CYTOLOGY

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◉ Sheet ○ Slides

Number

10

Done by:

Lana Khatib and Dena Kofahi

Corrected by:

Dena Kofahi

Doctor

Dr. Mamoun

DNA Repair

DNA needs to be free of all errors and mutations in order to function as the control center of the cell; especially in gametes which are inherited.

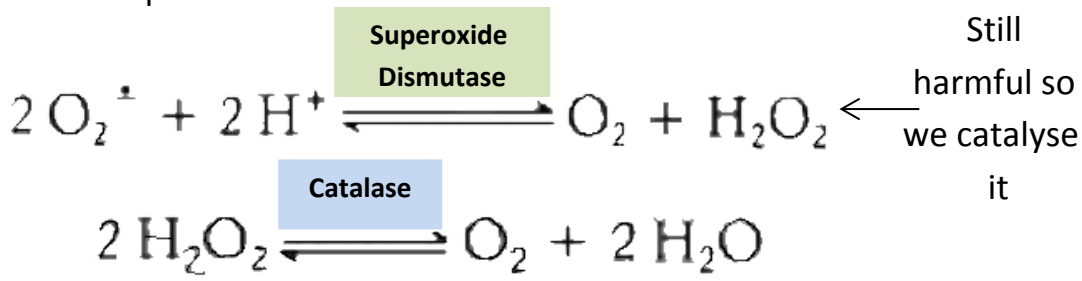
There are many ways in which DNA corrects these mistakes:

1. **Prevention of errors before they happen**
2. **Direct reversal of damage**
3. **Excision-repair pathways**
4. **Base Excision Repair**
5. **Mismatch Repair System**
6. **Translesion DNA synthesis**
7. **Recombination repair**

It must be noted that these different mechanisms can be used by the cell to fix the same mutation. The cell will not limit itself to only one mechanism.

Let's see how this happens:

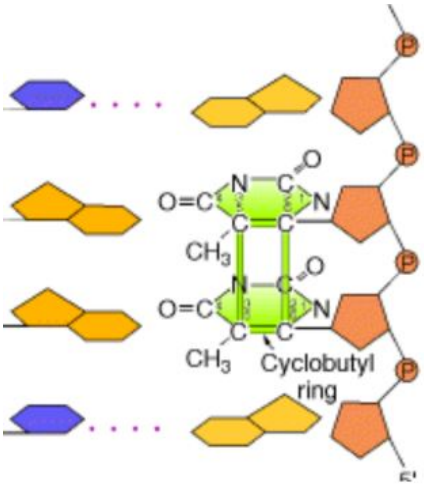
1. **<u>Prevention of Errors:</u>**
   The cell prevents damage from reactive oxygen species by enzymatically catalyzing them before they can reach DNA.
   This is done in two steps:



   - <u>Why are reactive oxygen series dangerous?</u>
     - They can easily oxidize/steal electrons from molecules such as DNA and damage it. Free radicals are an example of these reactive oxygen series and are especially dangerous as they have an unpaired electron and will strongly react.
   - <u>How does the cell end up with these reactive species in the first place?</u>
     - These reactive oxygen series are normally produced in cells due to metabolism. Additionally, they are used in immune cells to attack pathogens.

Cells can make reactive oxygen series more safe for the cell, but this is not enough to prevent mutations as there are still other mutagens. Cells must have other methods.

2. **<u>Direct Reversal of Damage:</u>**
   A quick reaction that occurs enzymatically to immediately and directly reverse the damage that occurs

