Genetics & molecular biology

◉ Sheet ○ Slide

Number:		5
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E. coli has at Least Five DNA Polymerases:

DNA polymerase 1(pol1)

For E-coli cells, scientists used to think that polymerase 1 is the major enzyme in the replication process and that there is no other enzymes involved in building the new strands of DNA until they found that:

1.The rate of added Nucleotides is 600N/min □its rate is slower than the rate of replication of E-coli.

□ Result: there are other polymerases that synthesize DNA.

2. DNA pol I has slow processivity: takes many brakes during the replication process

3. Genetic studies: found other proteins that may be involved in the replication process.

4. Another study□ mutation analysis (Un-functional polymerase 1) □the cell replicates normally □ other polymerases can lead the replication process.

-DNA polymerase 1 is important for DNA repair.

Comparison of DNA polymerases:

-Subunits: give us idea about polymerase complexity

- Important characteristics: 3′→5 ′exonuclease (proofreading) the ability to go back and remove wrong linked nucleotides (three polymerases can do that)

-polymerization rate (nucleotides/sec) pol3>pol2>pol1

-processivity (nucleotides added before polymerase dissociate):pol3>pol2>pol1

-5′→3′ exonuclease activity :(removal of the primer) pol1.

Features of DNA Polymerases

table 25–1

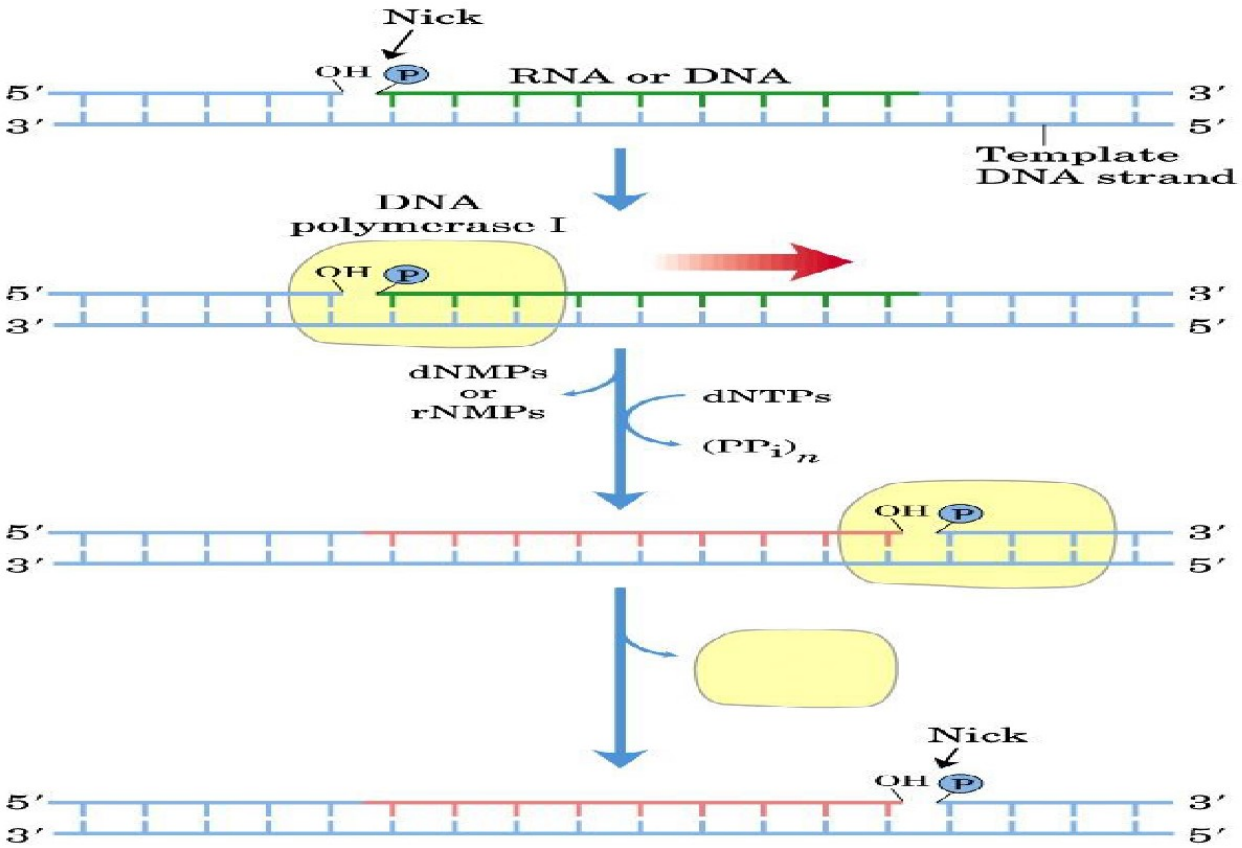
Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	88,000†	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dna*E is an earlier designation of the gene now referred to as *pol*C.

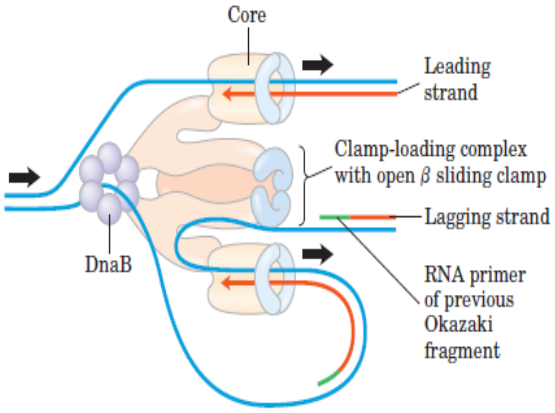
†Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the β, γ, δ, δ′, χ, and ψ subunits (see Table 25–2).

