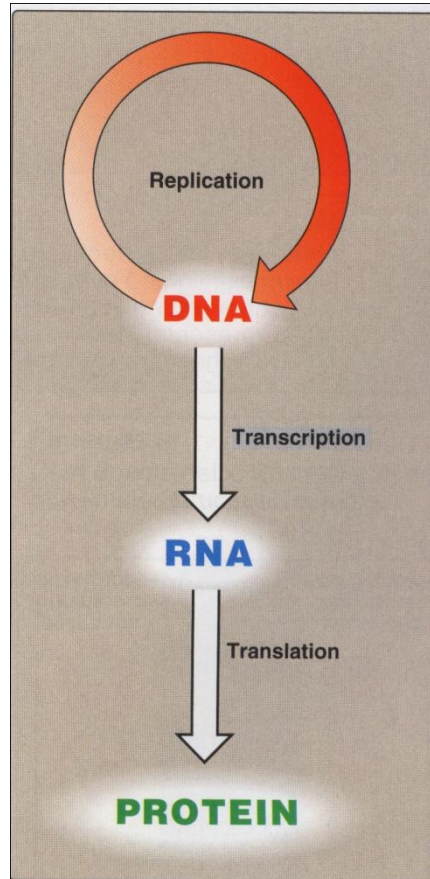


Protein Synthesis & the Genetic code

Genetic code: is the sum of all codons.

Codon: is a 3 letter code word consisting of the letters A,G,T,C

Genetic information Flows from DNA to RNA to Protein



- **Prokaryotes:** There is a linear correspondance between, Gene, mRNA and resulting Polypeptide.
- **Eukaryotes:** There is no linear corespondance. Primary transcripts (hnRNA) >>> from mRNA. hnRNA contains Introns + Exons while mRNA only contains Exons spliced together after removal of Introns.
- **Translation:** The machinery necessary to translate the information in mRNA (code) into amino acid sequense of the corresponding protein.
- **Adaptor molecule(tRNA):** can recognize (read) the **Genetic code** on the one hand, and a **specific amino acid** on the other hand.

mRNA Molecule consists of a series of codons that specify the Amino Acid sequence of the Encoded Protein

- There are 20 different Amino Acid, therefore there should be at least 20 different codon.
- A Codon consists of a sequence of 3 nucleotides, it is a Triplate code.
- The genetic code consists of 64 codon.

The Genetic code

First nucleotide	Second Nucleotide				Third Nucleotide
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Term	Term ²	A
	Leu	Ser	Term	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile ²	Thr	Lys	Arg ²	A
	Met	Thr	Lys	Arg ²	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

The Genetic code is Degenerate, UnAmbiguous, NonOverlapping, without Punctuation, & Universal

- **Nonsense codons:** there are 3 codons in the genetic code which do not specify amino acids they are used in the cell as stop codons (termination signals).
- The remaining codons ($64-3=61$) specify 20 amino acids.
- **Degeneracy** of the code: multiple codons must decode the same amino acid. Some amino acids are encoded by 6 codons (Serine), some have 1 codon (Tryptophan and methionine).
- **UnAmbiguosity** of the codon: for a specific codon only one amino acid is indicated.

- Degenerate but unambiguous code:

The codon of mRNA consisting of 3 nucleotides is recognized by Anticodon in tRNA by base-pairing rules, tRNA carries only one amino acid (unambiguity), but 3rd nucleotide in anticodon is not very specific therefore tRNA can recognize more than one code introducing same amino acid (degeneracy)

Example: UCU, UCC, UCA, UCG all are decoded as Serine (degeneracy). but for each of them only Serine is incorporated (unambiguity).

