
RAMA UNIVERSITY

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# FACULTY OF ENGINEERING &TECHNOLOGY
# DEPARTMENT OF BIOTECHNOLOGY

## DNA Replication

The only way to make new cells is by the division of pre-existing cells.

This means that all organisms depend on cell division for their continued existence.

DNA, as you know, carries the genetic information that each cell needs.

Each time a cell divides, all of its DNA must be copied faithfully so that a copy of this information can be passed on to the daughter cell. This process is called DNA replication.

Before examining the actual process of DNA replication, it is useful to think about what it takes to accomplish this task successfully.

❖The sheer number of nucleotides to be copied is enormous: e.g., in human cells, on the order of several billion.

❖A double-helical parental DNA molecule must be unwound to expose single strands of DNA that can serve as templates for the synthesis of new DNA strands.

❖This unwinding must be accomplished without introducing significant topological distortion into the molecule.

❖The unwound single strands of DNA must be kept from coming back together long enough for the new strands to be synthesized.



❖DNA polymerases cannot begin synthesis of a new DNA strand de novo and require a free 3' OH to which they can add DNA nucleotides.

❖DNA polymerases can only extend a strand in the 5' to 3' direction. The 5' to 3' extension of both new strands at a single replication fork means that one of the strands is made in pieces.

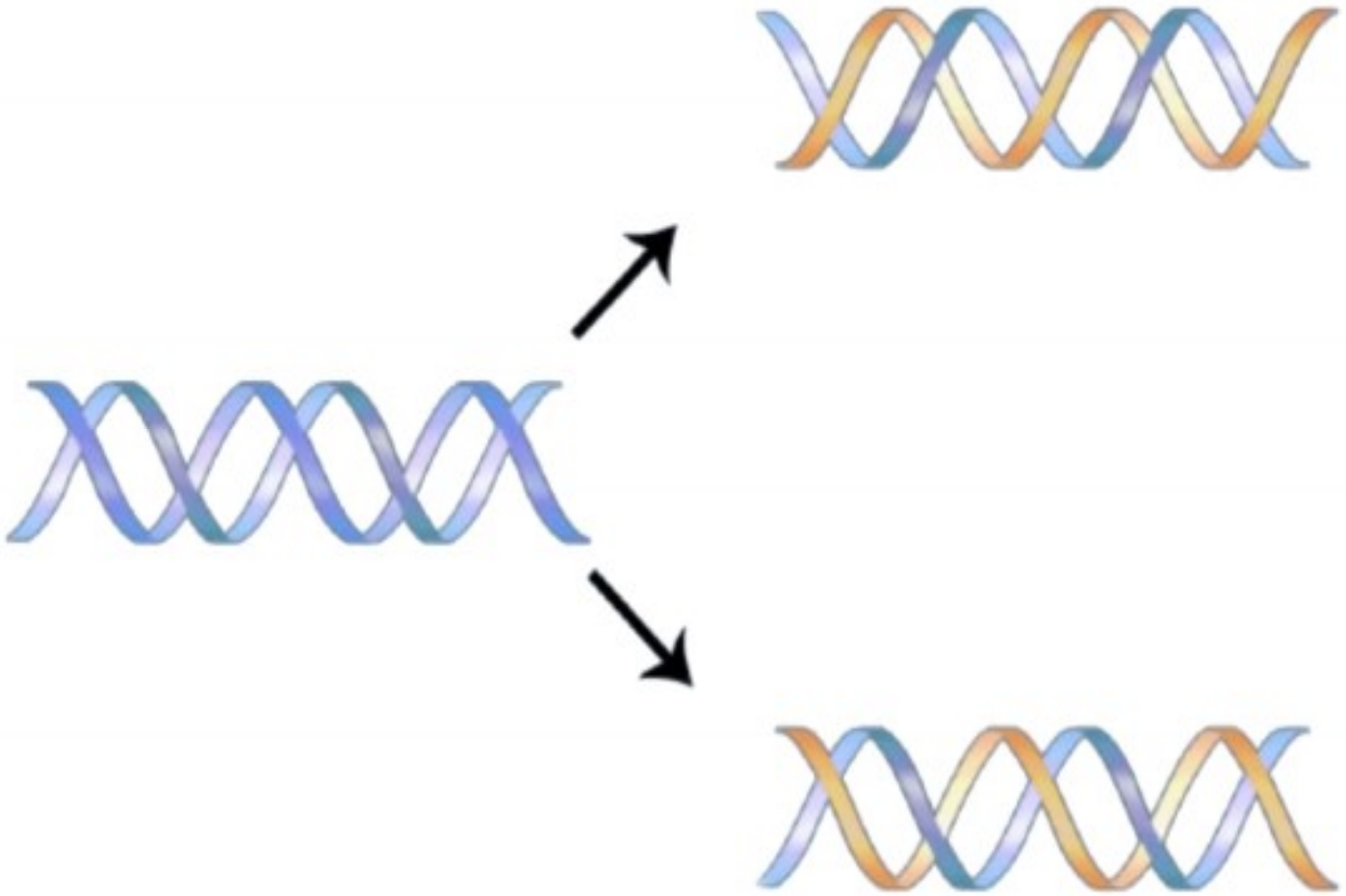
❖The use of RNA primers requires that the RNA nucleotides must be removed and replaced with DNA nucleotides and the resulting DNA fragments must be joined.
❖Ensuring accuracy in the copying of so much information.

