ENZYMATIC SYNTHESIS OF DNA

BY ARTHUR KORNBERG

C I B A LECTURES IN MICROBIAL BIOCHEMISTRY

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PREFACE

When enzymes in the test tube catalyze a reaction sequence of unimagined complexity, it is one of the happiest accidents in biochemistry. The biochemist stutters a description and then joins a rush of investigators to unravel it. These three chapters, presented as lectures, record our early and more recent encounters with the enzymatic synthesis of DNA. I am grateful to have had the companionship of I. R. Lehman, M. J. Bessman, my wife Sylvy, and E. S. Simms early in this work. J. Adler, J. Josse, S. B. Zimmerman, H. K. Schachman, C. M. Radding, H. V. Aposhian, A. D. Kaiser, M. N. Swartz, and T. A. Trautner have participated since then in our attempts to understand DNA biosynthesis.

I acknowledge with pleasure the hospitality of J. O. Lampen and the Institute of Microbiology at Rutgers and the generosity of S. Barkulis and CIBA Pharmaceutical Products Inc., who made this lecture series and publication possible.

ARTHUR KORNBERG

Department of Biochemistry
Stanford University School of Medicine
Palo Alto, California
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CHAPTER

1

REPLICATION OF DNA

We need not review the recent discoveries [1–3] that identify deoxyribonucleic (DNA) as the genetic substance. In its role DNA must have two functions: it must contain information, in chemical code, to direct the development of the cell according to its inheritance, and it must be reproducible in exact replica for the transmission of this inheritance to future generations.

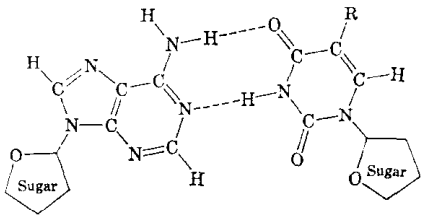
Are these two functions, the expression of the code (protein synthesis) and the copying of the code (preservation of the race), inextricably integrated or can they be studied separately? Encouraging studies are current in several laboratories on DNA-directed synthesis of a "messenger RNA" which may prove to direct protein synthesis.[4] What we have learned from our studies over the last several years is that the replication of DNA distinct from protein synthesis can be examined and at least partially understood at the enzymatic level.

Structure of DNA

First we shall review very briefly some aspects of DNA structure that are essential for this discussion. Analysis of samples of DNA from a variety of sources, by many investigators,[5] has revealed the remarkable fact that the purine content always equals the pyrimidine content. Among the purines, the adenine content may differ considerably from the guanine, and among the pyrimidines, the thymine from the cytosine. However, there is an equivalence of the bases with an amino group in the 6-position of the ring to the bases with a keto group in the 6-position. Watson and Crick,[6] in their masterful hypothesis of the structure of DNA, proposed that the 6-amino group of adenine is linked by hydrogen bonds to the 6-keto group of thymine and that guanine is hydrogen-bonded to cytosine, thus accounting for the equivalence of the purines to the pyrimidines (Fig. 1).

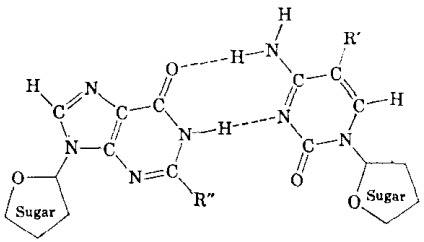
On the basis of models, X-ray crystallographic measurements by Wilkins et al.,[7] and chemical data, Watson and Crick proposed a structure for DNA in which two polynucleotide strands are wound about each other in a helical manner. Figure 2 is a diagram of a fragment of a DNA chain about 10 nucleotide units long. According to physical measurements, DNA chains are 10,000 or more units long. We see here the deoxypentose rings linked by phosphate residues to form the backbone of the chain; the purine and pyrimidine rings are the planar structures emerging at right angles from the main axis of the chain. Figure 3 is a space-filling molecular model [8] and gives a better idea of the packing of the atoms in the structure. The purine and pyrimidine bases of one chain are bonded to the pyrimidine and purine bases of the complementary chain by the hydrogen bonds described in Fig. 1. Extension of

