

IIT JAM

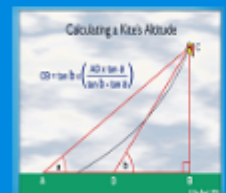
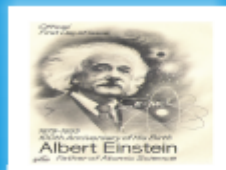
BIOTECHNOLOGY

SAMPLE THEORY



- * REPLICATION
- * TYPE OF DNA REPLICATION
- * REPLICATION IN PROKARYOTES
- * REPLICATION IN EUKARYOTES





IIT JAM - BIOTECHNOLOGY

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REPLICATION

- The process of replication involves the formation of two double helices which are exactly identical to that of original parental double helix.
- The process of replication occurs during interphase between the two mitotic cycles.
- It especially occurs during the S- phase of interphase. DNA replication is a semi conservative process which is later on proved by **Meselson & Stahl's** experiment in 1958.
- During semi conservative process the strand from the parent are such that one is entirely new & one is entirely old.
- For DNA replication a templet DNA, a primer, dNTP's, Mg ions, DNA unwinding proteins, super helix relaxin protein, modified RNA polymerase to synthesize RNA primer, products of dna A, dna B, dnaC, dnaD, dnaE, and dnaG gene, polynucleotide ligase [joining enzyme] are required.

TYPES OF DNA REPLICATION

- **Warburg** suggested that **Watson Crick Model** of DNA can be replicated by three methods:
 - a) Conservative Replication Method**
 - The conservative replication would mean that double stranded molecule is conserved as such and that a new copy is synthesized as such from the old molecule.
 - b) Dispersive Replication**
 - In this type of replication the old DNA molecules are disintegrated and two new molecules are synthesized.
 - c) Semiconservative Replication**
 - In this type of replication the two strands of DNA separates & maintains their integrity, each will synthesize from a pool of nucleotides, its complementary strand.

- The result would be that a newly synthesized molecule would carry or conserve one of the two strands from the parent molecule and the other strand will be entirely new.
- Finally **Taylor** demonstrated by **autoradiography** that both the chromatid of the chromosome has one half old and one half new material during prophase.

1) INITIATION OF REPLICATION

- The DNA chromosomes consist of many replicating units called as **replicon**.
- Replication is always initiated through these replicons only.

2) UNWINDING OR UNZIPPING

- The separation of the two chain of DNA is termed as unwinding or unzipping.
- In this process the double helix of DNA is progressively unwound to produce a single stranded DNA segments.
- It starts from a specific point called as **ori-c point** & enzymes **topoisomerase** is involved in producing a cut in a DNA strand.
- The ore-c region is used to produce a cut or unwinding of the DNA molecule because it consists of A=T base pair in a sufficient quantity & these bonds are easy to break-up.
- The unwinding of the DNA molecules is brought about by a specific unwinding protein called as DNA unwinding protein (i.e. helicase enzyme).
- In the process or unwinding Mg^{++} act as a co-factor. This unwinding always takes in an alkaline medium.
- The proteins bind particularly to the single strand of DNA promotes unwinding or denaturation of double helix
- The region of double helix where proteins binds form a Y-shaped structure called as **replication fork**.

- This replication fork moves down the double helix the parental strands undergo winding again however the strains imposed by unwinding is released by the action of super helix relaxin protein (S.S.B protein).

a) Separation of double helix

- This is brought about by a **helicase** enzyme. This enzyme leads to the separation of strands and binds single stranded binding protein (S.S.B) to the DNA molecule and prevents the double helix to wound again.

b) Synthesis of leading & lagging strands

- Synthesis of DNA by DNA polymerase occurs in 5'-3' direction only.
- As the two strands of double helix runs in the opposite direction, so there will be the formation of two types of strands.
- They are continuous or leading strand or 3'-5' DNA template, discontinuous or lagging strand or 5'-3' DNA template.

c) RNA priming

- Initiation of DNA synthesis requires RNA primer which is a short polynucleotide, chain with 5'-phosphate and 3'-OH group at their ends.
- The formation of RNA is catalyzed by an enzyme called as **RNA polymerase** .
- Synthesis of RNA primers takes place in 5'-3' direction.
- After the formation of new chain this RNA is removed and the gaps are filled with dNTP's provided by an enzyme **DNA polymerase-I**

