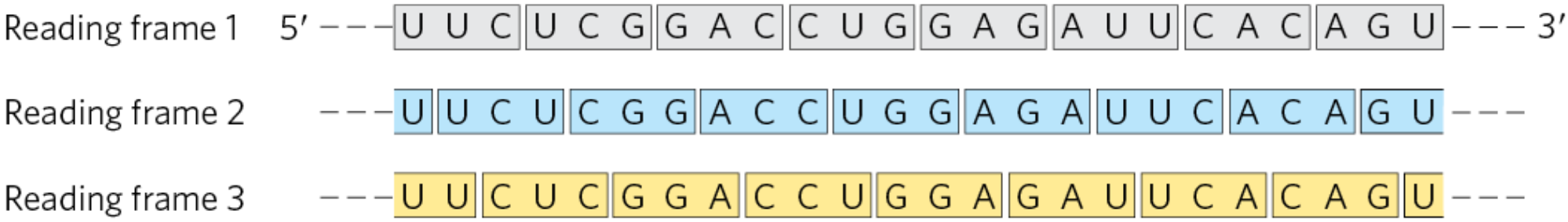
How to encode 20 amino acids from 4 nucleotides?

2 nucleotide code yields $4^2 = 16$ different combinations

3 nucleotide code yields $4^3 = 64$ different combinations



The Genetic Code

(almost)
•Universal

The same genetic code is used in bacterial, plants, fungal or animals genomes (exceptions)

•Degenerate

Multiple sequences code for the same AA

•Non-random

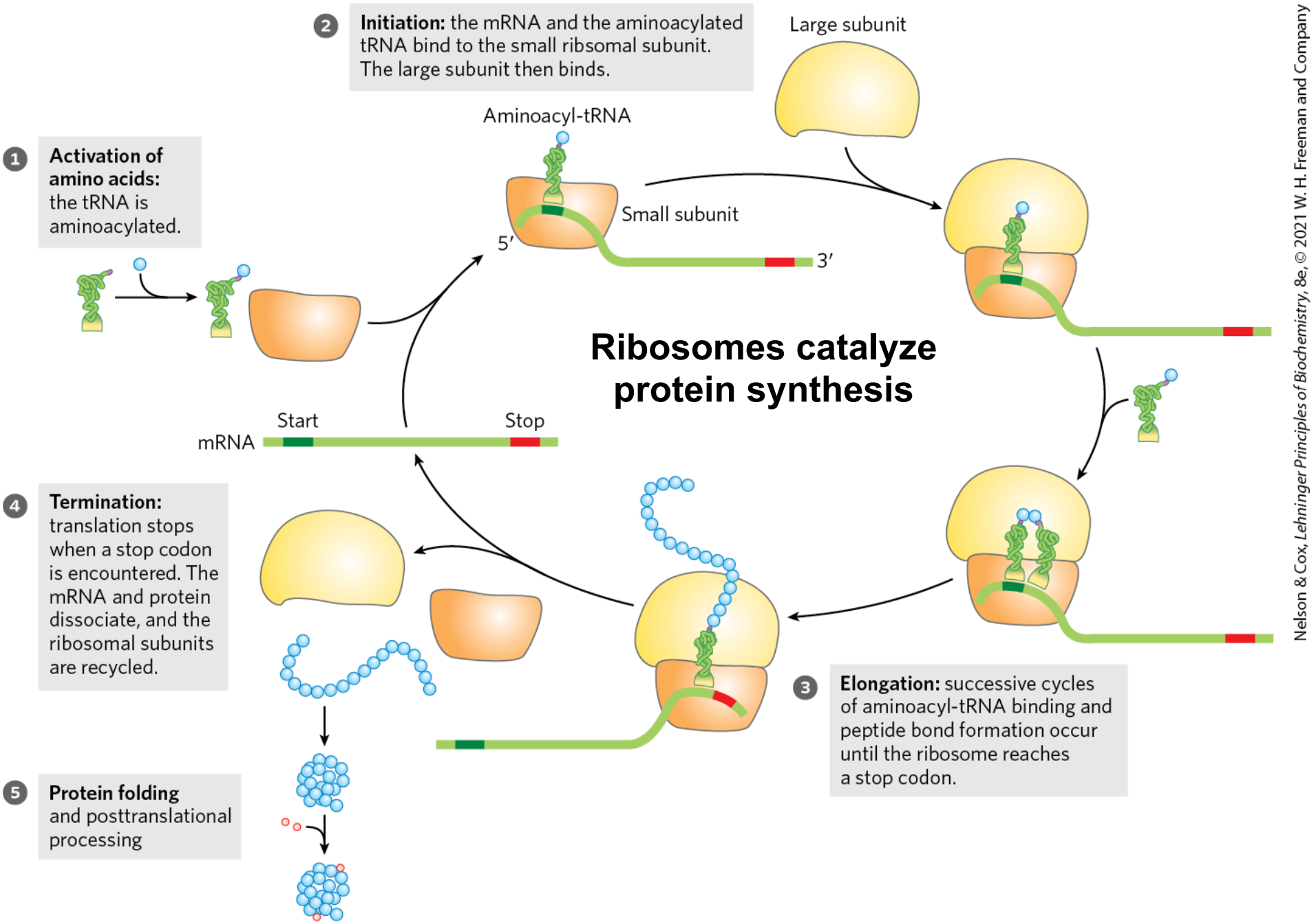
Single mutation of a codon is typically **neutral** (=> same AA) or **conservative** (results in a similar AA)

First position (5' end)	Second position U	Second position C	Second position A	Second position G	Third position (3' end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met[b]	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

[a]Nonpolar amino acid residues are tan, basic residues are blue, acidic residues are red, and polar uncharged residues are purple.

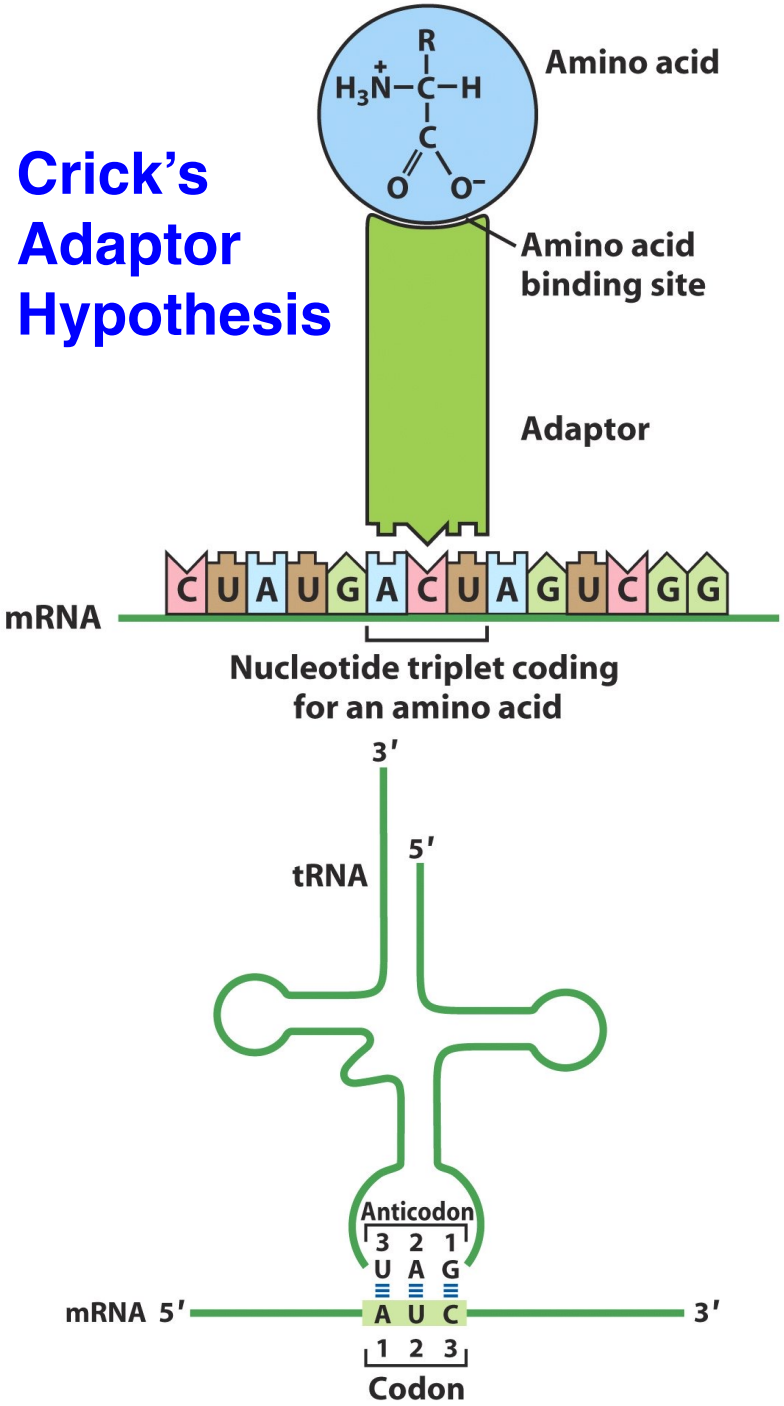
[b]AUG forms part of the initiation signal as well as coding for internal Met residues.

