CHAPTER 9

KORNBERG: ISOLATING DNA POLYMERASE

In 1956, Arthur Kornberg provided the field of genetics with two important findings. First, he isolated an enzyme called DNA polymerase, the enzyme required for the synthesis of DNA. Then he used his new enzyme to show that DNA is always constructed in a single direction.

THE POLYMERIZATION OF DNA

Arthur Kornberg isolated a new enzyme from *E. coli*, and called it "DNA polymerase" because of its ability to assemble nucleotides to manufacture strands of DNA. He could do this *in vitro* (in a test tube) by providing a pool of free nucleotides, a DNA primer (he used calf thymus DNA), a source of magnesium ions, and ATP. While the actual physiological role of this enzyme proved to be quite different from that originally supposed, studies of its DNA polymerizing activity have been crucial to the understanding of DNA chemistry.

Kornberg used DNA polymerase to verify one of the essential elements of the Watson-Crick model of DNA structure: DNA is always polymerized in the 5´ to 3´ direction (H-CH_2 sugar phosphate bonds to H-O sugar phosphate bond; new nucleotides are added at the 3´ end). He then used this property to demonstrate that the two strands of the DNA molecule were in fact antiparallel (going in opposite directions).

KORNBERG'S METHODS

To prove that DNA was consistently polymerized in the 5´ to 3´ direction, Kornberg provided himself with two essential tools. First, he used labeled nucleotides, which contained the radioactive phosphorus isotope ^{32}P in the phosphate group, and second, he used two different, very specific phosphodiesterase enzymes, which cleaved only O—P—O linkages (one breaks the DNA chain between the phosphate and the 5´ carbon, and the other breaks it between the phosphate and the 3´ carbon). Both enzymes always start at the 3´ end of the DNA chains and work inward. The first of these three enzymes releases the terminal phosphate group with the excised terminal nucleotide, while the second leaves it dangling at the end of the chain.

Kornberg was then set up to perform his experiment. He started up the DNA polymerization process *in vitro*, starting the reaction off using unlabeled cytosine as the nucleotide precursor. Once the process got going, he added radioactive (^{32}P) cytosine for a brief period, and then quickly stopped the reaction. He then digested the resulting DNA with one of the phosphodiester enzymes (figure 9.1).

KORNBERG'S RESULTS

As the reaction was permitted to proceed for a while before the labeled cytosine was added, most of the new DNA strand should be cold (not radioactive), and only the last base that was added would contain the ^{32}P label. If C-^{32}P was added to the 3´ position only, then all the radioactivity would be concentrated at the 3´ end. Because the phosphodiester enzymes started from the 3´ end, the radioactive label would show up in the cleavage products after even a brief digestion. (If an enzyme is used that breaks the chain at the 3´ carbon, ^{32}P will only show up on free cytosine; if it is cleaved at the 5´ carbon, ^{32}P will show up on other nucleotides.) If, on the other hand, Kornberg's polymerase was adding C-^{32}P to the 5´ end (for example, 3´ to 5´ replication) no labeled nucleotides should be released by a short phosphodiesterase digestion, as the label will be concentrated at the 5´ end while the enzymes act at the 3´ end.

