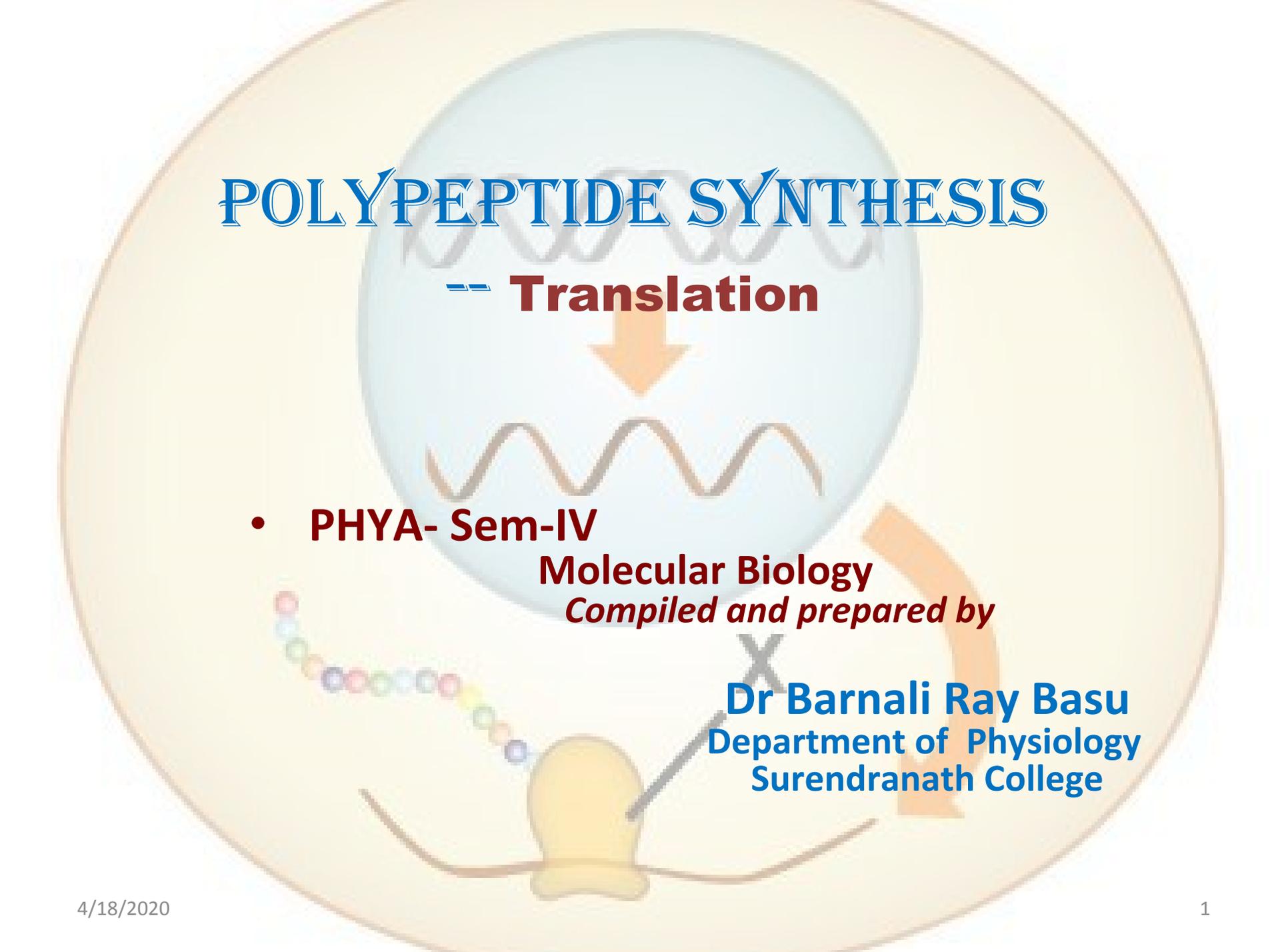


# POLYPEPTIDE SYNTHESIS



-- **Translation**

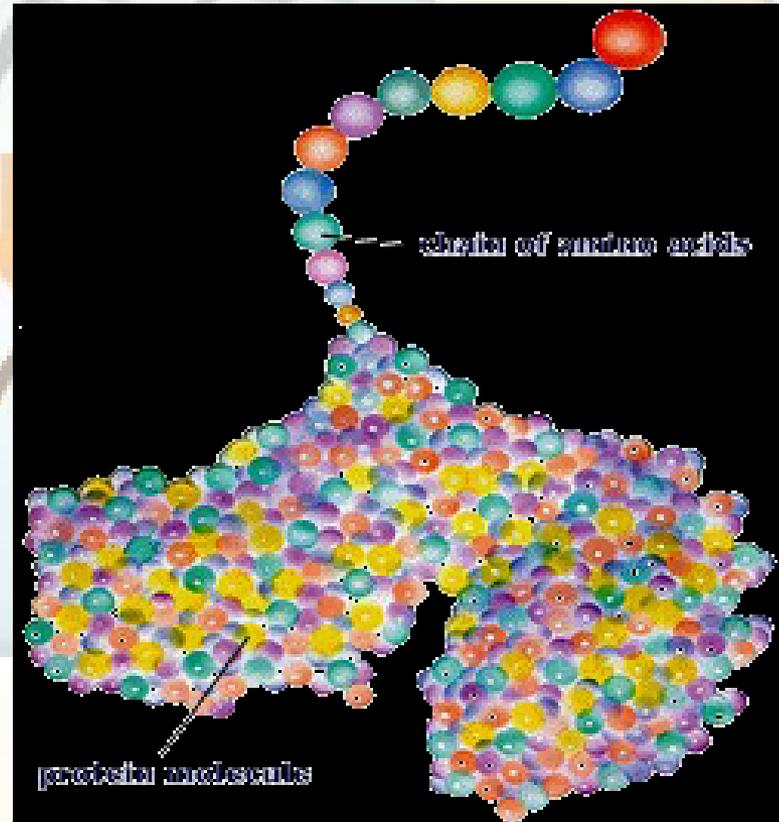
- **PHYA- Sem-IV**

**Molecular Biology**

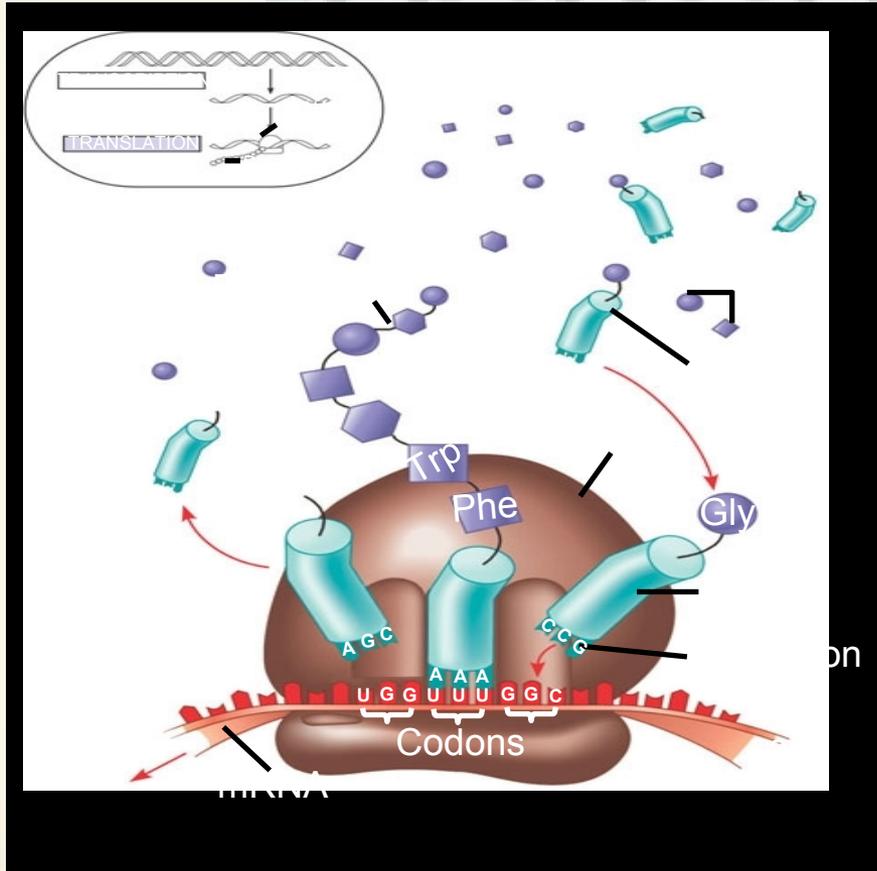
*Compiled and prepared by*

**Dr Barnali Ray Basu**  
Department of Physiology  
Surendranath College

- Proteins are composed of **amino acids** – there are **20** different amino acids
- Different **proteins** are **made by combining these 20** amino acids in different combinations



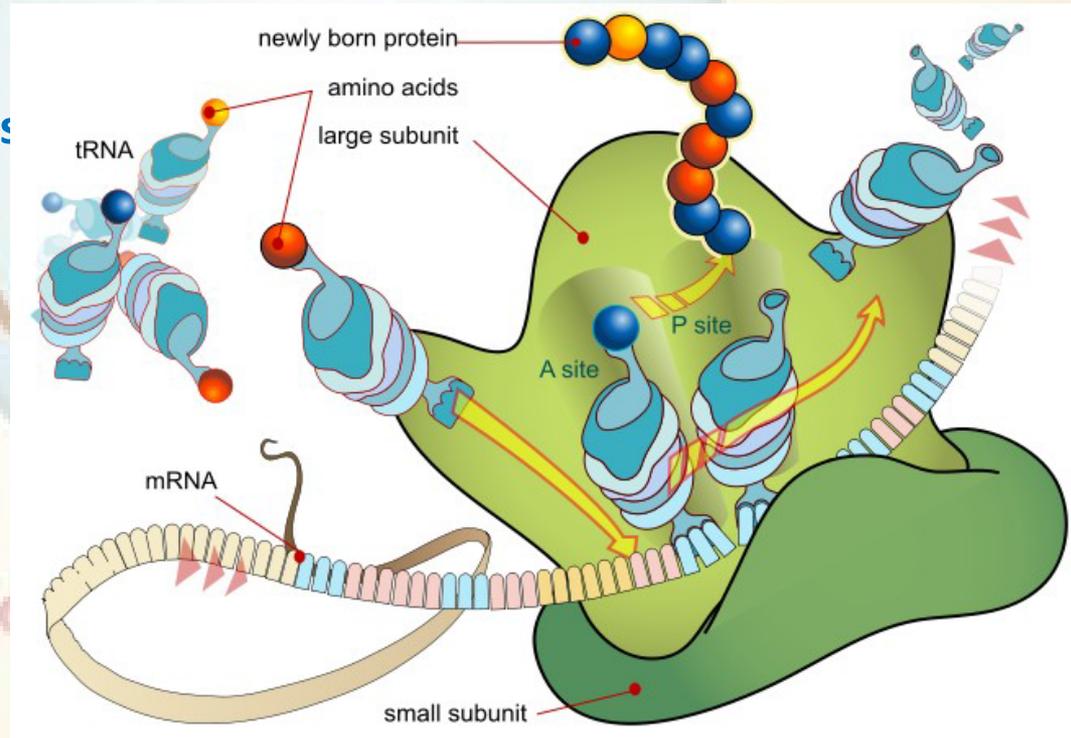
# Translation



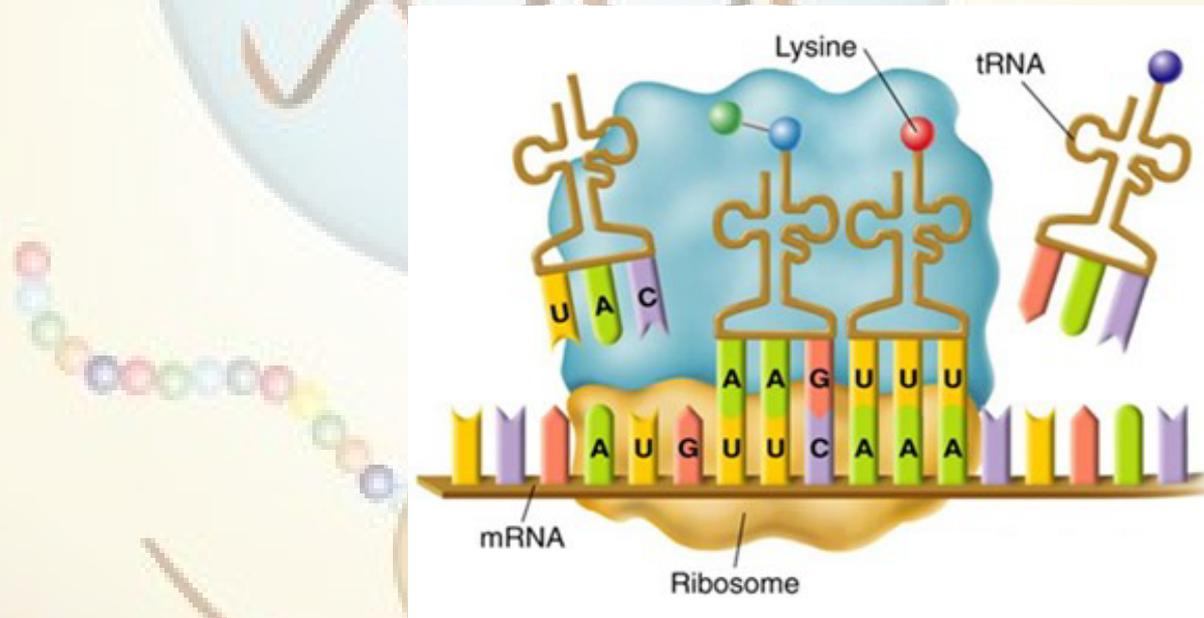
- Translation is the RNA-directed synthesis of a polypeptide

- Translation involves

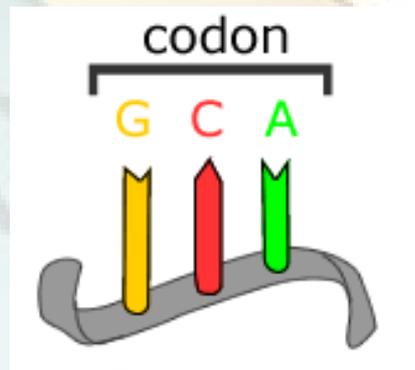
- mRNA
- Ribosomes - Ribosomal RNA
- Transfer RNA
- Genetic coding - codons



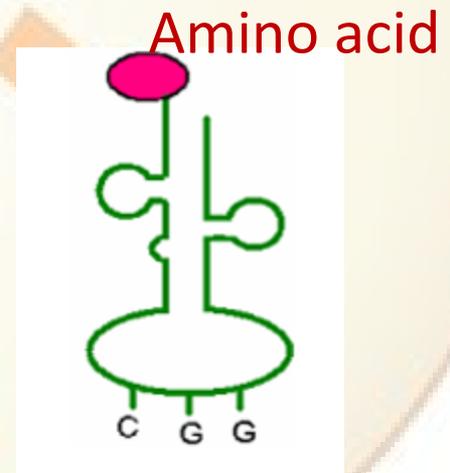
- **Making a Protein—Translation**
- **Decoding of mRNA into a protein is called Translation.**
- **Transfer RNA (tRNA) carries amino acids from the cytoplasm to the ribosome.**



- A series of **three adjacent bases** in an mRNA molecule codes for a specific amino acid—called a **codon**.



