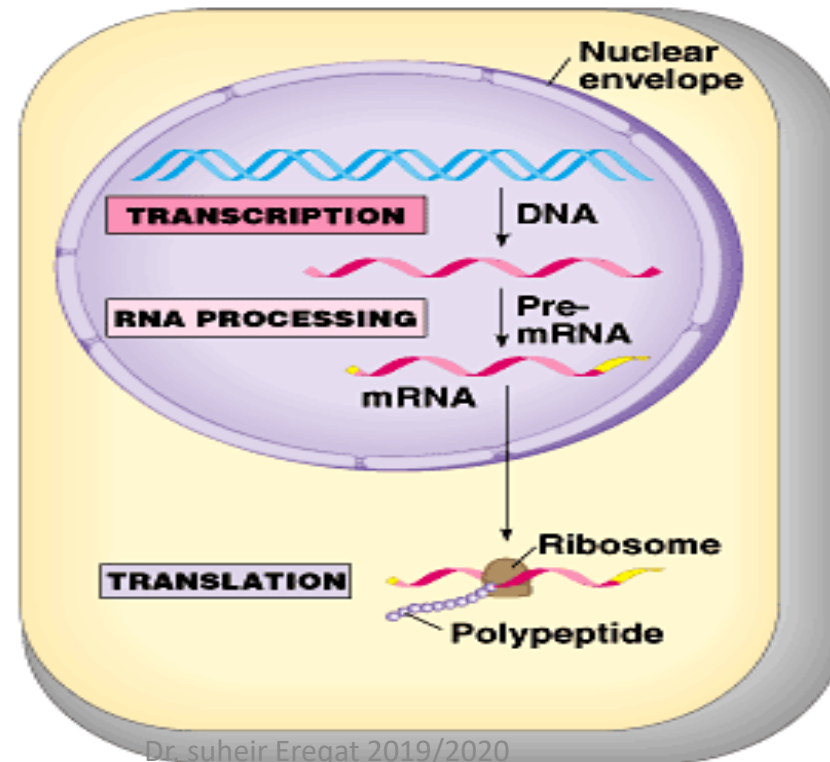
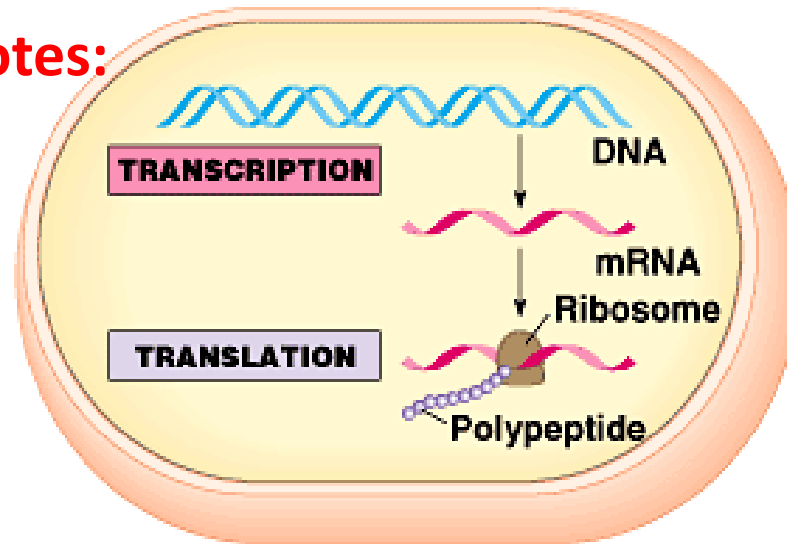


# - PROTEIN TRANSLATION –

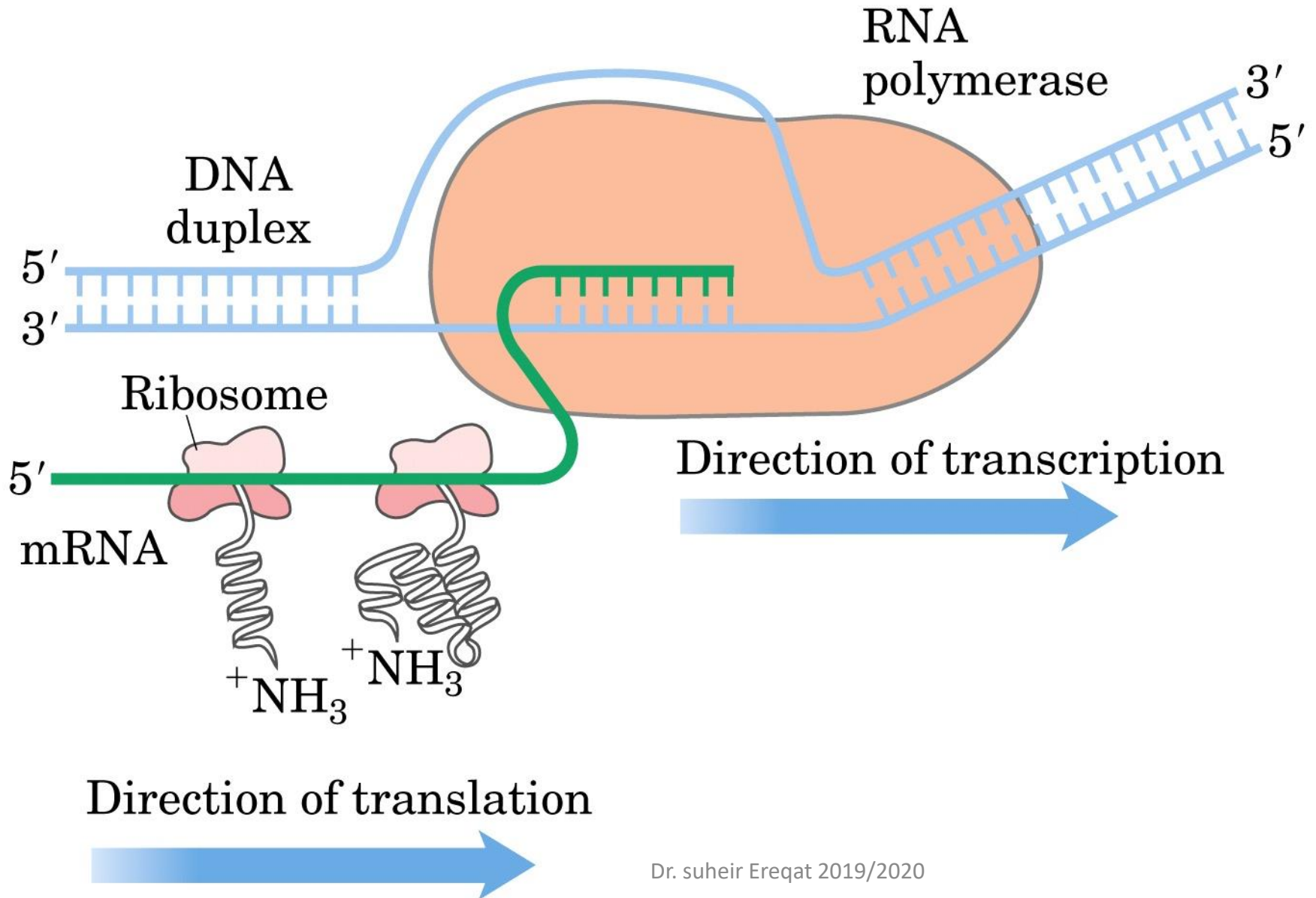
# Transcription and Translation in Prokaryotes vs. Eukaryotes:



