Genetics & molecular biology

◉ Sheet ○ Slide

Number:		7
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اليوم الموضوع هو عن الDNA repair ولهيك رح نبدأ بال mutations ورح ندرس عن كم نوع فقط والباقي رح يكون بمادة الgenetics

في اكثر من نوع من انواع DNA repair او (ورشات تصليح الحمض النووي)

اولا Mutations هي تغيير سلسلة ال nucleotides وبشرط هذا التغير يكون permanent لانو اذا كان لحظي وتصلح ما بكون طفرة، الطفرة هي الاشي الدائم وبكفي يكون بنيوكليوتايد واحد لنسميها طفرة.

انواع الطفرات

1. substitution mutation:

استبدال replacement لنيوكليوتايد واحد او اكثر (بدل ال G صار A)

2. insertion / deletion mutation:

يعني اضافة نيوكليوتايد واحد او اكثر للسلسة وهاذ باثر ع كل السلسلة.

او حذف نيوكليوتايد واحد او اكثر وبنعتبرها طفرة رغم انها اضافة او حذف واحد او اثنين لانو كل القراءة بالترجمة بتتغير (يعني بالعربي ممكن يصير كودون ايقاف مثلا ويوقف كل الترجمة او يغير كل الاحماض الامينية ويكون بروتين مختلف تماما عن المطلوب!! لانو القراءة بتكون ثلاثيات / codons)

3. silent mutation:

سايلنت بتاثيرها لانو ما في تغيير بالنتائج او الproducts مثلا بتغير النيوكليوتايد وبتغير الكودون فاذا الكودون القديم اعطى اسبارتيت وبعد التغير اعطى اسبارتيت برضو فهاي طفرة عديمة التاثير لانو الحمض الاميني يتم تشفيره باكثر من حمض اميني (وبرضو ممكن يكون التغير باماكن غير مهمة بالdna لانو في مناطق بالdna ما الها وظيفة او عبارة عن سلسلة لاتترجم!! والطفرة هناك تسمى سايلنت برضو)

طبعا الطفرات مرتبطة بالكانسر والcancer هي خلايا عندها بوتينشال عالي للانقسام والتكاثر في اوقات لازم ما تنقسم فيها وبرضو من telomerase كيف بساعد بعد موت الخليه السرطانية والي بميز الخليه السرطانيه انو بصير فيها accumulation وتجمع لطفرات كثير وهاي الطفرات ممكن تكون باماكن مهمة زي ال check point for cell cycle

وفي علاقة قوية بين الcarcinogens / يعني المواد المسببة للسرطان والmutagens / يعني المواد المسببة للطفرات بس مش كل ميوتاجين هو كارسينوجين لكن معظم الكارسينوجين هو ميوتاجين (بالعربي معظم السرطانات سببها طفرات لكن مش كل الطفرات بتسبب سرطانات؛ في طفرات صامته).

طبعا بعض السرطانات هي عبارة عن تجمع للطفرات فممكن خليه فيها عده طفرات لكن ما صار accumulations كافي ليكون سرطان وما وصل لمرحلة لخلايا تنتشر

(two hit model of cancer)

وفي برضو التو هيت موديل الي بقول لازم على الاقل عاملين يتواجدوا عشان يصير طفرة (ارجع للمحاضرة الي قبل)

لذلك 90% من السرطانات سببها طفرات

Dr. Imad

Today we will talk about:

1- Mutations and cancer.

2- Repair system.

Mutations and Cancer

Ames Test for Carcinogens:

Measures the potential of a chemical to induce mutations in bacteria (may act as a carcinogen)

مثال على الطعام: بنستخدم فيه بكتيريا السالمونيلا فمثلا لو بدنا نفحص مسحوق جلي اذا بسبب سرطان او طفرة بنحضر بكتيريا السالمونيلا الي عندها طفرة في انتاج حمض الاميني الهستدين الي هو ضروري لتنمو وتستمر بحياتها (يعني لازم احنا نحطلها هستدين برا في الوسط الي بدنا نحطه فيها عشان هي من جوا ما بتقدر تنتج هستدين) .. بس احنا بهاي التجربة ما بدنا نحطلها هستدين عشان نشوف تاثير المادة الي هي مسحوق الجلي ع السالمونيلا ,, ف اذا ماتت البكتيريا ف هو الاشي الطبيعي لانو ما حطينالها هستدين ومسحوق الجلي برضو ما اثر عليها (يعني المسحوق مش ميوتاجينك) // ولكن اذا عاشت السالمونيلا بالرغم من انه ما وفرنالها هستدين معناها شو؟ معناها المسحوق الي حطينا عمل فيها reverse mutation يعني عكس الطفرة الاصلية انها ما بتقدر تنتج هستدين فصارت تنتج هستدين وقدرت تضل عايشة ..من هون منكتشف انو المسحوق اثر على البكتيريا وعملها طفرة يعني هو mutagenic

وبنعمل دراسة ع المستعمرات البكتيرية (يعني كل ما كانت اكبر يعني المادة مسرطنة اكثر واذا ماتت بشكل اكبر المادة منيحة وهيكا!) طبعا هاذ الفحص اولي مش definitive.

***Carcinogen = mutagen

Salmonella typhimurium has a mutation that prevents it from synthesizing histidine, so it needs histidine to grow.

If we add a chemical (without histidine) to media that contains Salmonella typhimurium there are two options that could happen:

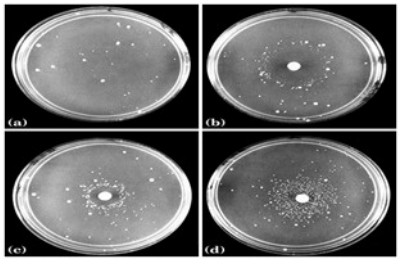
1- It will die

2- It will grow normally (which means that the chemical that was added is a mutagen and causes reverse phenotype which means producing histidine) = (the chemical made a mutation in the genes - originally there is a defect in the gene of this type- that is responsible for producing histidine).

أي أنه يقوم بعمل طفرة على طفرتها فتصبح قادرة أن تعيش دون إضافة الحمض الاميني الذي لا تقدر على صنعه بنفسها.

Back to Ames Test for Carcinogens:

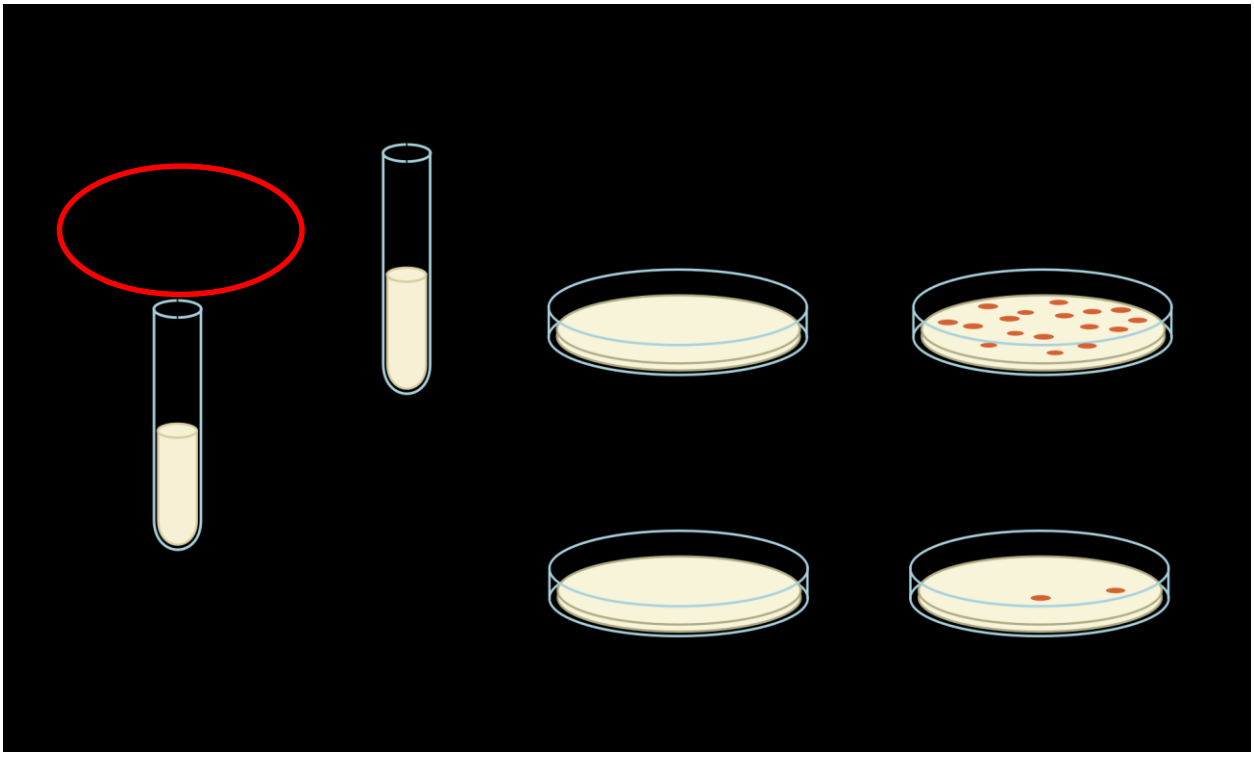
Look at the picture below, what do you observe?



- There are many substances which aren't Carcinogens, but when they exist in our body they become so. To know if a substance causes cancer or not, we introduce the substance to the body and see the effect of it. In the experiment above we put Salmonella in rat liver extract. Due to the proteins in the liver which do detoxification; it converts to be a Carcinogen.

هناك بعض المواد عندما تحدث لها عملية الأيض بعد دخولها الجسم تصبح مسرطنة مع أنها من قبل لا تكون كذلك. لذلك عند وضع بروتينات الكبد في التجربة سيقوم بالكشف لنا عنها هل تتحول لمسرطنة في جسمنا ام لا وبذلك نستطيع القيام بالفحص وبأخذ النتائج كاملة حول تلك المواد المرادة.