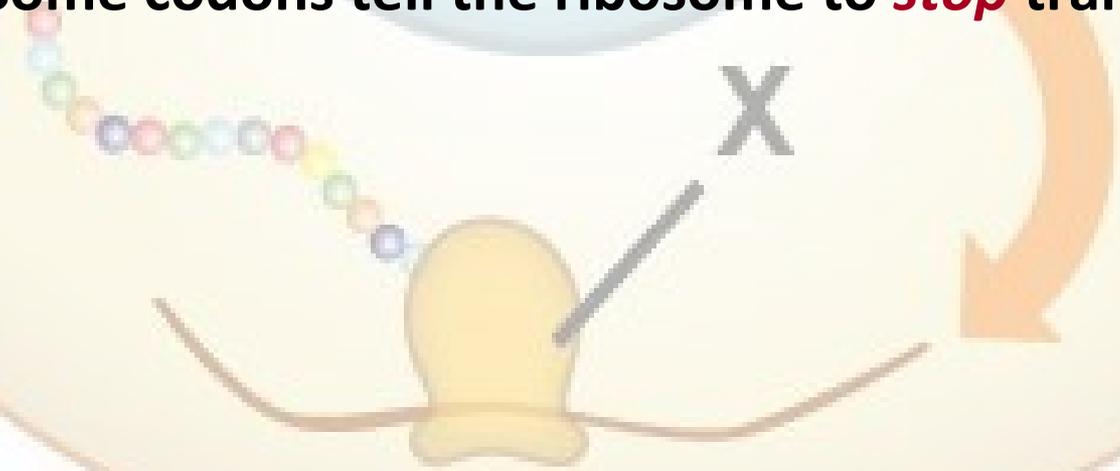
- A triplet of nucleotides in tRNA that is **complementary to the codon in mRNA**—called an **anticodon**.



- Each tRNA codes for a **different** amino acid.

# The Genetic Code

- A **codon** designates an **amino acid**
- An amino acid may have **more than one codon**
- There are 20 amino acids, but **64 possible codons**
- Some codons tell the ribosome to ***stop*** translating



# The genetic code

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

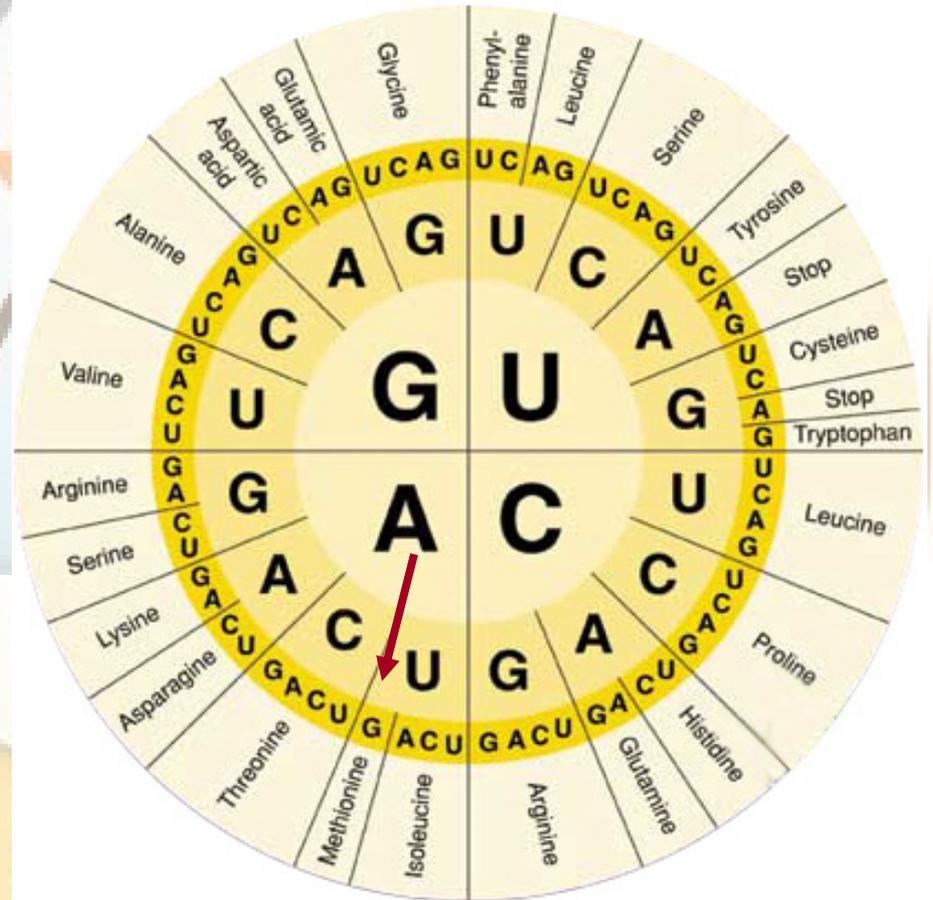
# The Genetic Code

- A codon in messenger RNA is either translated into an amino acid or serves as a translational start/stop signal

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

# The Genetic Code

- Use the code by reading from the **center to the outside**
- Example: **AUG** codes for **Methionine**

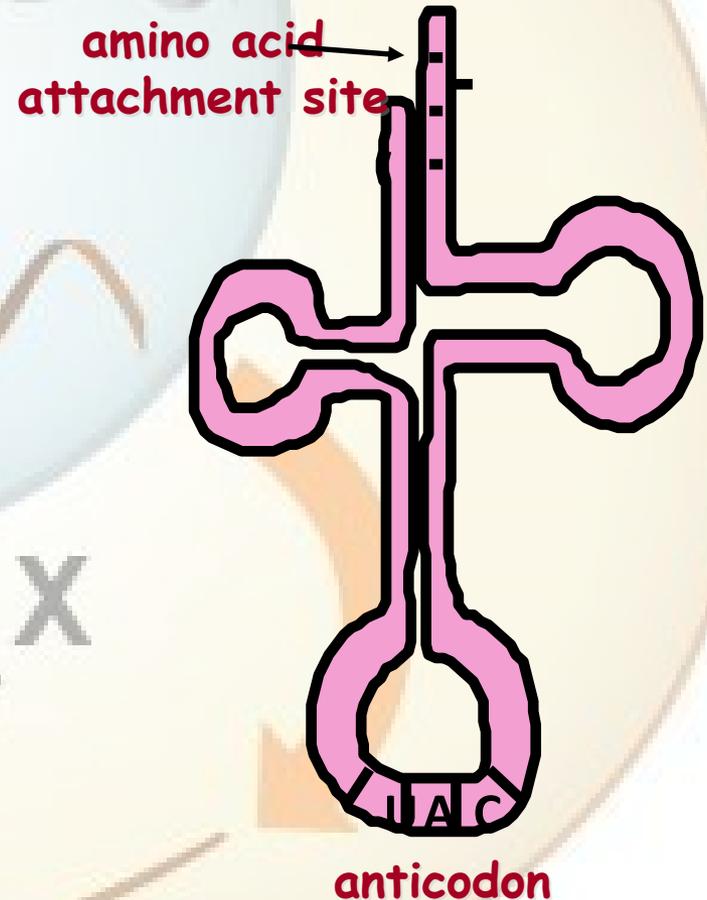


# The Genetic Code

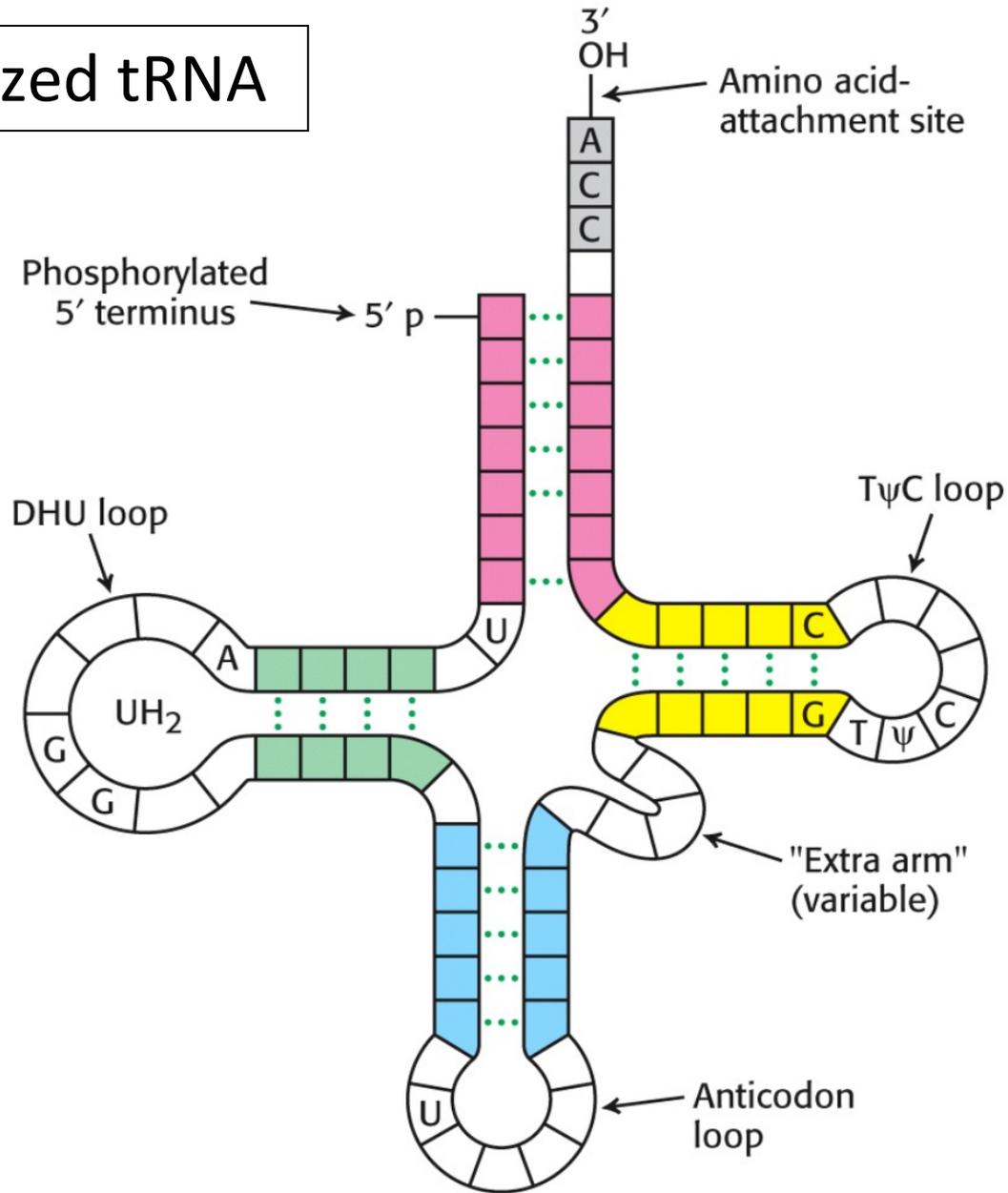
- Codons: 3 base code for the production of a specific amino acid, sequence of three of the four different nucleotides
- Since there are 4 bases and 3 positions in each codon, there are  $4 \times 4 \times 4 = 64$  possible codons
- 64 codons but only 20 amino acids, therefore most have more than 1 codon
- 3 of the 64 codons are used as STOP signals; they are found at the end of every gene and mark the end of the protein
- One codon is used as a START signal: it is at the start of every protein
- Universal: in all living organisms

# Transfer RNA (tRNA)

- **Clover-leaf** shape
- Single stranded molecule with attachment site at one end for an **amino acid**
- Opposite end has three nucleotide bases called the **anticodon**



