CHAPTER 10

OKAZAKI: DNA SYNTHESIS IS DISCONTINUOUS

In 1968, Reiji Okazaki determined that DNA synthesis was not a smooth, continuous process. Rather, fragments of DNA were synthesized discretely, and assembled later on.

THE PUZZLE IN THE DNA SYTHESIS MODEL

There is a fundamental problem implicit in the Watson and Crick model of DNA structure. The model requires, and Kornberg's work demonstrated, that the two DNA strands of a double helix are antiparallel, so that looking along one direction an investigator would see one strand going form 5′ to 3′, while the corresponding strand went from 3′ to 5′. At the end of the double helix the first strand stops with a free 3′ end and the other strand stops with a free 5′ end. The model also suggests, and subsequent work demonstrates, that replication proceeds by opening up the double helix so that each strand may act as a template for a new daughter strand. The problem is that all the DNA polymerases that have been discovered work only on free 3′ ends. Despite intensive searching during the 1960s, no investigator was able to demonstrate the existence of a polymerase that added bases to the 5′ ends of DNA strands. So it was not clear how DNA managed to replicate the 3′→5′ strand! And yet the strand is replicated. Its replication, while presenting no problem to the *E. coli*, presented major problems to geneticists trying to understand how it could occur. The only alternative to the apparently nonexistent 5′ polymerase seemed so outlandish, so out-of-keeping with the simplicity of the Watson-Crick model, that few wished to accept it. It was possible that normal 5′→3′ polymerases such as *poly*-III could successfully carry out the synthesis of the 3′→5′ strand—if the synthesis of this strand was discontinuous rather than continuous!

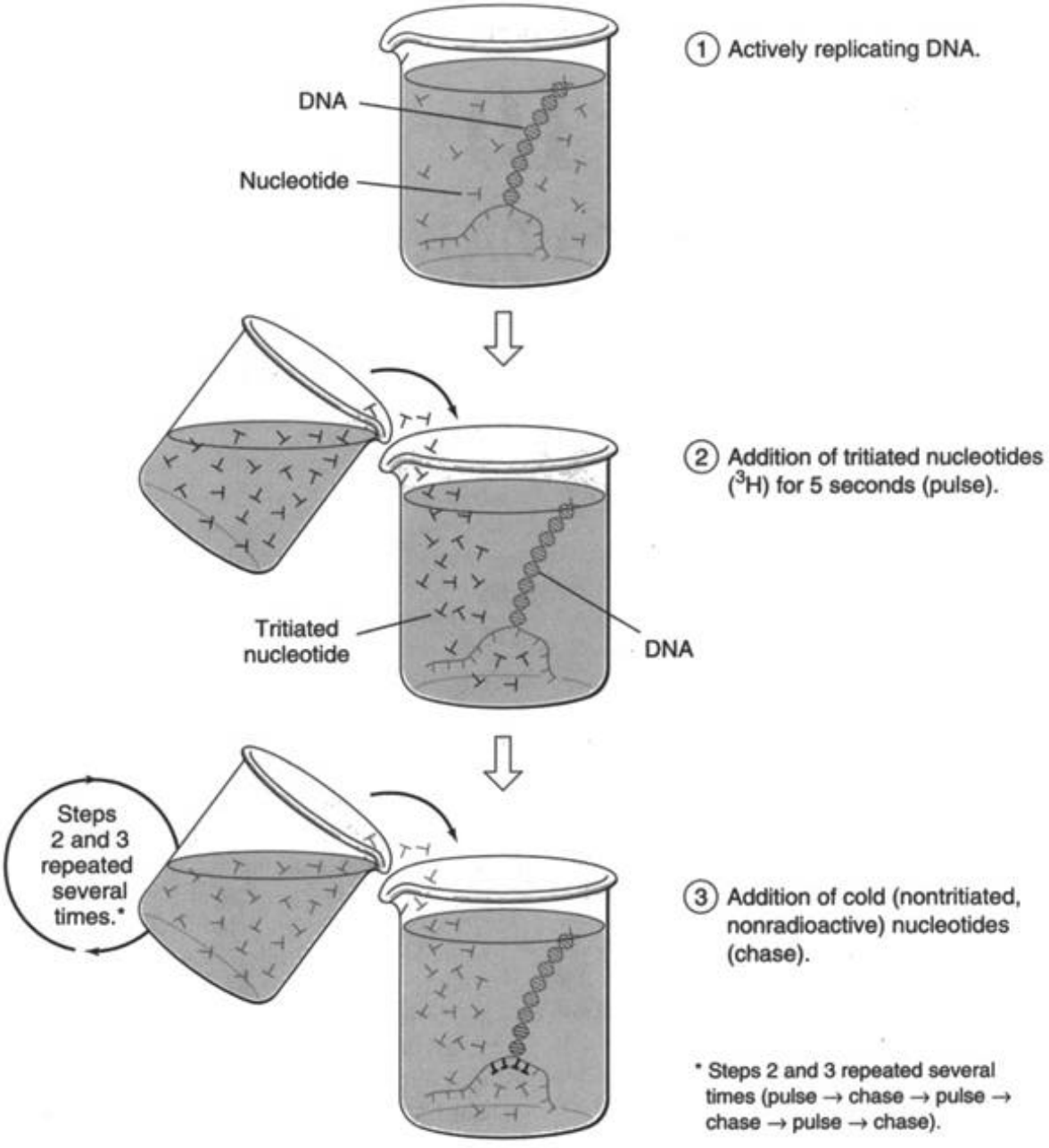
The idea was that as the 5′→3′ polymerase added bases to the free 3′ end, elongating the 5′→3′ strand along its template, the other template strand would be left naked, with no new daughter strand synthesized. Periodically, however, the polymerase could run down this naked strand in the 5′→3′ direction, using it as a template to synthesize a DNA fragment. The fragment could then be joined up to the growing strand by a ligase enzyme, producing the new 3′→5′ strand.

