Translation

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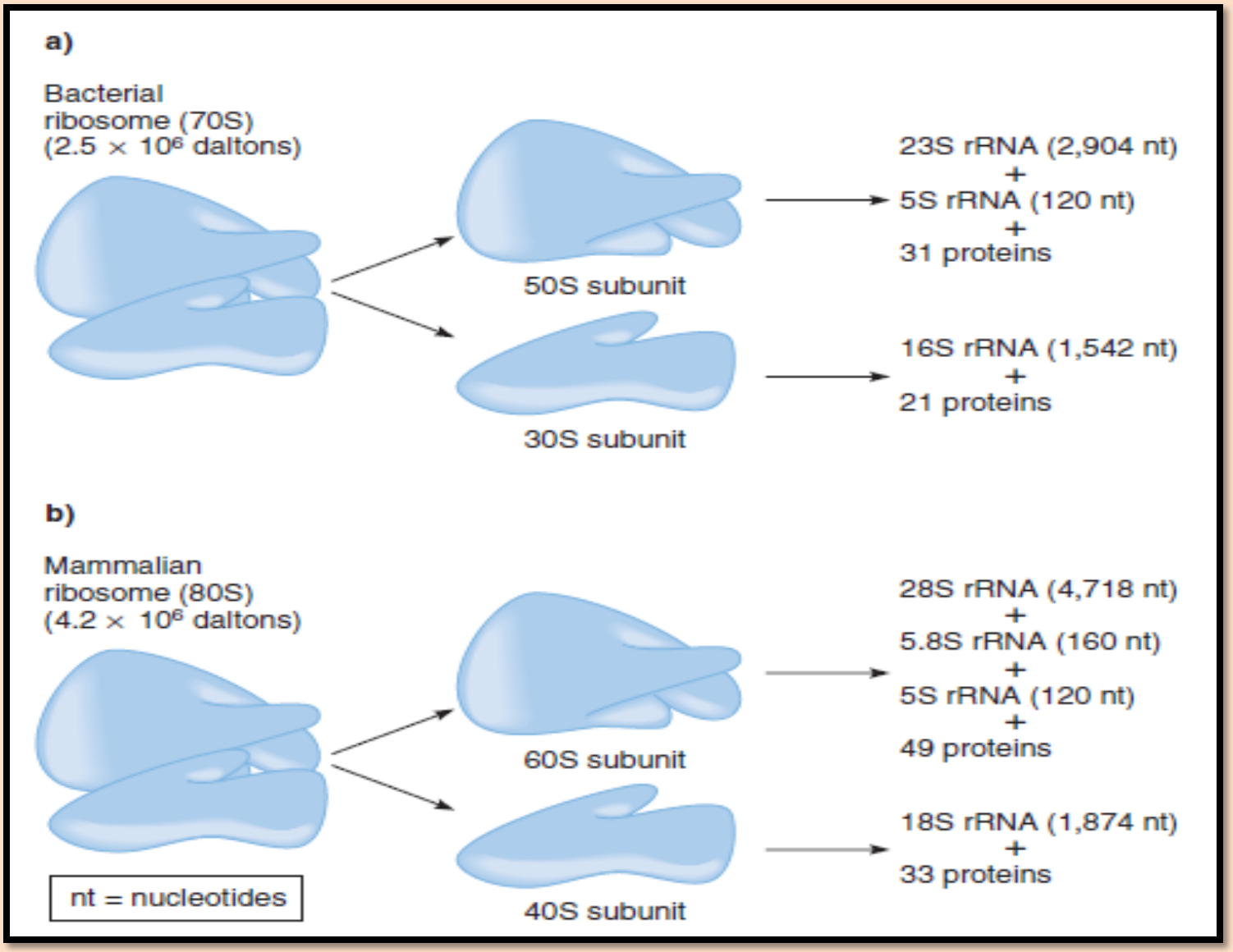
The fundamental process of **protein synthesis** is the formation of a **peptide bond** between the **carboxyl group of amino acid** at the end of a growing polypeptide chain and a **free amino group** on **another amino acid**. Proteins are made up from a set of **20 amino acids called standard amino acids**. Each of the 20 amino acids is specified by specific codons. One additional amino acid - selenocysteine present in some polypeptide is directed by a modified reading of the genetic code (5'UGA3). Polypeptide synthesis proceeds from **N-terminus to C-terminus** and ribosome read mRNA in **the 5' to 3' direction**.

Three kinds of RNA molecules perform different but cooperative functions in protein


a)
Bacterial
ribosome (70S)
(2.5 × 10⁶ daltons)
50S subunit
30S subunit
23S rRNA (2,904 nt)
+
5S rRNA (120 nt)
+
31 proteins
16S rRNA (1,542 nt)
+
21 proteins
b)
Mammalian
ribosome (80S)
(4.2 × 10⁶ daltons)
60S subunit
40S subunit
28S rRNA (4,718 nt)
+
5.8S rRNA (160 nt)
+
5S rRNA (120 nt)
+
49 proteins
18S rRNA (1,874 nt)
+
33 proteins
nt = nucleotides

Ribosomes

Polypeptide synthesis takes place on ribosomes, many thousands of which occur in each cell. Ribosomes bind to mRNA and facilitate the binding of the tRNA to the mRNA so that a polypeptide chain can be synthesized.

Ribosomal RNA and Ribosomes.

In both prokaryotes and eukaryotes, ribosomes consist of two unequally sized subunits—the **large and small ribosomal subunits**—each of which consists of a complex between RNA molecules and proteins. Each subunit contains one or more specific rRNA molecules and a large number of ribosomal proteins.

The bacterial ribosome has a size of 70S and consists of two subunits of sizes 50S (large subunit) and 30S (small subunit). The **50S subunit contains 23SrRNA(2904nucleotides) ,the small 5SRNA(120nucleotides) and 31 proteins.**

The **30S subunit consists of 16SrRNA(1542 nucleotides) and 21 r-proteins**.

Eukaryotic ribosomes are larger and more complex than their prokaryotic counterparts, and they vary in size and composition among eukaryotic organisms. Mammalian ribosomes, for example, have a size of 80S and consist of a large 60S subunit and a small 40S subunit. The **60Ssubunit contains 28SrRNA(4718 nucleotides),the small 5SrRNA(120 nucleotides),5.8SrRNA(160 nucleotides) and about 50 proteins**. The **40Ssubunit consists of the 18SrRNA(1874 nucleotides) and 33 rproteins.**

The rRNA molecules play a structural role in ribosome and have a functional role in several steps of translation.

The rRNA in the small subunit pairs with the shine dalgarno sequence in the mRNA and the rRNA in the large subunit has peptidyl transferase activity.

The 70S ribosome has three tRNA binding sites;
P-site: Psite, also called the peptidyl tRNA binding site holds the tRNA molecule that is linked to the growing end of the polypeptide chain.
A-site: a-sie,also called the aminoacyl tRNA binding site,holds the the incoming tRNA molecule charged with an amino acid.
E-site: Deacylated tRNA(lacking any amino acid)exits via the E site,also called the exit site.

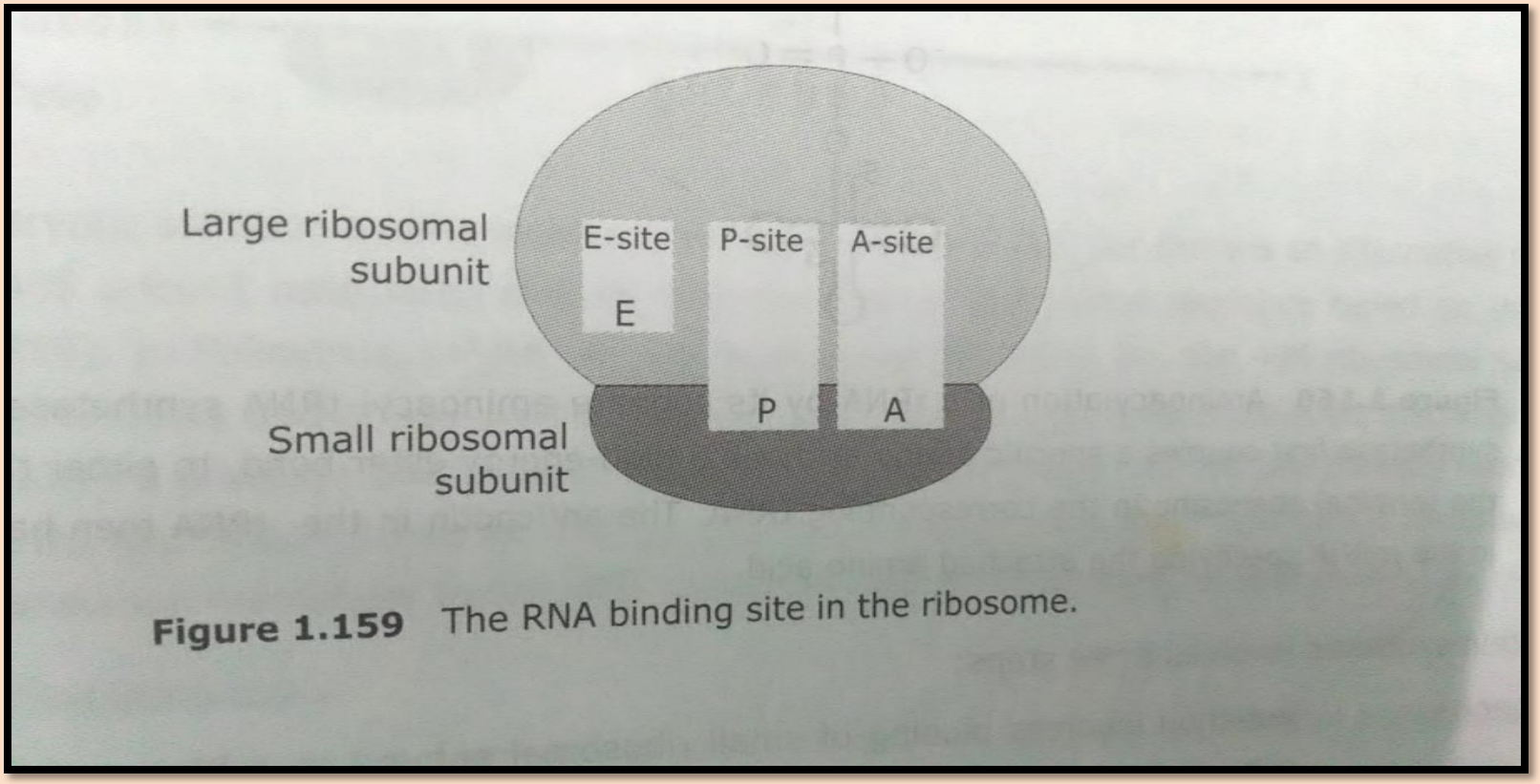


Figure 1.159 The RNA binding site in the ribosome.

mRNA

mRNA carries the genetic information copied from DNA in the form of a series of **three-base code words (codons**), each of which **specifies a particular amino acid**.

