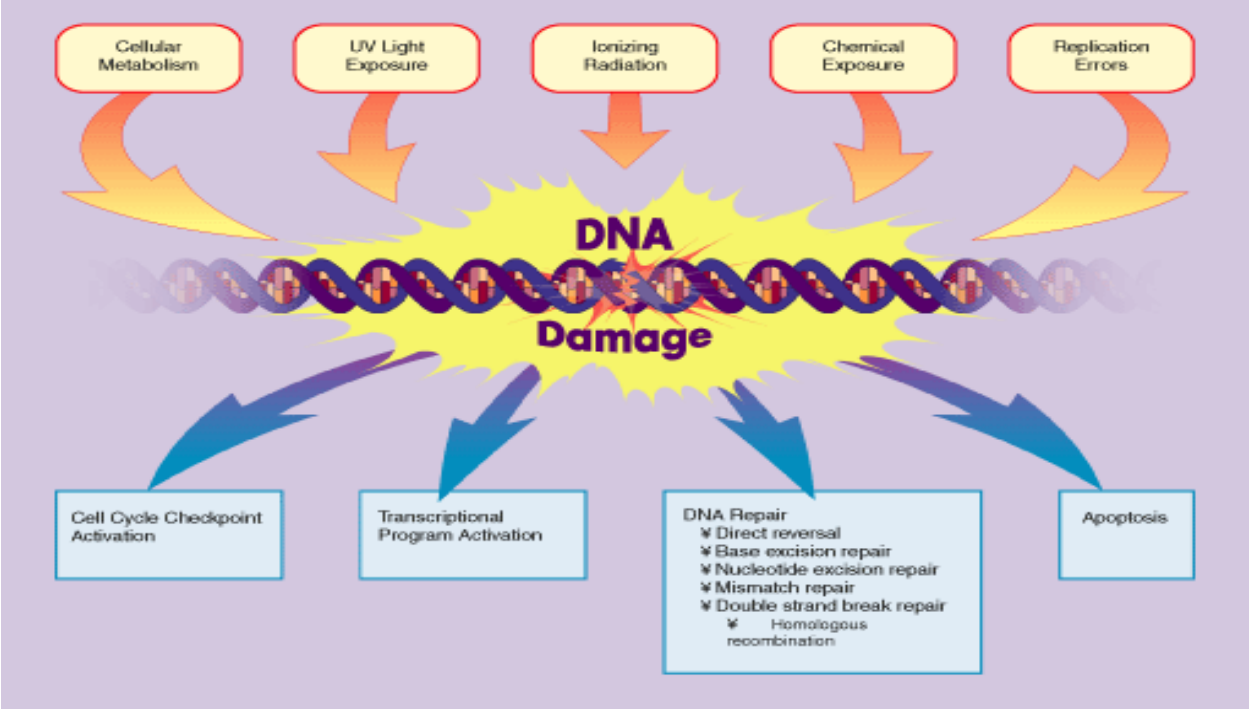
DNA DAMAGE, CELLULAR SENSING/RESPONDING AND DNA REPAIR

DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day.

While this constitutes only 0.000165% of the human genome's approximately 6 billion bases (3 billion base pairs), unrepaired lesions in critical genes (such as tumor suppressor genes) can impede a cell's ability to carry out its function and appreciably increase the likelihood of tumor formation.

- Failure to repair DNA lesions may result in blockages of transcription and replication, mutagenesis, and/or cellular cytotoxicity.

- In humans, DNA damage has been shown to be involved in a variety of genetically inherited disorders, in aging, and in carcinogenesis.



Sources of DNA Damage

DNA damage can be subdivided into two main types:

