DNA REPLICATION

- Various models of DNA replication
- Rolling circle replication
- θ (theta) mode of replication
- Replication of linear ds-DNA,
- Replication of the 5'end of linear chromosome
- Enzymes involved in DNA replication

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DNA REPLICATION

- Replication of duplex DNA is a complicated end over involving multiple enzyme complexes. Different activities are involved in the stage of initiation , elongation and termination. Before initiation can occur , however the super coiled chromosome must be relaxed . This occurs in segments beginning with the replication origin region. The alternation to the structure of the chromosome is accomplished by the enzyme topoisomerase. Replication can not occur on super coiled DNA , only the relaxed form.(lewins x)

VARIOUS MODELS OF DNA REPLICATION

❖Semi-conservative DNA replication

It is crucial that DNA is reproduced accurately , two polynucleotide strands are joined only hydrogen bonds, so they are able to separate without breakage of covalent bonds. The specificity of base pairing suggests that both of the separated parental strands could act as template strands for synthesis of complementary daughter strands. The sequence of the daughter strands is determined by the parental strand : an A in the parental strand causes a T to be placed in the daughter strand, a parental G directs incorporation of a daughter C and so on. Each of the daughter duplexes is identical in sequence to the original parent duplex, containing one parental strand and one newly synthesized strands. The structure of dna carries the information needed for its own replication. The consequences of this mode of replication called **semi-conservative replication.** (Lewins x)

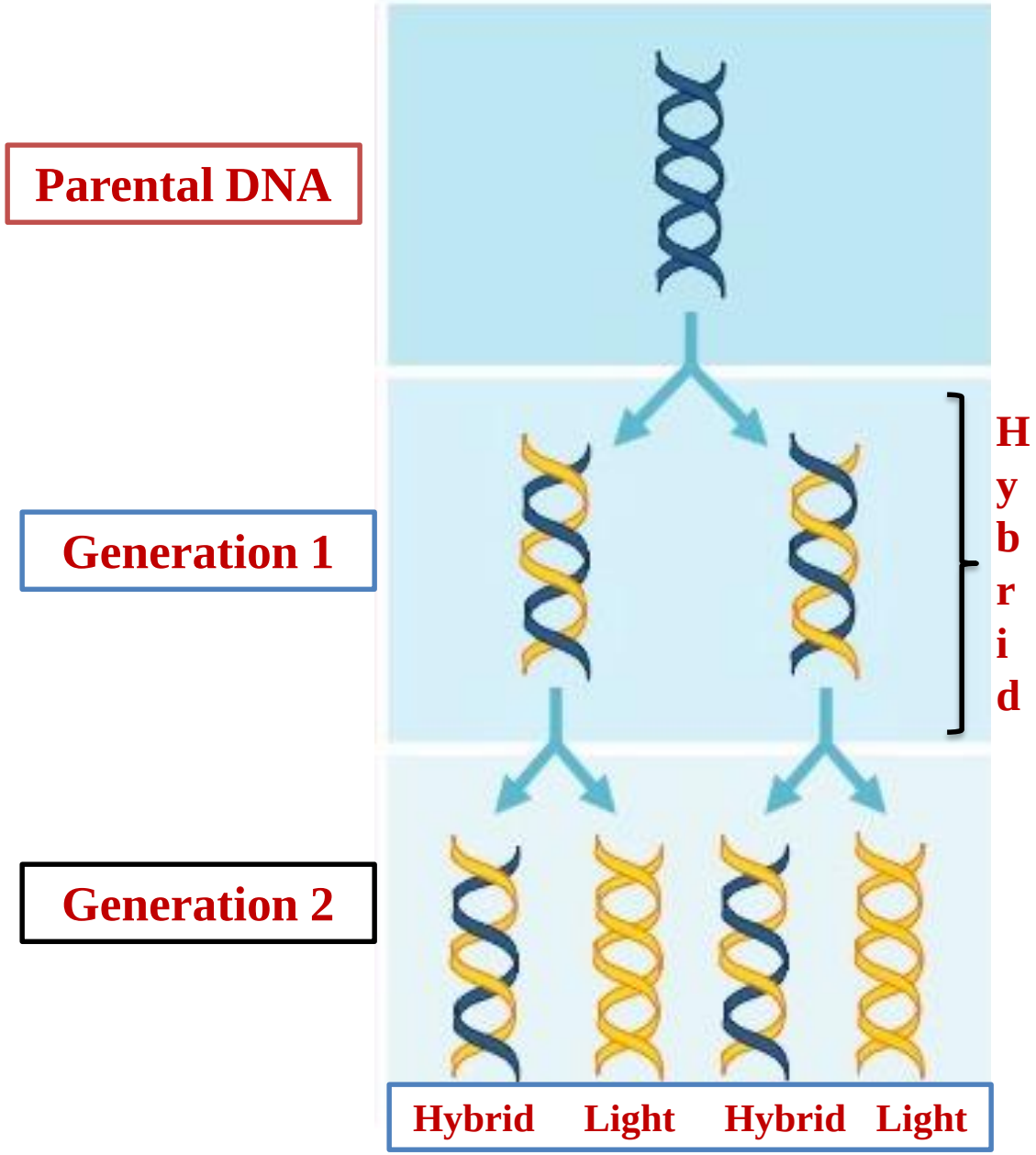


Figure illustrates a prediction of this model. If the parental DNA carries a **'Heavy'** density label. The parental DNA is duplex of two **heavy** strands(BLUE). After one generation of growth in **"light"** medium, the duplex DNA is **'hybrid'** density– it consists of one **heavy** parental DNA (BLUE) and one **'light'** daughter strand (YELLOW). After second generation , the two strands of each **hybrid** duplex have separated. Each strand gains a **light** partner , so that now one half of the duplex DNA remains **hybrid** and other half is entirely **light.** (YELLOW)

FIG : REPLICATION OF DNA IS SEMI-CONSERVATIVE

VARIOUS MODELS OF DNA REPLICATION

❖ Conservative DNA replication

The parental molecule directs synthesis of an entirely new double-stranded molecule, such that after one round of replication, one molecule is conserved as two old strands. This is repeated in the second round.

