Cellular & Molecular Biology
SQBS 1143

Chapter 2:
DNA Replication

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The Gene is the Fundamental Unit of Heredity

- The unit of heredity is known as a **gene**. Each gene is responsible for a single inherited property or characteristic of the organism.

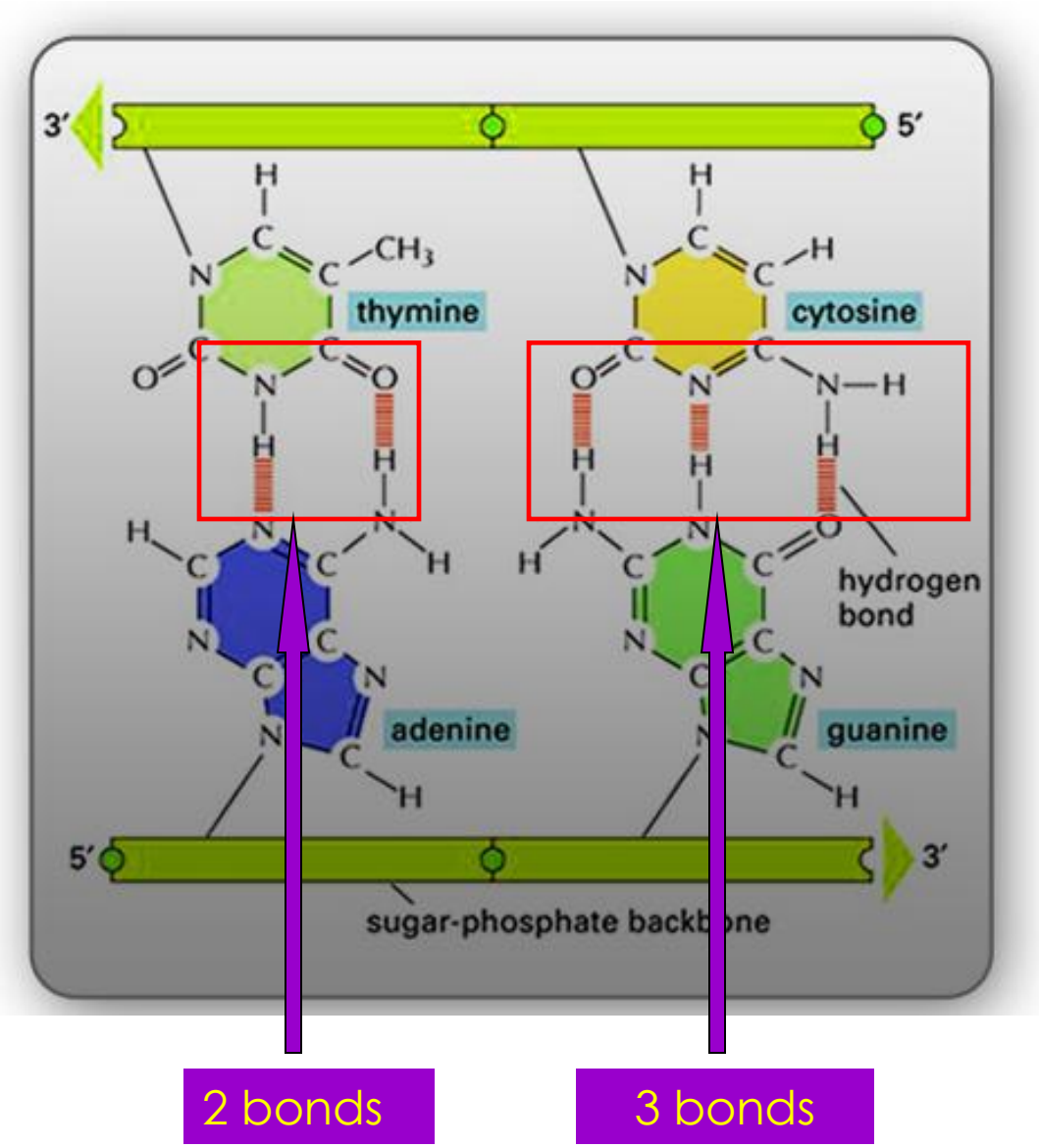
Inheritance of Genetic Information

- Since each cell needs a complete set of genes, it is necessary for the original cell to **duplicate its genes** before dividing.
- Because the genes are made of **DNA** and make up the **chromosomes**, this means that each chromosome must be accurately copied. Upon cell division, both daughter cells will receive identical sets of chromosomes, each with a complete set of genes.

Replication of DNA

- Replication of the DNA in molecular terms means that the DNA of the original, or mother, is **duplicated** to give **two identical copies**. This process is known as **replication**.

- Upon cell division each of the descendants gets one complete copy of the DNA. The original genes of the mother cell are on a double stranded DNA molecule so the first step in replication is to **separate the two strands** of the DNA double helix.



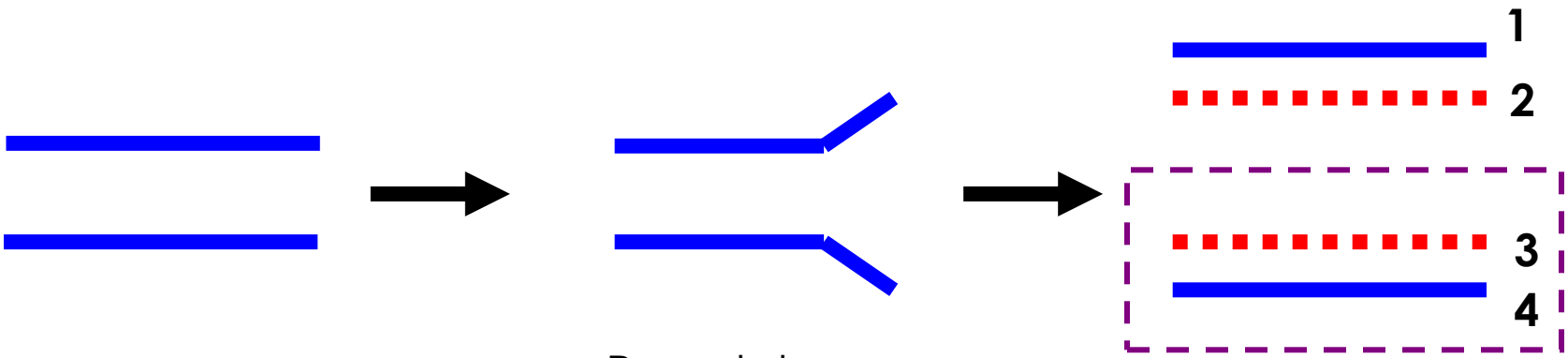
- The next step is to build a complementary strand on each of the two original strands. Since **A only pairs with T**, and since **G only pairs with C**, the sequence of each strand dictates the sequence of its complementary strand.

