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Bi4025en
Molecular Biology

Mgr. Jiří Kohoutek, Ph.D.

Lecture 9

- DNA repair mechanism and recombination.

Mutagenecity = Carcinogenesis

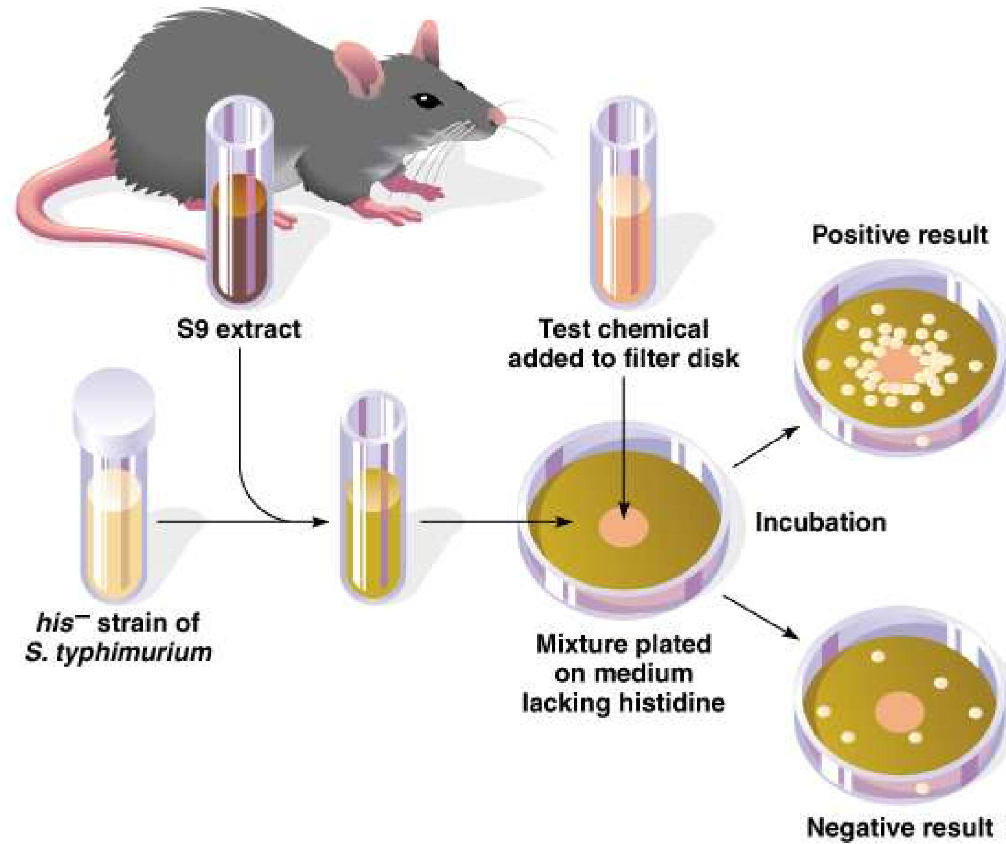
- Ames tests identified thousands of chemicals with ability to increase the frequency of mutation, result - catalog of substances,.
- Thus immeasurable becomes measurable.
- Important observation was made - mutagenic substances have tendency to be carcinogens.
- Also works for X-rays, tobacco smoke.

Ames test

- Ames Test is an inexpensive method used to screen possible carcinogens and mutagens.
- Histidine auxotroph *Salmonella typhimurium* (requires Histidine to grow) are mixed with rat liver enzymes and plated on media lacking histidine.
- Liver enzymes are required to detect mutagens that are converted to carcinogenic forms by the liver (e.g., procarcinogens).
- Test chemical is then added to medium.
- Control plates show only a small of revertants (bacteria cells growing without Histidine).
- Plates inoculated with mutagens or procarcinogens show a larger of revertants.
- Auxotroph will not grow without Histidine unless a mutation has occurred.

https://slidetodoc.com/chapter-7-a-dna-mutation-and-repair-mutation/

Ames test



https://slidetodoc.com/chapter-7-a-dna-mutation-and-repair-mutation/

Genetic stability depends on the DNA repair mechanisms

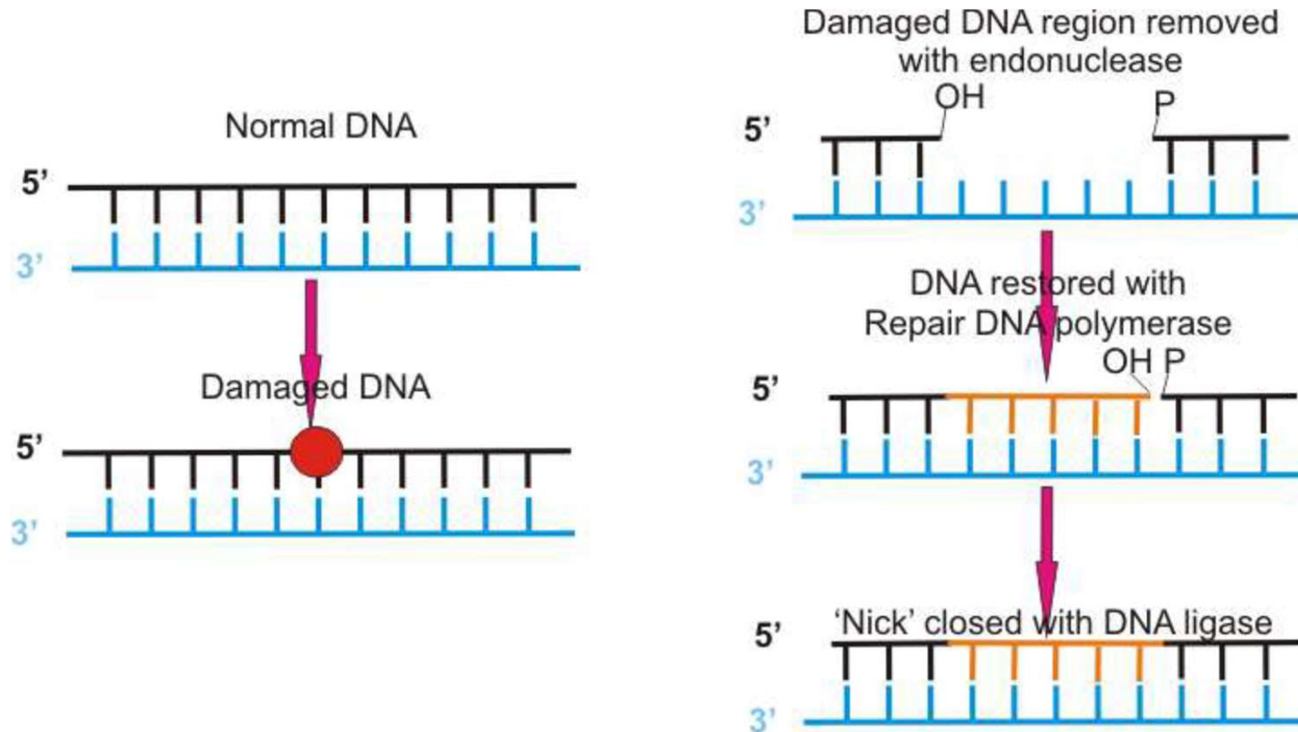
- DNA repair is a collection of cellular responses by which a cell identifies and corrects any damage to the DNA molecules that encode its genome.
- The frequency of mutations is kept to a minimum in healthy individuals.
- Extremely accurate DNA replication and existence of DNA-repair mechanisms contribute to keep frequency of mutations to minimimum.
- Most correction mechanisms use the existence of two copies of genetic information in the double helix of DNA.
- The damaged strand is identified by atypical structures and is repaired according to undamaged strand.
- Mutations in genes encoding proteins involved in DNA-repair increase the frequency of mutations, often lead to a predisposition to cancer.

DNA repair

For most repair mechanisms, there are three stages:

- Identification of the damaged DNA section and its removal.
  - Nucleases split covalent bonds between damaged nucleotides and the rest of the DNA strand.
  - The gap is created on one strand.
- Repair DNA-polymerase is attached to the 3´-OH end of broken strand and starts polymerization according to intact strand.
  - Filling the gap.
- Sugar-phosphate skeleton is healed by DNA-ligase.

Stages of DNA-repair



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Types of DNA-repair

Direct enzymatic repair
- Restoration to the original state.
- Without cleavage and resynthesis of DNA.
  - Photoreactivation.
  - Removal of methyl group - Dealkylation.

Indirect
- Excision repair.
  - Base.
  - Nucleotides.
  - Mismatch - controlled by methylation.

- Recombination/post replication.
  - Homologous recombination
  - Non-Homologous end joining

Inducible
- SOS-response.

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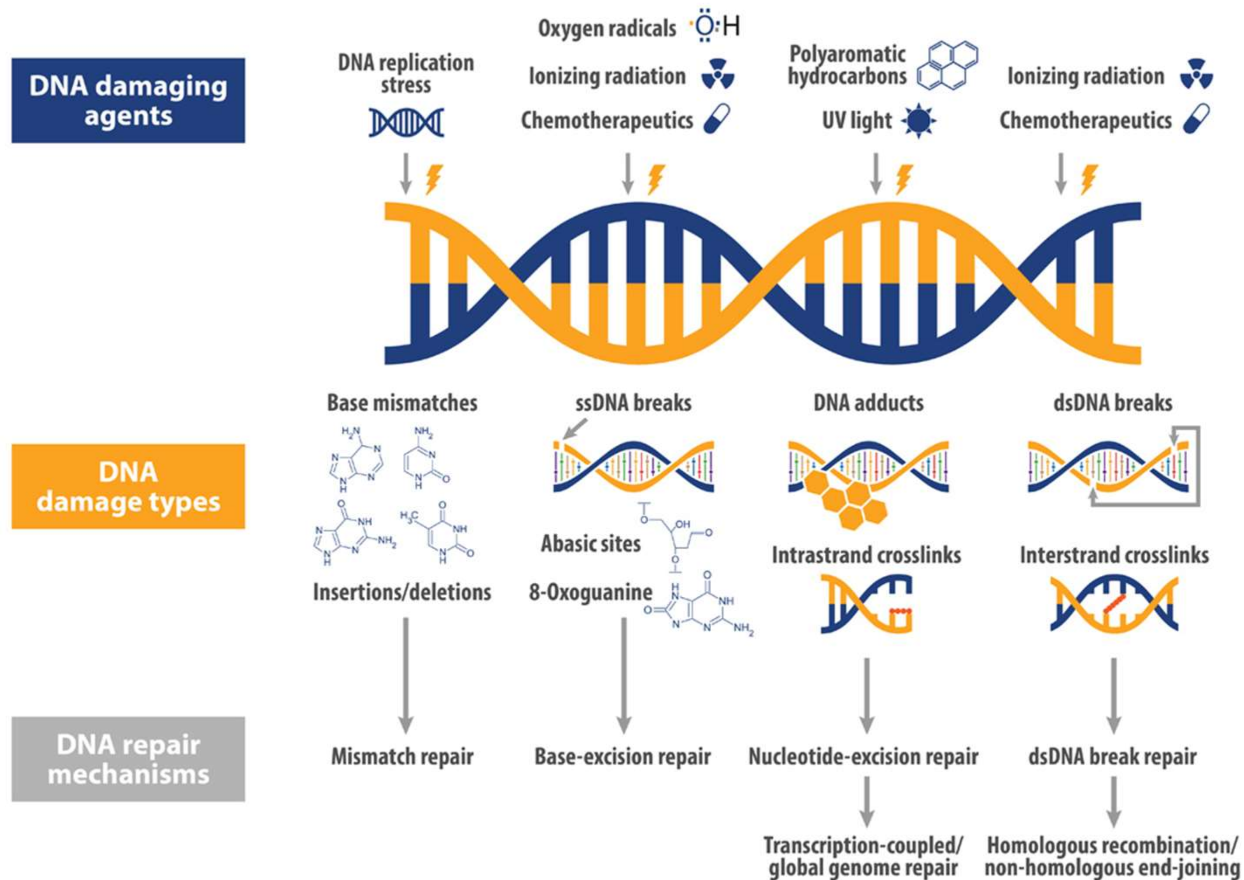
Types of DNA-repair



https://blog.crownbio.com/dna-damage-response

Mechanism of DNA-repairs

- Enzymes search for damaged DNA sites and activate repair.
- Genetic apparatus for DNA repair:
  - Very conservative, used by bacteria and humans.
  - About 100 genes.
  - Activates various mechanisms that can interconnect.

The most common types of DNA damage

- Depurination – removal of purine bases in DNA without disruption of the sugar-phosphate skeleton (the most common spontaneous mutation, 100 times more common than depyrimidination). Depurination prevents replication and transcription.

- Deamination – loss of the amino group of cytosine to form uracil.

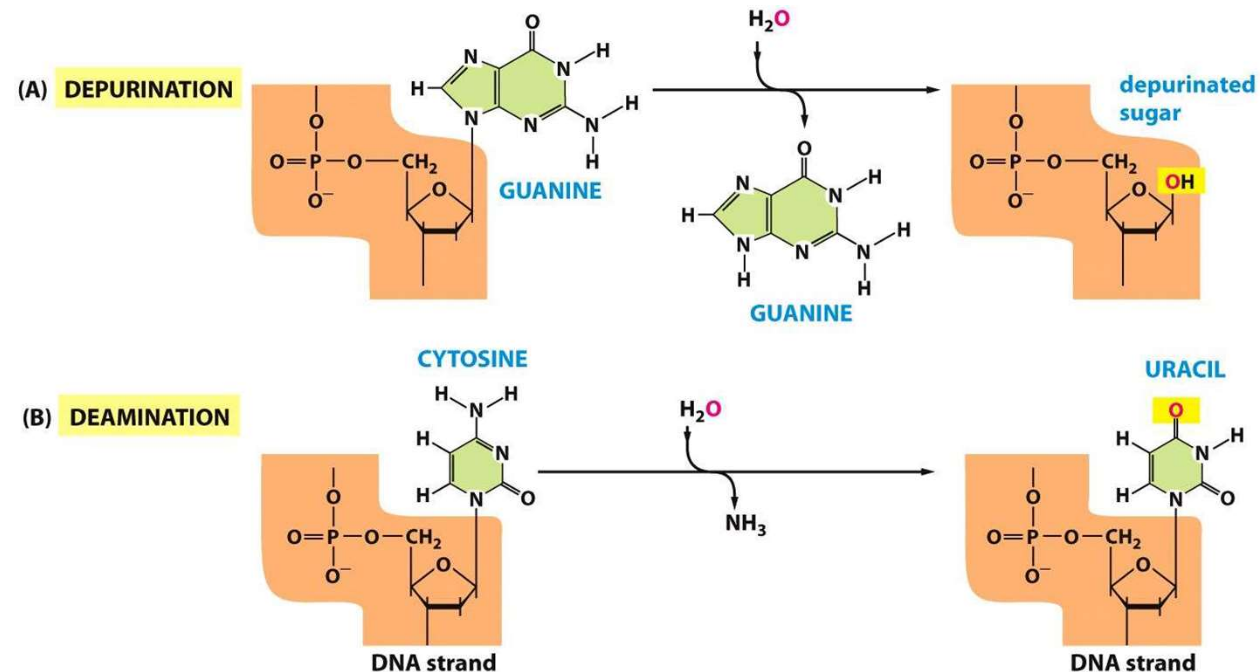


Figure 6-23 Essential Cell Biology 3/e (© Garland Science 2010)

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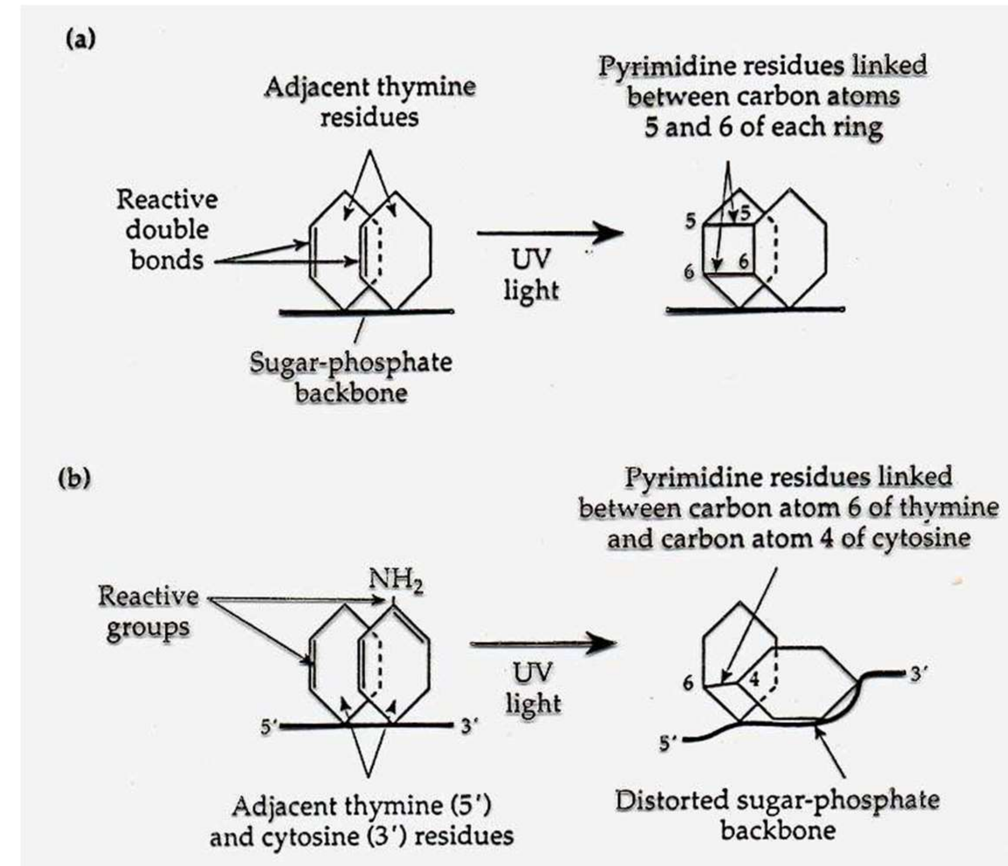
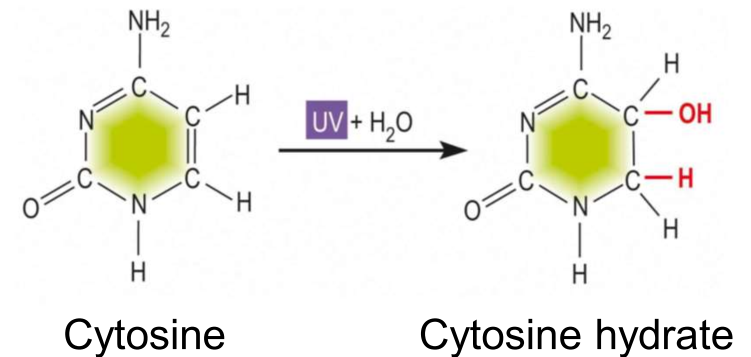
The most common types of DNA damage

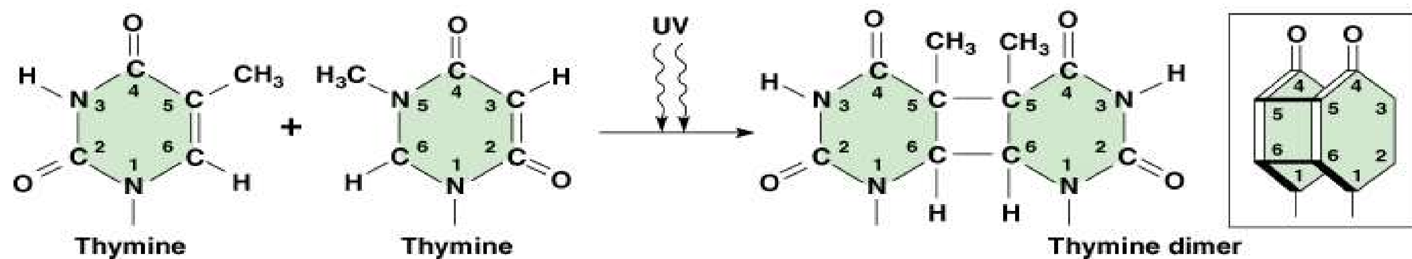
- Wrong base pairing – "mismatch„.
- Covalent connection of adjacent pyrimidines, consequence of UV radiation:
- Dimers thymine-thymine joined by aromatic (cyclobutane) circle.
- Less often appear dimers thymine - cytosine or cytosine – cytosine.



UV radiation - physical mutagens

- After absorption of UV, pyrimidines become very reactive and change themself to pyrimidine hydrates and then to the dimers.
- Pyrimidine dimers results in formation of the replication fork and block of replication.





Thymine dimers induced by UV light.

