



Genetics

***Subject* : Genetics**

***Lec no* : 19**

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وَقُلْ رَبِّ زِدْنِي عِلْمًا

→ severity of the mutation مسبب

Manifestations of Mutations

A. Lethal Mutations → mutations قاتلة لا يمكن أن تكون معنا ولكنها تكون فيه miscarriage ويحدث death فيصير embryo

❖ The alteration is incompatible with life of the cell or the organism. For example, mutation producing alpha-4 Hb is lethal, and so the embryo dies.

B. Silent Mutations → nucleotide يتغير nucleotide آخر ولكنه codon يبقى يعطي نفس a.a لذلك فإن protein الناتج له يتأثر.

❖ Alteration at an insignificant region of a protein may not have any functional effect.

Manifestations of Mutations

C. Beneficial Mutations

- Although rare, beneficial spontaneous mutations are the basis of evolution.

يوجد synthesised mutation احنا بنفعلها على نفس اوقات معينة
او فواى معينة امثال:

الذرة الطبيعية لا
تحتوي على
Tryptophan

- Such beneficial mutants are artificially selected in agriculture. Normal maize is deficient in tryptophan. Tryptophan rich maize varieties are now available for cultivation.

genetic modification
الذرة بتتغير
على انتاج
Tryptophane

- Microorganisms often have antigenic mutation. These are beneficial to micro-organisms (but of course, bad to human beings).

bacteria بتغير بصيرها mutation بحيث لا تسيب antibodys القضاء عليها (حتى انة يبيينا مرض بكتيريكي
بنة لاب antibodys لم يمود فعال)

Adaptation mutation
في evolution بتغير genetic mutation بحسب environment احنا موجودين فيها
يعني بصير لون عيون معين (phenotype معين)
التكيف مع stress معين

Genetic adaptations have occurred in many aspects of human life, including the adaptation to cold climate and high-altitude hypoxia, the improved ability of defending infectious diseases, and the polished strategy of utilizing new diet with the advent of agriculture.

Manifestations of Mutations

وهذا ما يحدث في cancer

بعض فيوت mutation

نوع proliferation

D. Carcinogenic Effect

cell cycle regulation → proteins ← cell cycle في
انما اصل regulatory function في mutation لهذه البروتينات فإنه يحدث مشاي في cell cycle

- The mutation **may not be lethal** but may **alter the regulatory mechanisms.**

regulatory proteins في mutation المنظمة لانقساماتها

- Such a **mutation in a somatic cell** may **result in uncontrolled cell division leading to cancer.**
- Any substance causing **increased rate of mutation** can also increase the probability of cancer. **Thus, all carcinogens are mutagens.**



DNA Damage & Repair

Nebras Melhem

Introduction

Introduction — هي عبارة عن كلام أخذناه المحاضرة السابقة .

- Most cells have only one or two sets of genomic DNA. ** أخذنا قبل انه ممكن يحصل repair عن طريقه DNA polymerase (proofreading) في اثناء replication ولكن يوجد mechanisms اخرى بقدر اتعلم من خلالها على errors التي بيصلها DNA polymerase اثناء replication*
- Damaged proteins and RNA molecules can be quickly replaced by using information encoded in the DNA, but DNA molecules themselves are irreplaceable.
- Maintaining the integrity of the information in DNA is a cellular imperative, supported by an elaborate set of DNA repair systems.
- DNA can become damaged by a variety of processes, some spontaneous, others catalyzed by environmental agents.
- Replication itself can very occasionally damage the information content in DNA when polymerase errors create mismatched base pairs (such as G paired with T).

Introduction

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- The genomic DNA in a typical mammalian cell accumulates many thousands of lesions during a 24-hour period. However, as a result of DNA repair, fewer than 1 in 1,000 become a mutation.
 - DNA is a relatively stable molecule, but in the absence of repair systems, the cumulative effect of many infrequent but damaging reactions would make life impossible.

Introduction

- The number and diversity of repair systems reflect both the importance of DNA repair to cell survival and the diverse sources of DNA damage.
- Some common types of lesions, such as pyrimidine dimers, can be repaired by several distinct systems.
- Nearly 200 genes in the human genome encode proteins dedicated to DNA repair. In many cases, the loss of function of one of these proteins results in genomic instability and an increased occurrence of oncogenesis.