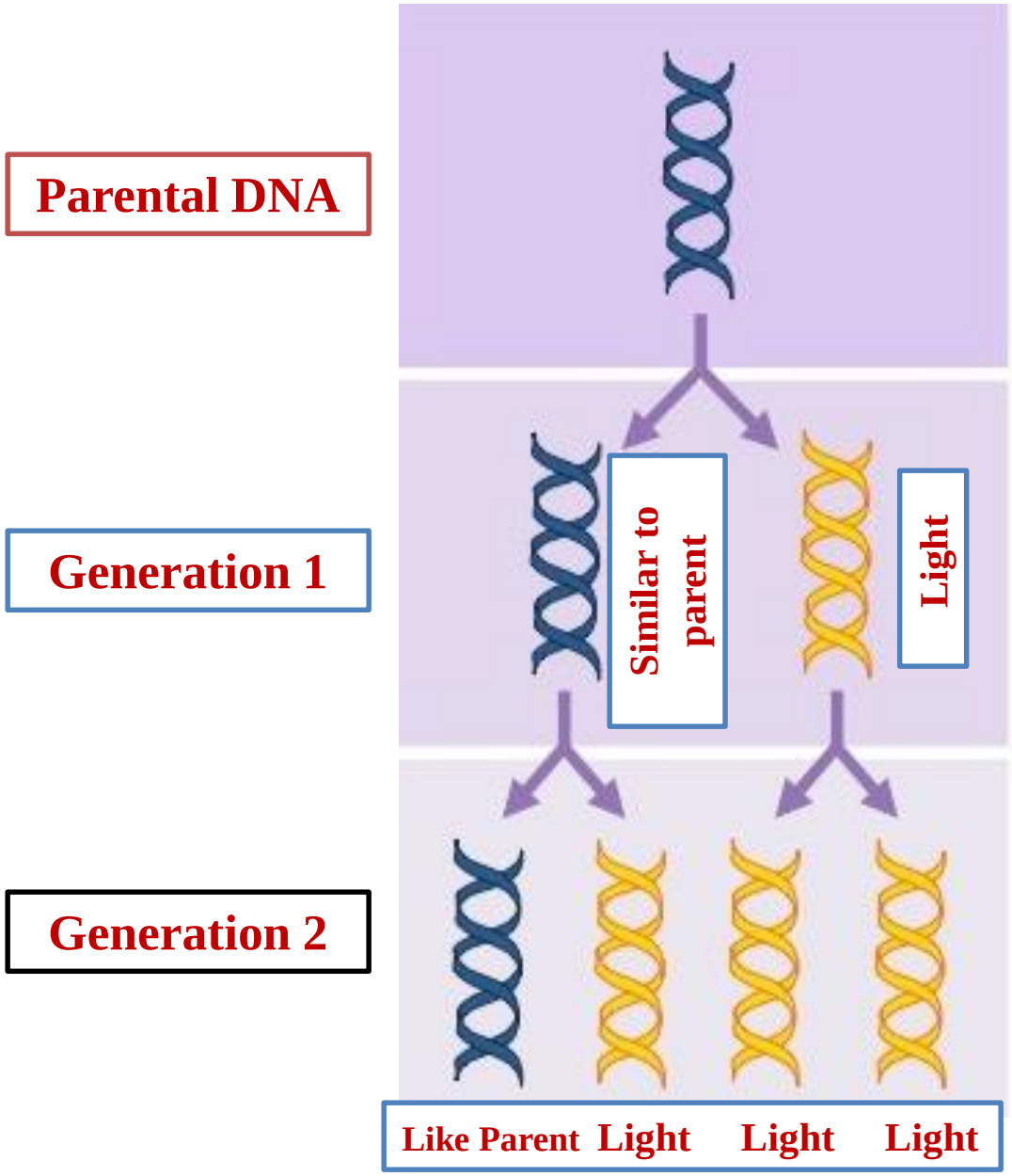


FIG : CONSERVATIVE DNA REPLICATION

VARIOUS MODELS OF DNA REPLICATION

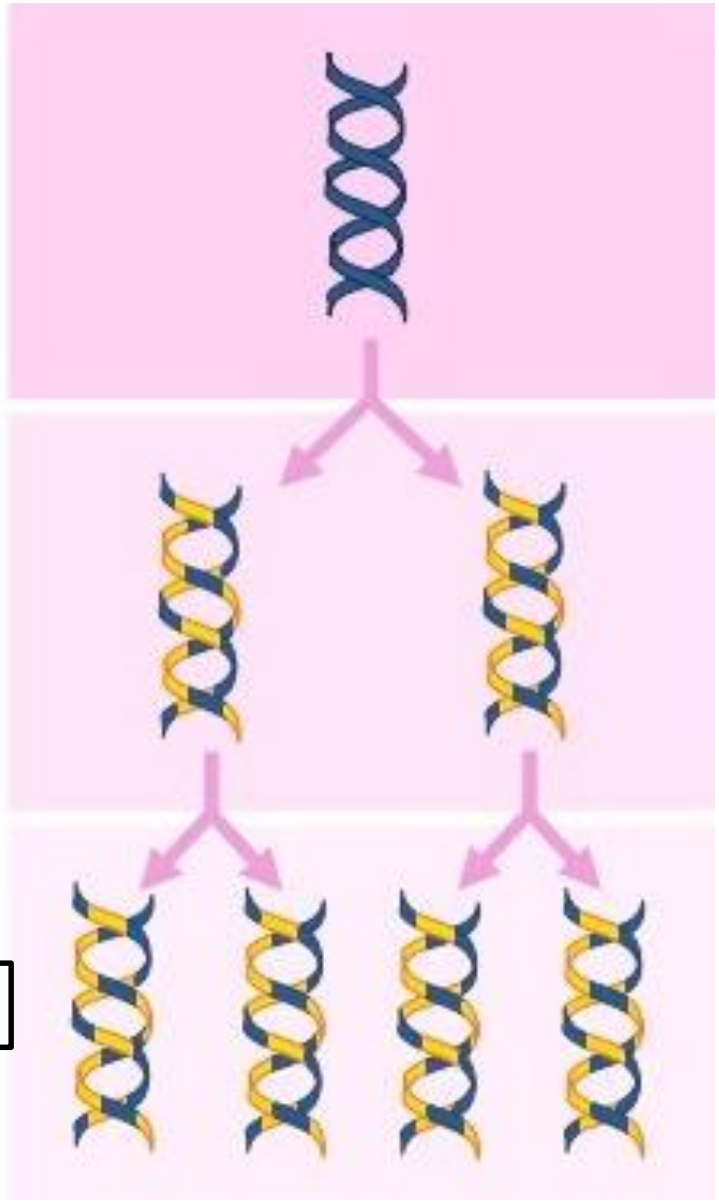
❖Dispersive DNA Replication

In the **dispersive** model, material in the two parental strands is distributed more or less randomly between two daughter molecules. In the model shown here, old material is distributed symmetrically between the two daughters molecules. Other distributions are possible.

Parental DNA

Generation 1

Generation 2



Each daughter DNA Molecule contains a mixture of old and new DNA

Each daughter DNA Molecule contains a mixture of old and new DNA

FIG : DISPERSIVE DNA REPLICATION

Rolling Circle DNA Replication

- It is also called unidirectional nucleic acid replication.
- This can rapidly synthesize multiple copies of circular molecules of DNA or RNA .

❖ **Steps :**

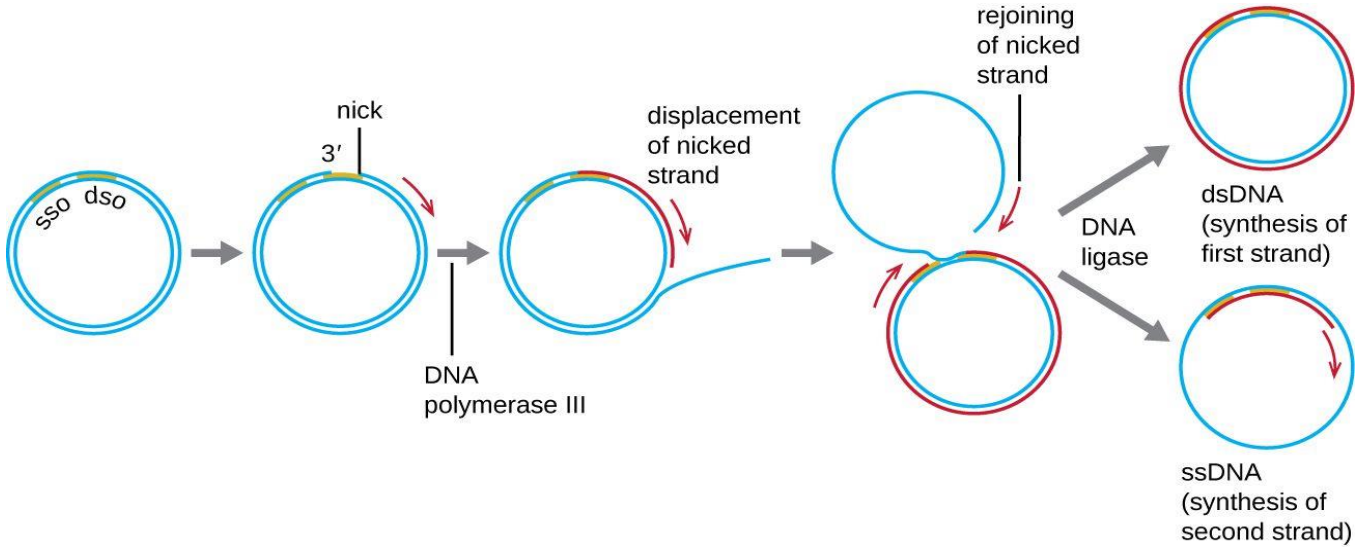
1. Circular DNA will be nicked.
2. 3’ End elongated – Leading strand
3. 5’ end displaced – Lagging strand
4. Replication of both un-nicked and displaced ss DNA
5. Displaced DNA circulates and synthesis its own complementary strand.

