
النادي الطبي
Genetics
Subject : Genetics
Lec no : 19
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وقل رب زدني علما

severity of the mutation حسب

Manifestations of Mutations

A. Lethal Mutations →

mutations قاتلة لا يمكن أن تكون معنا ولكنها تكون فيه embryo فيصير death ويحصل miscarriage

- 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 →

تتغير nucleotide الى nucleotide أخرى ولكن codon يبقى يعطي نفس a.a لذلك فإن protein الناتج لن 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.
- Such beneficial mutants are artificially selected in **agriculture**.
- Normal maize is deficient in tryptophan. Tryptophan rich maize varieties are now available for cultivation.
- Microorganisms often have antigenic mutation. These are beneficial to micro-organisms (but of course, bad to human beings).

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

D. Carcinogenic Effect

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

* أخذنا قبل انه ممكن يحصل repair عن طريق DNA polymerase (proofreading) في أثناء replication ولكن يوجد أخرى بقدر اتغلب من خلالها mecanisms على errors اللي بيعملها DNA polymerase أثناء replication

- Most cells have only one or two sets of genomic DNA.
- 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

- 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 يكون على مستوى repaire

هي دي نكمل هذه المحاضرة فقط عن أول mecanism

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

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

Mismatch repair

- 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.
- 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 N^6 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 كل 1000 nucleotides و 1000 nucleotides يوجد هذا
التسلسل GATC

الهدف من هذه sequence يحدث لـ methylation
على A سواء على parent strand أو daughter strand

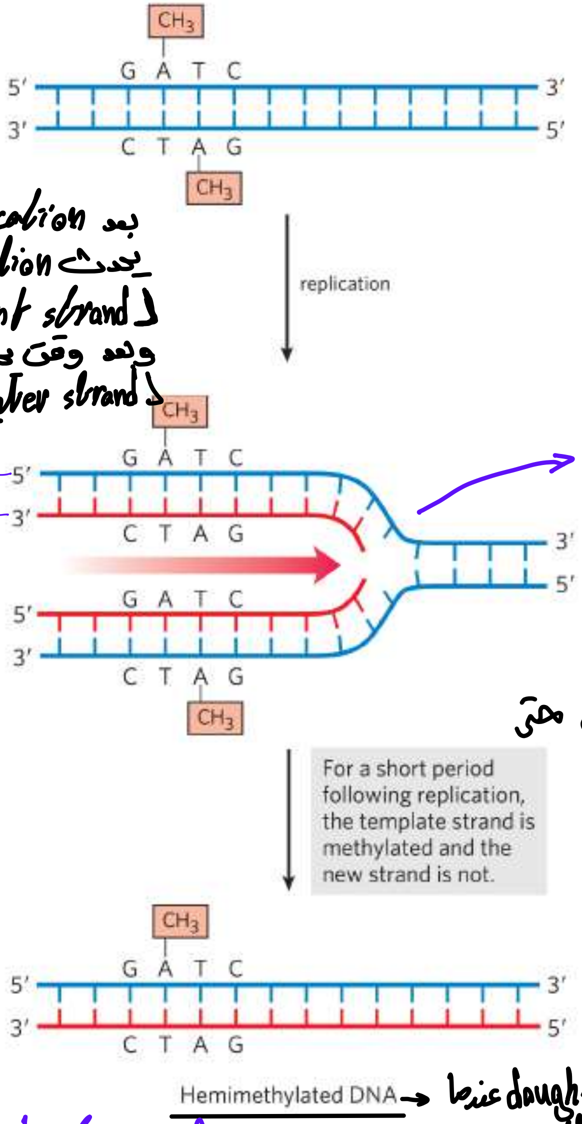
← تكون marker نبدأ من عنده عشان نشوف ما بين GATC و GATC الي بعدها أي mutation في nucleotides ذي بينهم.