هاي بروتينات الكبد الي منضيفها عالتجربة عشان تعمل detoxificationوالي من خلالها منعرف اذا المادة بتتحول ل مسرطنة اذا فاتت جسمنا او لا

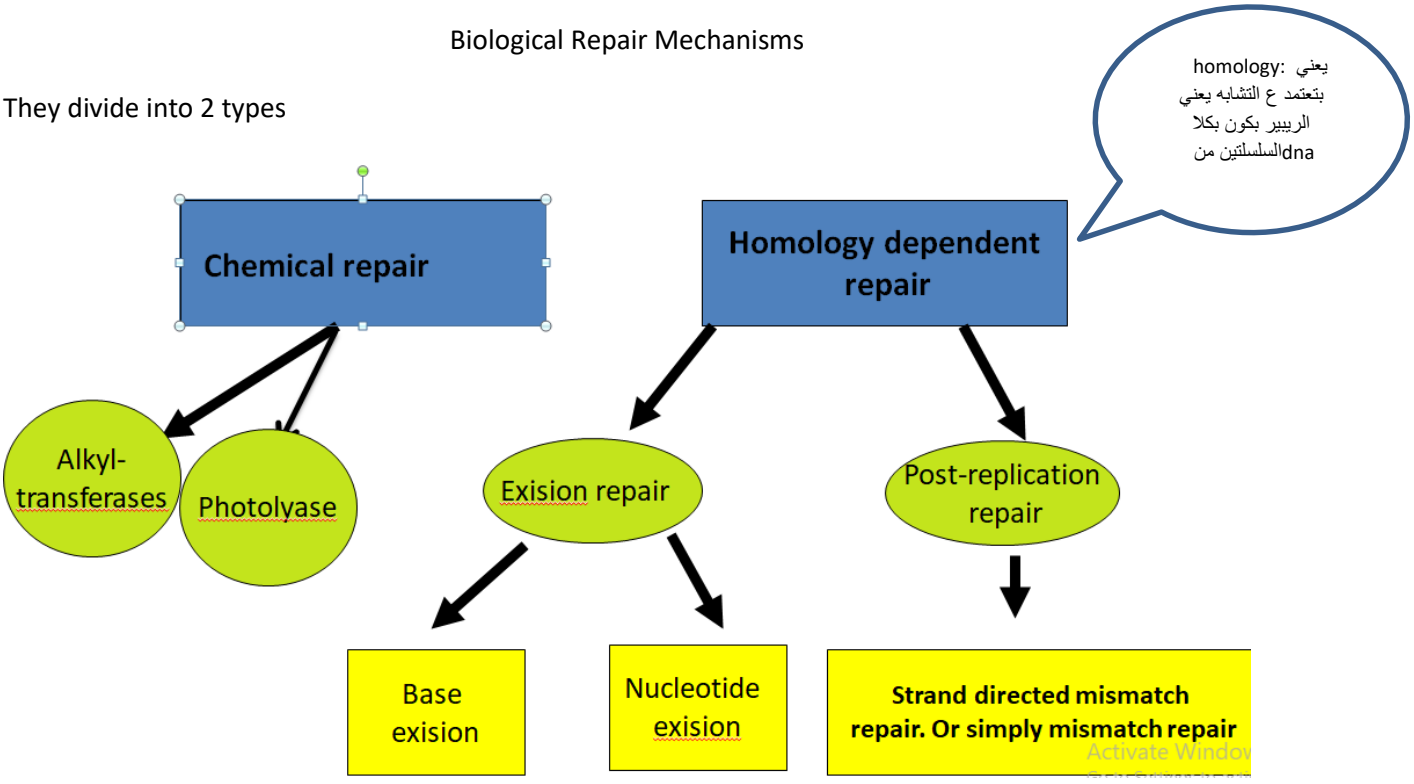


Mechanisms for maintaining DNA integrity:

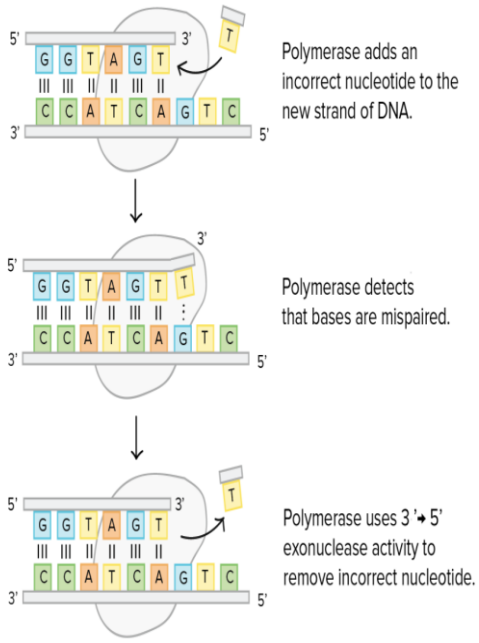
DNA damage and repair:

Biological Repair Mechanisms

They divide into 2 types



Remember: DNA polymerase exonuclease activity: During DNA replication, most DNA polymerases "check their work" with each base that they add. This process is called **proofreading**.



Types of DNA repair systems

TABLE 25–5 Types of DNA Repair Systems in *E. coli*

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	

TABLE 25-5 Types of DNA Repair Systems in *E. coli*

Enzymes/proteins	*Type of damage*
Nucleotide-excision repair	
ABC excinuclease	DNA lesions that cause large structural changes (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

Direct repair (chemical): Several types of damage are repaired **without removing a base or nucleotide and is independent from complimentary base pairing**= repairing doesn't depend on the complementary strand:

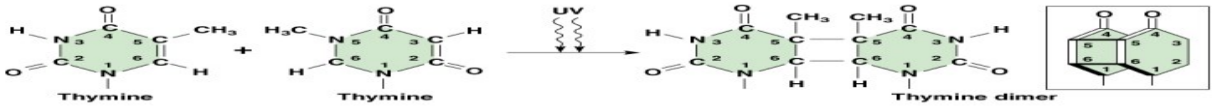
1. Photoreaction repair: exists mainly in plants and some bacteria and not in humans (there is an alternative type). Pyrimidine dimers result from a UV-induced reaction, and **photolyases** use energy derived from absorbed light to reverse the damage and this enzyme works only midday and uses the blue light and electrons taken from FADH- in lysis process, this is in bacteria not in humans which has an alternative way.

After **photolyases finishes its** work, it returns the electrons it took from FADH-.

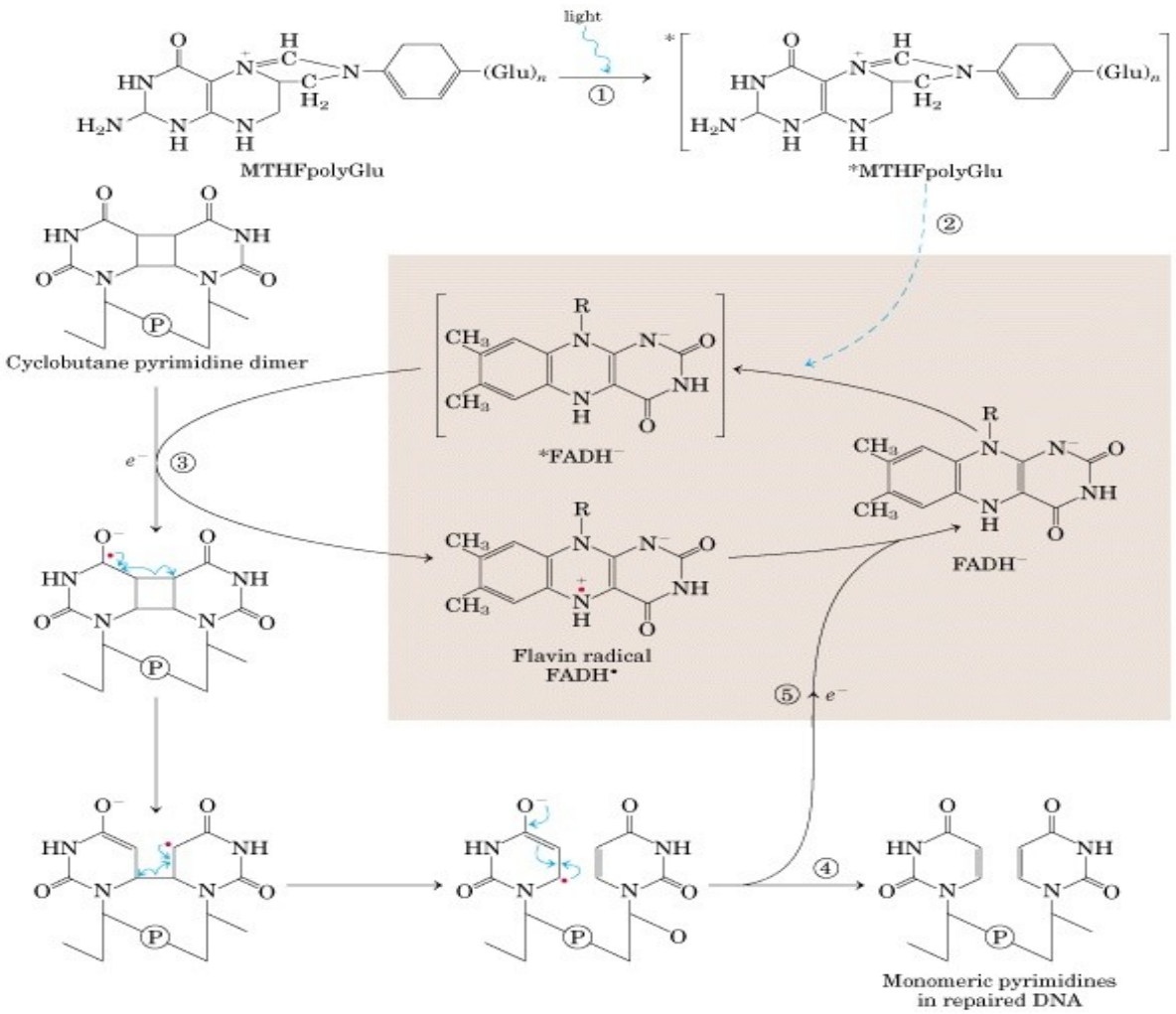
- Exposing UV treated cells to blue light results in a reversal of the thymine dimer formation.