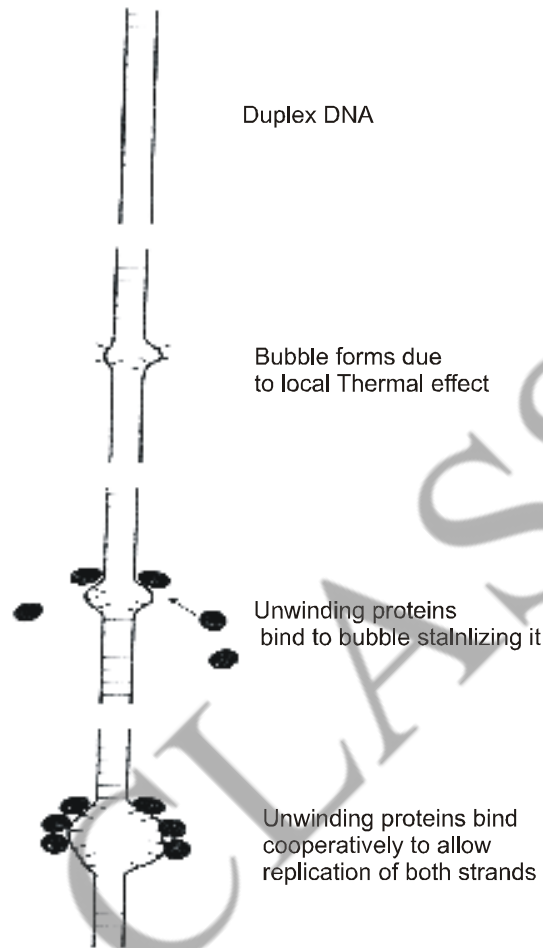


Fig: 1 Schematic representation of the action of unwinding proteins in separating strands of duplex DNA for replication.

3) CHAIN ELONGATION

- Synthesis takes place in 5' -3' direction on 3' -5' direction DNA template by enzyme **DNA polymerase-III** in the presence of ATP.
- The newly synthesized DNA possess primer RNA at the 5' position. This RNA primers are later on hydrolyzed by the 5'-3' exonuclease activity of the DNA polymerase-I and gap is later on filled by dNTP's provided the catalytic activity (5'-3' polymerase activity) of DNA polymerase-I.
- The synthesized DNA was later on joined by the activity of DNA ligase enzyme.

- As a result of chain elongation leading or continuous strand is synthesized in 3' - 5' DNA template whereas discontinuous or lagging strand is synthesized on 5' - 3' DNA template in the form of small **okazaki fragments**
- Later on these okazaki fragments are joined together by DNA ligase enzyme.

4) EXCISION OF RNA PRIMERS

- Once a small segment of okazaki fragments has been formed the nucleotide of RNA primers are removed from 5' end one by one by the action of 5' - 3' exonuclease activity of DNA polymerase.

5) LIGATION

- The final step is the joining of okazaki fragments together one by one by phosphodiester bonds.
- This step is catalyzed by polynucleotide ligase or DNA ligase which requires NAD (i.e. Nicotinamide adenine dinucleotide) for its activity.
- Ligase + NAD → Ligase-AMP + NMN
[adenylated ligase] [Nicotinamide mono nucleotide]
- The adenylated ligase then reacts with DNA having single strand break to produce repaired phosphodiester bond, ligase & AMP.
- Ligase – AMP + DNA (ss break) → Repair DNA + Ligase + AMP

NOTE

- Ramphampicin (drug) inhibits the process of replication.

PROKARYOTIC DNA REPLICATION

- It follows the replication process in which there is a formation of only one replication fork & therefore the replication is called as **unidirectional replication**.

1) Initiation of replication in a double strands of DNA

- For the initiation of replication from ori-c, dnaA gene which is a product of DNA A form complex with the ATP molecule (DnaA-ATP complex).
- Now this complex (DnaA-ATP complex) combines at ori-c region in order to promote the unwinding of DNA in a region of 13 base pair sequence.
- The opening occurs from the ori-c region to form a replication fork. This replication fork progresses in opposite direction to form an intermediate product called as **theta(s)fork**.
- During the unwinding the excessive super coiling of DNA also occurs ahead of the replication fork.
- In case of circular DNA molecules the problem of super coiling is overcome by topoisomerase enzyme.
- This enzyme is of two types:
Type -I Topoisomerase-It would produce a transient break in one of the DNA strand to remove excessive supercoiling.
Type-II Topoisomerase-This would produce a transient break in one of the DNA molecule to produce two daughter molecules.

