DNA Structure & Gene Expression

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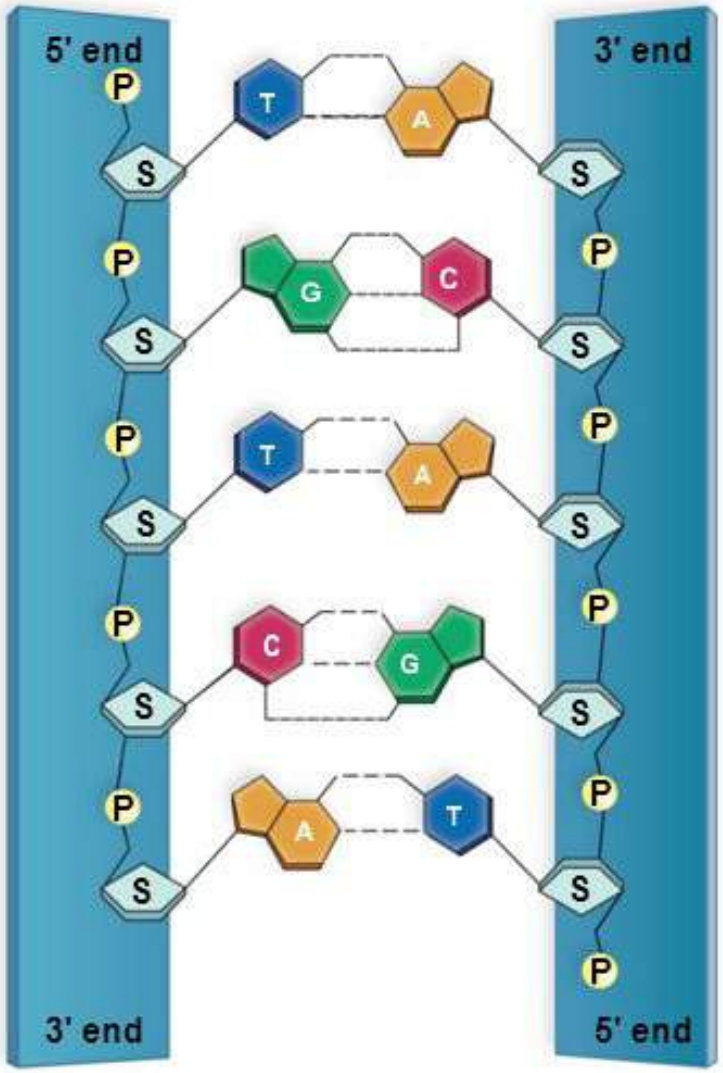
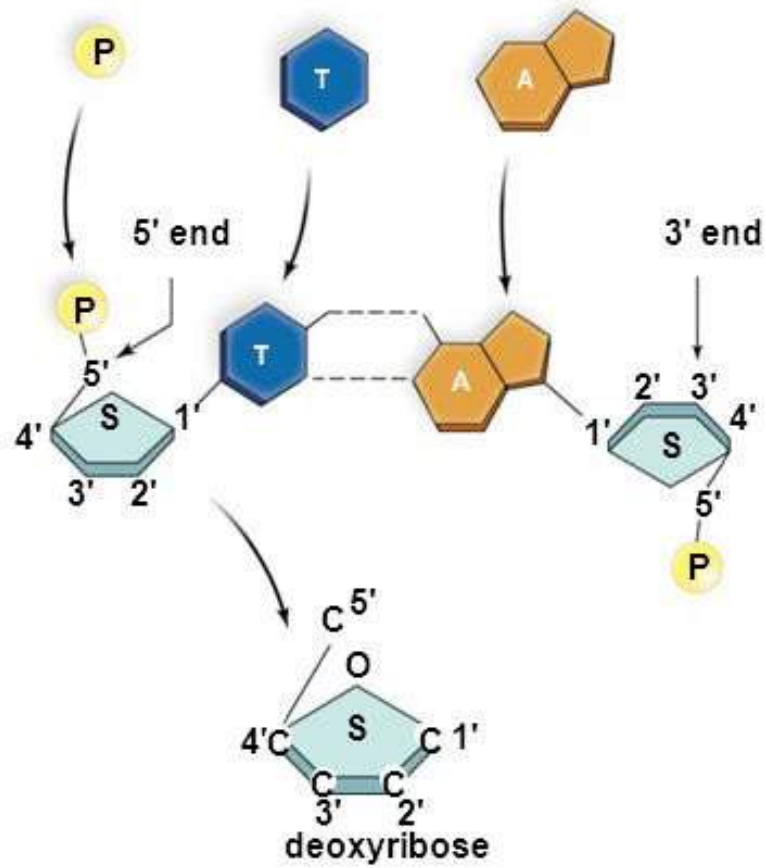
3.1 – DNA STRUCTURE

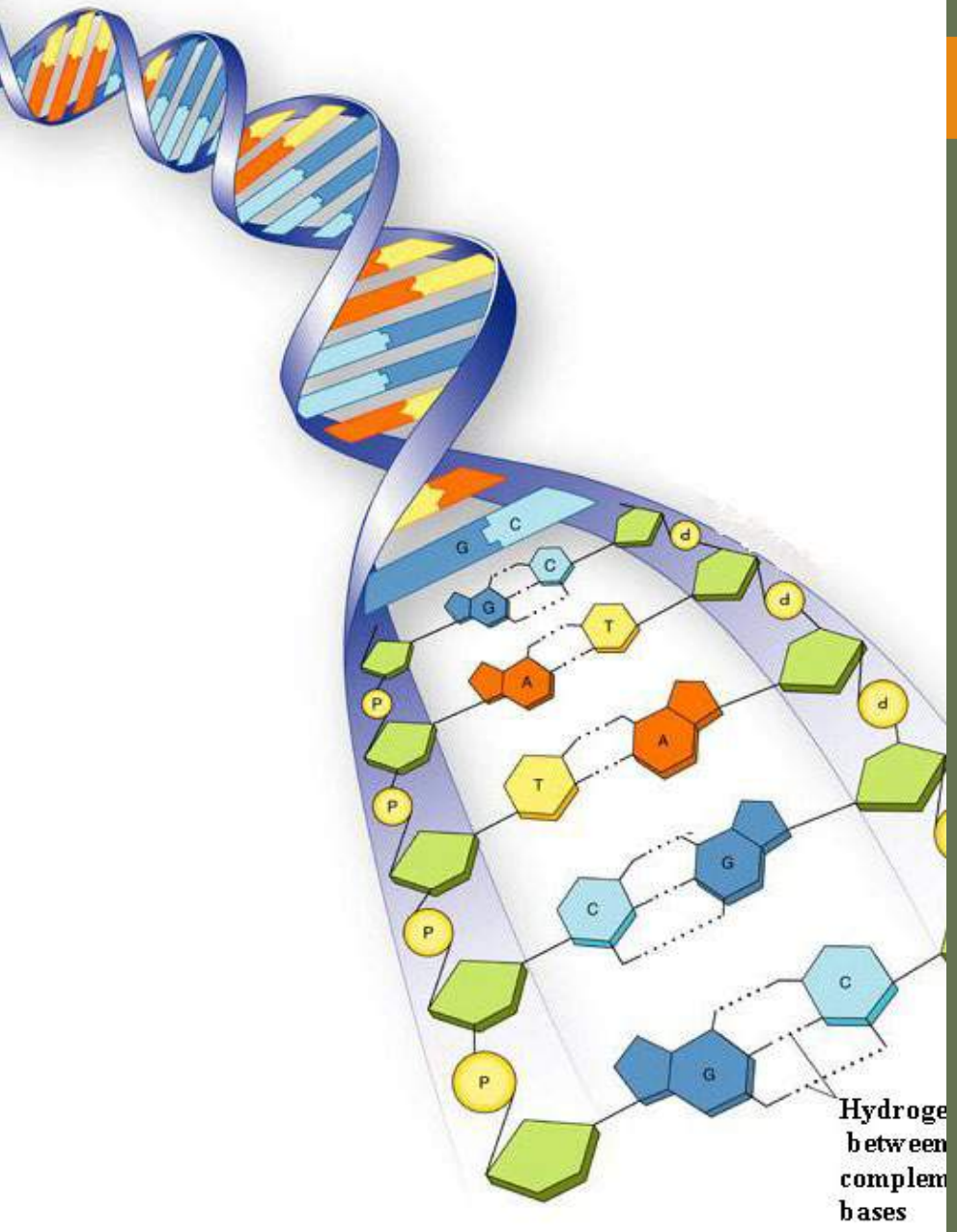
- DNA is a **chain of nucleotides.**
- Each nucleotide is made up of three subunits.
  - **Phosphoric acid** (phosphate group)
  - **Pentose sugar** (deoxyribose)
  - **Nitrogen containing base**
- There are 4 possible bases.
  - Two are **purines with a double ring**: Adenine (A) and Guanine (G)
  - Two are **pyrimidines with a single ring**: Thymine (C) and Cytosine (C)

- A DNA strand has a backbone made up of alternating **phosphate and sugar molecules.**
  - The bases are attached to the sugar but **project to one side.**
  - DNA's two strands twist about one another in the form of a **double helix.**
  - The strands are held together by hydrogen bonding between the bases: **A&T forming two hydrogen bonds**, and **G&C forming three hydrogen bonds.**

