Cycle 4: DNA Mutations and Repair

What is DNA damage?

DNA damage is a naturally occurring process in living organisms. DNA can be damaged through exogenous or endogenous sources.

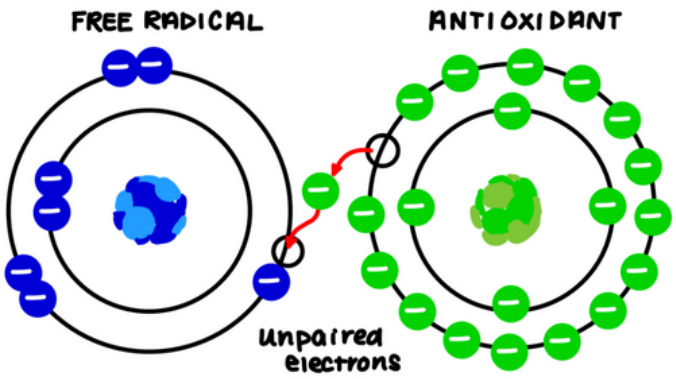
Exogenous: external factors that affect the living organism (ex. UV light, chemicals, ionizing radiation
Endogenous: factors that occur inside the cell (ex. DNA replication errors, reactive oxygen species)

Reaction Oxygen Species

Reactive oxygen species are free radical molecules in our bodies that can cause damage to DNA. For example: when ionizing radiation such as x-rays or gamma rays hit cells, the high energy rays can split molecules apart.

- For example, these rays can split O2 (•O•) in half in order to obtain O•
- This O• is unstable and highly reactive because it is missing an electron
- In search for another electron, O• will attack proteins or DNA to obtain an electron

As a result, the DNA can be damaged through a double-strand break. ROS can also come from single electrons traveling through the electron transport chain. Unpaired electrons can also attack O2, taking its electron and producing an oxide radical.



The Oxygen Paradox:

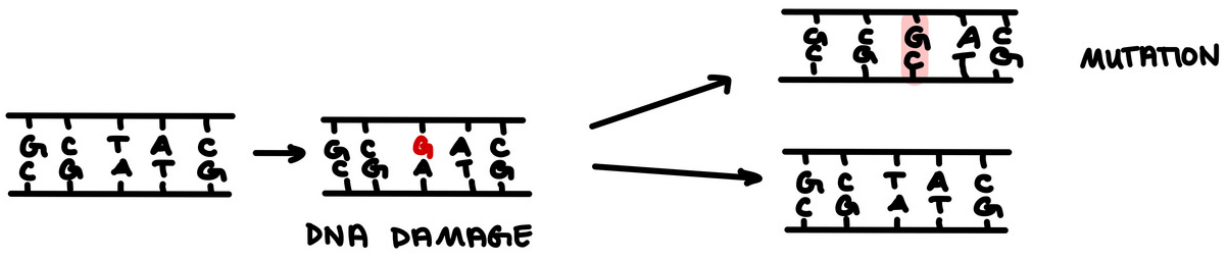
When our bodies have an excess amount of oxygen in our cells, it is referred to as **oxidative stress**. ROS is one cause of oxidative stress, however, free radicals can also form from diet, air pollution, toxins and more. Our bodies can help get rid of damaging free radicals by obtaining more antioxidants. **Antioxidants** will donate an electron to a free radical, making it stable again and preventing it from damaging DNA.

Damage vs Mutations:

Typically, DNA can be damaged by mutations but this is not always the case. DNA breaks can be classified by double or single stranded.

- **Mutation** = specifically a double stranded change in the base sequence of a region of DNA
- **DNA damage** = other changes that can occur in an organism's DNA that only affects one strand

DNA damage repair is important because if the damage persists, it can promote changes in the other DNA strand, causing mutation.

