Proving that DNA Replication is Semiconservative

The discovery that the structure of DNA is a double helix, containing two complementary strands of DNA, led to a number of hypotheses about how DNA might be replicated. Although the possible replication mechanisms were relatively easy to deduce, proving which occurs in vivo was a more difficult task. In 1958, Matthew Meselson and Franklin Stahl used the newly developed techniques of density-gradient centrifugation to show that DNA replication proceeds in a semiconservative fashion.

Background

During the 1950s, scientists uncovered many of the biological facts we now take for granted, beginning with the discovery that genetic information is passed on through deoxyribonucleic acid (DNA), and continuing through the elucidation of DNA’s three-dimensional structure. As the decade neared a close, biologists were ready to study how DNA passed on genetic information from the parental to the progeny generation.

James Watson and Francis Crick had hypothesized, based on their double-helical model of DNA, that replication occurs in a semiconservative fashion. That is, the double helix unwinds, the original parental DNA strands serve as templates to direct the synthesis of the progeny strand, and each of the replicated DNA duplexes contains one old (parental) strand, and one newly synthesized strand, often called the “daughter” strand. Another hypothesis proposed at the time was conservative replication, whereby after replication the parental strands formed one DNA duplex and the two daughter strands formed the second duplex.

When these hypotheses were first proposed, little experimental evidence was available to support one over another. In 1957, however, Meselson and Stahl, along with Jerome Vinograd, developed density-gradient centrifugation, a technique that can separate macromolecules exhibiting very small differences in density. The tools were now available for a definitive test to determine whether DNA replication occurs by a semiconservative or conservative mechanism.

The Experiment

Meselson and Stahl reasoned that if one could label the parental DNA in such a way that it could be distinguished from the daughter DNA, the replication mechanisms could be distinguished. If DNA replication is semiconservative, then after a single round of replication, all DNA molecules should be hybrids of parental and daughter DNA strands. If replication is conservative, then after a single round of replication, half of the DNA molecules should be composed only of parental strands and half of daughter strands.
To differentiate parental DNA from daughter DNA, Meselson and Stahl used "heavy" nitrogen (\(^{15}\text{N}\)). This isotope contains an extra neutron in its nucleus, giving it a higher atomic mass than the more abundant "light" nitrogen (\(^{14}\text{N}\)). Since nitrogen atoms make up part of the purine and pyrimidine bases in DNA, it was easy to label E. coli DNA with \(^{15}\text{N}\) by growing bacteria in a medium containing \(^{15}\text{N}\) ammonium salts as the sole nitrogen source. After several generations of growth, the bacteria contained only \(^{15}\text{N}\)-labeled DNA. Now that the parental DNA was labeled, Meselson and Stahl abruptly changed the medium to one containing \(^{14}\text{N}\) as the sole nitrogen source. From this point on, all the DNA synthesized by the bacteria would incorporate \(^{14}\text{N}\), rather than \(^{15}\text{N}\), so that the daughter DNA strands would contain only \(^{14}\text{N}\). As the bacteria continued to grow and replicate their DNA in the \(^{14}\text{N}\)-containing medium, samples were taken periodically, and the bacterial DNA was analyzed with the newly developed technique of equilibrium density-gradient centrifugation.

In this type of analysis, a DNA sample is mixed with a solution of cesium chloride (\(\text{CsCl}_2\)). During long periods of high-speed centrifugation the \(\text{CsCl}_2\) forms a gradient, and the DNA migrates to the position where the density of the DNA is equal to that of the \(\text{CsCl}_2\). If the DNA sample contains molecules of different densities, they will migrate to different positions in the gradient. Because \(^{15}\text{N}\) has a greater density than \(^{14}\text{N}\), \(^{15}\text{N}\)-labeled DNA has a greater density than \(^{14}\text{N}\)-labeled DNA. The higher-density (\(^{15}\text{N}\)) DNA will sediment to a different position than the lower-density (\(^{14}\text{N}\)) DNA. Hybrid DNA molecules, containing both \(^{15}\text{N}\) and \(^{14}\text{N}\), will sediment at an intermediate density, depending on the ratio of heavy nitrogen to light nitrogen.

Figure 12.1 illustrates the results obtained by Meselson and Stahl. Before any DNA replication had occurred in the \(^{14}\text{N}\)-containing medium, all DNA sedimented as a single species, corresponding to \(^{15}\text{N}\)-labeled DNA. As DNA replication proceeded, the amount of (\(^{15}\text{N}\))-DNA decreased, and a second DNA species, consisting of hybrid DNA molecules containing \(^{15}\text{N}\) - and \(^{14}\text{N}\)-labeled strands, appeared. DNA collected after completion of the first round of replication was found to sediment with the second species. When the DNA produced during a second round of replication was analyzed, two distinct species were observed. One corresponded to hybrid molecules; the other corresponded to \(^{14}\text{N}\)-labeled DNA. With each subsequent round of replication the proportion of hybrid DNA decreased as the amount of \(^{14}\text{N}\)-labeled DNA increased. As the diagrams in the figure show, the sedimentation patterns observed by Meselson and Stahl are consistent only with a semiconservative model of replication.

**Discussion**

For Meselson and Stahl to prove that DNA replication proceeds in a semiconservative manner, they not only had to design a clear, easily interpretable experiment, but also develop the technology to do it. The beauty of this classic experiment is that each of the possible models would produce distinctly different results, so that interpretation of the experimental data was unambiguous. This study remains a shining example of defining a problem and employing the proper methodology to solve it.

By demonstrating that DNA replication occurs in a semi-conservative fashion, Meselson and Stahl opened up the field of DNA replication for in-depth research. With the correct model in hand, researchers could now turn to unraveling the precise mechanism of DNA replication. In addition, equilibrium density-gradient centrifugation became a widely used tool for the analysis of complex mixtures of DNA.
Figure 12.1
Experimental demonstration by Meselson and Stahl that DNA replication is semiconservative. After several generations of growth in a medium containing “heavy” (15N) nitrogen, E. coli were transferred to a medium containing the normal “light” isotope (14N). Samples were removed from the cultures periodically and analyzed by equilibrium density-gradient centrifugation in CsCl₂ to separate heavy-heavy (H-H), light-light (L-L), and heavy-light (H-L) duplexes into distinct bands. The actual banding patterns observed were consistent with the semiconservative mechanism. [From H. Lodish et al., 1995, Molecular Cell Biology, 3rd ed. W. H. Freeman and Company. See M. Meselson and W. F. Stahl, 1958, Proc. Nat’l Acad. Sci. USA 44:671; photographs courtesy of M. Meselson.]