

Nucleic acid Metabolism

Nucleic acids are the polymers of nucleotides (polynucleotides) held by 3' & 5' phosphate bonds.

nucleotides are composed of a nitrogenous base, a pentose sugar & a phosphate. (nucleoside - base + sugar)
nucleotide - nucleoside + phosphate

→ Beside being the building blocks in the nucleic acids, they act as structural components of some coenzymes of B-complex vitamins (FAD, NAD⁺), in the energy reactions of cell.

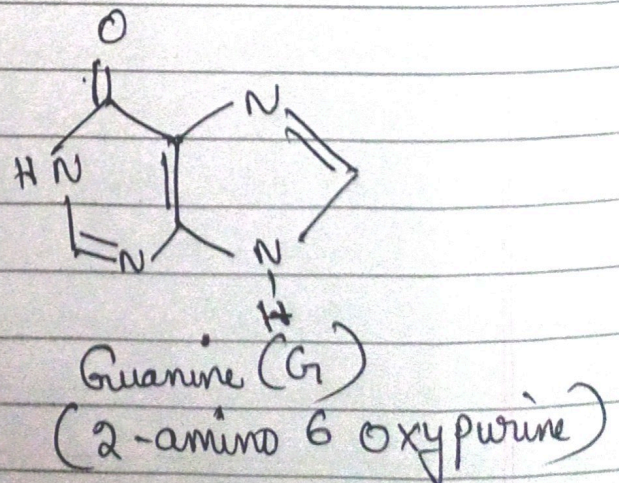
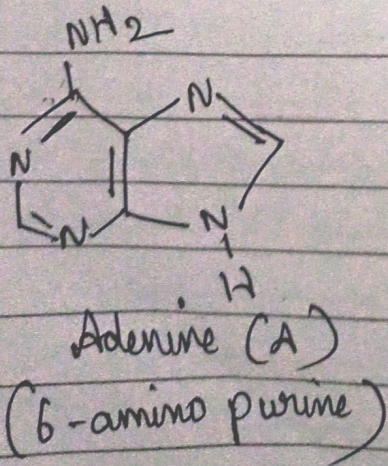
Purines & pyrimidines:-

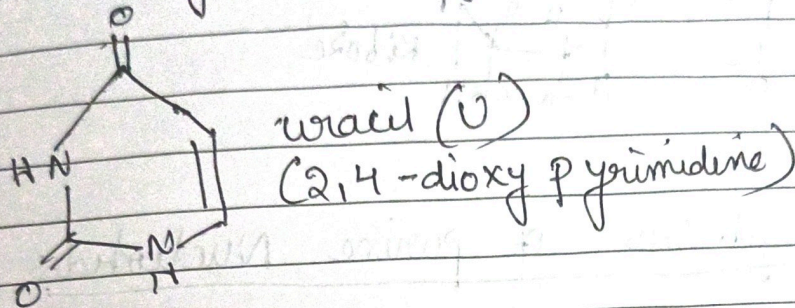
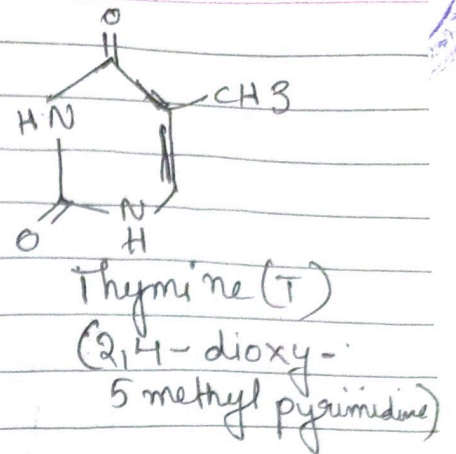
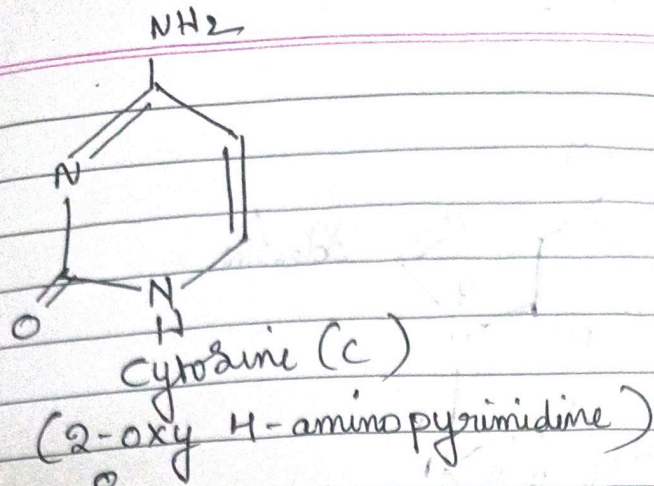
Nitrogenous base found in the nucleotides are aromatic heterocyclic compounds. Bases are of two types - Purines & pyrimidines.

DNA & RNA contain same purines - Adenine, guanine
Pyrimidines - cytosine - DNA & RNA

Second pyrimidine DNA - Thymine.
RNA - uracil.

→ The heterocyclic rings of purines & pyrimidines with oxo(-C=O) exhibit tautomerism.





If the sugar is a ribose in a nucleoside then a ribonucleoside is formed e.g. Adenosine, guanosine, cytidine & uridine are the ribonucleosides of A, G, C & U.

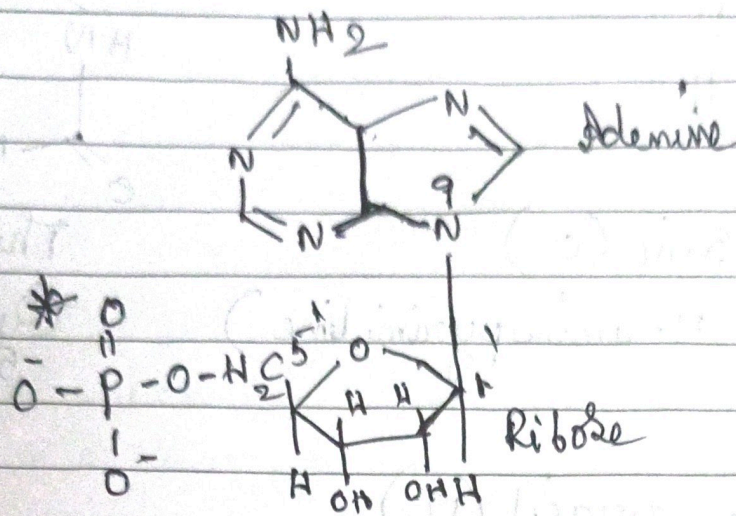
If the sugar is deoxyribose, then deoxyribonucleosides are formed.

→ Mononucleotide - Single phosphate moiety is added to nucleoside. e.g. - AMP Adenosine monophosphate
~~Adenosine~~ → Adenine + Ribose + phosphate

The pentoses are bound to nitrogenous bases by β -N-glycosidic bonds. N⁹ of a purine ring binds with C₁ of a pentose sugar to form a covalent bond. In pyrimidine nucleosides the covalent bond is formed b/w N¹ of pyrimidine & C₁ of a pentose.

The hydroxyl groups of adenosine are esterified with phosphates to produce 5' & 3' monophosphates.
e.g. AMP - adenosine 5' Monophosphate

Eg:- AMP

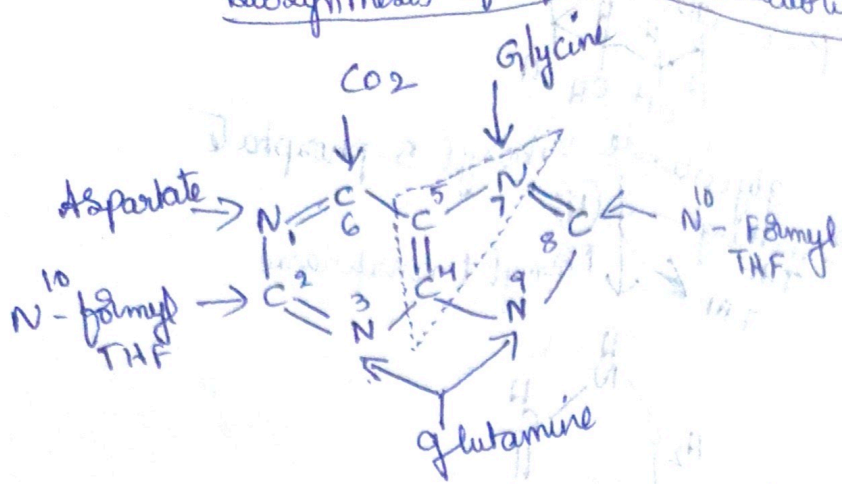


Biosynthesis of purine Nucleotides

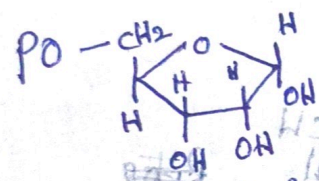
Protein synthesis
 Replication, types, errors
 DNA Repair, features
 Genetic code, features