Nick translation:
Aims of the process:
1.Primer removal by DNA polymerase 1
2.Complete the strand: DNA polymerase 1 works as exonuclease5'□3'replacing primer nucleotides one by one and building DNA nucleotides from 5'□3'direction. After replacing the primer by DNA strand, we need to link the replaced strand with the other DNA fragment. However, DNA polymerase 1 can't seal the nick.
3. Sealing of the nick: by ligase which links the OH with the phosphate group.
*nick: is a broken phosphodiester bond.
*In leading strand, pol1 can remove primer but it can't build or complete the strand because there is no 3' OH to link with.



DNA fidelity is maintained by:
1.Nitrogen base selection and it is mediated and facilitated by the correct hydrogen bond.
2. Proof reading activity: 3'□5'exonuclease activity.
3. Mismatch repair: 5'□3'exonuclease activity.

DNA pol III is more complex than DNA pol I(ten subunits):
-clamps: catch the DNA .
-core dimerization links the two cores.
-Beta subunits increase the processivity.



(a) Continuous synthesis on the leading strand proceeds as DNA is unwound by the DnaB helicase.

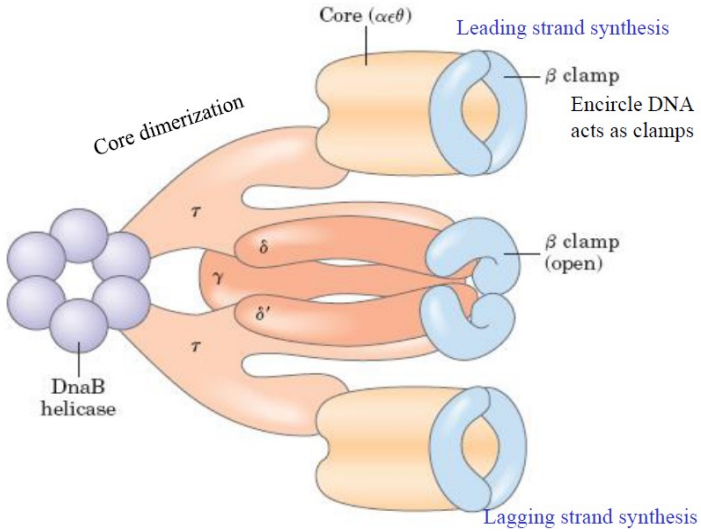
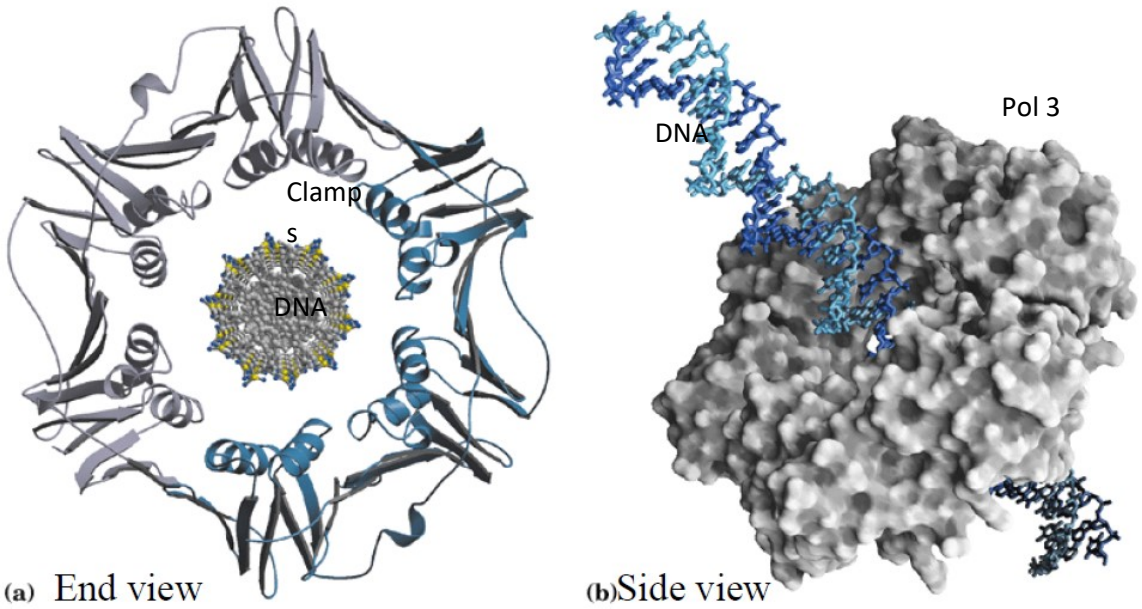


table 25–2

Subunits of DNA Polymerase III of *E. coli*

Subunit	Number of subunits per holoenzyme	M_r of subunit	Gene	Function of subunit	
α	2	132,000	*pol*C (*dna*E)	Polymerization activity	Core polymerase
ε	2	27,000	*dna*Q (*mut*D)	3′→5′ Proofreading exonuclease	Core polymerase
θ	2	10,000	*hol*E		Core polymerase
τ	2	71,000	*dna*X	Stable template binding; core enzyme dimerization	
γ	2	52,000	*dna*X*	Clamp-loading complex that loads β subunits on lagging strand at each Okazaki fragment	
δ	1	35,000	*hol*A		
δ′	1	33,000	*hol*B		
χ	1	15,000	*hol*C		
ψ	1	12,000	*hol*D		
β	4	37,000	*dna*N	DNA clamp required for optimal processivity	

*The γ subunit is encoded by a portion of the gene for the τ subunit, such that the amino-terminal 80% of the τ subunit has the same amino acid sequence as the γ subunit. The γ subunit is generated by a translational frameshifting mechanism (see Box 28–1) that leads to premature translational termination.



DNA replication (replisome):

Replisome: it is a replication system that is large and complex. It includes the requirements to access DNA strands:

1. Helicases: unwinds DNA double strands, it uses ATP to break hydrogen bonds between the two strands.
2. Topoisomerase**:** relieve stress or tension created while unwinding of the DNA two strands.
3. DNA binding proteins: stabilize the unwinding strands making them accessible.
4. Primase: enzyme that builds the primer to offer 3' OH.
5. Ligase: seals the gaps formed in the broken phosphodiester bond.
6. Single stranded DNA binding proteins: prevent formation of hydrogen bonds after it disassembled.