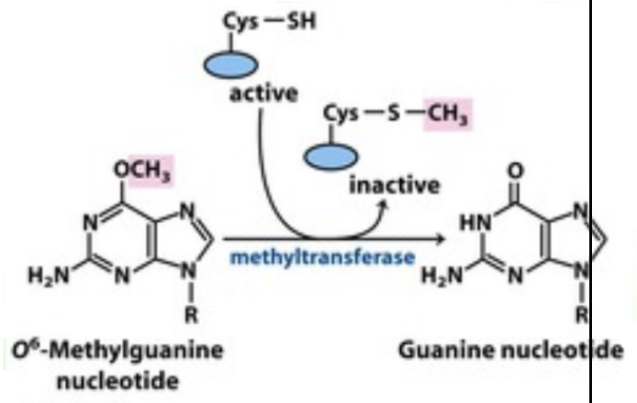


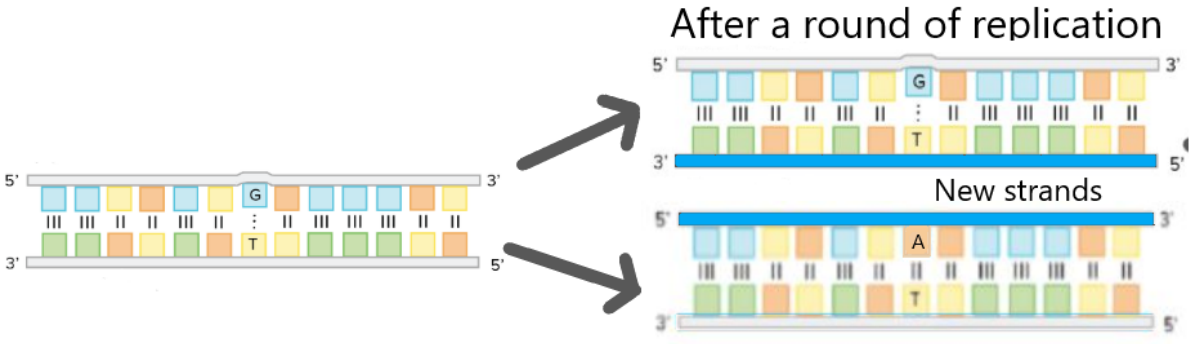
a) <u>Cyclobutane Pyrimidine</u>

- UV rays damage results in the formation of **cyclobutane pyrimidine dimers**, two adjacent pyrimidines of the same strand covalently bonded together. This results in lesions (=damage) that DNA and RNA polymerase cannot read, stopping synthesis. One example is thymine dimers (occurs most frequently).
- This mutagenic photodimer can be repaired by photolyase found *only* in bacteria. Humans must use other methods to fix photodimers (such as general excision repair or post-replication repair, to be discussed later).
- Note that the green bonds in the figure are abnormal covalent bonds between nitrogenous bases of pyrimidines.

b) <u>Repair of O6-Methylguanine</u>

- When methyl is bound to a guanine it forms **06-methylguanine,** which results in it pairing with thymine rather than cytosine. If a second round of replication occurs, it causes an adenine to take the place of guanine (due to the thymine).
- It is enzymatically removed by methyl transferase. The damage of the alkylating agent is now reversed.

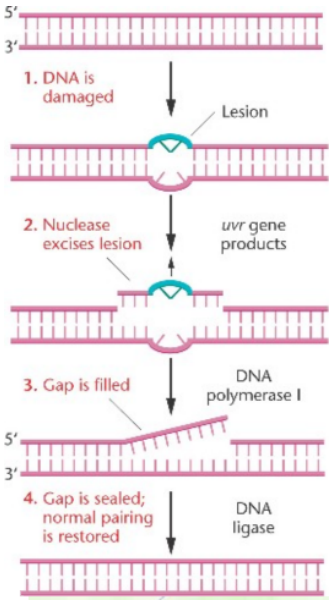




3. Excision Repair Pathways:

The excision repair pathways are different mechanisms with the same function: excise (cut) a region of DNA using enzymes as scissors. There are 4 types:

General Excision Repair, Coupling of Transcription and Repair, Specific Excision Pathways, and Mismatch Repair

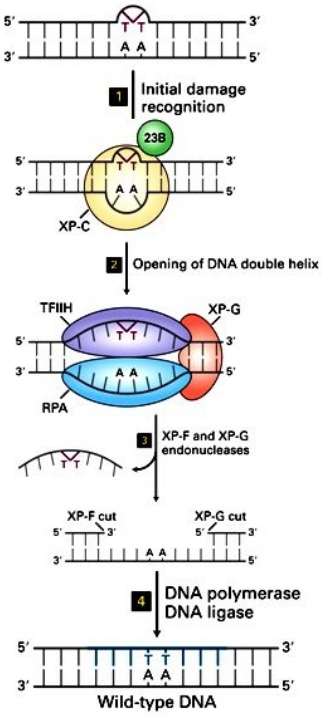


a) General or Nucleotide Excision Repair:

In bacteria; the process is controlled by the UVRabc proteins. When DNA is damaged, this complex of three proteins (UVRa, UVRb, and UVRc) is formed to:

i. Recognize the location of the damage
ii. Then, the segment (oligonucleotide) is excised.
iii. Helicase separates the strands.
iv. The gap is filled by DNA polymerase 1.
v. Finally it is connected by DNA ligase.