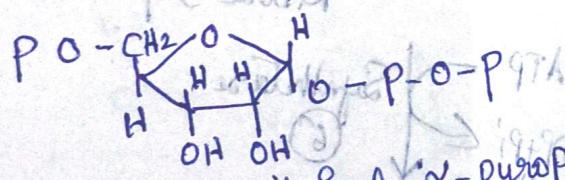
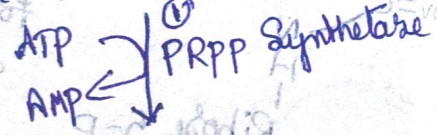
Biosynthesis of Purine Ribonucleotides



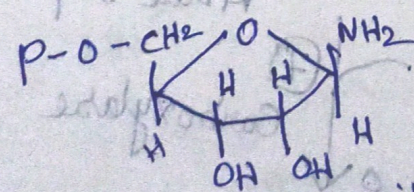
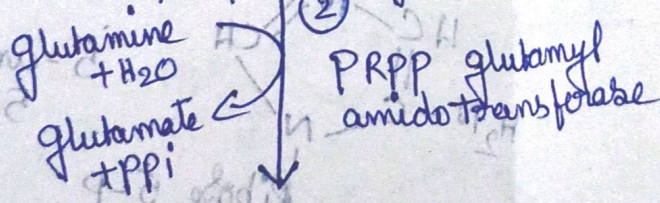
1. N₁ of purine is derived from amino group of aspartate.
2. C₂ & C₈ arise from formate of N¹⁰ formyl THF.
3. N₃ & N₉ are obtained from amide group of glutamine
4. C₄, C₅, & N₇ are contributed by glycine
5. C₆ directly comes from CO₂



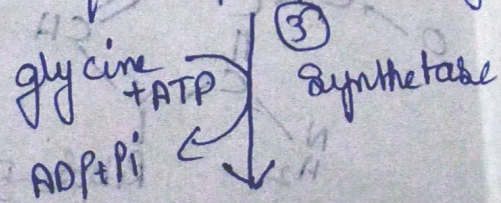
α -D-Ribose-5-phosphate

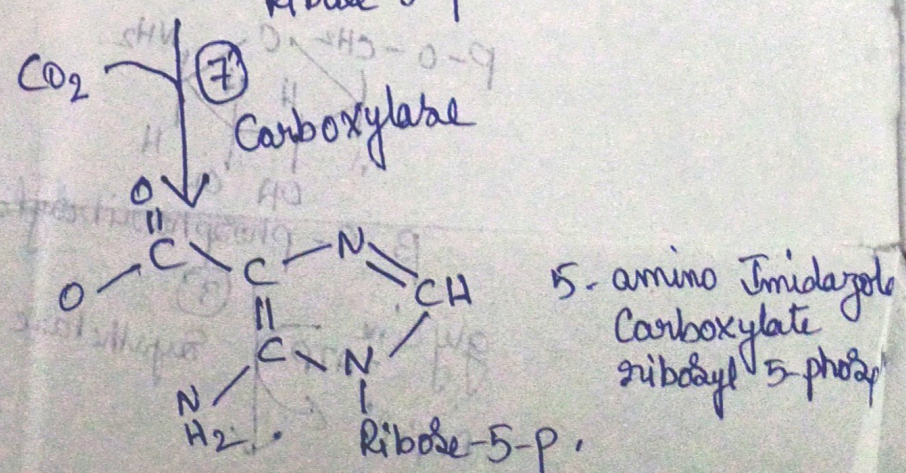
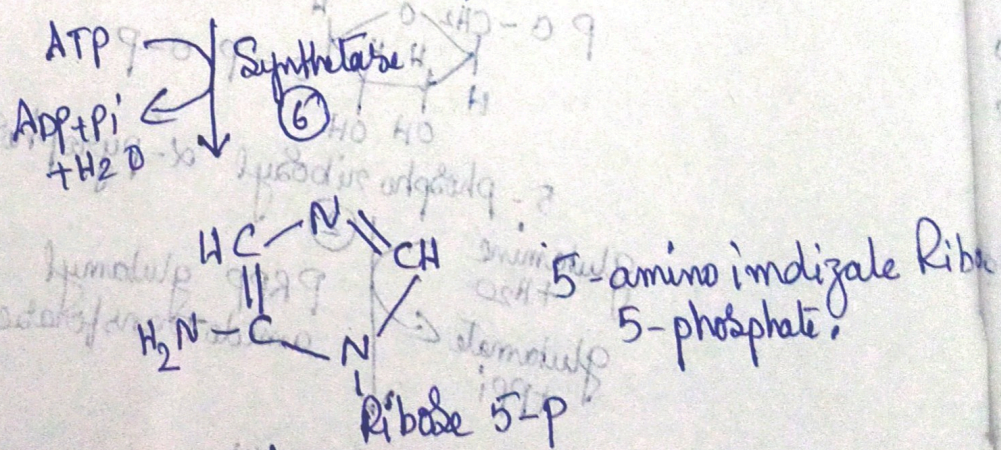
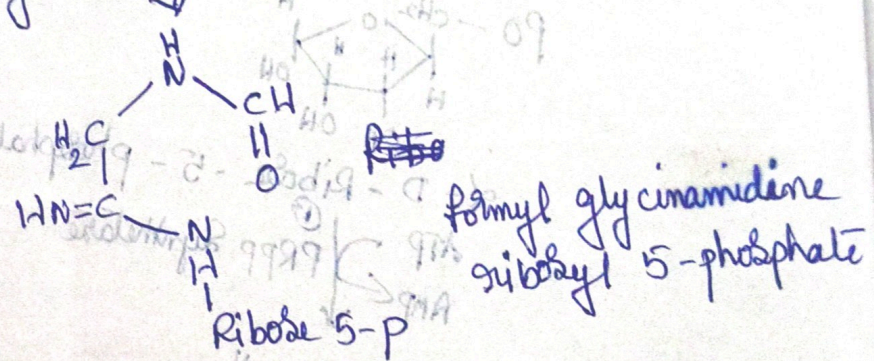
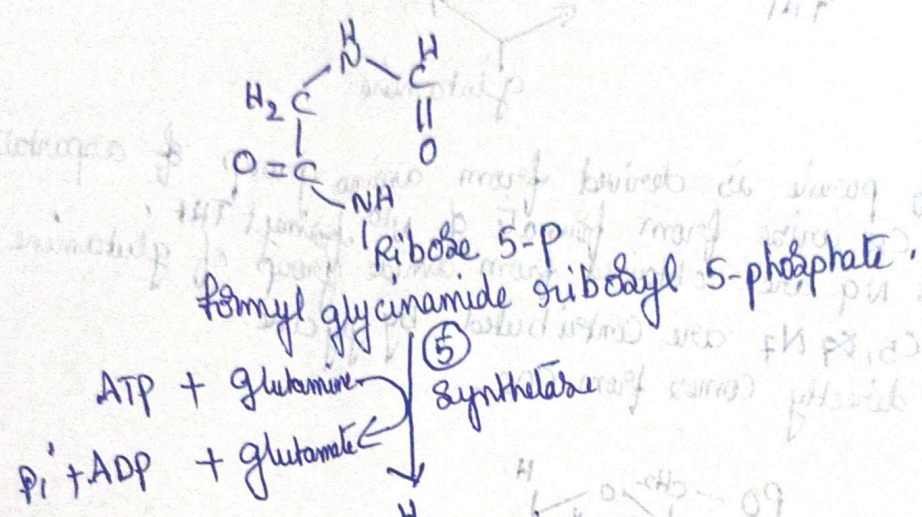
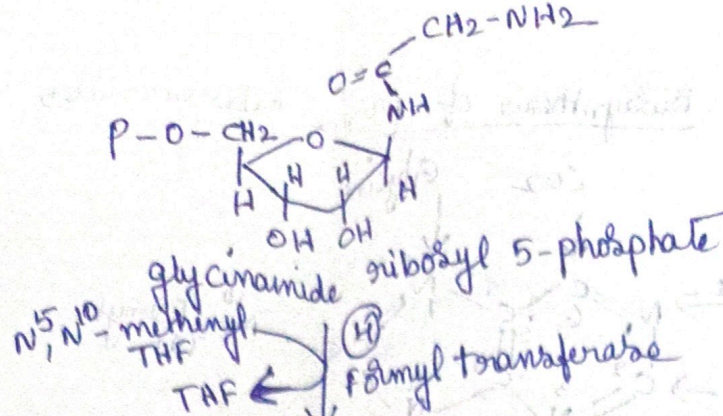


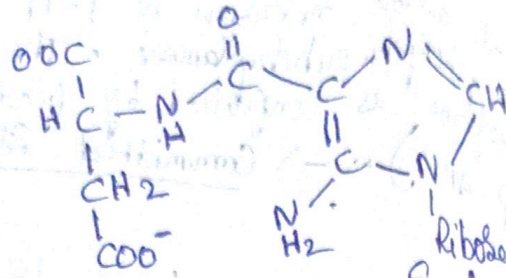
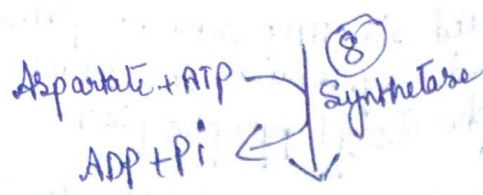
5-phosphoribosyl α -pyrophosphate



