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## Enzymatic synthesis of ribonucleic acid

*Nobel Lecture, December 11, 1959*

I am deeply conscious of the great distinction with which I have been honored and deem it a special privilege to review the recent studies of the biosynthesis of ribonucleic acid on this occasion.

The nucleic acids have considerable biological importance because of their role in cell growth and in the transmission of hereditary characters. As first suggested by the pioneer work of Caspersson and Brachet the former function is performed by ribonucleic acid (RNA) through its participation in the biosynthesis of proteins. The second is carried out by deoxyribonucleic acid (DNA), the main component of the nuclear chromosomes. It is of interest, however, that in certain viruses such as tobacco mosaic, influenza, and poliomyelitis virus, which consist of RNA and protein, RNA is the carrier of genetic information.

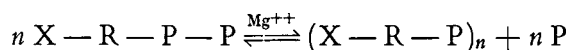
Most of the cell's RNA is present in the cytoplasm. There are two kinds of cytoplasmic RNA. One, of relatively small molecular size, is found in the cytoplasmic fluid and is, therefore, referred to as soluble RNA; the other, of much higher molecular weight, is a component of the microsomal ribonucleo-protein particles. Both play an essential role in protein synthesis. There is, in addition, a small amount of RNA in the cell nucleus, most of it located in the nucleolus. There are indications that most, if not all, of the cytoplasmic RNA is synthesized in the nucleus and subsequently transported to the cytoplasm. In transmitting genetic information, nuclear DNA is supposed to determine the nature of the nuclear RNA which, on entering the cytoplasm, determines in turn the nature of the proteins synthesized.

Although notable advances had been made in our knowledge of the way in which the nucleotides, the nucleic acid building stones, are synthesized, little was known until recently of the mechanism of synthesis of the giant molecules of the nucleic acids themselves. We owe our present information to the discovery of enzymes capable of catalyzing the synthesis of RNA and DNA in the test tube from simple, naturally occurring precursors. These precursors are the nucleoside di- and triphosphates, the nucleotide moieties

of which undergo polymerization with release of orthophosphate in the first case or of pyrophosphate in the second.

### *Polynucleotide phosphorylase*

In 1955 we isolated a bacterial enzyme capable of catalyzing the synthesis of high molecular weight polyribonucleotides from nucleoside diphosphates with release of orthophosphate<sup>1,2</sup>. The reaction, which requires magnesium ions and is reversible, can be formulated by the equation



where R stands for ribose, P - P for pyrophosphate, P for orthophosphate, and X for one or more bases including, among others, adenine, hypoxanthine, guanine, uracil, or cytosine. In the reverse direction the enzyme brings about a cleavage of polyribonucleotides by phosphate, i.e. a phosphorolysis, to yield ribonucleoside diphosphates. The reaction is similar to the reversible synthesis and cleavage of polysaccharides, catalyzed by phosphorylase; for this reason the new enzyme was named polynucleotide phosphorylase. Because of its reversibility, the reaction leads to an incorporation or "exchange" of orthophosphate into the terminal phosphate group of nucleoside diphosphates. It was through this exchange that polynucleotide phosphorylase was discovered by the use of radioactive phosphate. In our early work with Grunberg-Manago, polynucleotide phosphorylase was partially purified, by use of the radiophosphate exchange reaction, from the microorganism *Azotobacter vinelandii*. The enzyme has the unique feature of catalyzing not only the synthesis of RNA from mixtures of the four naturally occurring ribonucleoside diphosphates, but also that of non-naturally occurring polyribonucleotides containing only one, two, or three different kinds of nucleotides in their chains. The nature of the product depends on the kind and variety of the nucleoside diphosphate substrates utilized for the synthesis<sup>3,4</sup>. Table 1 lists the main types of polyribonucleotides which have been prepared with polynucleotide phosphorylase. The preparation of polyribothymidylic acid from synthetic ribothymidine diphosphate has recently been reported<sup>5</sup>.

*Structure of polynucleotides* - In joint experiments with L. A. Heppel<sup>6,8</sup> it has been established that the synthetic polyribonucleotides conform in all respects to the structural pattern of natural RNA. Thus, it was found by degradation with alkali, or such enzymes as snake venom and spleen phos-

phodiesterase or pancreatic ribonuclease, that they consist of linear chains in which the component nucleoside units are linked to one another through 3',5'-phosphodiester bridges. The structural identity with natural RNA can briefly be illustrated by the effect of pancreatic ribonuclease on the synthetic polymer poly AU. From what is known of the action of ribonuclease on RNA, poly AU would be degraded at the points indicated by the arrows in Fig. 1. Uridylic acid (uridine 3'-monophosphate) would be released as the

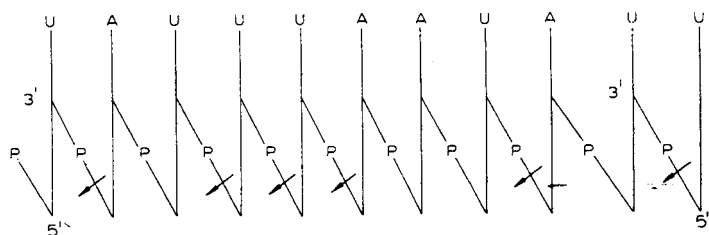


Fig. 1. Scheme of cleavage of poly AU by pancreatic ribonuclease. The vertical lines represent the ribose residues; A and U represent the bases adenine and uracil, respectively. The cleavage points are indicated by arrows<sup>3</sup>.

Table 1. Synthetic polyribonucleotides.