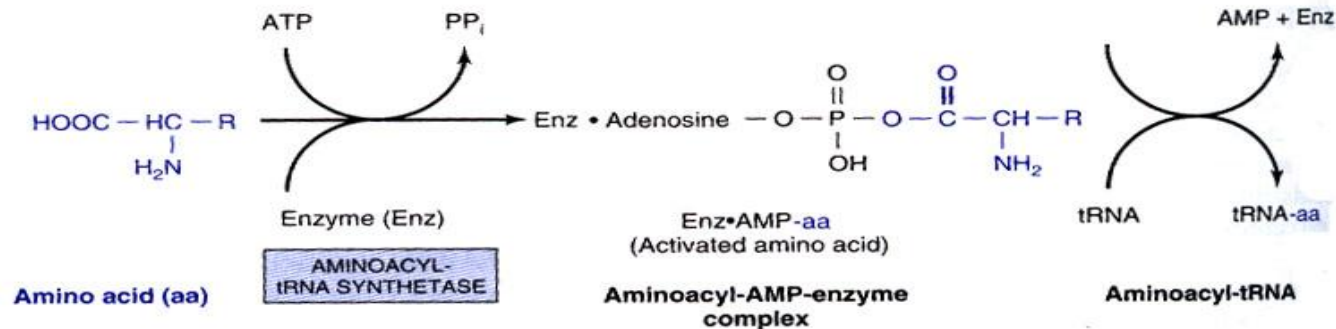
Given a specific codon, only a specific amino acid will be incorporated although given a specific amino acid more than one codon may be used.

- **NonOverlapping:**The reading of the Genetic code is nonoverlapping.
- There is no **punctuation** between codons in Genetic code, the message is read in a continuing sequence of nucleotide tripletes until a translation stop codon is reached .
- **Universal:**was thought that the Genetic code is Universal,but found that 4 of the mitochondria codons was read differently in same cell,even in humans.

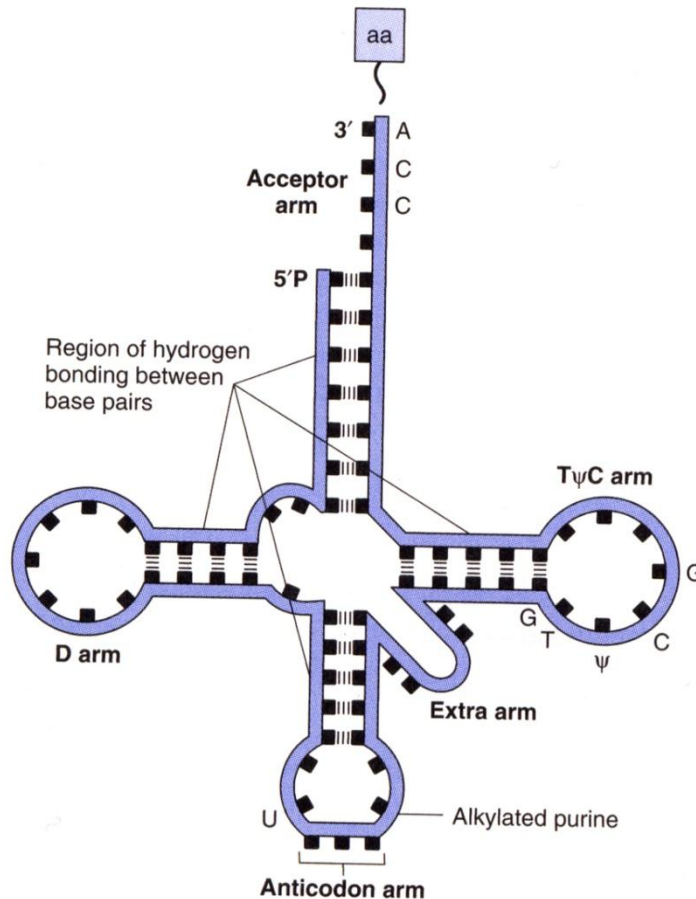
At least one species of tRNA exists for each of the 20 amino acids

- **Aminoacyl-tRNA synthetases:**

Responsible for recognition and attachment of each tRNA to its specific amino acid.



