Genetics & molecular biology

◉ Sheet ○ Slide

Number:		6
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To begin replication, the two strands of DNA must be methylated, otherwise it can't repair mistakes.

-replication fork يحدد اتجاه ال replication

Proteins at the *E. coli* Replication Fork

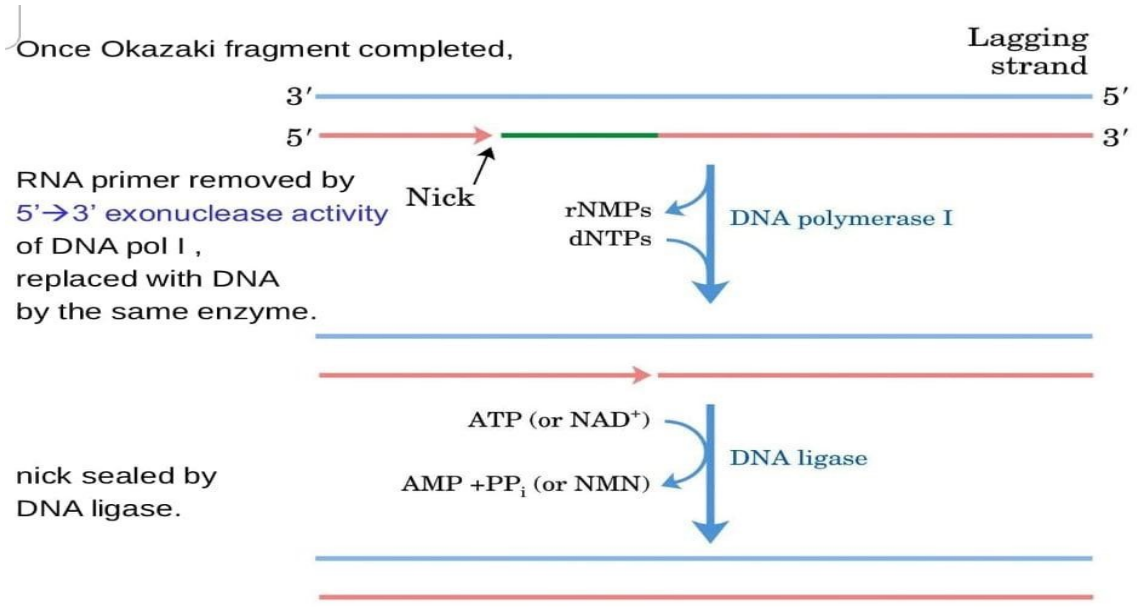
Protein	M_r	Number of subunits	Function
SSB	75,600	4	Binding to single-stranded DNA
DnaB protein (helicase)	300,000	6	DNA unwinding; primosome constituent
Primase (DnaG protein)	60,000	1	RNA primer synthesis; primosome constituent
DNA polymerase III	900,000	18–20	New strand elongation
DNA polymerase I	103,000	1	Filling of gaps, excision of primers
DNA ligase	74,000	1	Ligation
DNA gyrase (DNA topoisomerase II)	400,000	4	Supercoiling

Ligation: sealing of nicks.

DNA gyrase: protects against supercoiling

DNA polymerase 1:
1- From 3 to 5 (proofreading)
2- From 5 to 3 (removal of primer)- removes the primer and fills in the gaps at the same time

