REPLICATION

Upon proposing the double helical structure of DNA, Watson and Crick promptly introduced a model for DNA replication. According to their scheme, the two strands of DNA would separate, serving as templates for the synthesis of new complementary strands. Upon replication completion, each DNA molecule would consist of one parental strand and one newly synthesized strand. This model was coined as semiconservative DNA replication.

The Experimental Proof

Semi Conservative Mode of DNA Replication

The concept of semi-conservative DNA replication was initially proposed by Watson and Crick. Subsequently, in 1958, Matthew Meselson and Franklin Stahl experimentally verified this mode of replication, both in E. coli and in Vicia faba by Taylor.

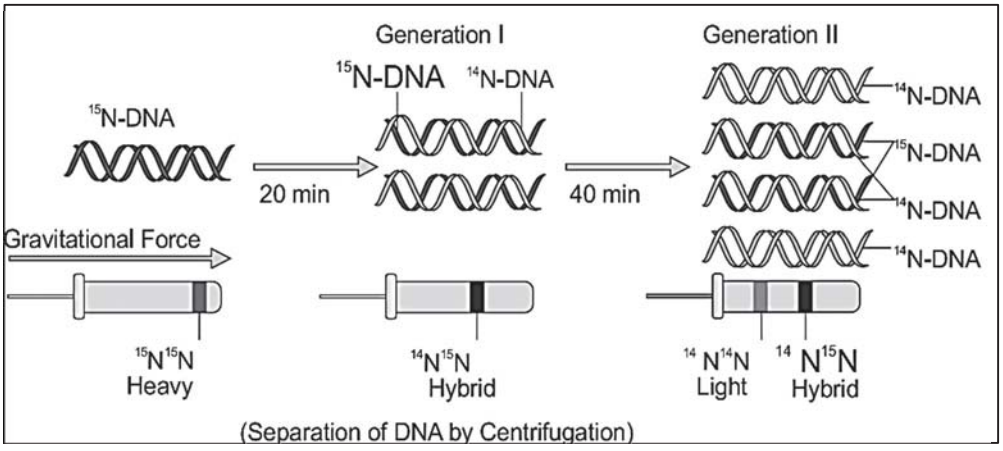
To validate this method, Taylor utilized the Radiotracer Technique, employing radioisotopes such as tritiated thymidine (3H). Meselson and Stahl, on the other hand, employed a heavy isotope of nitrogen (N^{15}).

The experiment conducted by Meselson and Stahl proceeded as follows:

(i) They cultured E. coli in a medium containing $^{15}NH_4Cl$, where ^{15}N served as the sole nitrogen source for numerous generations. The incorporation of ^{15}N into newly synthesized DNA was observed, distinguishing the heavy DNA molecule from normal DNA via centrifugation in a cesium chloride (CsCl) density gradient. (Note: ^{14}N is not a radioactive isotope and can only be separated from ^{15}N based on densities.)

(ii) Subsequently, the cells were transferred into a medium containing normal NH_4Cl, and samples were taken at specific time intervals as the cells multiplied. DNA was then extracted from the cells, which remained as double-stranded helices. These various samples were independently separated on CsCl gradients to measure DNA densities.

(iii) Consequently, DNA extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium (i.e., after 20 minutes, as E. coli divides in 20 minutes) exhibited a hybrid or intermediate density. After another generation (i.e., after 40 minutes, or the second generation), the DNA comprised equal amounts of hybrid DNA and "light" DNA.



The Machinery and the Enzymes

Replication within living cells necessitates a suite of enzymes, with the primary enzyme known as DNA-dependent DNA polymerase. This enzyme demonstrates exceptional efficiency, capable of polymerizing approximately 2000 base pairs per second. The critical requirement for these polymerases is not only speed but also accuracy in catalyzing the reaction. Any errors during replication could lead to mutations. Remarkably, the entire genome of Escherichia coli, consisting of

4.6 x 10 base pairs, is replicated within a span of 38 minutes. The process of DNA replication unfolds through the following steps:

Mechanism of DNA Replication

(1) Origin of Replication

DNA replication initiates at a specific site known as the origin of replication, often abbreviated as Ori. In prokaryotes, replication commences at a singular point, and the entire DNA strand participates in the process, resulting in a single replicon. Conversely, in eukaryotes, multiple replicons are present.
DNA replication in eukaryotes is characterized by being bidirectional, semi-discontinuous, and semiconservative.

(2) Activation of Deoxyribonucleotides

The nucleotides deAMP, deGMP, deCMP, and deTMP are initially found in an inactive state. However, when they encounter ATP in the presence of the phosphorylase enzyme, they undergo a reaction and are transformed into their active forms: deATP, deGTP, deCTP, and deTTP.

(3) Exposure of DNA helix

The helicase enzyme functions at the origin of replication (ori) site on the DNA template, where it facilitates the unwinding of the two DNA strands.
The single-stranded binding (SSB) protein plays a crucial role in preventing the recoiling of the uncoiled DNA strands.
Topoisomerase enzymes are responsible for creating nicks in one strand of the DNA, enabling the removal of coils, and subsequently resealing the same strand. Additionally, bacteria possess another enzyme known as DNA Gyrase, which, along with topoisomerase, has the ability to introduce negative supercoils.
The process of DNA unwinding does not occur all at once due to the substantial energy requirement involved. Instead, the point of separation gradually progresses from one end to the other, resulting in the formation of a Y-shaped structure known as the replication fork.

(4) RNA Primer

A short strand of RNA, typically comprising 5 to 10 nucleotides in length, is synthesized at the 5' end of the new DNA strand with the assistance of the enzyme Primase. The formation of this RNA primer marks the initiation phase of synthesis because the presence of the RNA primer is essential for the subsequent addition of nucleotides by DNA polymerase.