# DNA REPLICATION

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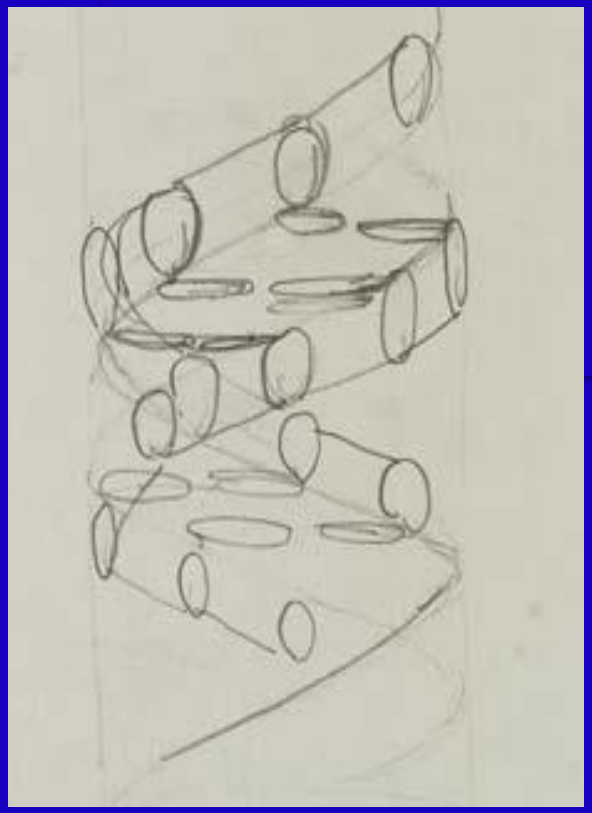
# Structure of a DNA molecule

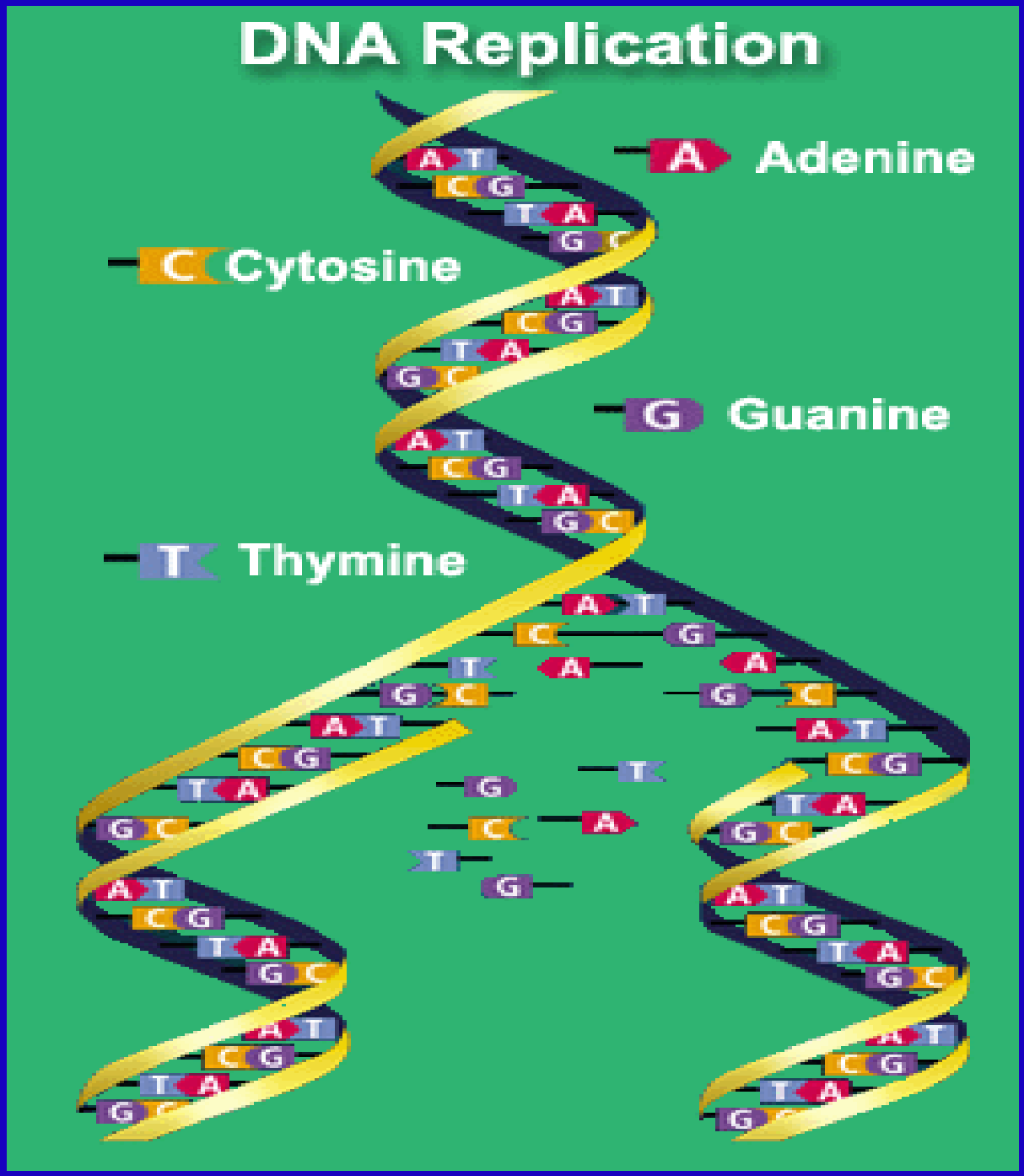
`It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material'