- ***In humans*** the process is more complex; however the basic steps are similar to those of E.coli. We have XP proteins, named from (XP)A to G, that take the role of the UVR complex.
- There are many functions for the XP proteins such as damage recognition and enzymatic functions (endonuclease and helicase activity). More XP proteins include:
  - TFIIH (Transcription factor 2H) which functions as a helicase that unwinds the cleaved strand.
  - Replication Protein A (RPA) which protects undamaged DNA.
    - How? RPA acts as a single stranded binding protein that prevents the undamaged strand from being cleaved, collapsing, or forming a hairpin structure.



- DNA Polymerase can fill in the gap and DNA Ligase can form phosphodiester bonds

- And if this process is not working it results in **xeroderma pigmentosum** (derma=skin, pigment=color), a skin cancer that results from UV rays causing mutations in genes that code for XP proteins.

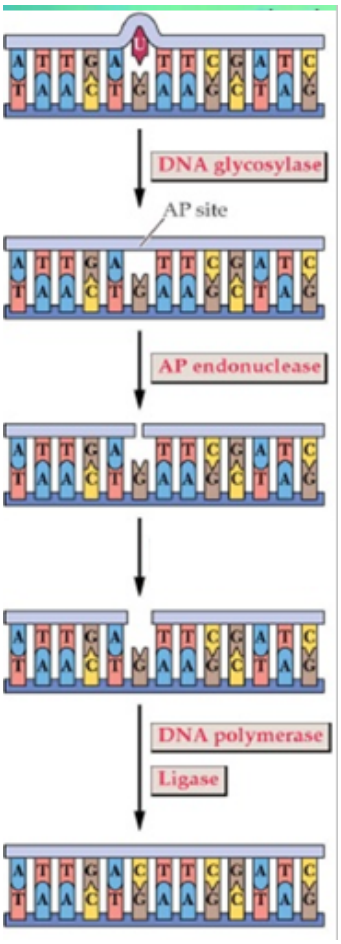
b) Coupling of Transcription and Repair

In both eukaryotes and prokaryotes, cells have a preference to repair the transcribed strand of DNA for actively expressed genes.

Cockayne's syndrome: This condition occurs when CSB protein is mutated. Normally, CSB recognizes that RNA polymerase has stopped transcription due to damaged DNA and recruits XPA, RPA, and TFIIH. When mutated, however, it cannot recognize there is a mutation and transcription can not be completed as the problem cannot be fixed.

Specific Excision Repair

Involves endonucleases and exonucleases that specifically target certain mutations and repair them.



4. DNA Glycosylase Repair Pathway (Base Excision Repair)

- Involves one of the most important proteins in DNA repair, **DNA glycosylase**, which cleaves the glycosidic bond (the bond between base and sugar).
- Numerous DNA glycosylases exist. Example: Uracil-DNA Glycosylase –> Removes Uracil from DNA
- Mechanism:
  - The glycosylase removes a damaged or inappropriate base (like Uracil in DNA) by cleaving the **N-glycosidic bond**. This releases the base and generates an apurinic or apyrimidinic site (both are called AP sites).
  - The resulting AP site is then repaired by an AP endonuclease repair pathway.
    - The AP endonuclease cleaves the **phosphodiester bonds** at AP sites. The sugar and phosphate are then removed.
    - DNA polymerase fills in the gap.
    - DNA ligase reforms the bond.
- What is the difference between this mechanism and nucleotide excision repair?
  - In base excision repair, we remove only the one mutated nucleotide. In nucleotide excision repair, we remove not just the mutated nucleotide but also the unmutated nucleotides around it.

How can Uracil become a part of DNA?

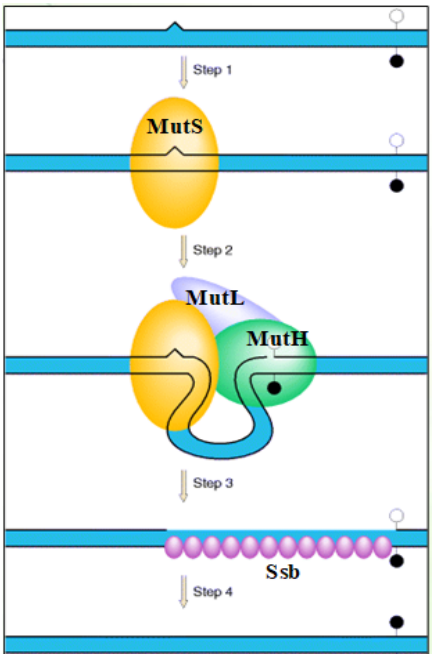
Uracil residues result from the spontaneous deamination of cytosine or if DNA polymerase makes a mistake and accidentally adds a uracil.