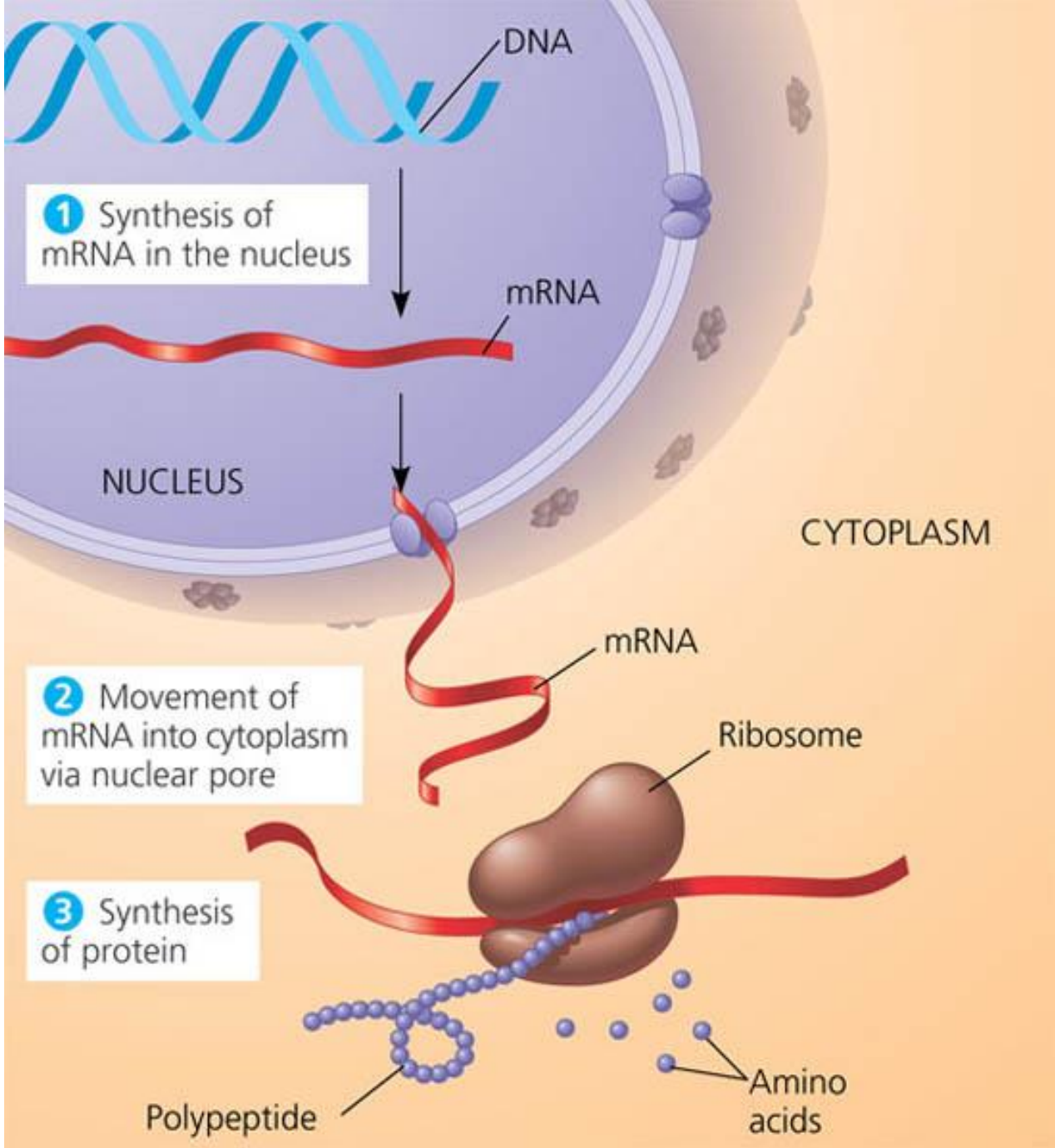
Dr. suheir Eregat 2019/2020

(b) Eukaryotic cell

# Prokaryotes



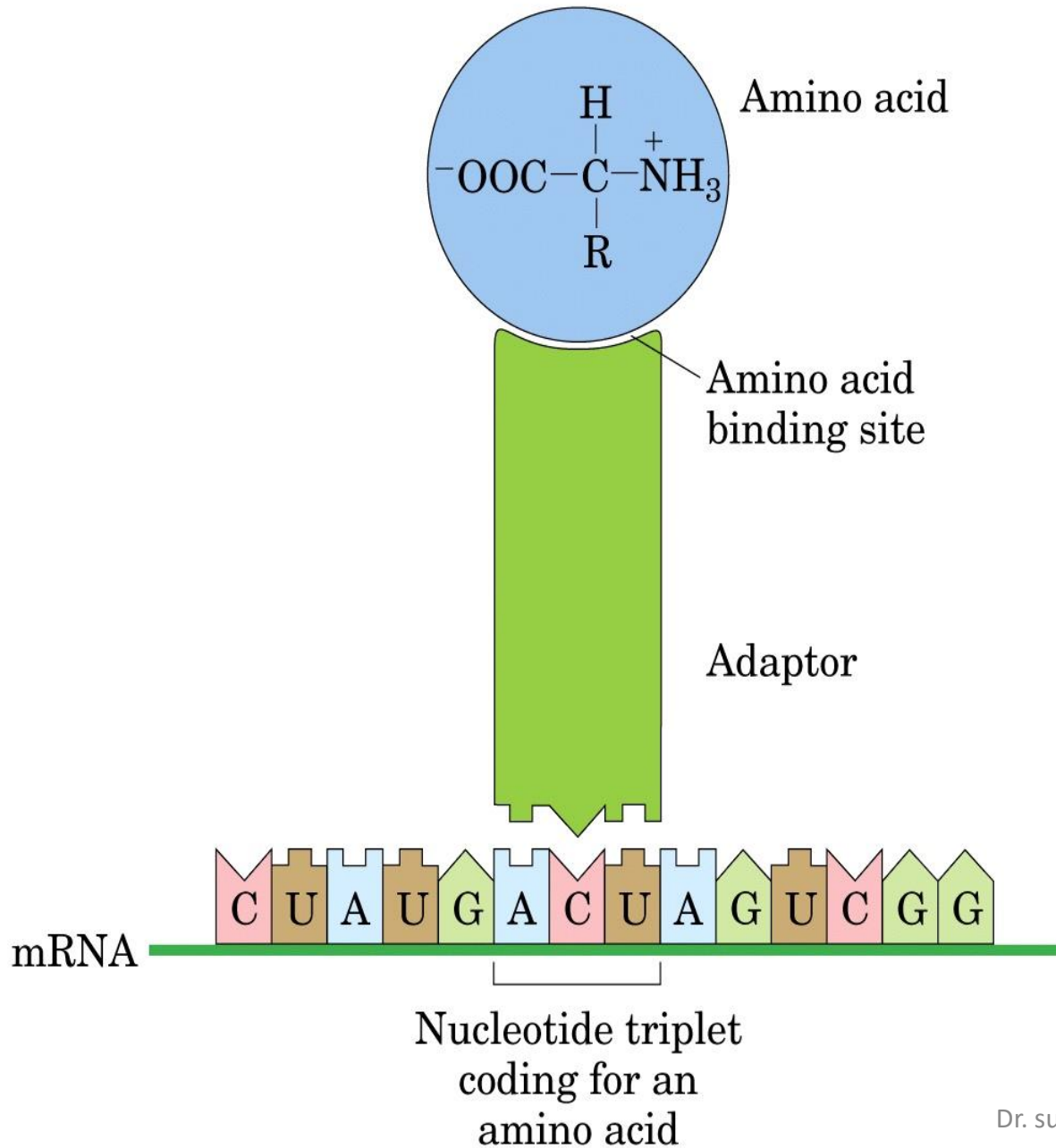
# Eukaryotes:



# Genetic code!

- The four code letters of DNA → encode 20 amino acids=codon (triplet of nucleotides)
- 64 combination
- No punctuation between codons for successive amino acid residues.
- Adaptor!

