# Lecture 10

## Protein Synthesis

Acknowledgements:
Leninger Chap 27
Scitable
Internet Resources

# Objectives of this Lecture

1. Stages of Protein Synthesis
2. The role of ribosomes
3. The role of tRNA
4. Details of each of the stages of protein synthesis

# Five Stage of Protein Synthesis in *E. coli*

1. Activation of Amino Acids
2. Initiation
3. Elongation
4. Termination
5. Folding and post translational processing

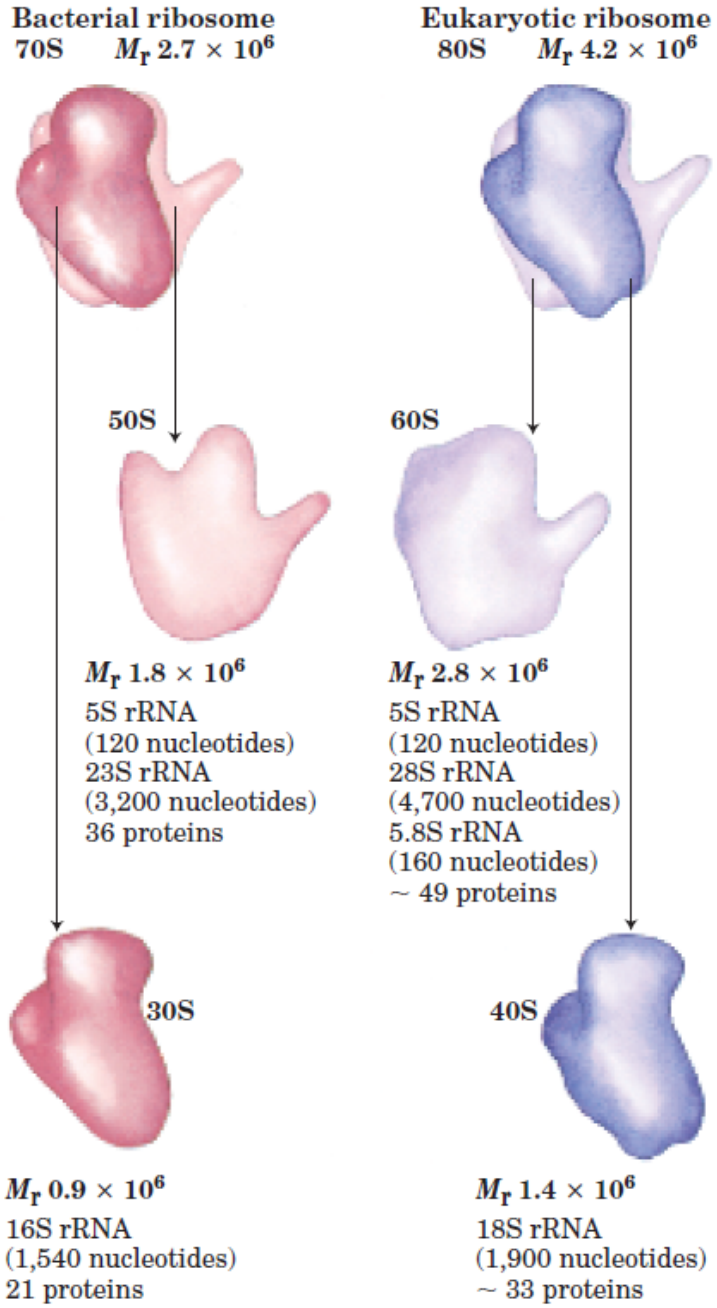
# Ribosome is a Complex Supramolecular Machine

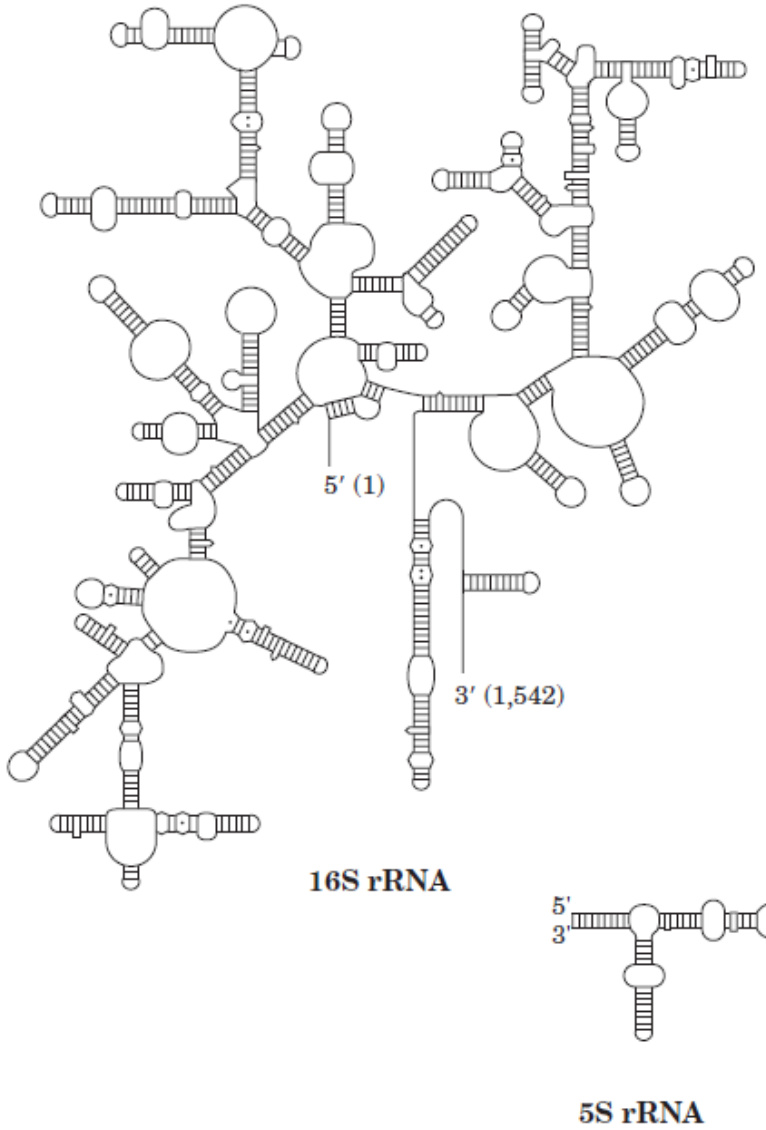
1. Each *E. coli* contains 15000 or more ribosomes (1/4 cell weight)
2. 18 nm is size
3. Two subunits  (i) 30S (ii) 50S combined 70S (S is the sedimentation coefficient)
4. Subunits are made of many proteins and at least one large rRNA
5. Bacterial ribosomes have 55 proteins with molecular weights varying from 6000 to 75000

