Molecular Genetics

The branch of genetics that deals with the ***molecules*** responsible for the transmission of traits from parent to offspring.

Discovering DNA as the material of heredity

DNA – Deoxyribonucleic Acid is the molecule that transmits traits (genes) from parent to offspring.

Scientists involved in the discovery of DNA.

1. **Miescher**
   - Isolated a substance known as "*Nuclein*" from white blood cells.
   - He said that nuclein was composed of an Acid portion (he called it nucleic acid) and an alkaline portion (later shown to be a protein)
   - Research after Meischer showed that nuclein was made up of a series of strand – like complexes of nucleic acids and proteins tightly bound together. – These strands were called ***Chromosomes.***

2. **Levene**
   - Isolated two types of Nucleic acids distinguished by type of sugar in them.
     i. RNA – Ribonucleic Acid (contains a 5 Carbon sugar called "Ribose")
     ii. DNA – Deoxyribonucleic Acid (contains a 5 carbon sugar called "Deoxyribose (deoxy = no oxygen))
   - Levene showed that nucleic acids are made up of long chains of individual units called **nucleotides.**
     i. **Nucleotide → A structure composed of a 5 carbon sugar, phosphate group and a Nitrogen base.**

   - There are **Four(4)** different nitrogen bases in each nucleic acid.

Nitrogen Bases in DNA	**Nitrogen Bases in RNA**
Adenine (A)	Adenine (A)
Guanine (G)	Guanine (G)
Cytosine (C)	Cytosine (C)
Thymine (T)	Uracil (U)

   - Said that DNA and RNA contained **equal** amounts of nucleotides. **THIS WAS AN ERROR. HE WAS LATER DISPROVED.**

3. **Griffith**
   - Studied bacteria and pneumonia.
   - Found that dead pathogenic bacteria passed on their pathogenic properties to live non-pathogenic bacteria.
     - Called this the ***Transforming Principle***

Transforming Principle: The idea that genetic information can be transferred from one organism to another.

4. **Avery, MacLeod, McCarty**
   - Did an experiment to try and prove Griffith's transforming principle by isolating what was causing it.

   - Here is what they did:
     - Treated pathogenic bacteria with protein destroying enzyme. The transformation still occurred.
     - Treated the pathogenic bacteria with DNA destroying enzyme. The transformation stopped.
     - Treated bacteria with RNA destroying bacteria, but not DNA, the transformation occurred.
   - **RESULTS:** ***They proved that DNA was the material responsible for the transforming principle.***

5. **Chargaff**
   - Overturned Levene's conclusion about nucleotides.
   - Chargaff found that the four nucleotides were NOT present in equal amounts, but in varying proportions.
     - The amount of nucleotides in DNA (A,C,T,G) varies from species to species.
     - The nucleotide composition from members of the same species is constant.
     - In any sample of DNA, the amount of Adenine = Thymine and Guanine = Cytosine. This is known as **Chargaff's Rule.**

Chargaff's Rule: In any sample of DNA the amount of Adenine = Thymine and Guanine = Cytosine.

6. **Hershey and Chase**
   - Scientists that gave the final proof that DNA and not proteins carried genetic information.
   - Used radioactive labeling in an experiment with a virus called a ***bacteriophage.*** Note: A bactriophage is a virus that infects a bacterium.
   - Here is what they did:
     - Ran two experiments the same time. Both on phages.
     - Added a radioactive marker to DNA of one phage.
     - Added a radioactive maker to Protein coat of another phage.
     - Allowed phages to infect bacteria (E. coli)
     - Two samples of bacteria were observed.
     - First sample (radioactive phage DNA) the bacteria became radioactive. Surrounding fluid did not.
     - Second sample (radioactive protein coat of phage) the bacteria did not become radioactive. The surrounding fluid did.
   - **CONCLUSION:** ONLY the DNA from the virus entered the bacterial cells, therefore transmission of genetic material from the virus to bacteria happened only because the DNA was injected into the bacteria.
   - DNA became known as the molecule that transmits genetic information.

7. **Wilkins and Franklin**
   - 1940's
   - Did work on determining the structure of nucleic acids and DNA.
   - Took X-rays of DNA that proved it had a "helical" or "twisted" shape.
   - Found that DNA had 2 distinct and repeating patterns.
   - Found that Nitrogenous bases were ***"hydrophobic"*** and contained within the center of the helical structure.
   - Found that the sugar –phosphate backbone was ***"hydrophilic***" and was located on the outside.

8. **Watson and Crick**
   - 1950's
   - Worked on different models of the "helical" structure using the knowledge of Wilkins and Franklin.
   - **Produced the structural model of DNA that is still in use today.**
   - **They called their model the "DOUBLE HELIX"**

The Structure of DNA

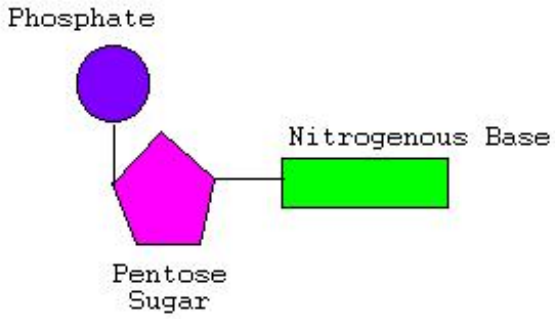
DNA – Deoxyribonucleic Acid.

