DNA Repair and Recombination

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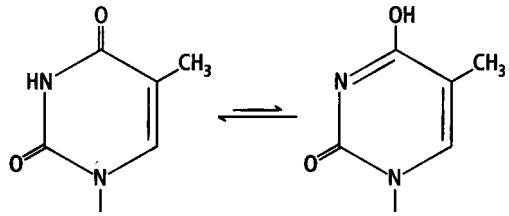
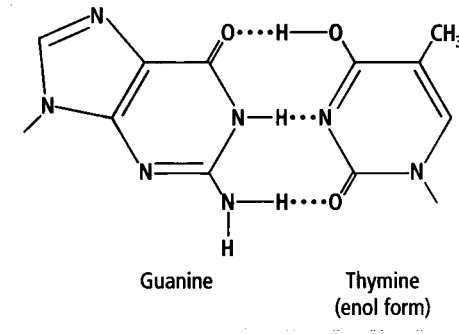
Mutations and their biomedical importance

- **Mutation** is defined as a permanent change in the DNA sequence.
- Mutations can occur spontaneously from faulty replication or repair of DNA, or can be induced by external agents, such as radiation, chemicals and viruses.
- Mutations in germ cells are transmitted to the offspring **("vertical" transmission** of hereditary disease).
- Mutations in somatic cells lead to an abnormal somatic cell clone **("horizontal" transmission**, within the same organism). Most dangerous somatic mutations are those that cause cells to grow at increased rates, leading to neoplastic diseases.
- **Homozygosity** is the condition in which an abnormal gene is present in both copies in a diploid cell, without its normal counterpart.
- **Heterozygosity** is the condition in which the abnormal gene is present in one copy together with its normal counterpart in a diploid cell. Heterozygotes for a normal and a defective gene usually produce both the normal and the defective proteins.
  - **Dominant inheritance pattern**: the heterozygous cariers of an abnormal gene are affected clinically.
  - **Recessive inheritance pattern**: the heterozygous cariers of an abnormal gene are not affected clinically.

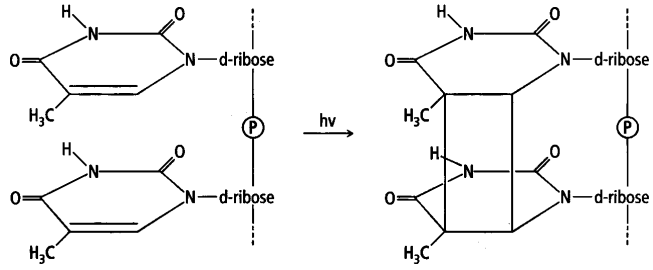
Types of DNA damage and their causes.

- **Tautomeric shifts** in the bases.
  - eg: Thymine, in normal keto form pairs with adenine. In the enol form it pairs with guanine.



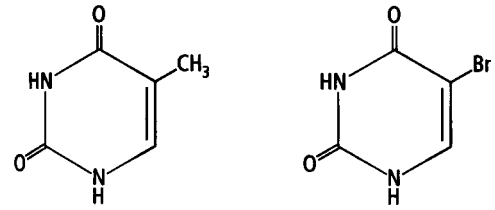


- **Pyrimidine dimers**. Mostly caused by UV radiation.



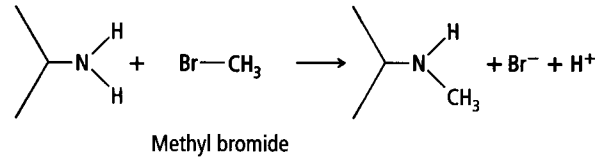
Types of DNA damage and their causes (cont)

- **AP sites (apurinic or apyrimidinic).** Caused by spontaneous hydrolysis of N-glycosidic bond between a purine or pyrimidine with 2-deoxyribose.
- **Base analogues**

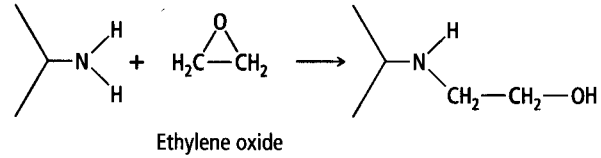


Eg. Bromouracil is incorporated into DNA in place of thymine. Although it can pair with adenine, it is very prone to tautomeric shifts.

