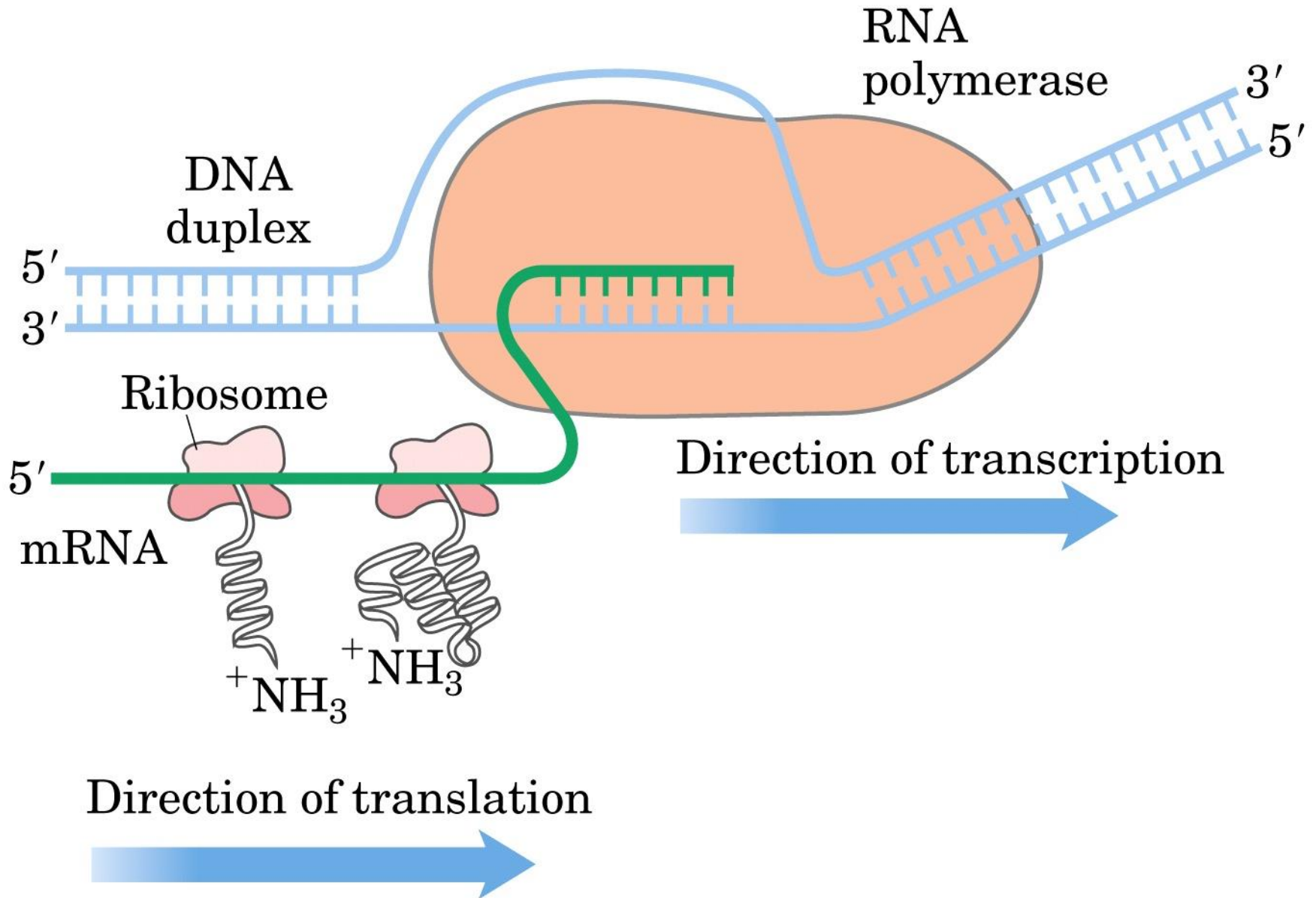


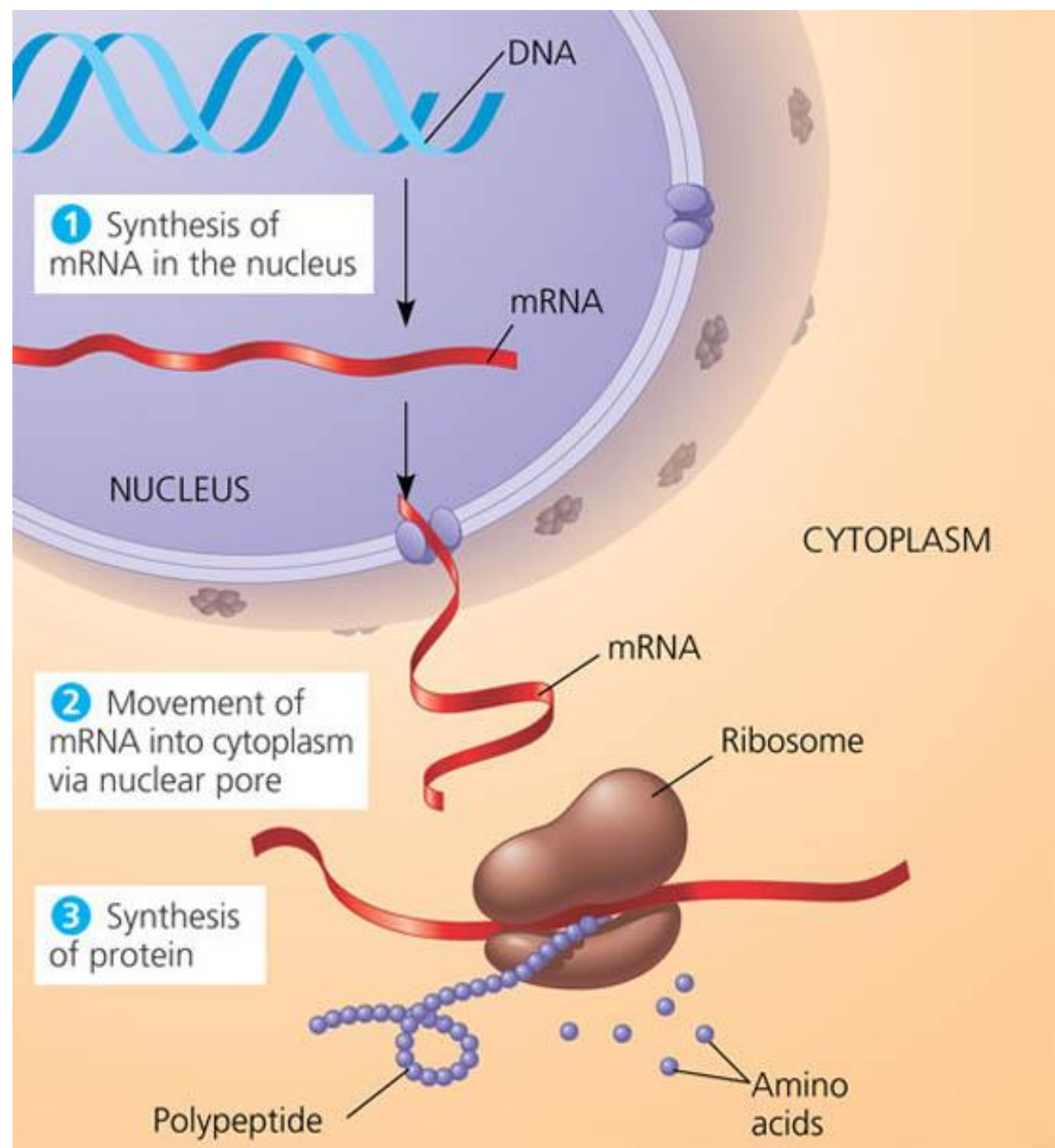
- PROTEIN TRANSLATION –

Dr. Rula Abdul-Ghani

Coupling of Transcription and Translation in prokaryotes



Eukaryotes:



The Genetic Code :

- Triplet Codons Code for a **Single Amino Acid**

UUU = Phe

- The Code is **Commaless**

AUGUUU = Met Phe

- 1 codon for Met, Trp

all other amino acids have 2-6 triplets

UCU Ser UCA Ser

UCG Ser UCC Ser

“Wobble” exists in the third position_The Wobble Hypothesis_
Wobble allows tRNA to recognize > 1 codon.

Silent mutation :

Base change results in no change in amino acid (wobble)

table 27-4

Degeneracy of the Genetic Code

Amino acid	Number of codons
Ala	4
Arg	6
Asn	2
Asp	2
Cys	2
Gln	2
Glu	2
Gly	4
His	2
Ile	3
Leu	6
Lys	2
Met	1
Phe	2
Pro	4
Ser	6
Thr	4
Trp	1
Tyr	2
Val	4

UUU is always Phe, not sometimes.

