The Enzymatic Phosphorylation of Nucleic Acids and Its Application to End-Group Analysis

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ABSTRACT   Polynucleotide kinase catalyzes the transfer of a phosphate group from ATP to the 5'-hydroxyl termini of polynucleotides. Selective labeling of the 5'-hydroxyl termini of DNA with polynucleotide kinase has been used to study the number and the identity of the 5'-terminal residues of bacteriophage DNA's, and to examine the nature of the phosphodiester bond cleavages produced by endonucleases and by sonic irradiation. The intact strands of T7 DNA bear 5'-phosphoryl end-groups; only deoxyadenylate and deoxythymidylate are present as 5'-terminal residues. The intact strands of native λ-DNA bear 5'-hydroxyl end-groups. M13 DNA, a circular molecule, cannot be phosphorylated. End-group labeling of DNA provides a method for determination of molecular weight; calibration against other DNA preparations is not required. The molecular weight of a single strand of T7 DNA, determined by end-group labeling, is 13.1×10^6; the molecular weight of a single strand of λ-DNA is 16.0×10^6. These values are in agreement with molecular weight estimates by sedimentation analysis and electron microscopy. Sonic irradiation of DNA has been shown to favor the production of polynucleotides terminated by 5'-phosphomonoester groups. All four deoxyribonucleotides are present as 5'-terminal residues of sonicated DNA.

The study of nucleic acid metabolism depends in large measure on our knowledge of the structure of DNA and RNA. In order to understand the metabolism of these macromolecules it is important to know the nature of their end-groups and the number and location of phosphodiester bond interruptions, as well as their three-dimensional structures. However, the great length of even the smallest DNA molecules isolated from natural sources has made it difficult to study these details of structure. For example, the determination of molecular weight and length by physical chemical procedures presents problems which are unique to polymers of such high molecular weight. Not only must the nucleic acid preparations be homogeneous, but the reagents

and analytical procedures must not alter the primary or secondary structure of the molecules.

The recent identification of a new enzyme, polynucleotide kinase (1, 2), has made the study of some of the problems of nucleic acid structure possible. Polynucleotide kinase, purified from extracts of *Escherichia coli* infected with
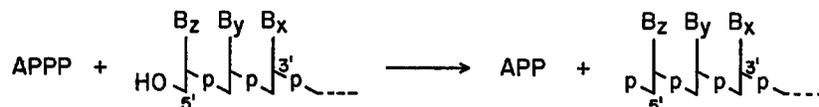

FIGURE 1.   The transfer of phosphate from ATP to the 5'-hydroxyl terminus of a polynucleotide by polynucleotide kinase.

T2 or T4 bacteriophages, catalyzes the transfer of one phosphate group from ATP to the 5'-hydroxyl terminus of a polynucleotide (Fig. 1). The products of the reaction are ADP and 5'-phosphoryl polynucleotides. This reaction results in the modification of nucleic acids at the polynucleotide level; i.e., preformed DNA and RNA molecules are phosphorylated. A rapid and sensitive assay for the reaction makes use of the acid solubility of ATP

TABLE I
REQUIREMENTS FOR KINASE ACTIVITY

Compound	Formation of acid-insoluble [32]P
	$m\mu mole$
Complete system	0.49*
Minus 5'-OH terminated DNA	<0.005
Minus Mg^{++}	<0.005
Minus 2-mercaptoethanol	0.01
Minus enzyme	<0.005

From Richardson (2). The complete incubation mixture for polynucleotide kinase contained 20 μmoles of Tris buffer (pH 7.6), 3 μmoles of $MgCl_2$, 5 μmoles of 2-mercaptoethanol, 80 mμmoles of 5'-hydroxyl–terminated salmon sperm DNA (micrococcal nuclease digest), 20 mμmoles of γ-^{32}P-ATP, and 0.5 unit of the phosphocellulose fraction of enzyme. Incubation was for 30 min at 37°C.
* Represents a turnover number of 2100 mμmoles of ^{32}P made acid-insoluble per minute per milligram protein of the phosphocellulose fraction.

and the acid insolubility of high molecular weight polynucleotides. If the terminal phosphate of ATP is labeled with ^{32}P, then any radioactivity transferred to the polynucleotide will become acid-insoluble. The sensitivity of the assay is limited only by the specific radioactivity of the ATP.