❖ Some eukaryotes also replicate their DNA by rolling circle mechanism, i.e bidirectional.

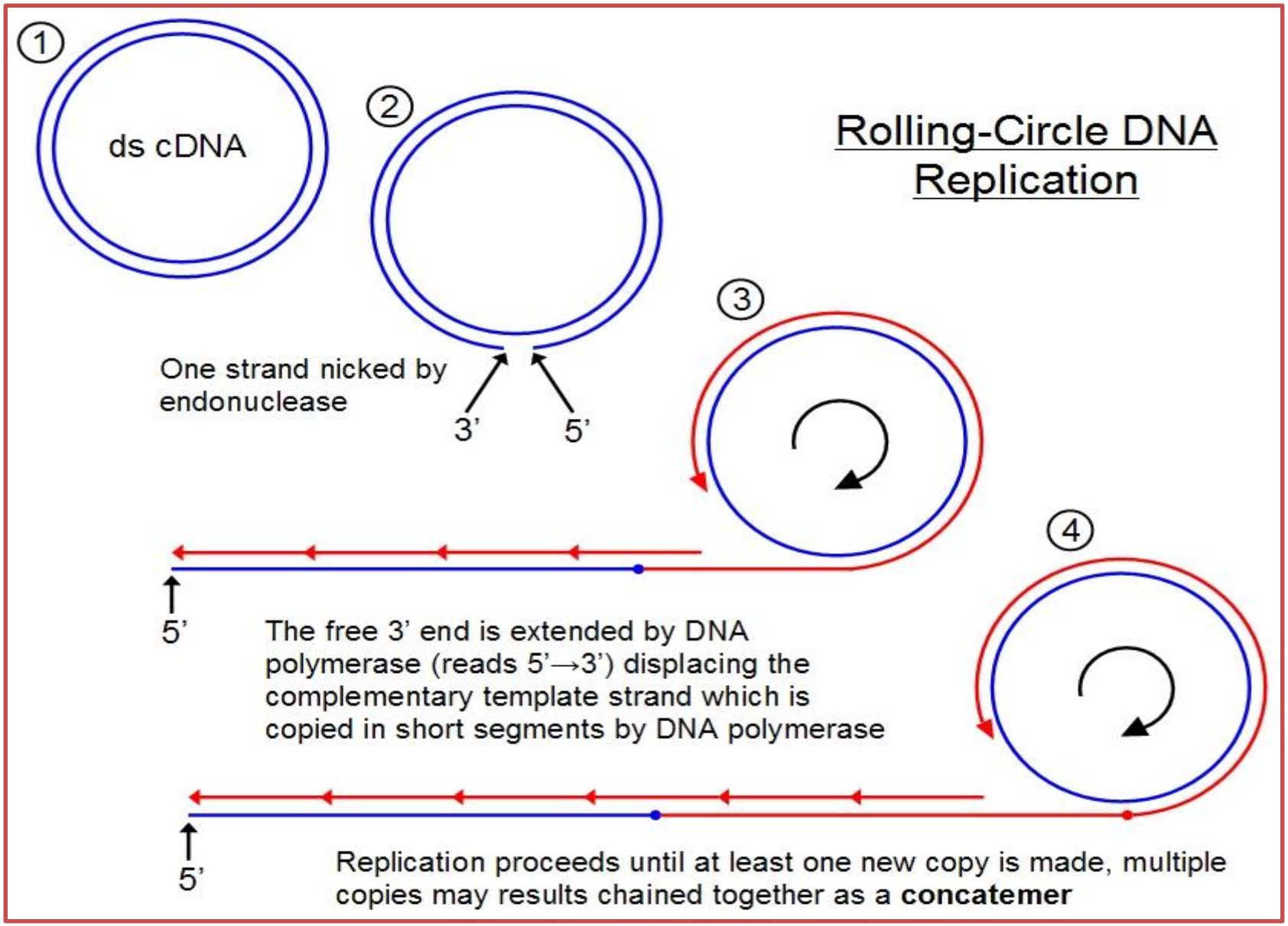
Rolling Circle DNA Replication

Three basic steps :

1. **Initiation :** Nick one of the two strand producing free 3-oh and 5 phosphate end by : helicase , topoisomerase, single strand binding protein (SSBPs)
2. **Elongation** : -OH group of broken strand with the unbroken strand as a template. This will move in a rolling circle model. Then 5' end will be displaced and depart similar to a thread.
3. **Termination :** At last DNA molecule is cleaved from the circle resulting circular double stranded DNA and single stranded linear DNA molecule.



Rolling Circle DNA Replication



Theta (θ)mode of DNA replication

- During replication in bacteria (*E.coli*) a bubble is initiated at the origin and circular DNA molecule exhibits a θ (theta like) shape.
- Replication is bidirectional and both strands of DNA are replicated.
- As the two DNA strands in circular chromosome uncoils it creates coiling , which is removed by topoisomerase.
- As strands separates positive super coils form elsewhere in the molecule.