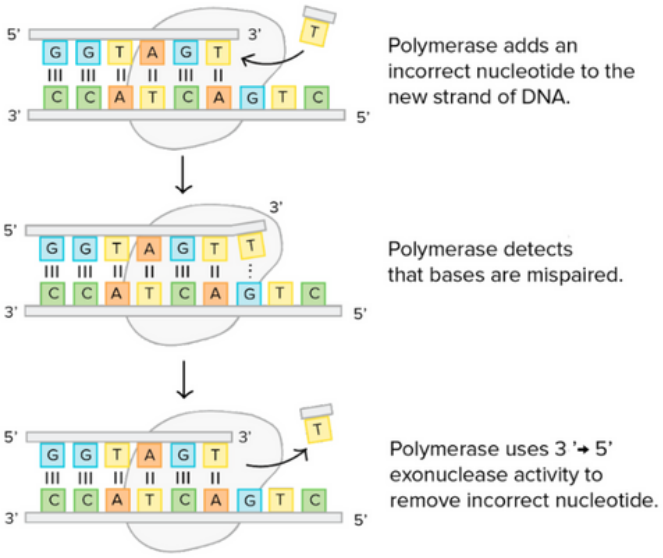


How is DNA damage recognized?

DNA damage will often cause a bulge in the backbone that is recognized by DNA polymerase. This will trigger DNA polymerase to fix its DNA synthesis mistake.

How is DNA damage repaired

Our body has many mechanisms of repairing damaged DNA. Four repair mechanisms are as follows:



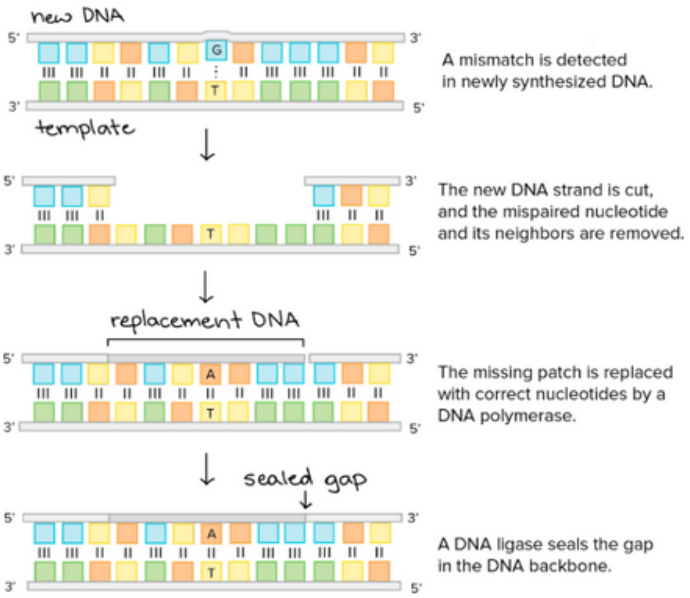
1. Proofreading: a mechanism for correcting errors made by DNA polymerase during replication

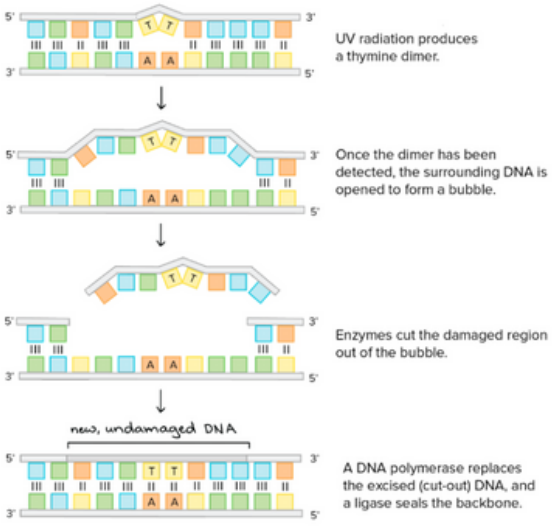
DNA polymerase's main mistake is base-pair mismatches (this is where the bases don't match)

- However, DNA polymerase has proofreading ability that allows it to correct base pair mismatches during DNA replication
- DNA polymerase can reverse its mistake by using a built-in 3'→5' exonuclease activity to remove mistakes
- DNA polymerase will then resume synthesis, inserting the correct nucleotide
- DNA polymerase III error rate is very low, therefore this mechanism is reliable