**Look up:** **PDB ID 1JJ2 and 1GIY**

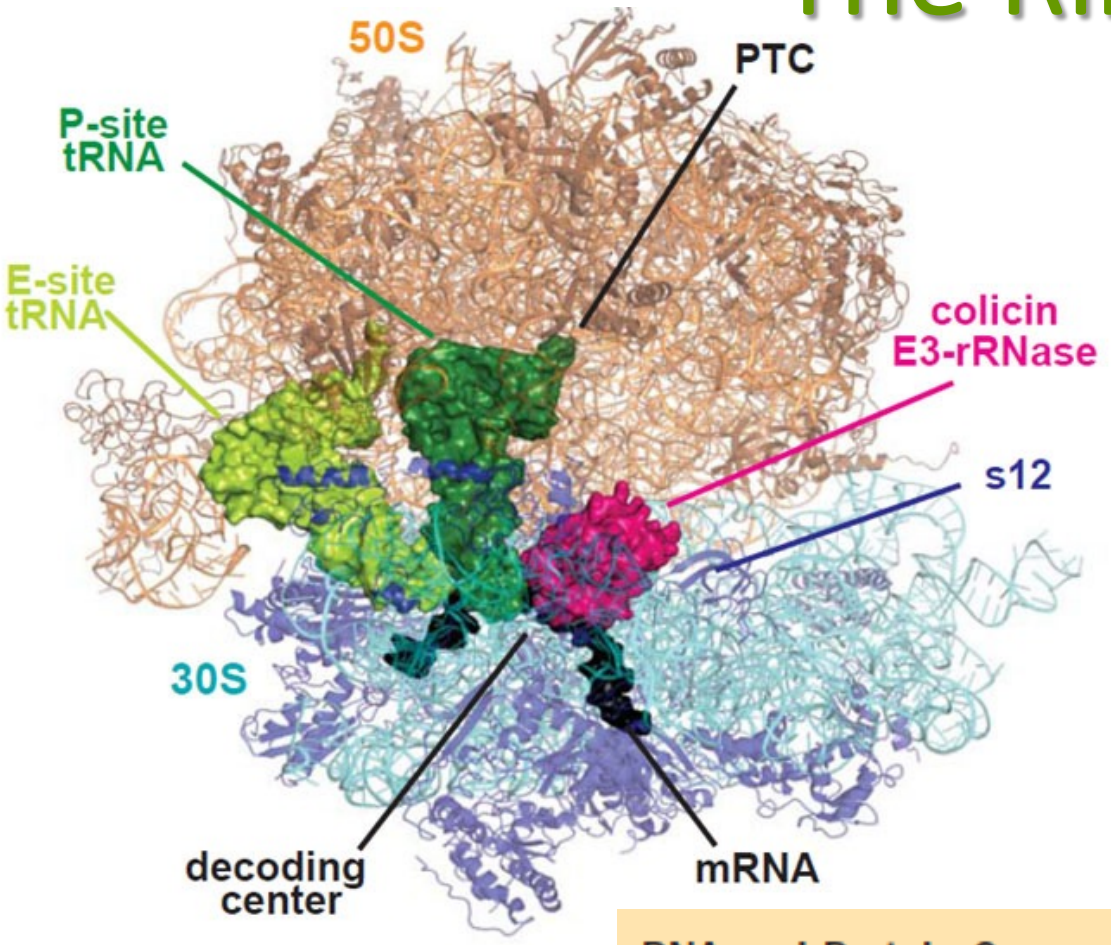
# Bacterial rRNA

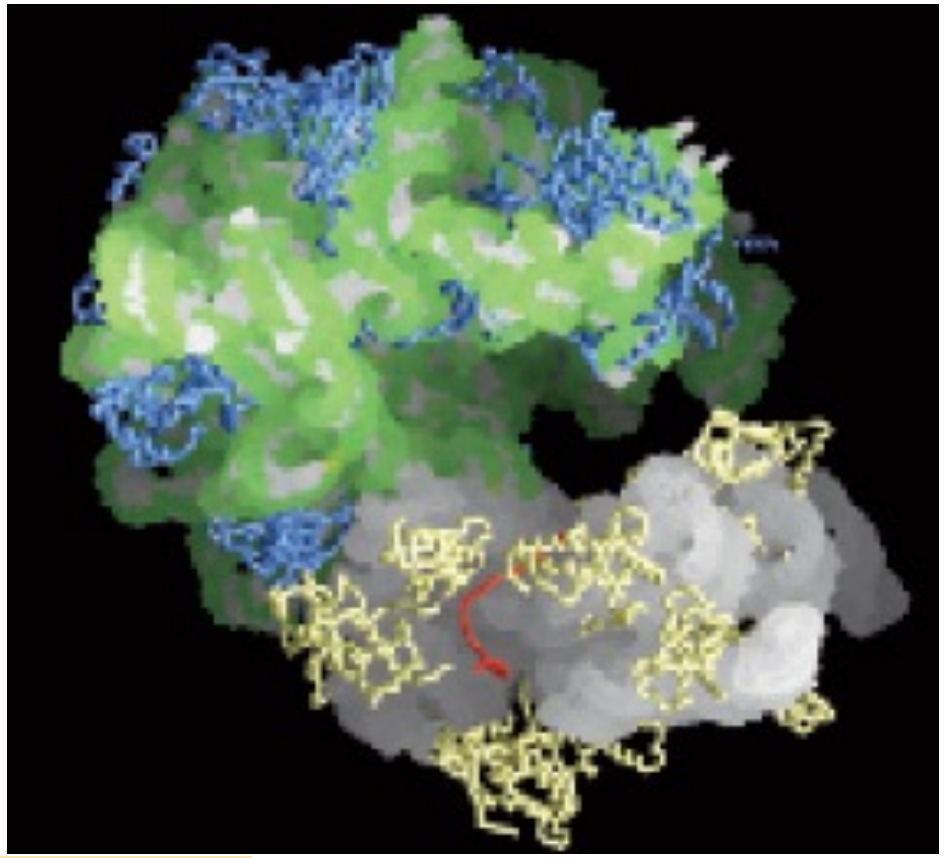
Secondary structure of *E. coli* 16S and 5S rRNAs. The first (5’ end) and final (3’ end) ribonucleotide residues of the 16S rRNA are numbered.