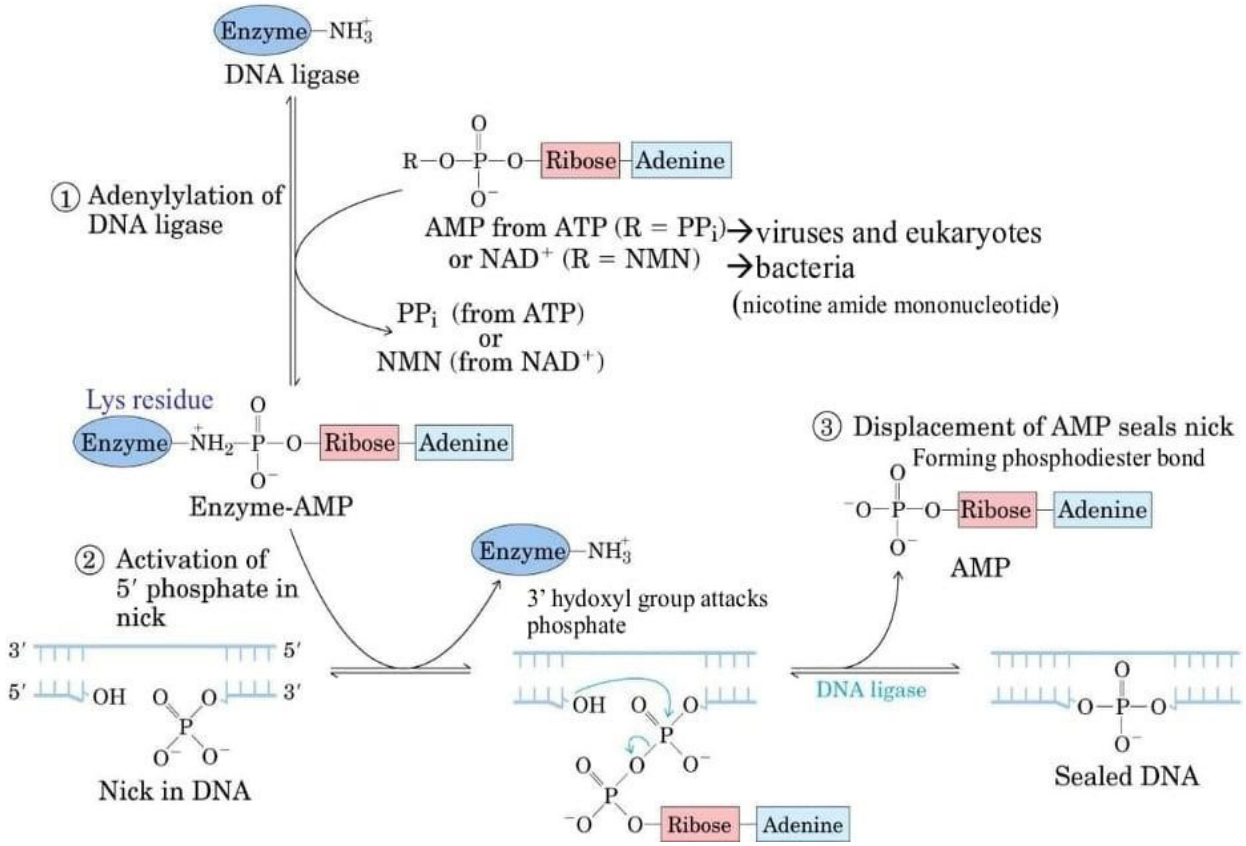
The difference between gaps and nicks:
Gap: missing nucleotides
Nick: broken phosphodiester bond but has all of the nucleotides.



The green part is a primer (5 to 3) (from the left).

Removal of primer: exclude rNMPs (ribose nu monophosphate), add new nu of DNA (dNTPs: deoxyribose nu triphosphate and then 2 pi will go out)
ATP or NAD+ (according to the organism) will activate ligase (in humans it is ATP).

DNA ligase catalyzes the formation of a phosphodiester bond bw a 3' hydroxyl at one end + 5'phosphate at the other.



Nick translation or (primer removal)
Ligase is an enzyme that has an amino group on lysine residue (this is the active site of ligase: lys + amino group)
Amino group of the enzyme connects with AMP (either with ATP or NAD+ depending on the organism) which will activate 5' phosphate to attach to 3' hydroxyl by nucleophilic attachment and then AMP will go out.

Termination:

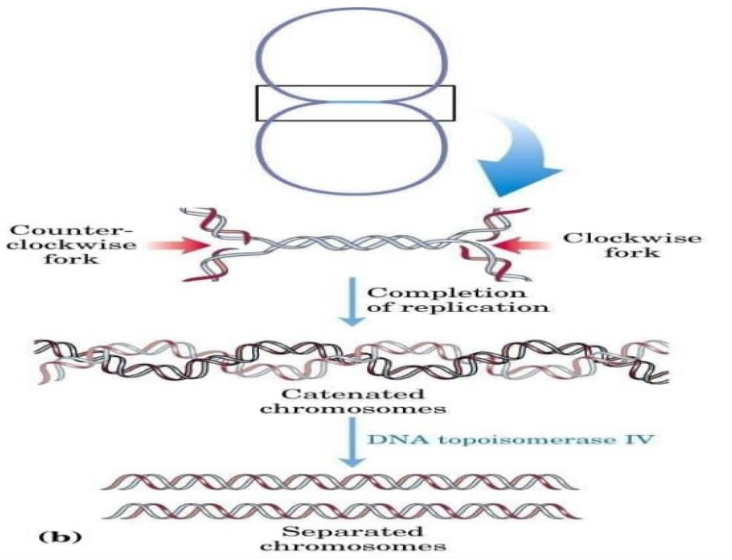
In eukaryotic cells: linear chromosomes that have ends (اطراف)
In prokaryotic cells: circular chromosomes which don't have ends; so there are terminus (almost opposite to the origin sequence) that are organized like a trap to prevent over replication (terminus bind to tus protein). And the replication goes bidirectional (clockwise fork + counter clockwise fork).
In eukaryotic cells there are no terminus.