2. Mismatch repair: a mechanism for correcting errors made during replication that escape proofreading

- Sometimes DNA polymerase proofreading ability doesn't catch all errors, however this is very rare
- DNA polymerase can employ another method of repair called **mismatch repair**
  - The mismatch repair mechanism corrects 99% of errors
- It removes a DNA chain segment and its replacement with a newly synthesized segment, complementary to template strand
- To correct post replication mismatch damage, **mismatch repair proteins** detect the incorrect base, cut new DNA strand on each side of the mismatch, and remove a portion of the chain
- Repair DNA polymerase fills the gap with new DNA
- Mismatch repair is completed by DNA ligase who seals nucleotide chain into a continuous DNA backbone





3. Excision repair: a mechanism for correcting various kinds of DNA damage like those caused by chemicals and radiation

- Thymine dimers are not a type of mismatch because the correct AT base pair was synthesized
- Thymine dimers are caused by UV light hitting adjacent thymine bases, causing them to chemically bond and distort the backbone
- Excision repair is used to repair this damage, which removes the **erroneous base** and replaces it with the correct one
- When thymine dimers occur, DNA polymerase can't synthesize past distortion, therefore stopping replication
- In excision repair, proteins recognize the distortion and remove the DNA segment with thymine dimers
- Repair DNA polymerase and DNA ligase will resynthesize the DNA and seal the gap
- Some organisms are able to repair thymine dimers using white light and an enzyme called **photolyase**
  - However, since humans do not have this enzyme, humans use excision repair

4. Non Homologous End Joining: a quick but sloppy repair mechanism for double strand breaks Unlike the previous DNA damages highlighted above, double strand breaks are a lot more damaging to the cell than mismatches or DNA bulging. Double strand breaks are often caused by ionizing radiation that breaks both strands. NHEJ is a quick way to repair the double strand break, but it often can introduce mutations because it is sloppy.

If DNA damage is not repaired when it occurs, it may cause further damage or mutations in the next rounds of replication. Each of these repair mechanisms will recognize DNA errors, replace damaged DNA using new DNA synthesis or will seal new DNA to old DNA using DNA ligase.

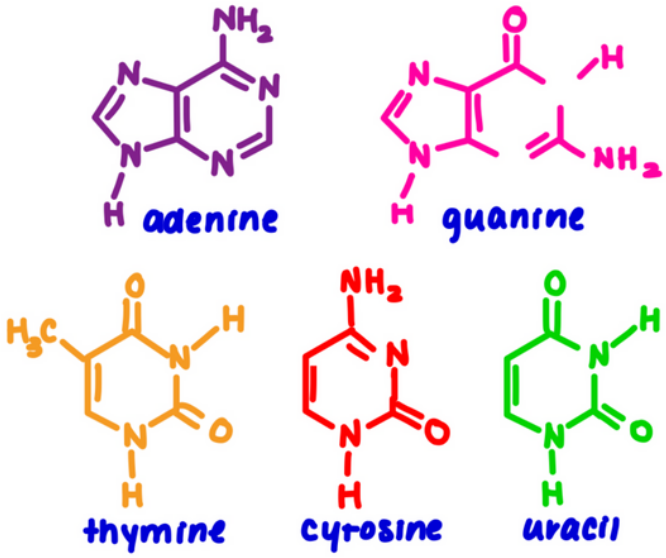
Summary of DNA Damage

Type of Damage	Mismatch Repairs	Thymine Dimers	Double Strand Breaks
Caused by	DNA synthesis mistakes	Damage by UV light	Ionizing radiation
Repair Mechanisms	proofreading repair, mismatch repair	excision repair	non homologous end joining

Differences Between Proofreading and Mismatch Repair

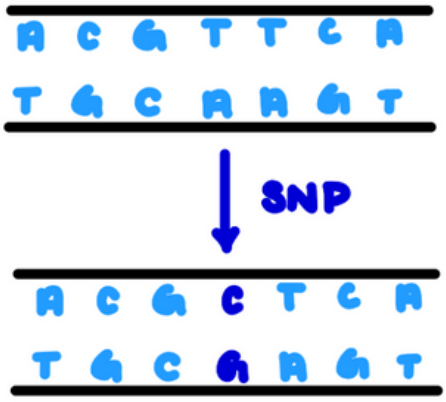
	Proofreading	Mismatch Repair
Who detects the error?	DNA Polymerase	Mismatch repair enzymes
Who fixes the error?	DNA Polymerase	DNA Polymerase
When does it do it?	Immediately	Immediately
How does it fit it?	- Uses its 3'-5' exonuclease activity - Reverses, removes base, corrects it and moves forward	- Enzymes cleave the backbone and remove it - Signal DNA polymerase to fill gap - Ligase seals nick