- Long molecule made up of TWO (2) strands of nucleotides bound together in the shape of a double helix (twisted Ladder)
- Found in the ***nucleus*** of cells.
- Each rung of the ladder is composed of repeating units called **Nucleotides.**

Nucleotide: Subunit of the DNA molecule made up of the following:

- Phosphate Molecule
- 5 carbon sugar (Deoxyribose in DNA, Ribose in RNA)
- Nitrogen base (A, T , C or G)

Structure of a Nucleotide:



Nitrogen Bases

- These are molecules containing nitrogen.
- They form the "rungs" in the DNA double helix.
- The bases are ***complementary***.
  - Adenine bonds with Thymine
  - Guanine bonds with Cytosine
- The nitrogen bases are found in one of two categories:
  - **Purines →** Nitrogen bases with a **double ring** structure. These are Thymine and Cytosine.
  - **Pyrimidines →** Nitrogen bases with a **single ring** structure. These are Adenine and Guanine.

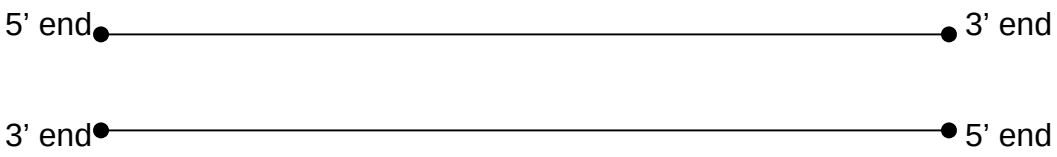
- **Purines = Thymine and Cytosine**
- **Pyrimidines = Adenine and Guanine**

Note: **In the DNA molecule 1 Pyrimidine bonds with 1 Purine (Hydrogen bonds keep them together)**

Antiparallel Nature of DNA

DNA is said to be 'antiparallel'. This means that the phosphate bridges (backbone) of the molecule run in opposite directions in each strand.

The 5' end of one strand is opposite the 3' end of the other strand and vice versa.



STRUCTURE OF RNA

RNA - Ribonucleic acid.

- **Single stranded** nucleic acid found inside and outside the nucleus of cells.
- Structure similar to DNA EXCEPT:
  - RNA has 5 carbon sugar called "**Ribose**"
  - RNA has 4 nitrogen bases → Adenine, Guanine, Cytosine , **URACIL** (no thymine in RNA)

Types of RNA

There are **three** types of RNA.

1. mRNA – Messenger RNA (found in the nucleus)
   - This is the molecule that carries the genetic message from the nucleus of a cell to the ribosomes.

2. tRNA – Transfer RNA (found in the cytoplasm)
   - The molecule that brings amino acids to the ribosome to make proteins.

3. rRNA – Ribosomal RNA (found at the ribosomes)
   - Molecule that helps to make up ribosomes.

Structure of a Chromosome

Chromosomes consist of the following:

- One linear double stranded DNA molecule. (The molecule may be very long)
- Proteins called ***histones.***
    - *The DNA is wrapped around the histones.*

The DNA is wrapped around a group of 8 histone molecules making a "bead – like" structure called a ***Nucleosome***.

The nucleosomes fold back upon themselves making a condensed structure called chromosome.

Compostion of a Chromosome

Chromosomes are composed of:

- 60 % protein
- 35% DNA
- 5% RNA

GENES and GENOME

Gene → Small segment of DNA found on a chromosome that codes for specific traits in organisms.

Genome → The sum of the entire DNA carried within the cells of an organism.

Structure of a Gene

Each gene is composed of **two** portions.

A. **Exons** → These are portions of DNA in a gene that are called "**Coding Regions"**. These portions code for specific proteins within a cell.

B. **Introns** → There are portions of DNA in a gene that are "noncoding". They are like spaces between the coding regions. Most of the structure of a gene is made up of these introns that were once called "junk" or "nonsense" DNA.