Overview of DNA Structure



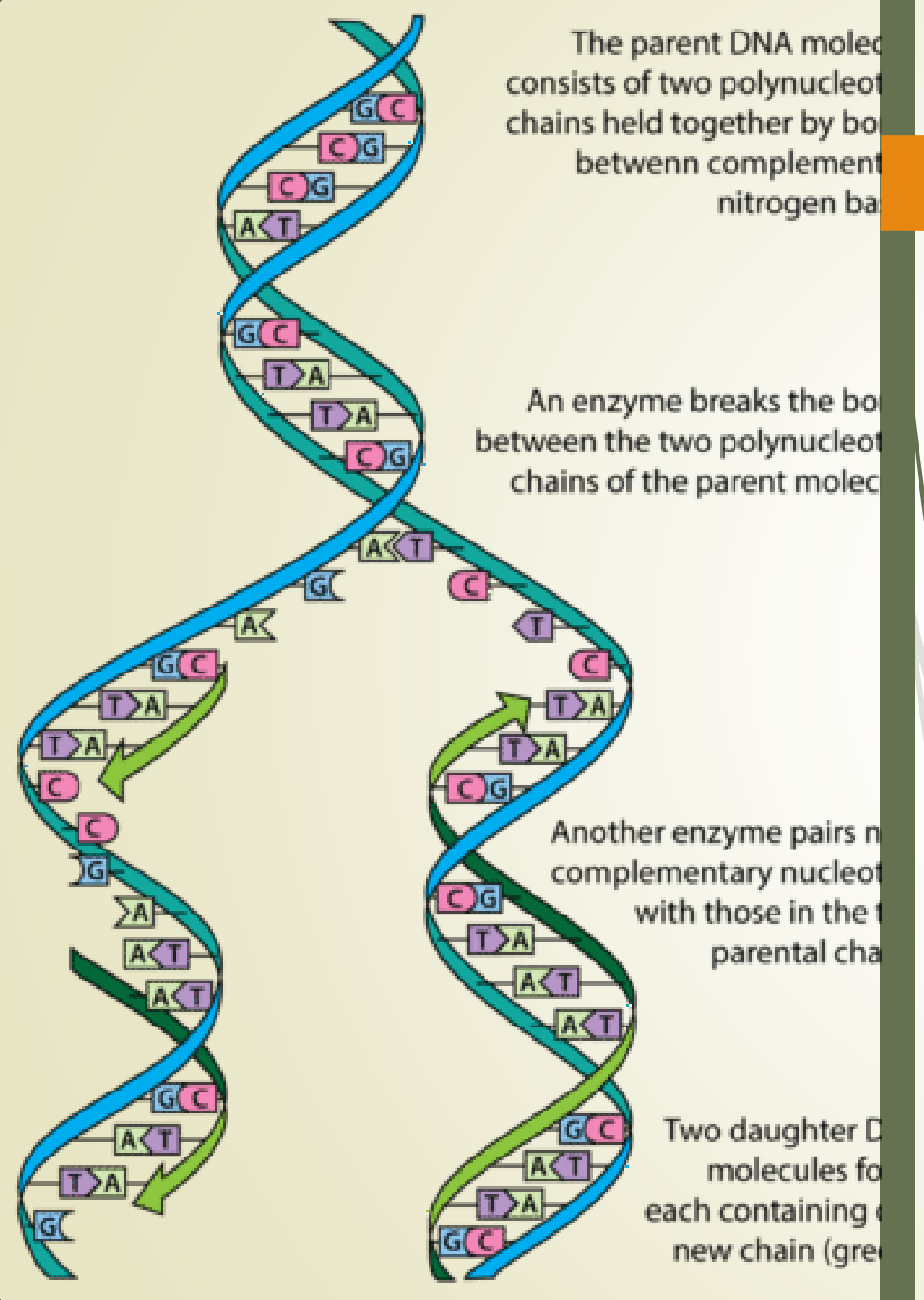




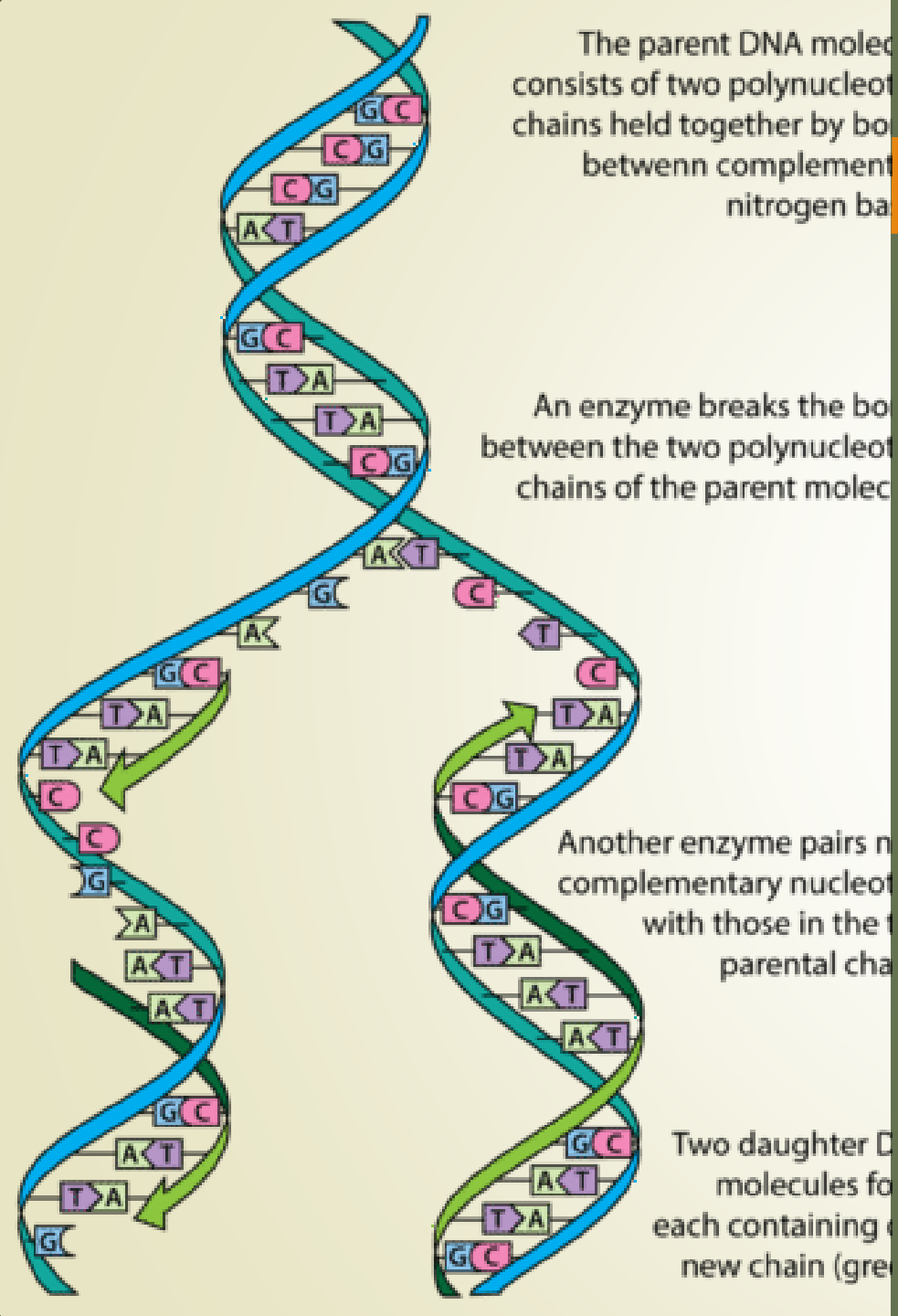
- Purine is always paired with a pyrimidine.
  - This is called **complementary base pairing.**
- When the DNA helix unwinds, it resembles a ladder.
  - The **sides of the ladder are the sugar-phosphate backbones**, and the rungs of the ladder are the **complementary paired bases.**
- The two DNA strands are antiparallel, meaning they are **oriented in the opposite direction.**

3.2 – DNA REPLICATION

- Each new cell requires an **exact copy of the DNA contained in the chromosomes.**
- DNA replication is the process of copying **one DNA double helix into two identical double helices.**
  - The process is carried out by an enzyme called **DNA polymerase.**
  - DNA polymerase uses each original strand as a template for the formation of a **complementary new strand.**
  - Because of this DNA replication is termed **semiconservative.**



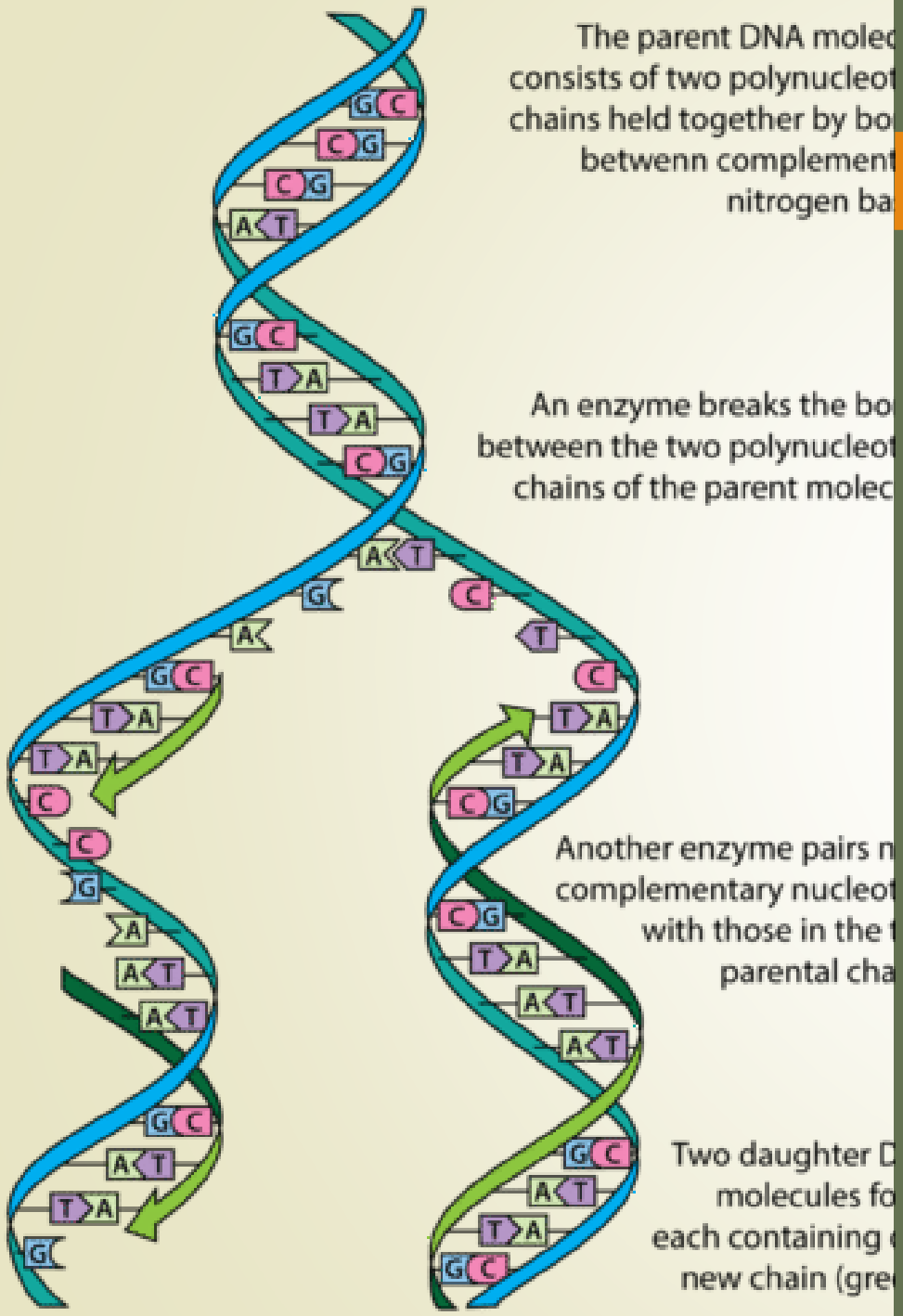
1. The enzyme DNA helicase unwinds and "unzips" **DNA by breaking the hydrogen bonds between the bases.**
2. New complementary DNA nucleotides fit into place by the process of the complementary base pairing. **Once positioned they are joined by DNA polymerase.**



3. DNA strands are oriented in an **antiparallel configuration**, therefore, DNA polymerase may add new nucleotides to **only one end of the chain.**

DNA synthesis occurs in the opposite direction.

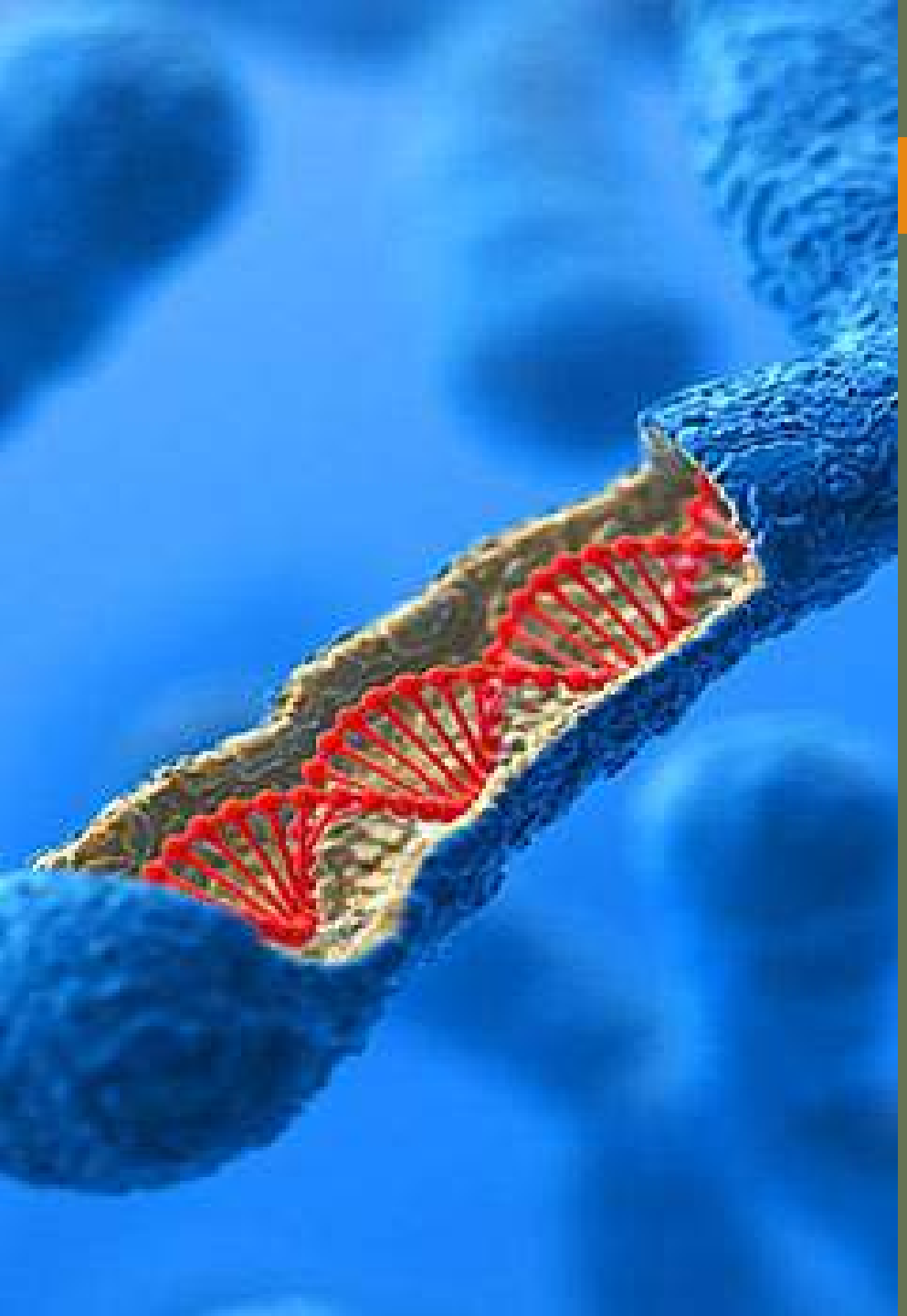
The **leading strand follows the helicase enzyme**, while the lagging strand forms short segments of DNA called **Okazaki fragments.**