The primer RNA must be removed from the strand, its length is between (10-60) nucleotide. Which enzyme removes the RNA primer? DNA polymerase one (repair).

We remove it for 2 reasons:

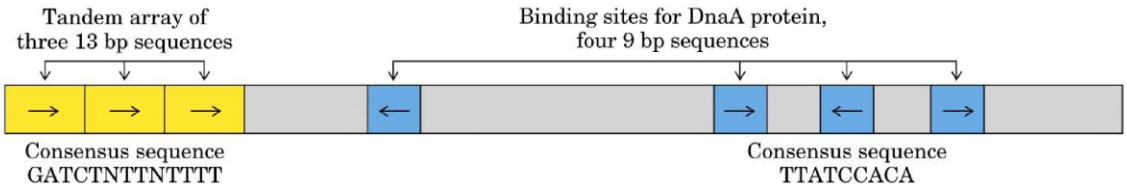
1) We can't imagine that there is DNA with RNA, they can't bind together.

2) The primase which puts the RNA primer is known for making mistakes while building the complementary strand. But polymerase doesn't.

The cancer cells are immortal.

Synthesis of DNA divided into 3 stages:
Initiation, Elongation, Termination
1.initiation :

Finding the origin: (245bp), contain two kinds of conserved repeats:
- a 13bp sequence repeated three times
- 9bp sequence repeated four times



At least 9 enzymes are involved in the Initiation process:
1. DNA A: recognizes the origin spot, partial unwinding of the double strands,
2.DNA B: acts as a helicase.
3. DNA C: leads DNA B to the origin point.
4. Histone-like enzymes
5. Primase to build a primer

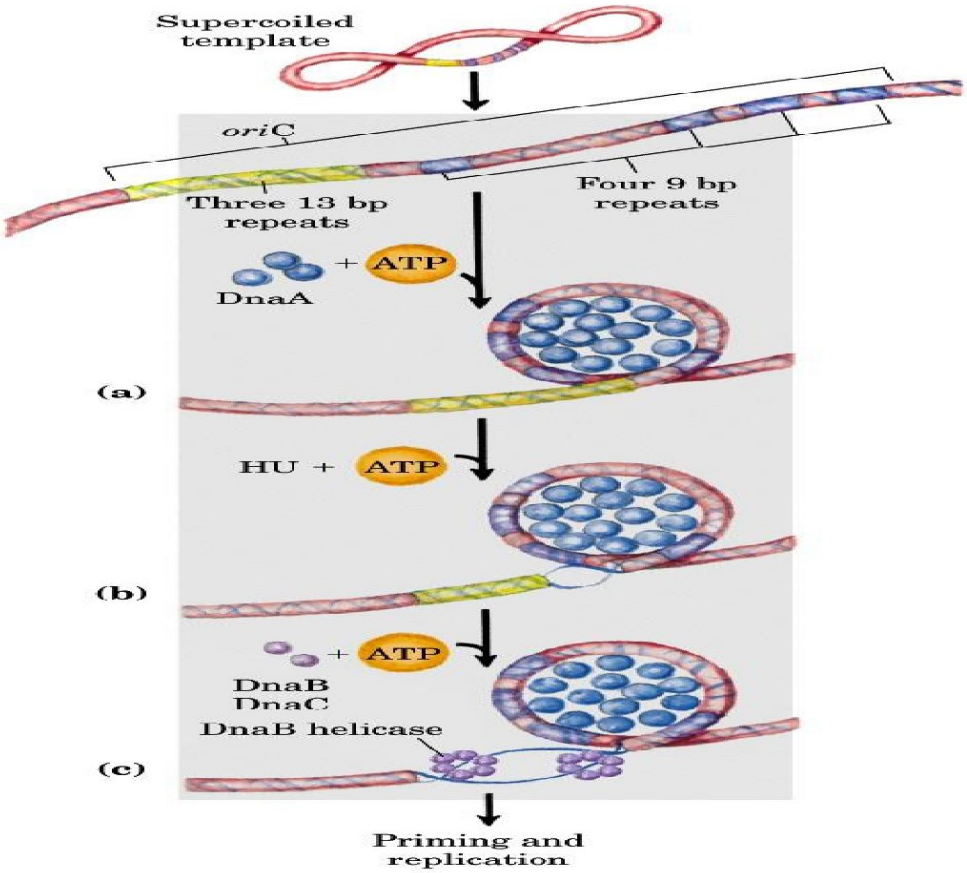
table 25–3

Proteins Required to Initiate Replication at the *E. coli* Origin

Protein	M_r	Number of subunits	Function
DnaA protein	52,000	1	Recognizes origin sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	29,000	1	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA bending protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,600	4*	Binds single-stranded DNA
RNA polymerase	454,000	5	Facilitates DnaA activity
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5′)GATC sequences at *oriC*

*Subunits in these cases are identical.

Initiation steps:
1.DNA A recognizes the origin point, it links to A=T rich region. A=T rich is easier for unwinding (due to the number of hydrogen bonds)
2.DNA C: uploads DNA B
3.DNA B: arranged as rings of six subunits.
4. Single stranded DNA binding proteins, stabilize separated strands
5. Topoisomerase: relieves stress.