# The Ribosome



RNA and Protein Components of the *E. coli* Ribosome

| *Subunit* | *Number of different proteins* | *Total number of proteins* | *Protein designations* | *Number and type of rRNAs* |
|---|---|---|---|---|
| 30S | 21 | 21 | S1–S21 | 1 (16S rRNA) |
| 50S | 33 | 36 | L1–L36* | 2 (5S and 23S rRNAs) |

# Nobel Prize in Chemistry 2009

Visit to KSBS Aug 2020

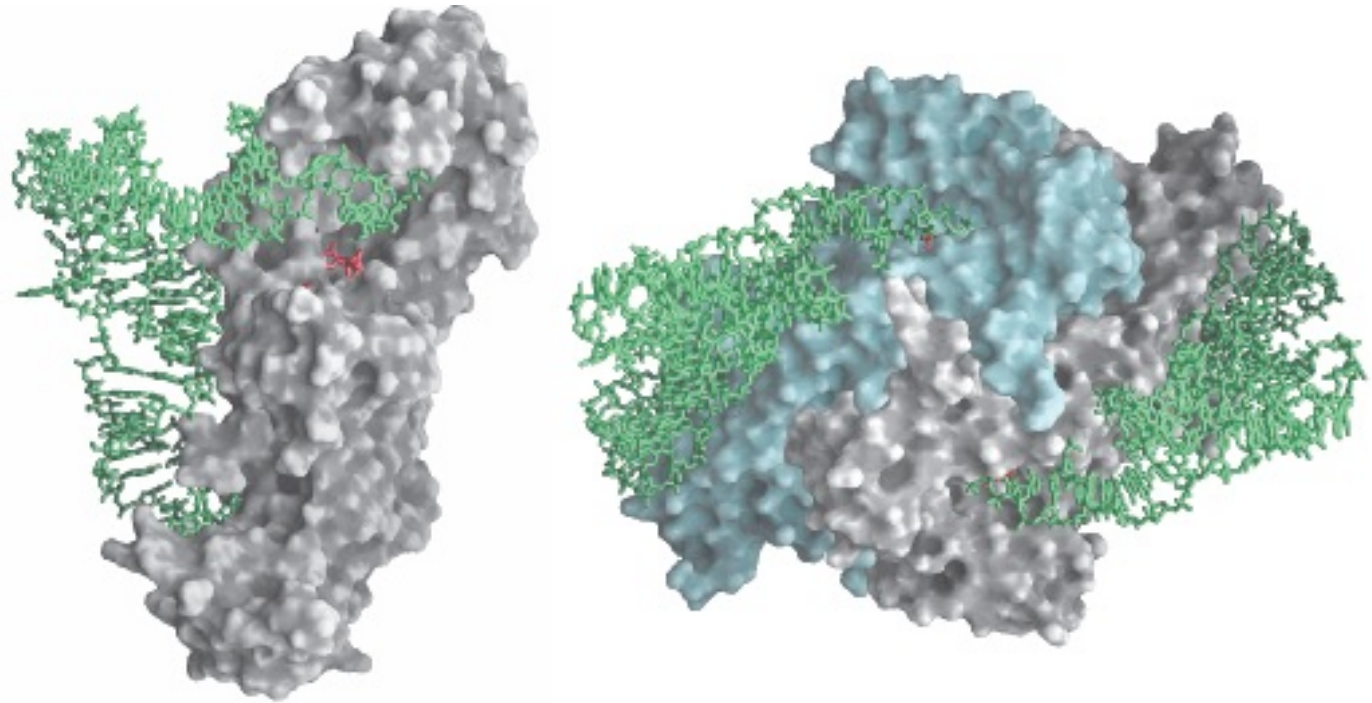
The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath "for studies of the structure and function of the ribosome"