Introduction

- Accurate DNA repair is possible largely because the DNA molecule consists of two complementary strands.
- Damaged DNA in one strand can be removed and replaced, without introducing mutations, by using the undamaged complementary strand as a template.
- We consider here the principal types of repair systems, beginning with those that repair the rare nucleotide mismatches that are left behind by replication.

Mechanisms of DNA repair

DNA repair يكون على مستوى DNA

هذه الخاصية فقط عن اول mechanism

1. Mismatch repair
2. Base excision repair
3. Nucleotide excision repair
4. Double strand break repair = Directe repair

The defective region in one strand can be repaired relying on the complementary information stored in the unaffected strand.

بما إنه الاصل أو الأناة (parent strand) لم يتغير فانا بقدر
 أنسى الخطأ في daughter strand وأرجع إليه بناءً على (parent strand)

* في replication يكون لدينا single strand (parent strand)
 وبتقينا daughter strand

Mismatch repair

parent strand ما يكون فيها mutation ولكن daughter strand هي التي يكون فيها mutation
 ← نبحر عن الخطأ في daughter strand (mutation mismatch) ونبشله ونعمل deletion
 DNA polymerase يقوم ببناء nucleotides الصحيح
 له وجود G في المكان الذي يقابل A في parent strand (الصح أنه يكون T وليس G)
 يوجد specific proteins target this place (mismatch)

- The mismatches are nearly always corrected to reflect the information in the old (template) strand, which the repair system can distinguish from the newly synthesized strand by the presence of methyl group tags on the template DNA.

لأنه حتى نتعرف على mismatch يجب وجود DNA methylated (methyl group على DNA)
 ← tagging of DNA فالتالي يعرف من وين تبدأ

- The methyl-directed mismatch repair system of E. coli efficiently repairs mismatches up to 1,000 bp from a hemimethylated GATC sequence.

Mismatch repair

- In bacteria, strand discrimination is based on the action of Dam methylase, methylates DNA at the N⁶ position of all adenines within (5')GATC sequences.
- Immediately after passage of the replication fork, there is a short period (a few seconds or minutes) during which the template strand is methylated but the newly synthesized strand is not.

Mismatch repair

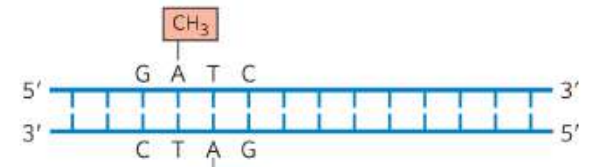
- The transient unmethylated state of GATC sequences in the newly synthesized strand permits the new strand to be distinguished from the template strand.
- Replication mismatches in the vicinity of a hemimethylated GATC sequence are then repaired according to the information in the methylated parent (template) strand.

Mismatch repair

- An endonuclease cuts the strand containing the mutation at GATC site adjacent to the defective site.
- An exonuclease then digests this strand from the site of the cut through the mutation, removing the mismatch area.
- A repair DNA polymerase then fills the gap and the DNA ligase seals the nick in the DNA.

* بين 500 nucleobides و 1000 nucleobides يوجد هذا

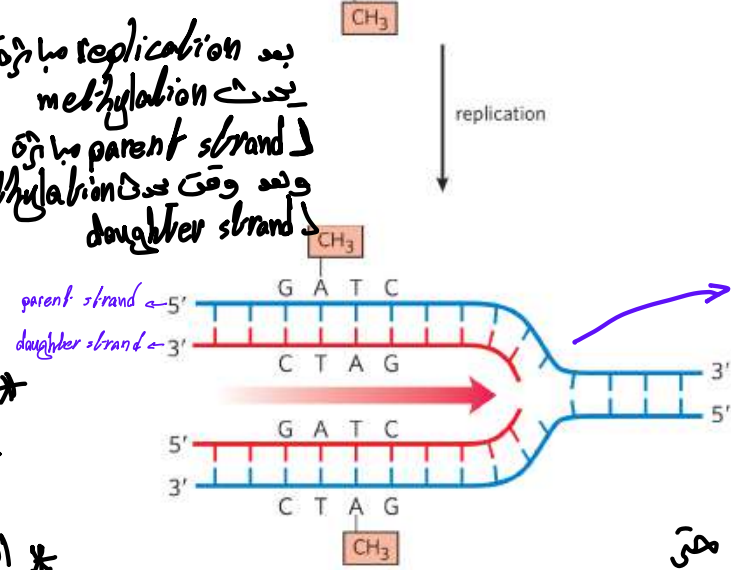
التسلسل GATC



methylation sequence يحدث في
على A يوجد على parent strand و daughter strand

بعد replication مباشرة
يحدث methylation
في parent strand
و بعد وقت يحدث methylation
في daughter strand

تكون marker بعد ان عنده عشان نشوف ما
بين GATC و GATC الي بعدها اي mutation في
nucleobides في بينهم.