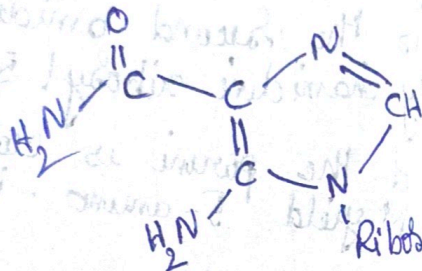
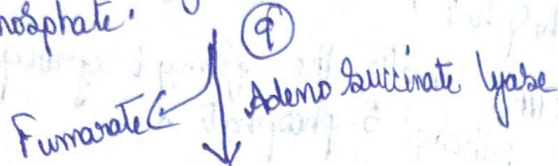
β -5-phosphoribosylamine



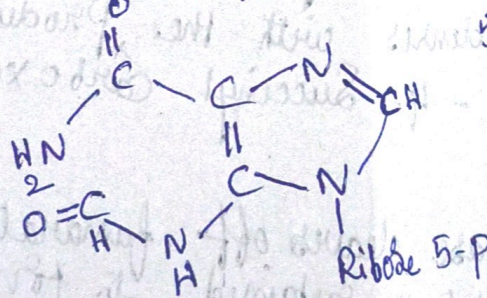
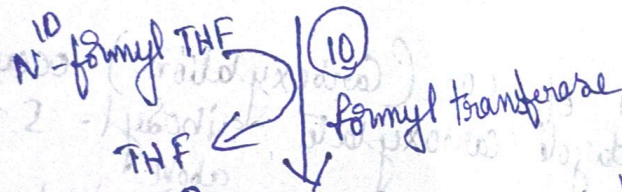




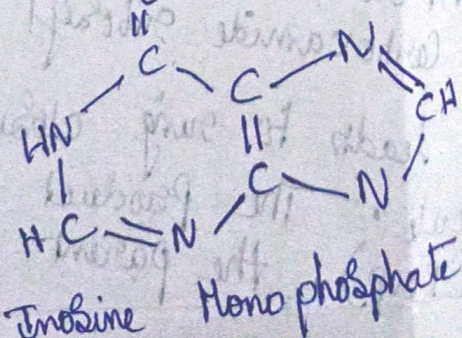
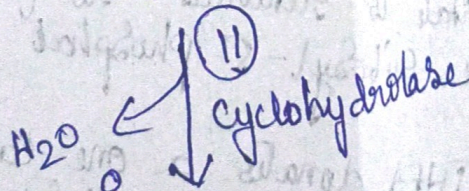
5-aminoimidazole-4-Succinyl Carboxamide ribosyl 5-phosphate



5-aminoimidazole 4-Carboxamide ribosyl 5-phosphate



5-formaminoimidazole 4-Carboxamide ribosyl-5-phosphate



Inosine Mono phosphate

imidine phosphate

azole Rib

imidazole 5-phosphate

- ① Ribose-5-phosphate produced in HMP shunt pathway is the starting material for purine synthesis. It reacts with ATP to form PRPP - phosphoribosyl pyrophosphate.
- ② Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate & produce 5-phosphoribosylamine. The enzyme PRPP glutamyl amidotransferase is controlled by feedback inhibition of nucleotides (IMP, AMP). $\rightarrow$ "Committed Step"
- ③ Phosphoribosylamine reacts with glycine to form glycinamide ribosyl 5-phosphate.
- ④ N^{10} -formyl THFA donates the formyl group to form formyl-glycinamide ribosyl 5-phosphate.
- ⑤ Glutamine transfers the second amide amino group to produce formylglycinamide ribosyl 5-phosphate.
- ⑥ The imidazole ring of the purine is closed in an ATP-dependent reaction to yield 5-amino-imidazole ribosyl-phosphate.
- ⑦ Incorporation of CO_2 (Carboxylation) occurs to yield aminoimidazole carboxylate ribosyl-5-phosphate.
- ⑧ Aspartate condenses with the ^{above} product to form aminoimidazole - 4-succinyl carboxamide ribosyl-5-phosphate.
- ⑨ Adenosuccinate lyase cleaves off fumarate & only the amino group of aspartate is retained to form aminoimidazole 4-carboxamide ribosyl-5-phosphate.
- ⑩ N^{10} -formyl THFA donates a one-carbon moiety to produce formylaminoimidazole 4-carboxamide ribosyl 5-phosphate.
- ⑪ Enzyme $\times$ Cyclohydrolase leads to ring closure with an elimination of water molecule. The product so formed is Inosine Monophosphate (IMP), the parent purine nucleotide.

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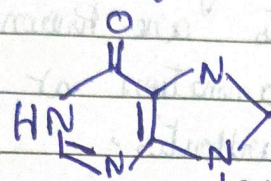
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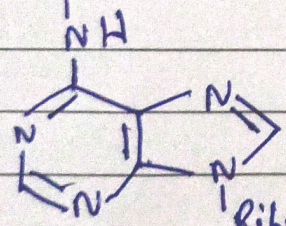
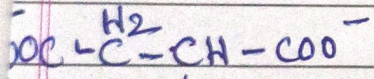
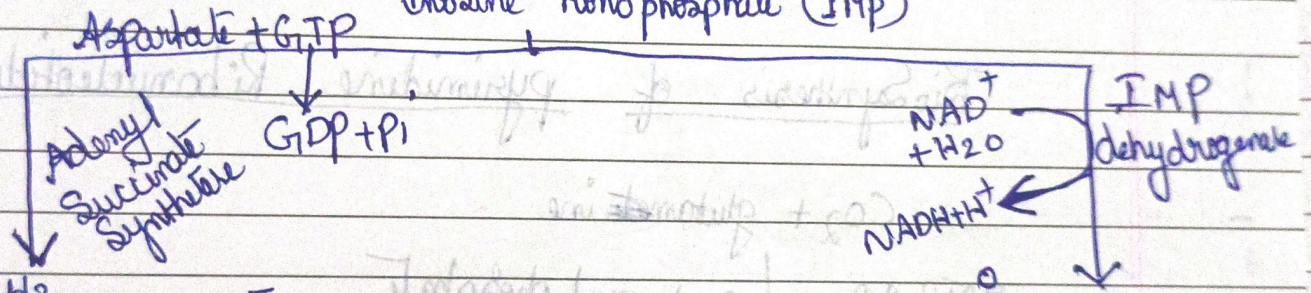
Synthesis of AMP & GMP from IMP

Inosine Monophosphate is the immediate precursor for the formation of AMP & GMP. Aspartate Condenses with IMP in the presence of GTP to produce adenyly Succinate which on cleavage forms AMP.

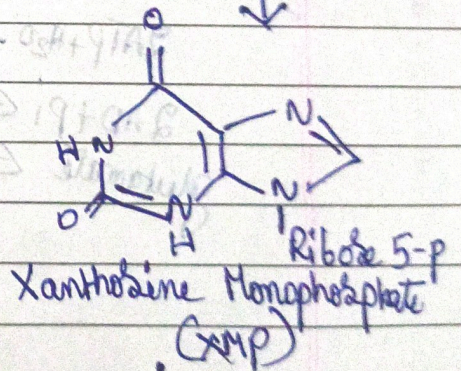
IMP undergoes NAD^+ dependent dehydrogenation to form Xanthosine Monophosphate. Glutamine then transfers amide nitrogen to XMP to form GMP.



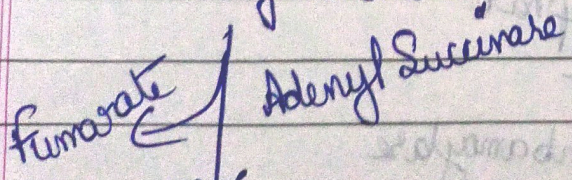
Inosine Monophosphate (IMP)



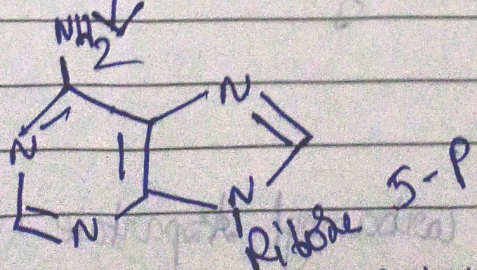
Adenyly Succinate



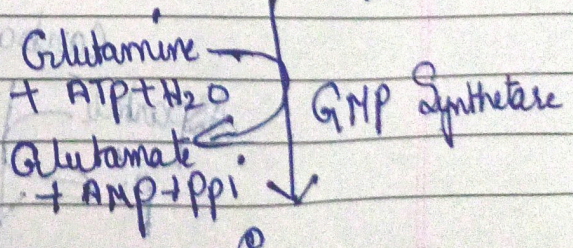
Xanthosine Monophosphate (XMP)



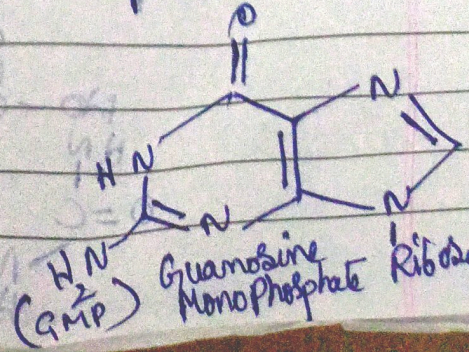
Fumarate



Adenosine Monophosphate (AMP)