A = T C ≡ G

- We now have two double stranded DNA molecules, both with sequences identical to the original one. One of these daughter molecules has the original left strand and the other daughter has the original right strand.

- This is known as **semiconservative replication** of the progeny, conserves half of the original DNA molecule.

Semiconservative Replication



Strand 1 is identical to strand 3
Strand 2 is identical to strand 4

Strand 1 is complementary to strand 2
Strand 3 is complementary to strand 4

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

How Does a Double Helix Separate into Strands?

- Because the two strands forming a DNA molecule are held together by **hydrogen bonding** and twisted around each other to form a **double helix**, they cannot simply be pulled apart.

- Worse still, the DNA inside a cell is also **supercoiled** to pack it into a small space. Before separating the strands , both the **supercoils and the double helix must be unwound**.

How Does a Double Helix Separate into Strands?

This is done in two stages :

1. First the supercoils are unwound by an enzyme known as **DNA gyrase (DNA topoisomerase)**. The gyrase cuts both strands of double stranded DNA to give a double stranded break.

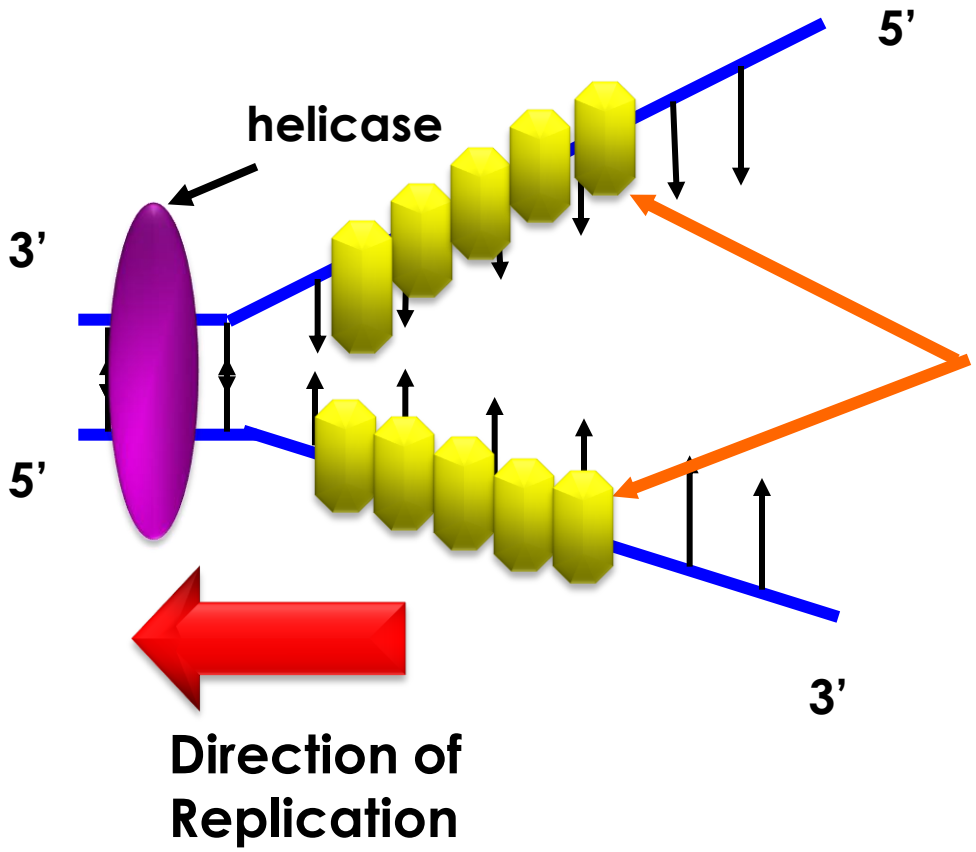
   However, it keeps hold of all of the cut ends. The two halves of the gyrase then rotate relative to each other and the ends are rejoined. This untwists the supercoils. Each rotation costs the cell a small amount of energy.

2. Once the supercoils have been untwisted, the double helix is unwound by the enzyme **DNA helicase**. Helicase does not break the DNA chain, it simply **disrupts the hydrogen bonds** holding the base pairs together.

How are the Parental Strands of DNA Kept Apart?

- The two separated strands of the parental DNA molecule are complementary to each other. Consequently all of their respective bases are capable of pairing off and binding to each other.
- In order to manufacture the new strands, the two original strands, despite their desire to cling together, must somehow be kept apart.
- This is done by means of a special "divorce" protein which **binds to the unpaired single stranded DNA and prevents the two parental strands from getting back together**. This is known as **Single Strand Binding protein (SSB)**