1. **Endogenous damage:**

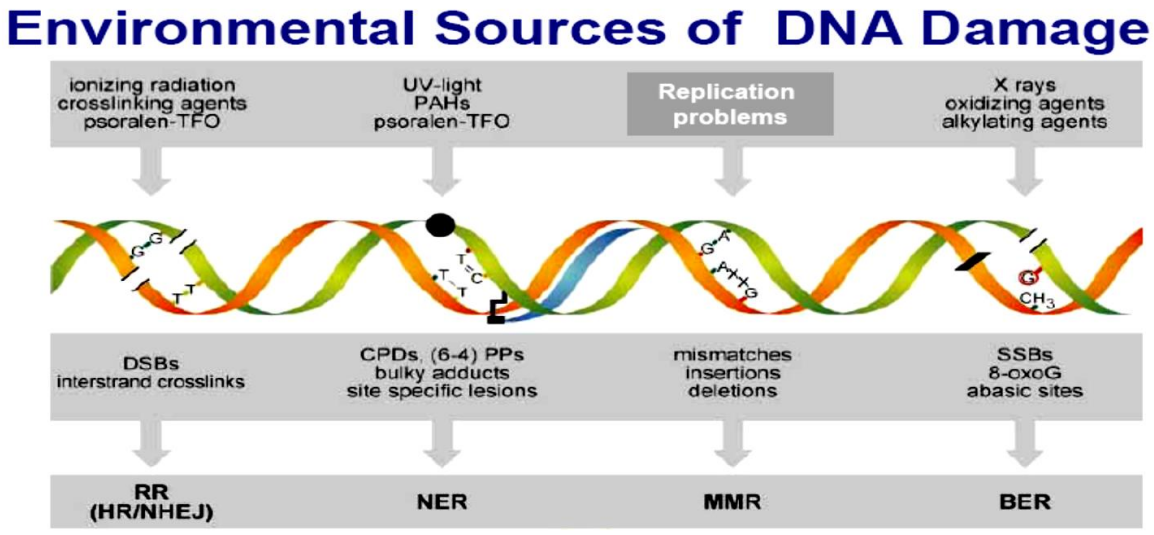
Such as attack by reactive oxygen species produced from normal metabolic byproducts (Spontaneous mutation), especially the process of oxidative deamination;

- also includes replication errors.

2.Exogenous damage

Caused by external agents such as

- ultraviolet [UV 200-300nm] radiation from the sun
- other radiation frequencies, including x-rays and gamma rays.
- human-made mutagenic chemicals, especially aromatic compounds that act as DNA intercalating agents
- Cancer chemotherapy and radiotherapy.



Types of Damage

The main types of damage to DNA due to endogenous cellular processes:

1. **oxidation** of bases [e.g., 8-oxo-7,8-dihydroguanine (8-oxoG)] and generation of DNA strand interruptions from reactive oxygen species.

2. **alkylation** of bases (usually methylation), such as formation of 7-methylguanine, 1-methyladenine, O6 methylguanine

3. **hydrolysis** of bases, such as deamination, depurination and depyrimidination.

4. **"bulky adduct formation"** (i.e., benzo[a]pyrene diol epoxide-dG adduct).

5. **mismatch of bases**, due to errors in DNA replication, in which the wrong DNA base is stitched into place in a newly forming DNA strand, or a DNA base is skipped over or mistakenly inserted.

DNA damage and mutation

It is important to distinguish between DNA damage and mutation, the two major types of error in DNA.

DNA damage and mutation are fundamentally different.

Damage is a physical abnormalities in the DNA, such as single and double strand breaks, 8-hydroxydeoxyguanosine residues and polycyclic aromatic hydrocarbon adducts.

In contrast to DNA damage, a mutation is a change in the base sequence of the DNA. A mutation cannot be recognized by enzymes once the base change is present in both DNA strands, and thus a mutation cannot be repaired.

At the **cellular level, mutations can cause alterations in protein function and regulation.**

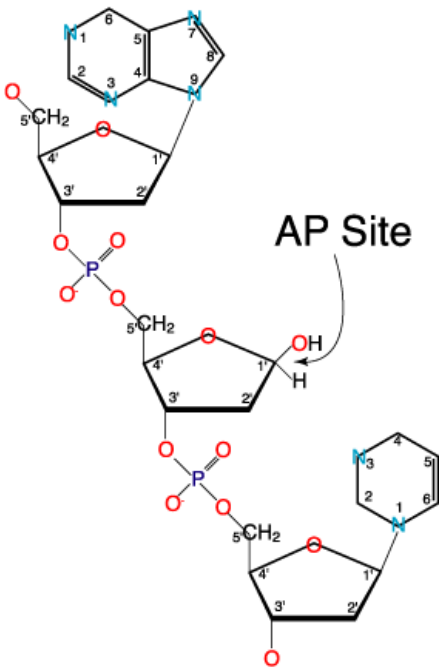
Mutations are replicated when the cell replicates. In a population of cells, mutant cells will increase or decrease in frequency according to the effects of the mutation on the ability of the cell to survive and reproduce.

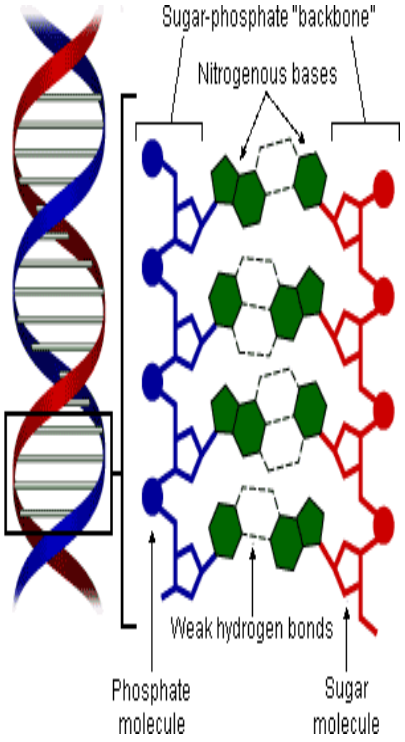
Types of DNA damage

1- Base loss

The glycosyl bond linking DNA bases with deoxyribose is labile under physiological conditions.