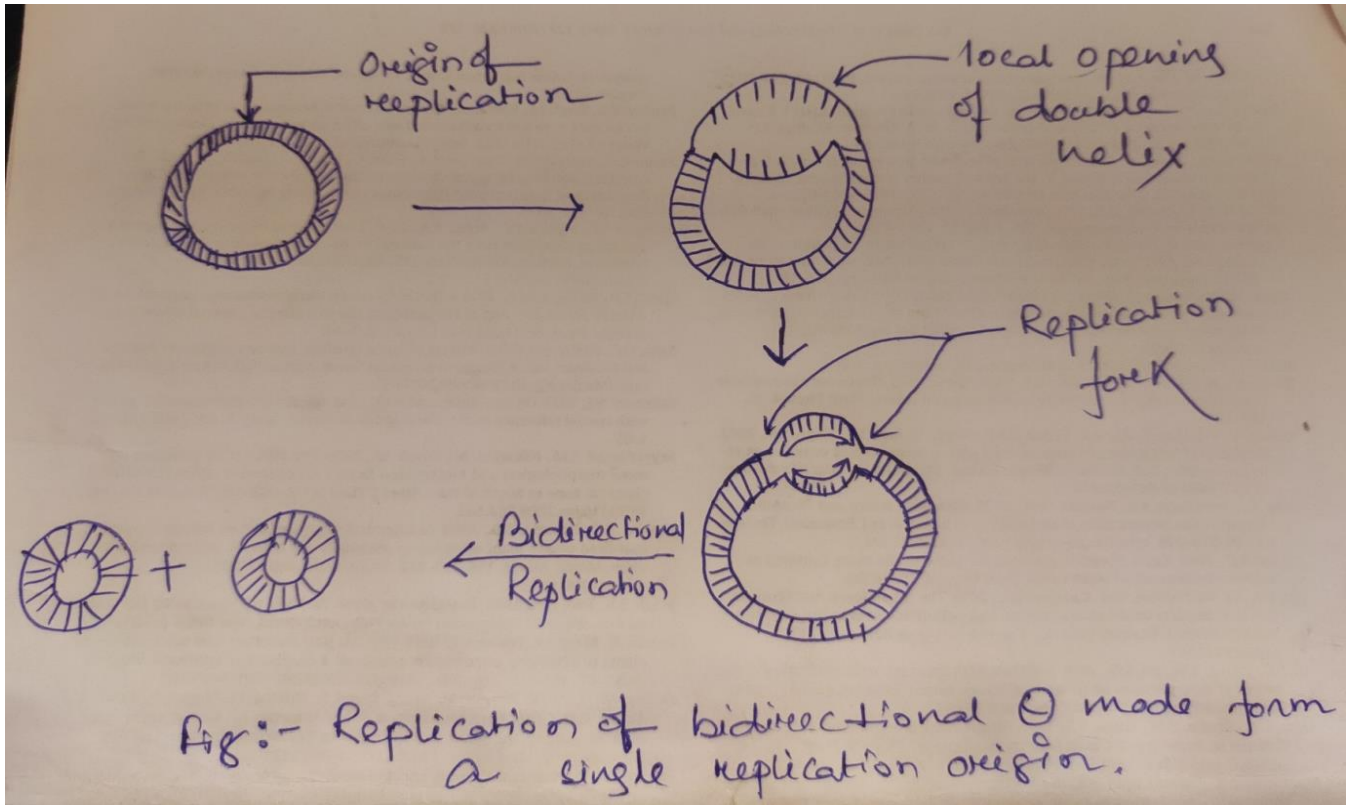
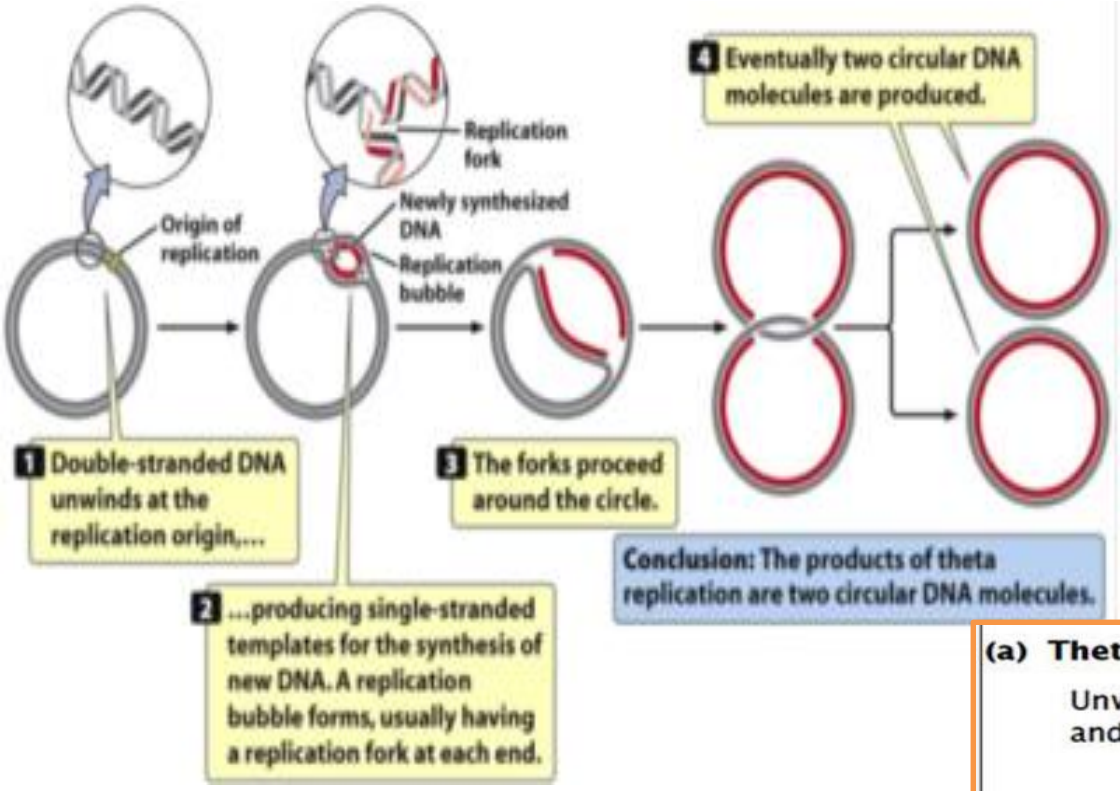
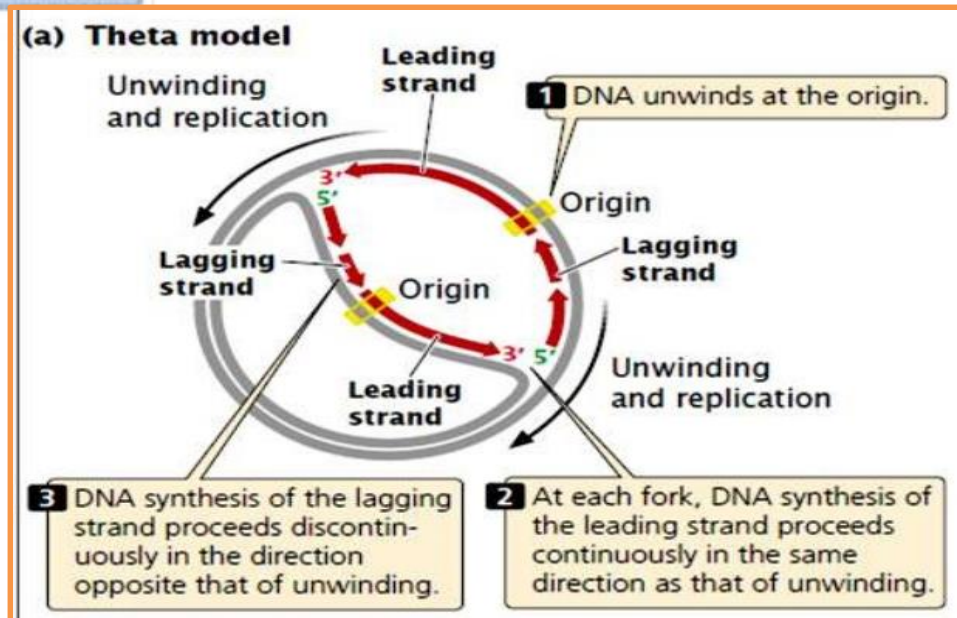


Fig:- Replication of bidirectional θ mode form a single replication oreigin.

