MOLECULAR BIOLOGY: TRANSLATION AND INHIBITORS OF PROTEIN SYNTHESIS

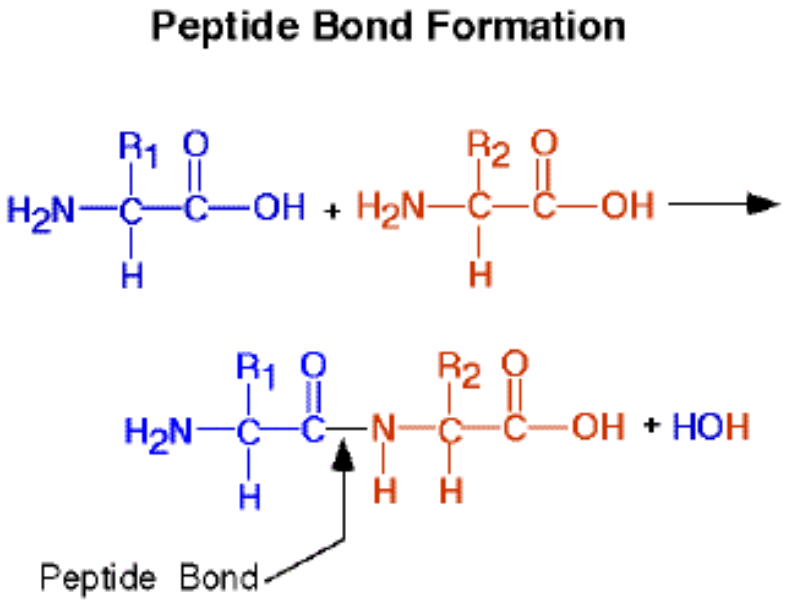
Lecture 4
Lecture slides

Protein Synthesis

- Proteins are composed of one or more polypeptides, plus (in some cases) additional small molecules (co-factors).
- Polypeptides are linear chains of amino acids. After synthesis, the new polypeptide folds spontaneously into its active configuration and combines with the other necessary subunits to form an active protein.
- Thus, all the information necessary to produce the protein is contained in the DNA base sequence that codes for the polypeptides.
- The sequence of amino acids in a polypeptide is known as its “primary structure”.

Amino Acids and Peptide Bonds

- There are 20 different amino acids coded in DNA.
- They all have an amino group (-NH_2) group on one end, and a carboxylic acid group (-COOH) on the other end. Attached to the central carbon is an R group, which differs for each of the different amino acids.
- When polypeptides are synthesized, the acid group of one amino acid is attached to the amino group of the next amino acid, forming a peptide bond.

