

Mechanism of DNA replication in eukaryotic cells :

The essential features of DNA replication are same in both eukaryotic and prokaryotes ~~are~~ has following differences :

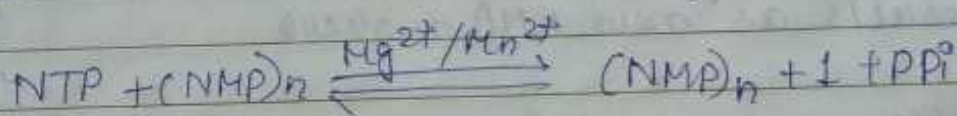
1. There are many origin of replication for each eukaryotic ~~and~~ chromosome. As it occurs at several point there are a no. of replication forks along the length of DNA.

2. There is a large no. of replication

DNA dependent RNA polymerase:

This enzyme was first characterised from rat liver by S. B. Weiss and extracts of E. coli by J. Hurwitz. In rat liver, enzyme is located in the cell nuclei. In plants it is associated with chromosomal DNA and in prokaryotes it occurs in cytoplasm and in eukaryote it also found in the nucleus.

The RNA poly from E. coli catalyzes the net synthesis of RNA with base sequence complementary to DNA strand and serve as template. It need no primer. The requirement of DNA dependent RNA poly are presence of the four ribonucleoside triphosphates: ATP, CTP, GTP, UTP by which divalent cation and RNA poly catalyze this reaction.



The enzyme from E. coli has been obtained in homogenous form by R. R. Saenger and his colleagues. The enzyme contains five separable unit of four types designated as α , β , β' and σ . The β' subunit is read for binding the RNA pol to the DNA template. β subunit is catalytic site. The σ sigma factor is correct to initiation of RNA synthesis.

2. The point on which DNA molecule from which transcription is start and stop:

It means binding of RNA pol to promoter, initiation, elongation and termination as like replication.

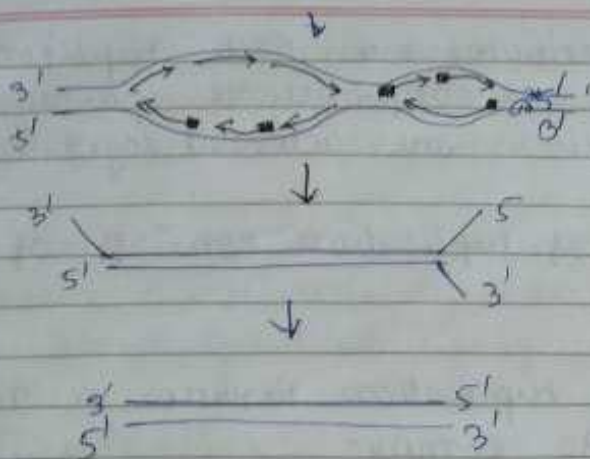


Fig: Diagram of DNA replication

Mutation:

Mutation is hereditary change in the genetic make up of an individual other than that which may be caused by simple recombination of genes. The term was introduced by Hugo de Vries.

Molecular basis of gene mutation:

Inaccuracy at any one of these levels introduces changes in the arrangement of nucleotides in a polynucleotide chain of a DNA molecule. The smallest change may involve the addition, deletion or substitution of a single nucleotide pair in DNA molecule.

Since these mutations include in triplet N bases segment of DNA, so called as point mutation.

1. Substitution mutation
2. Frame shift

Transcription of prokaryotes:

Transcription is the process by which the several steps of DNA based gene expression, in which particular segment of DNA is copied into RNA (mRNA) by some enzymatic reaction (RNA polymerase).

It has three steps —

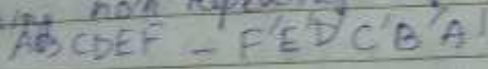
1. Enzymatic synthesis of RNA.
2. From the point on DNA molecule from which transcription start and stop.

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of template DNA. A newly synthesized RNA forms complementary produced RNA-DNA duplex. Once transcription begins the σ factor dissociate from holoenzyme to cover poly. This process continues along the DNA template until it gets signal for termination.

Termination: Specific termination signals necessary for termination and has many genes.

1. There is an inverted repeat base seq. containing non repeating segment in which A and A' are complementary base forming stem and loop configuration.



2. Near loop end having high GC content.

3. A stretch region is a seq. of A-T pairs in the RNA a seq. six to 8 U often folds by A.

Moreover having tRNA and rRNAs having specific lengths. Some oligomeric proteins such as ρ , kappa attach to enzyme blocking transcription, release RNA chain.

8. Types of transcription products (Post-transcriptional change in RNA).

In prokaryotes, certain modifiers enzyme occur in cytoplasm which shorten their lengths such as specific base of tRNA and mRNA such as methylation, acetylation, ~~amino~~ deamination, reduction.

