D1.3

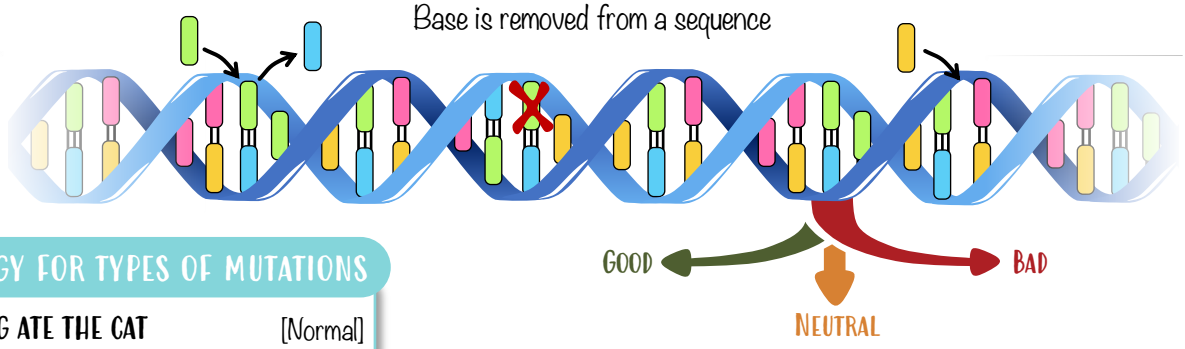
Mutations & Gene Editing

MUTATIONS – When one or more nucleotides (**A**, **C**, **T**, or **G**) of a DNA code are modified by mistake; may involve entire segments and even full chromosomes.

I. SUBSTITUTIONS
One base is changed for another

II. DELETIONS
Base is removed from a sequence

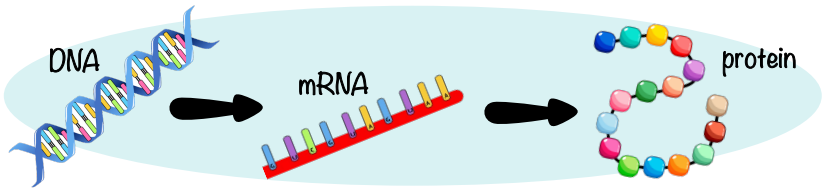
III. INSERTIONS
Base is added to a sequence



ANALOGY FOR TYPES OF MUTATIONS

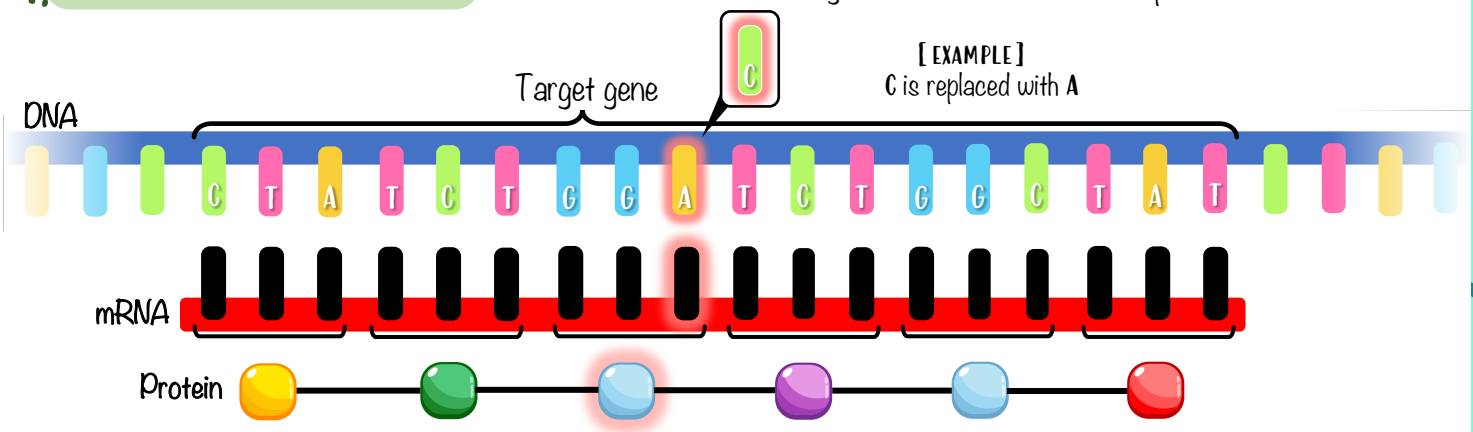
THE DOG ATE THE CAT	[Normal]
THE HOG ATE THE RAT	[Substitution]
THE DOG ATE THE BAD CAT	[Insertion]
ATH EDO GAT ETH ECA T_ _	[Insertion]
Frameshift (see page 4)	
HED OGA TET HEC AT_	[Deletion]