https://www.nobelprize.org/prizes/chemistry/2009/summary/

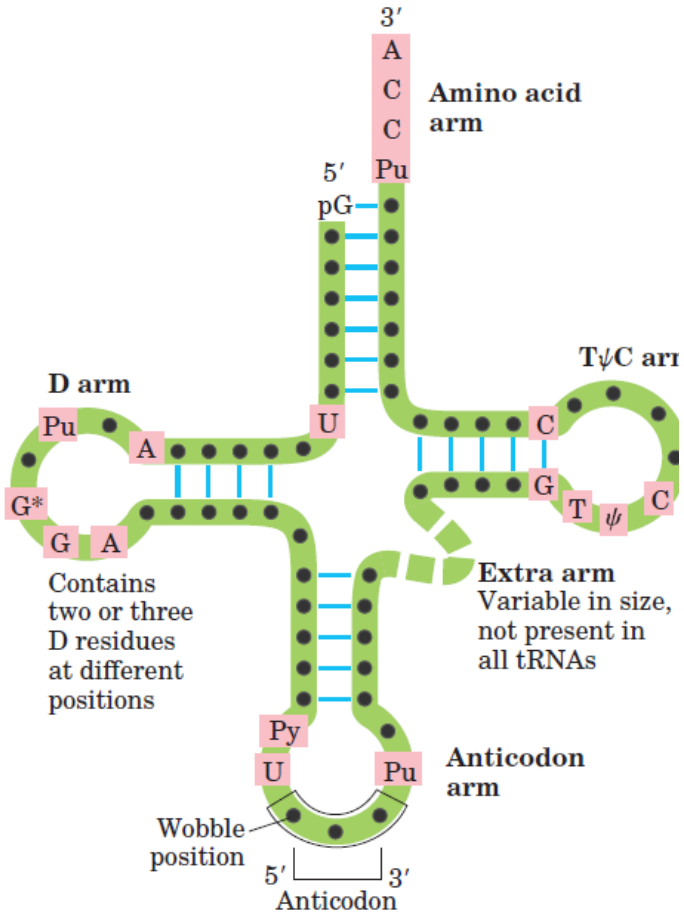
# Aminoacyl t-RNA Synthetase

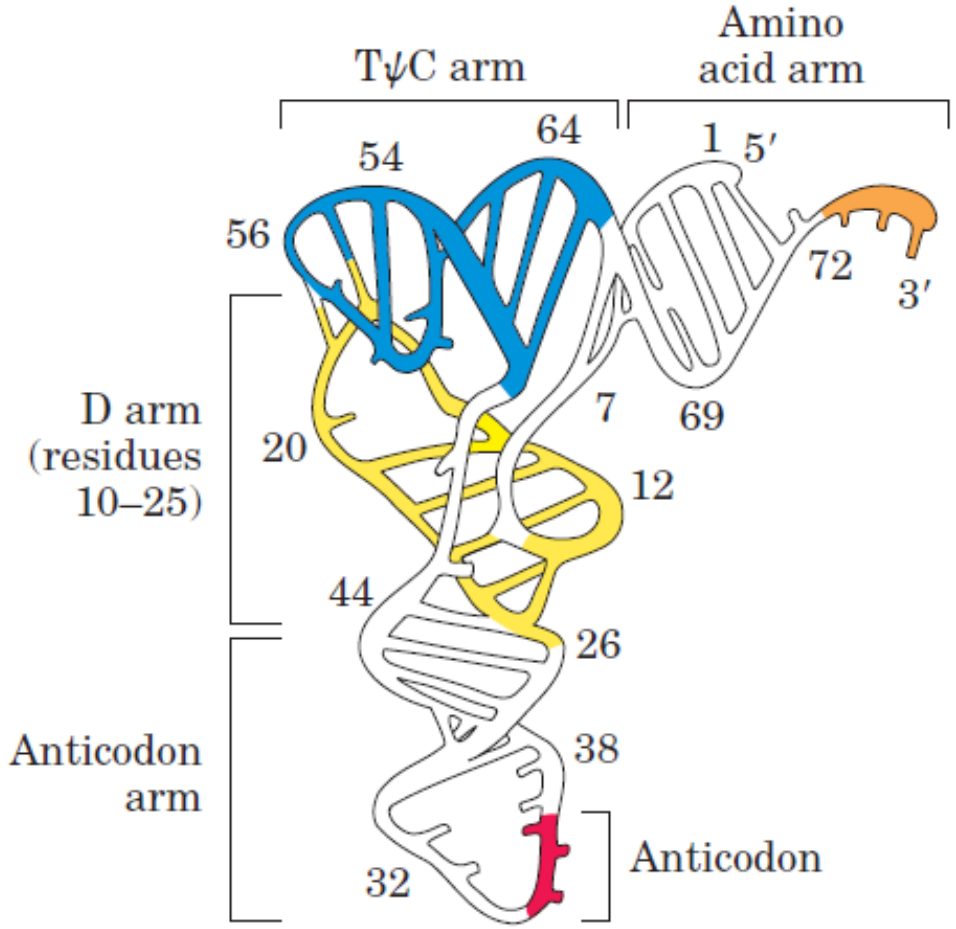
## Aminoacyl-tRNA Synthetases Attach the Correct Amino Acids to Their tRNAs

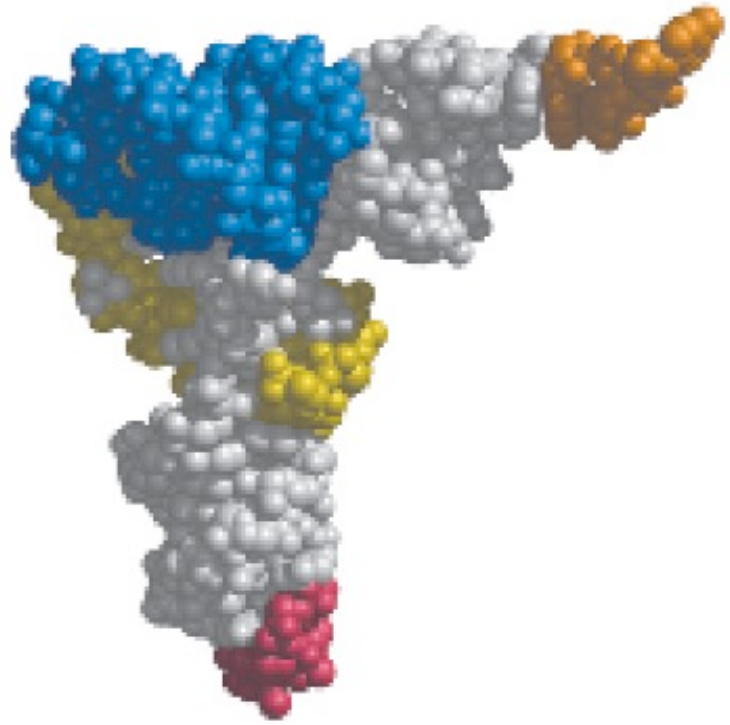
- aminoacyl-tRNA synthetases esterify the 20 amino acids to their corresponding tRNAs. Each enzyme is specific for one amino acid and one or more corresponding tRNAs
- Proofreading by Aminoacyl-tRNA Synthetases
- Interaction between an Aminoacyl-tRNA Synthetase and a tRNA



