Synthesis of Molecular and Cellular Structure

Edited by

DOROTHEA RUDNICK

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Sympo
sium

Synthesis of Molecular and Cellular Structure

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Foreword

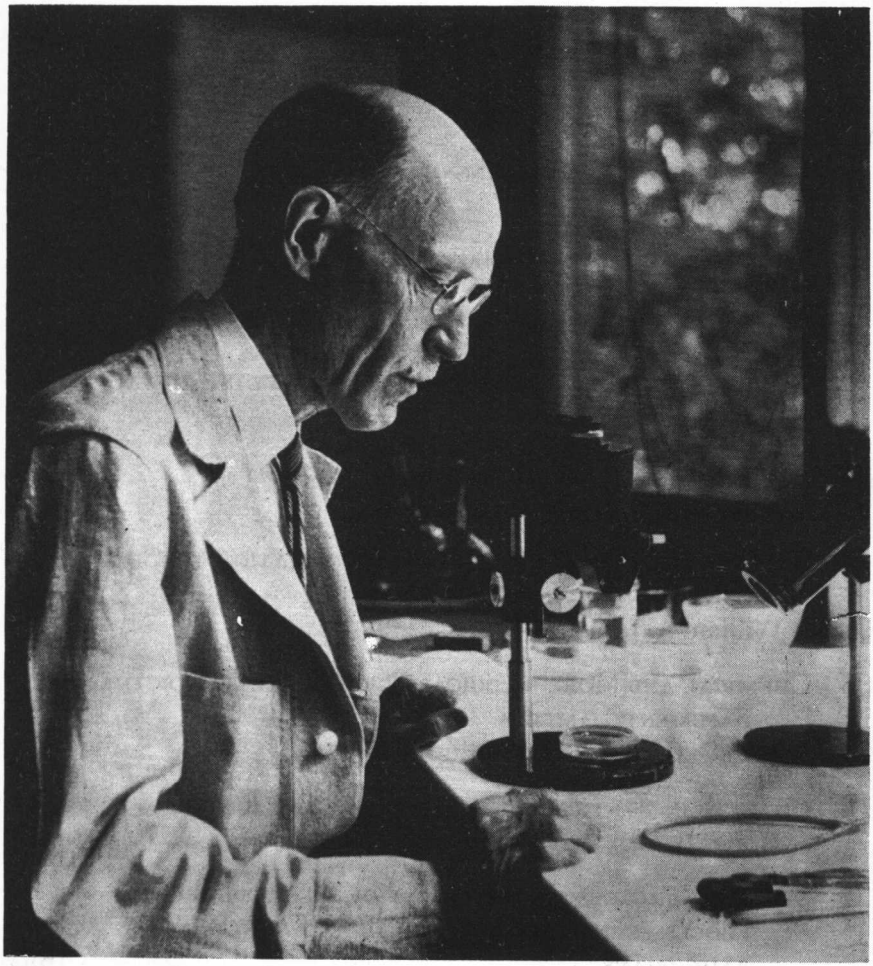
This volume embodies the written version of papers delivered at the Nineteenth Symposium of the Society for the Study of Development and Growth. The theme in the minds of the Executive Committee on this occasion was synthesis of molecular and cellular structure in development. The aim was to bring together a group of experimentalists who have recently made valuable contributions to our knowledge of various levels of organic differentiation—from molecule to organ system. The subjects of the several chapters accordingly range from behavior of nucleic acids and adaptive enzyme control, through analyses of cellular structures and differentiation processes in molecular terms, through regulation of tissue growth and differentiation *in vitro,* to control of organ regeneration in Amphibia.

The Symposium was held at Brandeis University in 1960. The Society records its thanks for the distinguished hospitality offered by this institution and for the thoughtful labors of the Local Committee. A grant from the National Science Foundation making the meeting possible is acknowledged with gratitude.

