Translation of mRNA

Translation is the process in which the sequence of codons within mRNA provides the information to synthesize the sequence of amino acids that constitute a polypeptide. One or more polypeptides then fold and assemble to create a functional protein.

The ability of mRNA to be translated into a specific sequence of amino acids relies on the **genetic code**. The sequence of bases within an mRNA molecule provides coded information that is read in groups of **three nucleotides** known as **codons**. The sequence of three bases in most codons specifies a particular **amino acid**. These codons are termed **sense codons.**

The codon **AUG,** which specifies methionine, **is used as a start codon**; it is usually the first codon that begins a polypeptide sequence. **The AUG codon** can also be used to specify additional methionine's within the coding sequence. Finally, three codons, **UAA, UAG, and UGA**, which are known as **stop codons,** are **used to end the process of translation. Stop codons are also known as termination codons or nonsense codons. The codons in mRNA are recognized by the anticodons in transfer RNA (tRNA) molecules**. Anticodons are three-nucleotide sequences that are complementary to codons in mRNA. The tRNA molecules carry the amino acids that are specified by the codons in the mRNA. In this way, the order of codons in mRNA dictates the order of amino acids within a polypeptide.

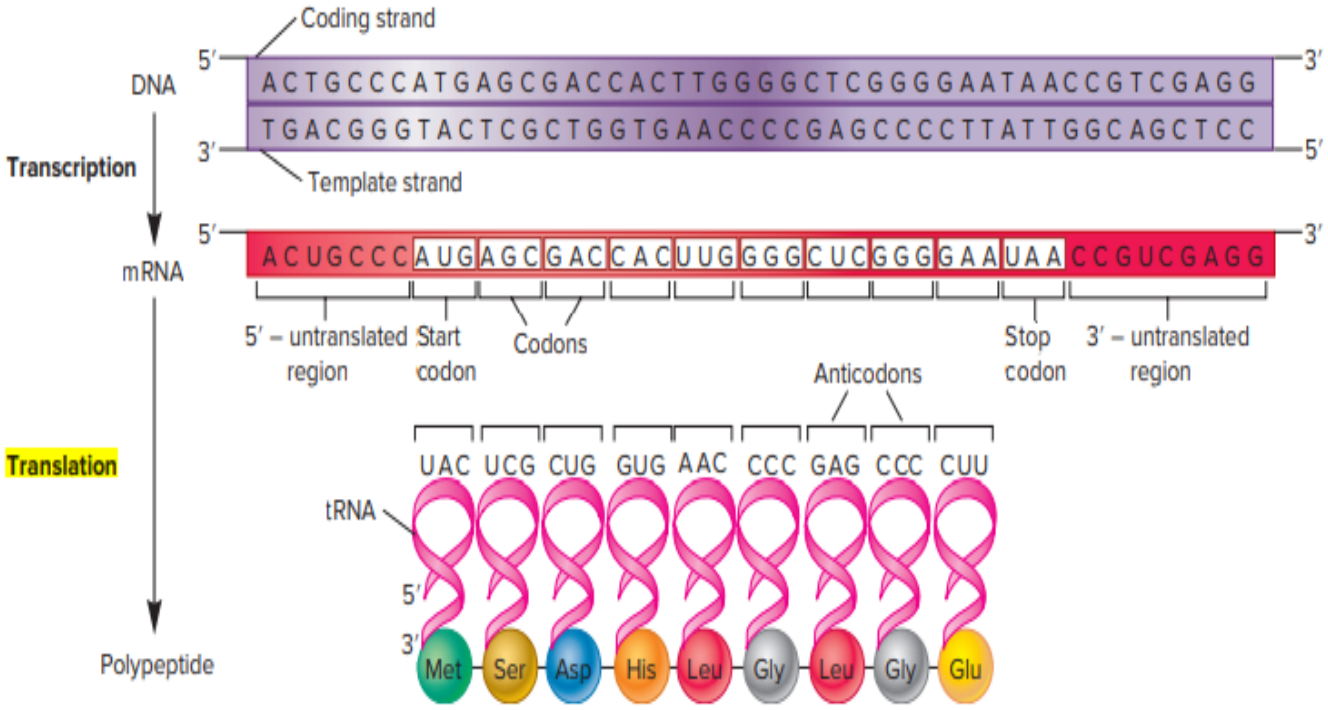

Figure 1: the relationship among the DNA, mRNA, tRNA, and amino acid sequence.

The genetic code

The genetic code is composed of 64 different codons, because polypeptides are composed of 20 different kinds of amino acids, a minimum of 20 codons is needed to specify all the amino acids. With four types of bases in mRNA (A, U, G, and C), a genetic code containing two bases in a codon would not be sufficient because it would specify only 4^2, or 16, possible types. By comparison, a three-base codon system can specify 4^3, or 64, different codons.

The characteristics of genetic code.