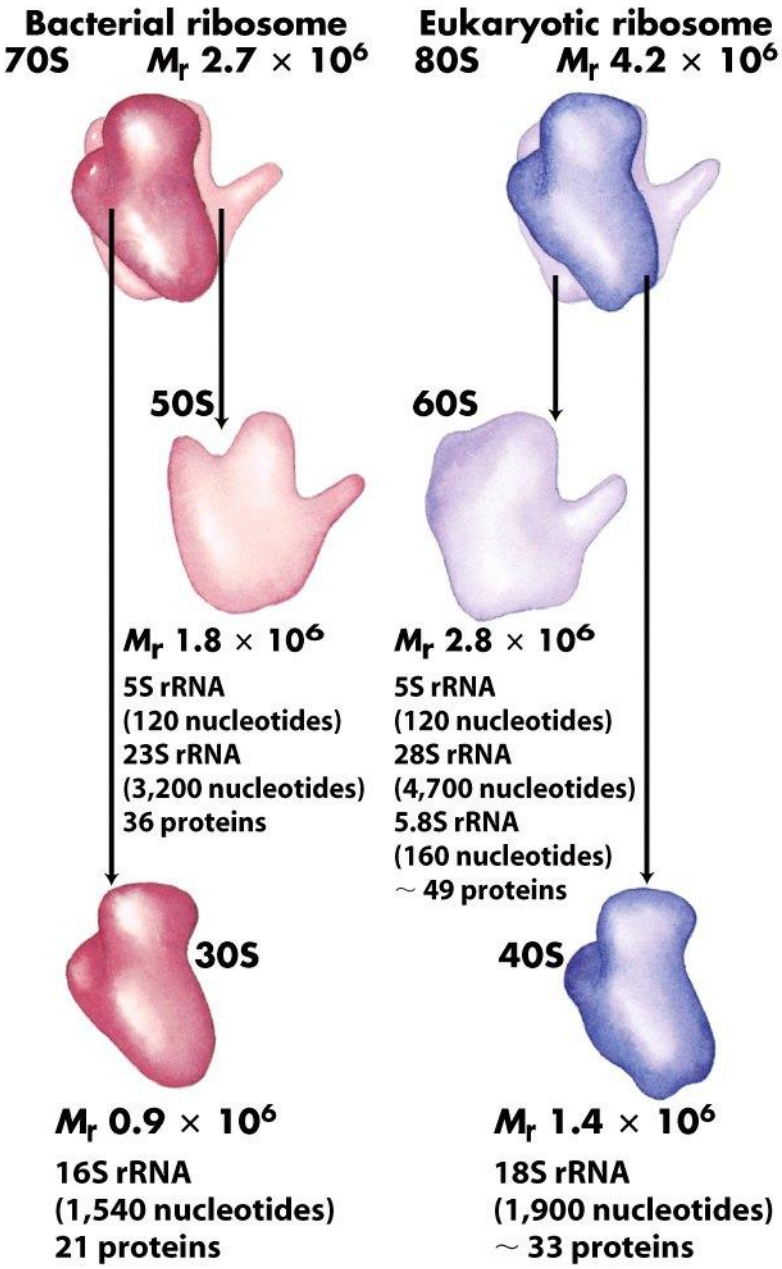


Translation

- Translation is the process in which the genetic information in mRNA molecule specifies the sequence of amino acids during protein synthesis.
- Translation of mRNA into protein is accomplished by the **ribosome**, an RNA (ribosomal RNA)/protein hybrid.
- Ribosomes are composed of 2 subunits, large and small.
- Ribosomes bind to the translation initiation sequence on the mRNA, then move down the RNA in a 5' to 3' direction, creating a new polypeptide.
- The first amino acid on the polypeptide has a free amino group, so it is called the "N-terminal". The last amino acid in a polypeptide has a free acid group, so it is called the "C-terminal".
- Each group of 3 nucleotides in the mRNA is a "**codon**", which codes for 1 amino acid. **Transfer RNA** is the adapter between the 3 bases of the codon and the corresponding amino acid.

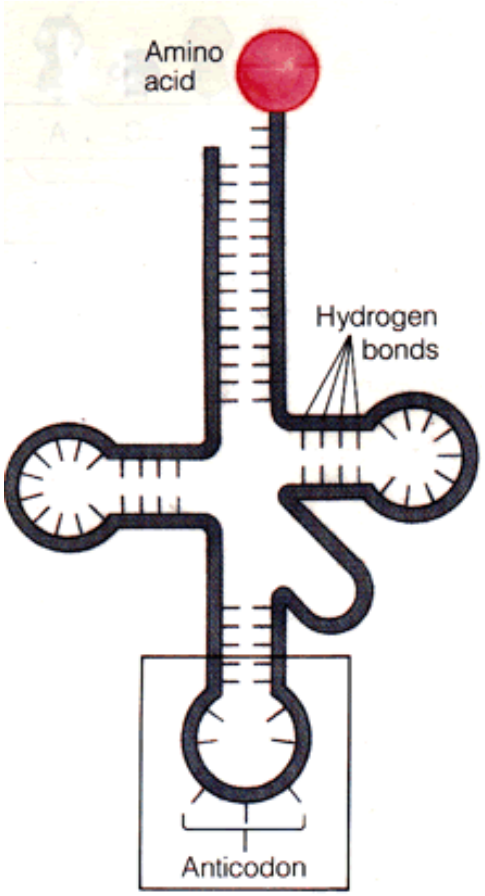
- Ribosomal subunits are identified by their S (Svedberg unit) values, sedimentation coefficients that refer to their rate of sedimentation in a centrifuge.

- The S values are not necessarily additive when subunits are combined, because rates of sedimentation are affected by shape as well as mass.



Transfer RNA

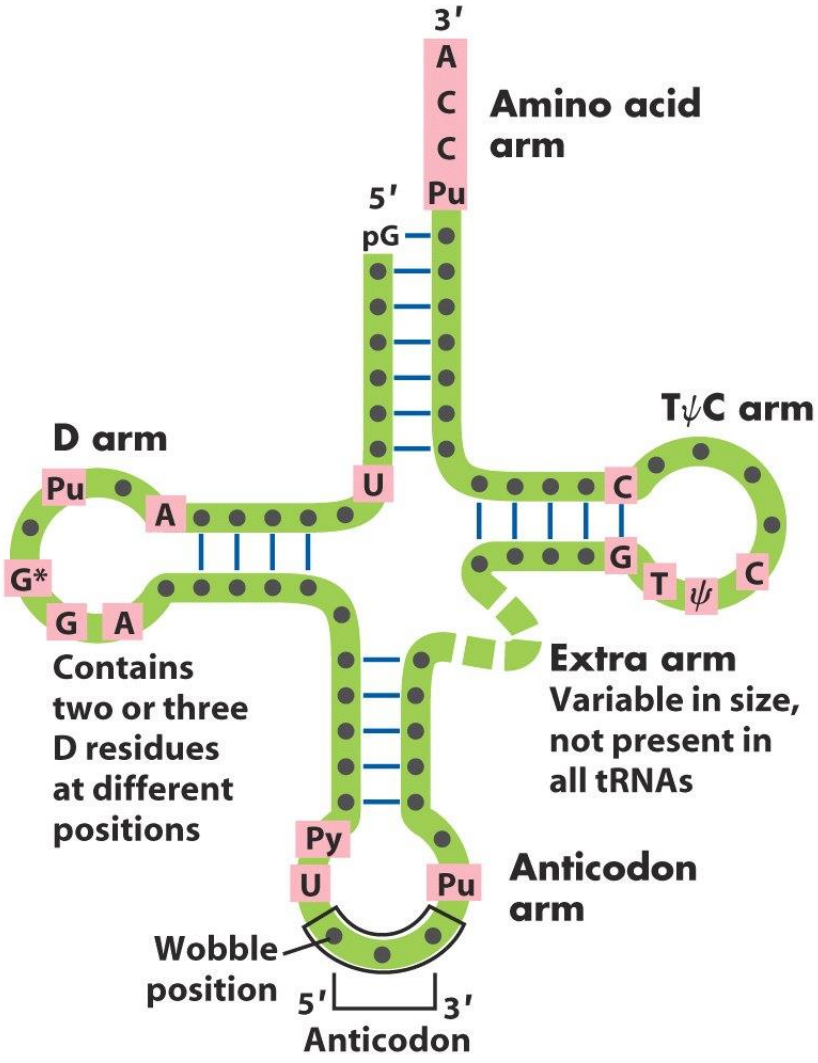
- Transfer RNA molecules are short RNAs that fold into a characteristic **cloverleaf pattern.**
- Some of the nucleotides are modified
- Each tRNA has 3 bases that make up the **anticodon**. These bases pair with the 3 bases of the codon on mRNA during translation.
- Each tRNA has its corresponding amino acid attached to the 3' end. A set of enzymes, the **"aminoacyl tRNA synthetases",** are used to "charge" the tRNA with the proper amino acid.
- Some tRNAs can pair with more than one codon. The third base of the anticodon is called the **"wobble position",** and it can form base pairs with several different nucleotides.