# Translation requires adaptor molecules, the tRNAs,



A minimum of 32 tRNAs are required to translate all 61 codons (31 to encode the amino acids and 1 for initiation).

## Nonoverlapping code:

Nonoverlapping  
code

A U A C G A G U C      
1                    2                    3

Overlapping  
code

A U A C G A G U C  
1  
            
2  
            
3

**Reading Frame:** all mRNAs have three potential reading frames,

**reading frame:** the series of nucleotides read in sets of 3 (codon)  
only 1 reading frame is correct for encoding the correct sequence of amino acids



a reading frame without a termination codon among 50 or more codons = **open reading frame (ORF).**



# The Genetic Code

**Marshall Nirenberg** identified the codons that specify each amino acid.

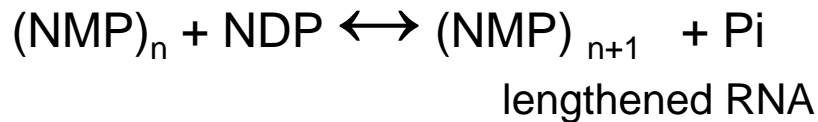
RNA molecules of only 1 nucleotide and of specific 3-base sequences were used to determine the amino acid encoded by each codon.

The amino acids encoded by all 61 possible codons were determined.

# Cracking the Genetic Code: Marshall Nirenberg experiment

The base sequences of the codons were deduced from experiments using synthetic mRNAs of known composition and sequence.

**Polynucleotide phosphorylase** (do NOT need template)



# 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 base”

# • “Dictionary” of amino acid code words in mRNAs

UUU is always Phe, not sometimes.  
 Nonsense Codons are Stop Points  
**UAA, UAG, and UGA**

The genetic Code is nearly **Universal**

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

# “Dictionary” of amino acid code words in mRNAs

First letter of codon (5' end)

Second letter of codon

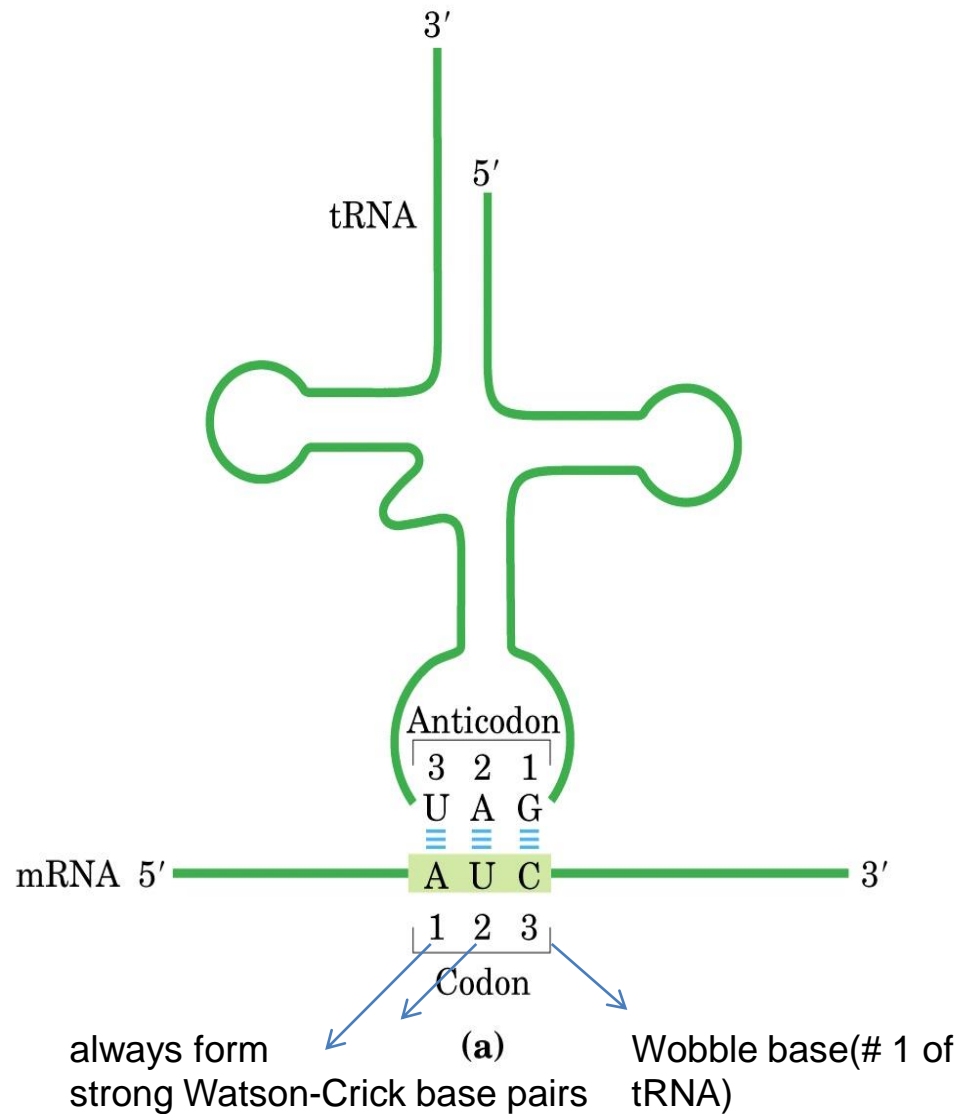
	U		C		A		G	
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
A	AUU	Ile	ACU	Thr	AAU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

Initiation codon=the most common signal for the beginning of a polypeptide in all cells

The genetic code is degenerate: it has multiple codons for almost every amino acid

TABLE 27–3		Degeneracy of the Genetic Code	
Amino acid	Number of codons	Amino acid	Number of codons
Met	1	Tyr	2
Trp	1	Ile	3
Asn	2	Ala	4
Asp	2	Gly	4
Cys	2	Pro	4
Gln	2	Thr	4
Glu	2	Val	4
His	2	Arg	6
Lys	2	Leu	6
Phe	2	Ser	6

# wobble hypothesis:



**TABLE 27-4**

**How the Wobble Base of the Anticodon Determines the Number of Codons a tRNA Can Recognize**

1. One codon recognized:

Anticodon	(3') X - Y - <b>C</b> (5')	(3') X - Y - <b>A</b> (5')
	- - -	- - -
Codon	(5') X' - Y' - <b>G</b> (3')	(5') X' - Y' - <b>U</b> (3')