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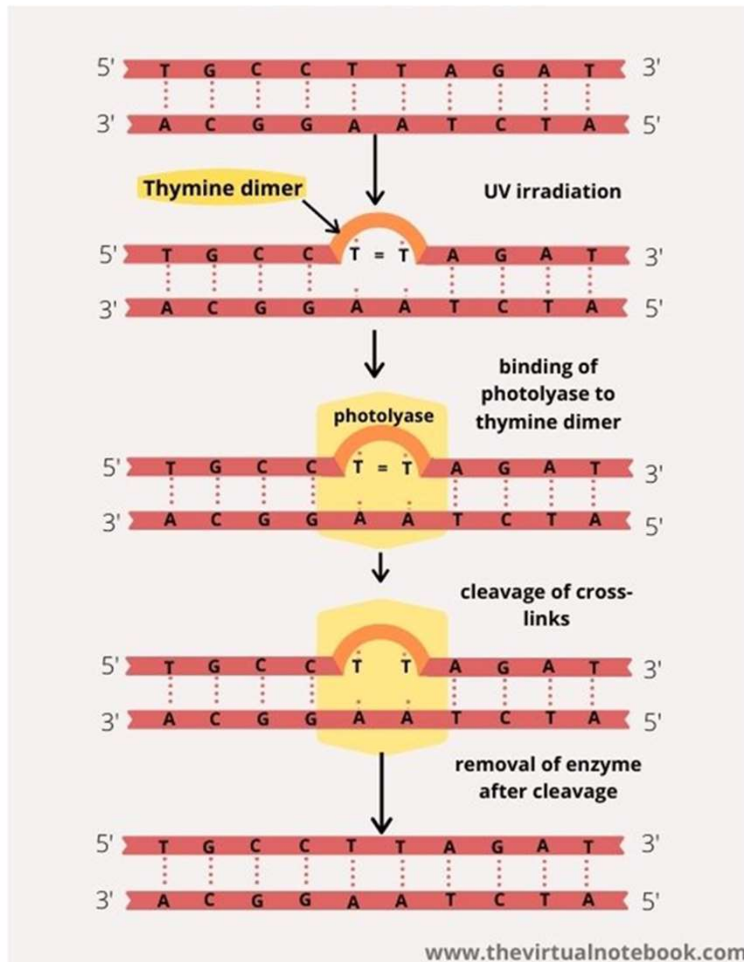
Photoreactivation – direct repair



- 1. Photoreactivation is a direct enzymatic cleavage of thymine dimers activated by visible light.
- Present in prokaryotes (e.g. *E.coli*) and plants exposed to sunlight.
- In mammals replaced by excision repair.
- Daylight activates the PHOTOLYASE and thus ensures cleavage of covalent cross-bond.
- Binding to dimers by photolyase occurs even in the dark.

https://www.thevirtualnotebook.com/dna-repair-definition-mechanism-types/

Photoreactivation – direct repair

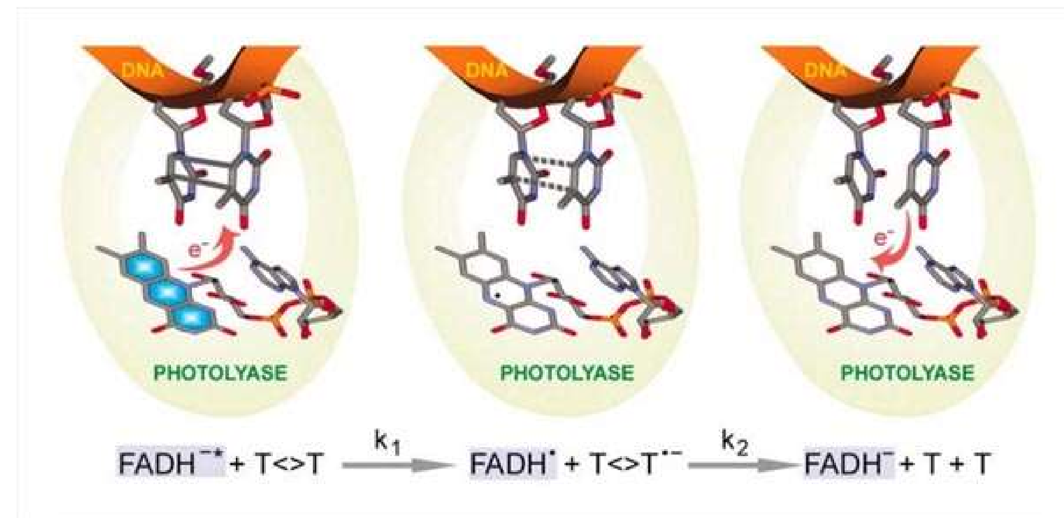
- Mechanism
- Enzyme photolyase (encoded by *phr* gene) binds to a pyrimidine dimer.

- Visible light (345 – 400 nm) shines on cell then FADH absorbs that light and release electron.

- Electron interact with dimer.

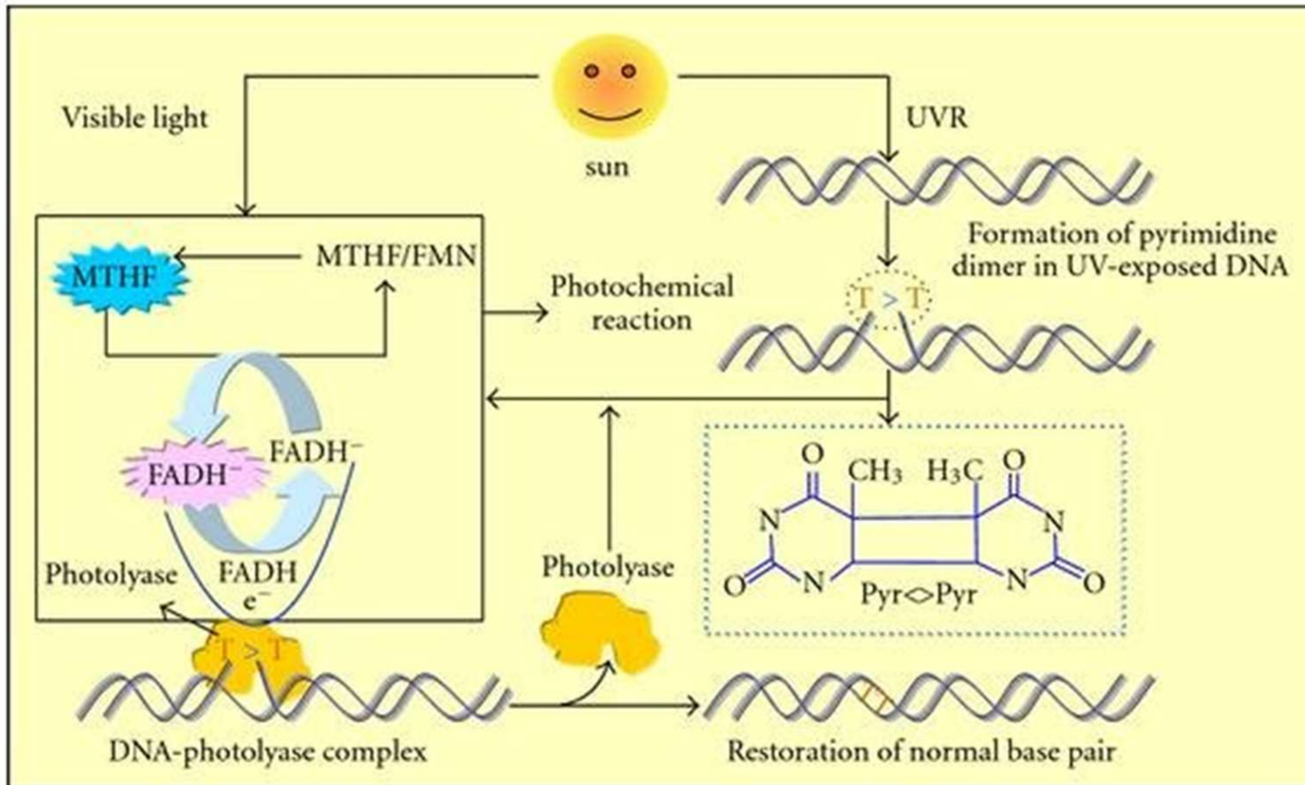
- Then splitting of cyclobutane ring in dimer due to electron interaction.

- Finally, enzyme leaves the DNA and the DNA structure returned to its prior state - the result is monomerization.



https://www.easybiologyclass.com/photoreactivation-dna-repair-uv-induced-dna-damages/

Photoreactivation – direct repair

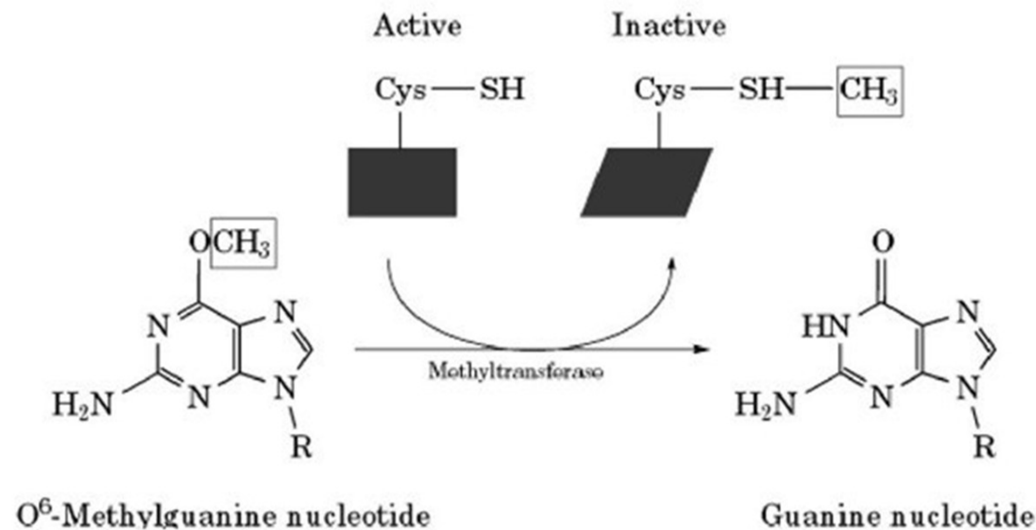


- The pyrimidine lesion, thymine dimers, are repaired by photoreactivating enzyme "photolyase".
- The light energy (>380 nm) is trapped by the antenna molecules of photolyase (such as MTHF/8-HDF/FMN) and transfers them to catalytic cofactor FADH− which becomes excited.
- FADH- transfers energy to the pyrimidine dimer and splits them into two monomeric unit, and then electron is transferred back to the flavin molecule.

Journal of Nucleic Acids, January 2010, (6551):592980

Repair of alkylated DNA

- Direct repair - dealkylation - adaptive response.
- The enzyme: alkyltransferase (demethylase) (O^6-metylguanin-DNA-metyltransferase = Ada-protein).
- Alkyltransferase takes the methyl group from methylated guanine (the acceptor is cysteine Ada-protein) or from phosphate groups of sugar-phosphate backbone.



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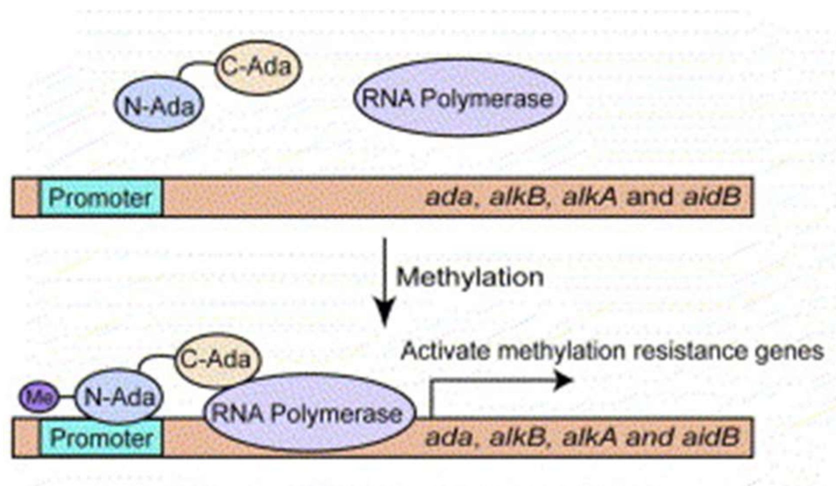
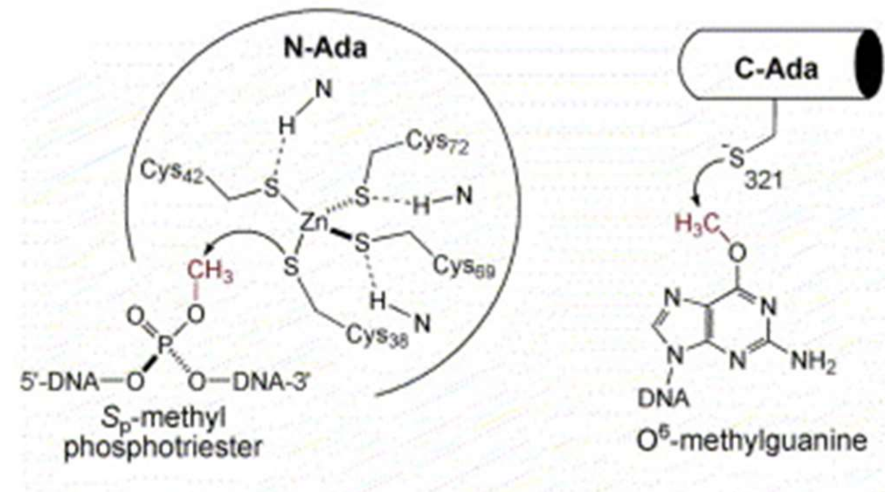
Repair of alkylated DNA

- Ada-protein is a <u>bifunctional</u> protein and uses a N-terminal Cys38 residue to remove a methyl group from Sp-methylphosphotriester and a C-terminal Cys321 residue to remove a methyl adduct from O^6-methylguanaine.

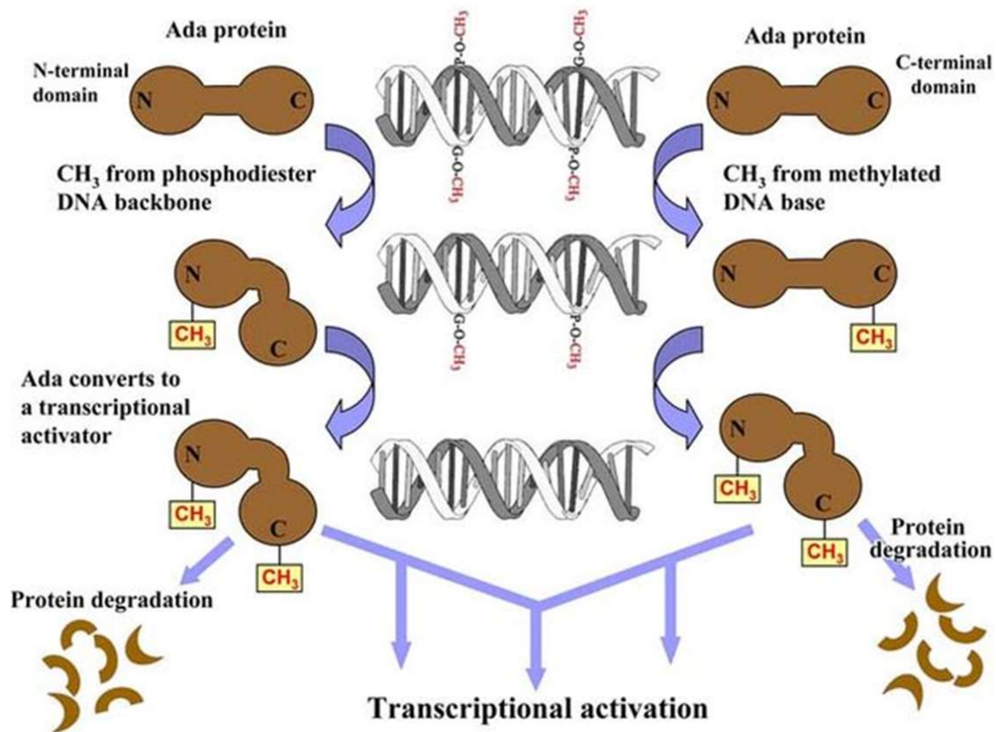
- Upon receiving a methyl group from the damaged DNA, Ada turns into a transcriptional activator and activates its own expression and transcription of genes repairing damaged caused by of alkylation, AlkB.

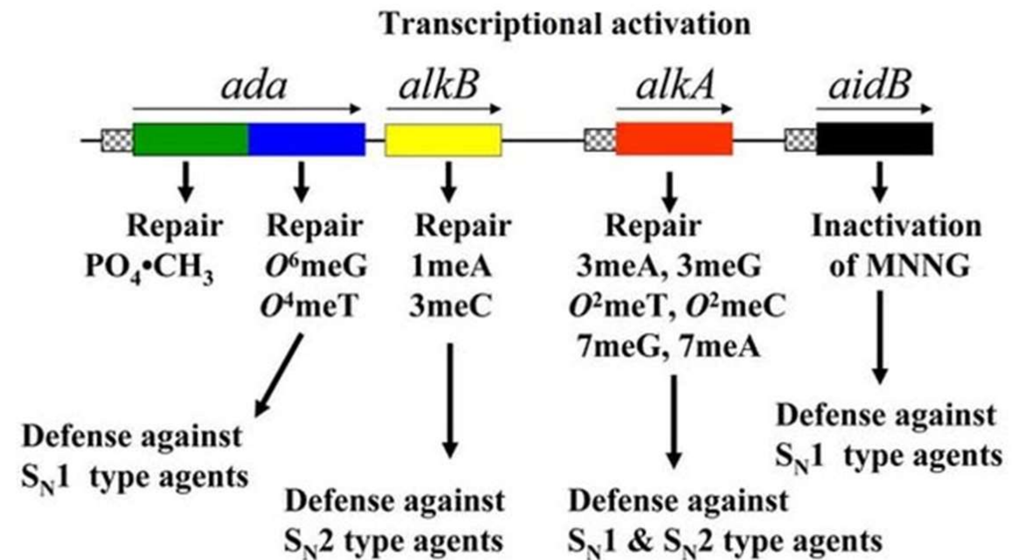




Journal of Inorganic Biochemistry, Volume 100, Issue 4, April 2006, Pages 670-678.

Repair of alkylated DNA





Direct DNA Lesion Reversal and Excision Repair in Escherichia coli , SOPHIE COUVÉ et al., February 19 , 2013.

Types of DNA-repair

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Inducible

- SOS-response.

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Excision repair mechanism

- Excision repair is a general mechanism of DNA repair.

- Various enzymes are involved that can sense DNA damage.

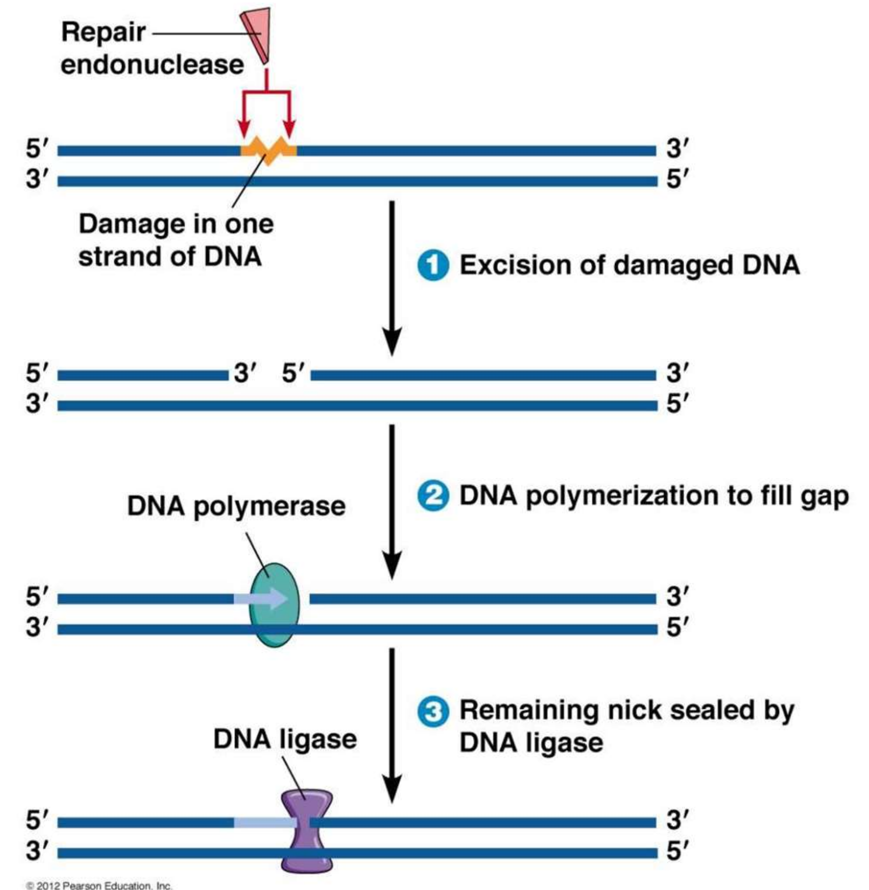
- During excision repair bases and nucleotides are removed from damaged strands.

- Gap is then patched using complementarity with the remaining strand.

- Excision repair is broadly categorized into:
  - Base excision repair.
  - Nucleotide excision repair.
  - Mismatch repair.

Excision repair mechanism

- Repair mechanisms that include nucleotide removal utilize a common four-step pathway:
- 1. Detection: The damaged section of the DNA is recognized.
- 2. Excision: DNA-repair endonucleases nick the phosphodiester backbone on one or both sides of the DNA damage and remove one or more nucleotides.
- Polymerization: DNA polymerase adds nucleotides to the newly exposed 3′-OH group by using the other strand as a template.
- Ligation: DNA ligase seals the nicks in the sugar-phosphate backbone.



https://www.thevirtualnotebook.com/dna-repair-definition-mechanism-types/

Base excision repair (BER) pathway

- Base excision DNA repair pathways remove abnormal or chemically modified bases from DNA.

- Base from the nucleotide within DNA can be removed in several ways:
  - either by direct action of an agent such as radiation,
  - or by spontaneous hydrolysis,
  - by an attack of oxygen free radicals, or
  - by DNA glycosylases.

- BER repairs DNA bases damaged by:
  - Alkylation.
  - Deamination.
  - Oxidation.
  - Lost of base – Depurination, Depyrimidation.

Base excision repair (BER) pathway

- In BER pathway, a modified base is first excised and then the entire nucleotide is replaced.

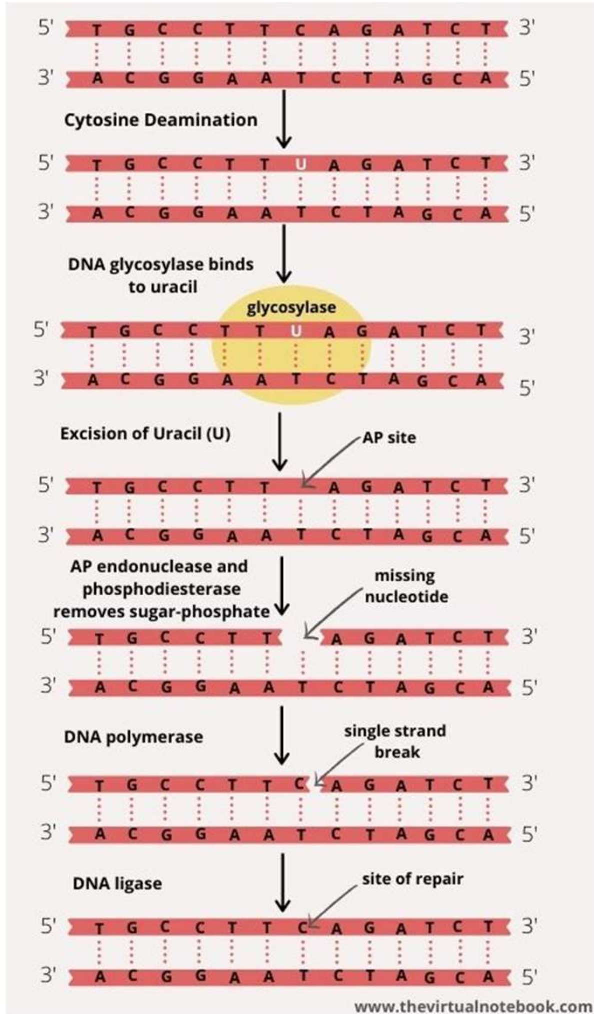
- The excision of modified bases is catalyzed by a set of enzymes called DNA glycosylases.

- Each of which recognizes and removes a specific type of modified base by cleaving the bond that links that base to the 1′-carbon atom of deoxyribose sugar.

- Each glycosylase recognizes a specific type of altered base, such as deaminated bases, oxidized bases, and so on.

https://www.thevirtualnotebook.com/dna-repair-definition-mechanism-types/

Base excision repair (BER) mechanism



- The glycosylases cleave the glycosidic bond between the abnormal, deaminated cytosine, base and 2-deoxyribose, creating apurinic or apyrimidinic sites (AP sites) with missing bases.

- An AP endonuclease then senses the minor distortion of the DNA double helix and initiates excision of the single AP nucleotide. Phosphodiesterase removes sugar-phosphate bond.