Theta (θ)mode of DNA replication



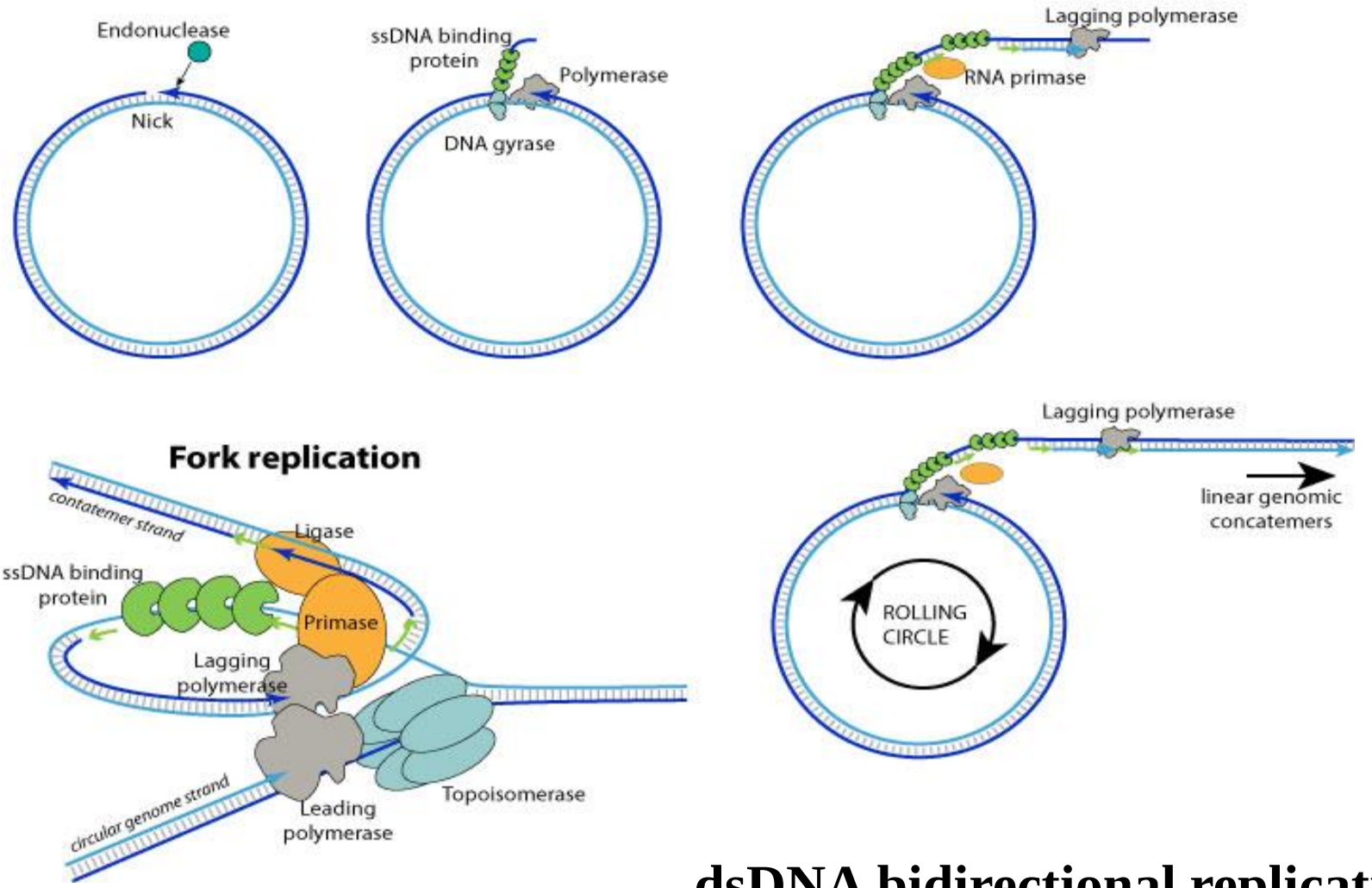


Linear ds-DNA replication

This kind of replication is used by all cellular organisms and some DNA viruses. It is the most classical way of replicating genomic nucleic acid.

1. DNA replication begins at specific locations in the genome, called “origins”. A topoisomerase unwinds the DNA double-strand at the origin of replication.
2. A primase synthesizes short RNA primers that are then used by the DNA polymerase to prime DNA synthesis. all DNA synthesis begins by 3’elongation of a RNA (bidirectional replication), DNA (strand displacement, nick translation), or by protein priming (virus only).
3. The DNA polymerase and associated factors begins to elongate the leading strand at the fork. For the lagging strand Okazaki fragment are elongated after sequential RNA primer synthesis by the primase.
4. The lagging strand RNA primer are removed and Okazaki fragments ligated.
5. The replication forks go on until they reach the end of linear genome, or until they meet at the opposite side of a circular genome.
6. After synthesis, topoisomerase allows separation of the two strands resulting from the replication.

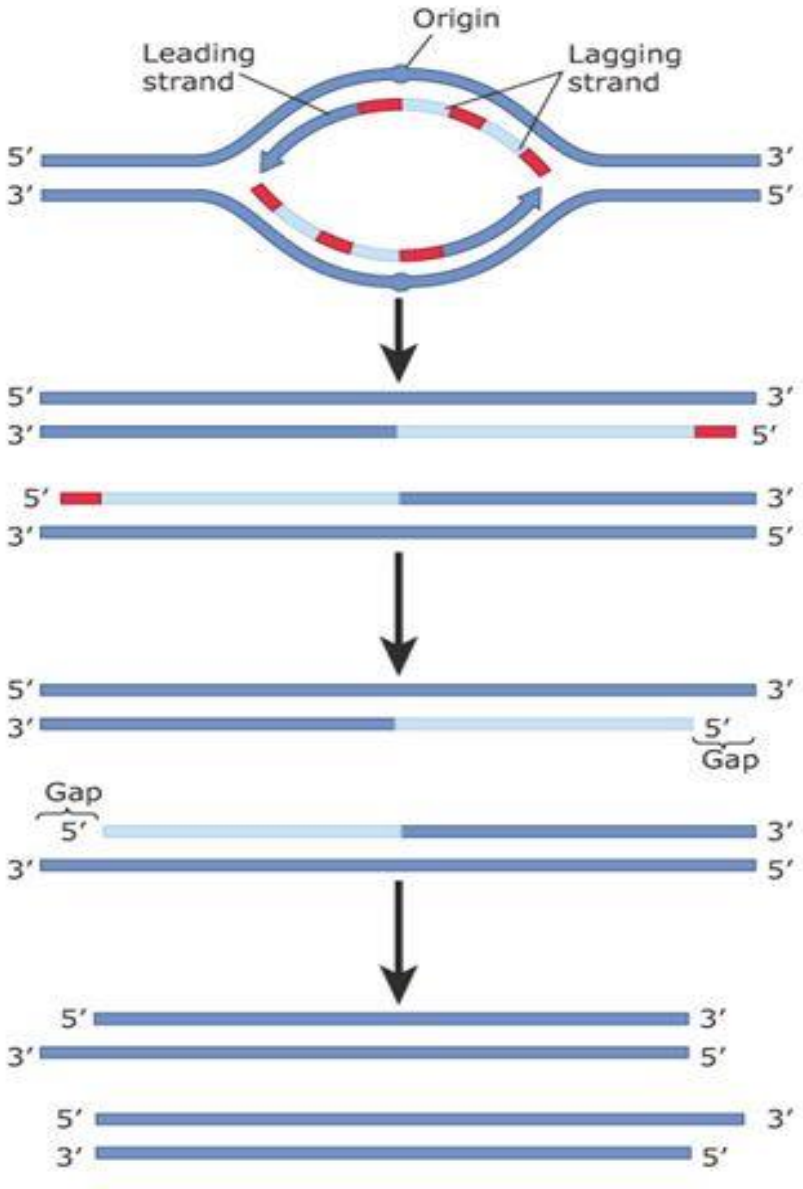
Linear ds-DNA replication



dsDNA bidirectional replication

Replication of 5′ end of linear chromosome

- Replication of a linear chromosome results in sequence loss from the chromosome end.
- Telomere are the structure at the end of the chromosomes comprised of numerous repeats of a short sequence.