Secondary Structure of tRNA

- The large dots on the backbone represent nucleotide residues; the blue lines represent base pairs.
- Characteristic and/or invariant residues common to all tRNAs are shaded in pink.
- At the end of the anticodon arm is the anticodon loop, which always contains seven unpaired nucleotides.
- The D arm contains two or three D (5,6 dihydrouridine) residues, depending on the tRNA.
- In some tRNAs, the D arm has only three hydrogen-bonded base pairs.

- Pu, purine nucleotide;
- Py, pyrimidine nucleotide;
- G*, guanylate *or 2'-O*-methylguanylate



Stages in protein synthesis

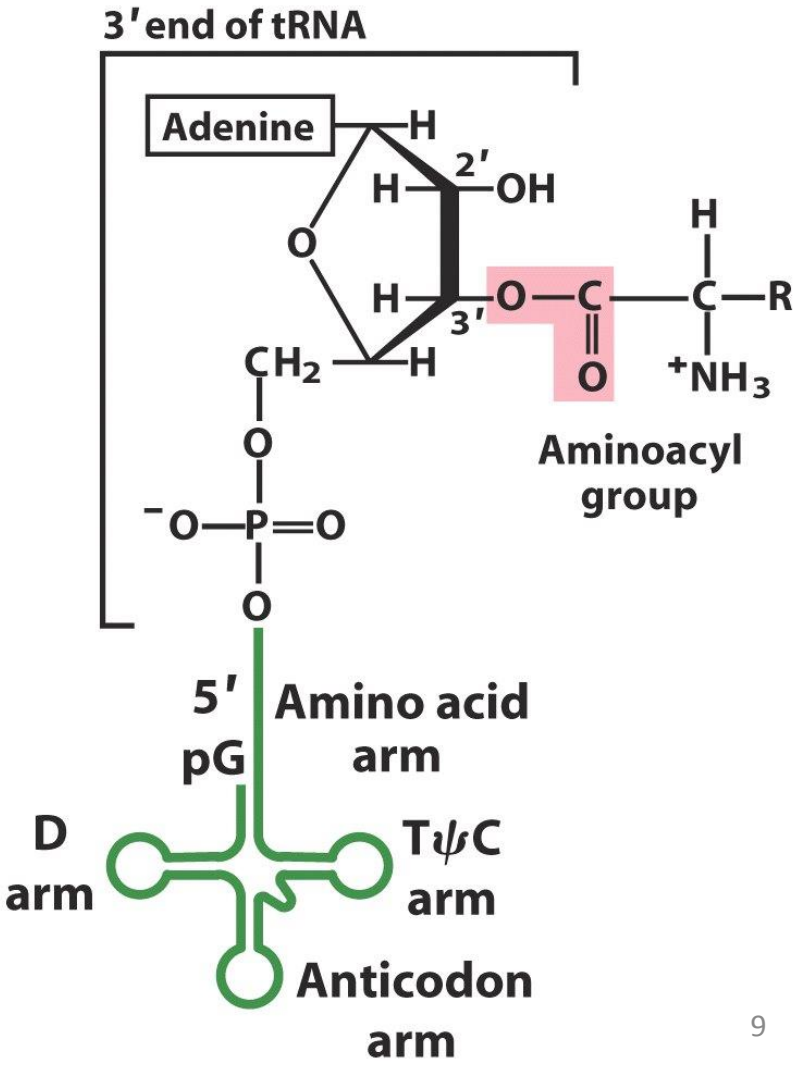
- Translation is the process whereby a base sequence of mRNA is used to create a protein

- There are four major stages in protein synthesis
  - activation
  - initiation
  - elongation
  - termination

Activation of Amino Acids

Amino acid + tRNA + ATP → aminoacyl-tRNA + AMP + PP_i

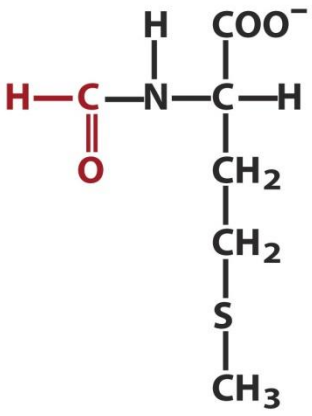
- Activation of amino acids occurs in the cytosol and the reaction is catalyzed by **Aminoacyl-tRNA Synthetases**
- Amino acid reacts with the corresponding tRNA in the presence of ATP.
- When attached to their amino acid (aminoacylated), the tRNA are said to be **"charged".**



Initiation of Translation

Prokaryotes

- Ribosomes bind to specific **translation initiation sites.**
- Bacteria have a large **50S** and a small **30S** ribosomal subunits.
- There can be several different initiation sites on a messenger RNA: a prokaryotic mRNA can code for several different proteins.
- Translation begins at an **AUG** codon, or sometimes a GUG.
- In Prokaryotes, the modified amino acid **N-formyl methionine** is always the first amino acid of the new polypeptide.



