DNA Replication

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Introduction

In their report announcing the structure of the DNA molecule, Watson and Crick observe, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." [1]. Some months later, Watson and Crick expanded upon this comment,

> "…our model for deoxyribonucleic acid is, in effect, a *pair* of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken and the two chains unwind and separate. Each chain then acts as a template for the formation onto itself of a new companion chain so that eventually we shall have two pairs of chains, where we only had one before. Moreover the sequence of the pairs of bases will have been duplicated exactly." [2].

This model for DNA replication is termed **semiconservative**. Under this model each copy of a DNA strand would contain one of the original chains and one new copy. The alternative **conservative** model would predict that the original template would reassociate and the copy would be entirely composed of copied strands (Figure 1A). In order to test which model for DNA replication was correct, Matthew Meselson and
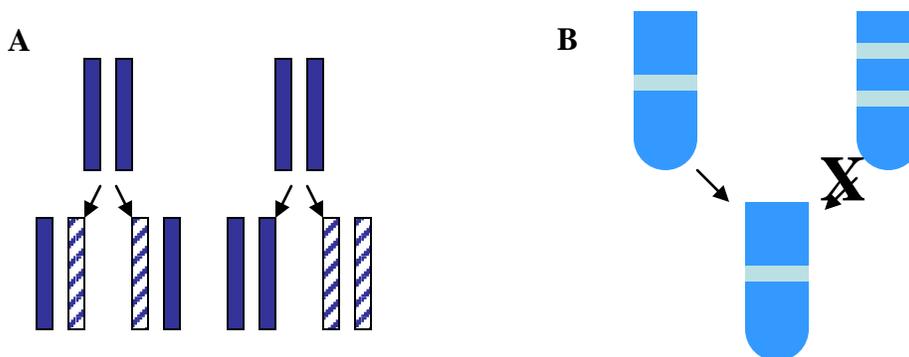

Figure 1. A. Semiconservative (left) and conservative (right) DNA replication models. B. The DNA sedimentation results obtained by Meselson and Stahl (1958). The two tubes on top represent the expected results for semiconservative (left) and conservative (right) DNA replication. The lower tube is the experimental observation.

Franklin Stahl carried out a definitive experiment [3]. They labeled DNA strands with the heavy isotope of nitrogen, ^{15}N. The DNA was then allowed to go through one cycle of replication with only ^{14}N as the nitrogen source. The DNA was centrifuged and the DNA bands were identified. If the conservative model was correct, the heavier ^{15}N DNA would clearly separate from the lighter ^{14}N DNA. If DNA replication was semiconservative, as Watson and Crick hypothesized, there would be only one DNA band occupying a position intermediate between the light and heavy band positions. The results of the Meselson and Stahl experiment confirmed that DNA replication was, in fact, semiconservative (Figure 1B).

The Mechanism of Replication

As with any other cellular process involving nucleic acids, DNA replication is an elegant, though complex, interplay of DNA, RNA, and protein. Regardless of the organism or the cell type within a multicellular organism DNA replication begins at a fixed point of origin or fixed points of origin and must proceed in the 5' to 3' direction. The reason for this restriction is shown in Figure 2. Looking at the sugar-phosphate backbone of a DNA
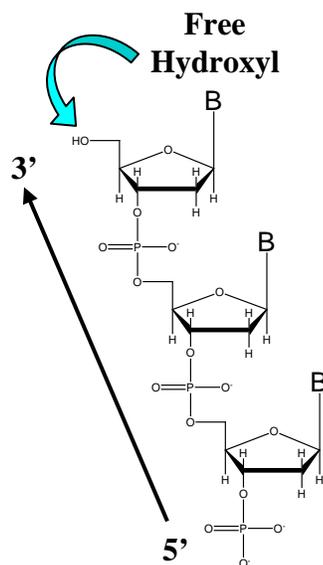

Figure 2. A schematic view of a short stretch of single stranded DNA showing the sugar-phosphate backbone and the 5' to 3' orientation of the strand.

strand, the addition of a new nucleotide requires that there is a free hydroxyl group present. Since the only free hydroxyl group is at the 5' end of the DNA strand, the addition of other bases must occur at that end. Further implications of this restriction will be seen below.