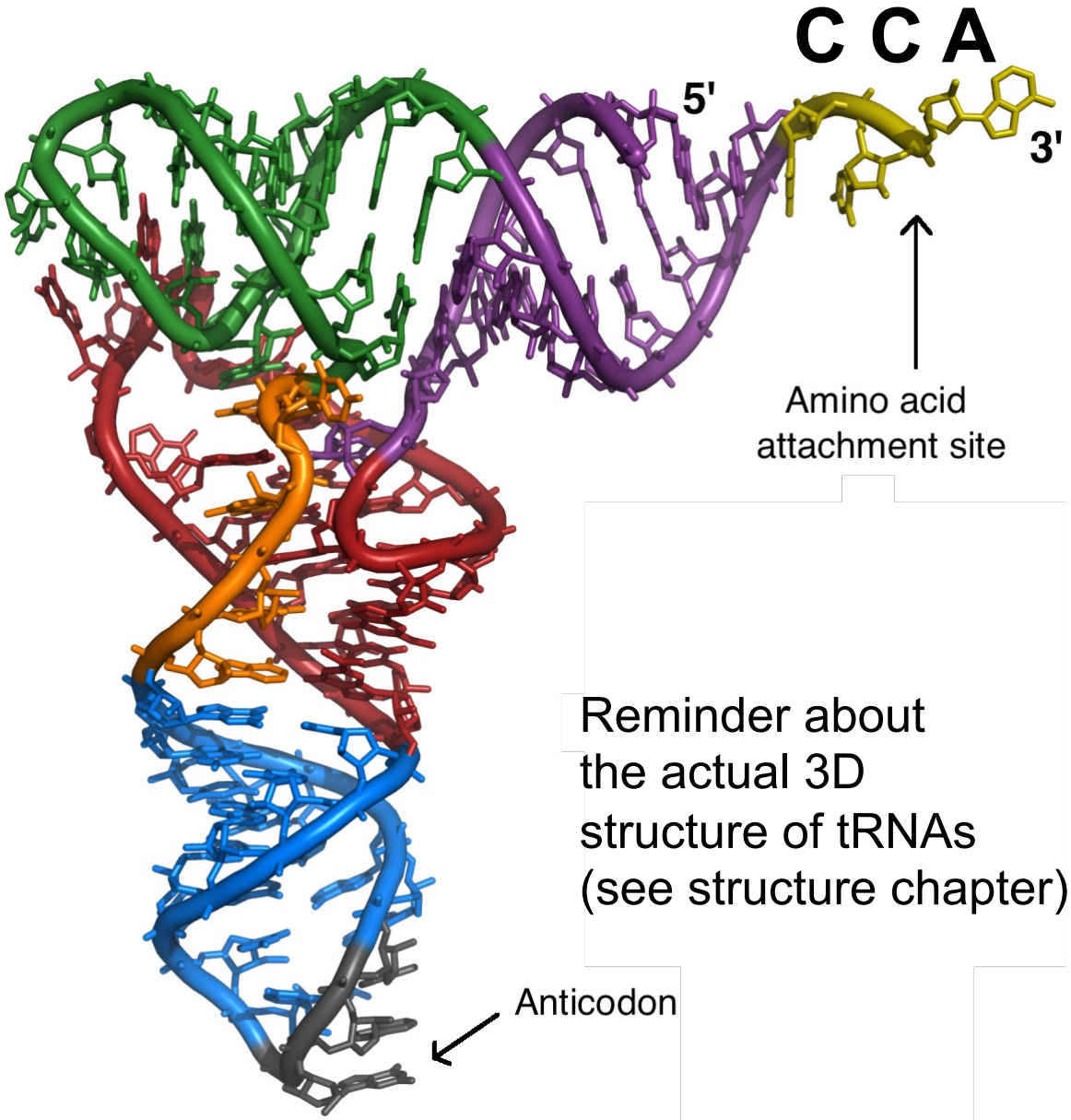
An overview of protein synthesis



tRNAs are adaptor molecules

Crick's Adaptor Hypothesis



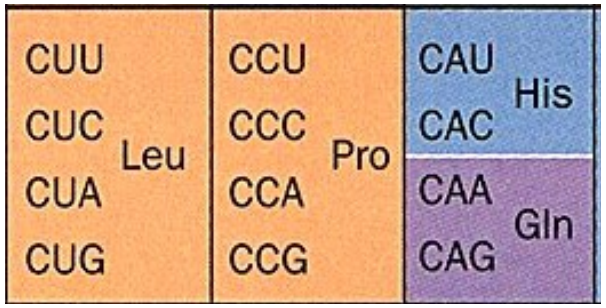


Reminder about the actual 3D structure of tRNAs (see structure chapter)

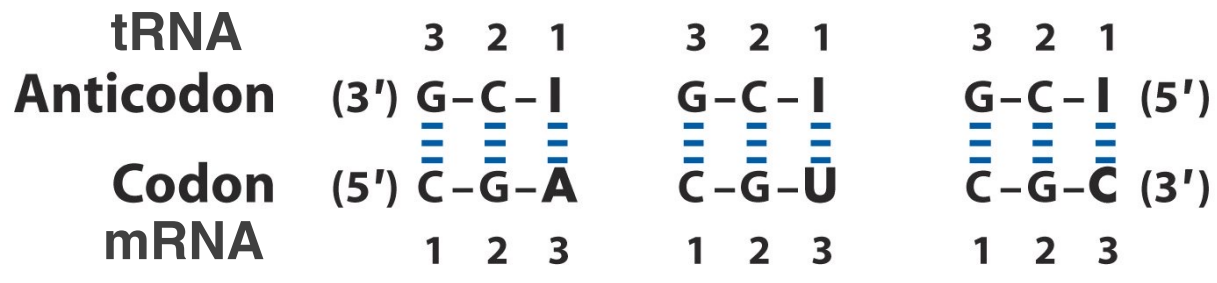
Khan academy

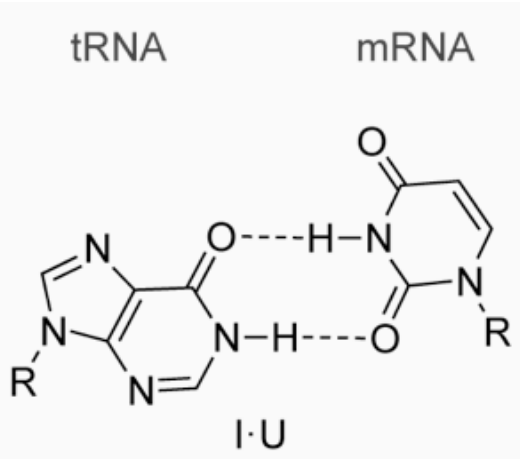
One tRNA can recognize several codons by flexibility of base pairing ("wobble") at position 3 of the mRNA codon

AntiCodon (1) (=tRNA)		Codon (3) (=mRNA)
C	can read	G
A	can read	U
U	can read	A,G
G	can read	C,U
I	can read	C,U,A

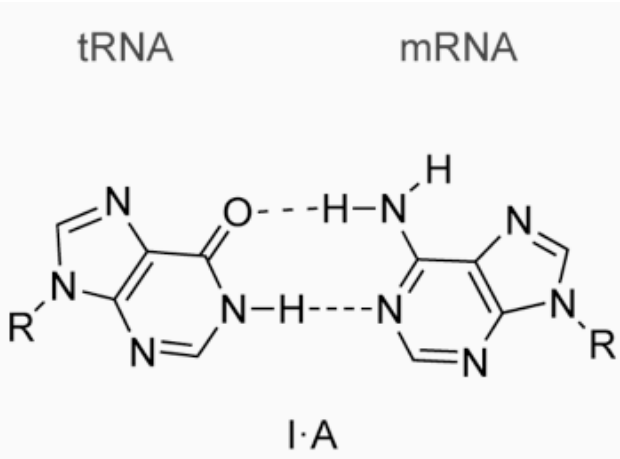


! The wobble rule only applies to position 3 of the codon NOT to Positions 1 and 2

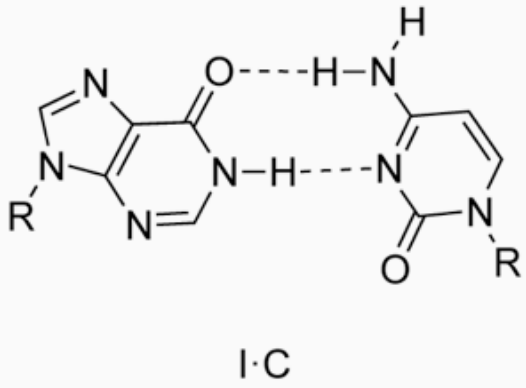




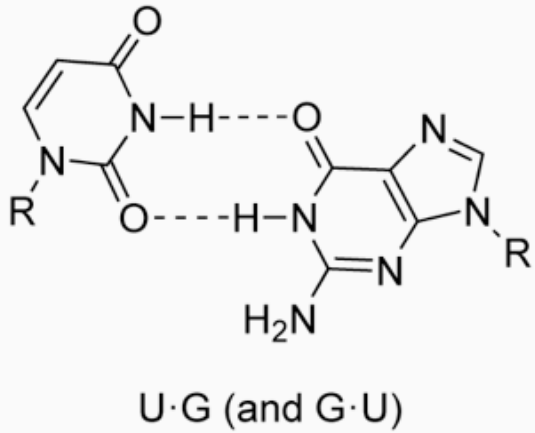
I·U



I·A



I·C



U·G (and G·U)

A tRNA has the anticodon 5’-GAA-3’. Which codons could it potentially recognize?