Glutamate + AMP + P_i



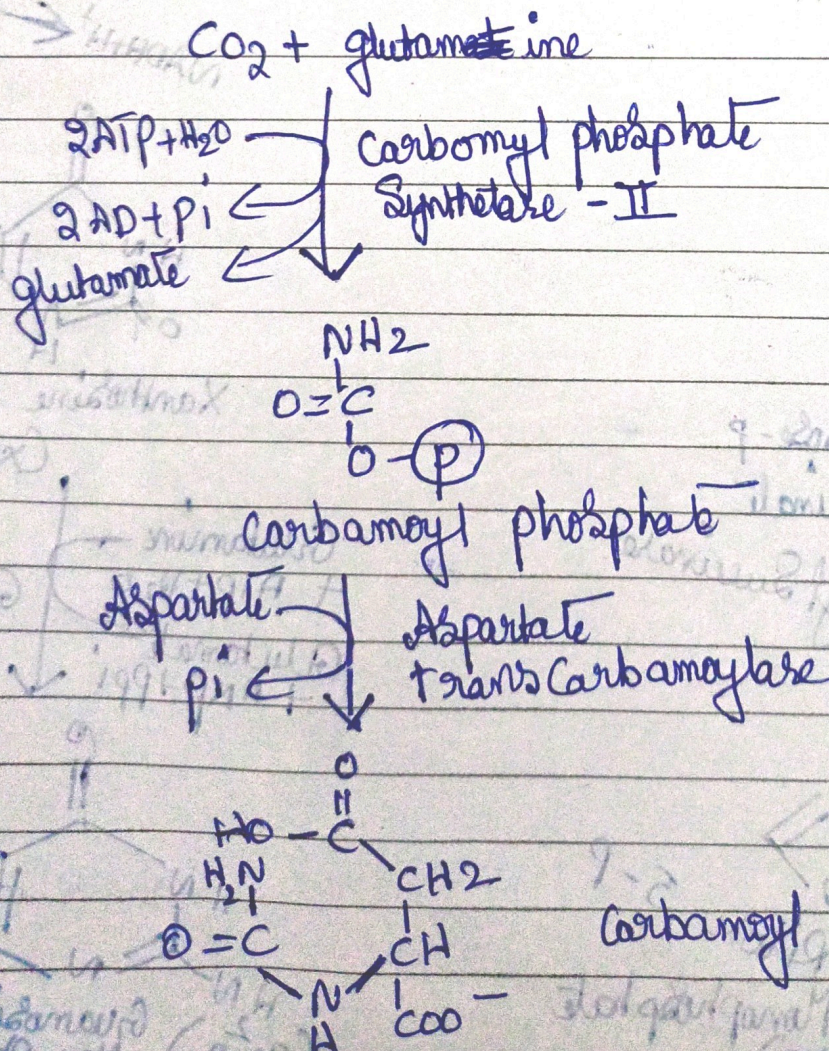
Guanosine Monophosphate (GMP)

"Gout" - (2M)

Uric acid is the end product of purine metabolism in humans. The normal concentration of uric acid in serum ranges from 3-7 mg/dl.

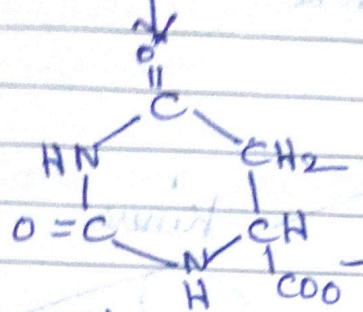
"Gout" is a metabolic disease associated with overproduction of uric acid. At the physiological pH, uric acid is found more soluble form as sodium urate. But in Hyperuricaemia crystals of sodium urate get deposited in soft tissues at joints. Such deposits are known as "Tophi". This deposition produces inflammation at the joints resulting in a painful gouty arthritis.

BioSynthesis of pyrimidine Ribonucleotides



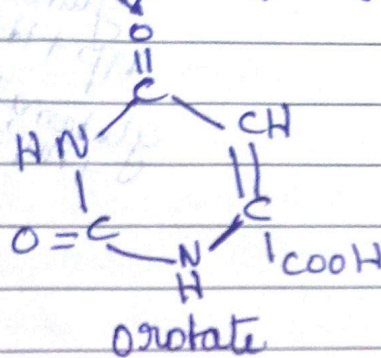
Carbamoyl Aspartate

$H_2O \leftarrow$ Dihydro Orotate

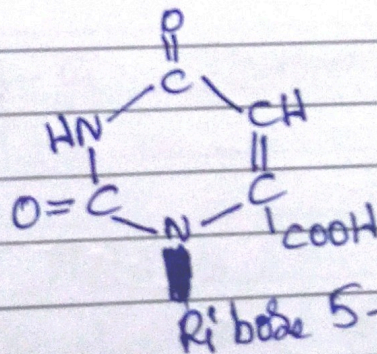


Dihydro orotate

$NAD^+ \rightarrow NADH + H^+$ Dihydro orotate dehydrogenase

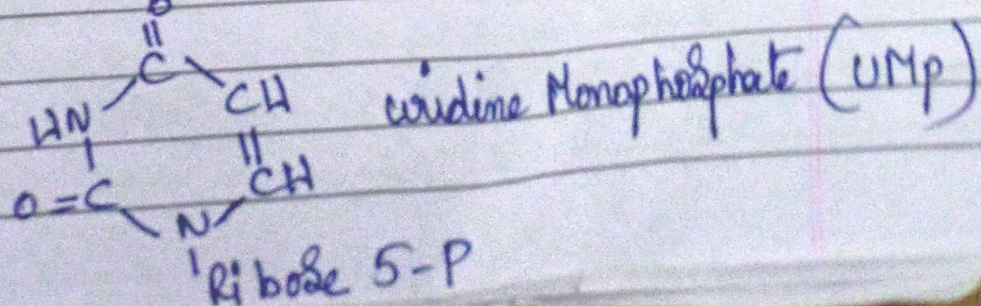


$PRPP \rightarrow PP_i$ Orotate phosphoribosyl transferase

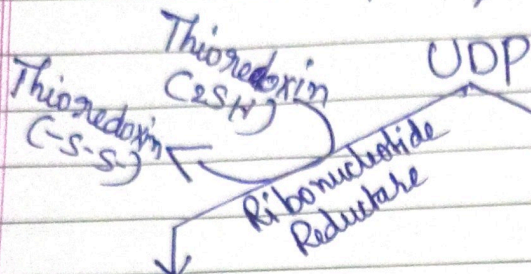
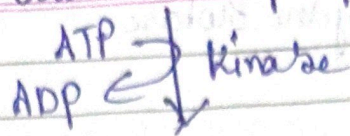


orotidine Monophosphate (OMP)

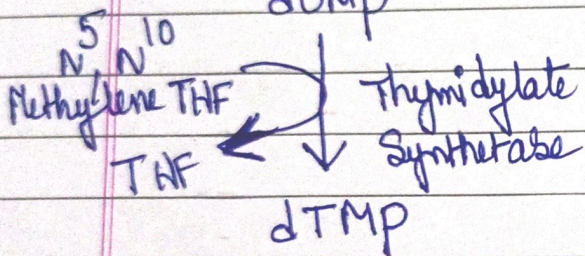
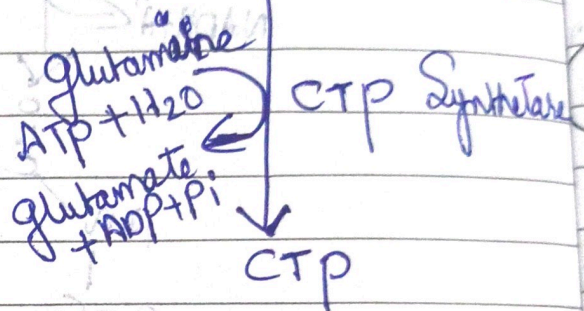
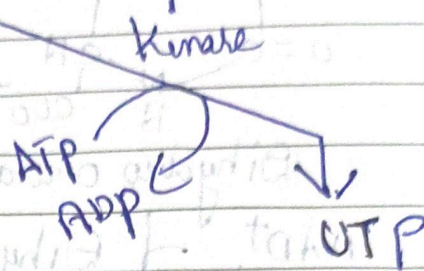
$CO_2 \leftarrow$ OMP decarboxylase



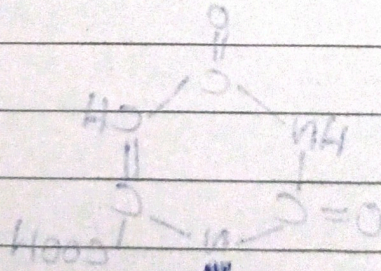
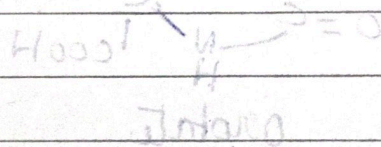
Uridine Monophosphate (UMP)



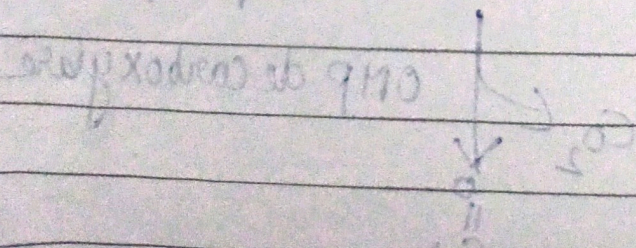
Deoxy uridine diphosphate (dUDP)



$\rightarrow$



(910) ...