A mutation can further affect downstream processes leading to mutated mRNA and mutated (abnormal) proteins. These may further have effect on the body which may be **GOOD**, **NEUTRAL** or **BAD**.



I. BASE SUBSTITUTION MUTATIONS

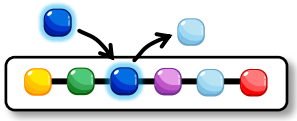
A mutation that results in a single base alteration: a base is replaced with another one.



Possible outcomes: (in coding regions)

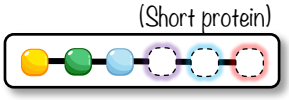
AMINO ACID CHANGE

Missense mutation
When a **DIFFERENT AA** is coded* for.



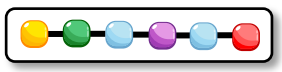
Nonsense mutation
When a **STOP CODON** is coded for. Cut of gene translation. Resulting in non-functional protein being made.

(Short protein)



NO AMINO ACID CHANGE

Same sense (Silent/Neutral) mutation
Sometimes a change in the sequence leads to **NO ALTERATION** in amino acid sequence because of the **DEGENERATE** nature of the DNA code.

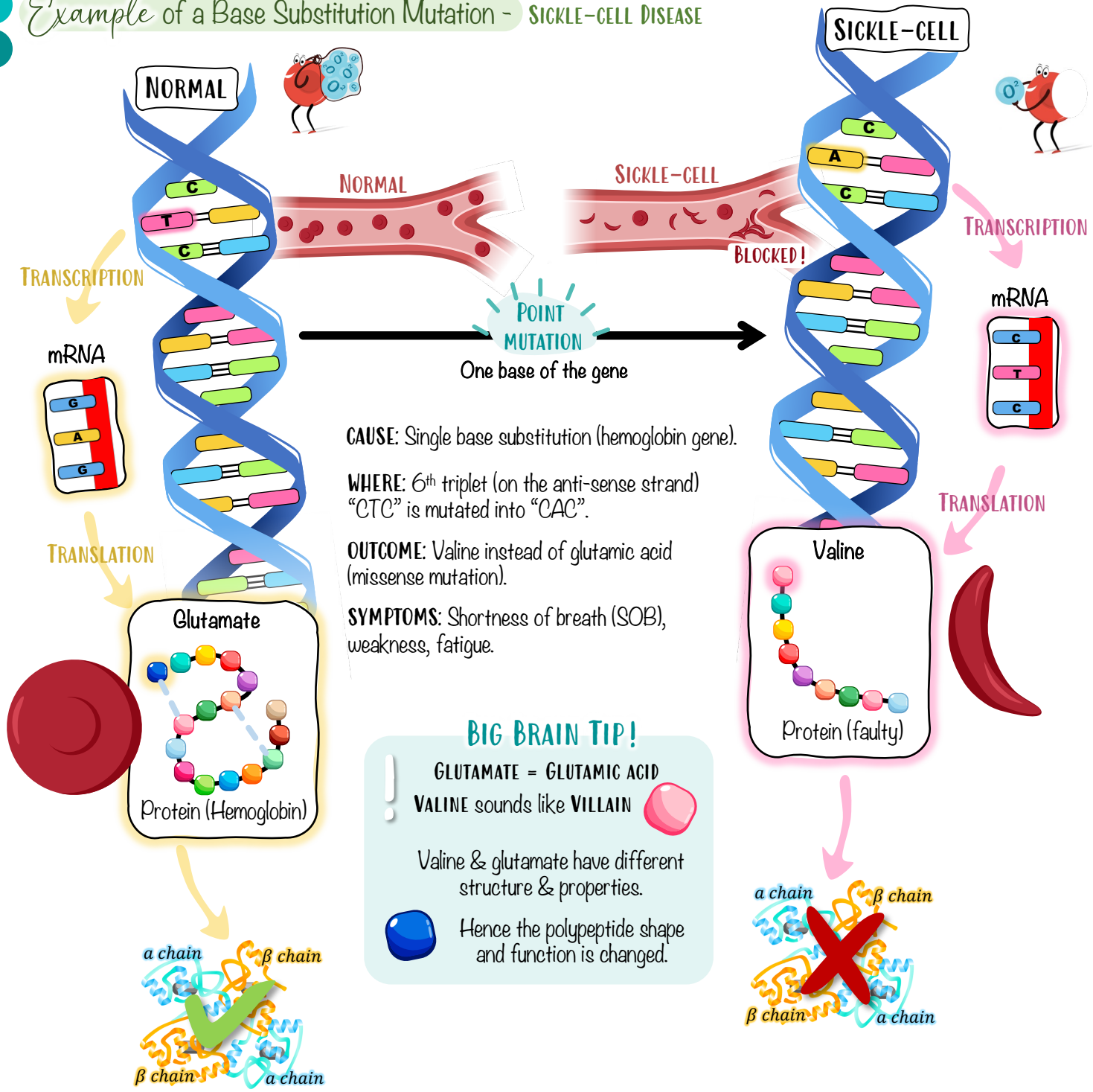


A variation in a single base pair in the DNA sequence:
SINGLE NUCLEOTIDE POLYMORPHISM (SNPs)

*see table in section D1.2 for genetic code

1.3 Mutations & Gene Editing