2) RNA PRIMERING

- Unwinding is followed by the synthesis of RNA primers by Dna-primase through its interaction with Dna-B and the primers are further elongated by DNA polymerase-III holoenzyme.

3) CHAIN ELONGATION

- The elongation of the chain involves loading and activation of Dna-B helicase enzyme because this enzyme will then activate a series of other enzymes such as DNA polymerase-III holoenzymes to promote chain elongation.
- DNA Polymerase-I holoenzyme is a large multiprotein complex made up of two components i.e core and accessory component.
- When both the components are linked together enzyme becomes functional.

- As a result of this process the two types of strands are synthesized out of which one is leading strand and the other is lagging strand.

4) CHAIN TERMINATION

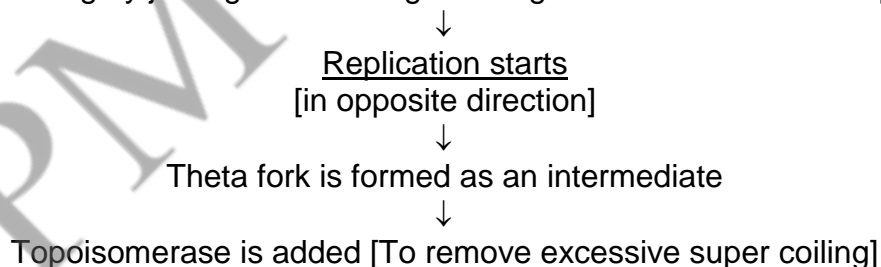
- The prokaryotic DNA strand contains large termination zone opposite to Ori-C region which can block the progress of the replication.
- E-coil chromosomes & various other plasmid contains specific sequence called as **terr site** where **TBP (Terr Binding Protein) or TUS protein binds**.
- TBP terr complex formed at terr site stops the migration of the replication fork by inhibiting the DNA helicase or DnaB protein and thus terminates the chain elongation.
- Even in the absence of terr site replication stops spontaneously by the meeting of the oppositely growing replication fork.