Single Strand Binding Protein



Making a New Strand of DNA

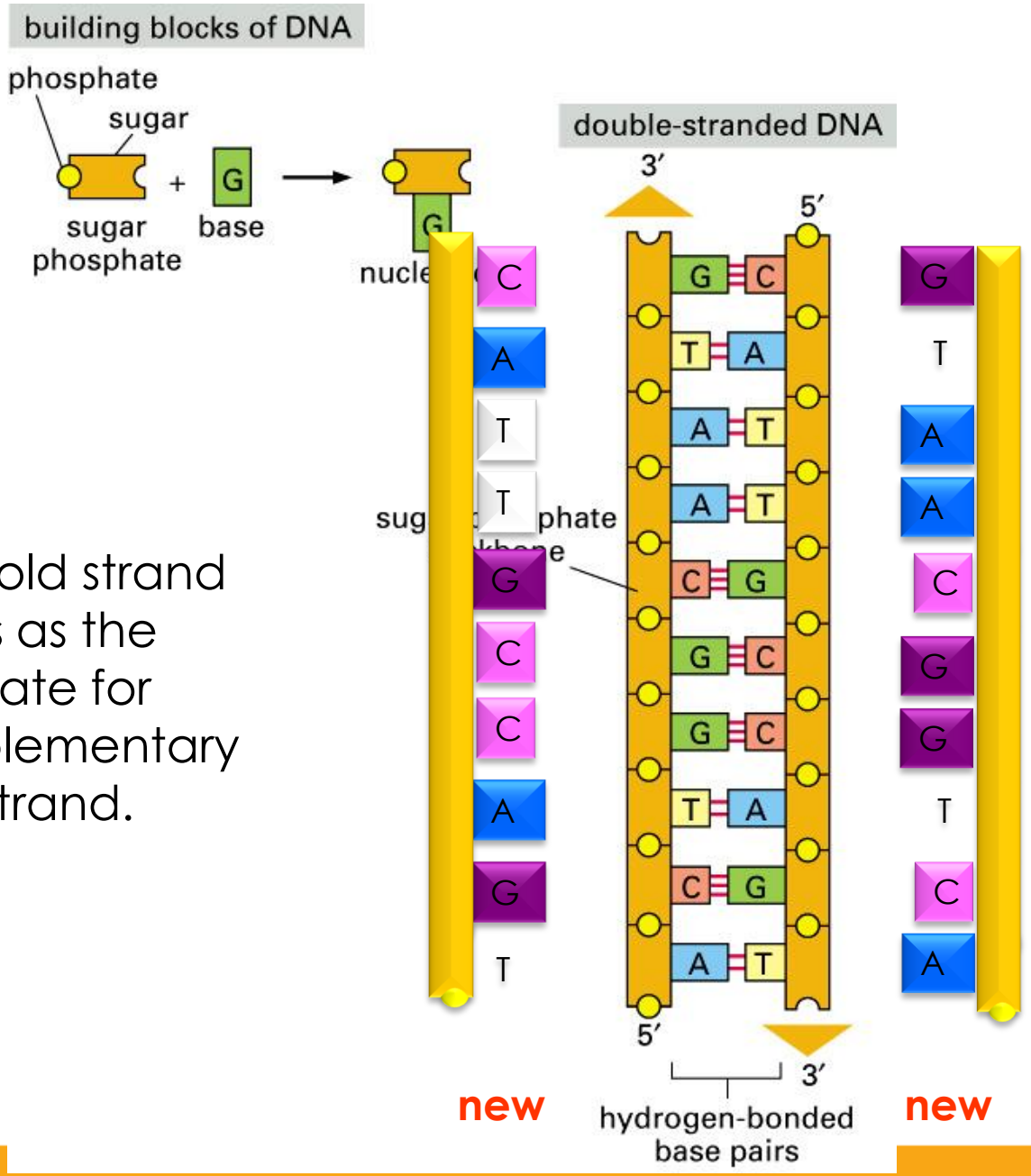
- The critical issue in replication is the base pairing of **A** with **T** and of **G** with **C**. Each of the separated parental strands of DNA serves as a template strand for the synthesis of a new complementary strand.

- The incoming nucleotides for the new strand recognize their partners by **base pairing** and so are lined up on the template strand. Actually, things are a bit more complicated.

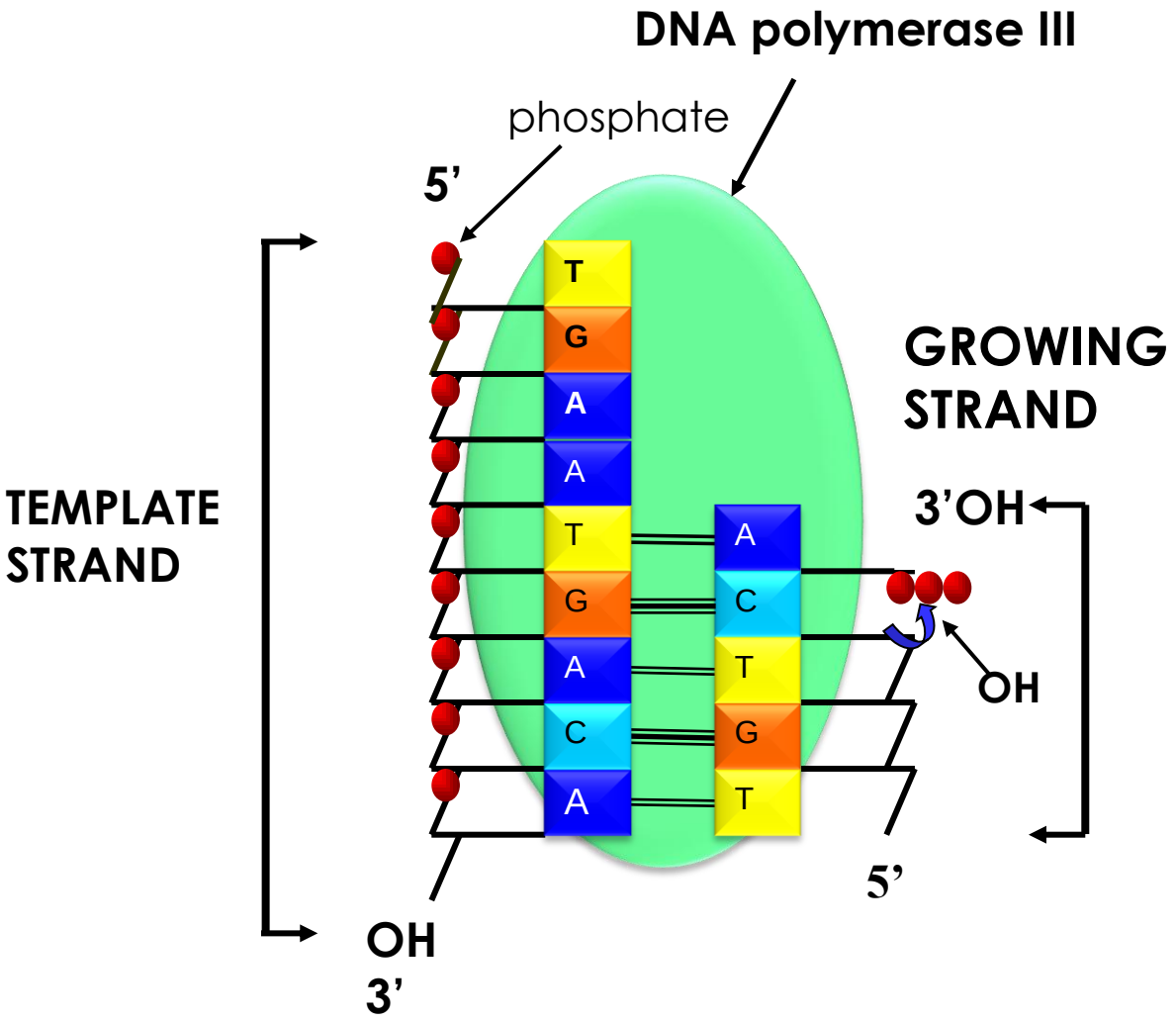
- Although hydrogen bonding alone would match bases correctly (**99%**) of the time this is not good enough.