Within a typical mammalian cell, several thousand purines and several hundred pyrimidines are spontaneously lost per diploid genome per day. Loss of a purine or pyrimidine base creates an **apurinic/apyrimidinic (AP)** site (also called an **abasic** site):





2- Base modification

2a. Deamination

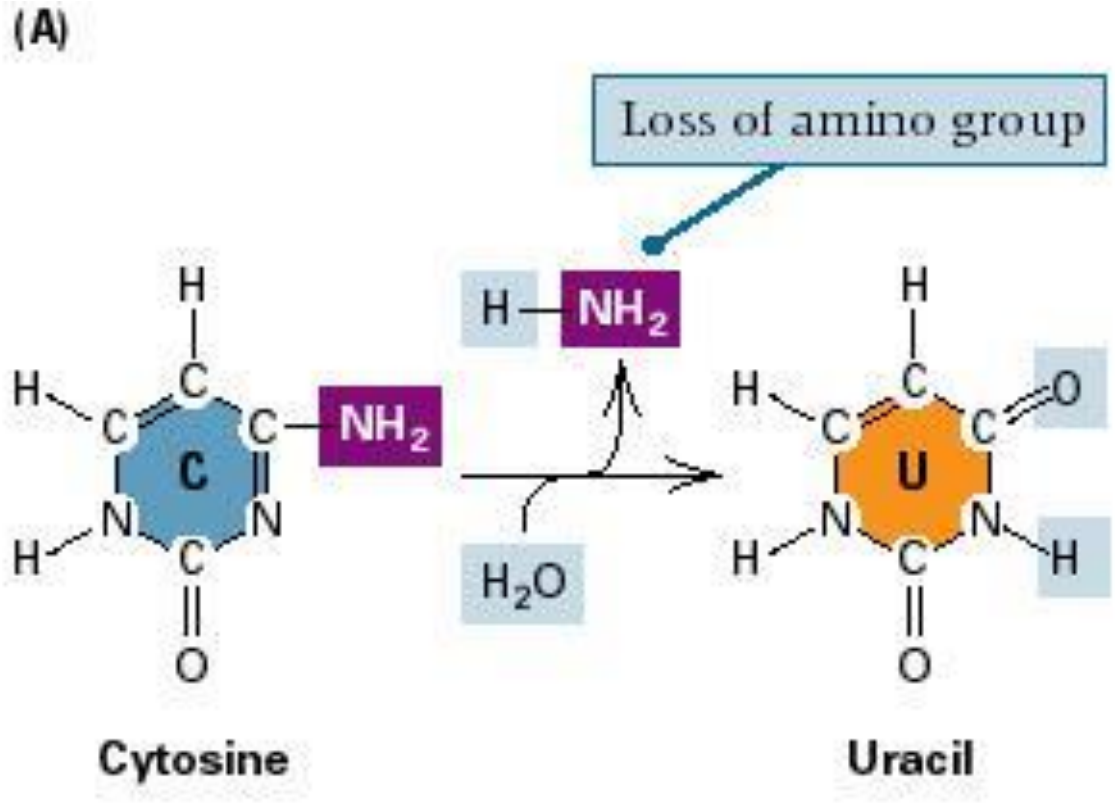
The primary amino groups of nucleic acid bases are somewhat unstable. The amino group is removed from the amino acid and converted to ammonia.

In a typical mammalian cell, about 100 uracils are generated per haploid genome per day in this fashion.

Other deamination reactions include conversion of adenine to hypoxanthine, guanine to xanthine, and 5-methyl cytosine to thymine.

Example: cytosine deamination

Spontaneous deamination is the hydrolysis reaction of cytosine into uracil, releasing ammonia in the process.