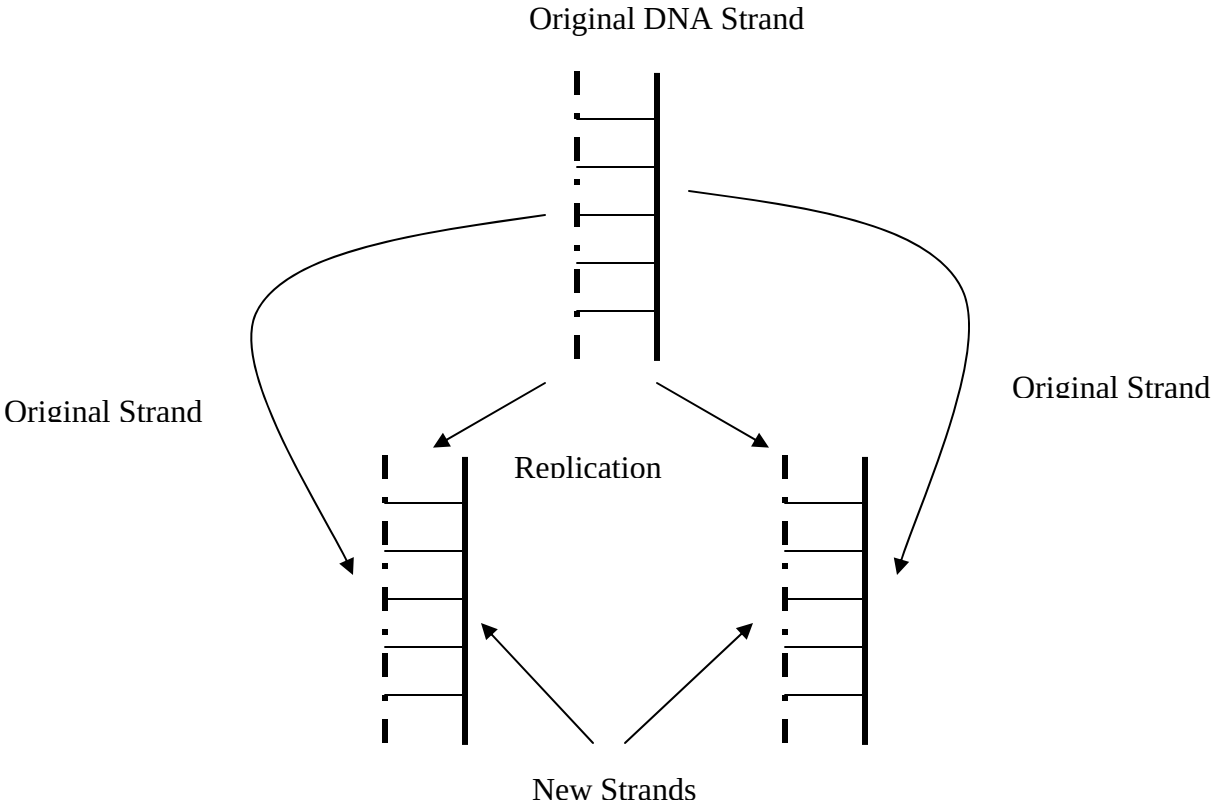
A Gene showing Introns and Exons

DNA REPLICATION

DNA Replication: Making a copy of the DNA molecule.

DNA Replication is ***Semi-conservative***.

This means that when DNA is copied, each new molecule created contains one strand of parental DNA and one strand of New DNA.



The Process of DNA Replication

DNA is copied through the following 4 steps.

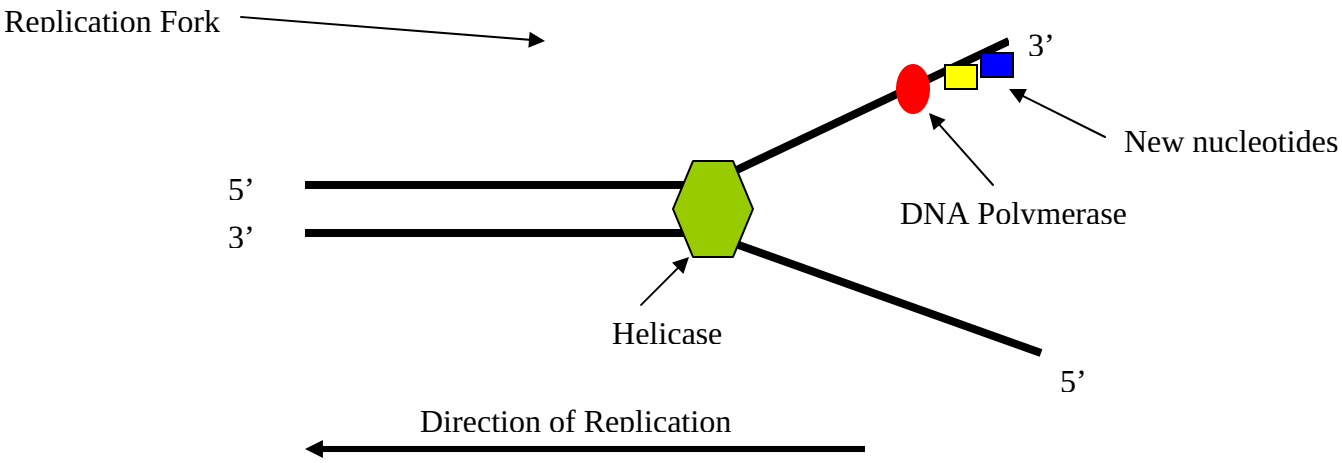
1. Initiation
2. Elongation
3. Termination
4. Proofreading and Correction

1. **Initiation**

This is the opening up (unwinding and unzipping) of the DNA molecule so new nucleotides can be inserted to eventually create 2 new strands.

Initiation happens according to the following steps.

a. At a specific sequence of nucleotides called the ***replication origin,*** enzymes called **helicases** unzip and open up the DNA creating ***Replication Forks.***
b. **DNA Polymerase** (an enzyme) inserts itself in the replication fork and begins to add NEW nucleotides, one at a time, to create a new complementary strand.



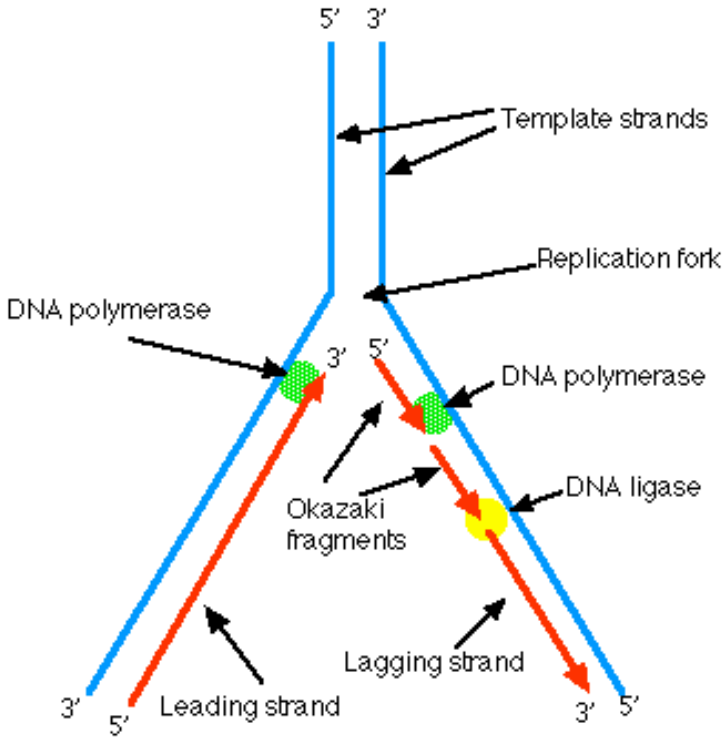
2. Elongation

During elongation, the new strand of DNA continues to grow at the replication forks. Elongation happens according to the following steps.