* نميز هذه sequence عن طريق methyl group الموجودة على Adenine

* الهدف من methyl group هو معرفة parent strand يعني ذلك ما في عليها mutation حيث واحنا بدنا نبني بناءً على parent strand ذلك ما في عليها mutation.

- طيب كيف بدنا نميز بين parent, daughter بما إنه الثنتين فيهم عليهم methyl group ?

بعد replication مباشرة يحدث methylation لـ parent strand مباشرة وبعد وقت يحدث methylation لـ daughter strand



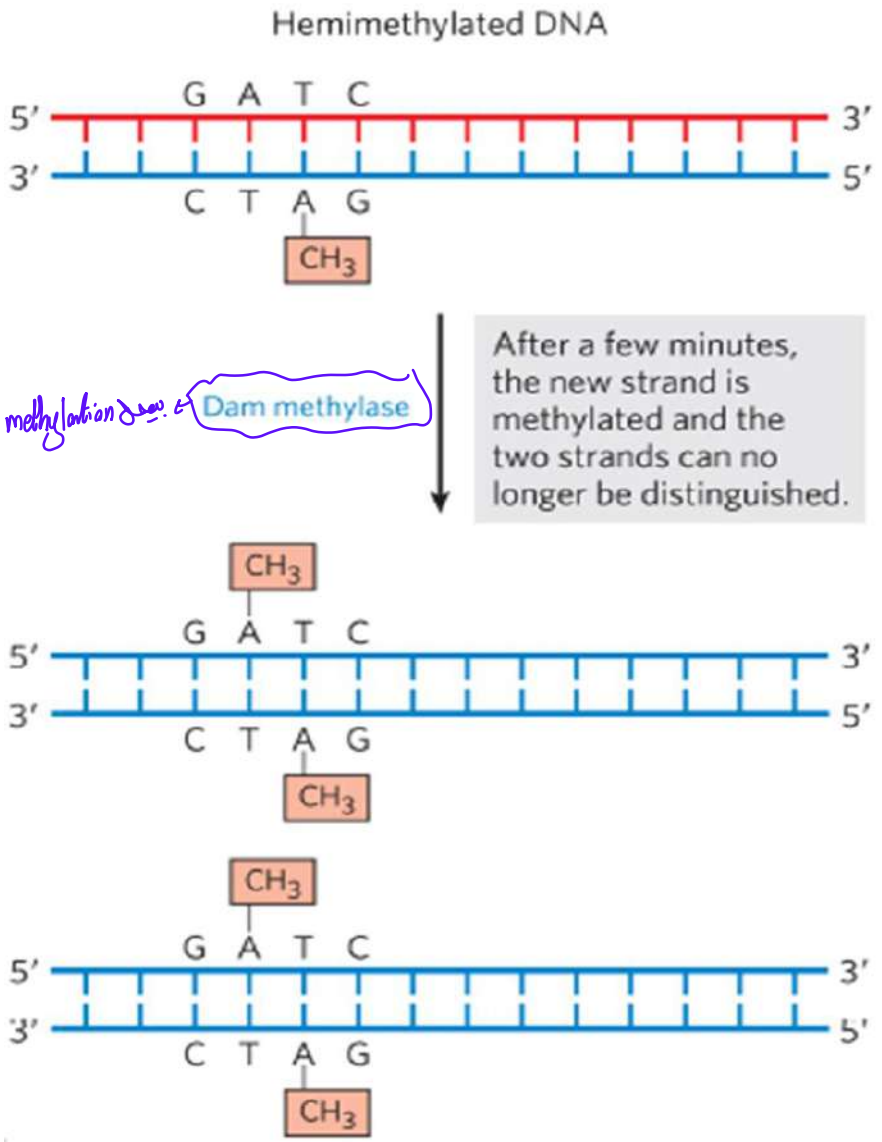
→ ماشي وقاعد replication forck

يعني replication بيرجع: إنه parent strand هي methylated بينما daughter لا.
لكن تحتاج ذلك وقت حتى تصبح methylated.

