Protein Synthesis 3&4

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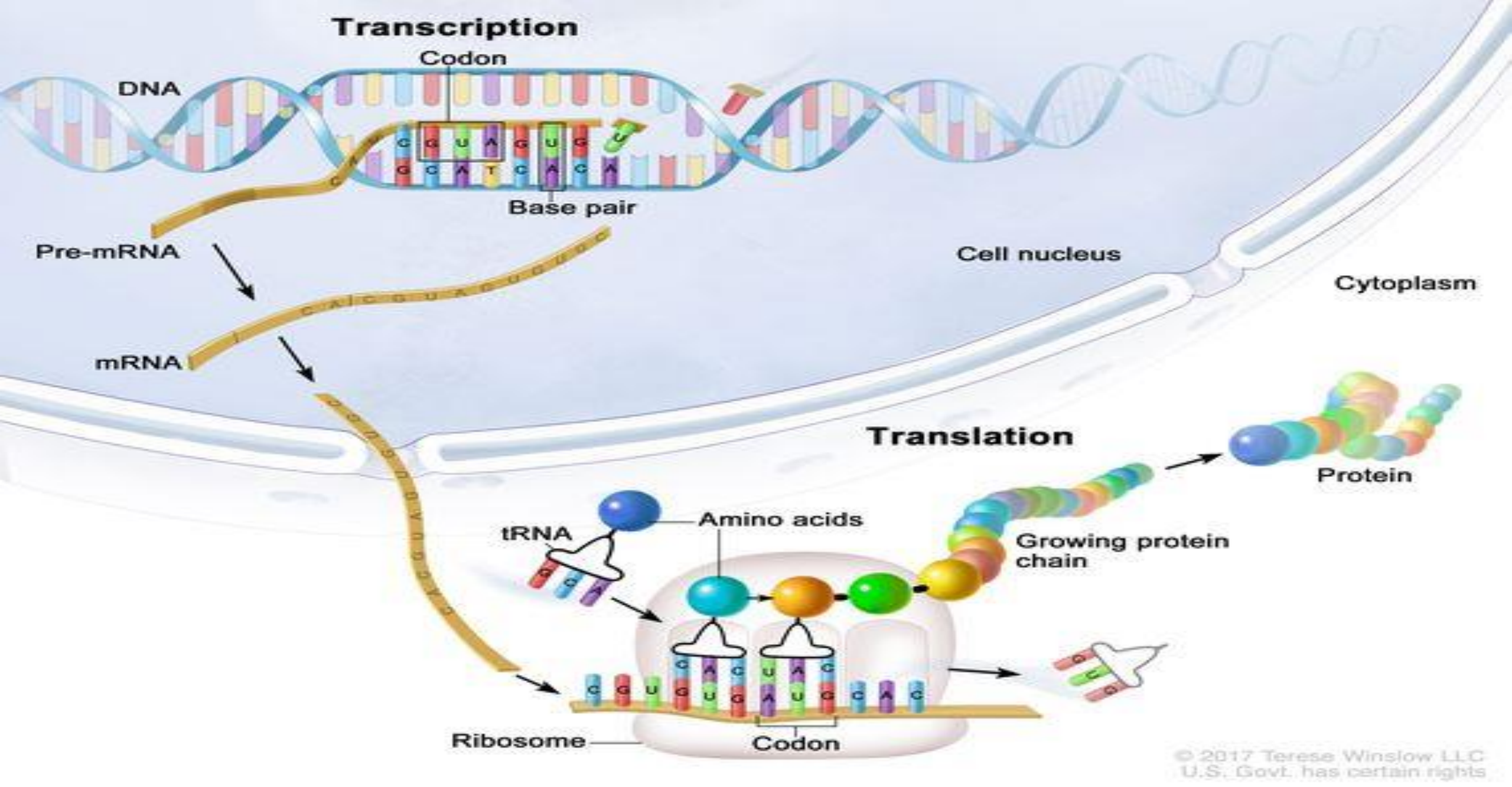
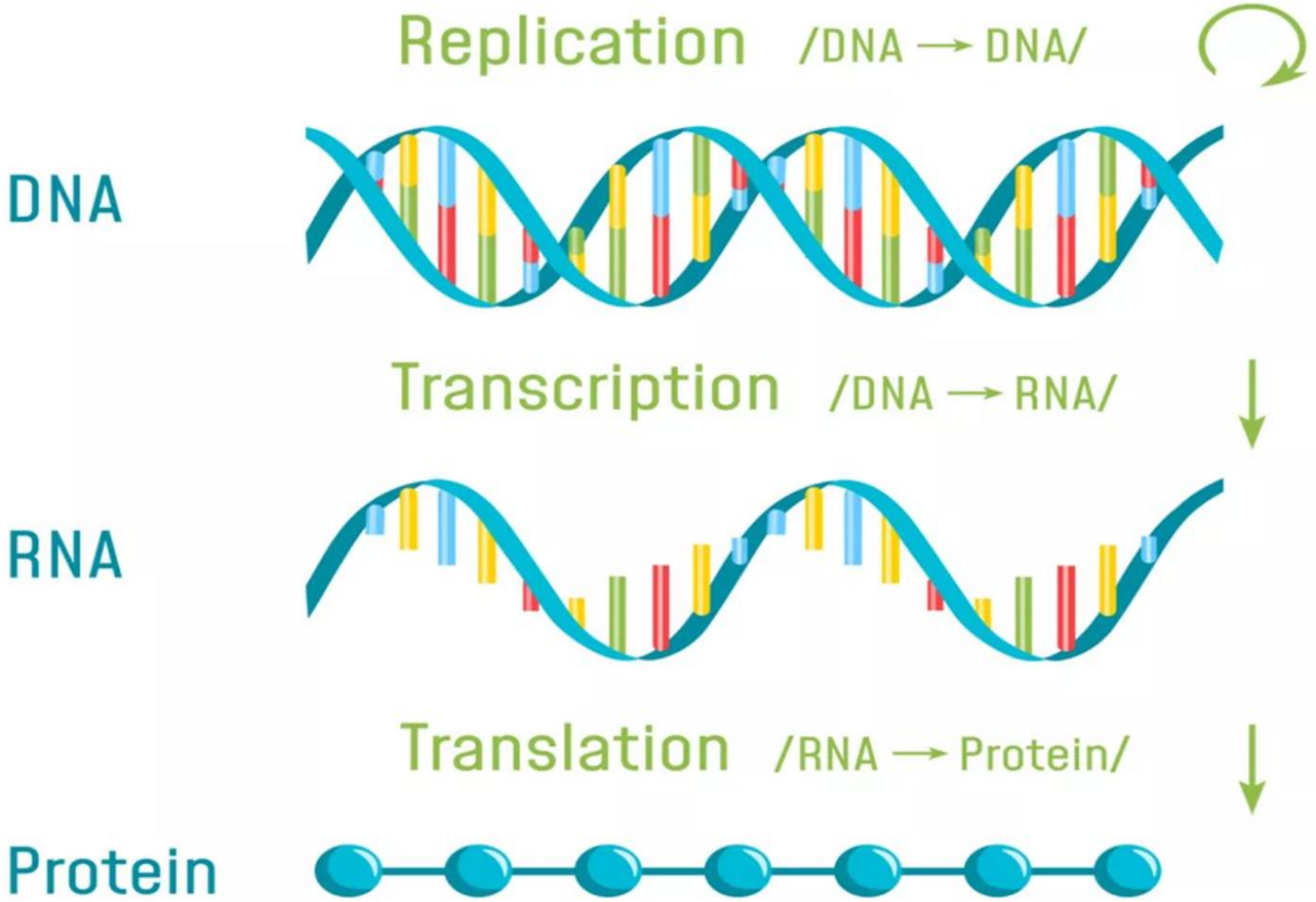
Definition

Protein synthesis is a core biological process, occurring inside cells, balancing the loss of cellular proteins (by degradation or export) through the production of new proteins.

Proteins perform a number of critical functions as enzymes and structural proteins or hormones.

Central dogma the process by which DNA is copied to RNA is called transcription, and that by which RNA is used to produce proteins is called translation.




Replication /DNA → DNA/
DNA
Transcription /DNA → RNA/
RNA
Translation /RNA → Protein/
Protein

Transcription

Transcription is the process by which DNA is copied (*transcribed*) to mRNA, which carries the information needed for protein synthesis. Transcription takes place in two broad steps.

First, *pre-messenger RNA* is formed, with the involvement of *RNA polymerase* enzymes. The process relies on Watson-Crick base pairing, and the resultant single strand of RNA is the reverse-complement of the original DNA sequence.

Second the pre-messenger RNA is then "edited" to produce the desired *mRNA* molecule in a process called *RNA splicing*.

- Partial unwinding of the double helix must occur before transcription can take place, and the *RNA polymerase* enzymes catalyzes this process according to the desired protein and gene .

- Unlike DNA replication, in which both strands are copied, only one strand is transcribed. The strand that contains the gene is called the *sense* strand, while the complementary strand is the *antisense* strand. The mRNA produced in transcription is a copy of the sense strand, but it is the antisense strand that is transcribed.
- *RNA polymerase* being the key enzyme in transcription ,joins the ribonucleotides together to form a pre-messenger RNA molecule. Transcription ends when the RNA polymerase enzyme reaches a triplet of bases that is read as a "stop" signal.
- The DNA molecule re-winds to re-form the double helix again after the transcription is done.
- In prokaryotes single type of RNA polymerase is responsible for synthesis of mRNA, rRNA and tRNA. In eukaryotes several types of enzyme required for synthesis of different types of RNA(RNA polymerase I,II, and III).

- Cells can be characterized by the spectrum of mRNA molecules present within them; this spectrum is called the ***transcriptome***.
- Whereas each cell in a multicellular organism carries the same DNA or genome, its transcriptome varies widely according to cell type and function.
- For example, the insulin-producing cells of the pancreas contain transcripts for insulin, but bone cells do not. Even though bone cells carry the gene for insulin, this gene is not transcribed.
- Therefore, the transcriptome functions as a kind of catalog of all of the genes that are being expressed in a cell at a particular point in time.

Steps of transcription

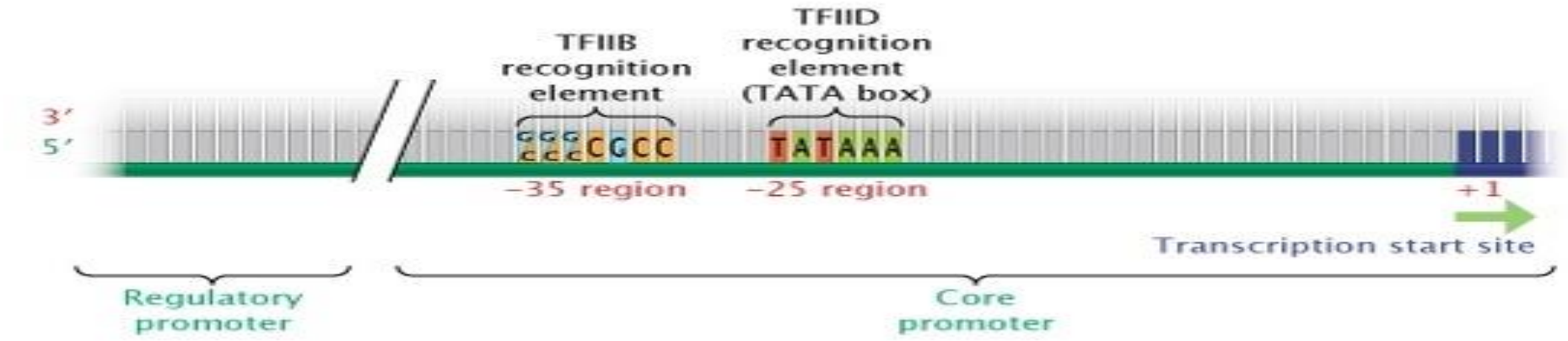
1-Formation of transcription complex. Is the beginning of transcription. It occurs when the enzyme RNA polymerase binds to a region of a gene called the **promoter.**

Sigma factor is required for recognition of the promoter by holoenzyme(RNA polymerase) and formation of complex of synthesis. This signals the DNA to unwind and important event will happen at this stage, the melting of DNA complex by enzyme after the dissociation of sigma factor, so the enzyme can “read” the bases in one of the DNA strands.

- Eukaryotic promoters are much larger and more complex than prokaryotic promoters, but both have a TATA box, which is located at approximately -30 relative to the initiation (+1) site which affects the transcription rate and determines location of the start site.
- The exact TATA box sequence is TATAAAA, as read in the 5′ to 3′ direction on the non template strand.
- The thermos stability of A–T bonds is low and this helps the DNA template to locally unwind in preparation for transcription.
- In eukaryotes, the "core" promoter for a gene transcribed by pol II is most often found immediately upstream (5′) of the start site of the gene.
- Many eukaryotic genes also possess enhancer sequences, which can be found at considerable distances from the genes they affect.
- Enhancer sequences control gene activation by binding with activator proteins and altering the 3-D structure of the DNA to help "attract" RNA pol II, thus regulating transcription.

- Because eukaryotic DNA is tightly packaged as chromatin, transcription also requires a number of specialized proteins that help make the template strand accessible.

