

# Chem\*4570 Applied Biochemistry Lecture 9

## Role of DNA repair in mutation

**Efficient repair mechanisms** normally eliminate lesions in the DNA. **Permanent mutations** are the results of errors in repair, and this can be promoted by the use of **error prone strains** of organisms.

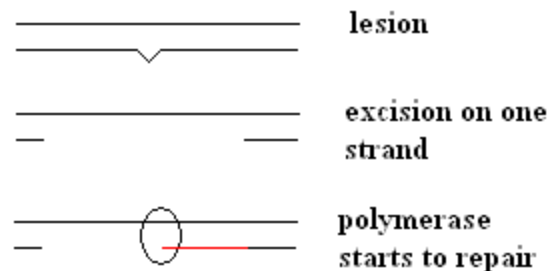
**There are several modes of repair: because repair is important there are many redundancies and alternative mechanisms for achieving the desired “fix” of errors or lesions in DNA.**

See Stryer pp. 810-826 or Lehninger pp. 949-967

There are two fundamental approaches to DNA repair, each used in appropriate circumstances:

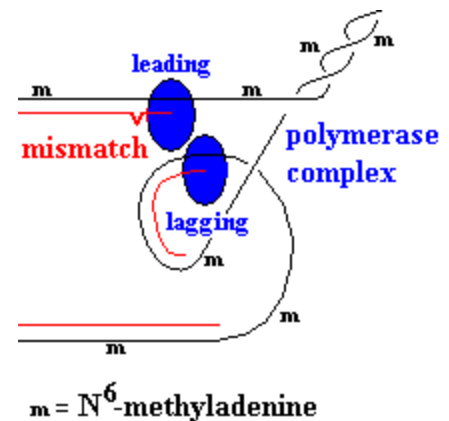
**Base excision repair (BER)** is used when a single base error is readily identified, e.g. the C to U mutation arising from deamination. A specific U-DNA glycosylase removes uracil leaving an apyrimidinic site (AP), a baseless deoxyribose. This is removed by the AP endonuclease, leaving a single nucleotide gap that can be filled in by DNA Polymerase I.

Nucleotide or strand excision repair is a more radical process, involving 1) identification of the lesion; 2) excision of strand containing the lesion; 3) copying from the undamaged strand by DNA polymerase, and finally resealing by DNA ligase.



**Mismatch repair:** replaces mismatched bases resulting from **errors in replication**. The lesion is a simple mismatch, not an altered base, so it is important that the cell can correctly identify which of the two opposed bases at the mismatch site is actually wrong. Since the error most likely arose from miscopying by polymerase, it is assumed to be in the newly synthesized strand.

Mature strands are marked by the **DAM methylase**, which tags adenine in GATC sites with an N<sup>6</sup>-methyl group (which does not cause base pairing errors). Therefore the error is assumed to be on the unmethylated strand.

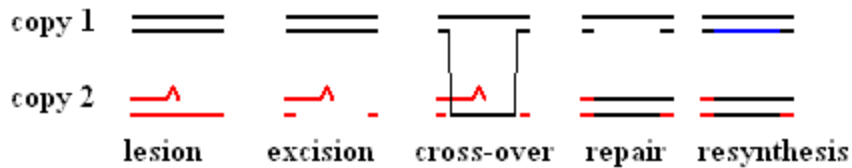


The mismatch repair process involves

- 1) factors MutL and MutS bind at the site of mismatch.
- 2) When a methyl tag is found by binding factor MutH, this identifies the old and new strands, the newer strand is cut at the CTAG opposite the methyl tag, and trimmed back to the lesion by exonuclease.
- 3) The gap is then refilled by repair polymerases, and sealed by DNA ligase.

Cells containing defective components of the mismatch repair process, particularly MutL, S or H (**mutator** phenotypes) have high spontaneous rates of mutation due to failed repairs, and these bacterial strains can be used as sources of mutants.

