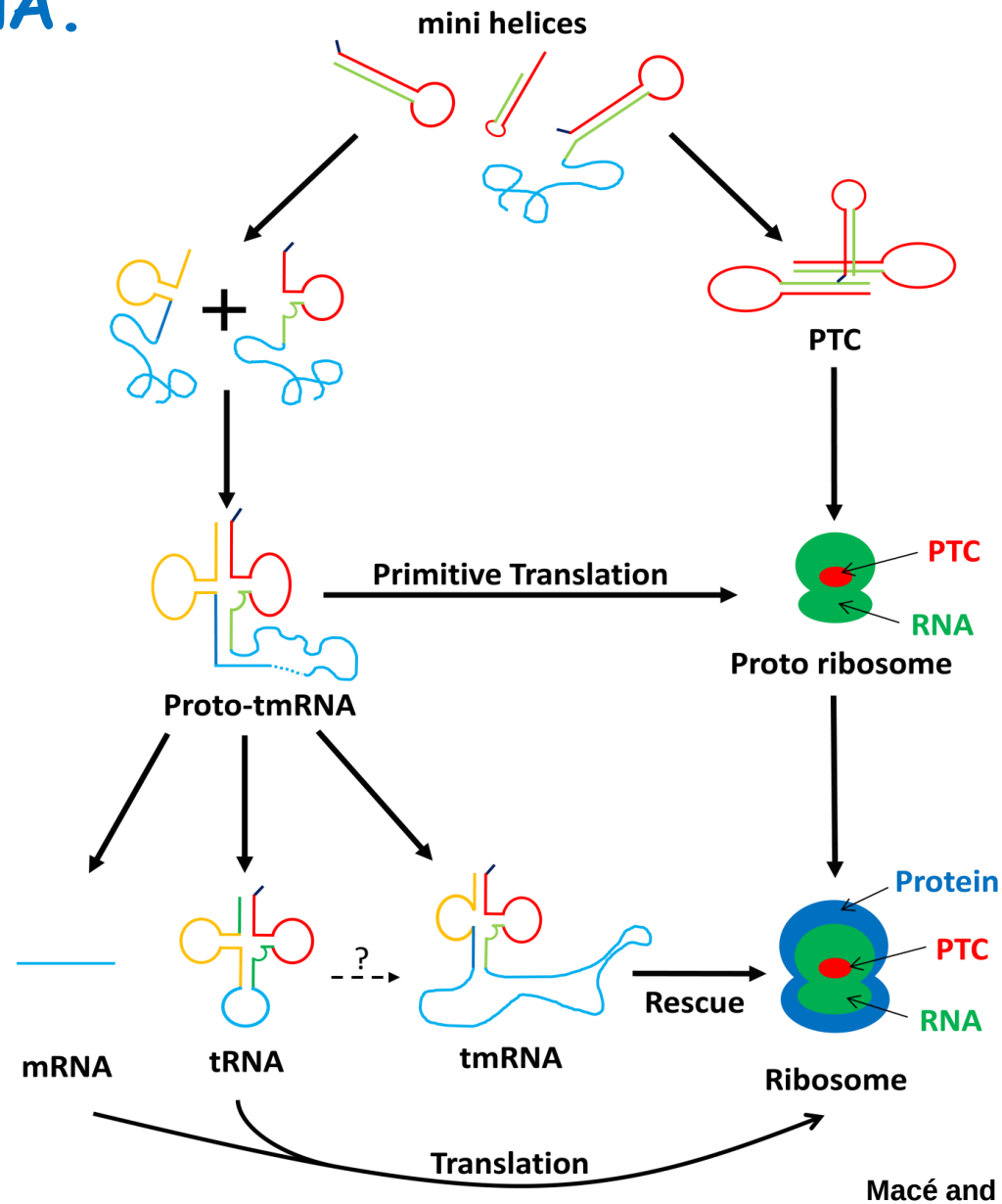
Driven by evolutionary forces, short RNAs would first have tended to pair with each other and/or fold into minihelices, forming hairpin structures.

In the second stage a primitive proto-tmRNA carrying a tRNA acceptor stem with a large intron was obtained by fusing two separate hairpin RNA.

These proto-tmRNAs might have rapidly interacted with the first forms of the early peptidyl transferase center (PTC) while the first genetic code was evolving.

We can assume that the proto-PTC might have emerged in the same way, by self-folding and dimerization of RNA chains, thus providing the first framework for stereochemistry favoring for peptide bond formation and substrate-mediated catalysis

In a subsequent stage, proto-tmRNAs eventually gave birth to tRNA and mRNA,



Macé and Gillet, NAR 2016

Institut
Génétique
et Développement
de Rennes

Université Rennes1,
UMR CNRS 6290

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