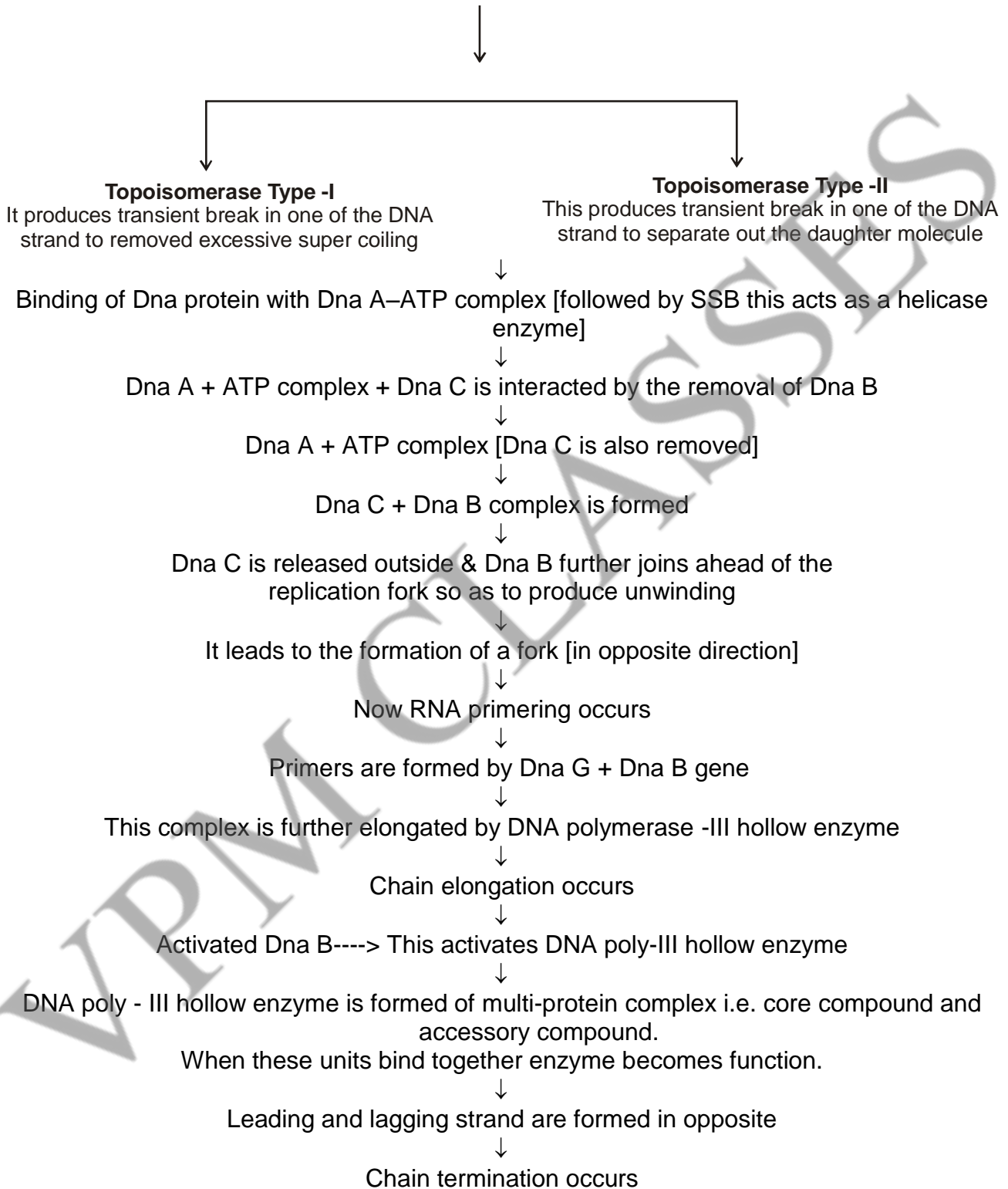
NOTE

- SSC binds in order to maintain the identity of single strands individually.
- Dna B helps in unwinding ahead of the replication fork.
- A=T base pairing is used because it contains double bonds only so it is easy to unwind them at low cost of energy

FLOW CHART REPRESENTING REPLICATION IN PROKARYOTES

[Produces unwinding by joining at ori - c region a region which is of 13 base pair sequence]





↓
Opposite to ori - c region termination zone is present

↓
E. coli & various other plasmids contains specific sequence called as terr side consisting of Terr Binding Protein [TBP]

↓
T.B.P stops the migration of the replication fork by inhibiting the DNA helicase Dna B protien.

Even in the absence of TBP chain elongation stops due to the meeting of the oppositely
Fig.2 Replication in prokaryotes

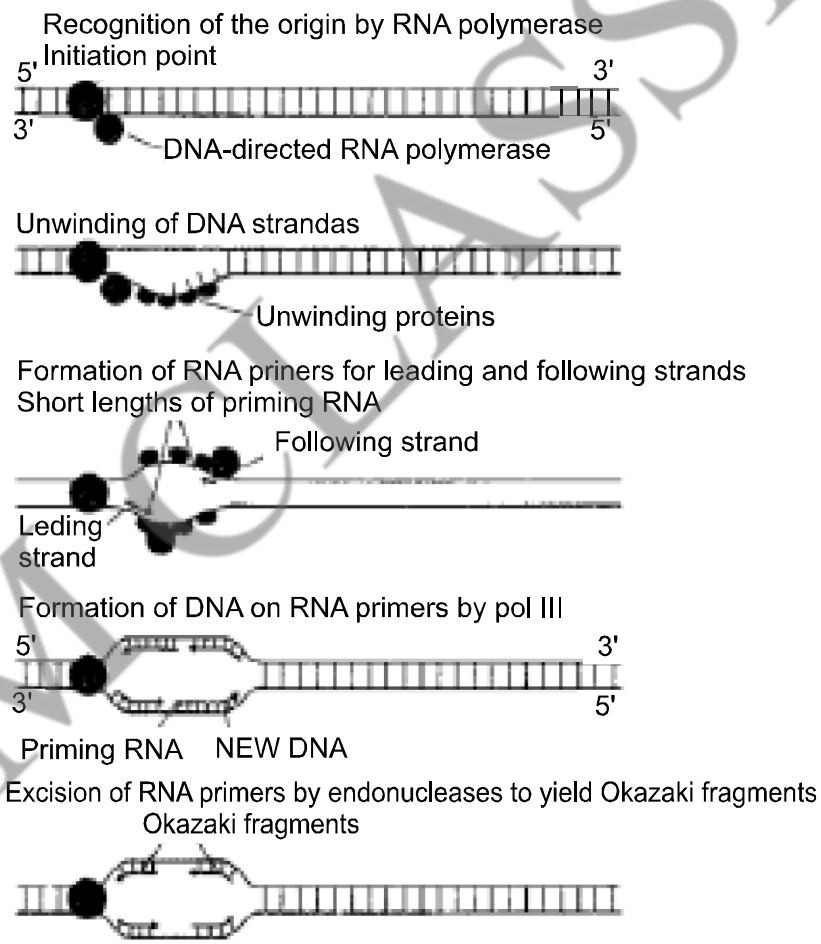


Fig. 3 hypothetical sequence of steps in the Replication of DNA Proceeding in one Direction to the from the initiation Point.