2. Two codons recognized:

Anticodon	(3') X - Y - <b>U</b> (5')	(3') X - Y - <b>G</b> (5')
	- - -	- - -
Codon	(5') X' - Y' - <b>A</b> (3')	(5') X' - Y' - <b>C</b> (3')

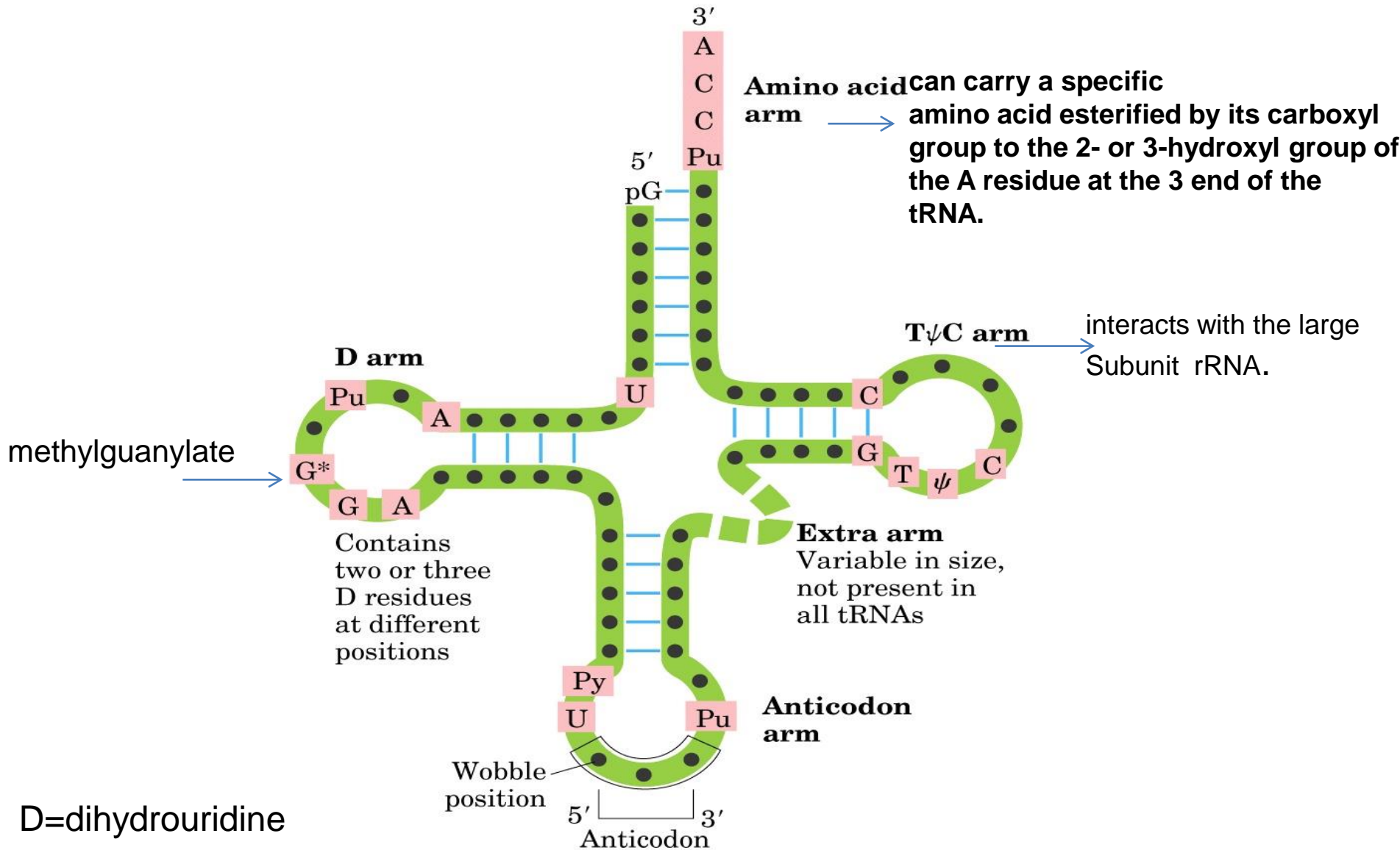
3. Three codons recognized:

Anticodon	(3') X - Y - <b>I</b> (5')
	- - -
Codon	(5') X' - Y' - <b>A</b> (3')
	- - -
	(5') X' - Y' - <b>U</b> (3')
	- - -
	(5') X' - Y' - <b>C</b> (3')

**Note:** X and Y denote bases complementary to and capable of strong Watson-Crick base pairing with X' and Y', respectively. Wobble bases—in the 3' position of codons and 5' position of anticodons—are shaded in pink.

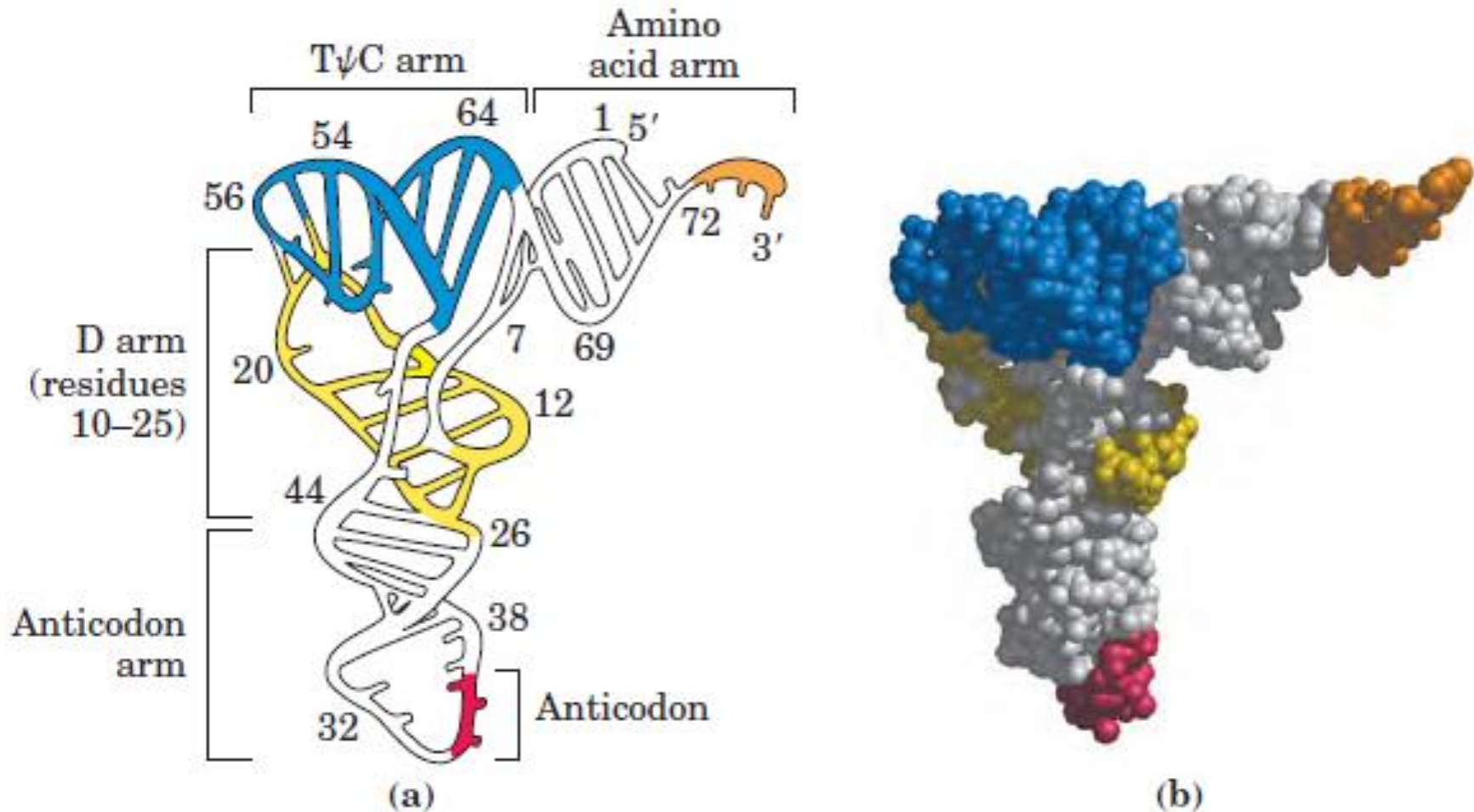
# Transfer RNAs Have Characteristic Structural Features

