DNA Replication

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DEFINATION:

In molecular biology, DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. DNA replication occurs in all living organisms acting as the most essential part for biological inheritance.

Mode of DNA Replication:

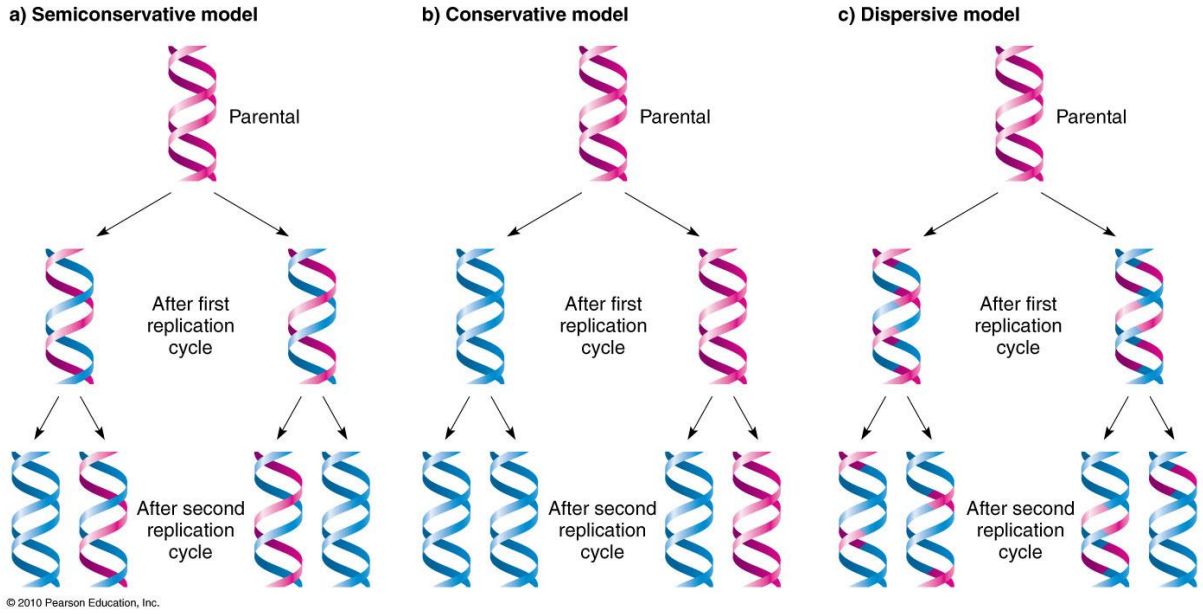

Figure 1 Source: Google

Semi-Conservative, Conservative, & Dispersive models of DNA replication

In the **semi-conservative** model, the two parental strands separate and each makes a copy of itself. After one round of replication, the two daughter molecules each comprises one old and

one new strand. Note that after two rounds, two of the **DNA** molecules consist only of new material, while the other two contain one old and one new strand.

In the **conservative** model, 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.

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.

The semi-conservative model is the intuitively appealing model, because separation of the two strands provides two templates, each of which carries all the information of the original molecule. It also turns out to be the correct one (Meselson & Stahl 1958).

The process of replication is divided into 3 steps:

1. **INITIATION:**

There are specific nucleotide sequences called **origins of replication** where replication begins. *E. coli* has a single origin of replication on its one chromosome, as do most prokaryotes. The origin of replication is approximately 245 base pairs long and is rich in AT sequences. This sequence of base pairs is recognized by certain proteins that bind to this site. An enzyme called **helicase** unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process because it requires energy. As the DNA opens up, Y-shaped structures called **replication forks** are formed. Two replication forks are formed at the origin of replication and these get extended bi-directionally as replication proceeds. **Single-strand binding proteins** coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.

2. **ELONGATION:**

The next important enzyme is **DNA polymerase III**, also known as DNA pol III, which adds nucleotides one by one to the growing DNA chain (Figure 2). The addition of nucleotides requires energy; this energy is obtained from the nucleotides that have three phosphates attached to them. ATP structurally is an adenine nucleotide which has three phosphate groups attached; breaking off the third phosphate releases energy. In addition to ATP, there are also TTP, CTP, and GTP. Each of these is made up of the corresponding nucleotide with three phosphates attached. When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the existing chain.