- **triplets.**
- **Comma -free code**
- **universal in all most organisms.**
- **Nonoverlapping code**
- **Directionality.**
- **Degeneracy (**the wobble rules)**:** which means that more than one codon can specify the same amino acid. Degeneracy usually occurs at the third position in the codon. For example, valine is specified by GUU, GUC, GUA, and GUG. In all four cases, the first two bases are always G and U. The third base, however, can be U, C, A, or G.

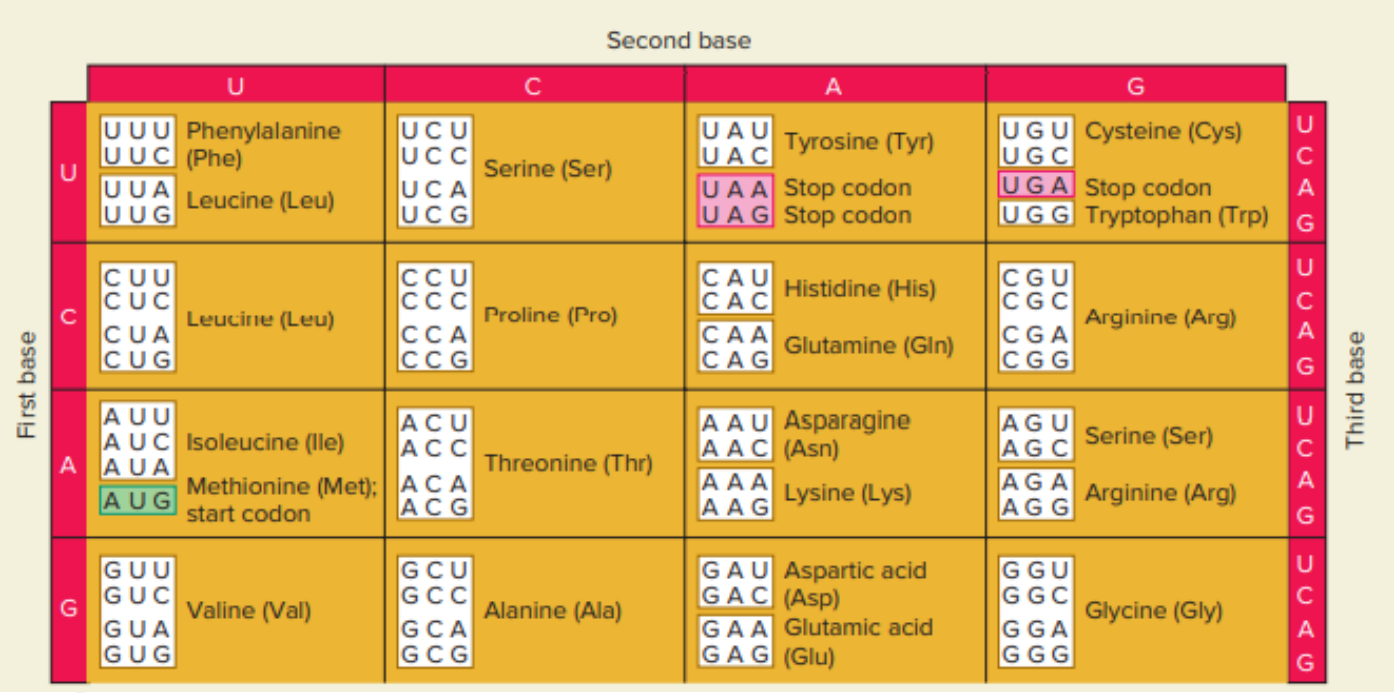

Figure2: The genetic code.

For example, the codons GGU, GGC, GGA, and GGG all specify the amino acid glycine. Such codons are termed **synonymous codons**. **The start codon (AUG)** defines the reading frame of an mRNA - a sequence of codons determined by reading the bases in groups of three, beginning with the start codon as a frame of reference.

Examples:

The mRNA sequence shown below encodes a short polypeptide with seven amino acids:

5′–AUG CCC GGA GGC ACC GUC CAA U–3′

Met – Pro – Gly – Gly– Thr – Val– Gln

If we remove one base (C) adjacent to the start codon, this changes the reading frame to produce a different polypeptide sequence:

5′–AUG CCG GAG CAC CGU CCA AU–3′

Met – Pro – Glu – Ala –Pro – Ser– Asn

Alternatively, if we remove three bases (CCC) next to the start codon, the resulting polypeptide has the same reading frame as the first polypeptide, though one amino acid (Pro, proline) has been deleted:

5′–AUG GGA GGC ACC GUC CAA U–3′

Met – Gly – Gly –Thr – Val– Gln

The UAG, UAA, UGA are stop codons are not recognized by a tRNA with a complementary sequence. Instead, they are recognized by proteins known as release factors.