# 2-D and 3-D structure of tRNA



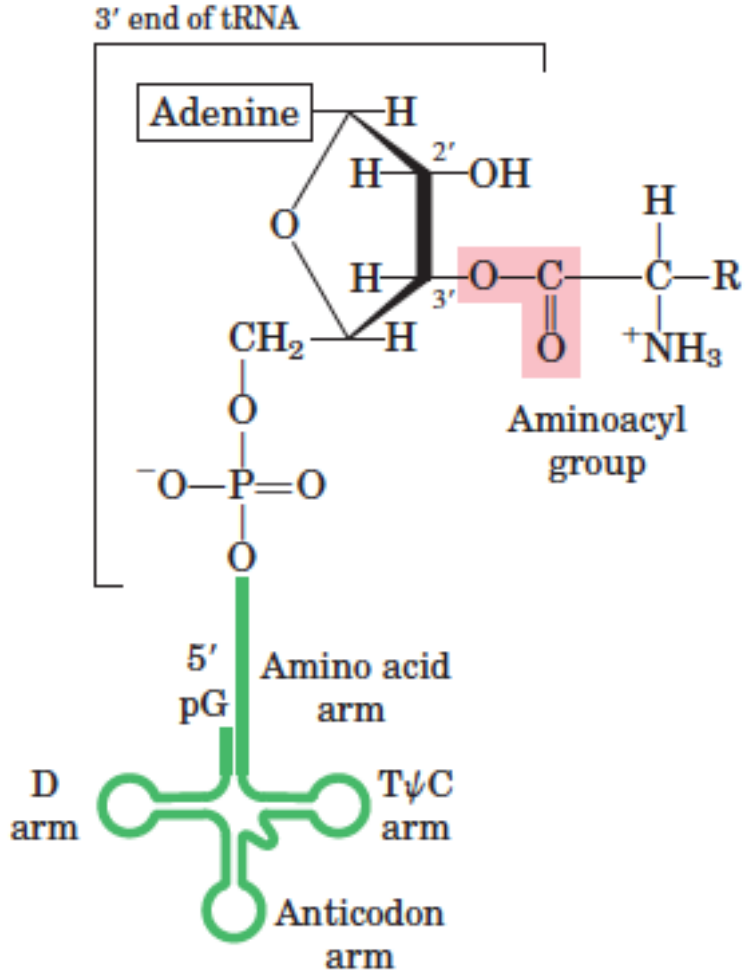



TWISTED "L" STRUCTURE

# Stage 1: Attaching the correct amino acid to the correct tRNA

in the cytosol, aminoacyl-tRNA synthetases esterify the 20 amino acids to their corresponding tRNAs.

- Each enzyme is specific for one amino acid and one or more corresponding tRNAs
- Most organisms have one aminoacyl-tRNA synthetase for each amino acid
- For amino acids with two or more corresponding tRNAs, the same enzyme usually aminoacylates all of them




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

$$\Delta G'^{\circ} \approx -29 \text{ kJ/mol}$$

# Proof Reading by Aminoacyl tRNA Synthetases

Aminoacylation of tRNA accomplishes

1. Activation of amino acid for peptide bond formation
2. attachment of amino acid to an adaptor tRNA for placement of amino acid

The amino acid attached is not checked on the ribosome!

**How is the fidelity assured?**

Consider Valine and Isoleucine - different by only – $CH_2$ –

In the case of Ile-tRNA synthetase

1. Activation of Ile is favored by a factor of 200
2. Binding is carried out in 2 steps (acts as filter)
3. Incorrect binding occurs at a second site that has a higher hydrolytic rate
4. In this case, overall process is 1:3000 in favor of the correct amino acid Ile

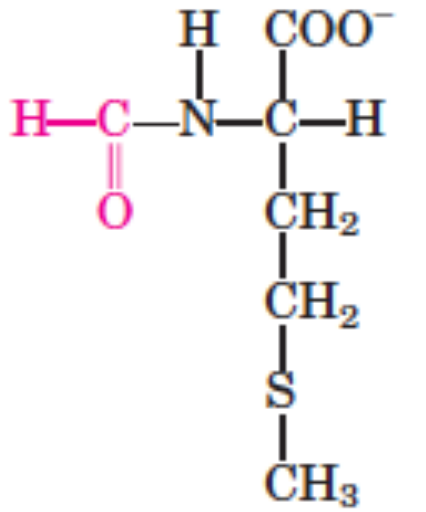
# Stage 2: A Specific Amino Acid Initiates Protein Synthesis

Although methionine has only one codon, (5')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
- The amino acid incorporated in response to the (5')AUG initiation codon is N-formylmethionine (fMet)

**Methionine + $tRNA^{fMet}$ + ATP → Met-$tRNA^{fMet}$ + AMP + PPi**

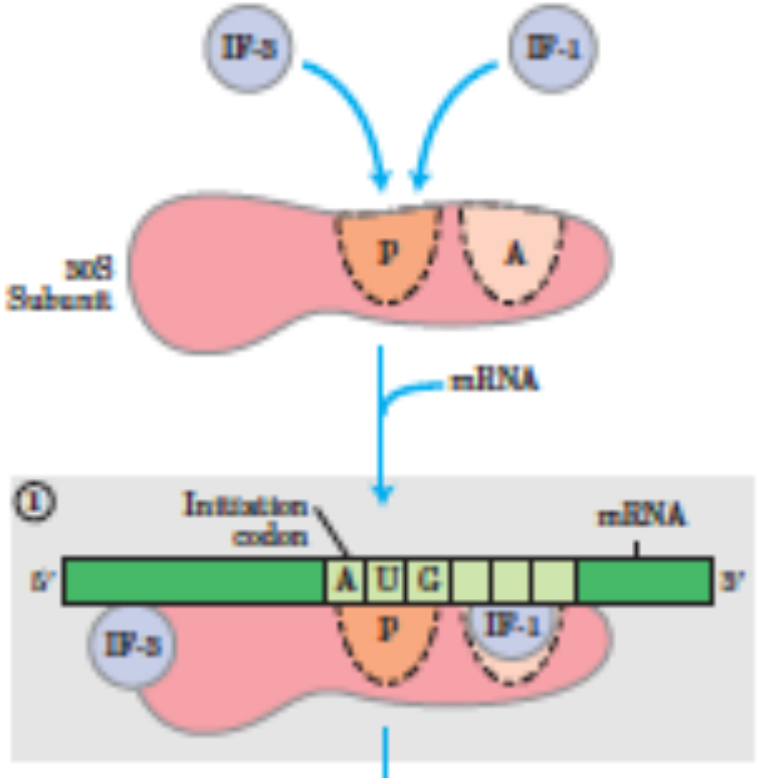
$N^{10}$-Formyltetrahydrofolate + Met-$tRNA^{fMet}$ ⟶
tetrahydrofolate + fMet-$tRNA^{fMet}$