At the general meeting of the Society, the members voted to dedicate the present volume to the memory of Ross Granville Harrison (1870–1959). There is a certain melancholy satisfaction in reflecting that the theme of this Symposium would have been particularly close to Dr. Harrison's heart.

DOROTHEA RUDNICK

Yale University



Ross G. Harrison

IN MEMORIAM

Ross Granville Harrison

1870–1959

When the formation of the Society for the Study of Development and Growth was first proposed, Dr. Harrison was a fervent supporter of the movement. His own studies had pointed the way to the integration of both the botanical and zoological approaches to problems of common interest to both branches of the field of biology. He frequently compared the relative simplicity of the botanical graft-scion relation with the obscure results so often obtained in animal transplantations. His reading of Sachs and others helped him to formulate his ideas concerning the study of tissues in nutritive supporting media. He had been stimulated in his study of growth by D'Arcy Thompson's *Growth and Form* and by Huxley's *Problems of Relative Growth.* Harrison was always looking for ways to express his findings mathematically, a probable outgrowth of his undergraduate studies in which he majored in biology and mathematics. He always planned his experiments with a mathematical slant. This is evidenced in the study of limb asymmetry: he calculated all possible asymmetric relations and combinations, and his operative sequence was derived from his over-all consideration.

Harrison planned the questions which he was asking the embryo with such precision that the embryo had to give an answer. The answers were interpreted in terms much different from those of the so-called classical embryology and certainly were thought of in the crystallographic analogy. Harrison invoked the paracrystalline analogy of Needham in his last personally published paper on the asymmetry problem. The carbon tetrahedron which had assisted him so much in the visualization of the limb axes was in his mind as he surveyed the ear and later limb experiments. This paper was published just before he became president of this Society in 1946–47.

At that time Harrison had just completed his chairmanship of the National Research Council which he had held from 1938–46. It was in a way his second retirement, and he welcomed the return to his laboratory and his active interest in biological problems. The pro-

gram for the period of his presidency of this Society was not only progressive and foreseeing but has served ever since as a model of the type and quality of program the Society needs for the edification of its members.

Professor Harrison's interest in growth and its problems was a continuous one persisting even after his unfortunate illness. A large manuscript on the relation of induction and limb asymmetry was found among his papers and is now being prepared for publication. This will be his last published work on this subject, but the impetus which he gave to this Society will both now and in the future attest to the stimulation which he made in our thinking. By his example he directed us toward a genuine understanding of the principles underlying the problem of growth.

J. S. NICHOLAS

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Synthesis of Molecular and Cellular Structure

1

The Transfer of Information Between the Nucleic Acids

ALEXANDER RICH [1]

We have a great deal of information today which strongly but indirectly suggests that there is some relation between the sequence of nucleotides which are found in the two major types of nucleic acid in the cell. Since we assume that one nucleic acid sequence copies from another, we describe this process as a "transfer of information."

It is quite clear that the deoxyribose nucleic acid (DNA) acts as the major carrier of genetic information. In addition to the fact that it is one of the major components of chromosomal material, it was shown by Avery over thirty years ago that DNA molecules, isolated from bacterial cells, have the property of transforming other bacteria in a manner which is inheritable. In more recent times an analysis of the infection of bacteria by bacteriophage virus has demonstrated that the infective agent is the DNA of the virus which is injected into the host cell. This infective DNA is capable of carrying all the genetic information contained in the original virus particle, and in addition it has the capacity to produce new progeny inside its host cell. At the present time a large number of viruses have been shown to contain infective nucleic acid which can be separated from the protein component of the virus.

The major mode for the expression of genetic potentialities is believed to be the synthesis of specific protein molecules. These are manufactured in the ribosomal particles which are found in the cytoplasm and which contain over 50 per cent ribonucleic acid (RNA). Again we believe that the information necessary to carry

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out the proper sequentialization of the amino acids in protein synthesis is embodied in the RNA component of the microsomal particles, although there is no clear proof as yet. This belief is reinforced by the observation that the protein component of ribosomal particles from widely different sources appears to be roughly the same even though they are synthesizing quite different proteins (Ts'o *et al.*, 1958).

