

DNA Repair

Importance

DNA in the living cell is subject to many chemical alterations (a fact often forgotten in the excitement of being able to do DNA sequencing on dried and/or frozen specimens. If the genetic information encoded in the DNA is to remain uncorrupted, any chemical changes must be corrected.

A failure to repair DNA produces a mutation.

The recent publication of the human genome has already revealed 130 genes whose products participate in DNA repair. More will probably be identified soon.

Agents that Damage DNA

- Certain wavelengths of **radiation**
 - ionizing radiation such as gamma rays and X-rays
 - **ultraviolet rays**, especially the UV-C rays (~260 nm) that are absorbed strongly by DNA but also the longer-wavelength UV-B that penetrates the ozone shield
- Highly-reactive **oxygen radicals** produced during normal cellular respiration as well as by other biochemical pathways.
- Chemicals in the **environment**
 - many hydrocarbons, including some found in cigarette smoke
 - some plant and microbial products, e.g. the aflatoxins produced in moldy peanuts
- Chemicals used in **chemotherapy**, especially chemotherapy of cancers

Types of DNA Damage

1. All four of the bases in DNA (A, T, C, G) can be covalently modified at various positions.

- One of the most frequent is the loss of an amino group ("deamination") — resulting, for example, in a **C** being converted to a **U**.
- 2. **Mismatches** of the normal bases because of a failure of proofreading during DNA replication.
 - Common example: incorporation of the pyrimidine U (normally found only in RNA) instead of **T**.
- 3. **Breaks** in the backbone.
 - Can be limited to one of the two strands (a single-stranded break, **SSB**) or
 - on **both strands** (a **double-stranded break (DSB)**).
 - Ionizing radiation is a frequent cause, but some chemicals produce breaks as well.
- 4. **Crosslinks** Covalent linkages can be formed between bases
 - on the same DNA strand ("intrastrand") or
 - on the opposite strand ("interstrand").

Several chemotherapeutic drugs used against cancers crosslink DNA.

Repairing Damaged Bases

Damaged or inappropriate bases can be repaired by several mechanisms:

- **Direct chemical reversal** of the damage
- **Excision Repair**, in which the damaged base or bases are removed and then replaced with the correct ones in a localized burst of DNA synthesis. There are three modes of excision repair, each of which employs specialized sets of enzymes.
 1. **Base Excision Repair (BER)**
 2. **Nucleotide Excision Repair (NER)**
 3. **Mismatch Repair (MMR)**

Direct Reversal of Base Damage

Perhaps the most frequent cause of point mutations in humans is the spontaneous addition of a methyl group (CH₃-) (an example of alkylation) to Cs followed by deamination to a T. Fortunately, most of these changes are repaired by enzymes, called glycosylases, that remove the mismatched T restoring the correct C. This is done without the need to break the DNA backbone (in contrast to the mechanisms of excision repair described below).

Some of the drugs used in cancer chemotherapy ("chemo") also damage DNA by alkylation. Some of the methyl groups can be removed by a protein encoded by our ***MGMT*** gene. However, the protein can only do it once, so the removal of each methyl group requires another molecule of protein.

This illustrates a problem with direct reversal mechanisms of DNA repair: they are quite wasteful. Each of the myriad types of chemical alterations to bases requires its own mechanism to correct. What the cell needs are more general mechanisms capable of correcting all sorts of chemical damage with a limited toolbox. This requirement is met by the mechanisms of **excision repair**.

Base Excision Repair (BER)

The steps and some key players:

1. removal of the damaged base (estimated to occur some 20,000 times a day in each cell in our body!) by a DNA glycosylase. We have at least 8 genes encoding different DNA glycosylases each enzyme responsible for identifying and removing a specific kind of base damage.
2. removal of its deoxyribose phosphate in the backbone, producing a gap. We have two genes encoding enzymes with this function.

3. replacement with the correct nucleotide. This relies on **DNA polymerase beta**, one of at least 11 DNA polymerases encoded by our genes.
4. ligation of the break in the strand. Two enzymes are known that can do this; both require ATP to provide the needed energy.

Nucleotide Excision Repair (NER)

NER differs from BER in several ways.

- It uses different enzymes.
- Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, NER removes a large "patch" around the damage.

The steps and some key players:

1. The damage is recognized by one or more protein factors that assemble at the location.
2. The DNA is unwound producing a "bubble". The enzyme system that does this is **Transcription Factor IIH, TFIIH**, (which also functions in normal transcription).
3. Cuts are made on both the 3' side and the 5' side of the damaged area so the tract containing the damage can be removed.
4. A fresh burst of DNA synthesis — using the intact (opposite) strand as a template — fills in the correct nucleotides. The DNA polymerases responsible are designated polymerase **delta** and **epsilon**.
5. A **DNA ligase** covalently inserts the fresh piece into the backbone.

Xeroderma Pigmentosum (XP)

XP is a rare **inherited** disease of humans which, among other things, predisposes the patient to

- pigmented lesions on areas of the skin exposed to the sun and
- an elevated incidence of skin cancer.

It turns out that XP can be caused by mutations in any one of several genes — all of which have roles to play in **NER**. Some of them:

- **XPA**, which encodes a protein that binds the damaged site and helps assemble the other proteins needed for NER.
- **XPB** and **XPD**, which are part of TFIIH. Some mutations in XPB and XPD also produce signs of premature aging.
- **XPF**, which cuts the backbone on the 5' side of the damage
- **XPG**, which cuts the backbone on the 3' side.