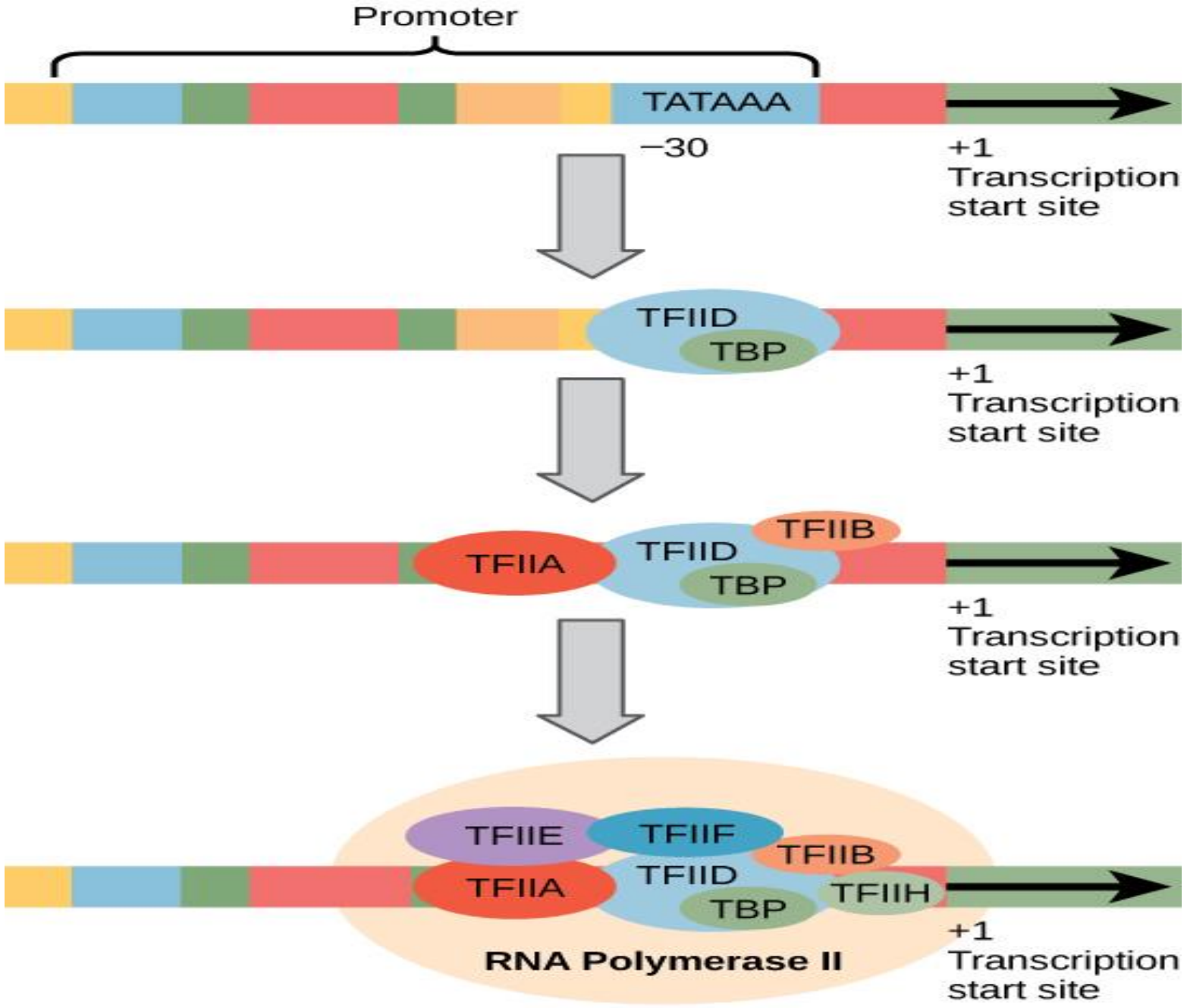
- Eukaryotic RNA polymerases use a number of essential cofactors (collectively called general transcription factors), and one of these, TFIID, recognizes the TATA box and ensures that the correct start site is used .
- Another cofactor, TFIIB, recognizes a different common consensus sequence, G/C G/C G/C G C C C, approximately 32 to 38 bases upstream .



- Together, the transcription factors and RNA polymerase form a complex called the transcription initiation complex. This complex initiates transcription, and the RNA polymerase begins mRNA synthesis by matching complementary bases to the original DNA strand.

- The terms "strong" and "weak" are often used to describe promoters and enhancers, according to their effects on transcription rates and thereby on gene expression.

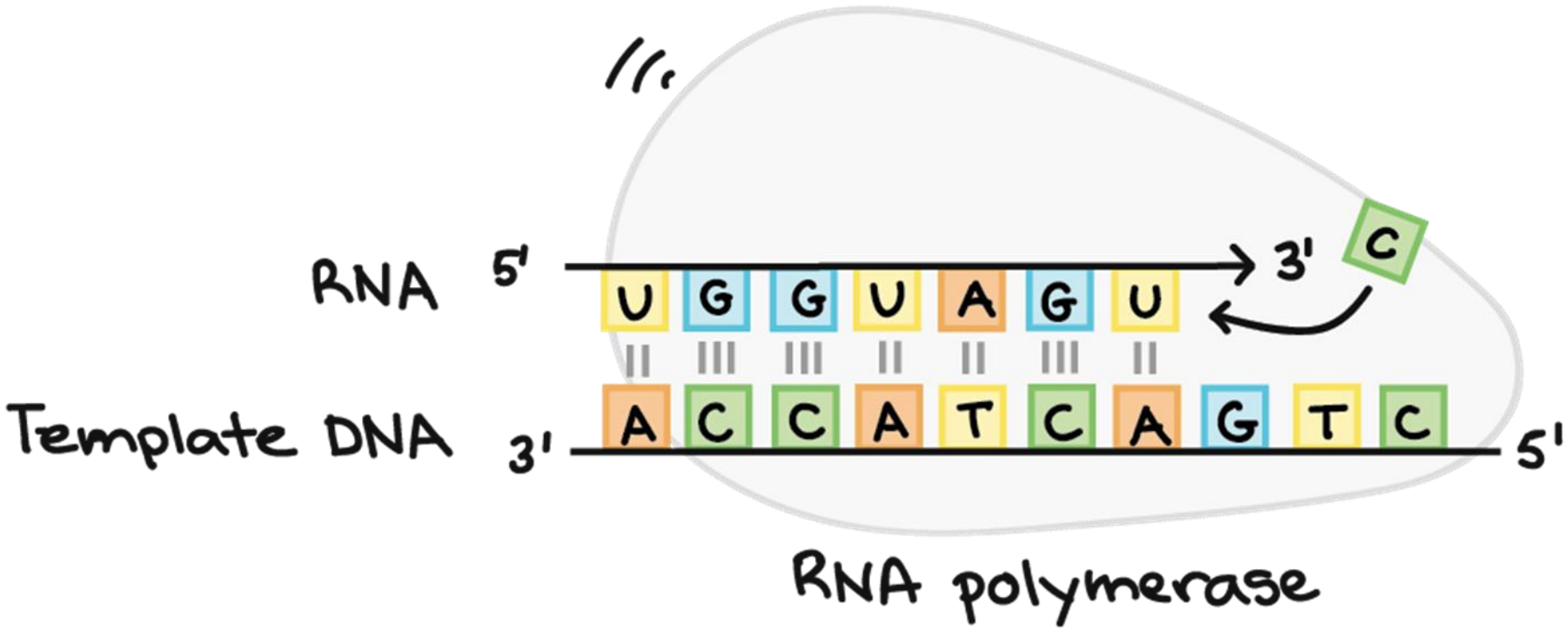
- Alteration of promoter strength can have bad effects upon a cell, often resulting in disease. For example, some tumor-promoting viruses transform healthy cells by inserting strong promoters in the nearness of growth-stimulating genes, while translocations in some cancer cells place genes that should be "turned off" in the proximity of strong promoters or enhancers.


Promoter
TATAAA
–30
+1
Transcription
start site
TFIID
TBP
+1
Transcription
start site
TFIIB
TFIIA
TFIID
TBP
+1
Transcription
start site
TFIIE
TFIIF
TFIIB
TFIIA
TFIID
TFIIH
TBP
RNA Polymerase II
+1
Transcription
start site

2-Initiation. RNA polymerase attaches to the DNA molecule and moves along the DNA strand until it recognizes a promoter sequence. The DNA double helix then unwinds and all the bases on each of the DNA strands are exposed. This acts as a template for a new mRNA strand. The enzyme is ready to make a strand of pre-mRNA with a complementary sequence of bases. The promoter is not part of the resulting mRNA.

3- Elongation is the addition of nucleotides to the mRNA strand by the enzyme with 3’-5’ direction of the coding strand.

4-Termination is the ending of transcription. As RNA polymerase transcribes the terminator, it detaches from DNA. The mRNA strand is complete after this step.


RNA
5'
3'
C
U G G U A G U
Template DNA
3'
A C C A T C A G T C
5'
RNA polymerase

Processing mRNA or post-transcriptional modifications

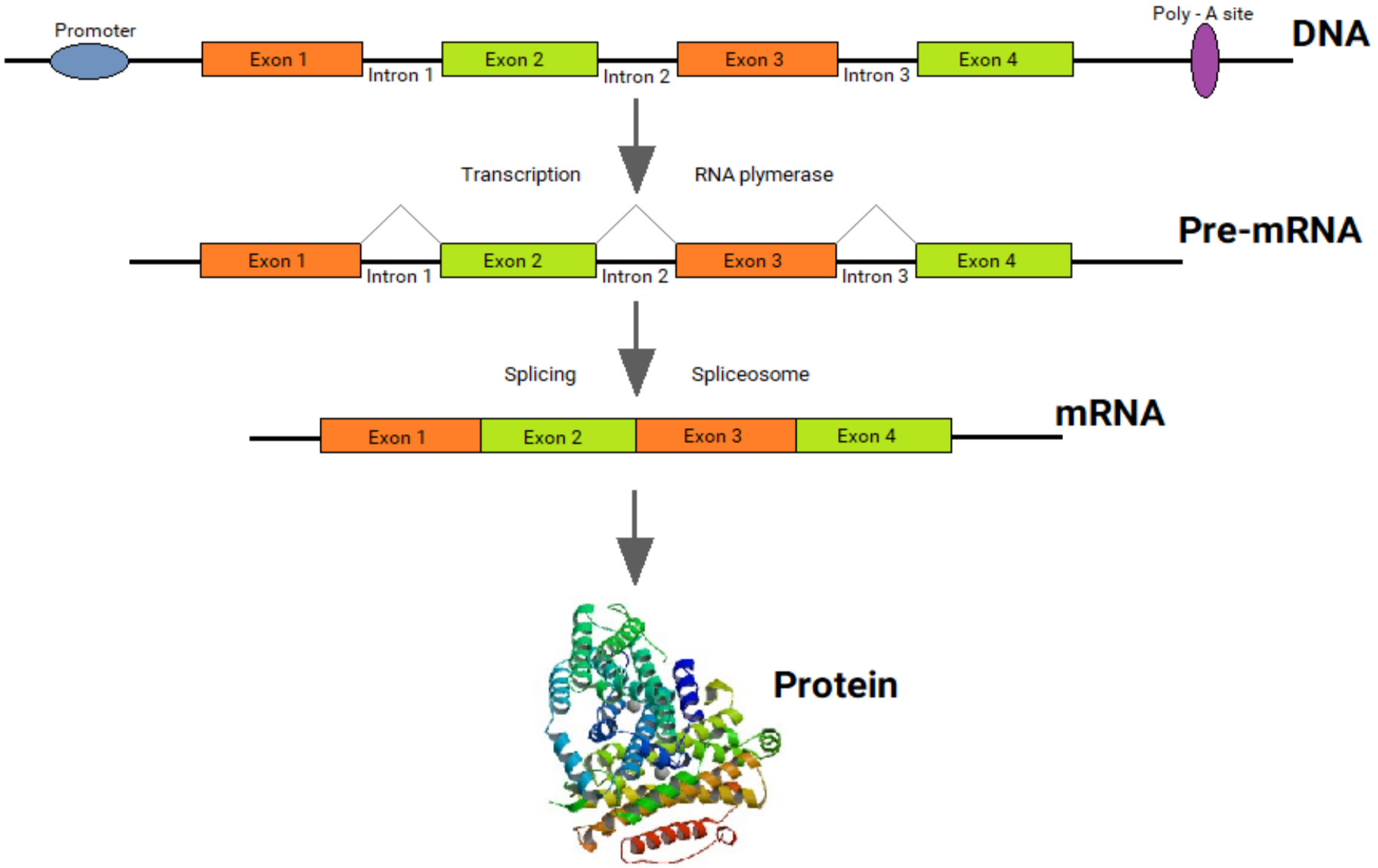
In eukaryotes, the new mRNA is not yet ready for translation. At this stage, it is called pre-mRNA, and it must go through more processing before it leaves the nucleus as mature mRNA to allow a single gene to be used to make more than one protein. The processing may include:

1- The addition of a 5' cap which protects mRNA in the cytoplasm from degradation, helps in the attachment of mRNA with the ribosome for translation, and enables mRNA to be differentiated from other RNAs in the cell. The 5' cap is added to the 5' end of the pre-mRNA molecule and is composed of a guanine nucleotide modified through methylation.

2-Polyadenylation adds a “tail” to the mRNA. The tail consists of a string of As (adenine bases). It signals the end of mRNA. It is also involved in exporting mRNA from the nucleus, and it protects mRNA from enzymes that might break it down. The 3' Poly(A) tail is added to the 3' end of the mRNA molecule and is composed of 100-200 adenine bases(A).

3-Splicing removes introns from the protein coding sequence of mRNA. Introns are regions that do not code for the protein. The remaining mRNA consists only of regions called exons that do code for the protein.