Example of a Base Substitution Mutation - Sickle-cell Disease



CAUSE: Single base substitution (hemoglobin gene).

WHERE: 6th triplet (on the anti-sense strand) "CTC" is mutated into "CAC".

OUTCOME: Valine instead of glutamic acid (missense mutation).

SYMPTOMS: Shortness of breath (SOB), weakness, fatigue.

Big Brain Tip!

Glutamate = Glutamic acid

Valine sounds like **Villain**

Valine & glutamate have different structure & properties.

Hence the polypeptide shape and function is changed.

Did you know?

Malaria is an infectious disease caused by the parasite *Plasmodium* and is transmitted by infected female *Anopheles* mosquitoes. The parasite attacks a person's red blood cells (causing symptoms such as high fever, chills and even death). Although sickle cell disease is a debilitating condition, those who have it are **highly resistant** to Malaria infection!

Mutations & Gene Editing

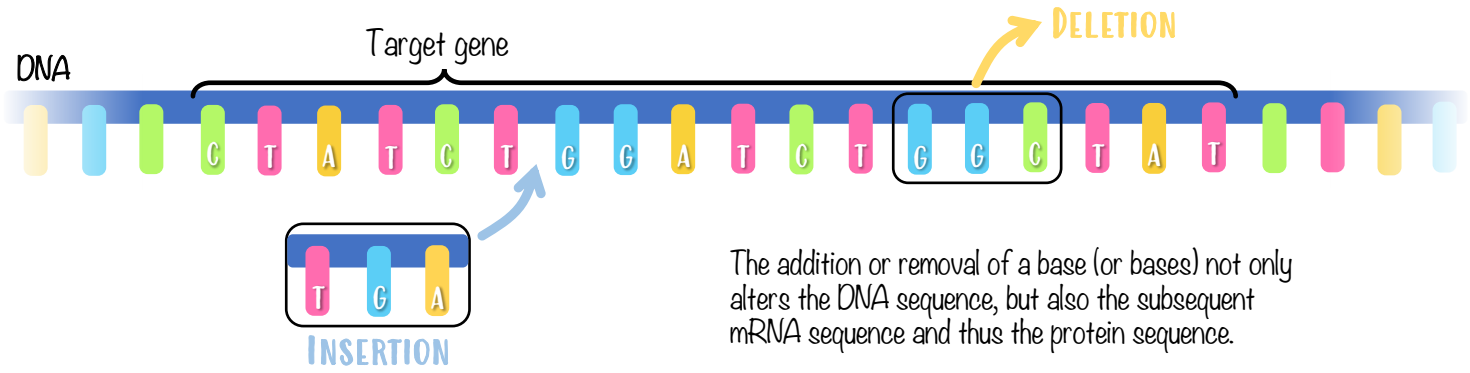
II. Deletions

Base(s) is/are removed from a sequence

III. Insertions

Base(s) is/are added to a sequence

Can be single or multiple nucleotides (here 3 are inserted and three and deleted)