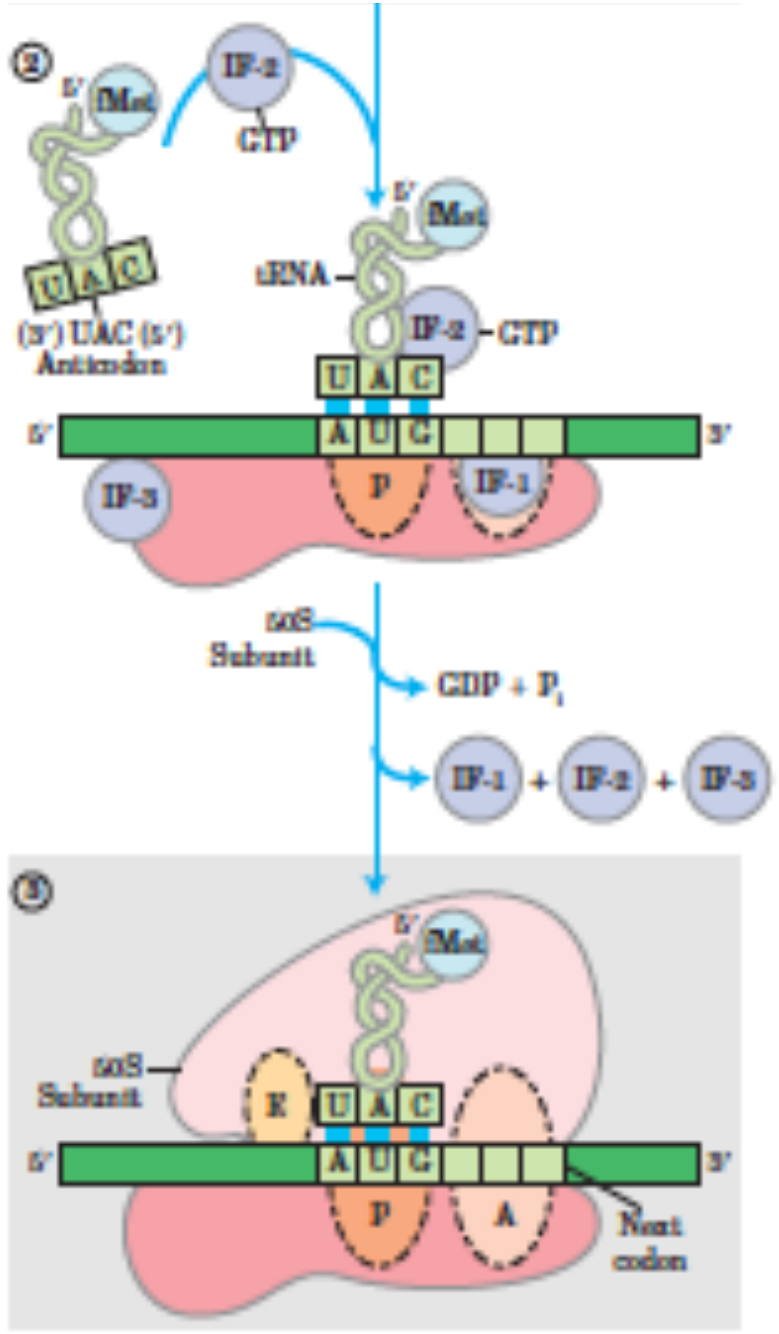


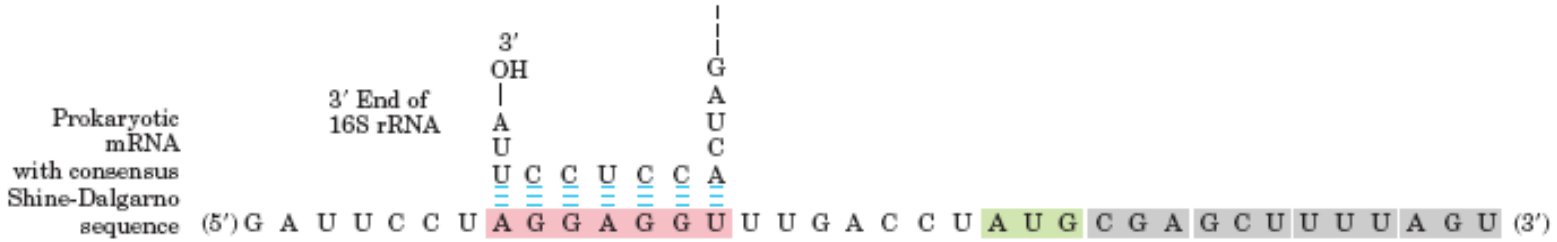
*N*-Formylmethionine

# Formation of the Initiation Complex

1. 30S ribosomal subunit
2. mRNA coding for the polypeptide to be made
3. Initiating fMet-tRNAfMet
4. A set of three proteins called initiation factors (IF-1, IF-2, and IF-3)
5. GTP
6. 50S ribosomal subunit
7. $Mg^{2+}$.