Comparison of the structures of prokaryotic and eukaryotic mRNA

Eukaryotic mRNAs are mostly **monocistronic**; having an average size of 1500 to 2000 nucleotides. It has **a 5' cap**. which is recognized by the **small ribosomal subunit**. Protein synthesis, therefore, begins at an initiation codon near the 5 end of the mRNA. Upstream of the initiation codon contains a non-translatable sequences called **5' UTR (5-untranslated region) or leader sequence**. Similarly non translatable sequences at the 3' end after stop codon is termed as **3' UTR (3-untranslated region) or trailer sequence**, which varies in length and sequence.

In prokaryotes, most of the **mRNAs are polycistronic**. In contrast to eukaryotic mRNAs, the 5 end has no cap-like structure, and there are **multiple ribosome-binding sites** (called Shine-Dalgarno sequences) within the **polycistronic mRNA chain**, each resulting in the synthesis of a **different protein**. Just like prokaryotic mRNA, eukaryotic mRNA also contains 5 UTR and 3' UTR.

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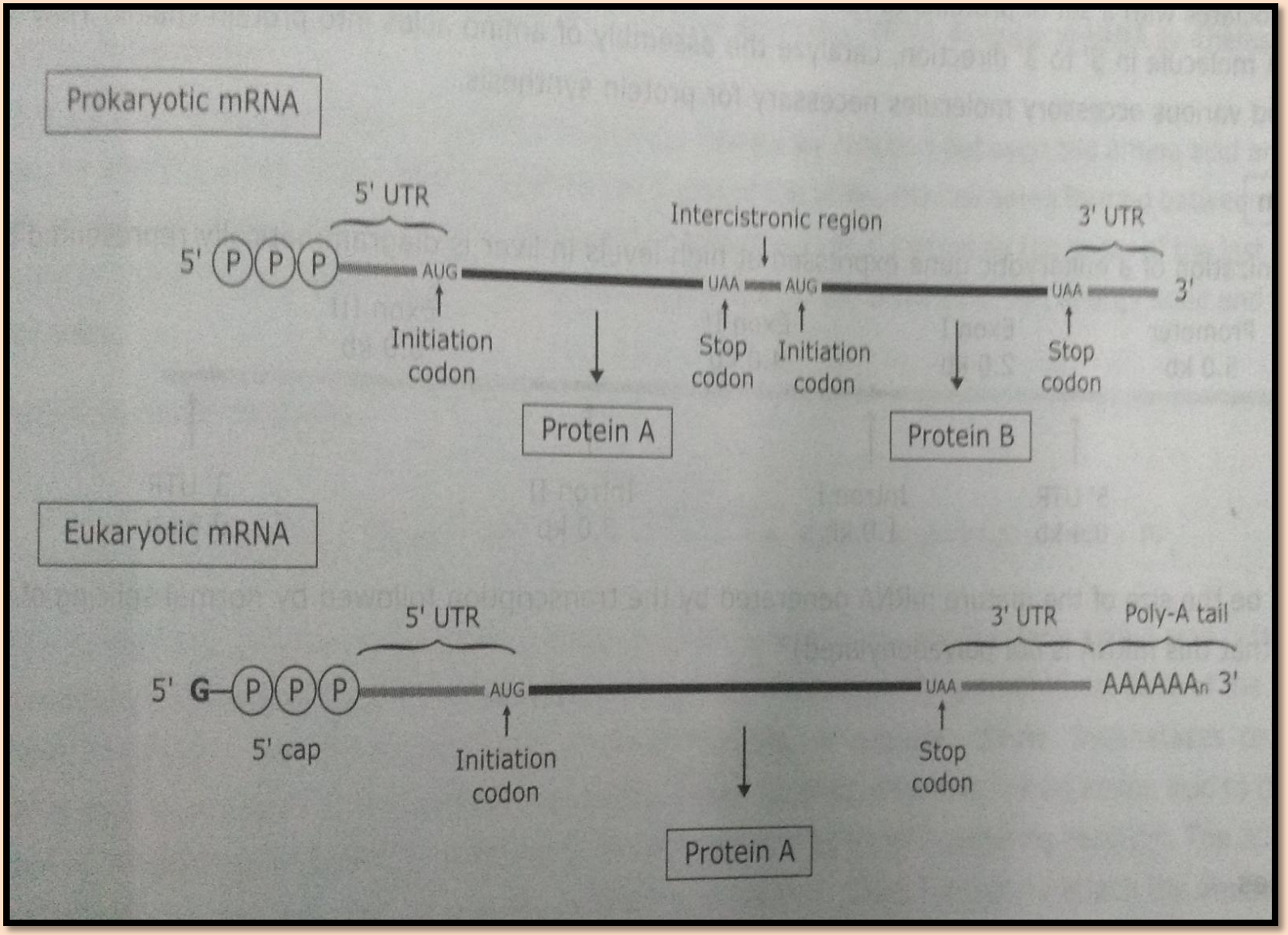

Prokaryotic mRNA
5' UTR
Intercistronic region
3' UTR
5'
P P P
AUG
UAA
AUG
UAA
3'
Initiation codon
Stop codon
Initiation codon
Stop codon
Protein A
Protein B
Eukaryotic mRNA
5' UTR
3' UTR
Poly-A tail
5'
G
P P P
AUG
UAA
AAAAAAn 3'
5' cap
Initiation codon
Stop codon
Protein A

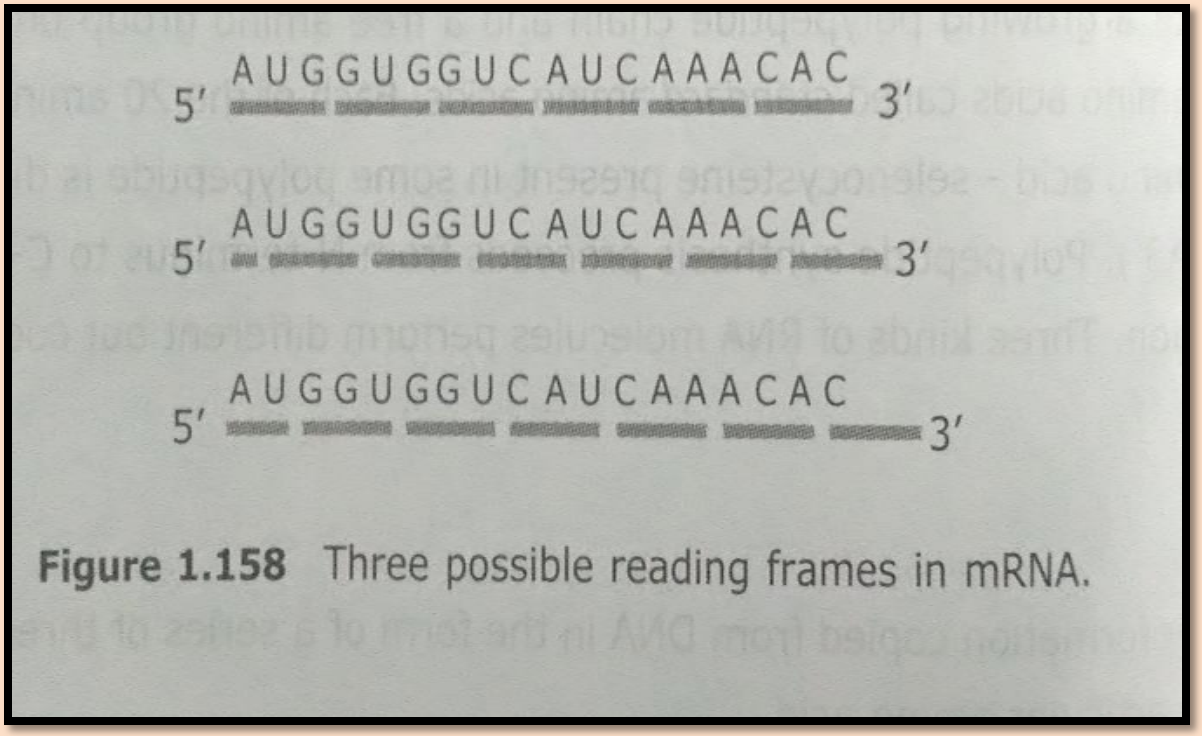


Figure 1.158 Three possible reading frames in mRNA.