**Watson & Crick**

***Nature (1953)***

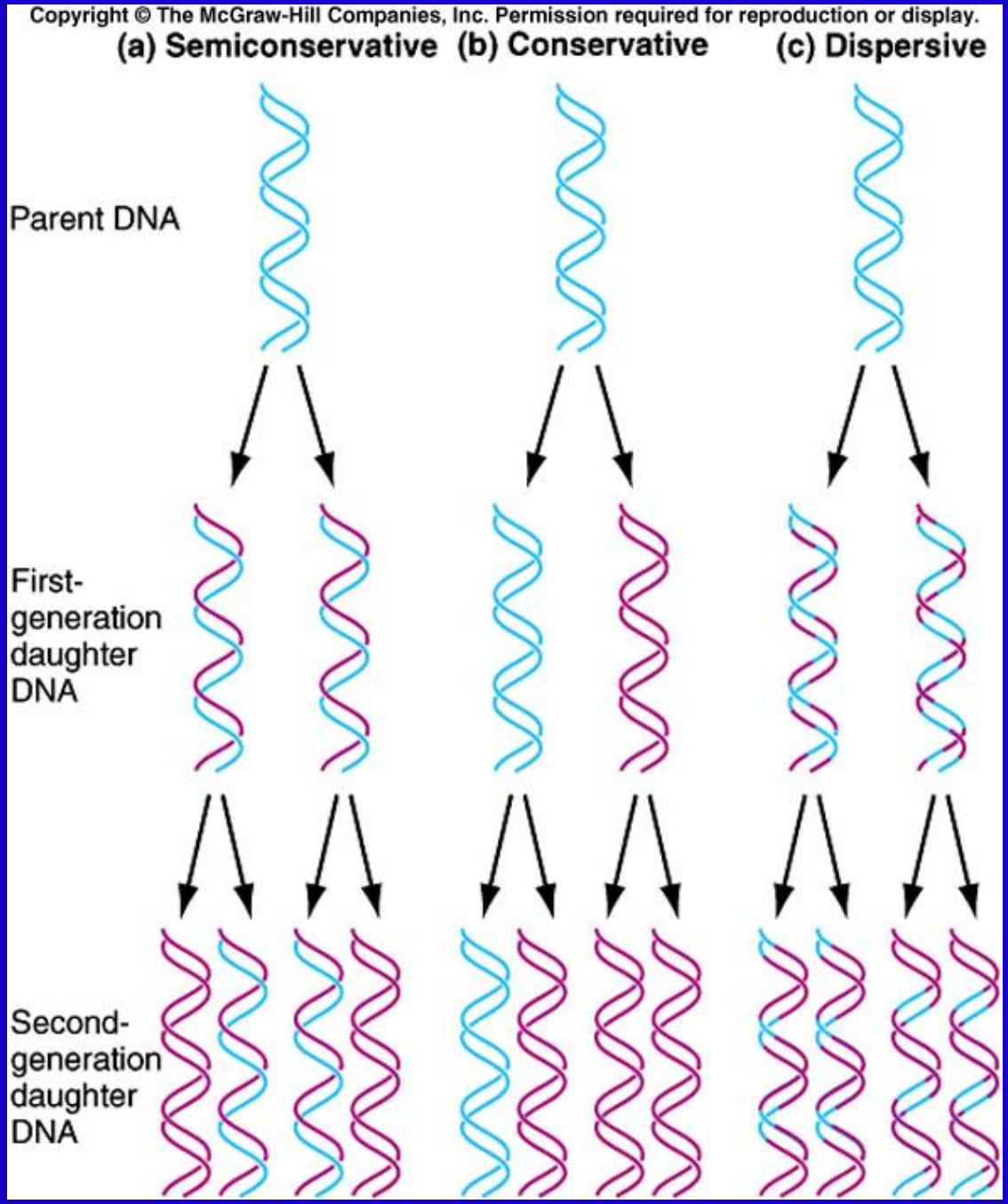


Original drawing by Francis Crick


DNA Replication
A Adenine
C Cytosine
G Guanine
T Thymine