Eukaryotic DNA replication

It is bi directional in nature

- DNA replication in eukaryotes involves the following steps:

(i) Chain initiation

- In the eukaryotic genome the origin of replication is also known as **autonomously replicating sequence (ARS)**.
- ARS contains 11 base pairs A=T rich consensus sequences or ACS domain [Autonomous replication consensus sequences].
- Unwinding occurs from the domain in order to form a replication fork. The replication fork in this case of eukaryotes has directional progress i.e. it is bidirectional in nature.
- The protein involved in the unwinding is **T-Ag protein** which requires the presence of **ATP**.
- The same protein is also associated with the **replication factor A (RF-A)** and the topoisomerase enzyme which performs the function of helicase & brings about the local unwinding of the DNA molecule.
- Just as soon as the replication fork is formed S.S.B binds so as to unwind single stranded DNA.

(ii) RNA primer

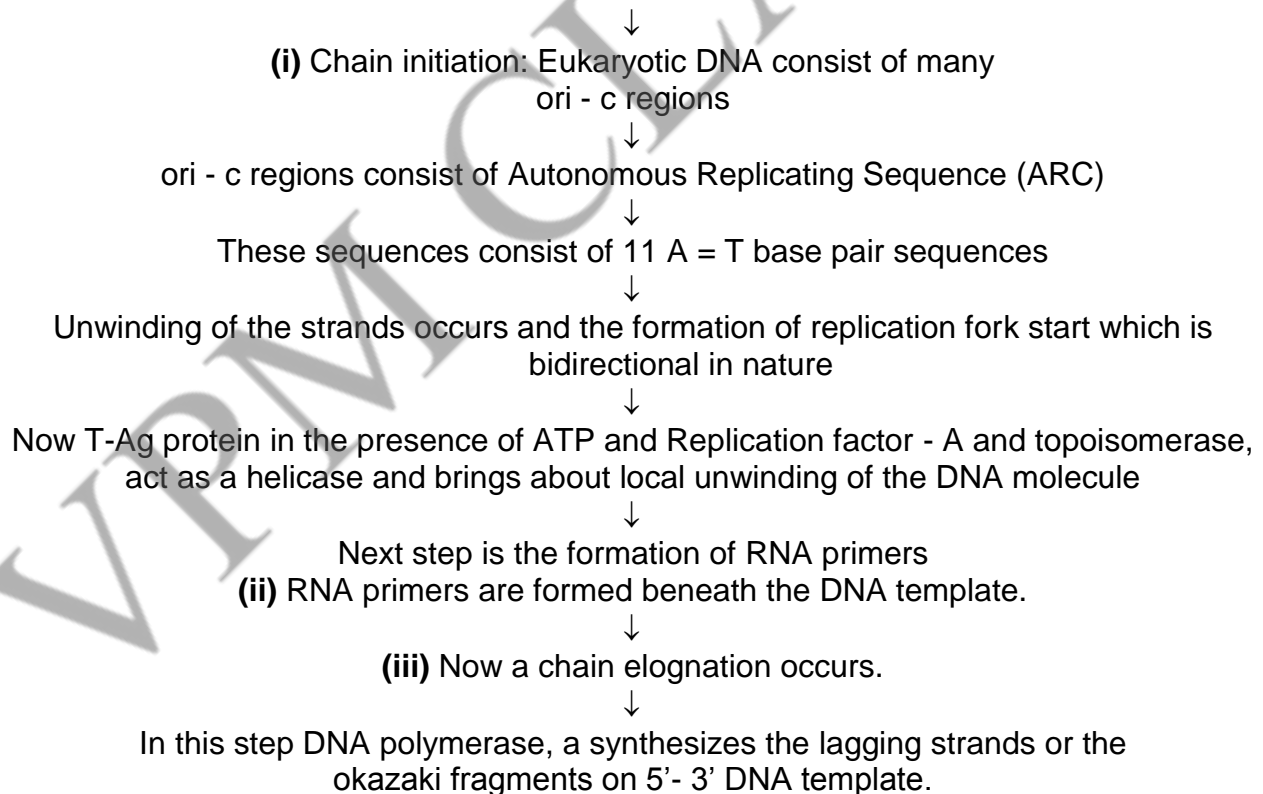
- Next step of eukaryotic replication is the RNA priming.
- In this step RNA primase synthesizes, the RNA primer beneath the DNA template or we can say it utilizes the DNA as a template to synthesize RNA primers.