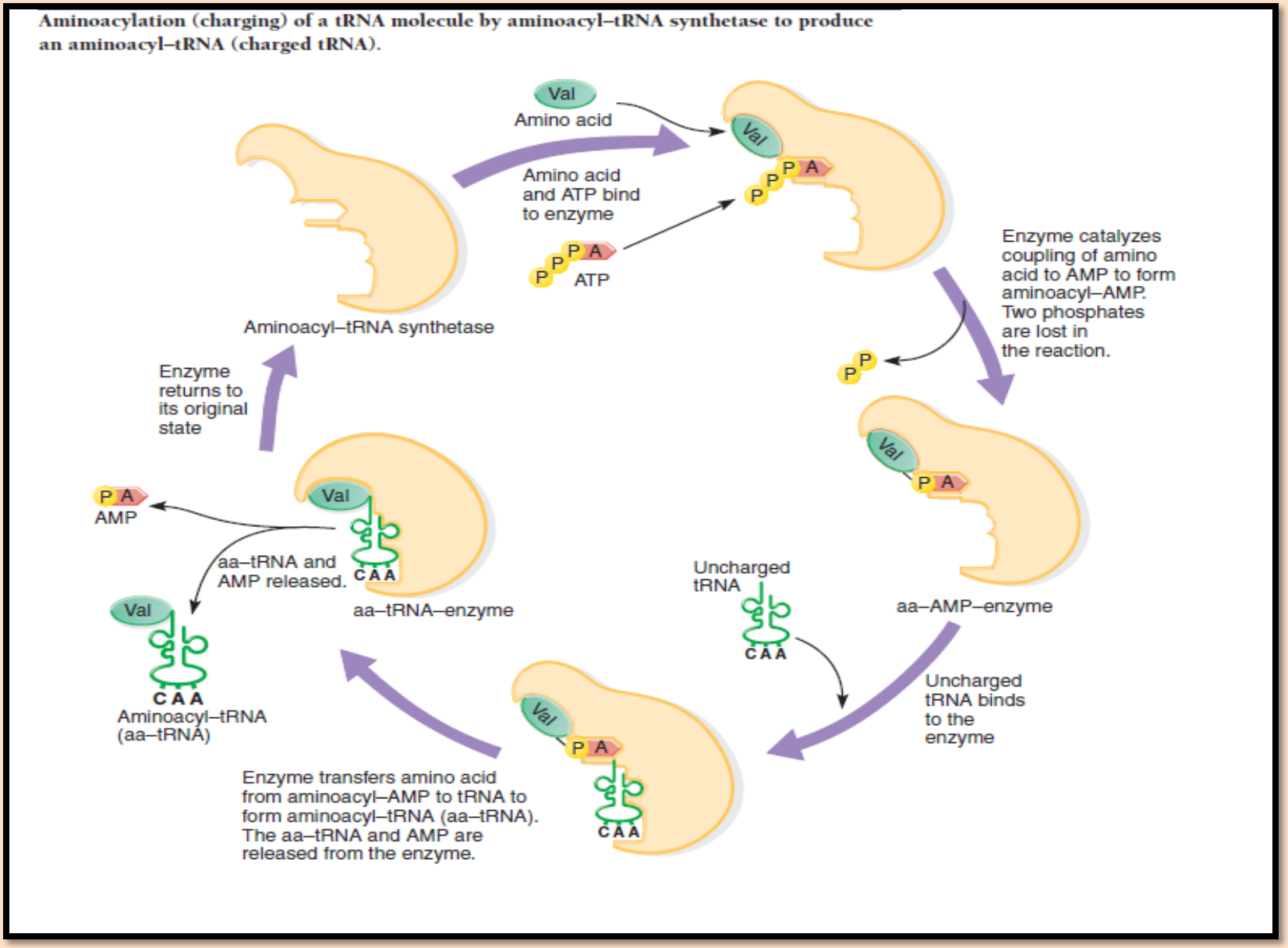
An mRNA can be translated in three different reading frames, depending on where the decoding process begins.

However, only one of the three possible reading frames in an mRNA encodes the required protein. Any sequence of bases (in DNA or RNA) that could, at least theoretically, encode a polypeptide, is known as an **open reading frame, often abbreviated to ORF.** The reading frame is open in the sense that it does not contain any stop codons that would interrupt its translation into termination a polypeptide chain.

The first stage of translation is the **binding of tRNA molecules to their appropriate amino acids, called tRNA charging**. Each tRNA is specific for a particular amino acid. All tRNAs have the sequence **5'CCA3'** at the 3′ end, and the **carboxyl group (COOH)** of the amino acid is attached to the **adenine nucleotide at the 3′ end** of the tRNA. **If each tRNA** is specific for **a particular amino acid** but all **amino acids are attached to the same nucleotide (A) at the 3′ end of a tRNA**, how does a **tRNA** link up with its **appropriate amino acid**? The key to **specificity between an amino acid and its tRNA** is a set of enzymes called **aminoacyl-tRNA synthetases.**

A cell has **20 different aminoacyl-tRNA synthetases**, **one for each of the 20 amino acids**. Each **synthetase recognizes a particular amino acid**, as well as **all the tRNAs** that accept

that **amino acid**. Recognition of the **appropriate amino acid** by a synthetase is based on the **different sizes, charges, and R groups** of the amino acids. The **recognition of tRNAs** by a synthetase depends on the **differing nucleotide sequences of the tRNAs** i.e, the **anticodon arm** and the **sequences in the acceptor stem**.



shows the charging of a tRNA molecule to produce valine–tRNA (Val–tRNA).

Adding an Amino Acid to tRNA.

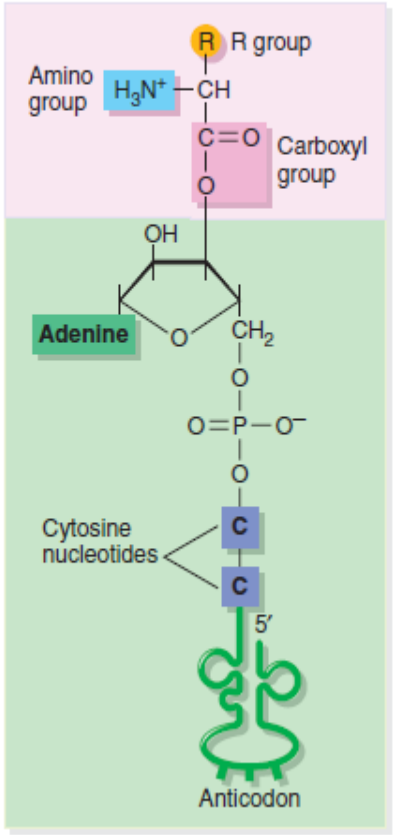
The correct amino acid is attached to the tRNA by an enzyme called **aminoacyl–tRNA synthetase**. The process is called aminoacylation, or charging, and produces an aminoacyl–tRNA (or chargedtRNA). Aminoacylation uses energy from ATP hydrolysis.

First, the **amino acid** and **ATP** bind to the **specific aminoacyl–tRNA synthetase** enzyme. The enzyme then catalyzes a reaction in which the **ATP is hydrolyzed to AMP**, which joins to the amino acid as AMP to form **aminoacyl–AMP.** Next, the tRNA molecule binds to the enzyme, which transfers the amino acid from the aminoacyl–AMP to the tRNA and displaces the AMP. The enzyme then releases the aminoacyl–tRNA molecule. Chemically, the amino acid attaches at the end of the tRNA by a **covalent linkage between the carboxyl group of the amino acid and the OH group of the ribose of the adenine nucleotide** found at the **end of every tRNA**.

Overall aminoacylation reaction:

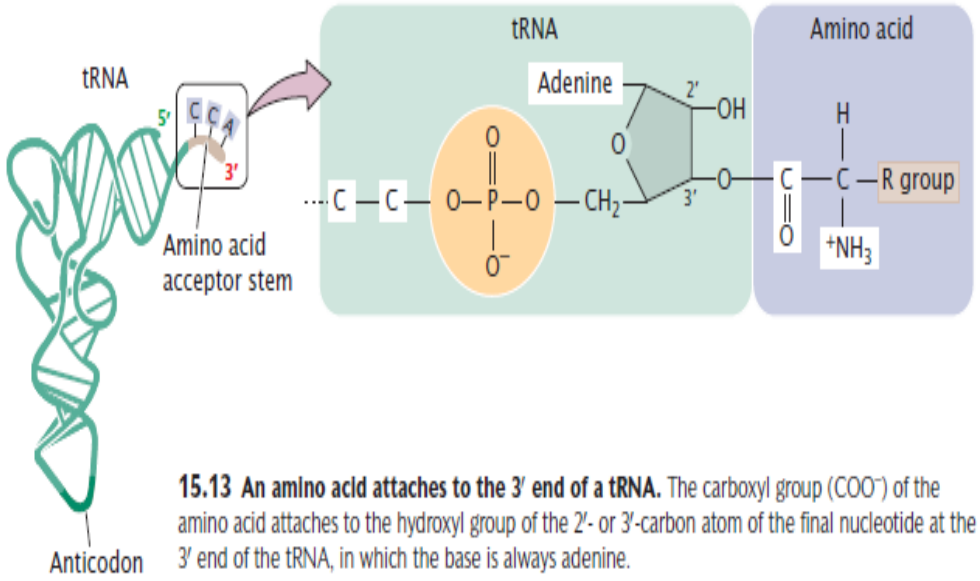
Amino acid+tRNA+ATP $\xrightarrow{\text{Amino acyl-tRNA synthetase}}$ Amino acyl-tRNA+AMP+Ppi

Attachment of an amino acid to a tRNA molecule. In an aminoacyl–tRNA molecule (charged tRNA), the carboxyl group of the amino acid is attached to the 3′-OH or 2′-OH group of the 3′ terminal adenine nucleotide of the tRNA.