Hydrogen bonding of adenine to thymine



R	
—CH_3	Thymine
—H	Uracil
—Br	Bromouracil
—F	Fluorouracil

Hydrogen bonding of guanine to cytosine



R'		R"	
—H	Cytosine	—NH_2	Guanine
—CH_3	Methylcytosine	—H	Hypoxanthine
—Br	Bromocytosine	=O	Xanthine
—F	Fluorocytosine		

Fig. 1.

a molecular model (manually) in Fig. 4 shows more clearly how the hydrogen-bonded base pairs are stacked in the double-helical structure.

The X-ray measurements have indicated that the space between the opposing chains in the model agrees with the calculated value for the hydrogen-bond linkage of a purine to a pyrimidine; it is too small for two purines and too large for two pyrimidines. Most rewarding from the biological point of view, the structure provides a useful model

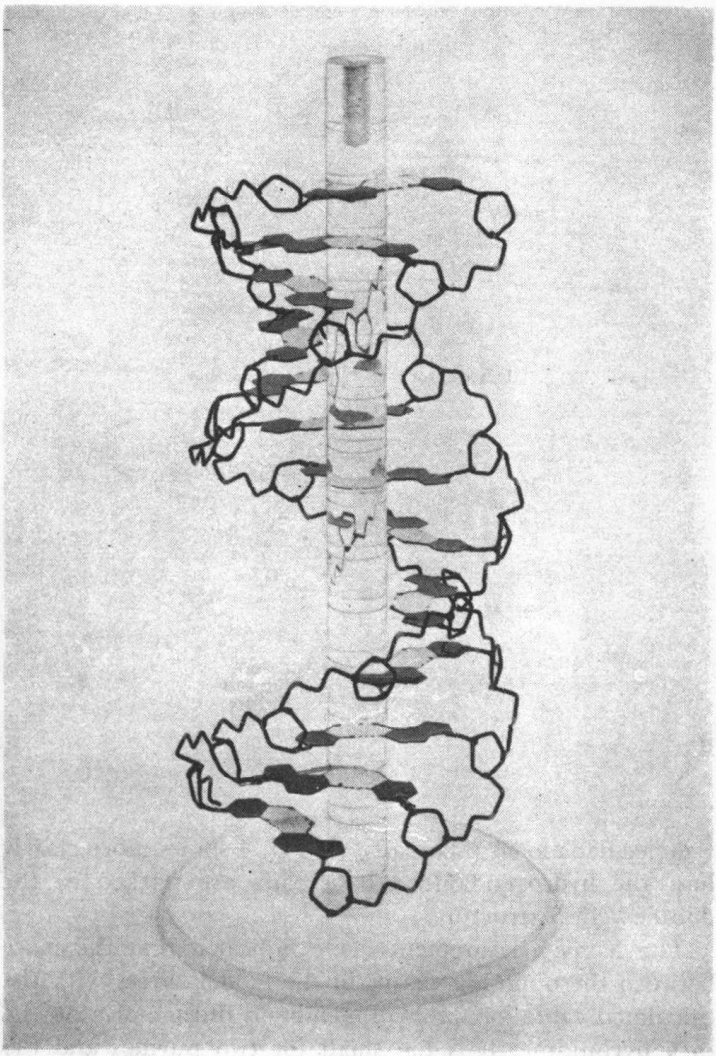


Fig. 2. Double helical structure of DNA. (Watson and Crick model.)