(iii) Chain Elongation

- DNA polymerase α synthesizes lagging strands or the okazaki fragments on 5'-3' DNA template.
- **RF-C** [Replicating Factor-C] & **PCNA** [Proliferating Cell Nuclear Antigen] or **cycline** helps in switching off DNA polymerase, so that poly- α is replaced by poly δ which will then continuously synthesize DNA as a leading strand in 3'-->5' direction

- Another okazaki fragment is then synthesized from the replication fork on the lagging strand by poly α primase complex.
- This step is repeated again and again till the entire DNA molecule is covered.
- The RNA primers are later on removed and the gaps are filled as in case of prokaryotic DNA replication. Thus, the chain elongation involves the switching between poly α and poly δ .
- Out of three polymerase discovered i.e. Poly- α poly δ and Poly β
- Poly α performs the dual functions as a primase, and also synthesizes a lagging strand.
- Poly δ performs single function of synthesizing a leading strand.
- Poly β only assist in the elongation of the strand.

FLOW CHART SHOWING THE PROCESS OF EUKARYOTIC REPLICATION



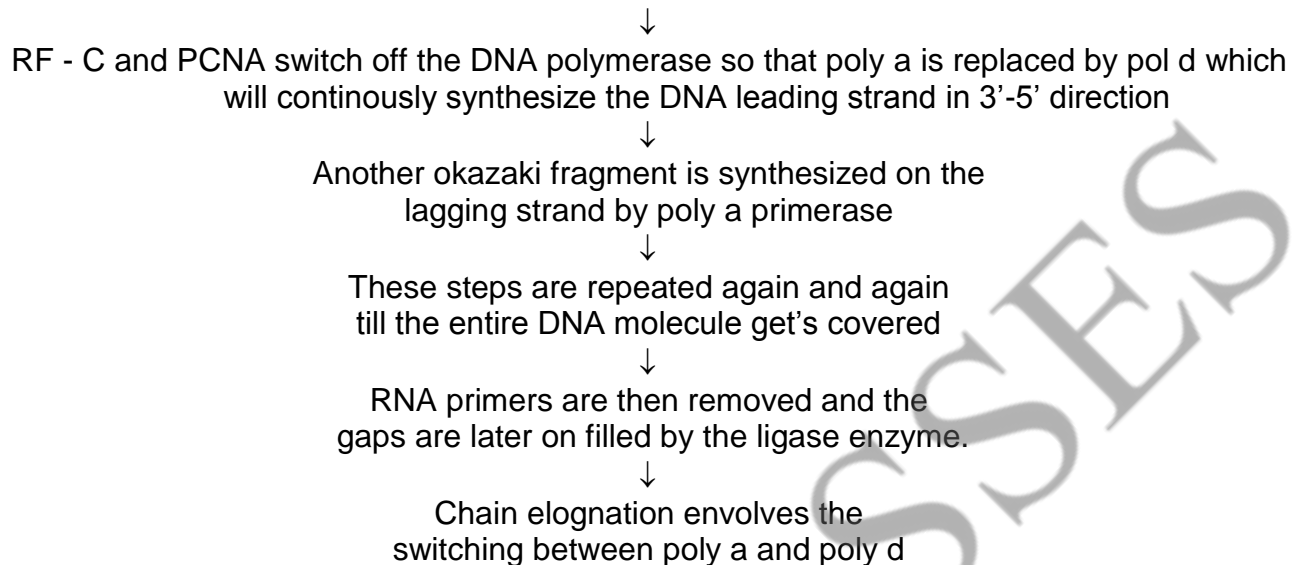


Fig. 4 Process of Eukaryotic Replication

- **Rolling Circle replication of DNA**
- Some of the small bacterias and eukaryotic viruses (e.g. bacteriophages) contains single stranded circular DNA called as **positive (+) strand**.
- These viruses have the capability that whenever they are injected into an appropriate host cells, the process of replication occurs in their chromosomes.
- **This process occurs in the following steps**
 - i) Production of replication from (RF)**
 - In this process the production of new strands occurs on to the positive strand which is complementary to it.
 - This complementary strand so formed is called as **negative (-) strand**.
 - This synthesis of negative strand is initiated by the synthesis of short RNA primers by the enzyme primase in combination with at least six primering proteins.
 - These protiens worked together as a group and are called as '**primosome**'.
 - Here DNA polymerase III catalyzes the covalent bond formation of deoxyribonucleotides to the 3' - OH of the RNA primer.