**cloverleaf secondary structure of tRNAs.**



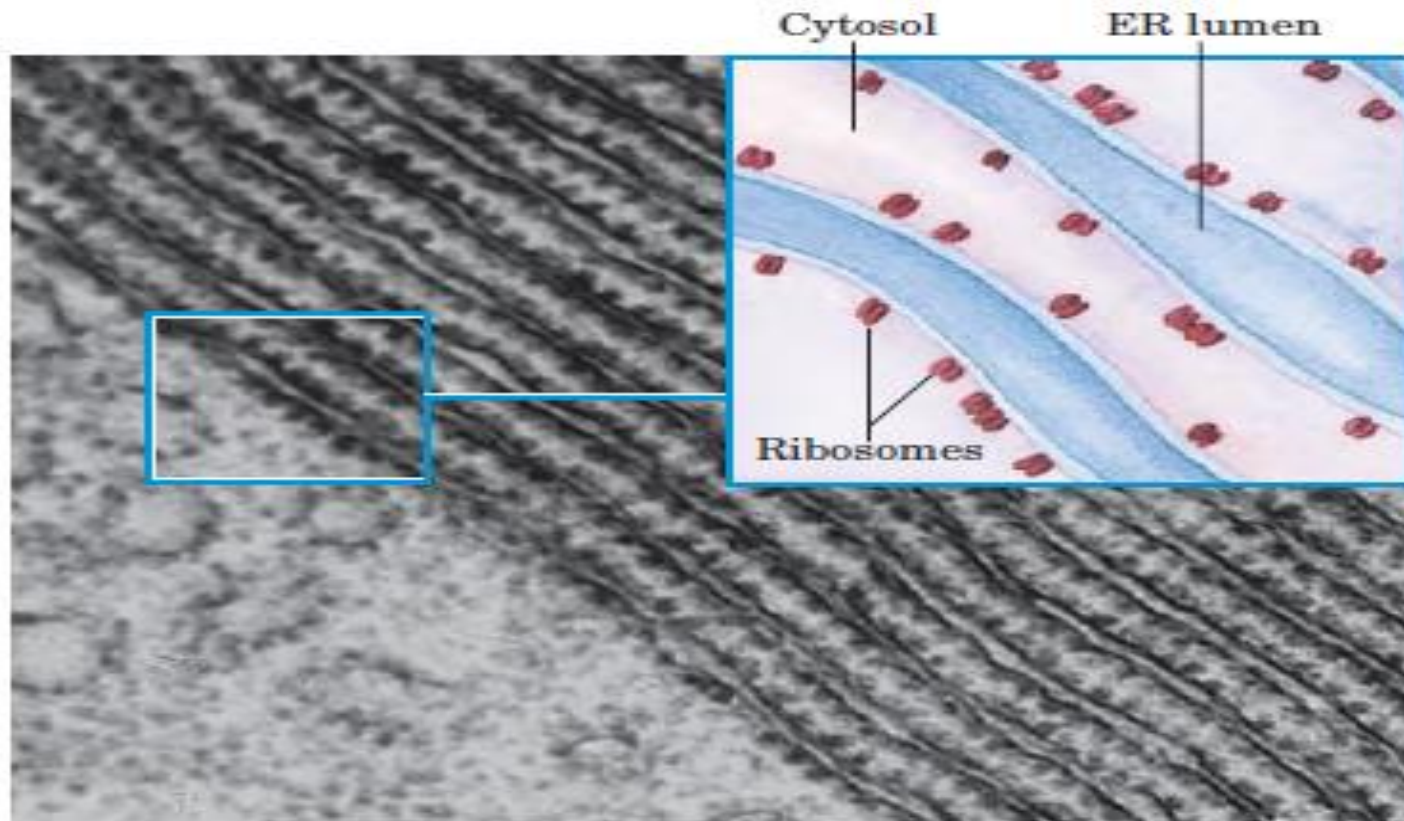


# Three-dimensional structure of yeast tRNA



translation of information encoded in mRNA=Protein; this process is carried out by ribosomes.

In eukaryotes, translation may occur on ribosomes in the cytoplasm or on ribosomes of the RER.



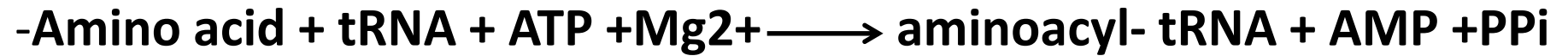
**Ribosomes and endoplasmic reticulum (ER):**