<i>Substrate</i>	<i>Polymer</i>
ADP	Poly A
GDP	Poly G
UDP	Poly U
CDP	Poly C
IDP	Poly I
Ribothymidine diphosphate	Polyribothymidylic acid
ADP + UDP	Poly AU
GDP + CDP	Poly GC
ADP + GDP + CDP + UDP	Poly AGUC (synthetic RNA)

only mononucleotide, along with a series of small oligonucleotides, each consisting of one uridylic acid residue and one or more adenylic acid residues. Fig. 2 is an ultraviolet print of a chromatogram illustrating the separation of mono-, di-, tri-, tetra-, and pentanucleotides each with decreasing *R<sub>f</sub>* values from a ribonuclease digest of poly AU. The individual spots were eluted and the respective oligonucleotides identified following hydrolysis with alkali. Digestion of synthetic RNA with ribonuclease yields, along with uridylic and cytidylic acids, mixtures of oligonucleotides which, as far as

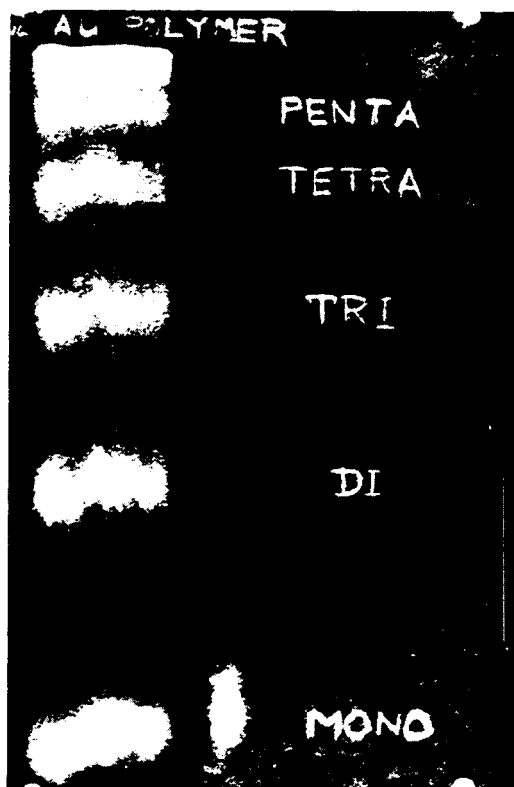


Fig. 2. Products of hydrolysis of poly AU by ribonuclease (L. A. Heppel). Origin is at top of chromatogram. Spot at bottom center is a marker of uridine 3'-phosphate<sup>3</sup>.

they have been identified, are identical to those obtained from natural RNA under the same conditions.

The question whether a given nucleotide species is linked to the various other nucleotides in the polynucleotide chains, as is the case with natural RNA, can be easily answered through degradation of synthetic RNA labeled with radioactive phosphate<sup>3</sup>. Fig. 3 presents the structure of a polynucleotide (poly A\*GUC), prepared from a mixture of adenosine diphosphate labeled with <sup>32</sup>P in the first phosphate group (adenosine-<sup>32</sup>P-P) and non-labeled guanosine-, uridine-, and cytidine diphosphates. If the labeled adenylic acid is randomly distributed as shown, hydrolysis of such a polymer with snake venom phosphodiesterase (Fig. 3A) will yield nucleoside 5'-monophosphates of which only adenosine 5'-monophosphate will be labeled. Hydrolysis with spleen phosphodiesterase, on the other hand (Fig. 3B) will release

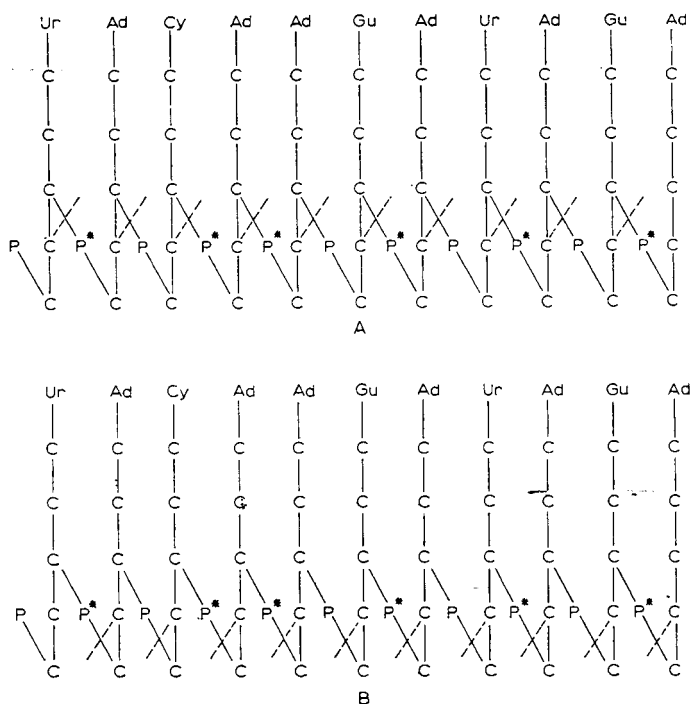


Fig. 3. Scheme of hydrolysis of  $^{32}\text{P}$ -labeled RNA (poly A\*GUC) by snake venom (A) or spleen (B) phosphodiesterase. The bonds hydrolyzed are indicated by dashed lines. The asterisk denotes  $^{32}\text{P}$  labeling. Ad, Gu, Ur and Cy represent the bases adenine, guanine, uracil and cytosine, respectively.

nucleoside 3'-monophosphates all of which will be labeled. That such is indeed the case is shown in Fig. 4. The separation of the hydrolysis products in this experiment was effected by ion-exchange chromatography.