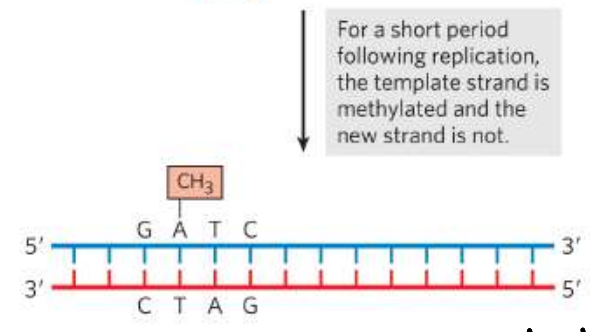


replication fork
بين replication
نلاحظ اننا على parent strand
مethylated بينما daughter لا.
لأنه يحتاج الى وقت حتى
تصير methylated.

* نميز هذا sequence عن طريق methyl group الموجود
على Adenine

* الهدف من methyl group هو معرفه parent strand
يعني الذي ما فيه عيبا mutation بين واحدنا
بنسب بناء على parent strand الذي ما فيه عيبا

• mutation
- طيب كيف بدنا نميز بين parent, daughter
بما انه المشيب في عيون methyl group?

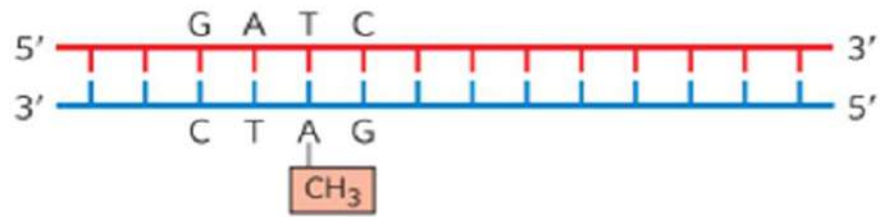


* اننا replication يجعل في البداية methylation في parent strands و بعدها بوقت في

في daughter strand
لا تكون methylated
(محدث replication تكون methylated parent)

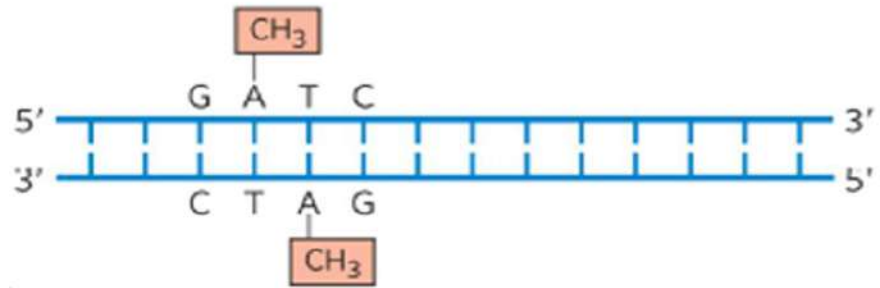
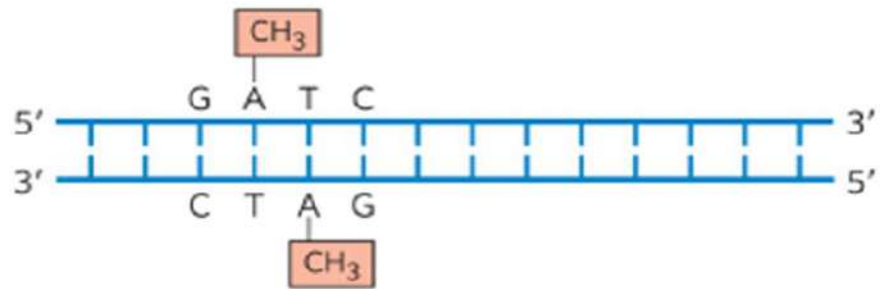
• daughter strand (بعد فتره زمنية)
* اننا replication يكون parent strand methylated بينما daughter لا.