Polynucleotide kinase provides an enzymatic reagent for studying end-groups in nucleic acids. The ability of the enzyme to specifically label the 5'-hydroxyl termini of polynucleotides provides a sensitive method for de-

termining the number and the identity of the 5'-end-groups in a DNA preparation. In this paper we wish, first, to describe some of the properties of polynucleotide kinase; in particular, those properties which are important to studies concerned with end-group labeling of polynucleotides. We will then describe our recent experimental attempts to examine the occurrence and the nature of end-groups in DNA, whether they occur naturally or are
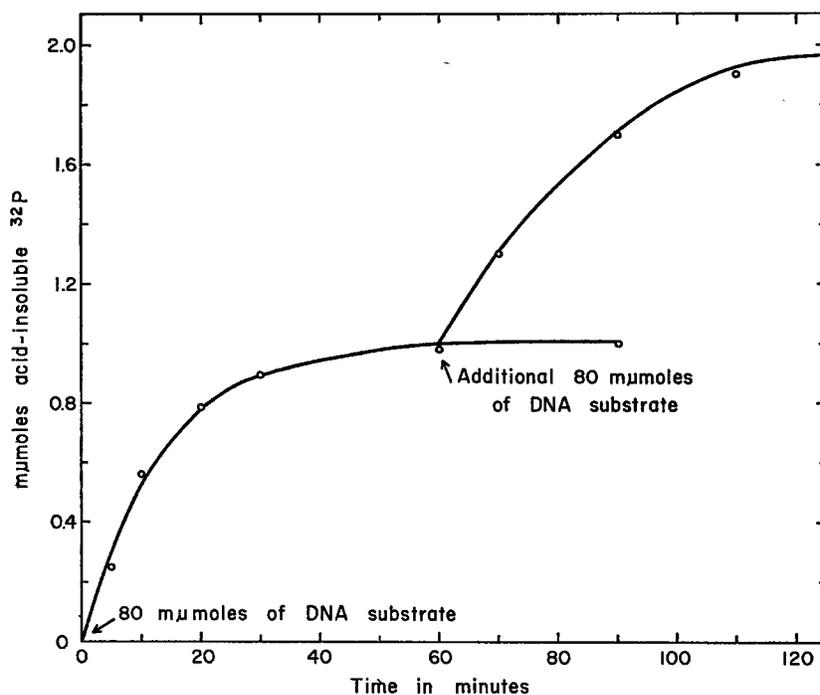

FIGURE 2. Extent of phosphorylation of 5'-hydroxyl–terminated DNA (2). Additional enzyme added at 90 and 120 min of incubation results in no further phosphorylation.

produced after isolation. Evidence will be presented to show that this method of end-group labeling can be used to determine the 5'-terminal residues and the molecular weight of native bacteriophage DNA's.

CHARACTERIZATION OF THE REACTION

Polynucleotide kinase activity can be detected in extracts of T4 phage–infected *E. coli* about 5 min after infection; a maximum level of activity is observed approximately 20 min after infection. The enzyme activity has been purified 1300-fold and is free of contaminating deoxyribonucleases, a necessary prerequisite for studies involving end-group labeling of high molecular weight DNA.

Properties of the Purified Enzyme The requirements for polynucleotide

kinase activity are shown in Table I. The reaction is dependent upon the addition of DNA or RNA containing 5'-hydroxyl end-groups. In order to increase the sensitivity of the assay, 5'-hydroxyl groups have been introduced into native DNA by incubation with micrococcal nuclease (3). This endonuclease specifically cleaves phosphodiester bonds to produce polynucleotides bearing hydroxyl groups at the 5'-termini. Optimal kinase activity is also dependent on the presence of Mg^{++} and a sulfhydryl compound.

