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1).

What is DNA replication

DNA, short for deoxyribonucleic acid, is the self-replicating material which is present in nearly all living organisms as the main constituent of chromosomes. It is the fundamental carrier of genetic information, present in virtually every cell in your body.

Double-helix DNA is made of two asymmetrical strands. Each strand is made of nucleotides lined up one after the other, and these nucleotides are bound to corresponding ones on the other strand to create a ladder-like structure. DNA is made up of four nucleotides — the building blocks of nucleic acids — which are composed of a nitrogenous base, a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group.

Adenine (A), Thymine (T), Guanine (G), and Cytosine (C) are called nucleotides. A and G are called Purines while T and C are called Pyrimidines. According to the rules of base pairing, A always pairs with T, and C always pairs with G.

Before a cell duplicates or divides, through either mitosis or meiosis, DNA must be replicated to ensure that each new cell receives the correct number of chromosomes. This process occurs in all living organisms and is the basis for biological inheritance.

DNA replication occurs in several steps that involve multiple proteins called replication enzymes, as well as RNA. DNA replication is vital for cell growth, repair, and reproduction in organisms.

DNA replication steps

There are three main steps to DNA replication: initiation, elongation, and termination.

In order to fit within a cell’s nucleus, DNA is packed into tightly coiled structures called chromatin, which loosens prior to replication, allowing the cell replication machinery to access the DNA strands.

Before DNA replication can begin, the double helix structure of the DNA molecules has to be ‘unzipped.’ Helicase, an enzyme, is integral to this process, breaking the hydrogen bonds that hold the complementary bases of DNA together (A with T and C with G). The separation creates a ‘Y’ shape called a replication fork and the two single strands of DNA now act as templates for making new strands of DNA.

Next, the Single-Stranded DNA Binding Protein (SSB Protein) binds to the now single-stranded DNA, preventing the separating strands from joining again.

The two strands of the double-helix DNA are joined together by cross-bars, twisted around. For this to work, each DNA strand runs in opposite direction.

Replication of leading and lagging strands of DNA. Credit: Genome Research Limited.

One of the strands is oriented in the 3’ to 5’ direction (towards the replication fork), this is the leading strand. The other strand is oriented in the 5’ to 3’ direction (away from the replication fork), this is the lagging strand.

Because the enzyme that carries out the replication, DNA polymerase, only functions in the 5′ to 3′ direction, this means that the daughter strands synthesize through different methods, one adding nucleotides one by one in the direction of the replication fork, the other able to add nucleotides only in chunks. The first strand, which replicates nucleotides one by one is the leading strand; the other strand, which replicates in chunks, is the lagging strand.

The notations 5′ and 3′ mean “five prime” and “three prime,” which indicate the carbon numbers in the DNA’s sugar backbone. These numbers indicate end-to-end chemical orientation, with the numbers 5 and 3 representing the fifth and third carbon atom of the sugar ring respectively. The 5′ carbon has a phosphate group attached to it and the 3′ carbon a hydroxyl (-OH) group. It’s this asymmetry that gives a DNA strand a “direction,” allowing for easy binding between nucleotides of the opposite strands.

It’s important to note that the two sides are replicated through two different processes in order to accommodate the directional difference.

Leading strand Lagging Strand

A short piece of RNA called a primer, which is produced by an enzyme called primase, binds to the end of the leading strand in the 5’ to 3’ direction. The primer acts as the starting point for DNA synthesis.

Enzymes called DNA polymerases generate new complementary nucleotide bases (the A,C, G, and T) and are responsible for creating the new strand by a process called elongation. In eukaryotic cells, polymerases alpha, delta, and epsilon are the primary polymerases involved in DNA replication.

This sort of replication is called ‘continuous.’

The lagging strand begins the replication process by binding with multiple RNA primers, tgenerated by the primase enzyme, at various points along the lagging strand.

Chunks of DNA, called Okazaki fragments, are added to the lagging strand between the primers, also in the 5’ to 3’ direction.

This type of replication is called ‘discontinuous’, as the Okazaki fragments will need to be joined up later.

After the formation of both the continuous and discontinuous strands, an enzyme called exonuclease removes all RNA primers from the original strands. The gaps where the primer(s) had been are then filled by yet more complementary nucleotides.

Another enzyme “proofreads” the newly formed strands in order to make sure there are no errors.

The enzyme DNA ligase then joins Okazaki fragments together, forming a single unified strand.

A special type of DNA polymerase enzyme called telomerase catalyzes the synthesis of telomere sequences at the ends of the DNA. Telomeres are regions of repetitive nucleotide sequences at each end of a chromatid, which protect the end of the chromosome from deterioration or from fusion with neighboring chromosomes. Think of shoelace caps. Telomeres are also a biomarker of aging, with telomeres shortening with each cellular division or, in other words, as you advance in age. As a cell’s telomeres shorten, it loses its ability to function normally. Basically, shorter telomeres make you more susceptible to a number of diseases, such as cancer or cardiovascular disease.