The first step in DNA replication is relaxation of the double helical structure. This is accomplished by an enzyme known as **DNA topoisomerase**. This enzyme will cause the DNA molecule to unwind to a point at which another enzyme called a **helicase** will begin to separate the two DNA strands. As the DNA strands separate, a structure is created called the **replication fork**. The replication fork is the site at which DNA replication actually starts. Since DNA replication is **bidirectional**, that is it proceeds in both directions from the origin (Figure 3), there are actually two replication forks for each replication origin.
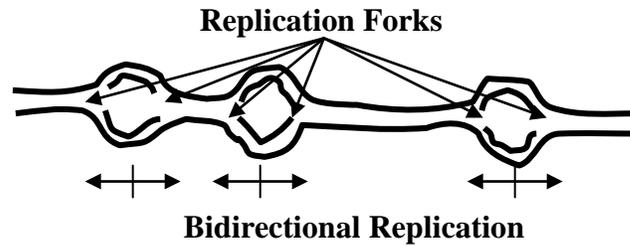

Figure 3. A representation of DNA replication proceeding from several different points of origin.

The actual structure of a replication fork is complex (Figure 4). As the helicase enzyme moves over the double-stranded DNA and opens it up, a protein called a **single-stranded binding protein** is bound to the newly single-stranded DNA to stabilize the integrity of the replication fork. This is necessary as single-stranded DNA is unstable and will degrade. At the replication fork there are two single stranded regions and each of them is replicated in a different way. The **leading strand** is oriented such that there are
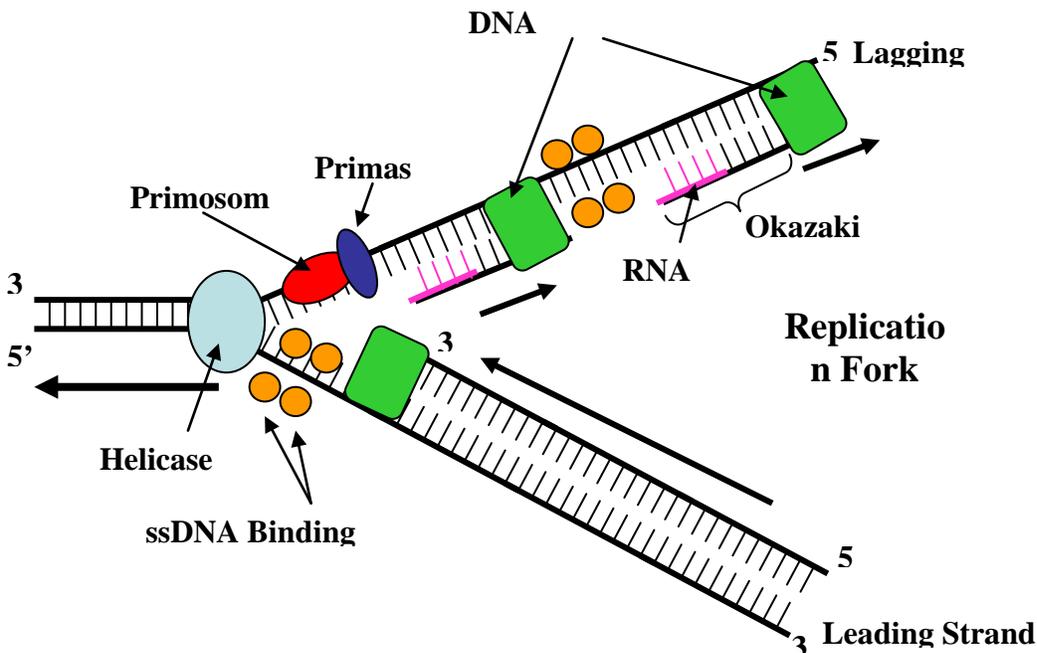

Figure 4. Anatomy and organization of the DNA replication fork.

available reactive hydroxyl groups that the polymerase can use and begin to synthesize the complementary copy immediately once a group of proteins called the **primosome** is bound to the template. The polymerase then simply follows along behind the helicase making the copy as it goes. The **lagging strand**, on the other hand, is not in the correct orientation for there to be available reactive hydroxyl groups that the polymerase can work from. Here, the synthesis reaction will also have a primosome but it also must be primed with a short stretch of nucleic acid oriented in the proper direction on the template. The primers used are RNA strands that serve to provide the necessary reactive hydroxyls on the 3' end. The DNA polymerase binds to the template and the RNA primer and begins to make a DNA copy of the template. The single stranded structure, composed of an RNA primer and a DNA extension, is called an **Okazaki fragment** [4].