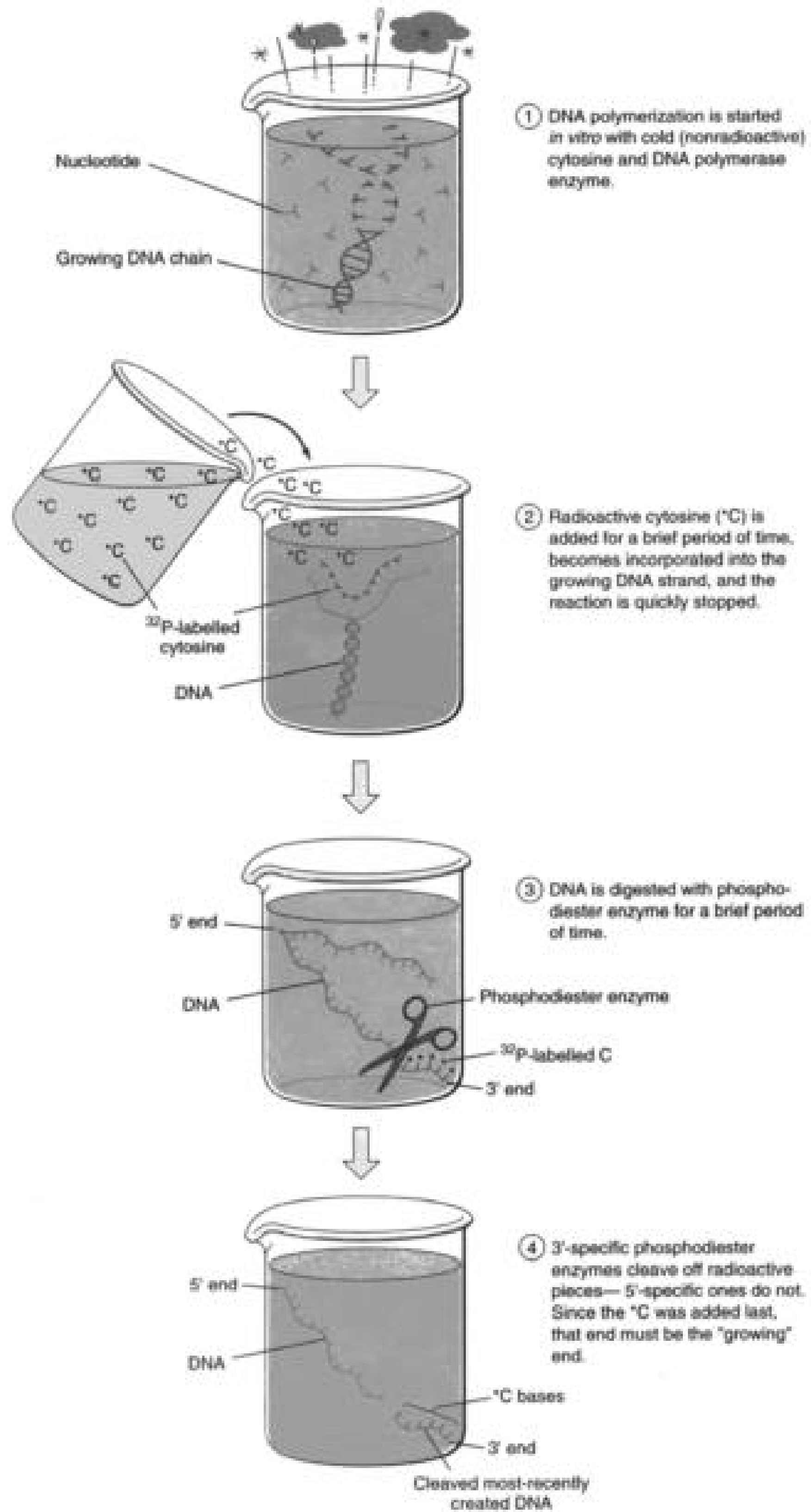
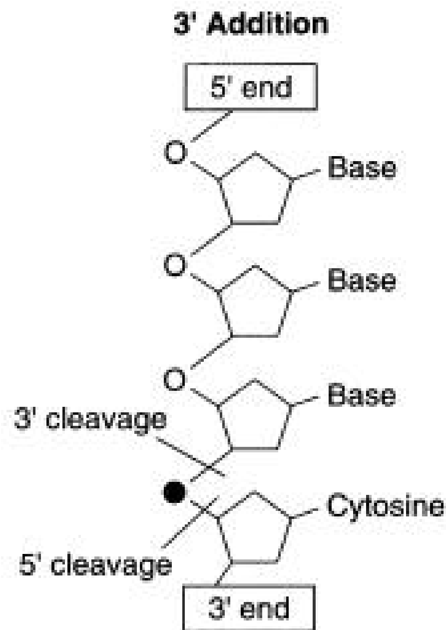
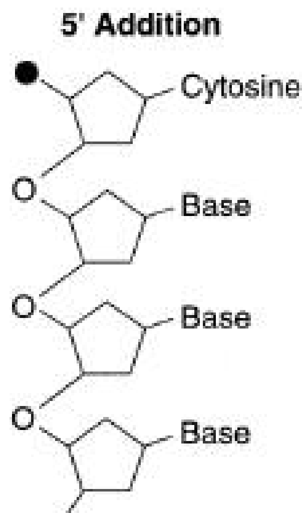


Figure 9.1
Kornberg's experiment.