(helicaseللblock بتعمل Tus-Ter complex بصير عنا منطقة اسمها ter يربط ع منطقة Tus لما بروتين)

Bidirectional replication of chromosome leaves the completed chromosome joined as:

catenanes = intertwined (but not covalent interaction)

topoisomerase II is used for the separation of catenanes.



Replication in eukaryotes is complex:

Variations: Essential features are the same, and the proteins' function + structure is conserved.

(Differences in the number and names of proteins)

DNA in eukaryotes >> bacteria + organized into nucleoprotein structures (chromatin).

1. Replication fork rate in eukaryotes is 50 nucleotides/s = 1/20 of E. coli. Solution = multiple origins, spaced 30,000-300,000 bp apart. (vary according to organisms) + bidirectional

2. Origins called Autonomously Replicating Sequences (ARS) / replicators. In yeast around 400 replicators / 16 chromosomes. Each around 150bp+conserved seq.

3. Initiation starts binding of a protein (recognition) (Origin Recognition complex=ORC) binds ARS. This is regulated by several proteins involved in cell cycle regulation. (similar to DnaA)

4. CDC6 (Cell Division Cycle), CDT1 (CDC10-Dependent Transcript 1) both bind ORC mediate loading of MCM2 to MCM7 (MiniChromosome Maintenance proteins) = heterohexamers forming ring shaped helicase analogous to bacterial DnaB helicase.

Role of CDC6 and CDT1 is similar to bacterial DnaC; loading MCM helicases onto replication origin.

*Replisome: the region between 2 different origins in eukaryotic DNA (its length 200,000-300,000 bp)

_في عنا مشكلة باليوكاريوت انو الخلية نفسها حجم الكروموسومات بتختلف بين بعضها، مثلا زوج رقم 1 هو اكبر زوج كروموسومات وزوج 32 هو اصغرهم، ف كيف رح تتم عملية الريبليكيشن الهم بنفس الوقت بالرغم من اختلاف الحجم؟؟؟

_الحل : في عنا اولوية مين يبلش ,,مش كلهم ببلشو مع بعض ,مثلا الكروموسومات الصغيرة بتبلش الريبليكيشن بعد بفترة من الكبيرة بالمحصلة كل الكروموسومات بخلصوا بنفس الوقت.

Several polymerases

DNA polymerase ما عنده خاصية النسخ العكسي بس - RNA polymerase

DNA polymerase α: multi-subunit enzyme, similar in all eukaryotes. - primase activity, (similar to primase) polymerization. - No proofreading 3'-5' exonuclease activity. (unsuitable for high fidelity DNA replication) - function: primer synthesis (RNA/DNA) for okazaki on lagging.

DNA polymerase δ: -function: extends the primers + 3'-5' exonuclease activity. - stimulated by PCNA (abundant in nuclei of proliferating cells). -PCNA similar to β-subunit in E. coli forming a circular clamp increasing processivity (indicates the speed of division).
-Pol δ carries out leading and lagging strand synthesis similar to bacterial DNA pol III.
-RFC: (replication factor C) = clamp loader of PCNA (similar to bacterial clamp loading γ complex).

DNA polymerase ε: -function: DNA repair + removing primers at replication fork

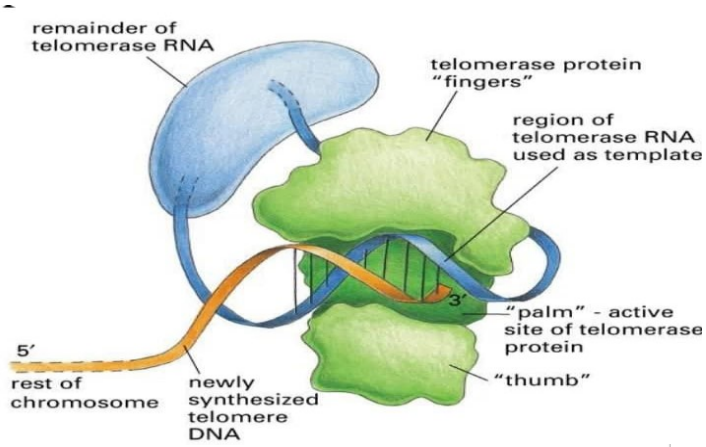
DNA gyrase (proteins that get rid of stress) it's similar to topoisomerase 1&2. We use antibiotics to inhibit DNA gyrase of bacteria ex: penodixin acid trough stopping proliferation in the cancer cell

Additional differences: - RPA: (replication protein A) = eukaryotic ssDNA binding protein (similar to SSB). Termination involves synthesis of telomeres. (Telomeres shorten with each S phase).