2b. Chemical modification

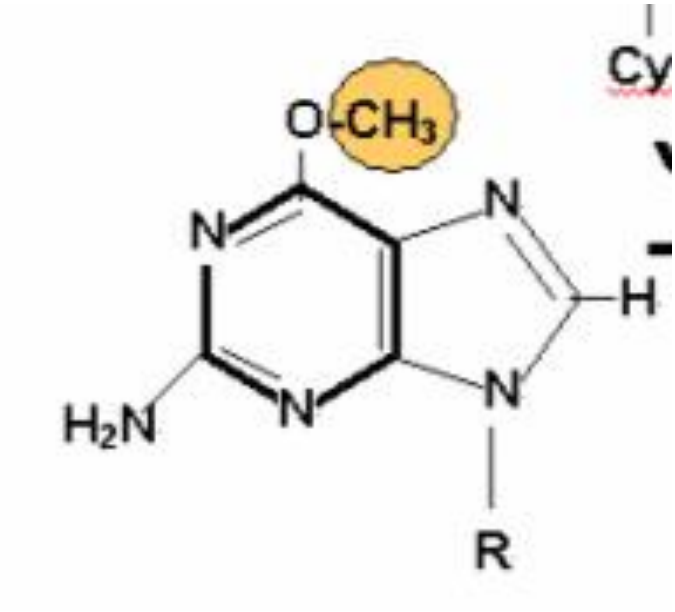
The nucleic acid bases are susceptible to numerous modifications by a wide variety of chemical agents.

For example, several types of hyper-reactive oxygen (singlet oxygen, peroxide radicals, hydrogen peroxide and hydroxyl radicals) are generated as byproducts

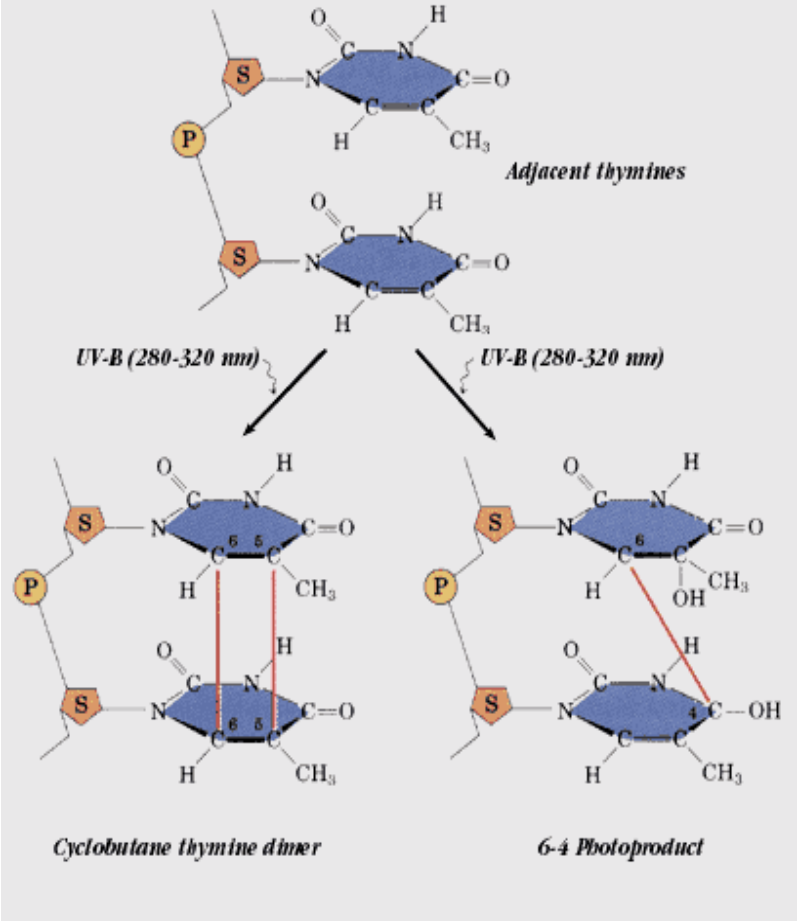
during normal oxidative metabolism and also by ionizing radiation (X-rays, gamma rays).

Another type of chemical modification: Methylation/alkylation

- Many environmental chemicals, including "natural" ones (frequently in the food we eat) can also modify DNA bases, frequently by addition of a methyl or other alkyl group (alkylation).



- **Photodamage:**
- Ultraviolet light is absorbed by the nucleic acid bases, and the resulting influx of energy can induce chemical changes.
- The most frequent photoproducts are the consequences of bond formation between adjacent pyrimidines within one strand, and, of these, the most frequent are cyclobutane pyrimidine dimers (CPDs).