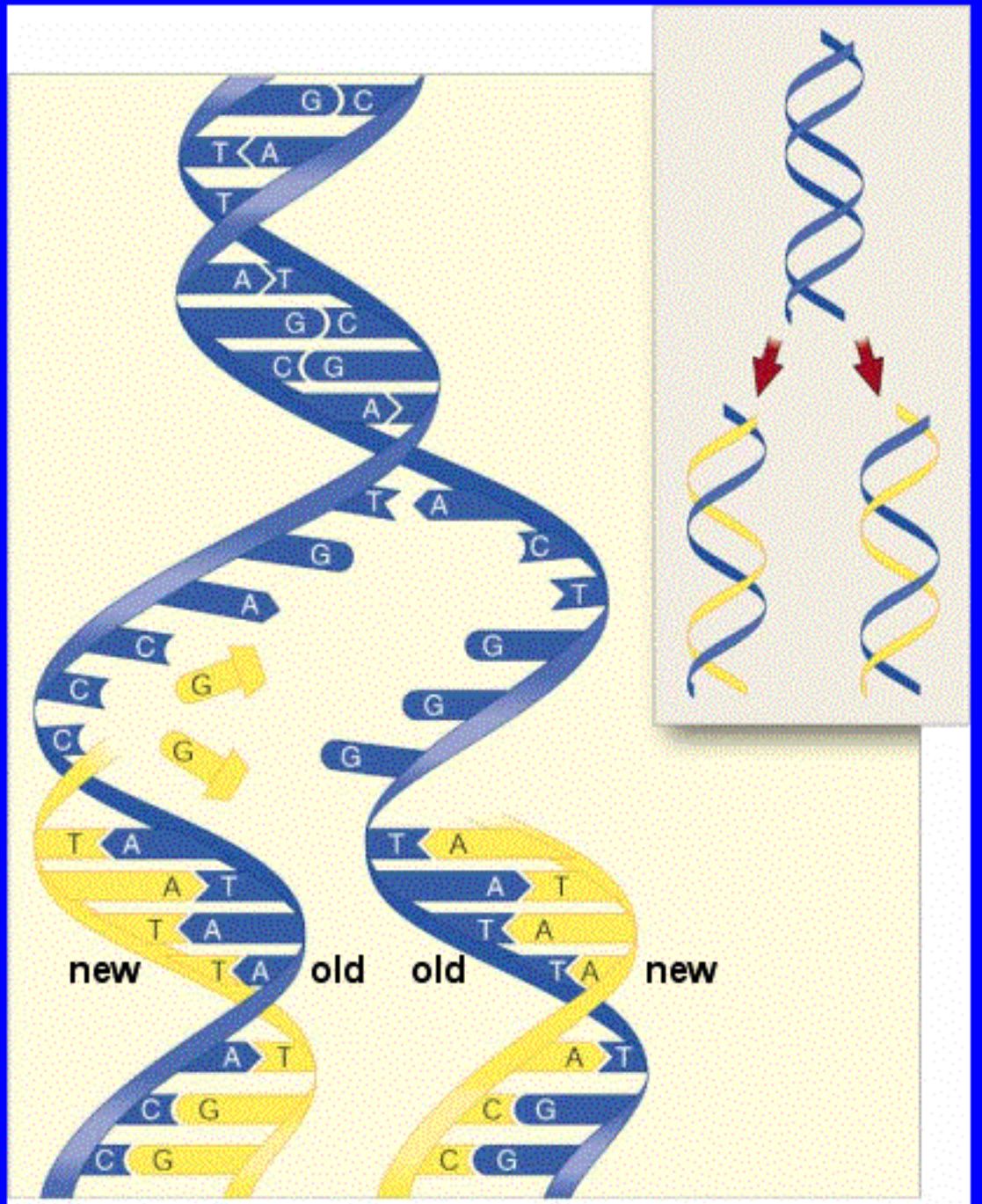
# DNA replication

## 3 possible models



# DNA Replication

- Each parent strand remains intact
- Every DNA molecule is half "old" and half "new"