- Nonsense Codons are Stop Points

UAA, UAG, and UGA

- The Code is not necessarily **Universal** mtDNA different code than nuclear.
- 64 codons, 61 represent amino acids, and three are stop signals

The Genetic Code:

- Marshall Nirenberg helped crack the code:
Ribosomes + synthetic mRNA + cell extract → product
- If mRNA = UUUUUUUUUUUUUUUUU...
Product is protein with only: Phe-Phe-Phe...
So, UUU encodes phenylalanine
- If mRNA = UAUUAUAUAUAUA...
Two kinds of 3-nucleotide “words”: UAU and AUA
Product is protein with only : Tyr-Ile-Tyr-Ile...
So:
UAU encodes tyrosine and AUA encodes isoleucine

We now know the complete genetic code

- 64 “words” or codons
- 61 represent an amino acid
- More than one codon for some amino acids
- AUG is the start signal and represents methionine
- UAG, UAA and UGA are the stop signals
- Universal
- Non-overlapping
- No spaces between codons

		Second mRNA base				
		U	C	A	G	
U	UUU	UCU UCC UCA UCG Ser	UAU	UGU UGC UGA UGG Cys Trp	U	
	UUC		UAC		C	
	UUA		UAA Stop		A	
	UUG		UAG Stop		G	
C	CUU	CCU CCC CCA CCG Pro	CAU	CGU CGC CGA CGG Arg	U	
	CUC		CAC		C	
	CUA		CAA		A	
	CUG		CAG		G	
A	AUU	ACU ACC ACA ACG Thr	AAU	AGU AGC AGA AGG Ser Arg	U	
	AUC		AAC		C	
	AUA		AAA		A	
	AUG Met or start		AAG		G	
G	GUU	GCU GCC GCA GCG Ala	GAU	GGU GGC GGA GGG Gly	U	
	GUC		GAC		C	
	GUA		GAA		A	
	GUG		GAG		G	

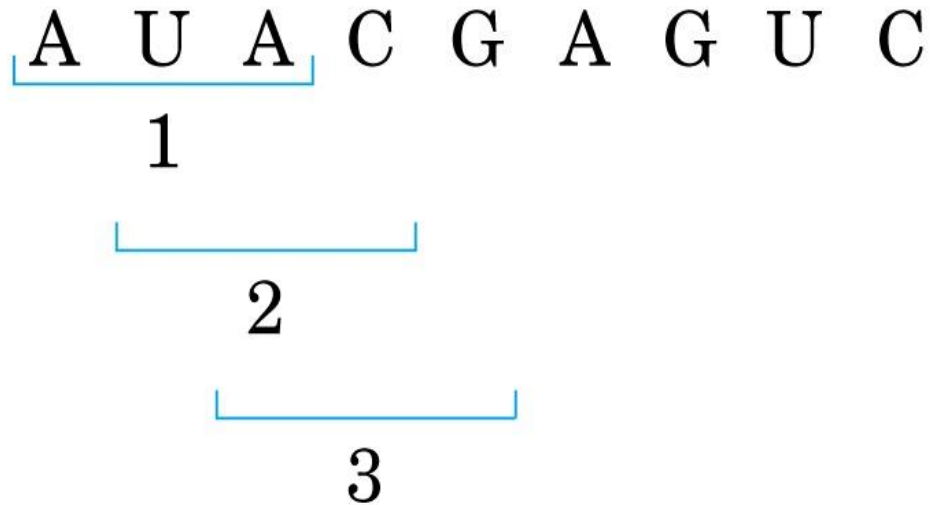
Nonoverlapping code:

codons don't share nucleotides.

Nonoverlapping
code



Overlapping
code



Reading Frame:

A specific 1st codon in the sequence establishes the reading frame.

In a triplet non overlapping genetic code all mRNAs have 3 potential reading frames.

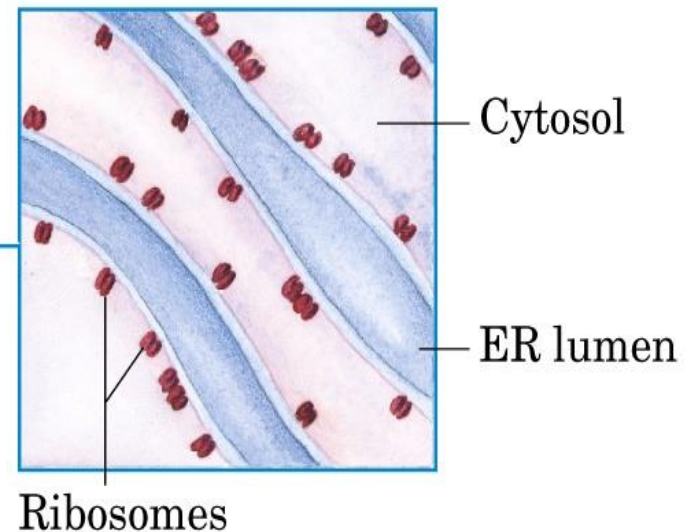
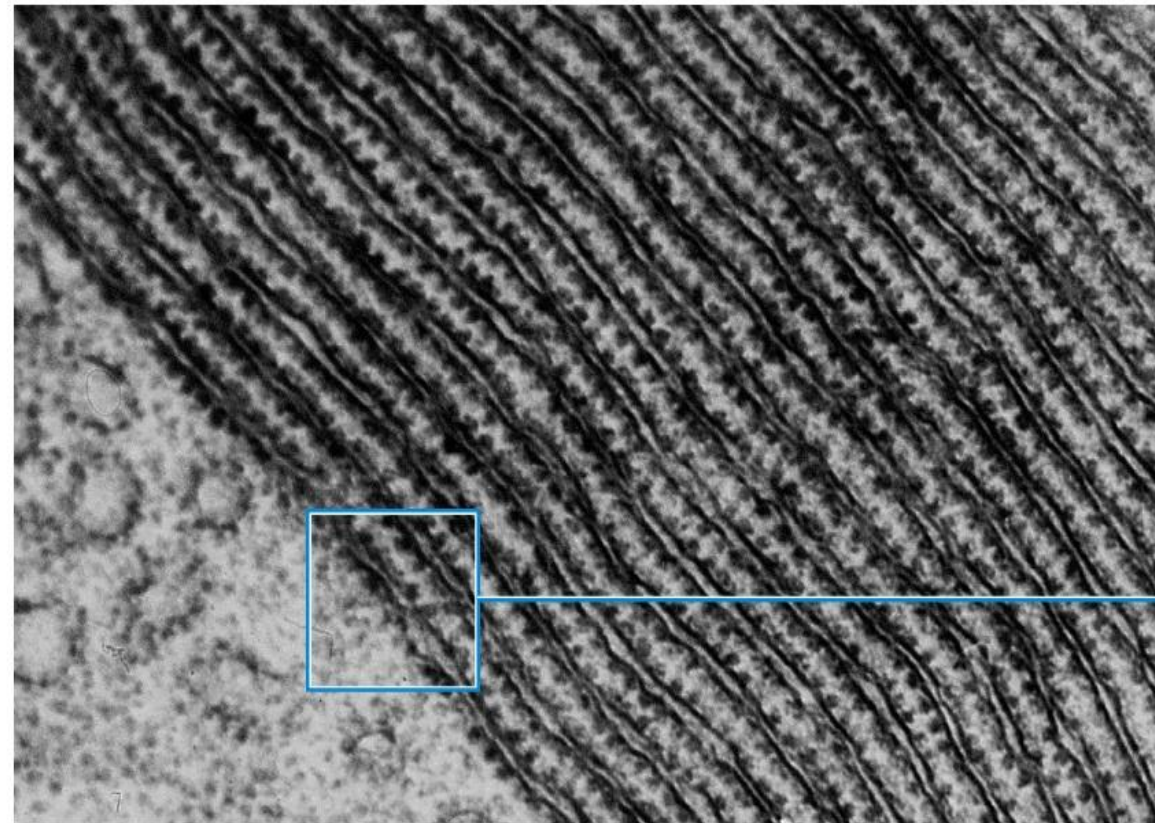


Ribosomes and endoplasmic reticulum (ER):

Two populations of ribosomes are evident in cells:

Free ribosomes mostly synthesize proteins that function in cytosol

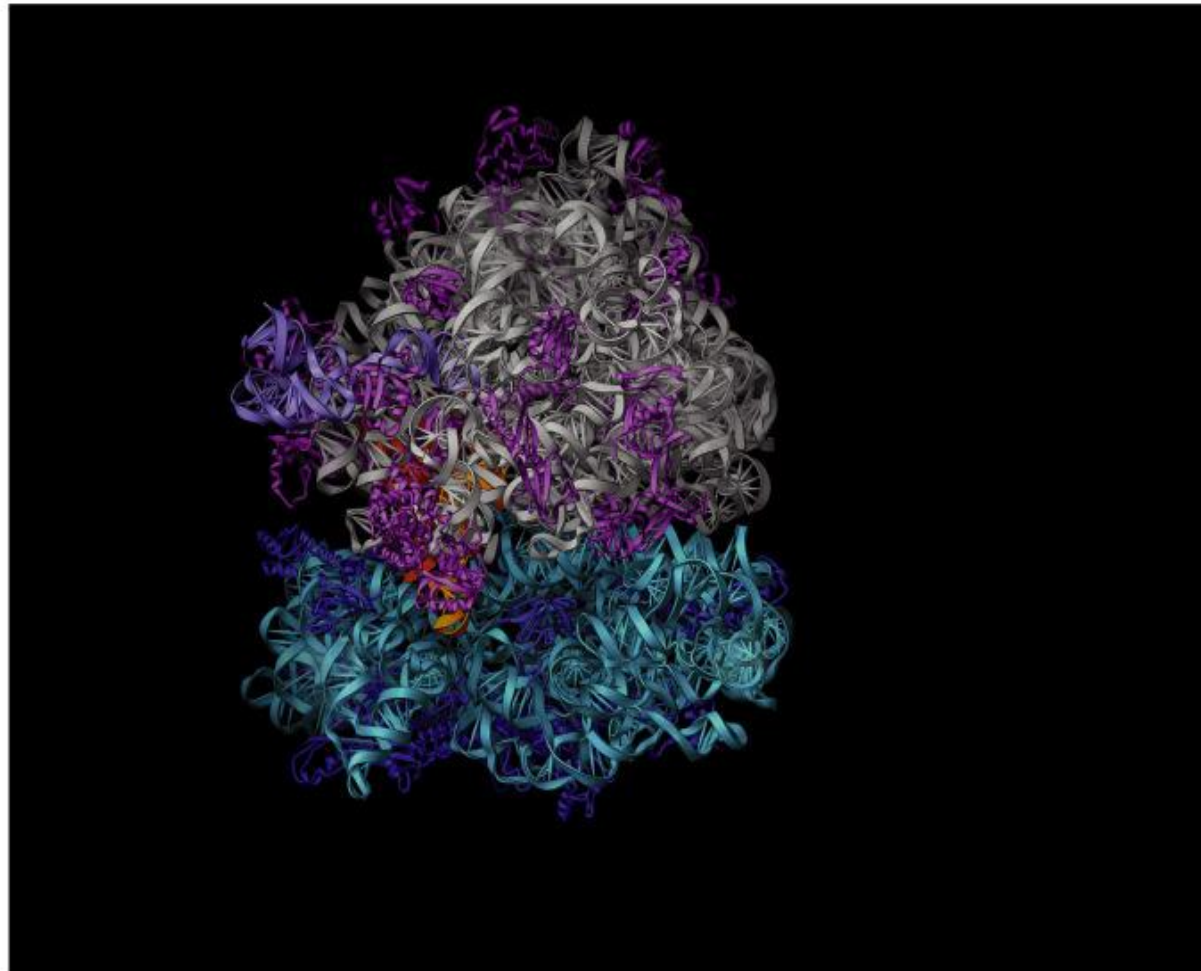
Bound ribosomes (attached to ER) make proteins that are secreted from the cell