The involvement of different parts of tRNA in protein synthesis



- TψC arm: binding of aminoacyl-tRNA to the ribosome.
- D arm: important for recognition of a given tRNA by its proper aminoacyl-tRNA synthetase.
- Acceptor arm: the site of attachment of a specific AA.
- Anticodon arm: 7 nucleotides, recognizes the 3 letter codon of mRNA.
- Wobble base: is the last nucleotide in codon, the base pairing between this nucleotide and the corresponding nucleotide in anticodon is not strictly by the Watson-Crick rules. Example AGA and AGG can both bind to UCU.

Mutations Result when Changes Occur in the Nucleotide Sequence

- **1-Point Mutations**: Single Base Changes.

A. Transitions: Pyrimidine ↔ Pyrimidine

T ↔ C

Purine ↔ Purine

A ↔ G

B. Transversions: Purine ↔ Pyrimidine

A ↔ C

T ↔ A

G ↔ T

C ↔ G

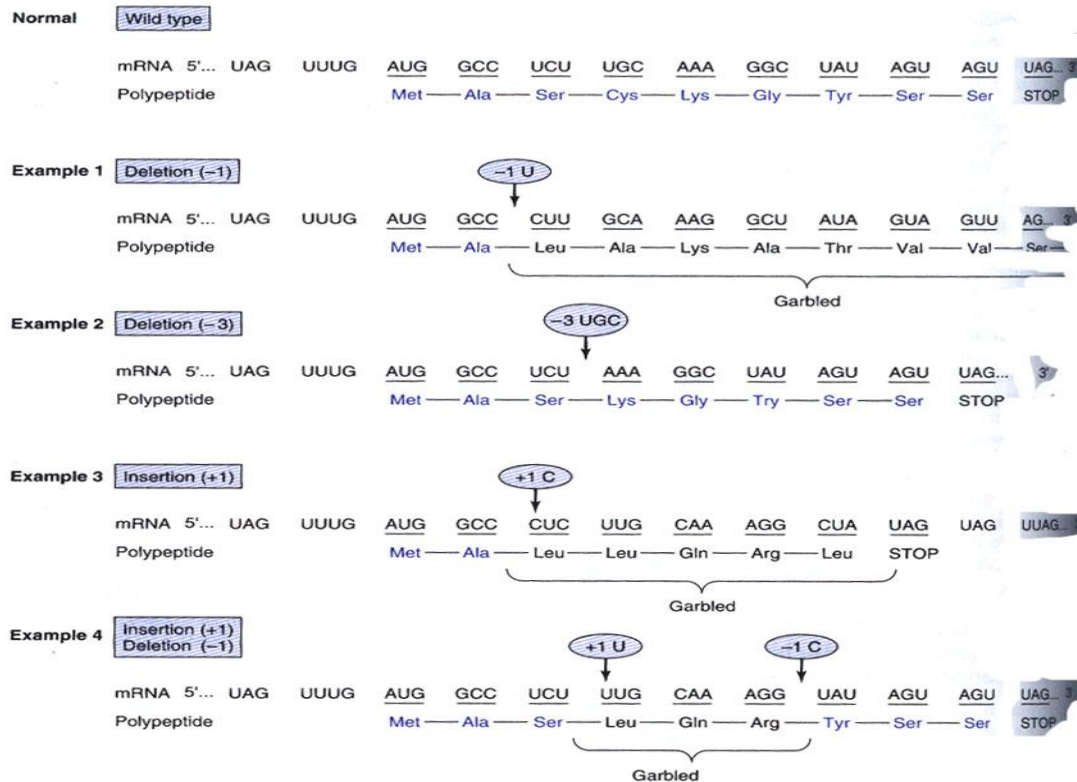
Consequences of point mutations

- **1.Silent Mutations**: No consequences, the changed base of mRNA occurs in 3rd nucleotide of codon (Wobble).
- **2.A missense effect**: When a different AA is incorporated in at the corresponding site in the protein molecule.
 - a-Acceptable: Resulting protein is not distinguishable.
 - b- partially acceptable:A protein with a partial but abnormal function
 - c- Unacceptable missense:The protein is not functional.
- **3.A nonsense codon**: A nonsense codon will appear that will result in the premature termination of a protein.