OKAZAKI'S RESEARCH

This sort of "back and fill" mechanism, while awkward and seemingly inefficient, has proven to represent the true state of affairs. Experiments by Reiji Okazaki (figure 10.1) and others in 1968 clearly showed the existence of 1000-to 2000-nucleotide fragments (called *Okazaki fragments*) during the course of DNA replication, fragments that later became incorporated into normal DNA strands. In later studies it was even possible to see with the electron microscope that one of the daughter strands behind the polymerase was single-stranded for about the postulated length of the DNA.

In order to follow the course of DNA replication, Okazaki and his colleagues exposed the replicating DNA to short pulses (about five seconds) of tritiated radioactive nucleotides, followed by the addition of an excess of normal cold (nonradioactive) nucleotides. This sort of *pulse-chase experiment* resulted in label being present only in the DNA that was synthesized during the short period of the pulse. Soon after the pulse, they isolated the DNA and separated the individual strands from one another in alkaline solution. The various pieces of DNA could then be sorted out by size: the alkaline solution of DNA was placed on a "sucrose gradient" and spun in an ultracentrifuge. The bigger pieces of DNA settled more rapidly in such a *sedimentation velocity* experiment as this (the sucrose served to stabilize the resulting separations until the investigator could look at them). The scientists then looked for the presence of label on the spun pieces of

DNA. Label occurred on *two* sizes, one very long, and the other only on small fragments of 1000 to 2000 nucleotides in length.



