

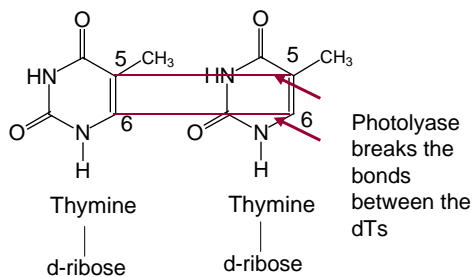
## Repair mechanisms

1. Reversal of damage
2. Excision repair
3. Mismatch repair
4. Recombination repair
5. Error-prone repair
6. Restriction-modification systems

## 1. Reversal of damage

- Enzymatically **un-do** the damage
  - a) Photoreactivation
  - b) Removal of methyl groups

## Photolyase breaks apart pyrimidine dimers

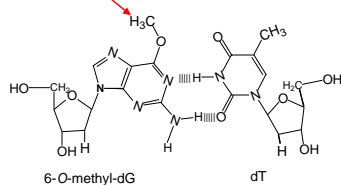


## 1a. Photoreactivation

- Photolyase: binds a pyrimidine dimers and catalyzes a photochemical reaction
- Breaks the cyclobutane ring and reforms two adjacent T's
- 2 subunits, encoded by *phrA* and *phrB*.

## Conversion of 6-O-methyl-dG back to dG

6-O-methylguanine methyltransferase : removes the mutagenic methyl group



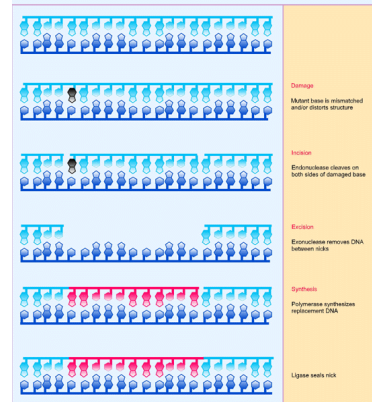
## 1b. Removal of methyl groups

- 6-O-methylguanine methyltransferase
- Recognizes 6-O-methylguanine in DNA, removes the methyl group
- Transfers the methyl group to an amino acid of the enzyme
- "suicide" mechanism
- Encoded by *ada* gene in *E. coli*

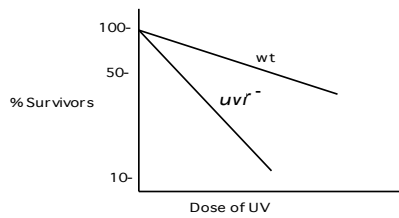
## 2. Excision repair

- **General Process:**
  - remove damage (base or DNA backbone)
  - ss nick/gap provides 3'OH for Pol I initiation
  - DNA ligase seals nick
- **Nucleotide excision repair:**
  - Cut out a segment of DNA around a damaged base.
- **Base excision repair:**
  - Cut out the base, then cut next to the apurinic/aprimidinic site, and let DNA Pol I repair

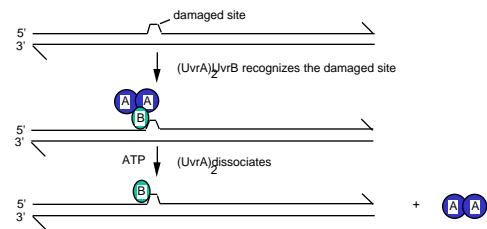
## Nucleotide excision repair



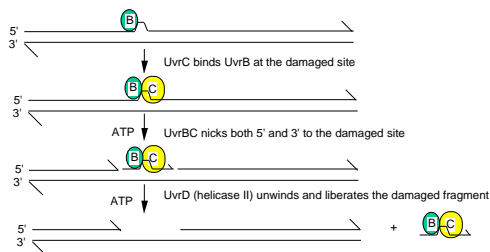
## Discovery of mutants defective in DNA repair



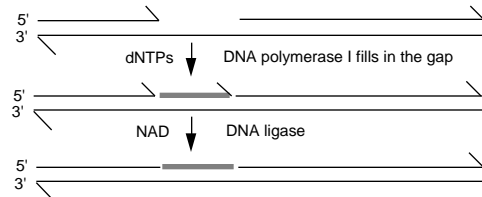
## UvrABC excision repair



## Cleavage and helicase



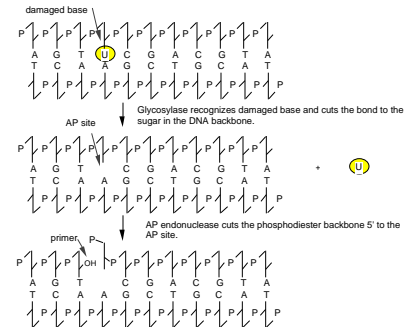
## Fill in with polymerase and ligate



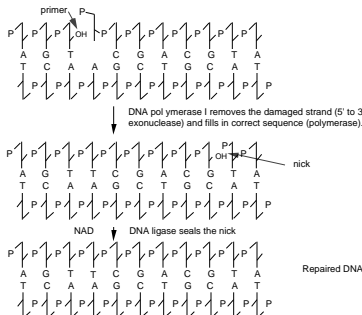
## Mutations in excision repair in eukaryotes can cause xeroderma pigmentosum (XP)

Human Gene	Protein Function	Analogous to <i>E. coli</i> :
XPA	Binds damaged DNA	UvrA/UvrB
XPB	Helicase, Component of TFIIH	UvrD
XPC	DNA damage sensor	
XPD	Helicase, Component of TFIIH	UvrD
XPE	Binds damaged DNA	UvrA/UvrB
XPF	Works with ERCC1 to cut DNA	UvrB/UvrC
XPG	Cuts DNA	UvrB/UvrC

## 2b. Base excision repair



## Excision and filling in by DNA Pol



## 3. Mismatch repair

- Action of DNA polymerase III (including proofreading exonuclease) results in 1 misincorporation per  $10^8$  bases synthesized.
- Mismatch repair reduces this rate to 1 change in every  $10^{10}$  or  $10^{11}$  bases.
- Recognize mispaired bases in DNA, e.g. G-T or A-C base pairs
- These do not cause large distortions in the helix: the mismatch repair system apparently reads the sequence of bases in the DNA.