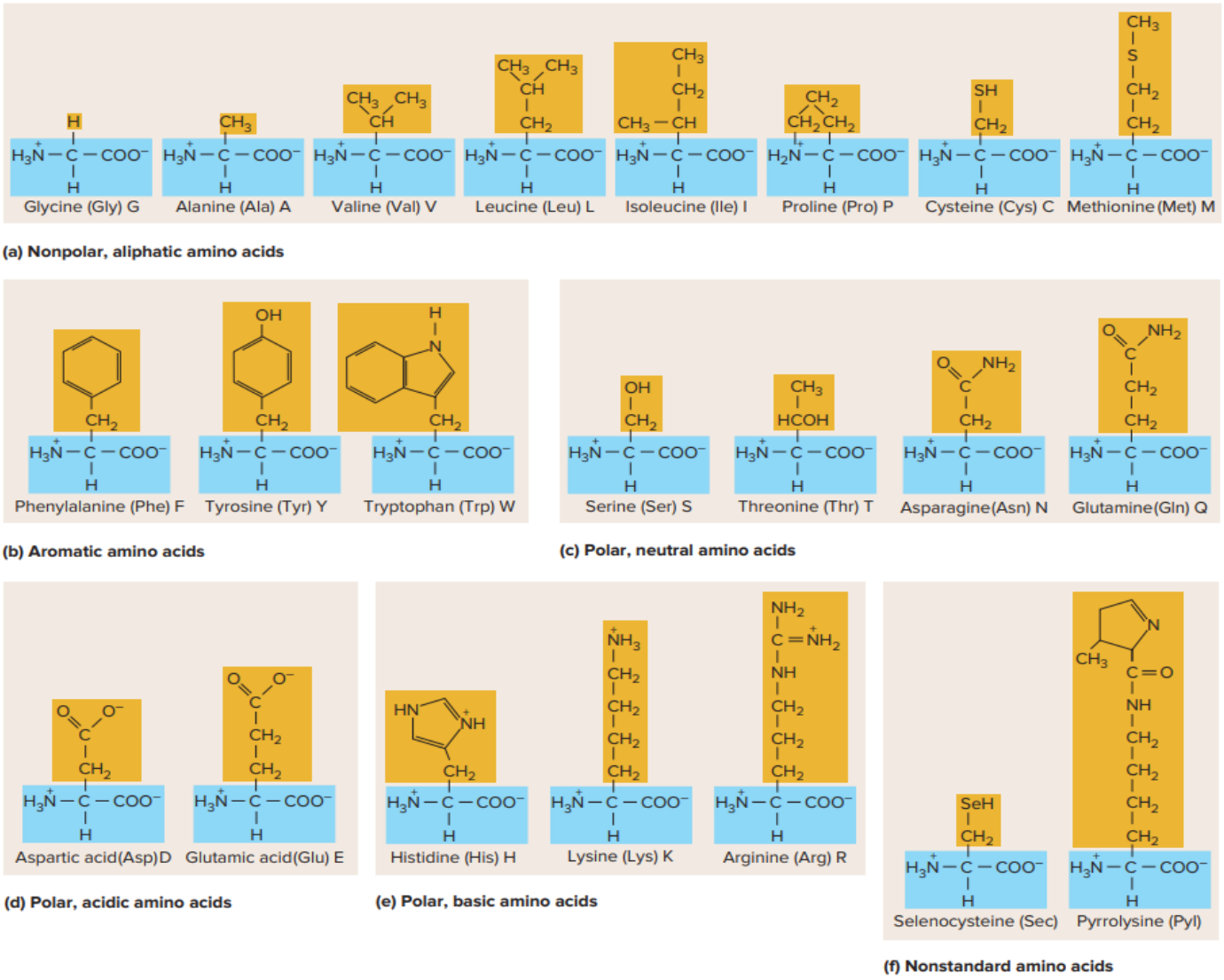

Figure3: the structures of the amino acids.

The directionality of the polypeptide chain

The polypeptide chain has a directionality that parallels the order of codons in the mRNA. As a polypeptide is made, **a peptide bond is formed between the carboxyl group in the last amino acid** of the polypeptide **and the amino group in the amino acid being added**. this occurs via a condensation reaction that releases a water molecule. The newest amino acid added to a growing polypeptide always has a free carboxyl group. The term **N-terminus** refers to the presence of a nitrogen atom (N) of an amino group ($NH3^{+}$) of the first amino acid that is specified by a codon that is near the 5′ end of the mRNA. By comparison, the last amino acid in a completed polypeptide is located at **the C-terminus,** or carboxyl-terminus. A carboxyl

group (COO^-) of the last amino acid that found in the end of the polypeptide chain and it is specified by a codon that is closer to the 3′ end of the mRNA