-Then in Initiation: the main things that occur are the recognition of the origin and making it accessible (DNA A partially unwinds the two strands to make it easy for DNA B)

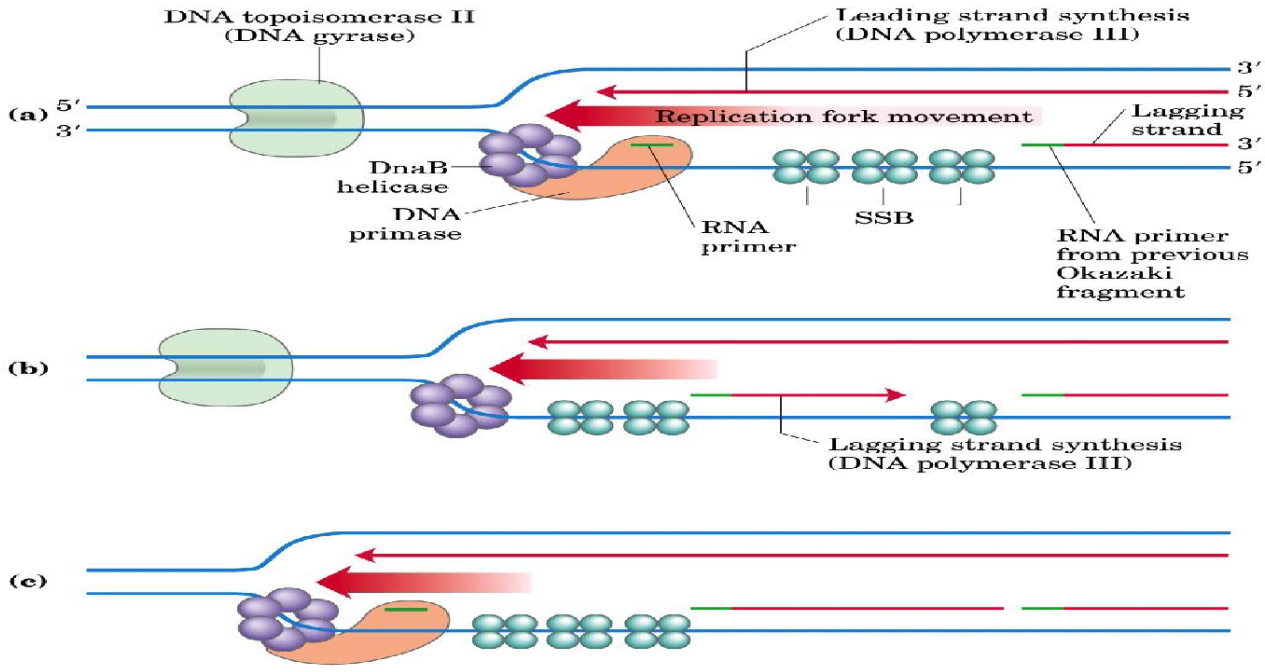
-Regulation only occurs in the first step of replication (Initiation), <u>how?</u>
Regulation of DNA replication is regulated by two things:
1.Phosphorilation: is the need of ATP for the replication process. As a first step, we need ATP to activate DNA A. What amount of ATP is needed to regulate DNA replication?

- The cell energy charge of the cell is low/ (high level of ADP) □ inactive DNA A □ No recognition of the **origin** □ No replication**.**
- The cell energy charge of the cell is high/ (low level of ADP) □ active DNA A □ Active DNA A recognizes the **origin** □ DNA replication starts normally.

2.Methylation: the **origin** region of E-coli is highly enriched in 5'-GATC-3' sequence it has 11 in its 245bp time.(5'-GATC-3' sequence)is the site where dam methylase enzyme adds a methyl group to adenine in this sequence. (Occurs in a lake period after replication). <u>How does this regulate DNA replication</u>? Replicated DNA can't enter another replication until all adenine (in 5'-GATC-3' sequences) bind to methyl groups.

Methylation: is a way to distinguish between the original strands (parent strands) of DNA and the new ones.
This helps in mismatch repair: in replication, DNA polymerase may add wrong nitrogen bases to new DNA sequences. Immediately after replication, the DNA is hemimethylated: the parent strands have methylated sequences but the newly synthesized strands do not. To repair these errors, they take the original strand as a base to choose the complementary nitrogen base.
-unrepaired DNA + Methyl □methylated DNA with mutation.
-In some states in Bacterial cells where the DNA is in contact with the plasma membrane, DNA can't enter another replication until it becomes free

2. Elongation: includes two distinct but related operations:
1. Synthesis of leading strand: it is able to be replicated continuously by DNA polymerase.
2. Synthesis of lagging strand: it undergoes replication discontinuously in small fragments.



Enzymes used while elongation:

table 25–4

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

Modified from Kornberg, A. (1982) *Supplement to DNA Replication*, Table S11–2, W.H. Freeman and Company, New York.

End replication problem:

1) We have RNA primer not DNA, if it was DNA we don't need to remove it.

2) All the enzymes can be functional only in one direction (5-> 3 ends)

The chromosomes extremities are called telomeres, and there is a centromere in the center. The DNA polymerase doesn't have a method to copy those extremities, therefore, with every cell division, telomeres brogan معناها انجرافThe chromosomes extremities will become shorter and shorter until they reach the DNA (loss of genetic material) -> the cell will be aging -> death.

What is the benefit of telomeres?

1) Protection from deterioration يعني تآكل

مهمة تأتي بالامتحان :

2) We suppose that if chromosomes are exposed to UV or X rays, it will be divided (broken) into 2 sections, and that the cell will have a connecting system to connect these 2 parts together, but in our bodies we have different chromosomes.

Q: why doesn't the cell connect them? Because there are telomeres

Q: What happens if there are no telomeres? All the 46 chromosomes will mix.

Q: The human originates from 2 cells, how can they can be 4 kg during 9 months?

There are Infinite strong divisions, so the fetus will have strong divisions

Q: why do not the telomeres of the fetus erode? Because he has a specific enzyme that will rebuild them, this enzyme called telomerase.

بعض الأطفال وهم بمرحلة الجنين التيلوميرز تبعهم بتآكل يعمل عندهم شيخوخة مبكرة .

Slide 26 , the form of sequence of telomeres الدكتور حكا رح يسأل ازا لازم نحفظهم أو لأ ...