The chromosomes remain divided and the telomeres shorten until they can't continue and one chromosome may connect by ligation with another chromosome, and this is a problem!!
We can know the sequence of telomeres by fluorescent that connects with complementary sequence of telomeres.
T-loop (for protecting the telomeres + bent)
Telomere biology: lead to aging
Telomere = molecular clock: scale for cell longevity

مثلا وضعنا خلية جديدة في وعاء مخبري مع وسط ملائم تبدأ بالانقسام بوتيرة عالية ويجب تغير الوسط بالبداية كل فترة قصيرة لأنها تخرج فضلات بسرعة وتنقسم بسرعة فيتم نقل عدد منها لوسط اخر ولكن مع مرور الوقت تقل سرعة الانقسام والعمليات الحيوية حتى تدخل الخلايا في مرحلة شيخوخة (senescence) ومن ثم تبدأ بالموت (apoptosis) وبعد فترة تبقى فقط الاوساخ.

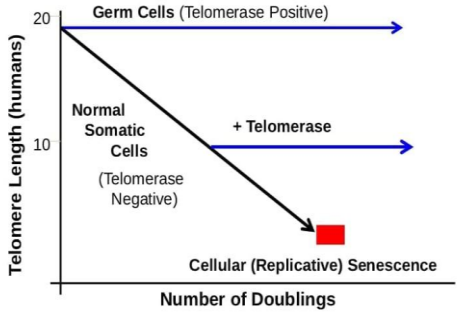
For experiments we use cancer cells or cell lines to elongate the age of cell by:
* Enzyme telomerase that elongates telomeres (build telomerase from RNA template) and this is reverse transcription (telomerase builds DNA from RNA)
Remember: transcription: build RNA from DNA
* Alternative lengthening of telomeres (ALT)
80-90% of cancer cells use telomerase and the other use ALT to elongate the cell's age.



Most normal cells (somatic) don't express telomerase (off position) they lose telomeres with each division

Telomerase are found in stem cells, cancer cells, follicular hair cells, germ cells, epidermal skin cells, and embryonic cells.

Telomere Length and Cell Division Potential



Telomerase- فقط ينسخ من اتجاه واحد

telomeres لازم يكون عليها بروتينات لحتى تحمي الكروموسوم

T-loop- حماية الاطراف الchromosome

TRF1. TRF2 امثلة على بروتينات تربط على التيلومير (Shelterin complex) تساهم في حماية اطراف الكروموسوم

Hay flick limit-: يعني عدد المرات الي بتنقسمها الخلية في حياتها قبل تموت (هي بتموت لانو كل ما انقسمت التيلوميرز عندها بقصر ومافي عنا بالسوماتيك سيلز انزيم تيلوماريز يعوضلنا النقص بالتيلومير ، بالتالي عند كل انقسام منصير نخسلر تيلوميرو منقرب ع الجينات المهمة ، فاذا خلص التيلومير بصير الضرر والنقص يصير من الجينات هاي فبتموت الخلية)

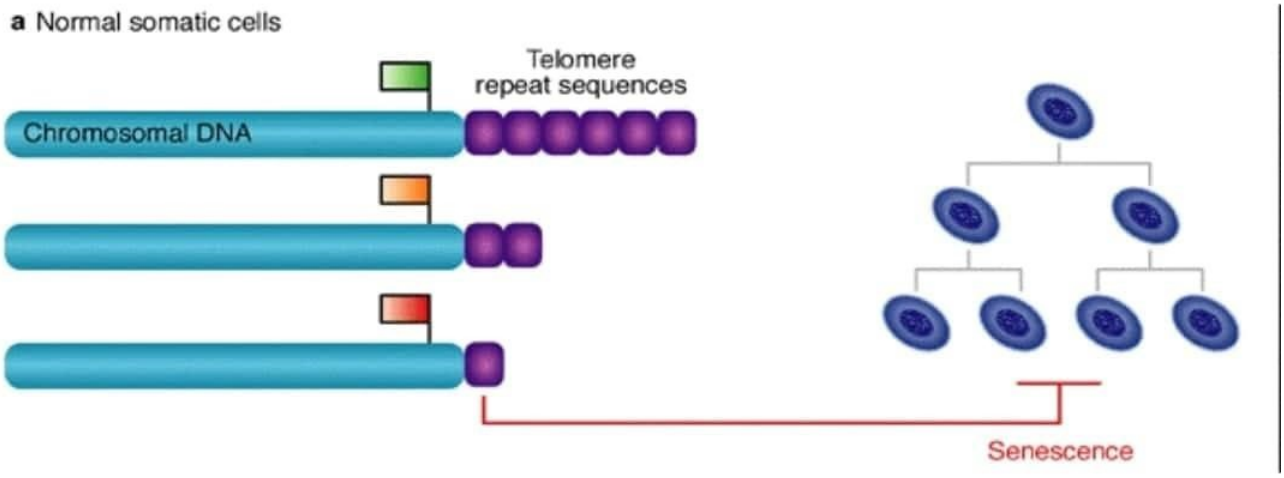
The objective of studying telomerase: Cancer therapy and aging

