

Repair of DNA

Photoreactivation

Organisms have evolved at least four processes for repairing UV damage in DNA: photoreactivation, excision, error-prone, and recombination repair. Depending on the type of organism and the nature of the UV damage, these processes may successfully repair damage, partially repair the damage and create a mutation, or fail to work at all.

The simplest process for repair of pyrimidine dimers is called photoreactivation which, as the name suggests, requires light. Photoreactivation is catalyzed by a single protein called photolyase, which uses the energy in a photon of light to break apart chemically a pyrimidine dimer in DNA. Photoreactivation probably represents the earliest type of UV repair system because many species, from bacteria through marsupials, share the enzyme responsible. Humans and other placental mammals do not seem to have a photoreactivation process, but the gene which codes for photolyase has been conserved and may have evolved to play a role in the excision repair process. The *PHR1* gene encoding photolyase is defective in the sensitive strain used in the experiments.

Excision repair

In excision repair, the region of DNA containing the dimer or other damage is physically cut out and then replaced by new DNA synthesis (Figure 1). Excision repair has more steps and requires more enzymes than photoreactivation, but it can work on damage created by agents other than UV and on lesions other than pyrimidine dimers. In *Escherichia coli* bacteria, excision repair requires six proteins: three are involved in finding the damaged region of the DNA and cutting the DNA strand around the lesion; one participates in removing the damaged bit; DNA polymerase replaces the portion which was removed; and a final enzyme called DNA ligase glues the new and old portions back together. Mutations in the genes coding for any of these proteins will interfere with the process and cause the mutant bacterium to be highly sensitive to killing and mutation by UV light. The excision repair system probably repairs a large amount of UV damage.

In yeast and other eukaryotes, DNA is wrapped up in more complicated structures than in bacteria, which may explain why these organisms seem to need more proteins to carry out excision repair. In yeast, at least twelve proteins may participate in excision repair. Researchers originally identified many of these by finding mutants unable to repair UV damage. We don't yet know the functions of all of these proteins, but scientists very recently found that the *RAD1* and *RAD10* gene products may act together in cutting DNA near dimers and that the *RAD3* gene product is needed to identify dimers to the other repair proteins. These genes have close counterparts in humans; for example, the protein made by the *RAD3* gene has the same sequence of amino acids in over 50% of the positions as the product of its human counterpart, *ERCC2*.

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People with mutations in *ERCC2* are very sensitive to sunlight and suffer from the disease xeroderma pigmentosum. Yeast with mutations in *RAD3* are very sensitive to UV and are killed or mutated by very low doses of UV. *RAD1* is mutated in the sensitive strain *G948-1C*.

Error-prone repair

The excision process described in the previous section is mostly accurate, or error-free. Sometimes, however, mistakes are made when a cell tries to repair a lesion in its DNA. In the case of pyrimidine dimers, mistakes may happen when two dimers are near each other on opposite strands of the DNA (Figure 2). If the cell tries to do excision repair, it won't know how to copy the dimer when it tries to carry out the repair DNA synthesis because the dimer is not a normal part of DNA. It might make a mistake rather than not repair the gap in the DNA. Sometimes, unfortunately, an error-prone process is the only way to repair DNA damage. Most mutations arising after UV treatment of cells are the result of error-prone repair of the DNA lesions. In yeast, we know of several genes whose products are required for error-prone repair; one of them, *RAD18*, is mutated in the sensitive strain used in the experiments.

Recombinational repair

When pyrimidine dimers block DNA replication in a eukaryotic chromosome, the polymerase can start replication at other places further downstream. The result of replicating a DNA molecule or chromosome containing a dimer is thus a gap in one strand of the DNA where the dimer blocked a portion from being copied (Figure 3). A gap in DNA means that one strand is missing information; the strand must be repaired before the cell divides. The most frequent way that cells fill such a gap is by genetic recombination with another DNA molecule or chromosome containing the same or similar information. The recombinational repair system is a fourth process involved in repair of UV damage to DNA. The genes which make the proteins functioning in this system have been identified because mutations in them block recombination. One important member of this group is the *RAD51* gene which makes a protein that can help DNA molecules find their similar partners and begin recombination.