- The enzyme, photoactivation repair enzyme (PRE) absorbs a photon of light (from blue light) and is able to cleave the bond forming the thymine dimer. Once the bond is cleaved, DNA is back to normal.

*UV is a Carcinogen.



Thymine dimers induced by UV light.



2. Alkylation of DNA by alkylating agents:

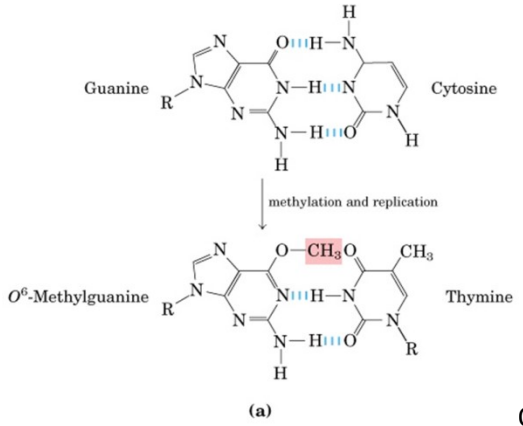
Example are **alkylating** mutagens. It adds an alkyl (**ethyl or methyl**) to nucleotides in various positions.

The most dangerous is when alkyl is added to oxygen at position 6 of G, creating **O-6-alkylguanine**, this will base with thymine.

Mustard gas is an alkylating agent, it kills and causes **Alkylation.**

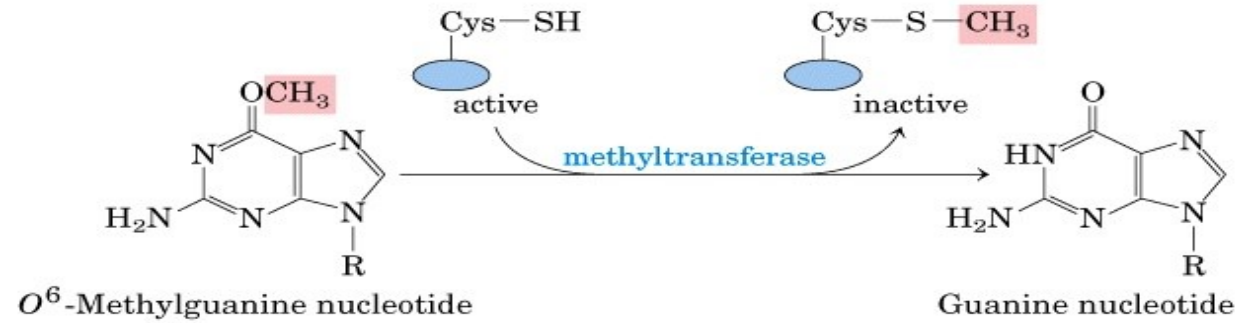
When DNA polymerase copies O-6-alkylguanine, it converts it to thymine; so it needs to be removed, if it isn't then it will produce a mutation. The enzymes in the body namely methyl transferase, which remove methyl groups and this enzyme has acceptor to it, so takes the methyl group on itself, then the enzyme becomes inactive (the cell regularly produces methyl transferase).

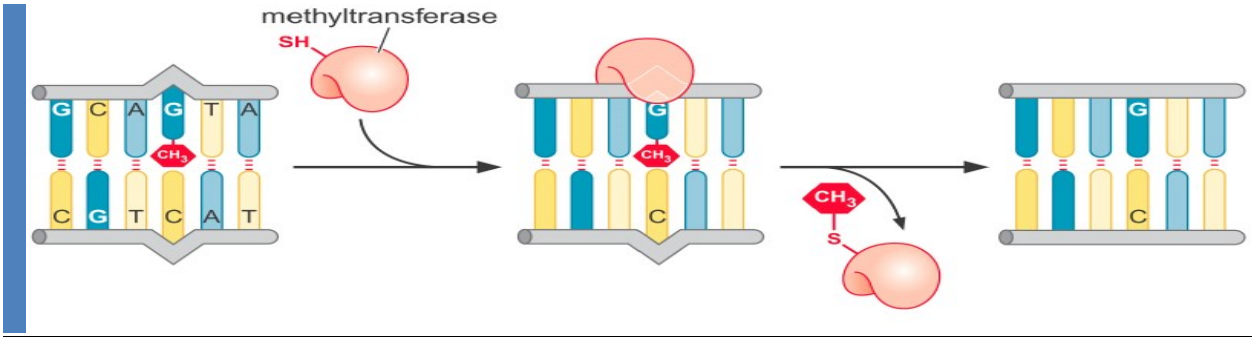
وكأن هذا الانزيم يضحي بنفسه.



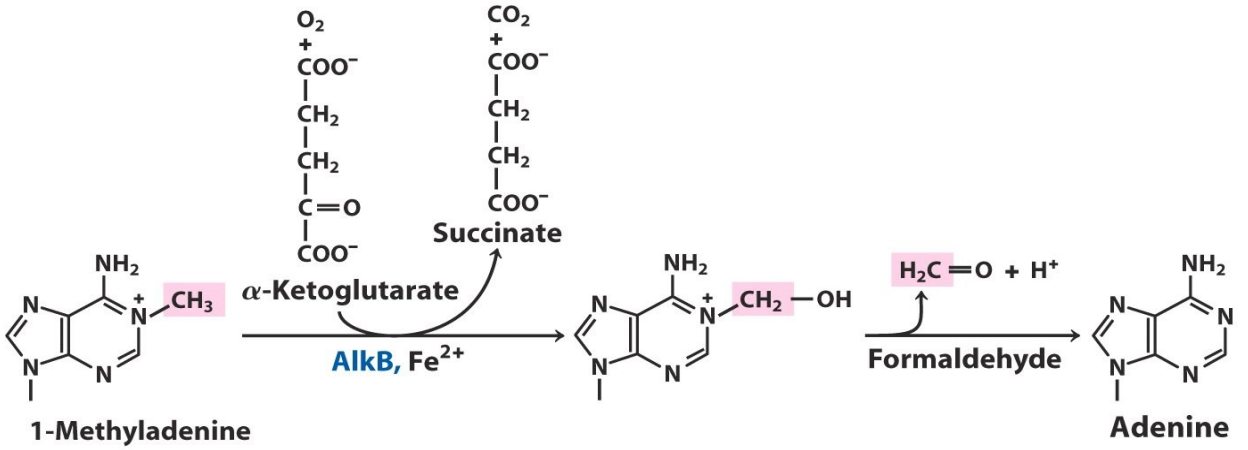
O6-methyl G, if not repaired, may produce a mutation

:Reversal of O6 methyl G to G by methyltransferase





In bacteria Oxidative demethylation of these alkylated nucleotides is mediated by the AlkB protein (α-ketoglutarate-Fe2–dependent dioxygenase)



High repetition of exposure to mutagen, can lead to methyltransferase losing its effectivity at removing alkyl groups.

•هنا الانزيم وصل حالة الاشباع•

The effectiveness of methyltransferase differs from person to another, and depends on their immune system, this explains why some people get cancer faster than others.

Homology dependent repair: post replication

This is one reason why DNA is double stranded(لو كان single كان معرفش انو فيه خطا بالتسلسل اصلا ع اي اساس بدو يعرف في خطا ولا لا؟. بس لما يكون double بعرف مثلا انو Tلازم ترتبط مع Aوهكذا فبعرف انو هون في خطا)

1. Mismatch repair: Mistake in nucleotides

- **Mismatch repair** هو انو nucleotide تربط ب nucleotide الغلط بالstrand الثانيه وفي فترة محددة لتصليح هاي الاخطاء بعد الانقسام (يعني بعد الانقسام الخليه بتعطيك عشر دقايق تشوف وضع nucleotides اذا فيها Mismatch صلحوا والا ما بتقدر تصلحوا بعدين وبتتحول لطفرة)

يعتبر غالي جداً على الخلية لانها بتستخدم فيه كثير انزيمات.

*** Mismatch repair can be divided into 4 phases:**

1- Recognition of a mismatch by MutS proteins (does scanning for mispairing) when it is placed on a mutation then (MutL) comes for breaking false pairs and around it. How does it differentiate the origin from the mutation? In bacteria there is always methyl on the origin strand but it delays the methylation process for the new strand to allow the repair system to do its work and then the methylation process occurs.