a. At the 3' exposed end of the DNA molecule (called the **Leading Strand**), DNA Polymerase adds new nucleotides. **This occurs in the direction from the 5' to 3' end only (New DNA)!** In order for DNA polymerase to know where to begin, a small piece of **RNA Primase** called a **primer** shows it where to begin. Once begun, the addition of nucleotides continues at a steady pace along the leading strand.

b. Because DNA polymerase lays down nucleotides from the 3' to 5' direction only, the 2nd strand of nucleotides are laid down in the ***opposite*** direction of the leading strand. This occurs when short copies of DNA are made in spurts called **Okazaki fragments.** This occurs slower than the leading strand and is known as the **Lagging strand.**
In order for this to happen, RNA primers are needed for each Okazaki fragment.

c. Another enzyme, **DNA Ligase**, stitches together the Okazaki fragments to make a complete strand of DNA.



3. **Termination**

This is the **stopping** of the copying of the DNA molecule. This normally happens when replication forks meet each other or the end of a linear piece of DNA is reached.

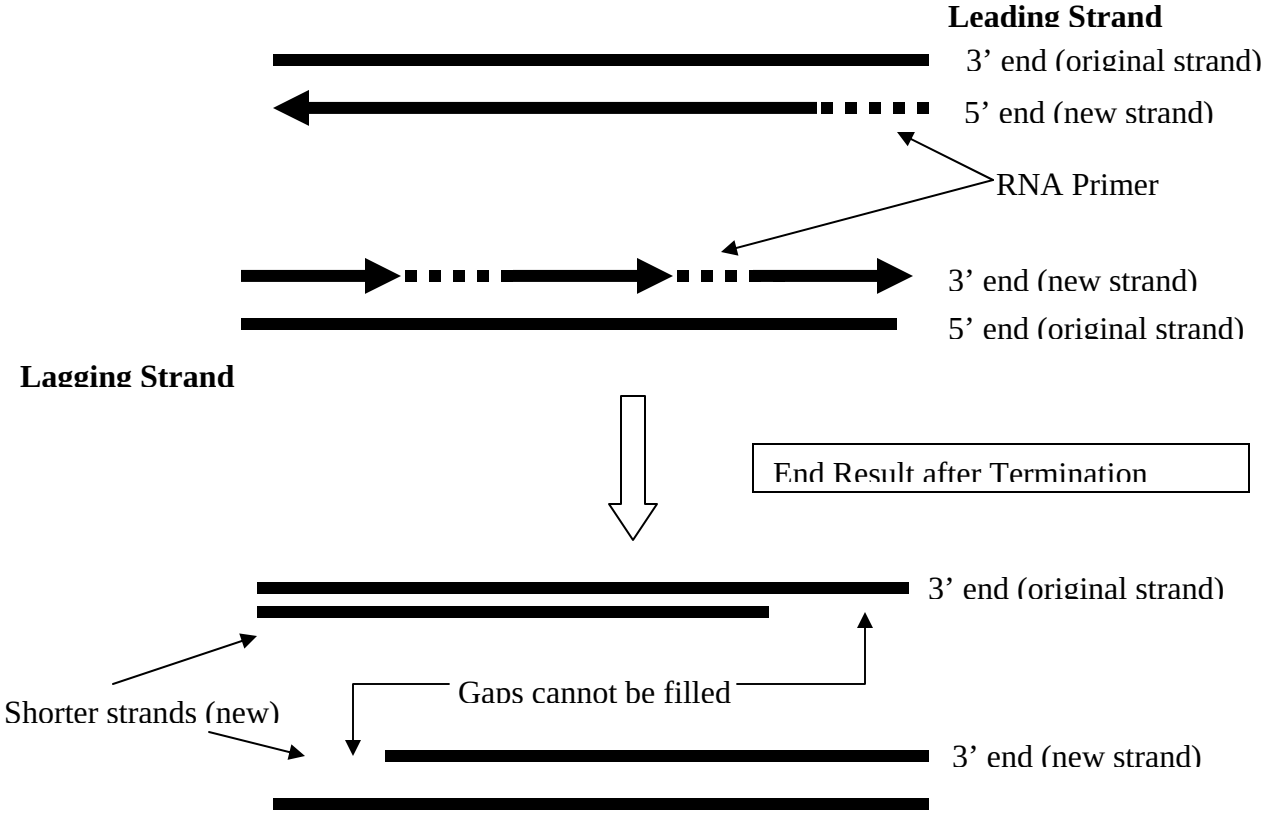
Termination results in strands of DNA that are SHORTER than the originals.

Why does this occur?

Recall

- At the leading strand an RNA primer is laid down to allow DNA polymerase to start placing new nucleotides. When this RNA primer is **removed** there is a **gap** at the 5' end of the New strand and it ends up being shorter that the original strand.
- At the lagging strand, RNA primers are laid down for each Okazaki fragment. When all of these are removed and the gaps filled, there is a **gap** at the 5' end of the new DNA that cannot be filled. This results in a strand that is shorter.

NOTE: In each successive replication of DNA (occurs each time the cell divides), the DNA (chromosomes) get shorter and shorter. This presents a problem for Eukaryotic cells (eukaryotic cells have linear DNA), but Prokaryotes do not have this problem as their DNA is circular.



How is the problem of shorter DNA strands fixed?

Fortunately, the ends of chromosomes contain specialized areas called ***Telomeres*** that guard against this.

Telomere: Segments of highly repetitive nucleotide sequences at the ends of chromosomes that do not contain coding genes.