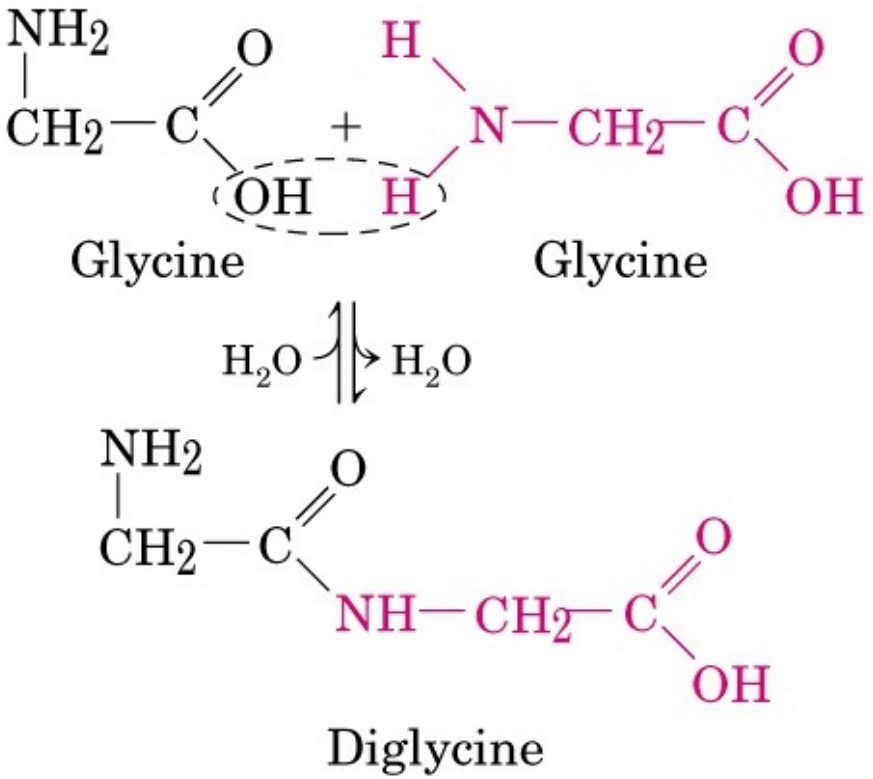
A: UUC only

B: UUU and UUC

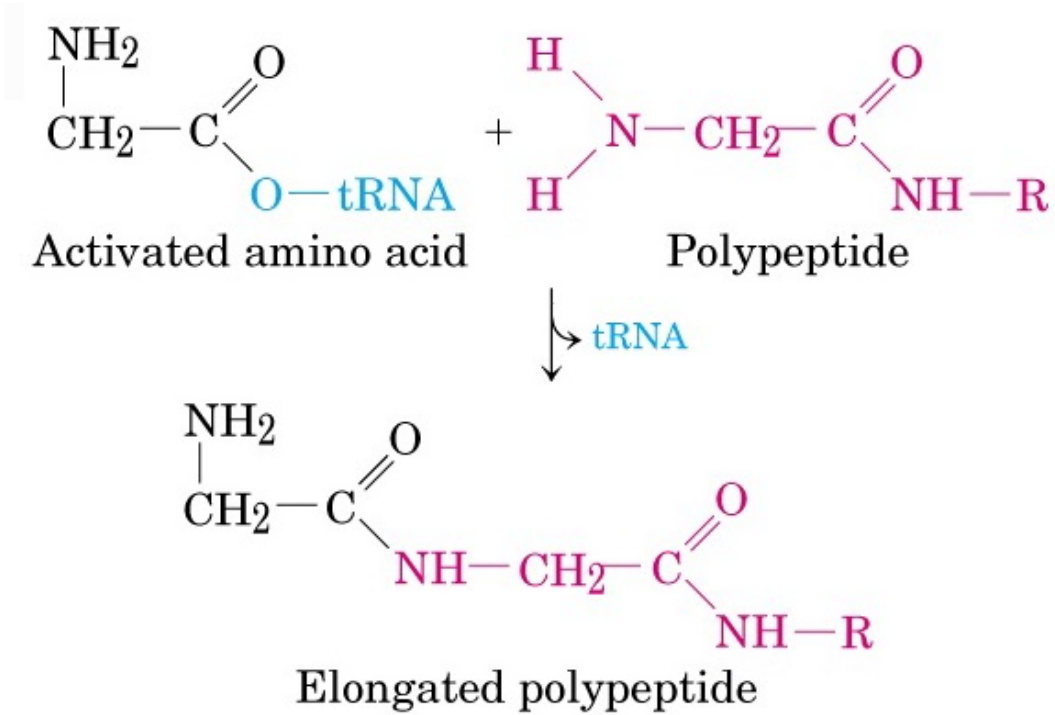
C: UUU, UUC, and UUA

D: UUC and UUG

Biological Peptide Formation is NOT a condensation reaction

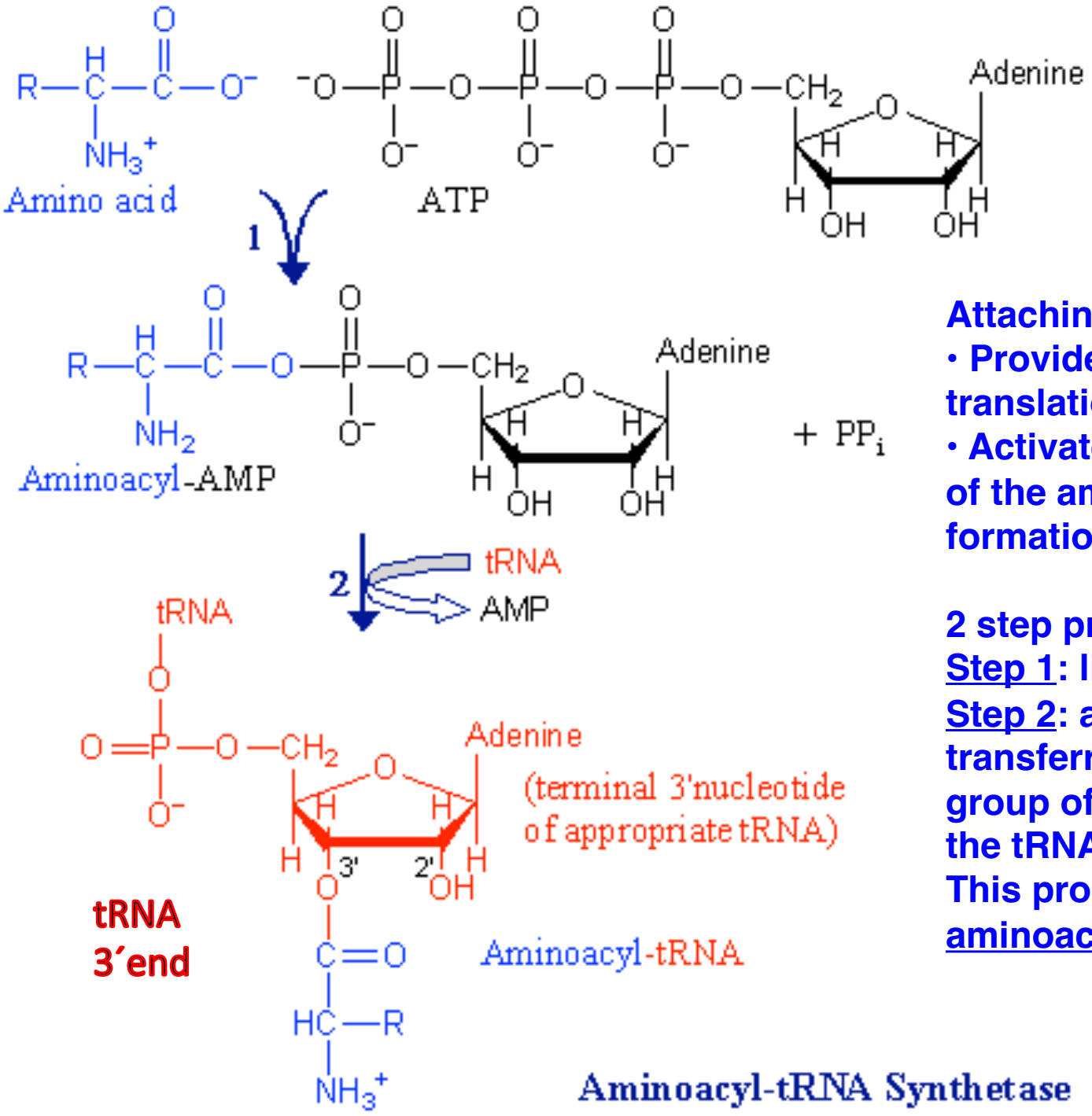


Condensation



Biological Peptide Bond Formation

Aminoacylation of tRNAs



Attaching amino acids to tRNAs:

- **Provides the adaptor for translation**
- **Activates the carboxyl group of the amino acid for peptide bond formation**

2 step process:

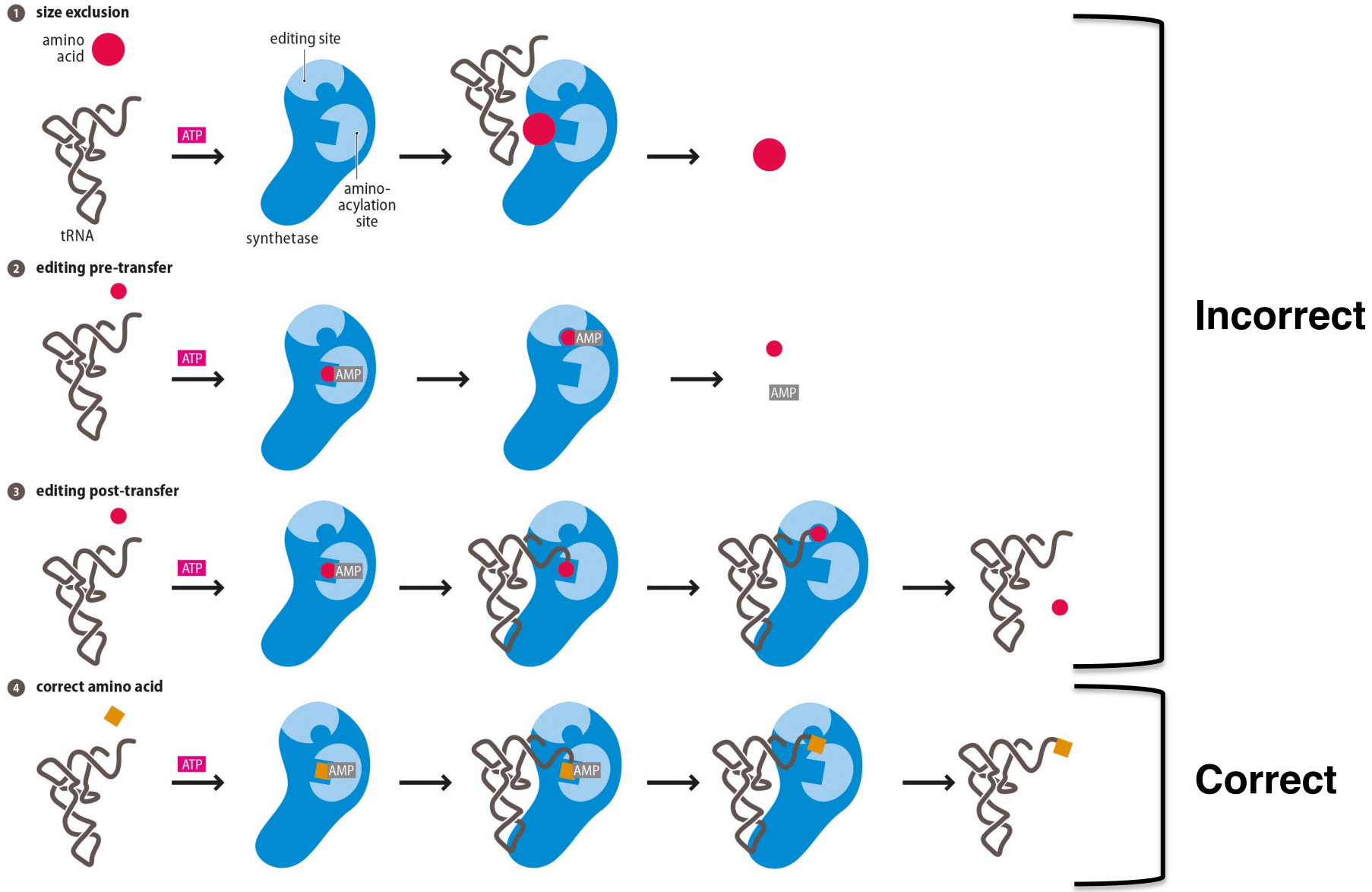
Step 1: link amino acid to AMP

Step 2: amino-acyl group is transferred to the 3' hydroxyl group of the terminal adenylate of the tRNA

This process is catalyzed by aminoacyl-tRNA synthetases

Fidelity of tRNA aminoacylation

- The ribosome is not going to check that the right AA is attached to a tRNA
- Discrimination of the "right" AA on a single step is not greater than ~1/100
- How do we get to the $1/10^4$ error rate of protein synthesis?
- Aminoacyl tRNA synthetases use multiple steps to ensure charging fidelity



Recognition of tRNAs and shuttling of the tRNA 3´-end between catalytic and editing domains provides a mechanism for proofreading

Catalytic Domain

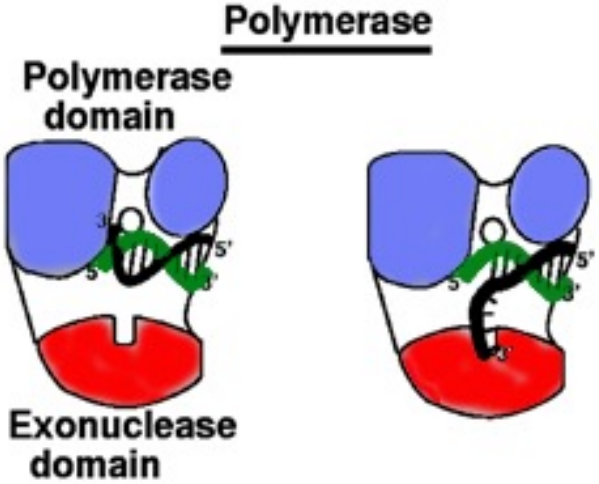
Editing Domain

Anticodon Binding Domain (important for charging specificity)

tRNA Substrate

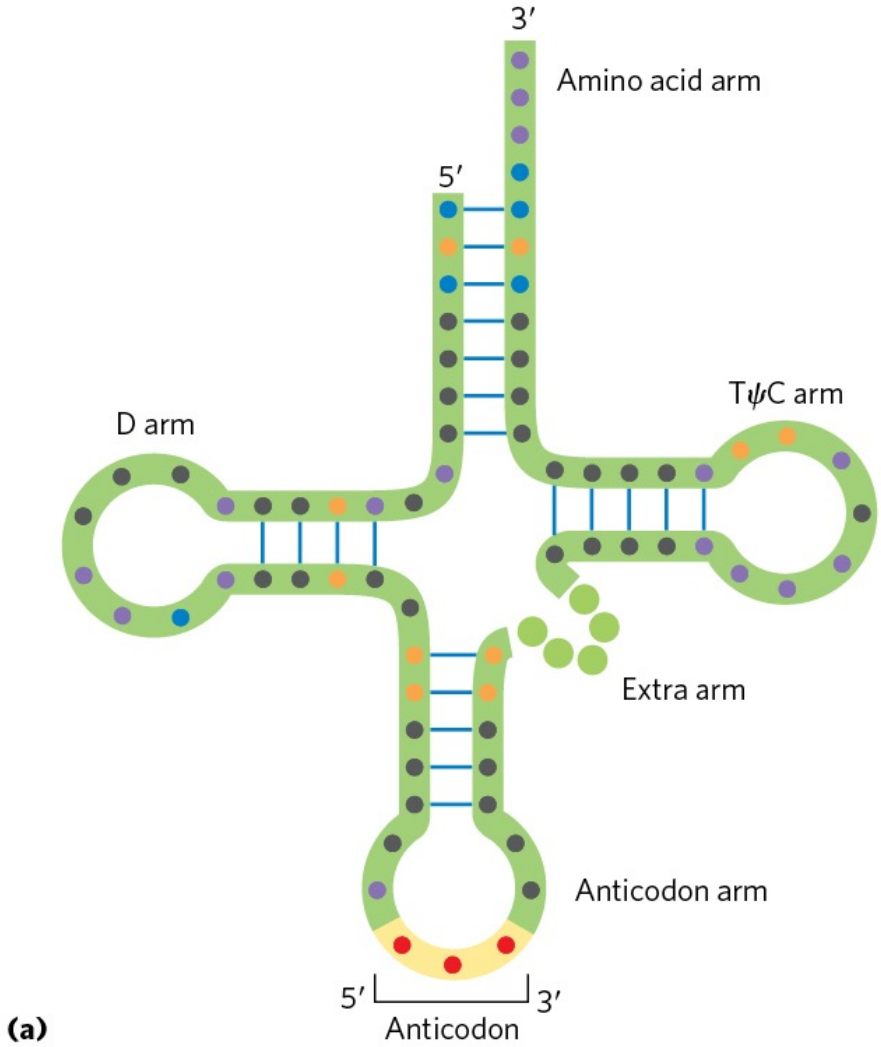
Dock-Bregeon et al. Mol. Cell 2004

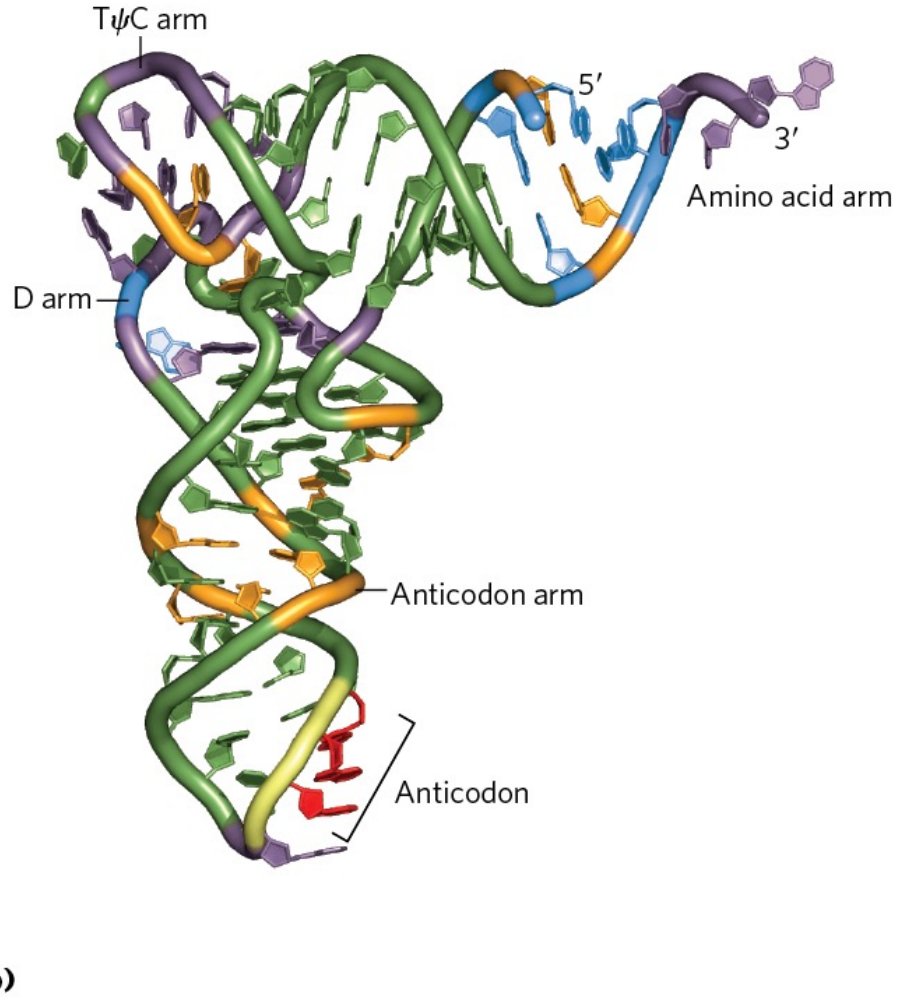
Functional similarities for editing in DNA Polymerases and tRNA synthetases