***N*-Formylmethionine**

Eukaryotes

- Ribosomes bind to the 5’ cap, then move down the mRNA until they reach the first **AUG**, the codon for **methionine.** Translation starts from this point.

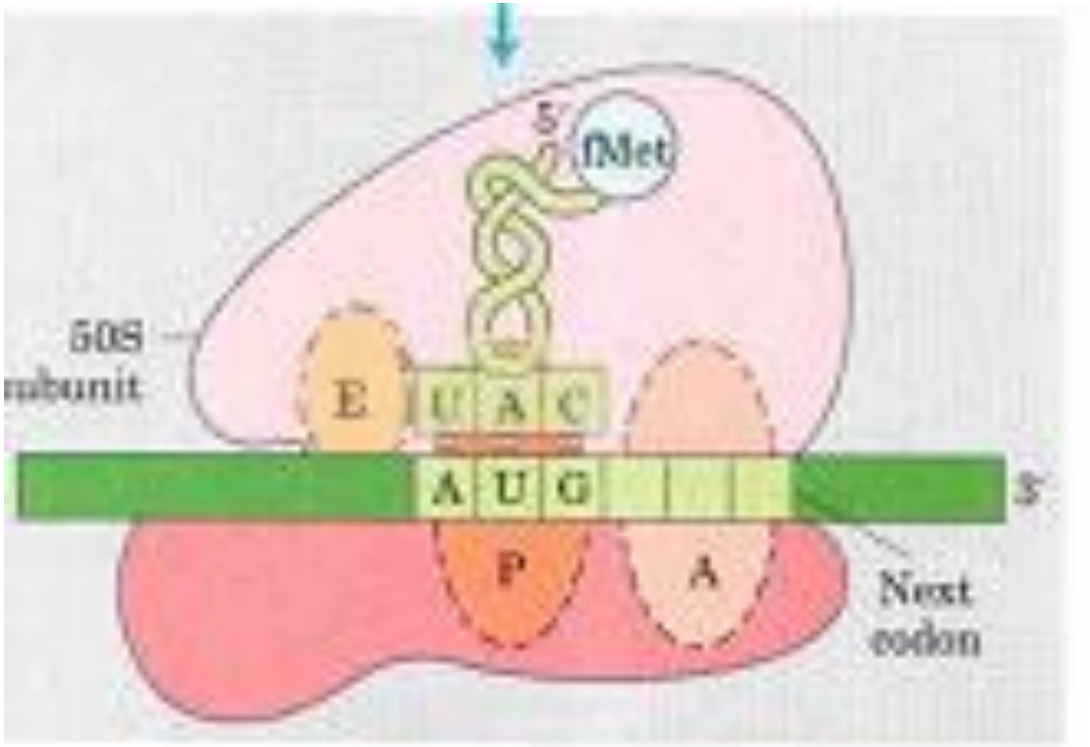
- Eukaryotes have a large **60S** and a small **40S** ribosomal subunits.

- Eukaryotic mRNAs code for only a single gene (although there are a few exceptions)

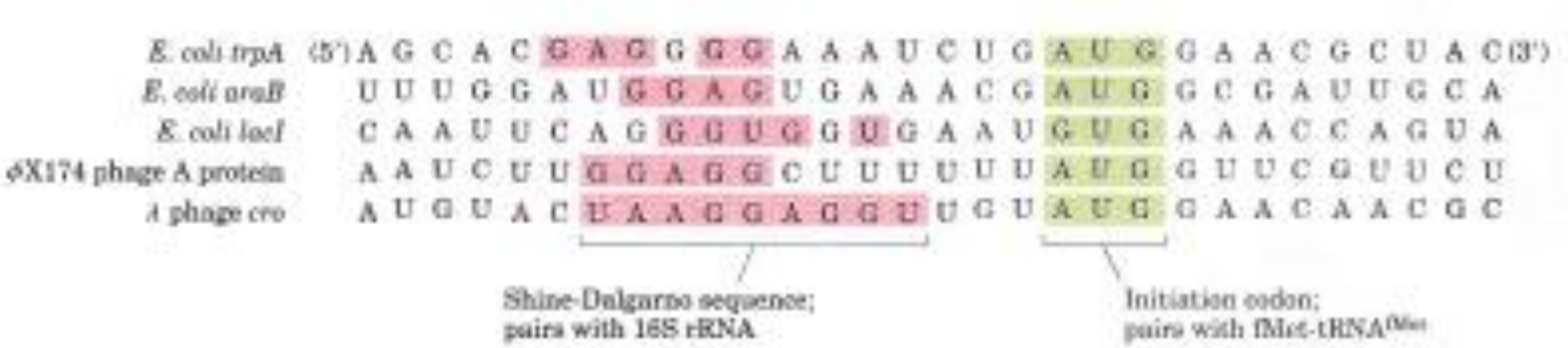
- Translation does not start at the first base of the mRNA.
- There is an **untranslated region** at the beginning of the mRNA, the **5’ untranslated region (5’ UTR).** Also there is untranslated region at the end, the **3’ untranslated region (3’ UTR)**