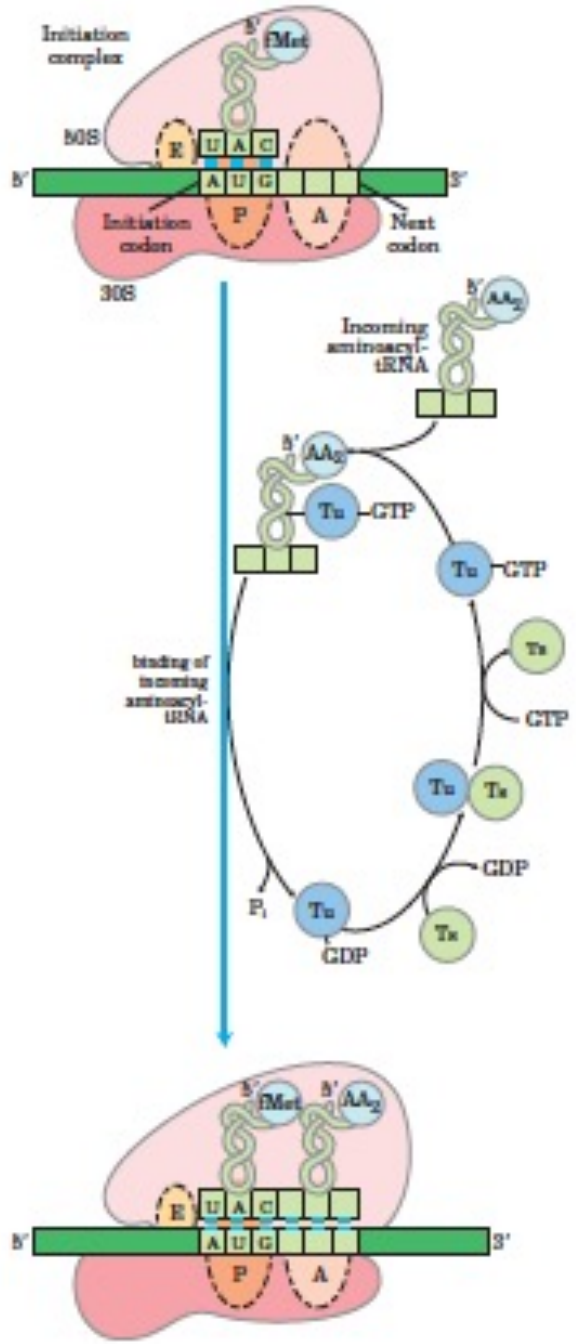



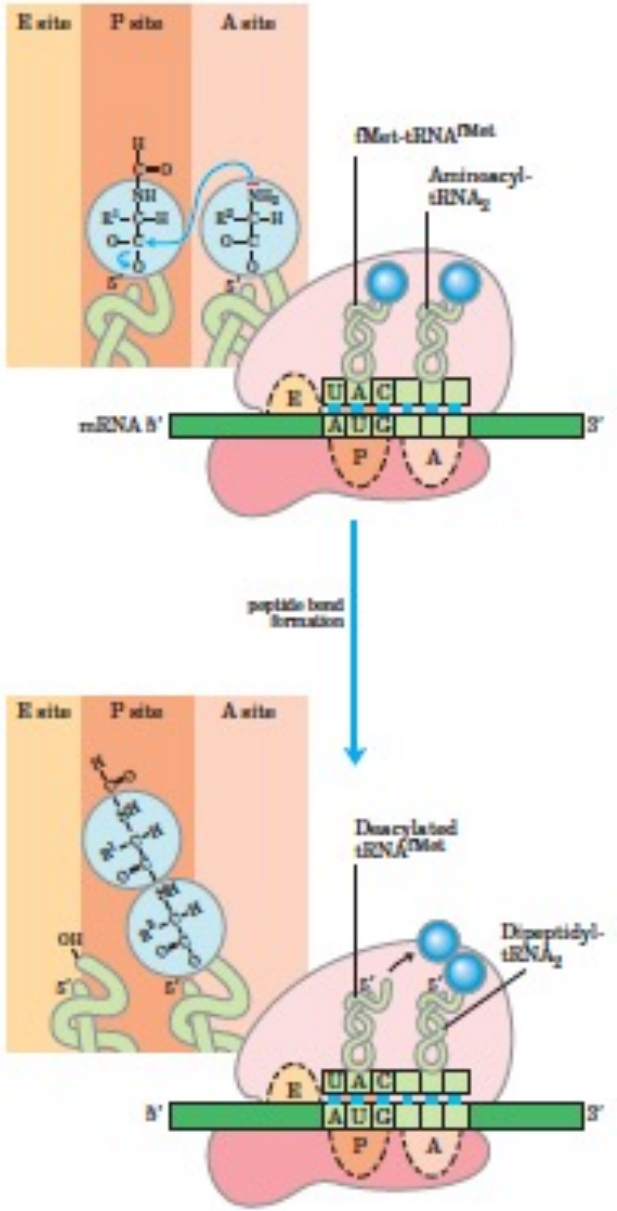



# Stage 3: Elongation of the Peptide Chain

## Elongation requires

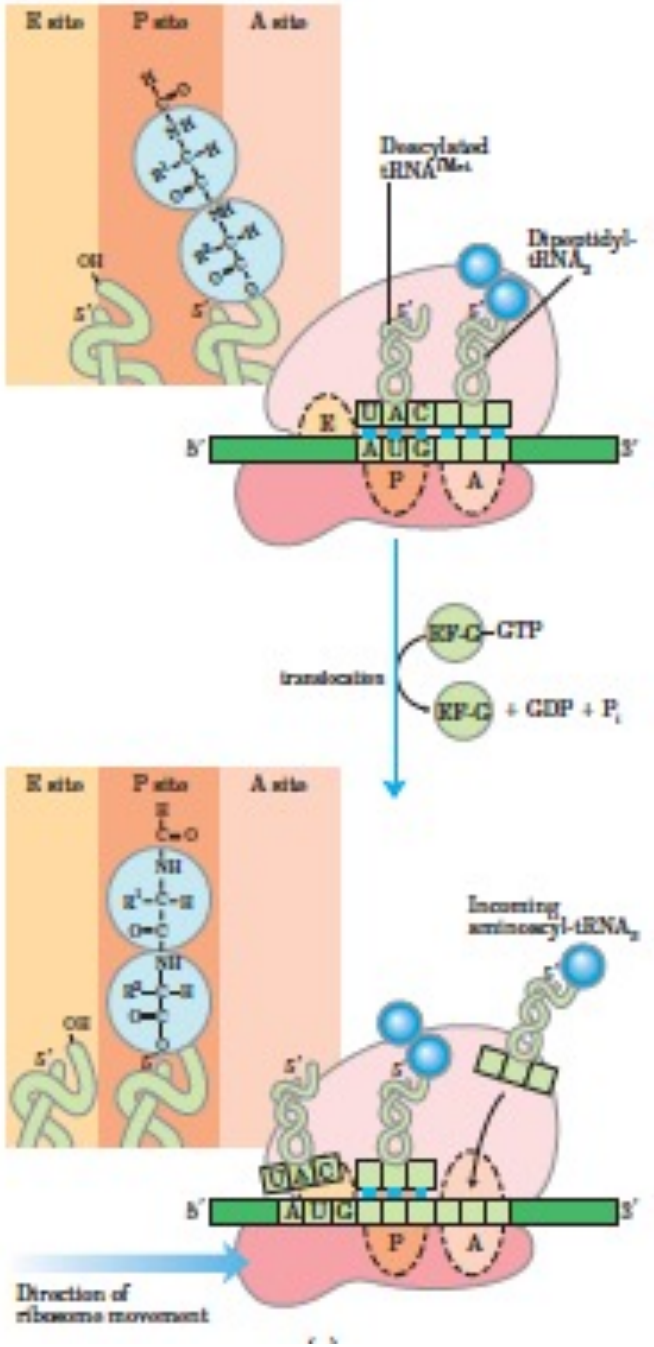
1. the initiation complex
2. aminoacyl-tRNAs
3. a set of three soluble cytosolic proteins called elongation factors (EF-Tu, EF-Ts, and in bacteria)
4. GTP