# Protein synthesis: 5 stages

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

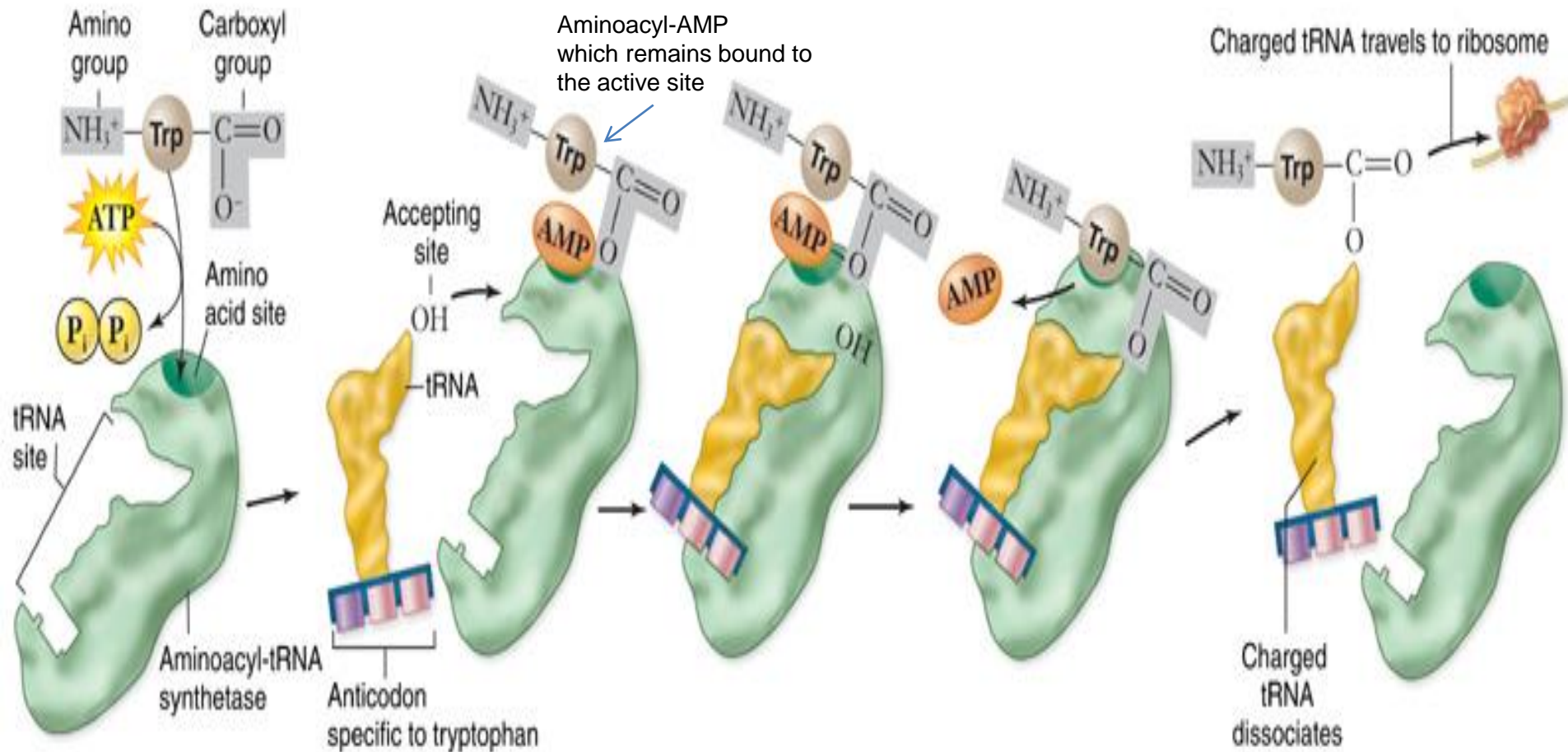
Stage	Essential components
1. Activation of amino acids	20 amino acids 20 aminoacyl-tRNA synthetases 20 or more tRNAs ATP Mg <sup>2+</sup>
2. Initiation	mRNA N-Formylmethionyl-tRNA Initiation codon in mRNA (AUG) 30S ribosomal subunit 50S ribosomal subunit Initiation factors (IF-1, IF-2, IF-3) GTP Mg <sup>2+</sup>
3. Elongation	Functional 70S ribosome (initiation complex) Aminoacyl-tRNAs specified by codons Elongation factors (EF-Tu, EF-Ts, EF-G) GTP Mg <sup>2+</sup>
4. Termination and release	Termination codon in mRNA Polypeptide release factors (RF <sub>1</sub> , RF <sub>2</sub> , RF <sub>3</sub> ) 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

## Activation of amino Acid: Aminoacyl tRNA synthetases



**This reaction takes place in the cytosol, not on the ribosome**

# aminoacyl- tRNA synthesis :Charging of tRNA



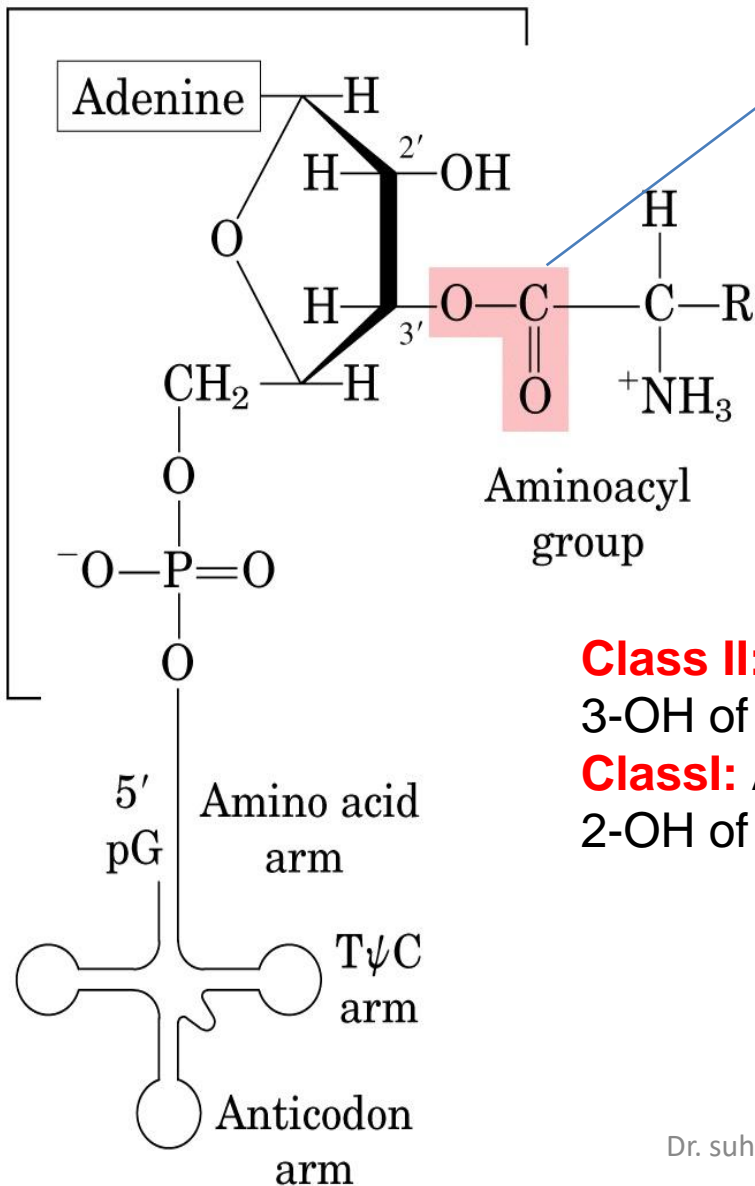
1. In the first step of the reaction, the amino acid is activated. The amino acid reacts with ATP to produce an intermediate with the carboxyl end of the amino acid attached to AMP. The two terminal phosphates (pyrophosphates) are cleaved from ATP in this reaction.

2. The amino acid-AMP complex remains bound to the enzyme. The tRNA next binds to the enzyme.

3. The second step of the reaction transfers the amino acid from AMP to the tRNA, producing a charged tRNA and AMP. The charged tRNA consists of a specific amino acid attached to the 3' acceptor stem of its RNA.