4. The enzyme DNA ligase connects the Okazaki fragments and **seals any breaks in the sugar phosphate backbone.**

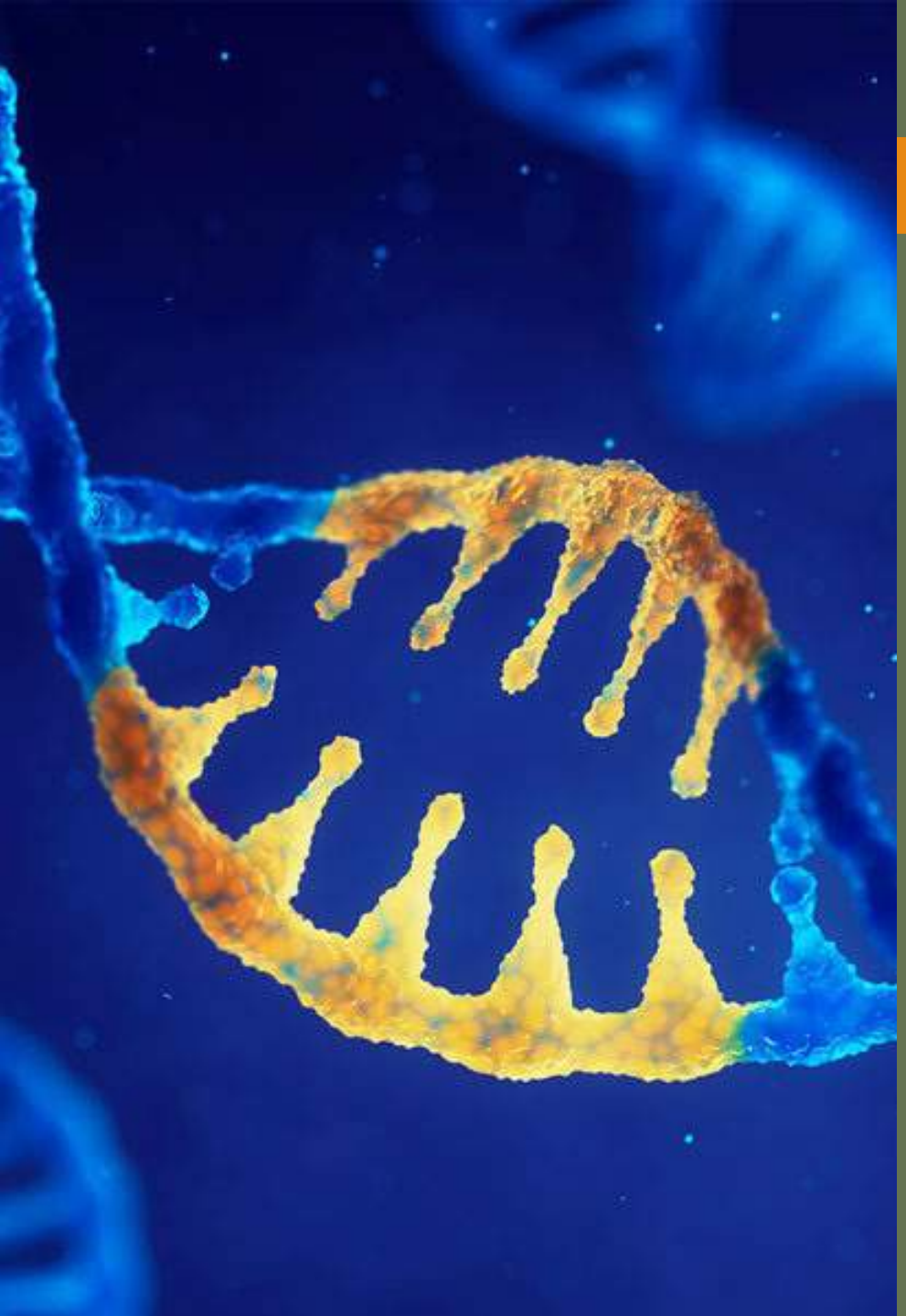
5. The two double helix molecules are **identical to each other.**

Molecular mechanisms of DNA replication – fig 4.5

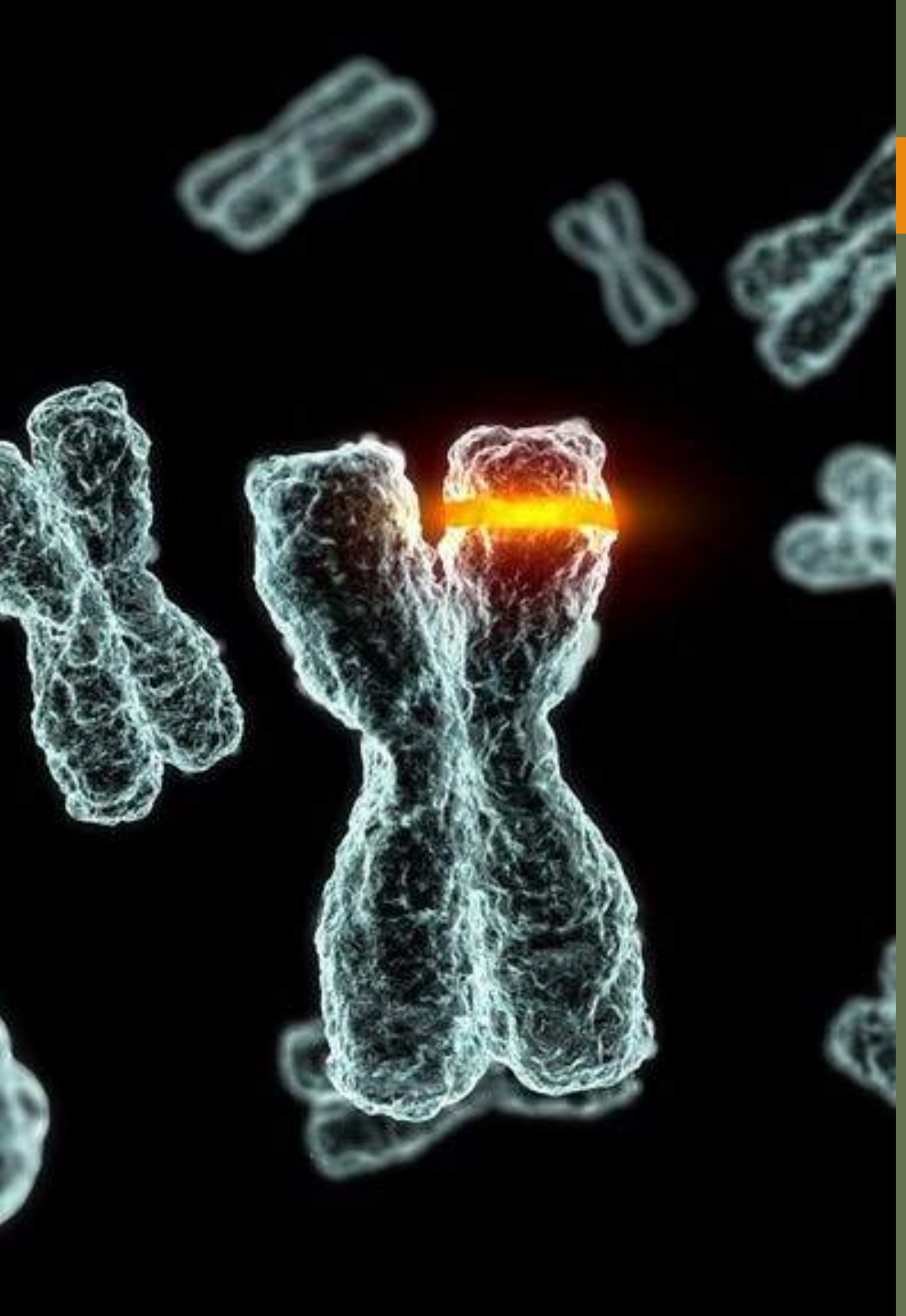


3.3 – GENE EXPRESSION

- Gene expression is the process of using a gene sequence to **synthesize (make) a protein.**
  - It relies on the participation of several different forms of RNA molecules including:
    - **Messenger RNA – mRNA**
    - **Transfer RNA – tRNA**
    - **Ribosomal RNA - rRNA**



- Gene expression requires two processes called **transcription & translation**.
  - Transcription takes place **in the nucleus** and translation takes place **in the cytoplasm.**
  - During transcription, a portion of DNA serves as a **template for mRNA formation.**
  - During translation, the sequence of mRNA bases determines the **sequence of amino acids in a polypeptide.**



- Genetic information lies in the **sequence of bases in DNA.**
  - mRNA determines the sequence of **amino acids in a protein.**
  - Transfer RNA assists mRNA during protein synthesis by **bringing amino acids to the ribosomes.**
  - Proteins determine the structure and function of cells and the **physical characteristics of the organism.**

Transcription

- During transcription, the gene serves as a **template for the production of an RNA molecule.**
  - Genes contain instructions for protein formation, and the **formation of mRNA, tRNA, and rRNA.**
  - We will focus on the formation of mRNA, **the first step in protein synthesis.**

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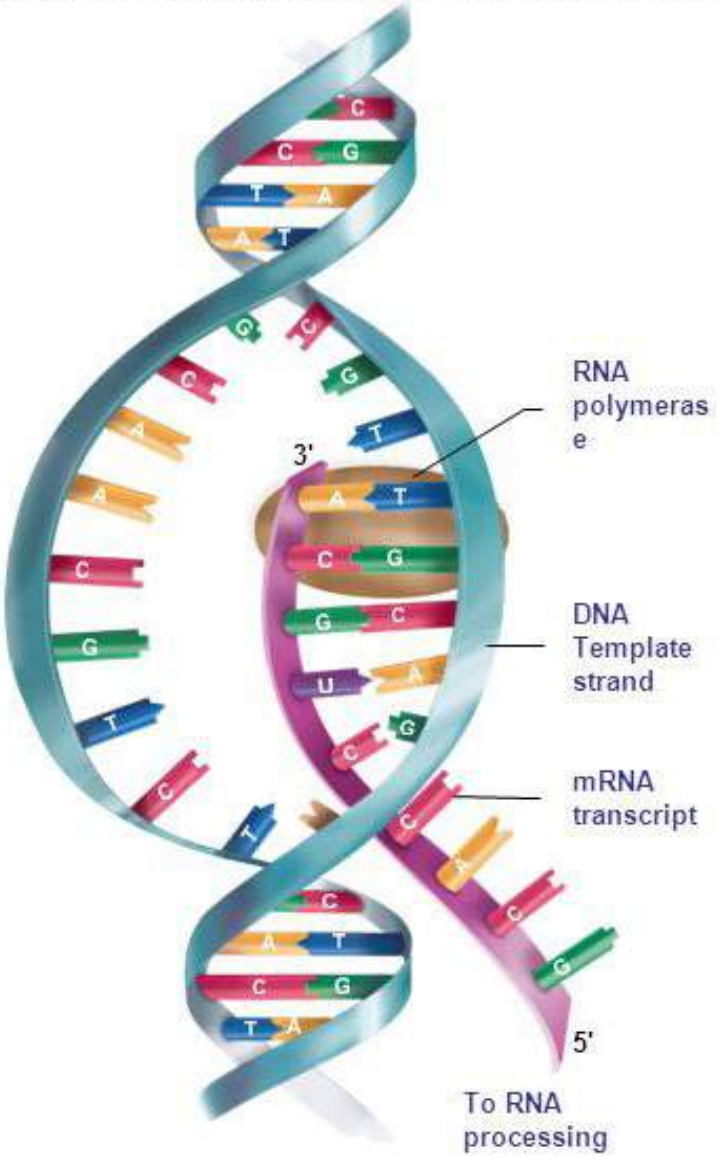
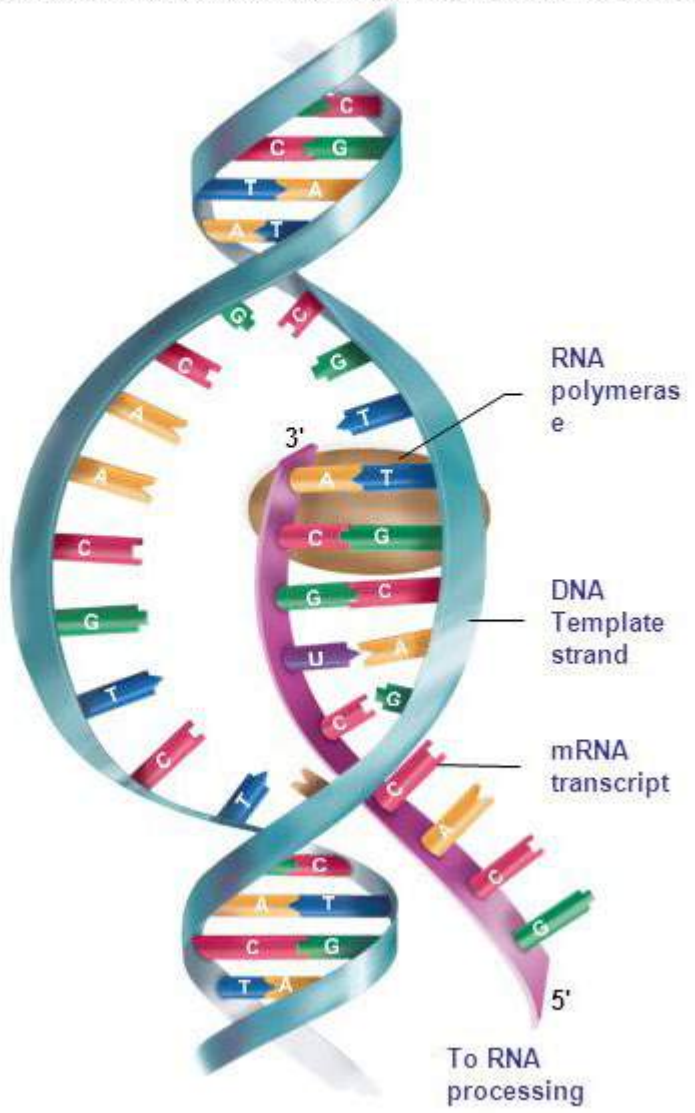


Messenger RNA

- The purpose of mRNA is to carry genetic information from the DNA **to the ribosomes for protein synthesis.**
  - mRNA is formed through **transcription which occurs in the nucleus.**
  - It begins when RNA polymerase binds tightly to a **promoter**.
  - This opens up the DNA helix so that complementary base pairing can occur in the **same way as DNA replication.**