Mutations :-

The ^q Mutations refers to a change in the DNA structure of a gene. The substances which can induce mutations are collectively known as Mutagens.

These mutations are replicated in replication, transcription & translation.

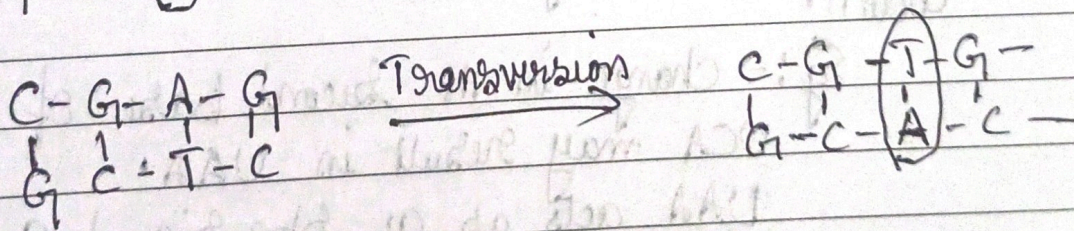
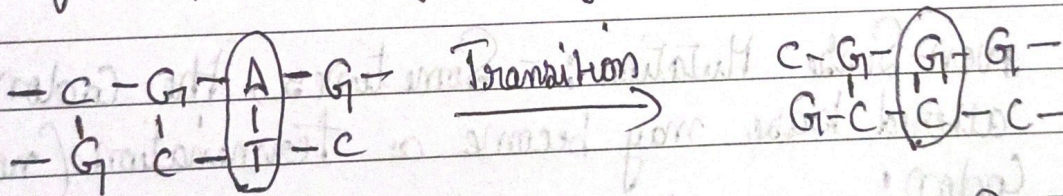
Types of Mutations :- Mutations mainly are of two types:-

① point mutations & frame shift mutations.

Point Mutations :- The replacement of one base pair by another results in point mutations.

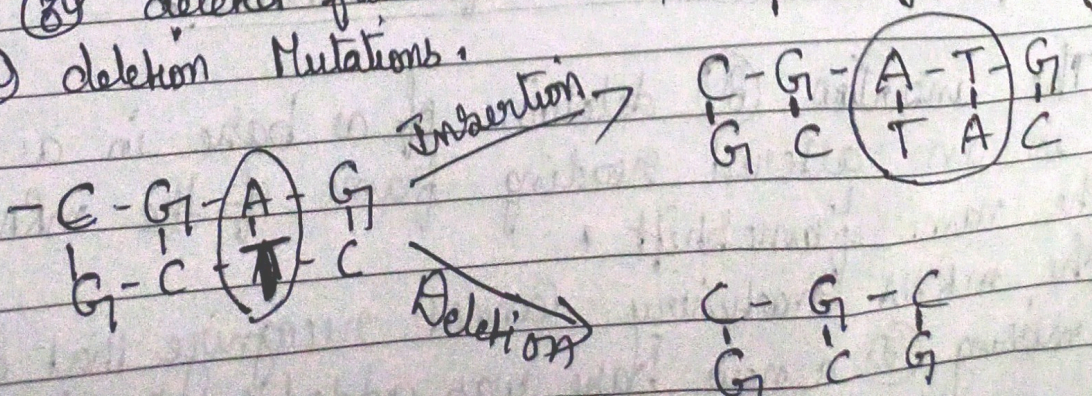
a) Transitions :- In this case, a purine (or pyrimidine) is replaced by another.

b) Transversions :- These are characterized by replacement of a purine by a pyrimidine @ vice versa.



Frame Shift Mutations :-

These occur when 1. More base pairs are inserted in 2. deleted from the DNA, respectively, causing insertion & deletion mutations.



Consequences of point Mutations :-

- ① ~~Silent~~ Silent Mutation :- The Codon (of mRNA) containing the changed base may code for the same amino acid.
for instance UCA codes - Serine } $\rightarrow$ This is due to degeneracy of genetic code.
change in 3rd base UCU^{SHV} codes - Serine }

So, therefore, there are no detectable effects in silent mutation.

- ② Missense Mutation :- In this case, the changed base may code for a different amino acid.

for Eg:- UCA codes - Serine
while ACA codes - Threonine

This mistaken amino acid may be acceptable, partially acceptable or unacceptable with regard to the function of protein molecule.
Eg:- Sickle Cell Anaemia.

- ③ non-Sense Mutation :- Sometimes, the Codon with the altered base may become a Termination (non Sense) Codon.

Eg:- Change in the second base of Serine Codon UCA may result in UAA.

UAA acts as a Stop signal & causes termination of protein Synthesis.

Consequences of Frame Shift Mutations :-

The insertion & deletion of a base in a gene results in an altered reading frame of the mRNA hence the name frameshift.

The mRNA machinery cannot recognize that a base is missing & a new base was added, the translation

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Continuous & results in altered protein synthesis with altered amino acids (a) premature termination of protein synthesis

Mutations & Cancer

These are permanent alterations in DNA structure, which are responsible for the pathogenesis of cancer.

Genetic Code :-

The three nucleotide (triplet) base sequences in mRNA that act as code words for amino acids in proteins form the genetic code.

- It is regarded as dictionary of nucleotide bases (A, G, C & U) that determines the sequences of amino acids in proteins.
- These four bases (A, G, C & U) produce 64 ~~amino acids~~ different combinations (4^3) of three base codons.
- The nucleotide sequence on mRNA is written from 5' end to 3' end.

→ 61 Codons code for 20 amino acids found in proteins.

→ UAA, UAG, UGA do not code for amino acids. They act as Stop Codons. → Stop Signals in protein synthesis. They are collectively known as Termination Codons @ non-Sense Codons.

→ UAG, UAA & UGA → referred as amber, ochre & opal Codon.

→ Codons AUG - GUG are initiating Codons.

characteristics of genetic Code :-

The genetic code is universal, Specific, non-overlapping & degenerate.

① universality :- The same Codons are used to code the same amino acids in all the living organisms. Thus, the genetic code is appropriately regarded as universal. However, ~~there~~ there are few exceptions.

Eg :- AUA - Codes Methionine in Mitochondria
AUA - Codes Isoleucine in cytoplasm.

② **Specificity** :- A particular Codon always codes for the same amino acid, hence the genetic code is highly specific.
Eg:- UGG - Tryptophan.

③ **Non-overlapping** :- The genetic code is read from a fixed point as a continuous base sequence.
It is non-overlapping, with out any punctuations.
For instance - UUCUUAAGAGGG

This is read as UUC | CUU | AGA | GGG.

Addition @ deletion of one @ two bases will radically change the message sequence in m-RNA & the protein synthesized from such m-RNA will be different & is commonly seen with frame shift mutations.

④ **Degenerate** :- Most of the amino acids have more than one codon.

Eg:- There are 61 Codons available for 20 amino acids.
For instance glycine can be coded by 4 codons.

So, those Codons which code same amino acids are synonyms. This is called degeneracy & mostly the Codons differ only in the third end (3' end) base of the codon.

DNA Replication

Replication is a process in which DNA copies itself to produce identical daughter molecules of DNA.

SemiConservative Method:-

The parent DNA has two strands Complementary to each other. Both the strands undergo simultaneous replication to produce two daughter molecules. Each one of the newly synthesized DNA has one-half of the parental DNA (one strand from original) & one half of new DNA. This type of replication is known as SemiConservative since half of the original DNA is conserved in the daughter DNA.

Initiation of Replication:-

The initiation of DNA Synthesis occurs at a site called Origin of Replication. In case of prokaryotes, only single site whereas in eukaryotes, there are multiple sites of origin. These sites mostly consist of a short sequence of A-T base pairs. A specific protein called dna A (20-50 monomers) binds with the site of origin for replication. This causes the double stranded DNA to separate.

Replication bubbles:-

The two complementary strands of DNA separate at the site of replication to form a bubble. Multiple replication bubbles are formed in eukaryotic DNA molecules.

RNA primer:-

For the synthesis of new DNA, a short fragment of RNA (5-50 nucleotides) is required as a primer. The enzyme primase along with a single stranded binding protein forms a complex called primosome & produces RNA primer.

The replication of DNA occurs in 5' to 3' direction simultaneously on both the strands of DNA. On one strand the DNA synthesis is continuous (leading strand) & on the other it is discontinuous (lagging strand) (20 Retrograde).

In the replication bubble, the DNA synthesis occurs in both the directions from the point of origin.

Replication Fork & DNA Synthesis:-

The separation of the two strands of parent DNA results in the formation of a replication fork. The active synthesis of DNA occurs in this region. The replication fork moves along the parent DNA as the daughter DNA molecules are synthesized.

DNA helicases:- These enzymes bind to both the DNA strands at the replication fork. Helicases move along the DNA helix & separate the strands. Their function is comparable with a zip opener.

DNA Synthesis Catalyzed by DNA Polymerase III:-

The synthesis of a new DNA strand catalysed by DNA polymerase III, occurs in 5' $\rightarrow$ 3' direction. This is antiparallel to all the parent template DNA strand. The presence of all the four deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, dTTP) is an essential for replication to take place.