# Generalized tRNA

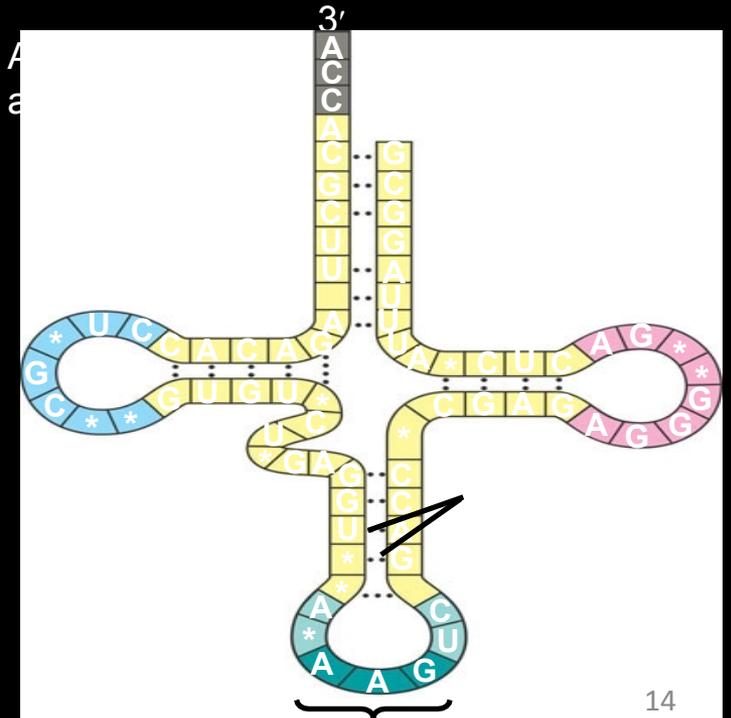


# Transfer RNA

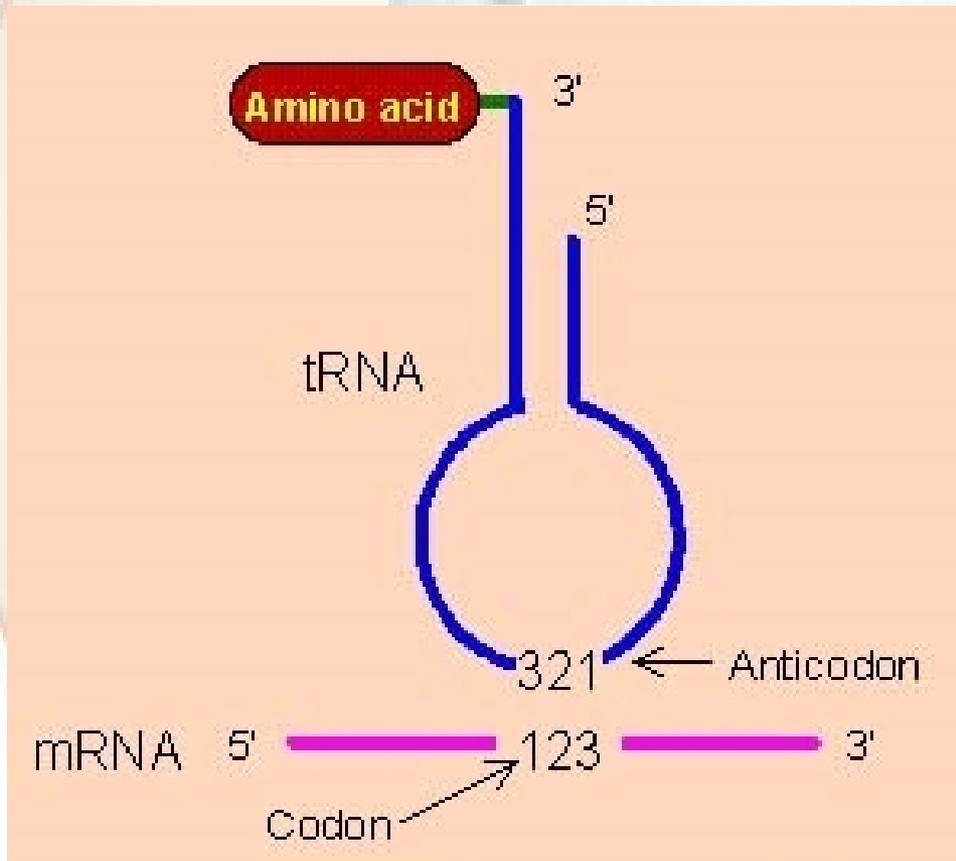
- Consists of a single RNA strand that is only about 80 nucleotides long
- Each carries a specific amino acid on one end and has an anticodon on the other end
- A special group of enzymes pairs up the proper tRNA molecules with their corresponding amino acids.
- tRNA brings the amino acids to the ribosomes,

The “anticodon” is the 3 RNA bases that matches the 3 bases of the codon on the mRNA molecule

**Two-dimensional structure.** The four base-paired regions and three loops are characteristic of all tRNAs, as is the base sequence of the amino acid attachment site at the 3' end. The anticodon triplet is unique to each tRNA type. (The asterisks mark bases that have been chemically modified, a characteristic of tRNA.)



# Codon-anticodon interaction

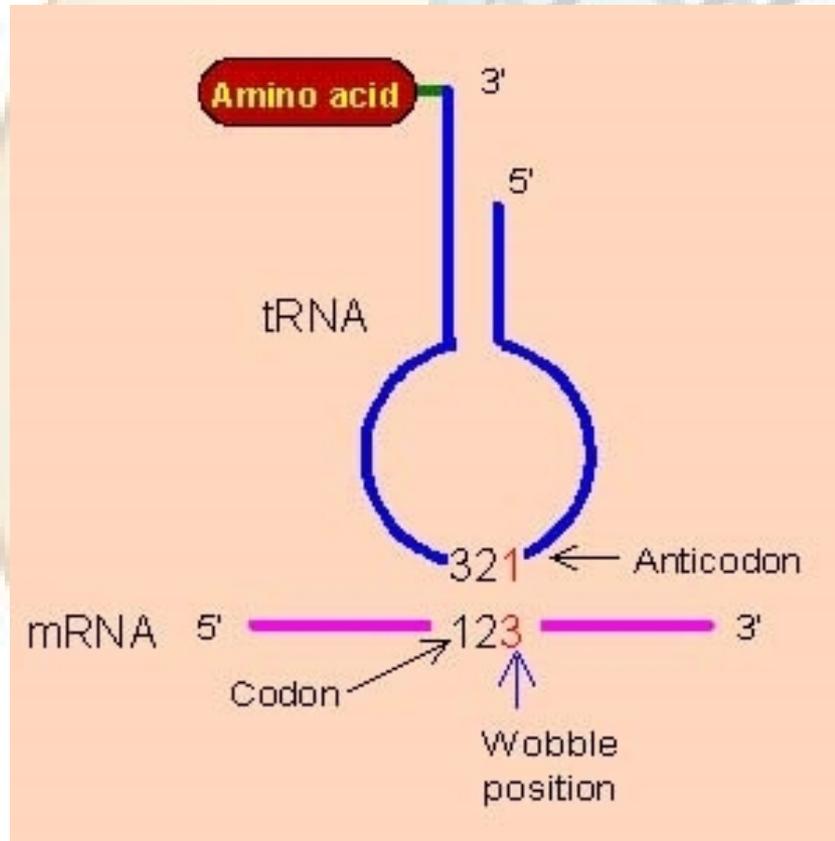


In the cleft of the ribosome, an anti-parallel formation of three base pairs occurs between the codon on the mRNA and the anticodon on the tRNA.

# Wobble

**move or cause to move unsteadily from side to side.**

To explain the redundancy of the genetic code. 18 aa are encoded by more than one triplet codons which usually differ at 5'-anticodin base



5'-anticodon base is able to undergo more movement than the other two bases and can thus form non-standard base pairs as long as the distances between the ribose units are close to normal.

## All possible base pairings at the wobble position

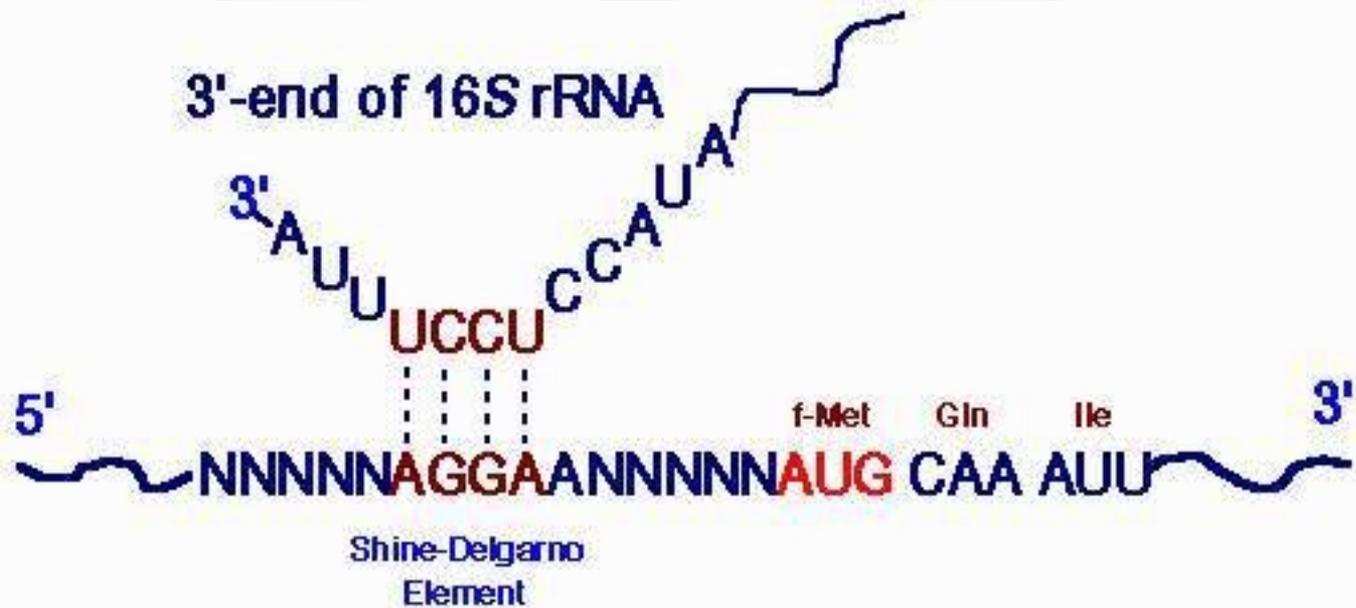
	Wobble bases						Wobble bases			
tRNA	C	A	G	U	I	mRNA	C	A	G	U
mRNA	G	U	C	A	C	tRNA	G	U	C	A
			U	G	A	I	I	U		I
					U					

**U is not found as 5'-anticodon base**

# Ribosome binding site (Shine-Dalgarno sequence)

- Solely for prokaryotic translation
- A purine-rich sequence usually containing all or part of the sequence 5'-AGGAGGU-3'
- Upstream of the initiation codon in prokaryotic mRNA
- To position the ribosome for initiation of protein synthesis

# Shine-Delgarno element

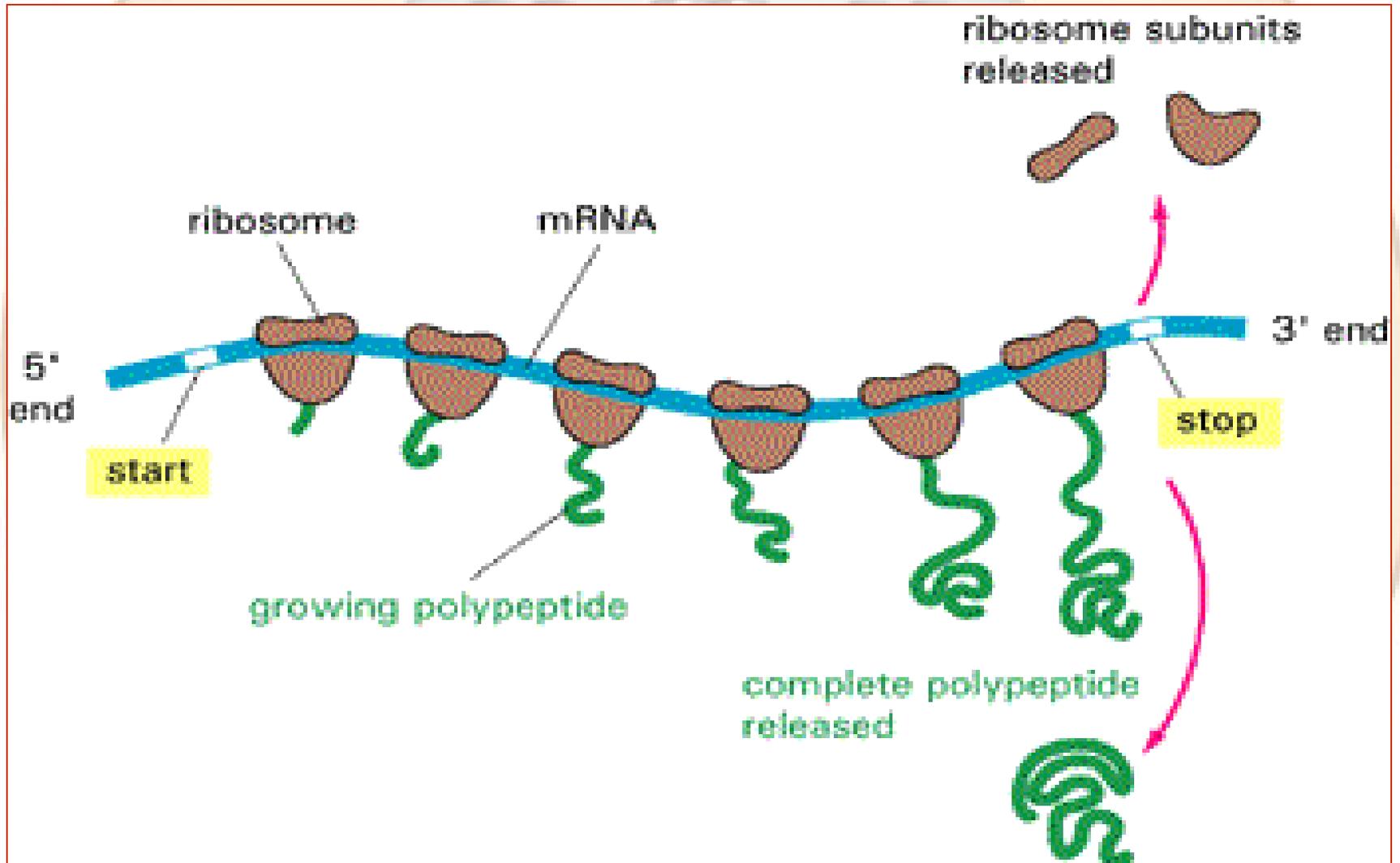


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# Polysomes

- Each mRNA transcript is read simultaneously by more than one ribosome.
- A second, third, fourth, etc. ribosome starts to read the mRNA transcript before the first ribosome has completed the synthesis of one polypeptide chain.
- Multiple ribosomes on a single mRNA transcript are called polyribosomes or polysomes.
- Multiple ribosomes can not be positioned closer than 80 nt.

# Polysomes

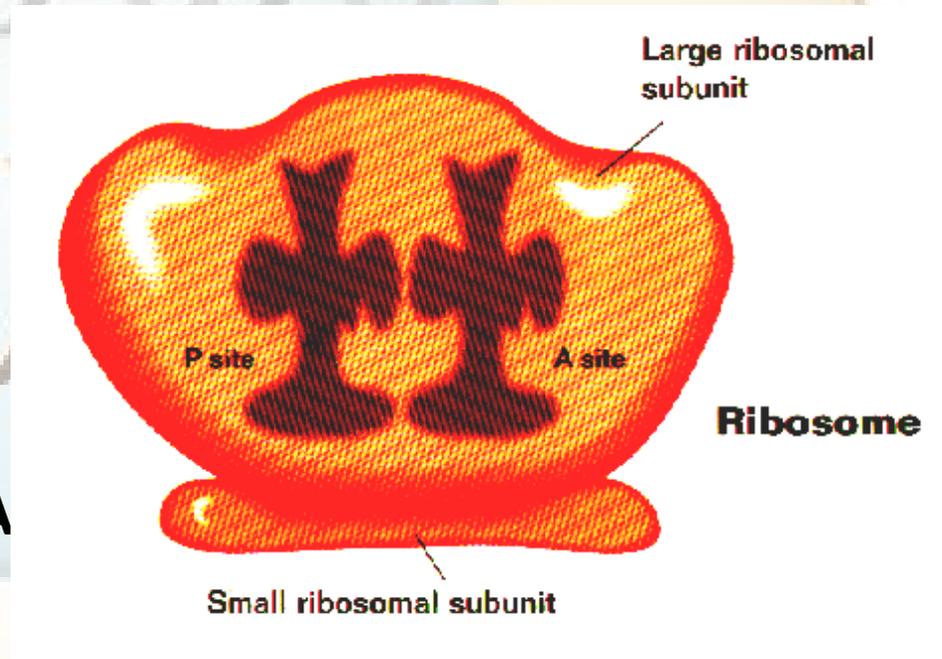


- Electron micrographs of ribosomes actively engaged in protein synthesis revealed by "beads on a string" appearance.