Finally, the parent strand and its complementary DNA strand coils into the familiar double helix shape. The result is two DNA molecules consisting of one new and one old chain of nucleotides. Each of these two daughter helices is a nearly exact copy of the parental helix (it is not 100% the same due to mutations).

The human genome — meaning the complete set of genes present in a cell’s nucleus — is comprised of 3 billion base pairs. Remarkably, it takes very little time for our biological machinery to copy something this exceedingly long. Every cell completes the entire process in just one hour

Step 1: Replication Fork Formation

Before DNA can be replicated, the double stranded molecule must be “unzipped” into two single strands. DNA has four bases called adenine (A), thymine (T), cytosine (C) and guanine (G) that form pairs between the two strands. Adenine only pairs with thymine and cytosine only binds with guanine. In order to unwind DNA, these interactions between base pairs must be broken. This is performed by an enzyme known as DNA helicase. DNA helicase disrupts the hydrogen bonding between base pairs to separate the strands into a Y shape known as the replication fork. This area will be the template for replication to begin.

DNA is directional in both strands, signified by a 5' and 3' end. This notation signifies which side group is attached the DNA backbone. The 5' end has a phosphate (P) group attached, while the 3' end has a hydroxyl (OH) group attached. This directionality is important for replication as it only progresses in the 5' to 3' direction. However, the replication fork is bi-directional; one strand is oriented in the 3' to 5' direction (leading strand) while the other is oriented 5' to 3' (lagging strand). The two sides are therefore replicated with two different processes to accommodate the directional difference.

Replication BeginsStep 2: Primer Binding

The leading strand is the simplest to replicate. Once the DNA strands have been separated, a short piece of RNA called a primer binds to the 3' end of the strand. The primer always binds as the starting point for replication. Primers are generated by the enzyme DNA primase

Step 3: Elongation

Enzymes known as DNA polymerases are responsible creating the new strand by a process called elongation. There are five different known types of DNA polymerases in bacteria and human cells. In bacteria such as E. coli, polymerase III is the main replication enzyme, while polymerase I, II, IV and V are responsible for error checking and repair. DNA polymerase III binds to the strand at the site of the primer and begins adding new base pairs complementary to the strand during replication. In eukaryotic cells, polymerases alpha, delta, and epsilon are the primary polymerases involved in DNA replication. Because replication proceeds in the 5' to 3' direction on the leading strand, the newly formed strand is continuous.

The lagging strand begins replication by binding with multiple primers. Each primer is only several bases apart. DNA polymerase then adds pieces of DNA, called Okazaki fragments, to the strand between primers. This process of replication is discontinuous as the newly created fragments are disjointed.

Step 4: Termination

Once both the continuous and discontinuous strands are formed, an enzyme called exonuclease removes all RNA primers from the original strands. These primers are then replaced with appropriate bases. Another exonuclease “proofreads” the newly formed DNA to check, remove and replace any errors. Another enzyme called DNA ligasejoins Okazaki fragments together forming a single unified strand. The ends of the linear DNA present a problem as DNA polymerase can only add nucleotides in the 5′ to 3′ direction. The ends of the parent strands consist of repeated DNA sequences called telomeres. Telomeres act as protective caps at the end of chromosomes to prevent nearby chromosomes from fusing. A special type of DNA polymerase enzyme called telomerase catalyzes the synthesis of telomere sequences at the ends of the DNA. Once completed, the parent strand and its complementary DNA strand coils into the familiar double helix shape. In the end, replication produces two DNA molecules, each with one strand from the parent molecule and one new strand.

DNA replication would not occur without enzymes that catalyze various steps in the process. Enzymes that participate in the eukaryotic DNA replication process include:

DNA helicase - unwinds and separates double stranded DNA as it moves along the DNA. It forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA.

DNA primase - a type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that act as templates for the starting point of DNA replication.

DNA polymerases - synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands.

Topoisomerase or DNA Gyrase - unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.

Exonucleases - group of enzymes that remove nucleotide bases from the end of a DNA chain.

DNA ligase - joins DNA fragments together by forming phosphodiester bonds between nucleotides.

DNA replication is the production of identical DNA helices from a single double-stranded DNA molecule. Each molecule consists of a strand from the original molecule and a newly formed strand. Prior to replication, the DNA uncoils and strands separate. A replication fork is formed which serves as a template for replication. Primers bind to the DNA and DNA polymerases add new nucleotide sequences in the 5′ to 3′ direction.

This addition is continuous in the leading strand and fragmented in the lagging strand. Once elongation of the DNA strands is complete, the strands are checked for errors, repairs are made, and telomere sequences are added to the ends of the DNA.

2).