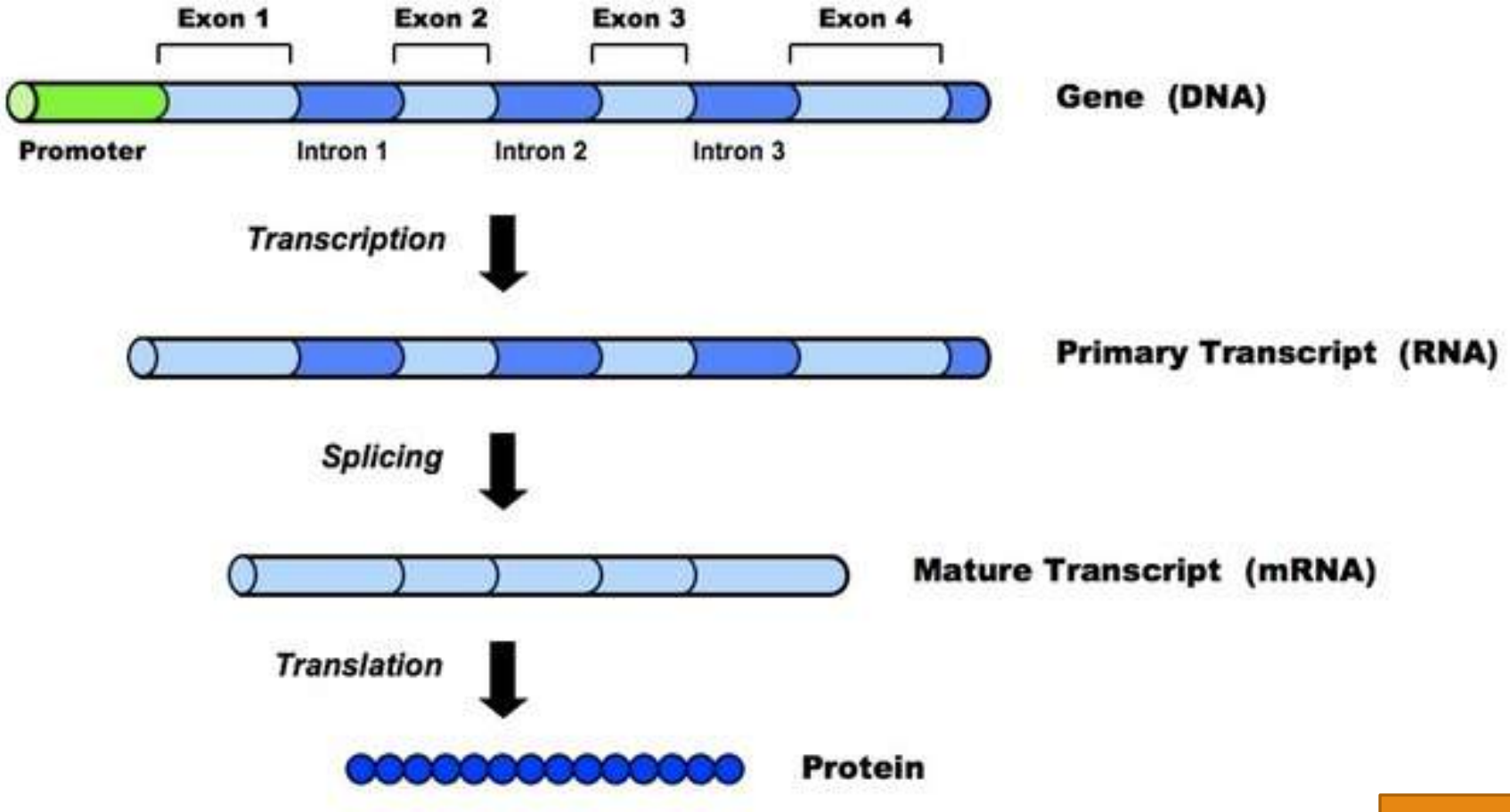
cription of DNA to Form



- RNA polymerase inserts the RNA nucleotides, and **an mRNA molecule results.**
- mRNA has a sequence of bases **complementary to that of the DNA.**
- The nucleotide thymine is replaced with **uracil in RNA strands.**

Processing of mRNA

- After the mRNA is transcribed, it must be **processed before entering the cytoplasm.**
- Most genes in humans are interrupted by segments of DNA that are **not part of the gene.**
- These portions are called **introns.**
- The other portions are called **exons because they are expressed.**
- **Only exons result in a protein product.**
- Ordinarily, processing **brings together** all the exons of a gene.


Exon 1
Exon 2
Exon 3
Exon 4
Promoter
Intron 1
Intron 2
Intron 3
Gene (DNA)
Transcription
Primary Transcript (RNA)
Splicing
Mature Transcript (mRNA)
Translation
Protein

Translation

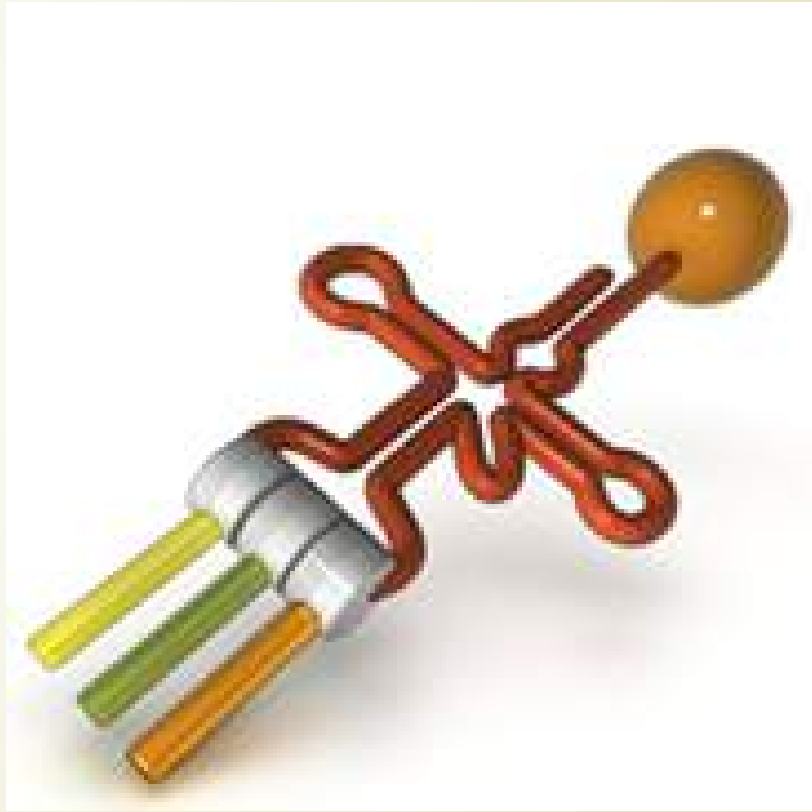
- This is the second process by which gene expression leads to protein synthesis.
  - Translation requires **several enzymes**, and several different types of RNA molecules, **including mRNA, tRNA, and rRNA.**

The Genetic Code

- The sequence of bases in DNA is transcribed into mRNA, which codes for a **sequence of amino acids to form a polypeptide.**
  - Each triplet of nucleotides is called a **codon.**
  - Most amino acids are coded for by **more than one codon.**
  - There are 64 codons, **61 code for amino acids**, the remaining are stop codons.

Transfer RNA

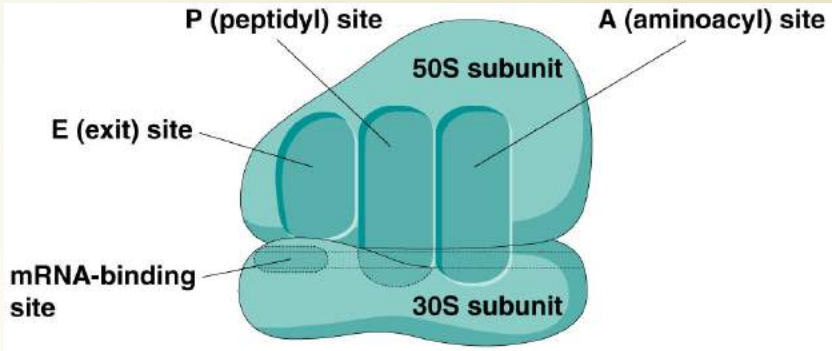
- Transfer RNA molecules **bring amino acids to the ribosomes**, the site of protein synthesis.
  - On one end of the tRNA molecule is the **amino acid**, and on the other end is an **anticodon.**
  - An anticodon is a triplet of **3 bases complementary to the codon of mRNA**.
  - When a tRNA-amino acid complex comes to the ribosome, its **anticodon pairs with an mRNA codon.**

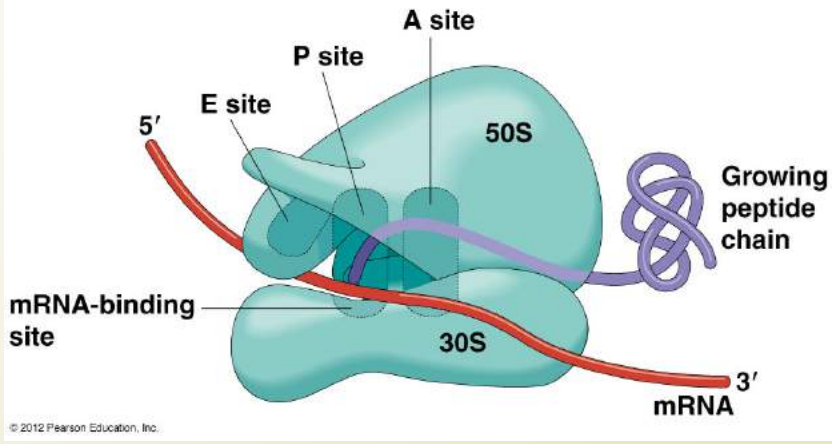


- During transcription, the **base sequence in DNA is copied into a sequence of bases in mRNA**.
- During translation, tRNA's bring amino acids to the ribosomes in the order **dictated by the base sequence of mRNA**.
- The sequence of amino acids form a polypeptide chain, aka **a complete protein.**

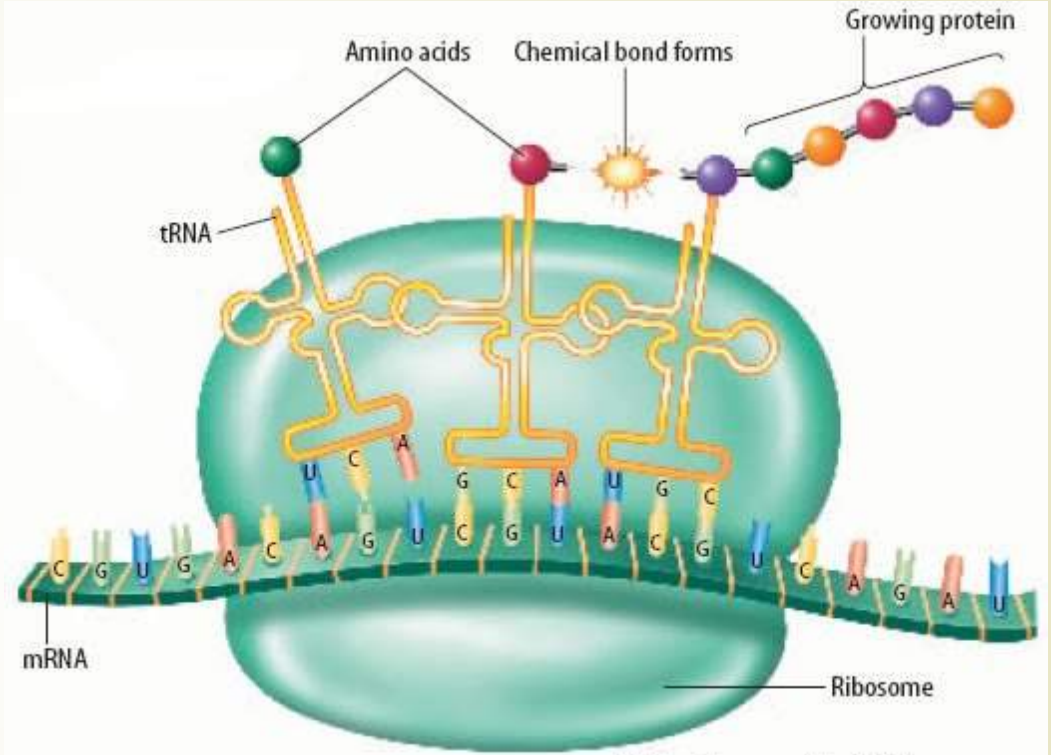
Ribosomes and Ribosomal RNA

- Ribosomes are found in the cytoplasm and on the ER where translation also occurs.
- Ribosomes are composed of many proteins and **several ribosomal RNA's.**
  - rRNA is produced in the **nucleolus within in nucleus.**
  - The rRNA joins with proteins manufactured in the cytoplasm to **form two ribosomal subunits.**





- A ribosome has a binding site for mRNA as well as for **3 tRNA molecules.**
  - These binding sites facilitate complementary base pairing between **tRNA anticodons and mRNA codons.**
  - As ribosomes moves down the mRNA molecule, new tRNAs arrive, **and a polypeptide forms and grows longer.**
  - Translation terminates once the polypeptide is fully formed and an **mRNA stop codon is reaches.**



At the ribosome, the RNA's message is translated into a specific protein.