Polynucleotide kinase can quantitatively phosphorylate the 5'-hydroxyl termini present in a nucleic acid preparation. The extent of phosphorylation can be shown to reach a limit by the addition of excess enzyme or with prolonged incubation (Fig. 2). Only when an additional amount of DNA substrate is added to the reaction mixture does further phosphorylation occur. The stoichiometry of the forward reaction has been measured and ADP, in

T A B L E I I

REQUIREMENT FOR 5'-HYDROXYL TERMINI

DNA preparation	Rate	Extent
	$m\mu moles/min/mg$	$m\mu moles$
Native T7 DNA	—	<0.001
5'-hydroxyl-terminated T7 DNA (micrococcal nuclease–treated)	2200	1.4
5'-phosphoryl-terminated T7 DNA (endonuclease I–treated)	—	<0.01
Phosphatase-treated 5'-phosphoryl-terminated T7 DNA	1600	1.1

Each DNA preparation (100 mμmoles of DNA-P) was tested in the standard assay. The extents of phosphorylation were determined in 1 hr incubations with the addition of 30 units of enzyme at 0 and 30 min of incubation. The small extents of incorporation of ^{32}P obtained with native T7 DNA and 5'-phosphoryl-terminated T7 DNA prevented a determination of the rates of phosphorylation of these compounds.

addition to 5'-phosphoryl polynucleotides, has been identified as a product. ADP and 5'-phosphoryl polynucleotides are formed in equal quantities and in amounts equal to the disappearance of ATP from the reaction mixture.

Specificity of Polynucleotide Kinase A preparation of native T7 bacteriophage DNA is phosphorylated to only a small extent relative to the same DNA after partial hydrolysis by micrococcal nuclease. As shown in Table II, hydrolysis of native T7 DNA with micrococcal nuclease to produce 5'-hydroxyl end-groups increases its capacity to accept phosphate at least 1000-fold. In contrast, hydrolysis of T7 DNA by *E. coli* endonuclease I (4) to produce 5'-phosphoryl end-groups does not increase its capacity to accept phosphate in the kinase reaction. However, if the 5'-phosphoryl DNA is treated with phosphatase, dephosphorylating the DNA and exposing 5'-hydroxyl groups, it becomes a substrate for polynucleotide kinase. Thus, a phosphate group at the 5'-terminus of a polynucleotide effectively prevents phosphorylation in the kinase reaction.

In addition to polydeoxyribonucleotides, the purified enzyme is able to phosphorylate a variety of other nucleic acid compounds. The rates of phosphorylation of several compounds are given in Table III. Heat-denatured DNA, sRNA, dinucleotides, and 3'-AMP are all phosphorylated, while nucleosides and nucleoside 2'-monophosphates are not. The product formed when 3'-AMP is used as a substrate has been identified as the nucleoside 3', 5'-diphosphate. Although not shown in Table III, the compounds which can serve as substrates are quantitatively phosphorylated with prolonged incubation or with an excess of enzyme.

TABLE III

SPECIFICITY OF POLYNUCLEOTIDE KINASE

Compound	Rate
	$m\mu moles/min/mg$
1. 5'-hydroxyl-terminated (micrococcal nuclease-treated)	
T7 DNA	2200
E. coli DNA	2000
Salmon sperm DNA	2200
Salmon sperm DNA (denatured)	1100
2. 5'-hydroxyl-terminated RNA	900
3. Dinucleotides	
T_pT	3600
A_pA	3000
C_pC	3200
4. 3'-AMP	2200
2'-AMP	<50
5. Adenosine, cytidine	<10

Each compound was tested in the standard assay. Polynucleotides were present in the incubation mixtures at a concentration of 0.004 mM (polymer concentration); other compounds were present at a concentration of 0.06 mM. The concentrations of polynucleotides may not represent concentrations which give maximum rates.

Location of ^{32}P *at the 5'-Terminus of the Polynucleotide* The acid-insoluble ^{32}P formed during the kinase reaction has been identified at the 5'-terminus of the polynucleotide by several different techniques. First, treatment of the ^{32}P-labeled kinase product with alkaline phosphatase from *E. coli* results in the release of more than 95% of the radioactivity as P_i, demonstrating that the polynucleotide has been terminally labeled. If the polynucleotide is hydrolyzed to its constituent nucleoside 5'-monophosphates by the action of snake venom phosphodiesterase, all the radioactivity is present in the nucleoside 5'-monophosphates. These results, considered together, are compatible only with the ^{32}P being present at the 5'-terminus of the polynucleotide.