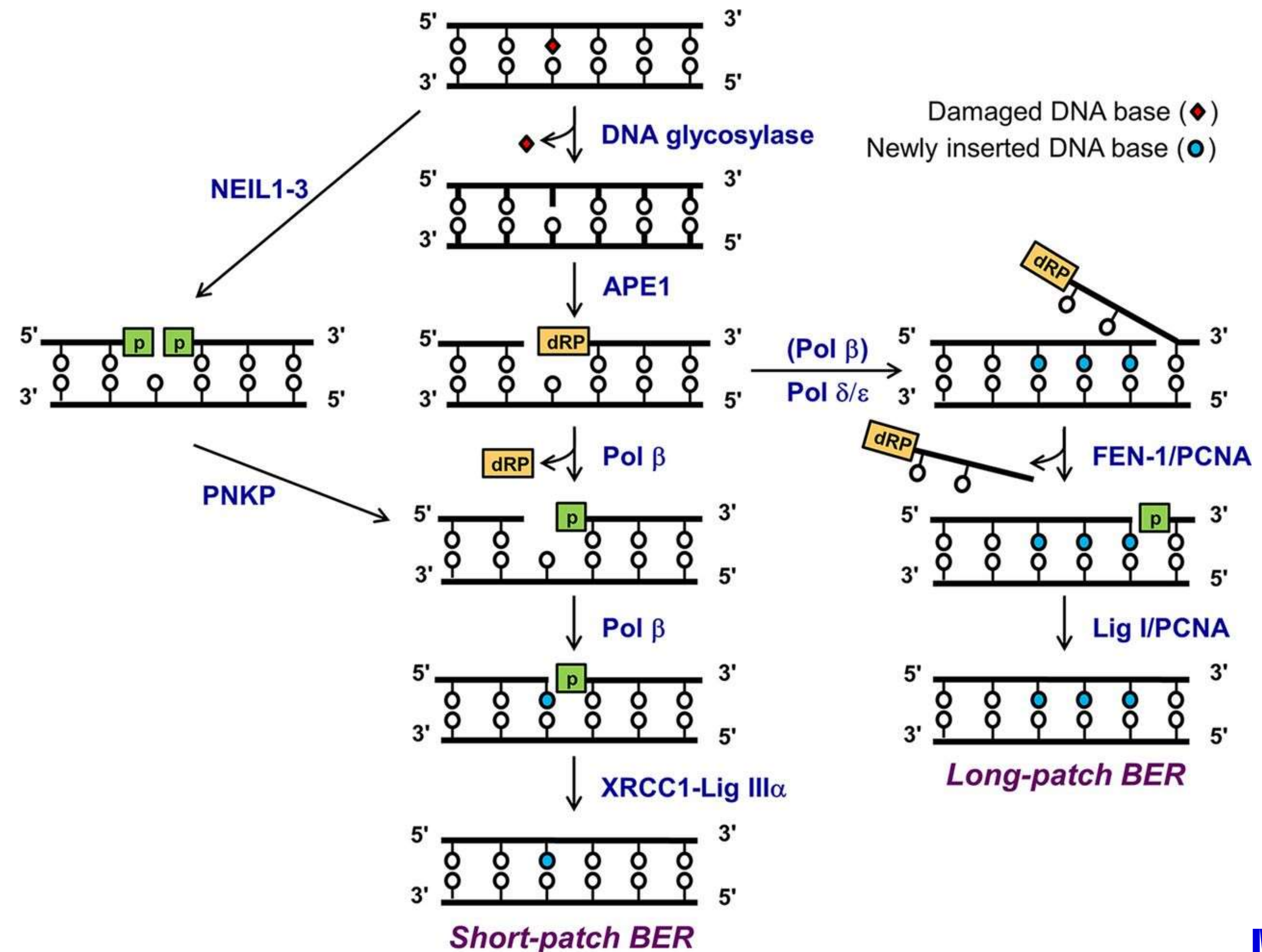
- DNA polymerase then replaces the missing nucleotide according to the specifications of the complementary strand.

- DNA ligase seals the nick.

https://www.thevirtualnotebook.com/dna-repair-definition-mechanism-types/

Base excision repair (BER) mechanism

- BER can remove
- Short-patch – nucleotide.
- Long-patch – couple of nucleotides.



Molecular and Cellular Biology, Volume 36, Issue 10, 15 May 2016, Pages 1426-1437.

Nucleotide excision repair (NER) pathway

- Nucleotide excision DNA repair pathways remove larger defects like thymine dimers and bases with bulky side-groups from DNA.
- NER is perhaps the most flexible mechanism in terms of the diversity of lesions that are recognized and repaired.
- The most significant of these lesions are pyrimidine dimers induced by UV light (cyclobutane pyrimidine dimers and 6-4 photoproducts), and other NER substrates include bulky chemical adducts, DNA intra-strand crosslinks, and some forms of oxidative damage.
- These types of lesions cause both a helical distortion of the DNA duplex and a modification of the DNA chemistry, both of which are hallmark features recognized by NER.

https://blog.crownbio.com/dna-damage-response

https://www.thevirtualnotebook.com/dna-repair-definition-mechanism-types/

Nucleotide excision repair (NER) pathway

- While mechanistically similar to BER, the NER pathway is more complex, requiring some thirty different proteins to carry out a multi-step 'cut-and-patch'-like mechanism.

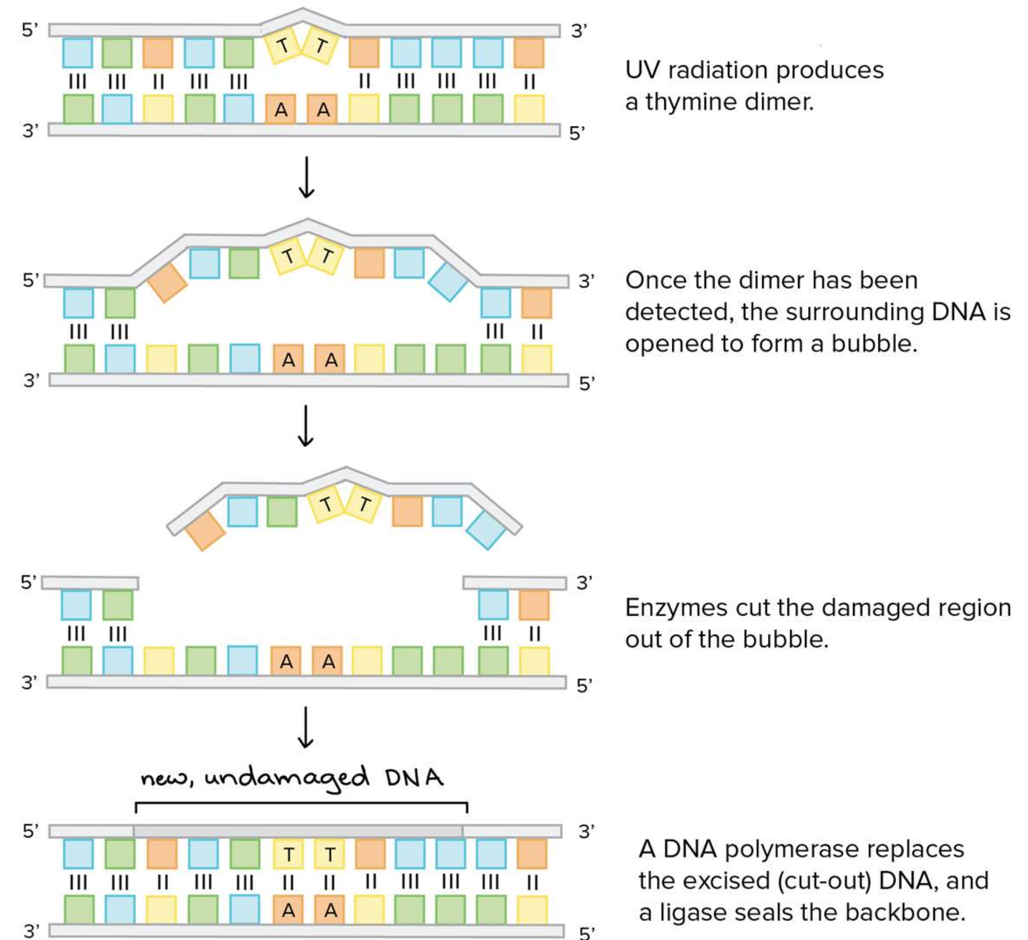
- The defects in NER cause several human genetic disorders, including:
  - Xeroderma pigmentosum
  - Cockayne syndrome
  - Trichothiodystrophy

- These all are characterized by extreme sun sensitivity.

- In addition, these diseases demonstrate overlapping symptoms associated with cancer, developmental delay, immunological defects, neurodegeneration, and premature aging.

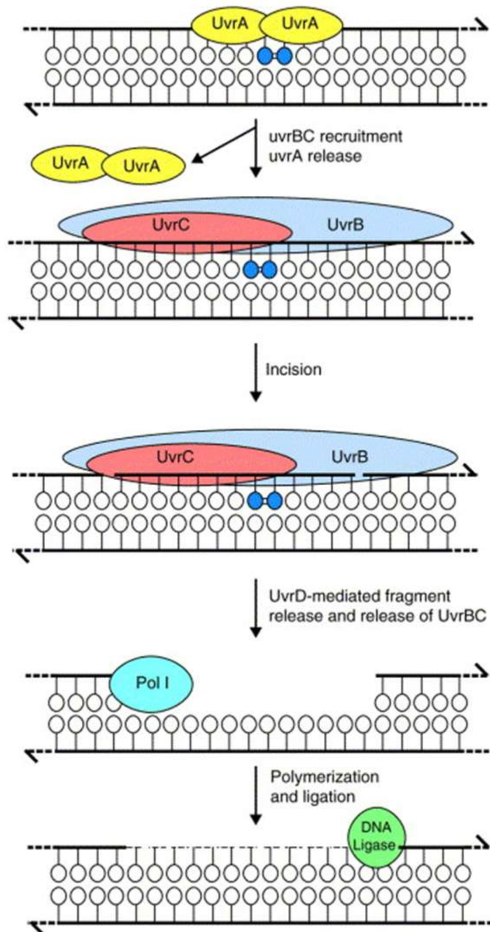
Nucleotide excision repair (NER) mechanism

- Thymine dimer is repaired by NER.
- Damaged nucleotide(s) are removed along with a surrounding patch of DNA.
- In this process, a helicase (DNA-opening enzyme) cranks open the DNA to form a bubble.
- DNA-cutting enzymes chop out the damaged part of the bubble.
- A DNA polymerase replaces the missing DNA, and a DNA ligase seals the gap in the backbone of the strand.



https://www.khanacademy.org/science/biology/dna-as-the-genetic-material/dna-replication/a/dna-proofreading-and-repair

Nucleotide excision repair (NER) in bacteria

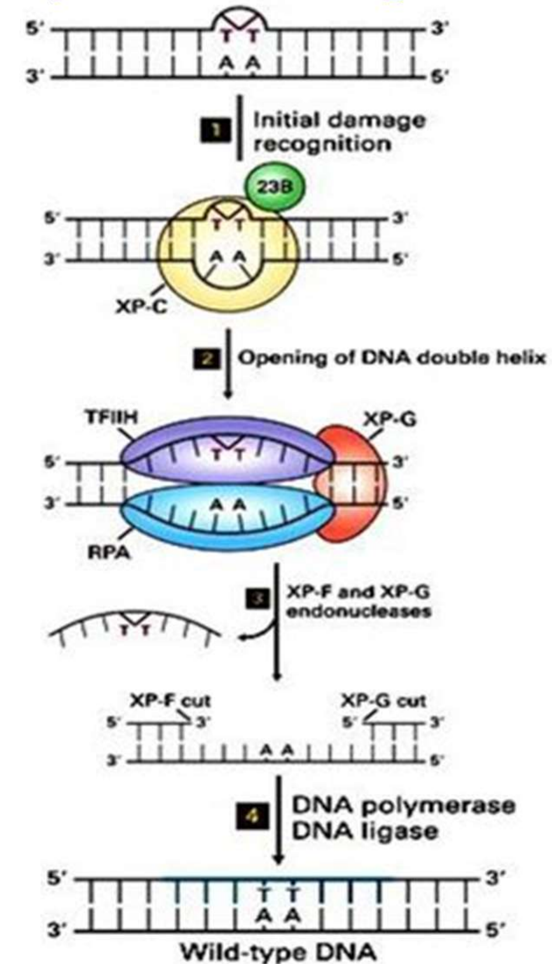


- UvrABC endonuclease is a complex in bacteria involved NER, is sometimes called an excinuclease. ABC excinuclease - subunits encoded by uvrA, uvrB and uvrC genes. It moves along DNA and detects Thymine dimers for excision endonuclease.
- UvrA and UvrB complex attach on distortion site then UvrA will dissociates.
- UvrB attracts UvrC and nicks 5 nucleotides at 3’ side of DNA while 8 nucleotides nicks at 5’ side of DNA will be produced by UvrC subunit.
- UvrD (DNA helicase II) removes 12 oligonucleotides.
- DNA polymerase I now fills in gap in 5‘ → 3' direction.
- DNA ligase seals the gaps.

Current Opinion in Microbiology, Volume 4, Issue 2, 1 April 2001, Pages 178-185

Nucleotide excision repair (NER) in eukaryotes

- Bulky DNA lesions that destabilize duplex DNA.
- Strongly distorting lesions are directly recognized by XPC-RAD23B, which binds the nondamaged strand opposite the lesion.
- TFIIH interacts with XPC-RAD23B and open DNA with RPA.
- The XPF recruitment makes incision 5´ to the lesion.
- Initiation of repair synthesis by Pol δ and Pol κ or Pol ε and, followed by 3′ incision by XPG.
- Sealing of the nick by DNA ligase IIIα/XRCC1 or DNA ligase I completes the process.



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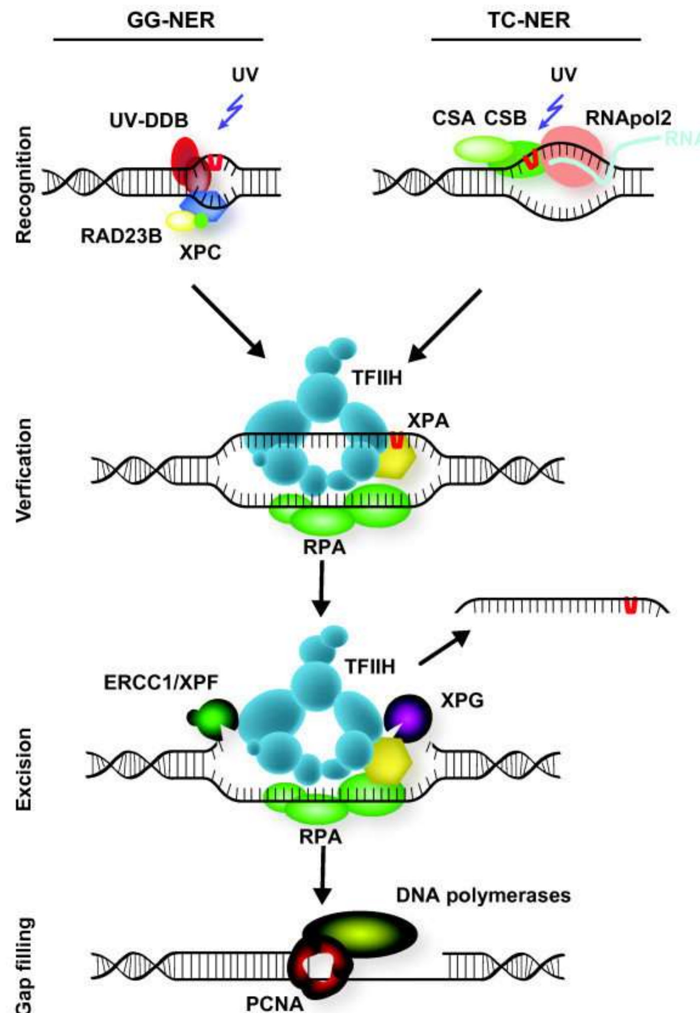
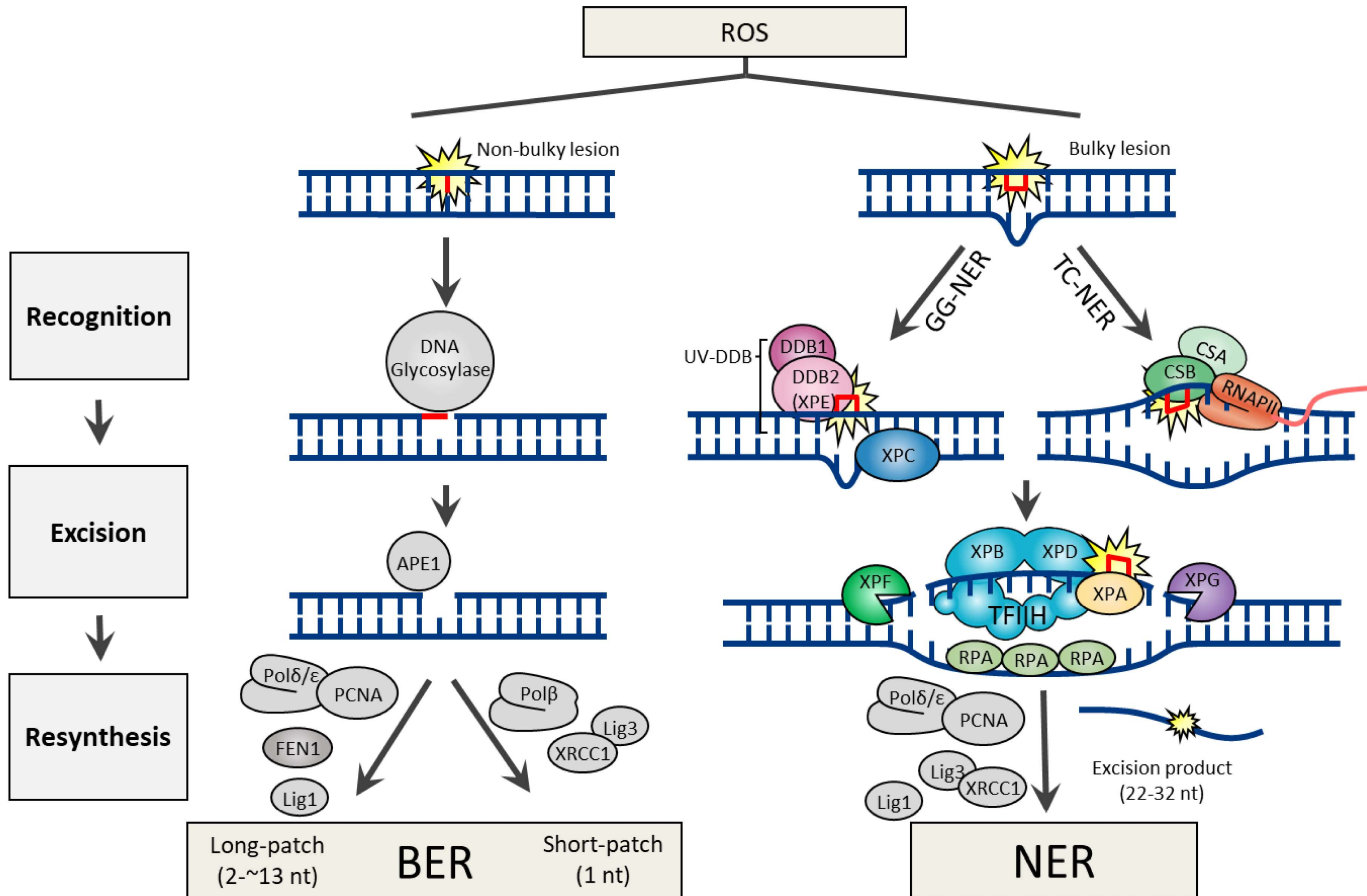
Nucleotide excision repair (NER) in eukaryotes



- NER is executed by two different damage-detection mechanisms, which utilize the same machinery to excise and repair the damage.
- Transcription-coupled NER (TC-NER) is initiated by stalling of RNA polymerase 2 on a lesion present in the transcribed strand of active genes, and depends on recruitment of the CSA and CSB proteins.
- Other lesions are removed by global genome NER (GG-NER), which is initiated by the UV-DDB ubiquitin ligase complex and the heterotrimeric XPC/RAD23/CETN2 complex.
- Following detection, the TFIIH complex is recruited, and unwinds a stretch of approximately 30 nucleotides around the damage, providing access for other repair factors.

Epigenetics & Chromatin, January 2012, 5(1):4

BER and NER comparison in eukaryotes



- DNA lesions:
- non-bulky (non-helix distorting)
- bulky (helix distorting) lesions.

- In human cells, BER and NER are responsible for the removal of non-bulky and bulky DNA lesions, respectively.

https://encyclopedia.pub/entry/176

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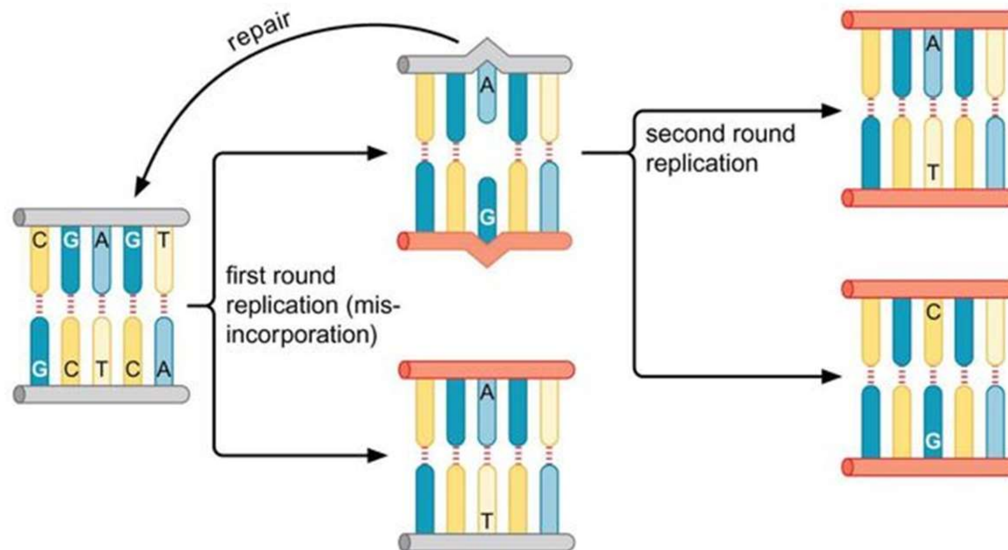
Mismatch repair (MMR) pathways

- A system of recognizing and repairing errors that occur during DNA replication and/or repairing errors caused by DNA damage.
- There are many different types of mismatch repair systems; however, mismatch repair mechanisms are strand-specific.
- Accounts for 99% of all repairs.