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The Synthesis of two new DNA strands, simultaneously takes place in the opposite direction - one is in a direction ($5' \rightarrow 3'$) towards the replication fork which is continuous, the other in a direction ($5' \rightarrow 3'$) away from the replication fork.

The incoming nucleotides are added one after another to 3' end of the growing DNA chain. A molecule of pyrophosphate (PP_i) is removed with the addition of each nucleotide. The base sequence of the newly synthesized complementary DNA is determined by template DNA.

Okazaki fragments :- The small fragments of the discontinuously synthesized DNA are called Okazaki pieces. These are produced on the lagging strand of the parent DNA.

They are later joined by DNA Polymerase-I & DNA ligase.

proof - Reading activity :-

DNA Polymerase III also has a proof reading activity. It checks the incoming nucleotides & allows only correctly matched bases to be added to the growing DNA strand. It edits the mistakes & removes the wrongly placed nucleotide bases.

Replacement of RNA primer by DNA :-

DNA Polymerase I removes the RNA primer & takes the position. DNA Polymerase I catalyzes the synthesis of a fragment of DNA ($5' \rightarrow 3'$) that replaces RNA primer.

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The enzyme DNA ligase catalyzes the formation of a phosphodiester linkage between the DNA synthesized by DNA polymerase I.

DNA polymerase - II, Participates in the DNA repair process.

Supercoils & DNA topoisomerases: -

As the double helix of DNA separates from one side & replication proceeds, supercoils are formed at the other side. Type - I DNA topoisomerase cuts the single DNA strand to overcome the problem of supercoils & then reseals the strand.

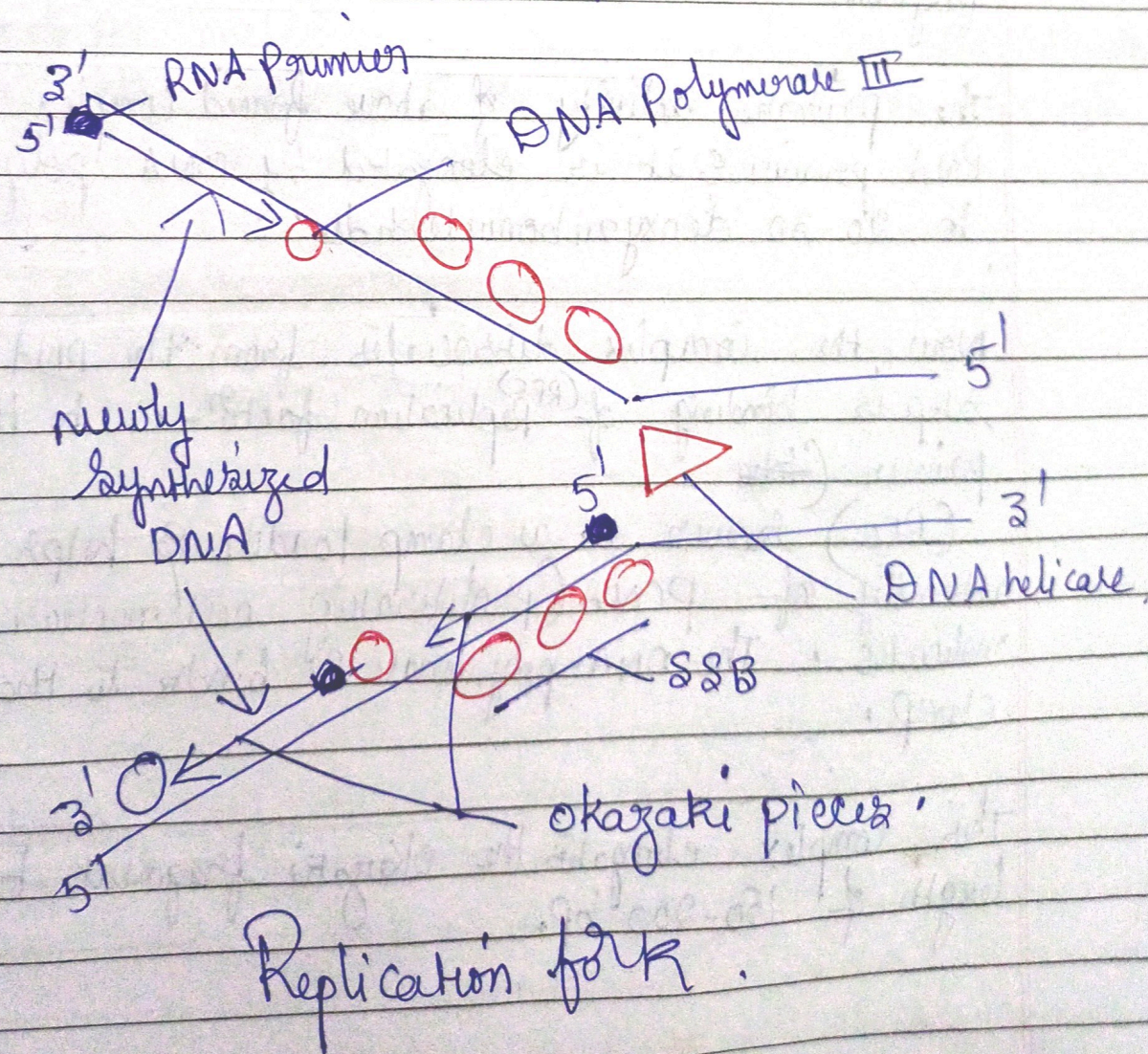
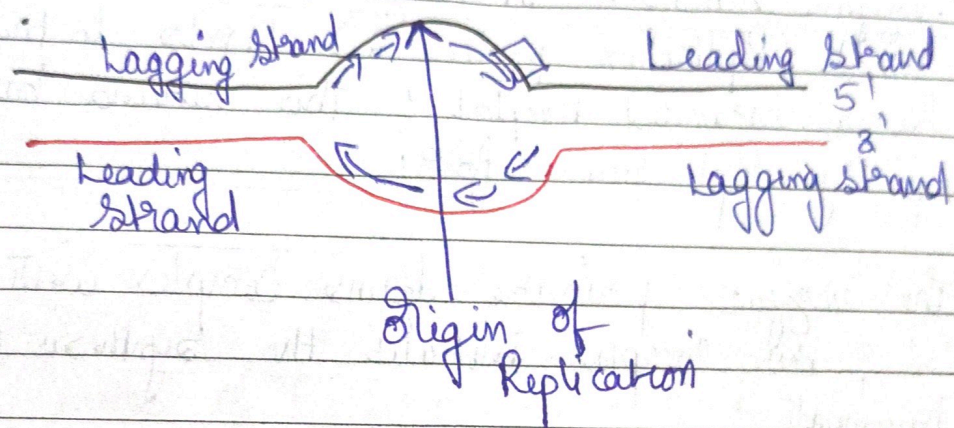
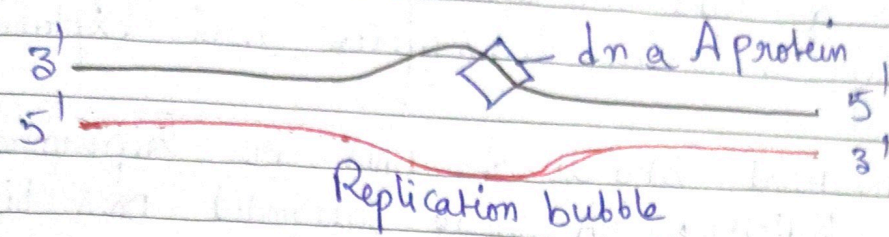
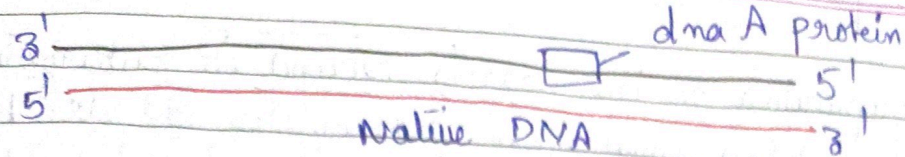
Type - II DNA topoisomerase cuts both strands & reseals to overcome the problem of supercoils.

Replication in Eukaryotes: -

Replication of DNA in Eukaryotes closely resembles that of prokaryotes. Multiple origins of replication is a characteristic feature of Eukaryotic cells & there are five distinct DNA polymerases known in Eukaryotes.

function

- ① DNA polymerase α - Synthesis of RNA primers
- ② DNA polymerase β - Repair of DNA.
- ③ " " γ - Replication of mitochondrial DNA.
- ④ " " δ - DNA replication, proof reading.
- ⑤ " " ϵ - proof reading of DNA.



process of Replication in Eukaryotes

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The replication on the lagging strand in Eukaryotes is more complex when compared to prokaryotes. Even the leading strands of Eukaryotes, the process of replication on lagging strand in Eukaryotes was explained below.

The parental strands of DNA are separated by the enzyme helicase. A single stranded DNA binding protein called (Replication protein A) binds to the exposed single stranded template. This strand has been opened up by Replication fork.

The enzyme primase forms complex with DNA polymerase α & this complex initiates the synthesis of Okazaki fragments.

