DNA RECOMBINATION, REPAIR, AND MUTATIONS

22. GENE MUTATION AND DNA REPAIR

Describe how DNA mutation occur

DNA is susceptible to spontaneous changes if left unrepaired. Guanine is more susceptible to oxidative damage. Uncontrolled methylation may occur in the bases.

Hydrolytic attack cleaves the chemical bonds in DNA. There are two types:

1. Depuration: loss of purines (G and A) from the DNA
2. Deamination: Converts C to U (other bases too)

Covalent linkage may occur between any two adjacent pyrimidine bases. This is damages caused by UV radiation from the sun. It may cause sunburn, melanin production, and melanoma.

Review how DNA is repaired

The double helical structure of DNA allows it to be repaired. When one strand is damaged, the complementary strand still has an intact copy of the same information. Thus, this can be used to restore the correct nucleotide to the damaged strand.

DNA can be repaired by excising the damaged nucleotide sequence, restoring the sequence via complementary strand, and sealing the break with ligase.

1. **Base excision repair**
   Base excision repair is used to remove SMALL non-helical, distorting lesions. ***DNA glycosylase*** will recognise the specific altered base, and catalyse its hydrolysis, hence breaking its bond with its complementary base. It will then flip out the altered nucleotide.
   AP endonuclease will recognise the missing tooth and cut the ***phosphodiester backbone***, thus allowing ***DNA Polymerase*** to add the correct nucleotide.
   DNA Ligase will seal the nick.

2. **Nucleotide excision repair**
   Nucleotide excision repair is used to remove LARGE changes to the DNA helix. These bulky lesions include pyrimidine dimers caused by UV radiation.
   A ***large multienzyme complex*** will scan the DNA for distortions in the double helix rather than for specific altered bases. Once found, it will cleave the ***phosphodiester backbone*** of the abnormal strand.
   DNA helicase will peel away the single stranded oligonucleotide containing the lesion, thus allowing repair by ***DNA Polymerase*** and ***DNA Ligase***.

DNA repair occurs **before transcription** to ensure the coding regions of the newly synthesised mRNA are correct. This is called ***transcription-coupled repair.***

Emergency repair occurs when DNA is heavily damaged. As we know, DNA Polymerase is highly accurate. When it encounters damage, it will stall, thus allowing ***translesion polymerase*** to replicate through the damage. They will only add a few nucleotides before they dissociate. This is very risky as it is responsible for many mutations.

What happens in a case where **both** strands are broken or defected. This severe break can be caused by ionising radiation, replication errors, oxidising agents, and other metabolites produced in the cell. If not repaired, the chromosome will break down, and the cell will break down. The problem is that there is no complementary strand to help restore the damaged strand.

Non-homologous end joining can be done by bringing broken ends together via **DNA ligation.** However, there is a loss of one or more nucleotides at the joining site.

Link repair defects and human disease
Nucleotide excision repair enzymes damaged = Xeroderma pigmentosum (autosomal, recessive disease which puts people at risk of developing skin cancers)

Defect in transcription-coupled repair = Cockayne syndrome (rare, autosomal, recessive congenital disorder, which causes growth retardation, neural retardation, severe sensitivity to sunlight, cell apoptosis). These RNA Polymerases become permanently stalled at sites of DNA damage.

23. DNA RECOMBINATION
Explain how double stranded breaks can occur
Environmental damage: Double stranded breaks occur from radiation and reactive chemicals
DNA replication: Most of the time, they arise from DNA replication forks that become stalled or broken (accidents occur during nearly every round of DNA replication).

One way this can happen is when a single stranded nick occurs. When the replication fork reaches the lesion, it falls apart, - leaving one broken and one intact daughter chromosome.

Homologous recombination can solve this by using the ***undamaged duplex as a template*** to restore the original DNA sequence. This takes place after DNA replication by before the cell divides.

Understand what homologous recombination is and what happens when things go wrong.
Homologous recombination refers to the exchange of genetic material between two homologous chromosomes. This process is crucial for repairing double-strand breaks. It can also be used for genetic exchange: alter versions of genes present, alter the timing and expression of genes, and produce genetic variation in offspring.

Repair can only take place after a DNA strand from one DNA helix is freed from pairing with its complementary strand. This occurs when the replication fork encounters a DNA nick and an ***exonuclease*** degrades the 5’ end, resulting in an unpaired single strand. To be honest, I don’t know exactly how that works, but all you have to know is:
DNA single strand nick = 5’ end of freed strand degraded, and 3’ end unpaired. How do we fix?
Strand Invasion