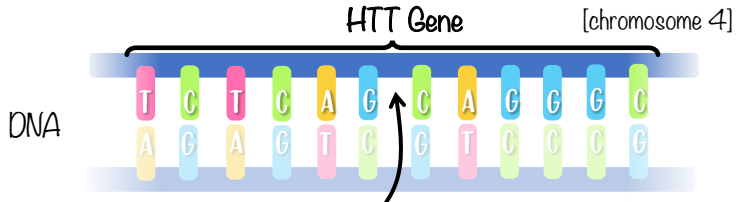
The addition or removal of a base (or bases) not only alters the DNA sequence, but also the subsequent mRNA sequence and thus the protein sequence.

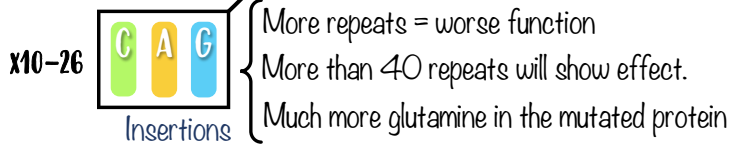
Example of an Insertion Mutation - Huntington's Disease

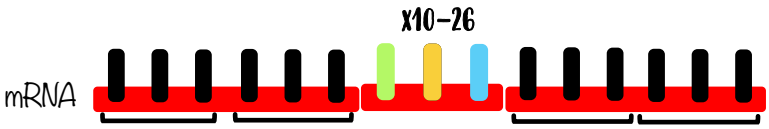
Trinucleotide repeat expansion

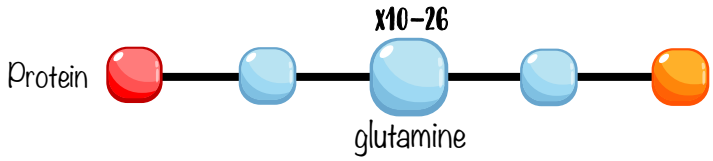
CAUSE: Insertion mutation. Multiple copies of **CAG** are inserted.
WHERE: Chromosome 4, specifically HTT gene.

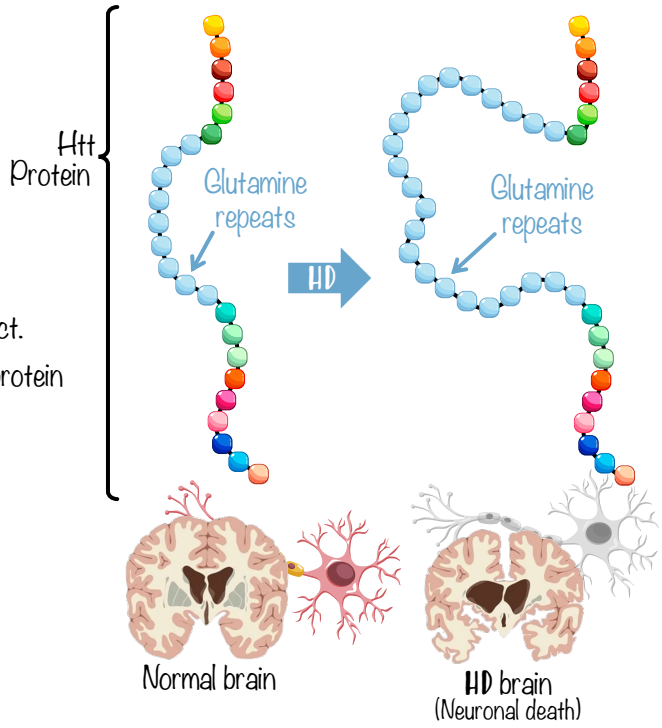
OUTCOME: Mutated protein (*mHtt*). Adverse effect on brain function.
SYMPTOMS: Difficulty walking, speaking and holding objects.