Amino acid attached by carboxyl group to ribose of last ribonucleotide of tRNA chain

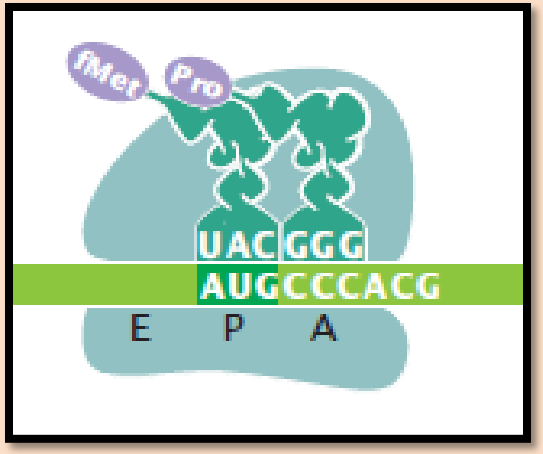
Last 3 nucleotides of all tRNAs are -CCA-3′

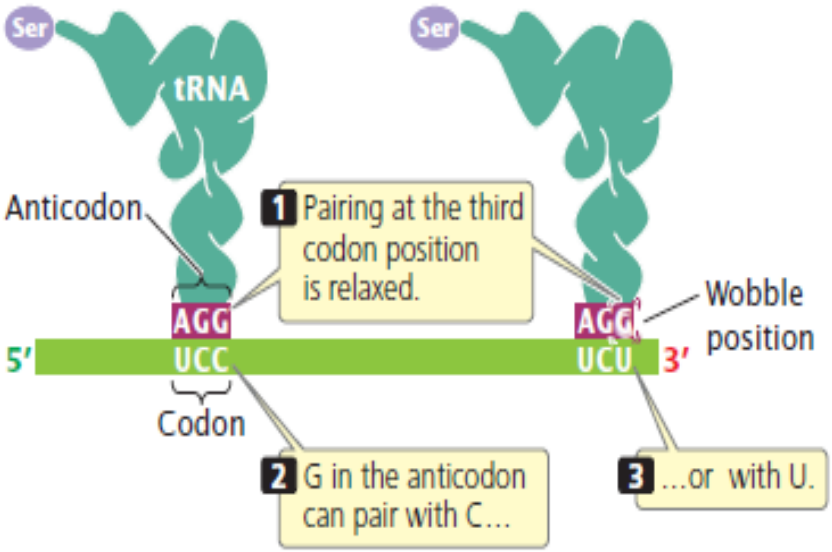


15.13 An amino acid attaches to the 3′ end of a tRNA. The carboxyl group (COO^-) of the amino acid attaches to the hydroxyl group of the 2′- or 3′-carbon atom of the final nucleotide at the 3′ end of the tRNA, in which the base is always adenine.

The 20 aminoacyl-tRNA synthetases fall into two distinct groups**, ClassI and ClassII**. **ClassI** attach the amino acid to the **2'OH** group of the terminal Adenine of the tRNA, whereas **ClassII** attach the amino acid to the **3'OH** group of the terminal Adenine of the tRNA.

Each tRNA molecule **brings a specific amino acid** to the ribosome to be added to the growing **polypeptide chain**. The amino acid is added to **a tRNA by an amino acid-specific aminoacyl–tRNA synthetase enzyme.**
The **anticodon of a tRNA is keyed to the amino acid it carries**, and it pairs with a **complementary codon** in an **mRNA molecule**.



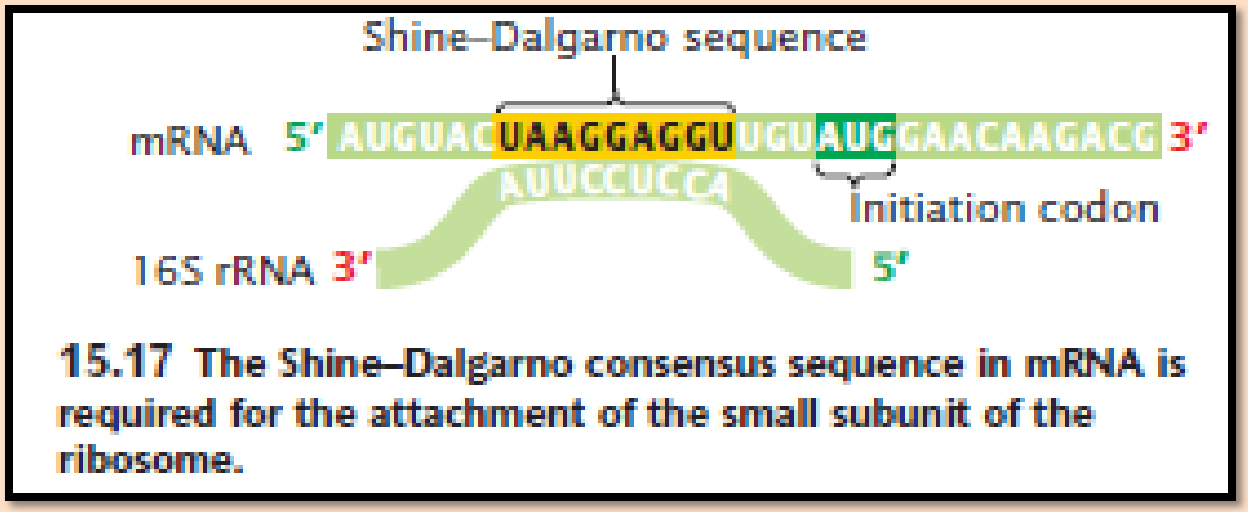


15.11 Wobble may exist in the pairing of a codon and anticodon. The mRNA and tRNA pair in an antiparallel fashion. Pairing at the first and second codon positions is in accord with the Watson-and-Crick pairing rules (A with U, G with C); however, pairing rules are relaxed at the third position of the codon, and G on the anticodon can pair with either U or C on the codon in this example.

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

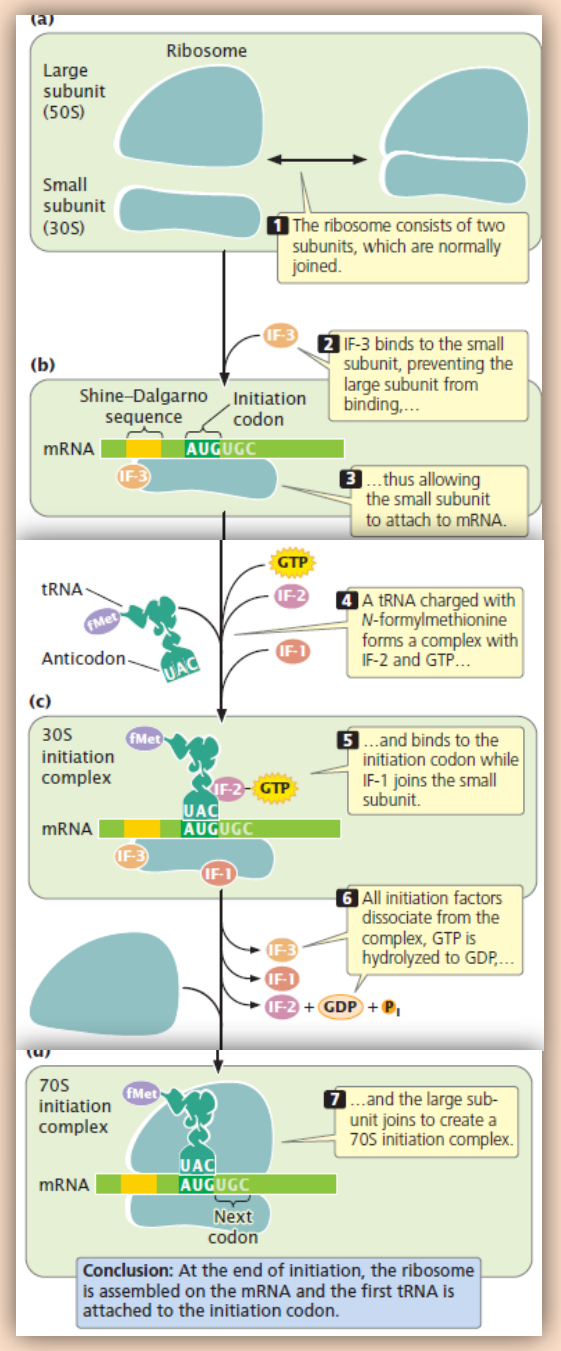
The Initiation of Translation

The second stage in the process of protein synthesis is initiation. At this stage, all the components necessary for protein synthesis assemble: (1**) mRNA; (2) the small and large subunits of the ribosome; (3) a set of three proteins called initiation factors; (4) initiator tRNA with *N-formylmethionine attached* (fMet-tRNAfMet); and (5) guanosine triphosphate (GTP**). Initiation comprises three major steps. **First, mRNA binds to the small subunit of the ribosome. Second, initiator tRNA binds to the mRNA through base pairing between the codon and the anticodon. Third, the large ribosome joins the initiation complex. Let's look at each of these steps more closely.**



15.17 The Shine–Dalgarno consensus sequence in mRNA is required for the attachment of the small subunit of the ribosome.

Initiation in bacteria The functional ribosome of bacteria exists as two subunits, the small 30S subunit and the large 50S subunit **. An mRNA molecule can** bind to the small ribosome subunit only when the subunits are separate. **Initiation factor 3 (IF-3) binds to the small**
subunit of the ribosome and prevents the large subunit from
binding during initiation.
Another factor, initiation factor 1 (IF-1), enhances the disassociation of the
large and small ribosomal subunits. **The sequence covered by the ribosome during initiation is from 30 to 40 nucleotides long and includes the AUG initiation codon**. Within the ribosome-binding site is the **Shine–Dalgarno consensus sequence**, which is **complementary** to a sequence of nucleotides at the **3′ end of 16S rRNA** (part of the small subunit of the ribosome). During initiation, the nucleotides in the **Shine–Dalgarno sequence pair with their complementary nucleotides in the 16S rRNA,** allowing the **small subunit of the ribosome to attach to the mRNA and positioning the ribosome directly over the initiation codon.**
Next, the initiator **tRNA, fMet-tRNAfMet**, attaches to the
initiation codon .This step requires **initiation factor 2 (IF-2), which forms a complex with GTP.**
At this point, the initiation complex consists of (1) the small subunit of the ribosome; (2) the mRNA; (3) the initiator tRNA with its amino acid (fMet-tRNAfMet); (4) one molecule of GTP; and (5) several initiation factors. These components are collectively known as the **30S initiation complex .**
In the final step of initiation, **IF-3 dissociates** from the small subunit, **allowing the large subunit of the ribosome to join the initiation complex**. The molecule of **GTP** (provided by IF-2) is **hydrolyzed** to guanosine diphosphate (GDP), and the **initiation factors dissociate**. When the large subunit has joined the initiation complex, the complex is called the **70S initiation complex.**