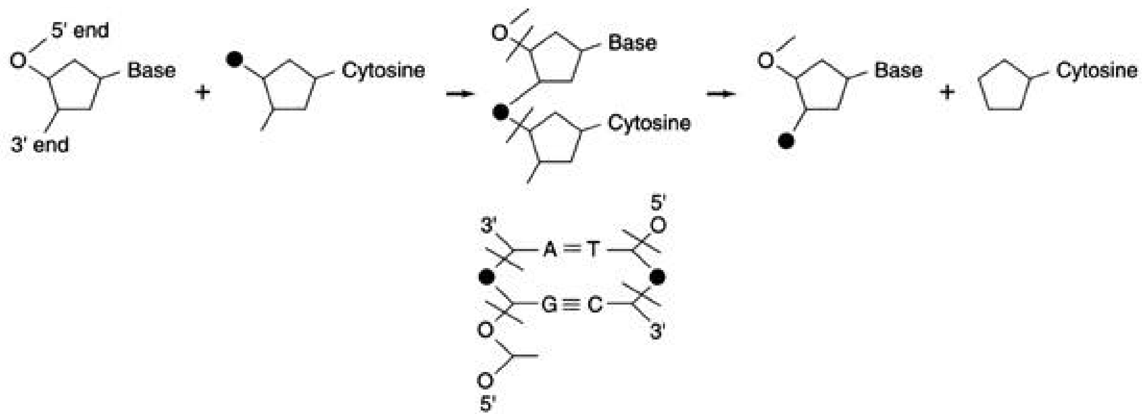
Kornberg did find that ^{32}P nucleotides were released from the new DNA by 3´ specific phosphodiesterases, and thus concluded that the enzyme that he had isolated polymerized DNA proceeding in the 5´ to 3´ direction:



Kornberg then went on to demonstrate that the two strands were antiparallel in the double helix, an absolute requirement of the Watson-Crick model. He made use of the simple fact that if nucleotides were added at the 3´ position, the ^{32}P will be transferred to its neighbor in the 5´ direction when the molecule is cleaved with a specific phosphodiesterase enzyme between the ^{32}P and 5´ carbon.



To determine the polarity of the two strands, the frequency of the *nearest neighbors* on each strand needed to be compared. 5´ phosphodiesterase cleavage could be used to demonstrate, for example, that the frequency with which T was the nearest neighbor to C in the 5´ direction is 0.061:



When C donates ^{32}P to T in the 5´ direction, the label on the opposite strand (also exposed to the same 5´–carbon-specific phosphodiesterase) must end up with G if the other strand is antiparallel. Thus, the frequency with which G is the nearest neighbor to A in the 5´ direction should be like C T. It is very close: 0.065. Note that if the second strand had been parallel, the label would have appeared with A, and the frequency with which A was the nearest neighbor to G in the 5´ direction should be like C T. It is not (actually, it is 0.045). These results clearly indicated that the two DNA strands were antiparallel.

DNA POLYMERASE I

Kornberg's enzyme, called DNA polymerase I, was the focus of a great deal of work in the early studies of DNA replication, and it soon appeared that it might not be the chief DNA-replicating enzyme after all. Very pure preparations of the *E. coli* enzyme failed to exhibit the expected levels of activity against purified *E. coli* DNA. Indeed, when care was taken not to fragment the bacterial DNA, the Kornberg polymerase had essentially no DNA synthesizing activity at all. More disturbingly, John Cairns went on to isolate a mutant of *E. coli*, which provided a clean test of the hypothesis: if DNA polymerase I (*poly*-I) is the principal replicating enzyme, then a *poly*-I negative mutant cell should not be able to replicate its DNA. Cairns succeeded in screening for a mutant of the Kornberg polymerase. *Poly*-I isolated from this mutant was not capable of carrying *in vitro* synthesis with calf thymus DNA primer, although normal *poly*-I could do it quite readily. However, these mutant cells replicated their own DNA in a normal fashion! This strongly suggested that some other enzyme carries out the primary replication function.

POLY-*II AND* POLY-*III*

Because of these results, there were concerted efforts to isolate the "true" polymerase. Several other polymerase-active fractions could be identified in *E. coli*, one of them in appreciable concentrations. This enzyme, *poly*-II, was like *poly*-I, not required for DNA replication. Later, a minor component of overall DNA polymerizing activity, *poly*-III, was isolated by Malcolm Gefter and Thomas Kornberg (Arthur Kornberg's son). The activity of *poly*-III proved incapable of cellular replication of DNA. *Poly*-III thus proved to be the polymerase whose activity was always essential for cell replication and DNA synthesis. *E. coli* temperature-sensitive replication mutants (cells that are normal at 37°C but cannot replicate at 42°C) had normal *poly*-I and *poly*-II enzymes, but their *poly*-III enzyme, normal at 37°C, often proved nonfunctional at 42°C. Thus, finally, *poly*-III was indeed "DNA polymerase." The other enzymes now appear to have role in the repair of DNA.