Model of Ribosome:

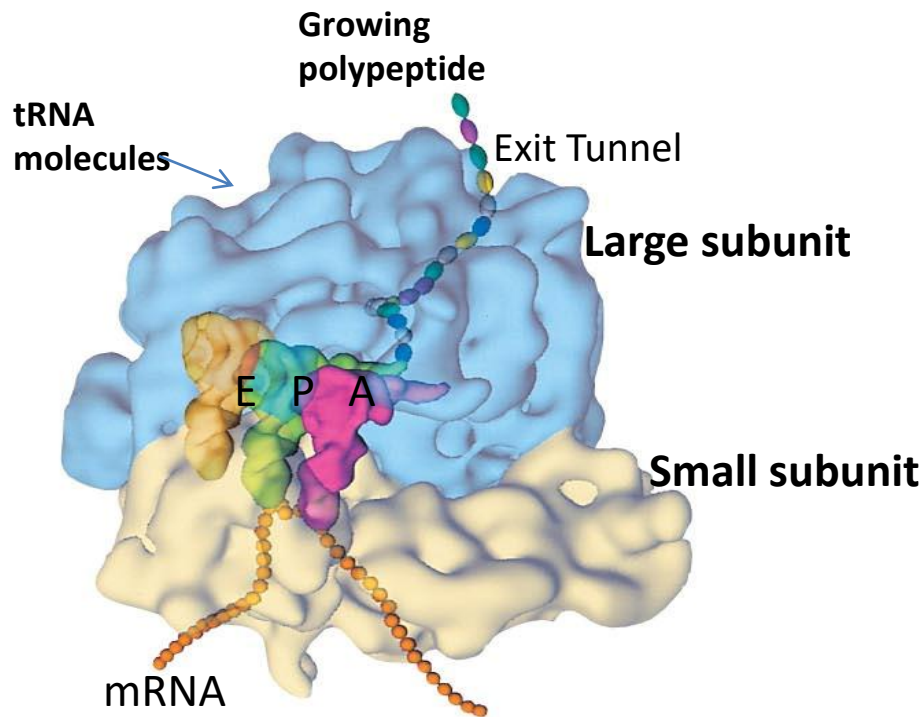
Two Subunits (large + small) composed of rRNA and Protein molecules.

Ribosomes = cellular organelle, constructed in nucleolus, function to facilitate specific coupling of mRNA codons to tRNA anticodons in protein synthesis.



A ribosome has three binding sites for tRNA:

- The **A site** (Aminoacyl site) holds the tRNA that carries the next a.a to be added to chain
- The **P site** (Peptidyl site) holds the tRNA that carries the growing polypeptide chain
- The **E site** (Exit site) where discharged tRNAs leaves the ribosome



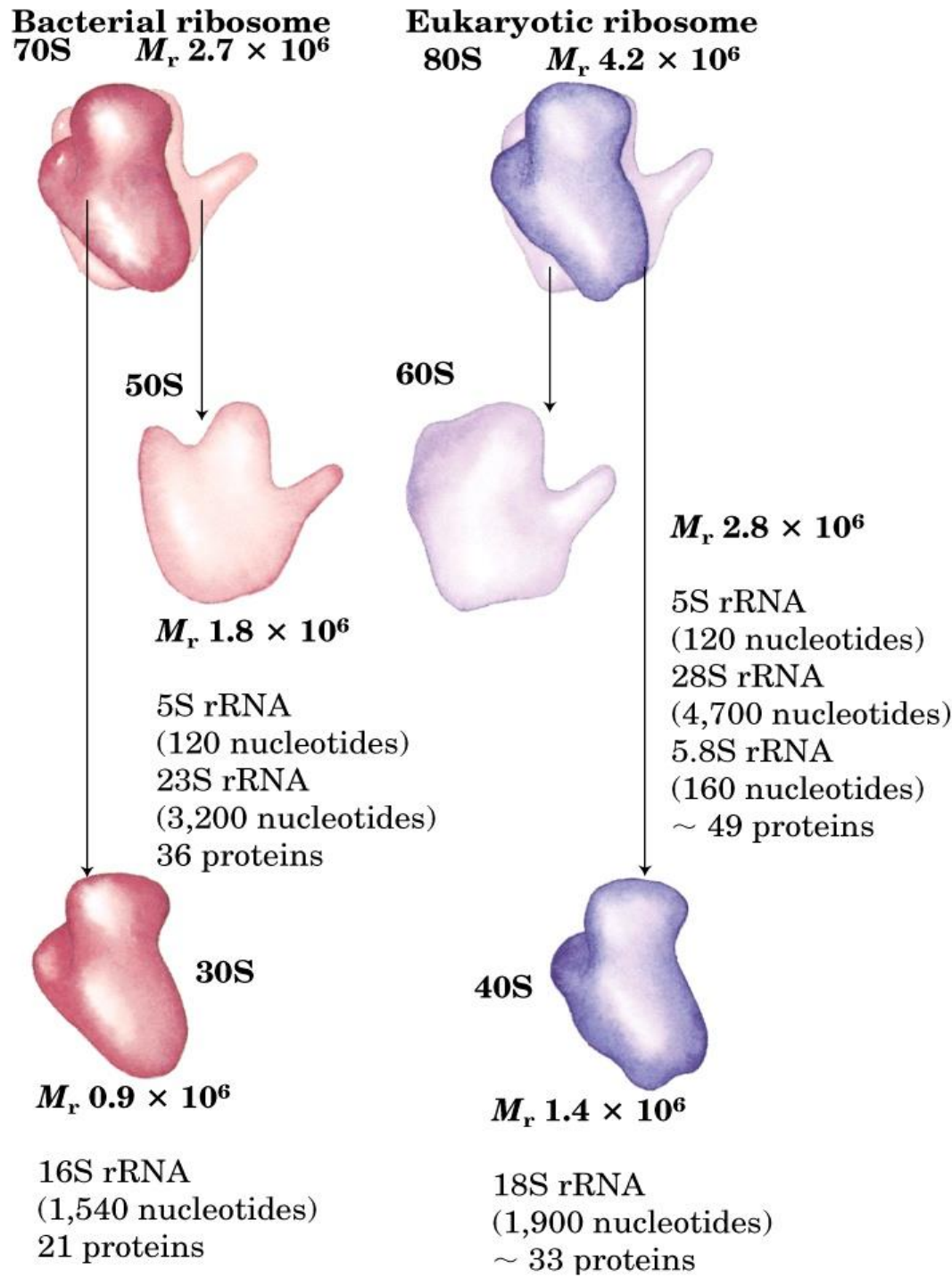
(a)

Ribosomes in prokaryotes vs. eukaryotes.

(Composition and mass)

S = Svedberg unit

(affected by shape and mass)



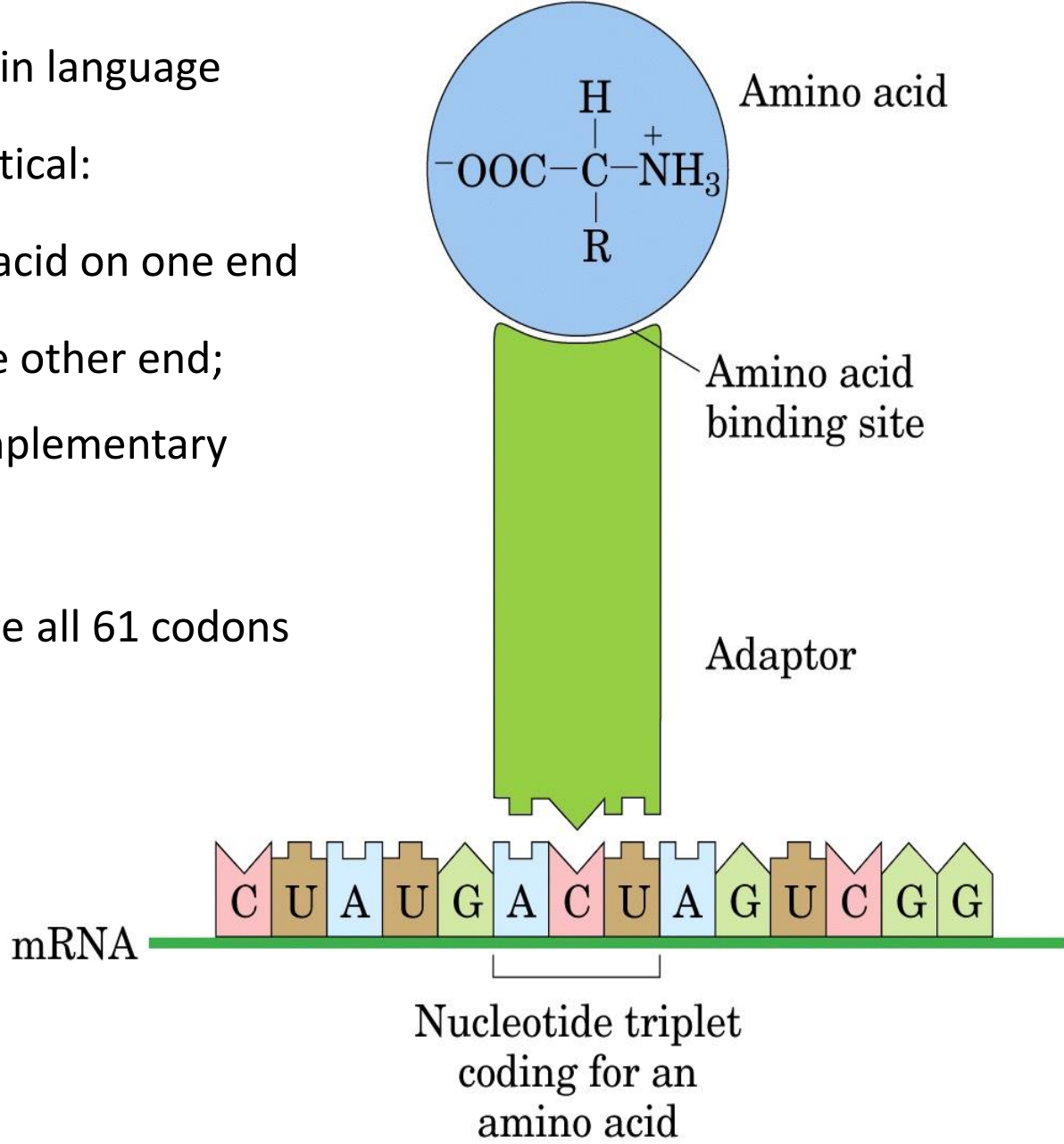
(b)