Hemimethylated DNA



Adenine → methylation → Dam methylase

After a few minutes, the new strand is methylated and the two strands can no longer be distinguished.



Mismatch repair

بوا: daughter or parent

بطلع على DNA بتشوف وين صار mismatch ويتوقف عنده

هذه البروتينات تتعرف على mutation ويتخلصوا منه.

• **MutS** scans the DNA and forms a clamp-like complex upon encountering a lesion. The complex binds to all mismatched base pairs. *MutH activation بعد*

بترتبط مع MutS ويتشكل complex

• **MutL** protein forms a complex with MutS protein, and the **MutSL** complex slides along the DNA to find a hemimethylated GATC sequence.

بعد ما حدد مكان mismatch يتحرك إلى MutH (GATC) ويعمل له activation

• **MutH** binds to MutL, and the MutSLH complex moves in either direction at random along the DNA. *مethylated GATC*

بتمسك بـ methylated GATC

• MutH has a site-specific endonuclease activity that is inactive until the complex encounters a hemimethylated GATC sequence. *في بعض inactive لأن بعد ارتباطه مع MutL, S عند موقع mismatch ويتحرك activation ويعملون*

• At this site, **MutH** catalyzes cleavage of the unmethylated strand on the 5' side of the G in GATC, which marks the strand for repair. *بعمل قطع صغير عند G of (MutH) endonuclease activity*

endonuclease activity

• **DNA polymerase** fills the gap, and **ligase** seals the nick. *بعد إكمال البناء يأتى ligase ويعمل phosphodiester bond يبنى مكان nucleotides و G of 5' وحتى مكان mismatch nucleotides ياتي DNA polymerase ياتي*

exonuclease ياتي بعد ذلك ياتي

بعد ذلك ياتي exonuclease ياتي nucleotides و G of 5' وحتى مكان mismatch ياتي DNA polymerase ياتي يبنى مكان nucleotides و G of 5' وحتى مكان mismatch ياتي ligase ويعمل phosphodiester bond ياتي

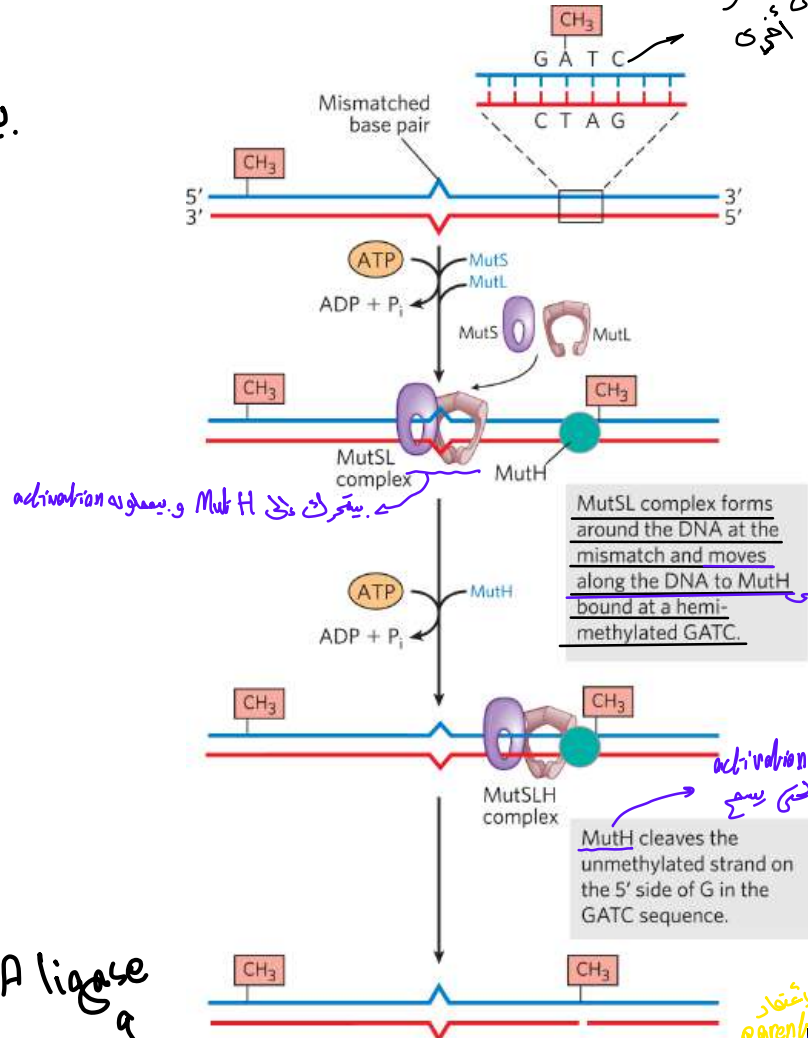
ملاحظة جارية:

endonuclease → from the middle of a DNA strand
يتم قطع

exonuclease → end of a DNA strand
يتم قطع من

* على القطع تكون من عند اقرب mismatch الى GATC

عن طريق Mut H methylated GATC
ننتجها على قدر replication لانها مرة اخرى بشكل صحيح



MutSL complex forms around the DNA at the mismatch and moves along the DNA to MutH bound at a hemimethylated GATC.

MutH cleaves the unmethylated strand on the 5' side of G in the GATC sequence.

نسي يموله activation

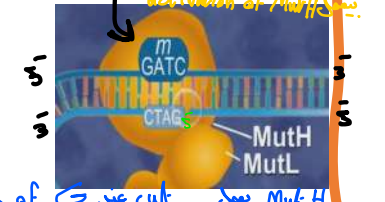
Mut H بعد ما يفرق نسي يمس exonuclease

has endonuclease activity

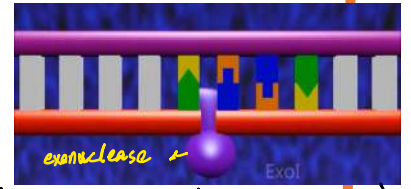
DNA polymerase III يقوم بالبناء والاختتام parent strand



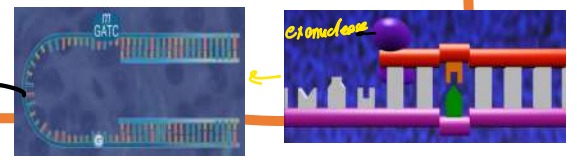
MutSL complex activation of MutH



5' of G عند cut بعد Mut H



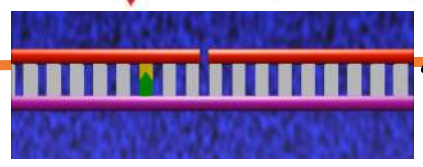
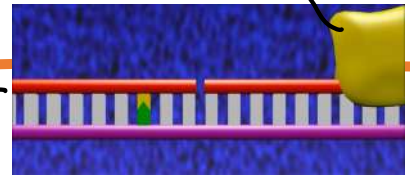
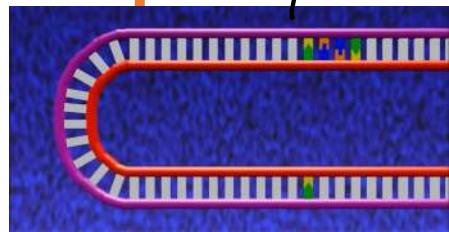
cut لا عنده Mut H يمس exonuclease في يمس ويكس GATC nucleotides. ناتج mismatch



exonuclease

انقاص الحجم

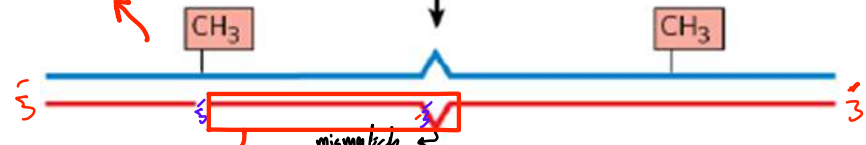
DNA ligase





MutS
MutL
MutH
ATP → ADP+P_i

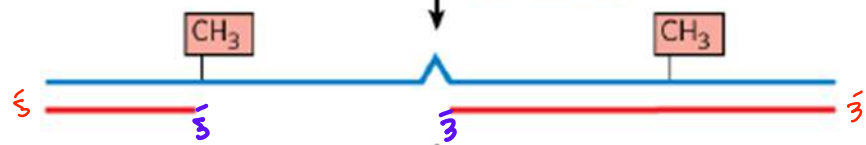
DNA helicase من 3' → 5'



mutS highlights this sequence
متمتع بتفرقة sequence التي كسرت

exonuclease يقوم في الاتجاهين من 3' → 5' و 5' → 3'
والتي DNA polymerase يمد في اتجاه واحد من 3' → 5'