Transcription-Coupled NER

Nucleotide-excision repair proceeds most rapidly

- in cells whose genes are being actively transcribed
- on the DNA strand that is serving as the template for transcription.

This enhancement of NER involves XPB, XPD, and several other gene products. The genes for two of them are designated **CSA** and **CSB** (mutations in them cause an inherited disorder called **Cockayne's syndrome**).

The CSB product associates in the nucleus with **RNA polymerase II**, the enzyme responsible for synthesizing **messenger RNA** (mRNA), providing a molecular link between transcription and repair.

One plausible scenario: If RNA polymerase II, tracking along the template (antisense) strand), encounters a damaged base, it can recruit other proteins, e.g., the CSA and CSB proteins, to make a quick fix before it moves on to complete transcription of the gene.

Mismatch Repair (MMR)

Mismatch repair deals with correcting mismatches of the **normal bases**; that is, failures to maintain normal Watson-Crick base pairing (A•T, C•G)

It can enlist the aid of enzymes involved in both base-excision repair (BER) and nucleotide-excision repair (NER) as well as using enzymes specialized for this function.

- **Recognition** of a mismatch requires several different proteins including one encoded by *MSH2*.
- **Cutting** the mismatch out also requires several proteins, including one encoded by *MLH1*.

Mutations in either of these genes predisposes the person to an inherited form of colon cancer. So these genes qualify as tumor suppressor genes.

Synthesis of the repair patch is done by **DNA polymerase delta**.

Cells also use the MMR system to enhance the fidelity of recombination; i.e., assure that only homologous regions of two DNA molecules pair up to crossover and recombine segments (e.g., in meiosis).

Repairing Strand Breaks

Ionizing radiation and certain chemicals can produce both single-strand breaks (**SSBs**) and double-strand breaks (**DSBs**) in the DNA backbone.

Single-Strand Breaks (SSBs)

Breaks in a single strand of the DNA molecule are repaired using the same enzyme systems that are used in Base-Excision Repair (BER).

Double-Strand Breaks (DSBs)

There are two mechanisms by which the cell attempts to repair a complete break in a DNA molecule:

- **Direct joining** of the broken ends. This requires proteins that recognize and bind to the exposed ends and bring them together for ligating. They would prefer to see some complementary nucleotides but can proceed without them so this type of joining is also called **Nonhomologous End-Joining (NHEJ)**.
- Errors in direct joining may be a cause of the various **translocations** that are associated with cancers.
- Examples:
 - Burkitt's lymphoma
 - the Philadelphia chromosome in chronic myelogenous leukemia (CML)
 - B-cell leukemia
- **Homologous Recombination**. Here the broken ends are repaired using the information on the intact
 - **sister chromatid** (available in G_2 after chromosome duplication), **or** on the
 - **homologous chromosome** (in G_1 ; that is, before each chromosome has been duplicated). This requires searching around in the nucleus for the homolog — a task sufficiently uncertain that G_1 cells usually prefer to mend their DSBs by **NHEJ**. **or** on the
 - **same chromosome** if there are duplicate copies of the gene on the chromosome oriented in opposite directions (head-to-head or back-to-back).

Two of the proteins used in homologous recombination are encoded by the genes **BRCA1** and **BRCA2**. Inherited mutations in these genes predispose women to breast and ovarian cancers.

Cancer Chemotherapy

- The hallmark of all cancers is continuous cell division.
- Each division requires both
 - the replication of the cell's DNA (in S phase) and
 - transcription and translation of many genes needed for continued growth.
- So, any chemical that damages DNA has the potential to inhibit the spread of a cancer.
- Many (but not all) drugs used for cancer therapy do their work by damaging DNA.

The table lists (by trade name as well as generic name) some of the anticancer drugs that specifically target DNA.

6-mercaptopurine	Purinethol®	<u>purine</u> analog. One effect: substitutes for G, inducing abortive <u>MMR</u> and strand breaks
Gemcitabine	Gemzar®	<u>pyrimidine</u> analog substitutes for C blocking strand elongation
Cyclophosphamide	Cytoxan®	<u>alkylating agents</u> ; form interstrand and/or intrastrand crosslinks
Melphalan	Alkeran®	
Busulfan	Myleran®	
Chlorambucil	Leukeran®	
Mitomycin	Mutamycin®	
Cisplatin	Platinol®	forms crosslinks
Bleomycin	Blenoxane®	cuts DNA strands between GT or

		GC
Irinotecan	Camptosar®	inhibit the proper functioning of enzymes (topoisomerases) needed to unwind DNA for replication and transcription
Mitoxantrone	Novantrone®	
Dactinomycin	Cosmegen®	inserts into the double helix preventing its unwinding

Sadly,

- the cancer patient has many other cell types that are also proliferating rapidly, e.g., cells of the
 - intestinal endothelium
 - bone marrow
 - hair follicles

and anticancer drugs also damage these — producing many of the unpleasant side effects of "chemo".

- Agents that damage DNA are themselves carcinogenic, and chemotherapy poses a significant risk of creating a new cancer, often a leukemia.

Here is an overview.