3. The types of transcription products and convention to the RNA molecule.

1. Enzymatic synthesis of RNA:

Synthesis of RNA are of following:

→ For synthesis of RNA four nucleotides 5'-triphosphate (NTP) - ATP, GTP, CTP, UTP. Each NTP, there has two OH groups - one each on the 2' and 3' C atom.

→ In the polymerization reaction 3'-OH group of one nucleotide reacts with the 5'-triphosphate of 2nd nucleotide phosphodiester bond is formed

→ For the bases C, T, G, A in DNA strand the base pair which is used by template of DNA is respectively G, A, C, U and results as new RNA molecule

→ The DNA molecule being transcribed is double stranded and serve as one template strand

→ The nucleotide added only to the 3'-OH and it is grow on same direction 5' → 3' such as DNA synthesis. By result RNA molecule terminated 5'-triphosphate and it is antiparallel to DNA.

→ RNA polymerase is depend of DNA polymerase are able to initiate growth of the chain -

→ Only ribonucleoside 5'-triphosphate participate RNA synthesis is a triphosphate

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an eukaryotic chromosome. Each replicon has about 50,000 to 2,00,000 nucleotide pairs. Clusters of 20-80 replicons are activated together.

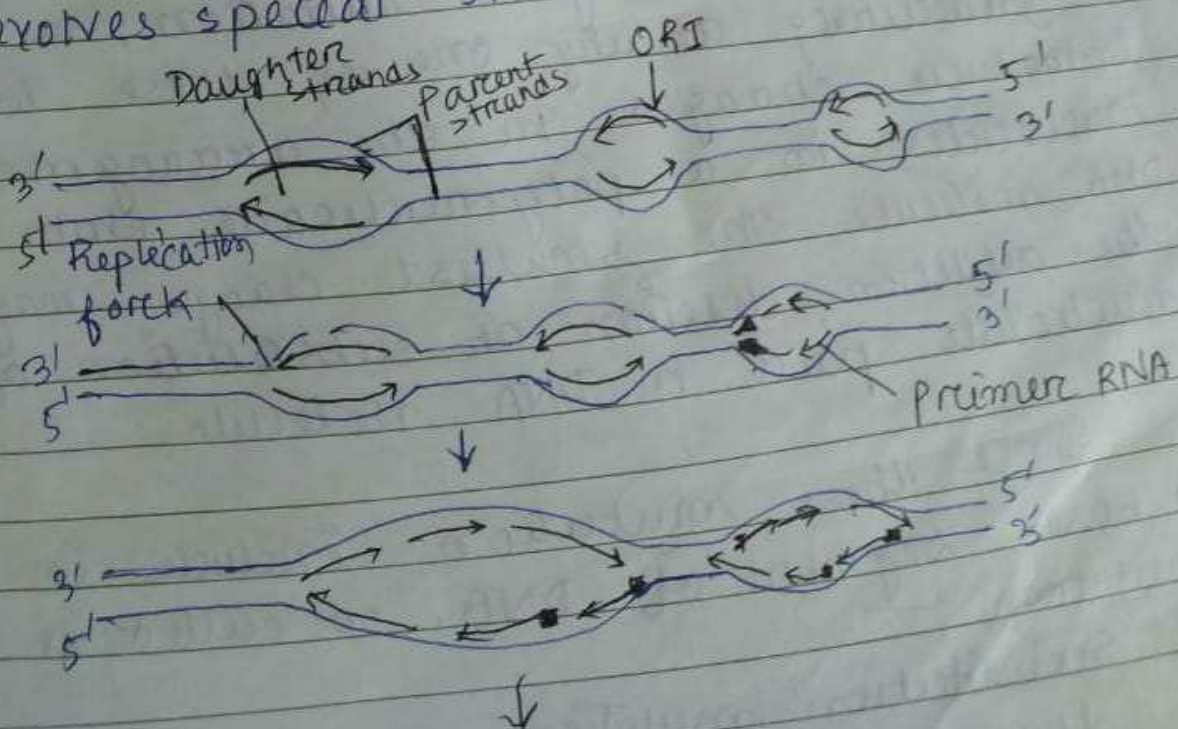
3. Each origin of replication consists of about 150 b.p.

