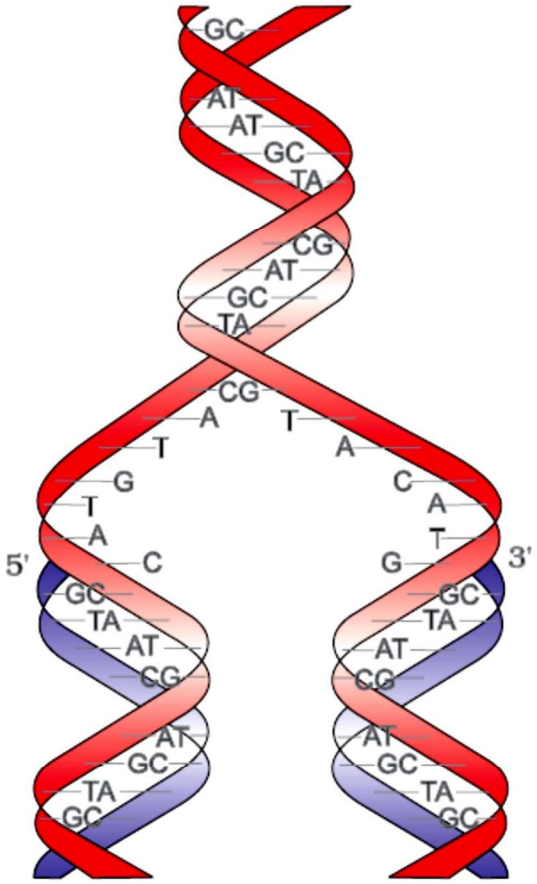
REPLICATION

Watson and Crick also proposed a mechanism for replication of DNA. Watson and Crick said, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" (Watson and Crick, 1953).

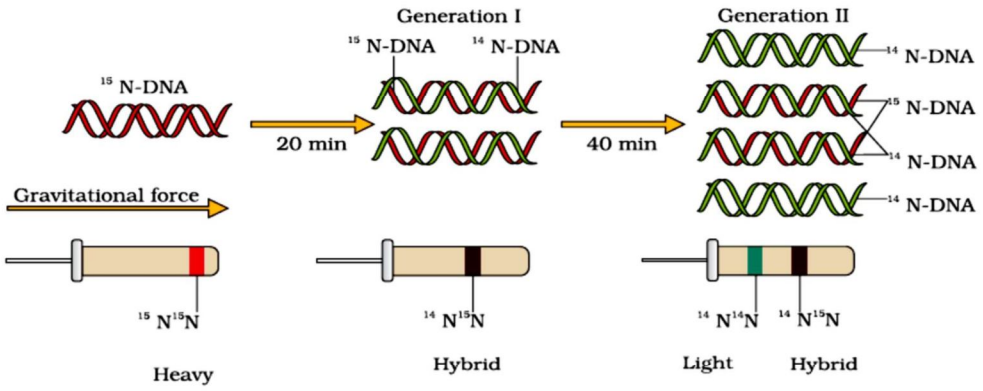


They suggested that the two strands would separate and act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesised strand. This called **semiconservative** method of DNA replication.

The Experimental Proof

Semiconservative method was shown first in *Escherichia coli* and subsequently in higher organisms, such as plants and human cells.

Matthew Meselson and Franklin Stahl performed the first convincing experiment in 1958:



(Separation of DNA by Centrifugation)

(i) They grew *E. coli* in a medium containing $^{15}NH_4Cl$ (^{15}N is the heavy isotope of nitrogen) as the only nitrogen source for many generations. The ^{15}N was incorporated into nascent DNA.

This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a **cesium chloride (CsCl) density gradient** (^{15}N is not a radioactive isotope, and it can be separated from ^{14}N only based on densities).

(ii) Then they transferred the cells into a medium with normal $^{14}NH_4Cl$ and took samples at various definite time intervals, and extracted the DNA. The various samples were separated independently on CsCl gradients to measure the densities of DNA.

(iii) Thus, the DNA that was extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20 minutes; *E. coli* divides in 20 minutes] had a hybrid or intermediate density. DNA extracted from the culture after another generation [that is after 40 minutes, II generation] was composed of equal amounts of this hybrid DNA and of 'light' DNA.

Very similar experiments involving use of radioactive thymidine to detect distribution of newly synthesised DNA in the chromosomes was performed on *Vicia faba* (faba beans) by Taylor and colleagues in 1958. The experiments proved that the DNA in chromosomes replicate semiconservatively.