# Aminoacyl-tRNA

3' end of tRNA



The ester linkage both activates the amino acid and joins it to the tRNA

Aminoacyl group

**Class II:** Aminoacyl group is transferred directly to the 3-OH of the A residue of tRNA

**Class I:** Aminoacyl group is transferred to 2-OH of A then to 3-OH

## 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.

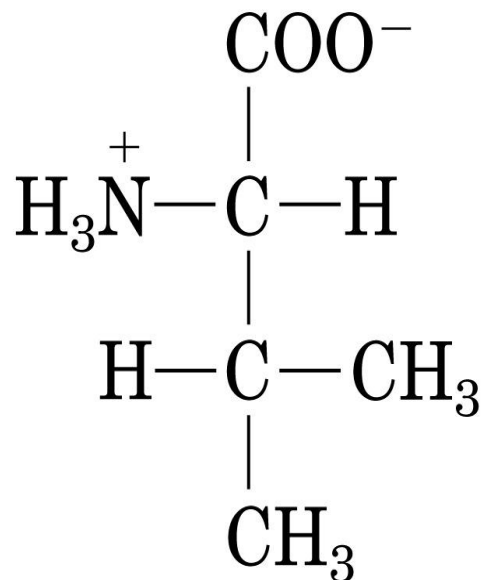
## Proofreading by aminoacyl-tRNA synthetase:

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

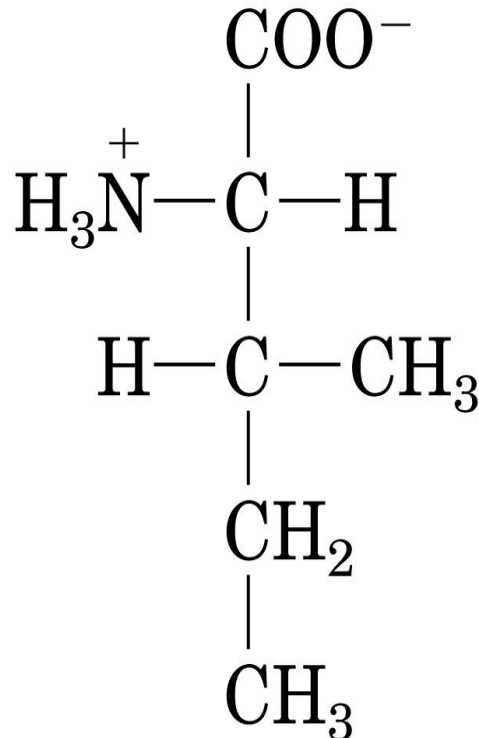
protein synthesis fidelity relies on accuracy of this rxn.