This had led to the hypothesis that the DNA molecule somehow has the ability to order ribonucleotides which are polymerized to form the RNA molecule. In this article we will discuss the probable mechanisms for this reaction.

Two-stranded Templates

The DNA molecule largely exists in the form of a two stranded complementary helix with the strands held together by hydrogen bonds between purine and pyrimidine residues. Thus one first asks whether it is possible for the two-stranded form of DNA to serve as a template for the manufacture of RNA. This idea has attracted a great deal of attention because of the fact that one of the helical grooves in a DNA molecule is just large enough to accommodate a third polynucleotide chain. This idea was further reinforced by the discovery that it is possible to make three-stranded helical polynucleotide structures in solution (Felsenfeld, Davies, and Rich, 1957). If DNA were to serve as a template for organizing an RNA molecule in such a configuration, then one would look for a series of specific sites on the DNA molecule which can act as recipients for oncoming ribonucleotides. Despite effort by many individuals there have been no satisfactory configurations discovered which will satisfy this template model (Rich, 1959). Although there is adequate room in a helical groove of DNA for a third polynucleotide chain, there do not appear to be an adequate number of hydrogen-bonding sites to maintain specificity for an oncoming ribonucleotide chain. Thus this mechanism for RNA synthesis does not appear promising, even though it cannot be disproved at the present time.

Single-stranded Templates

We might inquire about the possibility of an alternative synthetic mechanism. The work of Kornberg and his collaborators has clearly

shown that the synthesis of new DNA is dependent upon the presence of primer DNA which is itself replicated by the enzyme (Lehman *et al.*, 1958). However, in that system the ultimate primer is a single deoxypolynucleotide strand. This is shown quite clearly when the DNA from the bacteriophage ϕX-174 is used. This DNA acts as the best primer for the DNA polymerase enzyme, and it has been shown that this virus contains single-stranded DNA (Sinsheimer, 1959). Furthermore, it is possible to show that the rate of synthesis of new DNA is increased when the normal two-stranded primer DNA is heat denatured (Lehman *et al.*, 1958), a process which converts the material to the single-stranded form. The reaction therefore probably consists of two phases, an initial separation of the two strands of DNA, followed by a polymerization of the complementary strands of DNA.

We may ask whether the same conditions could prevail in the synthesis of RNA from template DNA; i.e., can a single DNA strand act as a template for making RNA? We can answer part of this question and show that such a reaction is *possible.*

In considering this question, we must bear in mind that, although the difference between the ribose and deoxyribose residues is not very great, there is a considerable difference in the stereochemistry of the two polymer chains. This is, of course, attributed to the oxygen on carbon 2 of ribose which has a van der Waals radius of 1.4 Å and thus through its bulk has considerable constraining effect on the flexibility of the ribose phosphate polymer chain. For example, if we compare the diameter of the DNA molecule with the diameter of the two-stranded helical molecule composed of polyriboadenylic acid and polyribouridylic acid, it is apparent that the latter molecule is approximately 6 Å greater in diameter, even though there is considerable similarity in the geometry of the two molecules, both of which are two-stranded and held together by similar types of hydrogen bonding (Rich, 1957). If we contemplate forming a two-stranded hybrid molecule, with one strand of DNA and one of RNA, we shall have to consider the possibility of forming a compromise two-stranded helical structure perhaps with a diameter intermediate between that which is seen in the two pure types of helices.

Synthetic polyribonucleotides have been used for many years to study various properties of the RNA-type backbone. In recent years small lengths of synthetic deoxyribonucleotides have been

prepared through the synthetic organic approach of Khorana and his collaborators (Tener *et al.*, 1958). He has prepared polydeoxyribothymidylic acid, and this material was generously made available by him to carry out experiments on formation of synthetic DNA-RNA hybrid helices (Rich, 1960).