- **Alkylating agents**: they alkylate nitrogen or oxygen atoms in the bases preventing correct base pairing. (eg. Ethylene oxide, methyl bromide)



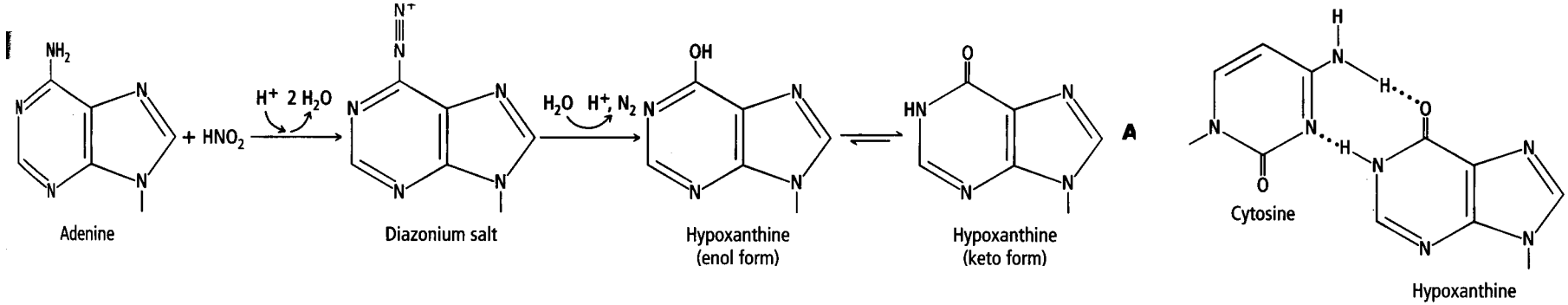
Methyl bromide



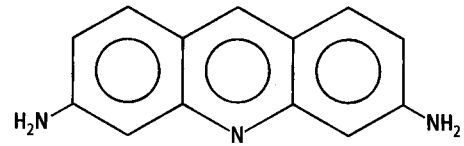
Ethylene oxide

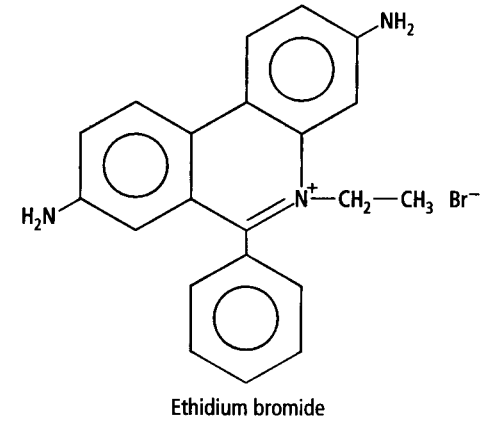
Types of DNA damage and their causes (cont)

- **Deaminating agents**: turn adenine, guanine, and cytocine into hypoxanthine, xanthine, and uracil, leading to errors during DNA replication. (eg. Hypoxanthine pairs with cytocine instead of thymine)