- The enzyme that links the nucleotides known as **DNA polymerase III** or **pol III** can also sense if the bases are correctly paired. If not, the mismatched base pair is rejected.

Base-pairing of DNA



Each old strand serves as the template for complementary new strand.



A Closer Look at Strand Assembly

Energy for strand assembly is provided by removal of two phosphate groups from free nucleotides

DNA POLYMERASE III MAKING DNA
DNA Polymerase III / Pol (III) enzyme that makes most of the DNA when chromosome are replicated

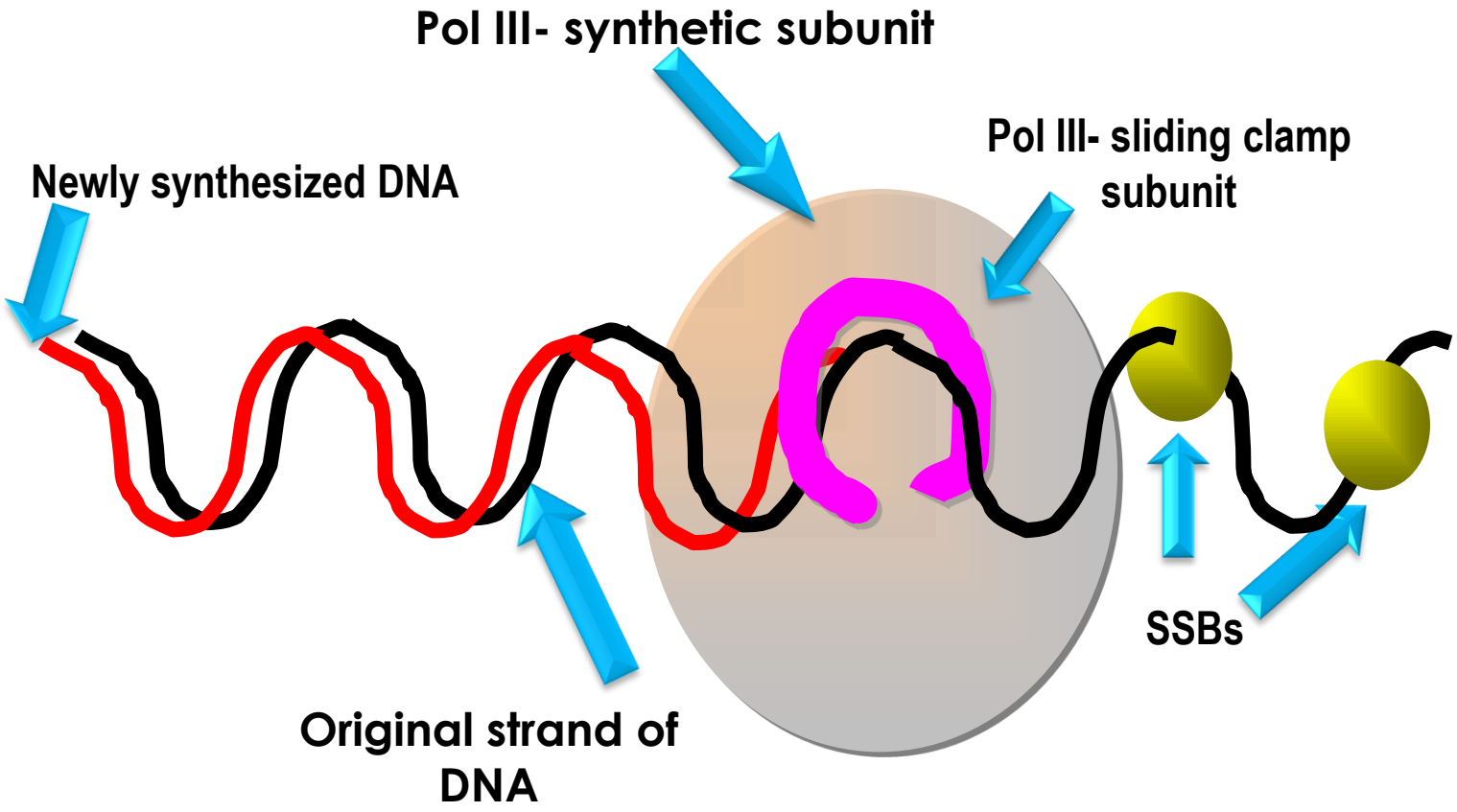
DNA polymerase III

The nucleotides are then joined together by the enzyme. This DNA polymerase has two subunits.

i. One of these is the **synthetic subunit** and is responsible for manufacturing new DNA.

ii. The other subunit is shaped like a doughnut and slides up and down like a curtain ring on the template strand of DNA. This **"sliding clamp" subunit** binds the synthetic subunit to the DNA.