Hemimethylated DNA → عندما daughter strand أي لا تكون methylated
(بعد replication تكون parent methylated)

* أثناء replication يحصل في البداية methylation لـ parent strand وبعدها بوقت لـ daughter strand (يوجد فاصل زمني)

* إذن بعد replication تكون parent strand methylated بينما daughter لا.



Adenine ی methylation میشه ← Dam methylase

Mismatch repair

- **MutS** scans the DNA and forms a clamplike complex upon encountering a lesion. The complex binds to all mismatched base pairs.
- **MutL** protein forms a complex with MutS protein, and the MutSL complex slides along the DNA to find a hemimethylated GATC sequence.
- **MutH** binds to MutL, and the MutSLH complex moves in either direction at random along the DNA.
- MutH has a site-specific endonuclease activity that is inactive until the complex encounters a hemimethylated GATC sequence.
- 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.

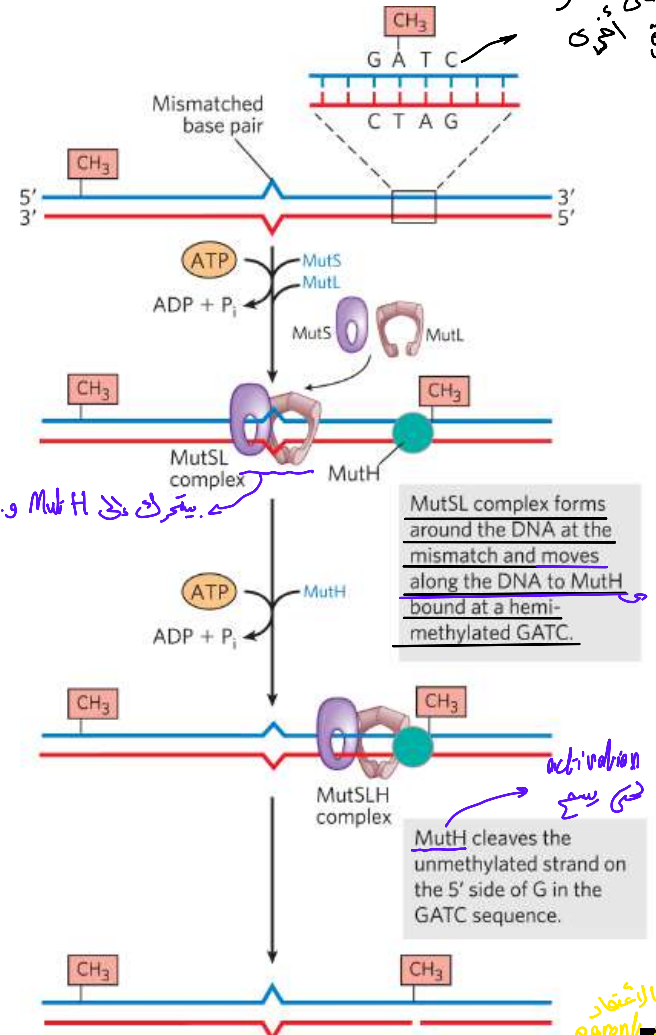
ملاحظة جانبية:

endonuclease → from the middle of a DNA strand يعمل قطع.

exonuclease → end of a DNA strand يعمل قطع من عند.

* عملية القطع تكون من عند اقرب GATC إلى mismatch.

Mut H عن طريق methylated GATC بنشتقوا حتى نقدر نفصل replication لأكلها مرة أخرى بشكل صحيح



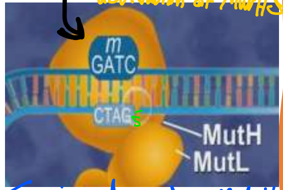
يتحرك لعند Mut H و بيعملوا activation

بيعملوا لحتى activation

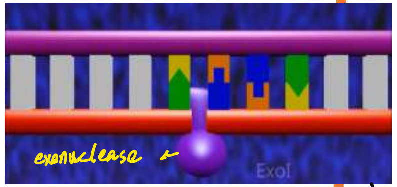
activation لحتى يسمح ل exonuclease يعمل cut عند 5 of G بعد ما يعملوا Mut H

has endonuclease activity

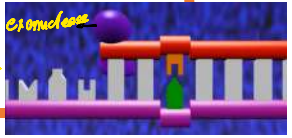
Mut SL complex يعمل activation of MutH.