Enzymes involved in DNA replication

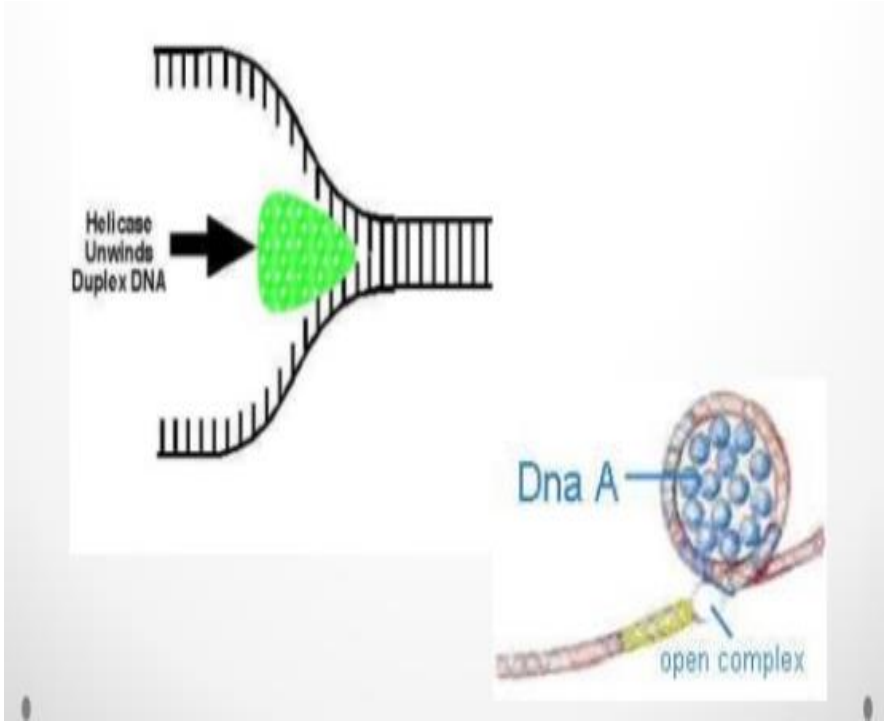
- **Helicase** (unwinds the DNA double helix)
- **Gyrase** (relieves the build up of torque during unwinding), A type II Topoisomerase
- **Primase** (lays down RNA primers)
- **DNA polymerase III** (main DNA synthesis enzyme)
- **DNA polymerase I** (replaces RNA primers with DNA)
- **Ligase** (fills in the gaps)

HELICASE

- Helicases are defined as a class of enzymes that catalyze the separation of duplex nucleic acids into single strands in an ATP-dependent reaction and function in DNA modification processing, including DNA replication, DNA repair, recombination, transcription, translation, and many other nucleic acid-related processes (Jankowsky & Fairman, 2007)

How does helicase separate DNA?

- **DNA helicase** is the enzyme that unwinds the **DNA** double helix by breaking the hydrogen bonds down the center of the strand. It begins at a site called the origin of replication, and it creates a replication fork by **separating** the two sides of the parental **DNA**.



GYRASE

- An enzyme that changes the number of times the two strands in a closed DNA molecule cross each other. It does this by cutting the DNA, passing DNA through the break, and resealing the DNA.
- It's belongs to a class of enzymes known as topoisomerases that are involved in the control of topological transitions of DNA.

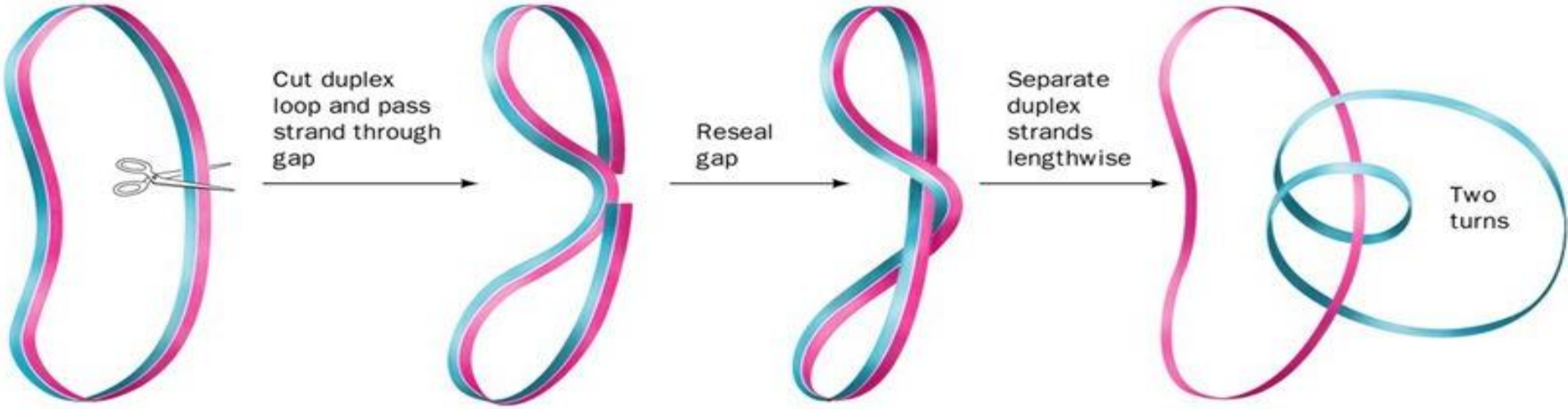
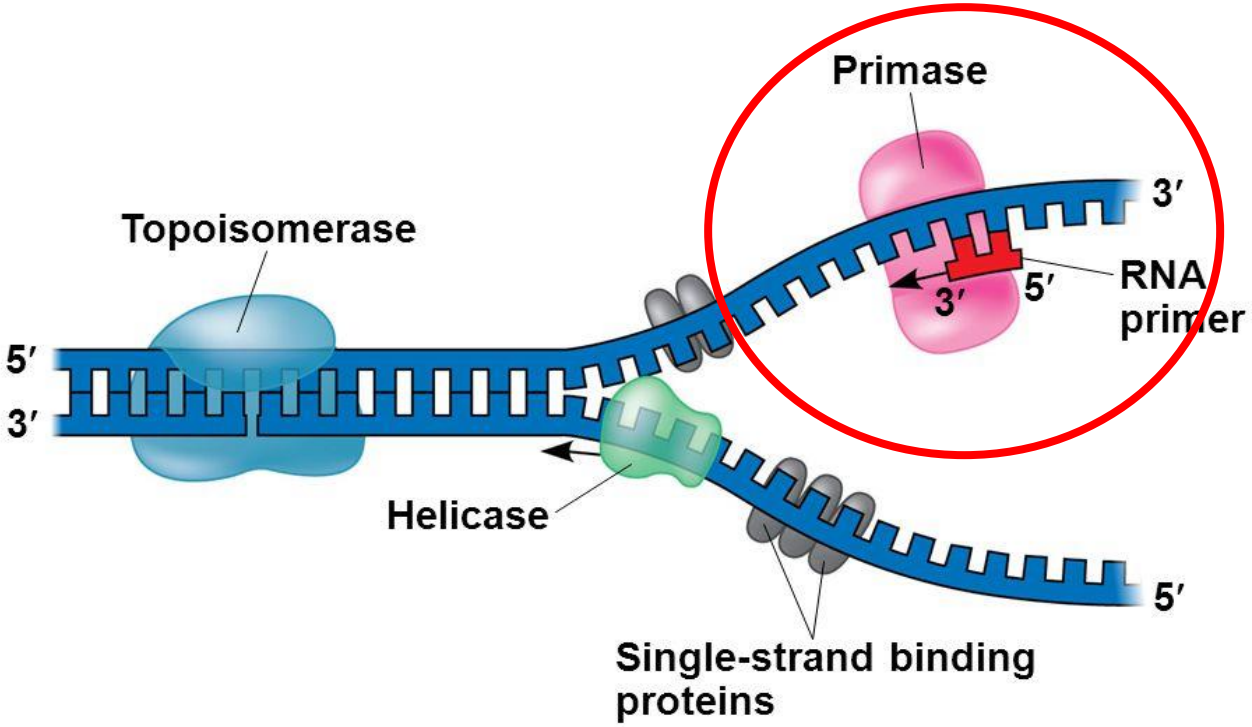