DNA Nitrogenous Bases



Single Nucleotide Polymorphisms (SNPS)

- Single nucleotide polymorphisms are a single base pair change in DNA
- Unlike DNA damage where only one base in the pair may be changed, a SNP is caused when an entire pair of bases is changed
- SNPs are the most common cause of genetic variation in people
- SNPs can give rise to new allele variations in individuals
- SNPs are associated with health and disease. A base pair change can cause:
  - Someone to respond differently to drugs
  - Susceptibility to toxins
  - Risk of developing certain diseases

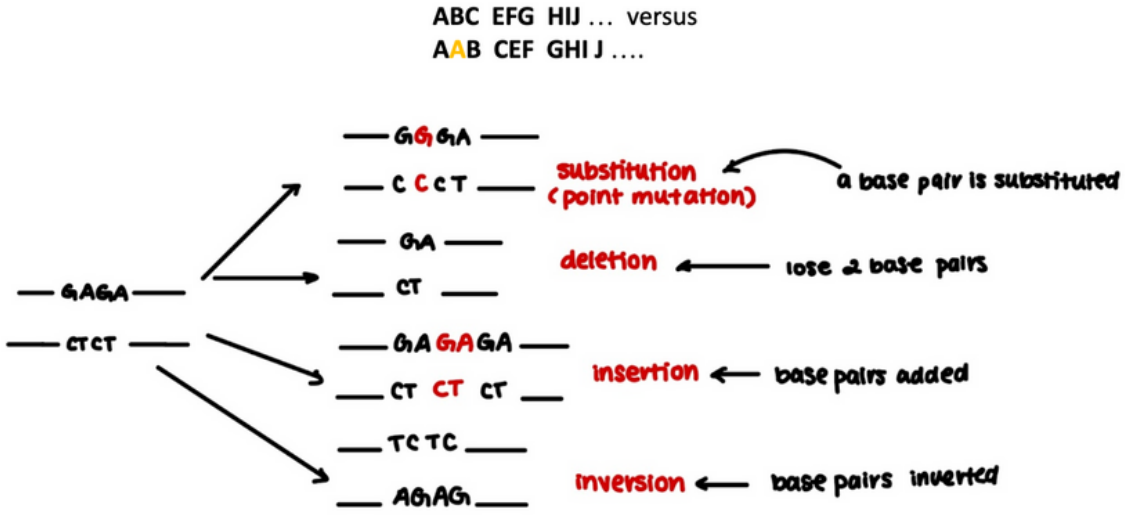


Types of DNA Mutations

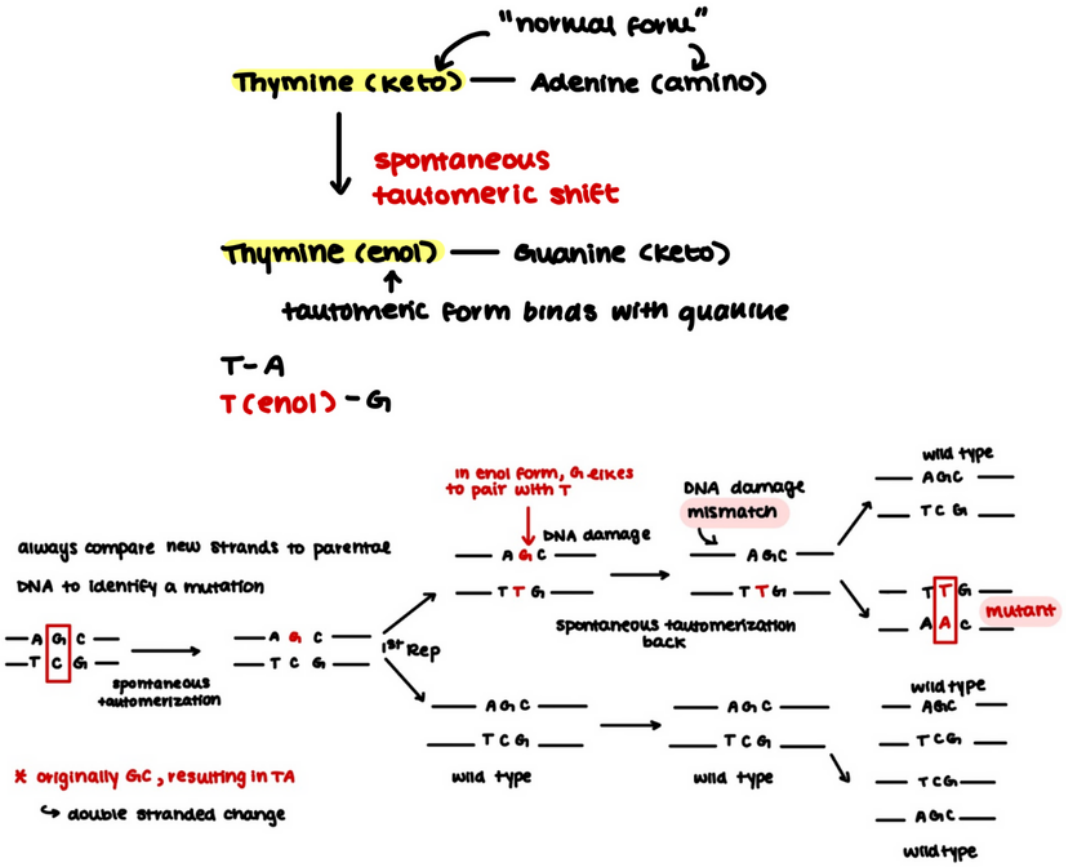
1. Substitution (point mutations)
   - Silent - a sense codon is changed to different sense codon, but the codon specifies the same amino acid as normal polypeptide so function is unchanged
   - Nonsense - a change in DNA codes for STOP codon (causes premature termination of protein). The location of the nonsense mutation will determine the effect
     - Translation of mRNA containing a nonsense mutation results in a shorter than normal polypeptide that will be only partially functional
   - Missense - a change in base pair codes for new amino acid