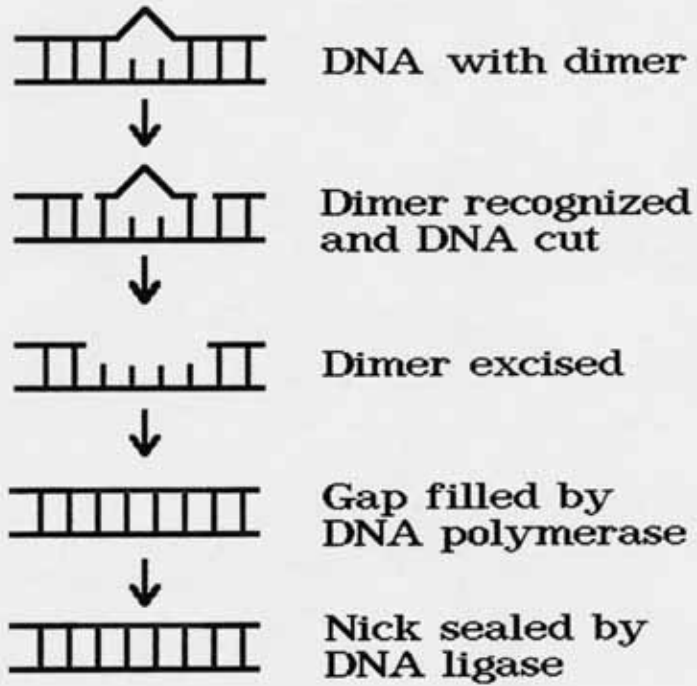


Figure 1: Steps in Excision Repair

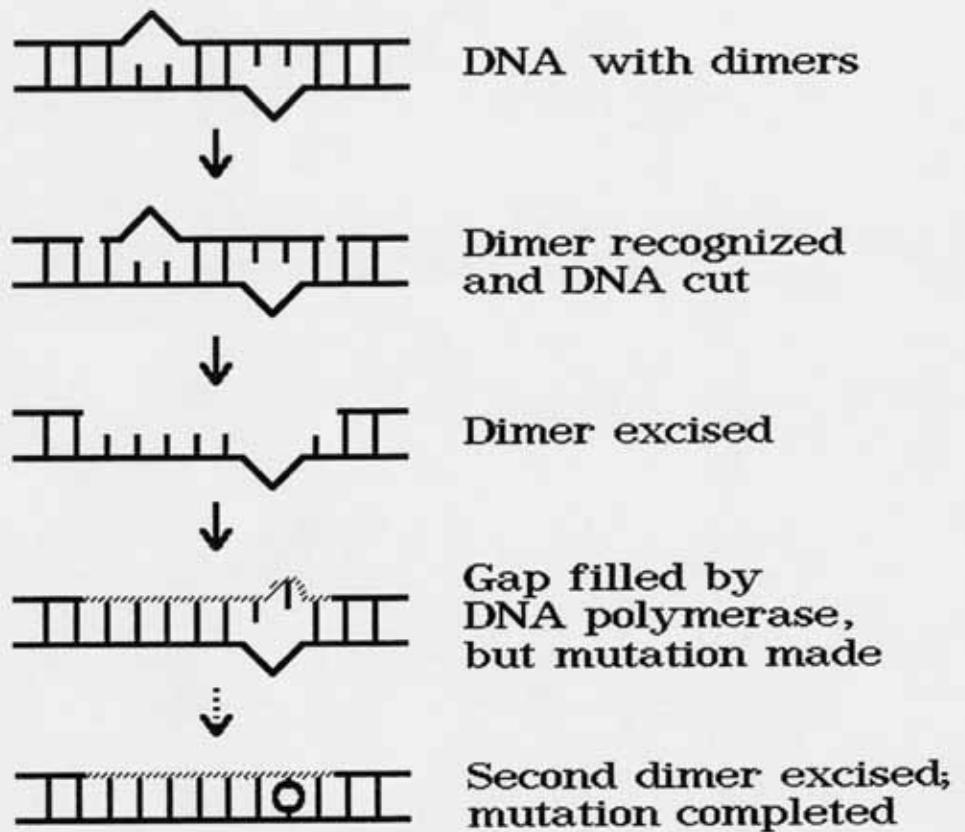


Figure 2: Steps in Error-prone Repair

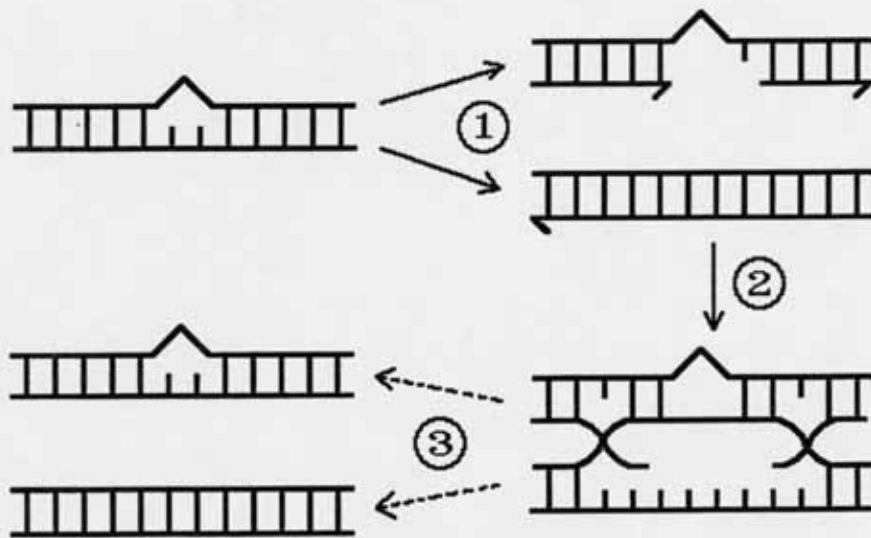


Figure 3: Steps in Recombinational Repair

- Step 1: DNA with dimer is replicated, leaving a gap in one daughter molecule.
- Step 2: Recombination with other daughter molecule fills gap by transfer of good strand.
- Step 3: DNA replication fills gap in donor daughter molecule.