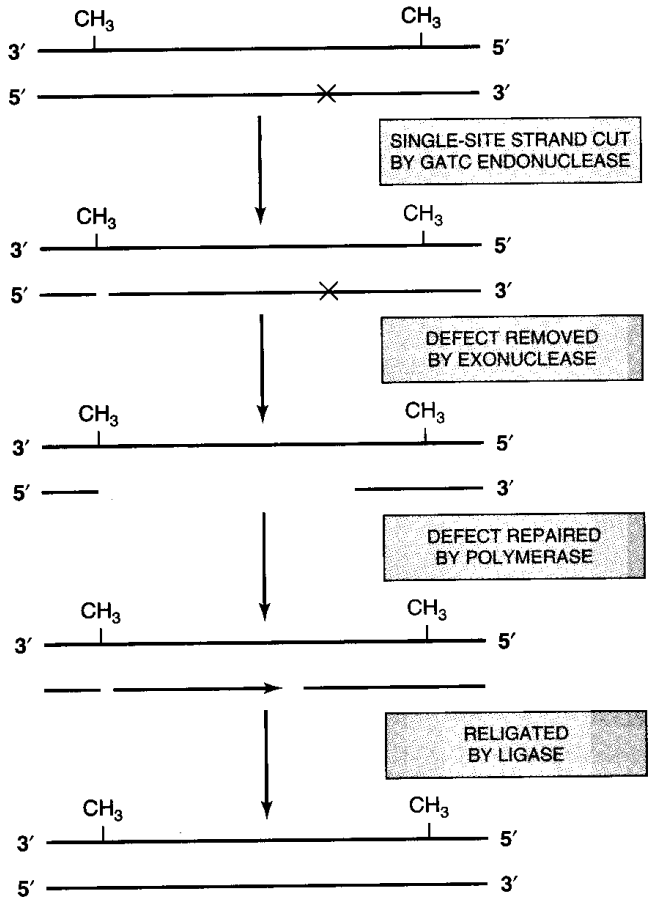


- **Intercalating agents**. They get inserted between stacked DNA bases causing frameshift mutations during DNA replication.





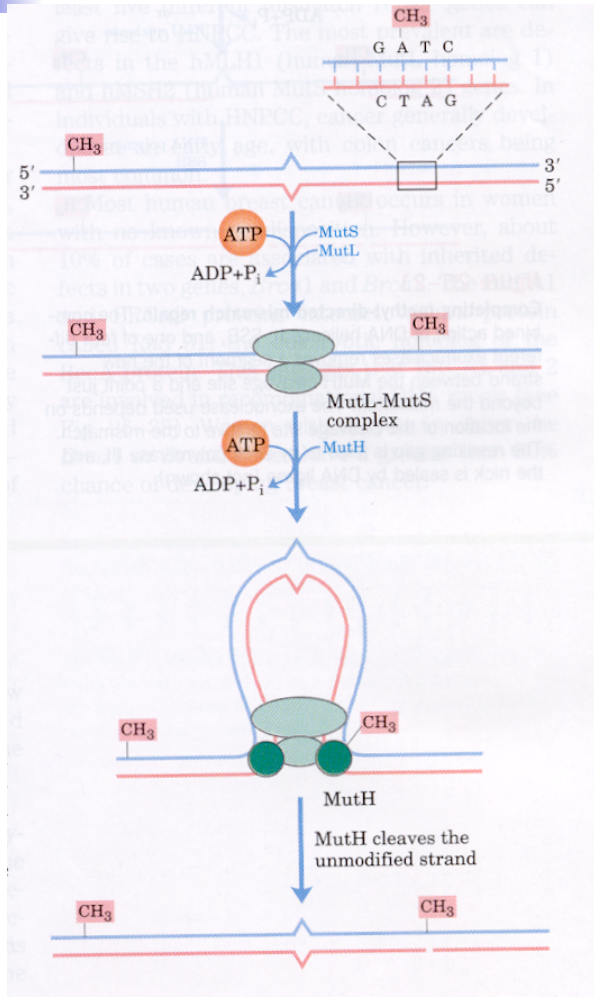
Mismatch repair of DNA



This mechanism corrects a single mismatch base pair, or a short region of unpaired DNA.

The defective region is recognized by an endonuclease that makes a single-strand cut at an adjacent methylated GATC sequence.
The strand is removed through the mutation, replaced and religated.

Specific proteins scan the newly synthesized DNA for mismatches using methylation within a GATC sequence as point of reference.



Mutations on hMSH2 protein, the human analogue of the *E.coli* MutS, lead to increased risk of developing HNPC (hereditary non polyposis colon cancer) in humans. Most other cases are associated with mutations of the hMSH1 protein, which is the human analogue of the *E.coli* MutL.