# Basic rules of replication

A. Semi-conservative
B. Starts at the ‘origin’
C. Synthesis always in the 5'-3'direction
D. Can be uni or bidirectional
E. Semi-discontinuous
F. RNA primers required

# What kind of enzyme synthesizes the new DNA strand?

1) RNA polymerase
2) DNA Polymerase
3) Primase
4) Helicase
5) Topoisomerase

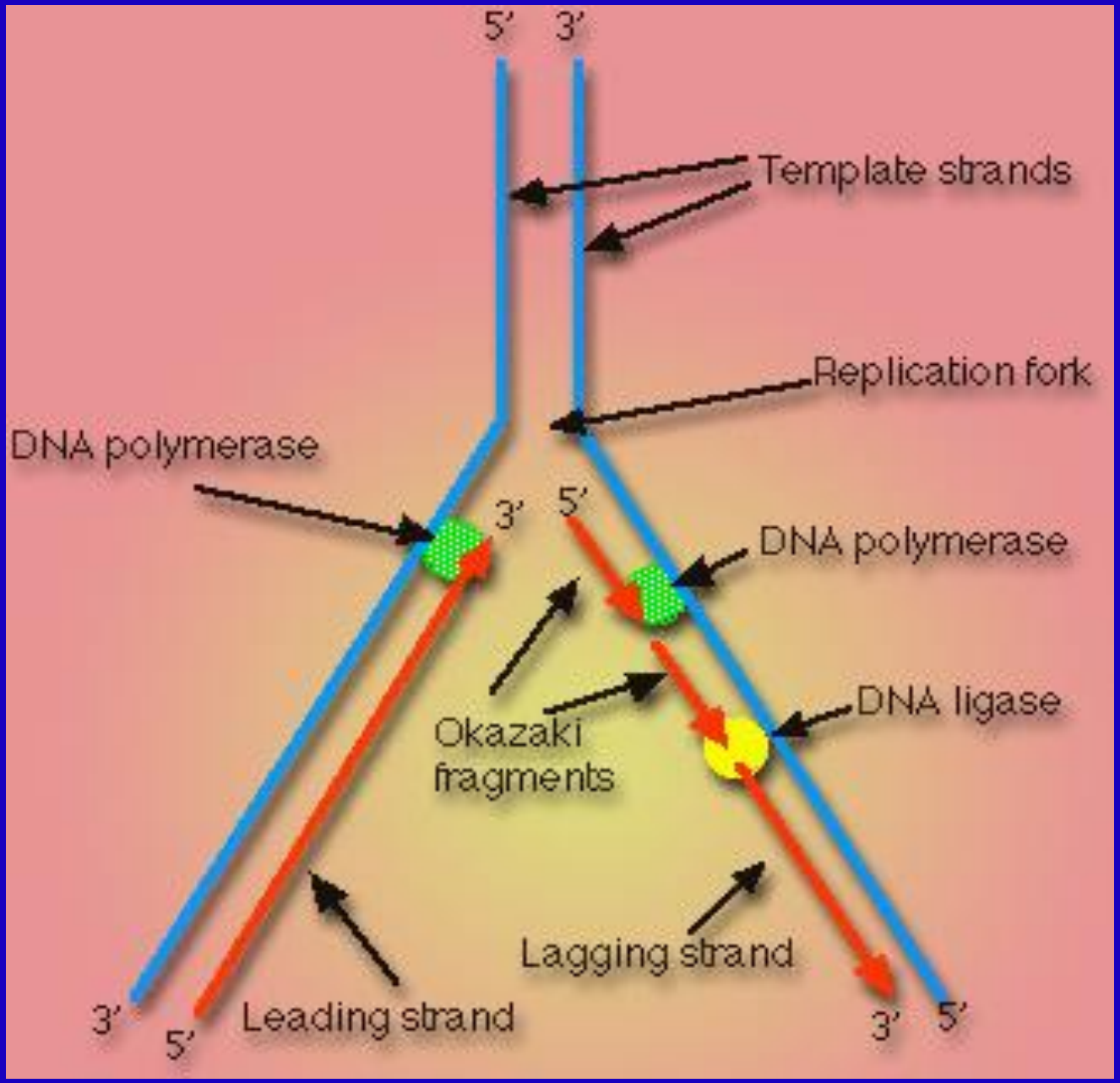
# *Core proteins at the replication fork*