     - The protein structure will only change if the missense mutation occurred in a functional part of the protein
     - If the amino acid change doesn't influence active, binding, or functional domains, the protein won't be affected
2. Insertion - a base is inserted into a DNA strand
3. Deletion - a base is removed from a DNA strand

Note: DNA slippage can cause insertions or deletions

4. Inversion - the top and bottom strands are inverted and switch spots
5. Frameshift - a single base pair deletion or insertion in the coding region of a gene alters the reading frame of the resulting mRNA
   - After point of mutation, the ribosome reads codons that are not the same as the normal mRNA, producing a different amino acid sequence in the polypeptide from then on
   - Resulting polypeptide is non-functional because of significantly altered amino acid sequence changes up the 3 base pair order of codons



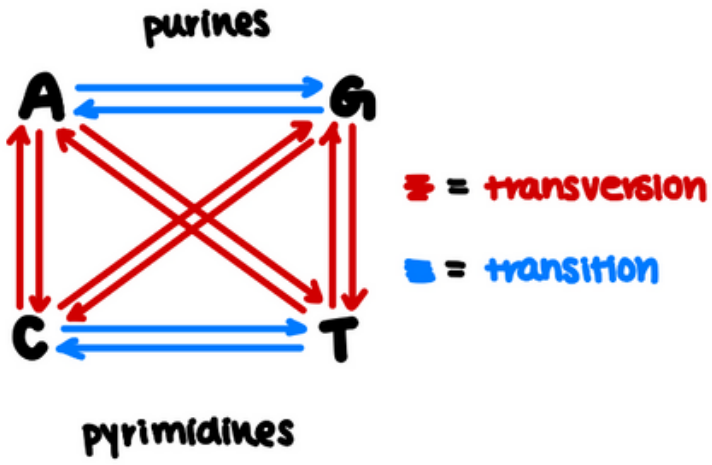
6. Tautomeric shifts - a tautomeric shift is where a nitrogenous base spontaneously rearranges into its isomer form
   - A nitrogenous base will go from the keto to enol form or amino to imino form
   - These spontaneous shifts can cause mismatching and mutation