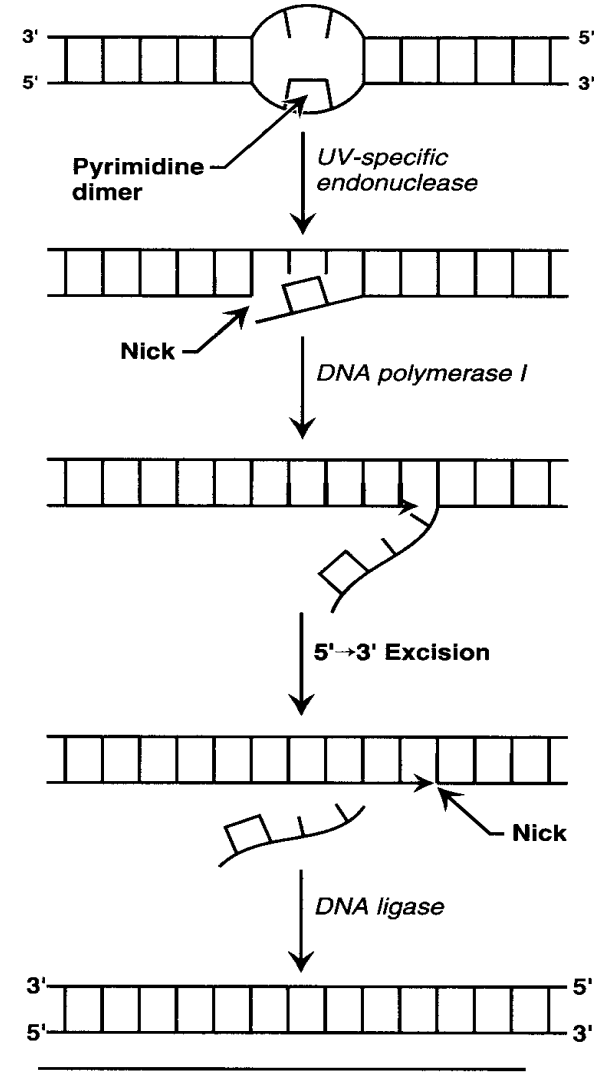
Repair of pyrimidine dimers

I. Nucleotide Excision repair

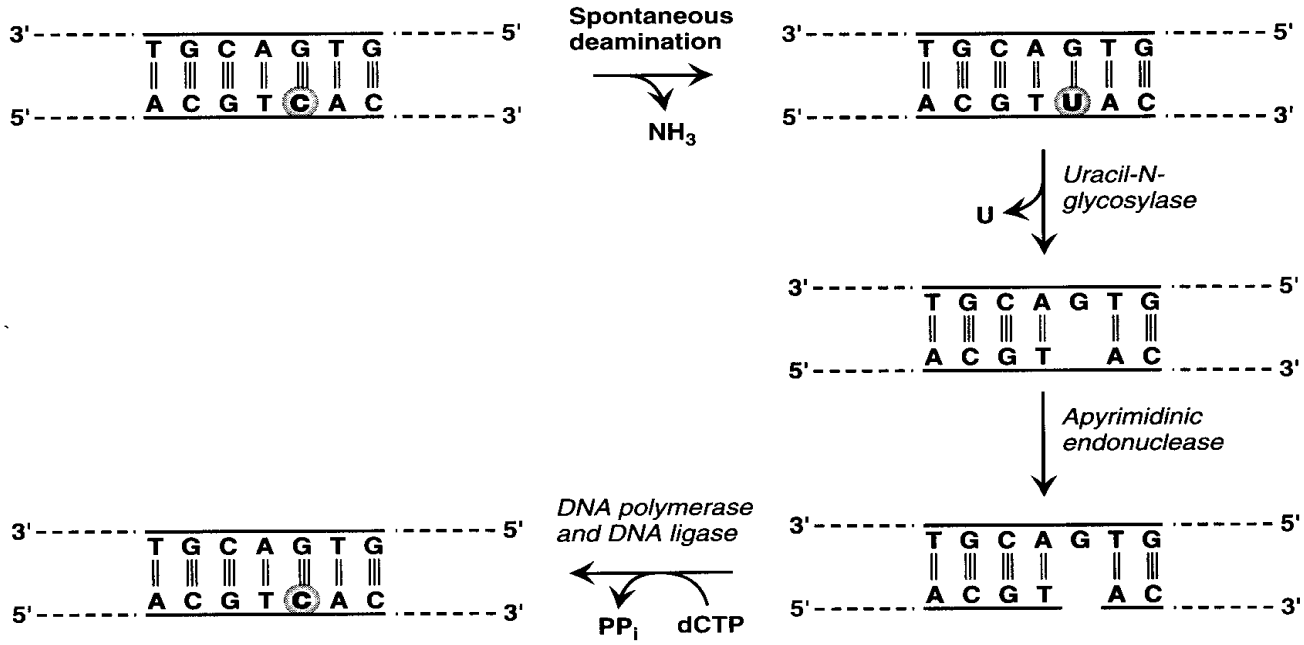
- A. Recognition of the dimer by UV-specific endocuclease (**UvrABC endonuclease** in *E. coli*) Cleavage of the damaged strand at the 5' side of the dimer. ATP-depended reaction.
- B. Excision of the damaged DNA by 5' exonuclease (DNA pol I)
- C. Repair of the DNA by 5' polymerase (pol I).
- Sealing of the repaired strand by ligase.