Although large variations in the relative proportions of the different nucleoside diphosphate substrates has a rather marked influence on the nucleotide composition of the resulting polymer, when synthetic RNA is prepared

Table 2. Base ratios of natural and synthetic RNA<sup>a</sup>.

Base	<i>Azotobacter</i> RNA	<i>Poly AGUC</i> (sample 1)	<i>Poly AGUC</i> (sample 2)
Adenine	1.00	1.00	1.00
Guanine	1.30	1.16	1.25
Uracil	0.73	0.66	0.69
Cytosine	0.90	0.72	0.73

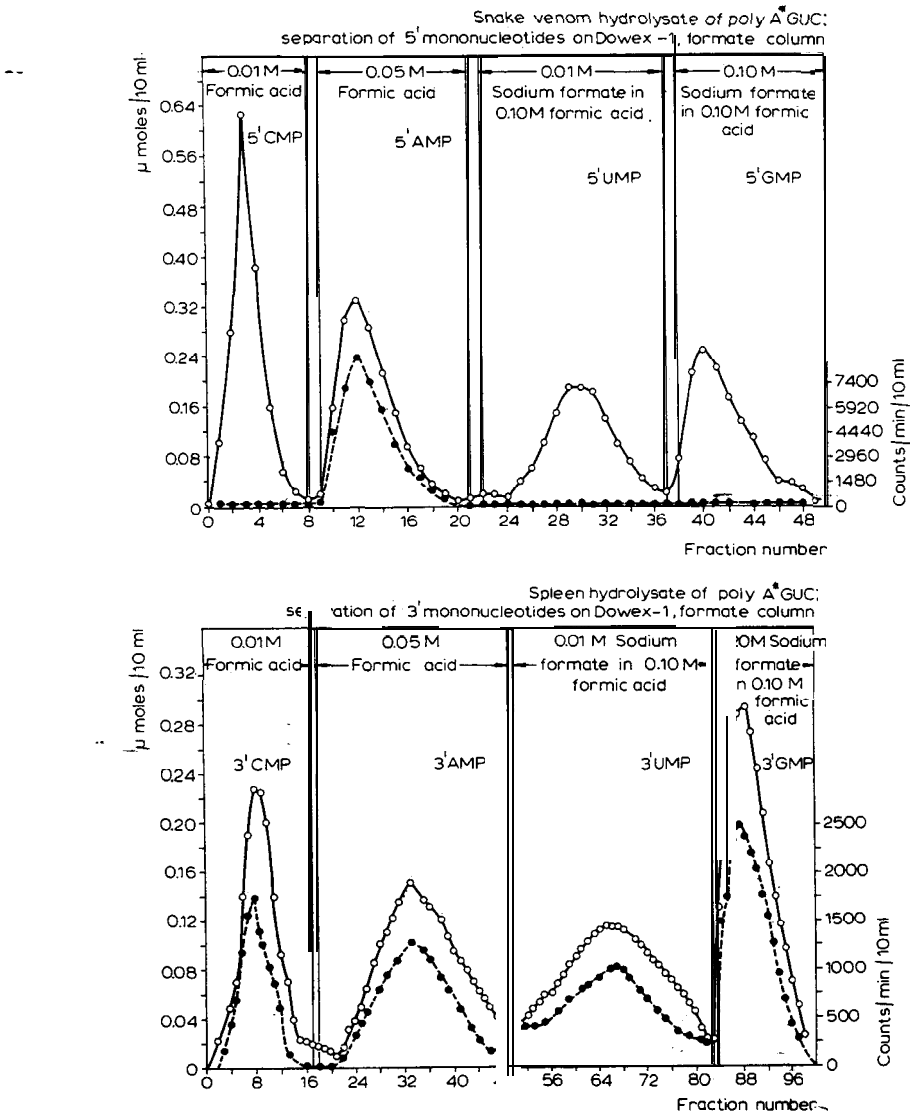


Fig. 4. Hydrolysis of poly A\*GUC with snake venom (top) or spleen (bottom) phosphodiesterase with separation of mononucleotides by ion-exchange chromatography. Plot of nucleotide concentration (solid lines -o-o-o-) and radioactivity (dashed lines -o-o-o-) against effluent fraction number<sup>12</sup>.

from equimolar mixtures of adenosine, guanosine, uridine, and cytidine diphosphate, the nucleotide composition of the product is very similar to that of natural *Azotobacter* RNA. This is shown in Table 2 which gives the

base ratios of *Azotobacter* RNA and of two different samples of the synthetic product. It is noteworthy that the base ratios differ widely from unity in spite of the fact that equimolar concentrations of the nucleoside diphosphate precursors were used.

The synthetic polyribonucleotides also resemble natural RNA in size. Their molecular weight varies between about 30,000 and one to two millions. The sedimentation constant of samples of synthetic RNA was similar to that of RNA isolated from whole *Azotobacter* cells. Polynucleotides containing only one kind of nucleotide unit, such as polyadenylic or polycytidylic acid, are often of very large size and confer high viscosity to their solutions. It is possible to follow the course of synthesis visually by the marked increase in viscosity that takes place on incubation of nucleoside diphosphates with a few micrograms of enzyme. Natural RNA- has a non-specific biological activity which is also exhibited by synthetic RNA. The latter is as effective as the former in stimulating the formation of streptolysin S (a lecithinase) by hemolytic streptococci<sup>10</sup>.