Figure 29-30 A demonstration that DNA gyrase acts by cutting both strands of a duplex, passing the duplex through the break, and resealing it.

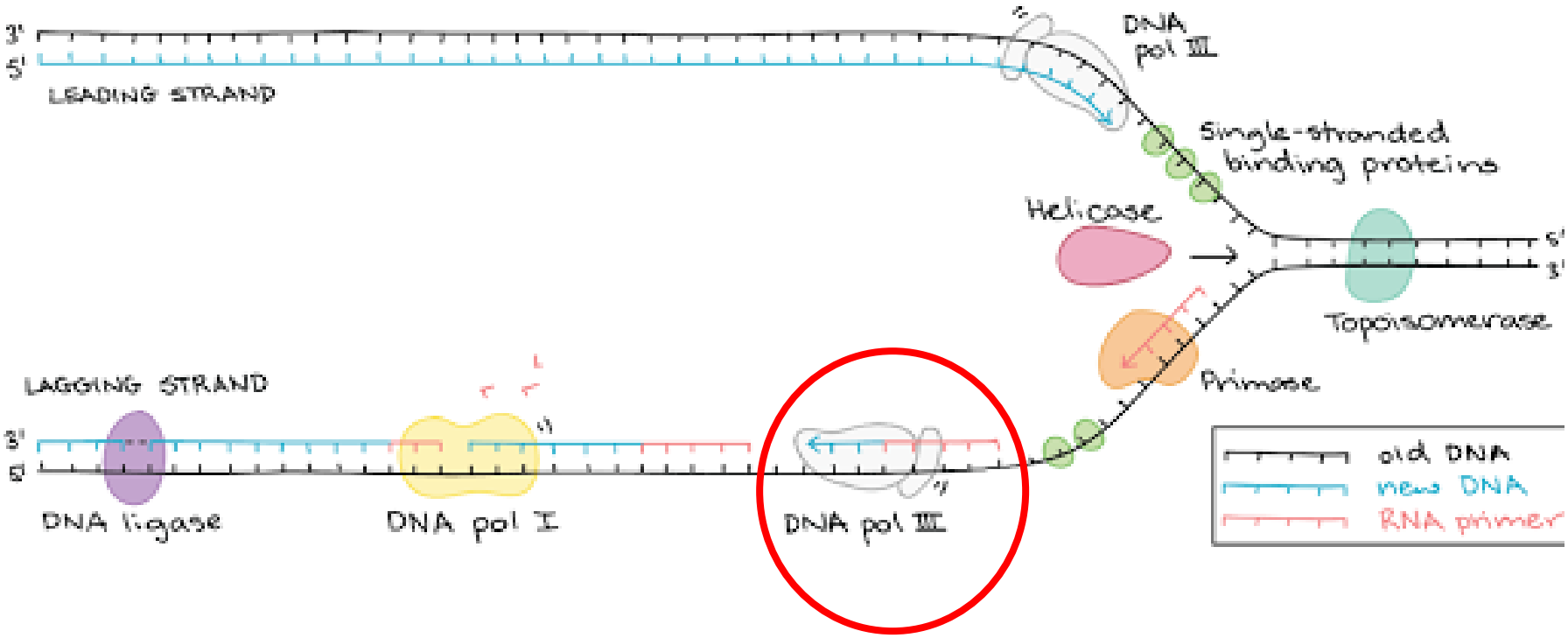
PRIMASE

- A type of RNA polymerase that synthesize short segments of RNA that will be used as primers for DNA replication.
- **Primase** is an enzyme that creates a primer on a **DNA** strand by adding RNA nucleotides to the strand according to the **DNA** template sequence. This process occurs during **DNA replication.**



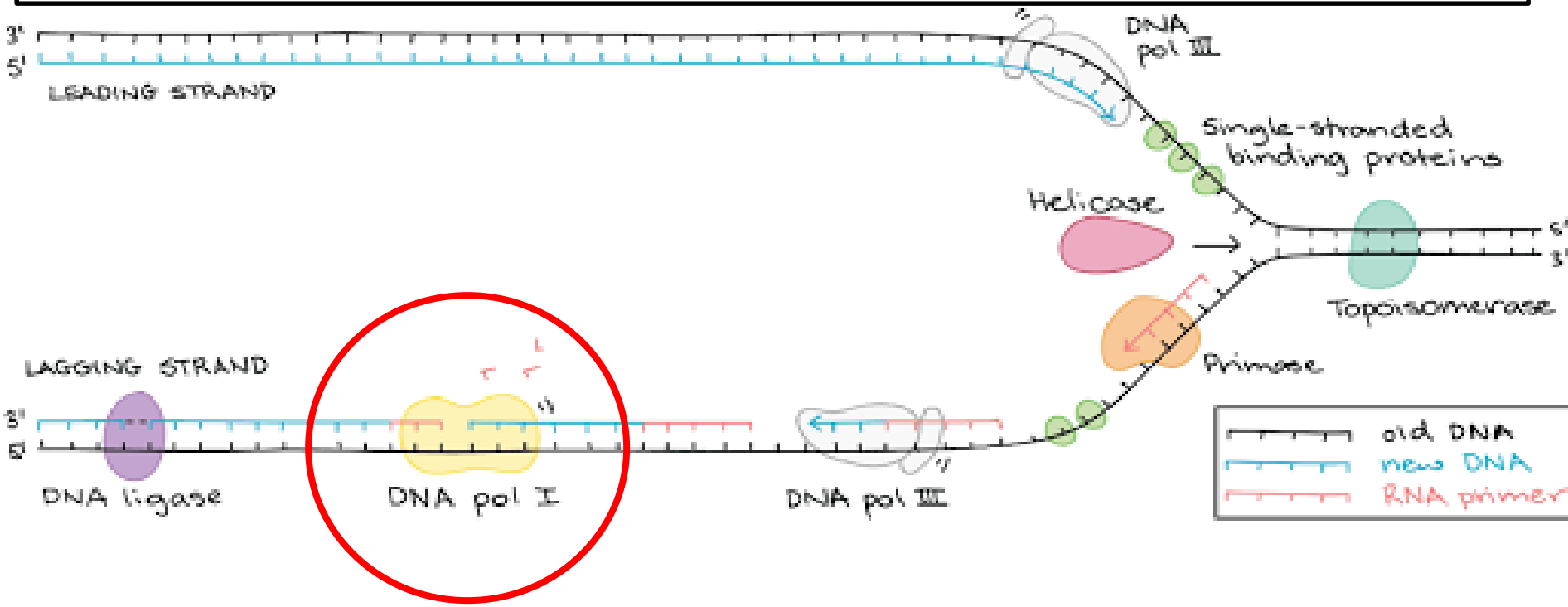
DNA polymerase III

- **DNA polymerase** III holoenzyme is the enzyme primarily responsible for replicative **DNA** synthesis in E. coli. It carries out primer-initiated 5' to **3**' polymerization of **DNA** on a single-stranded **DNA** template, as well as **3**' to 5' exonucleolytic editing of mis-paired nucleotides.