- **Xeroderma pigmentosum**: rare genetic condition (autosomal recessive) caused by absence in the UV-specific endoculease. Clinical appearance: numerous freckles, ulcer and cancer on sun-exposed skin. In some forms also neurological generation and growth retardation. No tx available. Pt needs to completely avoid sunlight exposure.

II. Direct reversal of the damage by Photolyase (photoreactivating enzyme): Light absorbing enzyme, splits the T-dimers.



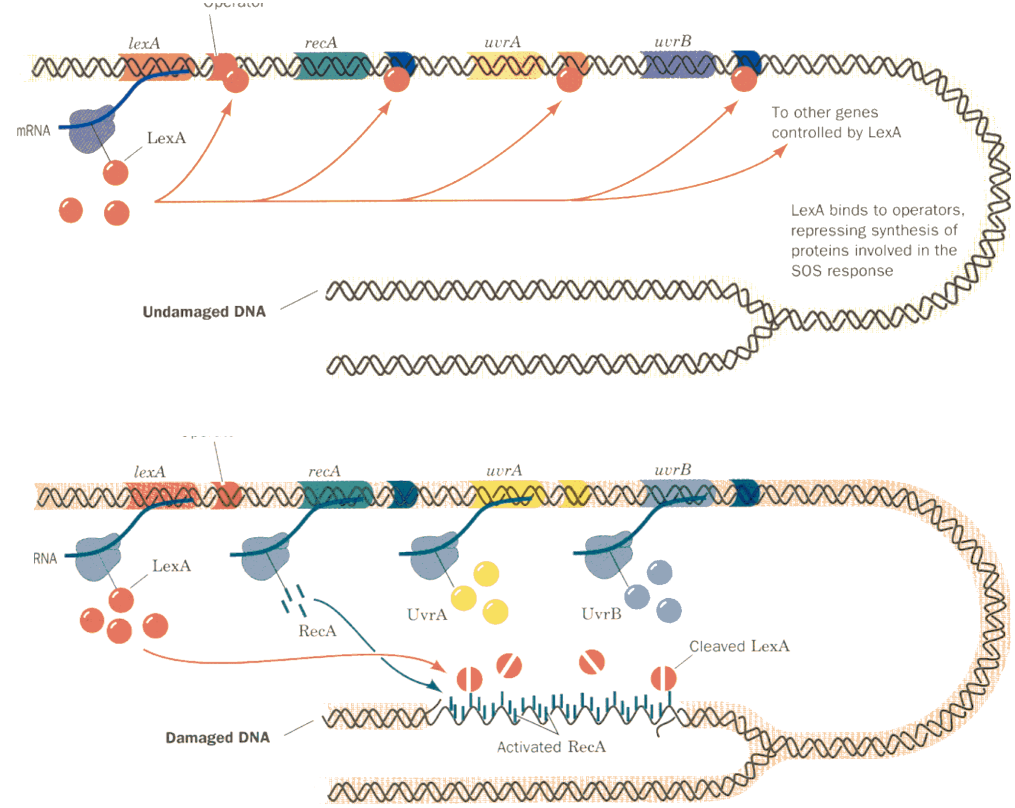
Correction of base alterations (Base excision-repair)



