

DNA Metabolism

- In DNA metabolism
 - ↓
 - Process of Replication
 - DNA Repair
 - Mutation
 - Reactions in processes that are related to DNA
- Q: Why process of replication/DNA metabolism requires (100s) of proteins, enzymes, so much supervision (every enzyme works diff. evently).
- Reason: → The DNA sequence should be same, there is no place for any error.
- Replication: → Making copy of genome.
- Prokaryotic DNA Replication:
- DNA is going to replicate (making same copy of DNA).
 - If there is no sequence to that may leads to various kinds of complications.
- Example:
(lipoproteins, conjugated lipid)
• phospholipid bilayer → associated with carbohydrate chain.

- Glycolipids → monosaccharides (Sugars)
↓
Oligosaccharides
(Glycopolysaccharide
side chain) → are associated with
cell membrane.

• Oligosaccharide's molecular arrangement coming from the information which is encoded in DNA.

• If this information is not disclosed properly to there's ~~no~~ problem start in Oligosaccharide.

• In this case they are required for "cell to cell recognition processes".

• The side chains of oligosaccharides involved in cellular recognition; (otherwise, the particular cell which lack the oligosaccharide chain will be treated as cancerous cell).

↓
Because there is no recognition, Oligosaccharide thinks that it is foreign particle. It's called metastases (begins to form into cancerous cells).

For cellular recognition (Required) :-

) Oligosaccharide side chain

↓ makes with

Enzymes

↓ bondings / cleavages (all of this
are encoded in enzymes).

all enzymes are protein in
nature & they are coming from
m-RNA

↓ m-RNA coming from DNA.

for cellular recognition they required
a small part of oligosaccharide, for this
the information needed.

↓ Enzyme → Synthesis from DNA

If there's even a minor error during
DNA metabolism → that may lead
to abnormality in that particular
enzyme, this abnormality if is on
active site so reaction doesn't catalyzed

- So there's no oligosaccharide like chitosan.
- There is no replication.

- Because of error 200s of function may be affected.

Basic Rules of Replication

O. Semi-Conservative

(one strand is from parent & one strand from daughter)

- It can be bidirectional. It can be unidirectional.
- Mostly bidirectional.

Ori-C: Origin of replication of E-coli

- Every organism have different origin point according to sequence.

- Direction is always Specific
"5' → 3'"

- Semi discontinuous process (one strand is continuous & other is in fragments)

↓
(leading strand)

↓
(lagging strand)

• Primers are required

(i.e.: required RNA fragment, if it is not available no replication stops)

- RNA fragment (about 40-50 nucleotide) required, that is removed by "DNA-I"
- Initially primers required.

- Proved by experiment of "The Meselson-Stahl".

• Nucleotides in nitrogen is (^{14}N).

• He used an isotope (^{15}N), synthesized Nucleotides, (he grown ~~nucleotides~~ cells on a media having ^{15}N nucleotides).

- After some generation we got a new species of E-coli with all ^{15}N DNA.

• Grown on normal media (^{14}N)

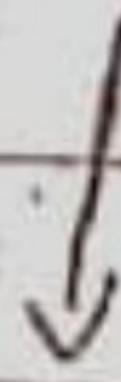
(alc. to semi-cons. mode of replication " ^{15}N grow on ^{14}N ").

• New genome hybrid of ^{15}N & ^{14}N .

After growing the generation, they were separated it on a "Sodium Chloride medium" \rightarrow a solution that separates on high speed centrifugation.

(e.g: Oil & water), separates in two phases.

- In this medium they have got a hybrid DNA (^{14}N & ^{15}N) in centre of the two phases.



- Then they again grows there cells on ^{14}N , then again same phenomena (one new, one old) then they have 2 types of DNA one is hybrid & the other ^{14}N .

^{14}N

hybrid

^{15}N

- Proven that Replication is semi-conservative

- Eucaryotic genome is much bigger in size as compare to prokaryotes, so process of replication is completed in many days.

- In eucaryotes origin of replication are multiple.

• DNA Polymerase •

- major enzyme.

- Required for process of Replication.

function is to bind one nucleotide to the nucleotide,

To make a phosphodiester bond.

Requirements of

Template : Because of accurate replication sequence that transfer parent to offspring
 $\text{AGCT} \rightarrow$ Complementary bases should be attached.

- Parent strand works as a Template.
new nucleotides add-up according to reading of parent strand.

(2) Primer ↓

(It has not ability to attack/ add first nucleotide)

? It's an oligonucleotide/ oligonucleotide fragment of RNA with a free 3-OH end, \rightarrow (called primer terminus)

(new nucleotide attached on P.T)

• DNA polymerase can't attack nucleotides if there's no 3-end (free 3-OH end).