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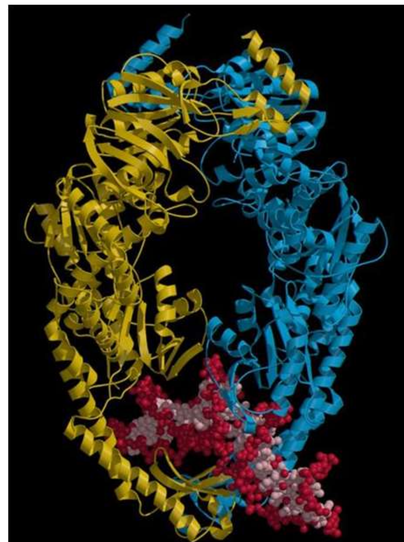
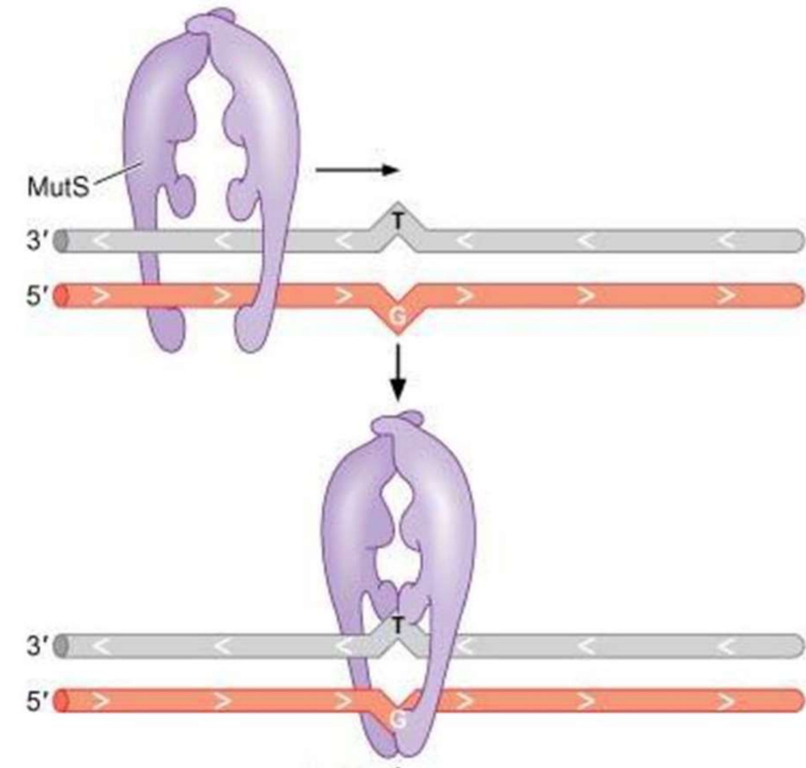
Mismatch repair (MMR) pathways

- Main challenges mismatch repair proteins face:
- 1. Must scan the genome for mismatches (sensed as a distortion of the double helix).
- 2. Must repair before the next round of replication.
- 3. Must repair the “mutant” nucleotide on the newly synthesized strand.





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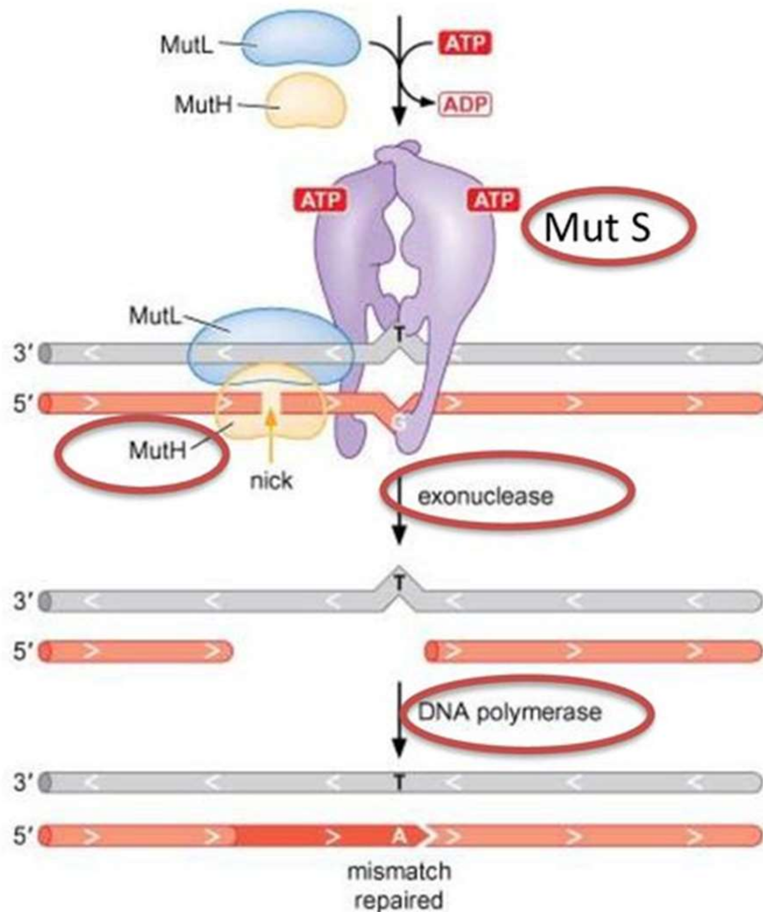
Mismatch repair (MMR) pathways in prokaryotes



- MMR removes single base pair errors that escape proofreading. Well studied in *E. coli*.

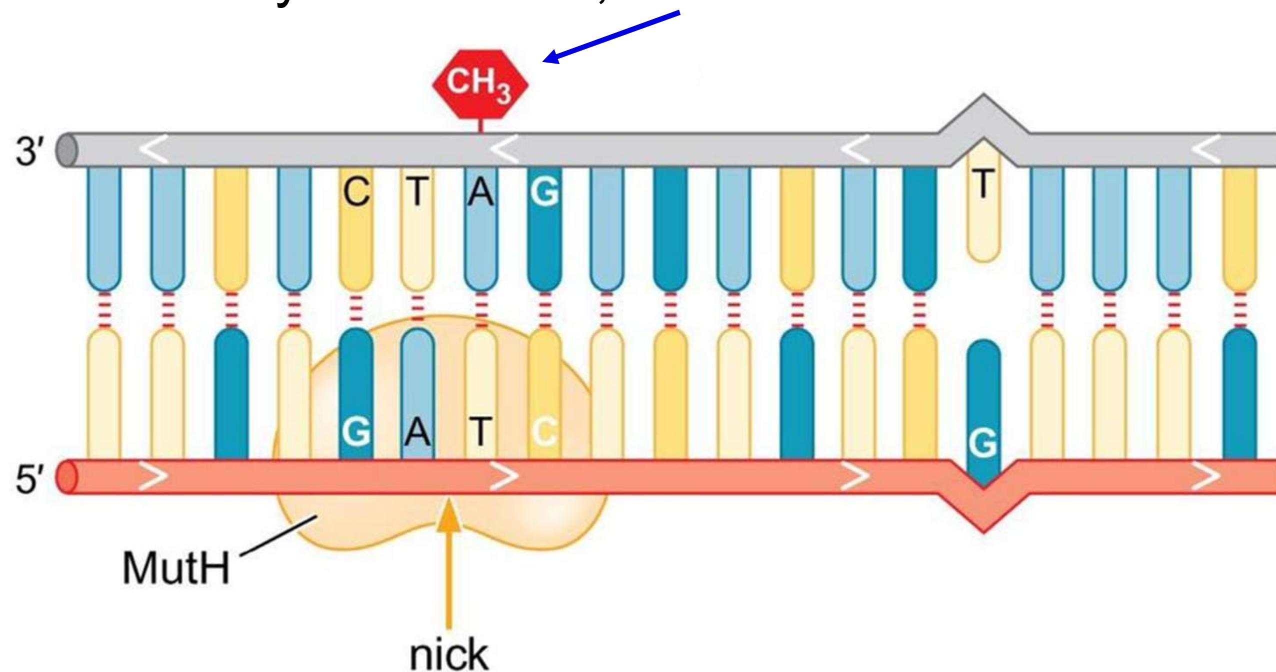
<u>Proteins are called the Mut proteins:</u>

- MutS - recognizes mismatches.
- MutH - nicks one strand ahead of the mutation.
- UvrD, DNA-helicase II, and exonuclease are recruited to remove base pairs including the mismatch.
- DNA Pol III fills in the gap and ligase sealed it.

https://slideplayer.com/slide/15878337/

Mismatch repair (MMR) pathways in prokaryotes

- How does the Mismatch Repair System “know” which strand has the mutation?
- It is because the newly synthesized strand of DNA is not methylated by the enzyme DNA methyltransferase, DAM.



- MutH does nicks the newly synthesized DNA strand.

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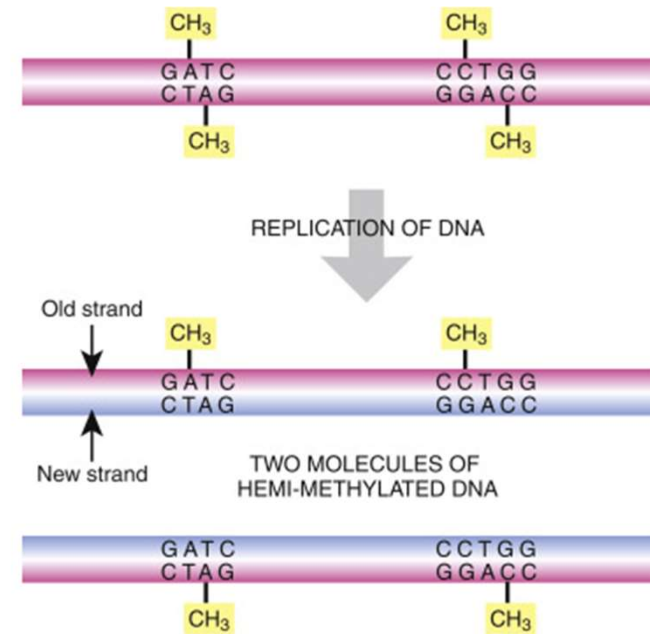
DNA methylation during replication in prokaryotes

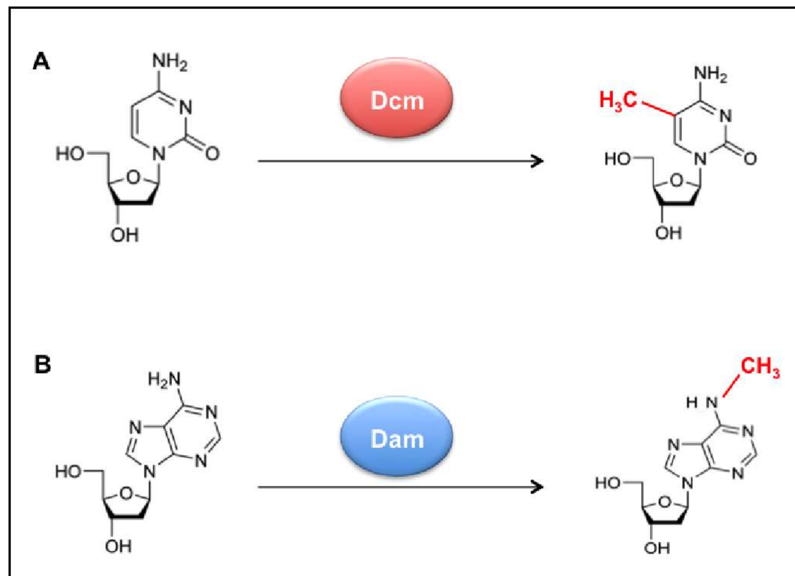
- When DNA is replicated, the old strand is methylated, thus the DNA double helix, as a whole, is “hemi-methylated“, but there is a delay in methylating the new strand and.
- Methylase works with a delay of 2 – 3 minutes.
- During this period, parent chains can be distinguished from newly synthesized.

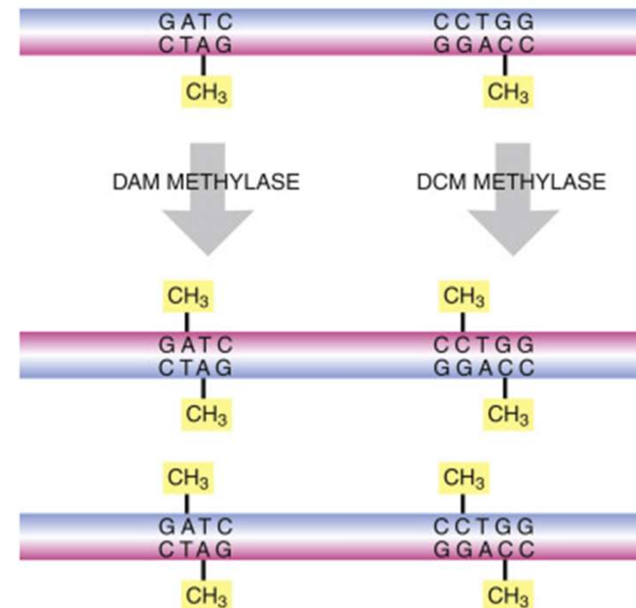


https://www.sciencedirect.com/topics/nursing-and-health-professions/dna-adenine-methyltransferase

DNA methylation during replication in prokaryotes

- Methylation is usually subject to adenine or cytosine, provided by enzymes – methylases.
- In bacteria eg. Dam-methylase methylates adenine in the GATC sequence.





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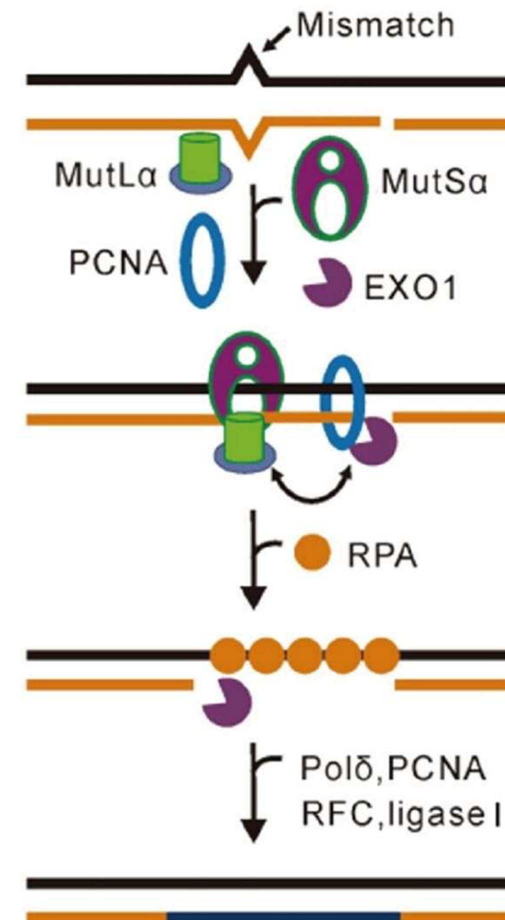
Mismatch repair (MMR) pathways in eukaryotes

- In eukaryotes the MutS Homologs (MSH) were discovered by analyzing for similarities in protein sequences.
- In eukaryotes the template DNA is not methylated.
- MMR in eukaryotes is coupled with DNA replication.
- The interaction of MutLα, member of MMR repair complex with PCNA provides the strand-discrimination signal for MMR, because RFC asymmetrically loads PCNA onto DNA at a nick.
- Mutations in MSH genes result in several malignant diseases, such as hereditary nonpolyposis colorectal cancers.

Mismatch repair mechanism in eukaryotes

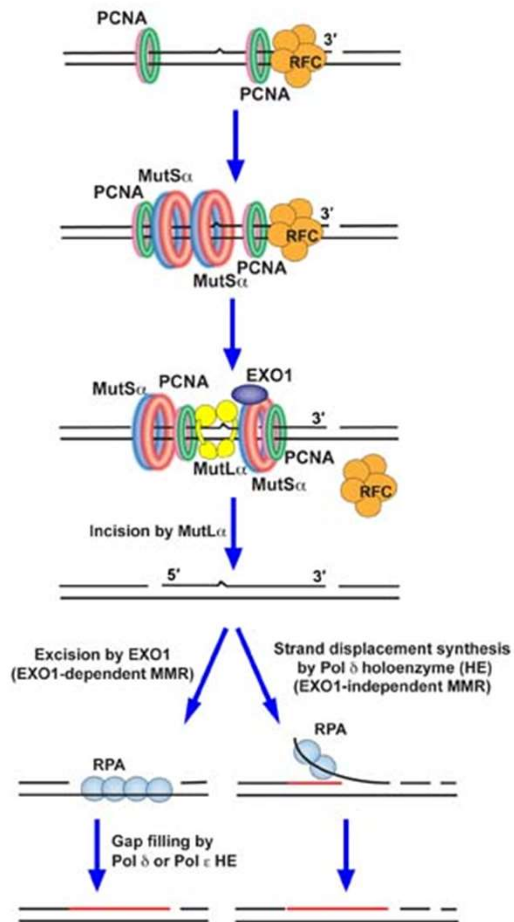
Factors required for mammalian DNA mismatch repair pathway:

- Primary mismatch recognition factor MutSα (MSH2-MSH6 heterodimer).
- MutLα endonuclease (MLH1-PMS2 heterodimer).
- Replicative clamp PCNA.
- The clamp loader RFC.
- The 5'→3' exonuclease EXO1.
- DNA polymerase δ (Pol δ).
- DNA Ligase I.



Science China. Life sciences, October, 2017, 60(10)

Mismatch repair mechanism in eukaryotes



- The MutSα recognizes the mismatch and cooperates with loaded PCNA to activate MutLα endonuclease.

- The activated MutLα endonuclease incises the discontinuous daughter strand 5' and 3' to the mismatch.

- An incision produced by MutLα 5' to the mismatch is utilized by MutSα-activated EXO1 to enter the DNA and remove the mismatch in a 5'→3' excision reaction modulated by RPA.

- The generated gap is repaired by the Pol δ holoenzyme.

https://biochem.siu.edu/faculty/Kadyrov.html

Types of DNA-repair

Direct enzymatic repair
- Restoration to the original state.
- Without cleavage and resynthesis of DNA.
  - Photoreactivation.
  - Removal of methyl group - Dealkylation.

Indirect
- Excision repair.
  - Base.
  - Nucleotides.
  - Mismatch - controlled by methylation.

- Recombination/post replication.
  - Homologous recombination
  - Non-Homologous end joining

Inducible
- SOS-response.

Double-srand DNA brakes

- A double-strand DNA break (DSB) occurs or arises when both strands of the DNA duplex is cut/disengaged, often as the result of ionizing radiation.

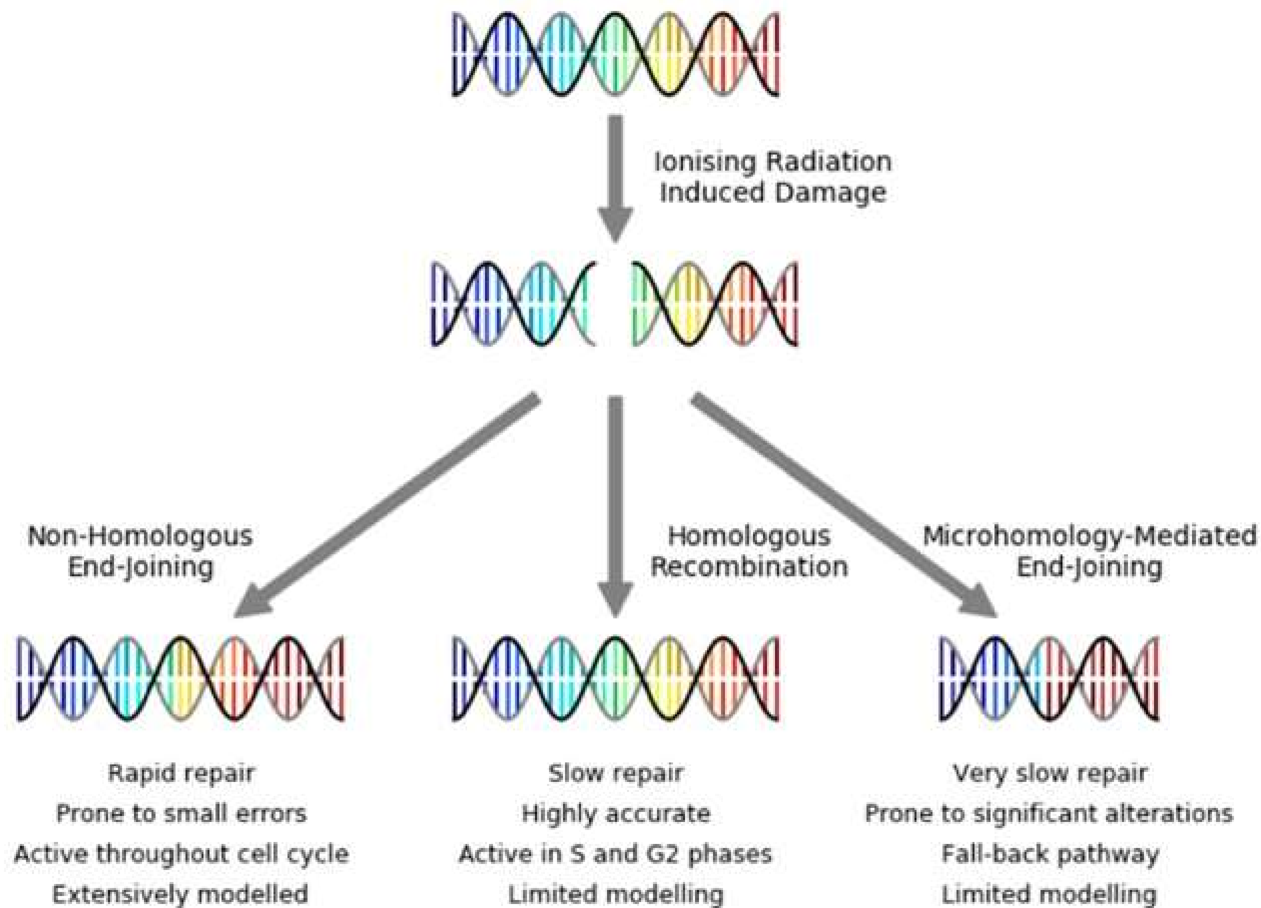
- DSB can also be caused by mechanical stress on chromosomes or when a replicative DNA polymerase encounters a DNA single-strand break or other type of DNA lesion.

- They are particularly deleterious as the DNA ends can promote potentially gene loss or lethal chromosomal rearrangements.