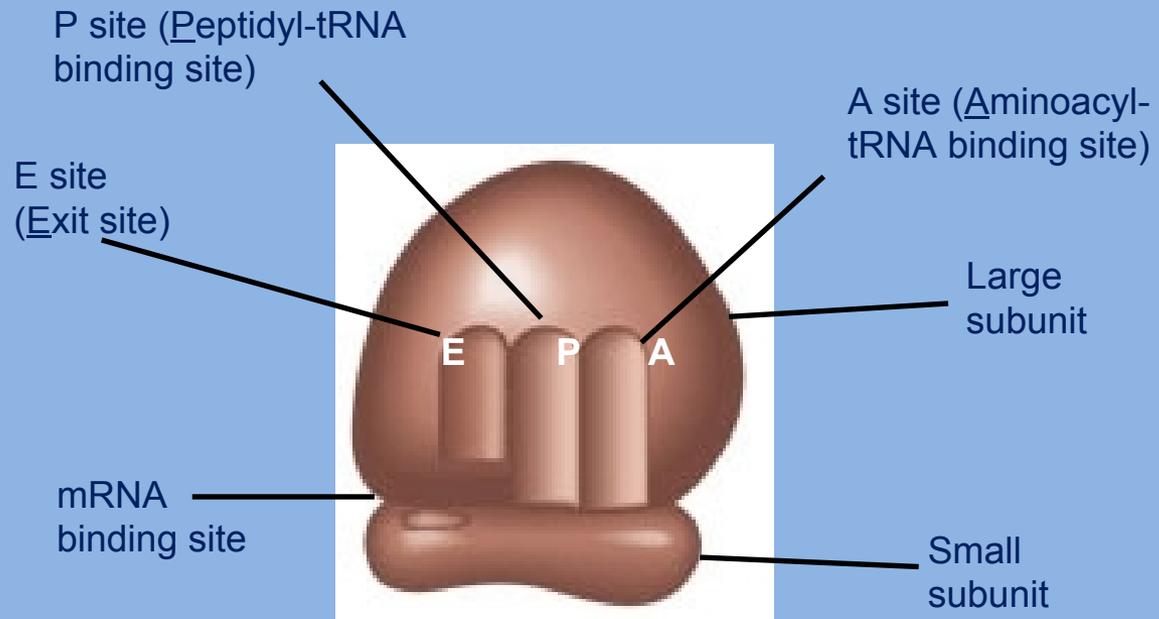
# Ribosomes

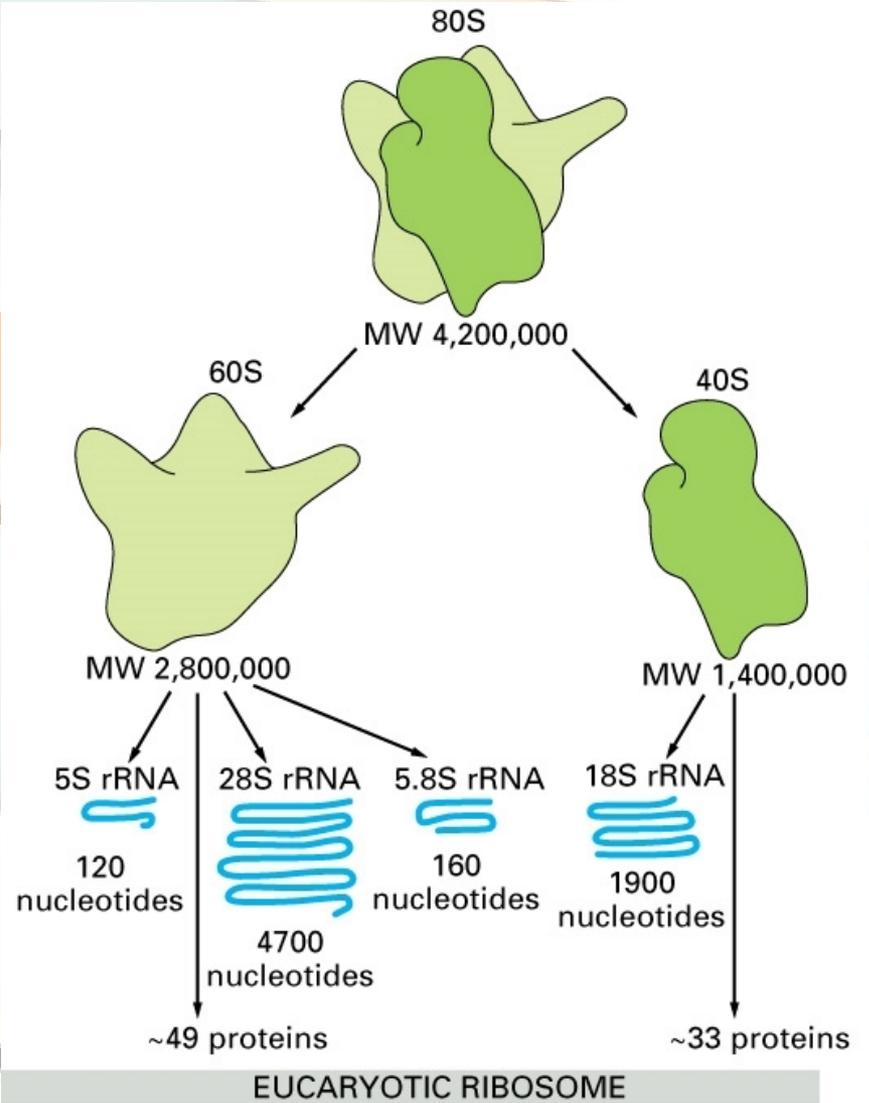
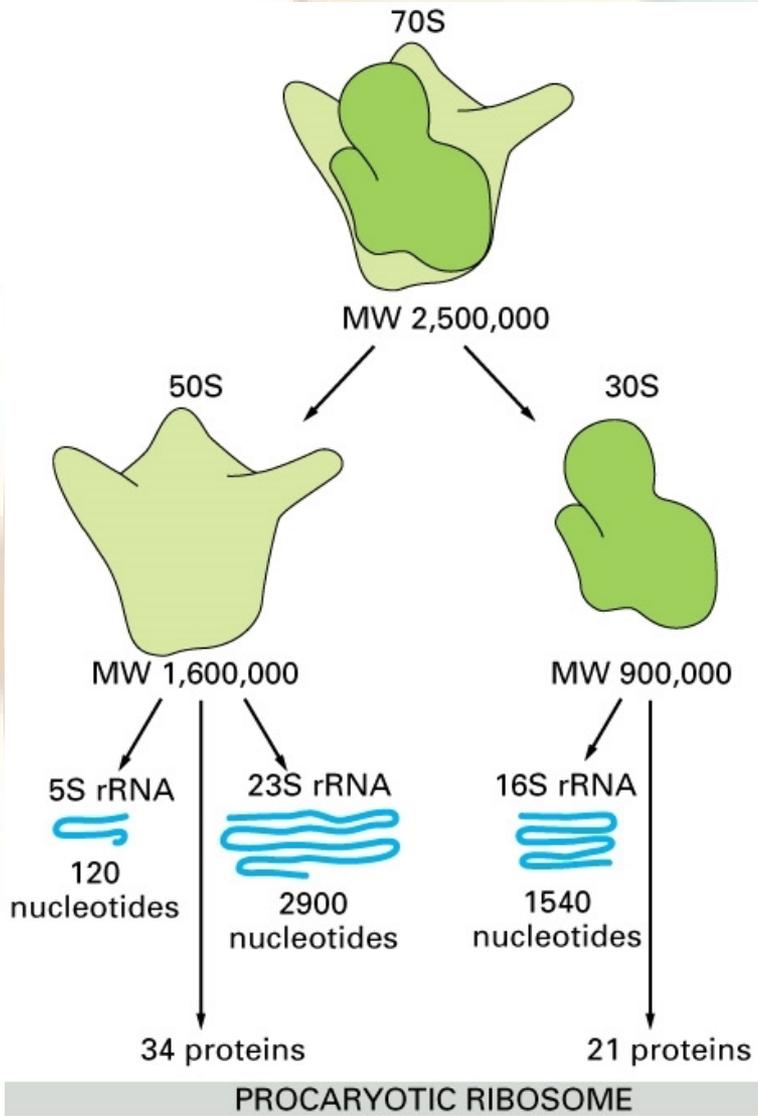
- Made of a **large and small** subunit
- Composed of **rRNA (40%)** and **proteins (60%)**
- Have **two sites** for tRNA attachment --- **P and A**



# Ribosome

- **The Prokaryotic ribosome has three binding sites for tRNA**
  - The P site
  - The A site
  - The E site





and mitochondria

# 23S rRNA secondary structure

(B)