Examples of missense mutations

	Protein molecule	Amino acid	Codons
Acceptable missense	Hb A, β chain ↓ Hb Hikari, β chain	61 Lysine ↓ Asparagine	AAA or AAG ↓ ↓ AAU or AAC
Partially acceptable missense	Hb A, β chain ↓ Hb S, β chain	6 Glutamate ↓ Valine	GAA or GAG ↓ ↓ GUA or GUG
Unacceptable missense	Hb A, α chain ↓ Hb M (Boston), α chain	58 Histidine ↓ Tyrosine	CAU or CAC ↓ ↓ UAU or UAC

Frameshift Mutations Result from Deletion or Insertion of Nucleotides in DNA that generate Altered mRNA



Normal

Wild type

mRNA 5'... UAG UUUG AUG GCC UCU UGC AAA GGC UAU AGU AGU UAG 3'

Polypeptide Met — Ala — Ser — Cys — Lys — Gly — Tyr — Ser — Ser STOP

Example 1 Deletion (-1)

mRNA 5'... UAG UUUG AUG GCC CUU GCA AAG GCU AUA GUA GUU AG 3'

Polypeptide Met — Ala — Leu — Ala — Lys — Ala — Thr — Val — Val — Ser

-1 U

Garbled

Example 2 Deletion (-3)

mRNA 5'... UAG UUUG AUG GCC UCU AAA GGC UAU AGU AGU UAG... 3'

Polypeptide Met — Ala — Ser — Lys — Gly — Try — Ser — Ser STOP

-3 UGC

Example 3 Insertion (+1)

mRNA 5'... UAG UUUG AUG GCC CUC UUG CAA AGG CUA UAG UAG UUAG 3'

Polypeptide Met — Ala — Leu — Leu — Gln — Arg — Leu STOP

+1 C

Garbled

Example 4 Insertion (+1)
Deletion (-1)

mRNA 5'... UAG UUUG AUG GCC UCU UUG CAA AGG UAU AGU AGU UAG 3'

Polypeptide Met — Ala — Ser — Leu — Gln — Arg — Tyr — Ser — Ser STOP

+1 U -1 C

Garbled

Protein Synthesis occurs in Three Phases: Initiation, Elongation & Termination

- Initiation: Involves tRNA, rRNA, mRNA and around 10 Initiation factors (IF), GTP, ATP and Amino acids.

Occurs in 4 steps:

- Ribosomal Dissociation.
- Formation of the 43s Preinitiation complex.
- Formation of the 48s Initiation complex.
- Role of the Poly (A) tail in Initiation.

