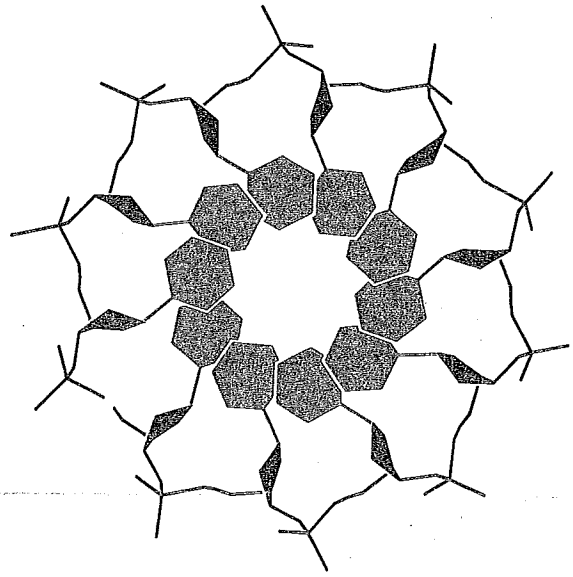
Biochemistry

FOURTH EDITION



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W. H. Freeman and Company

Library of Congress Cataloging-in-Publication Data

Stryer, Lubert.
Biochemistry/Lubert Stryer.—4th ed.
Includes index.
ISBN 0-7167-2009-4
1. Biochemistry. I. Title.
QP514.2.S66 1995
574.19'2—dc20 94-22832

Printed in the United States of America

Ninth printing, 2000

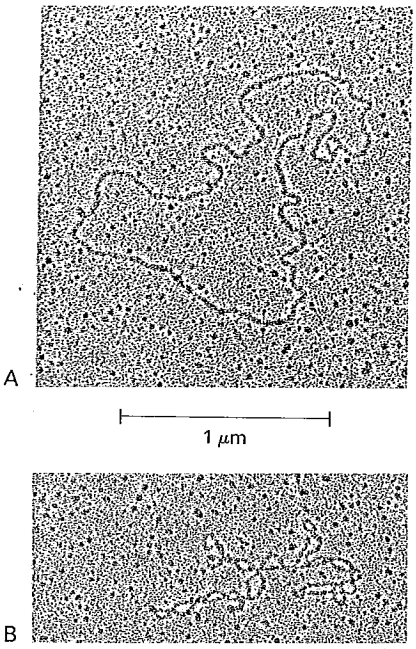

Figure 4-20
Electron micrographs of DNA from mitochondria: (A) relaxed circular form; (B) supercoiled circular form. [Courtesy of Dr. David Clayton.]

MANY DNA MOLECULES ARE CIRCULAR AND SUPERCOILED

Electron microscopy has shown that intact DNA molecules from many sources are circular (see Figure 4-19). The finding that *E. coli* has a circular chromosome was anticipated by genetic studies that revealed that *the gene-linkage map of this bacterium is circular.* The term *circular* refers to the continuity of the DNA chain, not to its geometrical form. DNA molecules in vivo necessarily have a very compact shape. Note that the length of the *E. coli* chromosome is about a thousand times as long as the greatest diameter of the bacterium.

Not all DNA molecules are circular. DNA from the T7 bacteriophage, for example, is *linear.* The DNA molecules of some viruses, such as the λ bacteriophage, *interconvert between linear and circular forms.* The linear form is present inside the virus particle, whereas the circular form is present in the host cell.

A new property appears in the conversion of a linear DNA duplex into a closed circular molecule. The axis of the double helix can itself be twisted to form a *superhelix.* A circular DNA without any superhelical turns is known as a *relaxed molecule.* Supercoiling is biologically important for two reasons. First, *a supercoiled DNA has a more compact shape than its relaxed counterpart* (Figure 4-20). Supercoiling is critical for the packaging of DNA in the cell. Second, *supercoiling affects the capacity of the double helix to unwind, and thereby affects its interactions with other molecules.* These topological features of DNA will be discussed further in a later chapter (p. 794).

DNA IS REPLICATED BY POLYMERASES THAT TAKE INSTRUCTIONS FROM TEMPLATES

We turn now to the molecular mechanism of DNA replication. In 1958, Arthur Kornberg and his colleagues isolated an enzyme from *E. coli* that catalyzes the synthesis of DNA. They named the enzyme *DNA polymerase;* it is now called *DNA polymerase I* because other DNA polymerases have since been found. DNA replication is mediated by the intricate and coordinated interplay of more than 20 proteins. We focus here on DNA polymerase I to illustrate some new principles.

DNA polymerase I is a 103-kd single polypeptide chain. *It catalyzes the step-by-step addition of deoxyribonucleotide units to a DNA chain:*

$$(\text{DNA})_{n\ \text{residues}} + \text{dNTP} \rightleftharpoons (\text{DNA})_{n+1} + \text{PP}_\text{i}$$

(The abbreviation dNTP denotes any deoxyribonucleoside triphosphate, and PP_i denotes the pyrophosphate group.) DNA polymerase I requires the following components to synthesize a chain of DNA (Figure 4-21):

1. All four of the activated precursors—the *deoxyribonucleoside 5′-triphosphates dATP, dGTP, dTTP, and dCTP*—must be present. Mg^{2+} ion is also required.