*(طبعا mutS لما يتعرف عالخطا ويروح يقعد هناك بغير شكله ,,تغيير شكله بحفز mutLيجي ويكسر)

**(احنا عنا DNA بتكون من سلسلة قديمة وسلسة جديدة . القديمة او الاصلية لانها جاهز بتكون زي مختومة ب ميثيل يعني صايرلها methylation بس الجديد لسا مش جاهزة ولا فاحصينها فبكون بعدها مش مختومة ب ميثيل ,,من هون الانزيم بميز انو الي عليها ميثيل هي القديمة ف بقربش عليها والي معليهاش ميثيل هي الجديدة الي عملية التصليح بدها تتم عليها)

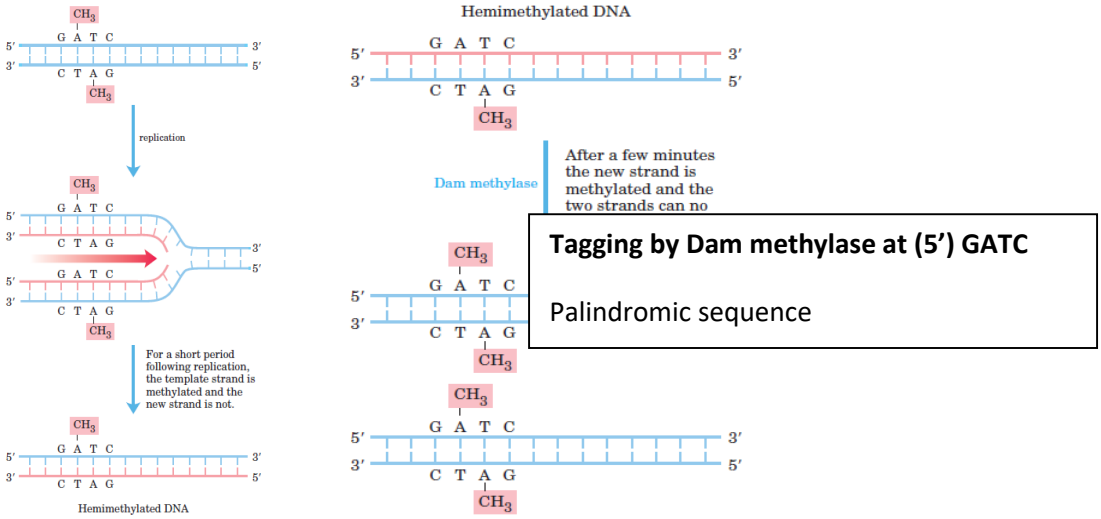
2- Recruitment of repair enzymes

3- Excision of the incorrect sequence (

احنا حكينا انو Mut L هو الي بكسر بس في كمان بروتين اسمه Mut Hهاد بحدد ل Mut Lحدود لوين يكسر فبعمل زي nick يعني لهون يا Mut Lكسر وبس(وهاد البروتين مش موجود عنا بس بالبكتيريا)

4- Resynthesis by DNA polymerase using the parental strand as a template.

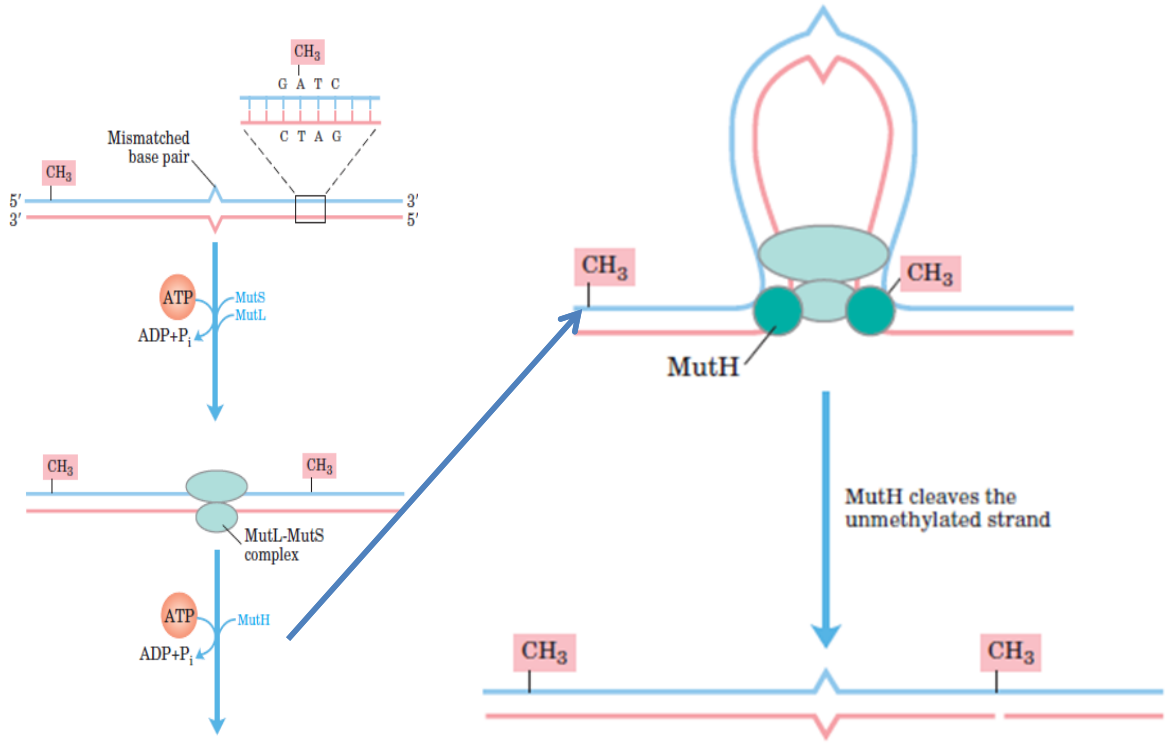
- Correction of the rare mismatches left after replication in *E. coli* improves the overall fidelity of replication by an additional factor of 10^2 to 10^3.
- Mismatch repair system must somehow discriminate between the template and the newly synthesized strand.
- Strand discrimination is based on the action of Dam methylase, which methylates DNA at the *N*6 position of all adenines within (5')GATC sequences. (هاي الي حكينا عنها فوق الي بتختم ميثيل بعد ما يجهز)
- If both strands are methylated at a GATC sequence, few mismatches are repaired; if neither strand is methylated, repair occurs but does not favor either strand.
- After DNA replication how can we differentiate between template and newly synthesized DNA?



A model of methyl-directed mismatch repair

Recognition of the sequence (5') GATC and of the mismatch by the **MutH** and **MutS** proteins, respectively.

- The **MutL** protein forms a complex with MutS at the mismatch.
- DNA is threaded through this complex such that the complex moves simultaneously in both directions along the DNA until it encounters a MutH protein bound at a hemi methylated GATC sequence.
- MutH cleaves the unmethylated strand on the 5 side of the G in this sequence.



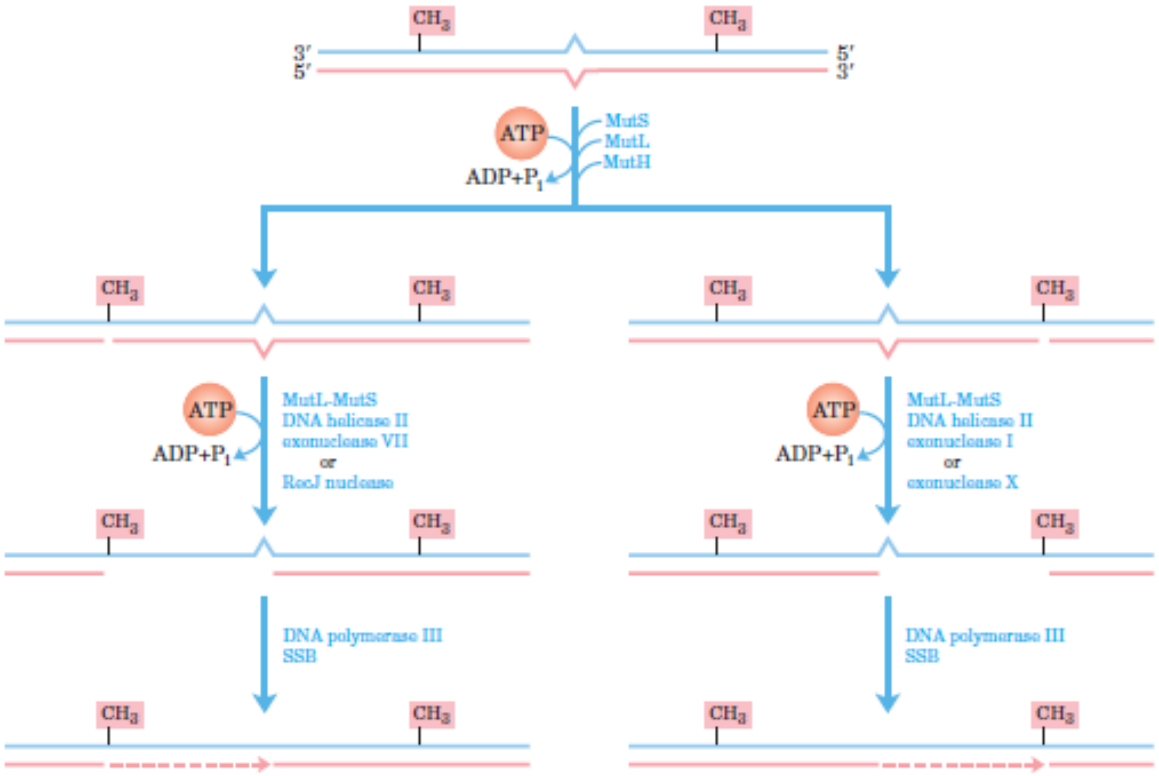
MutH: does a nick on non-methylated (new) strands, this means that the empty space is the signal for MutL. To stop breaking. Then DNA polymerase 1 –copies to fill in the blanks and ligase connects them.