| | |
|---|---|
| **Topoisomerases** | **- Prevents torsion by DNA breaks** |
| **Helicases** | **- separates 2 strands** |
| **Primase** | **- RNA primer synthesis** |
| **Single strand binding proteins** | **- prevent reannealing of single strands** |
| **DNA polymerase** | **- synthesis of new strand** |
| **Tethering protein** | **- stabilises polymerase** |
| **DNA ligase** | **- seals nick via phosphodiester linkage** |

# The mechanism of DNA replication

**Arthur Kornberg**, a Nobel prize winner and other biochemists deduced steps of replication

- Initiation
  - Proteins bind to DNA and open up double helix
  - Prepare DNA for complementary base pairing
- Elongation
  - Proteins connect the correct sequences of nucleotides into a continuous new strand of DNA
- Termination
  - Proteins release the replication complex


5′
3′
Template strands
Replication fork
DNA polymerase
3′
5′
DNA polymerase
DNA ligase
Okazaki
fragments
Lagging strand
3′
5′
Leading strand
3′
5′

# Steps involved in DNA Replication in Prokaryotes (*E.coli*)

In prokaryotes, the DNA is circular. Replication starts at a single origin (ori C) and is bi-directional and **semi-conservative**

The region of replicating DNA associated with the single origin is called a replication bubble or replication eye and consists of two replication forks moving in opposite direction around the DNA circle.

During DNA replication, the two parental strands separate and each acts as a template to direct the enzyme catalysed synthesis of a new complementary daughter strand following the base pairing rule.

Three basic steps involved in DNA replication are Initiation, elongation and termination.

# I. Initiation

**Step 1:** Binding of DNA around an initiator protein complex DNA-A ATP ~30-40.
The **DNA B** or helicase unwinds **ori C** (origin of replication) and extends the single stranded region for copying.

**Step 2:** Single strand binding protein (**SSB**) binds to this single stranded region to protect it from breakage and to prevent it from renaturing.
As the parental DNA is unwound by **DNA helicases** and SSB (travels in 5'-3' direction),

the resulting positive supercoiling (torsional stress) is relieved by topoisomerse I and II (DNA gyrase) by inducing transient single stranded breaks.

**Step 3:** The enzyme DNA primase (primase, an RNA polymerase) then attaches to the DNA and synthesises a short RNA primer to initiate synthesis of the leading strand of the first replication fork.

# II. Elongation

**Step 4:** DNA polymerase III extends the RNA primer made by **primase**. DNA polymerase possesses separate catalytic sites for polyme-risation and degradation of nucleic acid strands.
All DNA polymerases make DNA in 5'-3' direc-tion
**Leading strand synthesis:**
On the template strand with 3'-5' orientation, new DNA is made continuously in 5'-3' direction towards the replication fork. The new strand that is continuously synthesized in 5'-3' direction is the leading strand.

**Lagging strand synthesis:** On the template strand with 5’-3’ orientation, multiple primers are synthesized at specific sites by primase (primosome complex) and DNA pol III synthesizes short pieces of new DNA (about 1000 nucleotides long) new DNA is in 5’-3’ direction.

- These small DNA fragments that are discontinuously synthesises are called Okazaki fragments (named after the discoverer Reigi Okazaki). The new strand which is discontinuously synthesised in small fragments is called the lagging strand.

DNA polymerase III synthesizes DNA for both leading and lagging strands.

**Step 5:** After DNA synthesis by DNA pol III, DNA polymerase I uses its 5'-3' exonuclease activity to remove the RNA primer and fills the gaps with new DNA.

**Step 6:** Finally DNA ligase joins the ends of the DNA fragments together.

# III. Termination

**Step 7:** The two replication forks meet ~ 180 degree opposite to ori C, as DNA is circu-lar in prokaryotes. Around this region there are several terminator sites which arrest the movement of forks by binding to the tus gene pro-duct, an inhibitor of helicase (Dna B).

**Step 8:** Once replication is complete, the two double stranded circular DNA molecules (dau-ghter strands) remain interlinked. **Topoisomerase II** makes double stranded cuts to unlink these molecules.

