

CHAPTER 7

MESELSON/STAHL: DNA REPLICATION IS SEMICONSERVATIVE

In 1958, Matthew Meselson and Franklin Stahl labeled E. coli DNA with “heavy” nitrogen. When the labeled DNA was centrifuged, the labeled DNA would band out deeper in the test tube and was easily distinguished according to how heavy it was. By examining DNA from successive generations of bacteria, Meselson and Stahl were able to confirm the hypothesis that DNA replication is semiconservative.

SEMICONSERVATIVE REPLICATION

James Watson and Francis Crick, in suggesting that DNA had the structure of a double helix, hypothesized that replication of the DNA molecule occurs by unwinding the helix, followed by base-pairing to single strands. After one round of replication, each daughter DNA double helix would have one of the old strands and one newly-synthesized strand. This mode of DNA duplication is called *semiconservative* because the parental nucleotide sequence is preserved in the two progeny molecules, but in only *one* of their DNA strands. After a second round of such replication, the two original strands continue to be passed down, serving as templates again to produce two new *hybrid* double helices. The two new strands from the first round of replication also serve as templates, producing two double helices that contain only new DNA. Thus after two rounds of replication, *two hybrid* and *two new* DNA molecules are formed.

CONSERVATIVE REPLICATION

The alternative hypothesis was that DNA did not replicate itself directly at all, but rather transferred its information to some other intermediate that did not have to unwind and could more readily serve as a template for DNA synthesis. This alternative was more popular than the semiconservative suggestion of Watson and Crick because it was difficult to see how the DNA double helix unwound without breaking apart: DNA molecules are so long that unwinding an entire molecule without breaking it would produce enormous torque forces on the DNA, and would require a speed of rotation so great that the resulting heat should cook the cell! Replication by transferring information to an intermediate is not an unreasonable hypothesis from a biological viewpoint. Indeed, protein synthesis occurs in just this manner, with the ribosome complex reading the messenger RNA strand and producing a corresponding protein chain. Such an indirect mode of DNA replication has an important property: it implies *conservative* replication.

After one round of such indirect replication, one daughter DNA double helix could contain both of the original parental DNA strands, while both DNA strands of the other daughter double helix would be newly synthesized. The parental sequence would thus be fully conserved in one of the daughter double helices. After a second round of such replication, the two original parental strands would continue to be passed down together in the same double helix, never having been separated from one another. All of the other three DNA molecules would be newly synthesized, one in the first round of replication and two in the second. After two rounds of replication, *one old* and *three new* DNA molecules are obtained.

SEMICONSERVATIVE OR CONSERVATIVE?

Conservative replication predicted a different distribution of newly-synthesized DNA in F_2 (second generation) daughter strands than did *semiconservative* replication: one old and three new vs. two hybrid and two new. For several years, scientists tried frantically to examine the distribution of “new” DNA during replication using radioactive DNA precursors. The idea was to label the parental DNA with

radioactive ^{32}P or ^{14}C . This was done by growing cells on defined medium that contained only sugar, ammonium, potassium, magnesium salts, and trace elements, all dissolved in water, but with ^{14}C -labeled glucose substituted instead of the normal (^{12}C) sugar. All DNA made under these conditions would be radioactive, their nucleotides having ^{14}C -carbon skeletons. The investigator would then flood the cells with cold (nonradioactive, unlabeled) nucleotide precursors. DNA synthesized after this point would not be radioactive, as the radioactive DNA precursors would have been diluted out by their cold counterparts. Allowing two rounds of DNA replication after the addition of excess cold precursors, one could ask whether the ratio of labeled to unlabeled strands was 1:3 or 1:1. Unfortunately, the technical problems of measuring the minute amount of radioactivity in a single strand of DNA were too difficult to permit a clear distinction between the two possibilities using this approach.

MESELSON AND STAHL'S EXPERIMENT

The problem was solved in quite a different manner. In one of the classic experiments of genetics, Matthew Meselson and Franklin Stahl took a radically different approach (figure 7.1). Recently-developed centrifuges were capable of developing enormous g forces—forces so great that most molecules would pellet at the base of centrifuge tubes. Even heavy salts in solution showed displacement in their concentration, being more concentrated toward the base. Meselson and Stahl reasoned, "Why not use a *density label* to distinguish newly-synthesized DNA from parental DNA?" A solution of the heavy salt cesium chloride (CsCl), when spun at high speed in an ultracentrifuge, produced a range of densities down the centrifuge tube, a range that bracketed the density of naturally-occurring DNA. If they added DNA to a CsCl solution in such an ultracentrifuge, the DNA should sink in the tube until it reached a region of CsCl whose density was as great as that of DNA, and there the DNA should float as a discrete band. The key experimental opportunity lies in the fact that DNA that contained heavy isotopes would be more dense, and thus would sink further and band at a different region. If the experiment using radioactive nucleotide precursors was repeated using *heavy* rather than *radioactive* isotopes (growing bacterial DNA with ^{15}N as a source instead of ^{14}N), then band positions on the CsCl density gradient could be used to distinguish among parental, hybrid, and newly-synthesized DNA. The experiments succeeded brilliantly, and clearly showed that after two rounds of DNA replication, half of the DNA is hybrid and half is newly-synthesized. This established beyond reasonable question that DNA replicates in a semiconservative manner, as the Watson-Crick model of DNA had suggested.