4. Initiation of replication requires a multisubunit protein, the ORI complex.

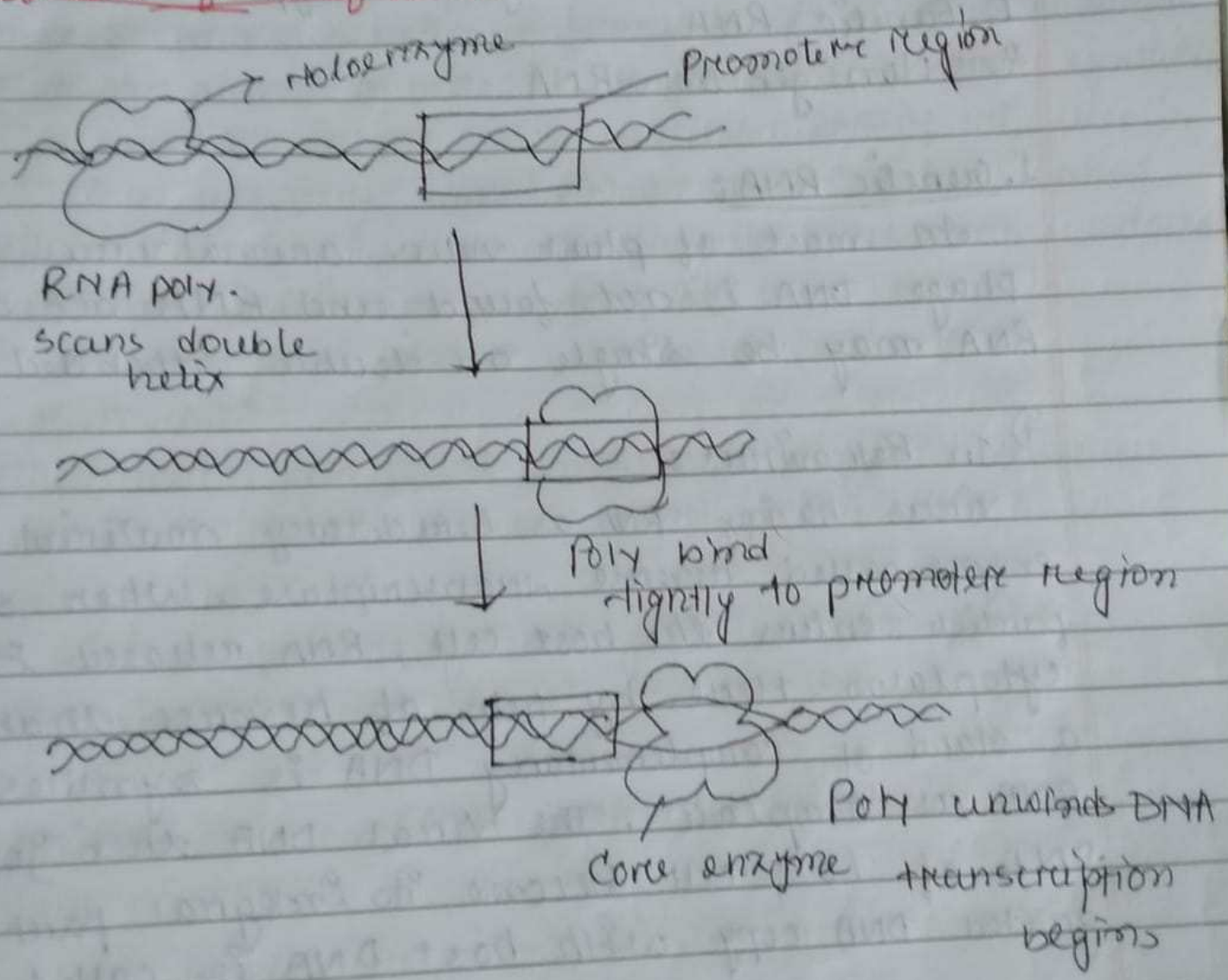
5. Replication bubbles are formed at origin due to separation of two strands of parent DNA at ORI.

6. In eukaryotic cell, DNA polymerase a multi subunit ~~pre~~ enzyme with similar structure in all eukaryotic cell. It lacks proof reading 3' to 5' exonuclease activity.

7. Termination of replication on linear chromosome involves special sites called as telomeres.



Different forms of RNA:



Eukaryotes ?

Initiation: The 1st initiating nucleoside 5' triphosphate or GTP is bound to the pol. at the promoter site to form initiation complex. In certain parts of chromosome only one of two strands of a DNA duplex is transcribed, this strand called the sense strand and the other non-transcribed is called antisense strand. The region of sense strand of DNA transcribed to RNA, called coding region. The first step in transcription is binding of RNA poly. to a DNA molecule. Two special regions have been identified that appear in all organisms. In a region of five to ten bases preceding the coding region as:

TATAATG: A seq. of minor variation is called consensus seq. b/ it is a seq. observed to occur with little variation in many different organisms. In bacteria region called the promoter box and in eukaryotes same region has the seq.

TATAAAT: It is called as Hogness box. This region is genetically referred to as the TATA box and is believed to orient RNA poly enzyme and synthesis proceed from left to right.

The first inter-nucleotide linkage or phosphodiester linkage formed after binding of the second nucleoside 5' triphosphate which is usually pyrimidine nucleoside CTP or UTP.

Elongation: The first 2 nucleotide residues have been joined, chain elongation proceeds rapidly in 5' → 3' direction and antiparallel to 3' → 5' strand.