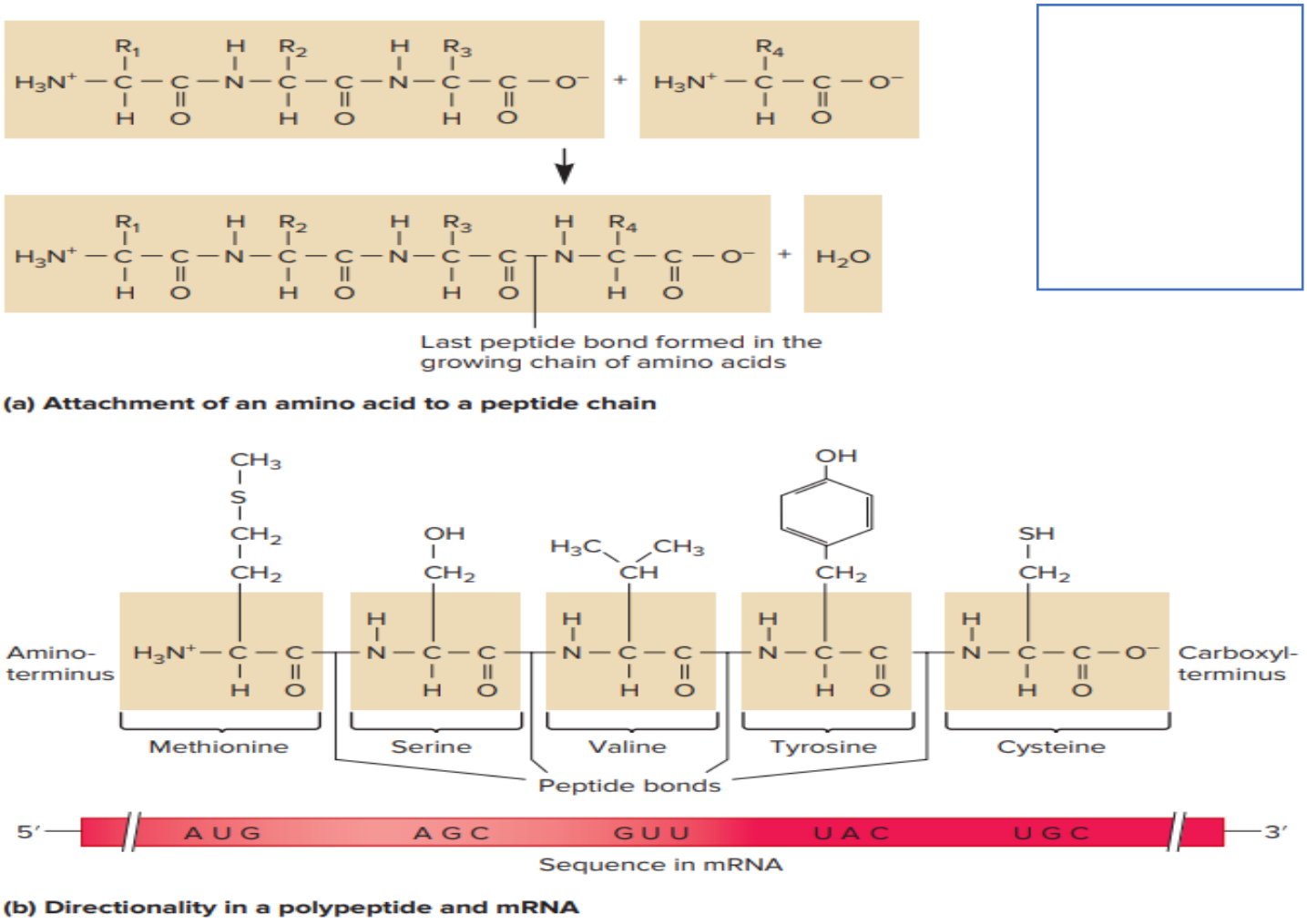

Figure 4: formation of the peptide ponds and the polypeptide chain synthesis and its direction.

The protein structural levels

Following gene transcription and mRNA translation, the end result is a polypeptide with a defined **amino acid sequence is known as the primary structure of a polypeptide**. The primary structure of a typical polypeptide may be a few hundred or even a couple of thousand amino acids in length. The newly synthesized chain is directly folded into 3-dimensional structure called **the secondary structure**. The two mains of the secondary structures are: **α helix and the β sheet** composed of regular repeated shapes that are formed by formation of hydrogen bonds between atoms that are located in the polypeptide backbone.

The short regions of secondary structure within a polypeptide are folded relative to each other to make **the tertiary structure** of a polypeptide. α-helical regions and β-sheet regions are connected by irregularly shaped segments to determine the tertiary structure of the polypeptide.

The tertiary conformation can usually occur spontaneously because the process is thermodynamically favorable. The structure is determined by various interactions, including the tendency of hydrophobic amino acids to avoid water, ionic interactions among charged amino acids, hydrogen bonding among amino acids in the folded polypeptide, and weak bonding known as van der Waals interactions.