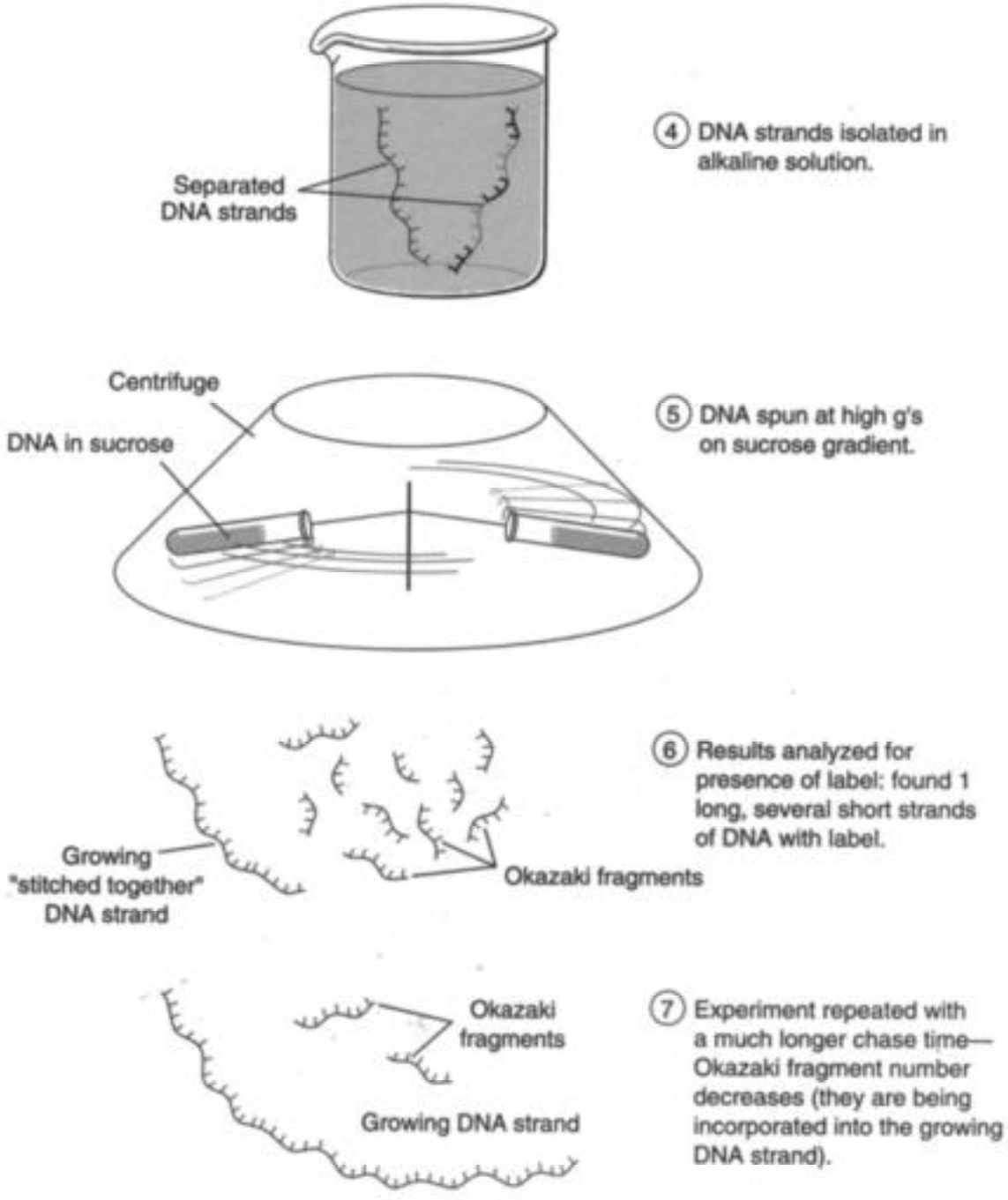


Figure 10.1
Okazaki's experiment.

Were the smaller fragments artificially induced breakdown products of normally larger pieces? No: when Okazaki extended the length of the exposure pulse to 30 seconds, a far greater fraction of the total label ended up in long DNA strands. A similar result was obtained if the period of "cold chase" was prolonged prior to isolation of the DNA. Clearly the fragments existed as such only temporarily, and soon became incorporated into the growing DNA strands.

As it turns out, normal 5 3 polymerases are responsible for the synthesis of these Okazaki fragments. Isolation of the fragments and digestion with 3 exonuclease revealed that the label was added at the 3 end of the fragments, as would be expected if the DNA fragments were synthesized by *poly*-III or another polymerase adding bases at the free 3 –OH end. Finally, the fragments *were* joined into DNA strands by a DNA ligase enzyme, and mutants that were ligase-negative (lack a functional ligase) failed to show the pulse-chase assembled into larger fragments.