A normal brain contains CAG or glutamine repeats but not so many.
In a Huntington's disease patient's brain, there is an excessive amount of CAG or glutamine repeats.

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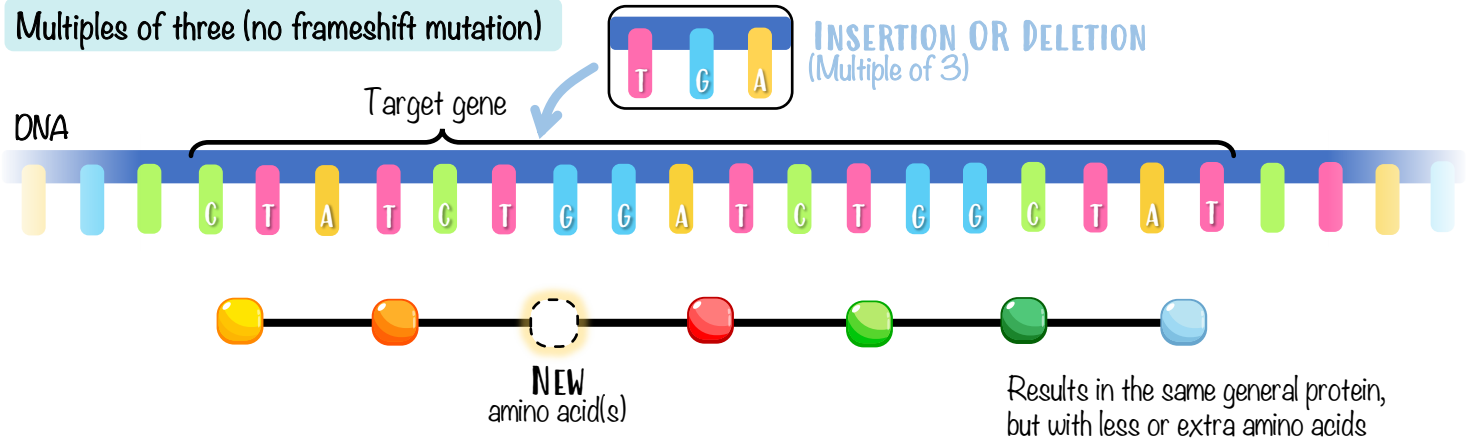
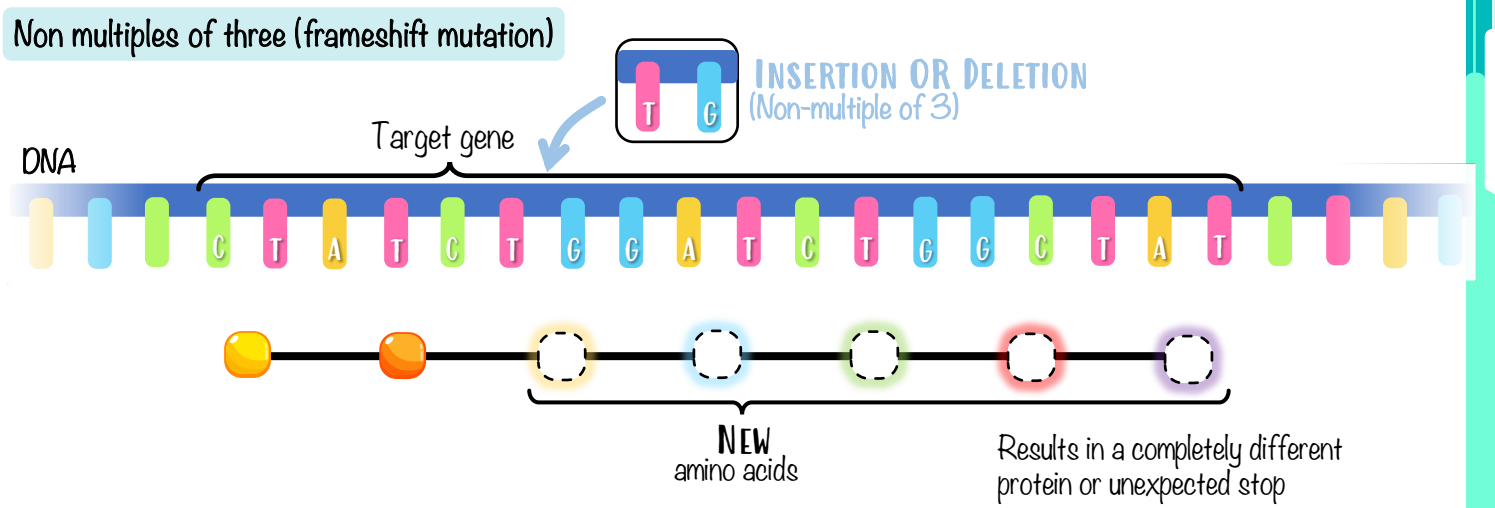
Frameshift mutations