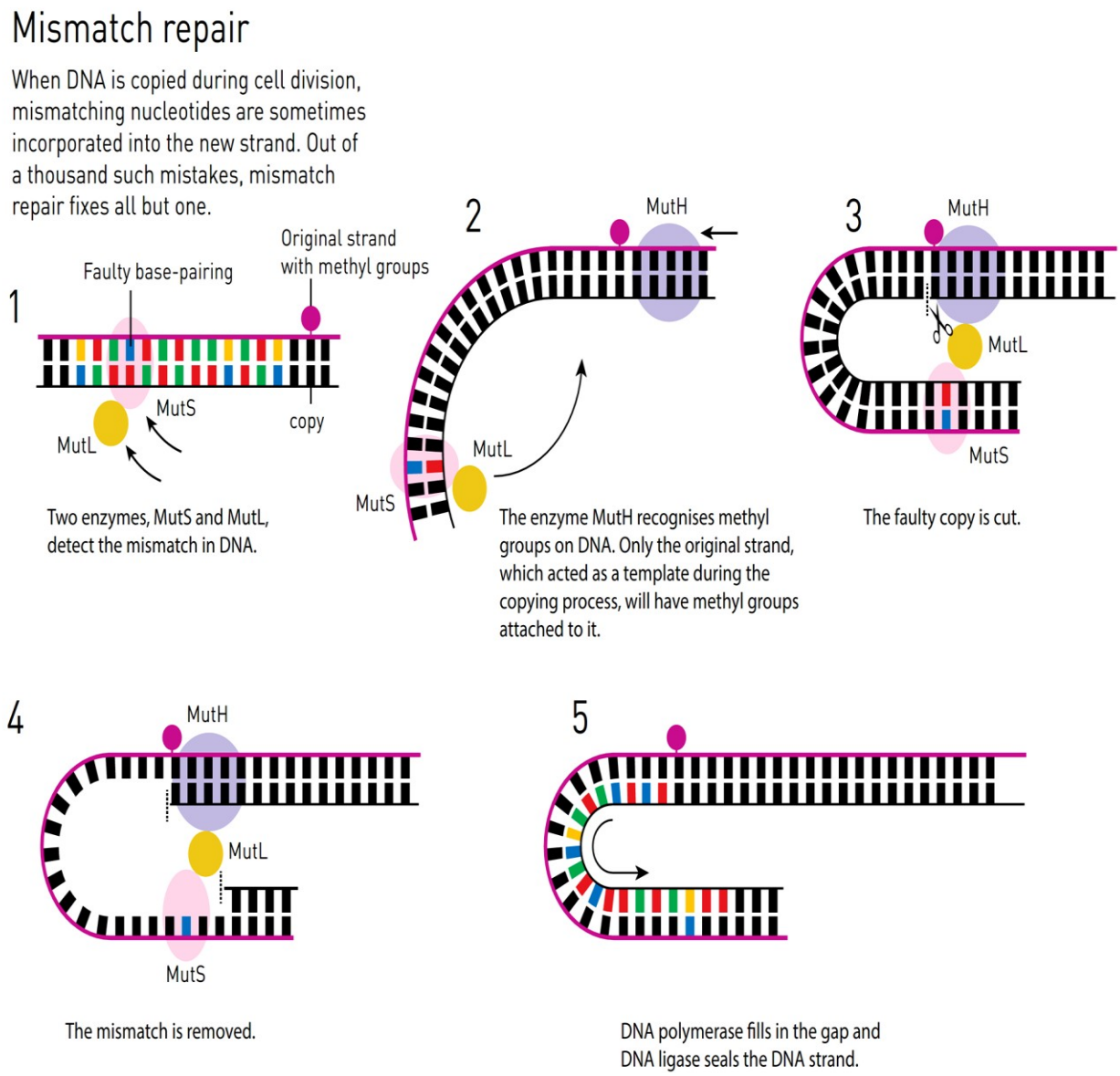
In our body there is no MutH and methylation, there are nicks in new DNA and these nicks determine for where MutL must stop breaking. Colon cancer is a popular example on a defect of mismatch repair.

يعني بجسمنا لا في ختم بميزلنا انو هاي قديمة او جديدة ولا في عنا بروتين mut h بعملنا nick انو هون اخلص تكسير !! شو الحل؟؟
العلماء بحكوا انو عنا بكنن nicks معمولات جاهزات بالسلسلة الجدة فما منحتاج نميز مين جديدة ولا بروتينmut h

Completing methyl-directed mismatch repair

- **A complex consisting of DNA helicase II and one of several exonucleases degrades the unmethylated DNA strand from that point toward the mismatch**


CH3
CH3
3′
5′
5′
3′
ATP
ADP+P1
MutS
MutL
MutH
MutL-MutS
DNA helicase II
exonuclease VII
or
RecJ nuclease
MutL-MutS
DNA helicase II
exonuclease I
or
exonuclease X
DNA polymerase III
SSB



A second protein complex cuts the DNA near the mismatch, and more enzymes chop out the incorrect nucleotide and a surrounding patch of DNA.

Mismatch repair in eukaryotes:

- Similar to MutS and MutL (but not MutH) proteins.
- MutS homologs for eukaryotes from yeast to humans.
- MSH2 (Muts homolog 2) MSH3, MSH6.
- Alterations in human genes encoding proteins of this type produce some of the most common inherited cancer-susceptibility syndromes

- We do not know the mechanism by which newly synthesized DNA strands are identified, although research has revealed that this strand identification does not involve GATC sequences
- Mutated in CANCER = increase mutation rate

Base- Excision Repair

1. Recognize and repair damage caused by environmental agents.

2. DNA glycosylase recognizes specific damaged base. Example; uracil-DNA glycosidase.

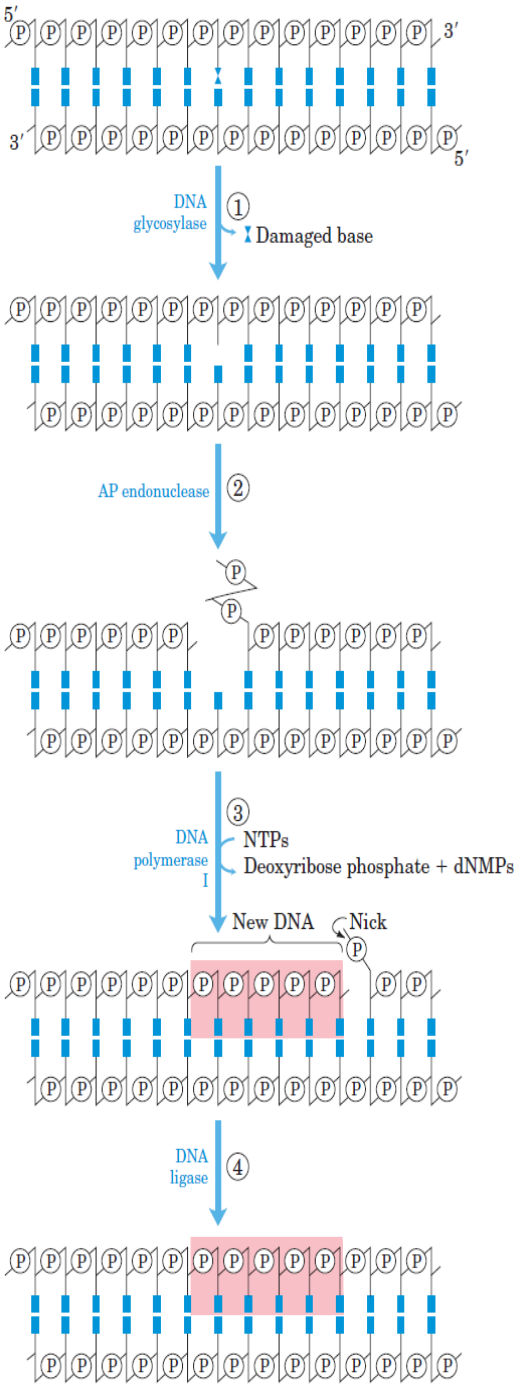
3. Cleaves glycosyl bond to remove base. cleaves base-sugar bonds creating apurinic or apyrimidinic sites.