- The process of the synthesis of a negative strand proceeds discontinuously till the synthesis is completed.
- Apparently these primosomes moves around the (+) positive strand and catalyzes the synthesis of RNA primers at variable intervals.
- Now by the activity of DNA polymerase - I these RNA primers are digested and the gaps are filled by the ligase enzyme.
- These are generally present in the form of small fragments called as okazaki fragments which are filled by the ligase enzyme to obtain double stranded replicative fork.
- The process is initiated for the synthesis of complementary strand on to the positive strand (which is called as negative strand).

FLOW DIAGRAM REPRESENTING THE PROCESS OF PRODUCTION OF RF

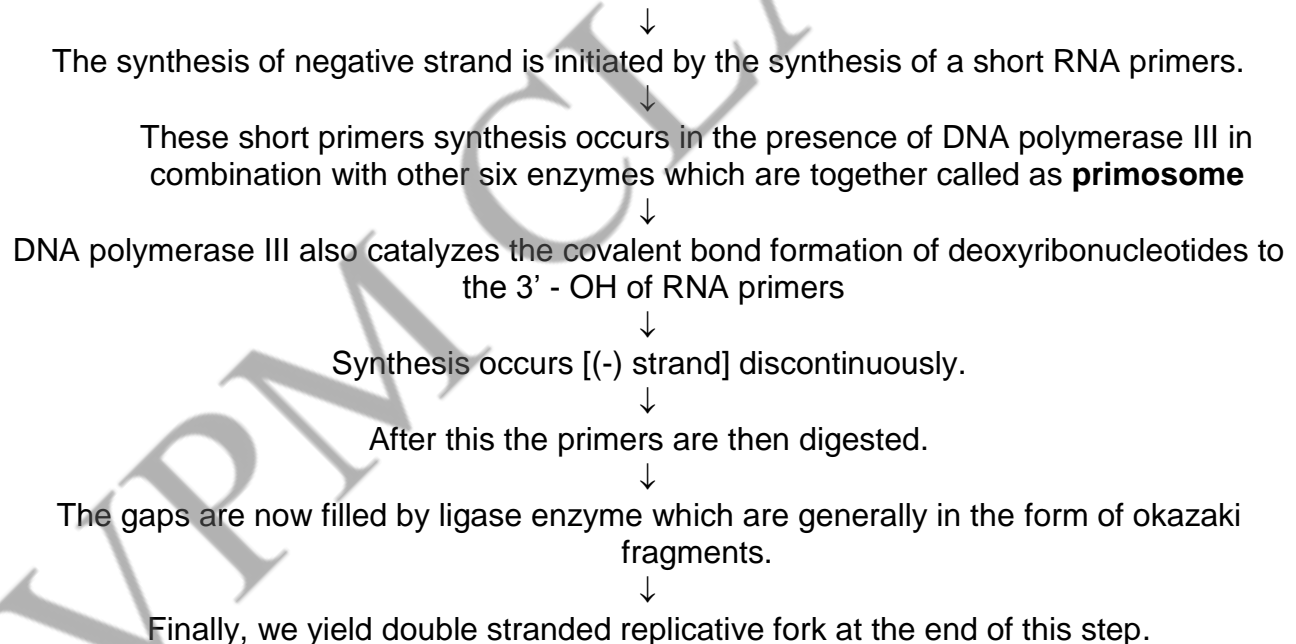


Fig. 5 Process of Production of RF

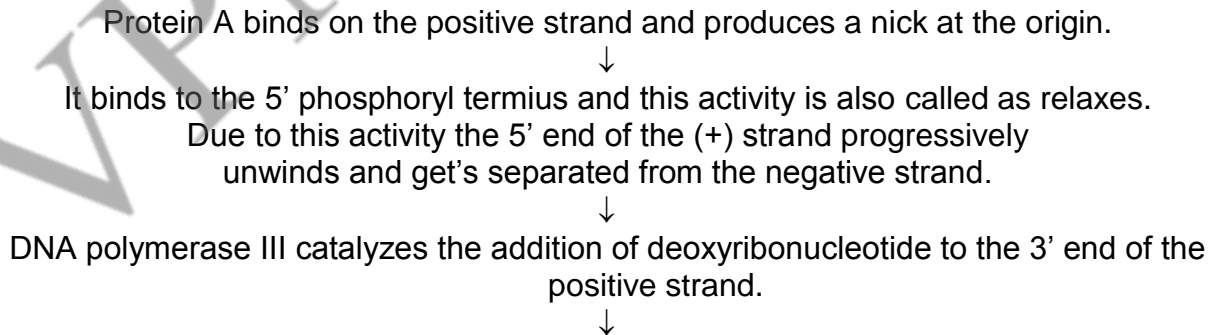
ii) Replication of Replicative Form

- This process of replication occurs through “rolling circle” replication.
- In this process the gene A whose product is protein A induces a nick in the positive strand of RF chromosomes at the origin and binds to the 5'-phosphoryl terminus.
- This type of enzyme activity is sometimes referred as **relaxes**.

NOTE

- The DNA is being nicked only when it is in the supercoiled form.
- The protein A synthesized by a genome can attach to the origin of that genome only.