(a)
Ribosome
Large subunit (50S)
Small subunit (30S)
1 The ribosome consists of two subunits, which are normally joined.
IF-3
2 IF-3 binds to the small subunit, preventing the large subunit from binding,…
(b)
Shine–Dalgarno sequence
Initiation codon
mRNA
AUGUGC
IF-3
3 …thus allowing the small subunit to attach to mRNA.
GTP
IF-2
IF-1
tRNA
fMet
Anticodon
UAC
4 A tRNA charged with N-formylmethionine forms a complex with IF-2 and GTP…
(c)
30S initiation complex
fMet
UAC
IF-2
GTP
mRNA
AUGUGC
IF-3
IF-1
5 …and binds to the initiation codon while IF-1 joins the small subunit.
6 All initiation factors dissociate from the complex, GTP is hydrolyzed to GDP,…
IF-3
IF-1
IF-2 + GDP + Pi
(d)
70S initiation complex
fMet
UAC
mRNA
AUGUGC
Next codon
7 …and the large subunit joins to create a 70S initiation complex.
Conclusion: At the end of initiation, the ribosome is assembled on the mRNA and the first tRNA is attached to the initiation codon.

Initiation in eukaryotes Similar events take place in the initiation of translation in eukaryotic cells, but there are some important differences. In bacterial cells, sequences in 16S rRNA of the small subunit of the ribosome bind to the Shine–Dalgarno sequence in mRNA. No analogous consensus sequence exists in eukaryotic mRNA. Instead, the **cap at the 5′ end of eukaryotic mRNA plays a critical role in the initiation of translation. In a series of steps, the small subunit**

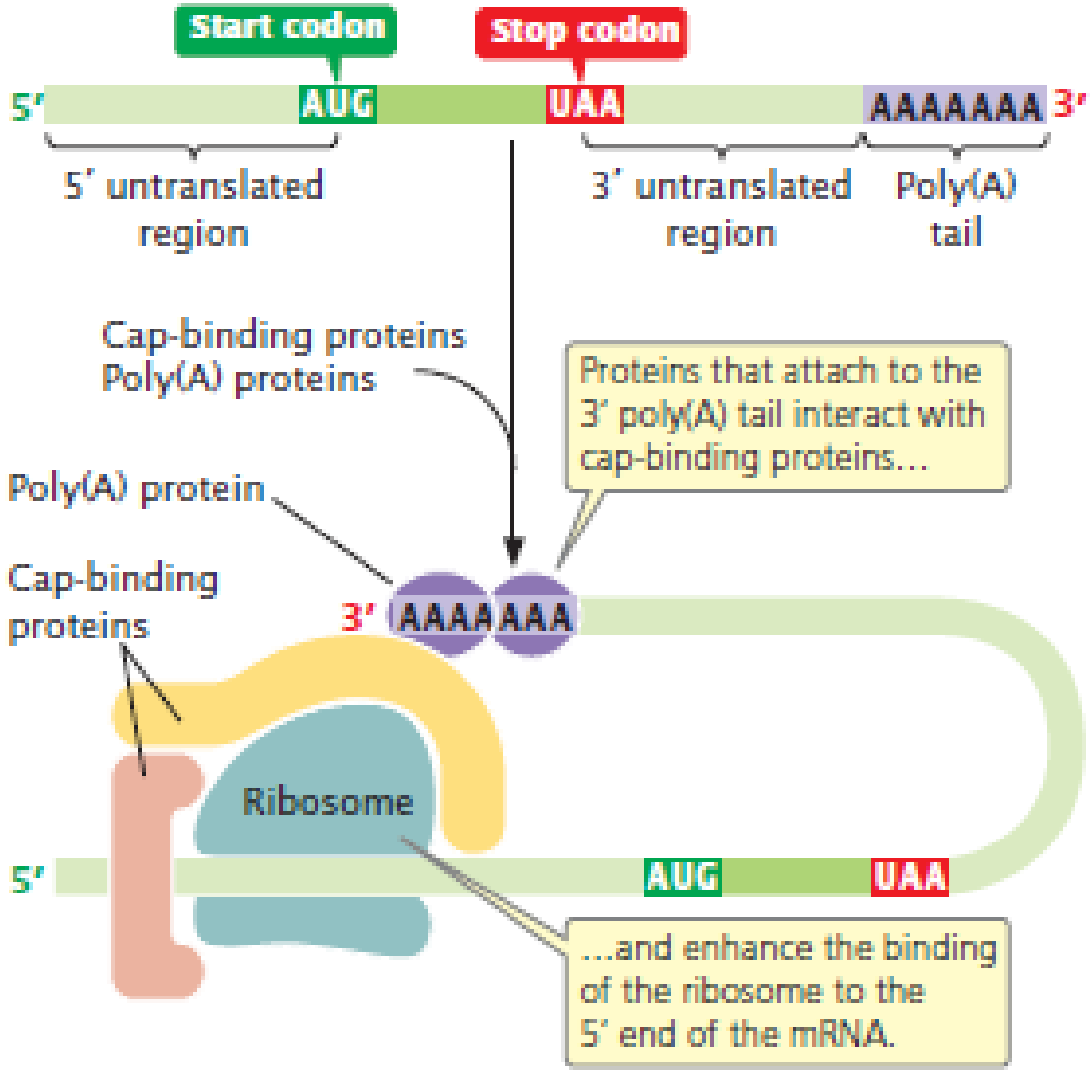
of the eukaryotic ribosome, initiation factors, and the initiator tRNA with its amino acid (Met-tRNAi Met) form an initiation complex that recognizes the cap and binds there. The initiation complex then **moves along (scans) the mRNA until it locates the first AUG** codon. The identification of the **start codon is facilitated** by the presence of a **consensus sequence** (called **the Kozak sequence**) that surrounds the start codon:

5 –ACC**AUG**G–3

Kozak sequence

Another important difference is that eukaryotic initiation requires at **least seven initiation factors.** Some factors keep the **ribosomal subunits separated**, just **as IF-3** does in bacterial cells. Others recognize **the 5′ cap on mRNA and allow the small subunit of the ribosome** to bind there.Still others **possess RNA helicase activity**, which is used to unwind secondary structures that may exist in the **5′ untranslated region of mRNA**, allowing the **small subunit to move down the mRNA until the initiation codon is reached**. Other initiation factors **help bring Met-tRNAi Met to the initiation complex.**

The **poly(A) tail** at the 3′ end of eukaryotic mRNA also plays a role in the initiation of translation. During initiation, **proteins that attach to the poly(A) tail interact with proteins that bind to the 5′ cap, enhancing the binding of the small subunit of the ribosome to the 5′ end of the mRNA.** This interaction indicates that **the 3′ end of mRNA bends over and associates with the 5′ cap** during the initiation of translation, forming a circular structure known as the closed loop



15.18 The poly(A) tail of eukaryotic mRNA plays a role in the initiation of translation.

Elongation

The next stage in protein synthesis is elongation, in which amino acids are joined to create a polypeptide chain. **Elongation requires (1) the 70S complex just described; (2)tRNAs charged with their amino acids; (3) several elongation factors; and (4) GTP.**

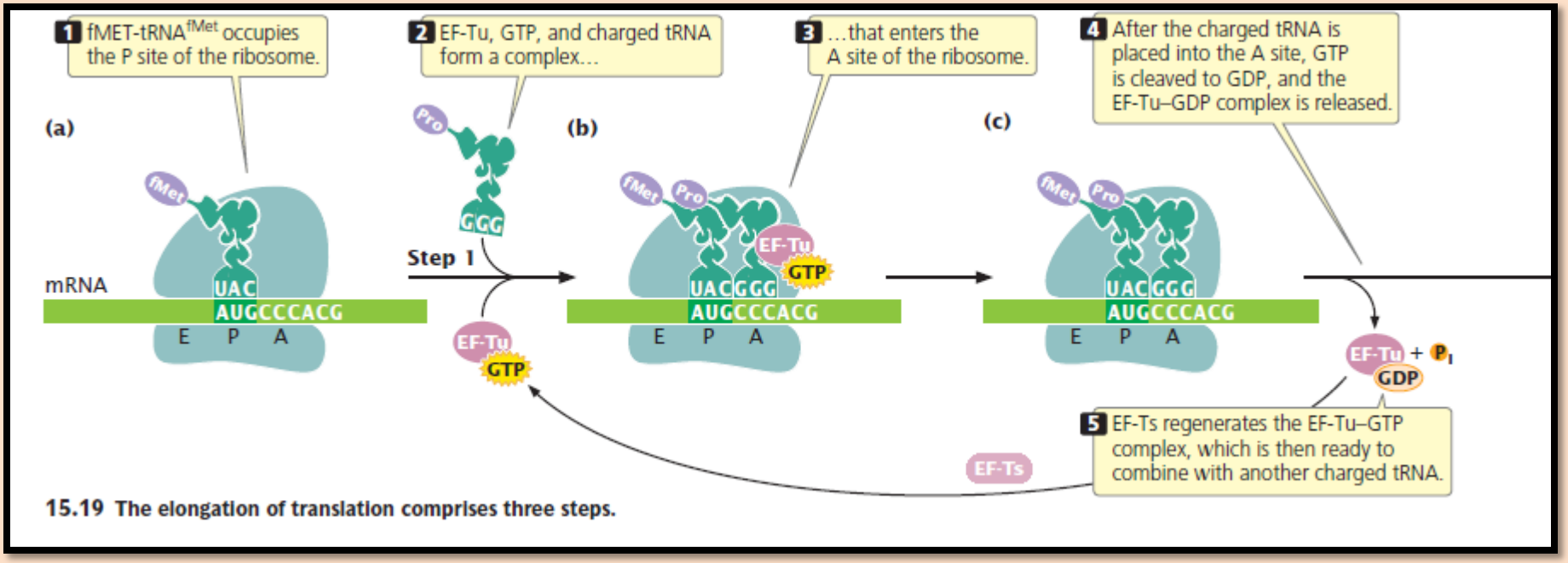
A ribosome has three sites that can be occupied by tRNAs; the **aminoacyl, or A, site, the peptidyl, or P, site,** and the **exit, or E, site.The initiator tRNA immediately occupies the P site (the only site to which the fMet-tRNAfMet is capable of binding), but all other tRNAs first enter the A site.** After initiation, the ribosome is attached to the mRNA, and fMet-tRNAfMet is positioned over the AUG start codon in the P site; the adjacent A site is Unoccupied.