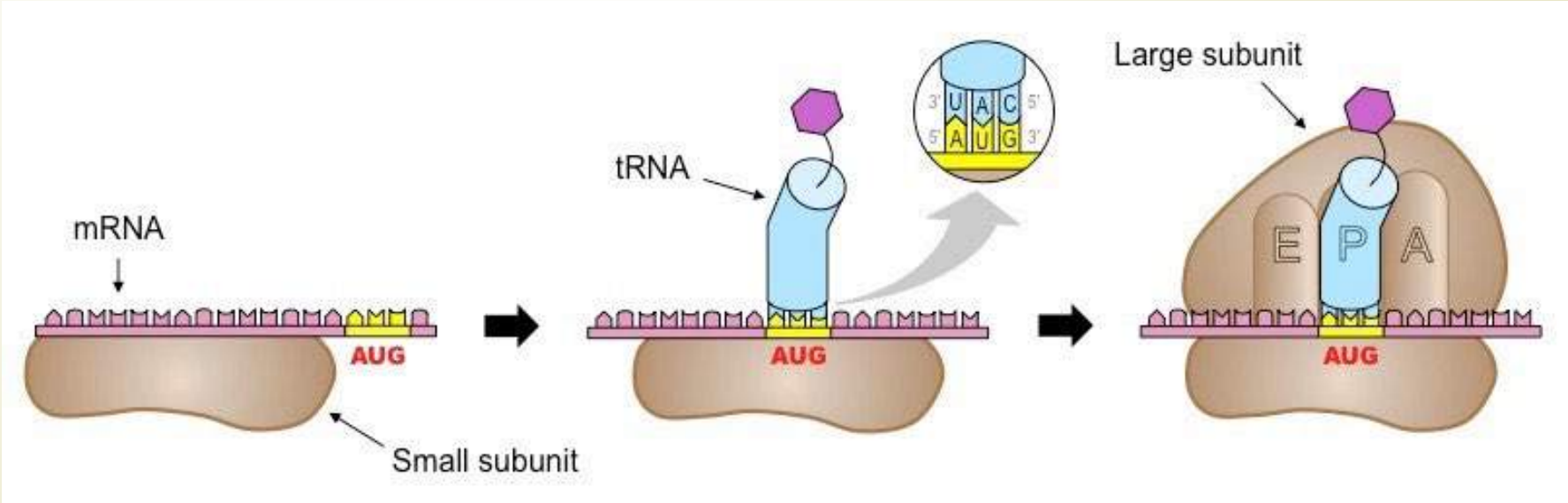
Translation Requires Three Steps

- During translation, the **codons of an mRNA base pair with the anticodons of tRNA molecules**, who are carrying a specific amino acid.
  - The order of the codons, determines the **sequence of amino acids in a polypeptide.**
- Protein synthesis involves three steps: **initiation, elongation, and termination.**

Initiation

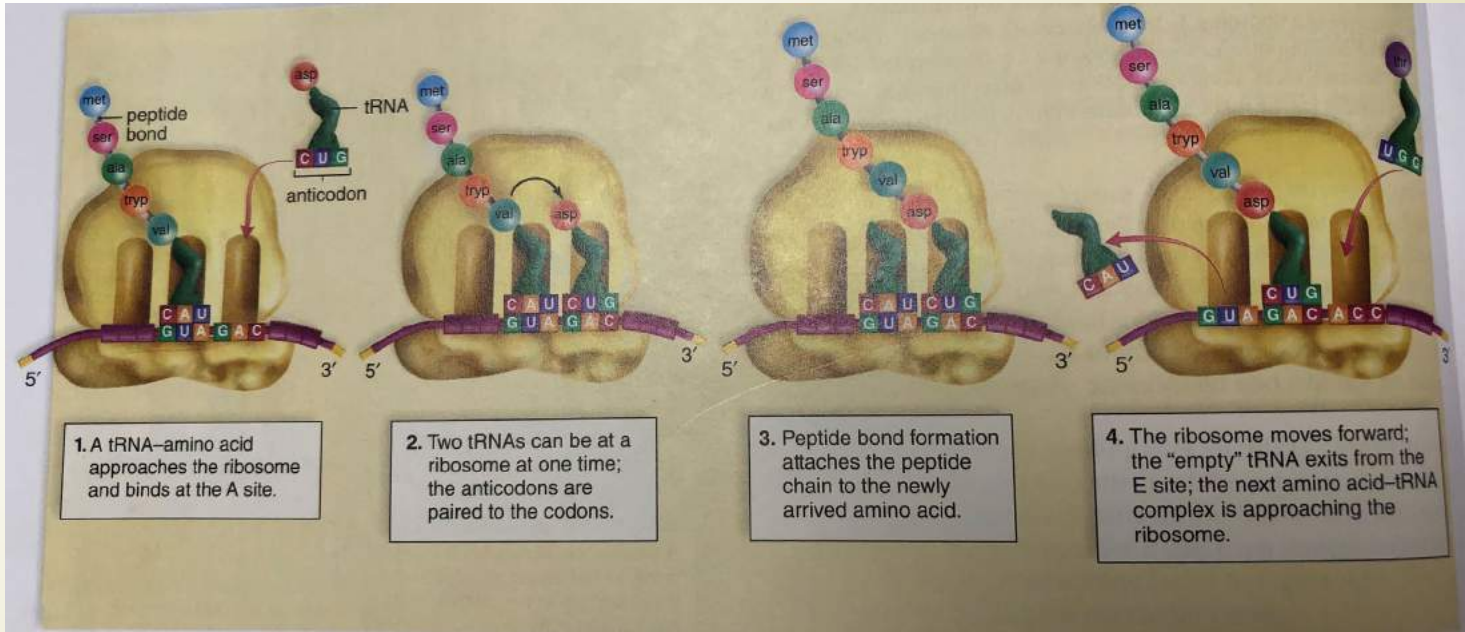
- Initiation is the step that brings all the translation components together.
  - Initiation factor proteins assemble the **ribosome subunits, as well as the mRNA, and initiator tRNA.**
  - The small ribosomal subunit attaches to the **mRNA start codon** (AUG).
  - The initiator anticodon UAC pairs with the start codon, and the **large ribosomal subunit joins the small.**

- A ribosome has three binding sites for tRNA.
  - P site – **peptide site**
  - A site – **amino acid site**
  - E site – **exit site.**
- The **initiator tRNA binds to the P site.**
- The A site is for tRNA carrying the **next amino acid.**

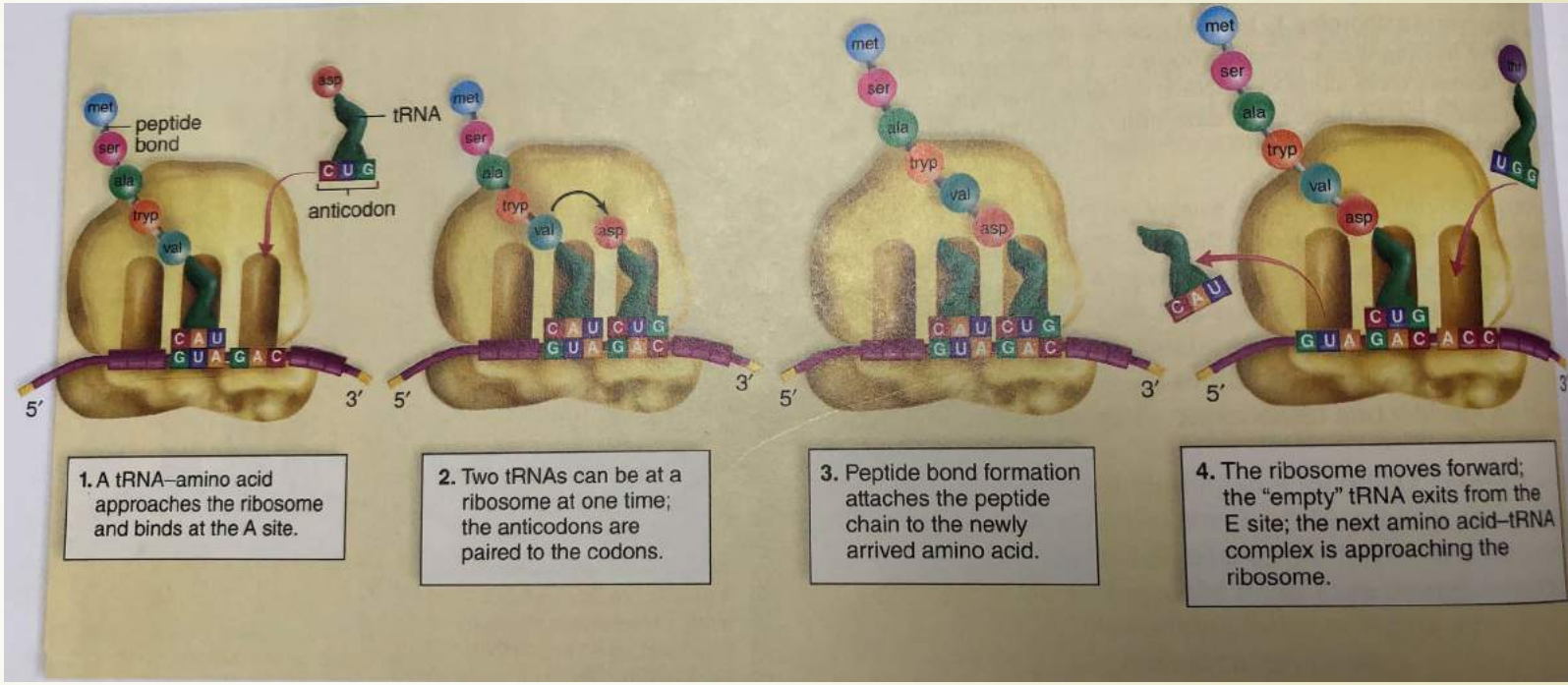


Elongation

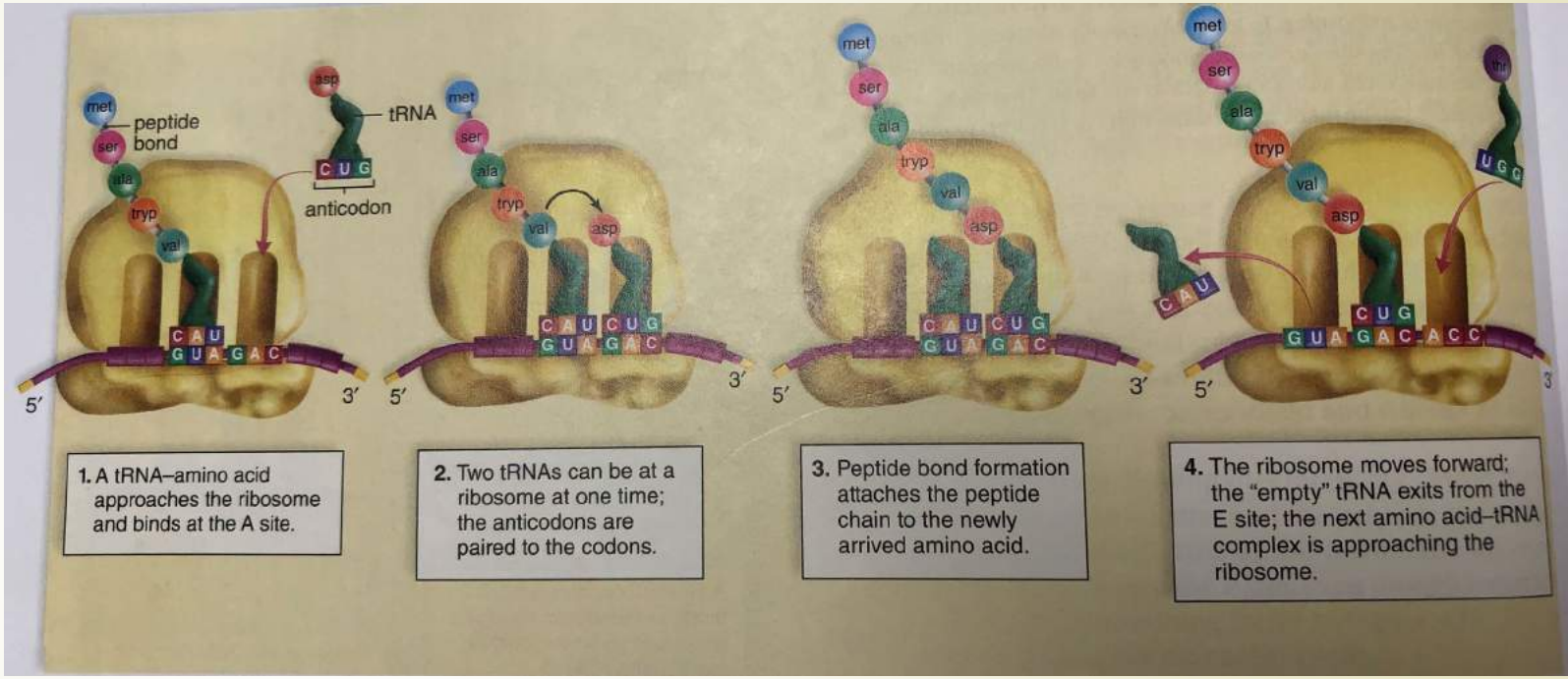
- This is the step in which a polypeptide **increases in length one amino acid at a time.**
- Elongation requires elongation factors, which facilitate the binding of **tRNA anticodons to mRNA codons at a ribosome.**
- Elongation is a series of 4 steps.