DNA Polymerase III – The Sliding Clamp



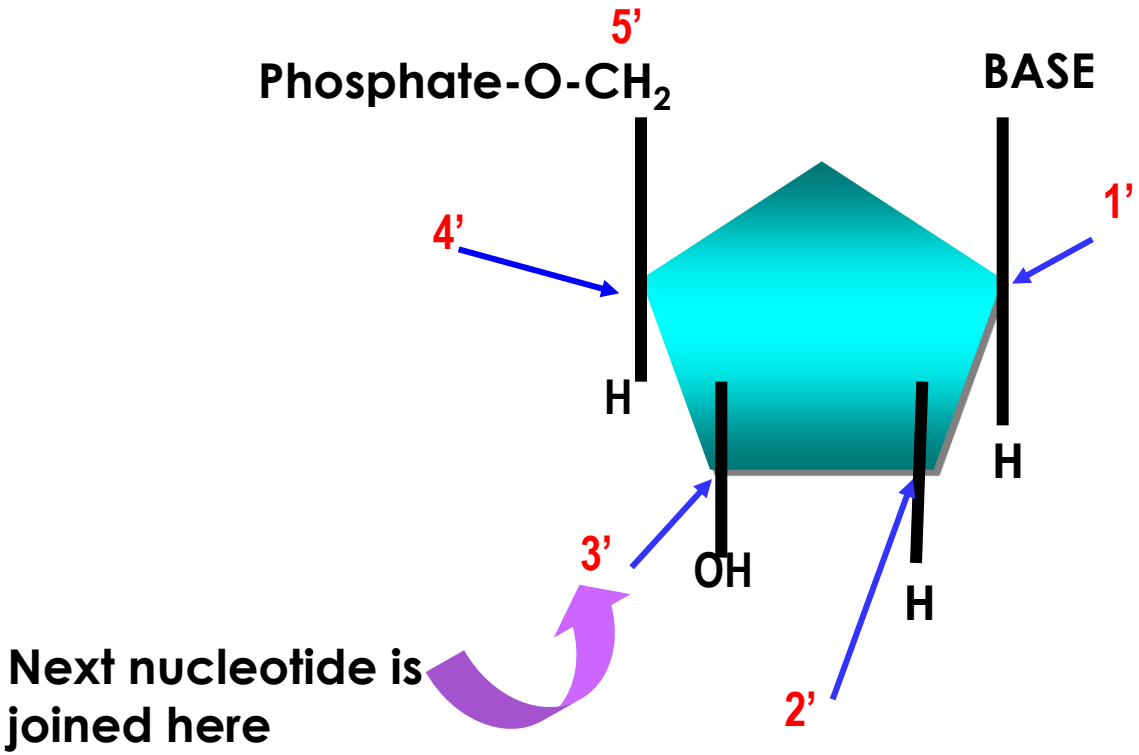
Synthesis Always Goes from 5' to 3'

As you know, nucleotides have three components:

a. **a phosphate group**
b. **sugar** and
c. **the base**.

- In DNA the sugar is **deoxyribose**, and is joined to the base at **position 1'** and to the phosphate group at **position 5'**.

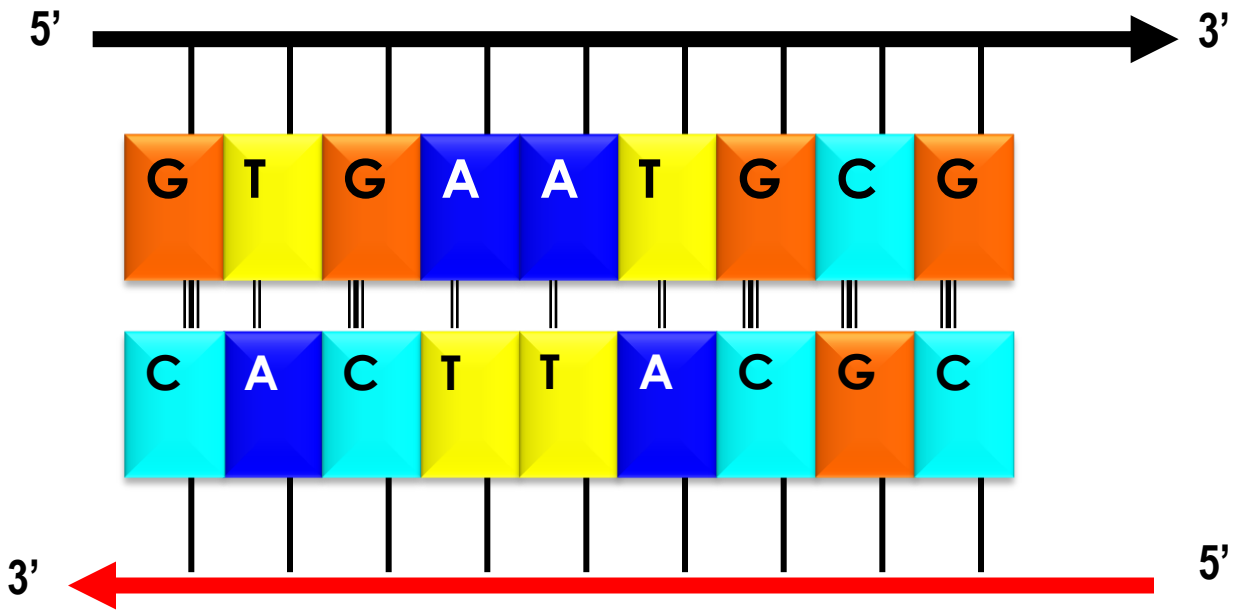
- (The carbon atoms of the deoxyribose sugar are numbered with prime marks to distinguish them from those of the base which have plain numbers)


5'
Phosphate-O-CH2
BASE
4'
1'
H
H
3'
OH
H
2'
Next nucleotide is joined here

Direction of DNA Synthesis

- When a new nucleotide is added it is joined, via its own phosphate group on position **5'** to the **3'** position as indicated by the arrow.
- New DNA strands always start at the 5' end and grow in the 3' direction. In fact, all nucleic acids, whether DNA or RNA, are always made in the 5' to 3' direction.
- However, DNA is normally double stranded, and it happens that the two strands run in opposite directions, that is, if one goes 5' to 3' then its complementary partner will run from 3' to 5'. The strands are said to be **anti-parallel**.