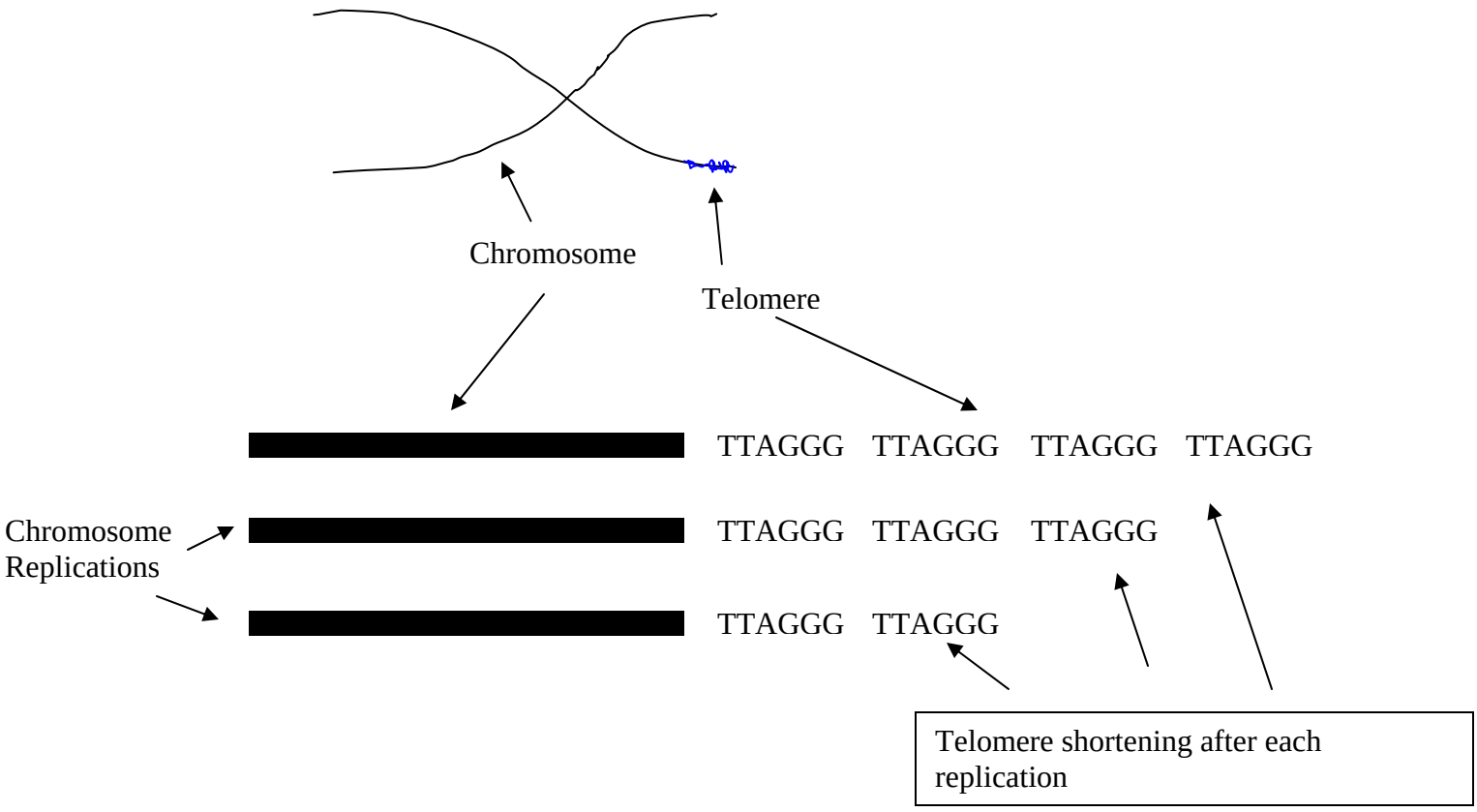
In humans, telomeres consist of the sequence TTAGGG repeated several thousand times.

As termination ends, the telomere ends are "cut off" making them shorter.

As more and more DNA replications happen, the length of telomeres shortens.

The shortening of telomeres directly relates to the death of a cell. This is why cells are unable to live forever.

Interesting point: An enzyme known as telomerase is related to keeping telomeres long. This helps to maintain the longevity of a cell. Cancer cells which divide much more than any other cell in the body have telomerase in them. This is believed to be related



4. **Proofreading and Correction**

This is the process whereby DNA polymerase moves along the newly completed DNA strand looking for mismatched nucleotides and corrects them by inserting the correct nucleotides.

How does proofreading and correction occur?

- DNA polymerase moves along the newly formed strands recognizing whether or not Hydrogen bonding is occurring. If Hydrogen bonding is not occurring, the DNA polymerase recognizes a mismatched set of nucleotides.
- The incorrect base is removed and a proper one is inserted.

PROTEIN SYNTHESIS

Protein Synthesis: This is the process whereby the instructions from DNA are used to create ***polypeptides*** that make up a protein. This process is also known as **Gene expression.**

Gene Expression: The transfer of genetic information from DNA to protein.

Polypeptide: Long chains of amino acids. 2 or more polypeptides joined together = a protein.

Why make Proteins?
Proteins are the important structural components of cells and your body as a whole. Proteins are what make you operate. For example, proteins are found in each of the following:

- Skin
- Hair
- Hemoglobin (blood)
- Enzymes
- Hormones

NOTE: DNA determines the sequence of amino acids in a polypeptide. This is determined by the sequence of nucleotides in the DNA.

The Process of Protein Synthesis

Protein Synthesis occurs in **two** steps.

A. Transcription – this is the process of changing DNA into mRNA.

B. Translation – this is the process of translating the DNA code into a protein.

How does Transcription occur?