The primase activity of above formed complex synthesizes RNA primers & it is elongated by DNA polymerase α to 20-30 deoxyribonucleotides.

Now, the complex dissociates from the DNA. The next step is binding of (RFC) Replication factor - C to the elongated primer. (~~the~~)

(RFC) serves as a clamp loader & helps in the assembly of PCNA (proliferating cell nuclear antigen) molecules. The DNA polymerase δ binds to the sliding clamp.

This complex elongates the Okazaki fragment to a final length of 150-200 bp.

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The elongated fragment now snatches the RNA primer of previous Okazaki fragment.

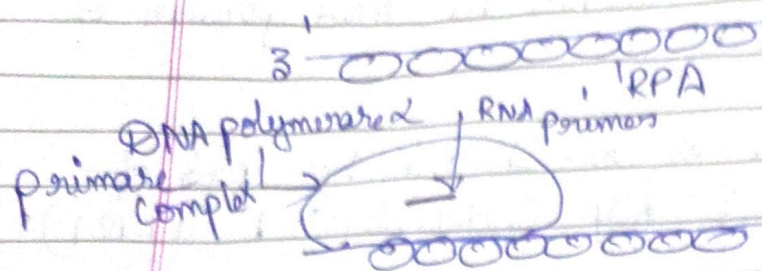
The RNA primer removal is carried out by pair of enzymes namely RNase H & flap Endonuclease-1 (FEN1).

The gap created by the removal of RNA primer is filled up by continuous elongation of new Okazaki fragment.

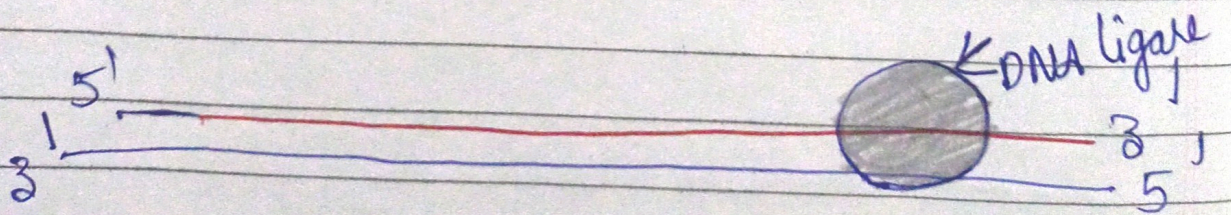
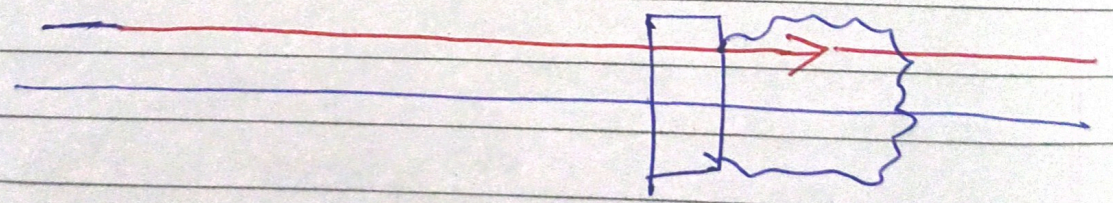
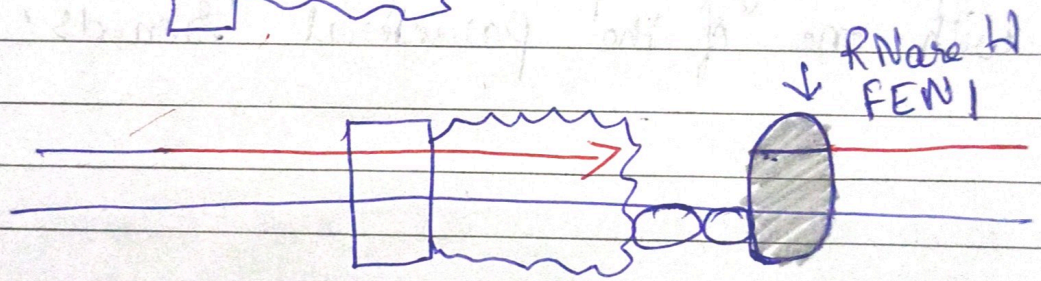
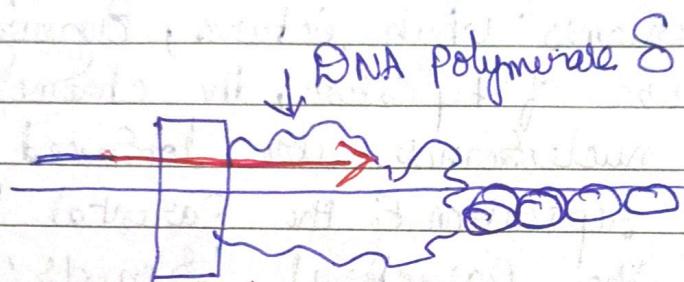
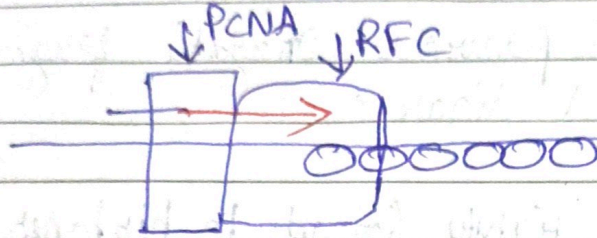
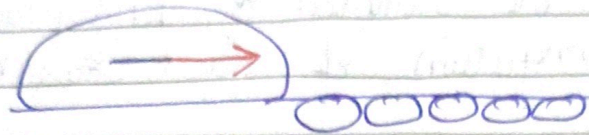
Finally the new & previous Okazaki fragments are sealed by DNA ligase.

Eukaryotic DNA is tightly bound to histones (basic proteins) to form nucleosomes which in turn, organize into chromosomes. During the course of replication, the chromosomes are relaxed & the nucleosomes are loosened. The DNA strands separate for replication & the parental histones associate with one of the parenteral strands.

Cor.
8x



3' 5'
↓ Template for lagging strand
RNA primers



Repair of DNA

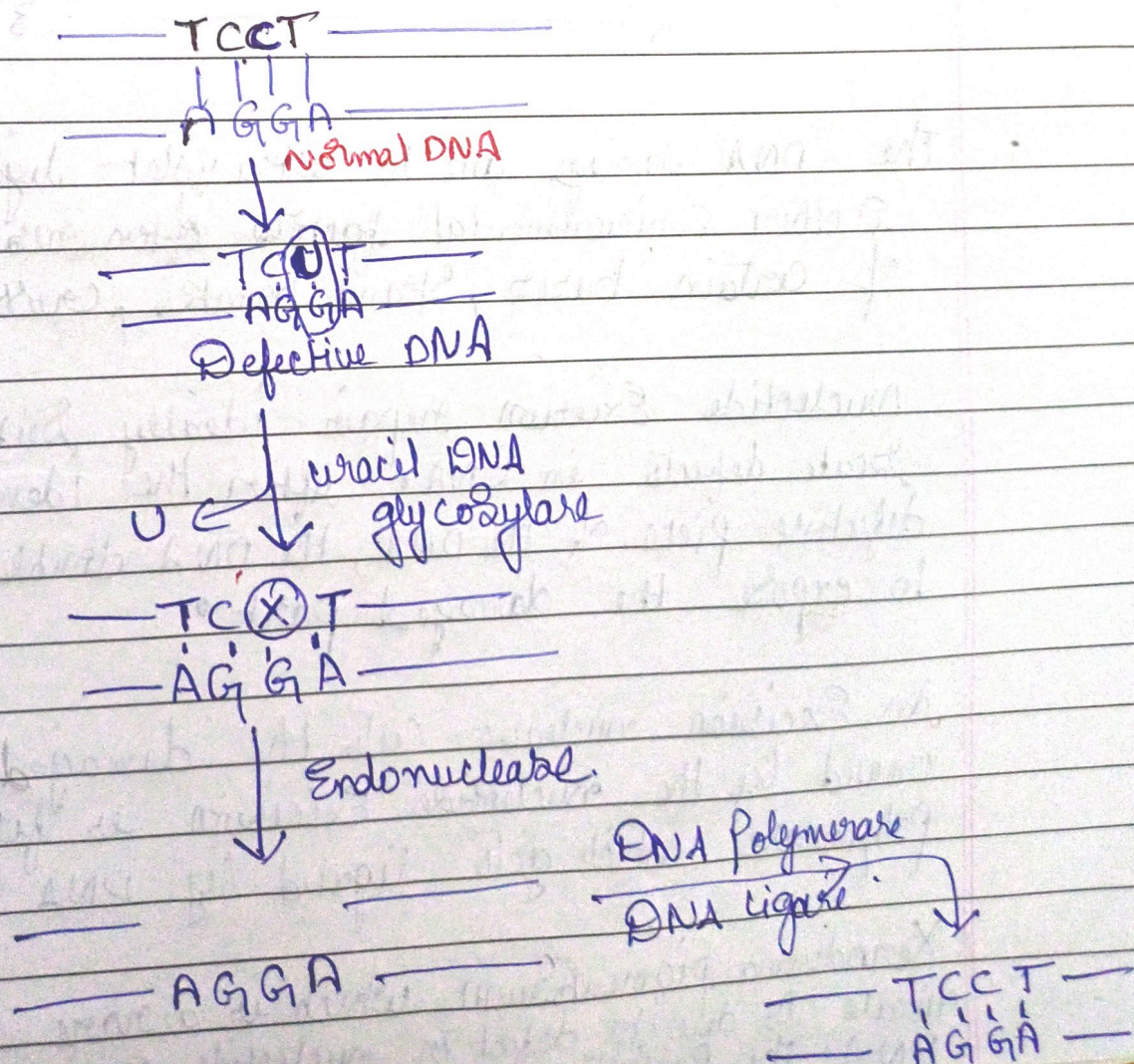
Damage to DNA caused by replication errors & mutations may have serious consequences.