\rightarrow P pole A \rightarrow (Synthesized DNA polymerase I)

pole B \rightarrow (Synthesized DNA poly. II)

pole C \rightarrow (Synthesized DNA poly. III).
(dnaE)

- Poly. I involved in primer removal.
- Poly II involved in DNA repair.
- Poly III involved in polymerization,
this is major enzyme.

\rightarrow Nucleases & Enzymes that cuts the DNA.

\rightarrow Type 2-

① Exonucleases & cut DNA on Terminal (specific direction).

② Endonucleases & Breaks DNA in fragments.

- In nucleoid's cases, they are specific.

(e.g. Restriction Enzymes are endonucleases.)

- Exonuclease cut (3'-5') if there's

any error during cutting so it cuts nucleotide in backaward direction.
(3' to 5') activity \rightarrow proofreading
(Because they have 3' to 5').
↓
2 sites

(There's no chance for any error)

- This ability is in DNA polymerase I, II & III.

- (5' to 3') activity \rightarrow for pioneer removal in DNA polymerase I only.

DNA Polymerase Mechanism

- Mechanism of forming a phosphodiester bond.

- New nucleotide come in triphosphate form. ~~form~~ ↓

- Join monophosphate by cutting & separate 2-phosphates

(How \rightarrow with the help of (-) non-electron pair, this pair disrupts the bond between nucleotide & triphosphate, be rejoin then release pyrophosphate), then monophosphate is going to attack with it.

- DNA polymerase reads through Template that it requires (A) so they gives it (T), because of A.

② Then it requires 3'-OH end (this is going to be provided by RNA primer)

③ Then attachment of nucleotides.

→ Polymerization

• makes structural variant, means (cytosine changes their physical structural
it looks like Thymine.)

• Changing in structure in form of solution.

(3' to 5') Exonuclease Activity

(proofreading ability)

• incorrect nucleotide (proper geometry doesn't match with other strand's nucleotide).



• DNA polymerase removes it by backward slide.



• Because/B enzymes have 2 sides

① Exonuclease activity

② Polymerization ability

Totomers

structural variants

(Structural Representation of DNA polymer)

- In bidirectional movement Replication, needs two polymerases, for both sides replication.

- DNA polymerase have 2 core polymerase, that moves with both strands.

Helicase:

- Flower like structure in centre \rightarrow (loads beta clamp) & it required for optimal activity (polymerization) speedly, for this activity B clamp is required

- B-clamp required by clamp loader, (In process, when lagging strand's fragment ends so after this, it leaves B-clamp over there & attached with new B-clamp);

(~~is~~ already present where new primer is made up, by the help of clamp loader)
(In lagging strand, primer - lag - pri - fig.)

⇒ Origin Of Replication

- Point where DNA duplex opens & starts replication, this point is specific / particular.
- There are 2 Imp things.

① DNA unwinding Element:

(This is the B-Bp sequence, in this sequence "T" is present in large quantity, A=T easily to break hydrogen bond).

- having multiple A=T residue, so this

can be destabilize easily in order to make a opened complex to start process.

② R-Repeats : They are multiple sequ. ($R_1 - R_r$) all are similar to each other. \rightarrow (in fig.)

• 9 Bp sequences, which repeats many times.

• Consensus Sequences

Those seq. which are common to Org. in Specie to Specie similar with minor diff. They may be nucleotides or amino acids.

Def & There are five representative sequences in which each nucleotide or amino acid occurs frequently. So all also similar in structure & function in various organism.

• R-repeats are specific sites where DNA - A protein binds.

• DNA - A protein is required for initiation.

\Rightarrow Replication

① Initiation : (25-12 Fig)

① Required DNA - A \rightarrow (to recognise R-repeat to recognise origin & bind with it)

• So it will form a right handed way.
② DNA itself is a left handed, so there cause (*)... (Table 25-3)

(After binding, that may be β -protein (helicase enzyme) break DNA polymerase bonds with D-nucleate \rightarrow (to unwind DNA)
(helps in DNA-C protein's binding)
(binding of DNA-D)

• FIS

• HU \rightarrow Facilitates initiation's process

• IHF

• Topoisomerase (Unwind DNA by removing supercoiling). On top

↓
• helicase (Unwind & separate strands)

↓
• SSB - (Stabilized)

↓
• DNA polymerase

• Dam Methylase (Methylation of adenine residue, on OriC this seq. is repeated many times GATC in this seq. if there is an adenine residue so it methylate by this enzyme), after methylation 1 strand is methylated if the other one is not so we can call it 'Hemimethylation'.