A mutation resulting in a **change in reading frame**, resulting in a completely different translation from the original.

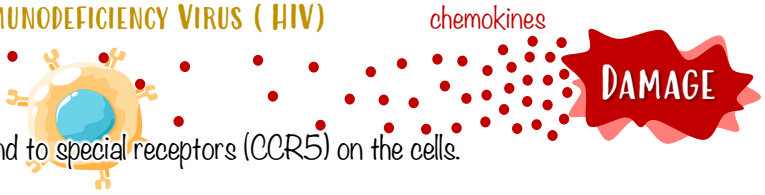
Multiples of three (no frameshift mutation)



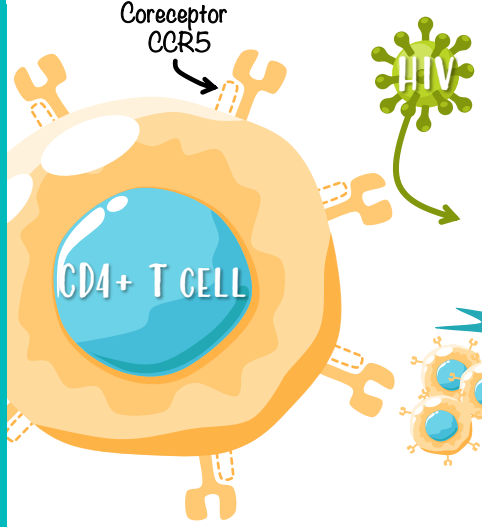
Non multiples of three (frameshift mutation)



Example of a Deletion Mutation - Human Immunodeficiency Virus (HIV)



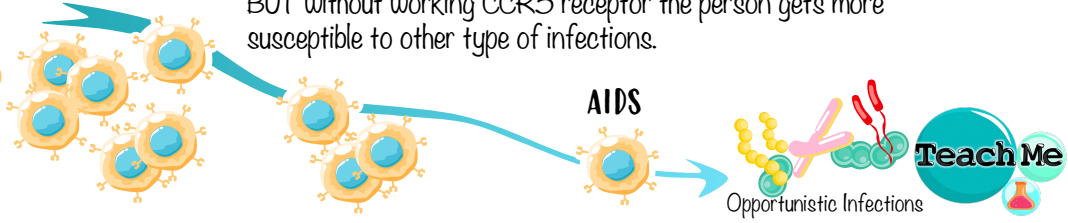
ROLE OF WBC: Migrate to sites of infection and fight infection.
HOW: Help of chemokines (molecules that attract WBC). They bind to special receptors (CCR5) on the cells.
DNA CODE: Gene on chromosome 3.



ENTRY OF HIV: HIV-1 can use CCR5 receptors as entry point (doorway) to infect and destroy CD4 T cells.

CONSEQUENCE: Slowly decrease in number of leucocytes. Eventually no longer able to fight off common infections: **AIDS** Stage.

MUTATION: Delta 32 mutation (CCR5-Δ32). Deletion of 32 nucleotides.
NOT multiple of 3, hence frameshift mutation (stop codon).
No proper CCR5 receptor --> Highly protected from HIV infection.
BUT without working CCR5 receptor the person gets more susceptible to other type of infections.