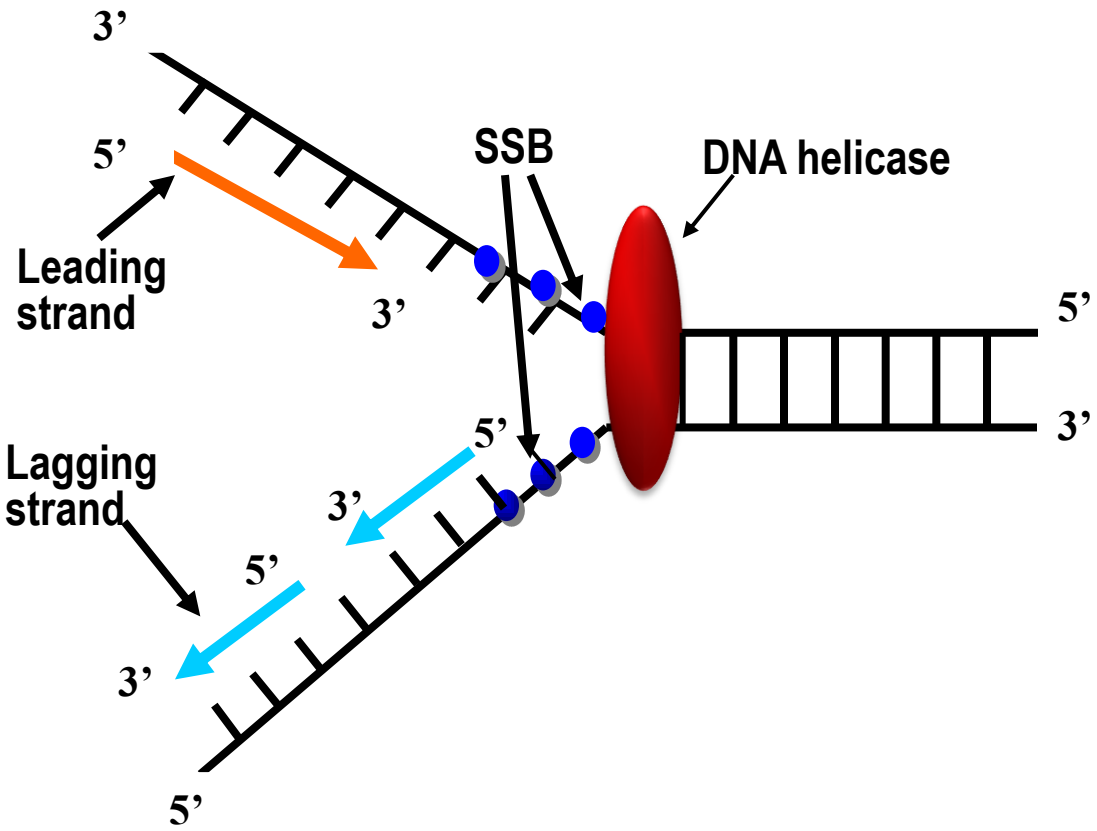
Double stranded DNA is antiparallel



The Replication Fork Is Where the Action Is!

- The replication fork is the total structure in the region where the DNA molecule is being duplicated.
- It includes the swivel where the DNA is being twisted by **DNA gyrase**, the **helicase** following right behind, and the stretches of single stranded DNA held apart by the **single strand binding protein**.
- It also has two molecules of **DNA polymerase III** which are busy making two new strands of DNA. Since DNA is always made in the 5' to 3' direction, and since the two strands of double helical DNA are **antiparallel**, this means that during DNA replication the two new strands must be synthesized in **opposite directions**.