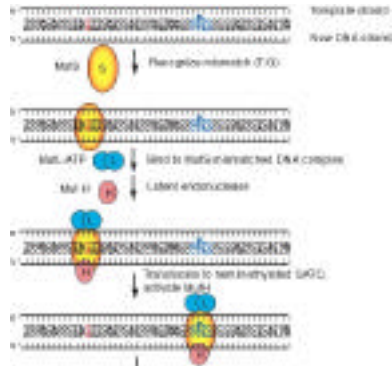
## Role of methylation in discriminating parental and progeny strands

- *dam* methylase acts on the A of GATC (note that this sequence is symmetrical or pseudopalindromic).
- Methylation is delayed for several minutes after replication.
- Mismatch repair works on the un-methylated strand (which is newly replicated) so that replication errors are removed preferentially.

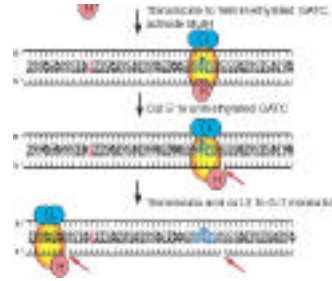
## Action of MutS, MutL, MutH

- **MutS**: recognizes the mismatch (heteroduplex)
- **MutL**: a dimer; in presence of ATP, binds to MutS-heteroduplex complex to activate MutH
- **MutH**: endonuclease that cleaves 5' to the G in an unmethylated GATC, leaves a nick

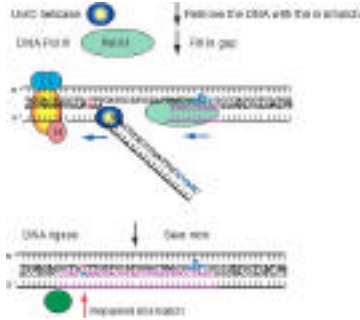
MutH, L, S action in mismatch repair #1



MutH, L, S action in mismatch repair #2



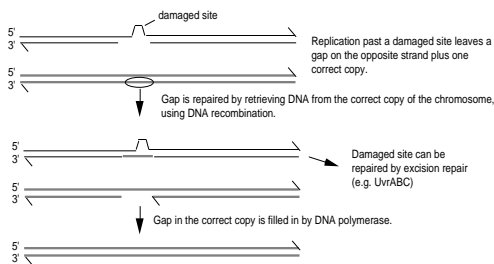
Mismatch repair: Excision of the misincorporated nucleotide



Eukaryotic homologs in mismatch repair

- Human homologs to *mutL* (hMLH1) and *mutS* (hMSH2, hMSH1) have been discovered, *because ...*
- Mutations in them can cause one of the most common hereditary cancers, hereditary nonpolyposis colon cancer (HNPCC).

4. Recombination repair: retrieval of information from an homologous chromosome



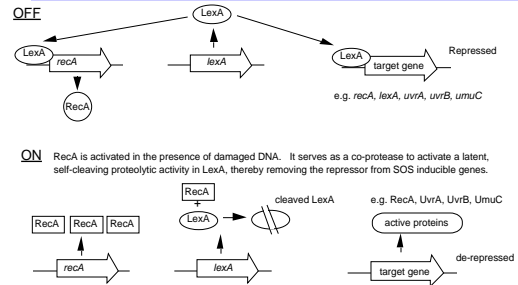
5. Error-prone repair

- **Last resort** for DNA repair, e.g when repair has not occurred prior to replication. How does the polymerase copy across a non-pairing, mutated base, or an apyrimidinic/apurinic site?
  - DNA polymerase III usually dissociates at a nick or a lesion.
  - But replication can occur past these lesions, especially during the **SOS response** ("Save Our Ship").
- This **translesion synthesis** incorporates random nucleotides, so they are almost always mutations (3/4 times)

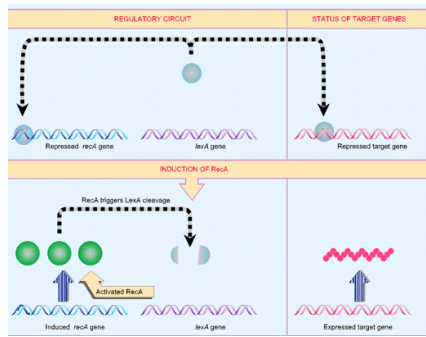
### Role of *umuC* and *umuD* genes in error-prone repair

- Named for the UV mutable phenotype of mutants with defects in these genes.
- Needed for bypass synthesis; mechanism is under investigation. E.g. these proteins may reduce the template requirement for the polymerase.
- UmuD protein is proteolytically activated by LexA.

### SOS response is controlled by LexA and RecA



### Lewin Figure 16-16



### 6. Restriction-modification systems