Transcription occurs within the **nucleus** of Eukaryotic cells and in the cytoplasm of prokaryotic cells (bacteria).
It occurs according to the following steps.

1. **RNA Polymerase** attaches to the DNA molecule inside the nucleus.
2. Only one of the strands of the DNA will be used to make the mRNA. This strand is called the **Anti-Sense Strand.** The other strand is called the **sense strand.** (It is not used)
3. **RNA Polymerase** attaches to the anti-sense strand and opens it up. It then begins reading the strand from the 3' to 5' end laying down RNA nucleotides as it moves along.

4. As the RNA Polymerase passes a region on the DNA molecule, the DNA zips back up. The mRNA separates from the anti-sense strand.
5. When the mRNA strand is completed it contains "exons" (coding regions) and "introns" (noncoding regions). The "introns" are removed and the exons are joined together. This is done by another series of enzymes.
6. The mRNA (single stranded) - called a "RNA sense strand" now leaves the nucleus and enters the cytoplasm.

How does Translation Occur?

Translation occurs within the cytoplasm of ALL cells. It occurs according to the steps below.

1. mRNA binds to an active Ribosome found in the nucleus.
2. The binding of the mRNA happens such that the first 2 **Codons** are exposed.

Codon: A sequence of 3 mRNA bases that code for a specific ***amino acid.***
Ex: AUG = a codon
CCC = a codon

3. A tRNA (transfer RNA) molecule carrying an amino acid base-pairs with the first exposed mRNA codon. This happens when the **anticodon** on the tRNA recognizes the complementary codon of the mRNA.

Anticodon: Sequence of 3 bases on the end of a tRNA molecule that recognizes the codons of the mRNA.

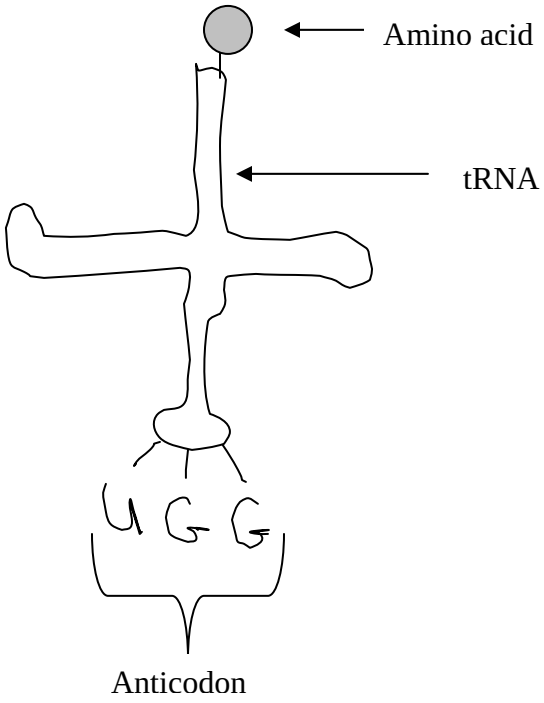
4. A second tRNA molecule carrying an amino acids arrives at the codon adjacent to the first tRNA.
5. Enzymes cause the amino acids to join together in a peptide bond.
6. The polypeptide chain is transferred from the first tRNA to the second one.
7. The ribosome moves one codon along the mRNA strand
8. The first tRNA molecule leaves to retrieve another amino acid.
9. Another tRNA molecule arrives at the exposed end next to the remaining tRNA molecule and the process repeats.
10. The process continues until a **"STOP"** codon is reached. At this point, the polypeptide is released from the ribosome.

What is tRNA?

- A clover – leaf shaped RNA molecule found within the cytoplasm of cells.
- Called ***transfer*** **RNA**

Function: Bring amino acids to the ribosome and recognize the codons on the mRNA so as to find the correct sequence of amino acids when making a polypeptide.

A tRNA molecule



Reading a Codon Table

Codon Table: A table that lists a series of **mRNA codons** that are read by the complementary tRNA molecule to determine the sequence of amino acids that make up a polypeptide.

Sample Codon table

FIRST	SECOND: U	SECOND: C	SECOND: A	SECOND: G	THIRD
U	phenyl-alanine	serine	tyrosine	cysteine	U
					C
	leucine		stop	stop	A
			stop	tryptophan	G
C	leucine	proline	histidine	arginine	U
					C
			glutamine		A
					G
A	isoleucine	threonine	asparagine	serine	U
					C
	* methionine		lysine	arginine	A
					G
G	valine	alanine	aspartic acid	glycine	U
					C
			glutamic acid		A
					G

* and start

Reading a Codon Table

To read a codon table perform the following steps:

a. Convert your DNA into an RNA codon.
b. Using the 3 letters from your RNA codon, look them up in the table above. Work from left to right using your three letters. In the table the word **FIRST** stands for the first letter in the RNA codon, the **SECOND** for second and so on.

c. When you have read all three letters of the RNA codon you should arrive at the name of an Amino acid. Continue to do this for each sequence of 3 bases from left to right (5' to 3' direction).

Ex: The DNA triplet AGG translates into RNA codon → UCC

First letter = U (you should be looking in the top row)
Second Letter = C (you should now be looking in the second column, first row)
Third letter = C (You should have identified the amino acid ***Serine*** as the correct amino acid.

Note: Sometimes you will be given the tRNA anticodon and have to find the DNA codon. Just work backwards in this case.

ALWAYS REMEMBER: The codon table is for mRNA ONLY!!!!!!

Regulating Gene Expression

All polypeptides come from the process of Protein synthesis. Controlling protein synthesis is a way of controlling the formation of certain polypeptides. Since the instructions for polypeptides comes from the genes, controlling the expression of genes can regulate what sorts of polypeptides are created in protein synthesis.