- Can be repaired by:
- Homologous Recombination (HR).
- Non-Homologous-End Joining (NHEJ).
- Microhomology-Mediated-End-Joining (MMEJ).

https://www.nature.com/subjects/double-strand-dna-breaks

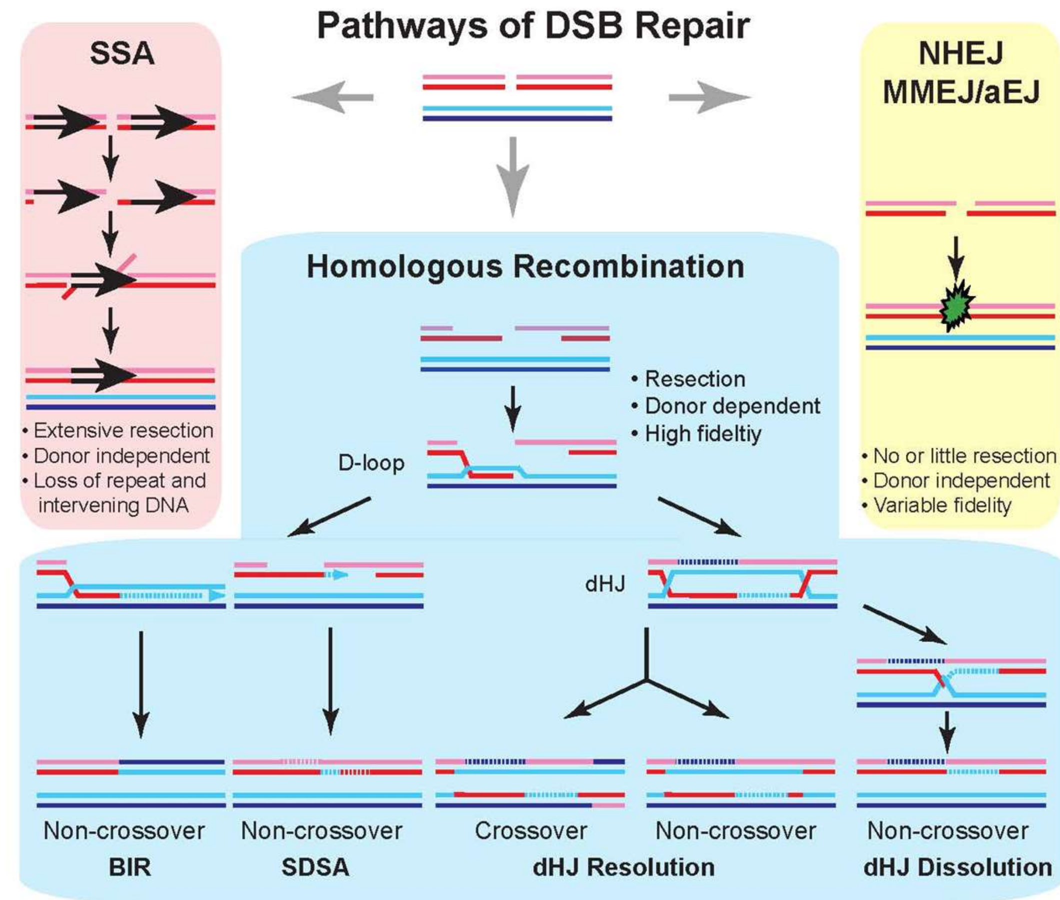
Double-srand DNA brakes



Cancers, February, 2019, 11(2):205

Double-srand DNA brakes

- DSBs can be repaired by several different mechanisms:
- nonhomologous end joining – NHEJ
- microhomology-mediated end joining – MMEJ
- double Holliday junction – dHJ
- double-strand break repair – DSBR
- synthesis-dependent strand annealing – SDSA
- break-induced replication - BIR
- single-strand annealing – SSA.



https://microbiology.ucdavis.edu/heyer/wordpress/research

Homologous recombination (HR)

- HR repair DNA breaks by retrieving sequence information from undamaged DNA.
- DSB are repaired mostly by HR and NHEJ pathways.

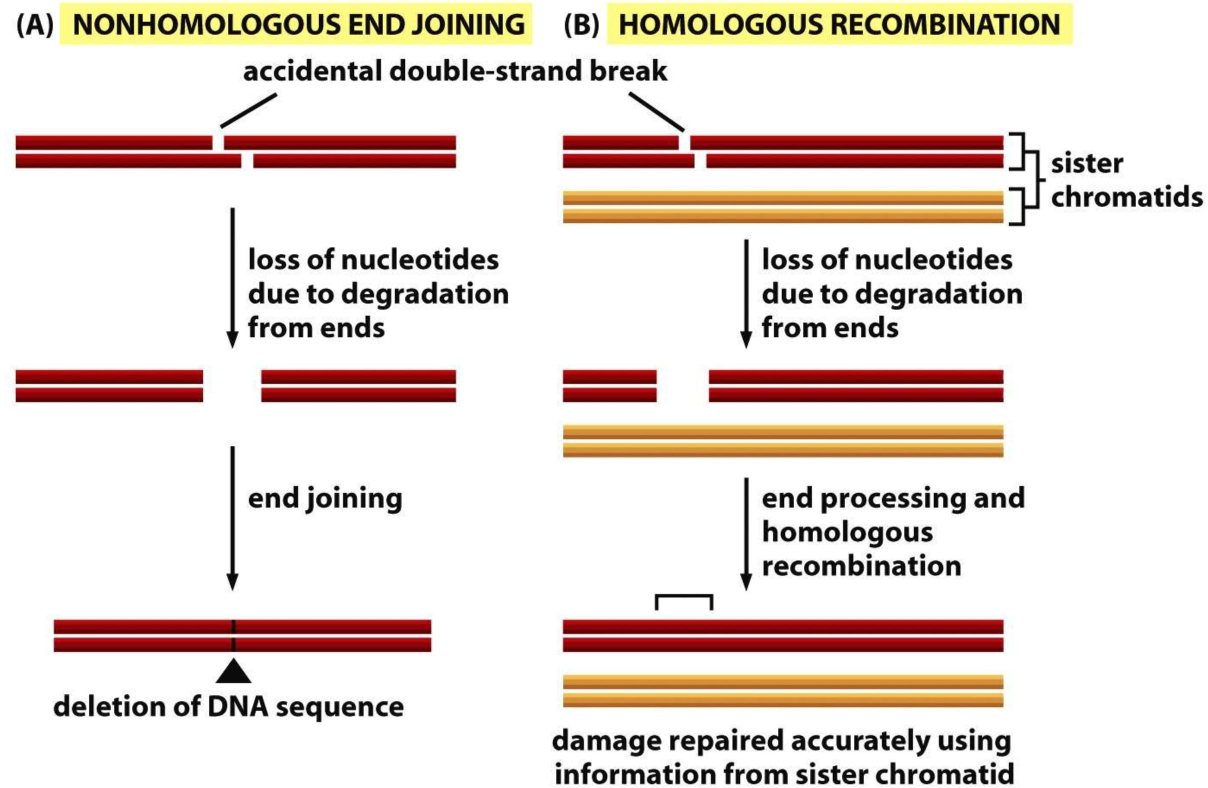
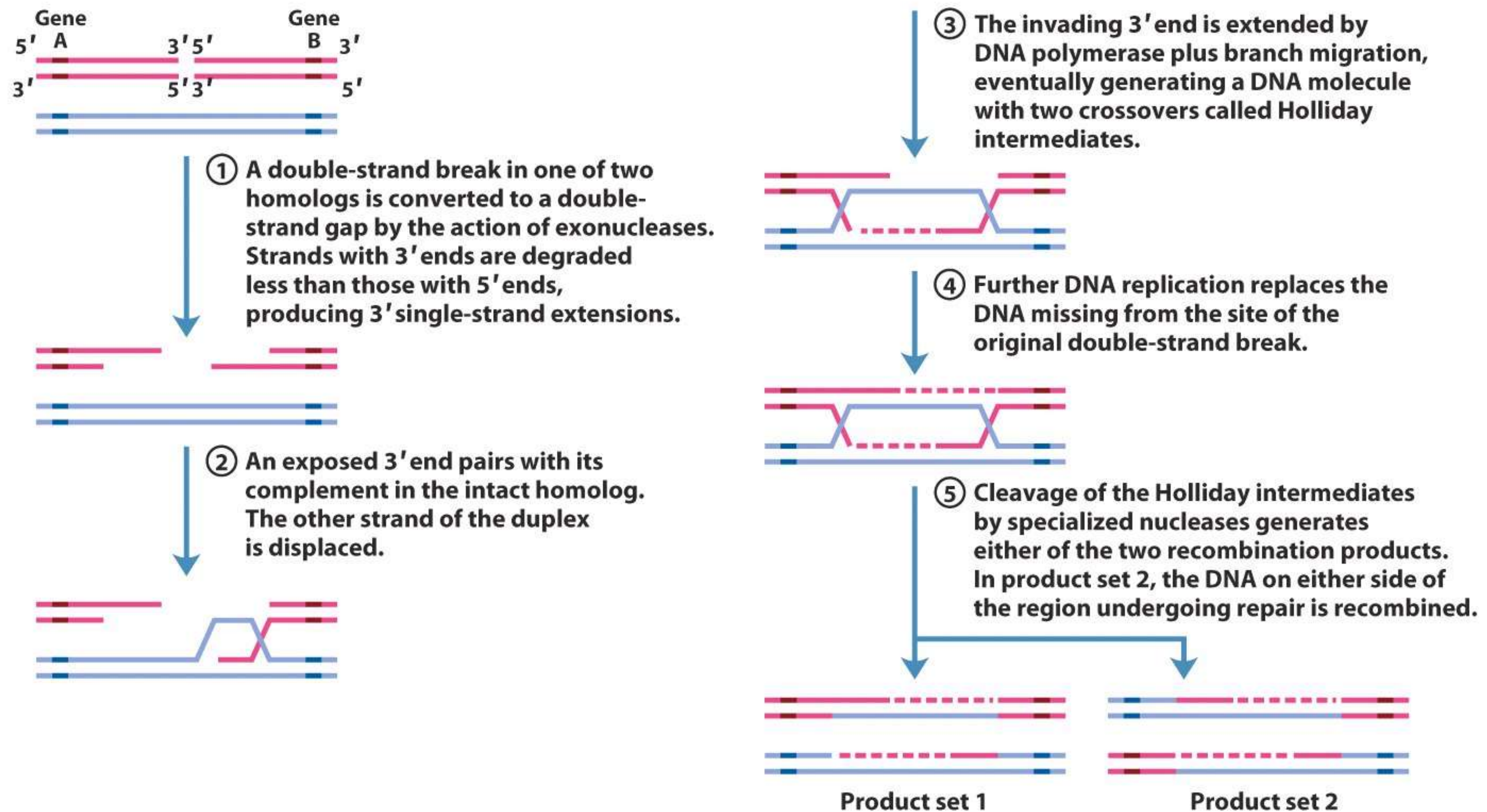


Figure 5-51 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Model of homologous recombination



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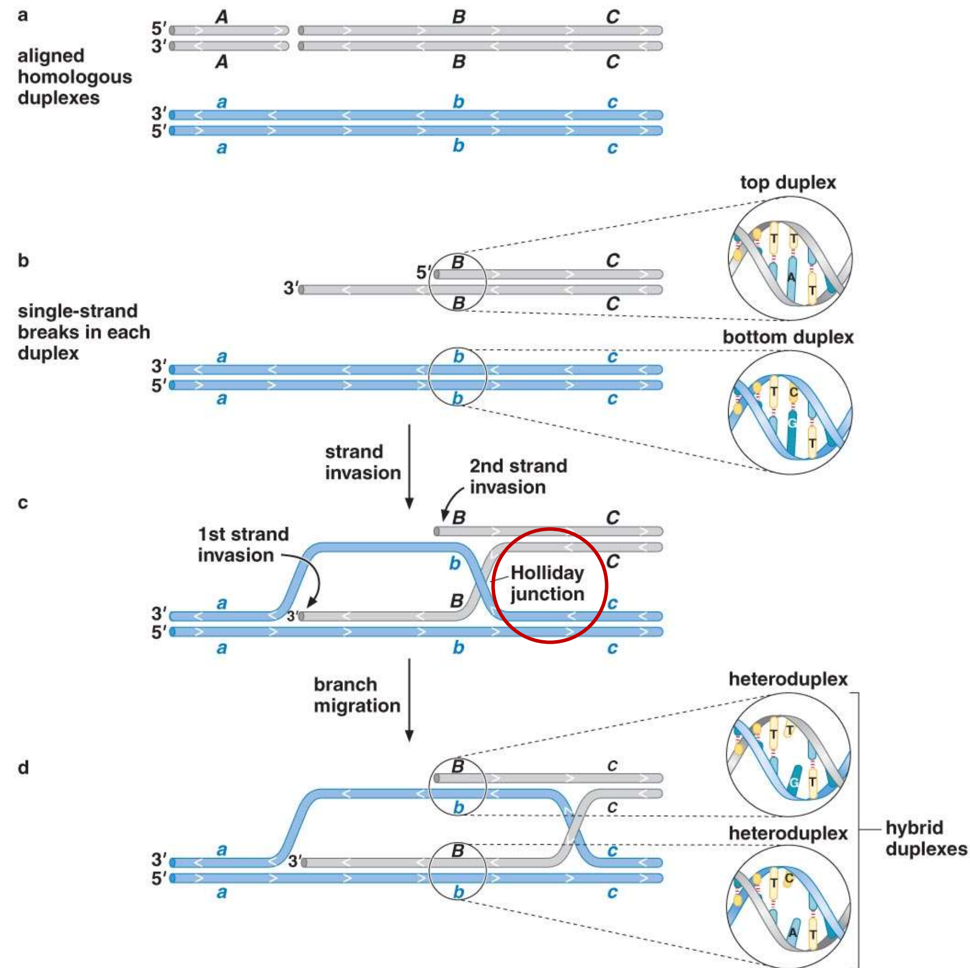
Model of homologous recombination

- Strand invasion (strand exchange) is a key step in homologous recombination.



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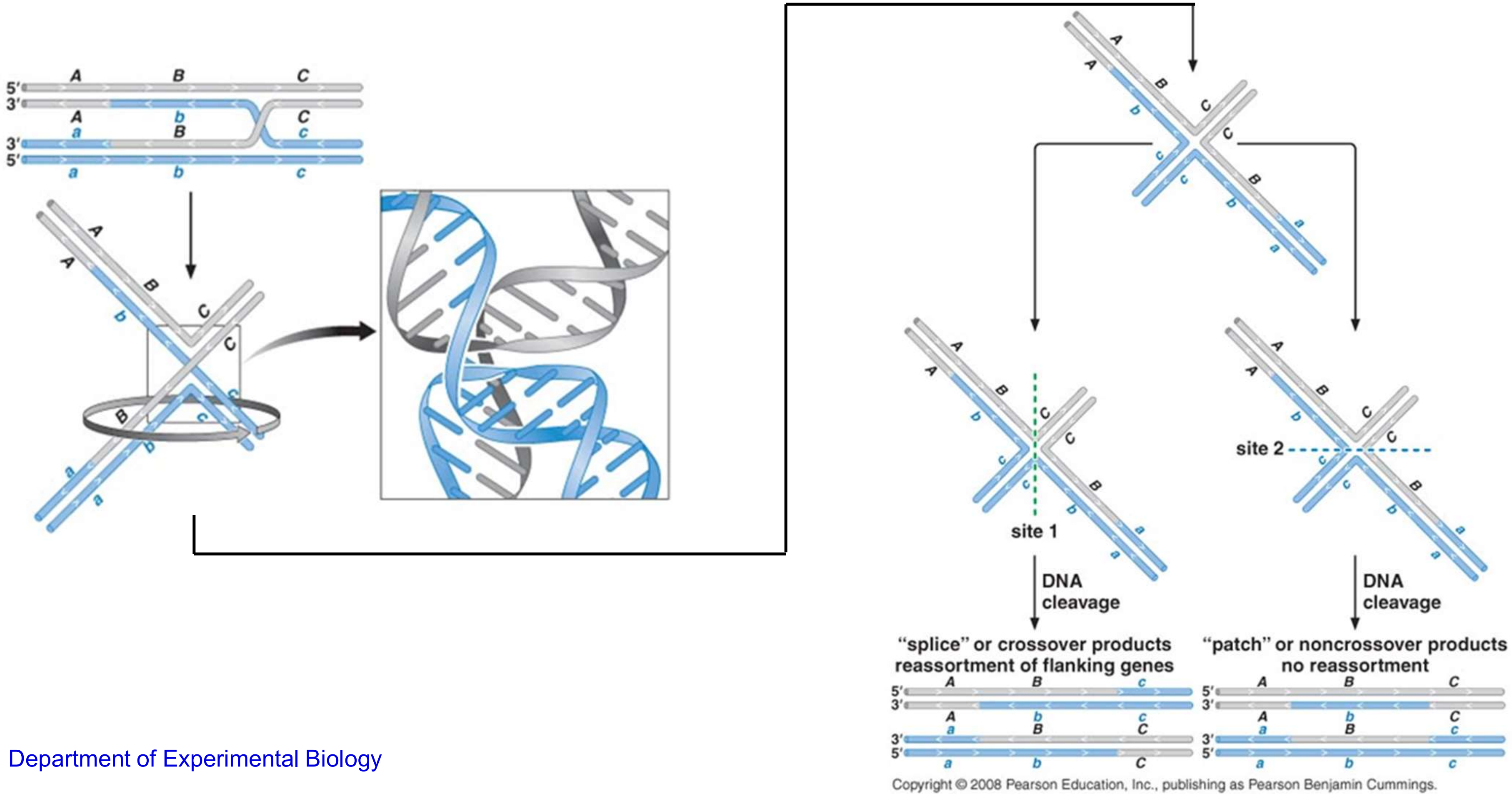
Model of homologous recombination

- Resolving Holliday junctions is a key step (final step) to finishing genetic exchange.



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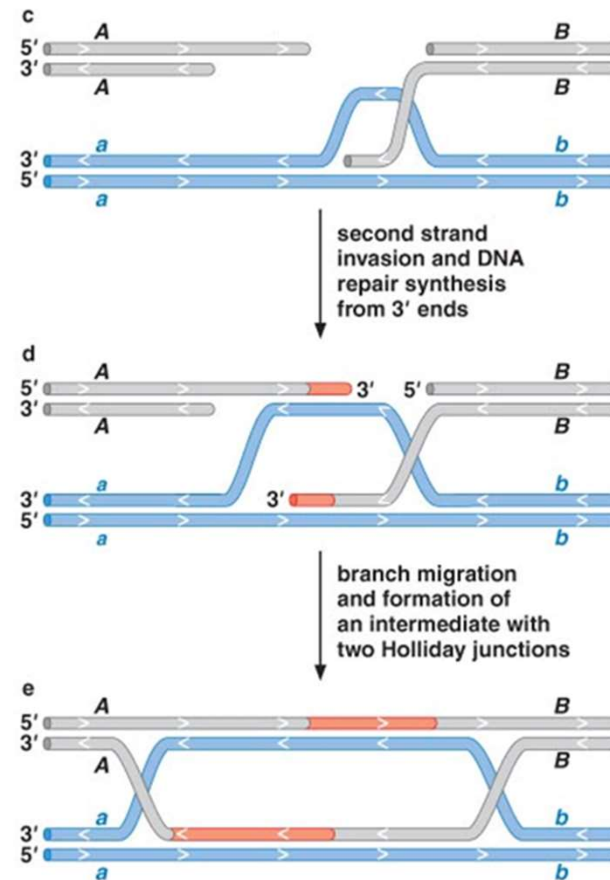
Model of homologous recombination

- The double-strand break-repair model describes many recombination events.



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Model of homologous recombination

- Prokaryotic and eukaryotic factors catalyzing recombination steps.

Recombination Step	*E. coli* Protein Catalyst	Eukaryotic Protein Catalyst
Pairing homologous DNAs and strand invasion	RecA protein	Rad51 Dcm1 (in meiosis)
Introduction of DSB	None	Spo11 (in meiosis) HO (for mating-type switching)
Processing DNA breaks to generate single strands for invasion	RecBCD helicase/nuclease	MRX protein (also called Rad50/58/60 nuclease)
Assembly of strand-exchange proteins	RecBCD and RecFOR	Rad52 and Rad59
Holliday junction recognition and branch migration	RuvAB complex	Unknown
Resolution of Holliday junctions	RuvC	Perhaps Rad51c-XRCC3 complex and others

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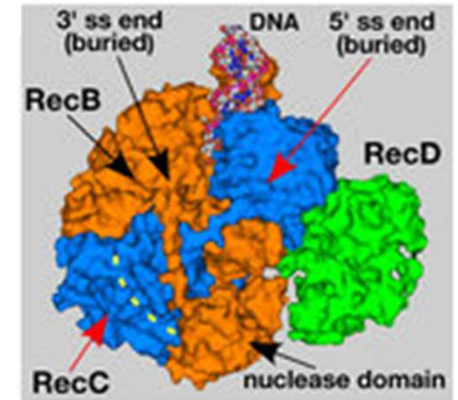
Prokaryotic homologous recombination

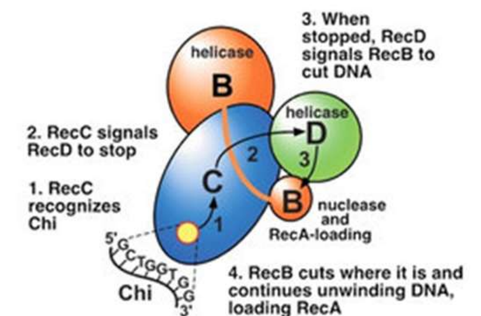
- The RecBCD enzyme of *Escherichia coli* is a helicase-nuclease that initiates the repair of double-stranded DNA breaks by homologous recombination.

- The RecBCD enzyme is, regulated by a cis-acting DNA sequence known as Chi (crossover hotspot instigator) that activates its recombination-promoting functions.

- When Chi is in the RecC tunnel,
  - RecC signals RecD to stop unwinding DNA;
  - RecD signals RecB to nick the DNA and to begin loading RecA.

- This enzyme is a prototypical example of a molecular machine: several autonomous functional domains that interact with each other to produce a complex, sequence-regulated, DNA-processing machine.

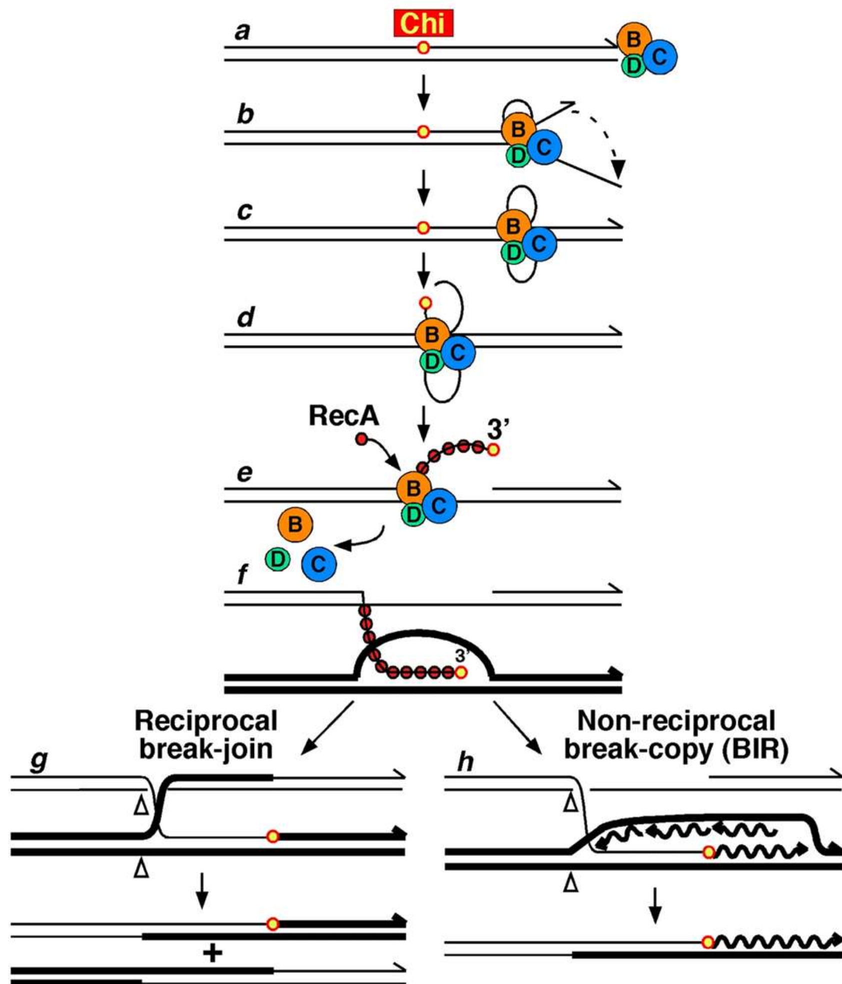




June 2020Nucleic Acids Research 48(14)

Microbiol Mol Biol Rev. 2008 Dec; 72(4): 642–671.