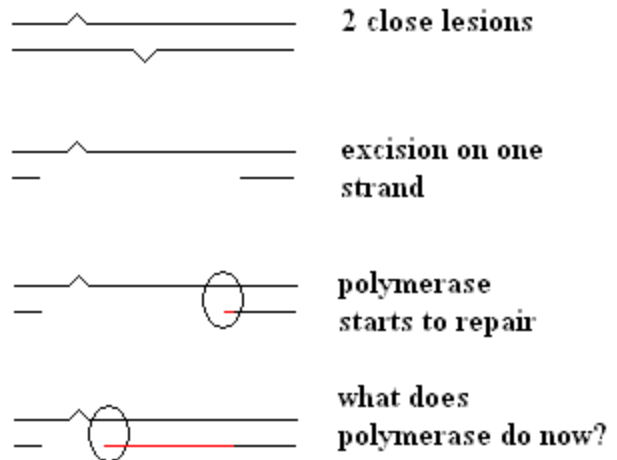
**Recombination repair:** after replication, 2 full duplex copies of DNA exist in a cell. If one duplex contains a lesion, a “good” copy can be obtained by crossover and recombination from the undamaged duplex.



**SOS response** is a special process normally induced only when damage to DNA appears to be very heavy.

**SOS repair allows for repair when gaps leave no “good” DNA strand to act as template. The SOS polymerase V components fill in nucleotides at random to bridge the gap.**

This is obviously **highly error prone**, but it’s better to try than to die.



SOS repair is an **inducible** system - normally in a **repressed** state so that accurate repair mechanisms have priority.

Cell strains in which accurate repair systems have been disabled, and **SOS components rendered constitutive are also good sources of mutants.**

## SOS components

**LexA** - repressor acting as **negative regulator of expression of SOS genes**. SOS response genes are dispersed over the genome, but are distinguished by the LexA binding sequence or **SOS-box** in their promoter region.

**RecA** - when bound to single stranded DNA, triggers **proteolytic destruction of LexA**  
**LexA** ↓ **triggers expression of SOS response genes:**

**These include**

***recA*** - single stranded DNA binding protein

***lexA*** - accumulation turns off SOS response

***uvrAB*** - excision enzymes

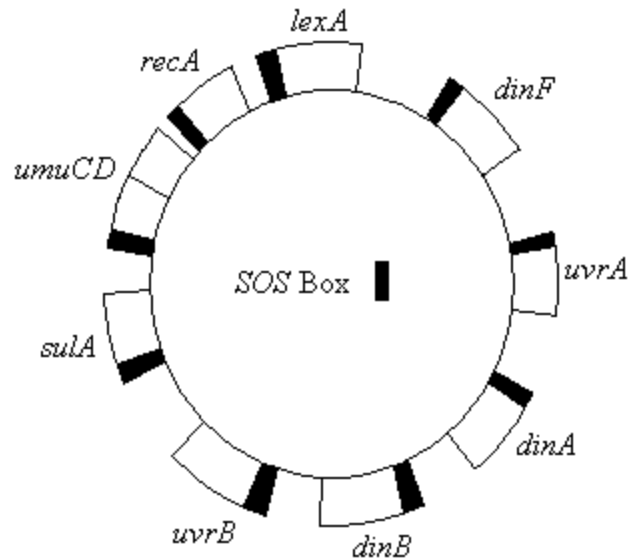
***din*** - repair polymerase

***umuC* and *umuD*** - polymerase components

**permitting passage through region where template is unreadable.**

***LexA*<sup>-</sup>** strains of *E. coli* have SOS response **constitutively active**, so have high frequency of permanent mutation due to overuse of error-prone repair.

**Deletion of LexA binding SOS box** in promoters for ***umuC* and *umuD*** causes these factors to be constitutively active.



**Success of random mutation** depends on the ability to obtain a good fraction of all possible mutations. This is possible because microbial cultures contain literally billions of cells, and each cell represents one mutational “experiment”.

e.g. *E. coli* genome is about  $4 \times 10^9$  bp. Substitution mutations result in any base being changed 3 possible ways. For each position, there are 4 possible insertions or one deletion, totalling  $32 \times 10^9$  possible changes. (Insertion or deletion of more than one nucleotide adds to this total).

If a treated culture contains  $10^{10}$  cells, and 10% contain a mutation, then we have potentially  $10^9$  mutations, a fairly good sample of the total range of possible changes. 10% mutation rate gives a reasonable frequency of mutation while avoiding too many double mutations.

What is needed next are efficient **selection methods** to isolate the few mutants from the population that actually do what is desired.