Synthetic mode --> Editing mode

Aminoacyl-tRNA synthetases must also be specific for the right tRNA

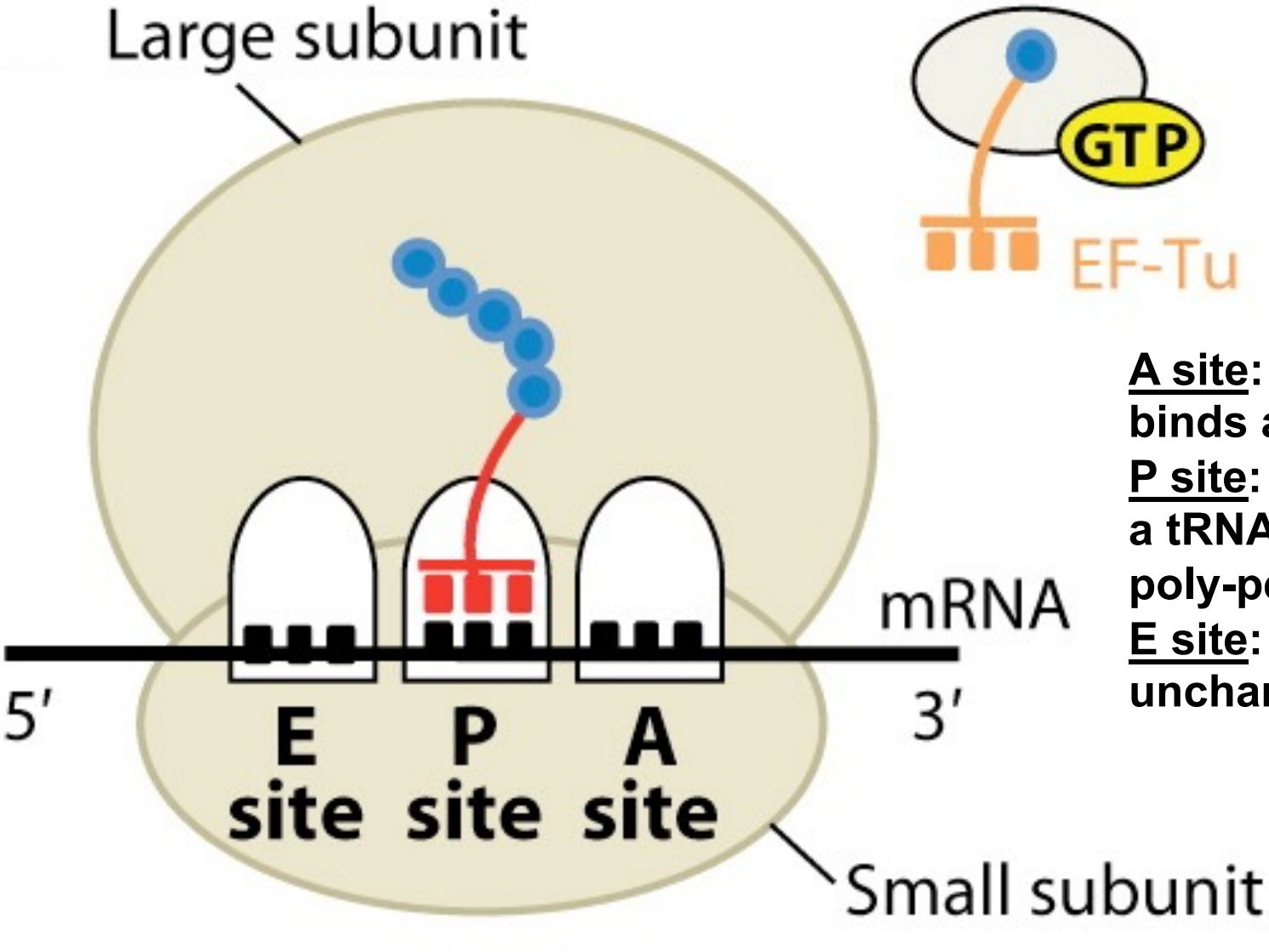




In addition to the anticodon sequence, orange and blue positions (above) are recognized by aminoacyl-tRNA synthetases

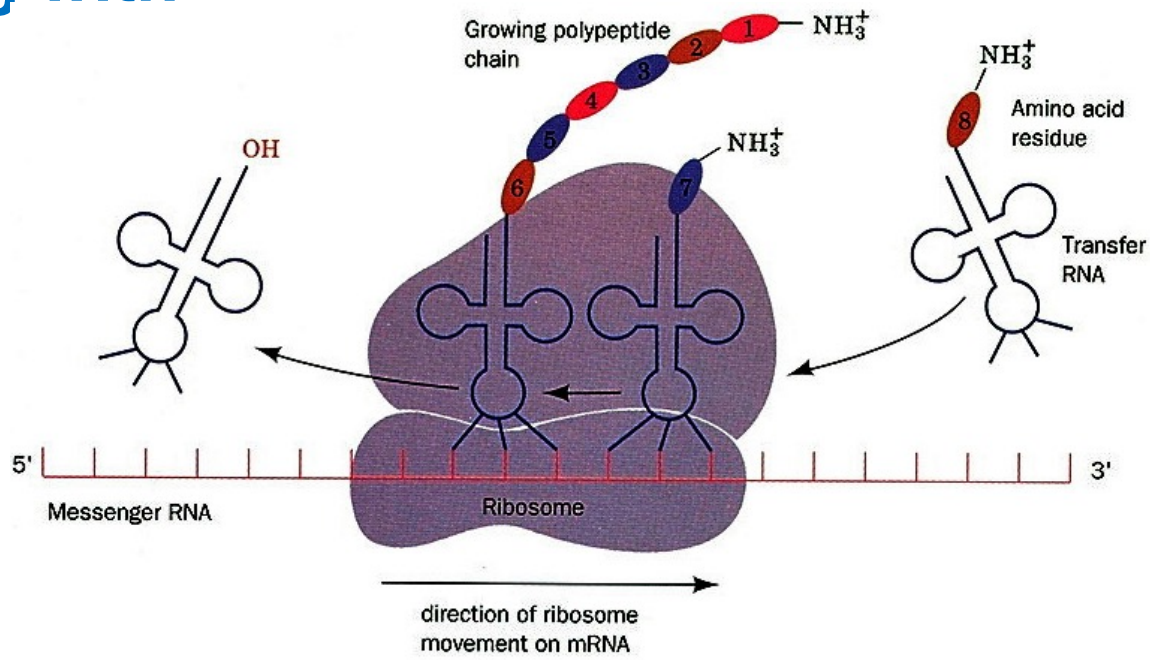
The Ribosome – simple version

tRNAs are loaded in the ribosome A-site by elongation Factor EF-Tu



A site: aminoacyl site, binds aminoacyl-tRNA
P site: peptidyl site, binds a tRNA connected to a poly-peptide chain
E site: exit site, binds uncharged tRNAs

What is wrong with this picture ?



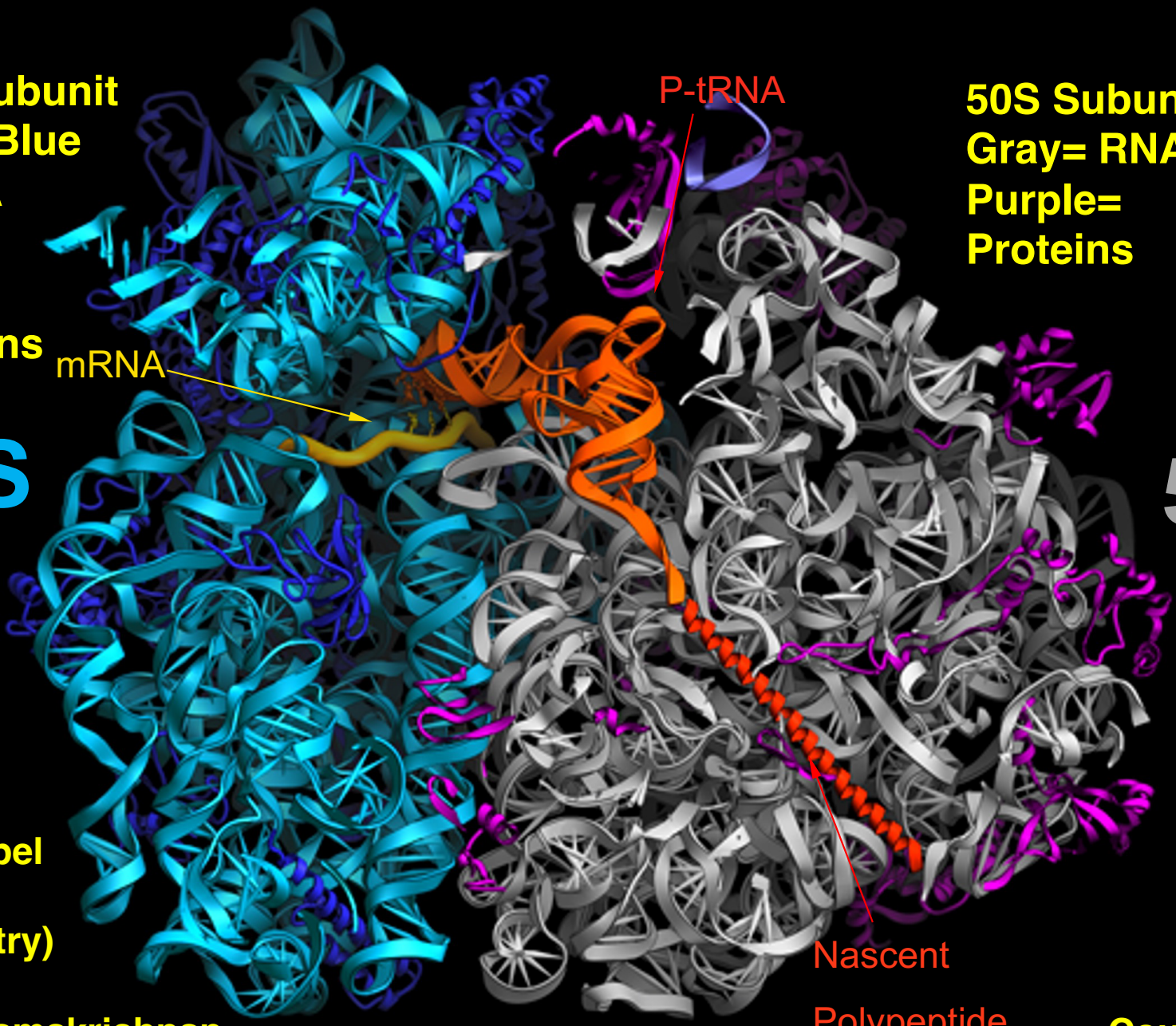
A: There is no Exit site shown on the ribosome