Prokaryotic homologous recombination model



- RecBCD binds a dsDNA end (a) and unwinds the DNA, producing loop-tail structures (b) that are converted into twin-loop structures (c) by annealing of the tails.
- At Chi, RecBCD nicks the 3′-ended strand (d) and loads RecA (e); later, the RecBCD subunits disassemble.
- The ssDNA-RecA filament invades intact homologous DNA to form a D-loop (f)
- D-loop is converted into a Holliday junction and resolved into reciprocal recombinants (g).
- Alternatively, the 3′-end in the D-loop prime synthesis of DNA and generate a non-reciprocal recombinant (h).

Scientific Reports volume 10, Article number: 19415 (2020)

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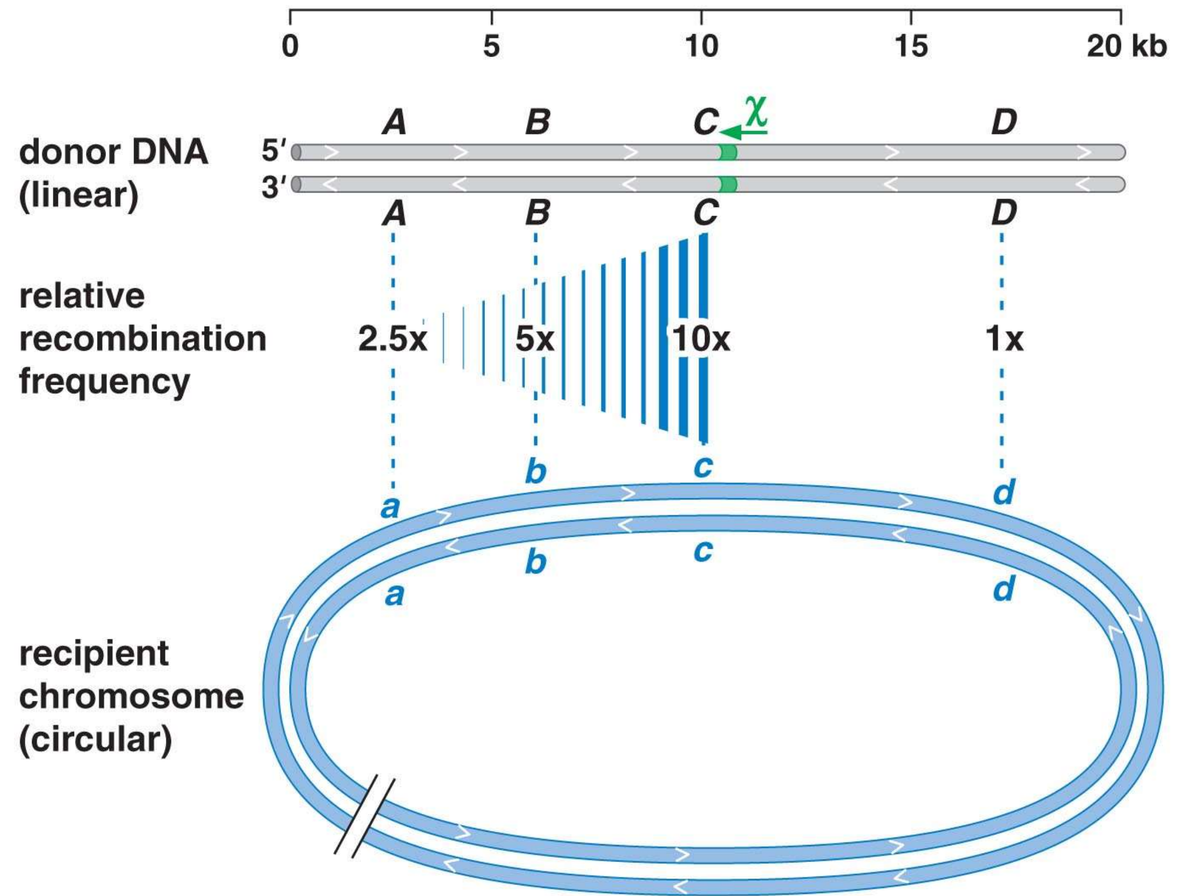
Prokaryotic homologous recombination model

- Chi sites controls RecBCD activity and relative recombination frequency.
- χ-site 5'-GCTGGTGG-3'.



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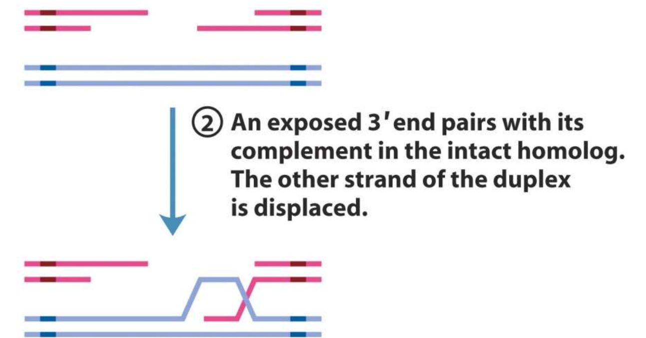
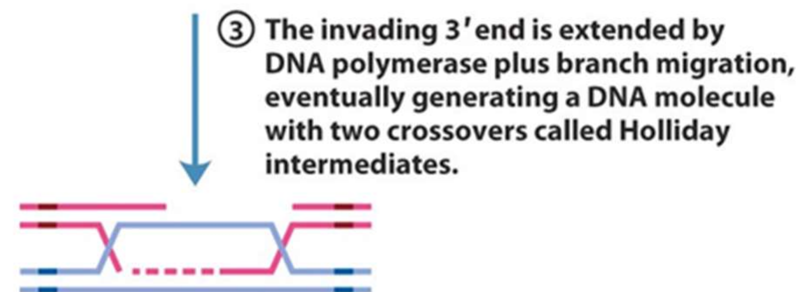
Prokaryotic HR strand exchange

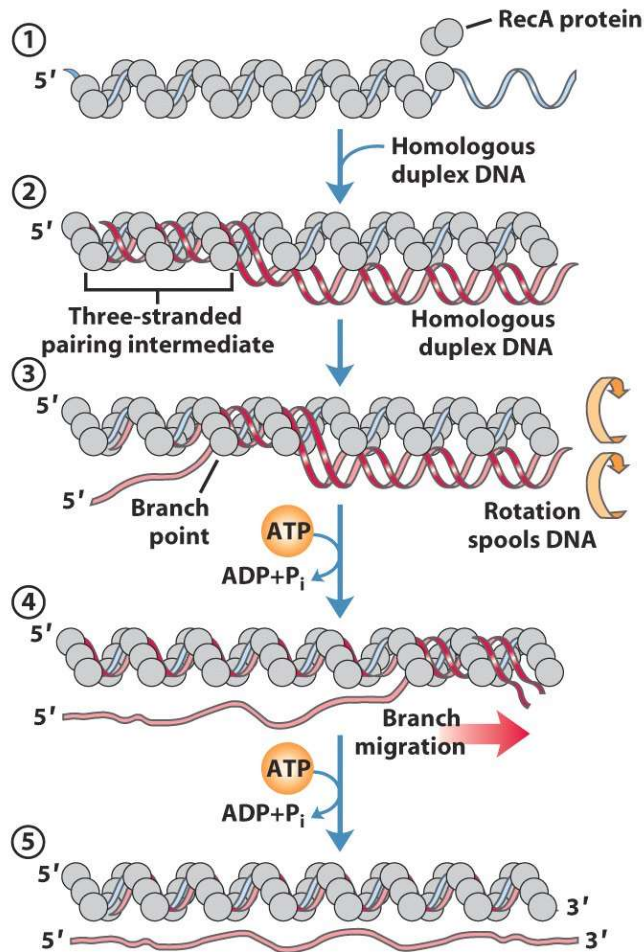
- Three steps.
- 1. Pre-synapsis - RecA coats single-stranded DNA, accelerated by SSB so get more relaxed structure.
- 2. Synapsis - alignment of complementary sequences in ssDNA and dsDNA.
- 3. Post-synapsis or strand-exchange – ssDNA replaces the same strand in the duplex to form new dsDNA, requires ATP hydrolysis.



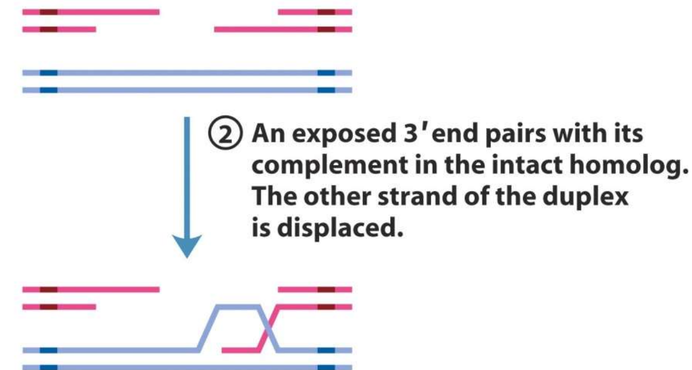


https://www.biologyonline.com/dictionary/mutagen

Prokaryotic HR pre-synapsis phase



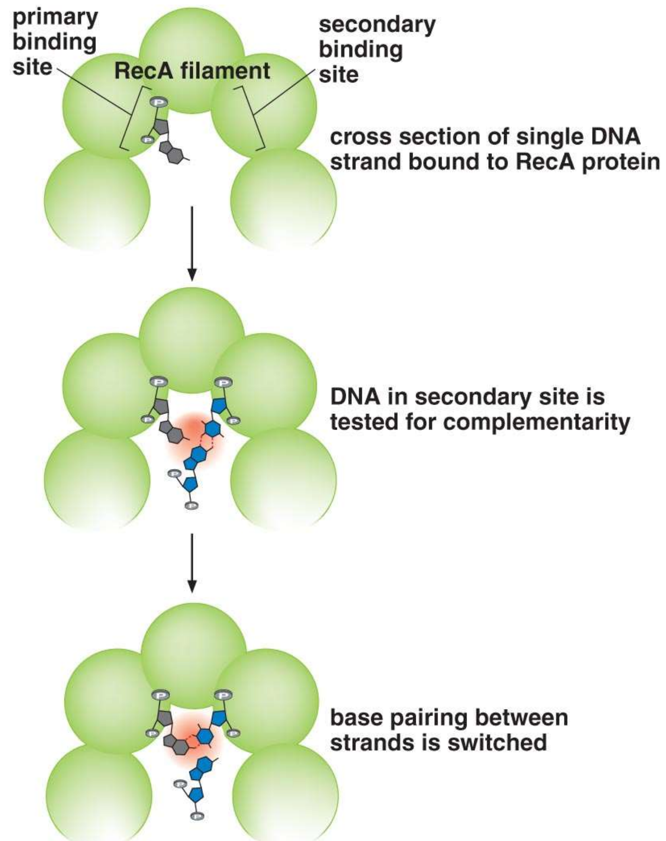
- RecA protein assembles on single-stranded DNA and promotes strand invasion.



Journal of Applied Biology & Biotechnology Vol. 9(04), pp. 162-169, July, 2021

Prokaryotic HR pre-synapsis phase

- Newly based-paired partners are established within RecA filament.



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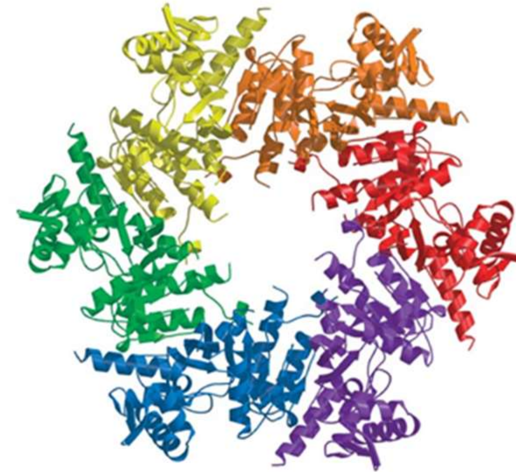
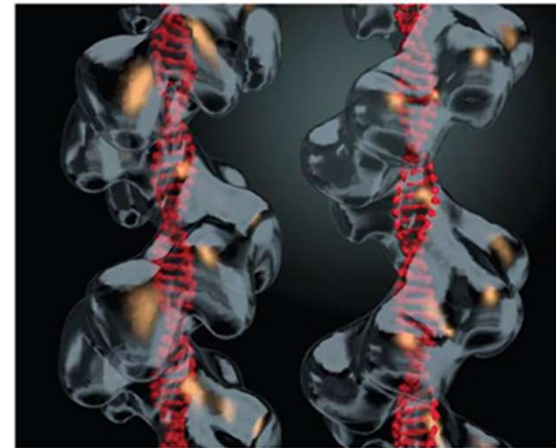
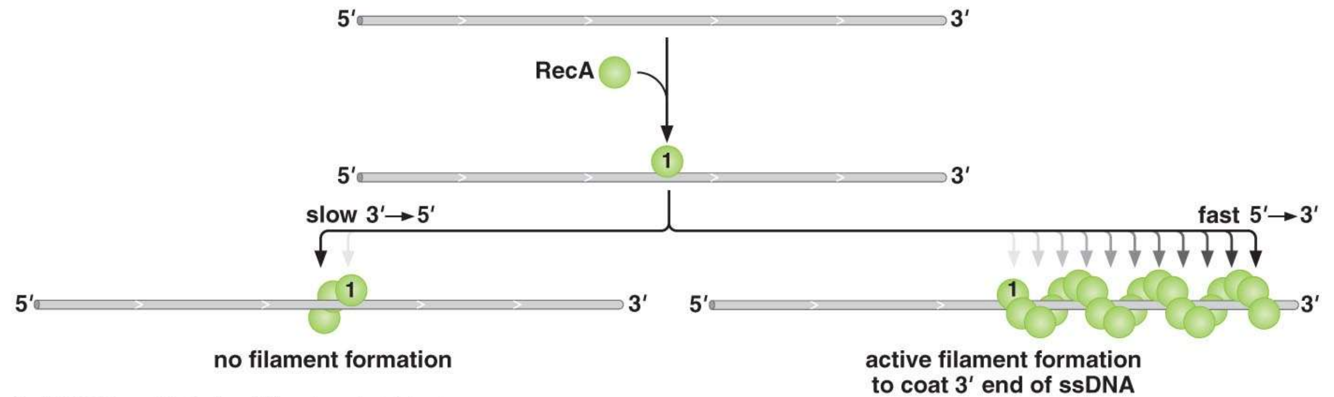
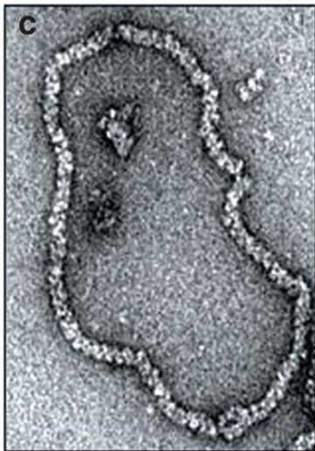




Prokaryotic HR synapsis phase

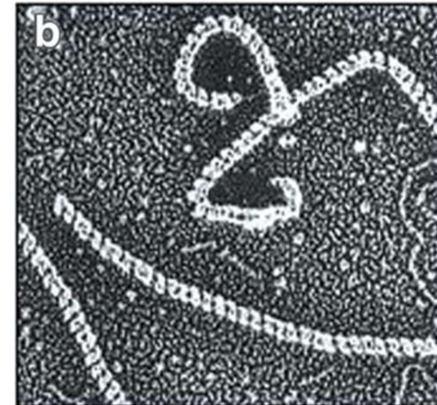
- Polarity of RecA assembly.





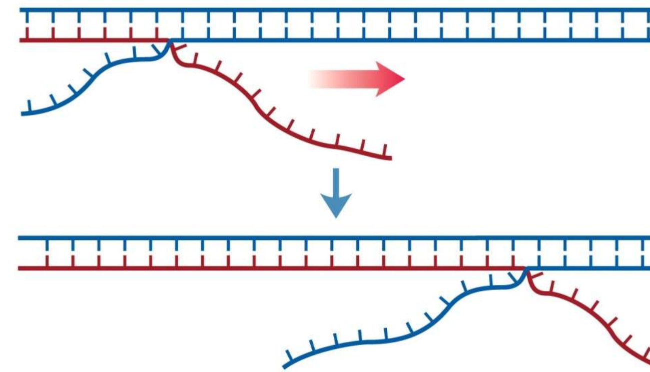
- Rad A Archaea

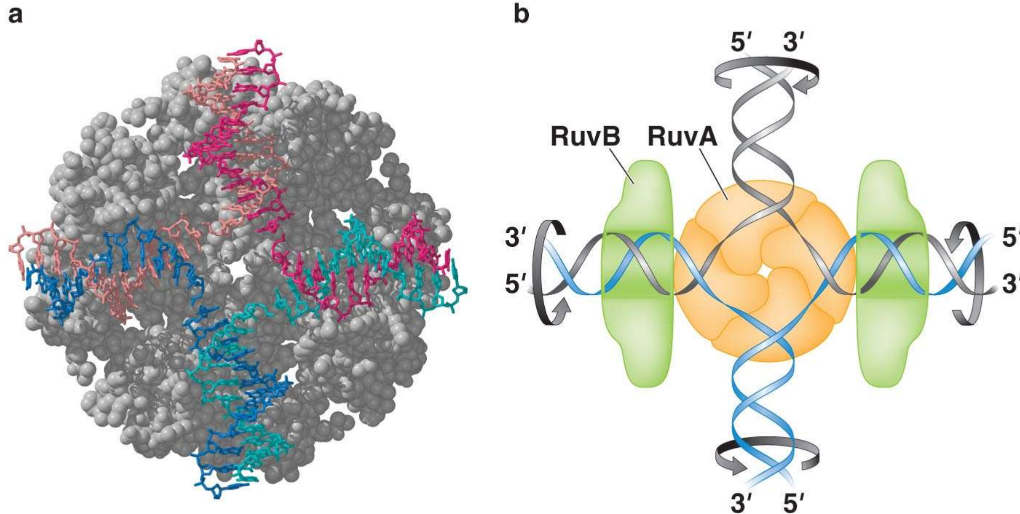
RecA



Prokaryotic HR strand-exchange

- The RuvAB complex specifically recognizes Holliday junctions (HJ) and promote branch migration.





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- Structure of RuvA and model of RuvAB bound to Holliday junction, HJ.

Prokaryotic HR strand-exchange

- The RuvA and RuvB – DNA helicase that catalyzes branch migration.
- RuvA - tetramer to HJ – each DNA helix between subunits, forces it into square planar conformation.
- RuvB – hexamer ring with ATP-dependent helicase activity. Two copies of RuvB associate with RuvA and DNA.
- Branch migration is in the direction of RecA mediated strand-Exchange.

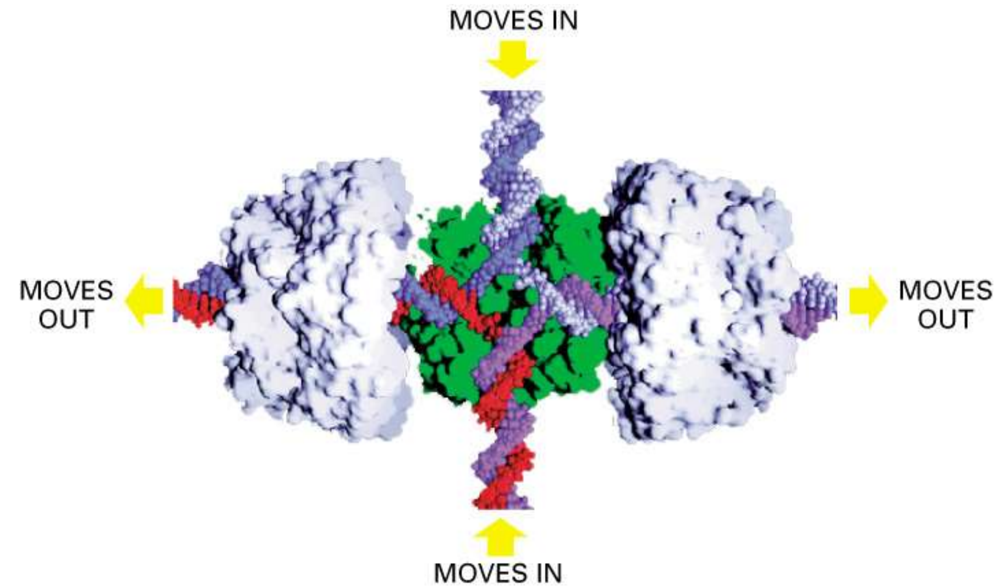
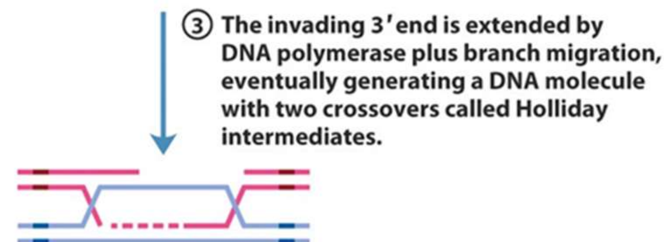


Figure 5–63. Molecular Biology of the Cell, 4th Edition.