(5')(T×Gy)n

(3')(A×Cy)n

Telomerase rebuild the deteriorated telomeres, it is functional in some places but nonfunctional in others, because we must die.

The places that telomerase is functional:

1) Embryo stage: if it isn't functional in this stage, early aging occurs

2) Germ cells: cells needed for reproduction

3) Stem cells: high division

4) Somatic cells: it is functional until birth, and then they become nonfunctional.

This enzyme is unique:

1) It is the only enzyme that consists of 2 subunits ازا الدكتور مش غلطان حسب ما بتوقع هو يعني

One subunit is protein, the other is RNA.

All the enzymes have 2 subunits: protein + protein except the later we mentioned above.

2) Reverse transcriptase like activity: Transcription is from DNA to RNA but from RNA to DNA we call it reverse transcription. Reverse transcriptase it is the enzyme that can manufacture DNA from RNA, like viruses.

This enzyme uses his RNA as a template to make DNA, what is strange in this situation?

The telomeres are made by this RNA; the DNA that is converted by the RNA template is the sequence of telomeres بصير عنا استطالة ل التيلوميرز

telomerase شغله مش بناء الناقص ، يطوّل الموجود

Primase makes RNA on the elongation part; DNA polymerase fills the empty part

Topoisomerases: We have 2 classes of topoisomerase:
Topoisomerase 1
Topoisomerase 2 (DNA Gyrase in E. coli)
They both release the topological stress during REPLICATION.

Q: Why do we have 2 types of topoisomerase?
Type I topoisomerases acts by transiently breaking one of the two DNA strands, passing the unbroken strand through the break and rejoining the broken ends; they change the linking number (*Lk*) in increments of 1.
Type II topoisomerases break both DNA strands and change *Lk* in increments of 2.

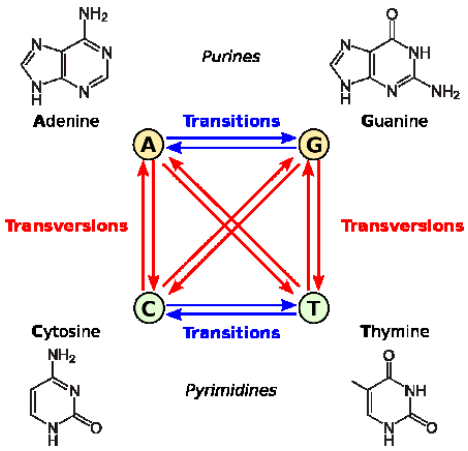
DNA Repair Mechanisms: During replication, whenever we have any mistakes, the DNA polymerase 3 itself repairs these mistakes through the 3'>5' exonuclease. Despite its fidelity and accuracy, the DNA pol does not recognize some mistakes during replication. Therefore, we have DNA repair Mechanisms after the replication. These repair mechanisms are for producing a non-mutant DNA and inheriting it to the next generation.

<u>Sources of damages that happen to the DNA strand</u>:
1- **Endogenous**: the source of this damage is from the inside of the body. For example, an error during the replication process that the DNA pol 3 didn't recognize, or during cellular respiration, a leakage for an electron might happen, this electron can bind to Oxygen giving radicals (Superoxide) , or (Hydroxyl radical) – reactive oxygen species. These came by accident from normal metabolism process. However, the body should have defensive mechanisms against them.
2-**Exogenous**: an external induction. For example, UV light, X-ray, chemicals, toxins, viruses –through binding its genome with the genome of the host cell. DNA intercalating agents: chemicals that bind to the DNA and restrict it from unwinding during replication.

To call damage in the DNA sequence a mutation, it has to be permanent. Therefore**, a mutation** is a permanent change in the nucleotide sequence and can be inherited.

Mutation are Categorized by the:

A) Nature of bases (Nature of nucleotides)



- Substitution mutation:

Transition, change in the base from purine to purine, or from pyrimidine to pyrimidine.
Transversion, change in the base from purine to pyrimidine or from pyrimidine to purine.

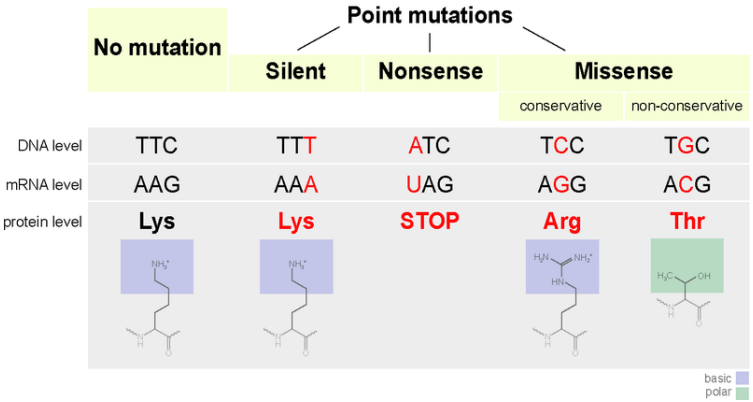
- Insertion or deletion: Removing or adding bases to the DNA strand.

B) Effect on coding sequence: each 3 nucleotides gives a codon which is transcribed and later translated into amino acids.

-Silent mutations do not alter the amino acid encoded: for example, AAG is translated in the ribosome into Lysine, if the base G is replaced with A giving the codon AAA, the amino acid given from AAA translation is Lysine, so there was no difference in the peptide chain.

-Missense mutation: change in the amino acid encoded which leads to changes in the peptide chain. One of the most common diseases that occur due to missense mutation in beta globin sequence is **sickle-cell disease**. People with this **disorder** have atypical hemoglobin molecules called hemoglobin S because the mutation gave GTG that encodes Valine instead of GAG that encodes glutamic acid.

-Nonsense mutation: Giving a stop codon, when a mutation produces a termination codon in the interior of a gene, translation is prematurely halted and the incomplete polypeptide is usually inactive and truncated.