*Reaction mechanism* - To study the mechanism of action of polynucleotide phosphorylase it was essential to obtain highly purified preparations of the enzyme. The last step (Fig. 5) was chromatography on a hydroxyl apatite column, a method developed by Tiselius and collaborators<sup>11</sup>. Only through chromatography was it possible to separate the enzyme from a contaminating yellow protein of unknown nature which, as seen in the figure, is eluted at higher buffer concentrations than is phosphorylase. The purification of the enzyme after chromatography is some six hundred fold over the initial extract of *Azotobacter* cells. The highly purified enzyme contains a firmly bound oligonucleotide<sup>12</sup> which cannot be removed by such methods as treatment with charcoal or ribonuclease. Since it has so far not been possible to remove the oligonucleotide without destroying the enzyme protein it remains undecided whether this compound, which represents about 3.5 per cent of the enzyme, is a prosthetic group or a contaminant. The oligonucleotide consists of about twelve nucleotide residues of adenylic, guanylic, uridylic and cytidylic acid in roughly the same molar ratios as in *Azotobacter* RNA. It can be isolated after denaturation of the protein with perchloric acid or phenol.

Since the *Aztobacter* enzyme can synthesize RNA as well as polynucleotides with only one nucleotide species, it is important to decide whether one is dealing with a mixture of enzymes, each reacting with a different nucleoside diphosphate, or with a single enzyme. Although this question cannot be

answered unequivocally, if the activity toward each of several nucleoside diphosphates increases to the same extent on purification, it is very likely that a single enzyme is involved. That this is so is shown in Table 3 in which the

Table 3. <sup>32</sup>P-exchange assay with different nucleoside diphosphates\* (S. Mii and S. Ochoa, unpublished).

Enzyme fraction	ADP	GDP	UDP	CDP	IDP
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> gel eluate	39	33	46	35	41
After chromatography	316	300	370	280	370
Purification ratio	8.1	9.1	8.0	8.0	9.0

\* Results, micromoles of <sup>32</sup>P-exchange per mg of enzyme protein, 15 minutes at 30°.

activity toward each of five nucleoside diphosphates, as assayed by the radioactive phosphate exchange method, is seen to increase approximately to the same extent between two advanced purification steps. The same was true for earlier stages of purification.

With partially purified preparations of polynucleotide phosphorylase po-

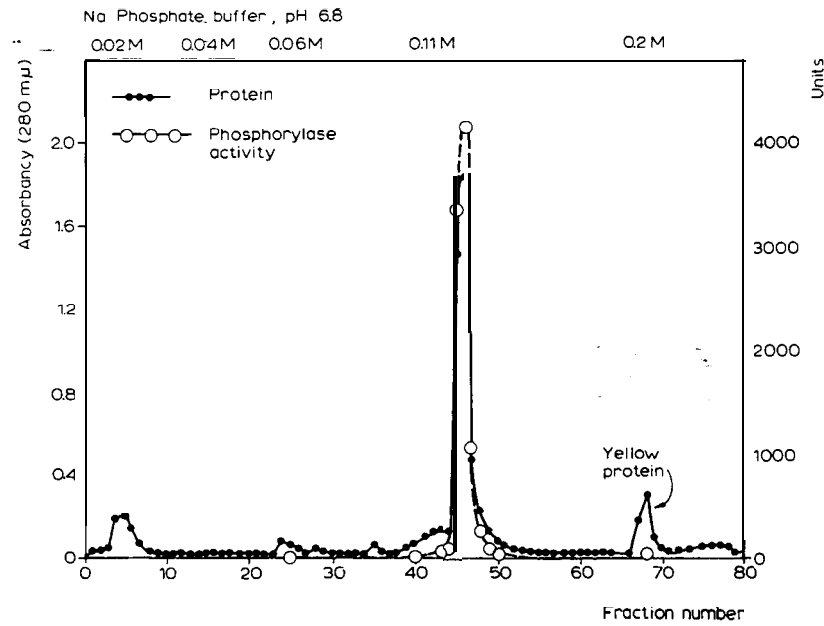


Fig. 5. Chromatography of *Azotobacter* polynucleotide phosphorylase on hydroxylapatite (S. Ochoa and S. Mii, unpublished).



lynucleotide synthesis starts immediately after adding the enzyme to an otherwise complete system. This is not the case, however, with highly purified preparations. In this case, there is mostly a more or less pronounced lag period although eventually the reaction starts and gradually increases in rate. Equilibrium is not reached even after many hours of incubation. The reaction rate is markedly stimulated by addition of small amounts of oligo- or polynucleotides which, as is the case with glycogen in polysaccharide synthesis from glucose-1-phosphate by (polysaccharide) phosphorylase, serve as primers of the reaction. The priming effect of oligoribonucleotides was discovered by Heppel and collaborators<sup>13</sup>, the priming by polynucleotides was disclosed in our laboratory<sup>14</sup>. The main oligonucleotide primers used have been di-, tri-, or tetraadenylic acids isolated as reaction products of hydrolysis of polyadenylic acid by a nuclease from liver nuclei<sup>15</sup>;

The priming by oligonucleotides is not specific. The oligoadenylic acids can prime the synthesis of polyadenylic and polyuridylic acid as well as that of RNA or any of the other polynucleotides. Priming by polynucleotides, on the other hand, shows a certain degree of specificity. Thus, polyadenylic acid primes only its own synthesis and the same is true of polyuridylic acid. On the other hand RNA, whether natural or synthetic, primes the synthesis of RNA as well as that of polyadenylic acid or polyuridylic acid. Very curious and completely unexplained is the effect of polycytidylic acid which primes the synthesis of all the polynucleotides so far tested (Table 4). Fig. 6 shows the priming of RNA synthesis with highly purified polynucleotide phosphorylase by triadenylic acid (TAA), liver RNA, and polycytidylic

Table 4. Specificity of priming by polynucleotides (Ref. 14, and unpublished data).