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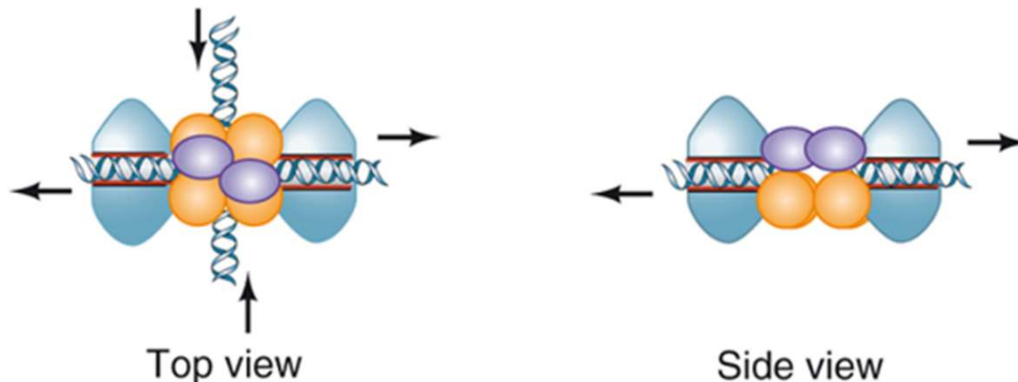
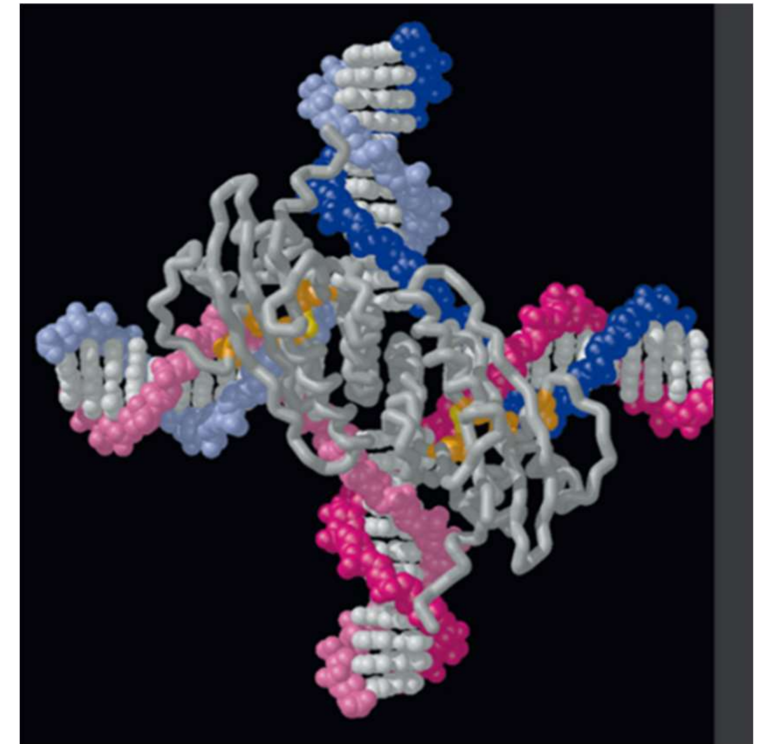
Prokaryotic HR resolving HJ junction

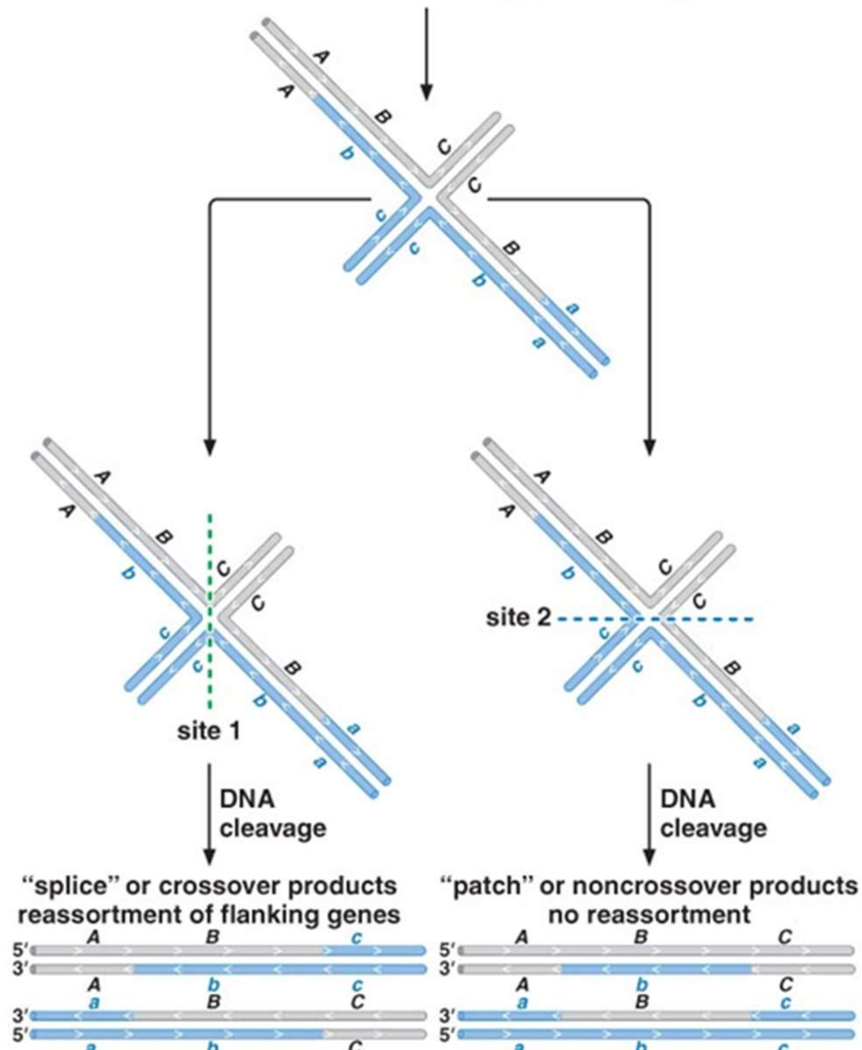
- The RuvC – resolvase
- Binds to HJ as dimer with bound RuvA/RuvB complex.

- Consensus sequence – (A/T)TT G/C)
  - Occurs frequently in *E. coli* genome.
  - Branch migration needed to reach consensus sequence.





Prokaryotic HR resolving HJ junction



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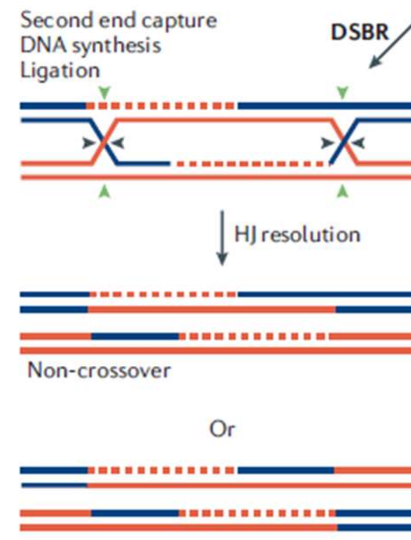
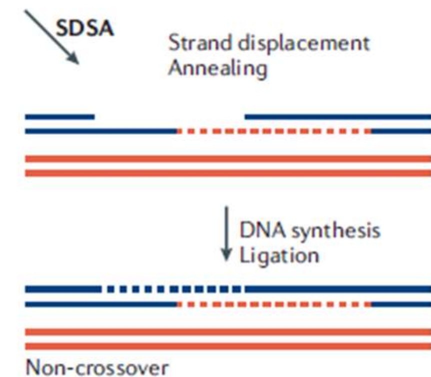
Eukaryotic homologous recombination mechanism

- DSB is repaired by double-strand break repair (DSBR) and synthesis-dependent strand annealing (SDSA).
- In both pathways, repair is initiated by resection of a DSB to provide 3′ single-stranded DNA overhangs.
- DSBR two Holliday junctions are fromed (HJs). After gap-repair DNA synthesis and ligation, the structure is resolved at the HJs in a non-crossover or crossover mode.
- SDSA is initiated by strand displacement, annealing of the extended single-strand end to the ssDNA on the other break end, followed by gap-filling DNA synthesis and ligation. The repair product from SDSA is always non-crossover.

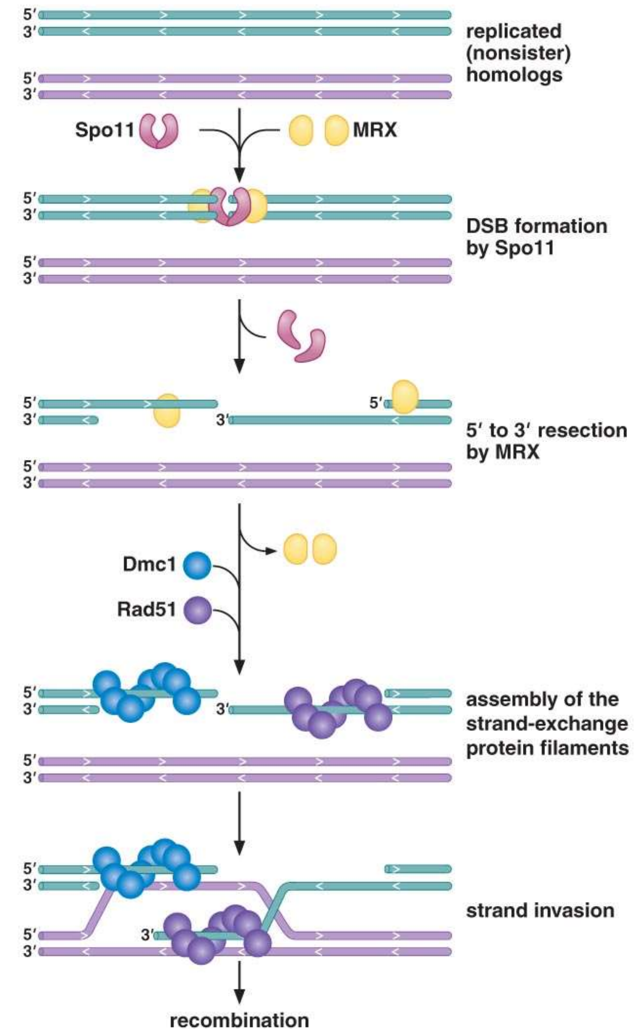




Molecular cell biology, October 2006, volume 7, 739.

Eukaryotic HR pre-synapsis phase

- MRX protein processes the cleaved DNA ends for assembly of the RecA-like strand exchange proteins - DMC1, Rad51.
- DMC1 specifically functions in meiotic recombination.



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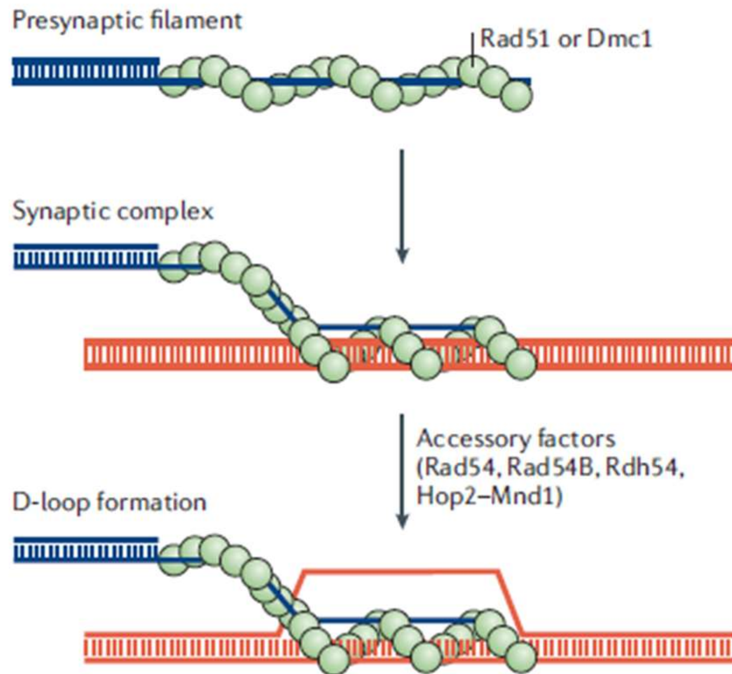
Eukaryotic HR pre-synapsis phase



- Branch migration needed to reach consensus sequence.
- The recombinases Rad51 or Dmc1 (green circles) assemble onto the ssDNA tails that form a helical protein filament, which is known as the presynaptic filament.
- The presynaptic filament binds duplex DNA to form the synaptic complex and ‘searches’ for DNA homology in the duplex DNA molecule. With function of Rad54, Rad54B, Rdh54 and Hop2–Mnd1.
- ssDNA invades the homologous region in the duplex to form a DNA joint, known as the displacement (D)-loop.

Molecular cell biology, October 2006, volume 7, 739.

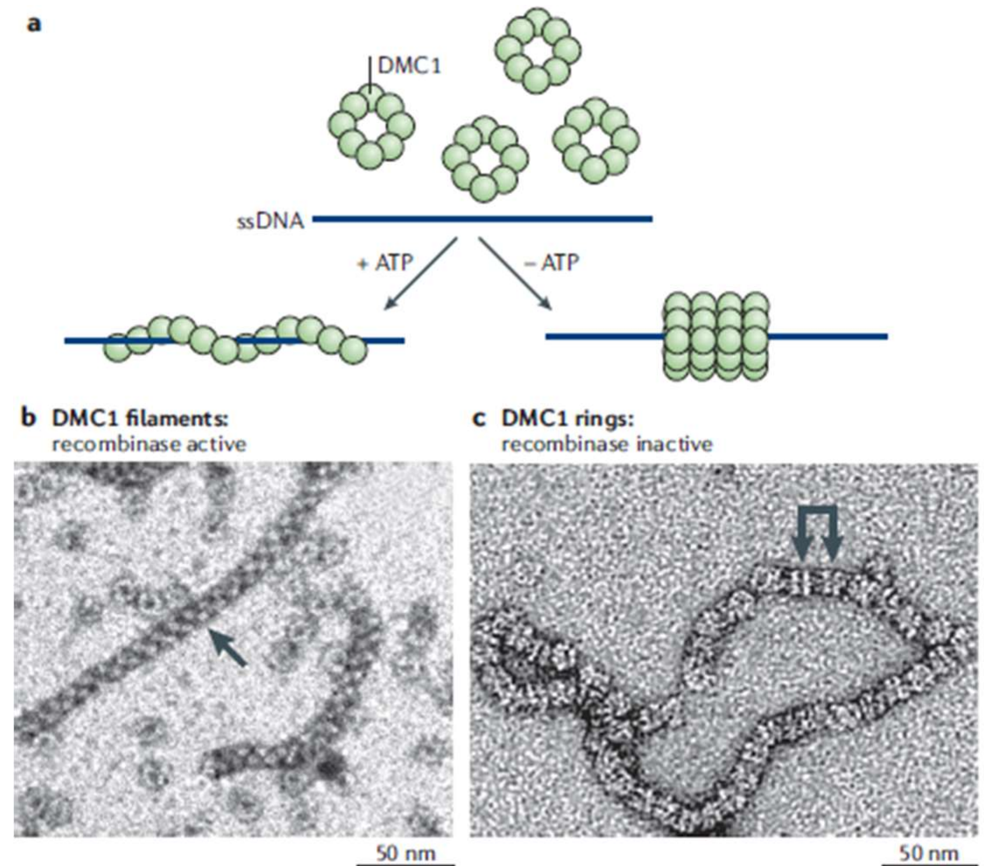
Eukaryotic HR pre-synapsis phase

- Human DMC1 can form either stacked rings or helical filaments on DNA, depending on whether ATP is present or not.

- Left electron micrograph shows helical filaments of DMC1 protein on ssDNA. These filaments are the catalytically active form of DMC1.

- Right electron micrograph shows stacked DMC1 rings (two such stacked rings are indicated by the double arrow) on ssDNA.

- These stacked DMC1 rings seem incapable of mediating the HR reaction.



Molecular cell biology, October 2006, volume 7, 739.

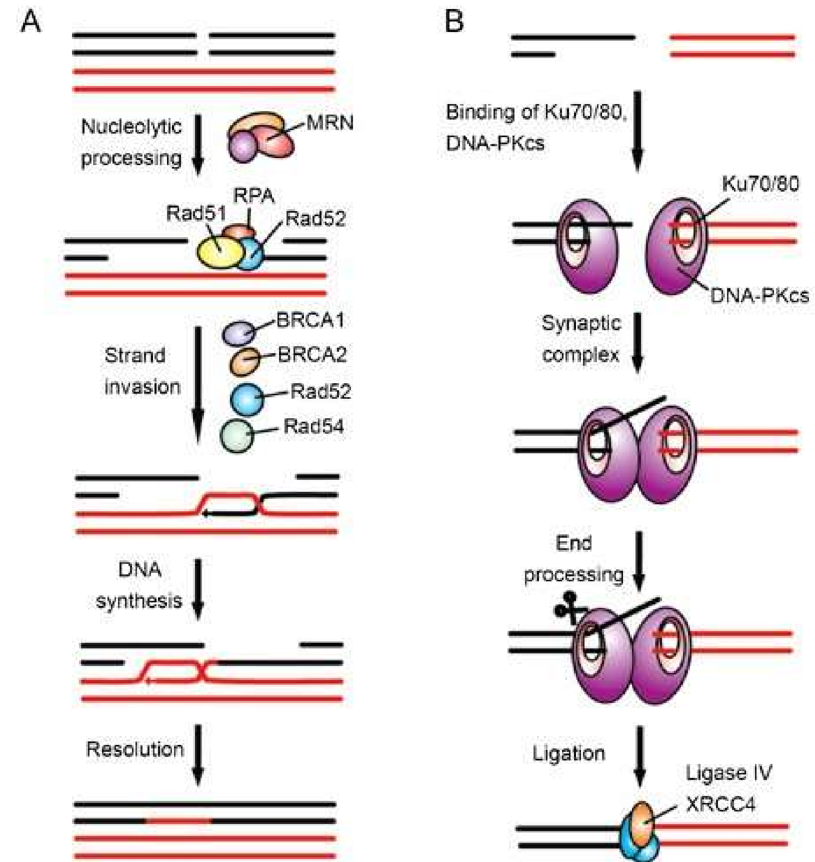
Non-homologous end-joining (NHEJ) pathway

- Non-homologous end-joining is a pathway that repairs double-strand breaks in DNA.
- We call it "non-homologous" because the break ends are directly joined without the need for a homologous template.
- This pathway often occurs when the cell is in G1 and a sister chromatid is not available for repair through homologous recombination.
- Nonhomologous end joining uses proteins that recognize the broken ends of DNA, bind to the ends, and then joins them together.
- Nonhomologous end joining is more error-prone than homologous recombination and often leads to deletions, insertions, and translocations.

https://www.thevirtualnotebook.com/dna-repair-definition-mechanism-types/

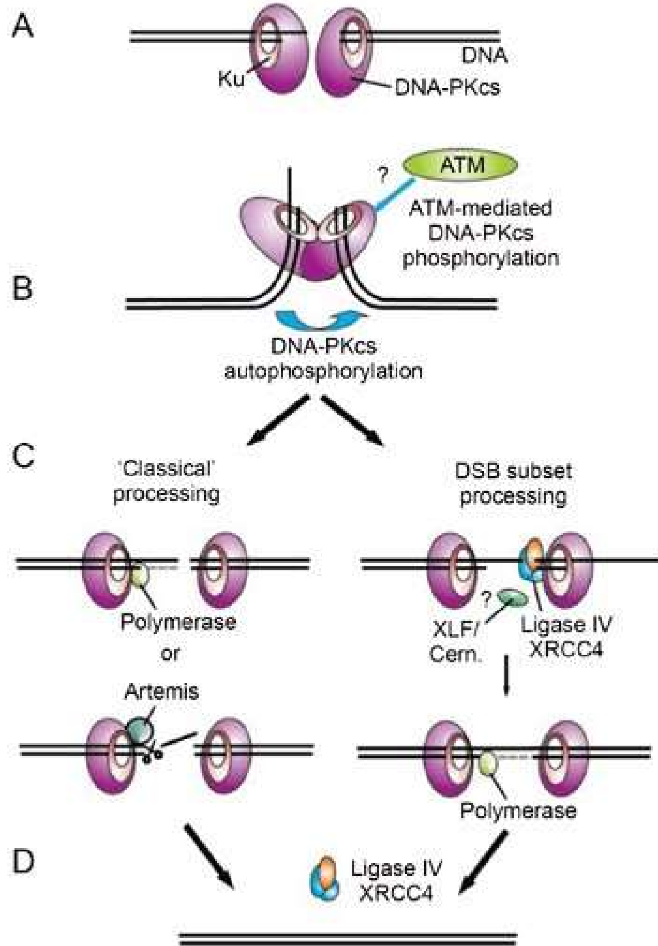
Non-homologous end-joining (NHEJ) pathway

- HR utilizes a homologous stretch on a sister chromatid and single-strand overhangs are created.
- Formation of a joint molecule with the damaged and undamaged strands.
- DNA synthesis complete repair of the DSB.
- NHEJ brings the ends of the broken DNA molecule together by the formation of a synaptic complex, consisting of two DNA ends, two Ku70/80 and two DNA-PKCS molecules.
- Non-compatible DNA ends are processed to form blunt termini, followed by repair of the break by the ligase IV/XRCC4 complex.