(*) becomes

— DNA destabilized to open / unwind often because of DNA-A bindings.

③ In opened area DNA C loads DNA - B,
Sc after DNA - B binding, DNA - C released.

- All these proteins (only coming for helicase binding)
- are ATP dependent.
- If ATP hydrolyzed to these proteins becomes denature & released.

④

DNA polymerase 3 bind with helicase
Sc initiation process completed.

- Now Before elongation, HDA - protein leaves DNA - A (lmp go further process). (Special protein, assemblies to remove these proteins).

Sc then Dam Methylase, methylate 'A' in GATC, to form a hemimethylated DNA.
(that is required to stop another round of DNA Replication) \rightarrow (Methylation).

② Elongation

- DNA polymerase starts to add Nucleotides with the help of 3-OH end,
- pyrophosphate released, monophosphate attached accordingly; with the help of template strand.

(Table 25-4)

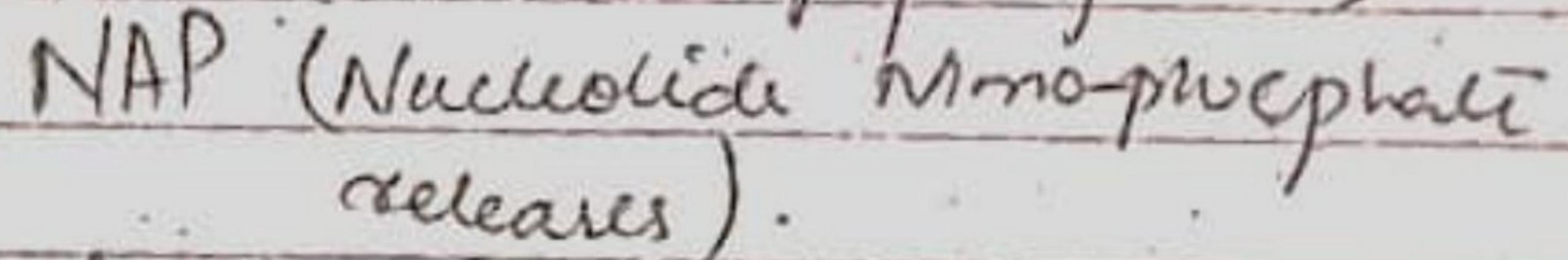
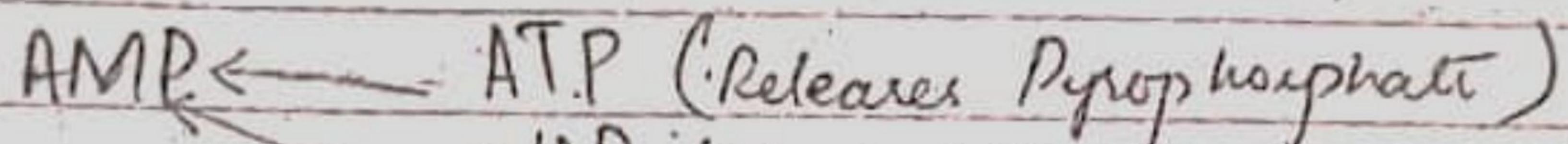
- SSB required (due to rejoining of DNA)
so DNA is stable in its single position.
- Required helicase, primase (because of synthesis of primer).
 - Helicase starts unwinding the SSB stable's DNA in its position, on the strand of DNA where SSB binds, the primer synthesized there by primase enzyme
 - Some times helicase attached with primase to make a complex (primosome) collectively primosome
 - Primase released
 - DNA polymerase starts to add nucleotide on newly syn. primer (opposite to the direction).
 - For this it makes a loop, after completed fragment it leaves the clamp & attached with other clamp.
(25-14 Fig).
 - The opening & closing of core-complex is ATP dependent.
 - Now we have leading & lagging strand. but there is proportion gap of RNA primers. they are going to be removed by DNA polymerase I, they remove the gaps & add new nucleotides.

(25-16.17 Fig)

- Se the nicks is going to be sealed by DNA ligase.

3 - Steps :-

- ① Enzyme activates (by attachment of AMP).
- ② Enzyme activates the phosphate which is present on that opening.
- ③ Phosphate makes bond to Adenine released.



- AMP displaced by Nick sealed.

③ Termination

- process is going to terminated by Ter sequences. (Origin), present multiple times.

- Protein binds with it which is TUS Terminus Utilization Sub.

- Replication fork moves towards the (Ter n. Tus) Complex, (so is complex k sate rene kriye krega us wahan rejaega jukt dura replication fork yahan aare meetups na kare).