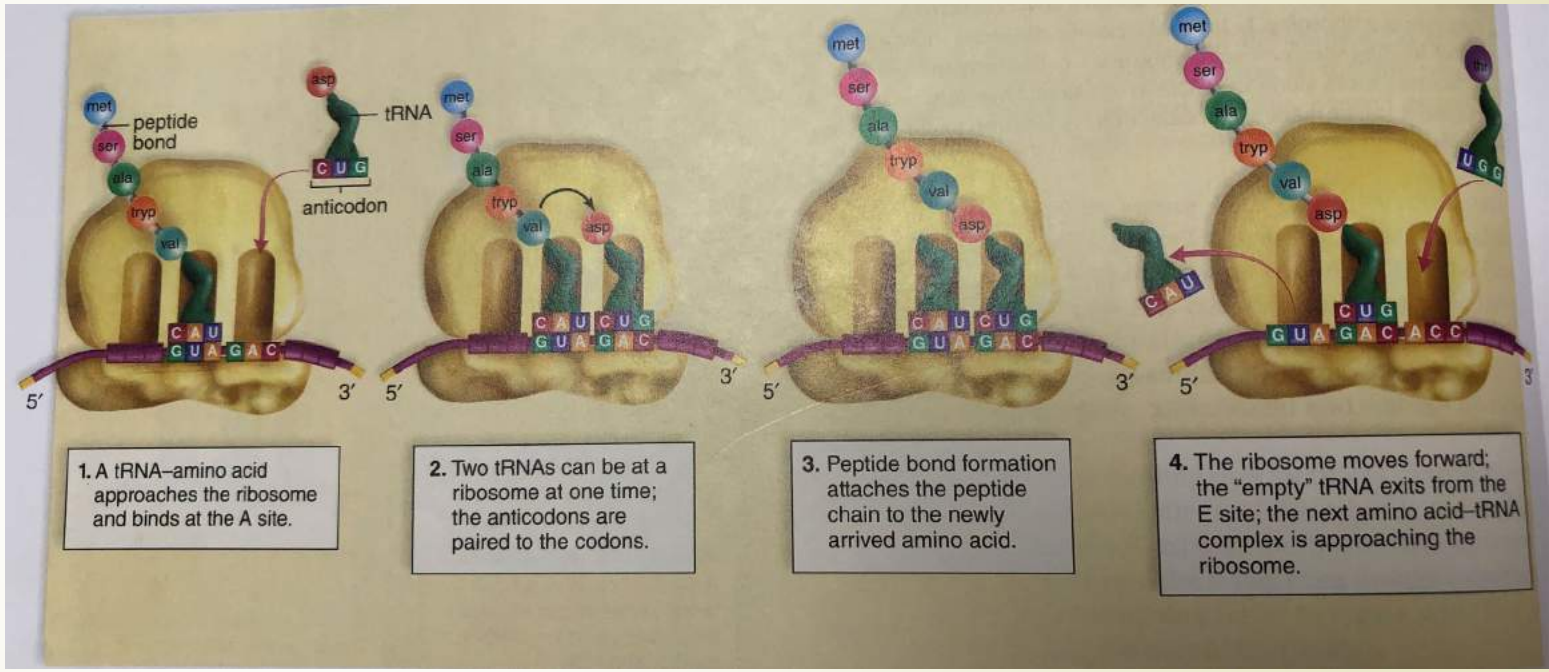
- 1. A tRNA with an attached peptide is already at **the P site.**
  - The tRNA carrying the **next amino acid in the chain is arriving at the A site.**



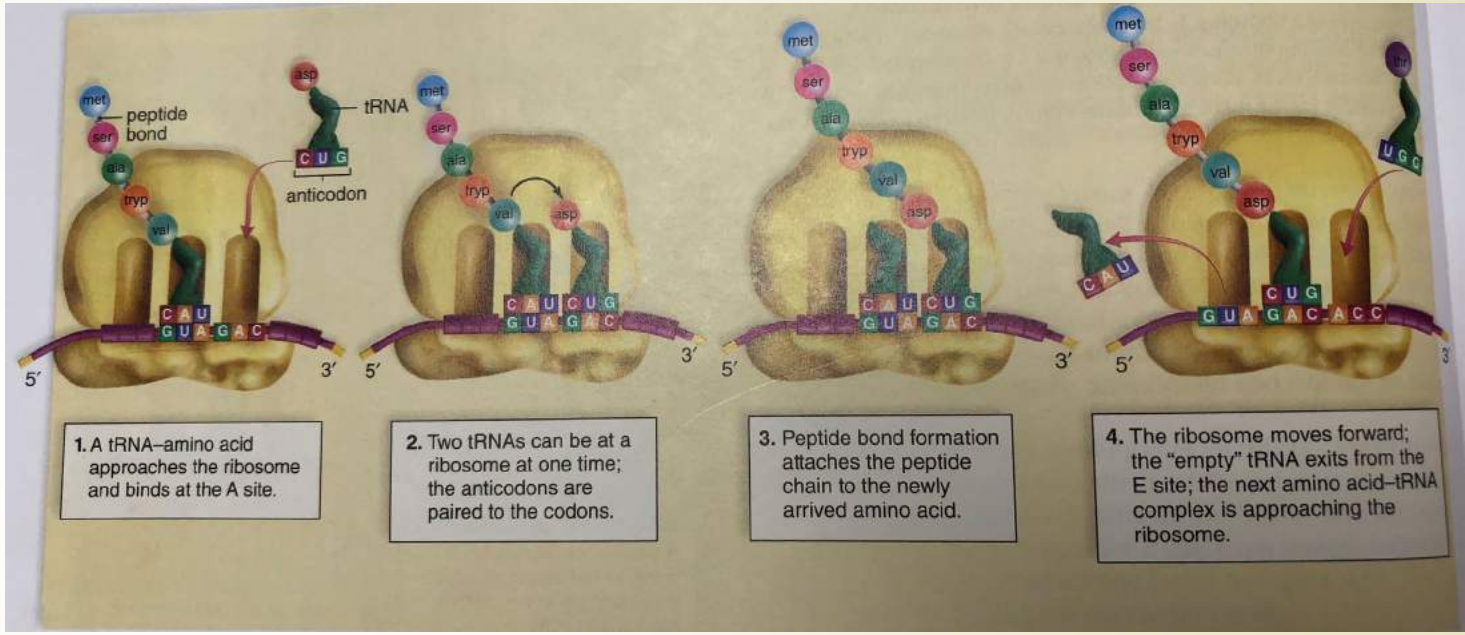
- 2. Once the next tRNA is in place at the A site, **the peptide chain will be transferred to this tRNA.**



- 3. energy is needed to make this transfer.
  - The energy forms a peptide bond, **which makes the peptide one amino acid longer by adding the peptide from the A site.**

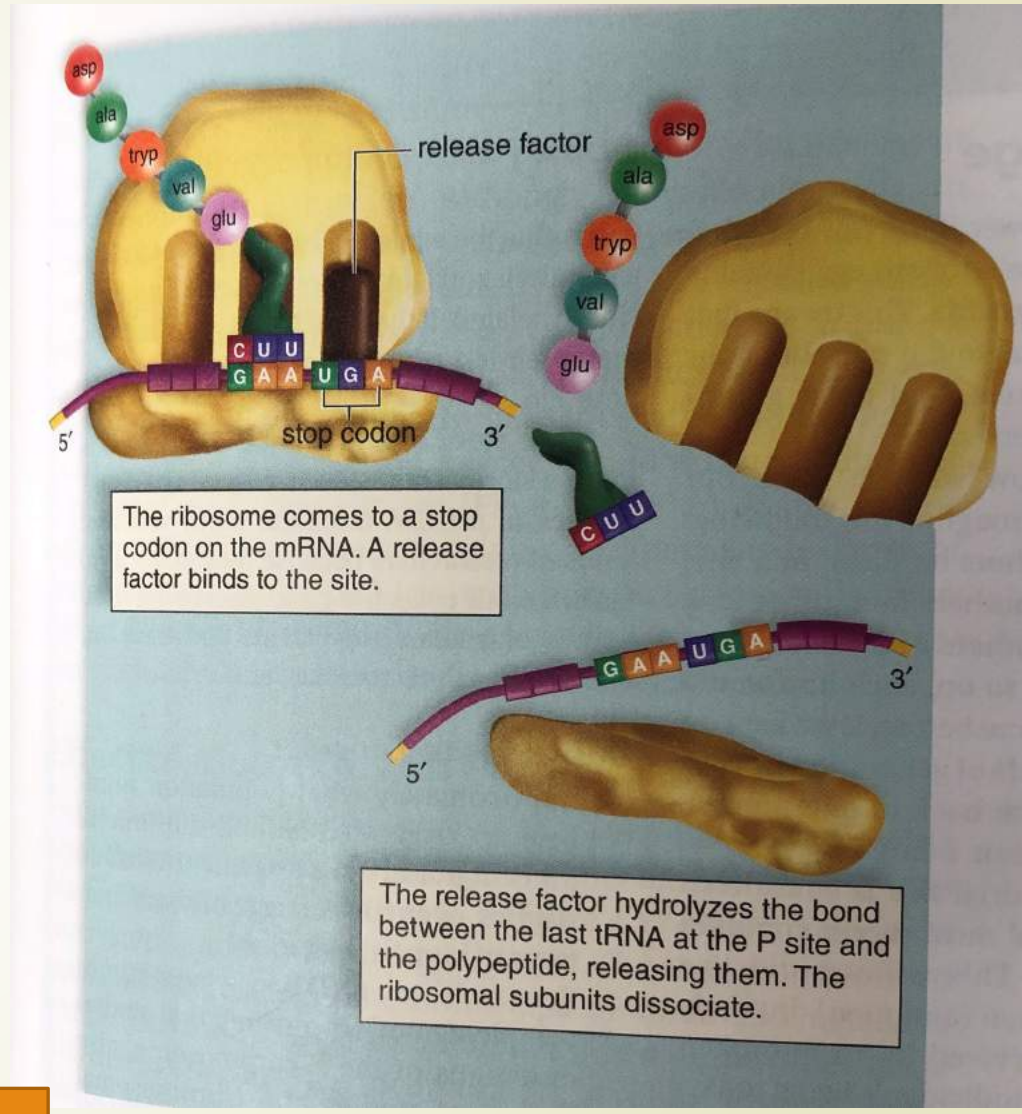


- 4. finally, translocation occurs.
  - The mRNA moves **forward one codon length.**
  - The peptide bearing **tRNA is at the P site.**
  - The "spent" tRNA now exits.
  - The new codon is at the site and is **ready to receive the next complementary tRNA.**



Termination

- This is the **final step in protein synthesis.**
  - Here the polypeptide and the assembled components are **separated from one another.**
  - Termination requires the stop codon and a protein called a **release factor which cleaves the polypeptide from the last tRNA**.
  - Then the polypeptide begins to take on its **3-D shape.**



Review of Gene Expression

- A gene is expressed when its protein product has **been synthesized.**
- Protein synthesis requires the process of **transcription and translation.**
- During transcription, a segment of a DNA strand serves as a template for the **formation of mRNA.**
- During translation, tRNAs bring attached **amino acids to the ribosomes.**
  - tRNA anticodons pair with codons, the amino acids become sequences in the order **originally specified by DNA.**

3.4 – GENE MUTATIONS & CANCER

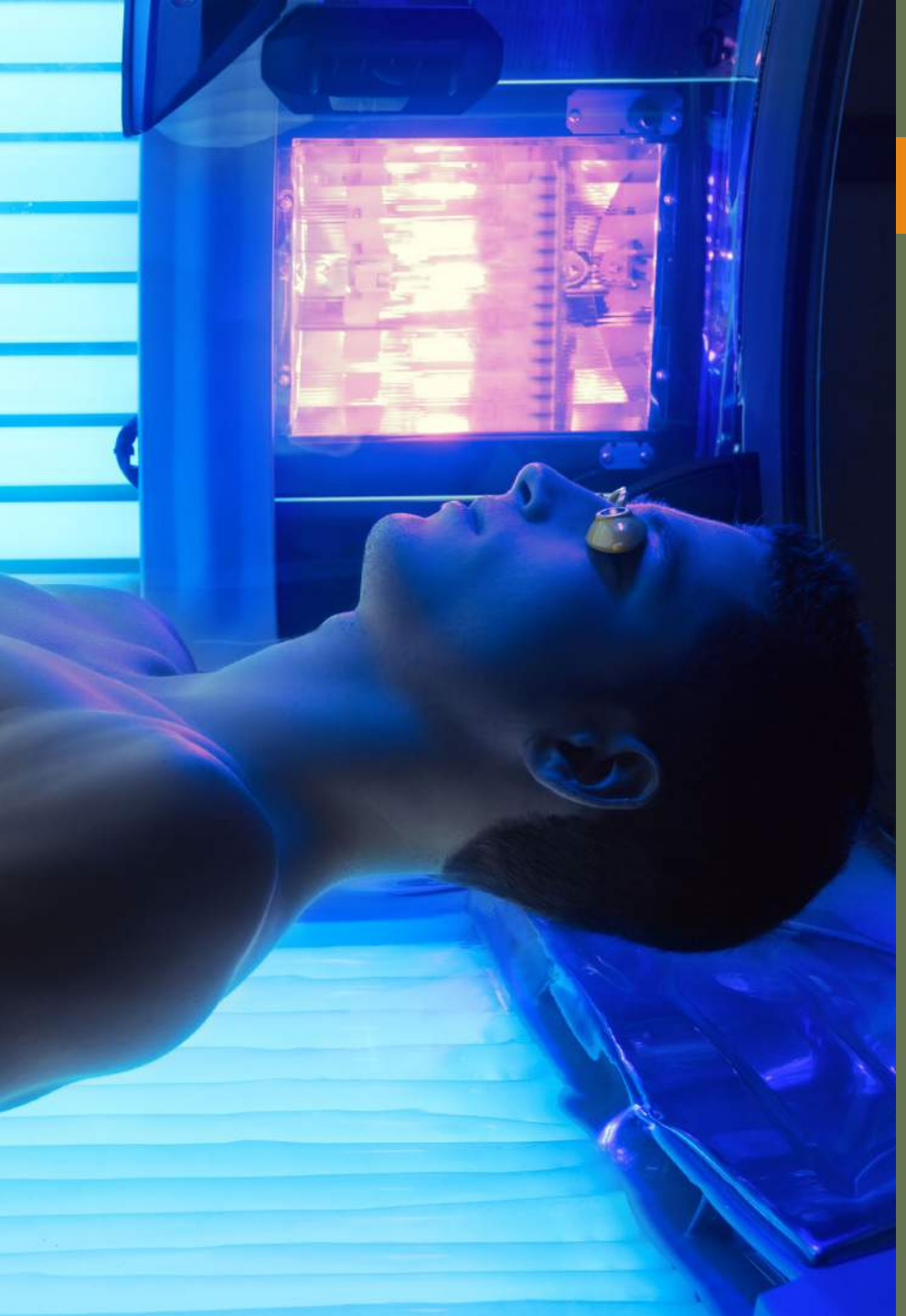
- A gene mutation is a **permanent change in the sequence of bases in DNA.**
  - The effect of this change can range from **no effect to complete inactivity.**
  - **Germ-line mutations occur in the sex cells** and can be passed to subsequent generations.
  - **Somatic mutations occur in the body's cells** are not passed on to future generations.