Note: not everything mutagenic is a carcinogenic, but every carcinogenic is a mutagenic

Ames test is for carcinogens: Measures the potential of a chemical to induce mutations in bacteria (may act as a carcinogen). In this experiment, they used (Salmonella typhimurium) bacteria possessing a point mutation that prevents the synthesis of histidine –amino acid. Therefore, it can't live in a media that doesn't have histidine. Logically, the bacteria shouldn't grow, but some colonies appeared on the plate, so they took a sample of the colonies and did a DNA extraction. They found that these bacteria had a back mutation and was able to produce histidine again. Nothing induced this mutation, so it was a spontaneous mutation –unknown cause.
Then, he took some of the mutant bacteria on another plate that had a filter disc in the middle and gave a mutagen. The areas that are close of the filter disc didn't have colonies, while the further areas did. In other plates he used the different concentrations of the mutagen and the number of the colonies increased. If we suspected that a chemical is a mutagenic, we use this test. It's used in the preparation of cleaning products and controlling the concentration of these chemicals.

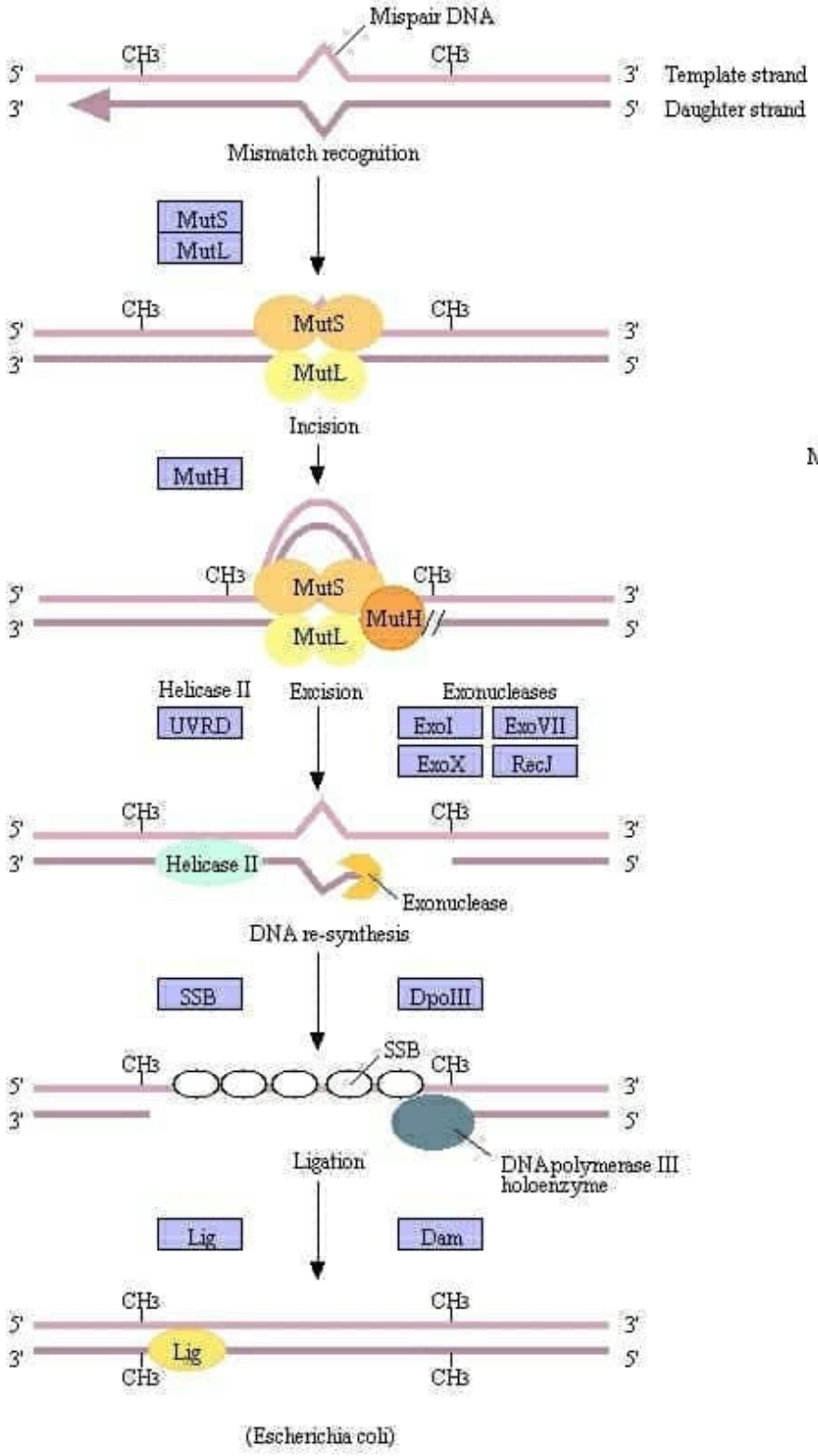
DNA repair system: AFTER REPLICATION!!!

1- **Methyl- directed Mismatch Repair**: It's used when there is an error in the DNA sequence and was noticed after finishing the replication immediately.
The parental strand - which is considered to be the origin-, can be distinguished from the new strand for a few minutes after the replication ends by the methyl groups. These groups attach to the Adenine in GATC palindromic sequence during the replication process by the enzyme DAM methylase. The tagged strand is the parental, and the untagged is the new one, and both are called the hemi-methylated DNA.
After few minutes, the new strand is methylated and the two strands can no longer be distinguished.
To fix this mutation, MutS enzyme binds to the site of the lesion –mismatch, which induces the binding of MutL to it producing MutS-MutL complex and it needs ATP. Then, MutH holds the strand around the mismatch from the complex side and from the palindromic sequence –where the methyl group is, DNA on both sides of the mismatch is threaded through the MutL-MutS complex, creating a DNA loop**. MutH** makes a nick in the new strand mismatch producing a broken phosphodiester bond, free OH and free Phosphate group. Then, **DNA helicase** breaks the Hydrogen bonds and opens the two strands and **SSBs** bind to the opened strands. Then **exonuclease** enzyme degrades the strand from, and **DNA pol 3** synthesizes the new strand from 5'>3'. Last, the nick is sealed with **DNA**

ligase.