The Replication Fork

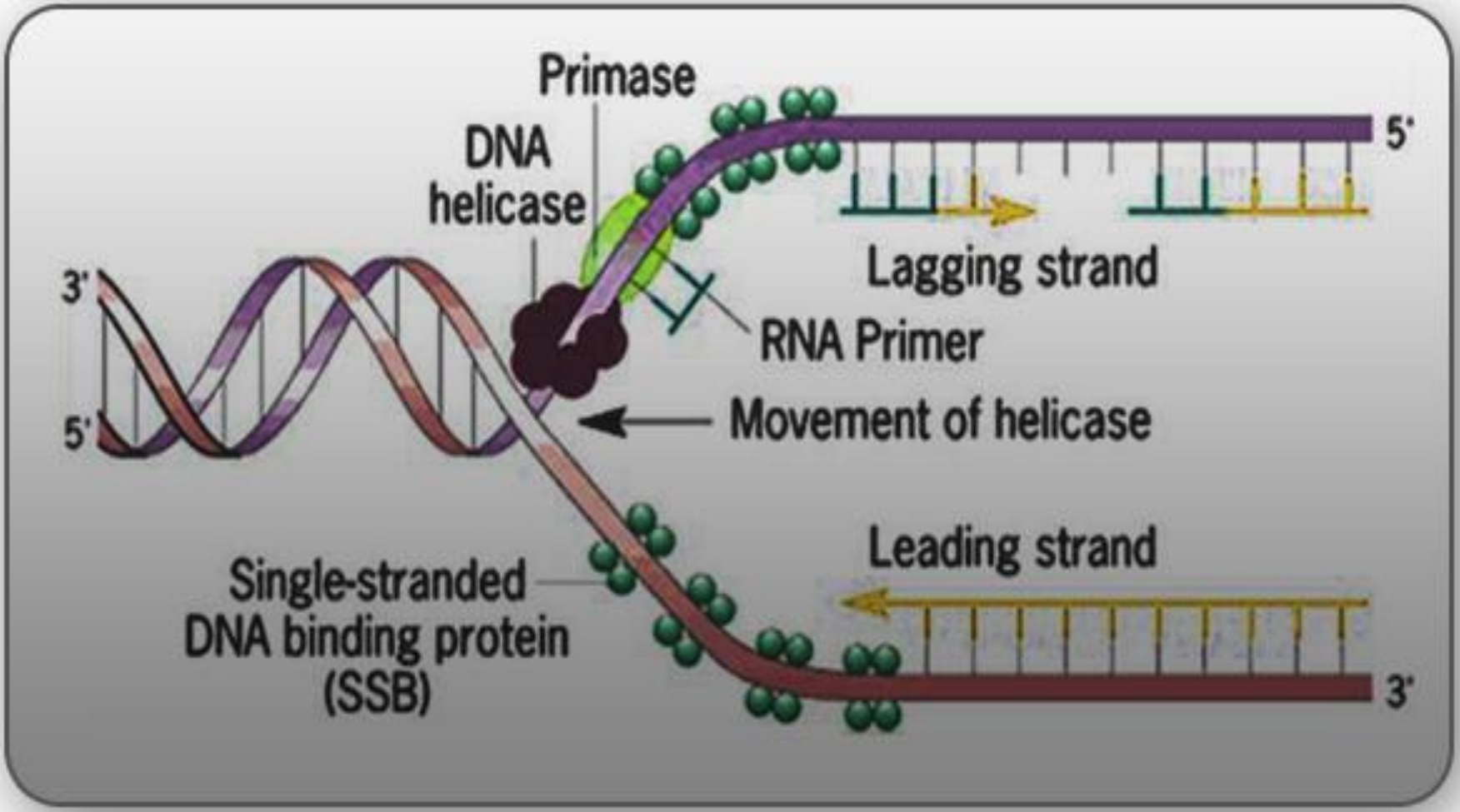


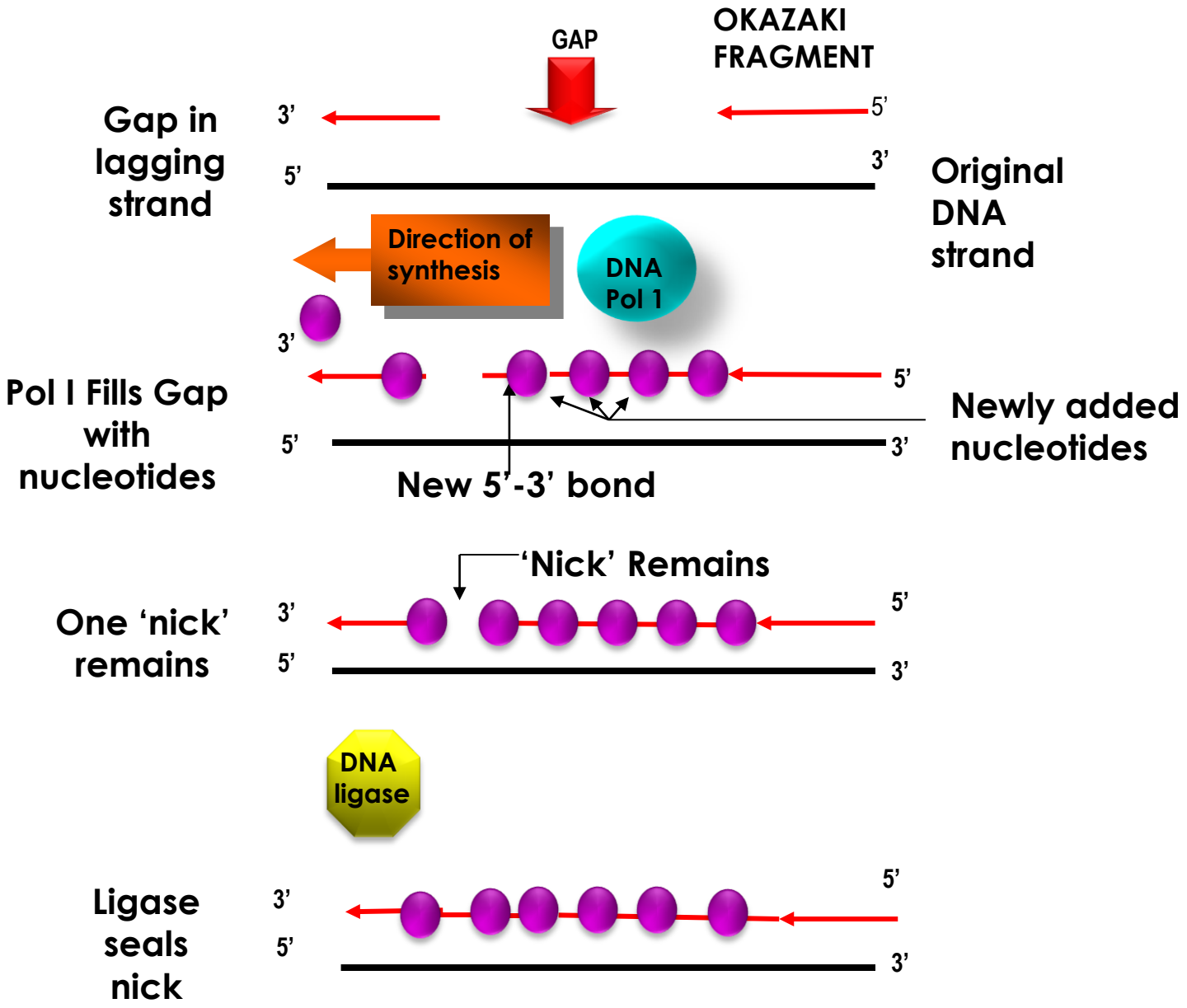
Completing the Lagging Strand

- Although the leading strand just keeps getting longer and longer, the lagging strand is handicapped.

- After the replication fork has passed by, the lagging strand is left as a series of short pieces with gaps in between. These newly made pieces of DNA are known as **Okazaki fragments** after their discoverer and must be joined together to give a complete strand of DNA.

Replication Fork Revisited




GAP
OKAZAKI FRAGMENT
Gap in lagging strand
3'
5'
5'
3'
Original DNA strand
Direction of synthesis
DNA Pol 1
3'
5'
Pol I Fills Gap with nucleotides
5'
3'
Newly added nucleotides
New 5'-3' bond
'Nick' Remains
One 'nick' remains
3'
5'
5'
3'
DNA ligase
Ligase seals nick
3'
5'
5'
3'

- This is accomplished by two enzymes working in succession: **DNA polymerase I** and **DNA ligase**. DNA polymerase I fills in the gaps and DNA ligase joins the gaps.

- DNA polymerase I was discovered before DNA polymerase III, hence the numbering.

- Both DNA polymerase I and DNA ligase have important uses in genetic engineering.

Starting a New Strand

- Up to now we have assumed that we have strands of DNA with free ends that can be elongated by DNA polymerase. But how do we get a new strand started?

- Although the leading strand only needs to be started once, the lagging strand is **made in short sections** and we **need to start again** every time we make a new **Okazaki fragment**.