MutSL
DNA helicase II
SSB
exonuclease VII
or
RecJ nuclease
ATP → ADP+P_i



DNA polymerase III
SSB
DNA ligase



تكملة الشئ

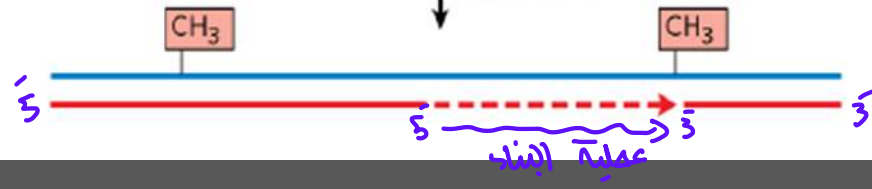


MutSL
DNA helicase II
SSB
exonuclease I
or
exonuclease VII
or
exonuclease X
ATP → ADP+P_i

DNA helicase من 5' → 3'



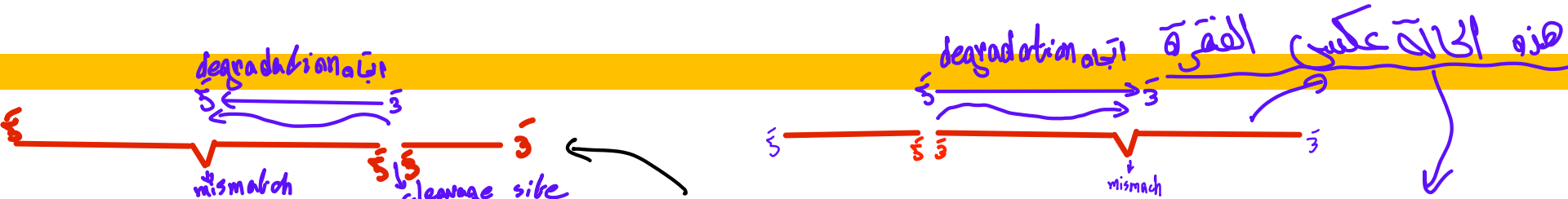
DNA polymerase III
SSB
DNA ligase



تكملة الشئ

Mismatch repair

اتجاه degradation من cleavage site إلى mismatch
 $\begin{matrix} & \swarrow & \searrow \\ 3 \rightarrow 5 & & 5 \rightarrow 3 \end{matrix}$



- When the mismatch is on the 5' side of the cleavage site, the unmethylated strand is unwound and degraded in the 3' → 5' direction from the cleavage site through the mismatch, and this segment is replaced with new DNA.

- This process requires the combined action of DNA helicase II (also called UvrD helicase), SSB, exonuclease I or exonuclease X (both of which degrade strands of DNA in the 3' → 5' direction) or exonuclease VII (which degrades single-stranded DNA in either direction), DNA polymerase III, and DNA ligase.

لأنه متى لا زوم نرفع بالتفصيل فقط نرفعه
 DNA polymerase III / Mut S, L, H

DNA helicase / SSB / exonuclease / DNA ligase

* كل ما تم شرحه كان على E. coli
 (ولكن هذه العملية تتم في كل organisms
 ولكن proteins تكون احياناً مختلفة)

← متى ضروري نعرف الازدحام فقط نعرف انه في exonuclease (من ١٥٠٠ I, VII)

Enzymes/proteins	Type of damage
Mismatch repair	
Dam methylase	} Mismatches
MutH, MutL, MutS proteins	
DNA helicase II	
SSB	
DNA polymerase III	
Exonuclease I	
Exonuclease VII	
RecJ nuclease	
Exonuclease X	
DNA ligase	
Base-excision repair	
DNA glycosylases	} Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; in some other organisms, pyrimidine dimers
AP endonucleases	
DNA polymerase I	
DNA ligase	
Nucleotide-excision repair	
ABC excinuclease	} DNA lesions that cause large structural change (e.g., pyrimidine dimers)
DNA polymerase I	
DNA ligase	
Direct repair	
DNA photolyases	Pyrimidine dimers
O ⁶ -Methylguanine-DNA methyltransferase	O ⁶ -Methylguanine
AlkB protein	1-Methylguanine, 3-methylcytosine