Elongation takes place in three steps. In the first step **a charged tRNA binds to the A site. This**

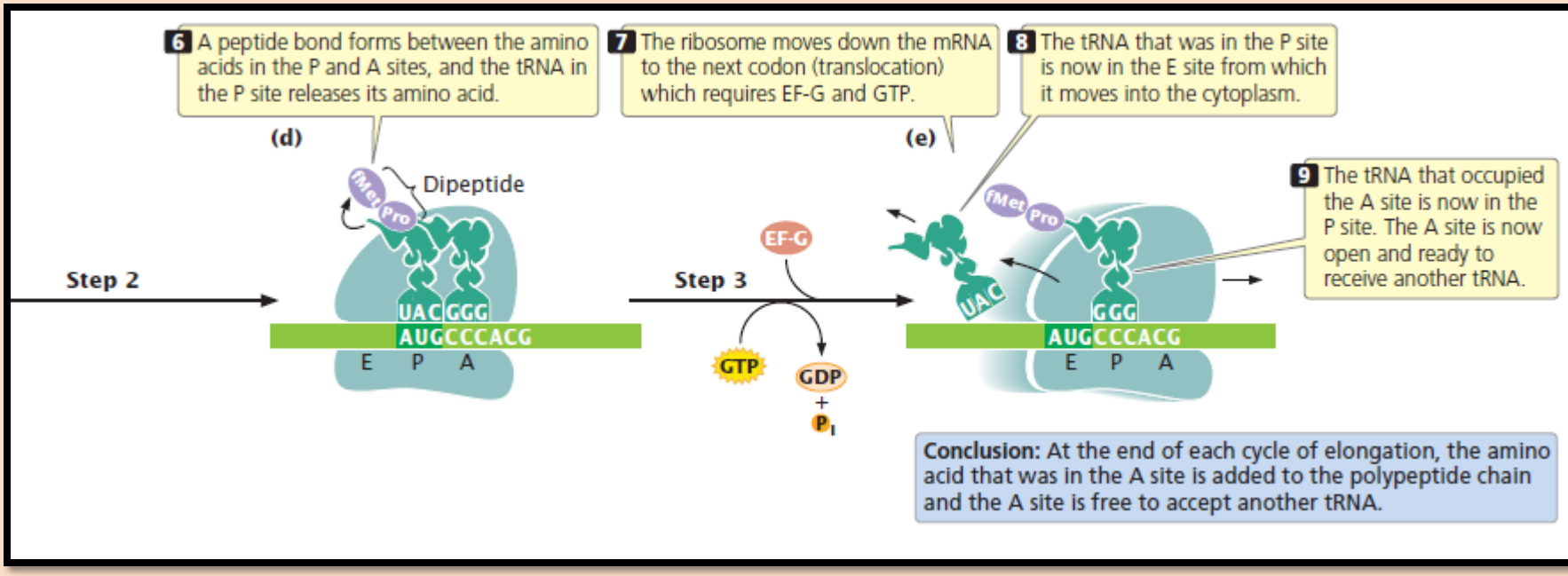
binding takes place when **elongation factor Tu (EF-Tu) joins** with GTP and **then with a charged tRNA to form a three-part complex**. This **complex enters the A site of the ribosome**,

where the **anticodon on the tRNA pairs with the codon** on the mRNA. After the charged tRNA is in the A site, **GTP is cleaved to GDP**, and the EF-Tu–GDP complex is released.**Elongation factor Ts (EF-Ts) regenerates EF-Tu–GDP to EF-Tu–GTP**. In eukaryotic cells, a similar set of reactions delivers the charged tRNA to the A site.

The **second step of elongation is the formation of a peptide bond** between the amino acids that are attached to tRNAs in the P and A sites .**The formation of** this peptide bond **releases the amino acid in the P site from its tRNA**. Evidence indicates that the catalytic activity is a property of the ribosomal RNA in the large subunit of the ribosome; this **rRNA acts as a ribozyme**

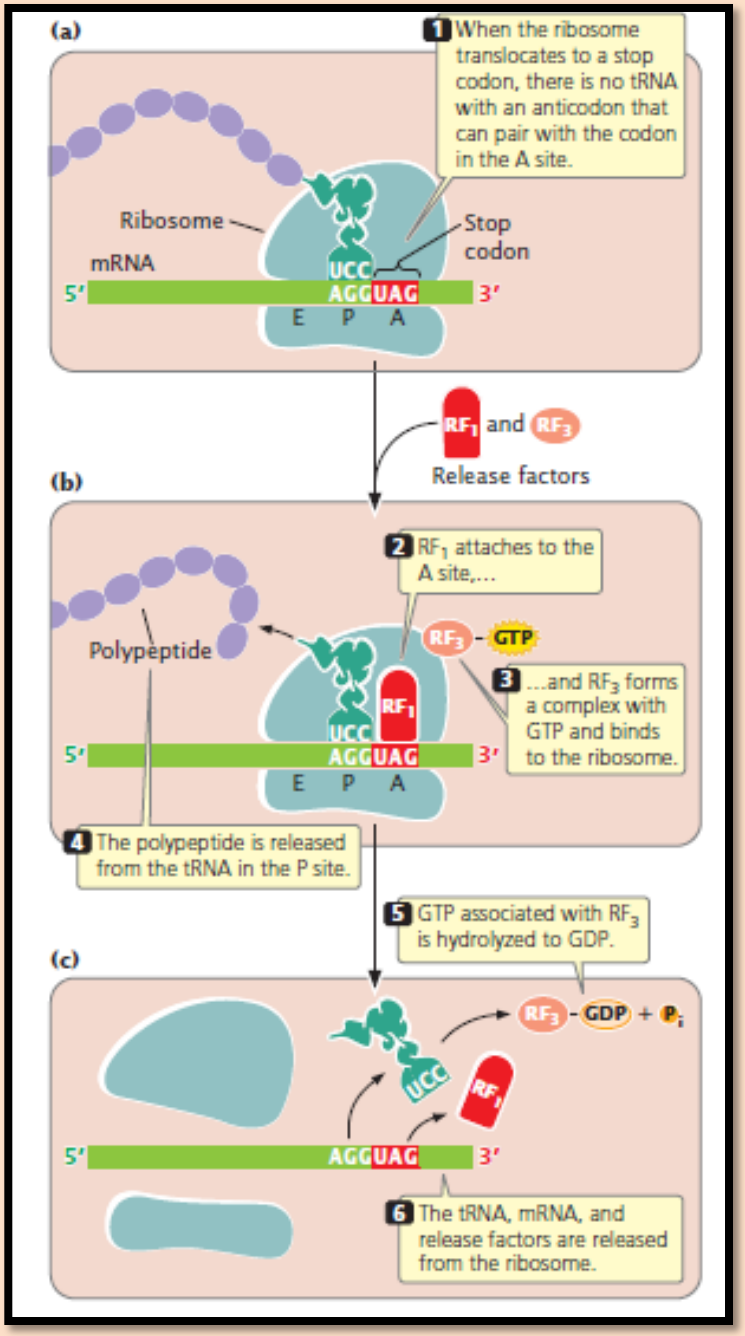


15.19 The elongation of translation comprises three steps.



The third step in elongation is **translocation the movement of the ribosome down the mRNA in the 5′→3′ direction**. This step **positions the ribosome over the next codon** and requires **elongation factor G (EF-G) and** the hydrolysis of GTP to GDP. Because **the tRNAs in the P and A sites are still attached to the mRNA through codon– anticodon pairing, they do not move with the ribosome as it translocates.** Consequently, the **ribosome shifts so that the tRNA** that previously **occupied the P site now occupies the E site**, from which it moves into the cytoplasm where it can be **recharged with another amino acid**. Translocation also causes **the tRNA that occupied the A site (which is attached to the growing polypeptide chain) to be in the P site, leaving the A site open**. Thus, the progress of each tRNA through the ribosome in the course of elongation can be summarized as follows:
cytoplasm → A site → P site → E site → cytoplasm. After translocation, **the A site of the ribosome is empty and ready to receive the tRNA specified by the next codon**.
The **elongation cycle repeats itself**: a charged tRNA and its amino acid occupy the A site, a peptide bond is formed between the amino acids in the A and P sites, and the ribosome translocates to the next codon.

Elongation in eukaryotic cells takes place in a similar manner. Eukaryotes possess at least three elongation factors, one of which also acts in initiation and termination. Another of the elongation factors used in eukaryotes, called elongation factor 2 (EF-2).