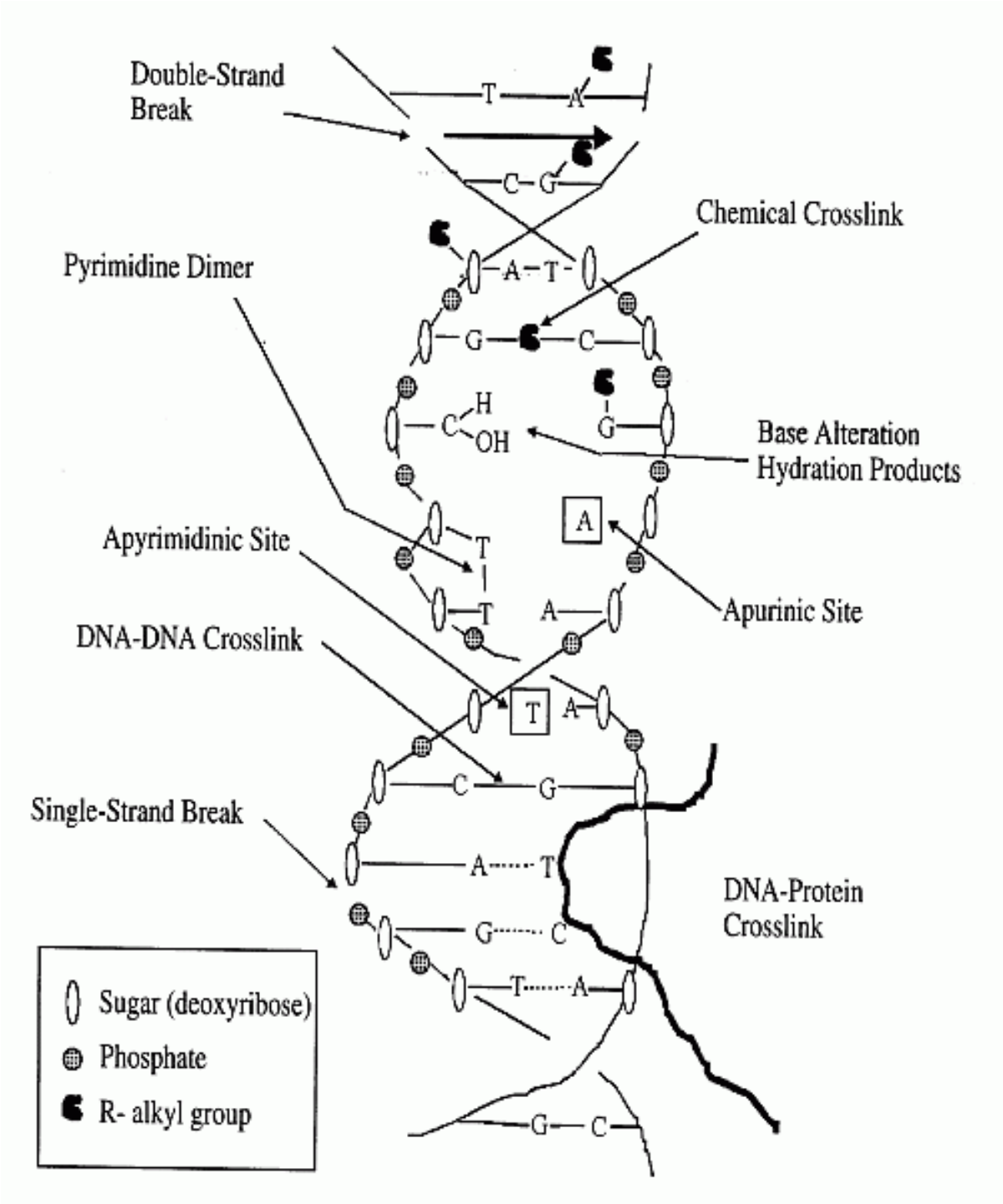
Ultraviolet light induces the formation of covalent linkages by reactions localized on the C=C

- **Inter-strand crosslinks**
- By attaching to bases on both strands, bifunctional alkylating agents such as the psoralens can cross-link both strands.
- Cross-links can also be generated by UV and ionizing radiation.
- **Strand breaks**

Single-strand and double-strand breaks are produced at low frequency during normal DNA metabolism by topoisomerases, nucleases, replication fork

"collapse", and repair processes.

Breaks are also produced by ionizing radiation.