- Replication Completed

If last Bases pair attached were each other be termed as (catenated chromosome or Catenated DNA).



- Released by reaction of DNA topoisomerase (4), it separates both DNA.

Q: What are the major requirements for replication?

- primer • Template • Enzymes (major enzyme → DNA polymerase).
To catalyze phosphodiester bond, by exposing nucleotide in the form of 2'-nucleotidyl triphosphate. It release free 2'-nucleotidyl phosphate in the form of pyrophosphate, later on pyrophosphatase enzyme act on it to convert it into inorganic pyrophosphate. It release lots of energy. This energy is utilized by DNA polymerase for bond formation b/w nucleotides.

• DNA polymerase properties:

- (3' to 5') (proofreading activity).

(i) (3' to 5') Exonuclease activity

(ii) (5' to 3') Exonuclease activity, particularly (Synth. both ends)

• DNA-polymerase - it is major enzyme.

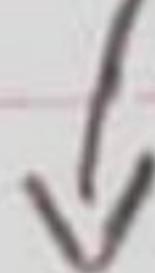
• if α is prime removed.

• β involved in DNA repair

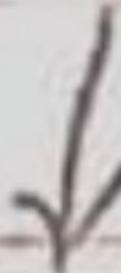
Because it is toward the direction

Conclusion & Replication

Inhibition: DNA protein binds with R. RepA (cause conformational change in DNA)



- cause the undirectional unwinding of DNA unwinding element.
- Helix Opens



- Helicase bind

Elongation



- Strands synthesized (leading towards the fork, lagging opposite) with the help of loop.



- Primers removed in poly-A, sc openings are sealed by DNA ligase.

Termination



- TUR protein binds with Tus; sc making a complex to arrested DNA fork.



- Replication completed.

- ## Eukaryotic DNA Replications
- large in size.
 - controlled mode of replication.

Cell Cycle

(complete/whole time or process in which one cell is divided into another)

- It is going to be divided in phases.
- Phases (S, G₁, G₂; M) G₀ is present/absent depending upon cell.

If cell is not going for replication, cell is in sleeping mode, so with the help of G₁ it becomes active to may be enter in G₁ phase.) So replication starts in S phase,

- Replication process depending upon some kinds of protein.

(e.g. Cyclin Dependent Kinase)

- There are the phosphorylation proteins.

Enzymes activate/deactivate.

- If these proteins are present (so there's no initiation, synth., Rep.)

If they are destroyed by some system known as (Ubiquitin Proteasome Sys)

- In the absence of these proteins the replication process is carried out.

① Cell is going to enter in M-phase when they have 2-genomes.

② When replication begins.

③ When they have starting material.

④ When synthesis begins.

All of the process starts when there's no (CDKs) / some regulatory proteins.

Some cells have lost their ability to enter in G₁, termed as Cancerous cell.

(They are differ from normal cells, because normal cell have ability to get enter in G₁/resting phase).

They don't have ubiquit p-S.

There is some sys/regulatory proteins who decides that cell should go in replicative phase or not, (cyclin & CDKs) are going to play an imp role.

Initiation

Required DNA unwinding etc. & R-repeats.

DNA-A protein binds

DNA duplex uncoated (from DNA - unwinding)
A-T rich region; DNA opens

• Helicase binds (Eukaryotic) (Dig: 25-30)
• Origin recognition complex (ORC) protein
responsible for recognizing origin.

↓
• Binding, later on ATPs come to
join this complex.

↓
• DNA duplex open by MCM 2-7 proteins
located (helicase like activity)

After loaded, ORC function is initiated that
will open the DNA duplex

↓
• going to replication ahead
(Depend on CDKs)

↓
• phosphorylation (Due to this MCV)
CDC6 phosphorylate
(maternal scenario)

↓
• Active due to Phos.
form a pre-

• Phospho. is carried replicative complex
out by CDKs by ADPs. (pre-RCs)

Reason for the permission to implement.

• Advancements, Cell has to compromise losing energy in replication process, Required ATP on every bond. Want complete nucleotides, then enter in pre-rep complex.

- 1. E. coli cell divide in 20-min, because it has small genome.

Fukayama cells are large in every cell takes up its own time. (2, 3 days etc).

- It should be defined by every type of cell.
- At certain age, replication stopped, by the age of time.

Process:

(MCM)

Dna_x opens \rightarrow helicase load

① Initiation completed by one with end.

② Elongation:

- leading strand continues Syn.
- lagging " by forming a loop.
- RPA are SSBs. (Single-strand binding).
- Ok (function in Slides).

(loop because it goes towards the

replication fork is opposite to the direction).