B: tRNAs are shown entering into the P site, not the A site

C: tRNAs form 5´-3´/5´-3´ anticodon/codon interactions

D: The ribosome moves in the wrong direction

3D structure of a bacterial Ribosome



Ribosome subunits functions

Small Subunit

Bacteria: 30S

16S rRNA

21 proteins

Eukaryotes: 40S

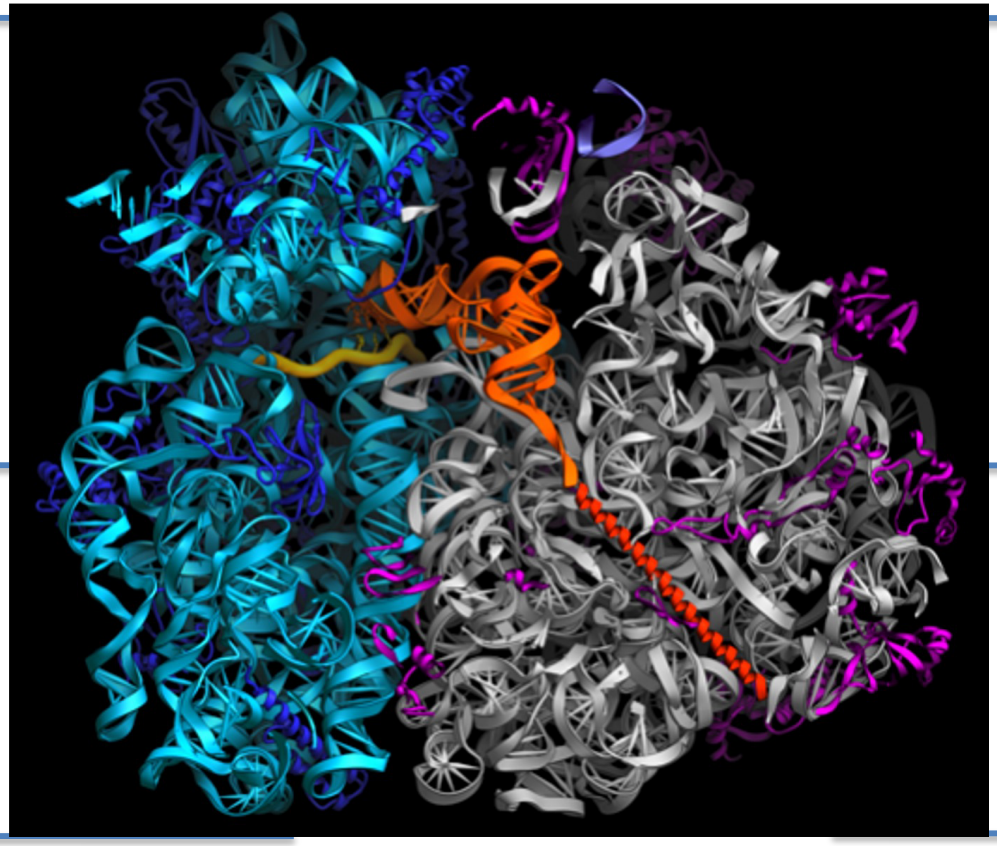
18S rRNA

33 proteins

Functions

mRNA binding

Codon/Anticodon interaction



Large Subunit

Bacteria: 50S

23S, 5S rRNAs

31 proteins

Eukaryotes: 60S

28S, 5S, 5.8 S rRNAs

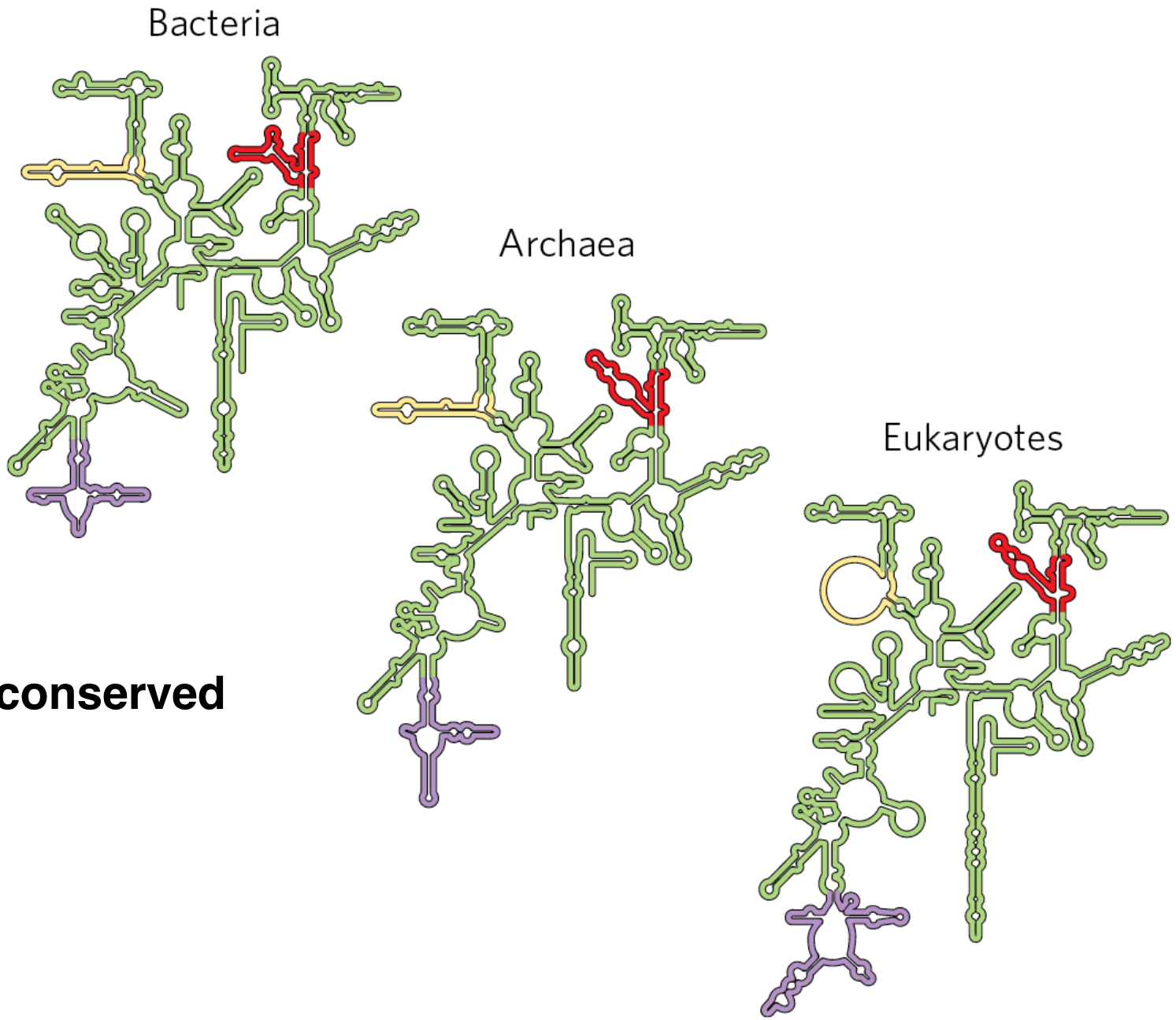
49 proteins

Functions

Peptidyl transferase