4. AP endonuclease cleaves backbone.

5. DNA Pol removes abasic site.

6. Replacement of base.

7. DNA ligase seals the gap.

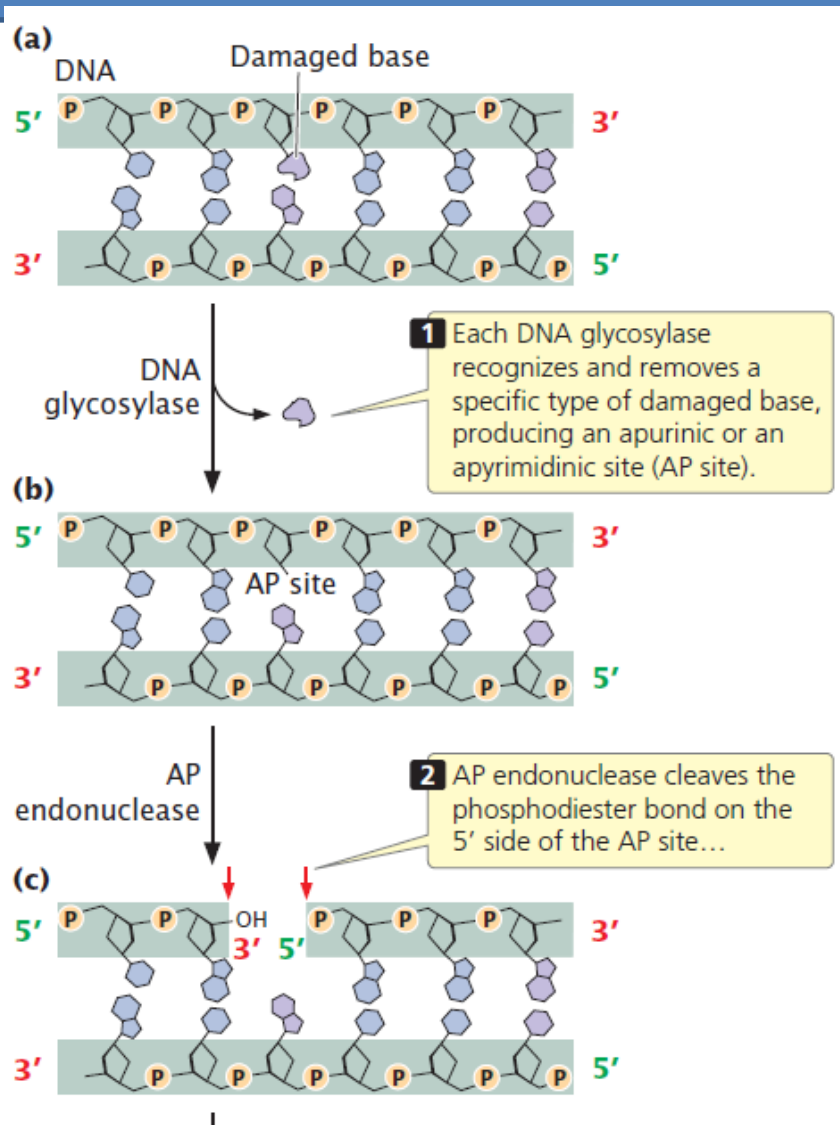


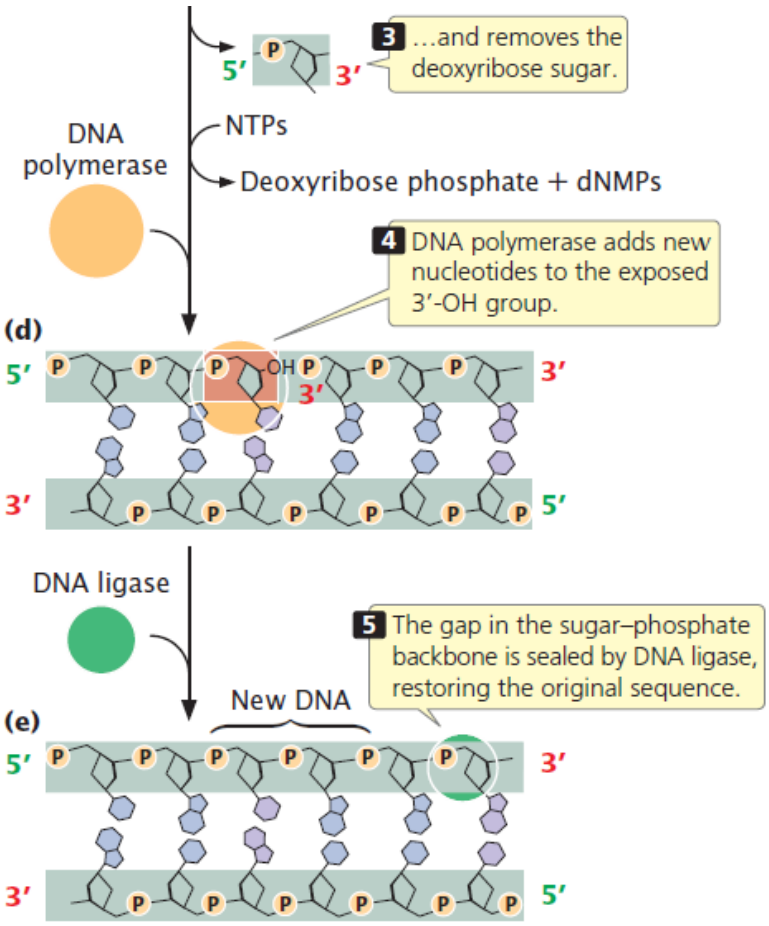
Base-Excision Repair

بالعربي : هاي الالية اسمها هيك لانه ببلش يشتغل من base .. مثلا احنا حكينا انو مش لازم يكون في عنا uفي dna واذا وجد هاد معناها صاير عندي deamination يعني طفرة ولازم اصلحها فبيجي عندي انزايم مختص ب u اسمه (uracil-DNA glycosidase)بيجي بفك القاعد النيتروجينية U عن السكر المربوط فيها(يعني زي كأنه قطع راس النيوكليتايد) ف ضل عندي سكر بس ناقص base يعني مقطوع راسه (abasic sugar, بيجي عندي انزيم اختصاصه يتعرف على abasic sugar. اسمه AP endonuclease)e)وبكسر السكر هاد مع شوية منطقة حواليه وبعدها بيجي بنسخلي نيوكليتايد جديد وبعدها ال ligaseبربط Pol 1

من هون بقدر اجاوب عالسؤال الثاني ليش ما بصير يكون عنا يوراسيل ب dna؟

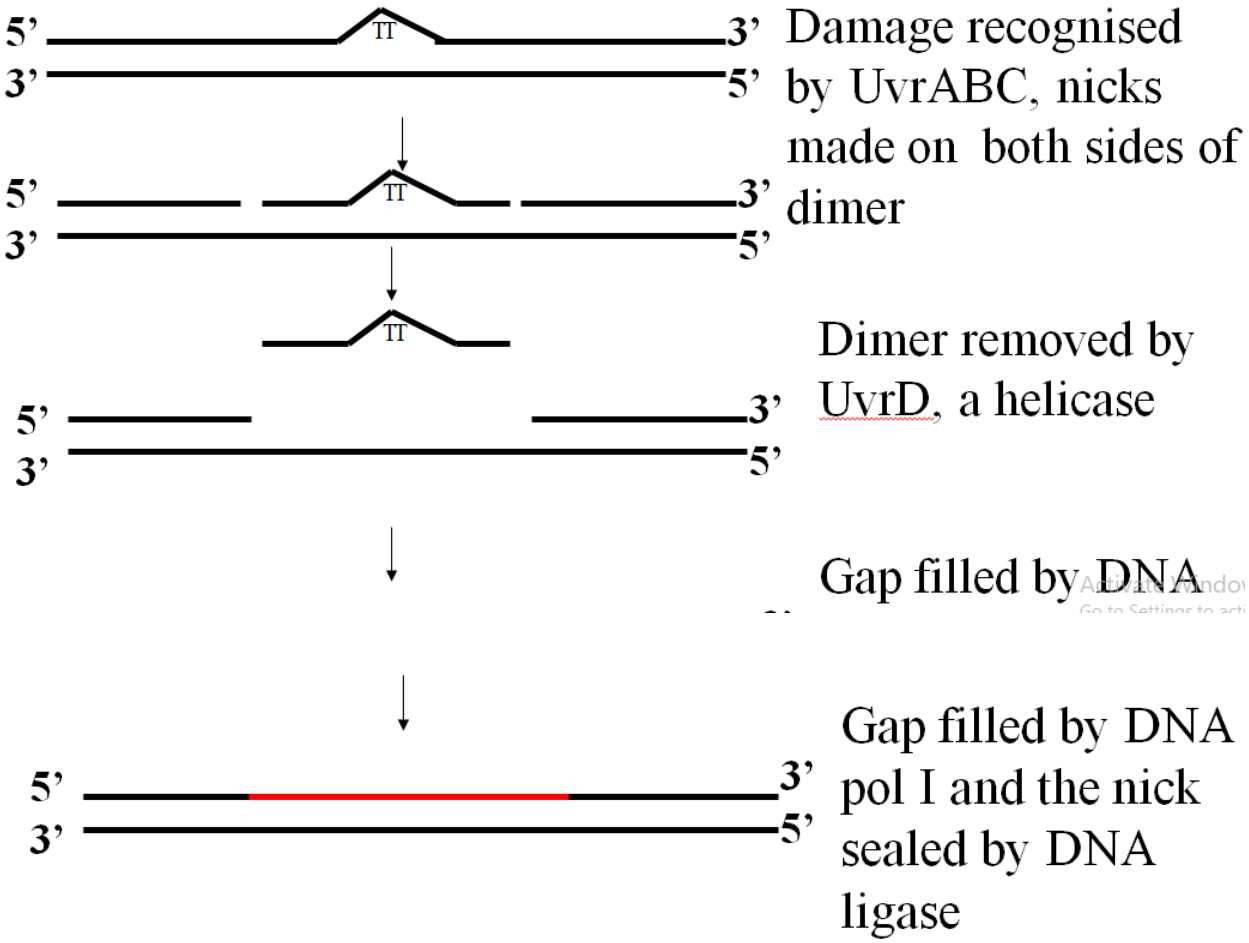
لانه بالعادة اذا وجد يوراسيل عنا ب dna تعتبر طفرة ناتجة عن deamination ف بروح يكسرها ويصلحها ..طب لوكان عنا بالاصل يوراسيل كيف بدو يميز انو هاي اليوراسيل هي طبيعية زيها زي الثايمين ولا ناتجة عن طفرة ال deamination؟؟؟