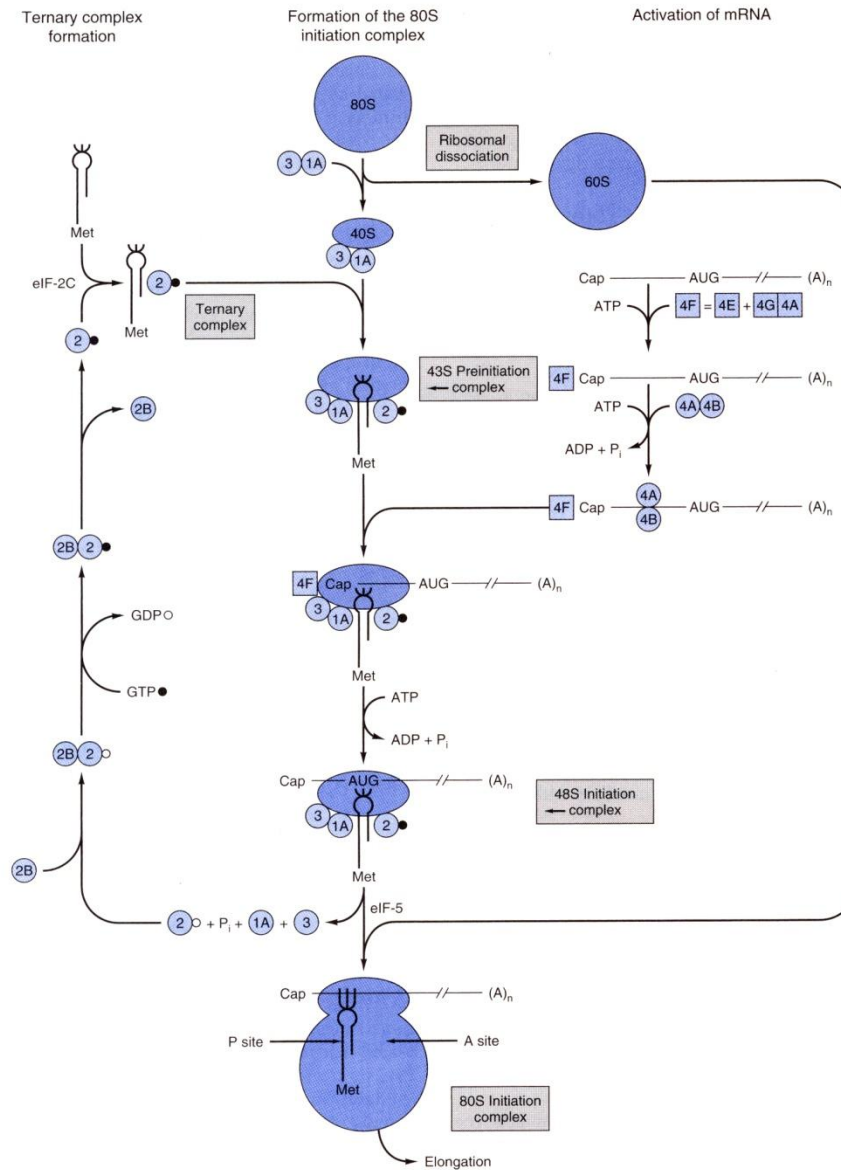
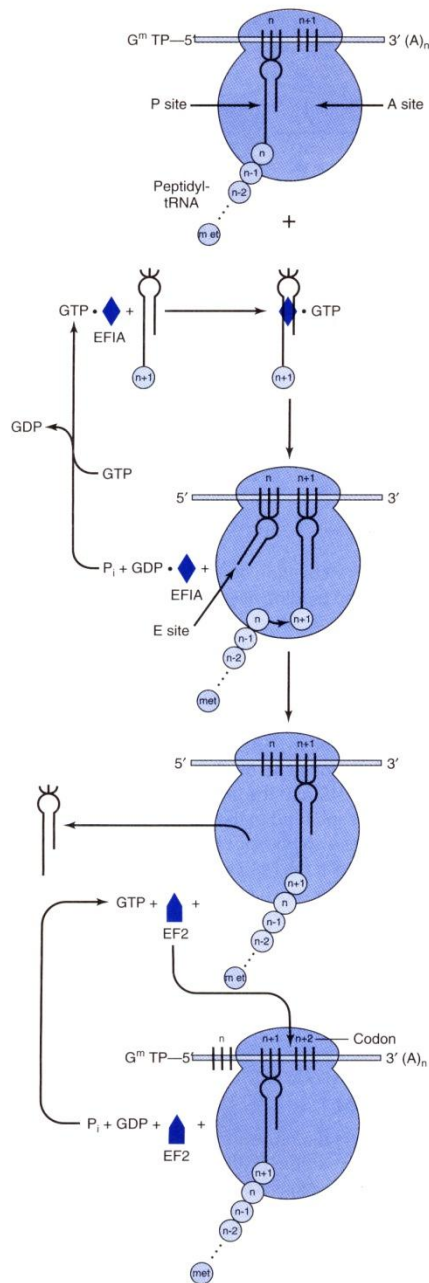