<i>Polymer synthesized</i>	<i>Effect of</i>					<i>RNA (natural or synthetic)</i>
	<i>Poly A</i>	<i>Poly U</i>	<i>Poly C</i>	<i>Poly I</i>	<i>Poly AU</i>	
Poly A	+	—	+	o	+	+
Poly U	—	+	+	o	+	+
Poly C	—	—	+			—
Poly G	o	o	+			
Poly I	—	o	+	+		
Poly AU					+	
Poly AGUC	o	o	+			+

+ Denotes priming, — denotes inhibition, o denotes no effect. Blank spaces, no information.

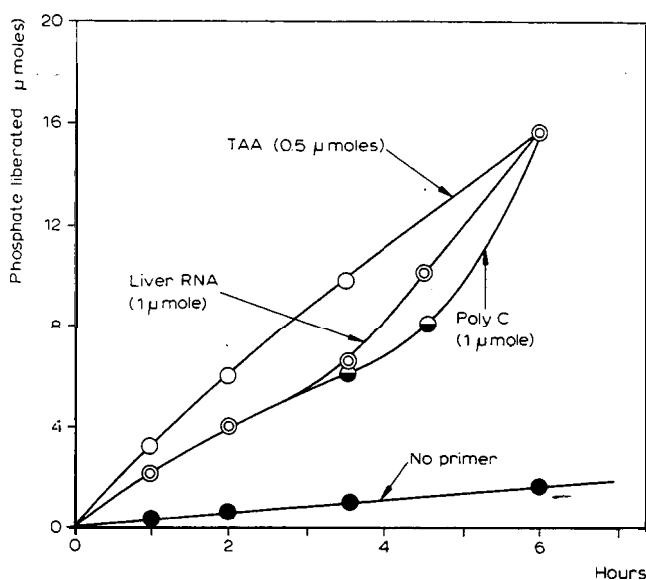


Fig. 6. Priming of RNA synthesis (S. Ochoa and Mii, unpublished).  
 Diphosphates (A+G+U+C),  $5 \mu$  moles each;  $Mg^{++}$ ,  $2.0 \mu$  moles; enzyme,  $140 \mu$  g;  
 TRIS, pH 8.1,  $150 \mu$  moles; volume, 1.0 ml.

acid (poly C). The effect of different polynucleotides on the synthetic reaction is illustrated in Table 4. It is to be noted that polyadenylic acid is not only without effect but actually inhibits the slow synthesis of polyuridylic acid which occurs in the absence of added primer; the converse is also true. It should further be noted that the synthesis of polycytidylic acid is primed only by polycytidylic acid itself.

The mechanism of priming by polynucleotides and the cause and significance of the specificity just described are as yet unexplained. The possibility that polyribonucleotides might function as templates for their own replication has not been substantiated experimentally. On the other hand, the mode of action of the oligonucleotide primers has been elucidated in elegant experiments by Heppel and his collaborators<sup>13,16</sup>. They proved that the oligonucleotides serve as nuclei for growth of the polynucleotide chains by successive addition of mononucleotide units. It may be recalled that polysaccharide phosphorylase acts in a similar way by catalyzing the successive addition of glucosyl residues to the terminal units of a polysaccharide primer. When the synthesis of polyuridylic acid is primed by di- or triadenylic acid, the new polynucleotide chains should consist of a number of uridylic acid residues preceded by two or three adenylic acid residues. This is shown schemat-

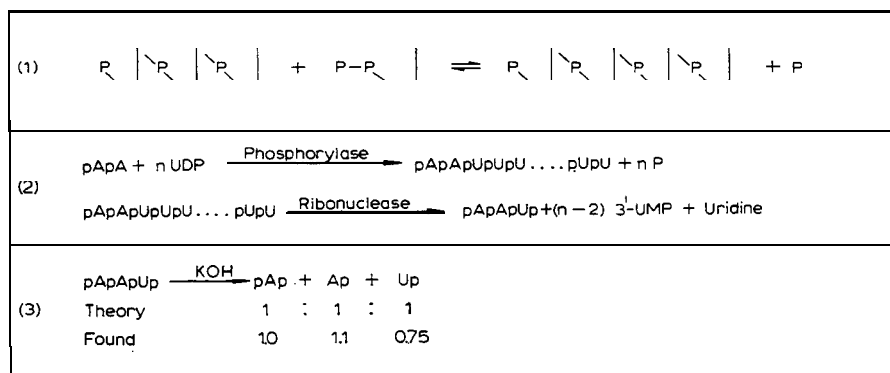


Fig. 7. Mechanism of priming by oligonucleotides (From data of M. F. Singer, L. A. Heppel, and R. J. Hilmoe<sup>13</sup>).

ically in the upper and middle portions of Fig. 7. That such is in fact the case was proved following ribonuclease digestion of polyuridylic acid synthesized in the presence of diadenylic acid as primer<sup>13,16</sup>. As shown in the middle portion of Fig. 7, ribonuclease would release a trinucleotide (pApApUp) from the origin, one molecule of uridine from the end, and a number of uridine 3'-monophosphate residues from the remainder of the chain. The trinucleotide was isolated by chromatography and digested with potassium hydroxide. As shown in the lower section of Fig. 7, this should yield equimolar amounts of adenosine 5',3'-diphosphate, adenosine 3'-monophosphate and uridine 3'-monophosphate. The figure also shows that the amounts actually recovered were in good agreement with the theory.