a. Recognition and removal of the abnormal base by specific glycosylase
b. Recognition and repair of the AP site

The SOS response in *E.coli*.

- When there is no DNA damage, LexA represses the synthesis of a number of proteins involved in DNA repair (SOS proteins: LexA, RecA, RecBCD, UvrABC, etc).
- When excessive DNA damage occurs, RecA gets activated by binding to damaged (single stranded) DNA.
- Activated RecA stimulates the repressor LexA to cleave itself, thus derepressing the SOS genes.



DNA recombination

- **Genetic recombination is defined as a rearrangement of genetic information among DNA molecules.**
  - **Homologous recombination**: genetic exchanges between any two DNA molecules that share an extended region of nearly identical sequence.
  - **Site specific recombination**: differs from the previous one in that the exchanges occur only at a particular DNA sequence.
  - **DNA transposition**: usually involves a short segment of DNA which has the ability to move from one location to another.

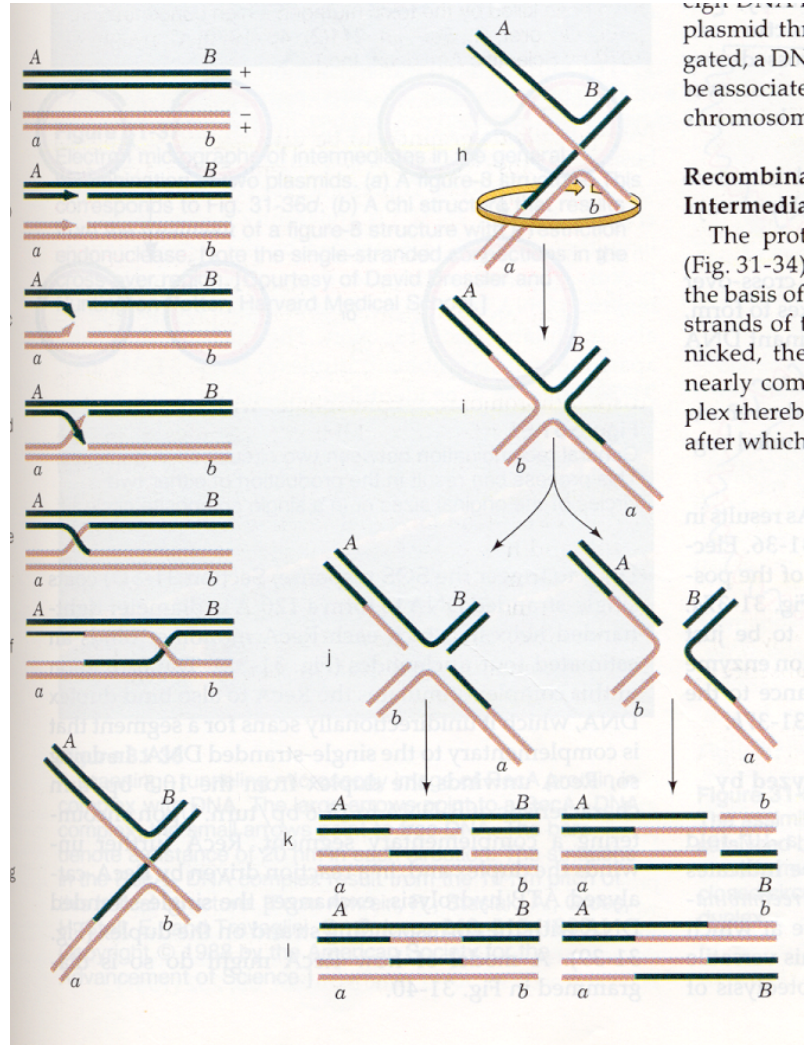
Recombination occurs via a cross-over intermediate