A protein is a functional unit that can be composed of one or more polypeptides. Some proteins are composed of a single polypeptide. Many proteins are composed of two or more polypeptides that associate with each other to make a functional protein with **a quaternary structure**. The individual polypeptides are called **subunits of the protein,** each of which has its own tertiary structure. The association of **multiple subunits** is the **quaternary structure of a protein.**

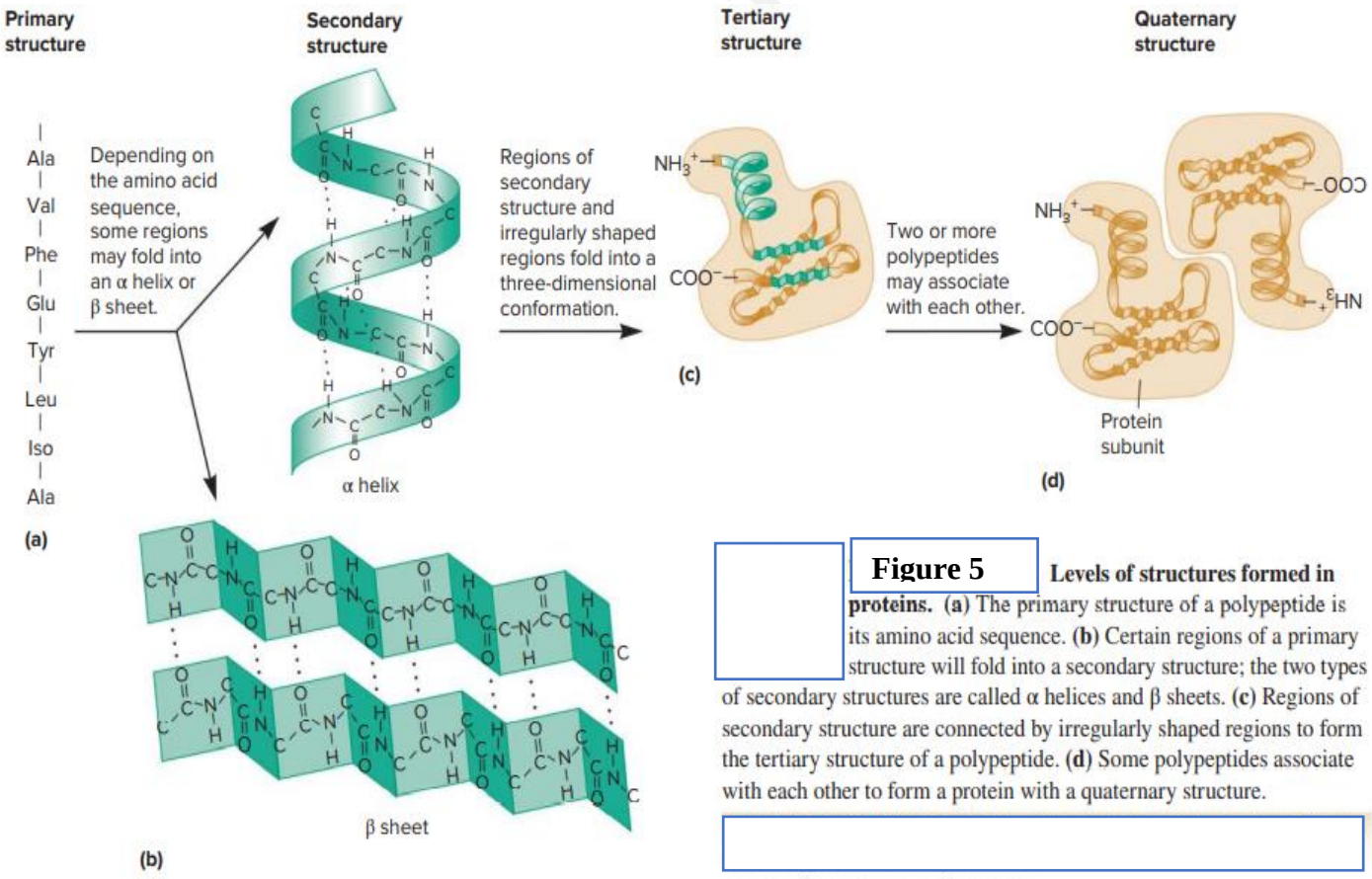

Figure 5 **Levels of structures formed in proteins.** **(a)** The primary structure of a polypeptide is its amino acid sequence. **(b)** Certain regions of a primary structure will fold into a secondary structure; the two types of secondary structures are called α helices and β sheets. **(c)** Regions of secondary structure are connected by irregularly shaped regions to form the tertiary structure of a polypeptide. **(d)** Some polypeptides associate with each other to form a protein with a quaternary structure.

Translation process

The translation is occurring in three stages: initiation, elongation, and termination. The translation process summaries by that:

During initiation, the ribosomal subunits, mRNA, and the first tRNA assemble to form a complex. The elongation stage starts after the formation of initiation complex, the ribosome slides along the mRNA in the 5′ to 3′ direction, moving over the codons. As the ribosome moves, tRNA molecules sequentially bind to the mRNA at the A site in the ribosome, bringing with them the appropriate amino acids. Therefore, amino acids are linked in the order commanded by sequence of the codon in the mRNA. Finally, termination of translation is signaled by the stop codon. At this point, disassociation of the ribosome subunits and mRNA occurs, and the newly made polypeptide is released.