Cell Research volume 18, pages114–124 (2008)Cite this article

Non-homologous end-joining (NHEJ) mechanism

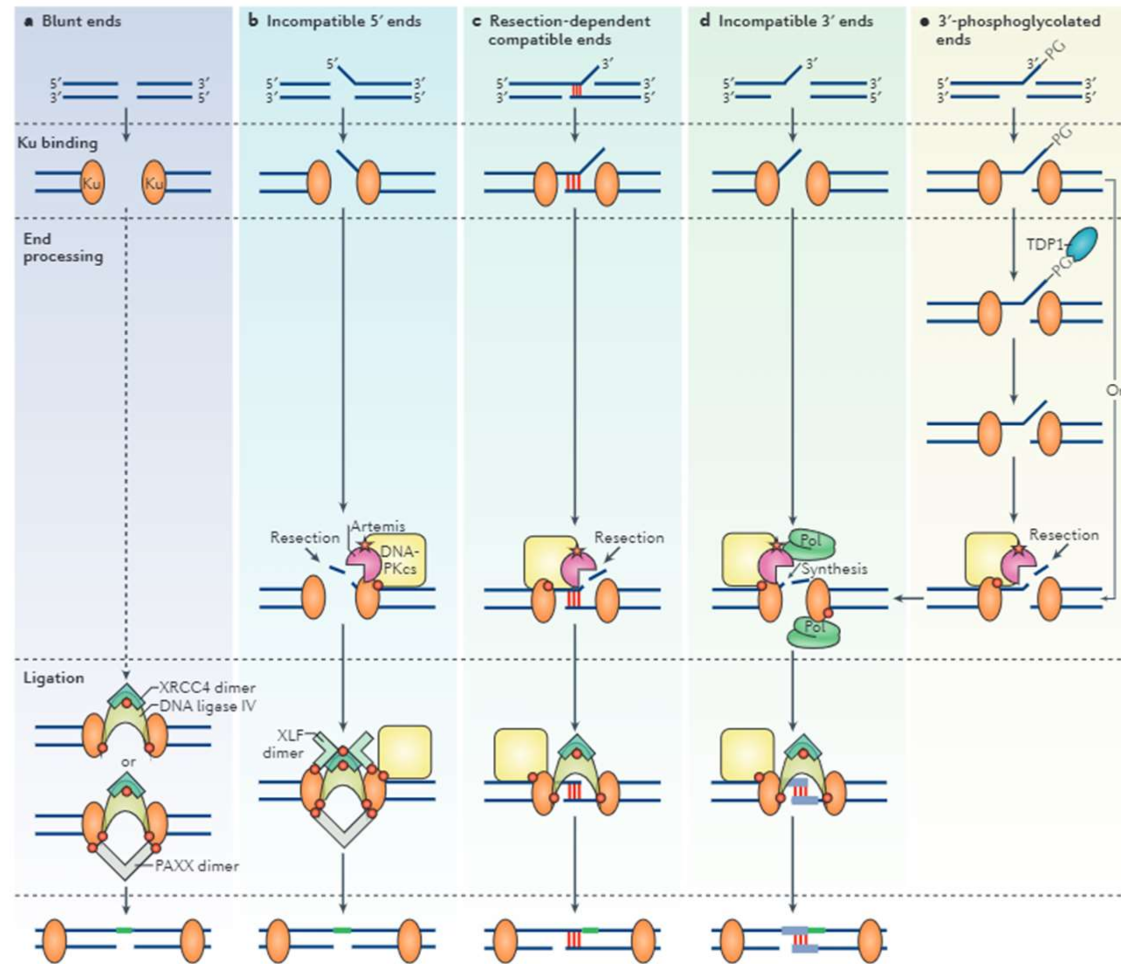


- A) The Ku70/80 heterodimer associates with the two ends of the broken DNA molecule. This DNA-Ku scaffold attracts DNA-PKCS, which protects the DNA termini against degradation and premature ligation.
- (B) The DNA-PKCS molecules on both DNA ends form a synaptic complex which tethers the DNA ends. Trans DNA-PKCS autophosphorylation then introduces a conformational change that makes the DNA termini accessible for other NHEJ enzymes.
- (C) Non-compatible DNA termini need to be processed before ligation can proceed. This can be done in the 'classical' way, by either filling (polymerases) or resection (Artemis) of single-strand overhangs.
- (D) ligation of the blunted ends by ligase IV/XRCC4.

Cell Research, 2008, volume 18, pages 114–124.

Non-homologous end-joining (NHEJ) pathway

- Various non-homologous end joining pathways.



Nat Rev Mol Cell Biol. 2017 Aug;18(8):495-506.

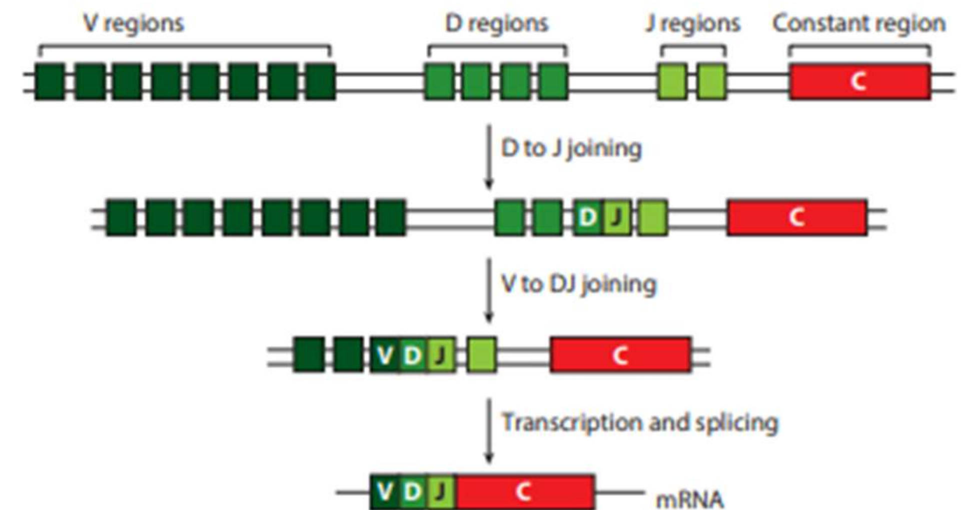
NHEJ in V(D)J recombination

- Genes that encode immunoglobulins or T-cell receptors are not present in an active form in developing B- and T-lymphocytes, but need to be formed by the combination of gene segments.

- This process is called V(D)J recombination.

- Gene segments are classified into three groups: variable (V), diversity (D), and joining (J) segments.

- In the case of an IgH gene, D and J segments are first joined, followed by the combination of the DJ assembly with a V segment.



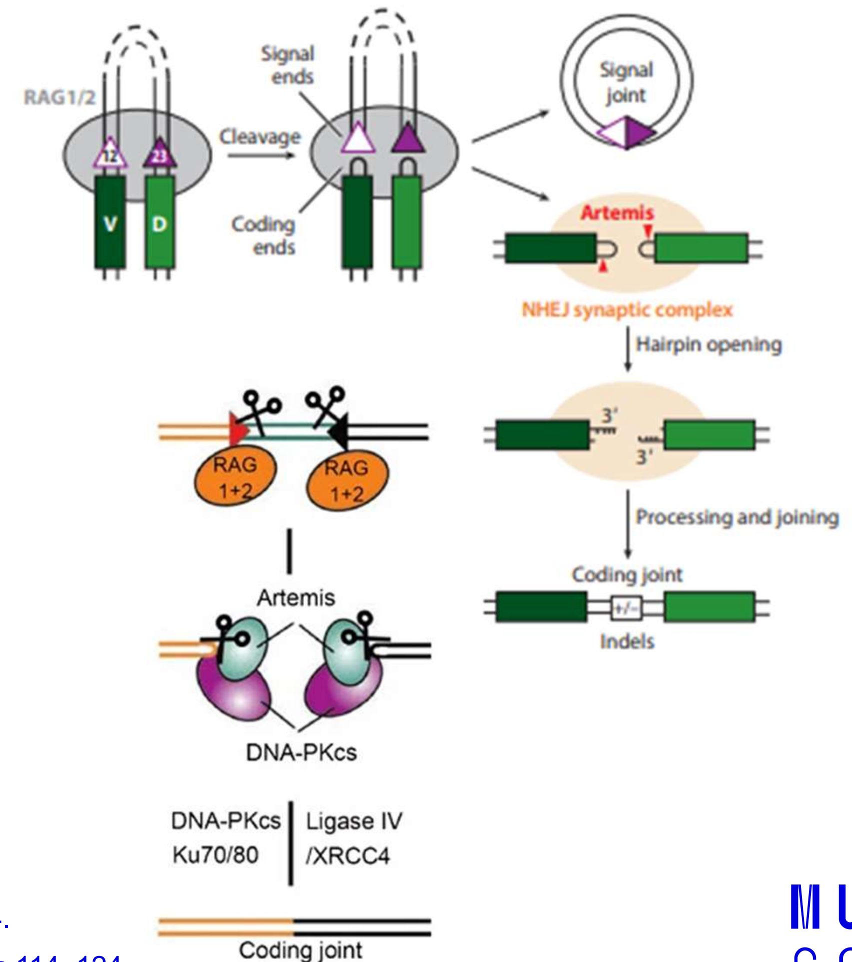
Annu. Rev. Biochem. 2021.90:137-164.
Cell Research, 2008, volume 18, pages 114–124.

NHEJ in V(D)J recombination

- Gene segments are joined by the introduction of a DSB at the edges of selected segments by the RAG1 and RAG2 proteins, followed by removal of the intervening DNA and ligation of the segments.

- Before ligation the typical hairpin structure of the coding ends needs to be opened by the endonuclease Artemis.

- V(D)J recombination requires the NHEJ core enzymes (DNA-PKCS, Ku70/80, ligase IV, and XRCC4), indicating that ligation of the gene segments is mediated by the NHEJ process.



Annu. Rev. Biochem. 2021.90:137-164.

Cell Research, 2008, volume 18, pages 114–124.

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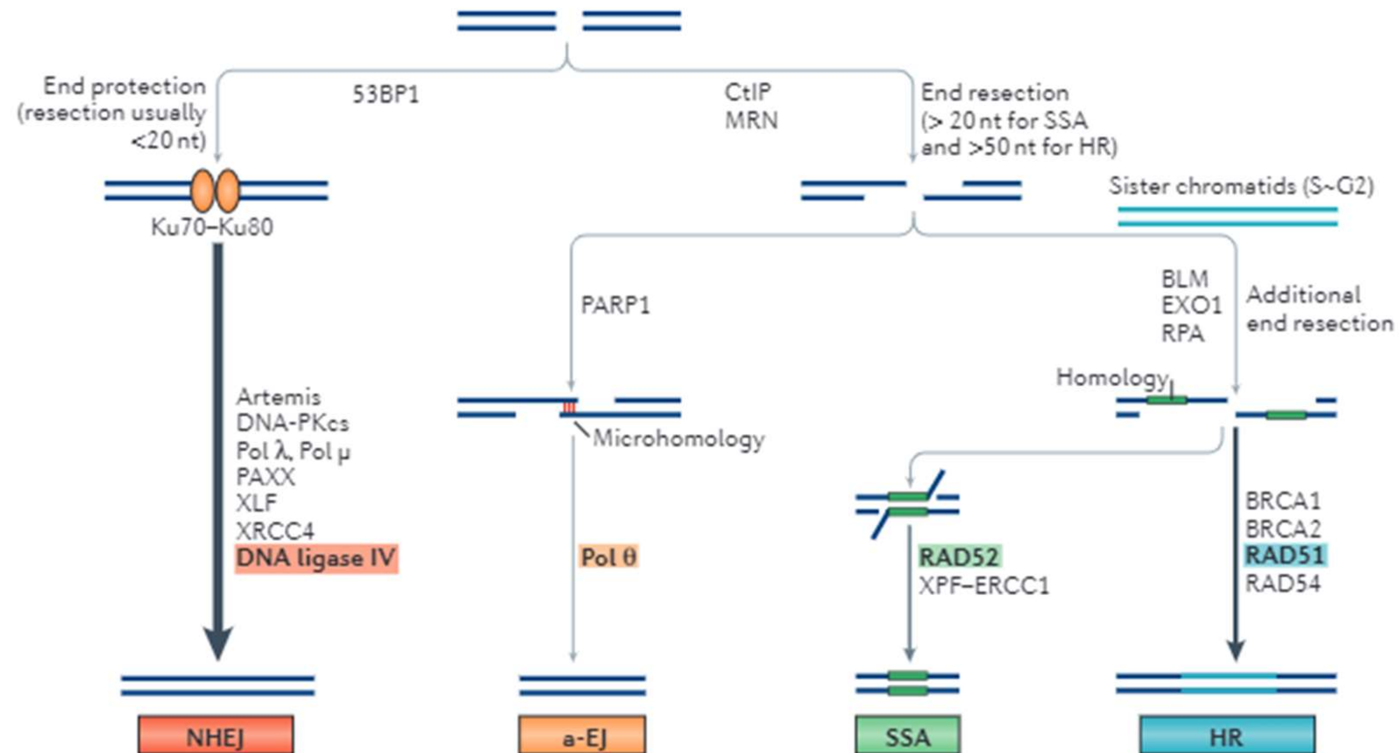
DNA strand break repair pathway choice



Nat Rev Mol Cell Biol. 2017 Aug;18(8):495-506.

Types of DNA-repair

Direct enzymatic repair
- Restoration to the original state.
- Without cleavage and resynthesis of DNA.
  - Photoreactivation.
  - Removal of methyl group - Dealkylation.

Indirect
- Excision repair.
  - Base.
  - Nucleotides.
  - Mismatch - controlled by methylation.

- Recombination/post replication.
  - Homologous recombination
  - Non-Homologous end joining

Inducible
- SOS-response.

The Error-prone Repair System (SOS response)

- The SOS repair is a complex set of processes that includes a bypass system that allows DNA replication to take place across pyrimidine dimers or other DNA distortions but at the cost of the fidelity of replication.

- Emphatically, SOS repair is an error-prone system for DNA repair.

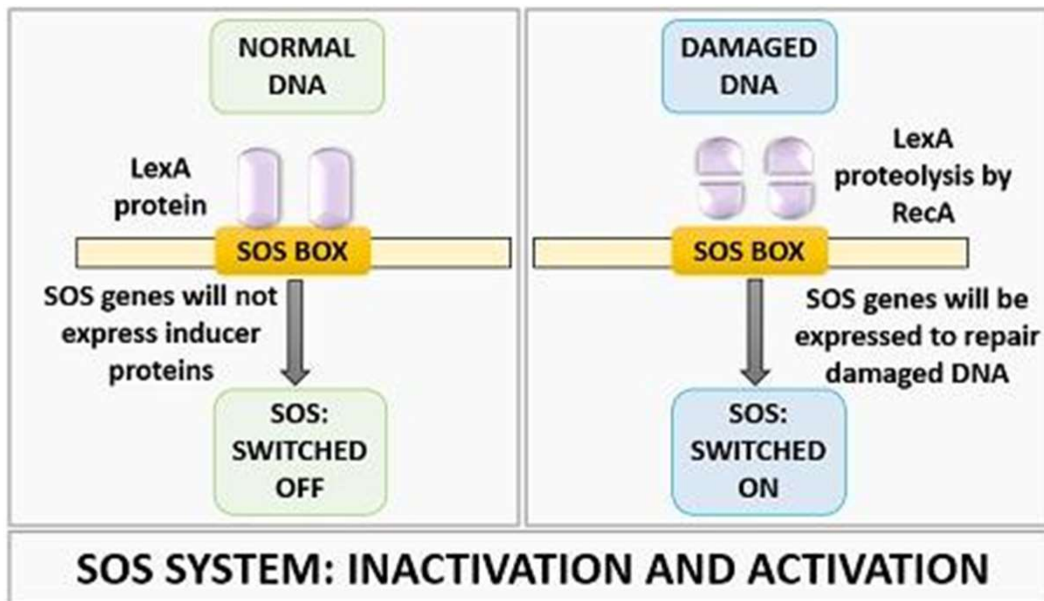
- “SOS repair” refers to a cellular response to UV damage.

- This error-prone repair system eliminates gaps in the newly synthesized strands opposite damaged nucleotides in the template strands but, in so doing, increases the frequency of replication errors.

- The SOS-response has been found in many bacterial species, but not in eukaryotic cells.

https://bio.libretexts.org/Bookshelves/Biochemistry/Book%3A_Biochemistry_Free_For_All_(Ahern_Rajagopal_and_Tan)

The Error-prone Repair System (SOS response)



- SOS system consists of more than 40 genes and is regulated by the LexA repressor protein. These include the uvr genes needed for nucleotide excision repair and recA, which is involved in homologous recombination.
- LexA binds to DNA sequence upstream of their coding region, called the SOS box.
- The SOS response can also induce the expression of translesion polymerases encoded by the dinA, dinB and umuCD genes.

https://biologyreader.com/sos-repair.html

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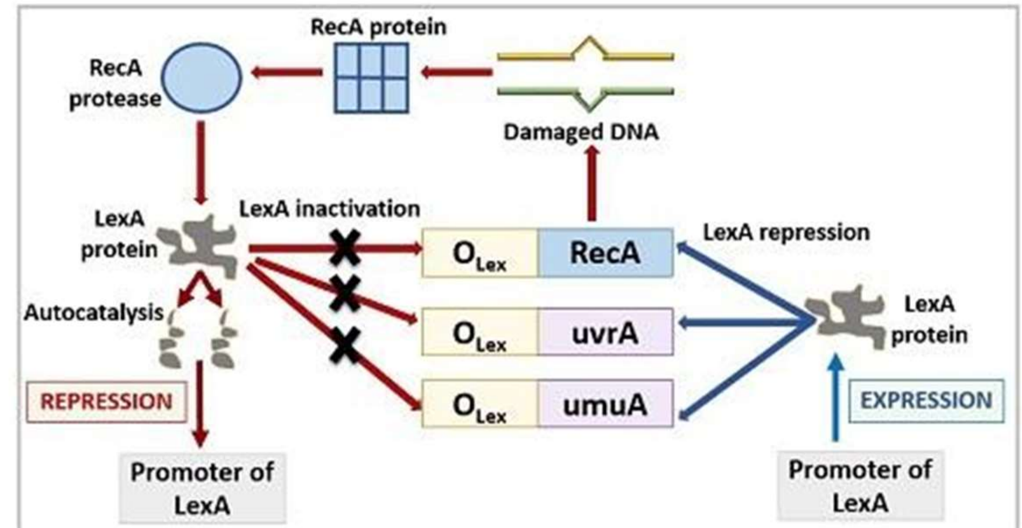
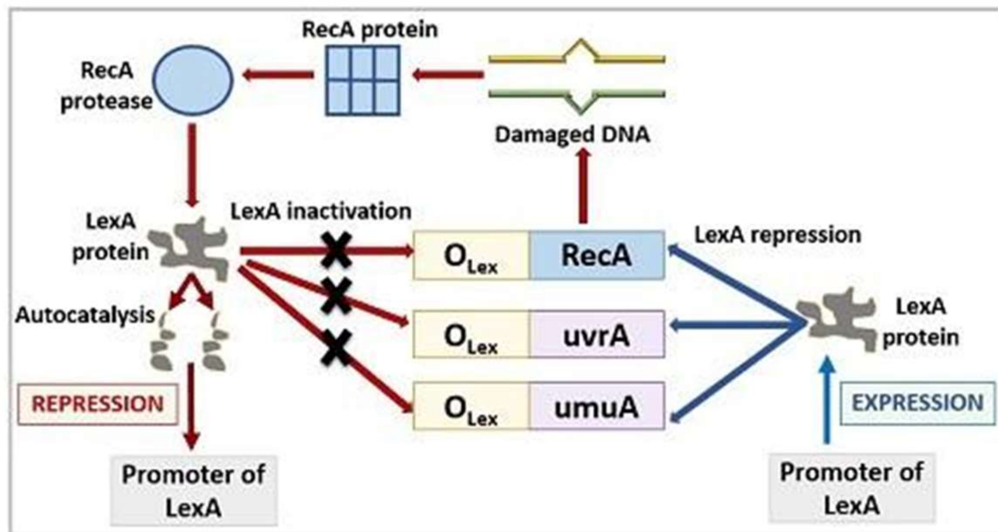
SOS response mechanism

- In case of excessive DNA damage, cell responds by activating signal or RecA protein.
- A RecA protein specifically binds to the single stranded DNA. On binding with the single stranded DNA fragments, RecA forms a filament-like structure around the DNA.
- Then, a LexA repressor comes in contact with the nucleoprotein filament assembled by the RecA protein. When RecA interacts with the repressor protein, it converts into RecA protease.



https://biologyreader.com/sos-repair.html

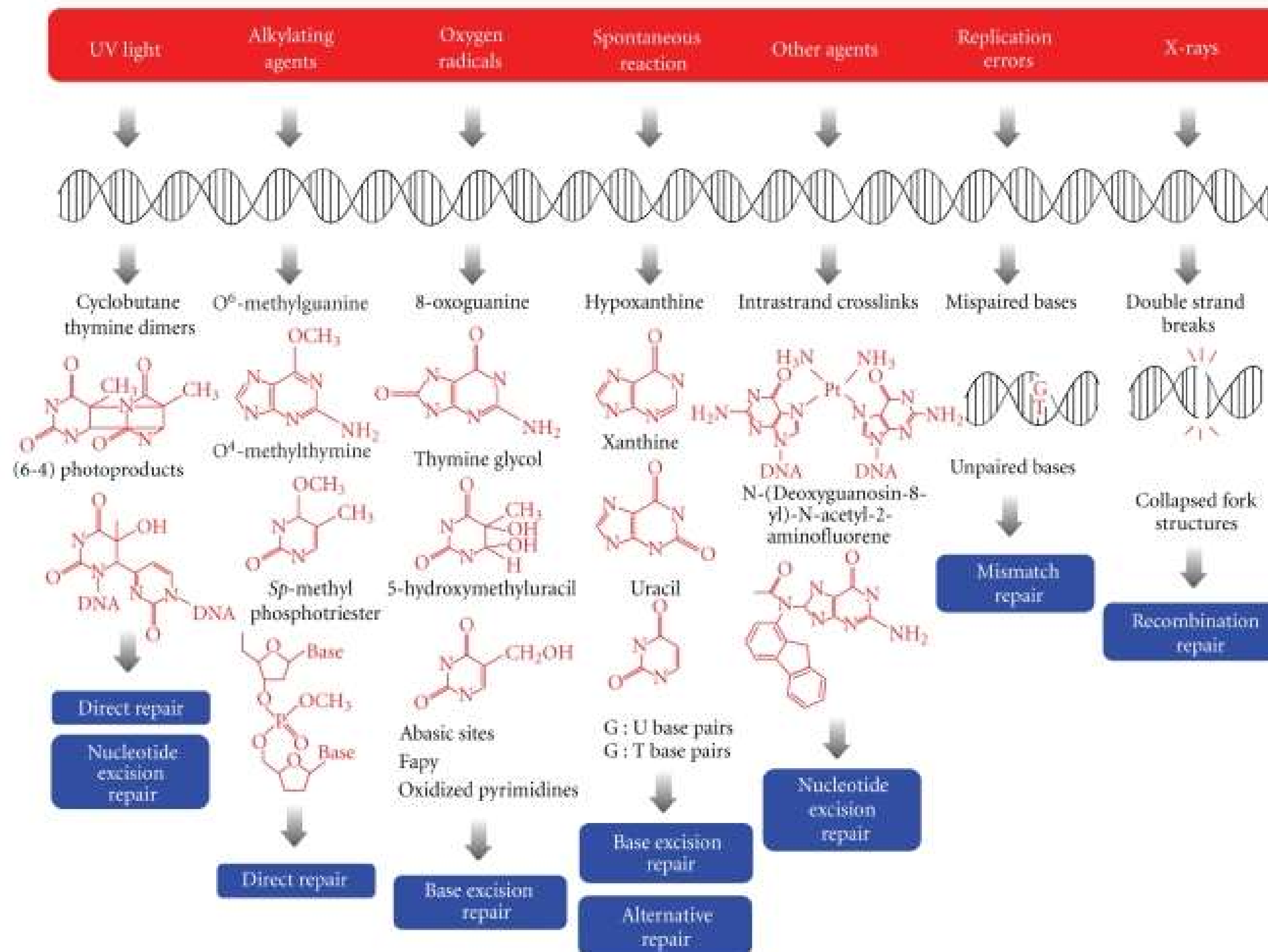
SOS response mechanism



- The formation of RecA protease causes autocatalytic proteolysis of LexA repressor protein. Thus, a LexA protein could not bind with the SOS operator.
- Inactivation of LexA protein activates the inducer proteins that repair the DNA damage but alters the DNA sequence.
- After DNA repair, the RecA protein loses its efficiency to cause proteolysis, and the LexA protein will again bind to the SOS operator or switch off the SOS system.

https://biologyreader.com/sos-repair.html

Different repair mechanism for principal DNA lesions



J Nucleic Acids. 2010; 2010: 179594.

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http://biocomicals.blogspot.com/2011/05/dna-repair.html