tRNA = RNA molecule that acts as interpreter

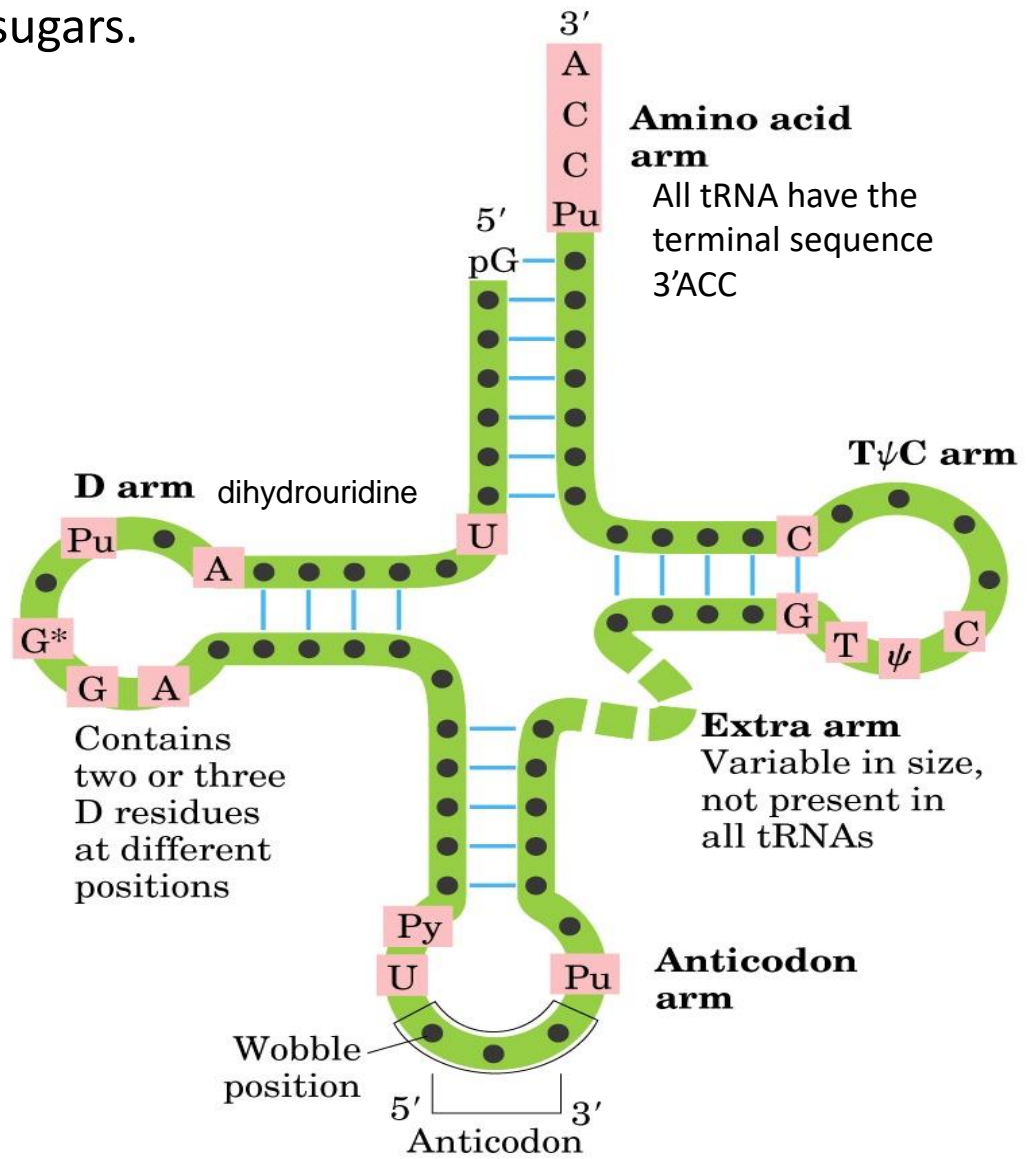
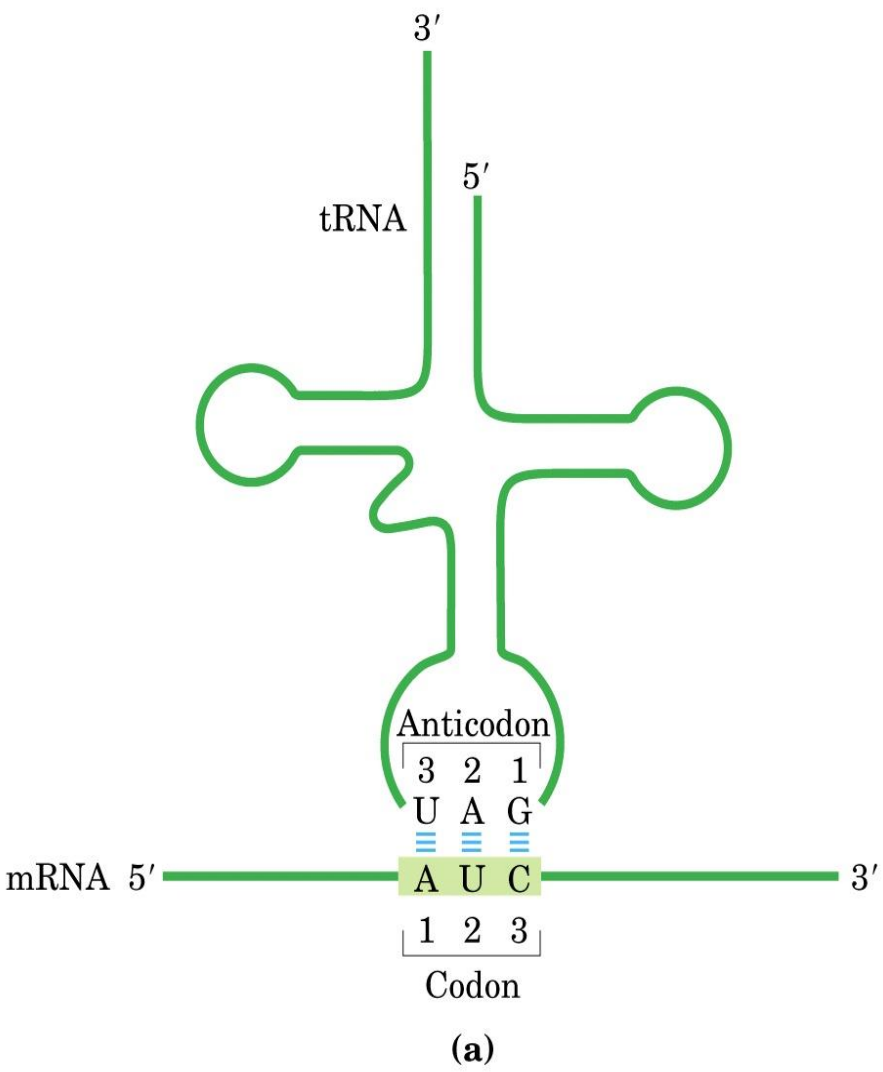
between nucleic acid and protein language

Molecules of tRNA are not identical:

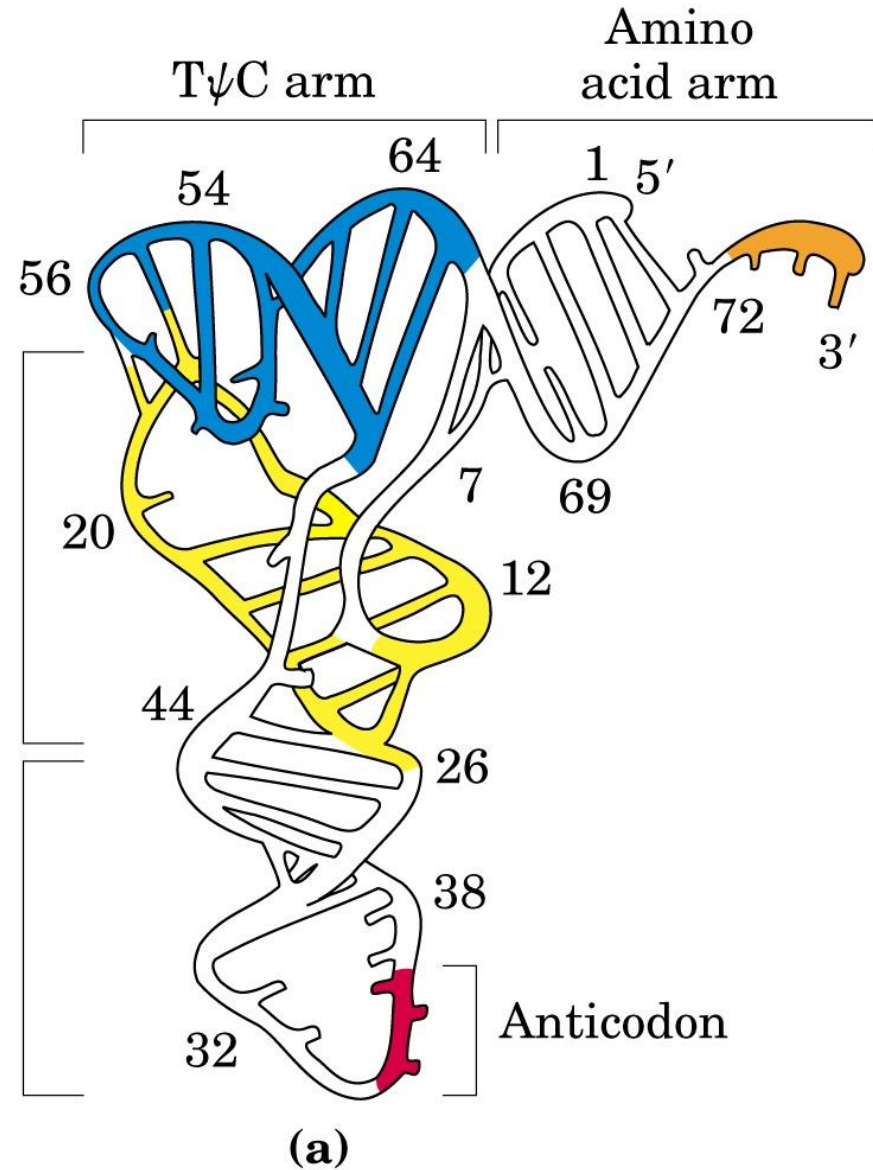
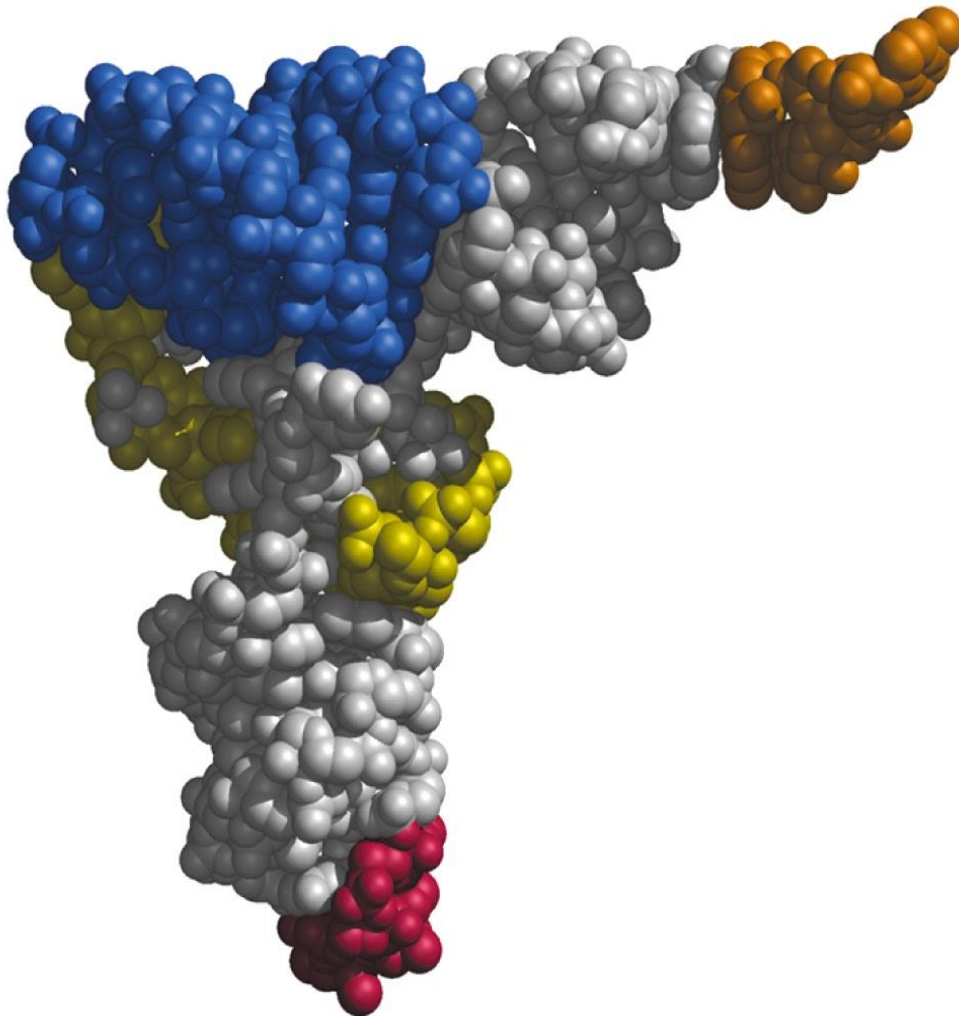
- Each carries a specific amino acid on one end
- Each has an anti-codon on the other end; which base-pairs with a complementary codon on mRNA.
- **32 tRNA** required to translate all 61 codons



tRNA single RNA strand contain **73-93** nucleotides:
 Essential for specificity of interaction bw Aminoacyl- tRNA and complementary mRNA codon.
 tRNA twists (due to H- bonds) and folds into a three-dimensional molecule.
 Contain 8 or more modified bases and sugars.



The Folded structure of tRNA: Twisted L



Translation

- The synthesis of protein from an RNA template.

- Five stages:

Preinitiation/activation of a.a

Initiation

Elongation

Termination and release

Post-translational modification

table 27-6

Components Required for the Five Major Stages of Protein Synthesis in *E. coli*

Stage	Essential components
1. Activation of amino acids	20 amino acids 20 aminoacyl-tRNA synthetases 20 or more tRNAs ATP Mg ²⁺
2. Initiation	mRNA N-Formylmethionyl-tRNA Initiation codon in mRNA (AUG) 30S ribosomal subunit 50S ribosomal subunit Initiation factors (IF-1, IF-2, IF-3) GTP Mg ²⁺
3. Elongation	Functional 70S ribosome (initiation complex) Aminoacyl-tRNAs specified by codons Elongation factors (EF-Tu, EF-Ts, EF-G) GTP Mg ²⁺
4. Termination and release	Termination codon in mRNA Polypeptide release factors (RF ₁ , RF ₂ , RF ₃) ATP
5. Folding and posttranslational processing	Specific enzymes, cofactors, and other components for removal of initiating residues and signal sequences, additional proteolytic processing, modification of terminal residues, and attachment of phosphate, methyl, carboxyl, carbohydrate, or prosthetic groups

Aminoacyl tRNA synthetases

- each one specific for 1 one amino acid, one or more tRNA.
- In cytosol not on ribosomes.
- In bacteria the initiating aminoacyl tRNA is **N-formylmethionyl tRNA**.
- In humans , **Methionine**. But mitochondria and chloroplast similar to bacteria.

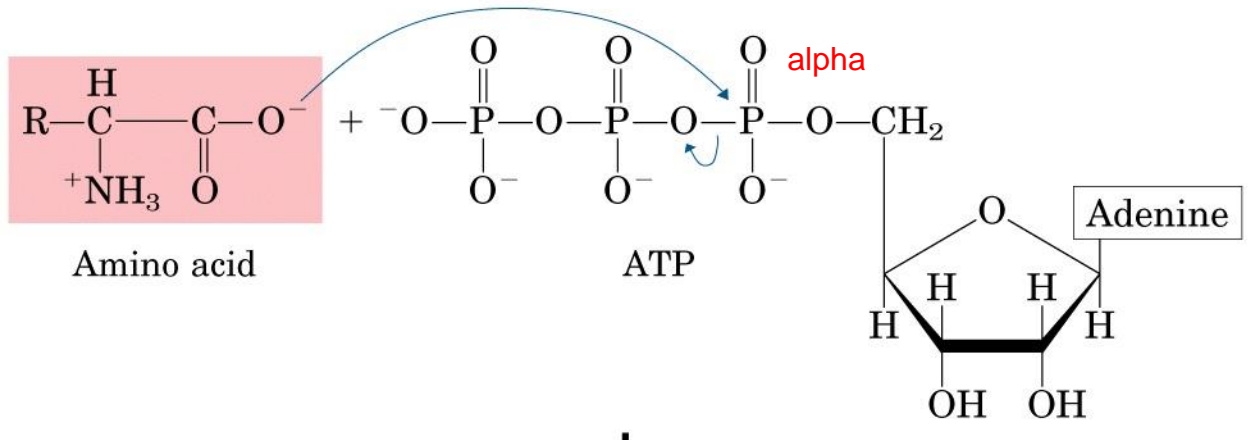
-Amino acid + tRNA + ATP $\xrightarrow{\text{Mg}^{2+}}$ aminoacyl- tRNA + AMP + PPI

charged tRNA = aminoacylated

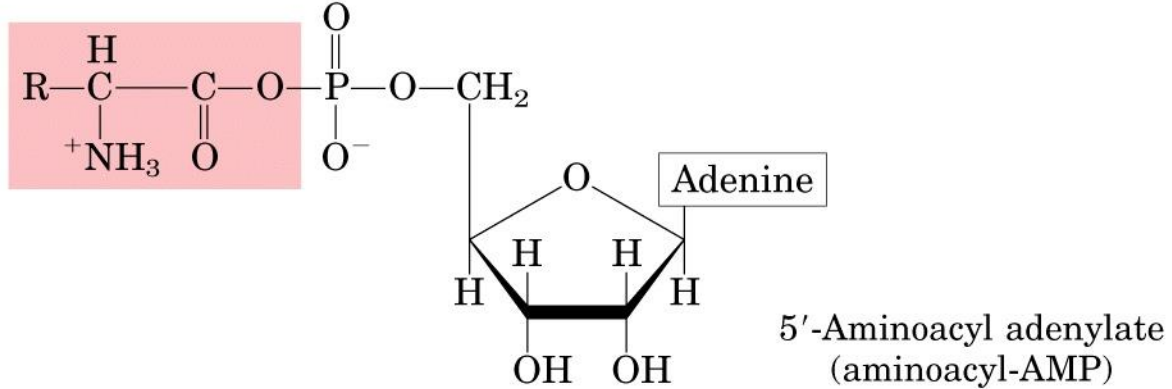
Aminoacyl - tRNA synthetase:

- 2 classes based on mechanism of action.

- Both present in all organisms.