Transition or Transversion Mutations

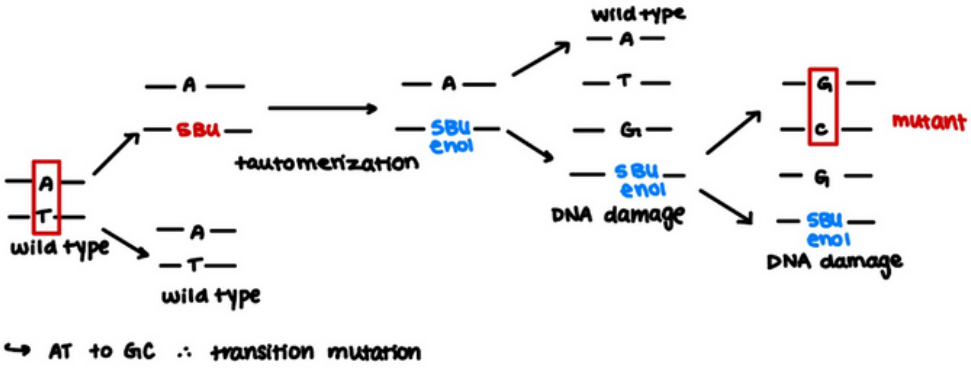
All mutations can be classified as a transition or a transversion mutation

- **Transition mutation** - a mutation where a purine (A to G) is changed to another purine, or a pyrimidine is changed to another pyrimidine (C to T)
- **Transversion mutation** - a mutation where a purine is changed to pyrimidine or pyrimidine is changed to a purine

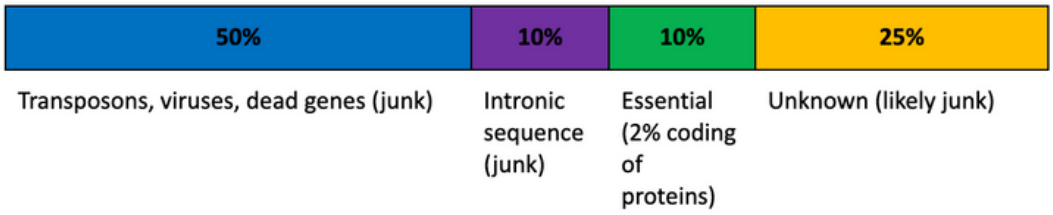


Spontaneous or Induced Mutations

1. **Spontaneous mutations**: mutations arising from small errors in DNA synthesis
2. **Induced mutations**: caused by physical, chemical or biological agents called mutagens. Production of mutagens in a lab by exposure to a mutagen is called mutagenesis and the treated organism is said to be mutagenized. One of the first mutagens used in research was high-energy ionizing radiation such as x-rays. Exposure to this type of radiation creates reactive oxygen species (ROS) in cells, causing double-stranded breaks in chromosomes. Repair of the breaks that are imperfect, resulting in mutation
3. **Synthetic chemical mutations**: caused by mutagens. For example: 5-bromouracil (5BU) is called a base analogue. A base analog is a molecule that is composed similarly to a base/ 5BU is a base analog for thymine, and is highly unstable causing it to switch between its regular and ionized form. This can lead to mutation



What does our DNA sequence encode?

