Biotechnology and dna replication processes

Technology

Replication thus helps DNA, in the transfer of genetic information generation after generation to daughter cells or organisms. The ability to reproduce is one of the most fundamental properties of all living systems. DNA replication, the basis for biological inheritance, is a fundamental process occurring in all living organisms to copy their DNA.

General mechanism of DNA replication:

In “ replication” each strand of the original double-stranded DNA molecule serves as template for the reproduction of the complementary strand. Hence, following DNA replication, two identical DNA molecules have been produced from a single double-stranded DNA molecule.

Structure Of DNA:

DNA usually exists as a double-stranded structure, with both strands coiled together to form the characteristic double-helix. Each single strand of DNA is a chain of four types of nucleotides:

- Adenine.
- Cytosine.
- Guanine.
- Thymine.

A nucleotide is a mono-, di- or triphosphate deoxyribonucleoside; that is, a deoxyribose sugar is attached to one, two or three phosphates

Chemical interaction of these nucleotides forms phosphodiester linkages, creating the phosphate-deoxribose backbone of the DNA double helix with the bases pointing inward. Nucleotides (bases) are matched between strands

through hydrogen bonds to form base pairs. Adenine pairs with thymine and cytosine pairs with guanine.

Process Of DNA Replication

DNA replication is a complicated process. To explain the process in a better way it is divided into three steps, i. e,

- Hypothesis of DNA Replication.
- Mechanism of DNA replication.
- Role of enzymes involved in DNA replication.

1. Hypothesis of DNA Replication:

The formulation of the structure of DNA by Watson and Crick in 1953.

The two strands are complementary to each other; each strand contains the information required for the construction of the other strand. Thus, once the strands are separated each can act as a template to direct the assembly of nucleotides required for the formation of the complementary strand and the restoration of the double stranded state.

Important Feature Of Watson And Crick Hypothesis:

The most important feature of Watson and Crick hypothesis from the genetic point of view is the postulation that the two strands of double helical DNA are complimentary and that replication of each to form new complimentary strands results in the formation of two duplex daughter DNA molecules, each of which receives one strand from the parental DNA and the second is synthesize new.

Three Main Hypotheses:

Three main hypotheses were suggested to explain the replication of DNA.

- Dispersive Hypothesis
- Conservative Hypothesis
- Semi conservative Hypothesis

Dispersive Hypothesis:

Such type of hypothesis in which parent helix is broken into fragments, dispersed, copied then assembled into two new helices. New and old DNA is completely dispersed.

Why it is rejected?

Due to lack of experimental evidences dispersive mechanism of DNA replication was rejected very early.

2. Conservative Hypothesis:

Such type of hypothesis in which parent strands transfer information to an intermediate, then the intermediate gets copied. The parent helix is conserved; the daughter helix is completely new.

Why it is rejected?

This hypothesis was also rejected due to lack of evidence.

3. Semiconservative Hypothesis:

Such type of hypothesis in which daughter DNA molecules contain one parental strand and one newly-replicated strand.

Meselson and Stahl’s Experiments

Experiments carried out by M. S. Meselson and F. W. Stahl in 1957, proved that in intact living E. Coli cells, DNA is replicated in semi conservative manner (as was postulated by Watson and Crick)

DNA Replication Mechanism

Replication of DNA, is a complicated bio-chemical process, which is divided into four stages, i. e,

Unwinding of parental double helical strands of DNA.

Synthesis of RNA primer.

Synthesis of DNA chain, i. e., Replication.

Excision of RNA primer from newly replicative DNA molecule and filling of the excision gaps and formation of the phosphodiester bonds.

Role of enzymes involved in replication.

Un Winding Of DNA

Replication of DNA is possible, only when the parent molecule uncoils (unwinds) and both of its strands get apart.

Involvement of Three Types of Enzymes

For unwinding and separation of the strands of the parent molecule, at the time of replication, three types of enzymes are involved. These are;

Gyrase Or Helicase

These enzymes uncoil replicating DNA and force I to become straight and uncoiled.

DNA Binding Proteins (DNA B-Protein)

DNA Binding Proteins (DNA B-Protein) or melting enzymes react with both strands of the parent DNA, and by breaking hydrogen bonds gap both strands apart.

3. Helix Destabilizing

Helix destabilizing proteins react with single stranded DNA and do not allow them to recoil during replication.

Creation of Origin Site:

By the activity of enzymes Gyrase, DNA b-proteins, and helix destabilizing proteins: origin site (single stranded region) is created at which replication starts bidirectionally

2. Synthesis Of RNA Primer:

DNA polymerase is the enzyme that catalyzes the replication of DNA.

Requirements of DNA polymerase:

This enzyme needs the presence of DNA single stranded template for copying the complementary sequence in the strand that is going to be synthesized.

The enzymes also require the presence of an oligomeric RNA segment as a primer.

RNA Primer synthesis:

RNA Primer is synthesized by the activity of an enzyme called DNA dependent RNA polymerase or primase. DNA polymerase catalyzes replication from a 5-3 direction.

Synthesis of DNA:

Leading strand: All DNA polymerases requires a free 3-hydroxyl end in the primer and continuous replication (growth) can occur in the 5-3 direction only strand of DNA called leading strand.

Lagging strand: Chain growth in the other strand (3-direction) called lagging strand is discontinuous.

Okazaki fragments: The discontinuous replication is executed with the help of Okazaki fragments. An Okazaki fragment is a primer fitted single strand of DNA.

Recent work, however, has proved that replication of both leading and lagging strands of DNA is of discontinuous nature and Okazaki fragments are used. Thus Okazaki fragments are attached by DNA polymerases at different origin sites of the template DNA. In this way newly replicated DNA appears as a hybrid of DNA and RNA, with gaps in the newly synthesized strands.

4. Excision of primer:

When replication of DNA molecule is completed newly synthesized strand has primer RNA. Three processes are involved that removes the primer. These are as following:

A ribonuclease removes the primer.

DNA polymerases fill the gaps by adding deoxyribotides in the place of the primer.

The enzyme ligase forms phosphodiester bonds between the nicks..

5. Role of Enzymes in the Replication:

Replication is catalyzed by number of enzymes, called DNA polymerases and ligases.

Three types of DNA polymerases are known to exist i. e. ,

- DNA polymerase I (Pol. 1)
- DNA polymerase II (Pol. II)
- DNA polymerase III (Pol. III)

DNA polymerases I and II are the minor enzymes which catalyze the addition of few nucleotides in gaps, removal of primer molecules and other minor corrections.

According to Kornberg DNA Pol. III during its activity changes to a more complex form called Pol. III*(read three star). Pol. III* is the biologically active form of Pol three star. Pol. III needs an auxiliary protein co polymerase III (copol III*).

Copoly III reacts with pol. III* to form Pol. III* – copoly III complex.