\downarrow PP_i Hydrolysis to P_i by inorganic pyrophosphatase

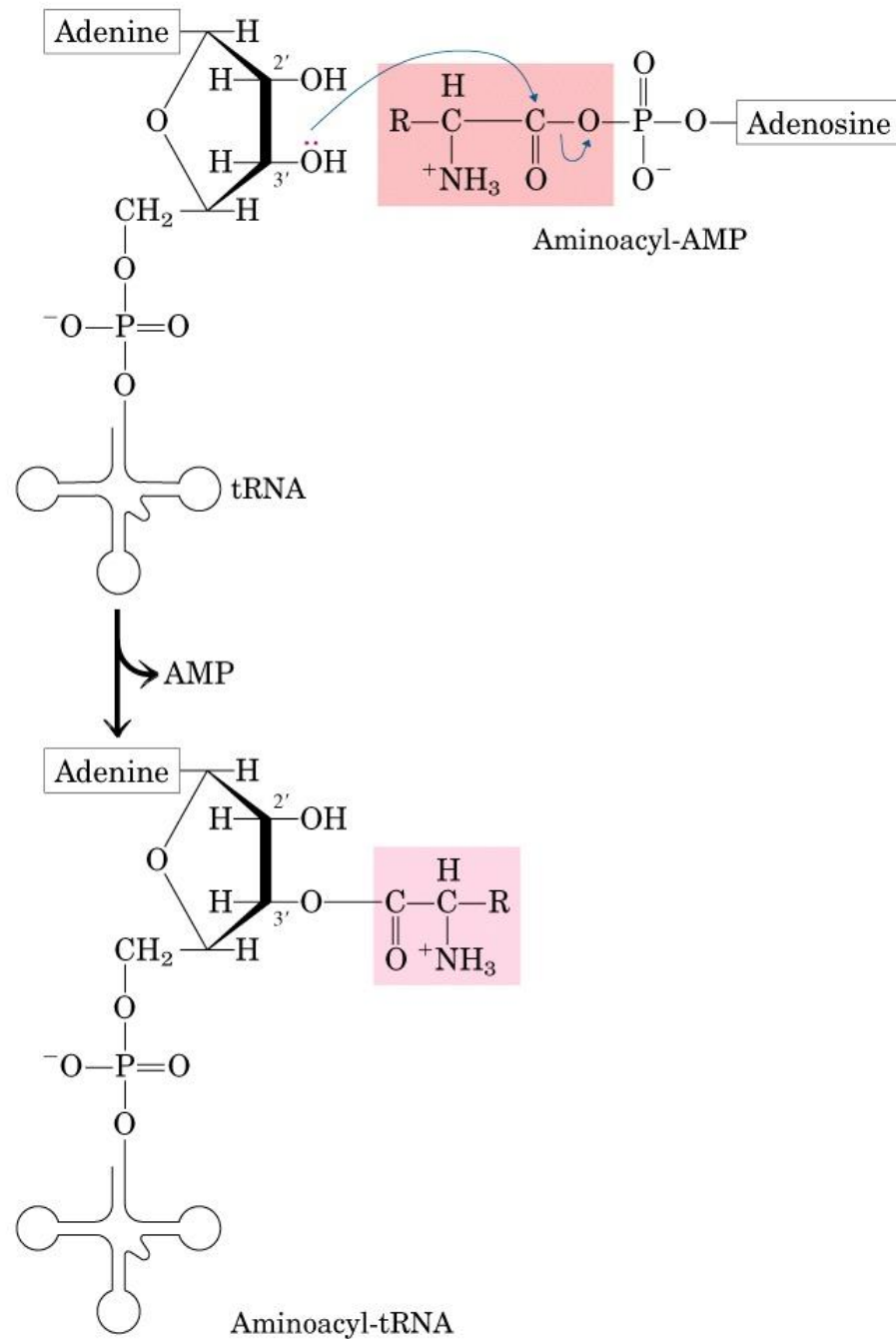
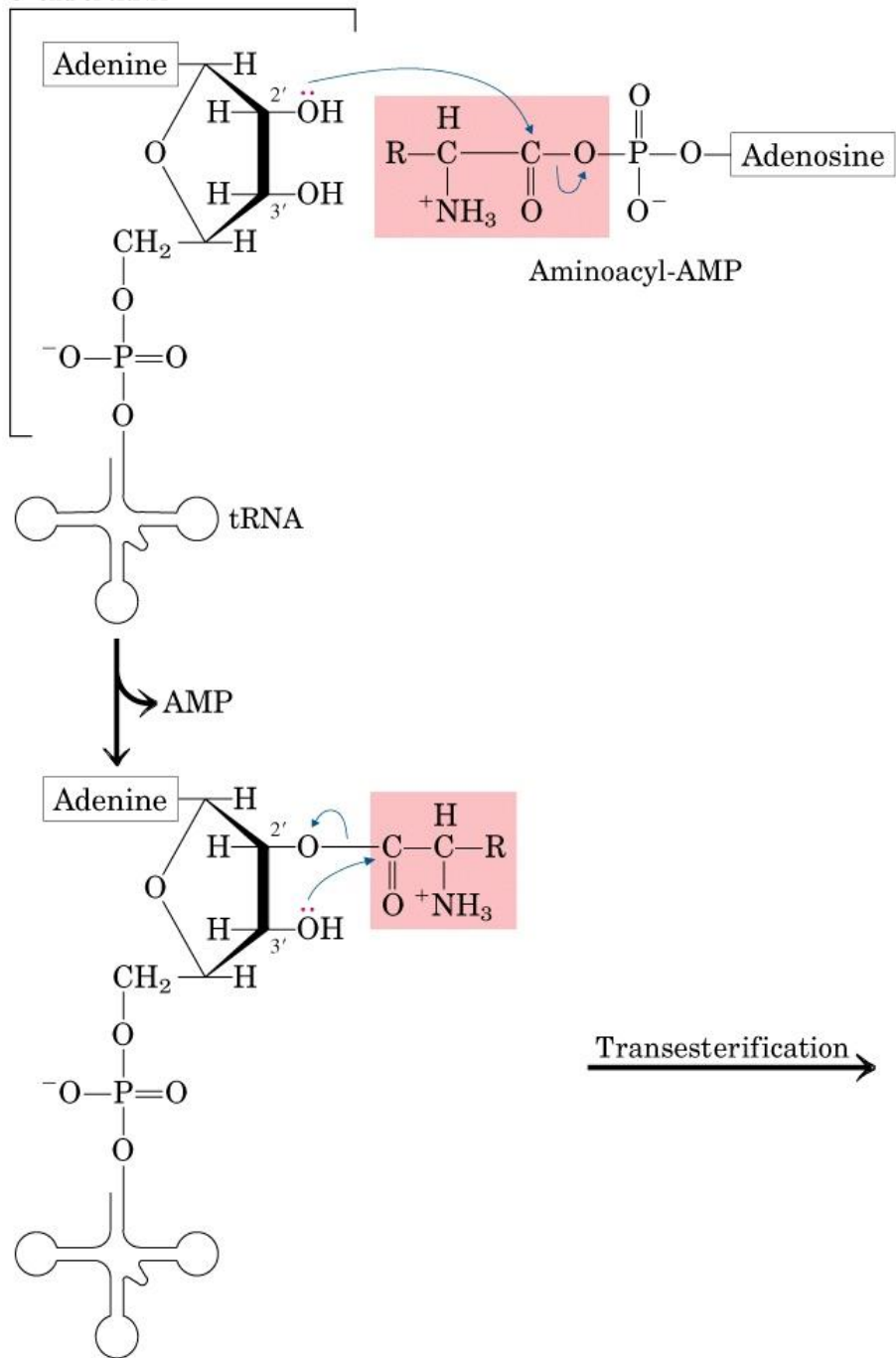


class I aminoacyl-tRNA synthetases

class II aminoacyl-tRNA synthetases



3' end of tRNA



Aminoacyl-tRNA

table 27-8

Two Classes of Aminoacyl-tRNA Synthetases*

Class I	Class II
Arg	Ala
Cys	Asn
Gln	Asp
Glu	Gly
Ile	His
Leu	Lys
Met	Phe
Trp	Pro
Tyr	Ser
Val	Thr

*Here, Arg represents arginyl-tRNA synthetase, and so forth. The classification applies to all organisms for which tRNA synthetases have been analyzed and is based on protein structural distinctions and on the mechanistic distinction outlined in Figure 27-16.

Proofreading by aminoacyl-tRNA synthetase:

Identity of a.a attached to tRNA not checked on ribosome.

protein synthesis fidelity relies on accuracy of this rxn (binding of a.a to tRNA)

Ile-tRNA synthetase:

Prefers Ile over Val (differ in one CH2 – group) by a factor of 200

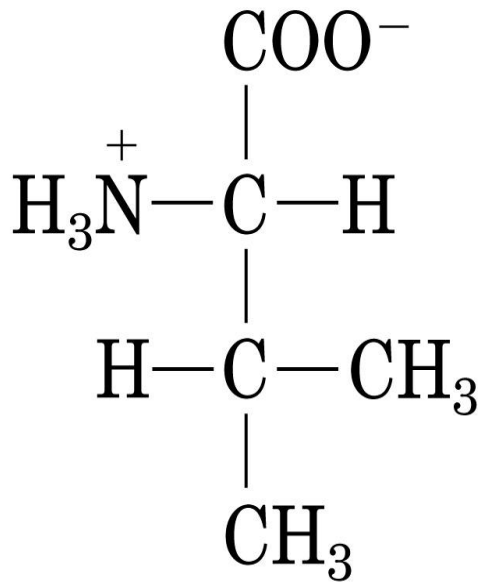
Expalnation: **PROOOOFREADING.**

Several filters:

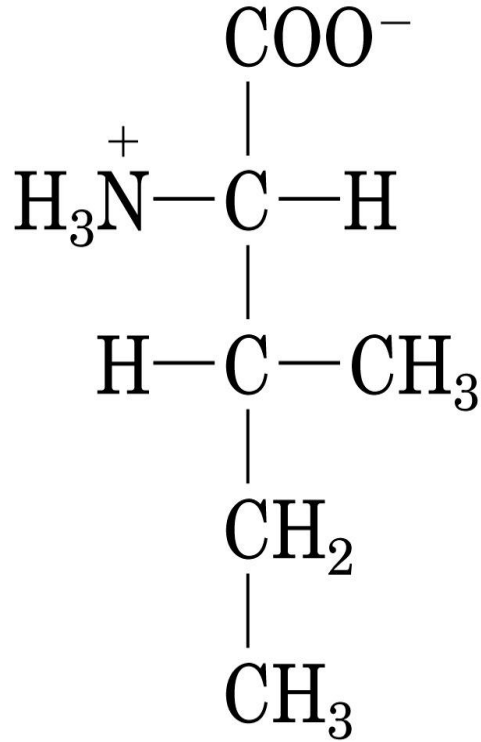
1) Binding of a.a + enzyme → aminoacyl-AMP

2) Incorrect aminoacyl-AMP in other site (proofreading site) →hydrolyzed = deacylation consuming GTP

3) Proofreading after aminoacyl-AMP Linked to tRNA



Valine



Isoleucine

Overall error= 1 mistake/10,000 a.a added

2nd genetic code:

Specificity not only for a single a.a but also to tRNA .