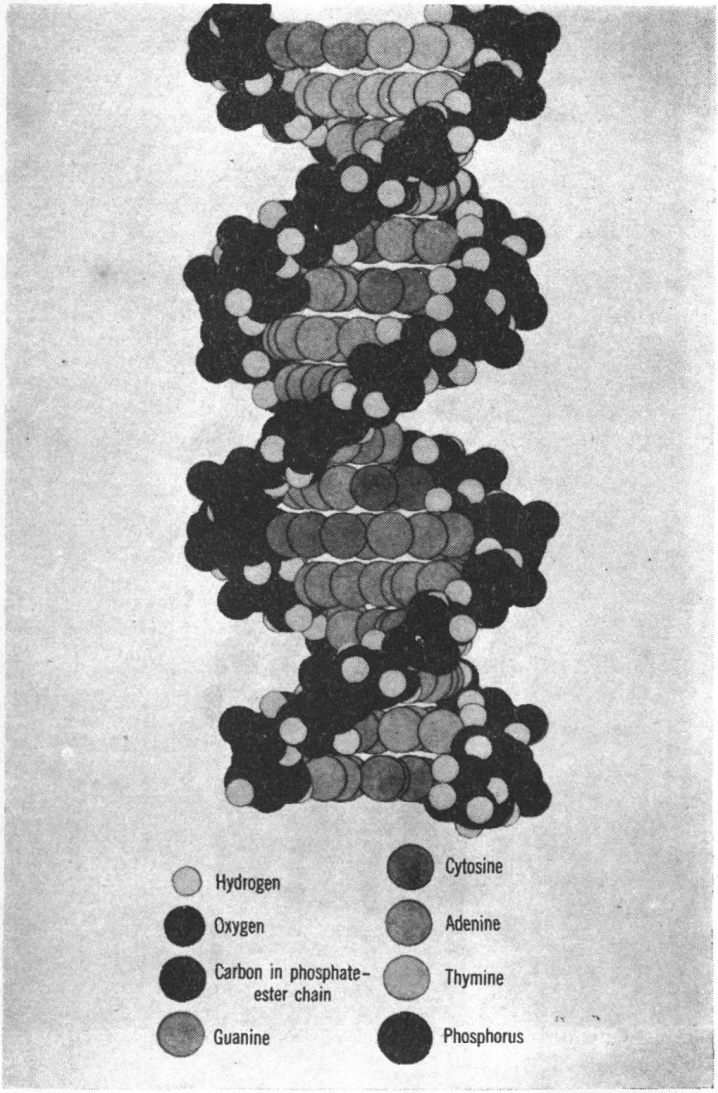


Fig. 3. Molecular model of DNA. (After M. Feughelman et al.[8])