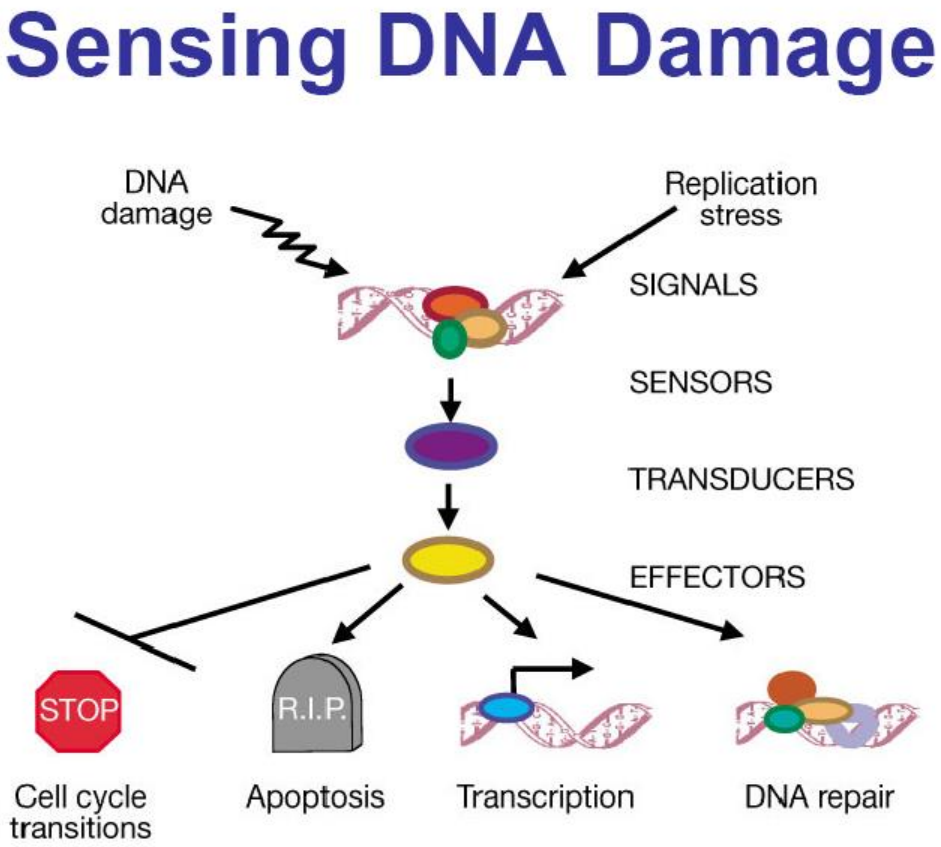

Double-Strand Break
Chemical Crosslink
Pyrimidine Dimer
Base Alteration Hydration Products
Apyrimidinic Site
Apurinic Site
DNA-DNA Crosslink
Single-Strand Break
DNA-Protein Crosslink
Sugar (deoxyribose)
Phosphate
R- alkyl group

WHAT CAN THE CELL DO TO PROTECT ITSELF?

DNA DAMAGE RECOGNITION

DNA damage is recognized by sensor proteins that then initiate a network of signal transduction pathways.

This ultimately results in the activation of effector proteins that execute the functions of the DNA damage response, including recruitment of DNA repair proteins, cell cycle arrest, damage induced transcription, or the induction of apoptosis.



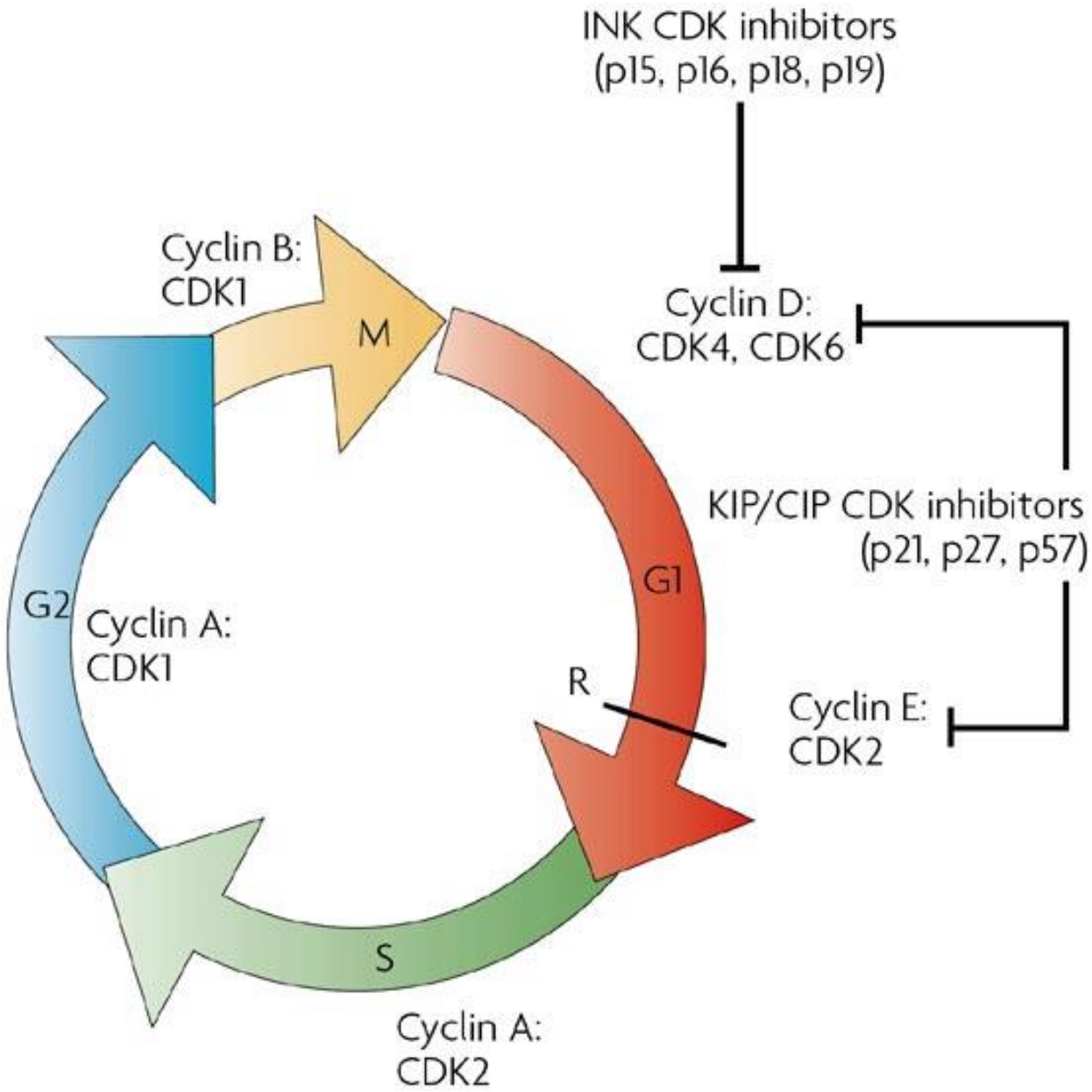
An option: DNA damage checkpoints:

- After DNA damage, cell cycle checkpoints are activated.
- Checkpoint activation pauses the cell cycle and gives the cell time to repair the damage before continuing to divide.

- The cell cycle of eukaryotic cells can be divided into four successive phases:
  - M phase (mitosis), in which the nucleus and the cytoplasm divide;
  - S phase (DNA synthesis), in which the DNA in the nucleus is replicated,
  - two gap phases, G1 and G2.

The G1 phase is a critical stage, allowing responses to extracellular cues that induce either commitment to a further round of cell division or withdrawal from the cell cycle (G0) to embark on a differentiation pathway.

- The G1 phase is also involved in the control of DNA integrity before the onset of DNA replication.
- Between S and M phases is the G2 phase during which the cell checks the completion of DNA replication and the genomic integrity before cell division starts.
- The transition from one phase of the cell cycle to the next is controlled by cyclin–CDK (cyclin-dependent kinase) complexes which ensure that all phases of the cell cycle are executed in the correct order.



- **DNA damage checkpoints occur at the G1/S and G2/M boundaries. An intra-S checkpoint also exists.**
- **Checkpoint activation is controlled by two master kinases, ATM and ATR.**
- **ATM responds to DNA double-strand breaks and disruptions in chromatin structure, whereas ATR primarily responds to stalled replication forks.**

- **These kinases phosphorylate downstream targets in a signal transduction cascade, eventually leading to cell cycle arrest.**

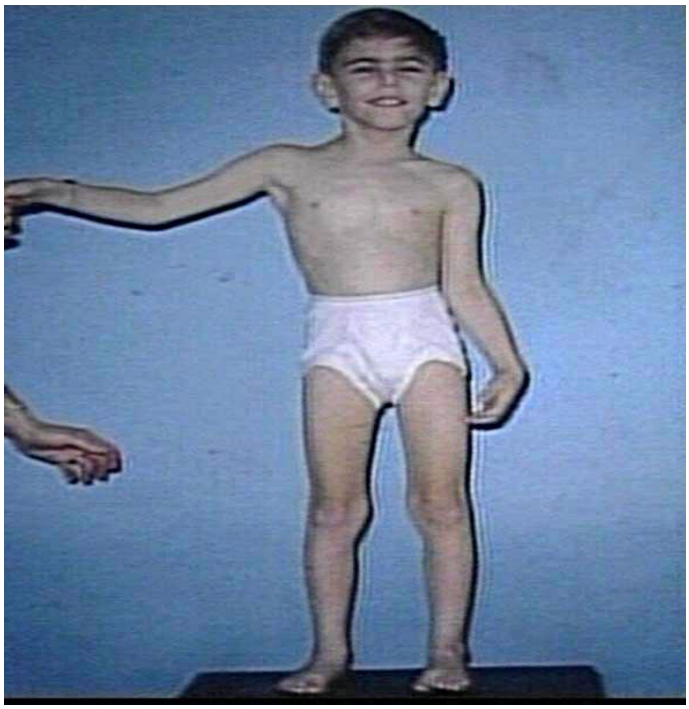
What happens if we have defective ATM??