The location of the ^{32}P on the product can be confirmed by utilizing a specific and unique property of *E. coli* exonuclease I, its *inability* to hydrolyze the phosphodiester bond of a dinucleotide (5). Exonuclease I, as schematically

illustrated in Fig. 3, initiates a stepwise hydrolysis from the 3'-hydroxyl terminus of a single-stranded DNA molecule, liberating 5'-mononucleotides and leaving the terminal 5'-dinucleotide. When 5'-^{32}P-phosphoryl DNA (kinase product) was digested with exonuclease I and the dinucleotides separated from the mononucleotides by chromatography on DEAE-cellulose, more than 95% of the radioactivity was present in the dinucleotide fraction.

The purified kinase is capable of phosphorylating all four of the nucleotides found in DNA, provided that their 5'-hydroxyl groups are terminally located. When a native DNA is fragmented by sonic irradiation or by hydrolysis with E. coli endonuclease I, an endonuclease having little base specificity (4),
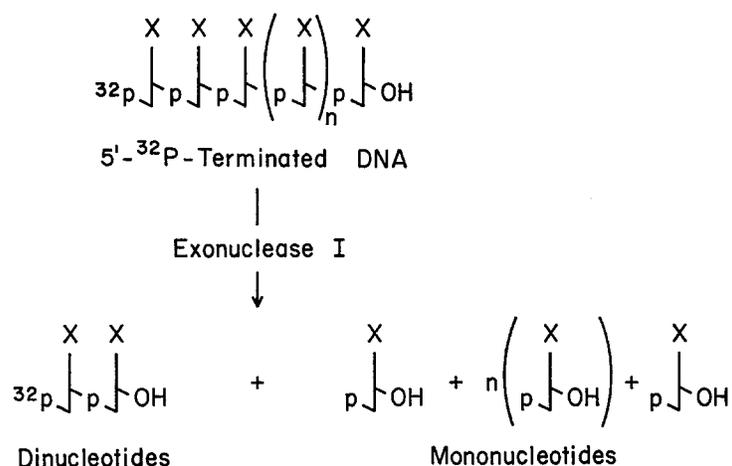

FIGURE 3.   Procedure for the identification of ^{32}P in the 5'-terminal dinucleotide of the polynucleotide kinase product. Exonuclease I initiates a stepwise hydrolysis from the 3'-hydroxyl terminus of the 5'-^{32}P-phosphoryl DNA, liberating 5'-mononucleotides and the terminal 5'-dinucleotide containing ^{32}P.

the newly formed terminal residues are phosphorylated by the kinase once the 5'-phosphoryl groups initially present have been removed. As shown in Table IV, all four of the nucleotides found in E. coli or T7 DNA are phosphorylated in the kinase reaction.

STUDIES ON PHOSPHODIESTER BOND CLEAVAGES

The specificity of polynucleotide kinase makes it a suitable reagent for studying the nature of strand interruptions in DNA produced by enzymatic or nonenzymatic cleavage. Not only can the number of 5'-hydroxyl end-groups be determined, but the identity of the terminal nucleotides can be established as well. Furthermore, the extent of phosphorylation of polynucleotides before and after phosphatase treatment permits a determination of the number of both 5'-phosphoryl and 5'-hydroxyl end-groups.

Enzymatic Hydrolysis of DNA As discussed above, polynucleotide kinase can be used to establish whether an endonucleolytic cleavage of a phosphodiester bond yields 5'-hydroxyl or 5'-phosphoryl terminated polynucleotides. Thus, micrococcal nuclease produces oligonucleotides which are phosphate acceptors while polynucleotides produced by endonuclease I are unable to function as phosphate acceptors in the kinase reaction (Table II).

End-group labeling provides a method for identifying the terminal 5'-nucleotides of the oligonucleotides produced by endonucleolytic cleavage. Endonuclease I for instance produced polynucleotides at whose 5'-termini all four nucleotides could be identified (Table IV). The technique was also used to characterize the polynucleotides formed when DNA was partially

TABLE IV

IDENTIFICATION OF THE ^{32}P-5'-TERMINAL NUCLEOTIDES
OF PHOSPHORYLATED DNA

Nucleotides	Treatment prior to phosphorylation		
	Sonication	E. coli endonuclease	Micrococcal nuclease
	% of total		
dAMP	28	14	44
dTMP	28	38	54
dGMP	22	17	<2
dCMP	22	31	<2