Corresponding strands of two aligned homologous DNA duplexes nicked

Nicked strands crossover to pair with the nearly complementary strands of the homologous duplex

Branch migration

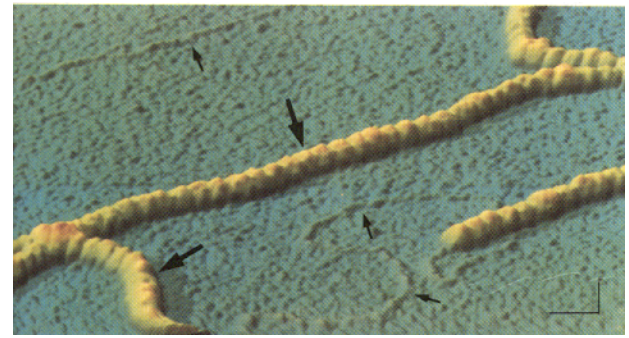
Holliday structure



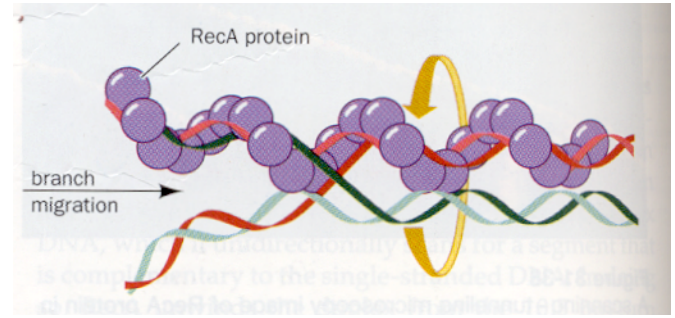
The Holliday structure can be resolved in two equally probable ways

General recombination in *E.coli* is catalyzed by RecA.

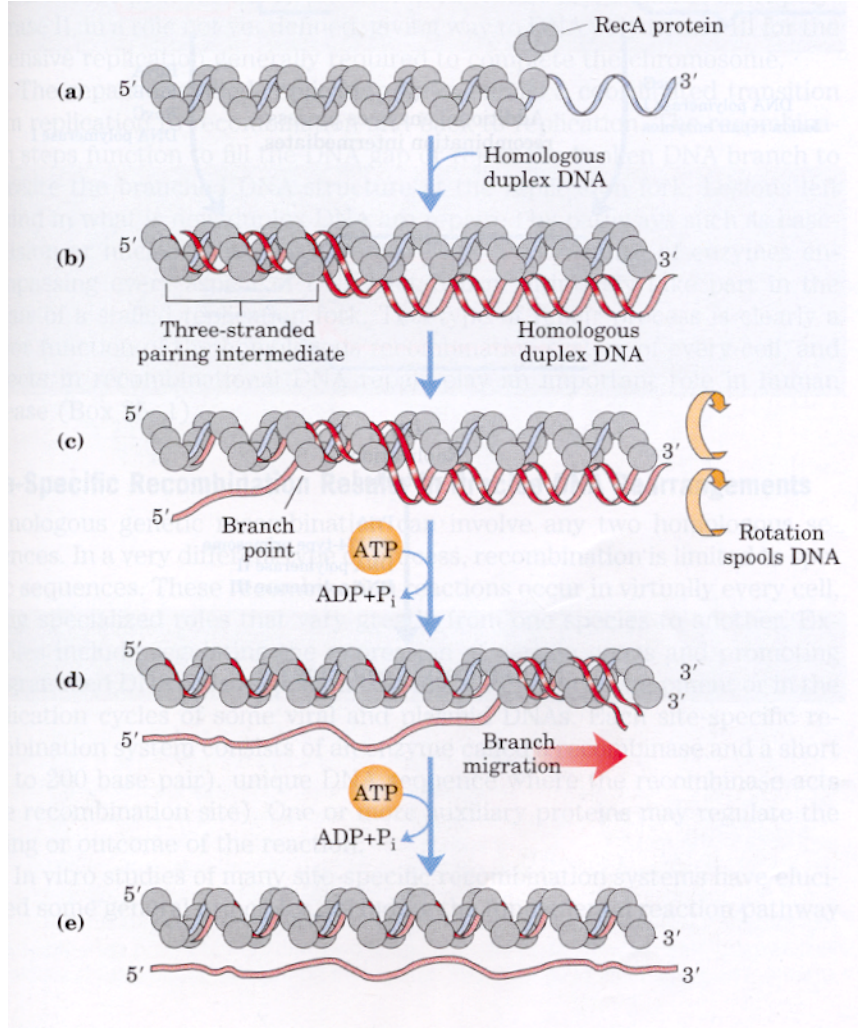
- The active form of RecA consists of several thousants of RecA monomers that cooperatively assemble on DNA to form a helical filament