حاولوا التفكير بال telomerase حتى يحلوا مشاكل مثل مرض السرطان, الجلد المحروق أو بناء من نسيج معين خلايا كثيرة ولكن اكتشفوا ان telomerase مرتبط مع خلايا سرطانية ففي احتمالية الاصابة بخطر السرطان

حاولوا يعملوا ليحاربوا السرطان drugs anti telomerase, reverse transcription inhibition

Two hit model:

التولميريز لحاله ما بكفي عشان يحول الخلايا من طبيعية لسرطانية ,يحتاج على الاقل طفرتين (ضربتين) بمكانين مهمين بالجينوم



Short telomeres may be a signal for cells to senesce (stop dividing)

The normal cell divides until it reaches a stage that is close to the important material and stops dividing but cancer cells continue to divide because telomerase builds new telomeres.

السرطان عدد لا نهائي من الانقسامات

العلاج الكيماوي يؤثر ايضا على proliferation للstem cells (لا يؤثر فقط على ال cancer cell فهاد يعتبر side effect)

telomerase- مش موجود بكل الخلايا مثلا مش موجود ب somatic cells

- Germ cells also have telomeres
- Cell transformation (from normal to cancerous)
- Full transformation of cells (by oncogenes): gene which causes cancer

- لاحظوا العلماء انه الخلايا تدخل في مرحلة الsenescence كعملية دفاعية لدخول oncogenes

- RAS: example of oncogene

DNA replication in eukaryotes & prokaryotes:

- Complexity (eukaryotes need a lot of proteins to fit its complexity) لانه ملفوف ع هستونات
- DNA amount (in prokaryotes there is only one origin of replication)

- Rate of synthesis (depends on polymerase speed). Prokaryotes are faster than eukaryotes (ex: bacteria 2000bases/s, Euk: 500-5000bases/s)

(pro سرعتها اقل من euk اغلب)

- DNA polymerase is located in the nucleus (euk) and in the cytoplasm (pro)

MUTATIONS

Endogenous: (background or spontaneous mutations)

Exogenous (UV, toxins, chemicals) which damages DNA. It's induced (caused by something/organism) and not naturally found.

endo:امثلة على ال

-Depurination : انه بتنفصل القاعدة النيتروجينية عن السكر ، لانه ابيورينز ثقال فيهن حلقتين مش وحدة فممكن تنكسر عن السكر وتسبب طفرة بصير منها الاف كل يوم)

-Deamination: amines lose their amino group مثلا السايتوسين بتخسر الامينو جروب تبعتها ف بتتحول ل يوراسيل فبتصير المتممة الها غلط وبتتشكل طفرة

-DNA polymerase: هو نفسه بعمل اخطاء و99.9% من اخطاؤه هو نفسه بصححهن

- ROS: احنا كل يوم منتنفس ومنعمل طاقة من المايتوكندريا بس مرات كثيرة بطلع اوكسجين ناقص الكترون فبصير كثير آكتف ويتفاعل مع اي اشي قدامه ولهيك بعملي طفرات

*mutation may occur in somatic or gremlin cells

- Point mutation: mutation in one nucleotide only. هي اصغر انواع الطفرات من حيث الحجم وليس التأثير

- Substitution mutation

Transition and Transversion

- Indel mutation (insertion and deletion)

- Substitution mutations could be in coding or non-coding sequences

#If it was in a non-coding sequence => mostly no damage

الا اذا مثلا اجت الطفرة ب اشارة البدء(promoter هيك بصير ضرر كبير)

#if it was in a coding sequence => the damage is different:

- Silent mutation/no effect

Different codes but give the same amino acid

- Missense mutation:

Conservative/non-conservative

- Conservative means that it turns into an amino acid that has the same chemical properties, so it doesn't have a large effect. But in non-conservative it turns into a wholly different amino acid which has dangerous effects (ex; stop codons).