E. coli DNA was partially digested with *E. coli* endonuclease I (and dephosphorylated with alkaline phosphatase) or with micrococcal nuclease. T7 DNA was reduced to a molecular weight of 4×10^5 and dephosphorylated with phosphatase. After phosphorylation in the kinase reaction with ^{32}P-ATP, the 5'-^{32}P-phosphoryl DNA's were hydrolyzed to mononucleotides by the action of pancreatic DNase and snake venom phosphodiesterase. The products were chromatographed and the per cent of the total radioactivity found in each of the four mononucleotides is given above.

degraded with micrococcal nuclease. This enzyme has been previously shown to yield only deoxyadenylate and deoxythymidylate as 5'-terminal nucleotides (6); only these two nucleotides could be identified as 5'-residues by end-group labeling in the kinase reaction (Table IV).

Sonic Irradiation of DNA Sonic irradiation reduces the molecular weight of native DNA. Richards and Boyer have shown that the degradation of DNA by sonic irradiation occurs almost exclusively by phosphodiester bond rupture and not by carbon-carbon bond rupture (7). The extent of phosphorylation of sonicated T7 DNA before and after phosphatase treatment permits a determination of the relative number of 5'-hydroxyl and 5'-phosphoryl end-groups in the DNA preparation. The results obtained when T7 DNA was sonicated and examined in this manner are summarized in Table V; sonication preferentially gives rise to 5'-phosphoryl-terminated polynucleotides

relative to 5′-hydroxyl-terminated polynucleotides, in a ratio of approximately 16 to 1. This result is consistent with the known relative stability of phosphate esters of primary alcohols as compared to secondary alcohols.

When T7 DNA was reduced to a molecular weight of 4×10^5 by sonication, treated with phosphatase, and then phosphorylated in the kinase reaction, all four nucleotides of the $5′-^{32}P$-phosphoryl sonicated DNA were found to contain radioactive material (Table IV). It appears that sonic irradiation has little, if any, base specificity with regard to sites of cleavage.

END-GROUP LABELING OF BACTERIOPHAGE DNA'S

Homogeneous preparations of DNA can be isolated from a variety of bacteriophages when precautions are taken to eliminate breakage by endo-

TABLE V

NATURE OF THE PHOSPHODIESTER BOND CLEAVAGE
PRODUCED BY SONIC IRRADIATION OF DNA

Experiment	Molecular weight (single strand)	End-groups	
		5′-P	5′-OH
1	2×10^5	0.95	0.05
2	4×10^5	0.94	0.06

From Richardson (8). T7 DNA was reduced to the molecular weights shown above by sonic irradiation. The extent of phosphorylation of the sonicated DNA before and after phosphatase treatment was used to calculate the relative number of 5′-phosphoryl (5′-P) and 5′-hydroxyl (5′-OH) end-groups.

nuclease and mechanical shearing. Selective labeling of the 5′-hydroxyl termini of DNA with polynucleotide kinase has been used to study the 5′-terminal residues of DNA's isolated from T7, λ, and M13 coliphages. In the studies to be discussed end-group labeling has been used to determine (a) the presence of 5′-hydroxyl groups in the native DNA preparations, (b) the number of nucleotides in the intact single strands of T7 and λ-DNA, and (c) the identity of the 5′-terminal nucleotides of T7 DNA.

(a) Studies with T7 DNA (8)

The DNA of a T7 bacteriophage is believed to be a single molecule (9), and monodisperse preparations of this DNA can be obtained (10–13). Subsequent studies have revealed that the two strands of this DNA, after denaturing treatments, disassociate and separate from one another (12, 14, 15). As a result of studies involving sedimentation analysis, it was found that the single strands of T7 DNA are polydisperse; from 20 to 50% of the denatured DNA sediments at a slower rate than do the intact single strands. Although the origin and the significance of the single-strand interruptions in this and other

DNA preparations are not known, T7 DNA preparations contain sufficient intact single strands for end-group analysis.