Causes of Mutations

- Gene mutations may be caused by:
  - **Errors in replication**
  - **Mutagens**
  - **The activity of transposons**

Errors in Replication

- These are a **rare source of mutations**.
  - DNA polymerase, the enzyme that carries out replication, **and proofreads the new strand against the old.**
  - Typically mismatched pairs are then **replaced with the correct nucleotide.**
  - There is typically only one mistake for every **1 billion pairs replicated.**

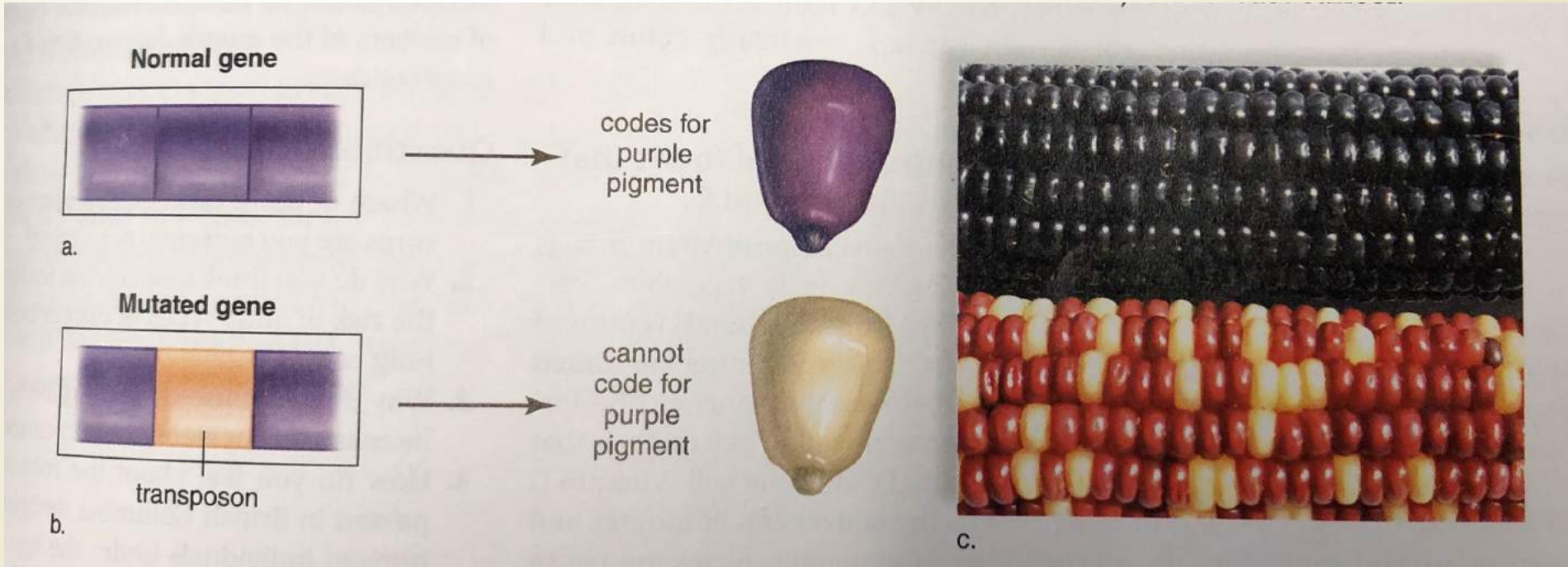


Mutagens

- These are **environmental influences that cause mutations in humans.**
  - This includes radiation and certain organic chemicals.
  - The rate of mutations resulting from mutagens is generally low because **DNA repair enzymes constantly monitor and repair any irregularities.**

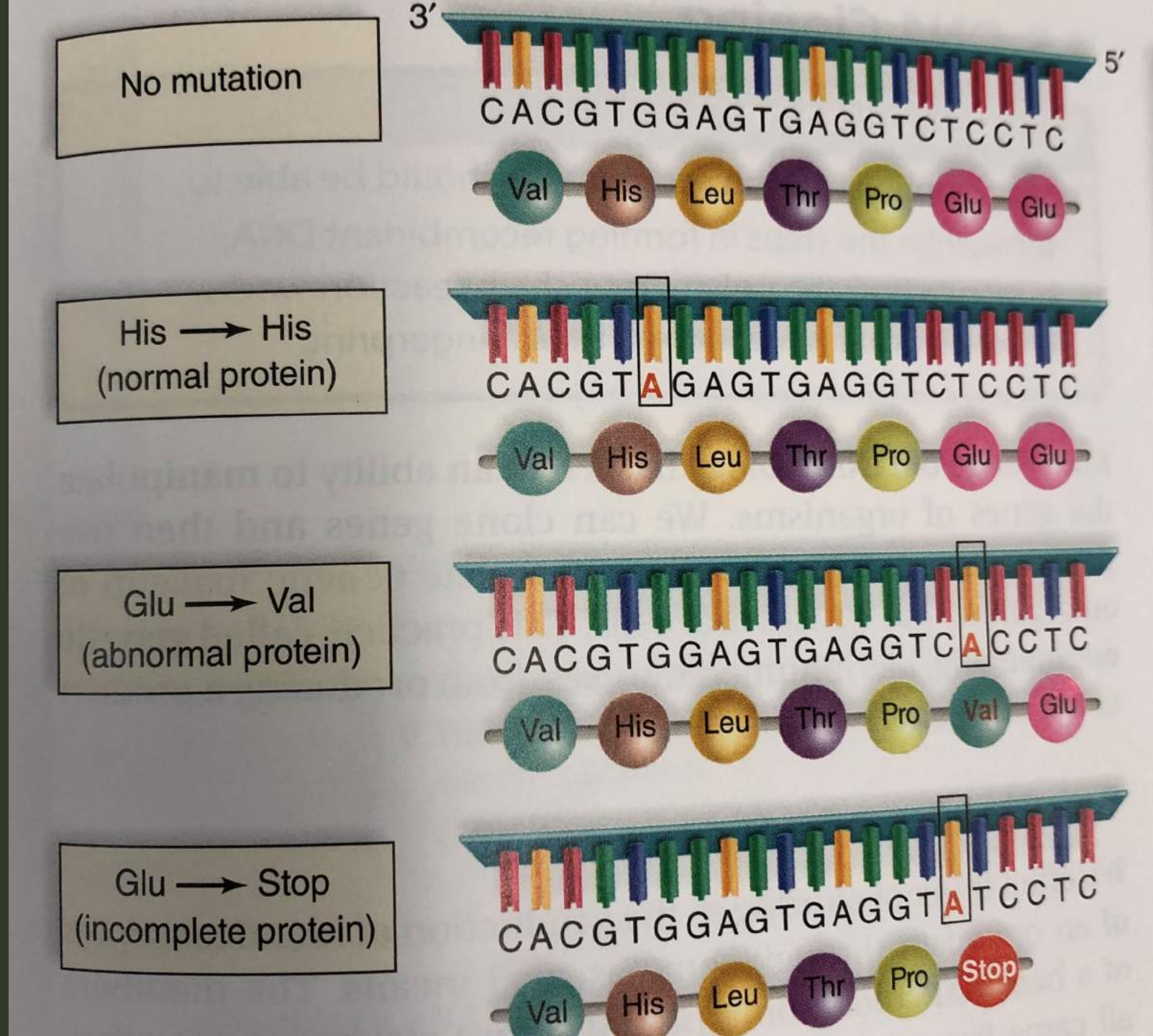
Transposons

- These are specific DNA sequences that have the ability to **move within and between chromosomes**.
  - This sometimes alters neighboring genes by **increasing or decreasing their expression.**



Effect of Mutations on Protein Activity

- Point mutations involve a change in a **single DNA nucleotide.**
  - Therefore a **possible change in an amino acid.**

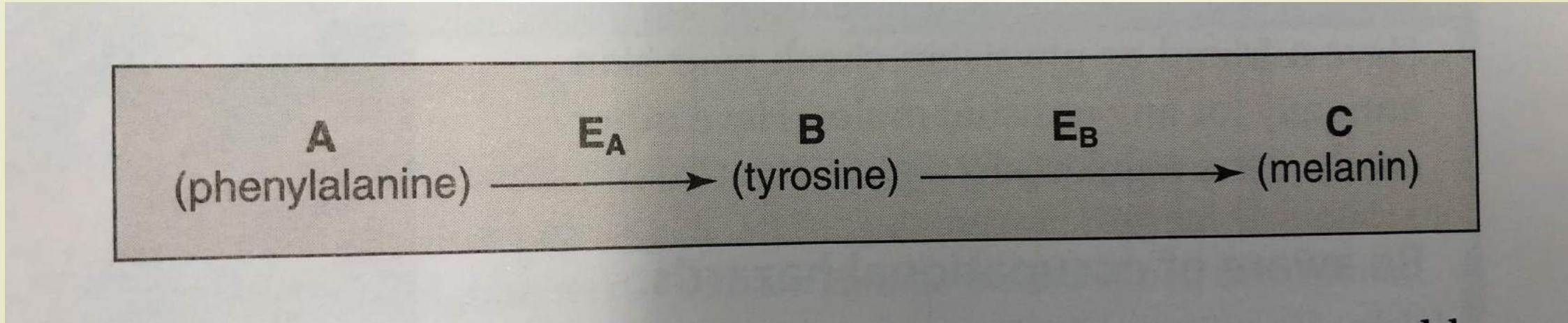


- Frameshift mutations occur most often because **one or more nucleotides are either inserted, or deleted from DNA.**
  - The result can be a completely new sequence of **codons and nonfunctional proteins.**
- Here is how this occurs – codons are read from a starting point as in the sentence:
  - **THE CAT ATE THE RAT**
- If the letter C is deleted, the reading frame is shifted:
  - **THE ATA TET HER AT-**

Nonfunctional Proteins

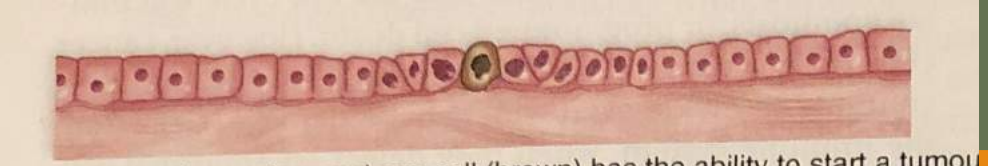
- A nonfunctioning protein can have a **dramatic effect on phenotype.**
  - Cell reactions that build up or break down biological molecules **operate in a sequential series.**
  - This is called a **metabolic pathway.**
  - Each step is regulated by the **activity of a particular enzyme.**

- If a faulty code for enzyme Ea is inherited, a person is not able to **convert molecule A to B.**
  - Molecule A (phenylalanine) builds up in the system, and can cause **mental impairment and other symptoms of PKU.**
  - Or, if a person inherits a faulty code for enzyme Eb, **this individual will develop albinism.**

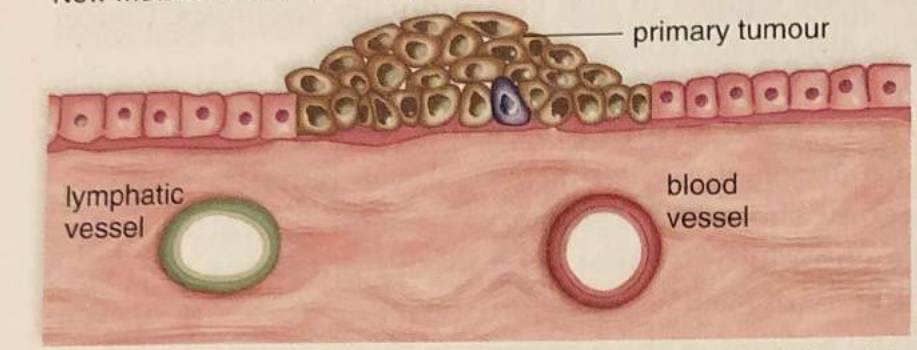


Mutations Can Cause Cancer

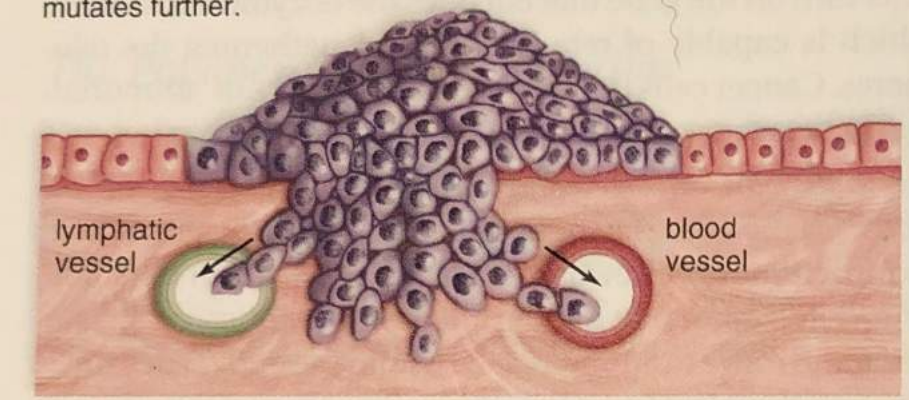
- The development of cancer involves a series of **accumulating mutations that an be different for each type of cancer.**
  - Tumor suppressor genes ordinarily **stop cell division**
  - Proto-oncogenes stimulate cell division, but are usually turned off in **fully differentiated non-dividing cells.**
    - When proto-oncogenes mutate, they become **oncogenes that are active all the time.**