The initiation stage:

A specific tRNA is required to recognize the start codon in the mRNA, called the **initiator tRNA**. In bacteria, the initiator tRNA, which is designated $tRNA^{fMet}$, carries a methionine which has been modified to N-formylmethionine

The initiation stage of translation in bacteria starts when the initiation complex composed of (mRNA, $tRNA^{fMet}$, and ribosomal subunits) is associated with each other.

- **In bacteria** the ribosome is composed of 2 subunits: the small subunit 30S and the large subunits 50S. The mRNA binds to the 30S subunit.
- The formation of this complex requires the participation of three initiation factors: IF1, IF2, and IF3. First, IF1 and IF3 bind to the 30S ribosomal subunit, which prevents the association of the 50S subunit.
- This binding is facilitated by a nine-nucleotide sequence within the bacterial mRNA called the **Shine-Dalgarno sequence** upstream the start codon. This sequence is complementary binding to the 16S rRNA in the 30S ribosomal subunit. After the mRNA and $tRNA^{fMet}$ have become bound to the 30S subunit, IF1, IF2 and IF3 are released.
- Then the 50S is binding to the complex of mRNA, $tRNA^{fMet}$, and 30S subunit.
- The ribosome is formed of 3 chambers **A site** (attachment of amino acid charged tRNA), **P site** (where the peptide bond is formed between the amino acid to form the peptide chain) and the **E site** (where the free t RNA is exit or released).

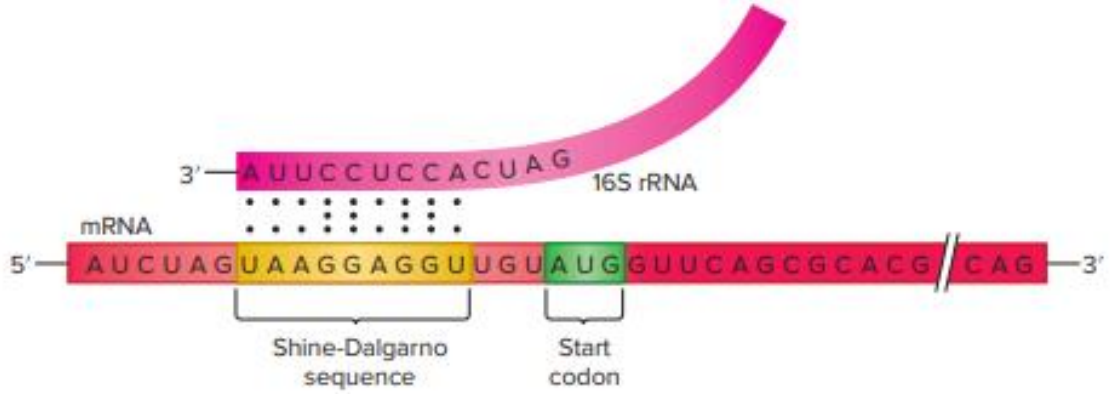

Figure 6: the Shine-Dalgarno sequence

- Then, the $tRNA^{fMet}$ binds to the start codon, which is typically a few nucleotides downstream from the Shine-Dalgarno sequence. The start codon is usually AUG.

In eukaryotes:

- In eukaryotes the ribosome is composed of 2 subunits (40S and 60S subunits).
- A eukaryotic initiation factor, eIF2, binds directly to $tRNA^{Met}$ and to the 40S subunit.
- The eukaryotic mRNAs do not have a Shine-Dalgarno sequence. Therefor mRNA is recognized by eIF4, which is a multiprotein complex. that recognizes the 7-methylguanosine cap at the 5′ end of the mRNA. eIF4 then facilitates the binding of the 5′ end of the mRNA to the 40S ribosomal subunit. Then the ribosome is assembled together.
- In eukaryotes the ribosome identifies the first start codon AUG in the 5′ end somewhere downstream from the 7-methylguanosine cap and then scans along the mRNA in the 3′ direction.
- eIF5 causes the release of the other initiation factors, which enables the 60S subunit to associate with the 40S subunit.
- Many eukaryotic mRNAs, were found that not all AUG codons near the 5′ end of mRNA can function as start codons. In some cases, the scanning ribosome passes over the first AUG codon and chooses an AUG farther down the mRNA. The sequence of bases around the AUG codon plays an important role in determining whether or not it is selected as the start codon by a scanning ribosome. The consensus sequence for optimal start codon recognition in complex eukaryotes.

Elongation stage

During the elongation stage of translation, amino acids are added, one at a time, to a growing polypeptide. Under normal cellular conditions, a polypeptide can elongate at a rate of 15–20 amino acids per second in bacteria and 2–6 amino acids per second in eukaryotes.