MISMATCH REPAIR

Prokaryotic type

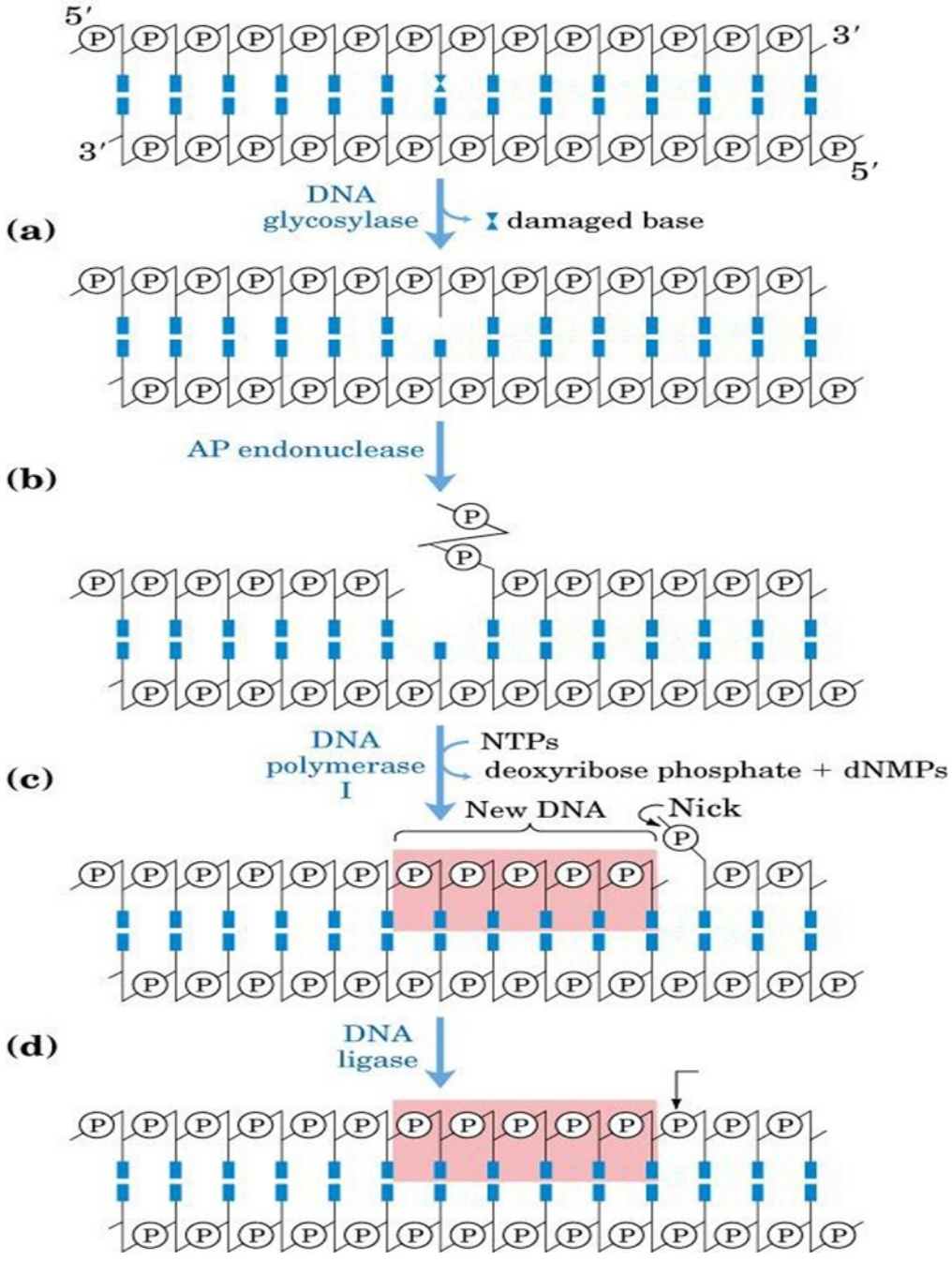


(Escherichia coli)

NOTE: if the mismatch was at the 5' side of the cleavage, the exonuclease (1 or 10) activity will be from 3'>5'. If the mismatch was at the 3' side of the cleavage, the exonuclease 7 activity will be from 5'>3'.
In human, we have MutS homologous (MSH2) instead of MutS and MutL.
In cancer cell, the genes of MSH2 are mutated so there's no mismatch repair mechanism, high rates of mismatch mutation.

2- **Base Excision repair:** When the back bone of the DNA is fine, but one of the bases is spontaneously deaminated. If Cytosine is deaminated, it turns to Uracil, along with Guanine to Xanthine, and Adenine to Hypoxanthine.
Every cell has a class of enzymes called DNA glycosylases that recognize particularly common DNA lesions and remove the affected base by cleaving the N-glycosyl bond. This cleavage creates an *apurinic or apyrimidinic* site in the DNA, commonly referred to as an AP site or abasic site. First, **DNA glycosylase** cleaves the N-glycosyl bond between the ribose sugar and the nitrogenous base and formes abasic side. Then, **AP endonuclease enzyme** (called endo not exo because we didn't break the back bone, the back bone is intact) cuts the DNA strand containing the AP site, then **the DNA pol 1** (it replaces a few adjacent nucleotides to the abasic site) adds nucleotides and the nick is sealed with **DNA**