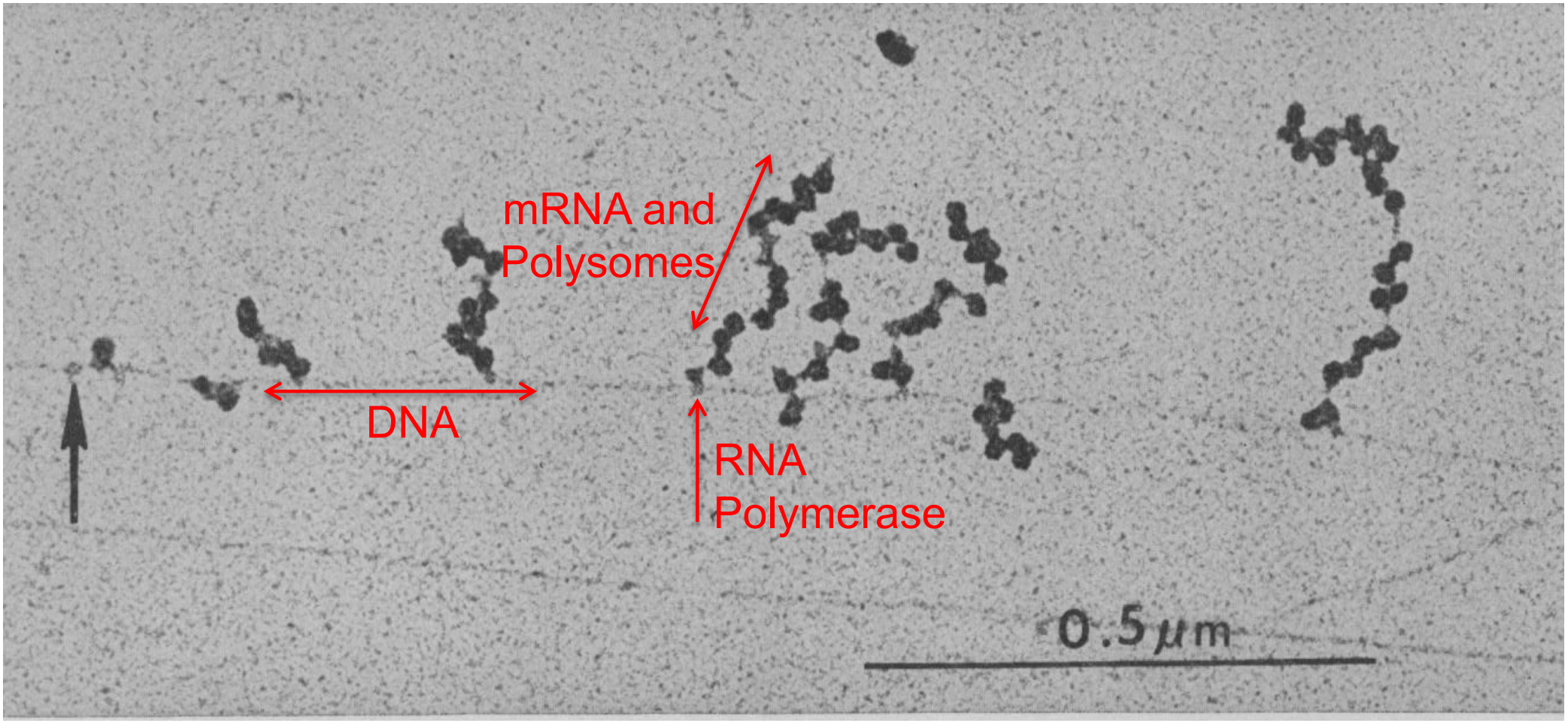
Exit tunnel for proteins

Conservation of rRNA secondary structure across 3 domains of life



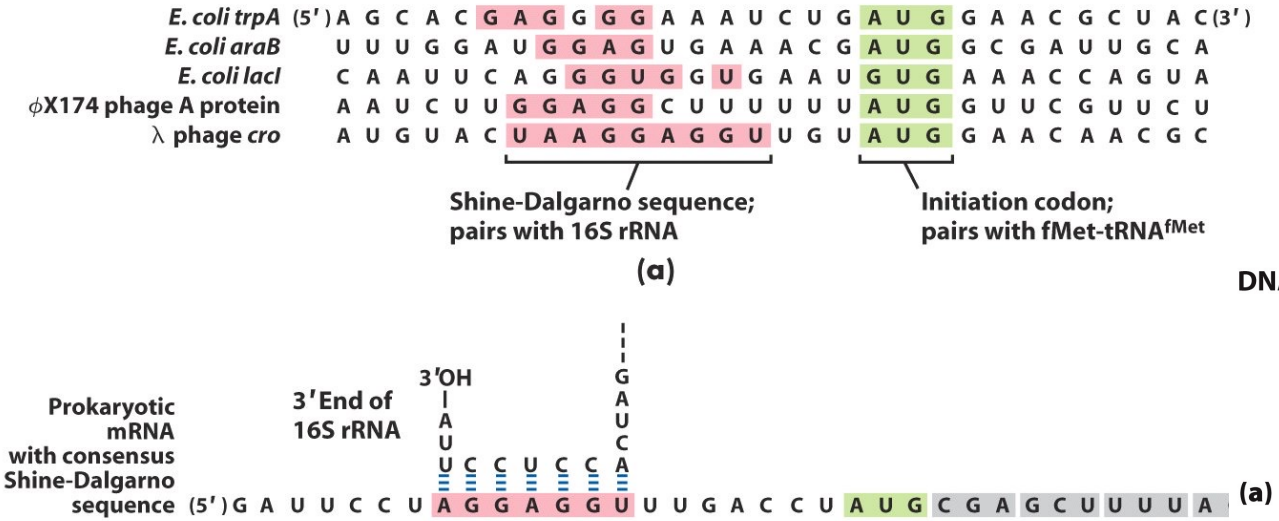
Green: conserved regions

Transcription and translation are coupled in bacteria

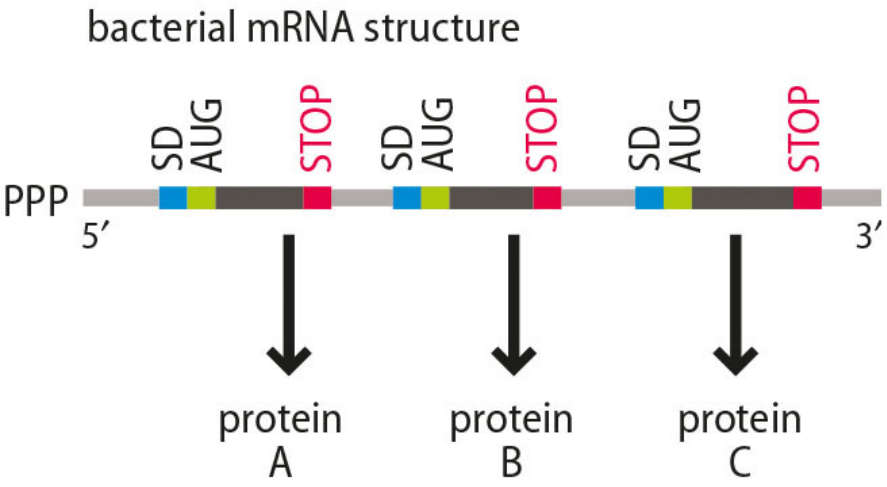


Miller et al., Science 1970

Bacterial translation initiation



Translation of a polycistronic mRNA with multiple ORFs in bacteria



Translation immediately follows transcription in bacteria (no nuclear/cytoplasmic compartmentalization)

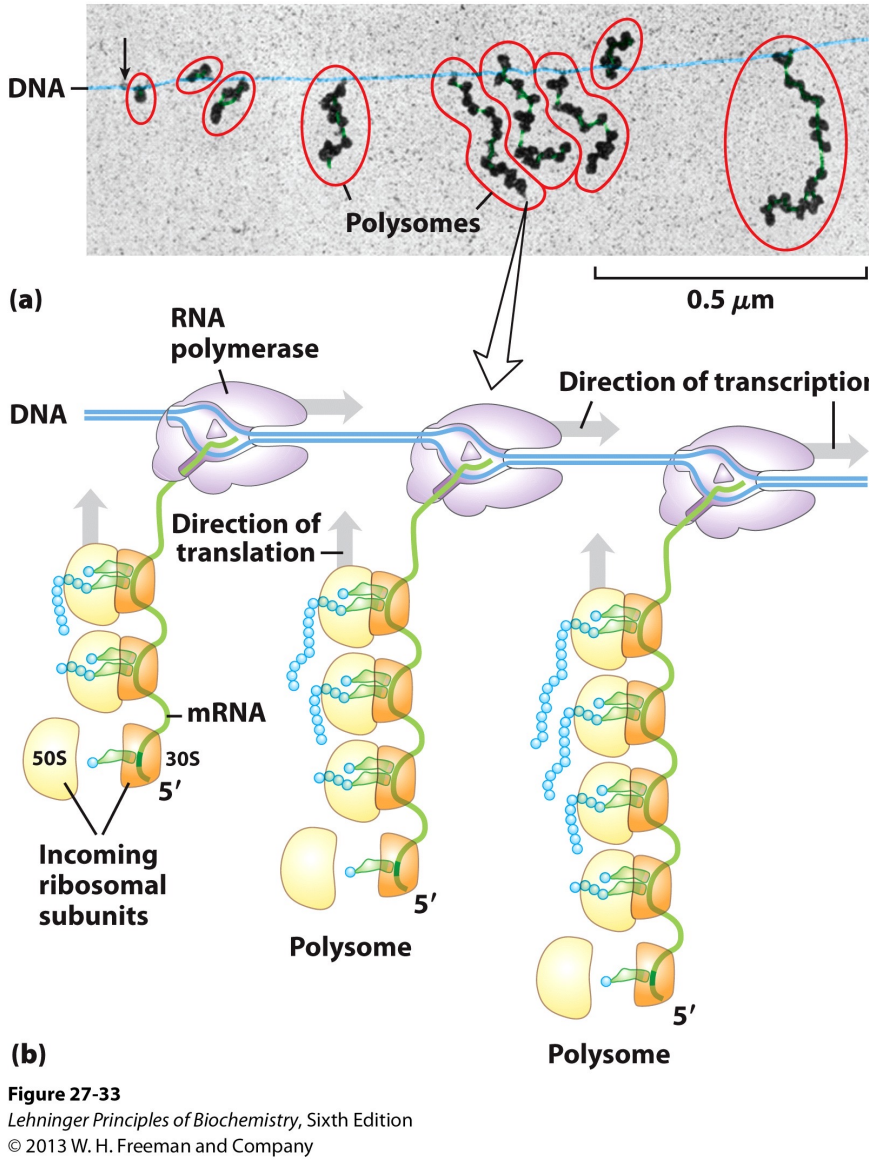
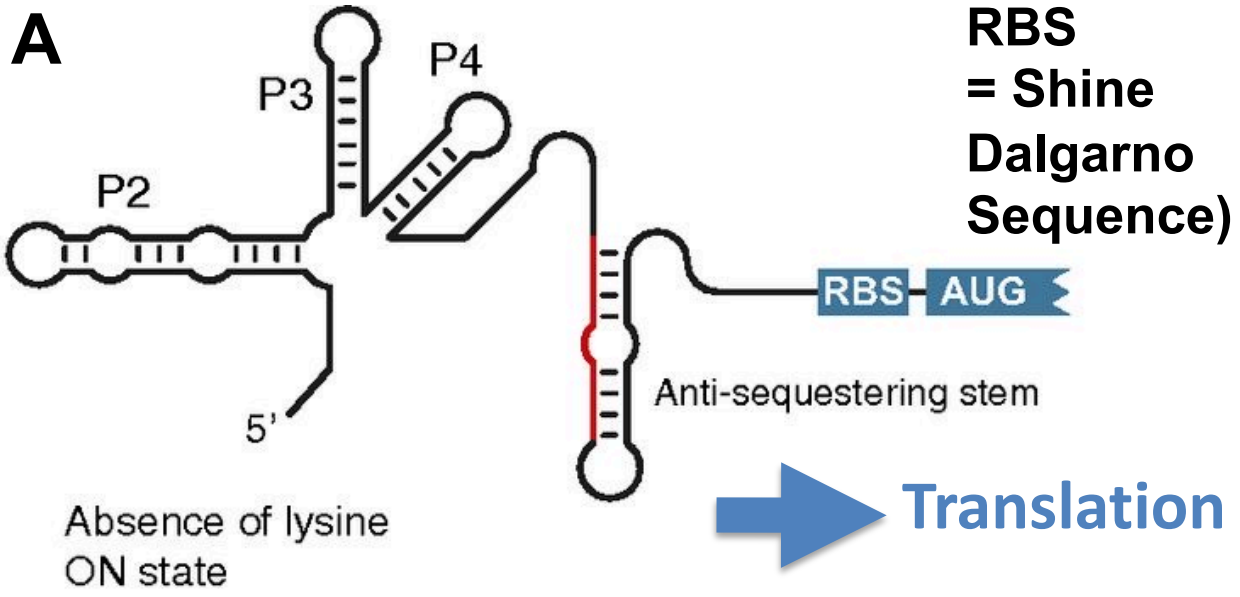