Nonsense mutations: very dangerous. For example: substitution mutation can stop codon.

كمان طفرة الاستبدال ممكن نحكي عنها **transition or transversion**

Transition: بكون استبدلنا **purine** ب **purine** (يعني الاستبدال من نفس العائلة)

Transversion: استبدلنا من عائلة مختلفة **P=>Py** او العكس

** **indel mutation (frame shift mutation)** اخطر اشي لانو كل السلسة ح تتغير

DNA repair mechanism

proofreading activity

by using DNA polymerase 3 segm) لا يوجد replication بالعكس ولكن يوجد تصحيح بالعكس

Exonuclease activity and DNA polymerase 3 should be during cell DNA replication but if it missed the repair during replication then there must be mechanisms to repair mutations after replication.

Repair mechanisms for after replication:

Nucleotide Excision Repair
Base- Excision Repair
Methyl- directed Mismatch Repair
Direct Repair

We talk about a mistake that could be a substitution (mismatch repair) or base damage (base-excision repair) but for nucleotide excision repair it is a bulky region mostly thymine dimer, so in our bodies the sole repair pathway for pyrimidine dimers is by nucleotide excision repair.

There are some people who have mutations in the genes that give the acquired proteins by nucleotide excision repair (Xeroderma pigmentosum -XP). And there are 7 genes responsible for these mechanisms in humans; so there are 7 classifications for these people, and there are differences in their symptoms, some people have a severe disease and some people could not be feeling it based on the mutations that exist and the amount of defects present in these mutations.

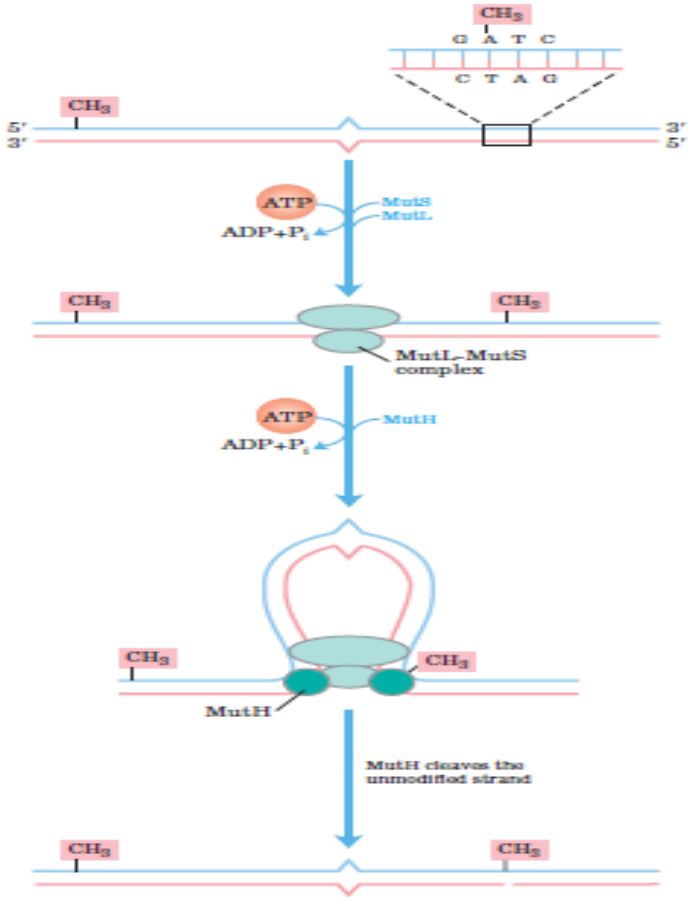
There is another disease (hereditary non-polyposis colorectal cancer) which is associated with defects in Mismatch repair. Symptoms include: rectal bleeding, stomach pain and most importantly a sudden weight loss with no known reason.

In **mismatch repair** Methylation is due to an exposure; changing the DNA but not the nucleotide sequence itself (epigenetic). The first recognition is due to MutL-MutS complex then MutH protein

that binds the complex with methyl causing a loop and cutting the new strand that has the mutation (repair on the template so a neck is formed for repair).

In humans there is mutation in MutL homologue 1 and MutS homologue 2 (MSH2).