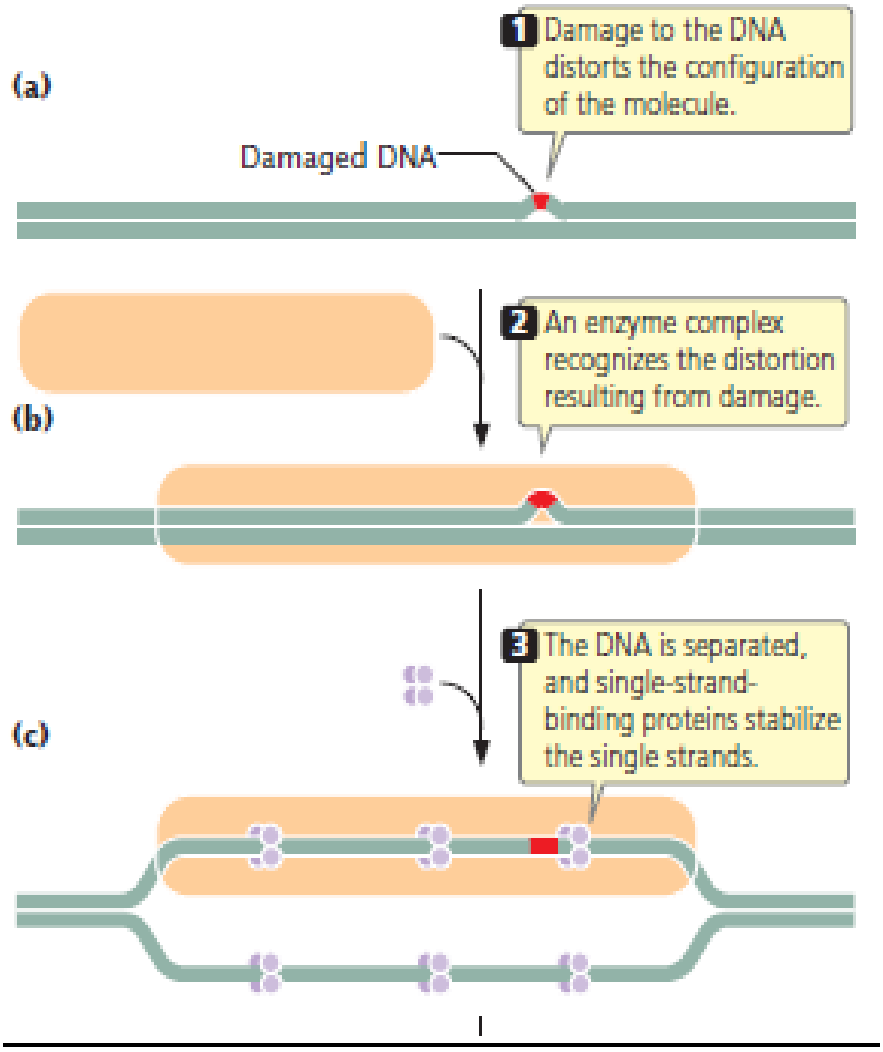


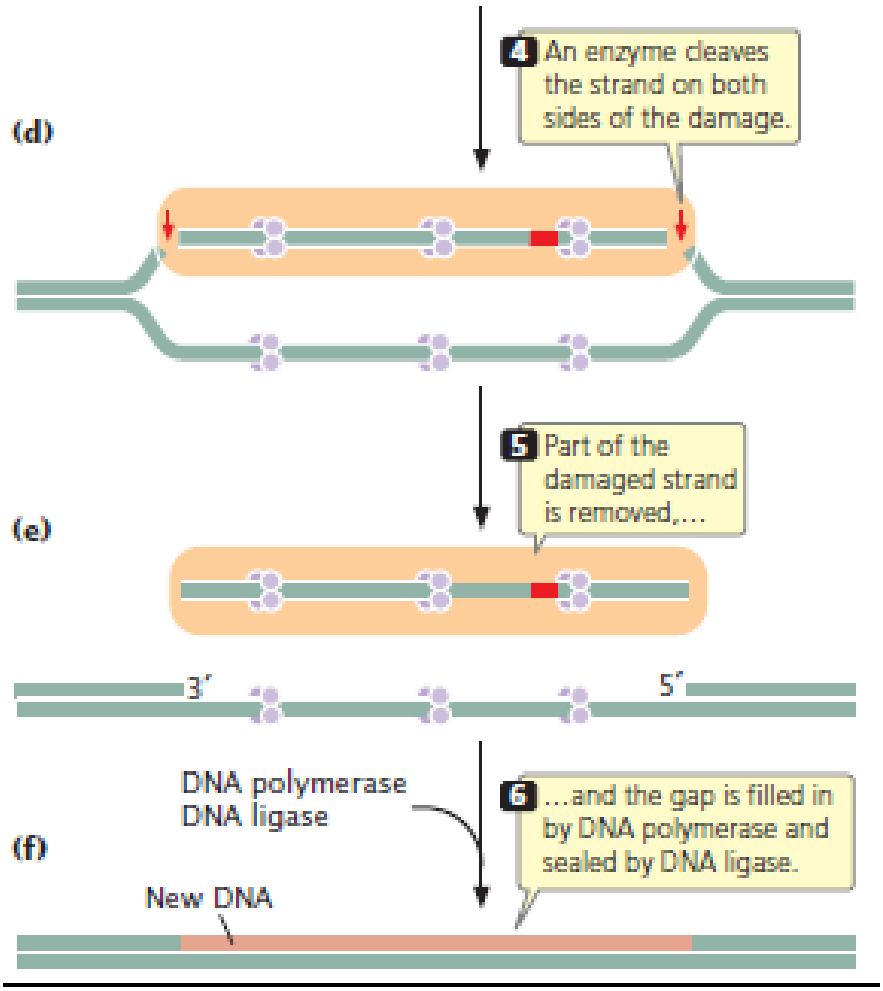
18.27 Base-excision repair excises modified bases and then replaces one or more nucleotides.

Nucleotide-excision repair in E.coli



Nucleotide-excision repair


(a)
1 Damage to the DNA distorts the configuration of the molecule.
Damaged DNA
(b)
2 An enzyme complex recognizes the distortion resulting from damage.
(c)
3 The DNA is separated, and single-strand-binding proteins stabilize the single strands.



Nucleotide-Excision Repair in E. coli and Humans

Nucleotide-Excision Repair:

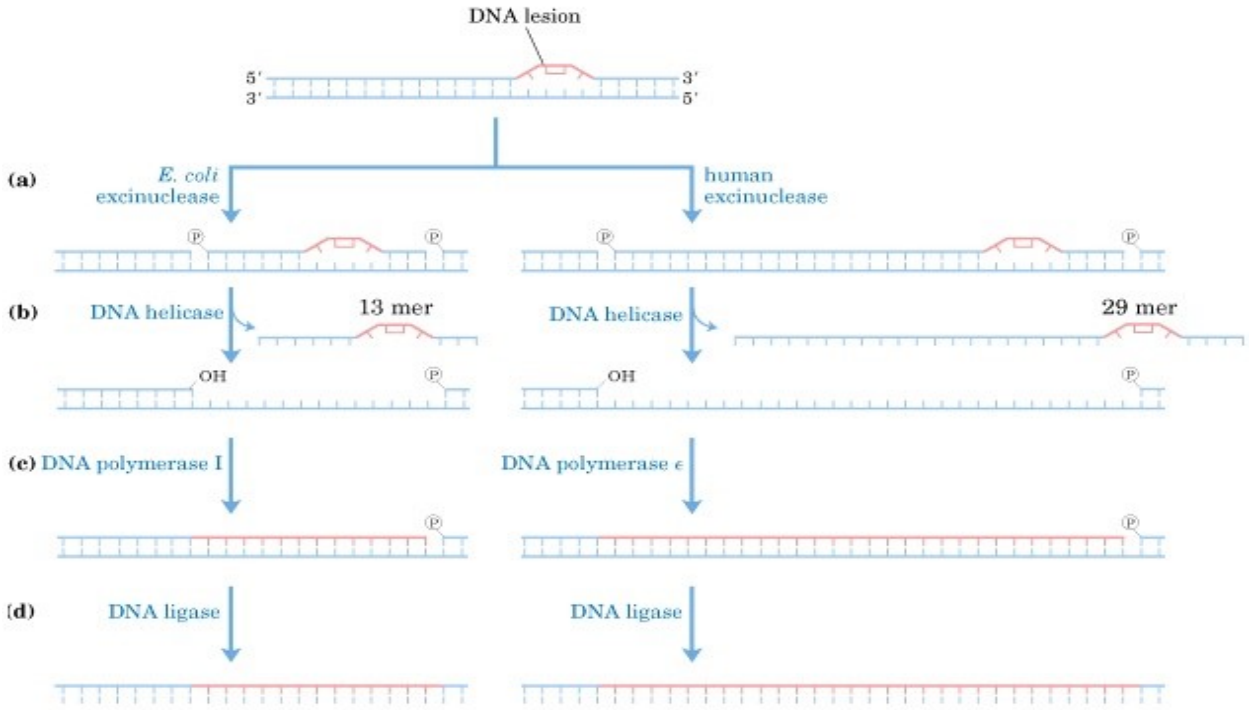
This pathway is the primary route for many lesion types:

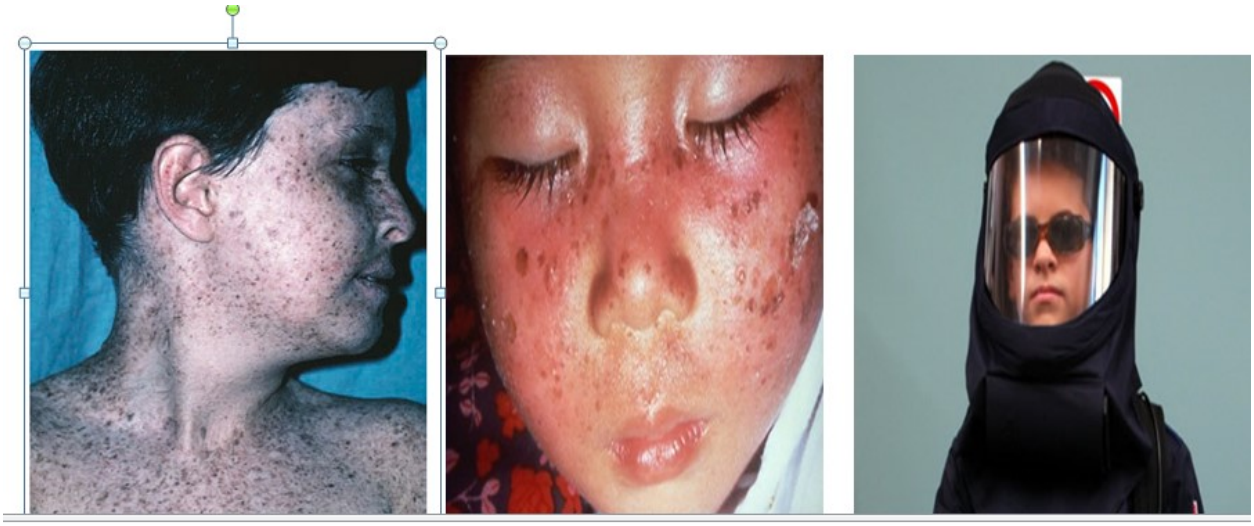
- pyrimidine dimers (T dimer).

- base adducts: benzo pyrene-guanine (formed in DNA by cigarette smoke).

Xeroderma pigmentosum (XP): rare inherited disease (pigmented lesions on skin + skin cancer) due to mutations in Nucleotide Excision Repair system (the sole repair pathway for pyrimidine dimers in humans).