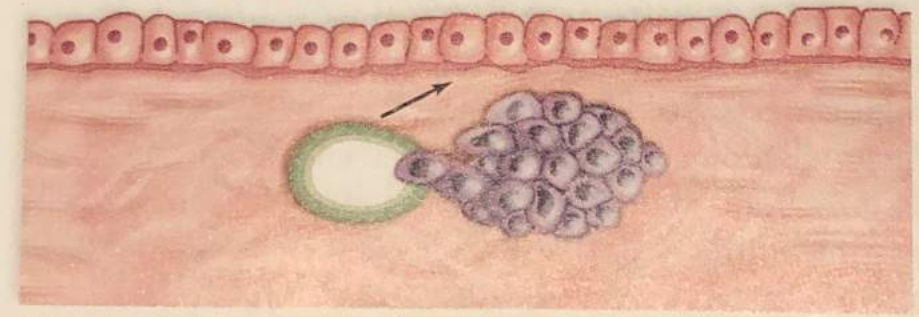
New mutations arise, and one cell (brown) has the ability to start a tumou



Cancer in situ. The tumour is at its place of origin. One cell (purple) mutates further.



Cancer cells now have the ability to invade lymphatic and blood vessels and travel throughout the body.



New metastatic tumours are found some distance from the primary tumo

- Carcinogenesis begins with the loss of **tumor-suppressor gene activity.**
  - When tumor suppressor genes are inactive and oncogenes active, cell **division occurs uncontrollably.**
- Cancers usually follow a common multistep progression.
  - Most begin as abnormal cell growth that is **benign, or noncancerous.**
  - Additional mutations may cause the **abnormal cells to fail to respond inhibiting signals.**
  - The growth is now malignant meaning is it **cancerous and has the ability to spread.**

Characteristics of Cancer Cells

- ***Cancer cells are genetically unstable*.**
  - Cancer cells appears to be linked to **mutagenesis.**
  - This allows the cell to continually divide until a **tumor forms.**
  - Tumor cells undergoes multiple mutations and also tend to have **chromosomal aberrations and rearrangements.**

- ***Cancer cells do not correctly regulate the cell cycle.***
  - The normal controls of the cell cycle do not operate to stop the cycle, and allow them to differentiate.
  - Therefore cancer cells are **nonspecialized**
  - Both the rate of **cell division and the number of cells increase.**

- ***Cancer cells escape the signals for cell death.***
  - A cell that has genetic damage or problems with the cell cycle will **initiate apoptosis.**
  - Cancer cells do not respond to internal signals to die, and continue to divide even with **genetic damage.**
  - Normal cells have a built-in limit to the number of times they can divide before they die.
  - Cancer cells turn on a gene that allows them to **divide incessantly**, exhibiting characteristics of "immortality."

- ***Cancer cells can survive and proliferate elsewhere in the body.***
  - Cancer cells travel through the blood and lymphatic vessels and **invade new tissue and form new tumors.**
  - This process is known as **metastasis.**
  - As the tumor grows it must increase the blood supply through **angiogenesis.**
  - This robs normal tissues of **nutrients and oxygen.**

3.6 – BIOTECHNOLOGY PRODUCTS AND GENE THERAPY

- Transgenic bacteria, plants, and animals are often called **genetically modified organisms** (GMO's)
  - The products they produce are called **biotechnology.**

Transgenic Bacteria

- Transgenic bacteria are grown in huge vats called **bioreactors.**
- The bacteria express the cloned gene, and the gene product is usually **collected from the medium the bacteria are grown in.**
  - The products found in the medium can include items such as **insulin, human growth hormone, and hepatitis B vaccine.**

- Transgenic bacteria have many uses including but not limited to:
  - GM bacteria can be used to **help strawberries be resistant to frost.**
  - GM bacteria have been developed to **eat oil that can help clean up oil spills.**

Transgenic Plants

- With the help of biotechnology, scientists have developed a plant known as the pomato.
  - Which produces **potatoes below ground and tomatoes above ground.**
- They have also developed soybeans that are **resistant to common herbicide that is sprayed to kill weeds that compete with soybeans**.

Transgenic Animals

- Techniques have been developed to **insert genes into the eggs of animals.**
- One application of this is **gene farming.**
  - This is the use of transgenic farm animals to **produce pharmaceuticals.**
  - The desired proteins appear in the animals milk where humans are able to **harvest proteins or hormones such as human growth hormone.**

Fig. 26.7b

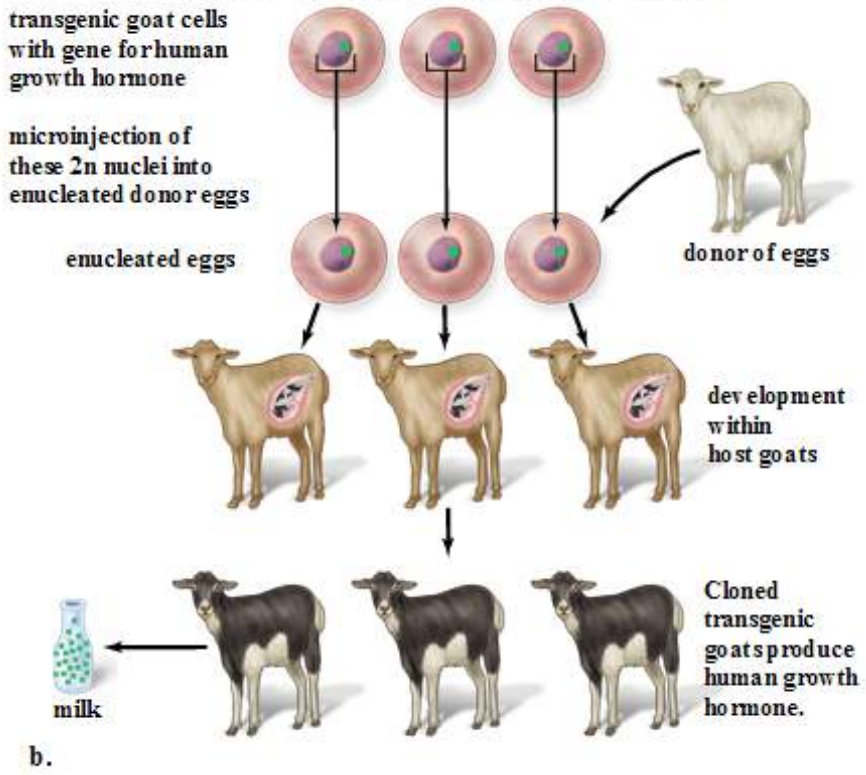
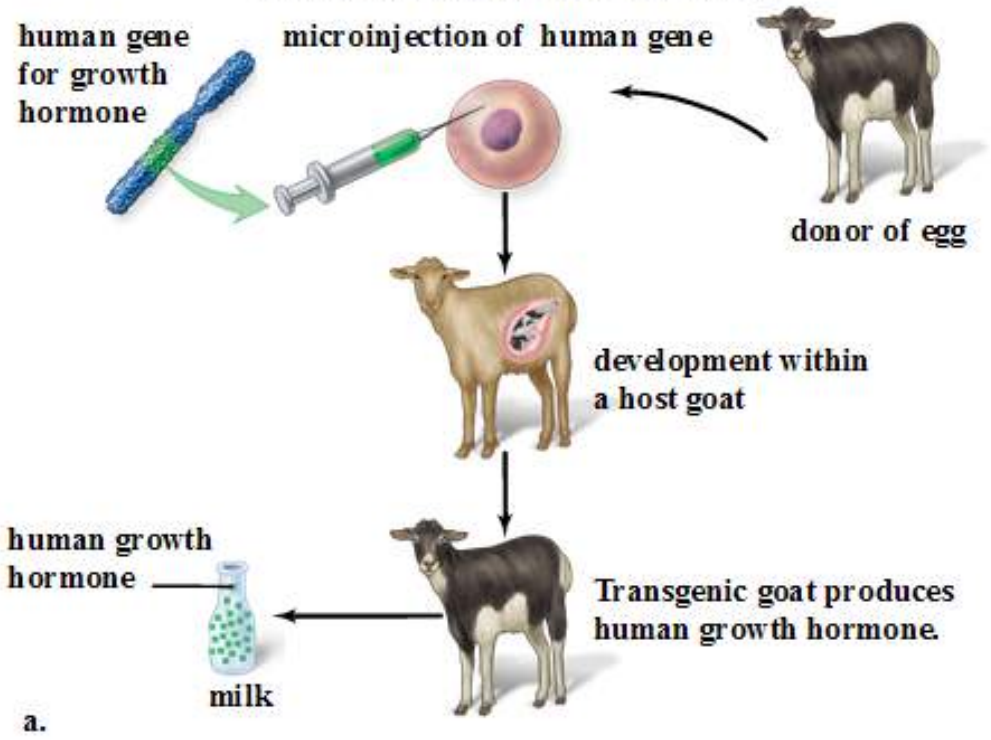


Fig. 26.7a



Gene Therapy

- If a genetic disorder is detected, gene therapy can sometimes be a course of treatment.
  - Gene therapy is the **insertion of genetic material into human cells for the treatment of genetic disorder or other illnesses.**
  - Viruses that are modified to be safe, can be used to **transport a normal gene into the body.**
  - Another way is to **inject the gene directly** into a particular region of the body.

Ex Vivo Gene Therapy

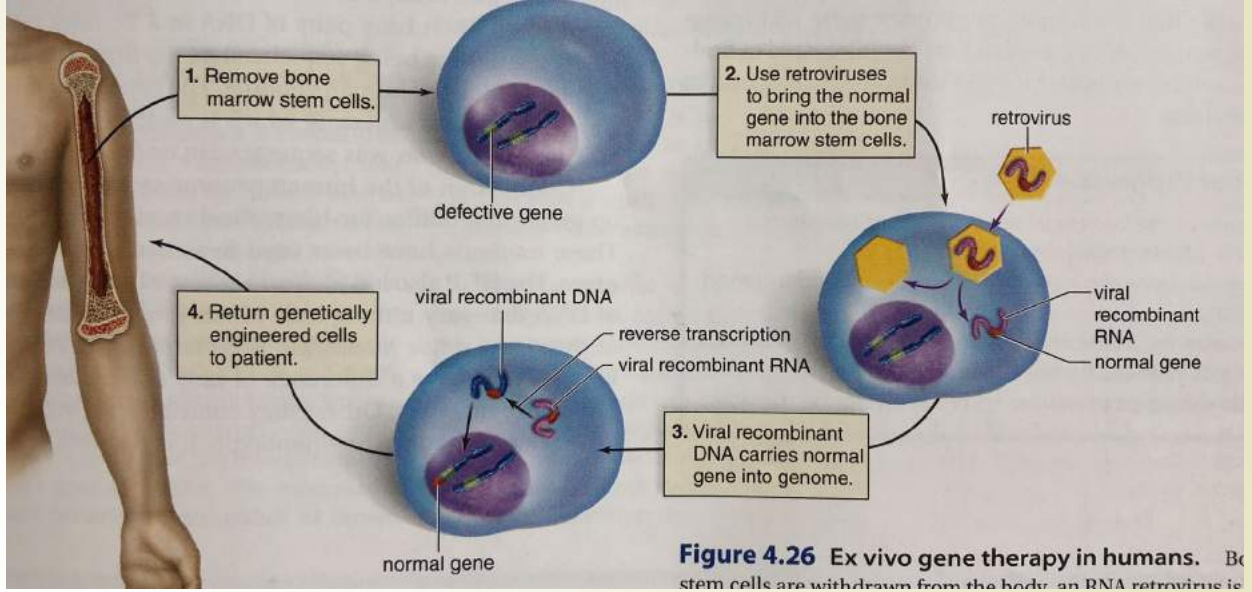


Figure 4.26 Ex vivo gene therapy in humans. Bo
stem cells are withdrawn from the body, an RNA retrovirus is

- One method of ex vivo gene therapy involves bone marrow stem cells being removed from the patient, and **infecting them with a virus that carries a normal gene, so it can be inserted into their DNA.**
  - The cells are then returned to the patient, where they will ideally produce **more cells with the normal gene.**

In Vivo Gene Therapy

- Gene therapy is increasingly relied upon as a part of cancer treatment.
  - Genes are being used to make healthy cells more **tolerant of chemotherapy**, **while making tumor cells more sensitive.**
  - This could significantly increase the effectiveness of the chemotherapy, without **as many adverse side effects.**