(a)
1 When the ribosome translocates to a stop codon, there is no tRNA with an anticodon that can pair with the codon in the A site.
Ribosome
mRNA
Stop codon
5′
UCC
AGGUAG
3′
E P A
RF1 and RF3
Release factors
(b)
2 RF1 attaches to the A site,...
Polypeptide
RF3 - GTP
3 ...and RF3 forms a complex with GTP and binds to the ribosome.
RF1
4 The polypeptide is released from the tRNA in the P site.
5 GTP associated with RF3 is hydrolyzed to GDP.
(c)
RF3 - GDP + Pi
6 The tRNA, mRNA, and release factors are released from the ribosome.

Termination

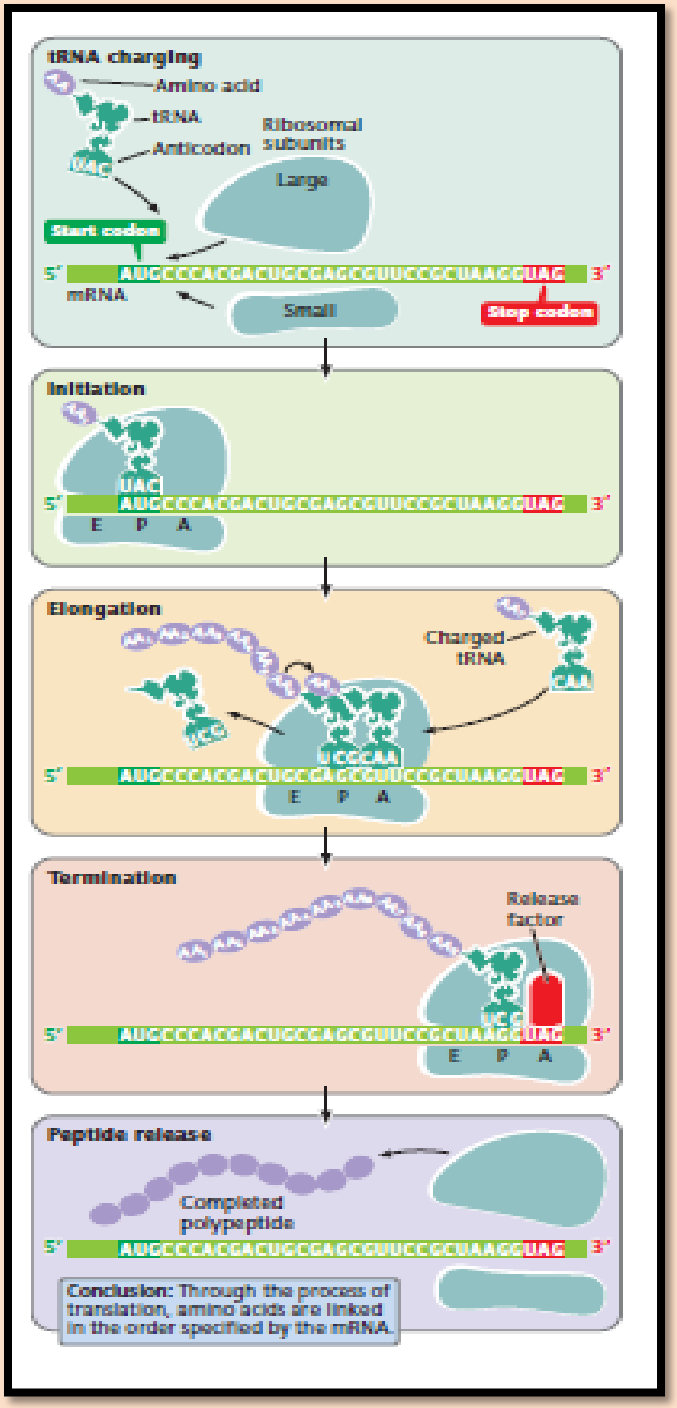
Protein synthesis terminates when the **ribosome translocates to a termination codon**. Because there are no **tRNAs with anticodons complementary to the termination codons, no**

tRNA enters the A site of the ribosome when a **termination codon** is encountered**. Instead, proteins called release factors bind to the ribosome. *E. Coli*** has three release **factors—RF1, RF2, and RF3**. Release factor **RF1** binds to the termination **codons UAA and UAG,** and **RF2**

binds **to UGA and UAA**. The binding of release **factor RF1 or RF2 to the A site of the ribosome promotes the cleavage of the tRNA in the P site from the polypeptide chain and the release**

of the polypeptide. **Release factor 3 binds to the ribosome and forms a complex with GTP**. This binding brings about **a conformational change** in the ribosome, **releasing RF1 or RF2 from the A site and causing the tRNA in the P site to move to the E site**; in the process, **GTP is hydrolyzed** to GDP. Additional factors help bring about the release of **the tRNA from the P site, the release of the mRNA from the ribosome, and the dissociation of the ribosome**

Translation in eukaryotic cells terminates in a similar way, except that there are two release factors: **eRF1, which recognizes all three termination codons, and eRF2, which binds GTP and stimulates the release of the polypeptide from the ribosome**.


tRNA charging
Amino acid
tRNA
Anticodon
Ribosomal subunits
Large
Small
Start codon
Stop codon
mRNA
5′
3′
AUGCCCACGACUGCGAGCGUUCCGCUAAGGUAG
Initiation
UAC
E P A
Elongation
Charged tRNA
UCCCAA
Termination
Release factor
Peptide release
Completed polypeptide
Conclusion: Through the process of translation, amino acids are linked in the order specified by the mRNA.

Fidelity of translation–
The **fidelity or accuracy** of protein synthesis **depends on the accuracy of the two mechanisms**: the **linking of each amino acid to its corresponding tRNA** molecule and the **base-pairing of the codons in mRNA to the anticodons in tRNA.** two fundamentally different **proofreading mechanisms** are used in the cell. Both are **active processes**.

One mechanism called **chemical proofreading** is used **to improve the accuracy of amino acid** attachment **to tRNA**. This is carried out by **aminoacyl tRNA synthetases**, which **recognize** an **incorrect amino acid** attached to **its tRNA molecule** and **remove** it by **hydrolysis**.

A **second** mechanism called **kinetic proofreading**, is used to **improve the fidelity of codon-anticodon pairing**. Once **tRNA molecules** have acquired an **amino acid**, they form a **complex with an elongation factor (EF)**. This **complex pairs** with the **appropriate codon** in an **mRNA molecule.** The **bound elongation factor** allows **correct codon-anticodon pairing** to occur, **but prevents** the **amino acid** from **being incorporated** into the **growing polypeptide chain**. The initial codon recognition, however, triggers the elongation factor to hydrolyze its bound GTP, whereupon the from the ribosome without its tRNA, allowing protein synthesis to proceed. **The elongation factor**, thereby, **introduces a short delay** between **codon anticodon base pairing** and **polypeptide chain elongation** which **provides an opportunity** for the bound **tRNA molecule** to **exit** from the ribosome. An incorrect **tRNA molecule forms a smaller number of codon anticodon hydrogen bonds than a correct one**; it, therefore, **binds more weakly** to the **ribosome** and is **more likely to dissociate** during this period. Thus the **delay introduced** by the **elongation factor causes most incorrectly bound tRNA molecules to leave the ribosome without being used for protein synthesis.**

Inhibitors of protein synthesis-Protein synthesis is a target of a wide variety of naturally occurring antibiotics and toxins. Mechanism of action of some common antibiotics and toxins, which inhibit protein synthesis in prokaryotes and eukaryotes, are described.**1 Prokaryotic inhibitors-**

Streptomycin- a basic trisaccharide, **binds with the 30S subunit** of the bacterial ribosome and **causes misreading of mRNA** at relatively low concentrations.

Chloramphenicol-binds to the **50S ribosomal subunit and blocks peptide bond** formation **through inhibition of peptidyl transferase** but does not affect the cytosolic protein synthesis in eukaryotes.

Tetracyclines- Tetracyclines **binds to the 30S ribosomal** subunit and **interferes with aminoacyl-tRNA binding.**

Erythromycin-Binds to the **50S ribosomal subunit and inhibits peptide chain elongation**

2.Eukatyotic inhibitors-

Cycloheximide blocks the **peptidyl transferase of 80S ribosome** but not that of 70S bacterial (and mitochondrial and chloroplast) ribosomes.

Diphtheria toxin, an **exotoxin of *Corynebacterium diphtheriae*** infected with a specific Lysogenic phage (Coryne Phage B), **stops the protein synthesis in eukaryotes by inactivating the elongation factor eEF2.**

Ricin-A toxic protein of the castor bean (*Ricinus communis*) that **inactivates the 60s subunit of eukaryotic ribosomes by depurinating a specific adenosine in 28S rRNA.**

Post translational modification of polypeptides-

1.Chemical modification

Primary translation products often **undergo a variety of modification reactions**, involving the **addition of chemical groups**, which are **attached covalently to the polypeptide**. This can involve **simple chemical modification like hydroxylation and phosphorylation** of the **side chains of single amino acids** or the **addition of different types of carbohydrates** or **lipid group**

Table 1.33 Examples of post-translational chemical modifications

Modification	*Amino acids that are modified*
Acetylation	Lys
Methylation	Lys, Arg
Phosphorylation	Ser, Thr, Tyr, Asp, His, Lys
Hydroxylation	Pro, Lys
Carboxylation	Glu (form γ-carboxyglutamic acid)
O-linked glycosylation	Ser, Thr
N-linked glycosylation	Asn
Acylation	Ser, Thr, Cys
Myristoylation	Gly
Palmitoylation	Cys
Farnesylation	Cys
Biotinylation	Lys
ADP ribosylation	At the nitrogen atom of His, Arg, Asn and Lys or at carboxyl group of Glu.