What happens if uracil remains?

First, what would've been a G (due to the original C) becomes an A (due to the new U that took C's place). Then, when another round of replication occurs, instead of having a C where it was originally, we now have a T (due to the A).

Post-replication Repair

The mutation stays as is during replication and is fixed after.



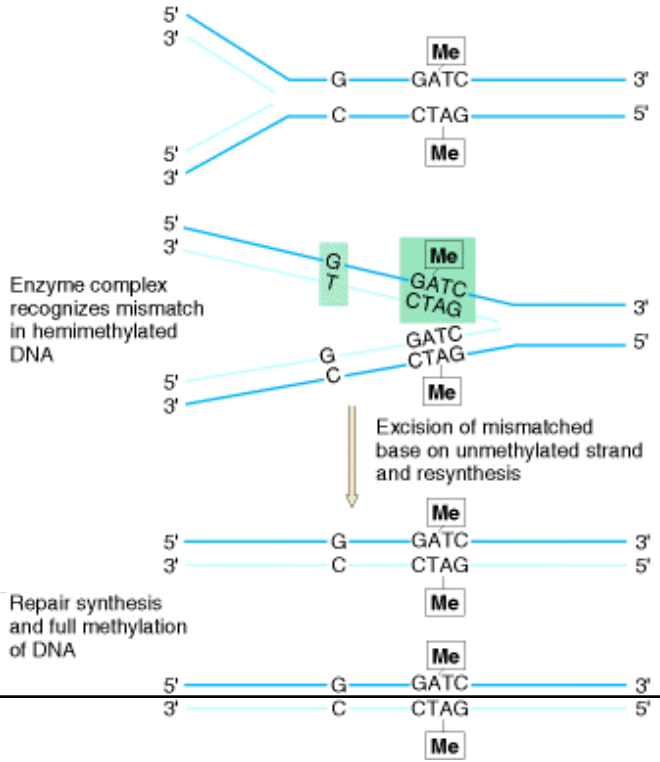
5. Mismatch Repair System (Prokaryotes)

- Mechanism
  1. **MutS** recognizes the mismatched base pairs and recruits other Mut proteins (MutL and MutH).
  2. The Mut proteins then work together to create a single nick within the DNA molecule through ***endonuclease*** activity.
  3. Then, ***exonuclease*** activity starts from the site of cleavage and removes/digests the lesion.
  4. Single stranded binding proteins keep the strand as is.
  5. DNA polymerase then fills the gap.

- How does the mismatch repair system determine which base is incorrect (like if I have a G and a T incorrectly paired, how does it know which is the mutation)?

Through DNA methylation! How?

Bacterial DNA has some of its adenines methylated following replication by the enzyme adenine methylase. However, it takes the adenine methylase several minutes to methylate the newly synthesized DNA. The mismatch repair system in bacteria takes advantage of this delay to repair mismatches.

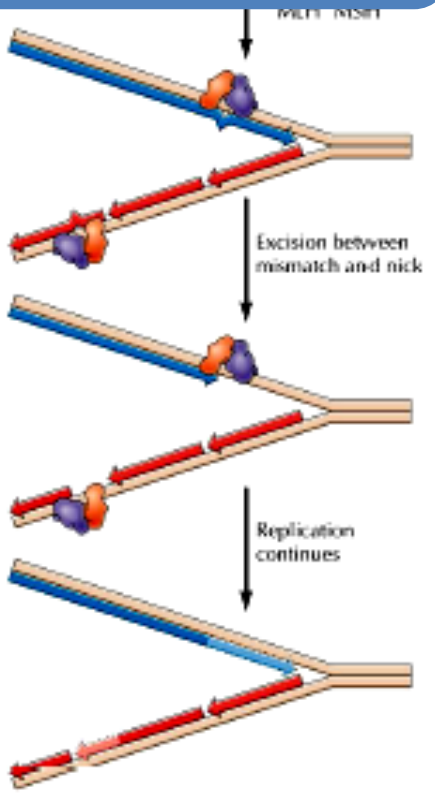


- The old strand already has it's A methylated.
- The newly synthesized strand is not methylated. So, when MutS recognizes the mutation, it will know this is the strand that has the nucleotide that must be removed.