Xeroderma pigmentosum results from defects in DNA repair

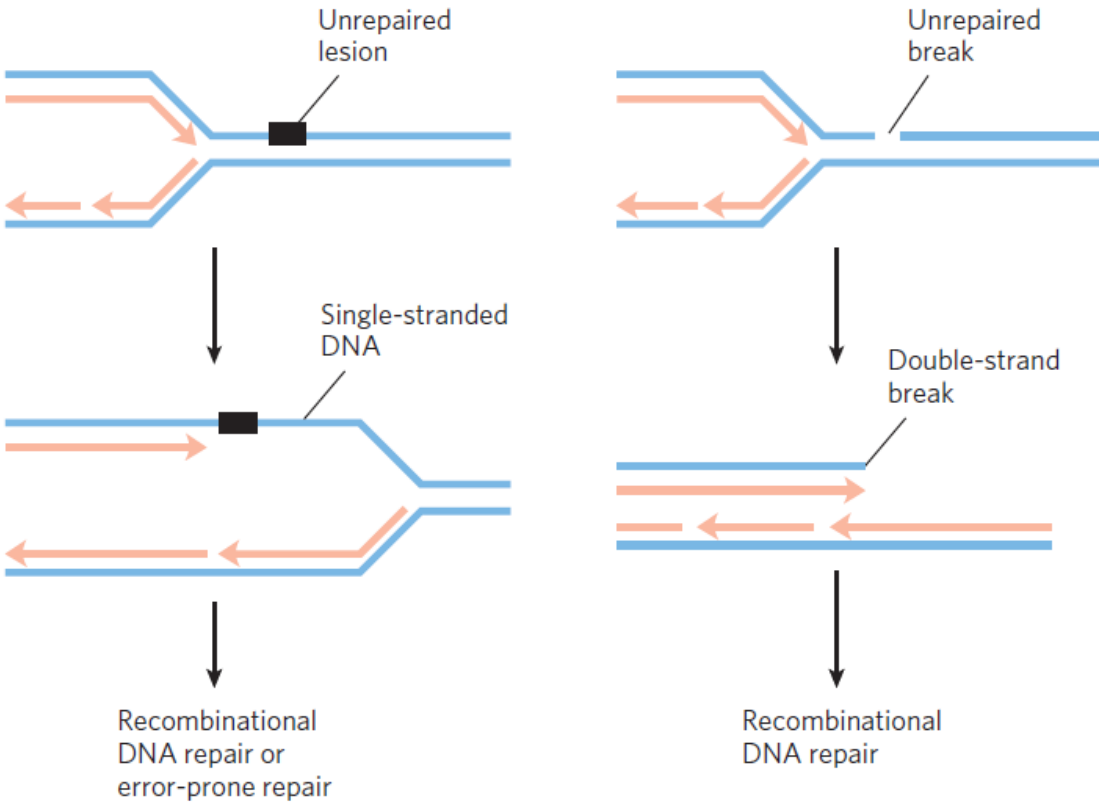




*** The disease is characterized by freckle-like spots on the skin (shown here) and a predisposition to skin cancer**

Models for recombinational DNA repair of stalled replication fork:

- In the absence of a second strand, the information required for accurate repair must come from a separate, homologous chromosome.
- The repair system thus involves homologous genetic recombination.
- Under some conditions, a second repair pathway, error-prone translesion DNA synthesis becomes available.
- In bacteria, error-prone translesion DNA synthesis is part of a cellular stress response to extensive DNA damage known, appropriately enough, as the SOS response.



SOS response

table 25–6

Genes Induced as Part of the SOS Response in *E. coli*

Gene name	Protein encoded and/or role in DNA repair
Genes of known function	
*pol*B (*din*A)	Encodes polymerization subunit of DNA polymerase II, required for replication restart in recombinational DNA repair
*uvr*A, *uvr*B	Encode ABC excinuclease subunits UvrA and UvrB
*umu*C, *umu*D	Encode DNA polymerase V
*sul*A	Encodes protein that inhibits cell division, possibly to allow time for DNA repair
*rec*A	Encodes RecA protein required for error-prone repair and recombinational repair
*din*B	Encodes DNA polymerase IV
Genes involved in DNA metabolism, but role in DNA repair unknown	
ssb	Encodes single-stranded DNA-binding protein (SSB)
*uvr*D	Encodes DNA helicase II (DNA-unwinding protein)
*him*A	Encodes subunit of integration host factor, involved in site-specific recombination, replication, transposition, regulation of gene expression
*rec*N	Required for recombinational repair
Genes of unknown function	
*din*D	
*din*F	

Note: Some of these genes and their functions are further discussed in Chapter 28.

In bacteria, error-prone translesion DNA synthesis is part of a cellular stress response to extensive DNA damage known, appropriately enough, as the SOS response.

- A large number of mutagens damage one or more bases. No specific base pairing is possible. This will lead to replication block.
- Unfortunately, such replication block in both eukaryotes and prokaryotes can be bypassed by inserting non-specific bases.
- Some repair mechanisms are themselves responsible for mutating DNA! Lets look at the SOS system in E. coli.

SOS system In E. coli:

- Induced as emergency response to prevent cell death in presence of significant damage.
- These genes induced by SOS system when bacteria is exposed to UV.

- Recently these genes were discovered to encode 2 error-prone DNA polymerases. “When Making a Mistake is the Only Way to Get Ahead”
- DinB encodes DNA pol IV. UmuC, UmuD’ encodes subunits of DNA pol.V. These polymerases overcome the block in replication by adding nucleotides to the strand opposite the damaged DNA.
- Error-prone polymerases were also discovered in diverse taxa in eukaryotes including human.

RecA protein induced by UV plays critical step in SOS system.

- When DNA pol III, stalled at site of damage, DNA ahead of polymerase continue to unwound, creating SSDNA bound to SS DNA binding protein. Next, RecA proteins (Rad51 in eukaryote) join the SSB and form a DNA-protein filament.
- In this situation RecA acts as a signal that lead to the induction of the error-prone polymerase and attracts it to the stalled fork.
- This system includes carcinogens (UV and aflatoxin B1).

Molecular - Soher

We have 3 types of recombination:

1. Homologous recombination: repairs DNA before the cell enters mitosis (M-phase). It occurs during and shortly after DNA replication.

Recombination: hybrid, recombinant

We use this recombination method when we want to exchange genetic material or when there's a breakage and it needs to be fixed.

How does it happen?

When the chromosomes align too close to each other; they stick together at a point which is named Chiasma, afterwards an enzyme cuts the cross over point causing a breakage and then they are rejoined again >>" recombination".

The mechanism of recombination:

1- Homologous chromosomes are aligned.

**Homologous chromosomes: are non identical but similar, different sequences, same order of genes, same number of genes.

For the exchange to happen there must be one intact chromosome and one non intact (with breakage) chromosome.

Intact chromosome: fixed chromosome with no breakage (cuts)

2- An enzyme called exonuclease recognizes the breakage\nick in the non intact chromosome, attaches itself and starts chewing the double strands which increases the gap\nick, it chews the 5' prime end more than the 3' which leads to an overhang on this side (3' prime end).

3- The overhang (exposed region) invades the intact DNA of the homologous chromosome and this is followed by the branch migration (searches for a complementary sequence, stops after finding no identical sequence). Afterwards the DNA polymerase fills the gap to create "Holliday Intermediate" which is a molecule of DNA with 4 strands and two cross overs.

4- The resolvase enzyme (resolves\cuts the Holliday intermediate) in two ways: horizontal – horizontal or vertical – horizontal creating recombinant structure\products.

* The flanking region is the area which isn't recombined.

* More genetically Diverse is the H-V.

(this is only a model or a theory, don't take it too seriously).

Branch Migration: a DNA strand is partially paired with its complement in a duplex to extend its pairing by displacing the resident strand with which it is homologous.

**The exonuclease that we mentioned above is named RecBCD. It has the ability of being both a helicase and a nuclease at the same time making it a specialized enzyme. It chews\degrades until reaching a chi sequence which is found on the 3', at which it stops chewing the 3' and continues chewing on 5' (when it stops chewing on 3' the enzyme starts counting a specific amount of nucleotides in the 5' and after that it dissociates).

The overhang is motivated by a protein called RecA to make the invasion of the intact chromosome. This protein is a dimer and it makes a filament over the strand by rolling over. It helps with the spooling and rotation of DNA leading to a displacement (branch point = first point when finding the complementary sequence)

Without RecA no exchange occurs.

2. Site Specific Recombination:

Unlike the homologous recombination, this type doesn't happen to fix any breaks\nicks randomly but only when recognizing a specific site formed by an enzyme called (Recombinase) is it able to fix. The recombinase is a tetamer (4 subunits which attach to four combination sites). It has endonuclease activity along with ligase. We have many types of this enzyme; different names depending on the active site but they do the same function.

The chromosomes do not need to be homologous

The mechanism happens in a few steps:

1- Enzyme should recognize the site.

2- Attaches itself on the recombination site.

3- Cutting one strand, immediately after that it holds into the phosphate group (to prevent the strands to connect again)

4- Ligase activity: connects the phosphate group of one strand with the OH group of the opposing strand creating Holliday intermediate.

5- Repeat of this process to the intermediate until we get the final product.

3. Transpositional Recombination:

Allows the movement of transposable elements (transposons) from one place on a chromosome (the donor site) to another on the same or a different chromosome (the target site).

Transposons as a structure are made of: 1- Gene. 2- Inverted Repeats (are on the sides of the genes and are complementary sequences to each other) 3- Direct Repeats (it comes from the target)

**look up the new slides

We have types and classes for this recombination:

The Types:

1. Simple Transposons: It only has the transposon gene which will be transformed into the transposase enzyme.

2. Complex Transposons: It has both a transposon gene plus an extra gene which gives the cell new features.

The Classes:

1. Retro Transposon: firstly, a transcription happens (copying) which transcripts DNA into RNA then a reverse transcription occurs by an enzyme which transforms the RNA to DNA, finally recombination, this product invades the DNA.

2. DNA transposons: a cut is made by a transposase enzyme and then recombination occurs.

**The cut that was made in the donor strand will either be repaired or degrade.

**Normally the sequence that will be recombined will be longer.