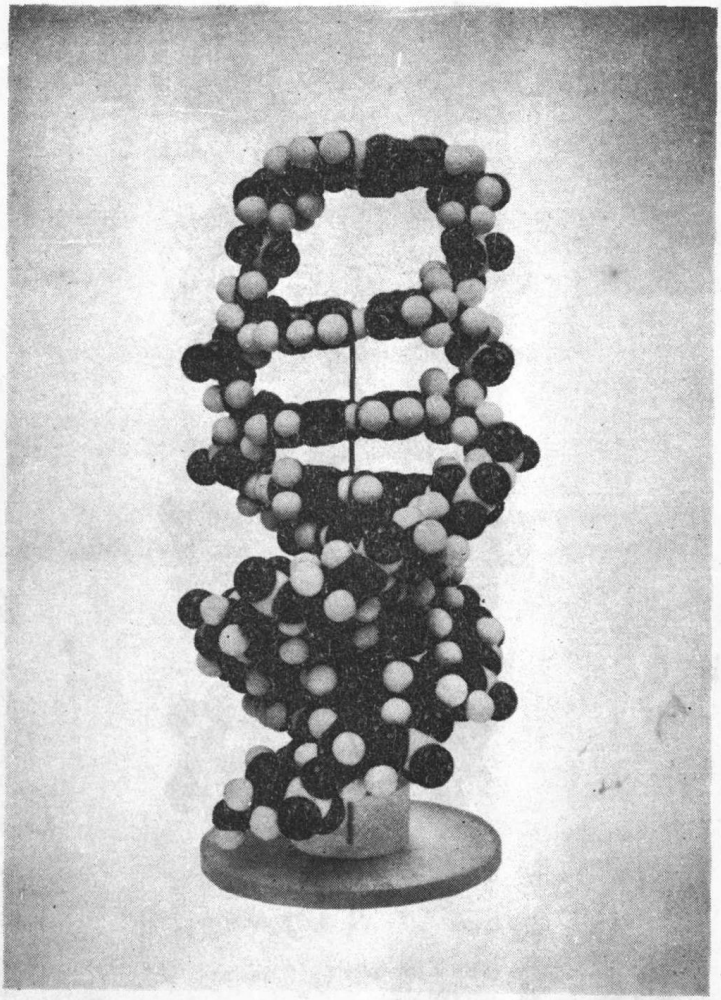


Fig. 4. Extended molecular model of DNA. (Courtesy of Dr. Alan Hodge.)

to explain how cellular replication of DNA may come about. For, if you imagine that these two chains separate and that a new chain is formed complementary to each of them, the result will be two pairs of strands, each pair identical to the original parent duplex (see Fig. 10).

Enzymatic Approach to Replication

Now that we have in the Watson and Crick proposal a mechanical model of replication, we may pose the question: What is the chemical mechanism by which this super molecule is built up in the cell? Observations by Meselson and Stahl [9] with dividing *Escherichia coli,* by Taylor [10] with growing bean plants, and by Sueoka [11] with the alga *Chlamydomonas* are all consistent with and lend support to the Watson and Crick replication model. However, these experiments with intact cells cannot by themselves provide the definitive proof for the precise chemical events, as illustrated by Cavalieri's recent objections.[12] Studies with broken-cell systems must therefore be pursued to try to elucidate the chemical reactions involved in DNA replication. Some 60 years ago the alcoholic fermentation of sugar by a yeast cell was considered to be a process inseparable from the living cell, but through the Büchner discovery of fermentation in extracts and the progress of enzymology during the first half of this century we understand fermentation by yeast as a sequence of integrated chemical reactions. The synthesis of DNA was also regarded for a long time in a "vitalistic" fashion: tampering with the genetic apparatus could produce nothing but disorder. This prediction was not warranted, nor should a similar pessimism govern our attitude toward the many unsolved problems of cellular structure and specialized function.

For an effective approach to the problem of nucleic-acid biosynthesis it is essential to understand the biosynthesis of the simple nucleotides and the coenzymes and to have these concepts and methodologies well in hand. It was from these studies that we developed the conviction that an activated nucleoside 5′-phosphate is the basic biosynthetic building block of the nucleic acids.[13] It will be recalled that the main pathways of purine and pyrimidine biosynthesis all lead to the nucleoside 5′-phosphate; [13] they do not usually include the free bases or nucleosides, except as salvage mechanisms. Although the 2′- and 3′-isomers of the nucleotides are known, they probably arise mainly from certain types of enzymatic degradation of the nucleic acids. It will also be recalled from the biosynthesis of coenzymes,[14] the simplest of the nucleotide condensation products, that it is adenosine triphosphate (ATP) that condenses with nicotinamide mononucleotide to form diphosphopyridine nucleotide, with riboflavin phosphate to form flavine adenine dinucleotide (FAD), with pantetheine phosphate to form the precursor of coenzyme A, and so forth. Uridine, cytidine, and guanosine coenzymes are likewise formed from the respective triphosphates of the nucleosides. The

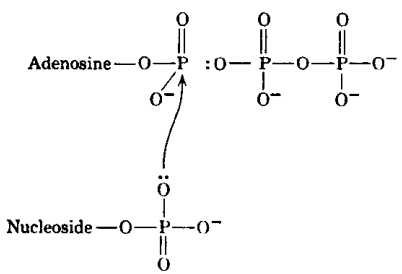


Fig. 5. Nucleophilic attack of a nucleoside monophosphate on ATP.