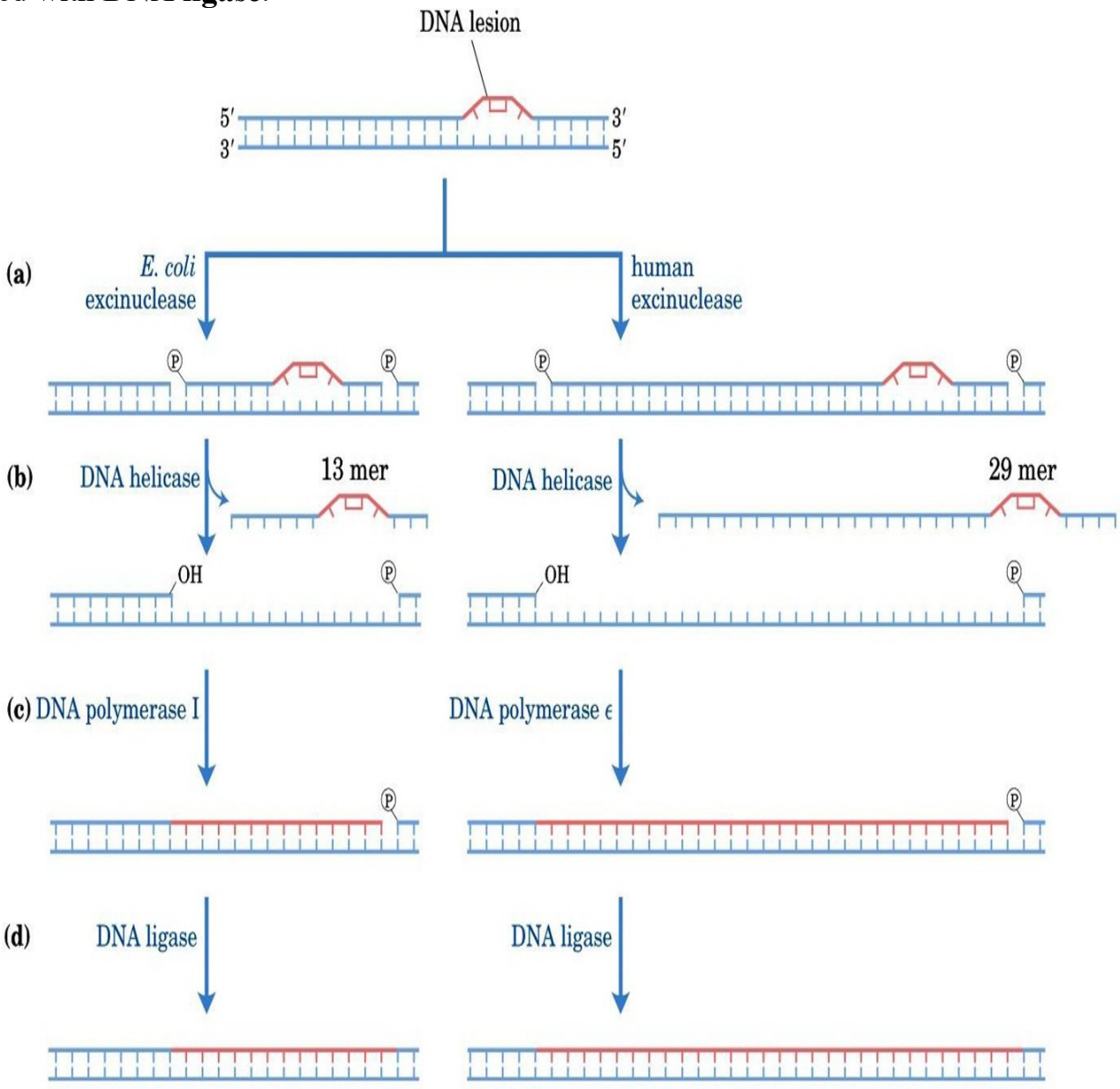
ligase.



3- **Nucleotide Excision repair**: Bulky lesion of Thymidine Dimer. Thymidine dimer is formed if 2 thymine bases were adjacent (T-T) and formed a Hydrogen bond instead with the fronting Adenine bases. This thymidine dimer causes a kink in the DNA strand –bulky lesion. Many Thymine bases can be repetitive, producing adjacent thymidine dimers. These bulky lesions can be caused by (C-T) or (C-C), but the most common is (T-T).

In E.coli, The **Exinuclease enzyme** recognizes the bulky lesion**,** makes a dual incision around it (from two sides) and cuts **13** mer from the back bone, then the **helicase enzyme** separates the two strands by breaking the hydrogen bonds. After

that, **DNA pol 1** fills this gap –short sequence do not require DNA pol 3. Last, the nick is sealed with **DNA ligase**.



In E.coli, The **Exinuclease** enzyme consists of 3 subunits (Uvr ABC) –Ultra violet repair. The genes of exinuclease are induced by the UV light which caused the bulky lesion. Then the exinuclease removes this lesion. The bacteria has another method to fix this lesion along with nucleotide excision repair called **(Photolyase mechanism)**, but humans only use nucleotide excision repair by an enzyme called **human exinuclease**, it makes dual incision around the lesion like the bacteria but for **29** mer, and the enzyme that fills the gap is **DNA pol ε**. The nick is sealed by the **DNA ligase** that uses ATP in human.

If the genes of the exinuclease enzyme were mutated, for example, this person will be very sensitive to the UV light that he can't go out in the sun, has skin rash, or skin cancer. This disease is called "Xeroderma Pigmentosum". It's classified into seven classes (XPA, XPB…XPG) according to the seven genes that were mutated –the human has more subunits than the bacteria.
PS: watch "Midnight Sun" ☺

Another reason for bulky lesion is base adduct –smoking-. Benzo pyrine is a substance that is available in the cigarettes, attaches to the Guanine base in the DNA, making a base adduct.
The liver is responsible for the detoxification process for the benzo pyrine, turning it into diol epoxide. This substance replaces the cytosine in the DNA sequence. When the replication process takes place and the polymerase reaches a diol epoxide, it doesn't recognize it as a base that can make a hydrogen bond with A, T, C or G, so it will be left empty. And this mutation might be transferred into the next generation through the gametes if they were affected.

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