Assignment: Read and make notes on formation of initiation complex

Initiation complex

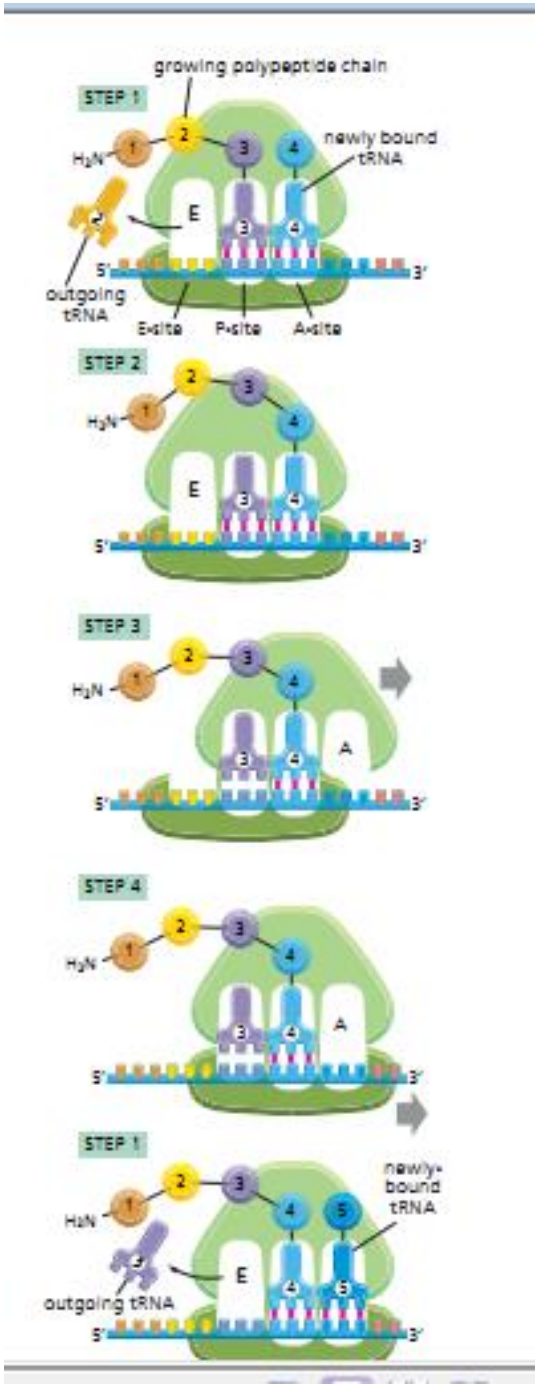


Sequences that serve as signals for initiation of protein synthesis in bacteria



Elongation

- The ribosome has 2 sites for tRNAs, called P and A. The initial tRNA with attached amino acid is in the P site.
- A new tRNA, corresponding to the next codon on the mRNA, binds to the A site. The ribosome catalyzes a transfer of the amino acid from the P site onto the amino acid at the A site, forming a new peptide bond; reaction catalyzed by **peptidyl transferase.**
- The ribosome then moves down one codon. The now-empty tRNA at the P site is displaced off the ribosome, and the tRNA that has the growing peptide chain on it is moved from the A site to the P site; a process requiring hydrolysis of GTP.
- The process is then repeated:
  - the tRNA at the P site holds the peptide chain, and a new tRNA binds to the A site.
  - the peptide chain is transferred onto the amino acid attached to the A site tRNA.
  - the ribosome moves down one codon, displacing the empty P site tRNA and moving the tRNA with the peptide chain from the A site to the P site.



Step 1: An aminoacyl-tRNA molecule binds to a vacant A-site on the ribosome and the spent tRNA molecule dissociates from the E-site

Step 2: A new peptide bond is formed catalyzed by peptidyl transferase

Step 3: The large subunit translocates relative to small subunit

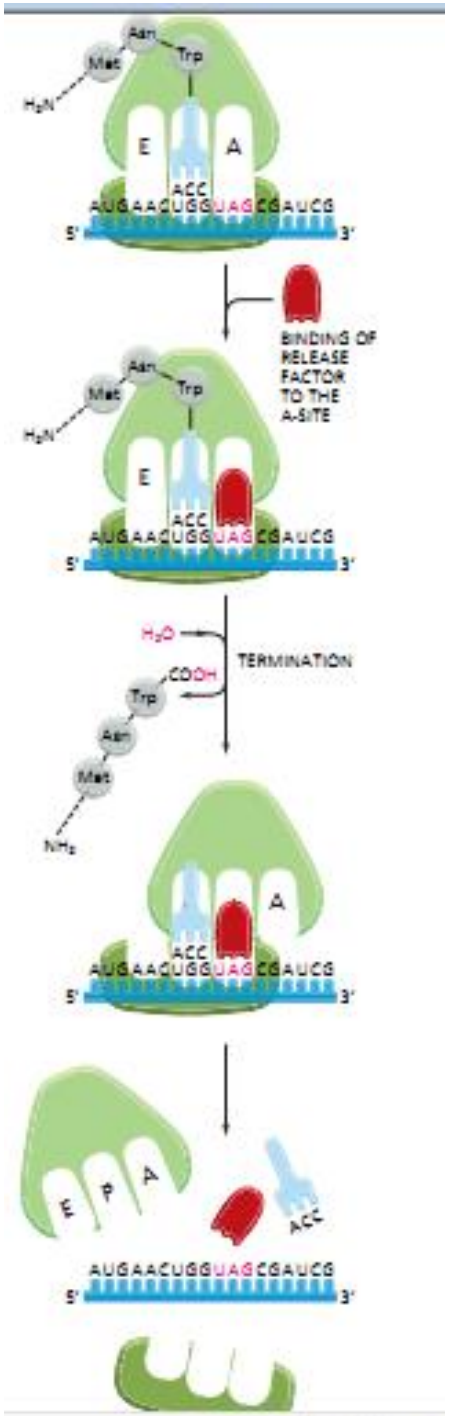
Step 4: The small subunit translocates carrying its mRNA a distance of three nucleotides (codon) through the ribosome. This resets the ribosome with a fully empty A-site ready for the next aminoacyl-tRNA molecule to bind.