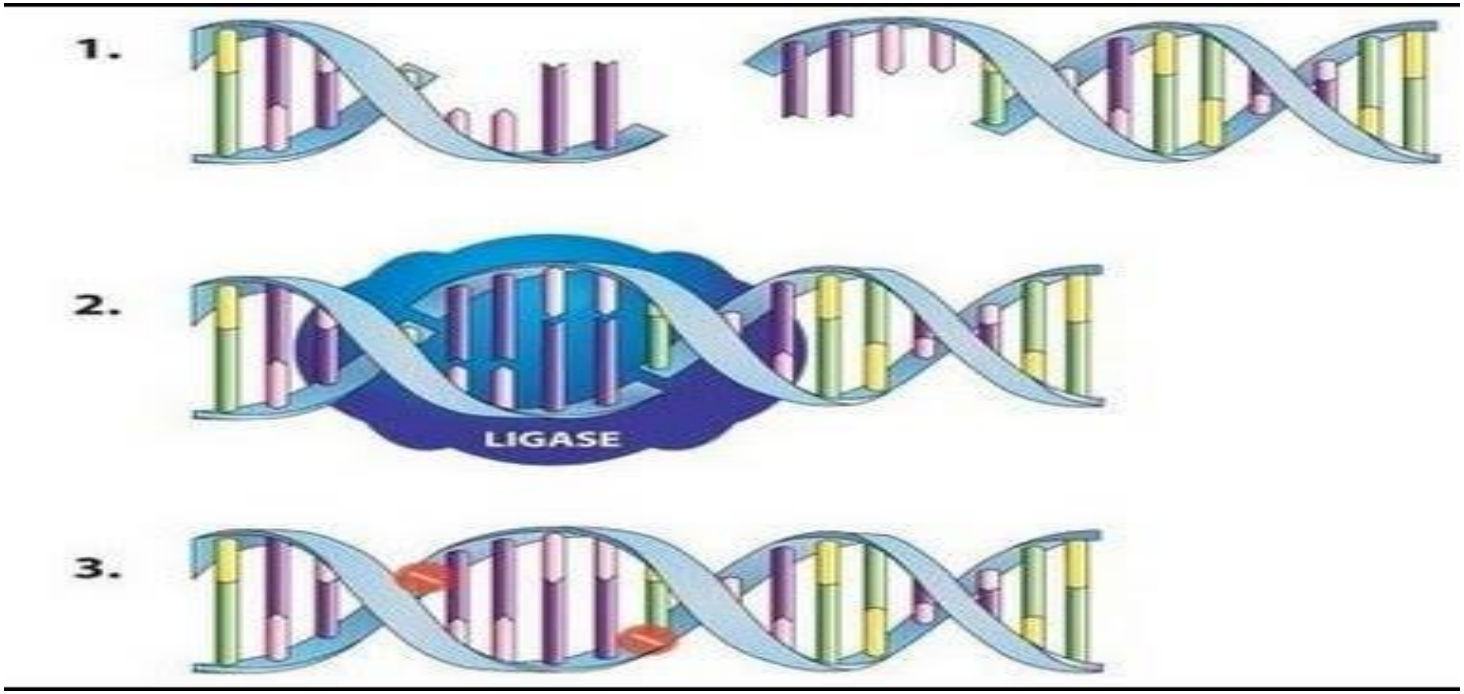
DNA polymerase I

- **DNA polymerase** I (or **Pol** I) is an enzyme that participates in the process of prokaryotic **DNA replication**. The physiological **function** of **Pol** I is mainly to repair any damage with **DNA**, but it also serves to connect Okazaki fragments by deleting **RNA** primers and replacing the strand with **DNA**.



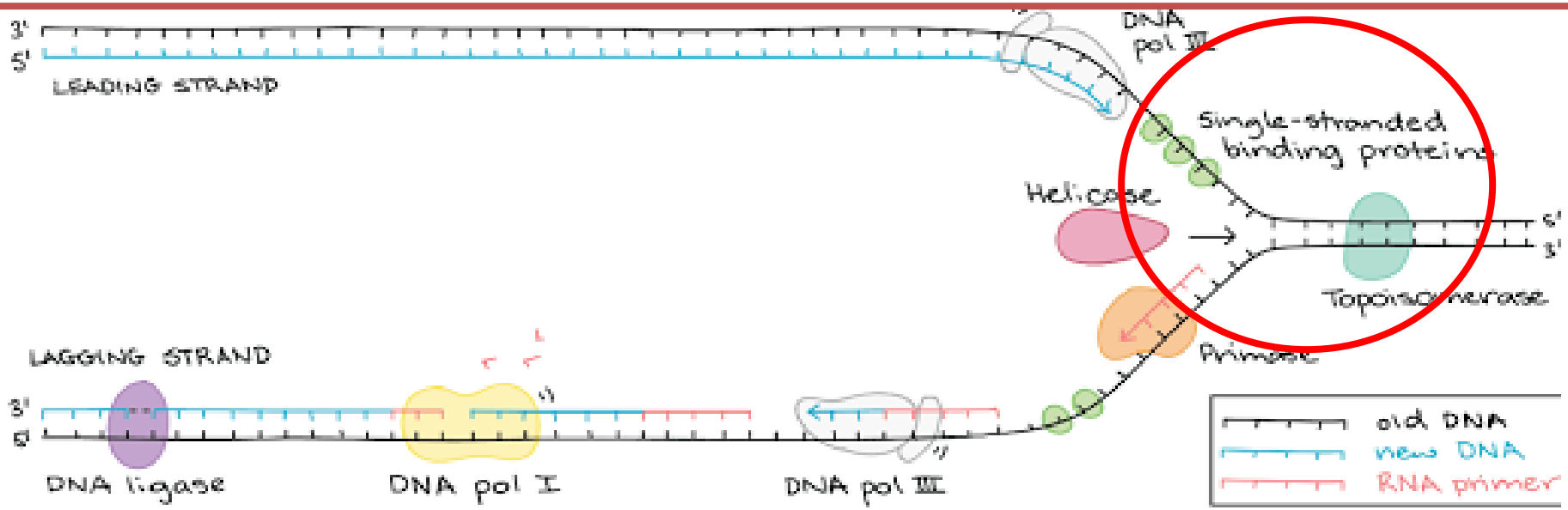
LIGASE

- Enzyme that function like molecular ‘glue’, Bonding two separate DNA together.
- DNA ligase seals the “nicks” between Okazaki fragments converting them to a continuous strands of DNA.
- Covalently closes the nicks in double stranded DNA.



Single Stranded Binding Protein (SSBPs)

- Accessory replication protein lacking enzymatic activity , but required for efficient activity of other enzymes in the replisome.
- It binds to the single stranded part of the each DNA strand preventing the strands from reassociating and shielding them from degradation by nuclease.
- In *E.coli* and eukaryotes protein is called SSB, and interacts with primase to help specific priming activity.
- The strands separated by holding them in place so that each strand can serve as a template for new **DNA** synthesis.



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