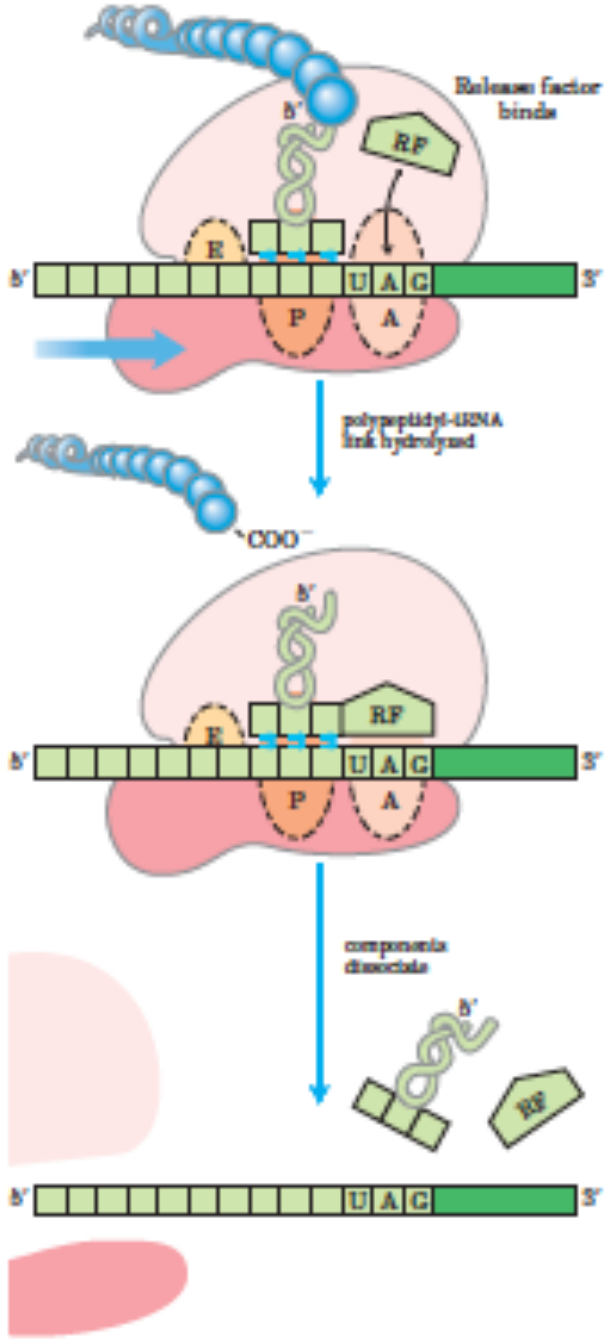
# Stage 3 : Translocation

1. The ribosome moves one codon toward the 3’ end of the mRNA
   This shifts the anticodon of the tRNA attached to the 2nd codon from the A→P site and the deacylated tRNA from P→ E site
2. This movement required EF-G (translocase) and the energy is provided by the hydrolysis GTP→GDP+Pi
3. The uncharged tRNA is dislocated from the E-site and a peptide bond is formed between the growing chain and the new amino acid carried by the tRNA at the A-site



# Stage 4: Termination of Synthesis

1. Elongation continues until the last amino acid in the sequence
2. Termination is signaled by the presence of one of the 3 stop codons UAA, UAG or UGA immediately following the final coded amino acid
3. Once the terminal codon occupies A-site, three termination or release factors RF1, RF2 and RF3 contribute to
   i. Hydrolysis of terminal peptidyl tRNA bond
   ii. Release of polypeptide from P-site
   iii. Dissociation of the 70S ribosome into the 30S and 50S subunits



# Summary of the 5 stages of protein synthesis

| *Stage* | *Essential components* |
|---|---|
| 1. Activation of amino acids | 20 amino acids<br>20 aminoacyl-tRNA synthetases<br>32 or more tRNAs<br>ATP<br>$Mg^{2+}$ |
| 2. Initiation | mRNA<br>*N*-Formylmethionyl-$tRNA^{fmet}$<br>Initiation codon in mRNA (AUG)<br>30S ribosomal subunit<br>50S ribosomal subunit<br>Initiation factors (IF-1, IF-2, IF-3)<br>GTP<br>$Mg^{2+}$ |
| 3. Elongation | Functional 70S ribosome (initiation complex)<br>Aminoacyl-tRNAs specified by codons<br>Elongation factors (EF-Tu, EF-Ts, EF-G)<br>GTP<br>$Mg^{2+}$ |
| 4. Termination and release | Termination codon in mRNA<br>Release factors (RF-1, RF-2, RF-3) |
| 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 |