Termination

- There are three codons (UAA, UAG and UGA) which code for no amino acid hence called “stop codons”. All protein-coding regions end in a stop codon.
- When the ribosome reaches a stop codon, there is no tRNA that binds to it. Instead, proteins called “Release Factors” (RFs) bind, and cause the ribosome, the mRNA, and the new polypeptide to separate.
- The new polypeptide is completed.
- Note that the mRNA continues on past the stop codon.
- The remaining portion is not translated: it is the 3’ untranslated region (3’ UTR).

- The process of termination begins when a stop codon on mRNA is encountered in the A site. **In bacteria,** recognition of the stop codon involves two release factors, RF1 and RF2.
- Both factors recognize **UAA**; however, **UAG** is recognized by RF1 while **UGA** is recognized by RF2.
- **In eukaryotes**, a single factor, eRF1, recognizes all three stop codons.

- The binding of RF1 or RF2 to a ribosome with the appropriate stop codon in the A site triggers the hydrolysis and release of the peptide chain from tRNA in the P site.
- RF3 promotes rapid dissociation of RF1 and RF2. Originally, it was thought that the binding of RF3 to the ribosome triggered its GTPase activity with concomitant release of RF1 or RF2.
- However, more recent work shows that the hydrolysis of peptidyl tRNA by RF1 or RF2 is required for binding GTP to RF3 on the ribosome.
- This in turn leads to a conformation of RF3 with high affinity for ribosomes and the dissociation of RF1 or RF2. The hydrolysis of GTP is required for subsequent dissociation of RF3.



Stop codon (UAG) is on A site

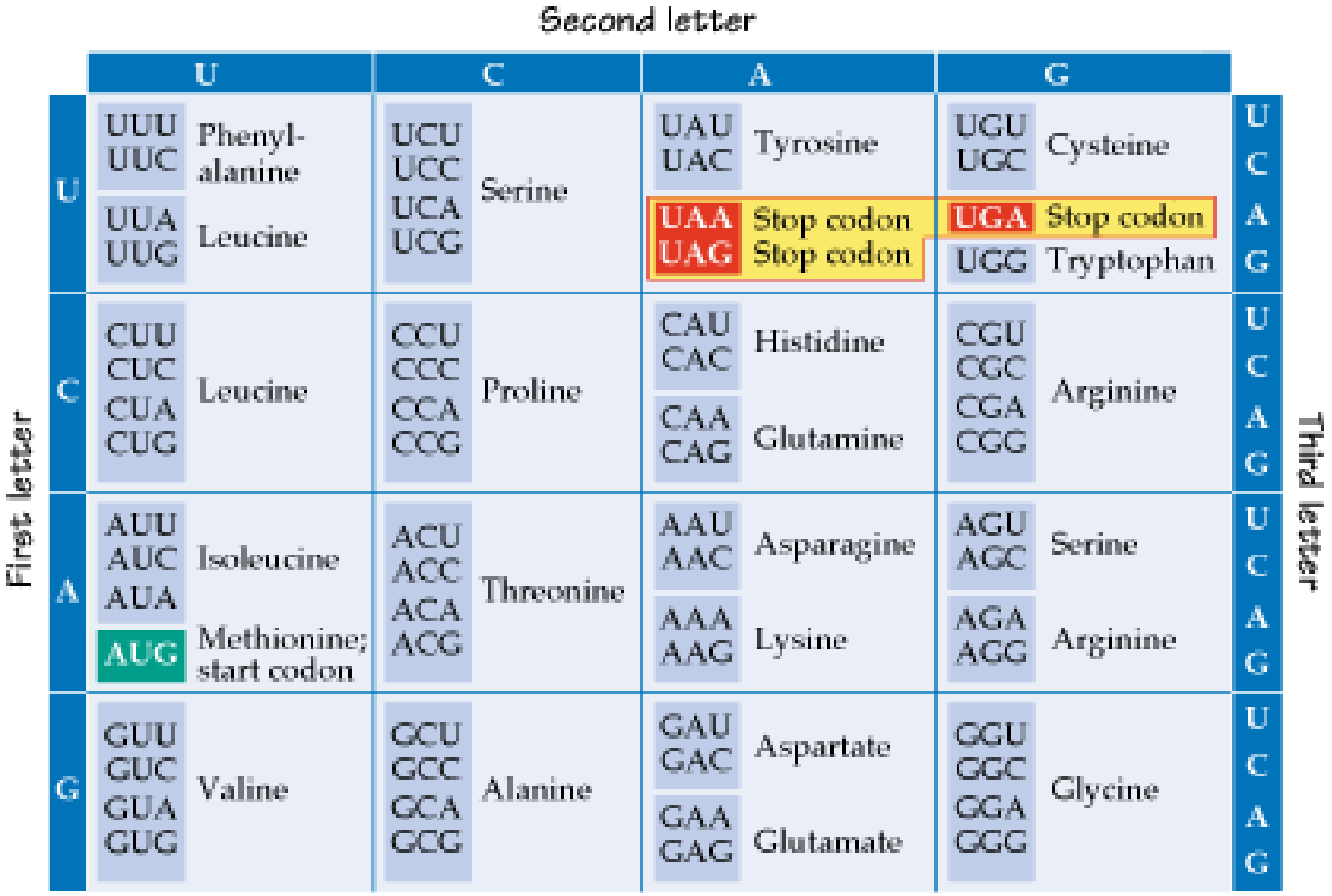
The binding of Release factor to an A site bearing a stop codon terminates translation.