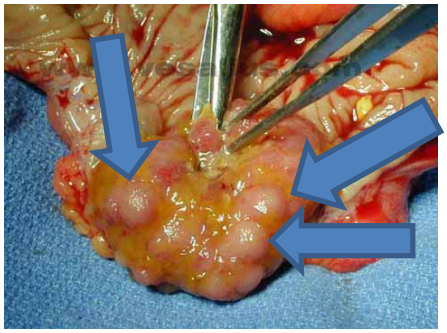
ensures mechanism proofreading the ,Basically. methylation <u>before</u> mistakes no theres

The "pimples" are tumors (abnormal masses of cells). They become cancerous if the cells become malignant.

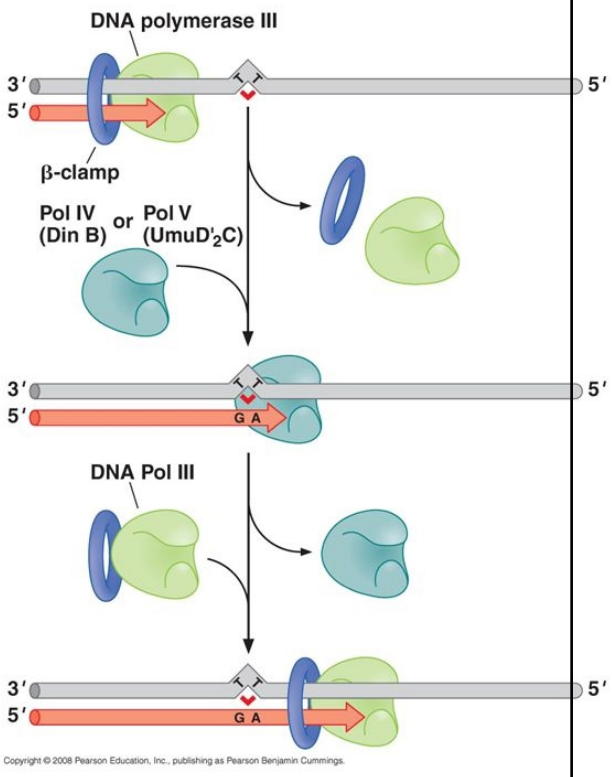
- <u>What about in humans?</u>
  - Humans have their own mismatch repair system.
  - Two of the proteins, hMSH2 and hMLH1, are very similar to their bacterial counterparts, MutS and MutL, respectively.
  - They work during DNA replication to recognize the mutation and make a nick. This is followed by exonuclease activity to remove the damaged portion and DNA polymerase can resynthesize the strand (whether it's from a leading stand or lagging strand).



- **Hereditary Nonpolyposis Colon Cancer (HNPCC)**
  - Occurs in 15% of colon cancer cases.
  - 50% of cases of HNPCC are caused by a mutation in MSH while most of the remaining cases are caused by mutated MLH.



6. Translesion DNA Synthesis (Prokaryotes and Eukaryotes)



If the normal DNA polymerase reaches a lesion, it will not know how to insert a nucleotide and stops. Therefore, it is released and specialized DNA polymerases "jump" over the lesion and synthesize DNA over the lesion

- Ex in E. Coli: DNA polymerase III stops synthesis and is released at the site of mutation and another DNA polymerase (like Pol IV or Pol V) can jump in and insert any nucleotide.

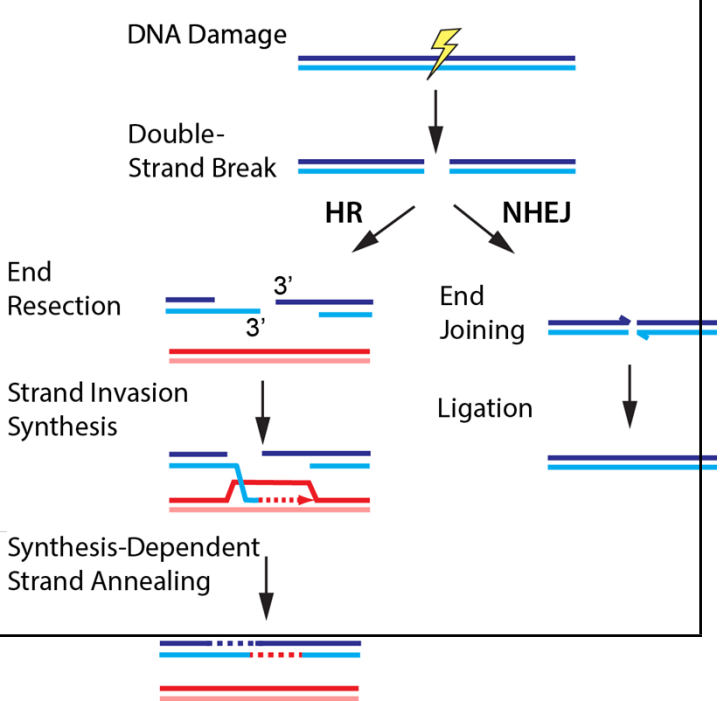
➢ <u>What's the problem with these specialized DNA polymerases?</u>
- Although they display some selectivity in base insertion, they have low fidelity and are error-prone.