❖With this in mind, we can begin to examine how cells deal with each of these challenges. Our understanding of the process of DNA replication is derived from studies using bacteria, yeast, and other systems, such as Xenopus eggs. These investigations have revealed that DNA replication is carried out by the action of a large number of proteins that act together as a complex protein machine called the replisome. Numerous proteins involved in replication have been identified and characterized, including multiple different DNA polymerases in both prokaryotes and eukaryotes.

❖Although the specific proteins involved are different in bacteria and eukaryotes, it is useful to understand the basic considerations that are relevant in all cells, before attempting to address the details of each system.

❖A generalized account of the steps in DNA replication is presented below, focused on the challenges mentioned above.
❖The sheer number of nucleotides to be copied is enormous.

❖For example, in human cells, the number of nucleotides to be copied is on the order of several billion. Even in bacteria, the number is in the millions. Cells, whether bacterial or eukaryotic, have to replicate all of their DNA before they can divide. In cells like our own, the vast amount of DNA is broken up into many chromosomes, each of which is composed of a linear strand of DNA. In cells like those of E. coli, there is a single circular chromosome.

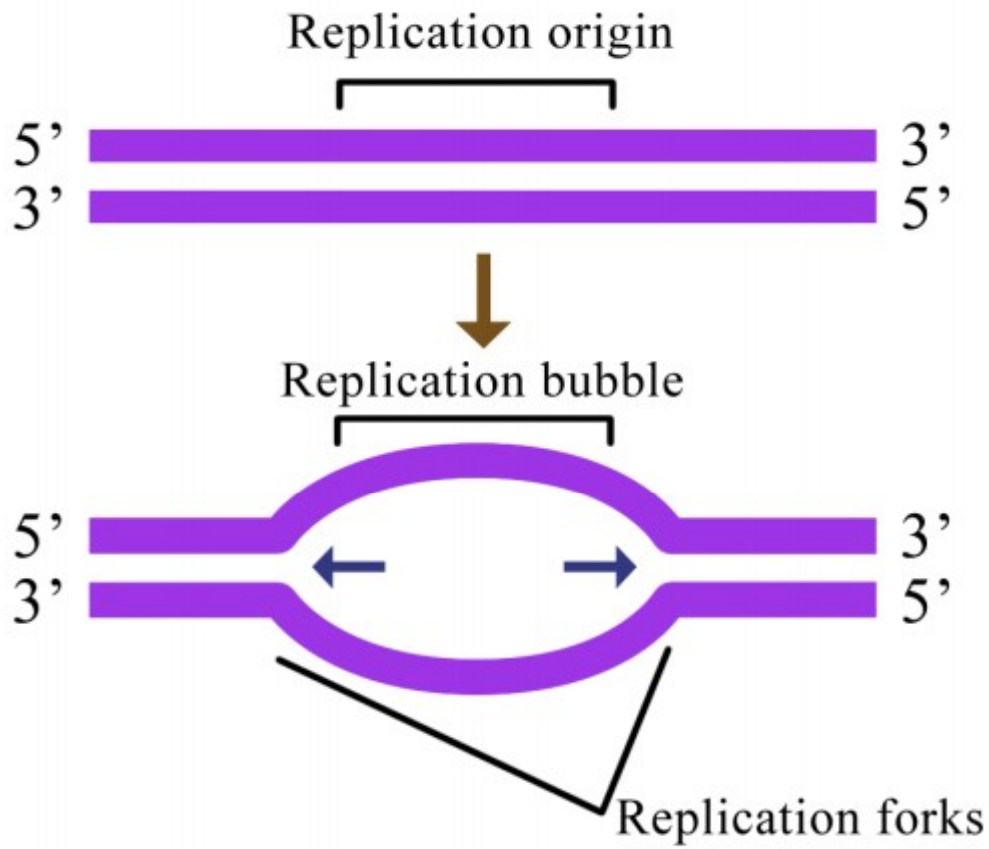


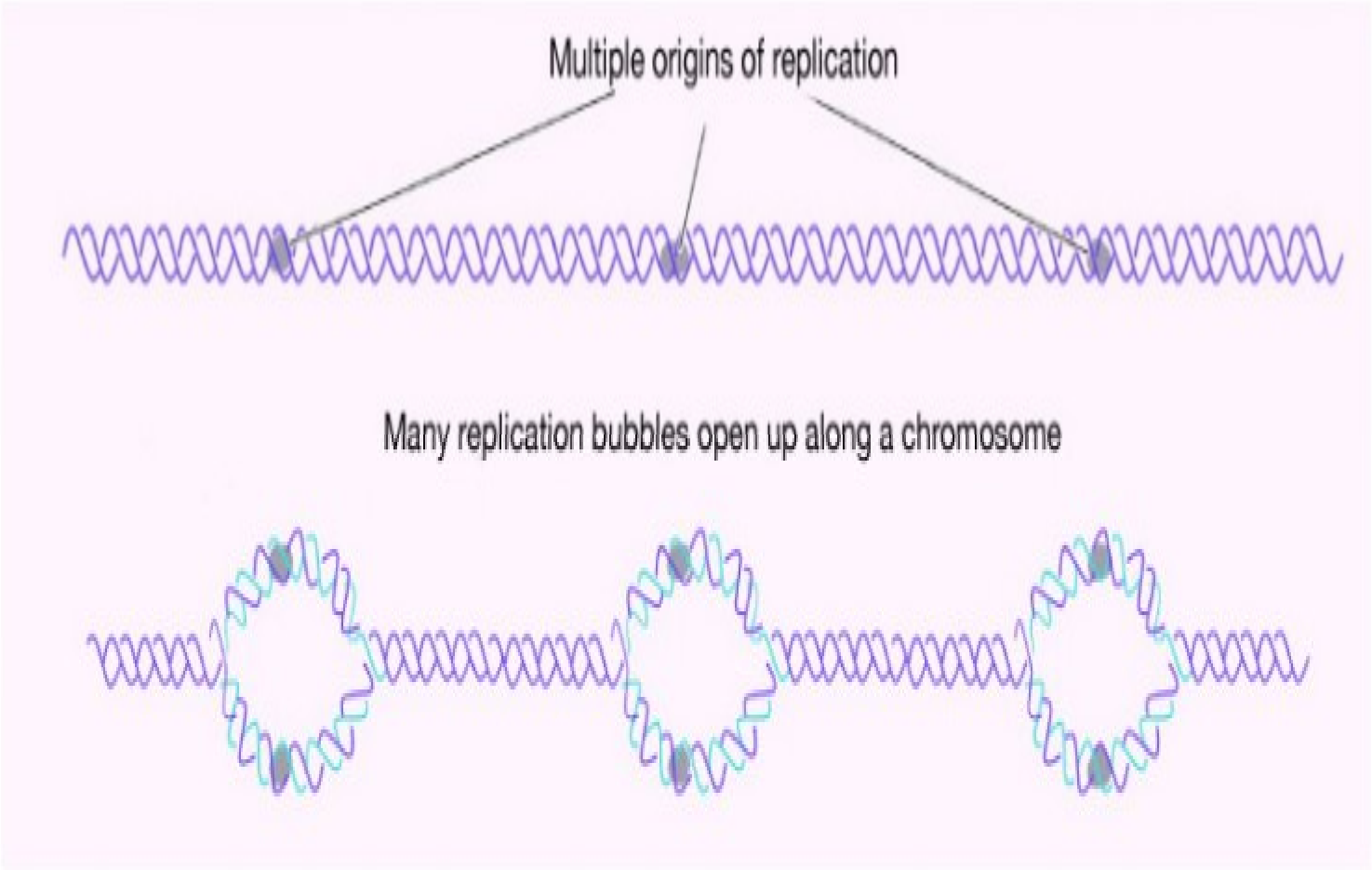
*Semi-conservative DNA replication*