The cells possess an inbuilt system to repair the damaged DNA. The repair mechanisms are of four types:-

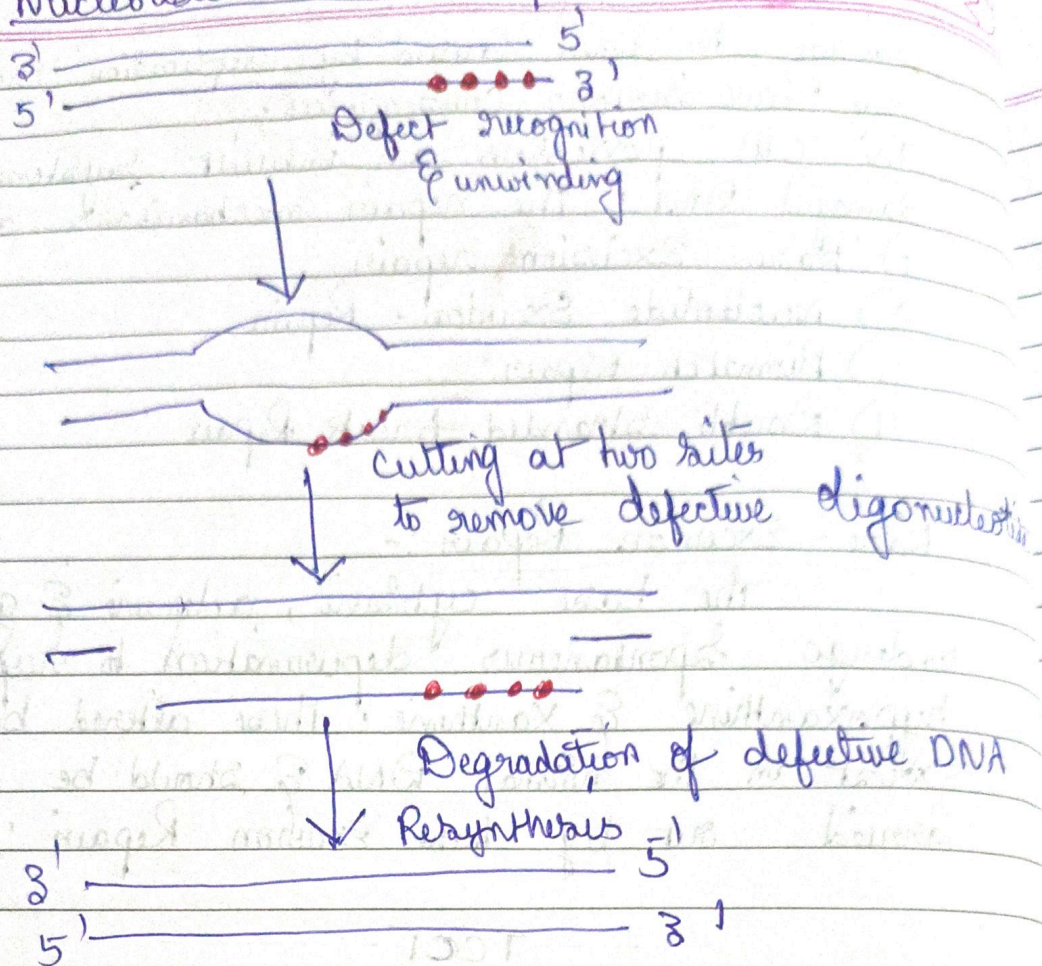
- 1) Base-Excision repair
- 2) Nucleotide Excision - Repair
- 3) Mismatch Repair
- 4) Double Stranded break Repair

Base-Excision Repair :-

The base cytosine, adenine & guanine can undergo spontaneous depurination to respectively form uracil, hypoxanthine & xanthine. These altered bases do not exist in the normal DNA & should be removed. This is carried out by base excision repair.



Nucleotide Excision Repair



- The DNA damage due to ultraviolet light, ionizing radiation & other environmental factors often results in the modification of certain bases, strand breaks, cross-linkages etc.

Nucleotide Excision repair is ideally suited for such large scale defects in DNA. After the identification of the defective piece of the DNA, the DNA double helix is unwound to expose the damaged part.

An Excision nuclease cuts the damaged DNA & the gap created by the nucleotide excision is filled up by DNA polymerase which gets ligated by DNA ligase.

Xeroderma pigmentosum which is a rare autosomal recessive disease is due to defect in nucleotide excision repair of damaged DNA. The patients are photosensitive & are susceptible for skin cancer.

Mismatch Repair: -

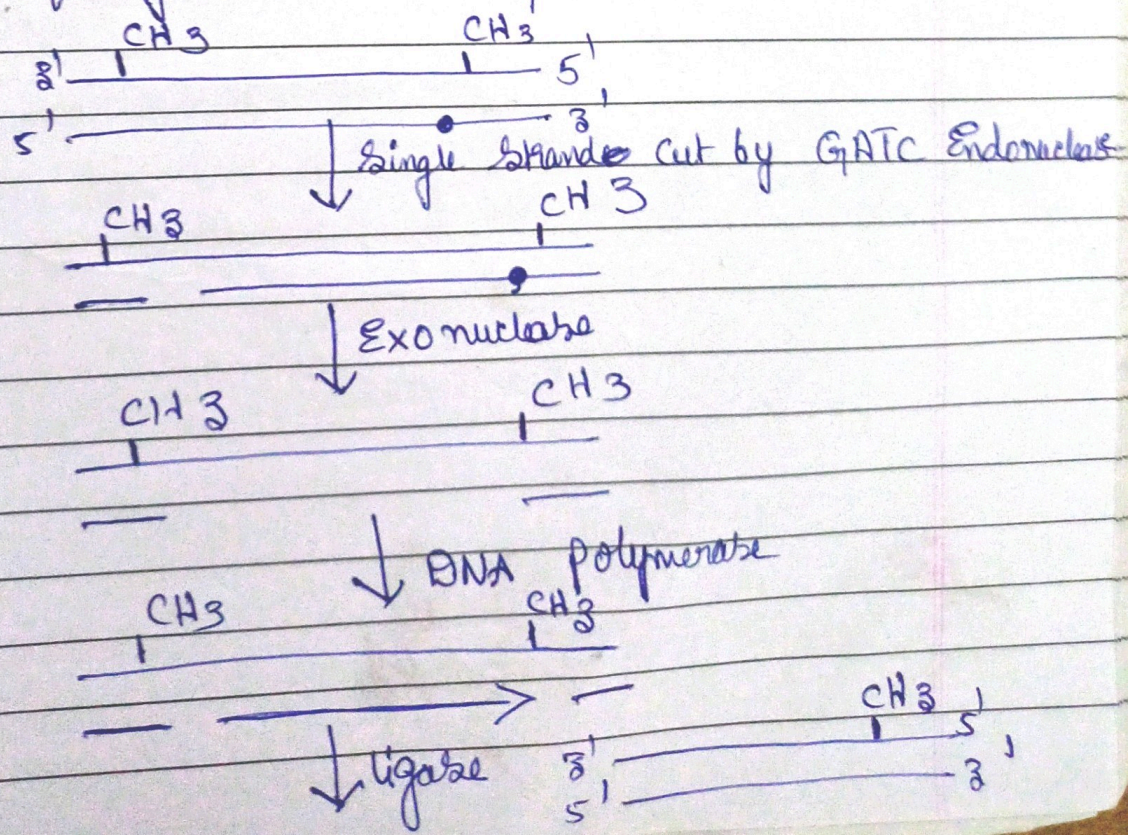
Despite high accuracy in replication, defects do occur when DNA is copied. For instance, cytosine (instead of thymine) could be incorporated opposite to adenine.

Mismatch repair corrects a single mismatch base pair eg:- C to A, instead of T to A.

The template strand of the DNA exists in a methylated form, while the newly synthesized strand is not methylated. This allows the recognition of new strands. The enzymes ~~GATC~~ GATC Endonucleases cut the strand at an adjacent methylated GATC sequence. This is followed by an exonuclease digestion of the defective strand, & thus its removal.

A new DNA strand is now synthesized to replace the damaged one.

Hereditary nonpolyposis colon Cancer (HNPCC) is one of the most common inherited cancers. This cancer is now linked with faulty mismatch repair.



Double - Strand break Repair

Double-strand breaks (DSBs) in DNA are dangerous. The result in genetic recombination which may lead to chromosomal translocation, broken chromosomes, & finally cell death.

DSBs can be repaired by homologous recombination (a) non-homologous end joining.