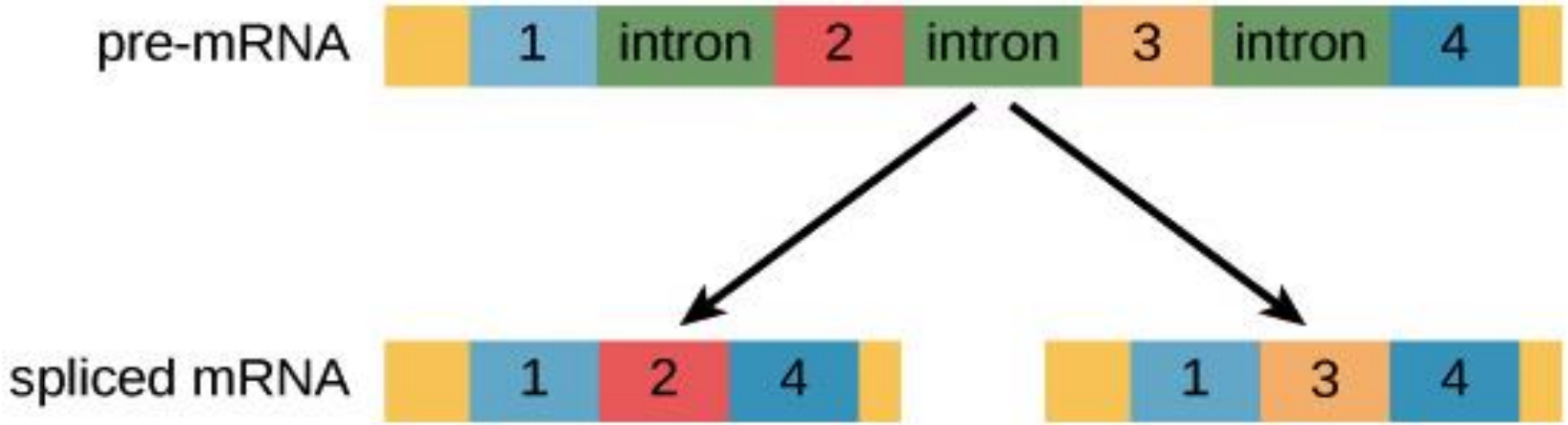
- These distinct mRNA modifications enable the cell to detect that the full mRNA message is intact if both the 5' cap and 3' tail are present


Promoter
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
Intron 3
Exon 4
Poly - A site
DNA
Transcription
RNA plymerase
Pre-mRNA
Splicing
Spliceosome
mRNA
Protein

4-Alternative splicing: In alternative splicing, individual exons(nucleotide sequences codes for protein) are either spliced or included, giving rise to several different possible mRNA products.

- Each mRNA product codes for a different protein isoform; these protein isoforms differ in their peptide sequence and therefore their biological activity.

- It is estimated that up to 60% of human gene products undergo alternative splicing.
- Alternative splicing contributes to protein diversity - a single gene transcript (RNA) can have thousands of different splicing patterns, and will therefore code for thousands of different proteins.
- Abnormal splicing patterns can lead to disease states including cancer.


pre-mRNA
1
intron
2
intron
3
intron
4
spliced mRNA
1
2
4
1
3
4

5-Editing changes some of the nucleotides in mRNA.

- For example, a human protein called **lipoprotein,** which helps transport lipids in the blood has two different forms because of editing. One form is smaller than the other because editing adds an earlier stop signal in mRNA of APOB gene.

❖ **Reverse transcription** This process consider as one of the modification of RNA is "reverse transcribed" into DNA. This process, catalyzed by *reverse transcriptase* enzymes, allows retroviruses, including the human immunodeficiency virus (HIV), to use RNA as their genetic material (replicate their viral genome).

- Reverse transcriptase enzymes have also found applications in biotechnology, allowing scientists to convert RNA to DNA for techniques such as **PCR**(polymerase chain reaction).

- The enzyme *reverse transcriptase* transcribes RNA to generate a single strand of complementary DNA (cDNA). The enzyme *DNA polymerase* converts the single-stranded (cDNA)into a double-stranded molecule as it does in DNA replication. Scientists also use reverse transcriptase processes to detect retroviruses.
- Eukaryotic cells also use reverse transcription to extend the end sections of chromosomes known as **telomeres**. The enzyme *telomerase reverse transcriptase* is responsible for this process. The extension of telomeres produces cells that are resistant to apoptosis, or programmed cell death, and become cancerous.

- The molecular biology technique known as reverse transcription-polymerase chain reaction (RT-PCR) is used to amplify and measure RNA. Since RT-PCR detects gene expression, it can also be used to detect cancer and in aid genetic disease diagnosis.

Translation

- The translation is the second part of the central dogma of molecular biology: RNA --> Protein.
- It is the process in which the genetic code in mRNA is read to make a protein.
- After mRNA leaves the nucleus, it moves to a ribosome, which consists of rRNA and proteins. In addition to the mRNA template, many other molecules contribute to the process of translation, such as ribosomes, tRNAs, and various enzymatic factors by translation process.
- Translation happens on the ribosomes floating in the cytosol, or on the ribosomes attached to the rough endoplasmic reticulum. The ribosome reads the sequence of codons in mRNA, and molecules of tRNA bring amino acids to the ribosome in the correct sequence.

The genetic code

The genetic code can be defined as the set of certain rules using which the living cells translate the information encoded within genetic material (DNA or mRNA sequences). The first step in decoding genetic messages is transcription, during which a nucleotide sequence is copied from DNA to RNA. The next step is to join amino acids together to form a protein.

- The order in which amino acids are joined together determine the shape, properties, and function of a protein. The four bases of RNA form a language with just four nucleotide bases: (A), (C), (G), and (U). The genetic code is read in three-base words called codons. Each codon corresponds to a single amino acid (or signals the starting and stopping points of a sequence).

- There are multiple molecules of tRNA. Each tRNA molecule has an **anticodon** for the amino acid it carries.
- An anticodon is complementary to the **codon** for an amino acid. A codon is a sequence of three nucleotides which together form a unit of genetic code in a DNA or RNA molecule.
- For example, the amino acid lysine has the codon AAG, so the anticodon is UUC. Therefore, lysine would be carried by a tRNA molecule with the anticodon UUC.
- Wherever the codon AAG appears in mRNA, a UUC anticodon of tRNA temporarily binds. While bound to mRNA, tRNA gives up its amino acid. With the help of rRNA, bonds form between the amino acids as they are brought one by one to the ribosome, creating a polypeptide chain.
- The chain of amino acids keeps growing until a stop codon is reached.

Properties of Genetic Code

1-Triplet code: The code is a triplet. The four bases of nucleotide (A, G, C, and U) are used to produce three-base codons. The 64 codons involve sense codons (that specify amino acids). These 64 codons are for 20 amino acids since every codon for one amino acid means that there exist more than code for the same amino acid.

2-Non-ambiguous and Universal: Non ambiguous is mean a specific codon will only code for a particular amino acid. Also, the same genetic code is seen valid for all the organisms so they are universal. For example GGA only codes for glycine.

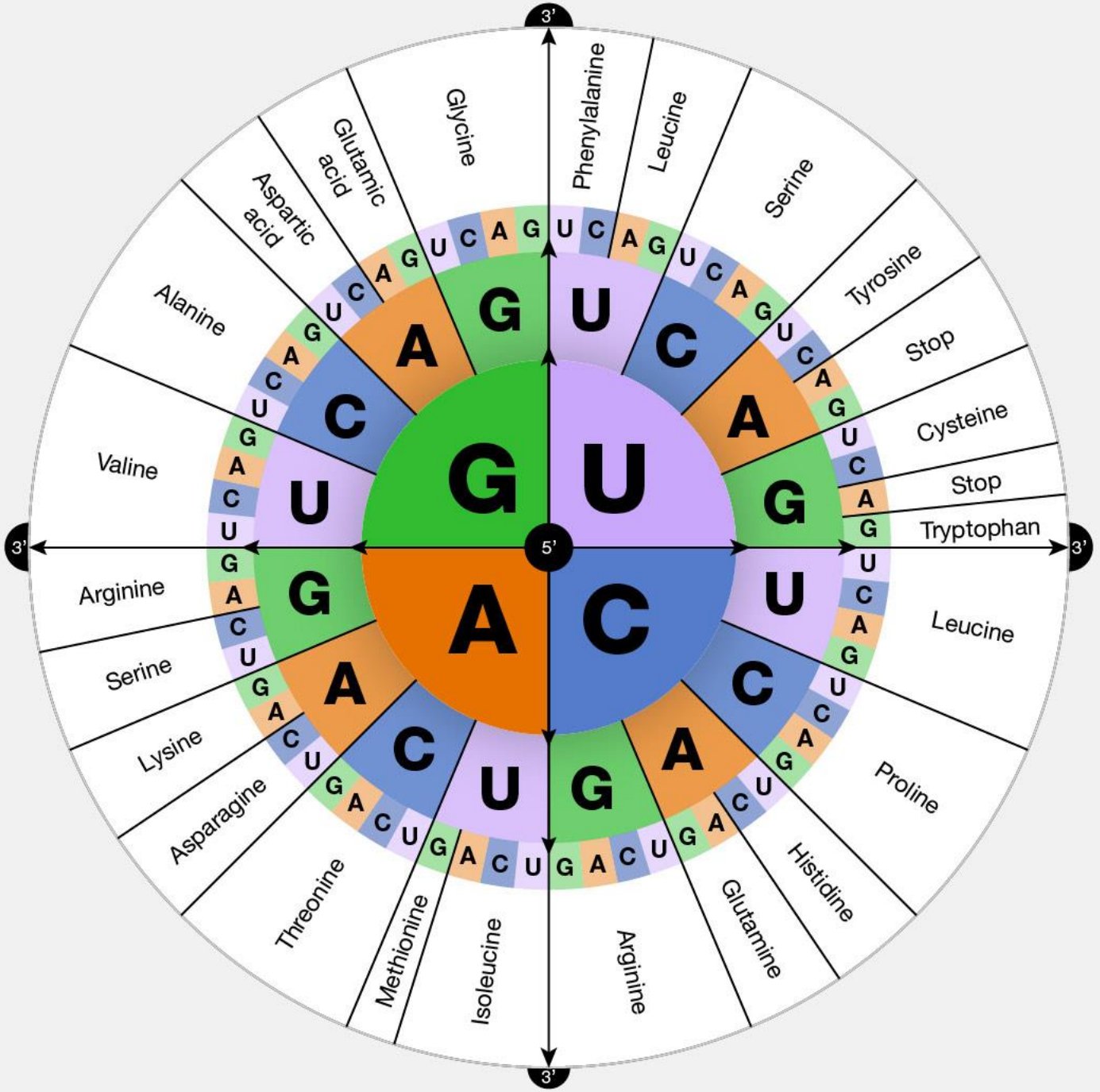
3-Degenerate code: Every amino acid except tryptophan (UGG) and methionine (AUG) is coded by various codons, a few codons are synonyms and this aspect is known as the degeneracy of genetic code. For example UCA,UCU,UCG and UCC all code for Serine amino acid.

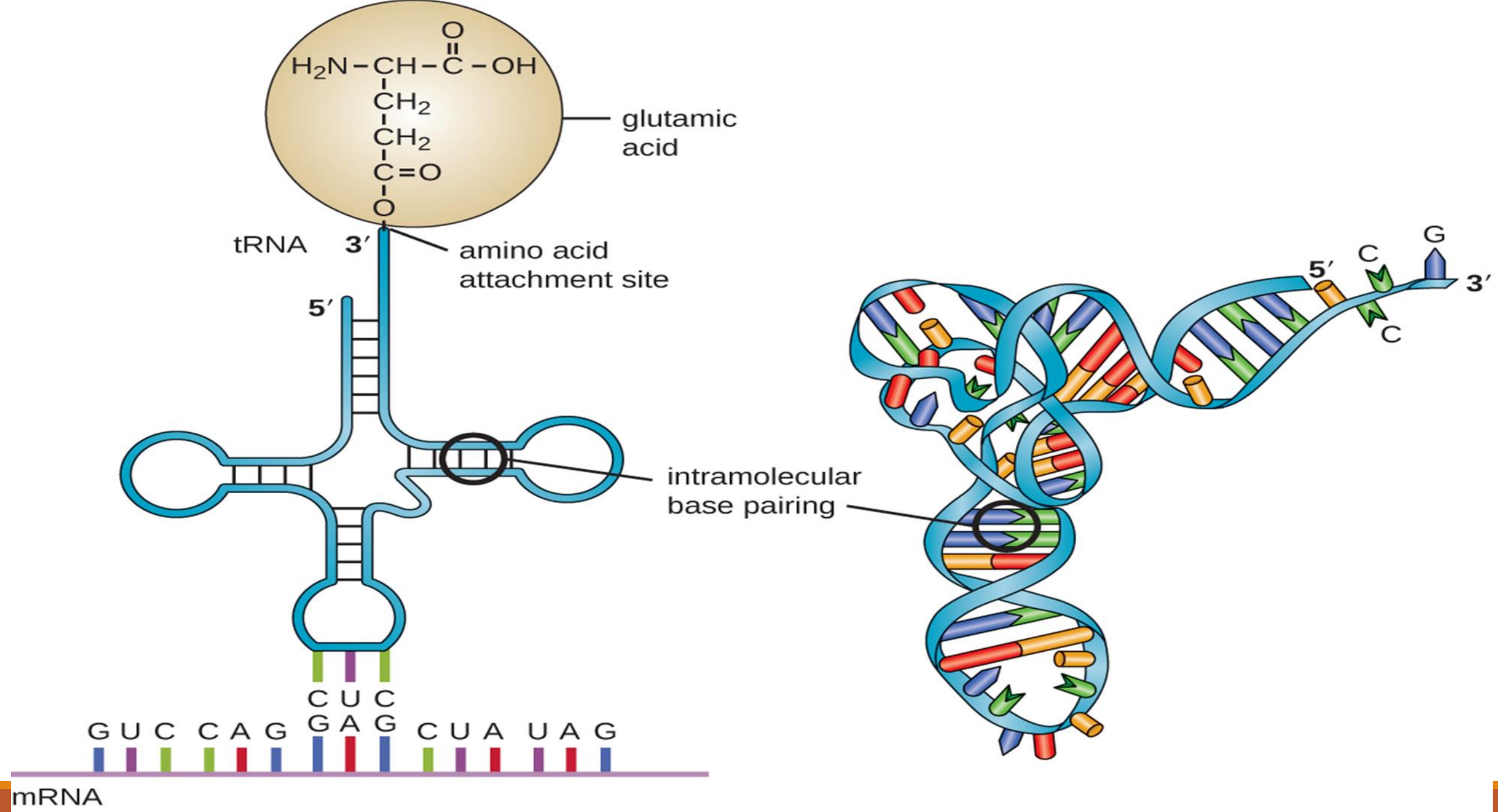
4-Nonoverlapping code: The code is read sequentially in a group of three and a nucleotide which becomes a part of triplet never becomes part of the next triplet. For example 5’-UCU-3’ codes for Serine and 5’-AUG-3’ codes for methionine.

