DNA Replication:1

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Genetic material is always nucleic acid and it is always DNA except some viruses. DNA is the storehouse of genetic information. This information is in the form of nucleotide sequence called genetic code. This information is copied and transcribed into RNA molecules. This information (genetic code) is for specific sequence of amino acids. The RNA then synthesizes proteins, which are specific sequence of amino acids, by a process called translation. In 1956 Francis Crick called this pathway of flow of genetic information as the Central Dogma.

Both transcription and translation are unidirectional. Proteins never serve as template for RNA synthesis. But sometimes RNA acts as a template for DNA synthesis (reverse transcription), Example is RNA viruses (HIV virus).

One of the most important properties of DNA is that it forms its additional identical copies. The process of forming its replica copy is called **replication**. Replication is the basis of evolution of all morphologically complex forms of life.

During interphase of cell division the number of DNA molecules doubles which at anaphase is separated into two daughter cells, and thus equal number of chromosomes is maintained.

However, replication does not occur during entire anaphase but is confined only to synthesis (S) phase. Only S phase involves replication process.

The Gl phase is most variable and in many eukaryotic cells it is completed within 3 to 4 hours or even months depending on physiological conditions. Mostly DNA synthesis is accomplished in 7 to 8 hours. In bacteria growing at log phase, DNA synthesis occurs from the time a cell originates to give rise to two daughter cells.

Enzymes Involved In DNA Replication:

Both the prokaryotic and eukaryotic cells contain three types of nuclear enzymes that are essential for DNA replication. These enzymes are nucleases, polymerases and ligases.

(i) Nucleases:

The polynucleotide is held together by phosphodiester bonds. The nucleases hydrolyse the polynucleotide chain into the nucleotides. It attacks either at 3′ OH end or 5′ phosphate end of the chain. The nucleases are of two types .

(a) Exonucleases:

The nuclease that attacks on outer free end of the polynucleotide chain is called exonuclease. It breaks phosphodiester bond either in direction (A) or in 3'→ 5′ direction (B). The enzyme moves in either cases stepwise along the chain and removes nucleotides one by one. Thus, the whole chain is digested.

(b) Endooudeases:

The endonucleases attack within the inner portion of one or the double strands. Therefore, a nick is made on double stranded DNA molecule. However, if the polypeptide chain is single stranded (e.g. in DNA viruses), the attack of endonuclease will render the chain into two pieces.

On double stranded DNA the nick contains two free ends that in turn act as template for DNA replication. Apart from this, the nicked double helix is distorted due to rotation of free molecules around its intact strand.

(ii) DNA Polymerases:

DNA polymerases carryout the process of polymerization of nucleotides and formation of polynucleotide chain. This enzyme is called replicase when it replicates the DNA molecules and inherited by daughter cells. In 1959, for the first time A. kornberg discovered an enzyme in E. coli which polymerized the deoxyribonucleotide triphosphate on a DNA template and produced complementary strand of DNA.

This enzyme was called DNA polymerase. Later on it was named as Kornberg polymerase kornberg enzyme after the name of discoverer, for demonstrating in vitro polymerization of DNA. For the catalysis of polymerization, it requires the four deoxyribonucleotide triphosphates e.g. dATP, dGTP, dTTP and dCTP, a DNA template, a primer for initiation of catalytic activity and Mg^{++}

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In prokaryotes, three types of DNA polymerases e.g. polymerase I (Poly-I), polymerase II(Pol II), and DNA polymerase III (Pol III) are found, whereas in eukaryotes three or four polymerases termed as α, β and γ polymerases and mitochondria (mit) DNA polymerase are present. The α and β polymerases are located in the nucleus. The β-polymerase copies a poly (A) or poly (C) template.

(a) Polymerase I (Pol I):

The Kornberg polymerase is known as Pol I. One atom of zinc (Zn) per chain is present, therefore, it is metalloenzyine. In E. coli, approximately 400 molecules of Pol I are present.

Early experiments carried out by Kornberg revealed that when artificially synthesized DNA template strands alternating A and T i.e. poly d(AT) were incubated with polymerase and four radio-labelled nucleoside triphosphate, radioactive DNA containing alternating A and T was synthesized.