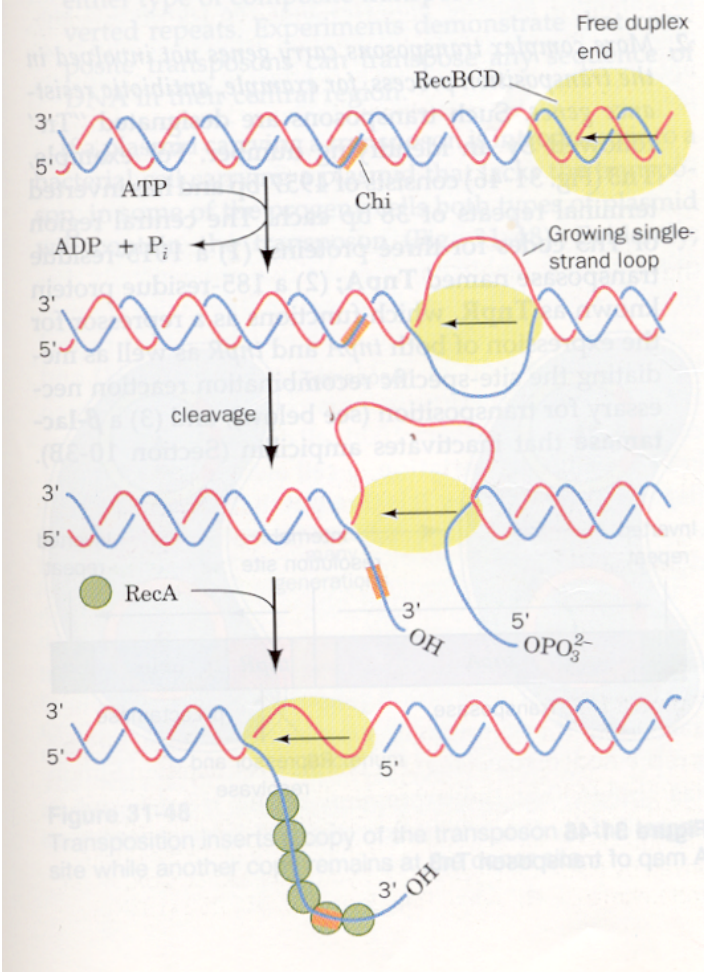
- Processes that are mediated by RecA:
  - Pairing of the two DNAs
  - Formation of Holliday intermediates
  - Branch migration



Model for DNA strand exchange mediated by RecA



The role of RecBCD in homologous recombination



- Has helicase and nuclease activities
- Activity changes (to nuclease) when it interacts with *chi* sequences (GCTGGTGG) (~1 every 10,000 bp)
- Initiates recombination by generating a potentially invasive single strand of DNA for RecA to bind.