5-Commaless code: No room for punctuation in between which indicates that every codon is adjacent to the previous one without any nucleotides between them.

6-Start and Stop Codons: Generally, AUG codon is the initiating or start codon. The polypeptide chain starts either with eukaryotes (methionine) or prokaryotes (N-formylmethionine).On the other hand, **UAG, UAA** and **UGA** are called as termination codons or stop codons. These are not read by any tRNA molecules and they never code for any amino acids.

7-Polarity: Each triplet is read from 5’ → 3’ direction and the beginning base is 5’ followed by the base in the middle then the last base which is 3’. This implies that the codons have a fixed polarity and if the codon is read in the reverse direction, the base sequence of the codon would reverse and would specify two different proteins.


3'
5'
3'
3'
3'
G
U
A
C
Phenylalanine
Leucine
Serine
Tyrosine
Stop
Cysteine
Stop
Tryptophan
Leucine
Proline
Histidine
Glutamine
Arginine
Isoleucine
Methionine
Threonine
Asparagine
Lysine
Serine
Arginine
Valine
Alanine
Aspartic acid
Glutamic acid
Glycine



tRNA 2-dimensional structure

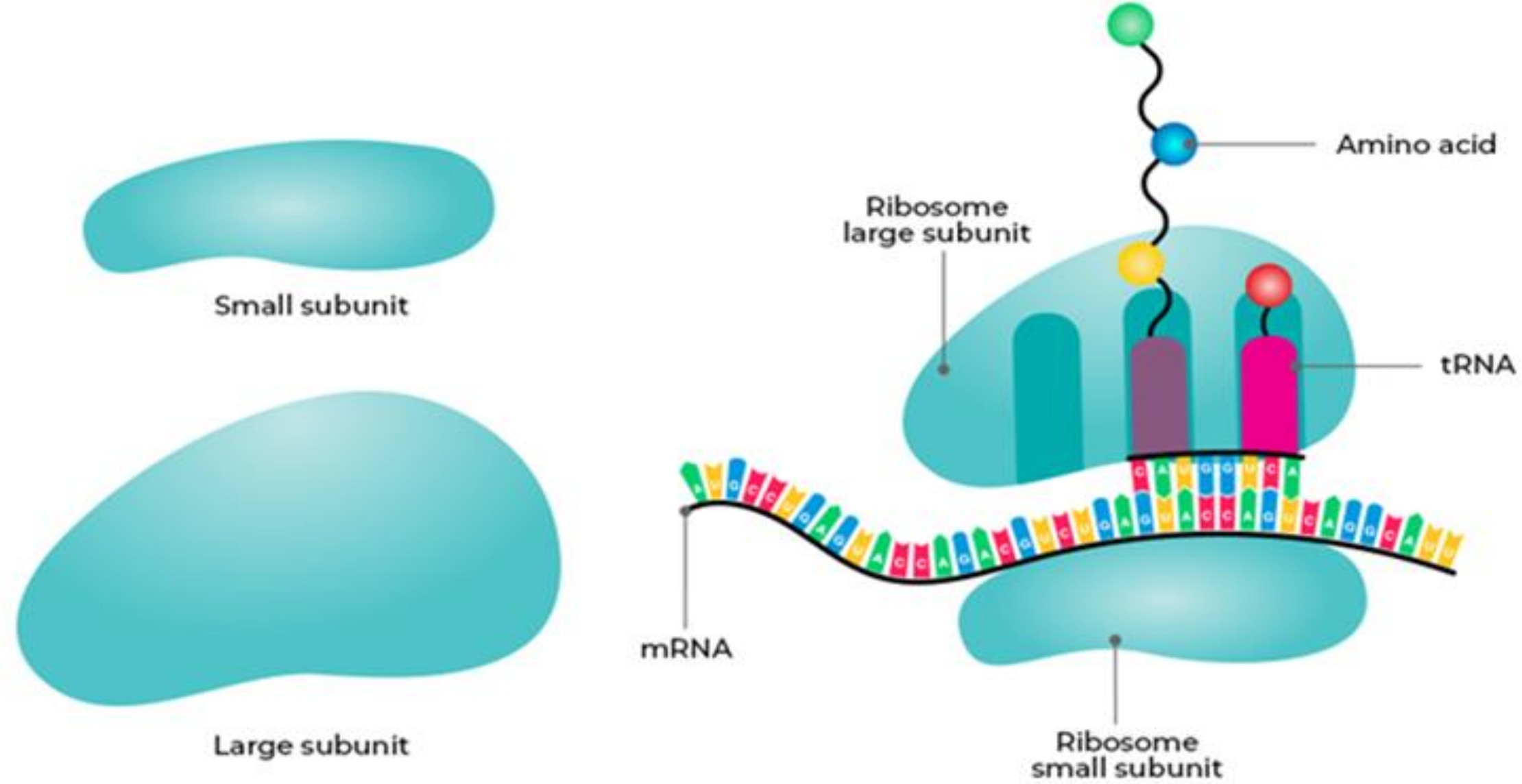
tRNA 3-dimensional structure

Ribosomes

- Ribosomes are the sites in a cell in which protein synthesis takes place. Cells have many ribosomes, and the exact number depends on how active a particular cell is in synthesizing proteins. For example, rapidly growing cells usually have a large number of ribosomes.

- Eukaryotic and prokaryotic ribosomes are different from each other as a result of divergent evolution. These differences are exploited by antibiotics, which are designed to inhibit the prokaryotic ribosomes of infectious bacteria without affecting eukaryotic ribosomes, thereby not interfering with the cells of the sick host, for example tetracyclin blocks binding of aminoacyl-tRNA to A-site of ribosome.

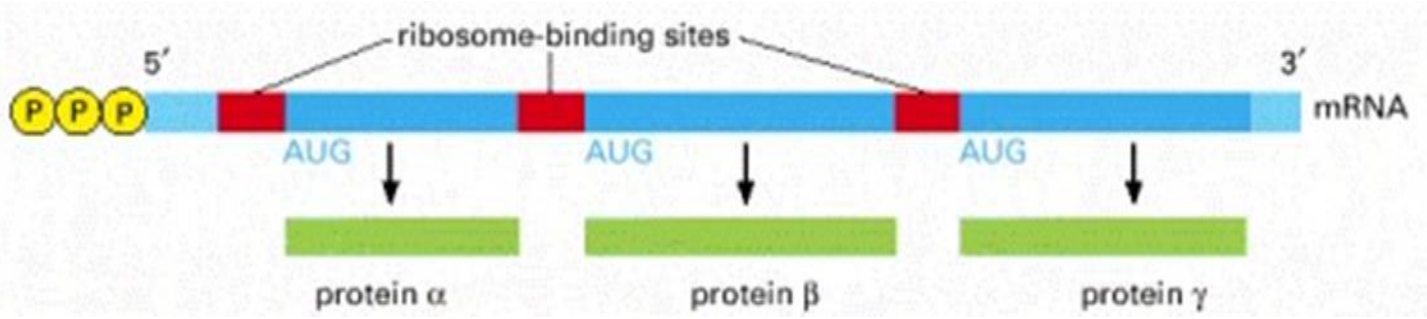
- Ribosomes, which are just made out of rRNA (ribosomal RNA) and protein, have been classified as ribozymes because the rRNA has enzymatic activity. The rRNA is important for the ***peptidyl transferase*** activity that bonds amino acids.
- Ribosomes have two subunits. **The small** subunit provides a framework on which the tRNAs can be accurately matched to the codons of the mRNA ,while **the large** subunit catalyzes the formation of the peptide bonds that link the amino acids together into a polypeptide chain.
- The large subunit has three active sites called E, P, and A sites. These sites are important in the catalytic activity of ribosomes.
- Ribosomes operate with remarkable efficiency: in one second, a single ribosome of a eukaryotic cell adds about 2 amino acids to a polypeptide chain; the ribosomes of bacterial cells operate even faster, at a rate of about 20 amino acids per second.


Small subunit
Large subunit
Amino acid
Ribosome
large subunit
tRNA
mRNA
Ribosome
small subunit

- When not actively synthesizing proteins, the two subunits of the ribosome are separate. They join together on an mRNA molecule, usually near its 5′ end, to initiate the synthesis of a protein.
- The mRNA is then pulled through the ribosome; as its codons encounter the ribosome's active site, the mRNA nucleotide sequence is translated into an amino acid sequence using the tRNAs as adaptors to add each amino acid in the correct sequence to the end of the growing polypeptide chain.
- When a stop codon is encountered, the ribosome releases the finished protein, its two subunits separate again. These subunits can then be used to start the synthesis of another protein on another mRNA molecule.

- The mechanism for selecting a start codon in bacteria is different.
- Bacterial mRNAs have no 5′ caps to tell the ribosome where to begin searching for the start of translation. Instead, each bacterial mRNA contains a specific ribosome-binding site (called the ***Shine-Dalgarno sequence,*** named after its discoverers) that is located a few nucleotides upstream of the AUG at which translation is to begin. This nucleotide sequence, with the consensus 5′-AGGAGGU-3′, forms base pairs with the 16S rRNA of the small ribosomal subunit to position the initiating AUG codon in the ribosome.
- A set of translation initiation factors operate this interaction, as well as the subsequent assembly of the large ribosomal subunit to complete the ribosome.

- Unlike a eukaryotic ribosome, a bacterial ribosome can therefore readily assemble directly on a start codon that lies in the interior of an mRNA molecule, so long as a ribosome-binding site precedes it by several nucleotides.
- As a result, bacterial mRNAs are often ***polycistronic***—that is, they encode several different proteins, each of which is translated from the same mRNA molecule. In contrast, a eucaryotic mRNA generally encodes only a single protein.



Steps of translation

- **1-Initiation**:The small subunit binds to a site upstream (on the 5' side) of the start of the mRNA.

- It proceeds to scan the mRNA in the 5'-->3' direction until it encounters the START codon (***AUG***). The large subunit attaches and the ***initiator tRNA, which carries methionine (Met),*** binds to the P site on the ribosome, (in bacteria, a modified form of methionine—***formylmethionine***—is used) so that all newly made proteins have methionine as the first amino acid at their N-terminal end, the end of a protein that is synthesized first.

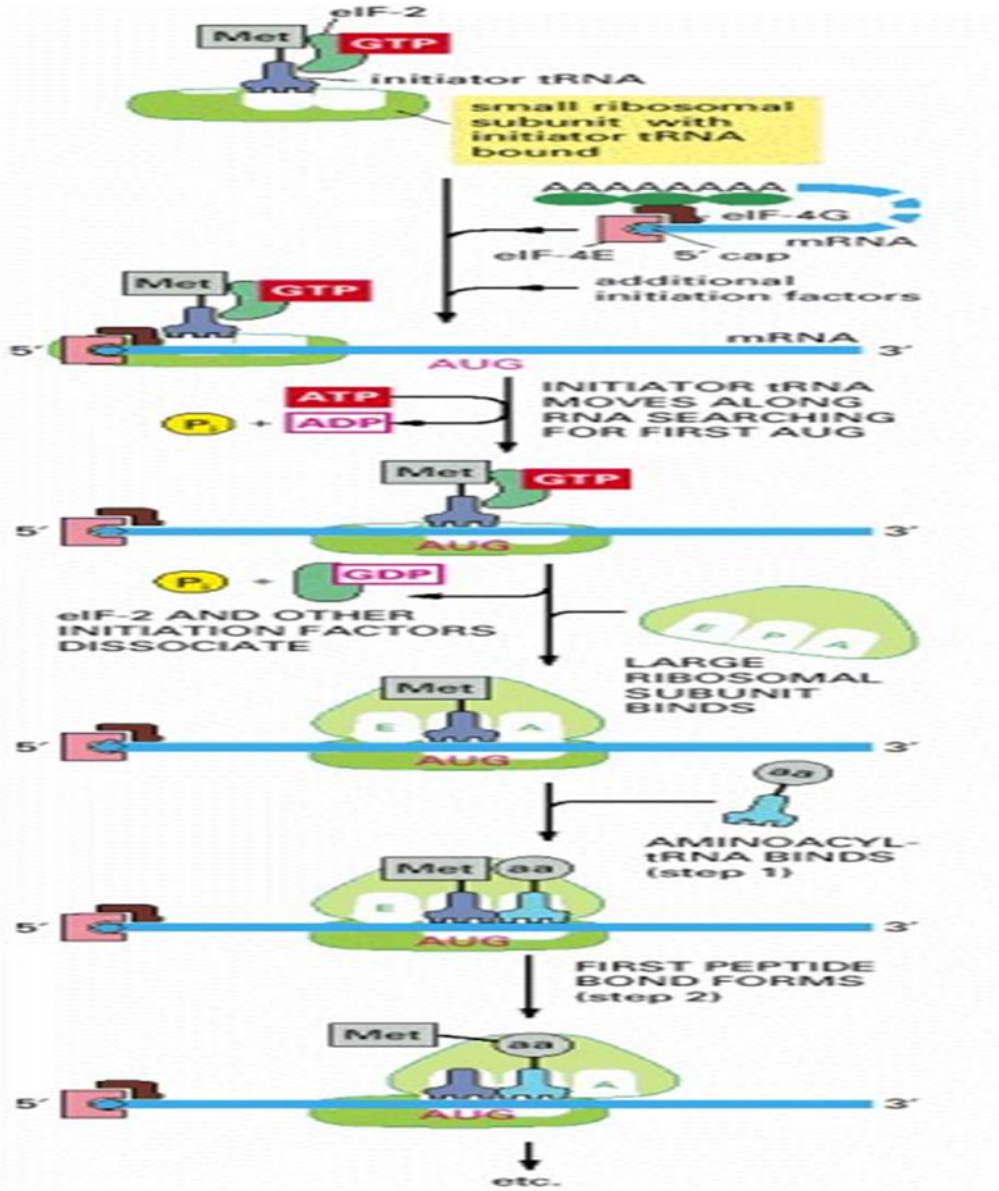
- This methionine is usually removed later by a specific protease.