A PHOPHATASE-SENSITIVE GROUP AT THE 5'-TERMINI OF T7 DNA When T7 DNA was incubated with the purified kinase and ^{32}P-ATP of high specific radioactivity, a small amount of ^{32}P was transferred to the acid-precipitable DNA. With excess enzyme and prolonged incubation, the acid-insoluble ^{32}P reached a limit. However, the ^{32}P transferred to T7 DNA was only 5 to 20% of the value expected if each strand of T7 DNA were unbroken and bore a free hydroxyl group at its 5'-terminus (calculated from a molecular weight of 13.2×10^6 for a single strand of T7 DNA, 12). If the native DNA was incubated with purified alkaline phosphatase from *E. coli* prior to incubation
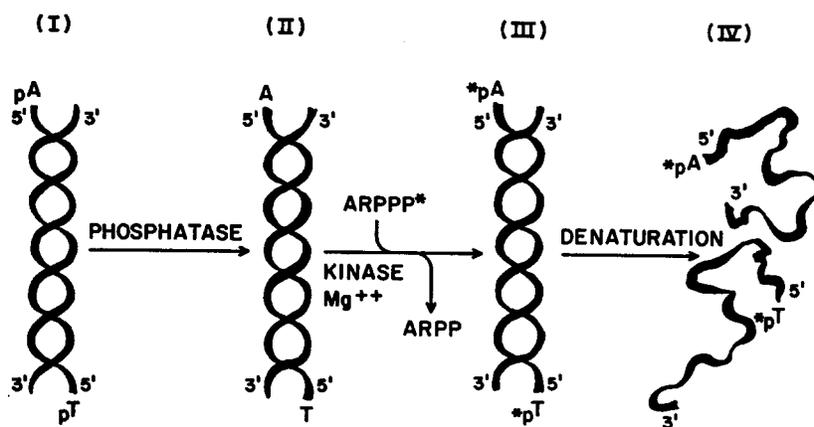

FIGURE 4. Summary scheme for determination of the 5'-termini of T7 DNA (8).

in the kinase reaction, the extent of phosphorylation increased 20-fold. The extent of phosphorylation after phosphatase treatment of the DNA was always 10 to 40% greater than would be expected for unbroken strands of T7 DNA. This value, as will be shown, reflects the phosphorylation of fragments of DNA as well as of intact strands.

It was previously shown that polynucleotides bearing 5'-phosphoryl end-groups will not serve as substrates in the kinase reaction (Table II). The failure of native T7 DNA to be phosphorylated in the polynucleotide kinase reaction is most easily explained by the presence of phosphoryl groups esterified to the 5'-hydroxyl groups of T7 DNA. The increase in the extent of phosphorylation after phosphatase treatment strongly supports this postulate (see Fig. 4).

ANALYSIS OF PHOSPHORYLATED DNA BY PREPARATIVE ZONE SEDIMENTATION
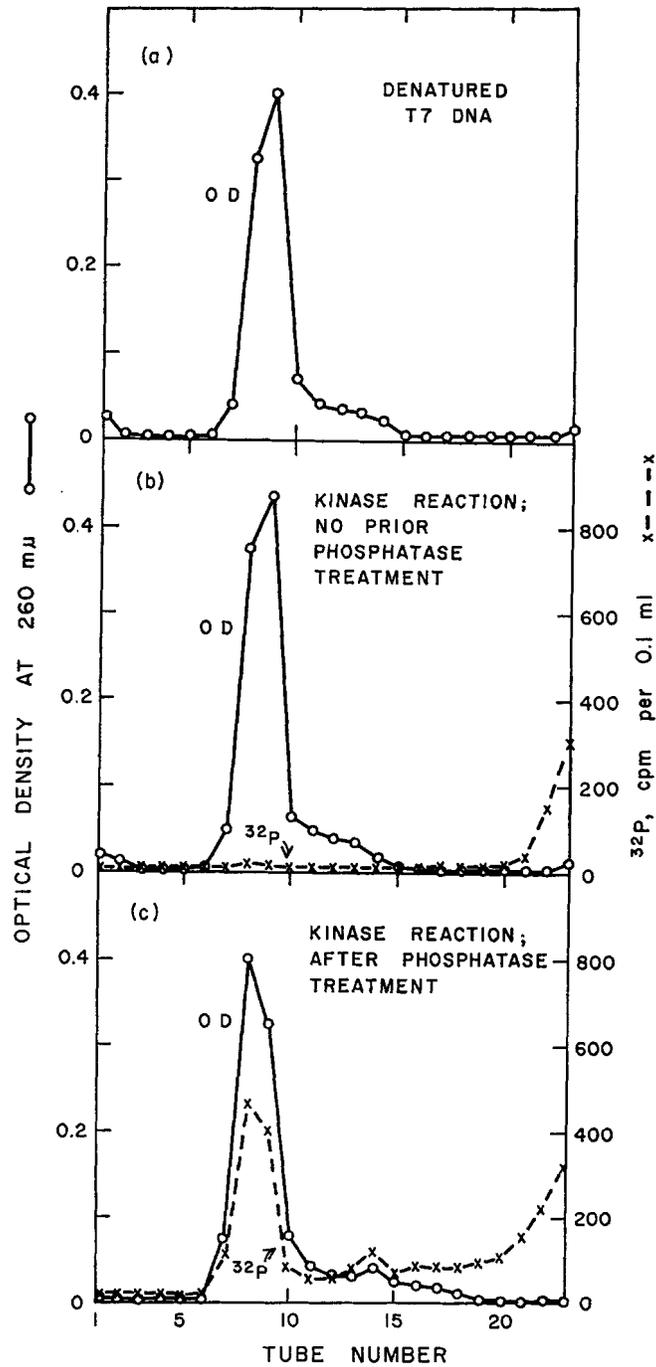In order to identify those molecules which had been phosphorylated in the