Pol I possesses several attachment sites such as:

(i) A template site for attachment to the DNA template,

(ii) A primer site of about 100 nucleotides contemporary to a segment of RNA on which the growth of newly synthesized DNA occur,

(iii) A primer terminus site containing a terminal 3'OH group at the tip, and

(iv) A triphosphate site for matching the incoming nucleoside triphosphates according to complementary nucleotide of DNA template.

Function:
Pol I plays a significant role in polymerization (synthetic) as well as degradation (exonucleolytic) process of nucleotides. The large fragement shows 3′ → 5′ exonuclease activity, and the small fragement shows 5′ → 3′ exonuclease activity. In E.coli the following three types of functions of Pol I have been found.

Polymerization:
Polymerization is a process of synthesis in 5′ → 3′ direction of short segments of DNA chain from deoxyribonucleoside triphosphate monomers to the 3′ -OH end of a DNA strand. It is not the main polymerization enzyme because it cannot synthesize a long chain. It synthesizes only a small segment of DNA.

It binds only to a DNA and forms nick in dsDNA. Therefore, it takes part in repair synthesis. The chief enzyme associated with polymerization is known as polymerase III.

Exonuclease activity:
3' → 5′ exonuclease activity:
Pol I catalyses the breaking of one or two DNA strands in 3' → 5′ direction into the nucleotide components i.e. the nucleotides are set free in 3′ → 5′ direction which is reverse to polymerization direction.

Therefore, it is called 3′ → 5′ exonuclease activity. Pol I correct the errors made during the polymerization, and edits the mismatching nucleotides at the primer terminus before the start of strand

synthesis. Therefore, the function of Pol I is termed as repair synthesis.

5′ → 3′ exonuclease activity:
Pol I also breaks the polynucleotide chain in 5′ → 3′ direction with the removal of nucleotide residues. Upon exposure of DNA to the ultraviolet light two adjacent pyrimidines such as thymines are covalently linked forming pyrimidine dimers. These dimers block the replication of DNA. Therefore, removal of pyrimidine dimers e.g. thymine dimers (T=T) is necessary.

Through 5′ → 3′ exonuclease activity, Pol I removes pyrimidine dimers. Secondly, DNA synthesis occurs on RNA primer in the form Okazaki fragments. Through 5′ → 3′ exonuclease activity Pol I remove RNA primer and seal the gap with deoxyribonucleotides. Its onward movement results in removal of ribonucleotides from the front portion followed by of deoxyribonucleotides behind it.

(b) Polymerase II (Pol II):
For several years Pol I was considered to be responsible for replicating in E.coli. but the work done during 1970s made it clear that Pol I is associated only with repair synthesis and the other enzymes, Pol II and Pol III are involved in polymerization process. Pol II is a single polypeptide chain that shows polymerization in 5’ → 3’ direction of a complementary chain.

It also shows exonuclease activity in 3’ → 5’ direction but not in 5’ → 3’ direction. The polymerization activity of Pol II is much less than Pol I in E.coli cells. About 50 nucleotides per minute are synthesized. E.coli cells contain about 40 Pol II molecules.

The 3′ → 5′ exonuclease activity of Pol II shows that it also plays a role in repair synthesis or DNA damaged by U.V. light just like Pol I. In the absence of Pol I, it can elongate the Okazaki fragments. Therefore, Pol II is an alternative to Pol I.

(c) Polymerase III (Pol III):

DNA polymerase III is several times more active than Pol I and Pol II enzymes. Pol III polymerises deoxyribonucleoside triphosphates in direction very efficiently.

Like Pol II, it cannot polymerize efficiently if the template DNA is too long but can do when ATP and certain protein factors are present. Synthesis of a long template also occurs when an auxiliary protein DNA (co-polymerase II) is linked with Pol III and produced Pol III-co Pol II complex. In addition Pol III also shows 3'→ 5′ exonuclease activity like Pol II.

The 5'→ 3′ exonuclease activity is absent. All the polymerases e.g. Pol I, Pol II and Pol III show 3'→ 5′ exonuclease activity, whereas besides Pol I, the other two polymerases (Pol I and Pol II) lack 5'→ 3′ exonuclease activity. However, some workers have shown both 3'→ 5′ and 5'→ 3′ exonuclease activity in Pol III.

(iii) DNA Ligases:

The DNA ligases seal single strand nicks in DNA which has 5'→ 3′ termini. It catalyses the formation of phosphodiester bonds between 3′-OH and 5′-PO_4 group of a nick, and turns into an intact DNA. There are two types of DNA ligases.