21.3 Mutations & Gene Editing

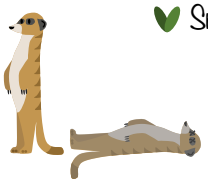
"MUTATIONS ARE ~~ONLY~~ BAD" → Disease → Not passed on (Evolution)

NEUTRAL → No effect

GOOD → Helpful adaptation → Better chance survival → Likely passed on

LONG TERM BENEFITS

- Mutations are important for evolution. needed for change.
- Needed as the world is constantly changing, we need to change to suit the environment for survival's sake.
- Having some individuals in a population that possess a slightly different genome can help species survive the changes (as a species).



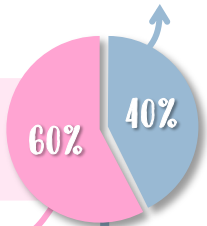
SHORT TERM BENEFITS

- CCR5-Δ32 (mutation preventing HIV)
- Lactose tolerance (see below)
- Smoking (some mutations can help reduce the harmful effects)

LACTOSE INTOLERANCE

100% Originally all mammals drank milk as infants and became lactose intolerent during adulthood as the body stops being able to break down the disaccharide lactose found in milk.

It is still the case for most people today, however as people depended more and more on dairy based agricultural products in the past 10,000 years, those who could handle lactose lived longer leading to their genetics being passed on.



MUTATIONS IN *Somatic cells* AND *Germ cells*

SOMATIC CELLS
(Body cells)

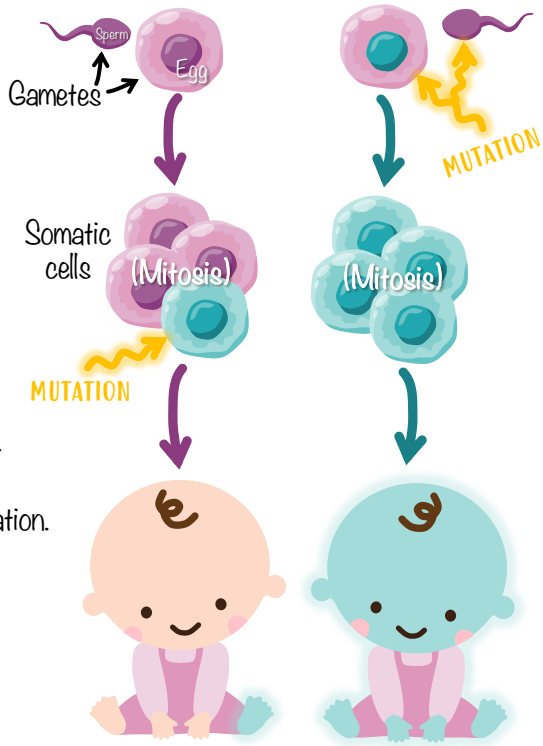
Mutation occurs in a somatic cell.

Mutated cell divides by mitosis.

Forming a clump of mutated cells. (tumor or cancer)

Affects the organ where mutated cell was found. It may metastasize.

NOT passed on to the next generation. (only affect the individual)



GERM CELLS
(Sperm & Egg)

Mutation occurs in a germ cell.
A cell that undergoes meiosis to make gametes.

Leads to the babies first cells (zygote) being mutated.

All the other cells of this baby will be generated from this cell.

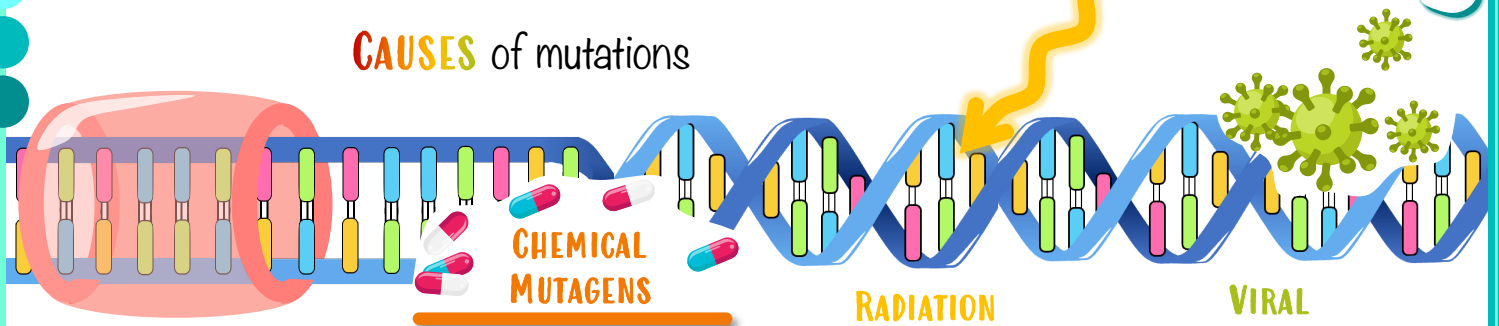
Passed on to the next generation (Inherited). Affect individual + offspring