If polyriboadenylic acid (polyribo A) is mixed with polydeoxyribothymidylic acid (polydeoxy T), evidence for a reaction is seen in the ultraviolet hypochromism of the resultant complex. Hypo-

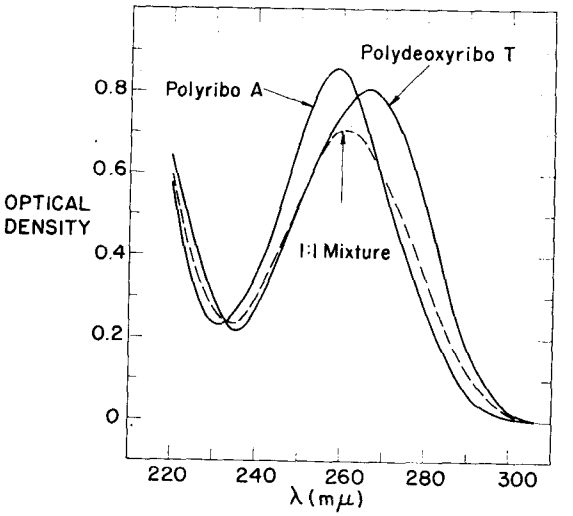

Fig. 1–1. Ultraviolet absorption spectrum of polyriboadenylic acid (polyribo A), polydeoxyribothymidylic acid (polydeoxyribo T), and a 1 : 1 mixture. The solutions are at pH 7 in 0.6 M NaCl, T = 24° C. The thymine polymer had between 11 and 13 residues, while the adenine polymer had near 2,000. The marked hypochromism can be seen near 260 mμ.

chromism (Fig. 1–1) is seen whenever an organized helix is formed from random chains, and it is associated with the orderly stacking of purine and pyrimidine bases. By measuring this hypochromism using the method of continuous variation, it can be shown that two types of complexes form, one involving the single strand of polyribo A and a single strand of polydeoxy T. Another kind of helix, polyribo A plus two polydeoxy T, will also form in analogy to the three-stranded complex of polyribo A plus two polyribouridylic acid (Felsenfeld *et al.*, 1957). Additional evidence for the formation of these complexes can be seen in the analytical ultracentrifuge which

shows a new peak migrating more rapidly than either of the uncombined polymer materials. Thus, we can say that it is possible for an RNA strand to wrap around a DNA strand and form a two-stranded helix held together by complementary hydrogen bonds, and this structure is similar to the two-stranded form of DNA.

Enzymatic Studies

To establish the mechanism for information transfer between the nucleic acids, studies must be made directly in a biological system. Fortunately there has been recent progress in this direction. Three different research groups have reported the partial purification of an enzyme system which appears to be DNA dependent and which incorporates ribonucleoside triphosphates into a polyribonucleotide linkage. The system reported by Weiss (1960) has been isolated from rat liver nuclei and is very sensitive to small amounts of DNase but not very sensitive to RNase. This strongly supports the interpretation that DNA may be acting as a primer in the system. Furthermore this system is dependent on the presence of all four nucleoside triphosphates which suggests a polymerization involving the manufacture of an RNA molecule. Hurwitz and his collaborators (1960) report the presence of a cell-free extract of *Escherichia coli* which will incorporate the nucleoside triphosphates into an RNA-like polymer. As in the case of the rat liver nuclei, this incorporation is dependent upon the presence of DNA and can be stimulated by the addition of DNA from a variety of sources, bacterial or otherwise. This suggests that the reaction has the kind of generality which has been found by Kornberg in the DNA polymerase system. Stevens (1960) has described what may be a similar enzyme system in *E. coli* which also polymerizes an RNA-like substance that appears to be incorporated into microsomal particles. This enzyme system is susceptible to both DNase and RNase digestion. One can interpret all these reports as indicating the presence of an enzymatic system capable of utilizing ribonucleoside triphosphates to polymerize an RNA molecule. The exact nature of the priming reaction of course needs to be clarified, although there is at the present time strong suggestive evidence that it is a DNA molecule.