- Binding of a charged tRNA to the A Site to begin elongation, a charged tRNA brings a new amino acid to the ribosome so it can be added to the end of the growing polypeptide at the P site. This binding occurs because the anticodon in the tRNA is complementary to the codon in the mRNA.
- The 16S rRNA in the 30S ribosomal subunit can detect when an incorrect tRNA is bound at the A site and will prevent elongation until the mispaired tRNA is released from the A site. This phenomenon, termed **the decoding function of the ribosome**, is important in maintaining high fidelity of mRNA translation.
- **Peptidyl Transfer Reaction** The next step of elongation is a reaction called peptidyl transfer. The polypeptide is removed from the tRNA in the P site and transferred to the amino acid at the A site. This transfer is accompanied by the formation of a peptide bond between the amino acid at the A site and the polypeptide, lengthening the polypeptide by one amino acid.
- The peptidyl transfer reaction is catalyzed by peptidyl transferase and rRNA. ribosome is a ribozyme which means the rRNA is responsible for the formation of the peptide bond not the protein.

- Then the ribosome moves to the next codon in the mRNA. This moves the tRNAs at the P and A sites to the E and P sites, respectively.

- Finally, the uncharged tRNA exits the E site.

- The new aminoacyl- tRNA can enter the empty A site, and the same steps are repeated several times. the new amino acid is added to the growing polypeptide chain.

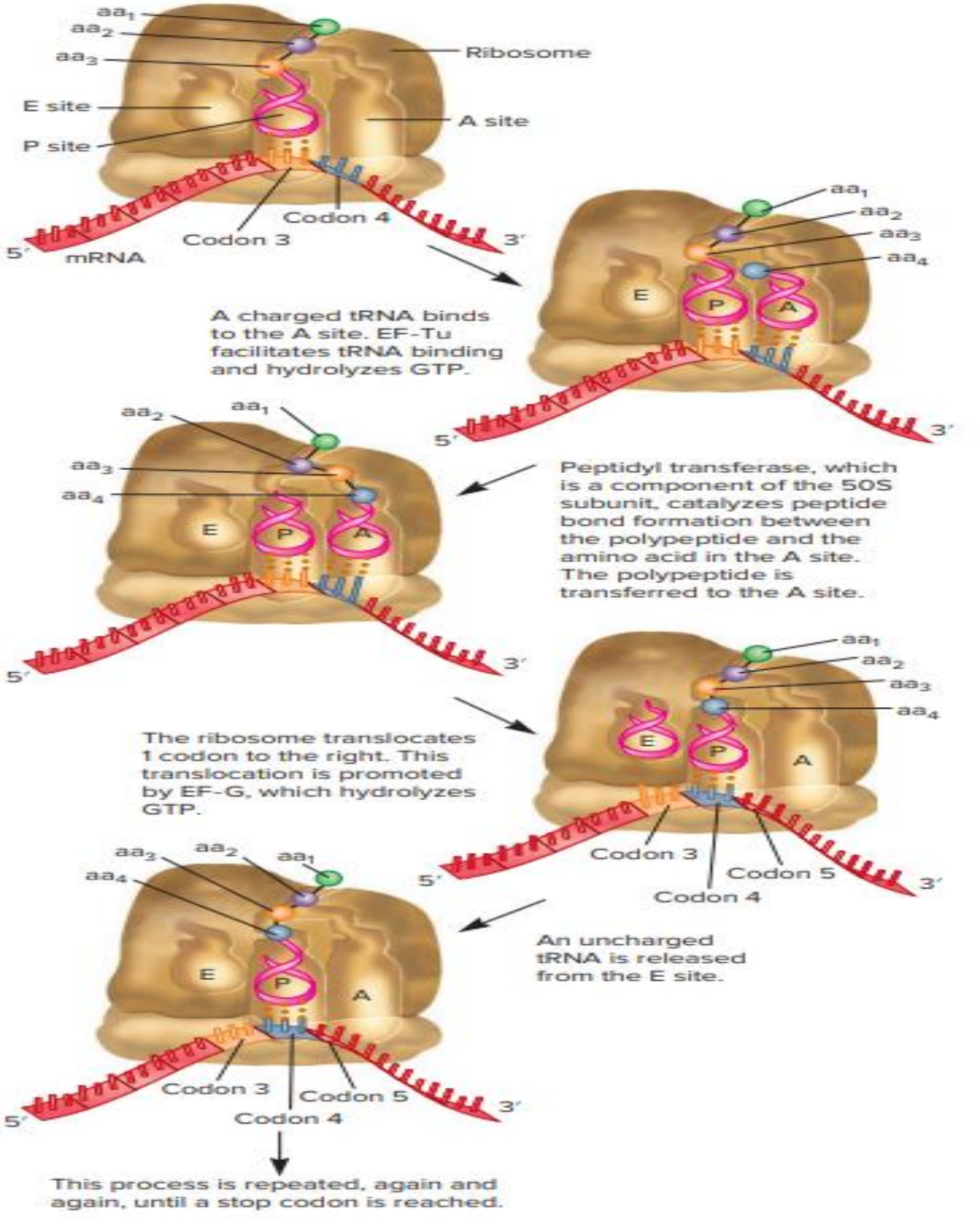

Figure 7: the elongation stage of translation.