BIG BRAIN TIP!

Every cell carries the mutation **BUT** only the cell that expresses that gene will be affected.

1.3 Mutations & Gene Editing

CAUSES of mutations



REPLICATION ERRORS

In DNA replication, DNA polymerase **PROOFREADS** to replace the incorrect bases with the correct ones.

BUT sometimes "**SPELLING MISTAKES**" can occur. Incorrect insertion of a nucleotide, missing nucleotides etc...

Potentially leading to **MUTATIONS** and thus altered gene function.

CHEMICAL MUTAGENS

Chemicals can interact with DNA molecules to cause mutations:

From WITHIN the cell
- Certain enzymes

From OUTSIDE the cell
- Benzene – Used to synthesize acetone, polystyrene & nylon.
- **TOXIC** and can cause leukemia (blood cancer). Industries need to minimize their employee's exposure. masks etc.

Chemicals also include smoking, alcohol...

RADIATION

IONIZING RADIATION

Example: x-rays, gamma rays

How: Cause formation of radicals which break DNA molecules.

NON-IONIZING RADIATION

Example: Ultraviolet (UV) light

How: Causes non-complementary bases to bind to each other. Leads to mutations and can increase the risk of skin cancer.

VIRAL INFECTIONS

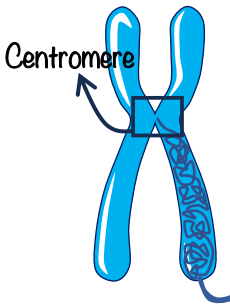
Some **VIRUSES** such as HBV, HCV, HPV, HIV can cause **MUTATIONS.**

HPV can insert a part of its genetic material into the host's genome which alters gene expression and can lead to uncontrolled replication of cells (known to cause cervical cancer).

Where are mutations found?
LOCATIONS OF MUTATIONS

(Mutations can occur anywhere that sequences of nucleotides can be found)

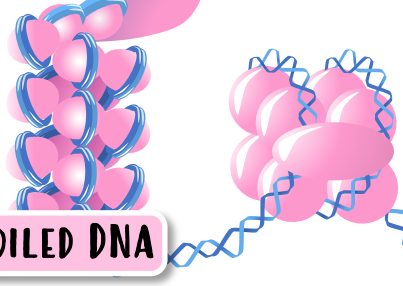
Mutation hotspots – zones where mutations are more frequent



CPG ISLANDS

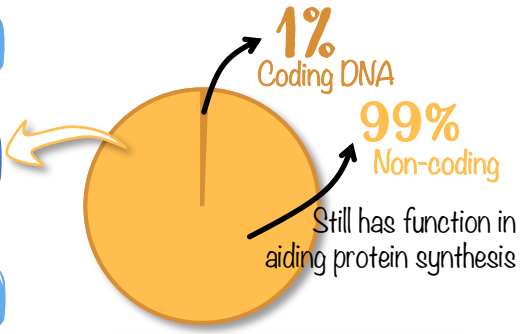
Cytosine followed by Guanine. Hotspots of repeat = CpG islands.

When methylation happens, the C can mutate into a T (associated with colorectal cancer).



UNCOILED DNA

Mutations are more likely, compared tightly coiled DNA (more exposed)



NON-CODING REGIONS

More likely for mutations to happen on the non-coding regions.

GENES WITH >1 COPY

>1 copy = greater change of being mutated. A gene that has two functioning copies can tolerate mutations in the extra copy without it influencing the cell as the original copy still exists.

notes