It appears justified to conclude that polynucleotide phosphorylase may be unable to start the synthesis of a polynucleotide chain from nucleoside diphosphates as the only reactants and that the presence of an oligonucleotide to serve as nucleus for growth of new polynucleotide chains is probably indispensable. If the enzyme could be obtained completely free of oligonucleotide primer material, it might prove to be completely inactive in the absence of added oligonucleotides.

*Biological significance* - Polynucleotide phosphorylase is widely distributed in bacteria. The enzyme has been partially purified from microorganisms other than *Azotobacter vinelandii*<sup>17-19</sup> and polyribonucleotides have been synthesized with these enzyme preparations. Indications have also been obtained for the presence of the enzyme in green leaves<sup>17</sup>. On the other hand, it has been difficult to detect the enzyme in animal tissues. Recently, however,

Hilmoe and Heppel<sup>20</sup> have reported the presence of polynucleotide phosphorylase in preparations from mammalian liver nuclei.

The occurrence of polynucleotide phosphorylase in nature appears to be widespread enough to warrant the assumption that this enzyme may be generally involved in the biosynthesis of RNA. This possibility appears to be strengthened by recent studies with ribonucleoside diphosphates containing different analogues of the naturally occurring bases. Thus j-bromouridine diphosphate which contains 5-bromouracil, an analogue of uracil or thymine which in experiments with intact bacterial cells is incorporated into DNA but not into RNA, is not a substrate of polynucleotide phosphorylase, while thiouridine diphosphate containing the uracil analogue thiouracil, which in similar experiments is incorporated into RNA but not into DNA, is a substrate for phosphorylase. In line with these observations is the fact that azauridine diphosphate containing the uracil analogue aza-uracil which is not incorporated *in vivo* into RNA, does not react with polynucleotide phosphorylase<sup>21</sup>. However, in spite of the fact that polynucleotide phosphorylase can bring about the synthesis of an RNA of the same nucleotide composition and molecular weight as that isolated from *Azotobacter*, and despite the intriguing specificity of priming by polyribonucleotides, there is so far no evidence that the enzyme is able to replicate the primer molecules as is the case with Kornberg's DNA polymerase. Since there must be mechanisms in the cell capable of synthesizing individual ribonucleic acid molecules with a determined nucleotide sequence, it is likely that enzymes capable of performing this function are still to be discovered.

Enzymes catalyzing the addition of a few nucleotide units to a preexisting RNA chain have recently been described from various laboratories<sup>22-24</sup>. These enzymes catalyze the transfer of cytidylic and adenylic acid residues from the corresponding nucleoside triphosphates to the end of polynucleotide chains with release of pyrophosphate. There are no indications that they can bring about a net synthesis of RNA. Other investigators<sup>25,26</sup> have described the incorporation of ribonucleotides in the interior of RNA chains by particulate cell fractions from animal tissues. In a recent report<sup>26</sup> a fraction from rat liver nuclei brought about this incorporation optimally from a mixture of all four ribonucleoside triphosphates of adenosine, guanosine, uridine and cytidine, and the reaction was markedly decreased after treatment with ribonuclease suggesting requirement for an RNA primer. These experiments suggest that an enzyme similar to Kornberg's DNA polymerase might be involved.

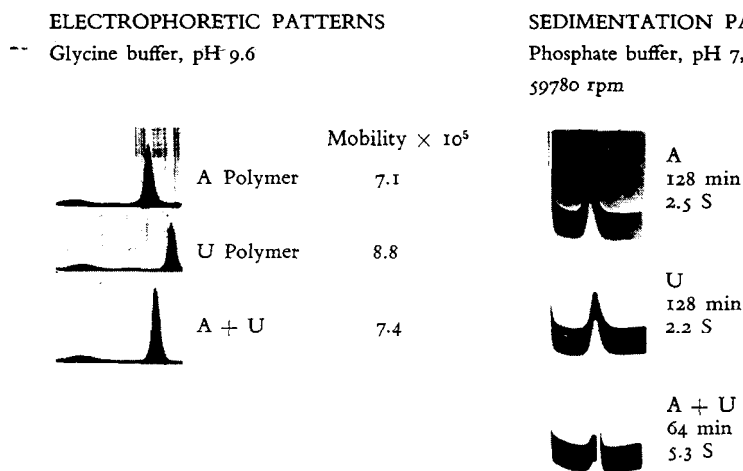


Fig. 8. Electrophoresis and sedimentation patterns of poly A, poly U and poly A + U<sup>27</sup>.

### *Polynucleotide interactions*

Physical-chemical studies on a variety of synthetic polyribonucleotides have thrown much light on their macromolecular structure and may greatly further our understanding of the biological properties of RNA and DNA. Warner, in our laboratory, found that polyadenylic and polyuridylic acids interact in solution to form a stable complex<sup>27</sup>. At suitable pH values this complex migrates on electrophoresis with a sharp single boundary of mobility intermediate between that of poly A and poly U. On ultracentrifugation, it has a higher sedimentation constant than that of the parent polynucleotides (Fig. 8). Warner further observed that formation of the complex is accompanied by a marked decrease of the absorption of ultraviolet light (Fig. 9). Formation of the complex can in fact be observed visually through the marked increase in viscosity that takes place on mixing solutions of poly A and poly U.