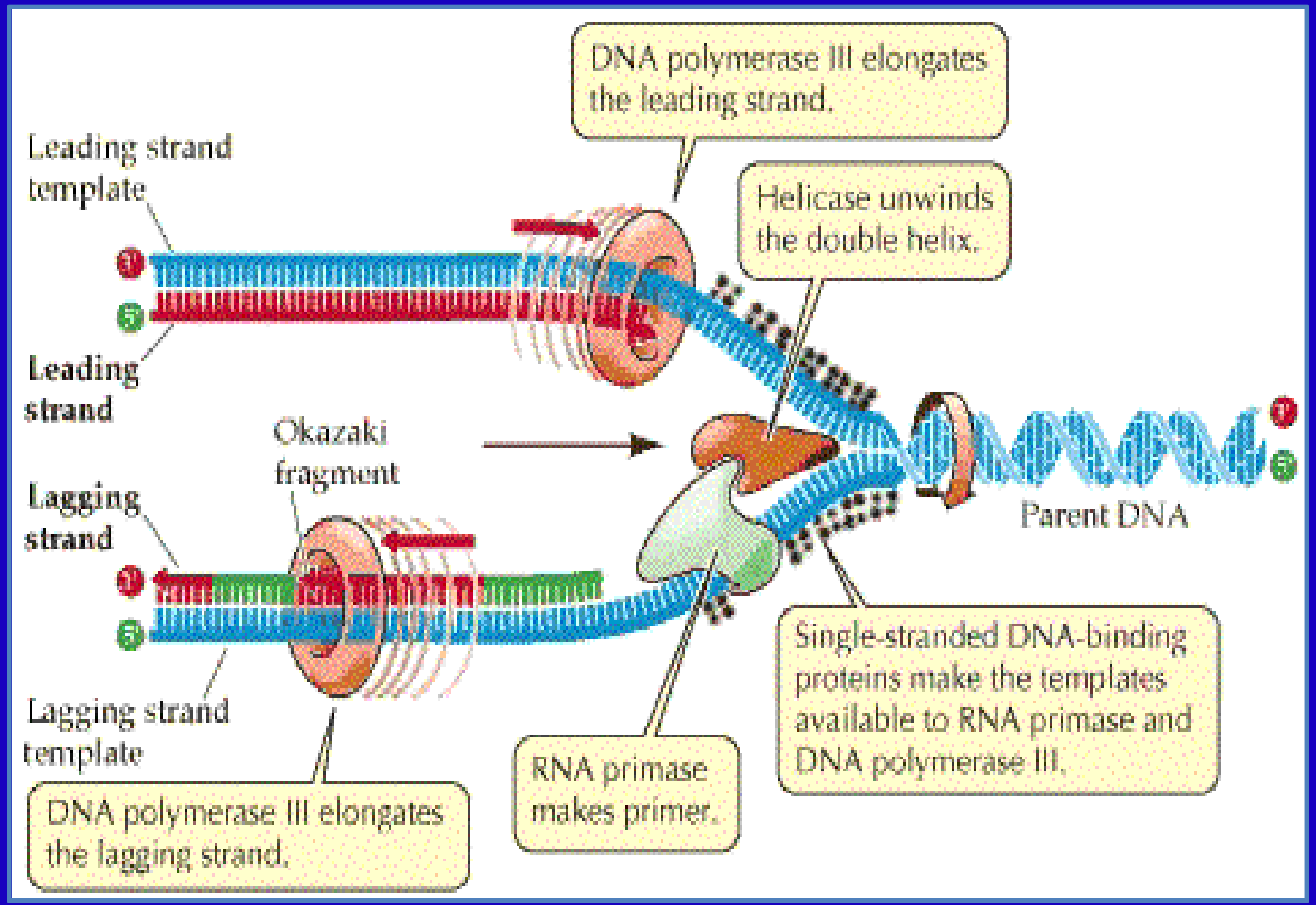
# Why is an RNA primer necessary for DNA replication?

A. The RNA primer is necessary for the activity of DNA ligase.

B. The RNA primer creates the 5' and 3' ends of the strand.

C. DNA polymerase can only add nucleotides to RNA molecules.

D. DNA polymerase can only add nucleotides to an existing strand


DNA polymerase III elongates the leading strand.
Leading strand template
Helicase unwinds the double helix.
3'
5'
Leading strand
Okazaki fragment
Lagging strand
3'
5'
Parent DNA
Single-stranded DNA-binding proteins make the templates available to RNA primase and DNA polymerase III.
Lagging strand template
RNA primase makes primer.
DNA polymerase III elongates the lagging strand.

# THANKS TO ALL