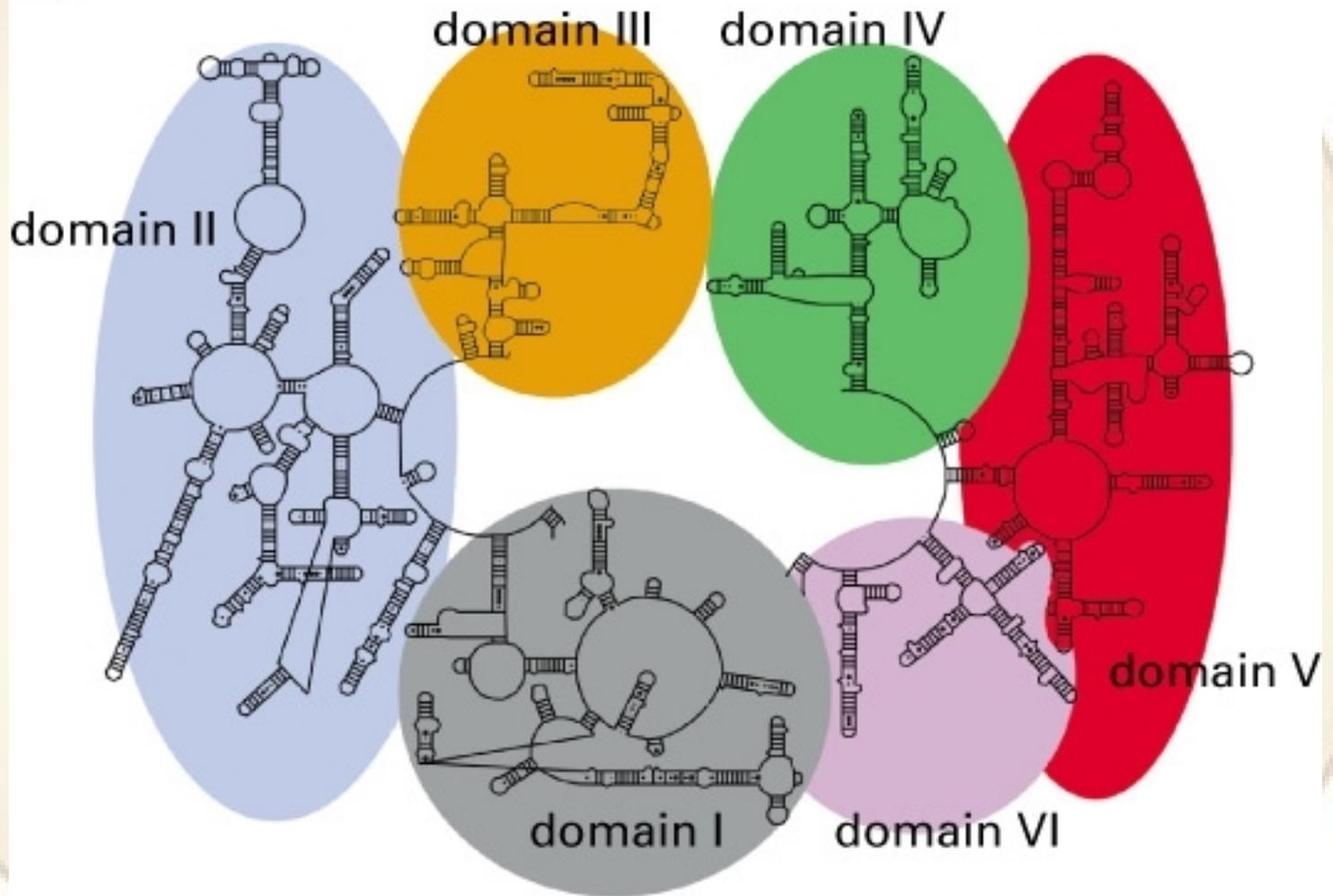


Figure 6-67 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

## 3D organization of the eukaryotic large subunit rRNA

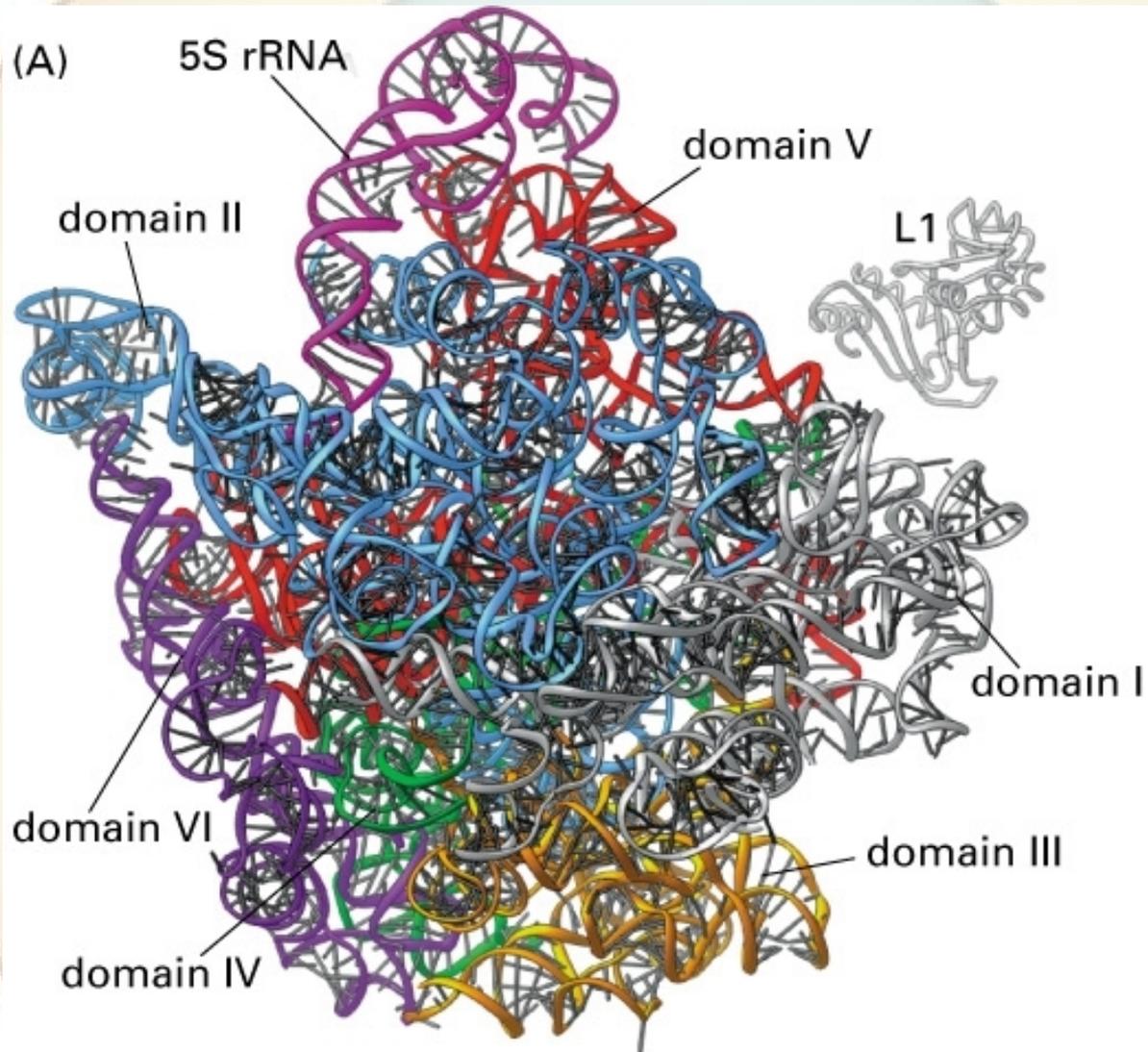
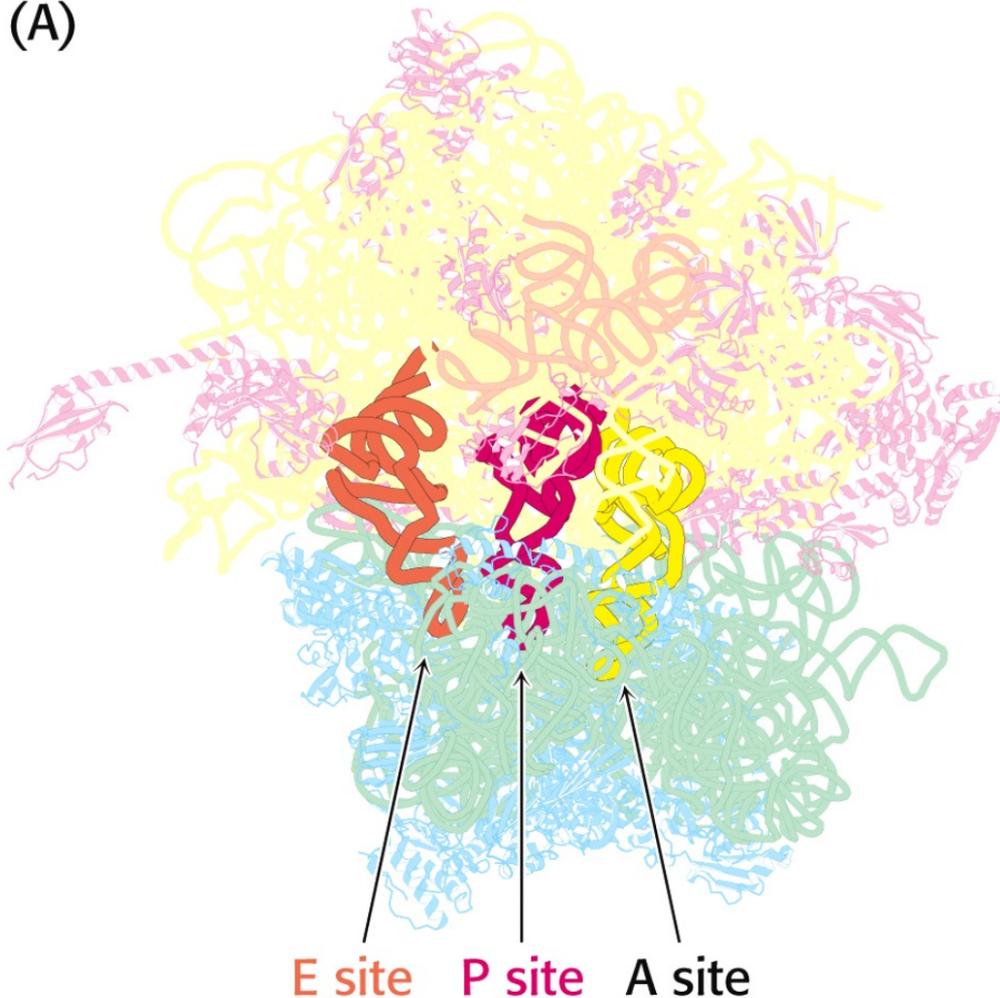


Figure 6-67 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

The association of the large and small subunits creates the structural features on the ribosome that are essential for protein synthesis

(A)



Three tRNA binding sites:

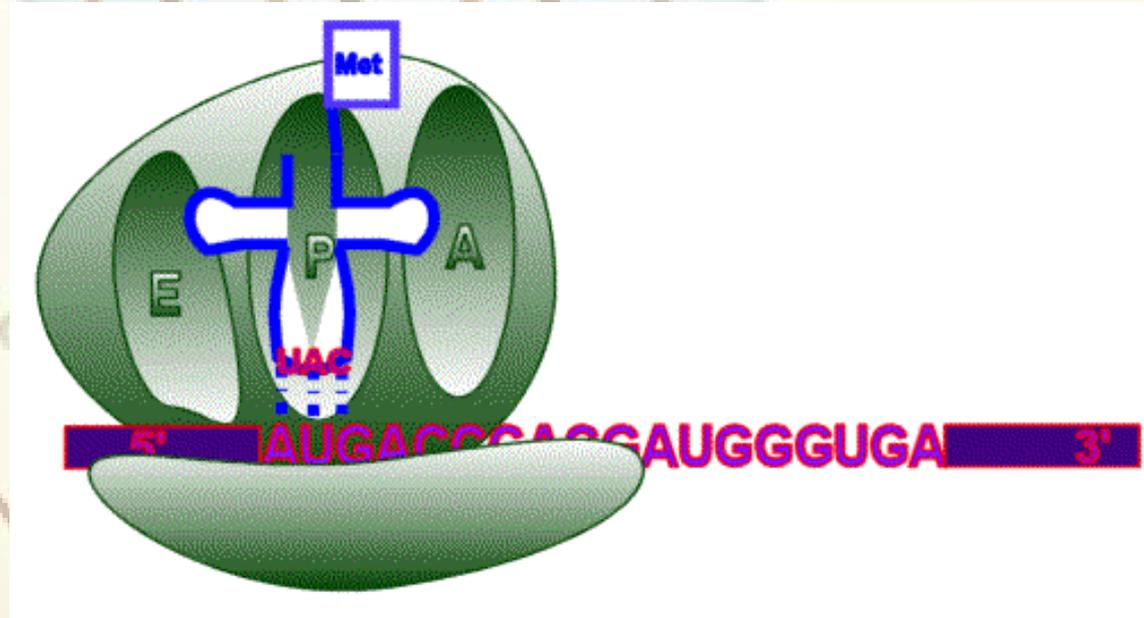
A site = amino-acyl tRNA binding site

P site = peptidyl-tRNA binding site

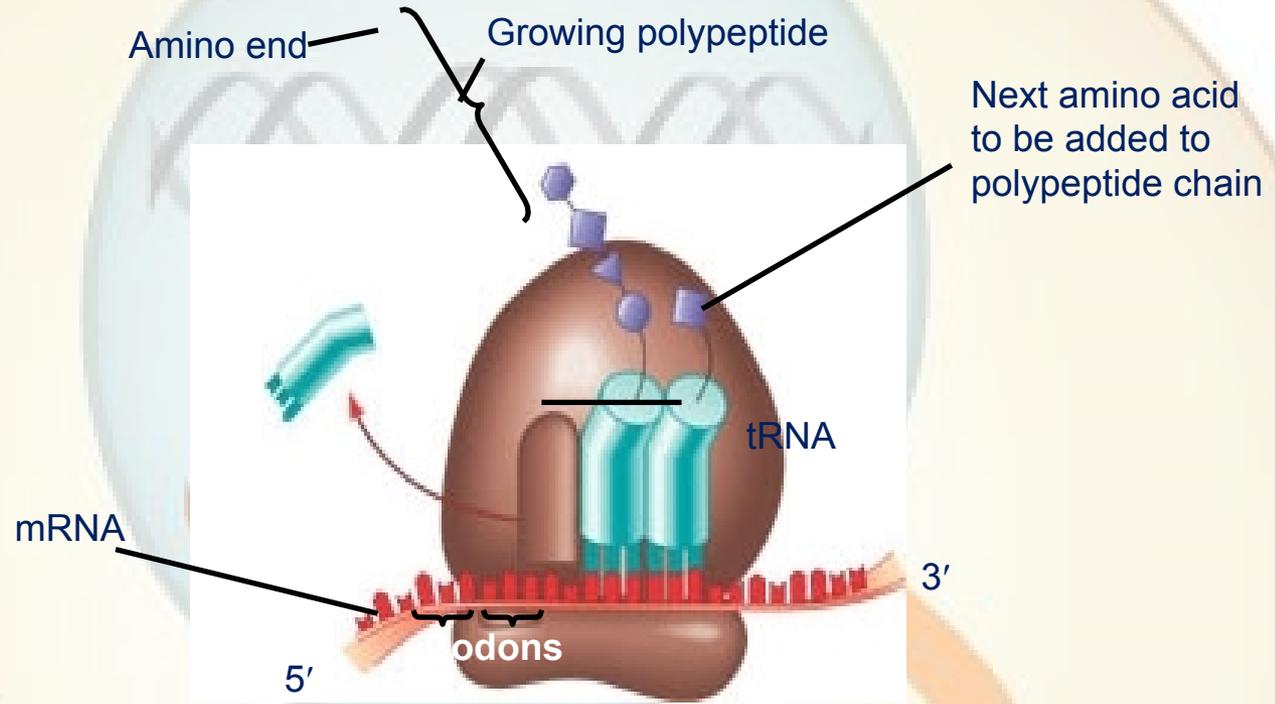
E site = exit site

# Making a protein

- mRNA from nucleus Through cytoplasm to the ribosome
- mRNA start codon AUG signals beginning of protein



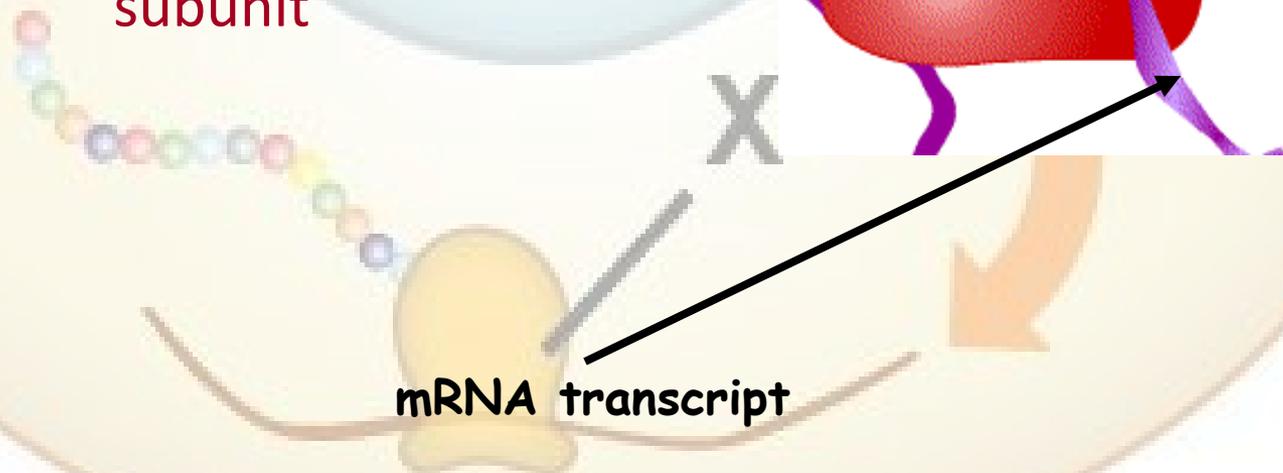
# Building a Polypeptide



Schematic model with mRNA and tRNA. A tRNA fits into a binding site when its anticodon base-pairs with an mRNA codon. The P site holds the tRNA attached to the growing polypeptide. The A site holds the tRNA carrying the next amino acid to be added to the polypeptide chain. Discharged tRNA leaves via the E site.