X-ray diffraction studies of Rich and collaborators<sup>28</sup> showed that fibers made from the poly A + U complex give rise to a crystalline pattern not unlike that of DNA<sup>29</sup>, indicating that this complex has a double-stranded helical structure. Further studies<sup>30</sup> demonstrated that the strands are held together by hydrogen bonds between the complementary pairs of bases adenine and uracil. These observations provided the first experimental dem-

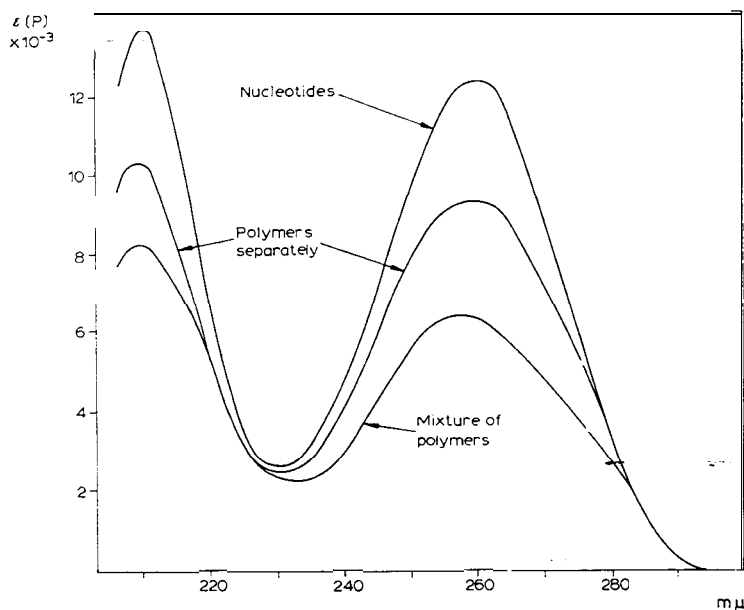


Fig. 9. Absorption spectrum of an equimolar mixture of poly A and poly U. The *upper curve* refers to the mononucleotides obtained by alkaline hydrolysis of the mixture. The *middle curve* is that calculated for the separately measured spectra of the individual polymers. The *lower curve* is the measured curve for the mixture of polymers<sup>27</sup>.

onstration that polynucleotides can interact to form double-stranded helical structures similar to that proposed by Watson and Crick for DNA and, moreover, that the same configuration may be assumed by RNA.

The decrease of ultraviolet light absorption accompanying polynucleotide complex formation has facilitated an extensive study of interactions between different polynucleotides by Rich and collaborators<sup>31-33</sup>. These investigators made the further important observation that triple-stranded helical polyribonucleotide structures can also be formed. As illustrated in Fig. 10, a complex consisting of one poly A and two poly U molecules is formed in the presence of  $Mg^{++}$ . The figure shows the optical density at 259 mμ on adding increasing amounts of a solution of poly U to one of poly A. In the absence of magnesium the absorbancy goes through a minimum when equimolecular amounts of the two polynucleotides are present; in the presence of magnesium, minimum absorbancy is reached when the solution contains 2 moles of poly U per mole of poly A. Fig. 11 illustrates the type of hydrogen bonding postulated by Rich for the poly A + U and the poly A + U + U

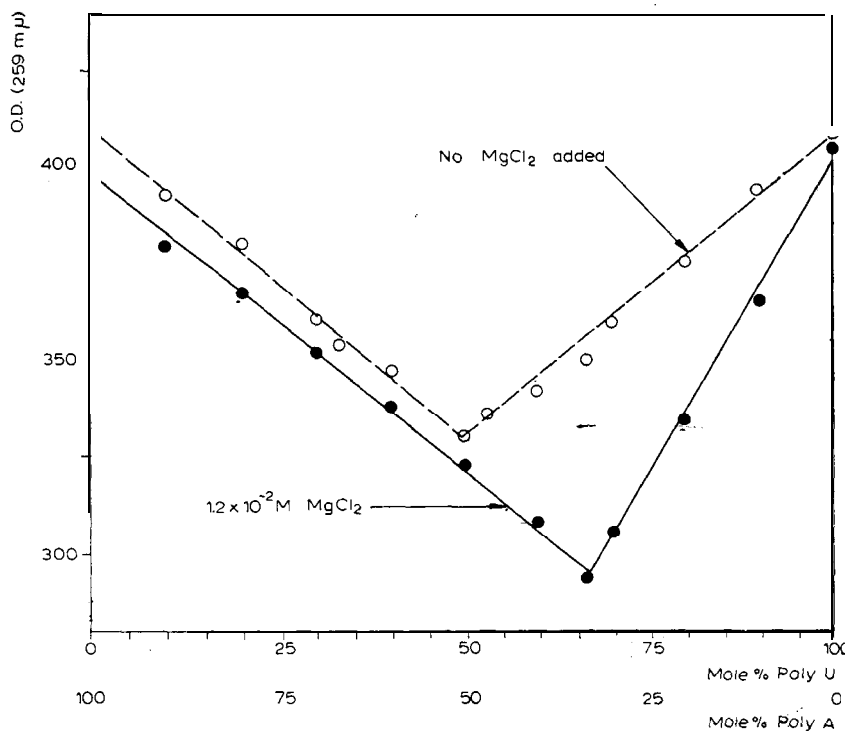


Fig. 10. Optical density ( $\times 10^3$ ) of various mixtures of poly A and poly U (From G. Felsenfeld, D. R. Davies, and A. Rich, *J. Am. Chem. Soc.*, 79 (1957) 2023.

complexes. A double-stranded helical complex between poly A and poly-ribo-thymidylic acid has more recently been obtained by Rich and collaborators. Its formation is illustrated diagrammatically in Fig. 12. I am greatly indebted to Dr. Rich for permission to use these illustrations. Double-stranded helical structures can be formed by poly-adenylic acid in solution. The elegant experiments of Doty and collaborators<sup>34</sup> have shown that, above

Table 5. Double- and triple-stranded polynucleotide complexes.

