

Highlights DNA Replication

1. DNA consists of a double helix. Each strand of the helix is a polymer of nucleotides joined together in phosphodiester linkages that have alternating sugar-phosphate-sugar-phosphate links. On the inside of the double helix are the complementary base pairs held together by hydrogen bonds. The arrangement of the double helix is in an 'anti-parallel' fashion, meaning that one strand oriented in the 5' to 3' direction is directly paired to a complementary strand oriented in the 3' to 5' direction. Phosphodiester bonds involve linkage between the 5' phosphate group of the incoming nucleotide and the 3' hydroxyl of the previous nucleotide in the chain.
2. DNA contains four bases - A,T,C, and G arranged with A paired with T and G paired with C on the internal portion of the double helix. Hydrogen bonds stabilize these base pairs - two for the A-T pair and three for the G-C pair. Thus, G-C pairs are harder to break than A-T pairs.
3. DNA has a major and a minor groove arising from asymmetric glycosidic linkages between the deoxyribose sugar and each base in the double helix.
4. DNA has three major forms - A,B, and Z. The A and B forms are right-handed helices, whereas the Z form is a left-handed helix. The B form of DNA is the most prevalent one and contains about 10.5 bases per turn of the helix.
5. Z-DNA may have roles in marking the location of genes in eukaryotic chromosomes.
6. Another DNA form is the A form (actually discovered by Rosalind Franklin), which is more "compressed" and is also a right handed helix. The A form is the form assumed by double strand RNA or RNA-DNA duplexes as well. RNA cannot exist in the B form due to steric hindrance arising from the oxygen on carbon number 2 of ribose, which is not present in the deoxyribose of DNA.
7. All DNA polymerases require a primer to start DNA synthesis. The primer is formed inside of cells by a special RNA polymerase known as primase. (RNA polymerase does not require a primer)
3. DNA replication proceeds by two distinct mechanisms (both 5'-3', however)- one on each strand. Leading strand and lagging strand synthesis occur by different mechanisms, but both are catalyzed by the same DNA replication complex (Pol III, in the case of *E. coli*).
4. Leading strand synthesis is continuous in the 5' to 3' direction. Lagging strand synthesis can only occur when the leading strand synthesis opens up a new single stranded region for replication. The 5' to 3' syntheses of the lagging strand are discontinuous. The many pieces of lagging strand synthesis are called Okazaki fragments.
5. Okazaki fragments must be combined together ultimately. First, the RNA primer must be removed from each one. The 5' to 3' exonuclease activity of DNA Polymerase I is needed to remove the initial RNA primer of leading strand synthesis, but is needed frequently to remove the

primers of lagging strand synthesis.

6. DNA ligase is an enzyme that creates phosphodiester bonds between adjacent nucleotides between Okazaki fragments. Biotechnologists use this enzyme to join DNA fragments together to create recombinant molecules.

7. *E. coli* DNA replication occurs at 1000 base pairs per second. At 10 base pairs per turn, this represents a machine turning at 5000 to 6000 rpm. *E. coli*'s helicase protein (DNA B - part of the BC complex) unwinds DNA at a rate of at least 5000 - 6000 rpm. The protein separates strands ahead of the DNA Pol III so as to make single strands accessible for replication. Unwinding of strands causes superhelical tension to increase ahead of the helicase. Topoisomerase II (gyrase) relieves the tension created by the helicase and is essential for replication to proceed efficiently.

8. DNA Polymerase III is very processive in its action, meaning that once it gets onto a DNA molecule, it stays on it for a long time replicating it. DNA Polymerase I is NOT very processive. The difference between the processivity properties of these two enzymes is because a protein called a sliding clamp (beta clamp in *E. coli*) hold DNA polymerase III to the DNA during replication, but it does not interact with DNA Polymerase I.

9. In *E. coli* DNA replication, a dimer of DNA Polymerase III is at the replication fork and performs most of the DNA replication in the cell. One portion of it replicates the leading strand and the other replicates the lagging strand. Leading strand synthesis is faster, so the lagging strand template sometimes loops out in a trombone-like fashion when the lagging strand replication falls behind.

10. Proteins at/near the replication fork and their functions include primase (makes RNA primers necessary for the DNA polymerase to act on), SSB (single stranded binding protein - protects single-stranded DNA and interacts with the replication proteins), DNA gyrase (topoisomerase II - relieves the superhelical tension created by helicase), Pol I (removes RNA primers), DNA ligase (joins DNA fragments together by catalyzing synthesis of phosphodiester bonds at nick sites), and helicase (unwinds double helix).

11. DNA Polymerase I is much more abundant in cells than DNA Polymerase III and its function is to remove RNA primers on Okazaki fragments and fill in the short regions where the RNA bases were with DNA.