Major Enzymes

The process of DNA replication is catalyzed by a type of enzyme called DNA polymerase (poly meaning many, mer meaning pieces, and –ase meaning enzyme; so an enzyme that attaches many pieces of DNA). Observe Figure 1: the double helix of the original DNA molecule separates (blue) and new strands are made to match the separated strands. The result will be two DNA molecules, each containing an old and a new strand. Therefore, DNA replication is called semiconservative. The term semiconservative refers to the fact that half of the original molecule (one of the two strands in the double helix) is “conserved” in the new molecule. The original strand is referred to as the template strand because it provides the information, or template, for the newly synthesized strand.

Stylized DNA replication fork with nucleotides matched, 5'->3' synthesis shown, no enzymes in diagram.

Figure 1. By Madprime(wikipedia) (DNA replication split horizontal) CC BY-SA 2.0

Diagram of a primer moving along the template strand of DNA.

Figure 2. Primer and Template

DNA replication relies on the double-stranded nature of the molecule. One double stranded DNA molecule, when replicated, will become two double-stranded molecules, each containing one original strand and one newly synthesized strand. You remember that the two strands of DNA run antiparallel: one from the 5′ to the 3′, and the other from the 3′ to the 5′. The synthesis of the new DNA strand can only happen in one direction: from the 5′ to the 3′ end. In other words, the new bases are always added to the 3′ end of the newly synthesized DNA strand. So if the new nucleotide is always added to the 3′ end of an existing nucleotide, where does the first nucleotide come from? In fact, DNA polymerase needs an “anchor” to start adding nucleotides: a short sequence of DNA or RNA that is complementary to the template strand will work to provide a free 3′ end. This sequence is called a primer (Figure 2).

How does DNA polymerase know in what order to add nucleotides? Specific base pairing in DNA is the key to copying the DNA: if you know the sequence of one strand, you can use base pairing rules to build the other strand. Bases form pairs (base pairs) in a very specific way. Figure 3 shows how A (adenine) pairs with T (thymine) and G (guanine) pairs with C (cytosine). It is important to remember that this binding is specific: T pairs with A, but not with C. The molecular recognition occurs because of the ability of bases to form specific hydrogen bonds: atoms align just right to make hydrogen bonds possible. Also note that a larger base (purine, A or G) always pairs with a smaller base (pyrimidine, C or T).

Diagram showing the hydrogen bonds between nucleotides. Adenine is bound to thymine, and cytosine is bound to guanine.

Basic Features of DNA Replication:

All genetically relevant information of any DNA molecule is present in its sequence of bases on two strands. Therefore the main role of replication is to duplicate the base sequence of parent DNA molecule. The two strands have complementary base pairing. Adenine of one strand pairs with thymine of the opposite strand and guanine pairs with cytosine. This specific complementary base pairing provides the mechartism for the replication.

The two strands uncoil and permanently separate from each other. Each strand functions as a template for the new complementary daughter strand. The base sequence of parent or old strand directs the base sequence of new or daughter strand. If there is adenine in the parent or old strand, complementary thymine will be added to the new strand. Similarly, if there is cytosine in the parent strand, complementary guanine will be copied into the new daughter strand. Maintenance of integrity of genetic information is the main feature of replication.

Mechanism of DNA Replication:

ADVERTISEMENTS:

Mechanism of DNA replication is the direct result of DNA double helical structure proposed by Watson and Crick. It is a complex multistep process involving many enzymes.

1. Initiation:

It involves the origin of replication. Before the DNA synthesis begins, both the parental strands must unwind and separate permanently into single stranded state. The synthesis of new daughter strands is initiated at the replication fork. In fact, there are many start sites.

2. Elongation:

The next step involves the addition of new complementary strands. The choice of nucleotides to be added in the new strand is dictated by the sequence of bases on the template strand. New nucleotides are added one by one to the end of growing strand by an enzyme called DNA polymerase. There are four nucleotides, deoxyribrnucleotide triphosphates dGTP, dCTP, dATP, dTTP present in the cytoplasm.

3. Termination:

All the end termination reactions occur. Duplicated DNA molecules are separated from one another.

The purpose of DNA replication is to create two daughter DNA molecules which are identical to the parent molecule.

DNA Replication is Semi-Conservative:

Watson and Crick model suggested that DNA replication is semi-conservative. It implies that half of the DNA is conserved. Only one new strand is synthesized, the other strand is the original DNA strand (template) that is retained. Each parental DNA strand serves as a template for one new complementary strand.

Enzymes of DNA Replication:

The enzymes which take part in replication are able to copy DNA molecules which may contain millions of bases. They perform this function with utmost accuracy and at high speed, even though DNA molecule is highly compact and is bound with proteins. Maintenance of integrity of genetic information is the main feature of replication.

In E. coli, two main enzymes that take part in polymerization are DNA polymerase I and DNA polymerase III. Of these DNA polymerase III is the main enzyme involved in replication. Polymerization involves addition of new nucleotides to a growing strand.

In addition, there is an enzyme DNA polymerase II which takes part in DNA repair. DNA polymerase IV and DNA polymerase V have also been discovered. DNA polymerases are capable of adding 1000 nucleotides per second. The speed of DNA synthesis is known as processivity.