Double-stranded	Poly A + Y
	Poly A + polyribo-thymidylic acid
	Poly I + C
Triple-stranded	Poly A + A
	Poly A + U + U
	Poly A + I + I
	Poly I + I + I

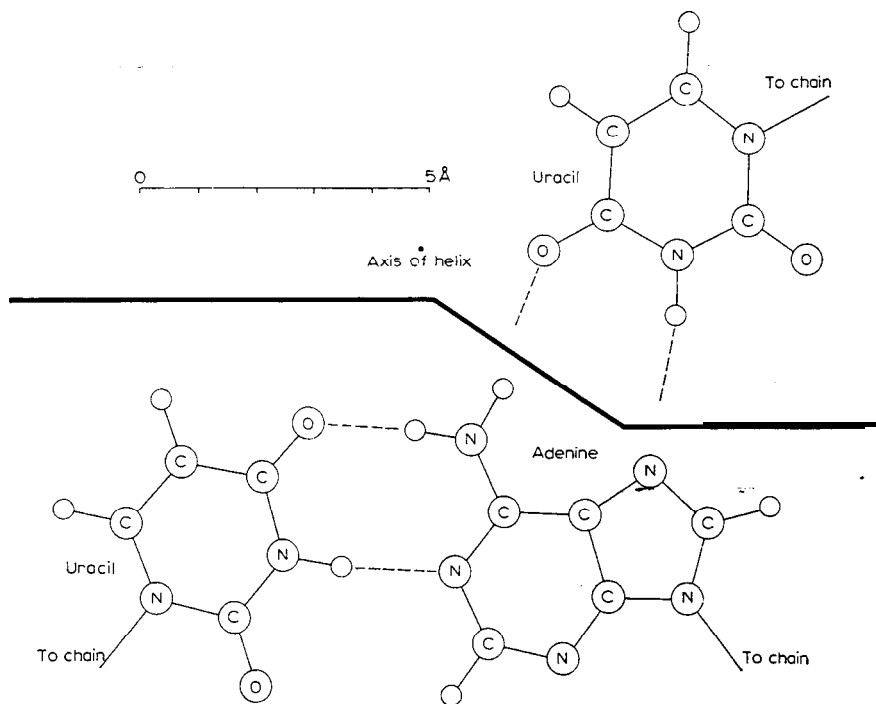


Fig.11. Hydrogen-bonding system in poly A + U and poly A + U + U. The hydrogen bonds are represented by dashed lines (Courtesy of A. Rich).

neutral pH, poly A exists in solution as a random coil but the chains are joined to form a helical complex at pH values below neutrality. This transformation is reversible and has a sharp transition point. The various types of polyribonucleotide complexes thus far obtained are listed in Table 5.

The above studies may be of importance for a better understanding of the physical-chemical interactions underlying the role of DNA in cell division. These interactions may also play a role in the biological behavior of RNA. Since there are good indications that the genetic information stored in DNA is first transmitted to RNA, it is believed that DNA may function as a template for RNA replication. Oncoming ribonucleotide residues could be linked into an organized polynucleotide chain growing in the helical groove of the native, double-stranded DNA molecule to form a triple-stranded helix. Alternatively, a single-stranded DNA template might be used to give a double-stranded DNA-RNA helix<sup>35-37</sup>.

The work of Kornberg and his collaborators<sup>38</sup> has given us deep insight



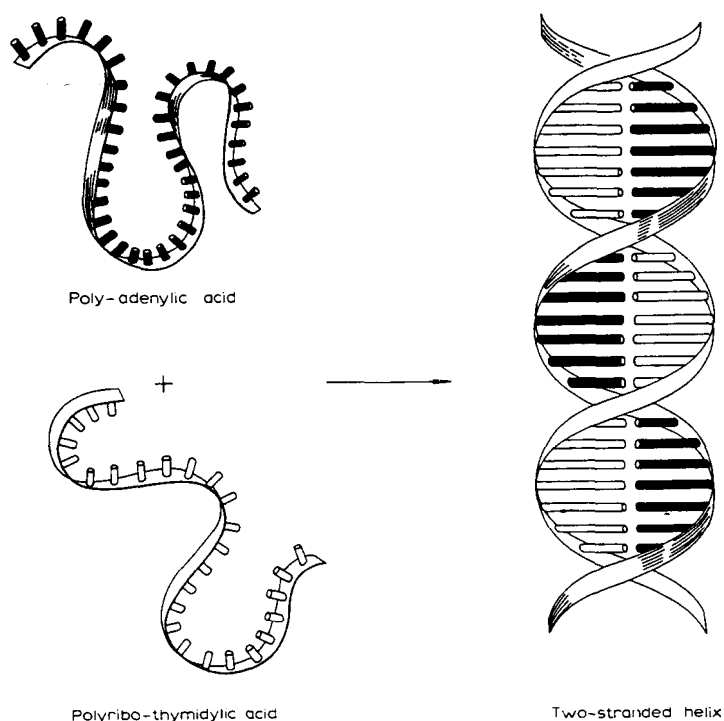


Fig. 12. Diagram showing the combination of two random coil molecules of poly-adenylic and polyribo-thymidylic acid to form a double-stranded helix. The bases are represented by short rods (Courtesy of A. Rich).

into the mode of replication of DNA and may lead in the not too distant future to the synthesis of genetic material in the test tube. Since RNA is the genetic material of some viruses, the work reviewed in this lecture may help to pave the way for the artificial synthesis of biologically active viral RNA and the synthesis of viruses. These particles are at the threshold of life and appear to hold the clue to a better understanding of some of its most fundamental principles.

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