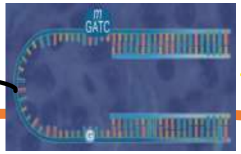


يعمل Mut H cut عند G of 5

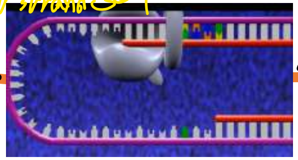


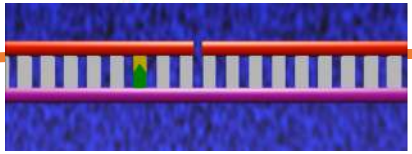
بعد cut يسمح MutH لل exonuclease انه يعمل ويكسر nucleotides باتجاه GATC mismatch



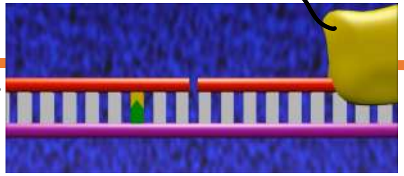


DNA polymerase III يقوم بالبناء بالاعتماد على parent strand

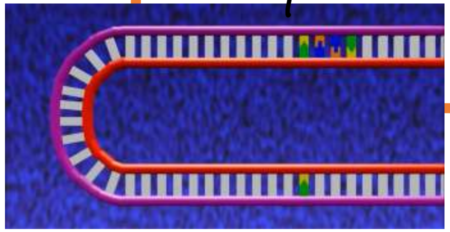


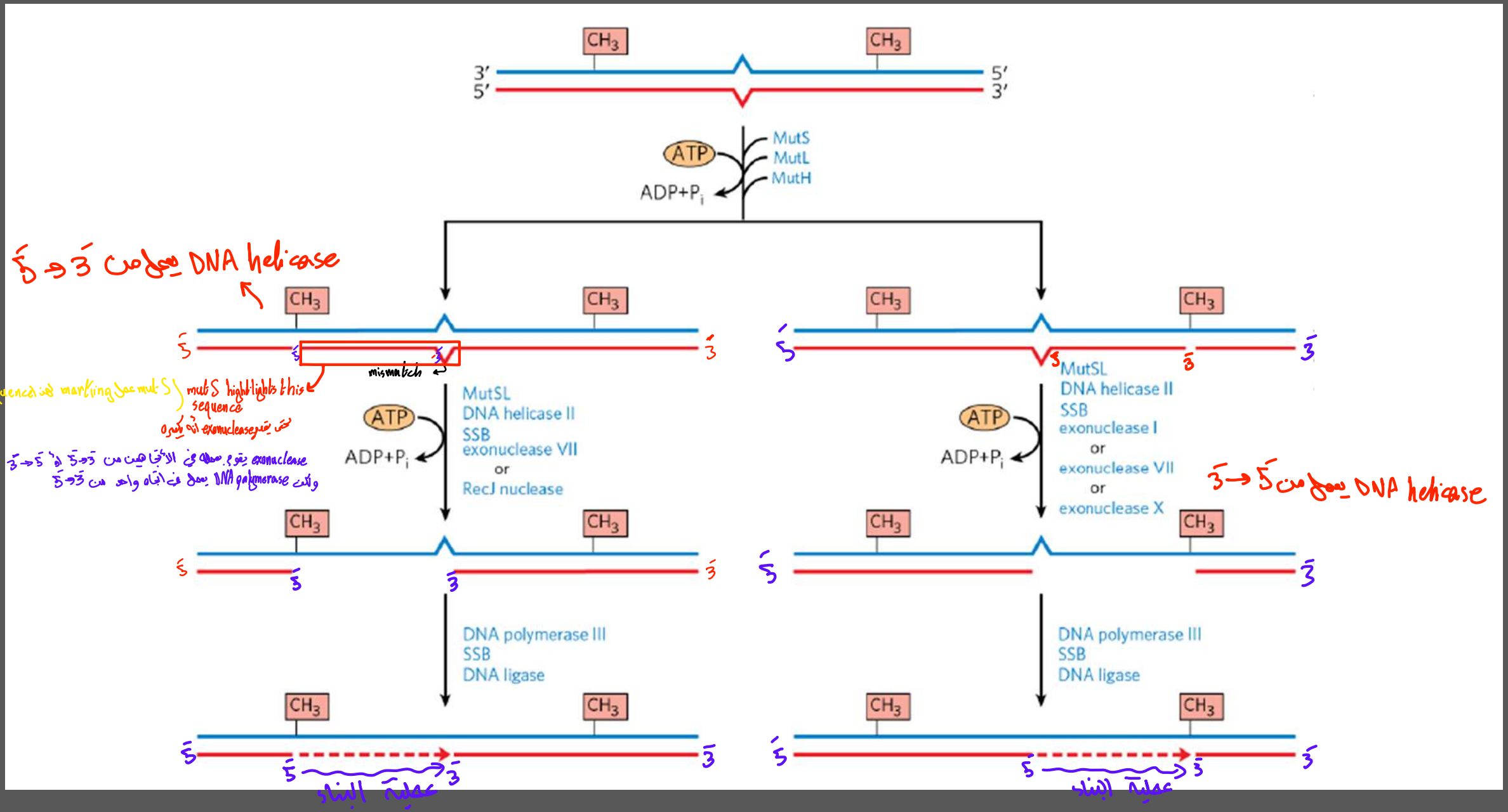


DNA ligase

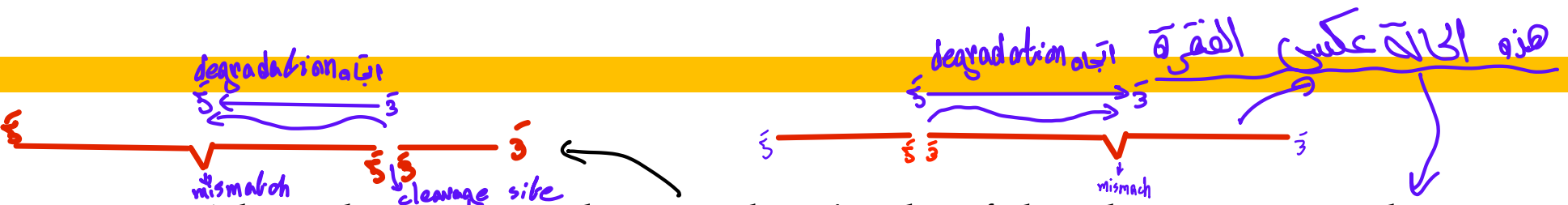


انتهت المهمة




CH3
CH3
3′
5′
5′
3′
ATP
ADP+Pi
MutS
MutL
MutH
mismatch
MutSL
DNA helicase II
SSB
exonuclease VII
or
RecJ nuclease
DNA polymerase III
SSB
DNA ligase
MutSL
DNA helicase II
SSB
exonuclease I
or
exonuclease VII
or
exonuclease X
5′
3′

Mismatch repair



- 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.

DNA polymerase III/ Mut S, L, H لـ مش لازم نروحلو بالتفصيل فقط نعرف

DNA helicase/ SSB/ exanuclease/ DNA ligase

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

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

← مش ضروري نعرف الأرقام فقط نعرف انه فيه exonuclease (I, VII مهم مش)