③ Termination:

- When chromatid is arrived on end at that time Telomerase will act on it.

• That is going to be syn. by Telomerase.

• In lagging strand every time we need a primer, but in last primer doesn't make up, so this part is taken up by Telomerase.

• usually last seq. is similar in all chromosomes

• In Eukaryotes origin of replication are multiple.

Comma are have both RNA polymerase
DNA is made up of nucleotide & the some
is similar to the DNA's end.
Also act on it & found to add nucleo-
tides on it along their seq. (100 Bp).

Telo removes few (100 Bp) it is in single
strand form.

Some times DNA pol binds with it & syn.
Some times remains some (Fig. - 26-39).

At the end of the chromosome, some
times single stranded (in daughter), going
to be cut by Telomerase.

DNA Repair :-

After replication if there is some change in
nitrogenous bases due to chemical
reactions \rightarrow (Decarboxylation Reaction is
common reaction). (DR)

e.g. Cytosine replaced by Uracil

D.R in nitrogenous base that may leads (unwanted DNA,
that is going to another compound be repaired)
that's DNA is not wanted

Known as 'DNA Repair' (e.g. in slides)

Hypoxanthine Base:

is part of IMP, (Inosine Mono-phov),
made up by deamination of adenine.

Depurination:

- In this process nucleophilic attack occurs.
- Catalyzed by (DNA glycosylase).

(e.g.: Guanine makes a Totomer in which there is minor variation in guanine structure due to this they can't make pair with cytosine.)

→ It's an O-Methyl Guanine (ortho-Methyl Guanine).

(It must be removed).

→ Tymine Dimer / Pyrimidil Dimer.

- Tymine pair with adenine
- In this case Tymine - Adenine bond pairing will break. So it will form a 'kink' in DNA.

(It must be removed)

- also include in DNA repair modifications.

→ Repair Mechanism: (Tab 25-5).

① Mismatches Repair

② Base excision Repair

③ Nucleotide excision Repair.

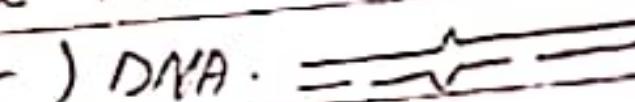
④ Direct Repair.

⑤ Mismatch Repair (Fig 25-23).

- triangle like structure (Error) it should be repair.

- carried out by Mut S, H etc.

MutS & MutL are going to recognize that mismatch & bind over there.
MutH binds with it (methylated strand) &
brings it closer so it will cut the unmethylated strand. (It recognized by GATC)
After cut this fragment is removed & new
synthesized.

- Error correct so that will take it in its original position.
- MutS → (25-24) Fig.
MutH forms a complex with MutL. methylated strand. It forms a cut.
↓
DNA helicase removes the cut. (unwind
the cut) DNA = 
- DNA exonuclease activity (removes the cut piece.) = 
- Then DNA pol III synthesizes it.
↓
DNA ligase brings to join it.

(2) Base Excision Repair

- Removing of complete base. (Damaged base).
① By enzyme DNA glycosylase ↓
(that will cause a A-purinoid base,
means there's no nitrogenous base).
- ② A-P endonuclease protein specifically cut,
where A-purine base present.
↓

③ DNA pol-I remove short fragment, cut & add new nucleotide, so the Nick is sealed by DNA ligase.
(fig- 25-25).

• DNA glycosylase has the ability to recognise damaged base.

Nucleotide excision Repair (25-26 fig).

• Removing of all nucleotide where the lesion (error) is occur. Example:

• This Knick is removed by exonuclease.

(cut at on two sides). T pair.

• In bacteria removed. Knick is predicted.

approx. (13 bp) frag.

• In Eukaryotes - cut (29-30 bp) frag.

• Helicase act on it. So remove it.

• It syn. by DNA pol-I

• Knick sealed by DNA ligase.

④ Direct Repair:

• Carried out by enzyme Photolyase.

(• It required 2-main molecule for its activity. (1) Methyltetrahydrofolate quinonate (MTHF).

⑤ FADH₂)

• This enzyme is used light energy.

MTHF is going to activated by your photon.

This MTHF activates FADH.

FADH gives their electron to thymine dimer

Due to these electron it breaks bond between (T-T) Thymine dimer.

So then Thymine dimer in its original position. (Fig 25-27).

- By Methyltransferase:

- Methylation \rightarrow Demethylation
- removing of methyl group
- Methyltransferase enzyme required.

(Convert active protein \rightarrow Inactive) by methylation.

It takes its methyl group & binds with protein (Enzyme protein).

Most expensive post process in term of protein.

After this process protein completely inactivated (methylated).