E.g. **Guanine always binds to cytosine** but if methylation happened to guanine like (O6-methyl guanine), due to this, binding with cytosine is not possible and it usually binds with thymine instead. (The danger is in replication as the two strands act as a template so the mutated template containing **methyl-guanine** instead of **guanine** will bind with thymine in the first replication and thymine in the other strand will bind with adenine in second replication and so on.) There is normal methylation {methyl group is put tagging on adenine temporarily in regulating sections or non-genetic sections for regulation but the methylation in guanine is permanent and causes mutations}



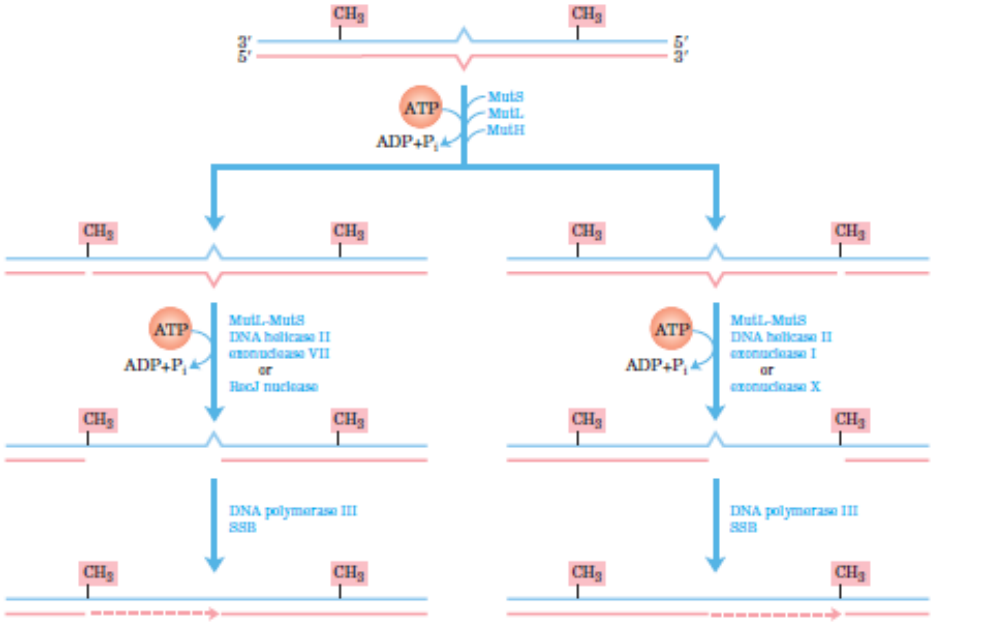


FIGURE 25–22 Completing methyl-directed mismatch repair. The combined action of DNA helicase II, SSB, and one of four different exonucleases removes a segment of the new strand between the MutH cleavage site and a point just beyond the mismatch. The exonuclease that is used depends on the location of the cleavage site relative to the mismatch. The resulting gap is filled in by DNA polymerase III, and the nick is sealed by DNA ligase (not shown).

Direct repair {no excision and no nucleotide removal} backbone is not affected.

A) - Photoreactivation by UV light.

E. g. E- coli when exposed to UV and sees thymidine dimer (kink) it will repair it by activating photolyase enzyme. UV light strike the cell DNA and converts 2 adjacent thymines to a thymine dimer then the photolyase will be activated so when excitation occurs to the photolyase, it will reach the MTHF domain and it will become hyperactive so it will lose electrons and give it to FADH converting it to FADH2 (high energy) which causes dimer breaking and DNA fixation.

Photolyase has two domains {MTHF and FADH domains}

In humans, thymine dimers are built by exposure to UV light and are fixed with nucleotide excision repair mechanism using excinuclease (a special type of endonuclease) which does a dual incision.

https://www.youtube.com/watch?v=CwuXOmtl_zI

B) - Repair of nucleotides with alkylation damage (very dangerous)