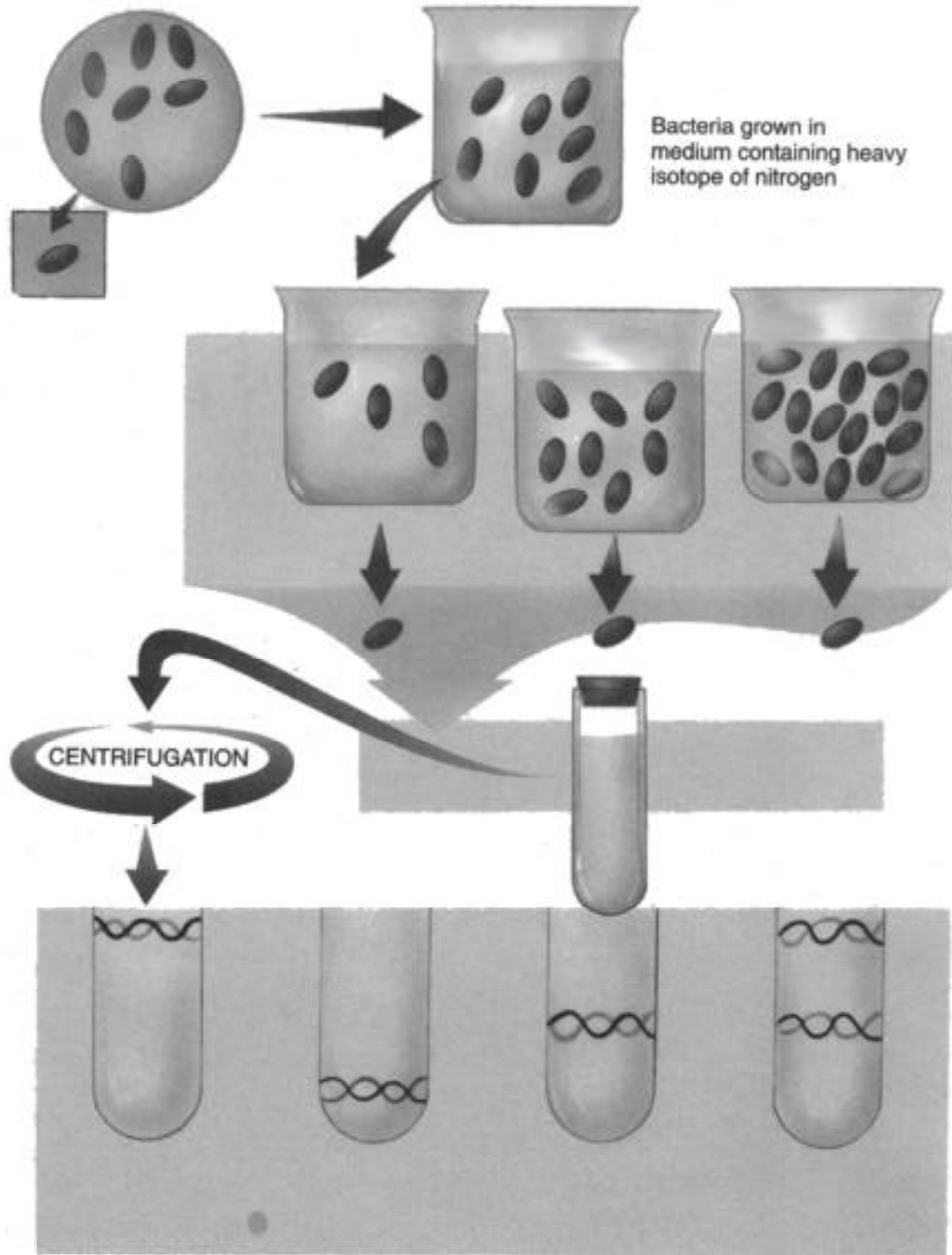


Figure 7.1
The Meselson-Stahl experiment.