7.3A: Basics of DNA Replication

DNA replication uses a semi-conservative method that results in a double-stranded DNA with one parental strand and a new daughter strand.

LEARNING OBJECTIVES

Explain how the Meselson and Stahl experiment conclusively established that DNA replication is semi-conservative.

KEY TAKEAWAYS

Key Points

- There were three models suggested for DNA replication: conservative, semi-conservative, and dispersive.
- The conservative method of replication suggests that parental DNA remains together and newly-formed daughter strands are also together.
- The semi-conservative method of replication suggests that the two parental DNA strands serve as a template for new DNA and after replication, each double-stranded DNA contains one strand from the parental DNA and one new (daughter) strand.
- The dispersive method of replication suggests that, after replication, the two daughter DNAs have alternating segments of both parental and newly-synthesized DNA interspersed on both strands.
- Meselson and Stahl, using *E. coli* DNA made with two nitrogen isotopes (\(^{14}N\) and \(^{15}N\)) and density gradient centrifugation, determined that DNA replicated via the semi-conservative method of replication.
Key Terms

- **DNA replication**: a biological process occurring in all living organisms that is the basis for biological inheritance.
- **Isotope**: any of two or more forms of an element where the atoms have the same number of protons, but a different number of neutrons within their nuclei.

Basics of DNA Replication

Watson and Crick’s discovery that DNA was a two-stranded double helix provided a hint as to how DNA is replicated. During cell division, each DNA molecule has to be perfectly copied to ensure identical DNA molecules to move to each of the two daughter cells. The double-stranded structure of DNA suggested that the two strands might separate during replication with each strand serving as a template from which the new complementary strand for each is copied, generating two double-stranded molecules from one.

Models of Replication

There were three models of replication possible from such a scheme: conservative, semi-conservative, and dispersive. In conservative replication, the two original DNA strands, known as the parental strands, would re-basepair with each other after being used as templates to synthesize new strands; and the two newly-synthesized strands, known as the daughter strands, would also basepair with each other; one of the two DNA molecules after replication would be “all-old” and the other would be “all-new”. In semi-conservative replication, each of the two parental DNA strands would act as a template for new DNA strands to be synthesized, but after replication, each parental DNA strand would basepair with the complementary newly-synthesized strand just synthesized, and both double-stranded DNAs would include one parental or “old” strand and one daughter or “new” strand. In dispersive replication, after replication both copies of the new DNAs would somehow have alternating segments of parental DNA and newly-synthesized DNA on each of their two strands.
To determine which model of replication was accurate, a seminal experiment was performed in 1958 by two researchers: Matthew Meselson and Franklin Stahl.

**Meselson and Stahl**

Meselson and Stahl were interested in understanding how DNA replicates. They grew *E. coli* for several generations in a medium containing a “heavy” isotope of nitrogen ($^{15}$N) that is incorporated into nitrogenous bases and, eventually, into the DNA. The *E. coli* culture was then shifted into medium containing the common “light” isotope of nitrogen ($^{14}$N) and allowed to grow for one generation. The cells were harvested and the DNA was isolated. The DNA was centrifuged at high speeds in an ultracentrifuge in a tube in which a cesium chloride density gradient had been established. Some cells were allowed to grow for one more life cycle in $^{14}$N and spun again.
Meselson and Stahl experimented with E. coli grown first in heavy nitrogen (\(^{15}\text{N}\)) then in lighter nitrogen (\(^{14}\text{N}\)). DNA grown in \(^{15}\text{N}\) (red band) is heavier than DNA grown in \(^{14}\text{N}\) (orange band) and sediments to a lower level in the cesium chloride density gradient in an ultracentrifuge. When DNA grown in \(^{15}\text{N}\) is switched to media containing \(^{14}\text{N}\), after one round of cell division the DNA sediments halfway between the \(^{15}\text{N}\) and \(^{14}\text{N}\) levels, indicating that it now contains fifty percent \(^{14}\text{N}\) and fifty percent \(^{15}\text{N}\). In subsequent cell divisions, an increasing amount of DNA contains \(^{14}\text{N}\) only. These data support the semi-conservative replication model.

During the density gradient ultracentrifugation, the DNA was loaded into a gradient (Meselson and Stahl used a gradient of cesium chloride salt, although other materials such as sucrose can also be used to create a gradient) and spun at high speeds of 50,000 to 60,000 rpm. In the ultracentrifuge tube, the cesium chloride salt created a density gradient, with the cesium chloride solution being more dense the farther down the tube you went. Under these circumstances, during the spin the DNA was pulled down the ultracentrifuge tube by centrifugal force until it arrived at the spot in the salt gradient where the DNA molecules’ density matched that of the surrounding salt solution. At the point, the molecules stopped sedimenting and formed a stable band. By looking at the relative positions of bands of molecules run in the same gradients, you can determine the relative densities of different molecules. The molecules that form the lowest bands have the highest densities.

DNA from cells grown exclusively in \(^{15}\text{N}\) produced a lower band than DNA from cells grown exclusively in \(^{14}\text{N}\). So DNA grown in \(^{15}\text{N}\) had a higher density, as would be expected of a molecule with a heavier isotope of nitrogen incorporated into its nitrogenous bases. Meselson and Stahl noted that after one generation of growth in \(^{14}\text{N}\) (after cells had been shifted from \(^{15}\text{N}\)), the DNA molecules produced only single band intermediate in position in between DNA of cells grown exclusively in \(^{15}\text{N}\) and DNA of cells grown exclusively in \(^{14}\text{N}\). This suggested either a semi-conservative or dispersive mode of replication. Conservative replication would have resulted in two bands; one representing the parental DNA still with exclusively \(^{15}\text{N}\) in its nitrogenous bases and the other representing the daughter DNA with exclusively \(^{14}\text{N}\) in its nitrogenous bases. The single band actually seen indicated that all the DNA molecules contained equal amounts of both \(^{15}\text{N}\) and \(^{14}\text{N}\).
The DNA harvested from cells grown for two generations in $^{14}\text{N}$ formed two bands: one DNA band was at the intermediate position between $^{15}\text{N}$ and $^{14}\text{N}$ and the other corresponded to the band of exclusively $^{14}\text{N}$ DNA. These results could only be explained if DNA replicates in a semi-conservative manner. Dispersive replication would have resulted in exclusively a single band in each new generation, with the band slowly moving up closer to the height of the $^{14}\text{N}$ DNA band. Therefore, dispersive replication could also be ruled out.

Meselson and Stahl's results established that during DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are synthesized. The new strand will be complementary to the parental or "old" strand and the new strand will remain basepaired to the old strand. So each "daughter" DNA actually consists of one "old" DNA strand and one newly-synthesized strand. When two daughter DNA copies are formed, they have the identical sequences to one another and identical sequences to the original parental DNA, and the two daughter DNAs are divided equally into the two daughter cells, producing daughter cells that are genetically identical to one another and genetically identical to the parent cell.