Figure 38-6. Diagrammatic representation of the initiation of protein synthesis on the mRNA template containing a 5' cap (G^mTP-5') and 3' poly(A) terminal [3'(A)_n]. This process proceeds in three steps: (1) activation of mRNA; (2) formation of the ternary complex consisting of tRNA^{Met}, initiation factor eIF-2, and GTP; and (3) formation of the active 80S initiation complex. (See text for details.) GTP, ●; GDP, ○. The various initiation factors appear in abbreviated form as circles or squares, eg, eIF-3 (③), eIF-4F (ⓁF). 4•F is a complex consisting of 4E and 4A bound to 4G (see Figure 38-7). The constellation of protein factors and the 40S ribosomal subunit comprise the 43S preinitiation complex. When bound to mRNA, this forms the 48S preinitiation complex.



A. BINDING OF AMINOACYL-tRNA TO THE A SITE

In the complete 80S ribosome formed during the process of initiation, the A site (aminoacyl or acceptor site) is free. The binding of the proper aminoacyl-tRNA in the A site requires proper codon recognition. **Elongation factor EF1A** forms a ternary complex with GTP and the entering aminoacyl-tRNA (Figure 38–8). This complex then allows the aminoacyl-tRNA to enter the A site with the release of EF1A•GDP and phosphate. GTP hydrolysis is catalyzed by an active site on the ribosome. As shown in Figure 38–8, EF1A-GDP then recycles to EF1A-GTP with the aid of other soluble protein factors and GTP.

B. PEPTIDE BOND FORMATION

The α -amino group of the new aminoacyl-tRNA in the A site carries out a nucleophilic attack on the esterified carboxyl group of the peptidyl-tRNA occupying the P site (peptidyl or polypeptide site). At initiation, this site is occupied by aminoacyl-tRNA met¹. This reaction is catalyzed by a **peptidyltransferase**, a component of the 28S RNA of the 60S ribosomal subunit. This is another example of ribozyme activity and indicates an important—and previously unsuspected—direct role for RNA in protein synthesis (Table 38–3). Because the amino acid on the aminoacyl-tRNA is already “activated,” no further energy source is required for this reaction. The reaction results in attachment of the growing peptide chain to the tRNA in the A site.

C. TRANSLOCATION

The now deacylated tRNA is attached by its anticodon to the P site at one end and by the open CCA tail to an **exit (E) site** on the large ribosomal subunit (Figure 38–8). At this point, **elongation factor 2 (EF2)** binds to and displaces the peptidyl tRNA from the A site to the P site. In turn, the deacylated tRNA is on the E site, from which it leaves the ribosome. The EF2-GTP complex is hydrolyzed to EF2-GDP, effectively moving the mRNA forward by one codon and leaving the A site open for occupancy by another ternary complex of amino acid tRNA-EF1A-GTP and another cycle of elongation.

Figure 38–8. Diagrammatic representation of the peptide elongation process of protein synthesis. The small circles labeled $n - 1$, n , $n + 1$, etc., represent the amino acid residues of the newly formed protein molecule. EF1A and EF2 represent elongation factors 1 and 2, respectively. The peptidyl-tRNA and aminoacyl-tRNA sites on the ribosome are represented by P site and A site, respectively.