## Step 1- Initiation

- mRNA **transcript** start codon **AUG** attaches to the **small ribosomal subunit**
- Small subunit attaches to **large ribosomal subunit**



# Ribosomes

Large subunit

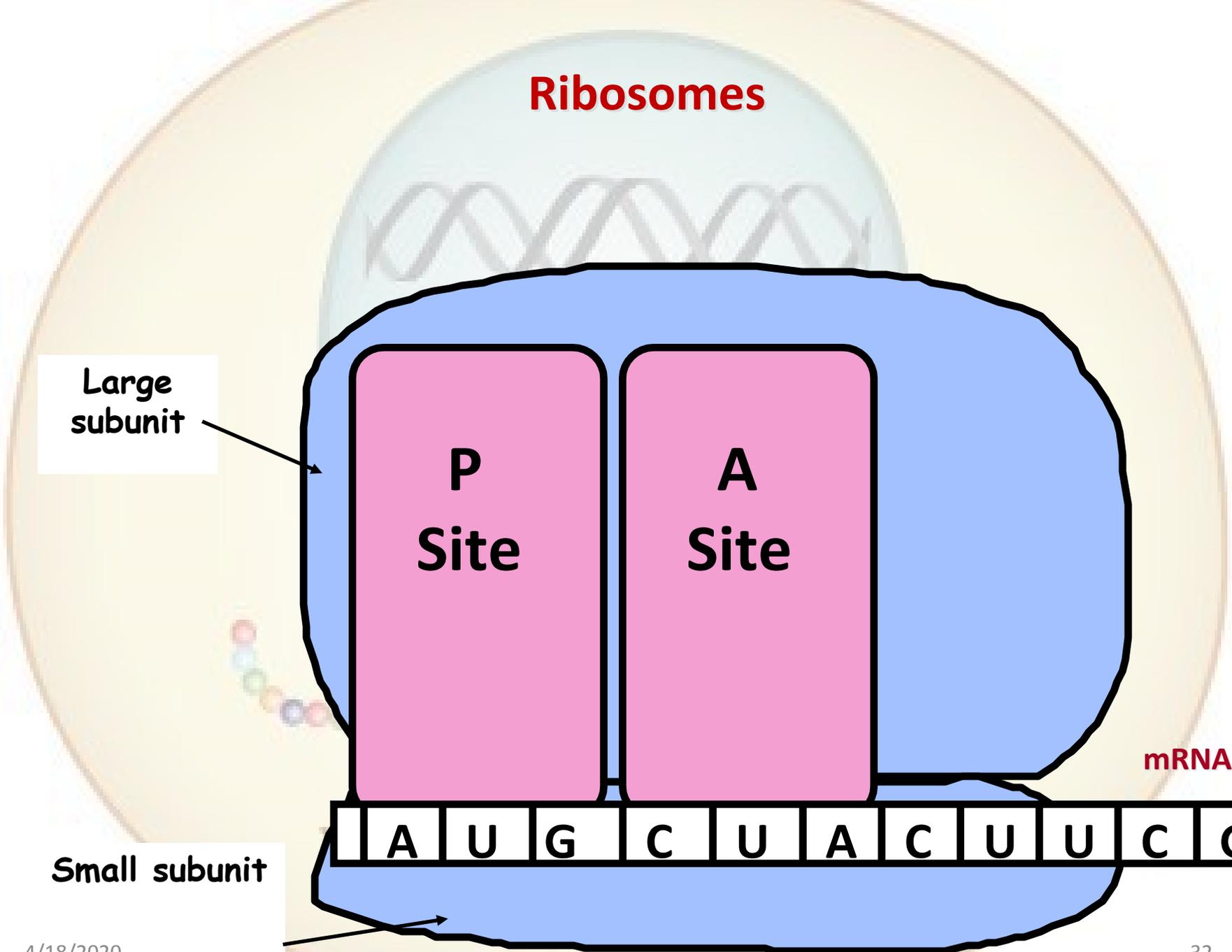
P Site

A Site

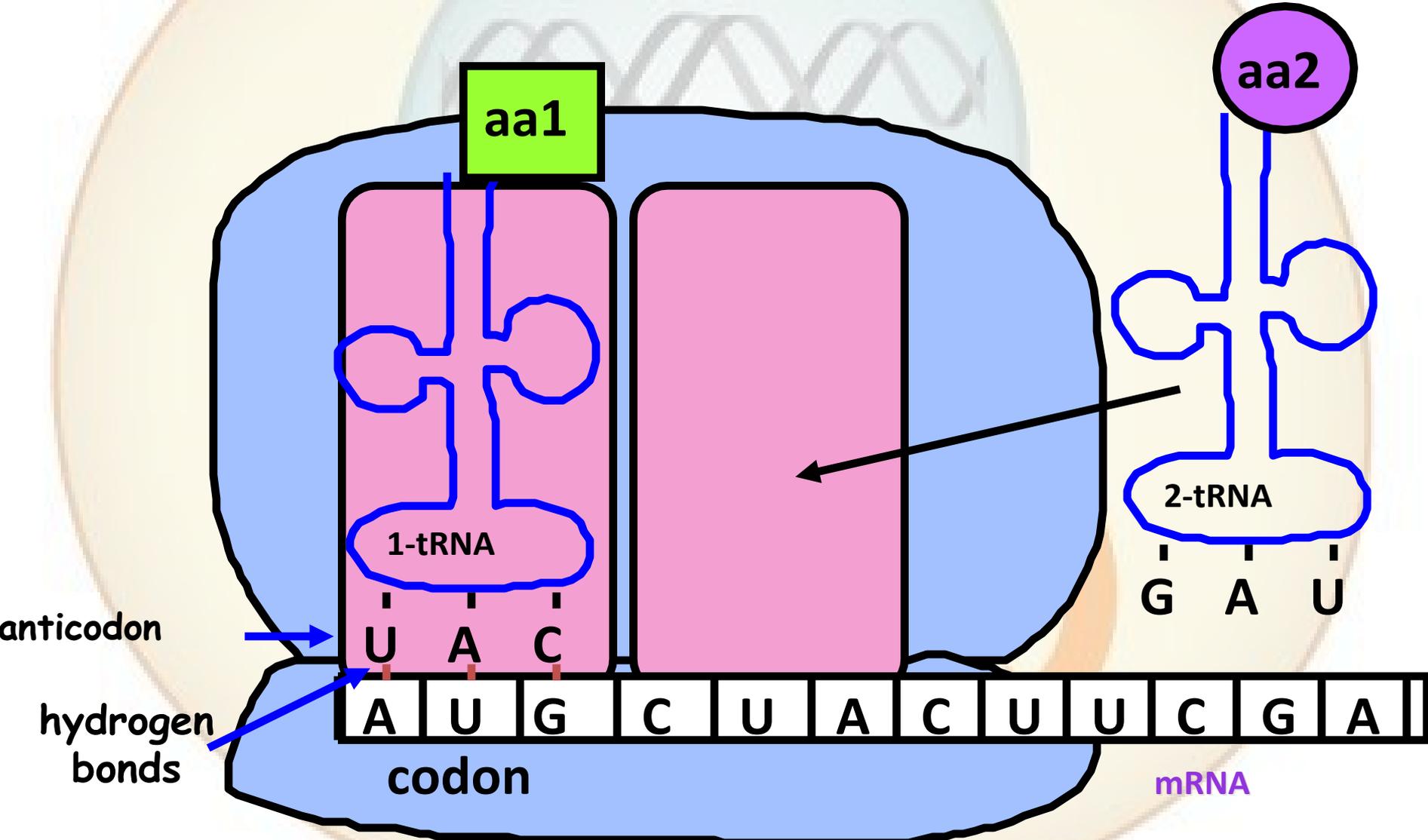
mRNA

Small subunit

A U G C U A C U U C G



# Initiation



## Building a Molecule of tRNA

- A specific enzyme called an aminoacyl-tRNA synthetase joins each amino acid to the correct tRNA

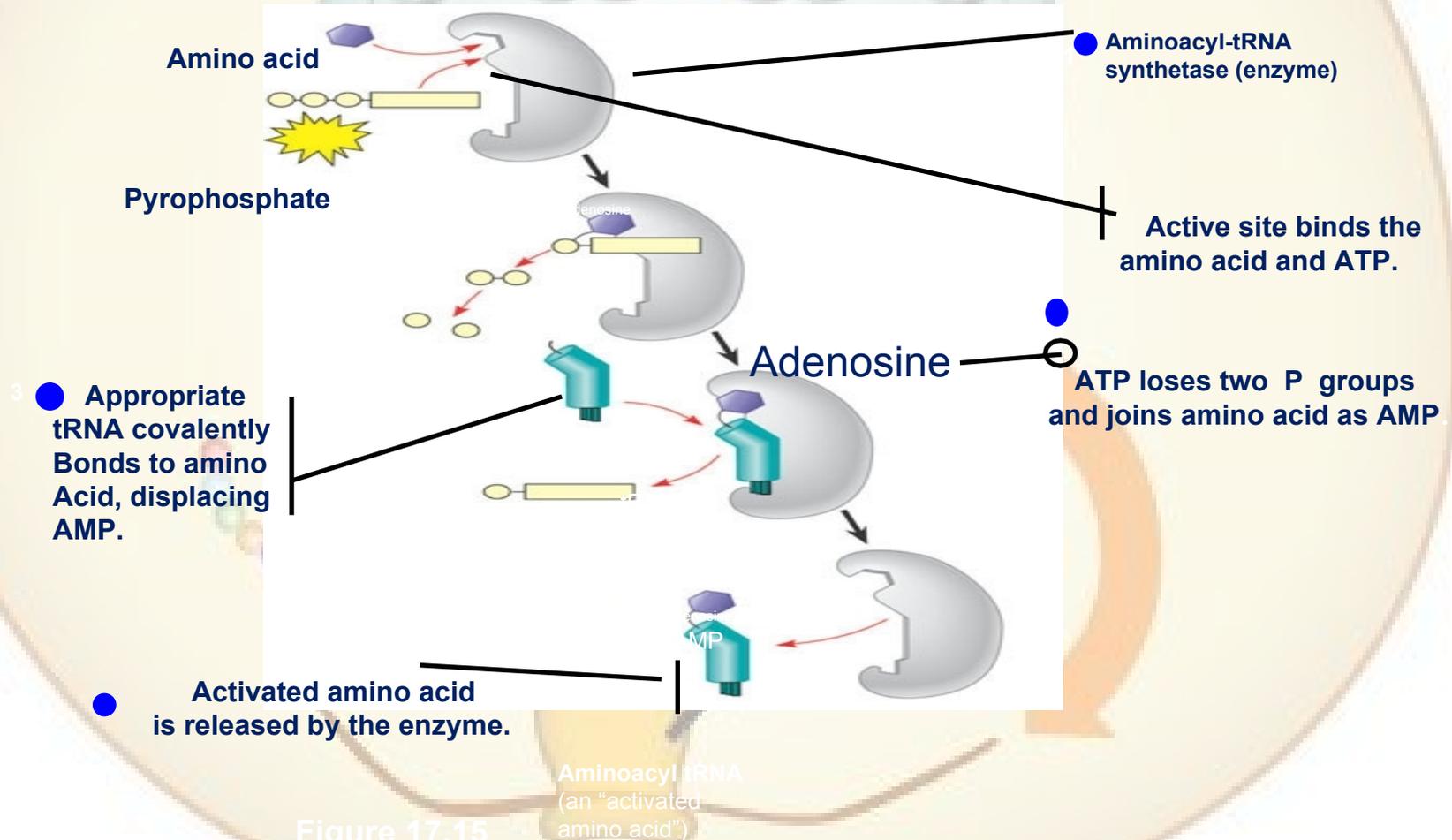


Figure 17.15

## tRNAs are activated by amino-acyl tRNA synthetases

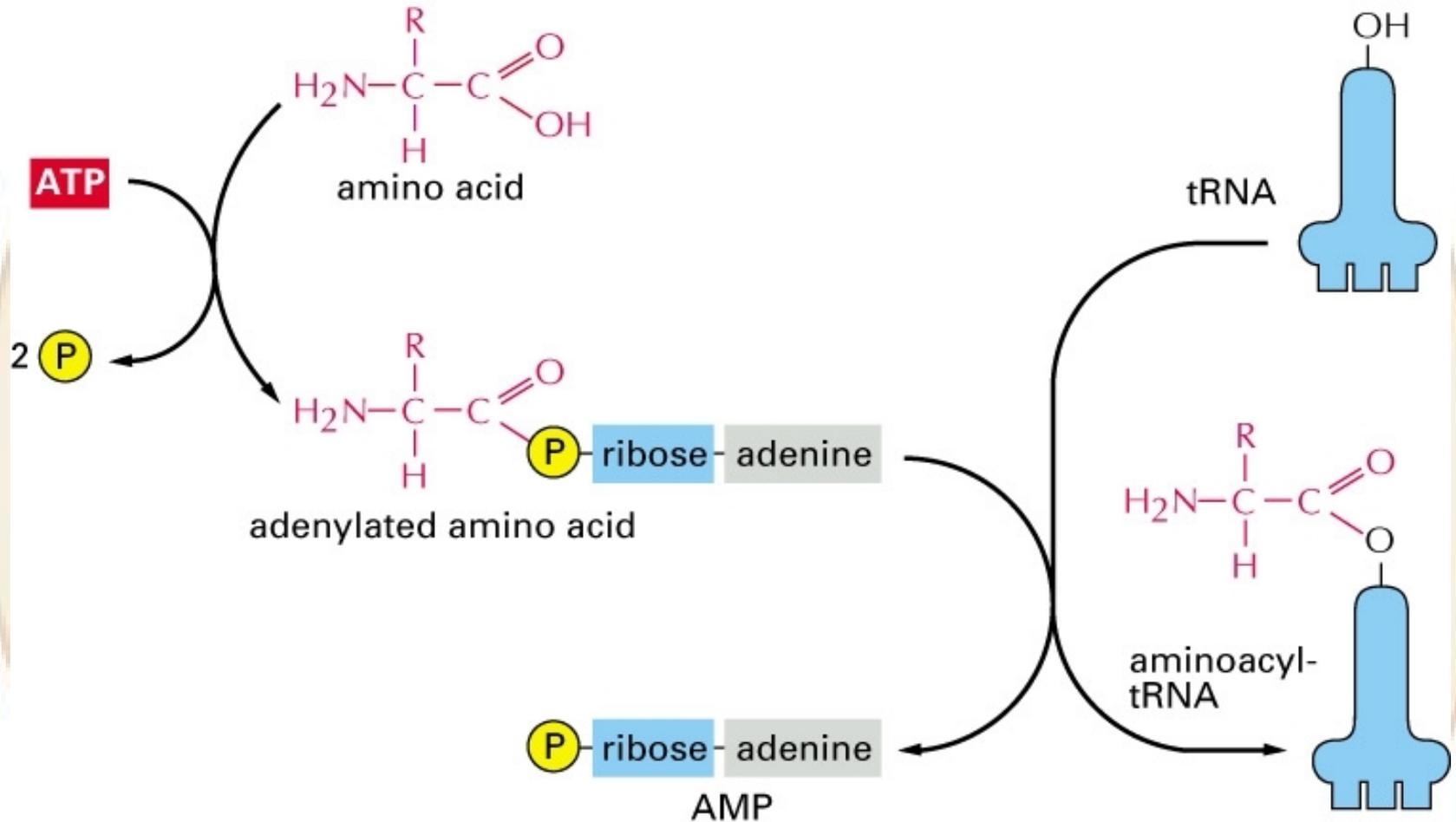
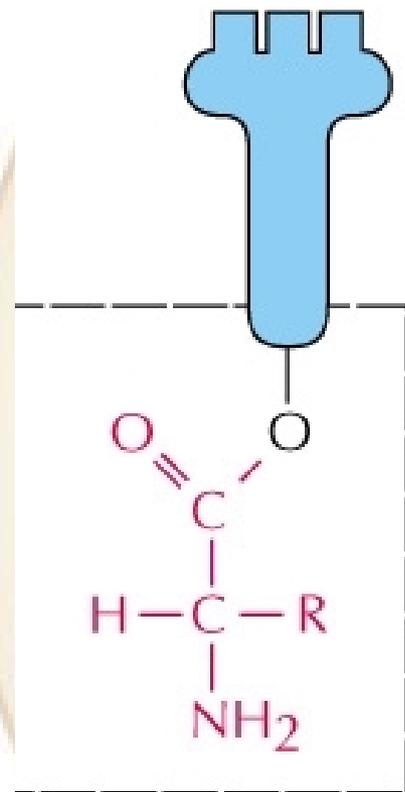


Figure 6-56. Molecular Biology of the Cell, 4th Edition.