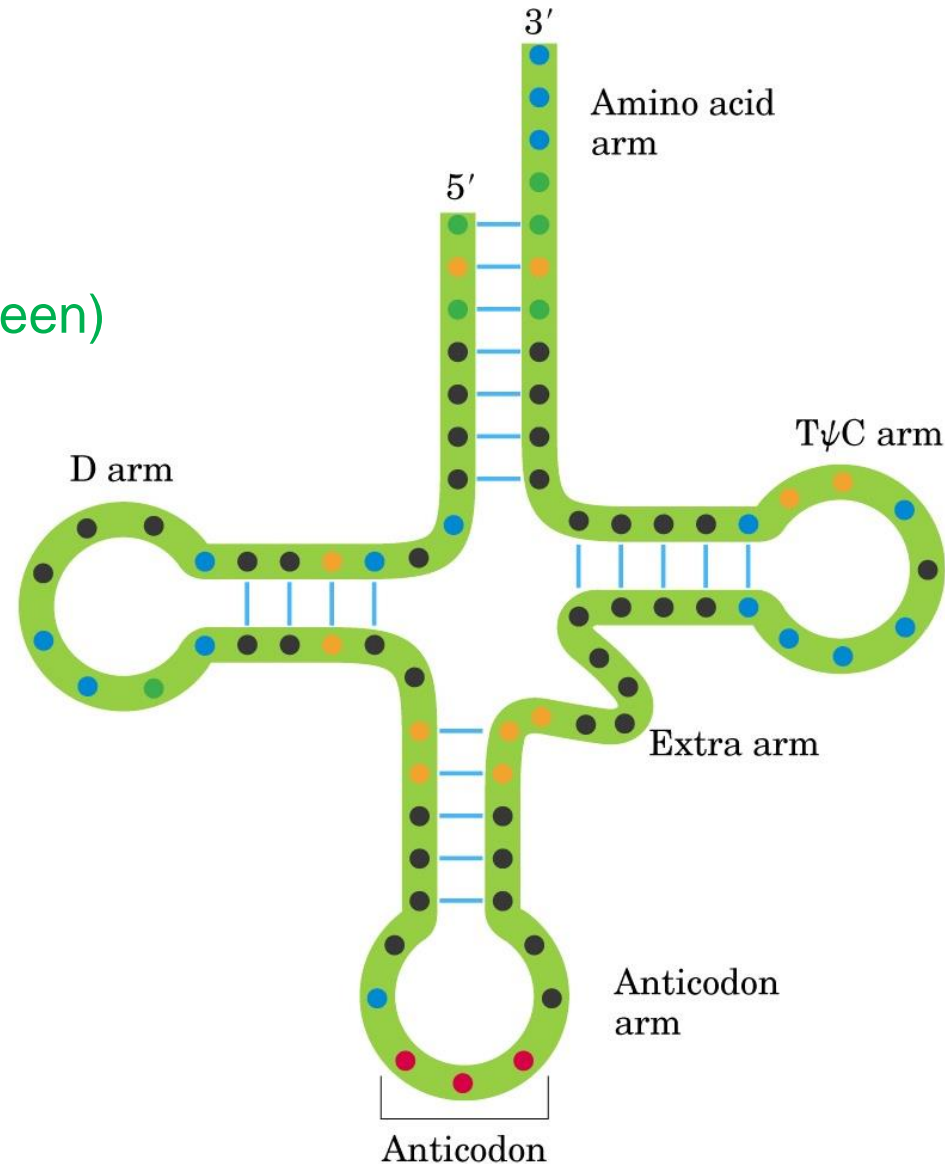
Choosing correct tRNA Important for fidelity as choosing correct a.a

Nucleotides present in all tRNA (blue)

Recognition points for one (orange)

or more aminoacyl-tRNA synthetase (green)

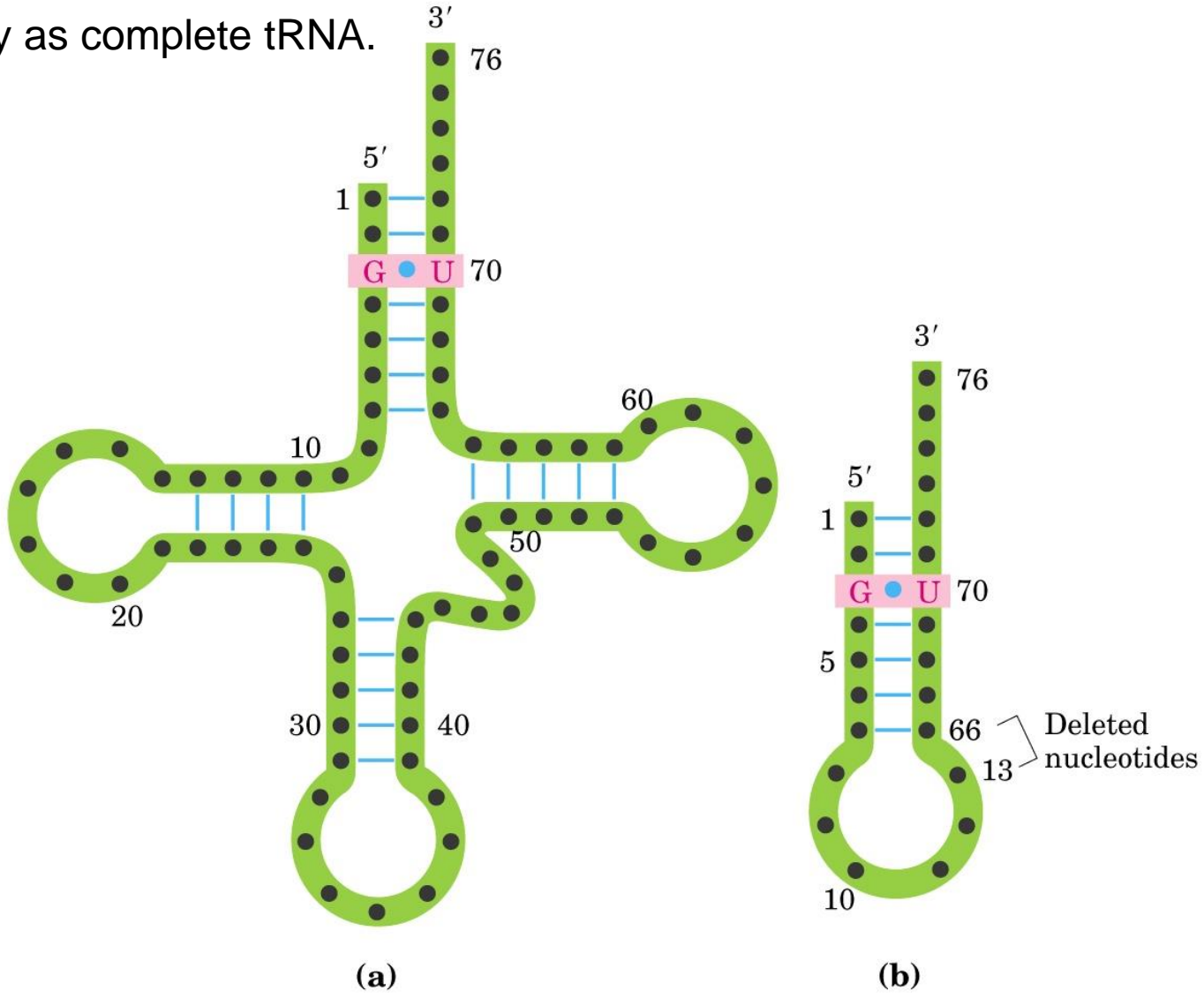
Around 10 nucleotides necessary
for recognition of a tRNA to its
aminoacyl-tRNA synthetase.



tRNA^{ala} :

Determinant of tRNA by its specific Ala-tRNA synthetase is a single G-U in a.a arm.