2. DNA polymerase I adds deoxyribonucleotides to the 3′-hydroxyl terminus of a preexisting DNA chain. In other words, a *primer chain* with a free 3′-OH group is required.

3. A *DNA template* is essential. The template can be single- or double-stranded DNA. Double-stranded DNA is an effective template only if its sugar-phosphate backbone is broken at one or more sites.

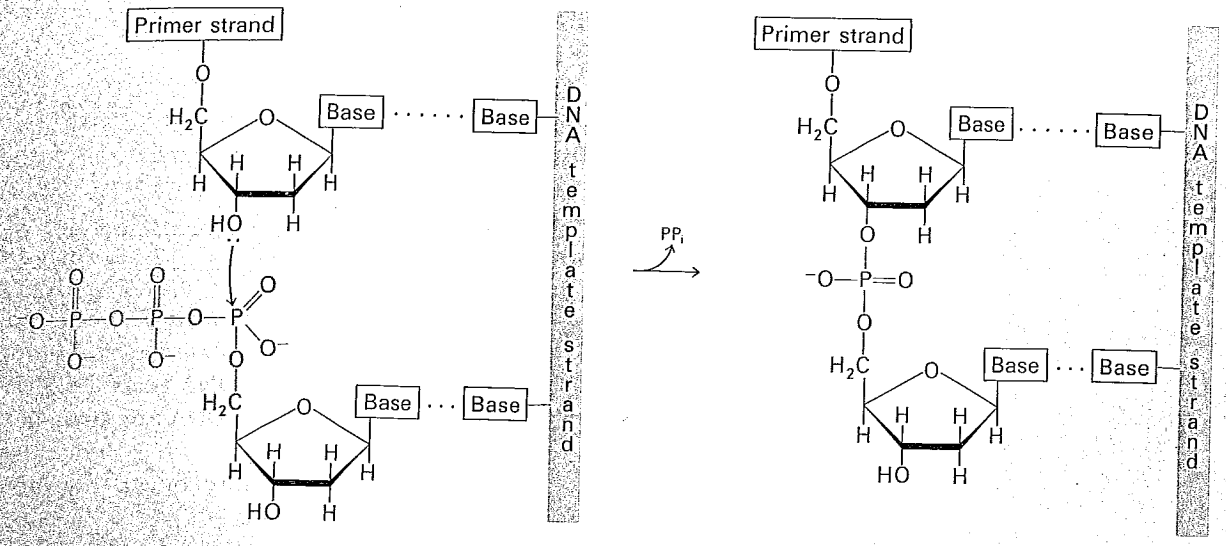

Figure 4-21
Chain-elongation reaction catalyzed by DNA polymerases.

The chain-elongation reaction catalyzed by DNA polymerase is *a nucleophilic attack of the 3′-OH terminus of the primer on the innermost phosphorus atom of a deoxyribonucleoside triphosphate.* A phosphodiester bridge is formed and pyrophosphate is concomitantly released. The subsequent hydrolysis of pyrophosphate by inorganic pyrophosphatase, a ubiquitous enzyme, drives the polymerization forward. *Elongation of the DNA chain proceeds in the 5′ → 3′ direction* (Figure 4-22).

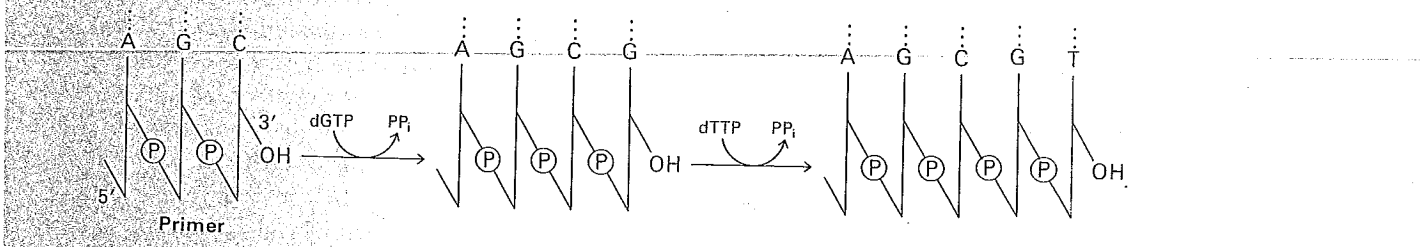

Figure 4-22
DNA polymerases catalyze elongation of DNA chains in the 5′ → 3′ direction.

DNA polymerase catalyzes the formation of a phosphodiester bond only if the base on the incoming nucleotide is complementary to the base on the template strand. The probability of making a covalent link is very low unless the incoming base forms a Watson-Crick type of base pair with the base on the template strand. Thus, DNA polymerase is a *template-directed enzyme.* The enzyme takes instructions from the template and synthesizes a product with a base sequence complementary to that of the template. Indeed, DNA polymerase I was the first template-directed enzyme to be discovered. Another striking feature of DNA polymerase I is that it corrects mistakes in DNA by removing mismatched nucleotides. These properties of DNA polymerase I contribute to the remarkably high fidelity of DNA replication, which has an error rate of less than 10^{-8} per base pair (p. 801).