The complete polypeptide is released.

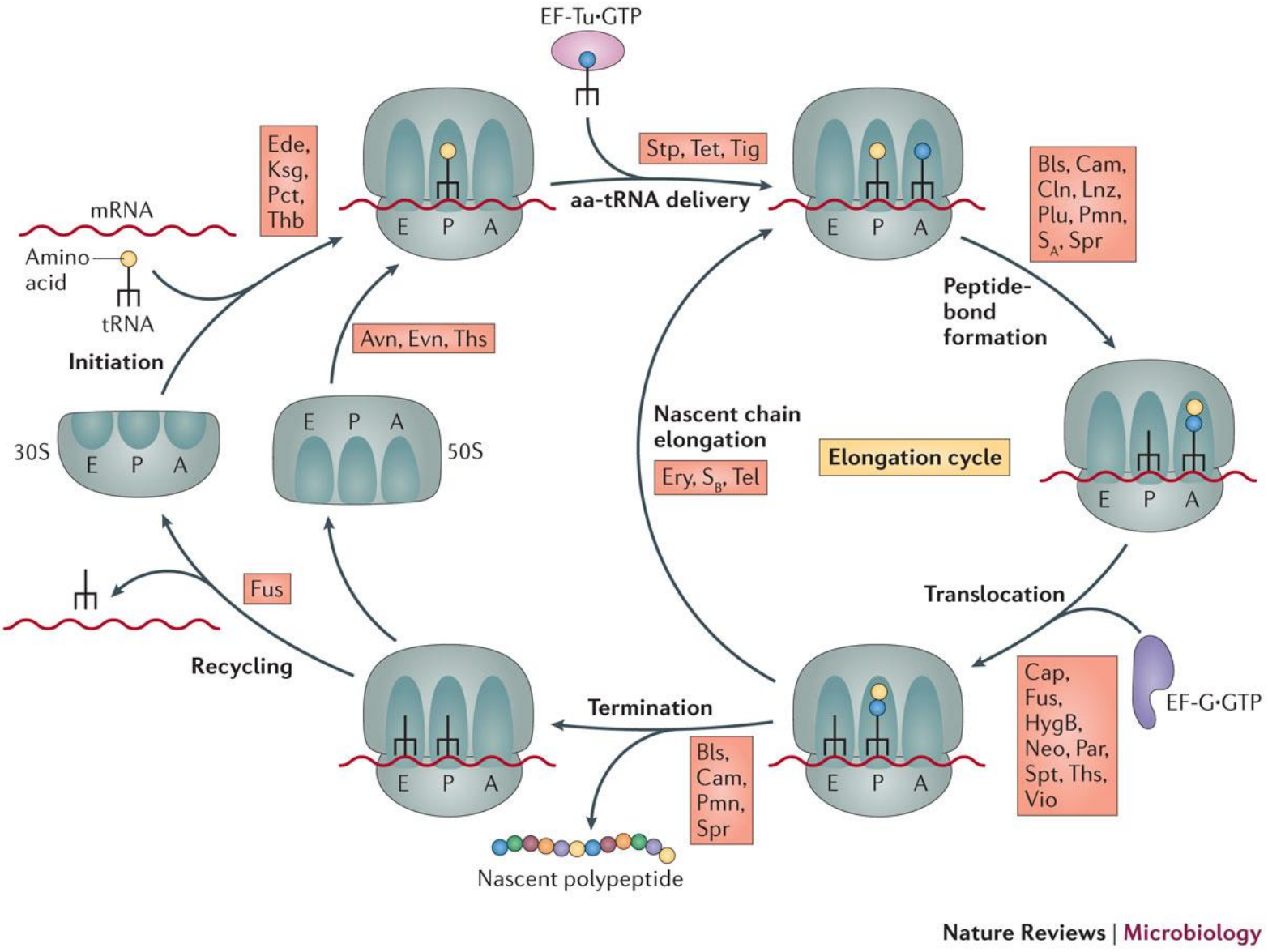
In a series of reactions that require additional proteins and GTP hydrolysis, the ribosome dissociates into its 2 separate subunits. Also mRNA, tRNA and RF are released.

The Genetic Code

- Each group of 3 nucleotides on the mRNA is a codon. Since there are 4 bases, there are $4^3 = 64$ possible codons, which must code for 20 different amino acids.
- More than one codon is used for most amino acids: the genetic code is “degenerate”. This means that it is not possible to take a protein sequence and deduce exactly the base sequence of the gene it came from.
- In most cases, the third base of the codon (the wobble base) can be altered without changing the amino acid.
- AUG is used as the start codon. All proteins are initially translated with methionine in the first position, although it is often removed after translation. There are also internal methionines in most proteins, coded by the same AUG codon.
- There are 3 stop codons, also called “nonsense” codons. Proteins end in a stop codon, which codes for no amino acid.