Factors affecting gene expression

1. **Changes in Temperature and Light**
   - Some plants germinate when it is warm (proteins created causes plant to germinate)
   - Bright lights cause some proteins to be created causing particular behaviours such as wakefulness.

2. **Presence or absence of nutrients in the environment**
   - *E. Coli* responds to presence of lactose by increasing the rate of enzyme production used for synthesizing lactose.
   - *E. Coli* react to the presence of the amino acid tryptophan by reducing their production of enzymes that synthesize tryptophan.

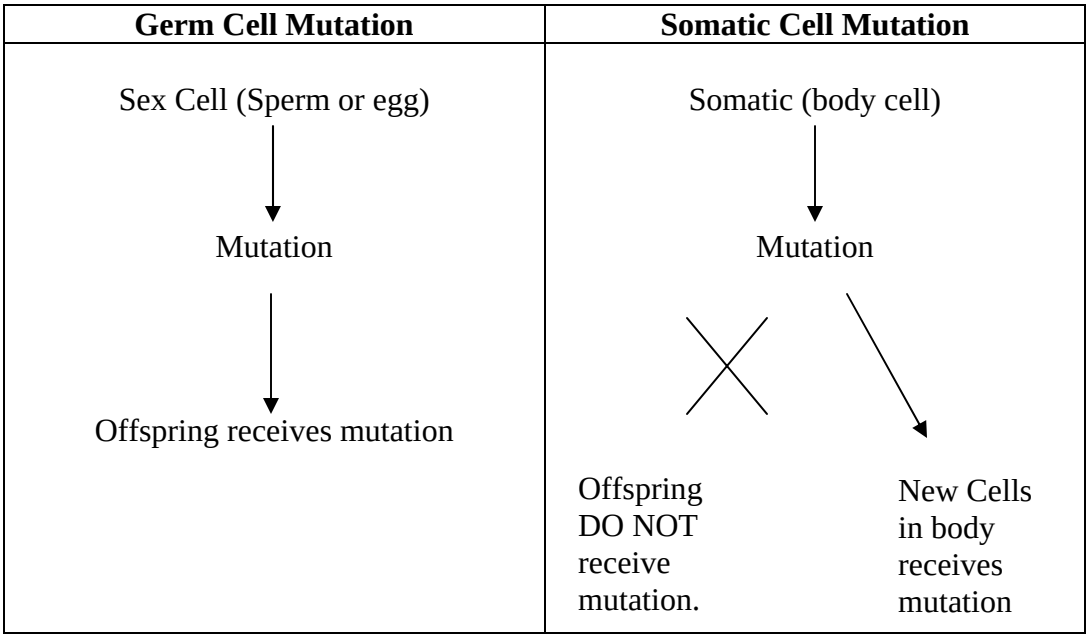
3. **Presence of Hormones in the body**
   - Hormones, which are proteins, trigger protein synthesis in other cells.
     - i.e. hormones (proteins made by protein synthesis) cause cells to create other proteins by protein synthesis.

Mutations

Mutation: A permanent change in the genetic material of an organism.

Notes about mutations

- All Mutations are heritable. That is, they will be copied during DNA replication.
- Not all mutations are passed on to the next generation.
- ONLY **GERM CELL MUTATIONS -** mutations in the sex cells, will be passed on to future generations.
- **Somatic cell mutations** -- mutations occurring in body cells. Will not be passed on to offspring. Instead, these are passed on to NEW cells created in the organism in which it occurs.



Types of Mutations

There are two main types of Mutations.

a. Gene Mutations.

b. Chromosome mutations

A. Gene Mutations

These are mutations that occur when **one** or a **few nucleotides** are changed within a gene. These are often referred to as ***Point Mutations***.

There are **Two (2)** types of Point Mutations.

i) **Substitution**
This is the replacement of one nucleotide for another.
Ex: CATCAT becomes CATTAT (notice the second C is substituted by a T)

Original	The fat cat ate the wee rat.
Substitution	The fat **h**at ate the wee rat.

Substitution mutations may or may not have an effect on cells. It depends on where and what nucleotide is substituted.

There are three possible effects/types of substitution mutations.

1) Silent Mutation → A substitution that has **no** impact on the cell's metabolism.

2) Mis-sense Mutation → A substitution that creates a slightly altered but still functional protein.
These mutations can be harmful.
Ex: A single nucleotide mutation in the protein that makes up hemoglobin results in a disease known as "sickle cell anemia".

3) Nonsense Mutation → A substitution that renders a gene unable to code for any functional protein. This usually occurs when the start signal is erased or the stop signal is premature inserted.
Nonsense mutations are always harmful.

ii) **Frameshift**

This is the **insertion** or **deletion** of one or two nucleotides within a sequence of codons. This causes the entire reading frame of the gene to be altered. It causes the sequence of amino acids in a protein to be changed.

There are two types of Frameshift Mutations

1) Insertion → This is when an extra nucleotide is inserted into the DNA sequence.

2) Deletion → This is when a nucleotide is "removed" from the DNA sequence.

Ex:

Original	The fat cat ate the wee rat.
Frame Shift (deletion)	The fat ca**a tet hew eer at**.

B. Chormosomal Mutations

These are mutations affecting entire chromosomes, parts of chromosomes or multiple genes on the same or different chromosomes. These mutations lead to the *rearrangement* of the genetic material in chromosomes.

Types of Chromosome Mutations

i) Transposons (Jumping Genes) → First discovered by Barbara McClintock.
These are genes that are able to move (jump) from one area in the DNA to another. This leads to tremendous variety in organisms.

Ex: Indian Corn has a variety of colours because of transposons.