What type of additional experimental evidence will be necessary to establish the mechanism? One easy test would be to determine whether the primer for the ribonucleotide incorporation is native or

heat-denatured DNA. If denatured DNA is a better substrate than native DNA, it would suggest that single polynucleotide strands are acting as the template material. The nature of the hydrogen bonding between the template and substrate could be determined most easily through the use of synthetic substrates such as the DNA-like AT copolymer produced by the DNA polymerase enzyme. Using this material as primer, one should be able to produce a synthetic RNA material containing only guanine and cytosine. It is quite likely that the purification of this enzyme system will directly lead to a clarification of the synthetic mechanism involved in the production of RNA directly from DNA.

One or Two RNA Strands

If a single strand of DNA serves as a template for the manufacture of RNA, we are faced with the following problem: is one strand of the DNA molecule used to polymerize a new RNA molecule, or are both strands of DNA active? Some preliminary information about this can be obtained through considering the experiments of Volkin and Astrachan (1957) on the RNA which is synthesized after bacteriophage infection. They performed an experiment in which they infected *E. coli* with the T_2 bacteriophage virus and at the same time added P^{32} to the medium. Subsequent to viral infection, the host RNA synthesis stopped, and another kind of RNA synthesis was carried out in conjunction with the manufacture of new phage particles. The new RNA could be analyzed by hydrolyzing the total RNA and simply counting that part which had incorporated the P^{32} label subsequent to phage infection. The results were striking in that the newly synthesized RNA had a base ratio which which was complementary or equal to the base ratio of the invading viral DNA. However, its composition was quite unlike that of the DNA or the RNA of the host. In the T_2 DNA, adenine equals thymine, while in the labeled RNA adenine equaled uracil with the same proportion as in the T_2 DNA. Likewise, T_2 guanine equaled the RNA guanine, and similarly with cytosine. Volkin and Astrachan tried to see how universal this result was by applying it to another bacteriophage (T_7) infection, and the results were substantially the same in that new RNA again had the same composition as the infecting T_7 DNA. Recently some short pulse experiments carried out by Yčas and Vincent (1960) in yeast yielded similar

results. Their results suggested that part of the yeast RNA turned over rapidly and that it had a composition complementary or equal to the over-all DNA composition of the yeast but quite different from the bulk composition of yeast RNA. Astrachan (personal communication) has recently carried out similar short pulse experiments in bacteria. His results suggest strongly that the base composition of the RNA that is rapidly turning over mirrors the composition of the bacterial DNA, even though he selected bacterial strains which had widely differing ratios of $\frac{G+C}{A+T}$.

All these results can be explained by assuming that both strands of DNA act as sites for the synthesis of new RNA. However, there is another possibility. Suppose a single strand of DNA had a gross composition which was the same as both strands together. If this were true, then the results described above would not be due to both DNA strands acting as template sites. There is only one example in which we have adequate information. In the small virus ϕX-174, the DNA isolated from it is single-stranded, and in this case the total composition of the single strand is distinctly far from being self-complementary (Sinsheimer, 1959), that is, A is not equal to T, and G is not equal to C. If individual strands of DNA from the much larger bacteriophage viruses or bacterial cells are that far from complementary, the results could not be explained simply by assuming that one strand of DNA reads out its sequence information. Instead, one would have to assume either that two strands read out the information or alternatively that a single RNA strand is made from a DNA and then proceeds to act as a template for making its own complement. We cannot differentiate either of these alternative mechanisms at the present time.

It may be possible to answer this question by direct experimentation. Thus, by heat denaturing the DNA molecule, we can prepare a solution which is largely single-stranded. If these two strands can be separated either by column fractionation or using the density gradient centrifugation method, we should then be able to analyze for the gross composition of the two complementary strands of DNA.

Let us assume for the moment that two strands of RNA are made from DNA. These two RNA strands are, of course, complementary to each other. What is the physiological role of these two complementary RNA strands? It does not seen likely that both of these