- Curiously, DNA polymerase **cannot start a new strand by itself, it can only elongate**!

- New strands are started with short stretch, not of DNA itself, self, but of RNA!

- These **short RNA pieces** known as **primers** and the enzyme that starts synthesis of new chains by making the RNA primers is called **primase**.

- So every time a new fragment of DNA is made, primase sneaks in and lays down a short RNA primer to get things going. Only then can DNA polymerase get to work elongating the strand.

Recoiling the DNA into a Helix

- As the two new strands of DNA are synthesized, two double DNA molecules are produced, each with one old and one new strand.
- Once the replication fork has moved past, the double stranded DNA molecule automatically rewinds into a helix.

Enzymes involved in DNA Replication- details

- **Two DNA polymerase molecules** are active at the fork at any one time. One moves continuously to produce the new daughter DNA molecule on the leading strand, whereas the other produces a long series of short 'Okazaki DNA fragments' on the lagging strand.

- Both polymerases are anchored to their template by polymerase **accessory proteins**, in the form of a sliding clamp and a clamp loader.

- A **DNA helicase**, powered by ATP hydrolysis, propels itself rapidly along one of the template DNA strands (here the lagging strand), forcing open the DNA helix ahead of the replication fork.

Enzymes- details

- The helicase exposes the bases of the DNA helix for the leading-strand polymerase to copy. **DNA topoisomerase** or **DNA gyrase** enzymes facilitate DNA helix unwinding.

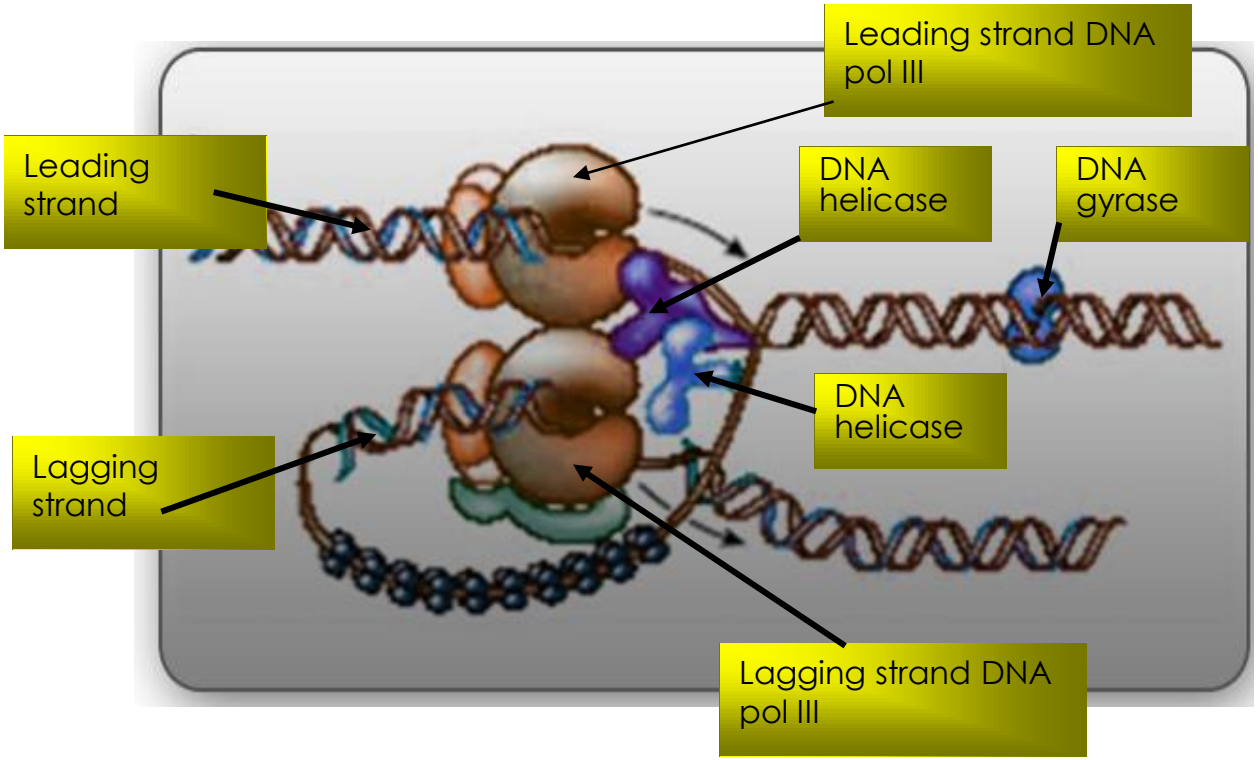
- In addition to the template, DNA polymerases need a pre-existing DNA or RNA chain end (**a primer**) onto which to add each nucleotide.

- For this reason, the lagging strand polymerase requires the action of a **DNA primase** enzyme before it can start each Okazaki fragment.

More stuff on the enzymes for replication

- The primase produces a very short RNA molecule (an **RNA primer**) at the 5' end of each Okazaki fragment onto which the DNA polymerase adds nucleotides

- Finally, the single-stranded regions of DNA at the fork are covered by multiple copies of a **single-strand DNA-binding protein**, which hold the DNA template strands open with their bases exposed.



- In the folded fork structure shown in the insert, the lagging-strand DNA polymerase remains tied to the leading-strand DNA polymerase. This allows the lagging-strand polymerase to remain at the fork after it finishes the synthesis of each Okazaki fragment.

Binary Fission in Bacteria

- Replication of chromosomal DNA in bacteria starts at a specific chromosomal site called the **origin** and proceeds **bidirectionally** until the process is completed.

- When bacteria divide by binary fission after completing DNA replication, the replicated chromosomes are partitioned into each of the daughter cells.

- The origin regions specifically and **transiently associate with the cell membrane** after DNA replication has been initiated, leading to a model whereby **membrane attachment** directs separation of daughter chromosomes (the replicon model).

- These characteristics of DNA replication during bacterial growth fulfill the requirements of the genetic material to be reproduced accurately and to be inherited by each daughter cell at the time of cell division.

References:

- Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2009). Brock Biology of Microorganisms: Pearson Education, USA.

- Clark, D.P. and Russel, L.D. (2000). Molecular Biology Made Simple and Fun: Cache River Press, USA.