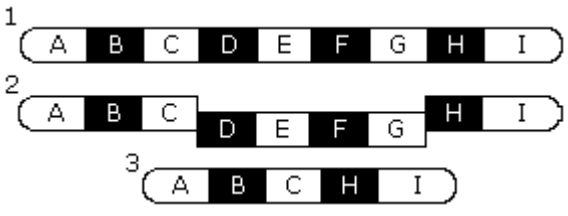
ii) Deletion → A portion of a chromosome is **lost.** A part of a chromosome often breaks off and is lost.
These can be caused by viruses, radiation, chemicals.

Ex: Chromosome # 5 - When a piece is lost, children are born mentally handicapped and with a cat – like voice. This is called ***cri –du-chat*** syndrome.

Ex:

Original	The fat cat ate the wee rat.
Deletion	The fat ate the wee rat.

A chromosome deletion →



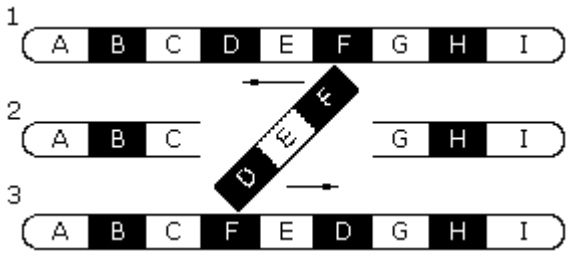
iii) Inversion → A segment of a chromosome may break free and "reverse" its orientation and then become reconnected to the chromosome.

Ex: Autism is believed to be caused by chromosome inversions.

Ex:

Original	The fat cat ate the wee rat.
Inversion	The fat **tar eew eht eta tac**.

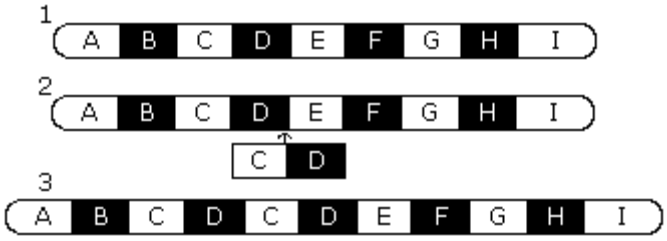
A chromosome Inversion →



iv) Duplication → A gene sequence may duplicate itself one or more times within one or several chromosomes. Repeats are not always harmful but can sometimes affect the functioning of a gene.

Ex: **Fragile X Syndrome** – This is a situation where the X chromosome in some females and males have several repeating units of nucleotides. (700 of them)

A chromosome Duplication →

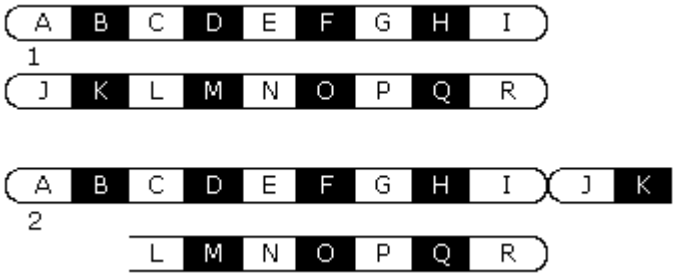


v) Translocation → A part of one chromosome changes places with another part of the same chromosome or with a part of another chromosome (non homologous). This often results in one longer and one shorter chromosome.

Ex: Cancer may occur in individuals when part of Chromosome # 14 exchanges places with chromosome #8.

Some Down's syndrome is related to translocation between Chromosome # 14 & 21.

A chromosome translocation →



vi) Nondisjunction → This is the failure of homologous to separate during meiosis. The result is a cell with either too many or two few chromosomes.

Of the cells created, one will have an "extra" chromosome while the other cell will have a "missing" chromosome.

There are two main types of Nondisjunction

a) **Trisomy** – When an extra chromosome is inherited in a cell resulting in 3 chromosomes of the same # in a cell.

Ex: Trisomy 18 (Three # 18 chromosomes) causes **Down's Syndrome**

b) **Monosomy --** When only one chromosome is inherited instead of a pair in a cell.

Human Genetic Diseases

These are diseases/syndromes caused by either chromosomal or gene mutations.

1. **Down's Syndrome (Trisomy 18)**
   - Caused by nondisjunction of chromosome # 18.

   Effects → Mild to moderate mental impairment, large tongue, short stocky stature.

2. **Turner Syndrome (XO)**
   - When a person receives an X chromosome and no Y chromosome.

   Effects→ Females born with external genitalia, no ovaries and no menstrual period. She will be **infertile.]**

3. **Klinefelter Syndrome (XXY)**
   - Nondisjunction of the X chromosomes causes a **Male** to have XXY chromosomes.

   Effects → Males have immature sex organs and will not grow facial hair. Some breast development occurs as well.

4. **Jacobs Syndrome (XYY)**
   - When a male receives an extra Y chromosome.

   Effects → Have similar features of Klinefelter's patients. Also have speech and reading problems as well as persistent acne.

5. **Triple X Syndrome (XXX)**

- When a female receives three X chromsomes because of nondisjunction.

Effects → Most women are normal. They are fertile, but may have menstrual irregularities. Some women have learning disabilities.

Karyotyping

Karyotype: A picture showing the size and arrangement of chromosomes inside the cell of an organism.

Chromosomes are arranged into pairs based upon size and banding patterns. The banding pattern comes from the unique sequence of genes within each chromosome.

Normally, there are 22 identical pairs of chromosomes in a karyotype and one set of "dissimilar" chromosomes. These are the **sex** chromosomes (X and Y)

Karyotypes are used to diagnose genetic disorders such as Trisomy 18 etc.