2.Proteolytic cleavage

Proteolytic cleavage of polypeptide chains after synthesis is a **common occurrence** with **certain classes of protein** For example, **proteolytic enzymes in the digestive tract** are **produced in inactive forms** that are generally termed **zymogens**. After **selective proteolysis**, these **enzymes are converted into active forms**. One **best known example** of **proteolytic cleavage** is the **conversion of preproinsulin into insulin**. **First, removal** of the **signal peptide** from the **newly synthesized peptide**, **preproinsulin**, generates the **proinsulin precursor molecule**. Finally, the **removal of C-peptide** moiety of **proinsulin gives insulin.**

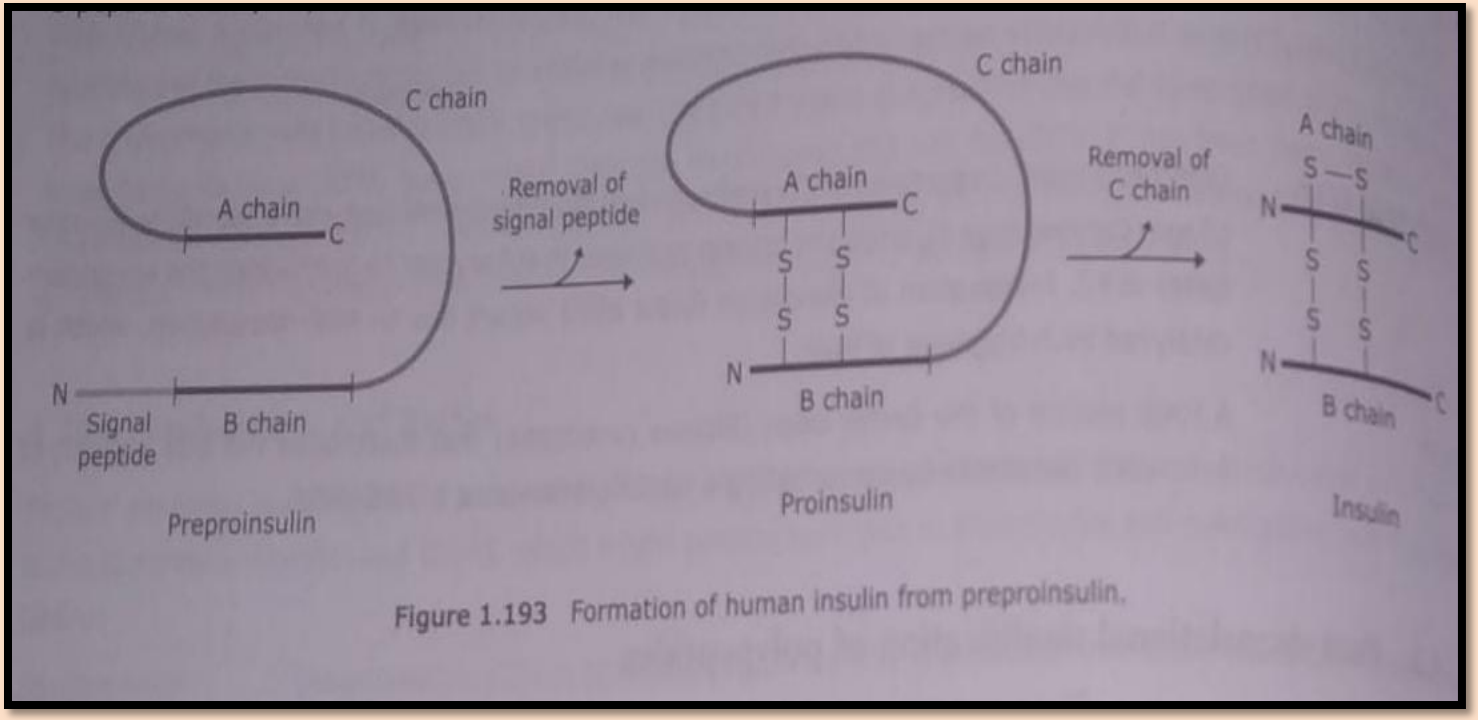


Figure 1.193 Formation of human insulin from preproinsulin.

Protein splicing -Occasionally, the primary translation product of a gene contains one or more short amino acid sequences, called **inteins** that excise themselves from the nascent polypeptide. The sequences that are represented in mature polypeptides are termed **exteins**. **Inteins** occur in both **eukaryotic and prokaryotic polypeptides**. An **intein** has the ability to **catalyze it's own removal** from primary translation products. The **autocatalytic process** of **protein splicing** involves **bond transfer reactions** and **no input of energy** is needed. According to the most accepted theory, the standard protein splicing mechanism of following four steps-

First step; N-O Shift A typical intein begins with Ser or Cys and end in Asn. The first residue in the C-extein is Ser,Thr or Cys. These residues often function as nucleophiles. N-O shift is the transition of the peptide band between the amino end of the intein and N-extein into ester or thioester bond. This transition depends on a nucleophillic attack of the bond by the side chain of the Ser or Cys residues at the amino terminal end of the intein (-OH or-SH respectively), This reaction is termed N-O when the attacking atom is an oxygen and N-S when this atom is sulfur.(refer to the last page of ppt for fig)

Second step: Transesterification In this step, the side chain of the first residue of the C-extein attacks the ester (or thioester) bond at the amino end of the intein, Here too the attack is by a polar side chain of a Ser, Thr(both –OH) or Cys (-SH). This leads to a transesterification and formation of thioester or ester bend between N-extein and C-extein. (refer to the last page of ppt for fig)

Third step Asn Cyclization Cyclization of the Asn side chain leads to cleavage of the peptide bond between the intein and the C extein. (C-terminal splice junction). This reaction removes intein from the ligated exteins, which are linked together via the ester bond. (refer to the last page of ppt for fig)

Fourth step O-N shift This step of protein splicing is spontaneous. The reverse N-O or N-S shift takes place and peptide bond formation occurs between N - and C-exteins. Some inteins show sequence specific endonuclease activity also. Such inteins cut DNA in the intein-minus gene at a specific point and allow a copy of a DNA sequence coding intein to integrate. This event is similar to intron homing and termed as intein homing. (refer to the last page of ppt for fig)

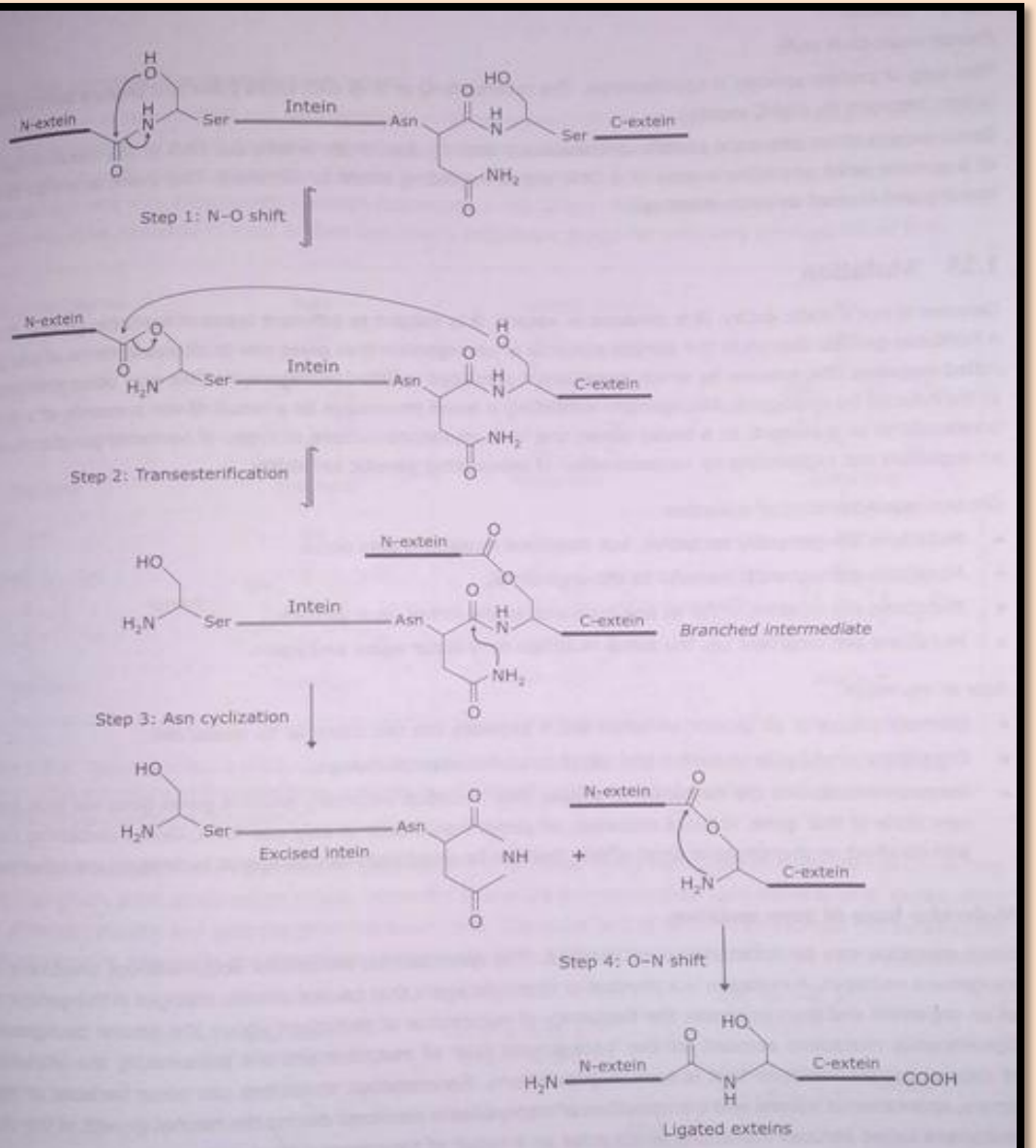


Figure 1.194 The mechanism of intein-mediated protein splicing.

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