Figure 27-33
Lehninger Principles of Biochemistry, Sixth Edition

https://www.youtube.com/watch?v=u9dhO0iCLww

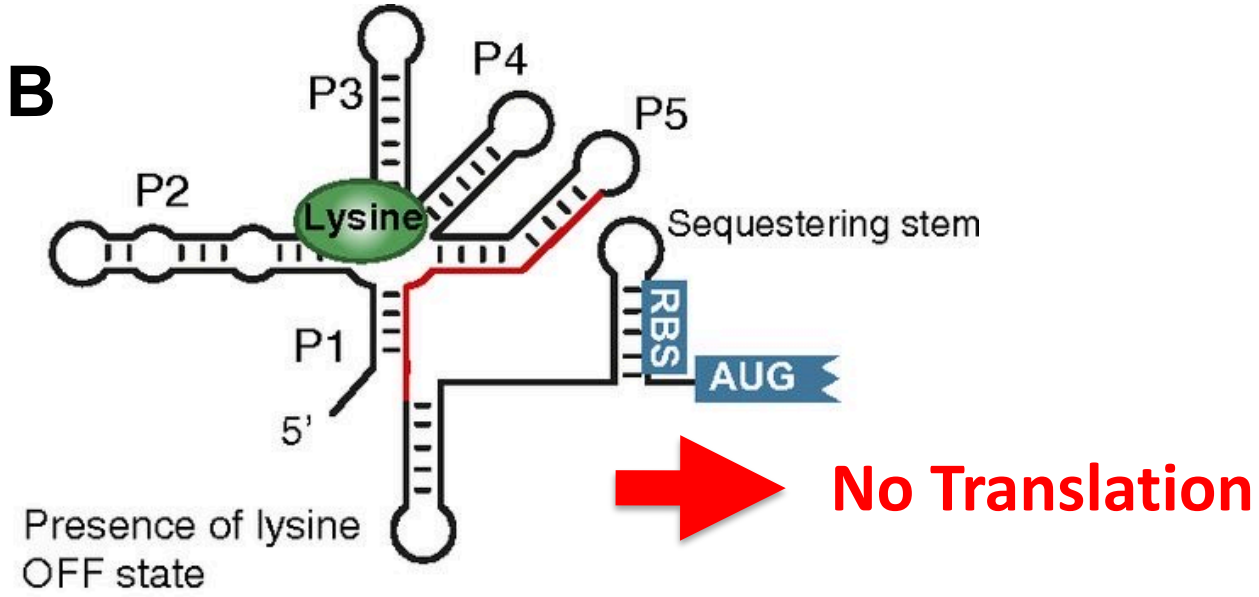
Regulation of bacterial translation initiation by RNA Structures that bind metabolites and change structure upon metabolite binding (aka Riboswitches): example of a Lysine-responsive riboswitch

A – Low cellular Lysine levels -> Translation of Lysine metabolic genes because the RBS is available



RBS = Shine Dalgarno Sequence)

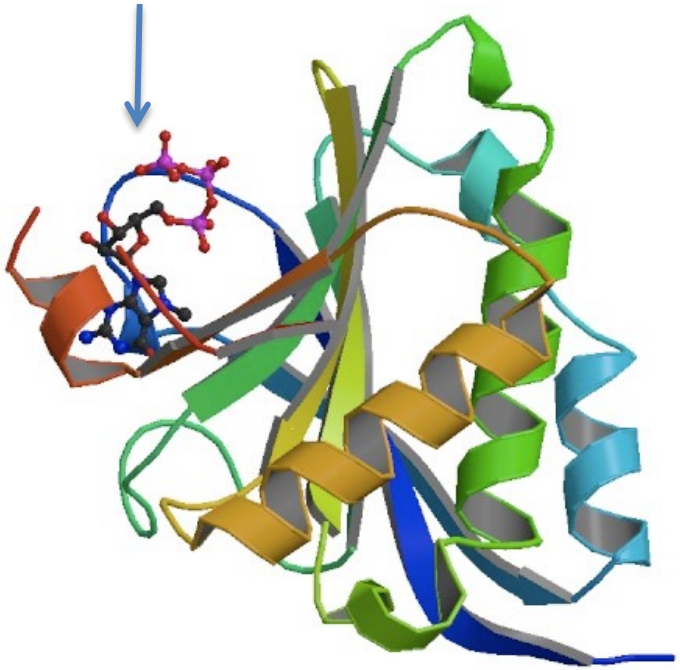
B – High cellular Lysine levels -> Repression of translation of Lysine Metabolic Genes because the RBS is sequestered

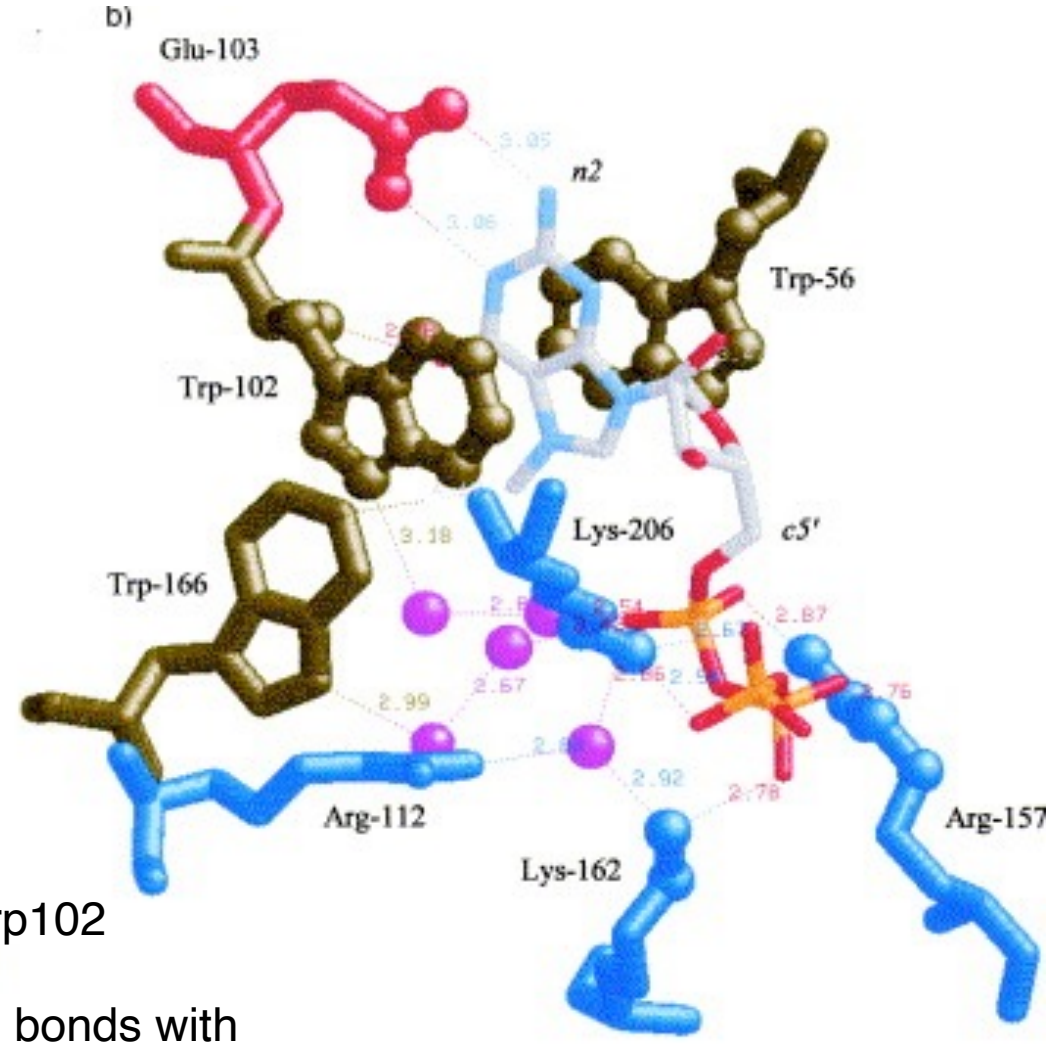


Recognition of the 5´-cap structure of eukaryotic mRNAs by eIF4e

Cap binding pocket is on the concave surface of the protein – allows easy entry of the mRNA 5´-end

PDB ID = 1L8B
Niedzwiecka et al. J.Mol.Biol. 2002





= H2O

- base sandwich-stacking between Trp56 and Trp102
- formation of three Watson–Crick-like hydrogen bonds with Glu103 and backbone NH of Trp102
- Hydrophobic interaction of the 7-methyl group with Trp166

PyMol: eIF4e_Cap_Complex.pse