- The initiator tRNA has a nucleotide sequence distinct from that of the tRNA that normally carries methionine.

- The initiator tRNA is first loaded into the small ribosomal subunit along with additional proteins called ***eucaryotic initiation factors,*** or **eIFs** . Of all the aminoacyl tRNAs in the cell, only the methionine-charged initiator tRNA is capable of tightly binding the small ribosome subunit without the complete ribosome present.

- Next, the small ribosomal subunit binds to the 5′ end of an mRNA molecule, which is recognized by its 5′ cap and its two bound initiation factors, **eIF4E** (which directly binds the cap) and **eIF4G**.

- The small ribosomal subunit then moves forward (5′ to 3′) along the mRNA, searching for the first AUG. This movement is facilitated by additional initiation factors that act as ATP-powered helicases, allowing the small subunit to scan through mRNA. At this point, the initiation factors dissociate from the small ribosomal subunit to make way for the large ribosomal subunit to assemble with it and complete the ribosome.
- The initiator tRNA is now bound to the P-site, leaving the A-site vacant. Protein synthesis is therefore ready to begin with the addition of the next aminoacyl tRNA molecule.


eIF-2
Met
GTP
initiator tRNA
small ribosomal subunit with initiator tRNA bound
AAAAAAAA
eIF-4G
mRNA
eIF-4E
5′ cap
additional initiation factors
Met
GTP
5′
mRNA
3′
AUG
INITIATOR tRNA MOVES ALONG RNA SEARCHING FOR FIRST AUG
ATP
ADP
Pi
Met
GTP
5′
3′
AUG
Pi
GDP
eIF-2 AND OTHER INITIATION FACTORS DISSOCIATE
LARGE RIBOSOMAL SUBUNIT BINDS
Met
5′
3′
AUG
aa
AMINOACYL-tRNA BINDS (step 1)
Met
aa
5′
3′
AUG
FIRST PEPTIDE BOND FORMS (step 2)
Met
aa
5′
3′
AUG
etc.

2-Elongation: The ribosome shifts one codon at a time, catalyzing each process that occurs in the three sites. With each step, a charged tRNA (with anticodon sequence) enters the complex , the polypeptide becomes one amino acid longer, and an uncharged tRNA departs.

- The energy for each bond between amino acids is derived from GTP (Guanosin triphosphate), a molecule similar to ATP.

- Briefly, the ribosomes interact with other RNA molecules to make polypeptide chains, due to the peptide bond that forms between individual amino acids.

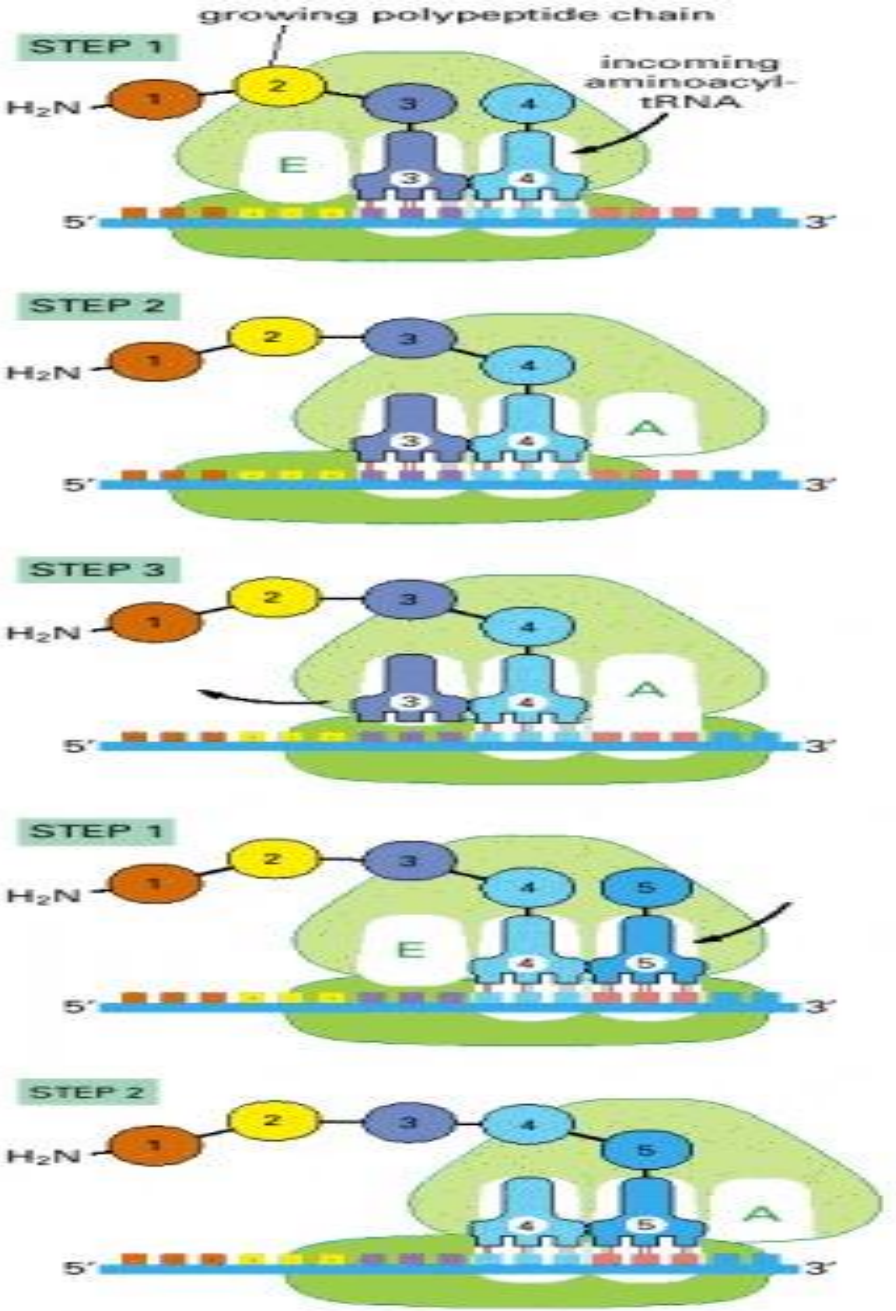
- *Two elongation factors* (**EF-Tu** and **EF-G**) enter and leave the ribosome during each cycle, each hydrolyzing GTP to GDP and undergoing conformational changes in the process. Under some conditions, ribosomes can be made to perform protein synthesis without the aid of the elongation factors and GTP hydrolysis, but this synthesis is very slow, inefficient, and inaccurate.

- In addition to helping move translation forward, EF-Tu is thought to increase the accuracy of translation by monitoring the initial interaction between a charged tRNA and a codon.
- Charged tRNAs enter the ribosome bound to the GTP-form of EF-Tu.
- To read the genetic code in DNA, cells make a series of different tRNAs.
- Recognition and attachment of the correct amino acid depends on enzymes called aminoacyl-tRNA synthetases, as first adapter which covalently couple each amino acid to its appropriate set of tRNA molecules.
- This stage include steps:

step 1, a tRNA carrying the next amino acid in the chain binds to the ribosomal A-site by forming base pairs with the codon in mRNA positioned there, so that the P-site and the A-site contain adjacent bound tRNAs.

step 2, the carboxyl end of the polypeptide chain is released from the tRNA at the P-site (by breakage of the high-energy bond between the tRNA and its amino acid) and joined to the free amino group of the amino acid linked to the tRNA at the A-site, forming a new peptide bond. This central reaction of protein synthesis is catalyzed by a peptidyl transferase catalytic activity contained in the large ribosomal subunit.

step 3, another series of conformational changes moves the mRNA exactly three nucleotides through the ribosome and resets the ribosome so it is ready to receive the next aminoacyl tRNA. Step 1 is then repeated with a new incoming aminoacyl tRNA, and so on.

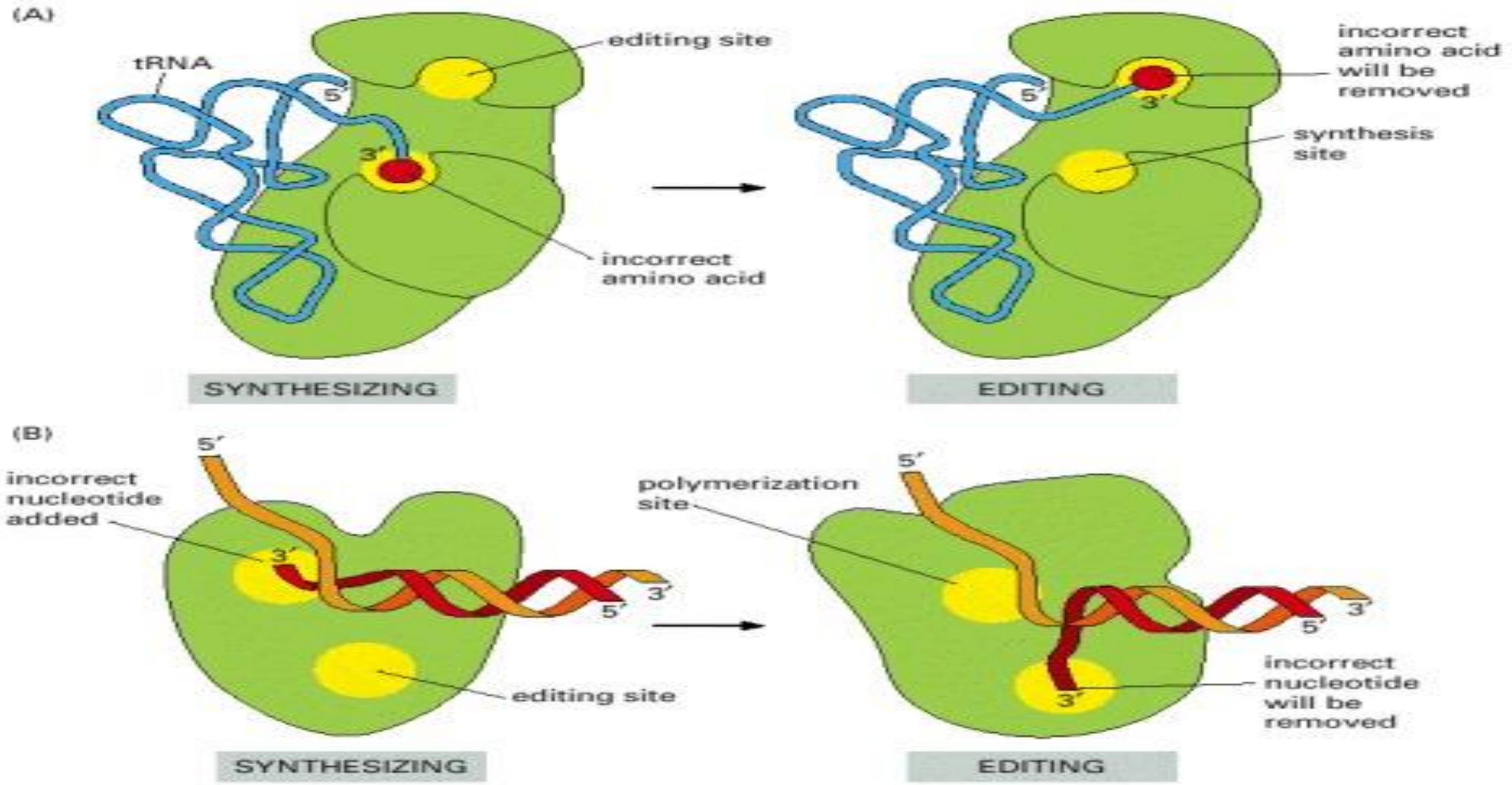


Editing by RNA Synthetases Ensures Accuracy

- Several mechanisms working together ensure that the tRNA synthetase links the correct amino acid to each tRNA.
- The synthetase must first select the correct amino acid, and most do so by a two-step mechanism.