Second letter
U
C
A
G
First letter
Third letter
U
UUU UUC Phenyl-alanine
UUA UUG Leucine
UCU UCC UCA UCG Serine
UAU UAC Tyrosine
UAA Stop codon
UAG Stop codon
UGU UGC Cysteine
UGA Stop codon
UGG Tryptophan
C
CUU CUC CUA CUG Leucine
CCU CCC CCA CCG Proline
CAU CAC Histidine
CAA CAG Glutamine
CGU CGC CGA CGG Arginine
A
AUU AUC AUA Isoleucine
AUG Methionine; start codon
ACU ACC ACA ACG Threonine
AAU AAC Asparagine
AAA AAG Lysine
AGU AGC Serine
AGA AGG Arginine
G
GUU GUC GUA GUG Valine
GCU GCC GCA GCG Alanine
GAU GAC Aspartate
GAA GAG Glutamate
GGU GGC GGA GGG Glycine

Antibiotic target sites during bacterial protein synthesis.



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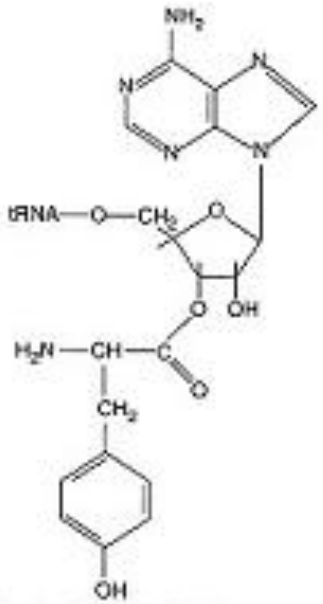
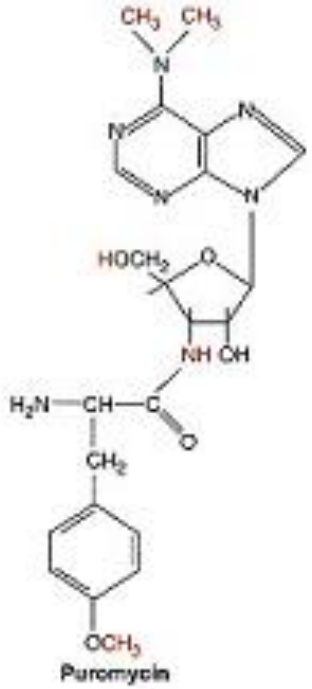
Antibiotics	Process inhibited
Edeine (Ede), kasugamycin (Ksg), pactamycin (Pct) , thermorubin (Thb), avilamycin (Avn) , evernimicin (Evn) and thiostrepton (Ths)	Inhibition of Initiation of protein synthesis
Streptomycin (Stp), tetracyclines (Tet) , glycylcyclines (tigecycline (Tig)).	The elongation cycle
Blasticidin S (Bls), chloramphenicol (Cam), lincosamides (clindamycin (Cln)), oxazolidinones (linezolid (Lnz)), pleuromutilins (Plu), puromycin (Pmn), streptogramin A (S_A) and sparsomycin (Spr).	Peptide-bond formation between the A- and P-site tRNAs
Capreomycin (Cap) , viomycin (Vio), aminoglycosides hygromycin B (HygB), neomycin (Neo), paromomycin (Par), fusidic acid (Fus) and Spectinomycin (Spt)	Translocation by inhibiting EF-G
Macrolides (erythromycin (Ery)), streptogramin B (S_B), ketolides and (telithromycin (Tel)).	Elongation of the nascent chain
Bls, Cam, Pmn and Spr,	Termination of the polypeptide synthesis

Antibiotic	**Mechanism of Protein Synthesis Inhibition**
Fusidic acid	Inhibits translocation in prokaryotes by inhibiting EF-G
Puromycin	Its structure is very similar to the 3' end of an aminoacyl-tRNA, (see Figure below) enabling it to bind to the ribosomal A site and participate in peptide bond formation, producing peptidyl-puromycin . However, because puromycin resembles only the 3' end of the tRNA, it does not engage in translocation and dissociates from the ribosome shortly after it is linked to the carboxyl terminus of the peptide. This prematurely terminates polypeptide synthesis.
Tetracyclines	Inhibit protein synthesis in bacteria by binding directly to ribosomes and blocking the A site on the ribosome, preventing the binding of aminoacyl-tRNAs.
Streptomycin	Binds small subunit of prokaryotic ribosomes, interferes with the initiation of protein synthesis and causes misreading of mRNA. It also interferes with elongation by inhibiting elongation factor Tu (EF-Tu)
Chloramphenicol	Inhibits protein synthesis by bacterial ribosomes by blocking peptidyl transfer i.e. peptidyltransferase; it does not affect cytosolic protein synthesis in eukaryotes.
Erythromycin	Is a macrolide antibiotic that interferes with translocation on prokaryotic ribosomes.

Toxin	Mechanism of Protein Synthesis Inhibition
Diphtheria toxin	Inhibits eukaryotic translocation by catalyzing ADP-ribosylation and inactivation of elongation factor 2(EF-2). ADP-ribose is attached to EF-2 as posttranslationally modified histidine residue known as diphthamide.
Ricin (from castor beans)	An extremely toxic protein, inactivates the 60S subunit of eukaryotic ribosomes by depurinating a specific adenosine in large subunit rRNA.



3' end of tyrozyl-tRNA



Puromycin