The Termination

The termination of building the peptide chain occurs when a stop codon is reached in the mRNA. In most species, the three stop codons are UAA, UAG, and UGA. The stop codons are not recognized by a tRNA with a complementary sequence. Instead, they are recognized by proteins known as release factors (RFs) which mimic the tRNA in their structure. Release factors can specifically bind to a stop codon sequence.

the termination stage of translation in bacteria.

- When the completed polypeptide is attached to a tRNA in the P site. A stop codon is located at the A site.
- In the first step, RF1 or RF2 binds to the stop codon at the A site and RF3 binds at a different location on the ribosome.
- the bond between the polypeptide and the tRNA is hydrolyzed.
- The polypeptide and tRNA are then released from the ribosome.
- The final step in translational termination is the disassociation of ribosomal subunits, mRNA, and the release factors.

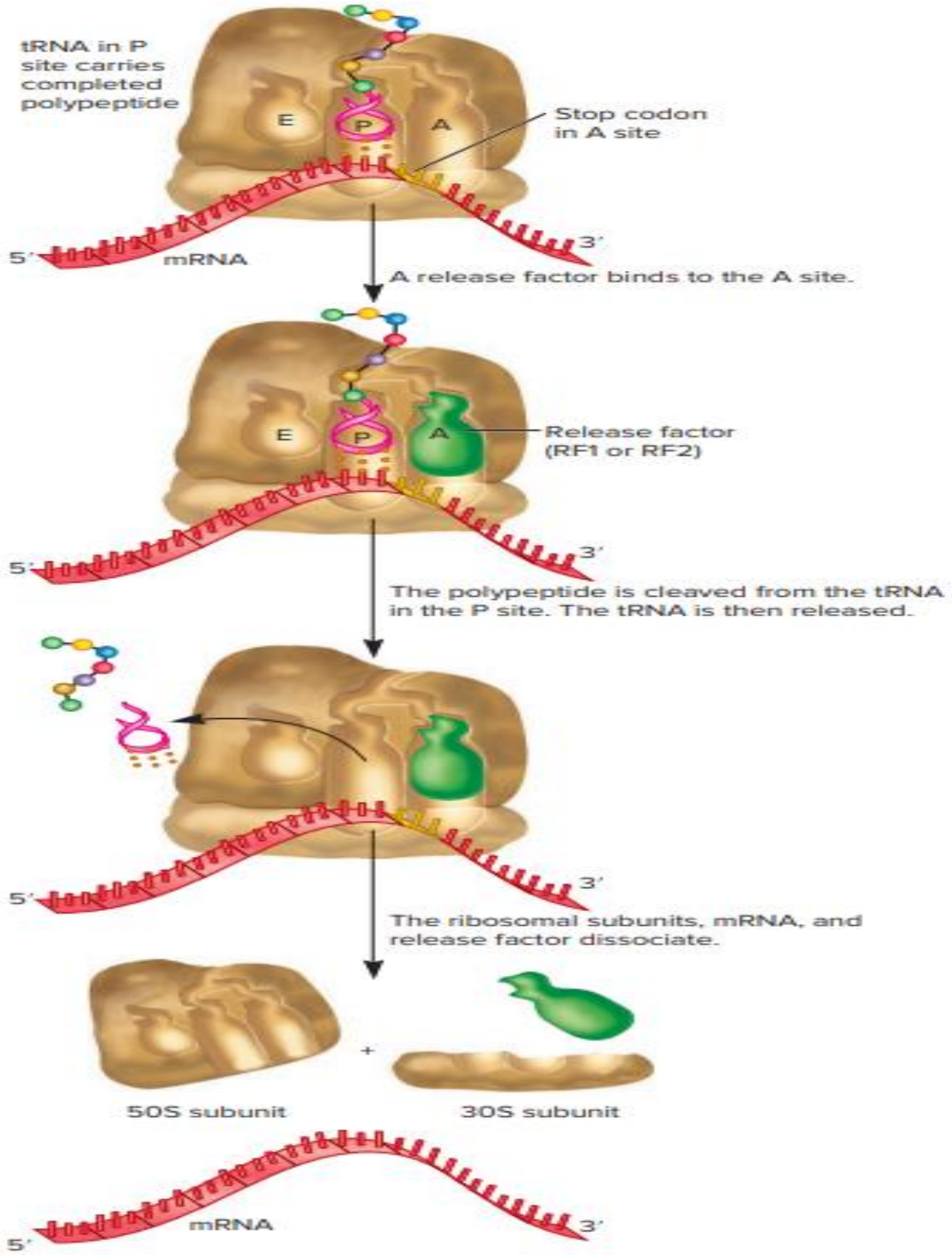

Figure 8: the termination stage of translation.