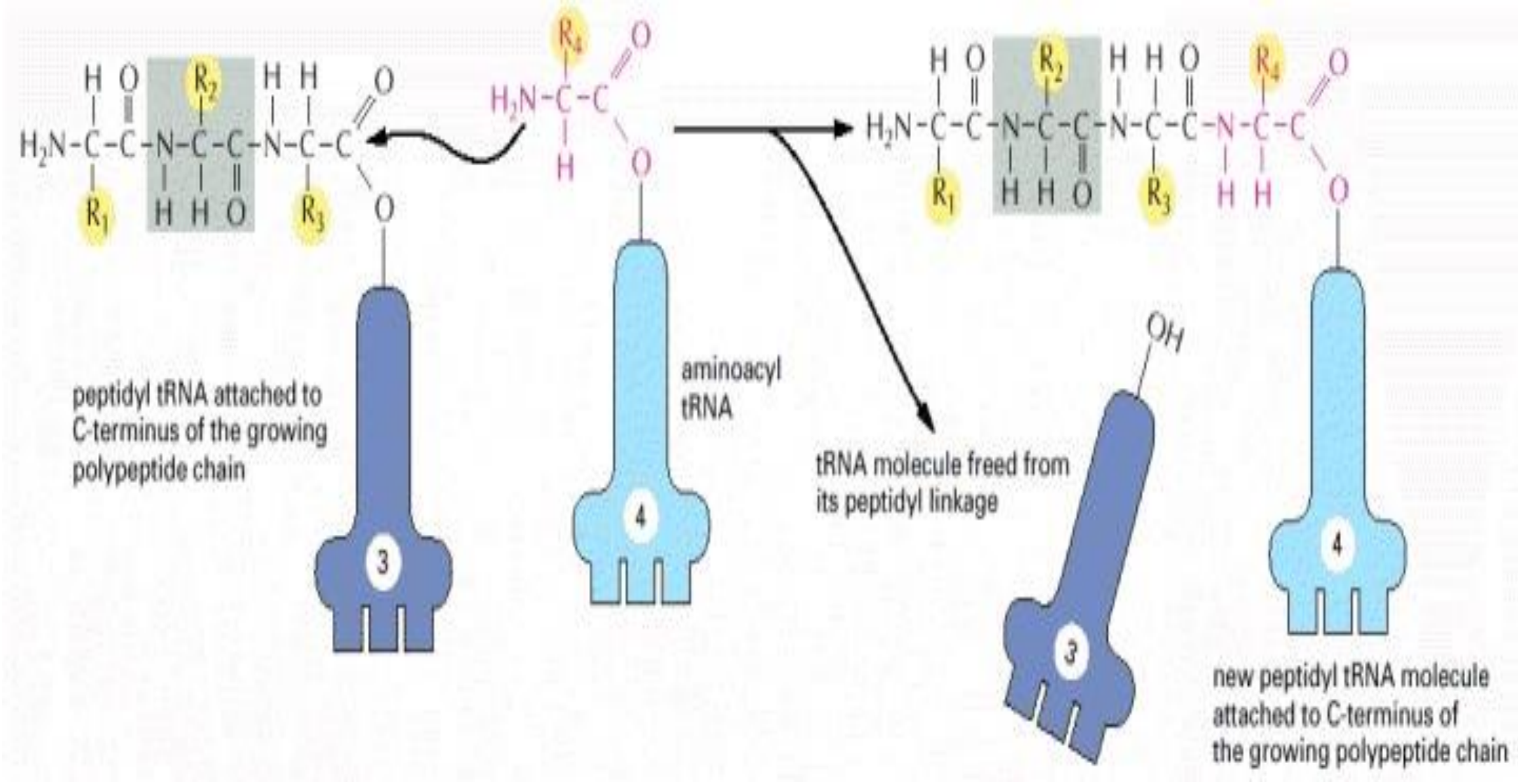
- **First**, the correct amino acid has the highest affinity for the active-site pocket of its synthetase and is therefore favored over the other 19. However, accurate discrimination between two similar amino acids, such as isoleucine and valine (which differ by only a methyl group), is very difficult to achieve by a one-step recognition mechanism.

- **Asecond** discrimination step occurs after the amino acid has been covalently linked to AMP(Adenosine monophosphate). When tRNA binds the synthetase, it forces the amino acid into a second pocket in the synthetase. Once an amino acid enters this editing pocket, it is hydrolyzed from the AMP (or from the tRNA itself if the aminoacyl-tRNA bond has already formed) and released from the enzyme.


(A)
tRNA
editing site
5′
3′
incorrect amino acid
SYNTHESIZING
incorrect amino acid will be removed
synthesis site
EDITING
(B)
5′
incorrect nucleotide added
3′
5′
3′
editing site
SYNTHESIZING
polymerization site
5′
5′
3′
3′
incorrect nucleotide will be removed
EDITING

Amino Acids Are Added to the C-terminal End of a Growing Polypeptide Chain

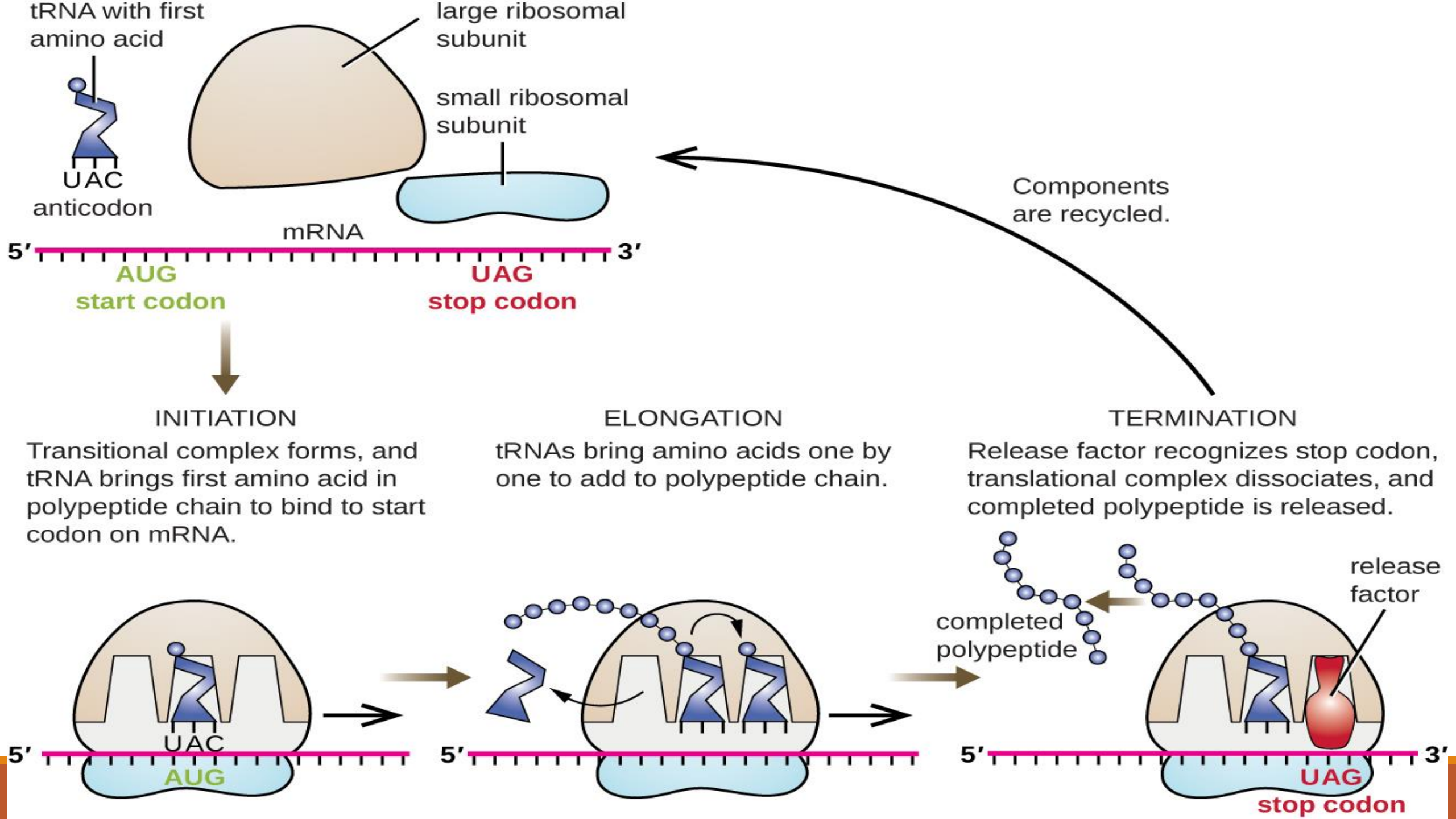
- The fundamental reaction of protein synthesis is the *formation of a peptide bond* between the carboxyl group at the end of a growing polypeptide chain and a free amino group on an incoming amino acid.
- Consequently, a protein is synthesized stepwise from its N-terminal end to its C-terminal end. Throughout the entire process the growing carboxyl end of the polypeptide chain remains activated by its covalent attachment to a tRNA molecule (a peptidyl-tRNA molecule).
- This high-energy covalent linkage is disrupted during each addition but is immediately replaced by the identical linkage on the most recently added amino acid.
- In this way, each amino acid added carries with it the activation energy for the addition of the next amino acid rather than the energy for its own addition.

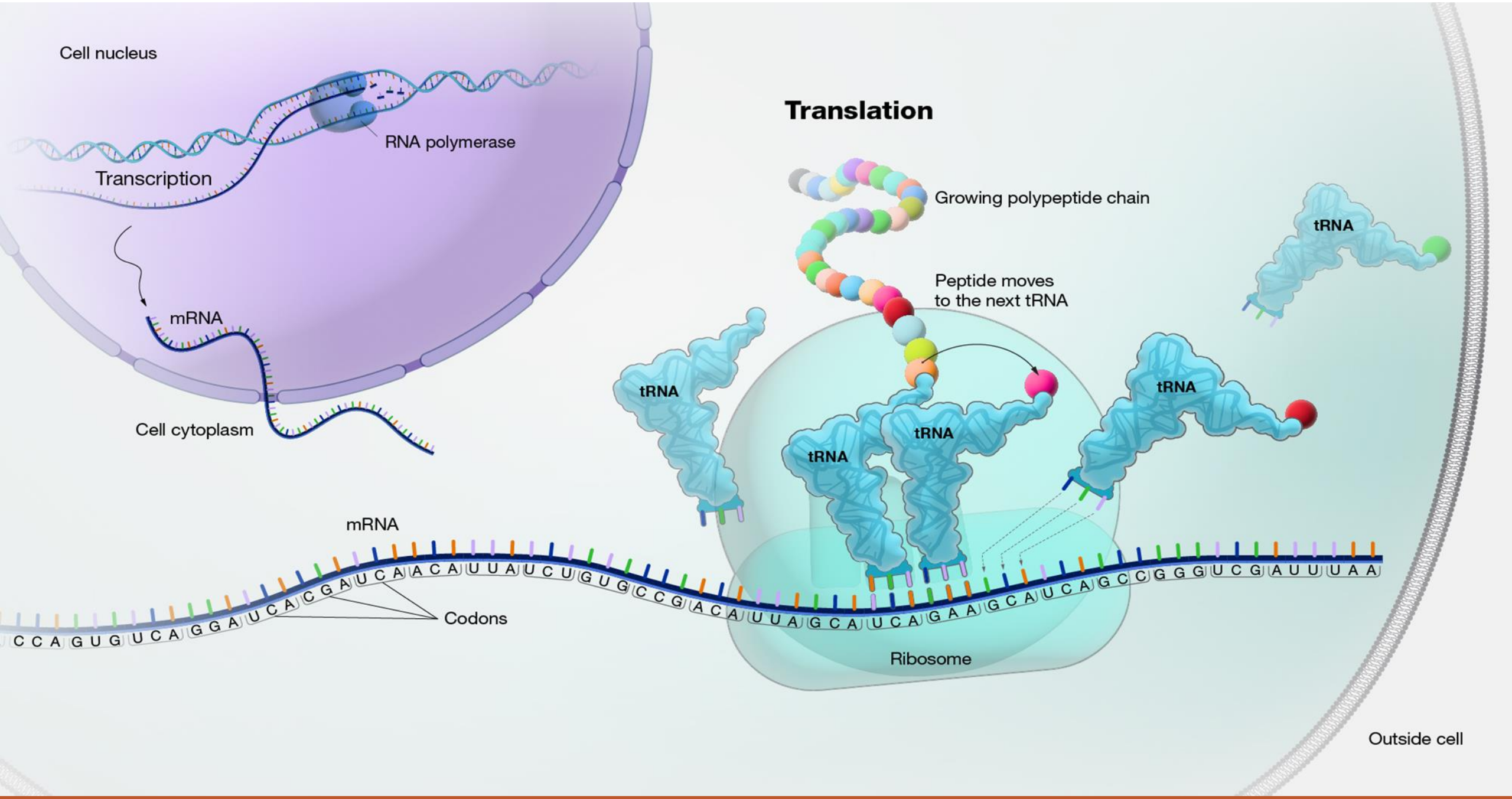

peptidyl tRNA attached to C-terminus of the growing polypeptide chain
aminoacyl tRNA
tRNA molecule freed from its peptidyl linkage
new peptidyl tRNA molecule attached to C-terminus of the growing polypeptide chain
3
4
3
4
OH

3-Termination:The end of the protein-coding message is signaled by the presence of one of three codons (**UAA, UAG, or UGA**) called stop codons . These are not recognized by a tRNA and do not specify an amino acid, but instead signal to the ribosome to stop translation.