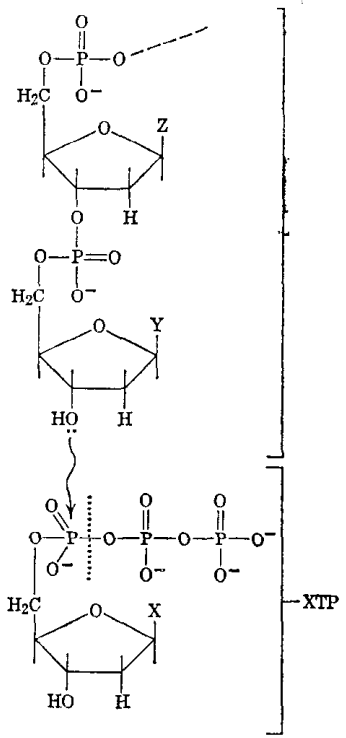


Fig. 6. Postulated mechanism for extending a DNA chain.

activation of fatty acids and amino acids is an example of the same pattern of reaction mechanism.

This mechanism (Fig. 5), in which a nucleophilic attack [15] on the pyrophosphoryl-activated adenyl group by a nucleoside monophosphate leads to the formation of a coenzyme, was adopted as a working hypothesis for studying the synthesis of a DNA chain. As illustrated in Fig. 6, it was postulated that the basic building block is a deoxynucleoside 5′-triphosphate which is attacked by the 3′-hydroxyl

group at the growing end of a polydeoxynucleotide chain; inorganic pyrophosphate is eliminated, and the chain is lengthened by one unit. The results of our studies of DNA synthesis, as shown below, are in keeping with this type of reaction.

Properties of the Enzyme: Polymerase

First let us consider the enzyme and comment on its discovery.[13,16] Mixing the triphosphates of the four deoxynucleosides which commonly occur in DNA with an extract of thymus, bone marrow, or *E. coli* would not be expected to lead to the net synthesis of DNA. Instead, as might be expected, the destruction of DNA by the extracts of such cells and tissues was by far the predominant process, and one had to resort to isotopic tracer methods to detect such a biosynthetic reaction. We used C^{14}-labeled substrate of high specific radioactivity and incubated it with ATP and extracts of *E. coli,* an organism that reproduces itself every 20 minutes. The first positive results represented the conversion of only a very small fraction of the acid-soluble substrate into an acid-insoluble fraction (50 or so counts out of a million added). Although this was only a few micromicromoles of reaction, it was something. Through this crack we tried to drive a wedge, and the hammer was enzyme purification.[17]

This has been and still is a major preoccupation. Our best preparations are several thousandfold enriched with respect to protein over the crude extracts, but still present are one or more of the several varieties of nuclease and diesterase activities that occur in the *E. coli* cell. The occurrence of what appears to be a similar DNA-synthesizing system in animal cells as well as in other bacterial species has been observed.[18] We must wait for more exten-

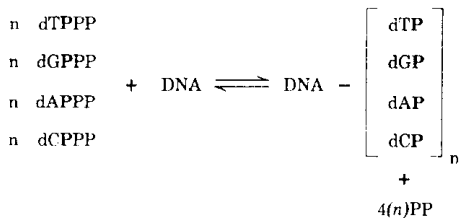


Fig. 7. Equation for enzymatic synthesis of DNA.

sive purification of the enzymes from these sources in order to make valid comparisons with the *E. coli* system.

The requirements for net synthesis of DNA with the purified *E. coli* enzyme[19] are shown in the equation in Fig. 7. All four of the deoxynucleotides which form the adenine-thymine and guanine-cytosine couples must be present. The substrates must be the tri- and not the di-phosphates, and only the deoxy sugar compounds are active. DNA must be present; DNA from animal, plant, bacterial, or viral sources serves equally well in the synthesis, provided the molecular weight is high. The product of the synthesis, which is discussed further below, accumulates until one of the substrates is exhausted and may be 20 or more times greater in amount than the DNA added; thus it is derived to the extent of 95% or more from the substrates added to the reaction mixture. Inorganic pyrophosphate is released in quantities equimolar to the deoxynucleotides converted to DNA.

If one of these substrates were omitted, the extent of the reaction would be diminished by a factor of more than 100. It turns out that when one of the deoxynucleotide substrates is lacking, an extremely small yet significant quantity of nucleotide is linked to the DNA primer. My co-workers and I have described this so-called "limited reac-