➢ <u>So why does the cell let them add the base?</u>
- If a base isn't added, the cell will have to kill itself. To prevent that, the polymerase will add the base and hope it's corrected later.

7. Recombinational Repair

If DNA is severely damaged, like when hit by an radiation (ex: X-ray), a double-strand break may occur. When this happens, recombinational repair takes place by one of two mechanisms:

1. **Non-Homologous End Joining (NHEJ)**
   - This fixes the DNA by joining the two ends together.



- The issue is it creates mutations (insertions and deletions).

2. **Homologous repair with the undamaged chromosome**

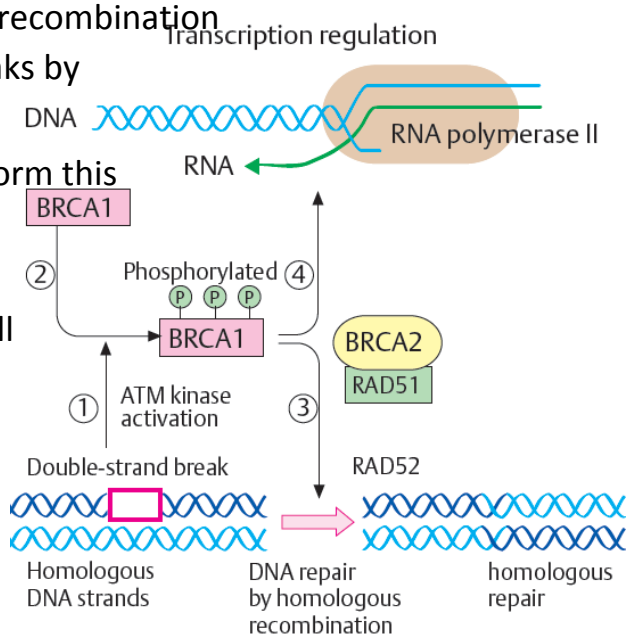
More Accurate

- *Only* occurs when homologous chromosomes are lined up next to each other.
- It involves a protein called Rad51.
- The undamaged chromosome is translocated (moved) so that the damaged chromosome could use it as a template that can be filled.

➢ **Breast Cancer**

BRCA = Breast Cancer

- BRCA1 activates homologous recombination repair of DNA double-stranded breaks by recruiting Rad51 to the ssDNA.
- When mutated, it cannot perform this function and this causes an accumulation of mutations in the DNA.
- Mutations in BRCA1 account for 2% of all breast cancer cases (Here, it is a hereditary breast cancer) and, at most, 5% of ovarian cancers.
- BRCA1 is also involved in transcription and transcription-coupled DNA repair.



Additional Notes and Extra Information

The Controversial Issue

A scientist wanted to do research on gametes and zygotes. She wanted to use a bacterial system, Cas9, that allows for the movement of certain regions of DNA and replacement of it with other regions. She wanted to understand the genes that are important for human development in the early stages. Controversial because it involves manipulation of human embryos.

The Effect of Reactive Oxygen Series on Different Molecules

If reactive oxygen series damage sugars or proteins, it's fine because the cell can replace them. However, if membrane lipids are oxidized, this can damage the cell membrane and kill the cell. Finally, we know that any damage on DNA is dangerous.

General Excision Repair and the Bonds Broken

When the endonuclease creates a nick, it's breaking phosphodiester bonds.

When helicase unwinds the strands, it breaks the hydrogen bonds between the region of the strand we want to remove and the complementary strand.

DNA Methylation In Humans

In humans it is normal for some (not all) cysteines to be methylated. We have a certain methylation pattern, but if this pattern is disrupted then it can cause disease.

A Transforming Cell

When we say a cell is transforming, it means its changing from a normal cell to an abnormal, cancerous cell.