(A)

aminoacyl-  
tRNA



(B)

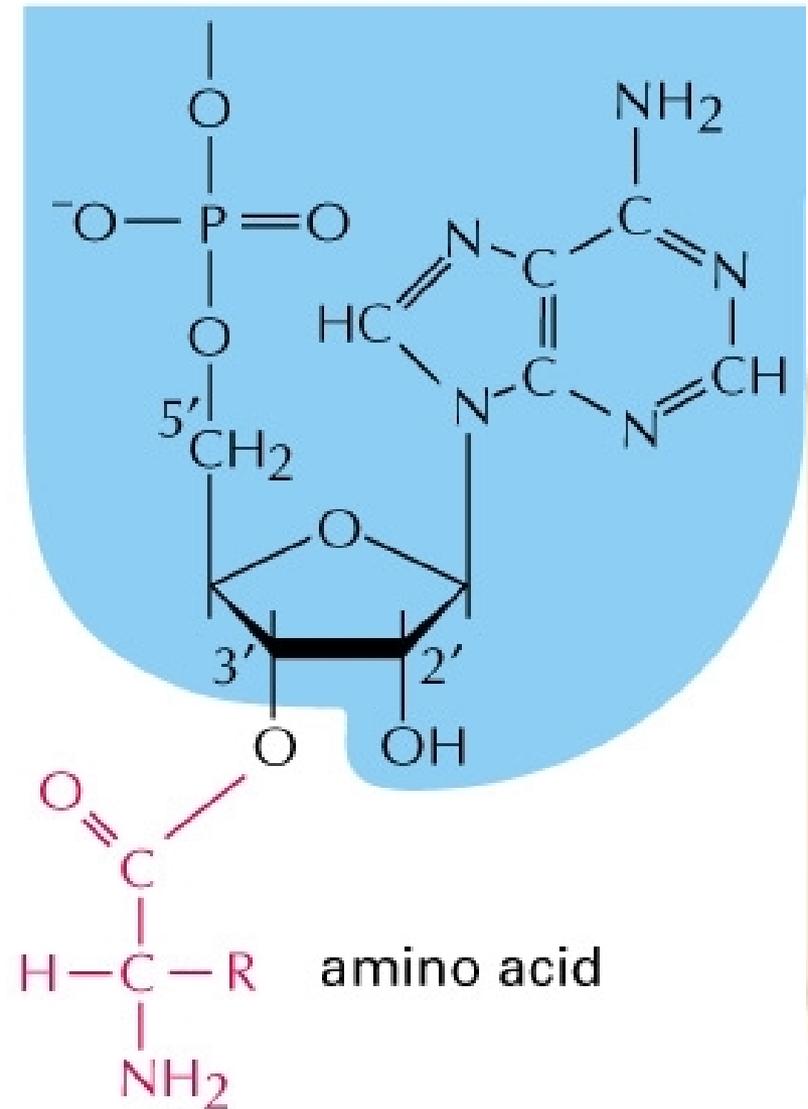


Figure 6-57. Molecular Biology of the Cell, 4th Edition.

## Structure of an amino acyl-tRNA synthetase bound to a tRNA

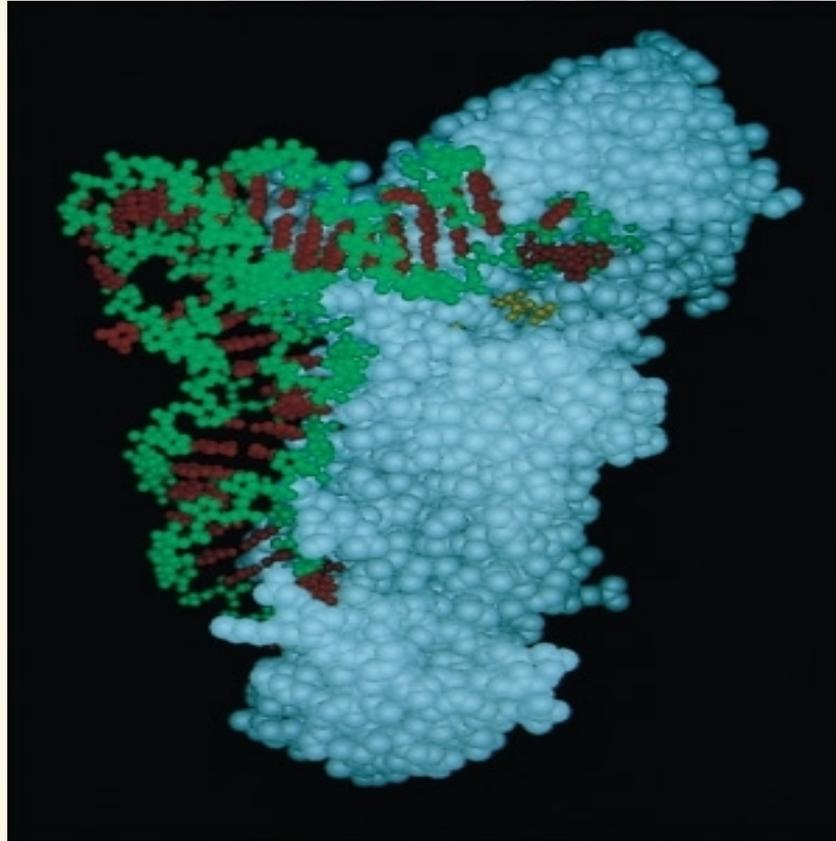


Figure 6–60. Molecular Biology of the Cell, 4th Edition.

**One mechanism for maintaining high fidelity of protein synthesis is the high fidelity of aa-tRNA synthetases**

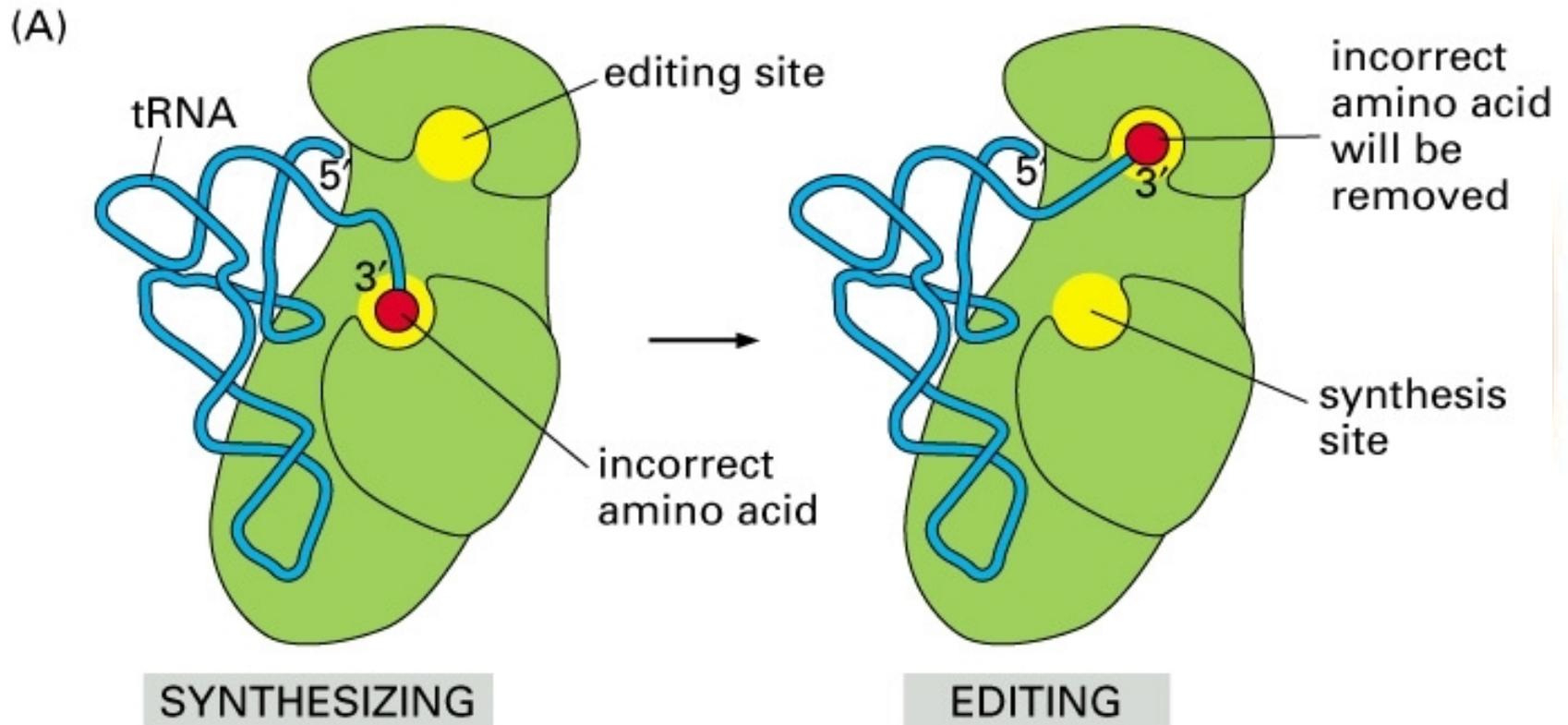
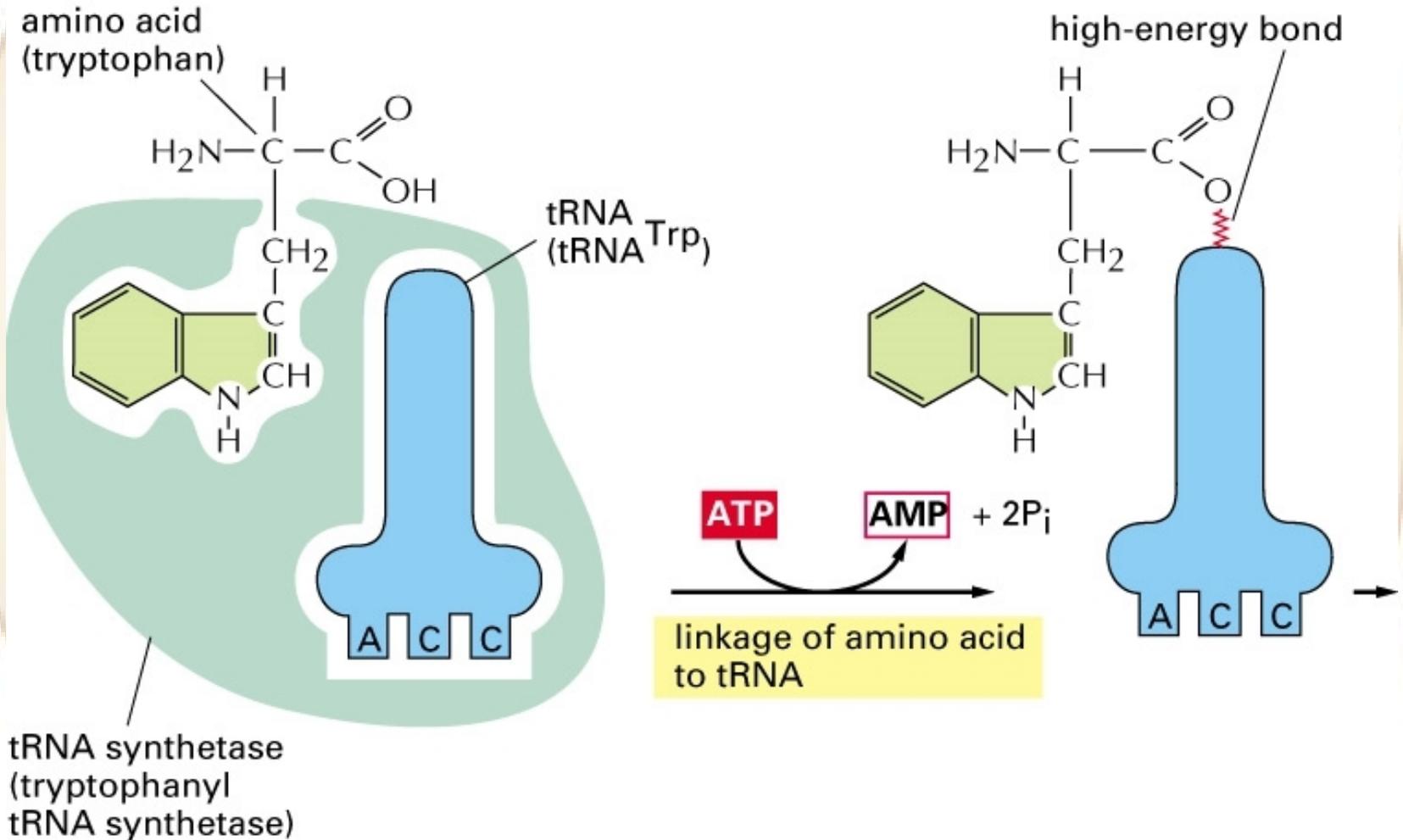


Figure 6-59 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

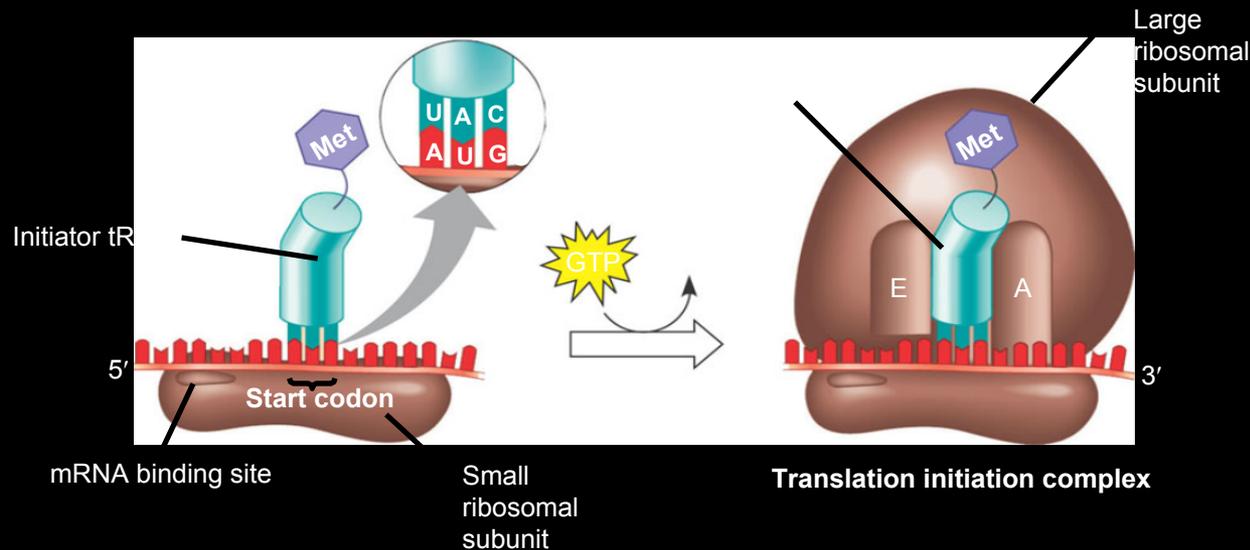
Two levels of control to ensure that the proper amino acid is incorporated into protein: 1) Charging of the proper tRNA





# Initiation of Translation

- The initiation stage of translation brings together mRNA, tRNA bearing the first amino acid of the polypeptide, and two subunits of a ribosome



**1** A small ribosomal subunit binds to a molecule of mRNA. In a prokaryotic cell, the mRNA binding site on this subunit recognizes a specific nucleotide sequence on the mRNA just upstream of the start codon. An initiator tRNA, with the anticodon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine (Met).