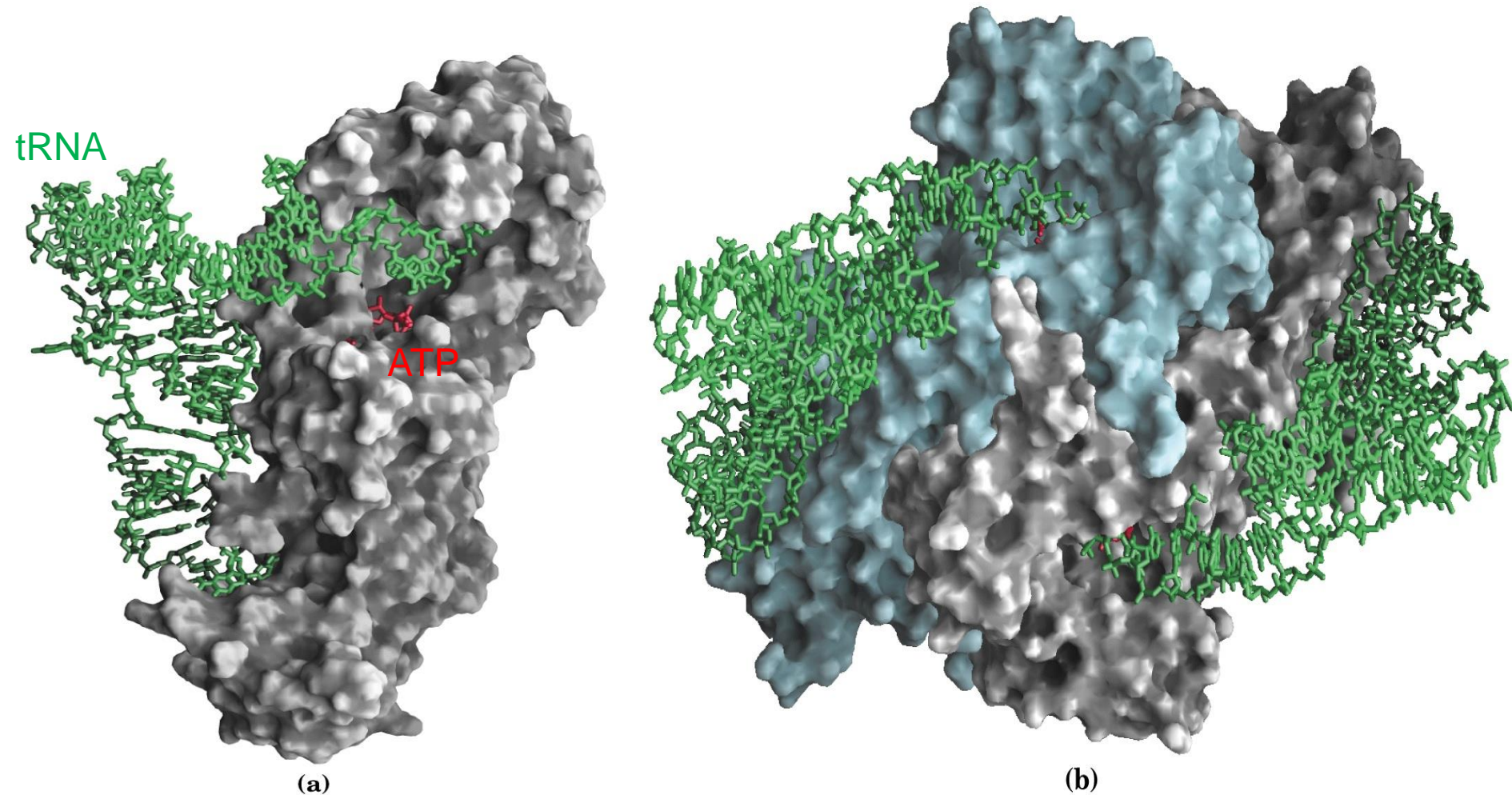
A short synthetic RNA containing the G-U specifically aminoacylated with Ala in the same efficiency as complete tRNA.



Aminoacyl tRNA synthetase bound to its tRNA:

Gln-tRNA synthetase: typical monomeric type I

Asp-tRNA synthetase : dimeric type II



Initiation of prokaryotic protein synthesis occurs at the Shine Delgarno site

The untranslated region or 5' end of prokaryotic mRNAs contain a ribosome binding site (rbs) or **Shine Delgarno site** located upstream of the AUG.

mRNA: 5'AGGAGGU.....AUG

Initiation codon:

Amino terminal residue= Met coded by 1 codon, 1 synthetase but 2 tRNA

→ one exclusively for the start / initiation codon 5` AUG

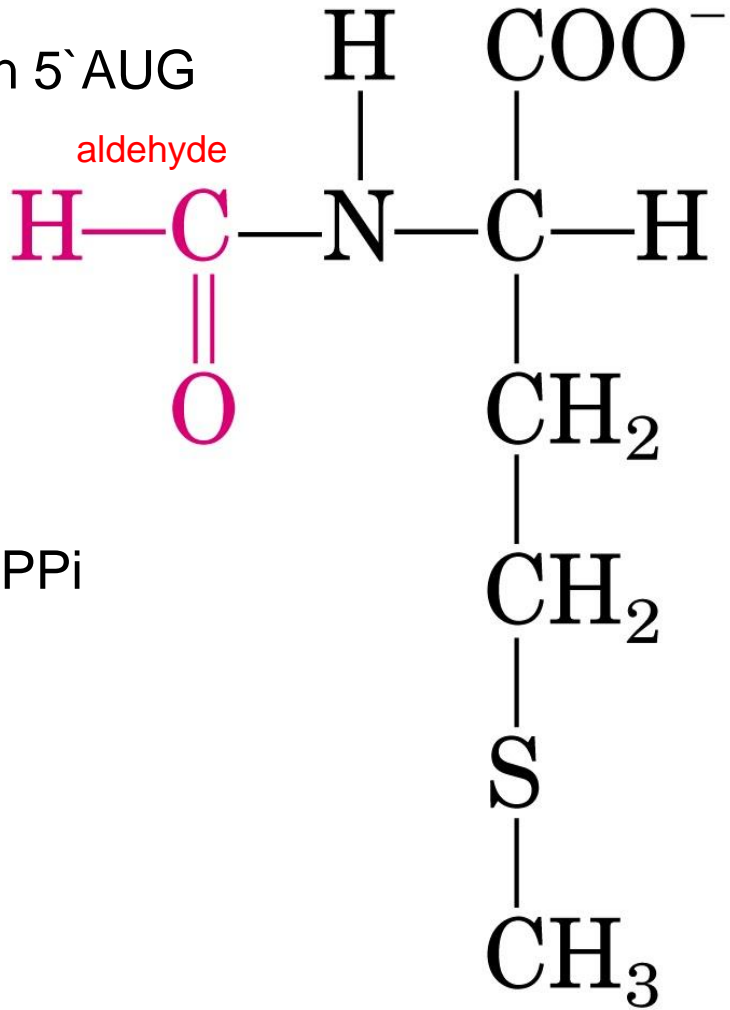
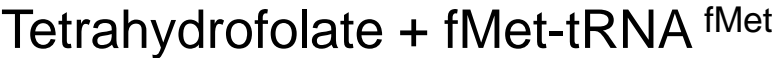
→ other for interior Met
tRNA^{Met} and tRNA^{fMet}

Formed in 2 steps:

1) Met-tRNA synthetase



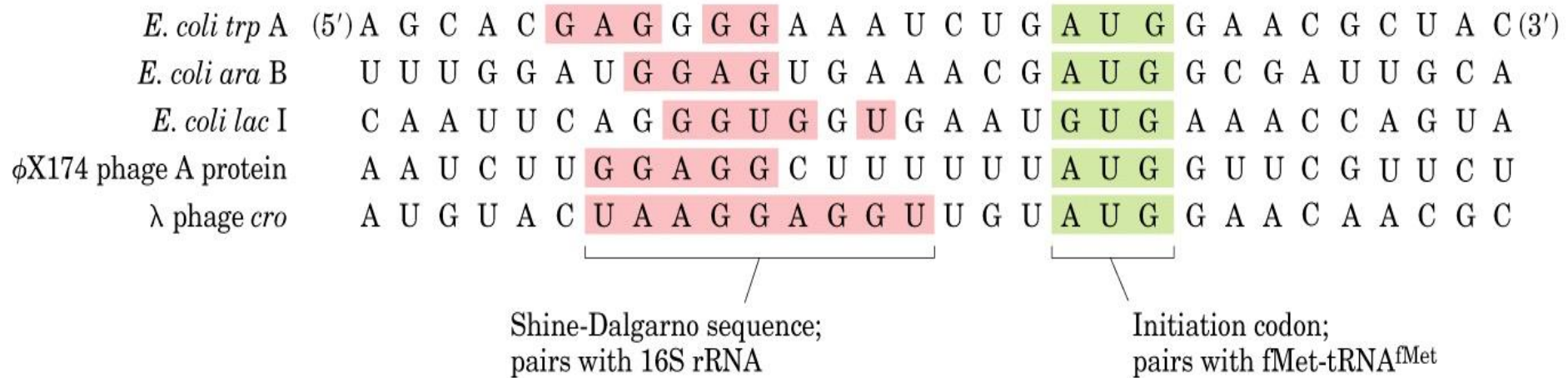
2) Transformylase (selective >> synthetase)



N-Formylmethionine

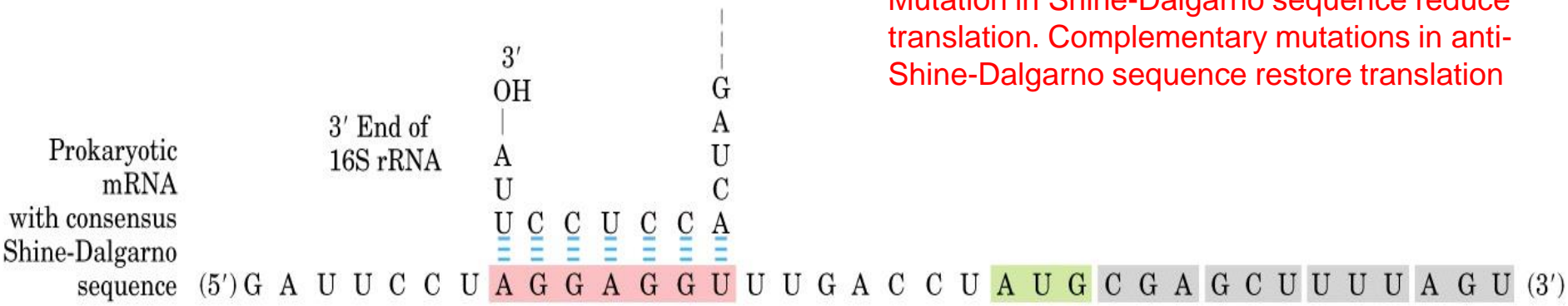
Shine Dalgarno sequence:

8-13 bp to the 5` side of the initiation codon



(a)

Shine Dalgarno base pairs with a sequence near the 3` end of 16S rRNA of 30S ribosome



(b)

Summary of Protein Synthesis

1. Binding of mRNA to ribosome
2. Charged, amino-acylated initiator tRNA binds to **P** site of ribosome and is base paired through tRNA anticodon to codon on mRNA
3. A second amino-acylated tRNA fills **A** site and anticodon H-bonds with second codon on mRNA
4. Amino acids in P and A site are joined by a peptide bond.
 - tRNA in **E** site is released.
 - tRNA (with 2 amino acids joined) in **A** site moves to **P** site
 - A new amino-acylated tRNA moves into **A** site by anticodon-codon pairing
5. Step (4) is repeated until codon in **A** site is a stop codon; peptide is released.