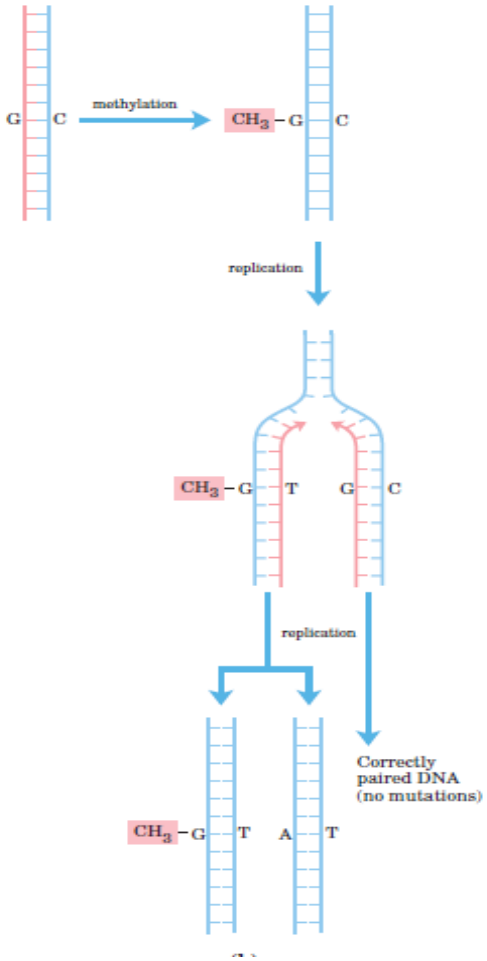
E.g. O6 methyl guanine should be fixed before replication by using methyltransferase enzyme that contains sulfhydryl (sulfnyl) group bound with cysteine (active state).

the enzyme binds with o6 methyl guanine and transfers the methyl group from guanine to its own cystine and gets inactivated and afterwards gets broken down. (The enzyme is lost permanently)

C) - Direct repair by the AlkB protein: (AlkBH1, AlkBH2...AlkBH9 and FTO in humans)

AlkB works on 1-methyadenine or 3-methylcytocine by doing coupling (demethylation of 3-methylcytosine and decarboxylation of alpha-ketoglutarate), steps are:

Hydroxylation of methyl group.
Decarboxylation of alpha-ketoglutarate producing CO2 and succlnyl.
Demethylation of 1-methylcytosine producing formaldehyde
Cleavage of formaldehyde and producing normal cytosine



There are other repairing pathways that the cell could do:

Recombination DNA repair (continuing a missing or broken chromosome from its other similar chromosome pair (homologous to it))
Error prone (sensitive) translesion DNA synthesis (TLS) (fixing with mistakes and errors)
SOS response (save our lives)

If there is a bulky region with mistakes, nucleotide excision repair is the first pathway to be taken but when there are **several** regions with damage the nucleotide excision repair is not capable of fixing it so the cell does induction to enzymes that fix with errors (SOS RESPONSE).

SOS response to replication damage: after cell cycle arresting.

Lex A is a dimer protein bounded with operon operator (operon is composed of promoter, operator and SOS genes).
Lax A prevents SOS genes to be expressed by RNA polymerase as long as there is no DNA damage, when damage occurs Rec A is responsible for initiation of proteolysis and separation of Lex A dimer leading to the activation of SOS genes which will be responsible for the synthesis of DNA repair enzymes.

If nucleotide excision repair is not sufficient (Proper base pairing is nearly impossible) the repair will occur with **Error prone DNA.**

UmuD complex with UmuC are part of (DNA polymerase V induced under SOS response) which is responsible of repairing with errors but not all SOS genes repair with errors (like Uvr genes). SOS activates UmuD +UmuC only when all replication forks are blocked {as a result of extensive DNA damage}.

DNA polymerase IV also repairs with errors (proofreading exonuclease activity absence.

In humans DNA polymerase eta and iota exist instead of DNA pol. IV and V in bacteria.

DNA POL.V and DNA POL.IV fix with errors under Translesion DNA Synthesis (TLS) category.

DNA RECOMBINATION review

DNA recombination (variation) refers to when segments of DNA moves from one place to another

DNA recombination could be:

Homologous recombination: DNA crossing over
site-specific recombination
trans-positional recombination

Genetic recombination is used to:

DNA repair: when there is a double stranded break we need homologous recombination to fix it.
Maintenance of genetic diversity: (different organs from zygote, different siblings look.)

Crossing over (exchange of genetic material) occurs in prophase I, first tetrad is formed (homologous chromosomes meet at a hotspot (chiasma)) then an enzyme does a double strand break on chiasma and genetic material is exchanged between two not sister chromatids (2 homologous chromosomes).

Double strand break could be spontaneous and will be fixed with homologous recombination:

Exonuclease will bind to both strands and will chew the 5prime end to the 3prime end faster but doesn't chew it all and then 3 prime end uses the homologous or intact chromosome by invading it to find the complementary strand and start migration and hydrogen bonding until it finds a non-identical nucleotides it will stop, then DNA polymerase copy the sequence on intact chromosome until we get the (Holliday junction or intermediate)
Holyday intermediate is a four stranded molecule with two crosses that is disassociated with resolvase enzyme either horizontal-horizontal disassociation causing recombinant DNA regions or other ways like vertical-horizontal.