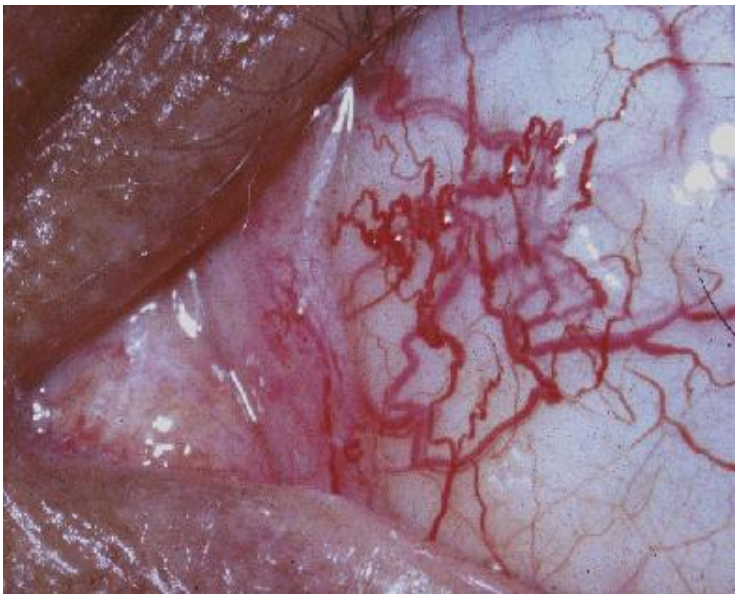
Disease: Ataxia-telangiectasia

Ataxia-telangiectasia is a rare, childhood neurological disorder that causes degeneration in the part of the brain that controls motor movements and speech.

Its most unusual symptom is an acute sensitivity to ionizing radiation, such as X-rays or gamma-rays. The first signs of the disease, which include delayed development of motor skills, poor balance, and slurred speech, usually occur during the first decade of life.

Telangiectasias (tiny, red "spider" veins), which appear in the corners of the eyes or on the surface of the ears and cheeks, are characteristic of the disease, but are not always present and generally do not appear in the first years of life.





About 20% of those with A-T develop cancer, most frequently acute lymphocytic leukemia or lymphoma.

Many individuals with A-T have a weakened immune system, making them susceptible to recurrent respiratory infections.

ATM MUTATIONS ARE ASSOCIATED WITH BREAST CANCER

- Researchers have found that having a mutation in one copy of the ATM gene in each cell (particularly in people who have at least one family member with ataxia-telangiectasia) is associated with an increased risk of developing breast cancer.
- About 1 percent of the United States population carries one mutated copy of the ATM gene in each cell. These genetic changes prevent many of the body's cells from correctly repairing damaged DNA.

LECTURE QUESTIONS SHEET

1. What is the typical number of uracils generated per haploid genome per day in mammalian cells?

- A) 50
- B) 100
- C) 200
- D) 300

2. What type of damage is caused by external agents such as radiation?

- A) Endogenous damage
- B) Exogenous damage
- C) Spontaneous damage
- D) Genetic damage

3. Which of the following is an example of exogenous damage?

- A) Replication errors
- B) Ultraviolet radiation

- C) Reactive oxygen species

- D) Cytosine deamination

4. What is the process of spontaneous deamination of cytosine?

- A) Hydrolysis into thymine

- B) Hydrolysis into uracil

- C) Hydrolysis into adenine

- D) Hydrolysis into guanine

5. Which of the following is NOT a cause of endogenous damage?

- A) Reactive oxygen species

- B) Replication errors

- C) Ultraviolet radiation

- D) Oxidative deamination

6. What is a common byproduct of normal metabolic processes that can lead to DNA damage?

- A) Water

- B) Reactive oxygen species

- C) Carbon dioxide

- D) Glucose

7. Which type of radiation is considered exogenous damage?

- A) X-rays

- B) DNA replication

- C) Cytosine deamination

- D) Ammonia release

8. What is the result of cytosine deamination?

- A) Formation of thymine

- B) Formation of uracil

- C) Formation of adenine

- D) Formation of guanine

9. How does oxidative deamination primarily occur?

- A) Through external radiation

- B) Through metabolic byproducts

- C) Through replication errors

- D) Through chemical exposure

10. What is the role of ammonia in the deamination process?

- A) It is a byproduct

- B) It is a reactant

- C) It is a catalyst

- D) It has no role

11. What is the primary mechanism of cytosine deamination?

- A) Hydrolysis

- B) Methylation

- C) Phosphorylation

- D) Acetylation

12. Which type of damage is associated with replication errors?

- A) Exogenous damage

- B) Endogenous damage

- C) Environmental damage

- D) None of the above

WITH OUR BEST WISHES