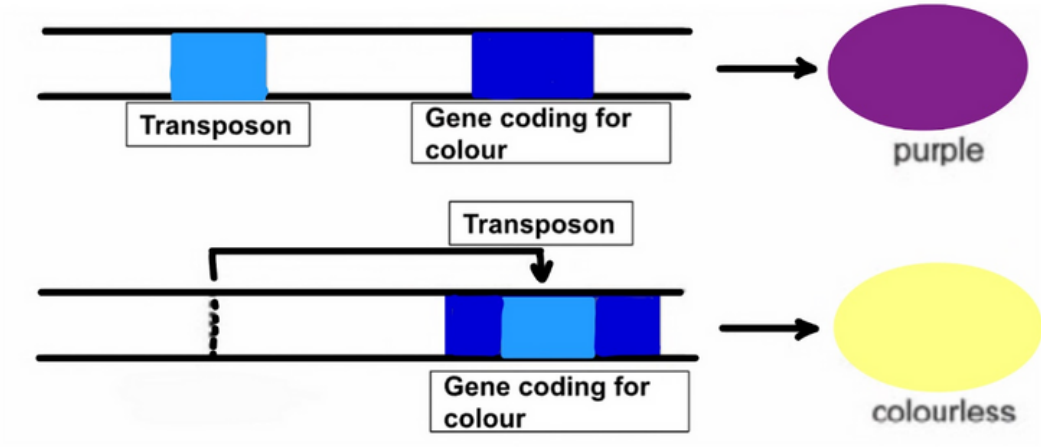


Transposable Elements

As seen above, a large part of our genome encodes for transposable elements

1. **Transposable elements:** small regions of the genome that can 'jump' from one location of the genome to the other
2. **Transpositions:** the process of moving from one area of the genome to another

Transposable elements were found by an individual named Barbara McClintok. McClintok did research on corn, and was wondering why some corn kernels were different colours. McClinktok observed that genes coding for kernel colour were interrupted by transposable elements, causing some kernels to be different colours. It was found that when a transposon jumps and inserts itself into the colour gene, you obtain light coloured kernels. When a transposon excises itself out of the gene, the gene can then code for colour and will create dark kernels.



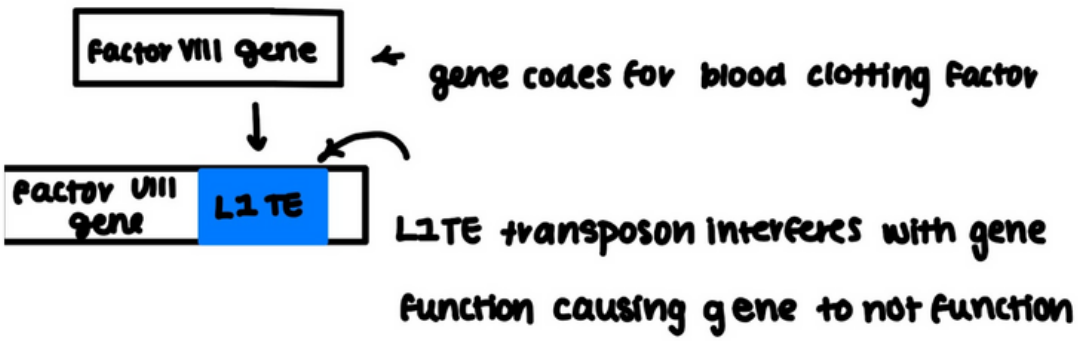
Transposable Elements Increase Genome Size

The majority of DNA sequences are transposable elements, making up most of our genome. For example, 50% of the human genome consists of transposable elements. Other organisms that have very large genomes are primarily transposable elements. Transposable elements will tend to insert themselves in “safe havens” which are places that do not interfere with gene function. However, they may also insert themselves into gene coding regions which can cause the gene to not function. It is found that most transposable elements are “dead” due to mutations in the transposable elements that inactivate them and prevent them from transposing

Transposable Elements Are Seen as Mutagens

A TE can act as a mutagen depending on where it inserts itself in the genome. If a TE lands in a non coding region, the TE usually does not have an effect. However, if a TE lands in a protein coding region, it can cause issues.

Example 1: if a TE inserts itself in the factor VIII gene coding for a blood clotting factor, the TE will disturb the gene and the individual will not be able to create blood clots
Example 2: a TE can lead to gene shuffling → when a TE excises itself to move from one gene to another, it can take some of the gene with it
Example 3: a TE can cause genes to be under the control of different promoters → when a TE inserts itself into a gene, it can cause the gene to be controlled by a different promoter, which may express the gene more or less



Diseases are Multifactorial

Many of the situations addressed above can contribute to disease in organisms. It is important to note that not one single mutation will cause a disease, but often multiple contributing factors. Diseases are multifactorial in that many different SNPs can cause them.