While leading strand replication is continuous, lagging strand replication is discontinuous being composed of hundreds of Okazaki fragments that need to be linked together. The first step in the process of linking the Okazaki fragments together is removal of the RNA primers. Most of the primer is removed by the enzyme **RNase H** and any remaining RNA nucleotides are "cleaned up" by an enzyme called **flap endonucleases** as well as by the DNA polymerase that is trailing just behind. Once the RNA nucleotides are removed, the DNA polymerase fills in the gaps with DNA nucleotides. Finally, the Okazaki fragments, that are now composed of DNA only, are joined by an enzyme called **DNA ligase**.

DNA Replication Rates

The classic picture presented above conveys an image of a sedate process with the topoisomerase moving along unwinding the DNA, the helicase following behind unzipping the double stands, accessory proteins binding and, finally, the stately march of the DNA polymerase along the template synthesizing the complementary strand. In reality, nothing could be further from the truth. DNA replication is a high speed, dynamic affair involving some novel protein structures in addition to those discussed above.

The speed of a DNA polymerase enzyme is referred to as **processivity**. In the bacterial chromosome, the processivity of DNA polymerase is about 1000 bases per second! In the human genome, DNA polymerase moves a relative crawl of 50 bases per second. At both ends of the polymerase speed range, however, there is a unique structure composed of numerous proteins acting in concert that keeps the polymerase on the track. One of these is called a **sliding clamp** or proliferating cell nuclear antigen (PCNA). This protein encircles the DNA and allows the polymerase to move along the DNA strand without falling off even at very high speeds. These molecules have been found in all living organisms. Bowman et al. note that sliding clamps also help the polymerase cope with the "considerable torque that results from the production of double-helical DNA,

by allowing them to relax and regain their hold on DNA without losing their place at the replication fork [5]. In essence, sliding clamp proteins are "molecular seat belts." Even more impressive is the fact that sliding clamps hold on to the polymerases and keep them properly oriented by spinning at a rate equal to the helical turns per unit distance. The DNA double helix completes one full turn every ten base pairs. Thus, at a processivity rate of 50 bases per second, the clamp is spinning at a rate of five rotations per second. In the very fast world of bacterial DNA replication, this rotational speed is several hundred times a second. In scale terms, this is equivalent to driving a car 300 miles per hour while spinning like a top along the direction of movement.

Bowman et al. also note that, in order for polymerases generating lagging strand copies to keep up with the continuous leading strand replication rate, there must be an efficient method of delivering sliding clamps to the Okazaki fragments. They have determined that another protein complex, called a **clamp loader**, is responsible (Figure 5). The clamp loader is composed of five proteins that complex with the clamp protein and can recognize primed DNA, delivering the clamp and setting it in place. The DNA clamp-clamp loader complex is able to recognize the point at which double stranded DNA becomes single stranded and the clamp loader opens the clamp, places at the rear of a DNA polymerase, and closes the clamp around the first available section of double stranded made available by the polymerase synthesis of a complementary strand.
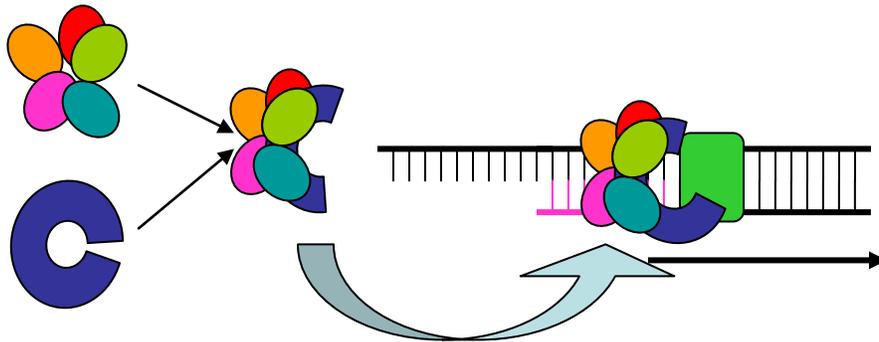

Figure 5. Schematic representation of a sliding clamp-clamp loader complex delivering the clamp to a DNA molecule in the process of replication. The five-subunit clamp loader complexes with the clamp and opens it up. The clamp-clamp loader complex then binds to the DNA polymerase. The clamp closes and the polymerase speeds off to carry out replication.

References

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