In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. DNA pol III is the enzyme required for DNA synthesis; DNA pol I is used later in the process and DNA pol II is used primarily required for repair (this is another irritating example of naming that was done based on the order of discovery rather than an order that makes sense).

DNA polymerase is able to add nucleotides only in the 5′ to 3′ direction (a new DNA strand can be only extended in this direction). It requires a free 3′-OH group (located on the sugar) to which it can add the next nucleotide by forming a phosphodiester bond between the 3′-OH end and the 5′ phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3′-OH group is not available. Then how does it add the first nucleotide? The problem is solved with the help of a **primer** that provides the free 3′-OH end. Another enzyme, **RNA primase**, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA. RNA primase does not require a free 3′-OH group. Because this sequence primes the DNA synthesis, it is appropriately called the primer. DNA polymerase can now extend this RNA primer, adding nucleotides one by one that are complementary to the template strand.

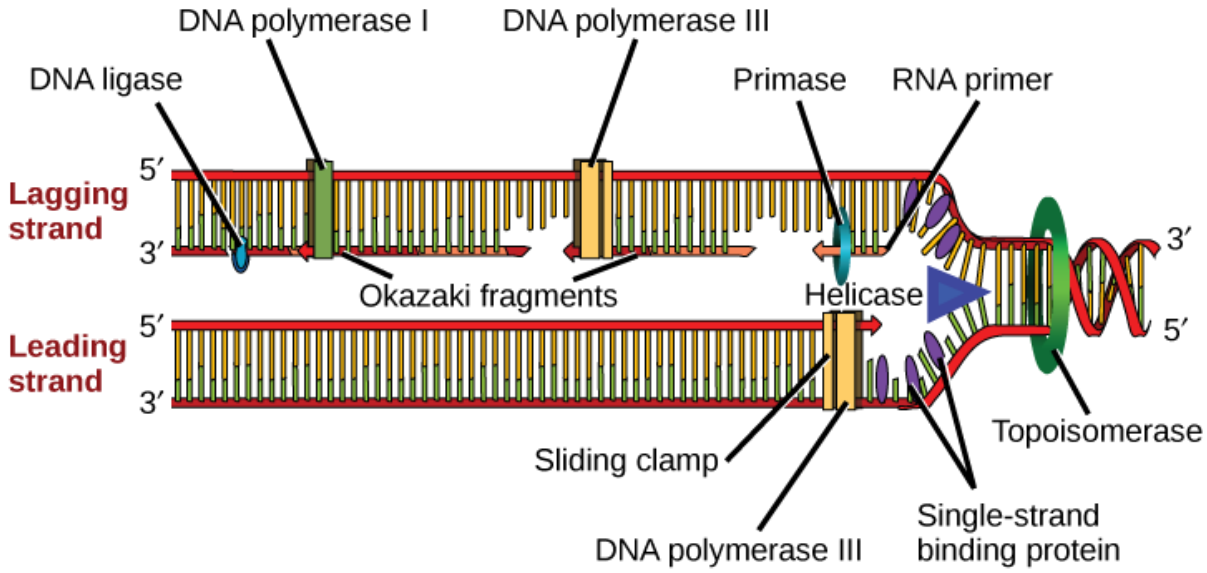

Figure 2 Source: Google

The replication fork moves at the rate of 1000 nucleotides per second. DNA polymerase can only extend in the 5′ to 3′ direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel; that is, one strand is in the 5′ to 3′ direction and the other is oriented in the 3′ to 5′ direction. One strand, which is complementary to the 3′ to 5′ parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the **leading strand**. The other strand, complementary to the 5′ to 3′ parental DNA, is extended away from the replication fork, in small fragments known as **Okazaki fragments**, each requiring a primer to start the synthesis. Okazaki fragments are named after the Japanese scientist who first discovered them. The strand with the Okazaki fragments is known as the **lagging strand**.

The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3’ to 5’, and that of the leading strand 5’ to 3’. A protein called the **sliding clamp** holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. **Topoisomerase** prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA pol I, which breaks down the RNA and fills the gaps with DNA nucleotides. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme **DNA ligase** that catalyzes the formation of phosphodiester linkage between the 3’-OH end of one nucleotide and the 5’ phosphate end of the other fragment.

3. **TERMINATION:**

Ter binding proteins binds to termination sequence present on DNA and prevent DNA polymerase from further of DNA and replication process stops.