## Ile-tRNA synthetase:

Prefers Ile over Val by a factor of 200



Valine

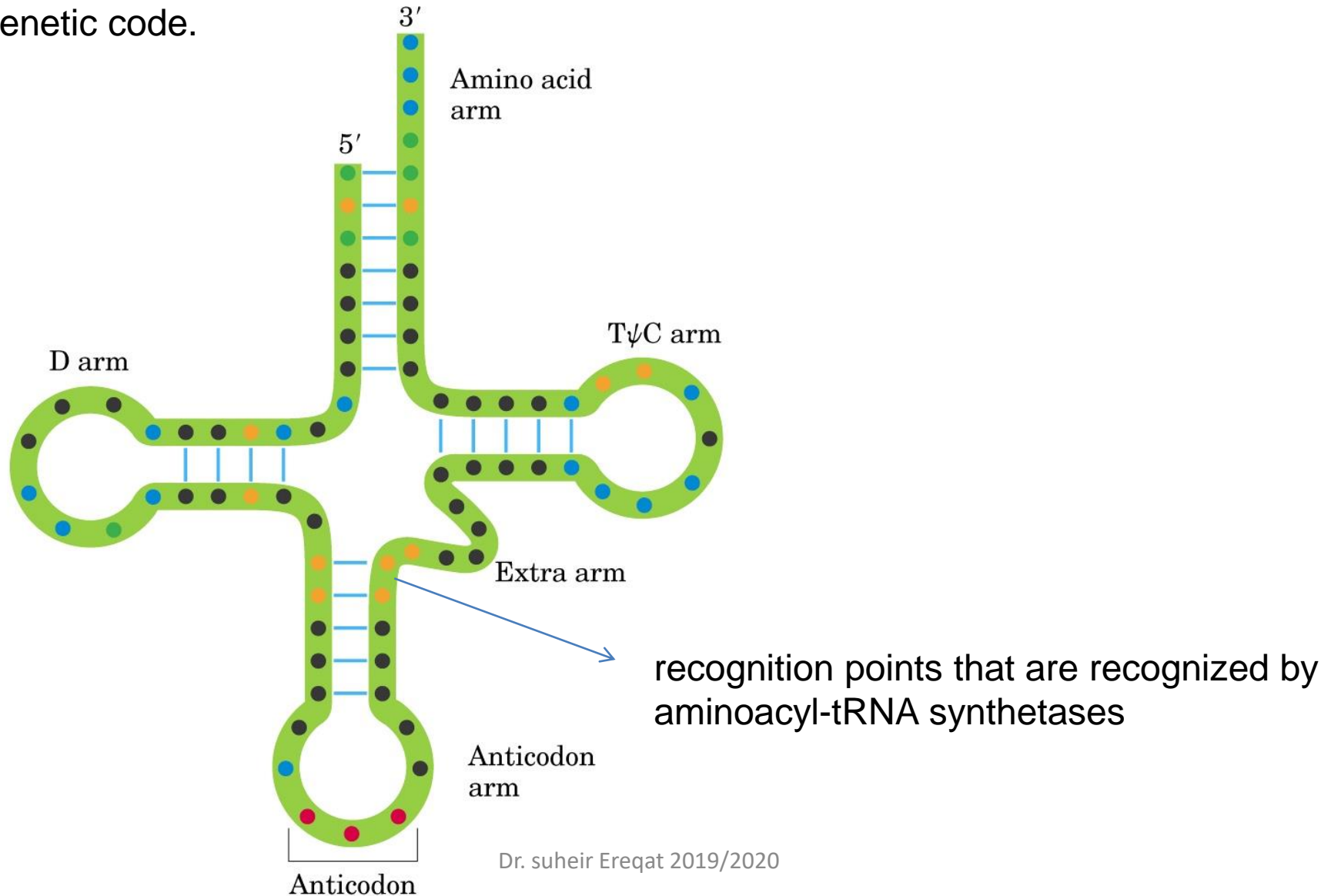


Isoleucine



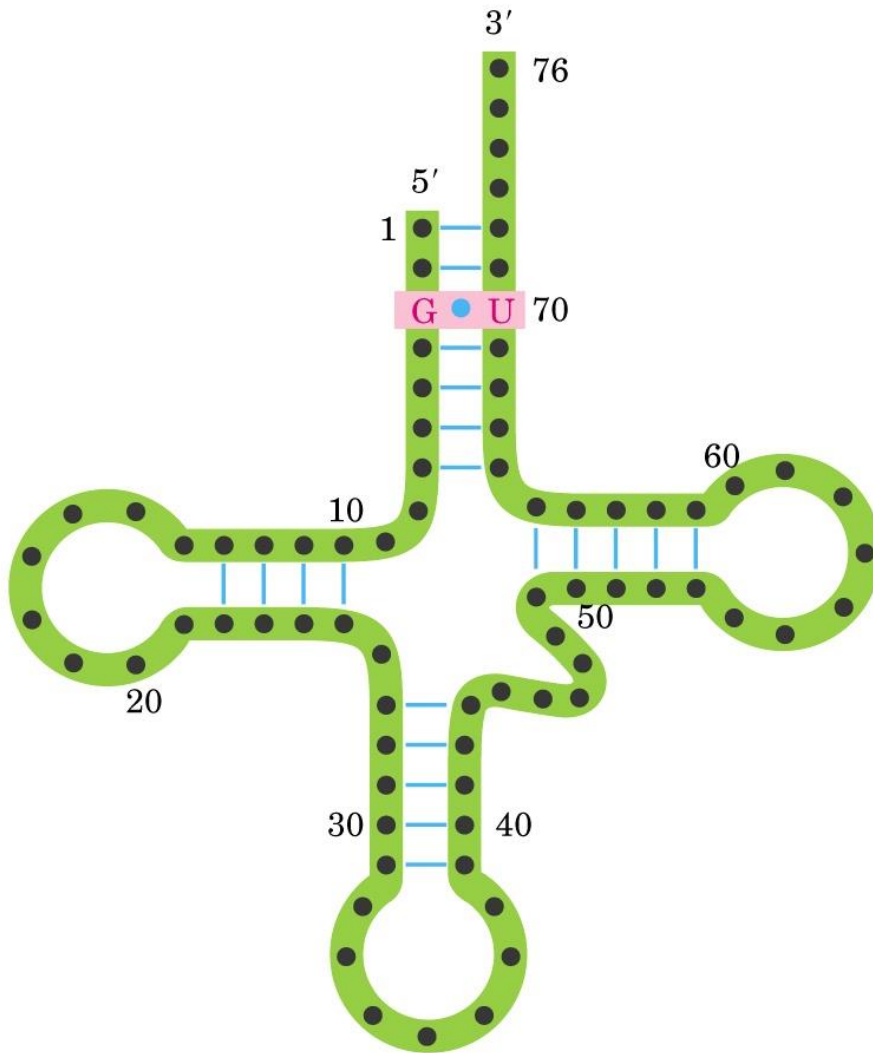
## 2<sup>nd</sup> genetic code: Specificity not only for a single a.a but also to tRNA .

The interaction of aminoacyl-tRNA synthetases and their cognate tRNAs is critical to accurate reading of the genetic code.

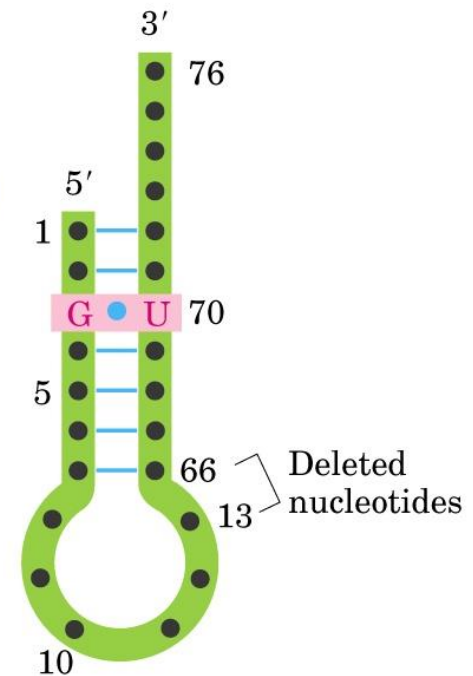


tRNA<sup>ala</sup> :

same efficiency as complete tRNA.

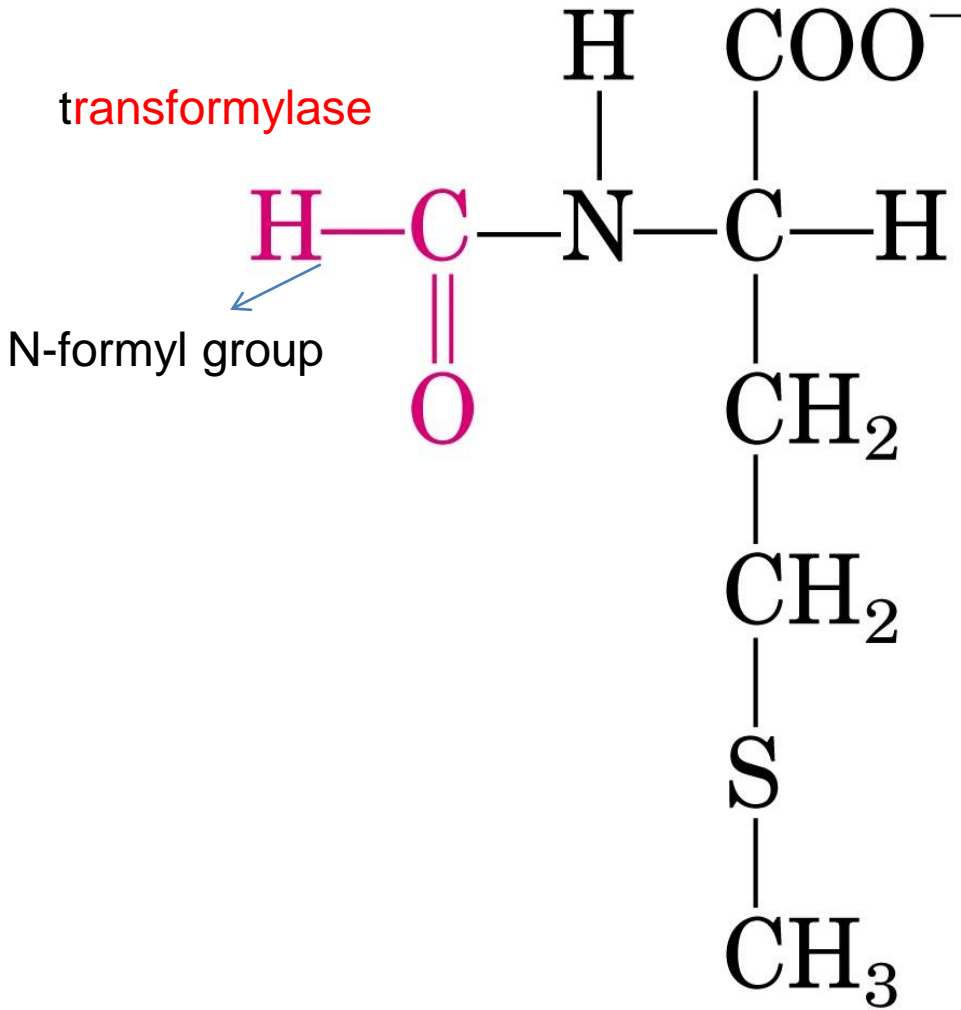


(a) Dr. suheir Ereqat 2019/2020



(b)

**Initiation codon(AUG):**, all organisms have two tRNAs for methionine. One is used exclusively when (5)AUG is the initiation codon for protein synthesis. The other is used to code for a Met residue in an internal position in a polypeptide.



Addition of the N-formyl group to the amino group of methionine by the **transformylase** prevents fMet from entering interior positions in a polypeptide while also allowing fMet-tRNA<sup>fMet</sup> to be bound at a specific ribosomal initiation site that accepts neither Met-tRNA<sup>Met</sup> nor any other aminoacyl-tRNA.

**N-Formylmethionine (Prokaryotes)**

**How can the single (5)AUG codon determine whether a starting N-formylmethionine (or methionine, in eukaryotes) or an interior Met residue is ultimately inserted?**

## Shine Dalgarno sequence:

The mRNA binds to the 30S subunit. The initiating (5)AUG is guided to its correct position by the consensus sequence; **Shine-Dalgarno sequence** (initiation signal)