- After many ribosomes have completed translation, Proteins known as *release factors* bind to any ribosome with a stop codon positioned in the A site, and this binding forces the peptidyl transferase in the ribosome to catalyze the addition of a water molecule instead of an amino acid to the peptidyl-tRNA .This reaction frees the carboxyl end of the growing polypeptide chain from its attachment to a tRNA molecule, and since only this attachment normally holds the growing polypeptide to the ribosome, the completed protein chain is immediately released into the cytoplasm.

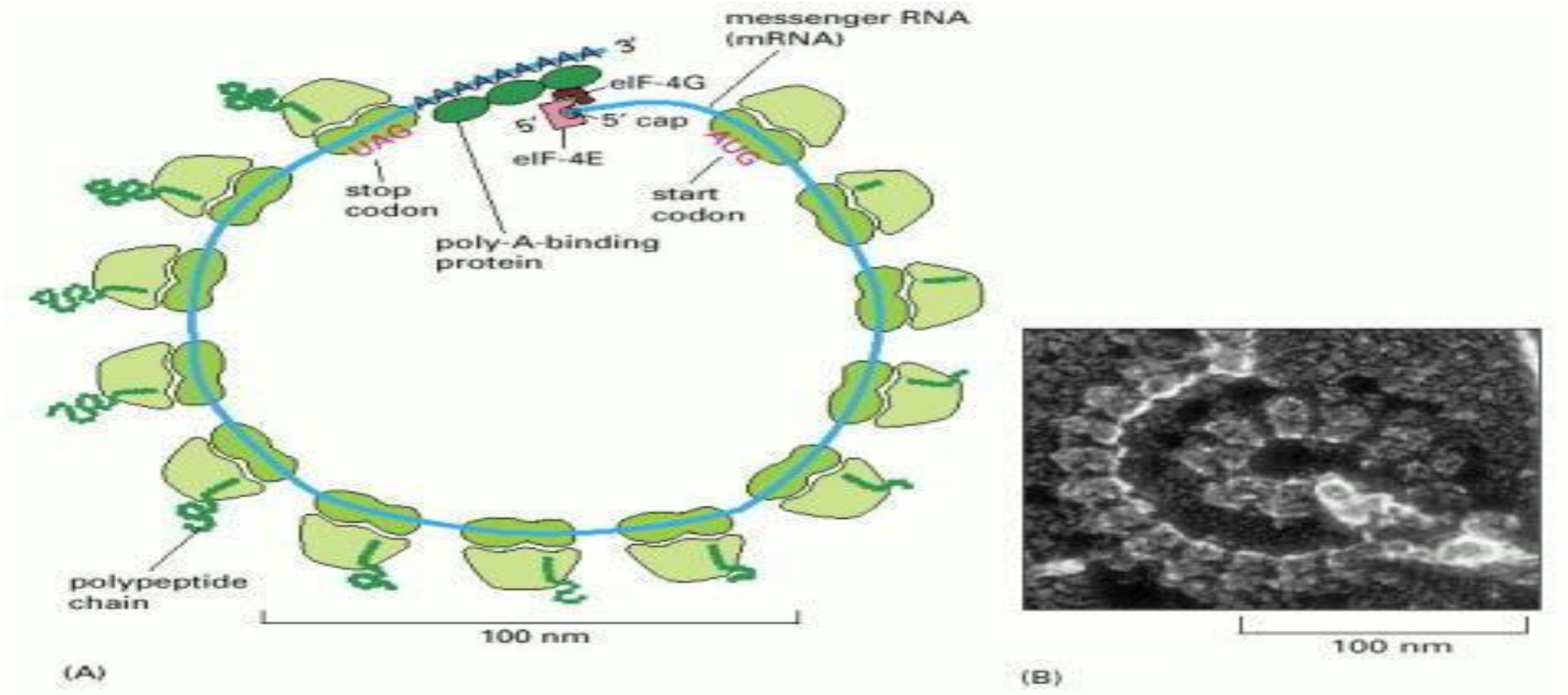
- The ribosome then releases the mRNA which is degraded so the nucleotides can be reused in another transcription reaction, and separates into the large and small subunits, which can assemble on another mRNA molecule to begin a new round of protein synthesis.


tRNA with first amino acid
large ribosomal subunit
small ribosomal subunit
UAC
anticodon
mRNA
5′
3′
AUG
start codon
UAG
stop codon
Components are recycled.
INITIATION
Transitional complex forms, and tRNA brings first amino acid in polypeptide chain to bind to start codon on mRNA.
ELONGATION
tRNAs bring amino acids one by one to add to polypeptide chain.
TERMINATION
Release factor recognizes stop codon, translational complex dissociates, and completed polypeptide is released.
UAC
AUG
completed polypeptide
release factor
UAG
stop codon


Cell nucleus
RNA polymerase
Transcription
mRNA
Cell cytoplasm
Translation
Growing polypeptide chain
Peptide moves
to the next tRNA
tRNA
tRNA
tRNA
tRNA
tRNA
mRNA
C C A G U G U C A G G A U C A C G A U C A A C A U U A U C U G U G C C G A C A U U A G C A U C A G A A G C A U C A G C C G G G U C G A U U U A A
Codons
Ribosome
Outside cell

Proteins Are Made on Polyribosomes

- The synthesis of most protein molecules takes between 20 seconds and several minutes. But even during this very short period, multiple initiations usually take place on each mRNA molecule being translated.
- As soon as the preceding ribosome has translated enough of the nucleotide sequence to move out of the way, the 5′ end of the mRNA is threaded into a new ribosome.
- The mRNA molecules being translated are therefore usually found in the form of *polyribosomes* (also known as *polysomes*), large cytoplasmic assemblies made up of several ribosomes spaced as close as 80 nucleotides apart along a single mRNA molecule.
- These multiple initiations mean that many more protein molecules can be made in a given time than would be possible if each had to be completed before the next could start.



(A) series of ribosomes can simultaneously translate the same eucaryotic mRNA molecule. (B) Electron micrograph of a polyribosome from a eucaryotic cell.

Protein Folding

- Following translation the polypeptide chain must fold to form a functional protein; for example, to function as an enzyme the polypeptide chain must fold correctly to produce a functional active site.
- The process of gene expression is not over when the genetic code has been used to create the sequence of amino acids that means a protein, this protein to be useful to the cell this new polypeptide chain must fold up into its unique three-dimensional conformation, bind any small-molecule cofactors required for its activity, be modified by **protein kinases or other protein-modifying enzymes**, and assemble correctly with the other protein subunits with which it functions.

- Proteins frequently assemble to form larger structures to perform important cellular functions. For example, the tail of a human sperm is a structure that is composed of different types of proteins that collectively work together to perform a single action, here they form into a network of rotary engine which propels the sperm forward.
- This folding is missed due to some reasons such as, when an individual human possess a gene mutation that changes an amino acid in the protein to find its suitable fold.

How a protein folds

The wide variety of conformations is due to the *huge amount of different sequences of amino acid residues*. Protein folding occurs in four stages namely:

1-primary structure is the sequence of residues of amino acids in the polypeptide chain.

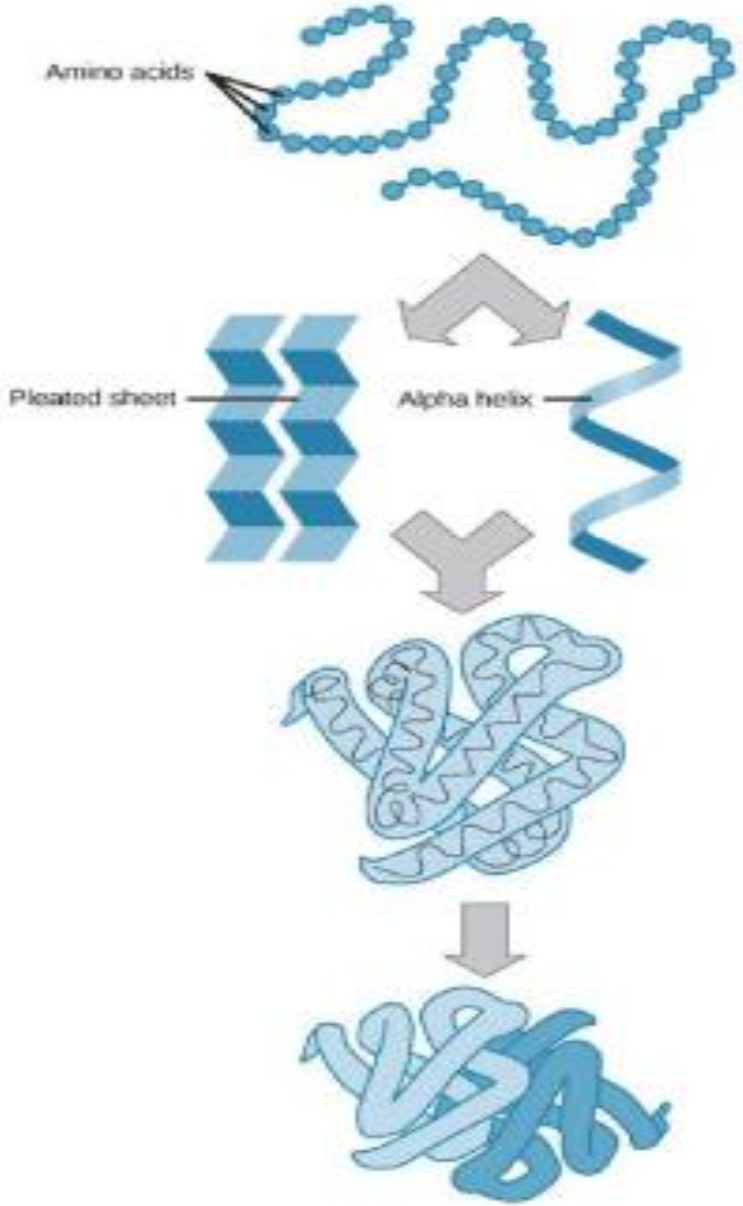
2-Secondary Structure is a local regularly occurring structure in proteins and is mainly formed through hydrogen bonds between backbone atoms. So-called *random coils*, *loops or turns* do not have a stable secondary structure.

There are two types of stable secondary structures, *the Alpha helices* and the *beta-sheets*. **Alpha-helices** involves intramolecular hydrogen bonding. In an alpha helix, the carbonyl (C=O) of one amino acid is hydrogen bonded to the amino H (N-H) of an amino acid that is four down the chain. In **a Beta-sheet**, two or more segments of a polypeptide chain line up next to each other, forming a sheet-like structure held together by hydrogen bonds.

3-Tertiary structure expresses the packing of alpha-helices, beta-sheets and random coils with respect to each other on the level of one whole polypeptide chain.

4-Quaternary structure only exists, if there is more than one polypeptide chain present in a complex protein. Then quaternary structure expresses the spatial organization of the chains. Example of quaternary structure - haemoglobin or DNA polymerase.

- Once correctly folded, the protein can undergo further maturation through different post-translational modifications. Post-translational modifications can alter the protein's ability to function, where it is located within the cell (e.g. cytoplasm or nucleus) and the protein's ability to interact with other proteins.

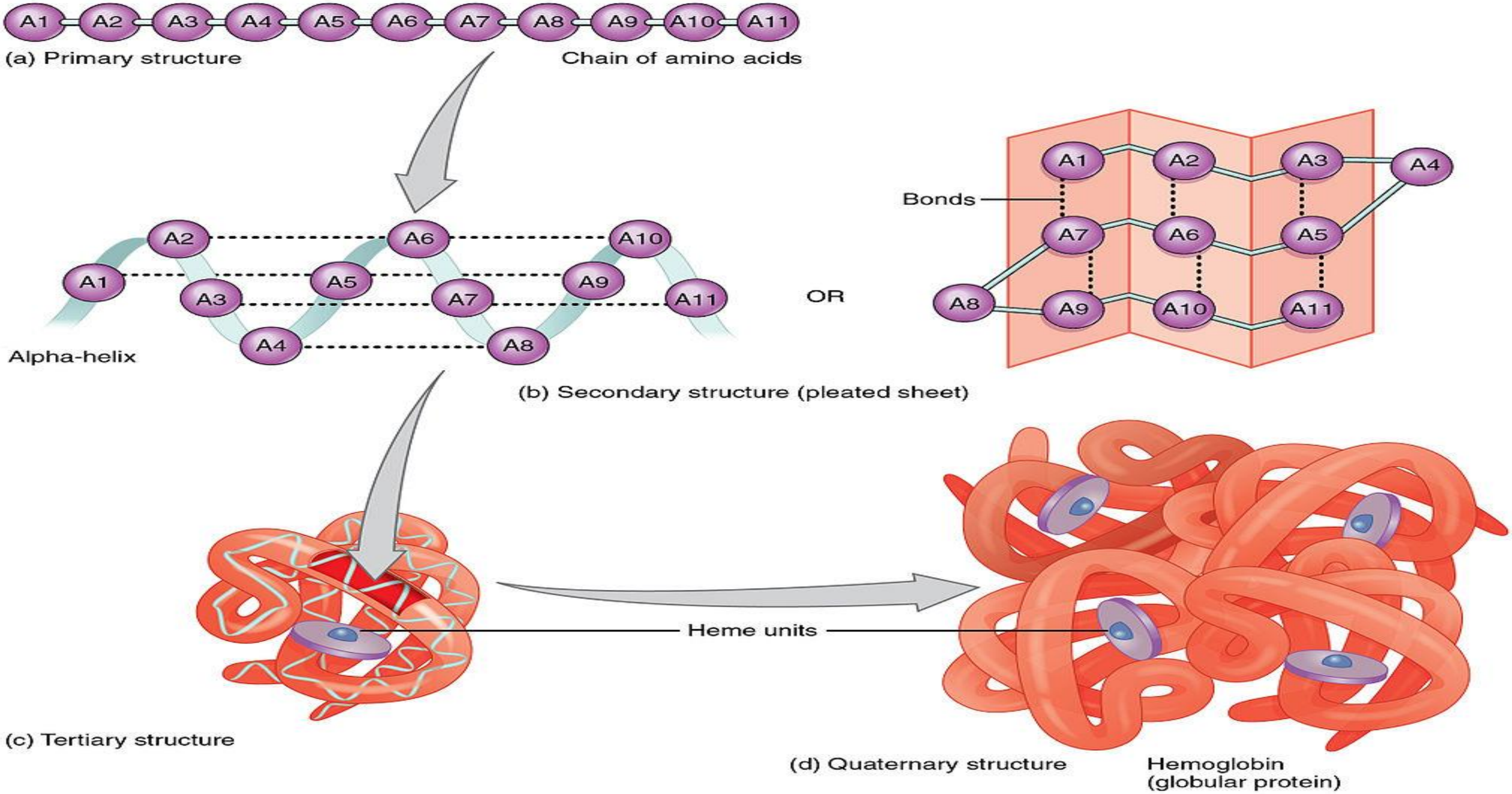


Primary protein structure
sequence of a chain of amino acids

Secondary protein structure
hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern

Tertiary protein structure
three-dimensional folding pattern of a protein due to side chain interactions

Quaternary protein structure
protein consisting of more than one amino acid chain


A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
(a) Primary structure
Chain of amino acids
Alpha-helix
Bonds
OR
(b) Secondary structure (pleated sheet)
Heme units
(c) Tertiary structure
(d) Quaternary structure
Hemoglobin
(globular protein)

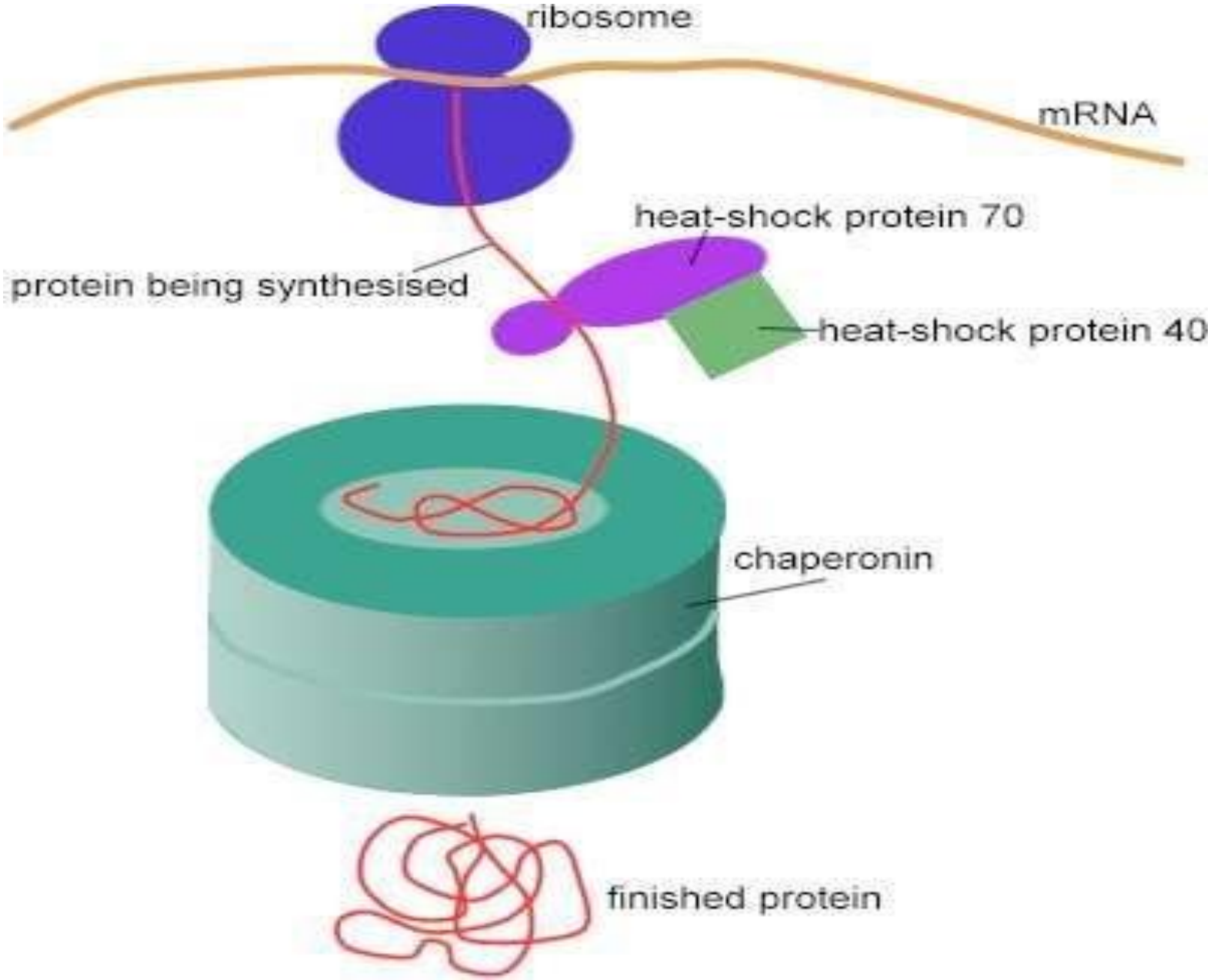
Proteins important in folding

Since protein folding occurs frequently, the cells have to control any dysfunction. The cells have several systems in place to refold or destroy aberrant protein formations.

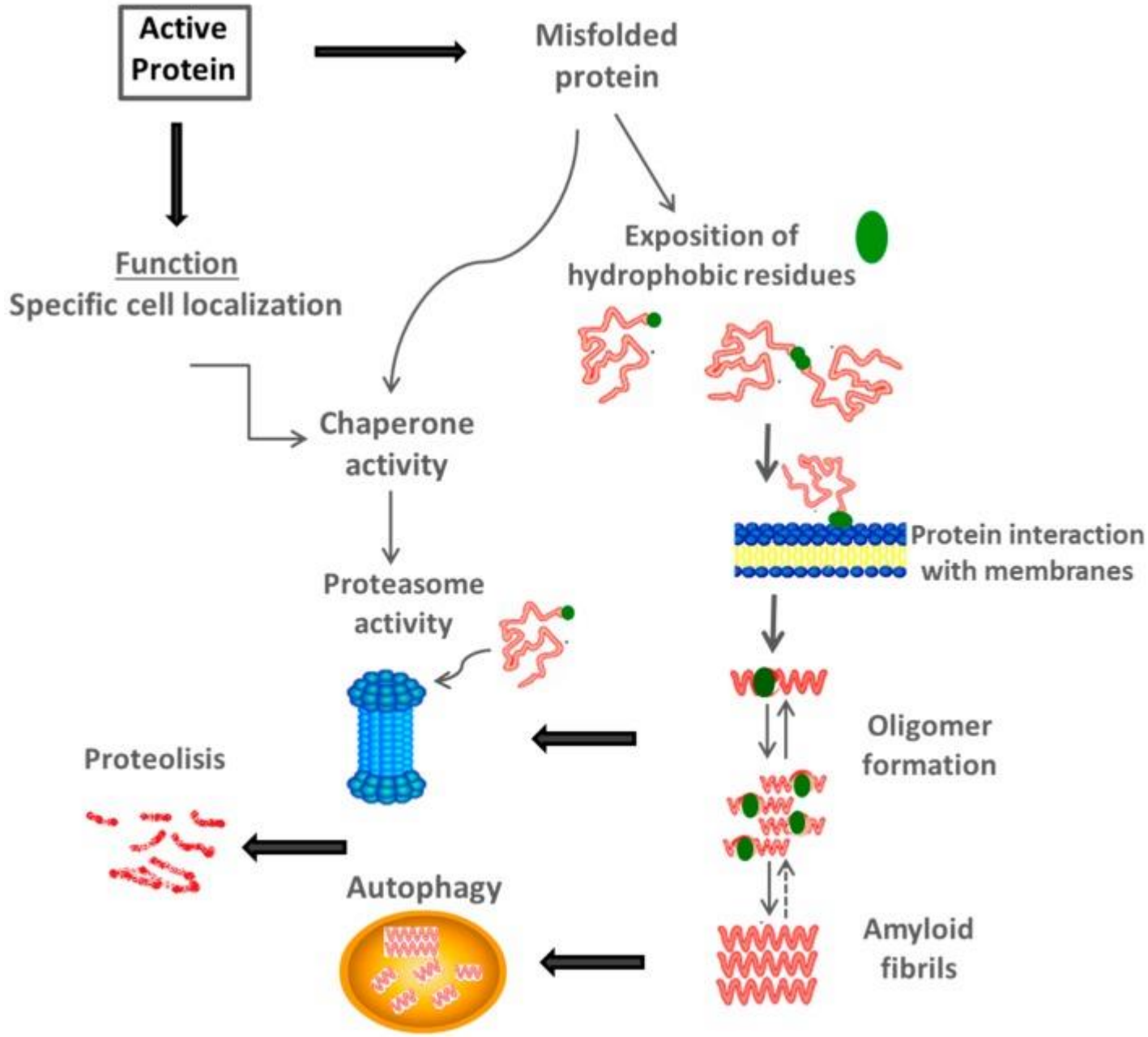
The first in line of defense are the system called **Chaperones** are proteins themselves, they partner with the proteins through the entire folding process, thereby improving the chances of a protein to fold suitably and also providing another chance for misfolded proteins along the way to refold again. There are various types of chaperones. Some serve *specifically* in helping a particular type of protein fold, while the remaining act *generally.*

- The production of the chaperones is boosted in number when a cell is encountered with high temperatures or other extreme conditions thereby making the protein folding a more difficult process, so it called **“heat shock proteins.”(hsp) .**

- These chaperones, known as **hsp60** and **hsp70**, recognize exposed hydrophobic patches on proteins and serve to prevent the protein aggregation that would compete with the folding of newly synthesized proteins into their correct three-dimensional conformations.
- The hsp70 machinery acts early in the life of many proteins, binding to a string of about seven hydrophobic amino acids before the protein leaves the ribosome.
- In contrast, hsp60-like proteins form a large barrel-shaped structure or large hollow cubicles that acts later in a protein's life, after it has been fully synthesized. This type of chaperone forms an “isolation chamber” which offer the protein a safe space to settle in, isolated from other molecules, where they can fold and misfolded proteins are fed, preventing their aggregation and providing them with a favorable environment in which to attempt to refold.


ribosome
mRNA
heat-shock protein 70
protein being synthesised
heat-shock protein 40
chaperonin
finished protein

- Another **second** such in line for cell defense against the misfolded proteins are called **Proteasome**. If misfolded proteins in the cell with abnormally exposed hydrophobic patches , they are most likely to be targeted for destruction and picked up by this machine that chews them up and spits them out as small fragments of amino acids.
- The proteasome is like a recycling center that allows the reuse of amino acids to produce more proteins within the cell. In this case, ***ubiquitin*** is covalently added to a misfolded protein by a ***ubiquitin ligase***, and the resulting multi ubiquitin chain is recognized by the cap on a proteasome to move the entire protein to the interior of the proteasome for proteolytic degradation.
- A closely related proteolytic mechanism, based on special degradation signals recognized by ubiquitin ligases, is used to determine the lifetime of many normally folded proteins. By this method, selected normal proteins are removed from the cell in response to specific signals.


Active Protein
Misfolded protein
Function
Specific cell localization
Exposition of hydrophobic residues
Chaperone activity
Protein interaction with membranes
Proteasome activity
Oligomer formation
Proteolisis
Autophagy
Amyloid fibrils

Diseases caused due to protein miss functioning

- Proteins can miss folding for several reasons. When a protein errors at folding it can cause denaturation of the protein. Denaturation is the loss of protein structure and function. This miss folding does not always result in complete lack of function, but there is a partial loss of function. This miss functioning of proteins can lead to different diseases in the human body foe example:
- **Alzheimer's disease** (AD) is a progressive neurological disorder that cause the brain to shrink (Atropy) and brain cells to die. AD is the most common cause of dementia and a continuous decline in several mental functions.
- The major risk factors of Alzheimer's disease are age, family history, and heredity. AD results in dense plaques in the brain that are comprised of fibrillary -amyloid proteins along with the sheet secondary structure.
- AD has been established as a case of misfolded proteins the brain, where the misfolded protein is directly related to the formation of plaques in the brain .There was several theories that point out to the oxidative stress in the brain as the triggering stage.

Cystic fibrosis (CF) is a hereditary disease that affects the lungs and digestive system of the patient. The body produces thick and sticky mucus that can clog the lungs and obstruct the pancreas which is life-threatening as it prevents proper food processing.

CF is also a result of protein missfolding. The missfolding leads to some changes in protein which is known as **Cystic Fibrosis Transmembrane Conductance Regulator (CFTR**) that results in a possible fatal disease. In the CFTR, the phenylalanine is deleted at position of 508. This depletion of Phe508 is in direct connection of CF formation.