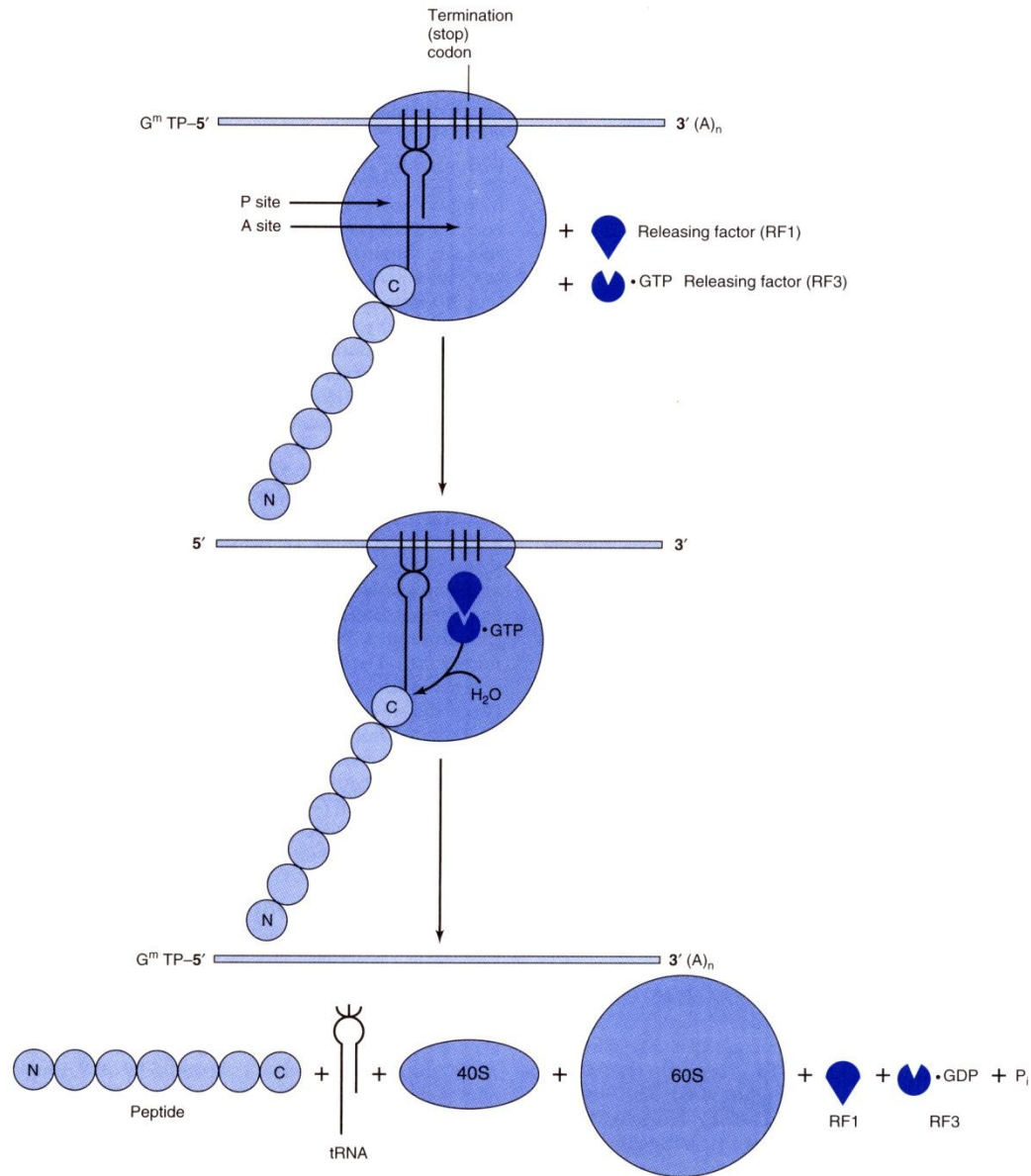


Figure 38-9. Diagrammatic representation of the termination process of protein synthesis. The peptidyl-tRNA and aminoacyl-tRNA sites are indicated as P site and A site, respectively. The termination (stop) codon is indicated by the three vertical bars. Releasing factor RF1 binds to the stop codon. Releasing factor RF3, with bound GTP, binds to RF1. Hydrolysis of the peptidyl-tRNA complex is shown by the entry of H₂O. N and C indicate the amino and carboxyl terminal amino acids, respectively, and illustrate the polarity of protein synthesis.