- In this process the 5' end of the positive strand progressively unwinds and gets separated from the (-) strand.
- DNA polymerase III catalyzes the addition of deoxyribonucleotide to the 3' end of the positive strand.
- Thus due to this activity the (+) strand proceeds from 5'-3' direction.
- The protein A remains bound to the 5' end of the positive strand as well as replication fork.
- Here protein A performs the following functions:
 - 1) Cleaves the new origin.
 - 2) Ligase the 3' and 5' ends of the free positive strand.
 - 3) Again binds to the 5' phosphoryl terminus of the newly synthesized strand of the RF chromosome.

FLOW DIAGRAM REPRESENTING THE PROCESS OF REPLICATION OF RF



Thus the synthesis of positive strand proceeds in 5'-3' direction while the negative strand is not synthesized.



When a complete positive strand has been synthesized and then a new origin is produced in it.

3) Production of A Positive Strand from (RF) Chromosomes

- It also takes place in the same manner as the replication of RF i.e. through rolling circle replication.
- In this case when positive strands are produced viral coat protein are also synthesized, which binds to the (+) strand.
- As a result of this circularization occurs and the circularized strand are unable to serve as a template for the synthesis of (-) strand so that they may remain as single stranded.

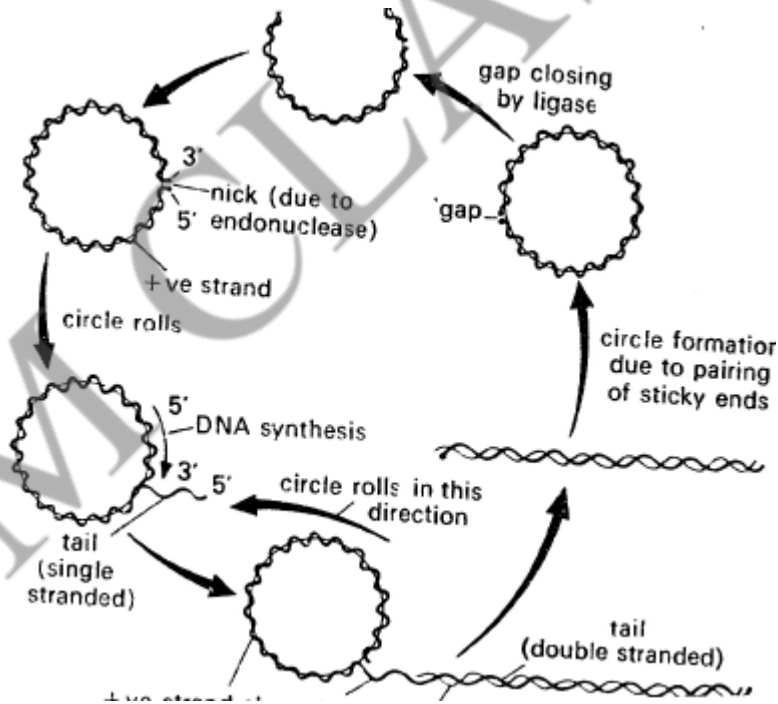


FIG: 6 ROLLING CIRCLE MODEL OF DNA REPLICATION