**2** The arrival of a large ribosomal subunit completes the initiation complex. Proteins called initiation factors (not shown) are required to bring all the translation components together. GTP provides the energy for the assembly. The initiator tRNA is in the P site; the A site is available to the tRNA bearing the next amino acid.

- ◆ As **mRNA** binds, **IF-3** helps to correctly position the complex such that the  $tRNA^{fMet}$  interacts via base pairing with the mRNA initiation codon (AUG). A region of mRNA upstream of the initiation codon, the **Shine-Dalgarno sequence**, base pairs with the 3' end of the **16S rRNA**. This positions the 30S ribosomal subunit in relation to the initiation codon.

- ◆ As the **large ribosomal subunit** joins the complex, **GTP** on IF-2 is **hydrolyzed**, leading to dissociation of IF-2-GDP and dissociation of IF-1. A domain of the **large ribosomal subunit** serves as **GAP** (GTPase activating protein) **for IF-2**.

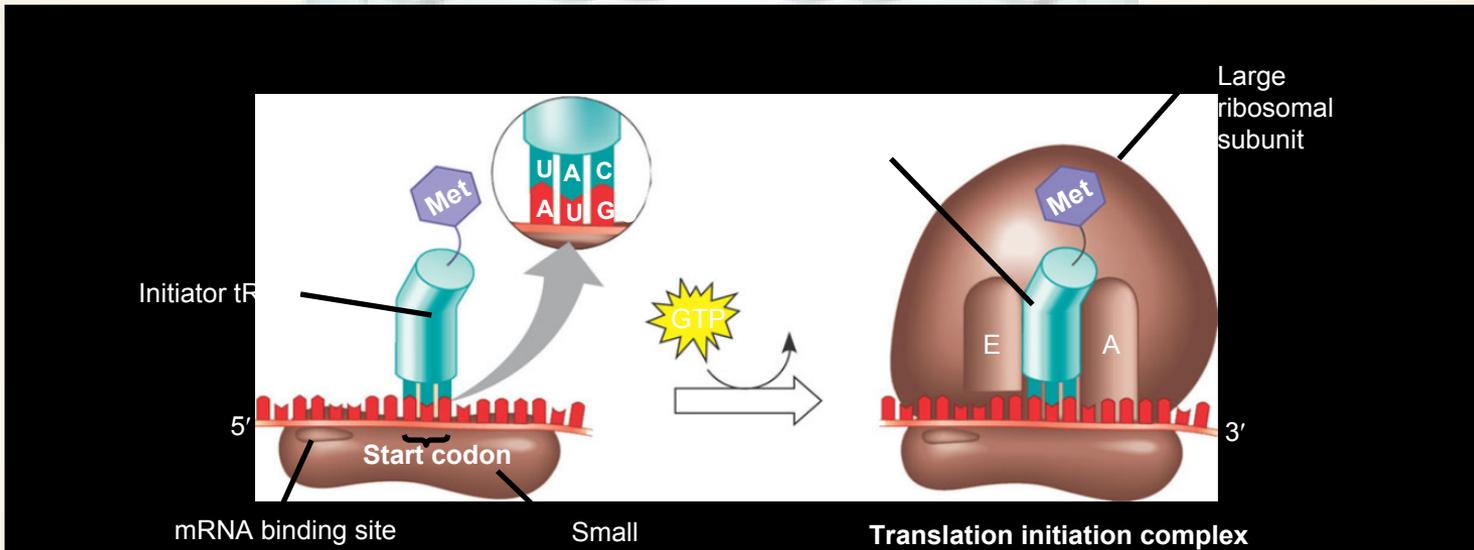
- ◆ Once the two ribosomal subunits come together, the **mRNA** is threaded through a curved channel that wraps around the "neck" region of the small subunit.

**Initiation of protein synthesis in E. coli requires initiation factors IF-1, IF-2, & IF-3.**

- ◆ **IF-3** binds to the **30S ribosomal subunit**, freeing it from its complex with the 50S subunit.
- ◆ **IF-1** assists binding of **IF-3** to the 30S ribosomal subunit. **IF-1** also **occludes the A site** of the small ribosomal subunit, helping insure that the initiation aa-tRNA  $fMet$ -tRNA<sup>fMet</sup> can bind only in the P site & that no other aa-tRNA can bind in the A site during initiation.
- ◆ **IF-2** is a small **GTP-binding** protein. **IF-2-GTP** binds the initiator **fMet-tRNA<sup>fMet</sup>** & helps it to dock with the small ribosome subunit.

# Initiation of Translation

- The initiation stage of translation brings together mRNA, tRNA bearing the first amino acid of the polypeptide, and two subunits of a ribosome

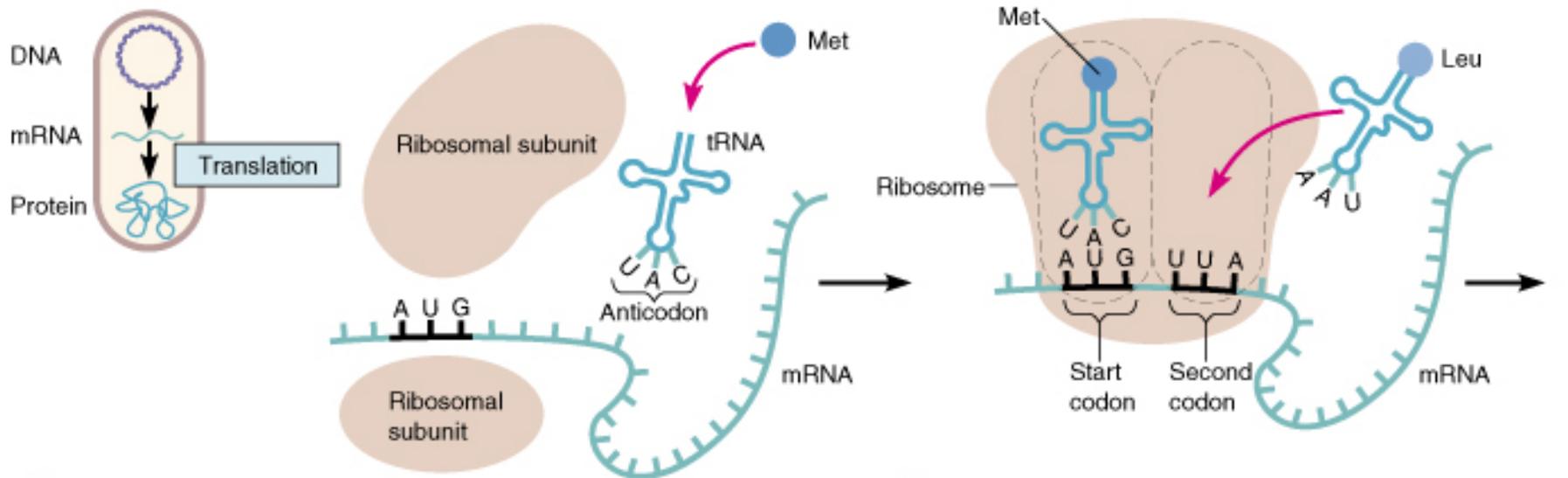


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# Translation: Initiation

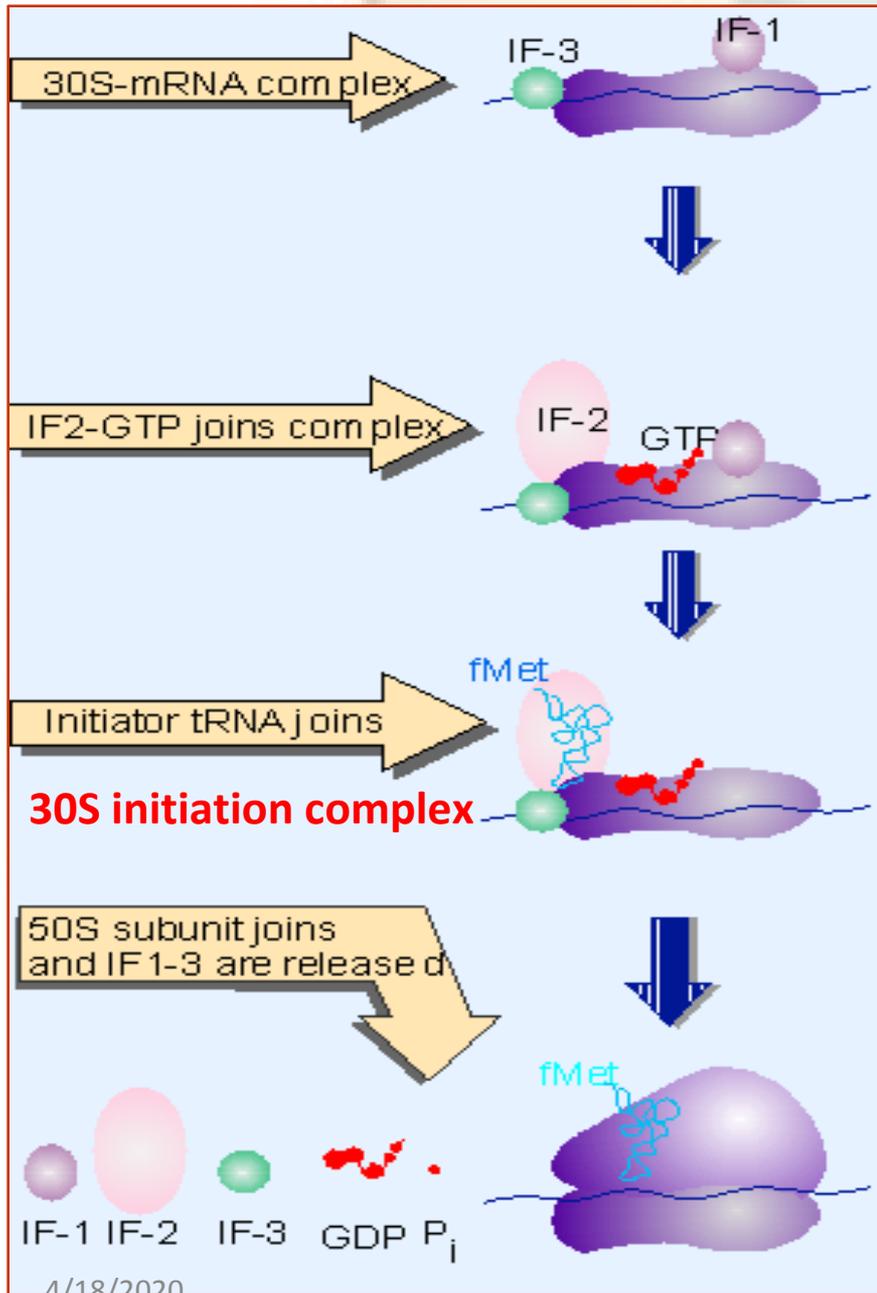
- mRNA binds to a ribosome, and the transfer RNA corresponding to the START codon binds to this complex. Ribosomes are composed of 2 subunits (large and small), which come together when the messenger RNA attaches during the initiation process.



1 Components needed to begin translation come together.

2 On the assembled ribosome, a tRNA carrying the first amino acid is paired with the start codon on the mRNA. A tRNA carrying the second amino acid approaches.

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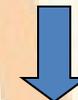
IF1 and IF3 bind to a free 30S subunits.



IF2 complexed with GTP then bind to the small subunits, forming a complex at RBS.



The initiator tRNA can then bind to the complex at the P site paired with AUG codon.



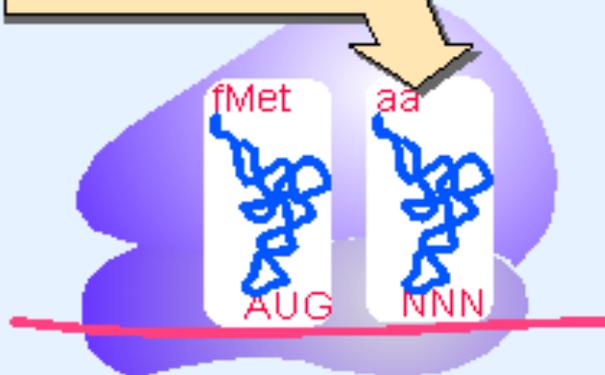
The 50S subunits can now bind. GTP is then hydrolyzed and IFs are released to give the **70S initiation complex**

Only fMet-tRNA<sub>f</sub> enters partial P site on 30S subunit bound to mRNA



50S subunit

Only aa-tRNA enters A site on complete 70S ribosome



The assembled ribosome has two tRNA-binding sites, which are called **A-** and **P-site**, for aminoacyl and peptidyl sites respectively.

Only **fMet-tRNA<sup>fMet</sup>** can be used for initiation by 30S subunits; all other aminoacyl-tRNAs are used for elongation by 70S ribosomes.