In either situation, DNA replication is initiated at sites called origins of replication. These are regions of the DNA molecule that are recognized by special origin recognition proteins that bind the DNA. The binding of these proteins helps open up a region of single-stranded DNA where the synthesis of new DNA can begin.

In the case of E. coli, there is a single origin of replication on its circular chromosome. In eukaryotic cells, there may be many thousands of origins of replication, with each chromosome having hundreds.

DNA replication is thus initiated at multiple points along each chromosome in eukaryotes. as shown in Electron micrographs of replicating DNA from eukaryotic cells show many replication “bubbles" on a single chromosome.


Replication origin
5’
3’
3’
5’
Replication bubble
5’
3’
3’
5’
Replication forks


Multiple origins of replication
Many replication bubbles open up along a chromosome

This makes sense in light of the large amount of DNA that there is to be copied in cells like our own, where beginning at one end of each chromosome and replicating all the way through to the other end from a single origin would simply take too long. This is despite the fact that the DNA polymerases in human cells are capable of building new DNA strands at the very respectable rate of about 50 nucleotides per second.

A double-helical parental molecule must be unwound to expose single strands of DNA that can serve as templates for the synthesis of new DNA strands.
Once a small region of the DNA is opened up at each origin of replication, the DNA helix must be unwound to allow replication to proceed. How are the strands of the parental DNA double helix separated?

The unwinding of the DNA helix requires the action of an enzyme called helicase. Helicase uses the energy released when ATP is hydrolyzed to unwind the DNA helix. Note that each replication bubble is made up of two replication forks that "move" or open up, in opposite directions. At each replication fork, the parental DNA strands must be unwound to expose new sections of single-stranded template.

This unwinding must be accomplished without introducing topological distortion into the molecule.

What is the effect of unwinding one region of the double helix? Unwinding the helix locally causes over-winding or topological distortion of the DNA ahead of the unwound region. The DNA ahead of the unwound helix has to rotate, or it will get twisted on itself.

How is this problem solved? Enzymes called topoisomerases can relieve the topological stress caused by local unwinding of the double helix. They do this by cutting the DNA and allowing the strands to swivel around each other to release the tension before rejoining the ends. In E. coli, the topoisomerase that performs this function is called gyrase.

The unwound single strands of DNA must be kept from coming back together long enough for the new strands to be synthesized.
Once the two strands of the parental DNA molecule are separated, they must be prevented from going back together to form double-stranded DNA. To ensure that unwound regions of the parental DNA remain single-stranded and available for copying, the separated strands of the parental DNA are bound by many molecules of a protein called single-strand DNA binding protein (SSB).