FIGURE 5. Analysis of phosphorylated T7 DNA by preparative zone sedimentation (8).

kinase reaction, the 5'-^{32}P-phosphoryl T7 DNA (kinase product) was denatured with alkali and subjected to zone sedimentation in a sucrose gradient. With this technique it is possible to identify molecules containing either double or single strand interruptions as the material sedimenting more slowly than the main boundary of intact single-stranded molecules. When DNA, prior to any enzymatic treatment, was denatured and analyzed by this procedure, the major component of the DNA showed a narrow distribution; only about 20% appeared as trailing material (Fig. 5 *a*).

Fig. 5 *b* shows the results of an experiment in which T7 DNA, isolated from the kinase reaction mixture, and not previously treated with phosphatase, was denatured and analyzed by preparative zone sedimentation. First, it should be noted that there was no significant increase in the amount of material of lower molecular weight, indicating that relatively few endonucleolytic scissions occurred during the incubation with kinase. Second, there was no detectable ^{32}P present in the major component of unbroken molecules; all the radioactivity was found associated with material of lower molecular weight, presumably polynucleotides of relatively short length.

It was noted previously that, following the phosphatase treatment of T7 DNA, there was a 10- to 20-fold increase in the extent of phosphorylation by polynucleotide kinase. The 5'-^{32}P-phosphoryl T7 DNA isolated from such a reaction mixture was also denatured and examined by preparative zone sedimentation. A peak of radioactivity was found which coincided with the peak of intact single strands (Fig. 5 *c*). Furthermore, the ratio of ^{32}P material with OD_{260} was constant in these fractions.

Identical results were obtained when each of the three samples of DNA shown in Fig. 5 was analyzed by preparative zone sedimentation in alkali, where renaturation could not occur. These findings confirm that groups sensitive to phophatase are present at the 5'-termini of native T7 DNA. However, it should be emphasized that this finding does not distinguish between either one or several phosphoryl groups esterified to the 5'-hydroxyl group of a strand of DNA. For example, RNA synthesized in vitro by the DNA-dependent RNA polymerase has been shown to be terminated by a nucleoside triphosphate (16). Whether such end-groups occur naturally is not known.

THE NUMBER OF NUCLEOTIDE RESIDUES IN A SINGLE STRAND OF T7 DNA   In several experiments, the ratio of ^{32}P to material with OD_{260} in the major component of DNA isolated by preparative zone sedimentation remained constant. This enabled a determination of the number of nucleotides in an intact strand of T7 DNA. The 5'-^{32}P-phosphoryl single strands of T7 DNA were isolated by preparative zone sedimentation, and the number of moles of ^{32}P and total phosphorus determined. As shown in Table VI, an average of one mole of ^{32}P was found per 4.0×10^4 moles of nucleotide phosphorus

in a series of five experiments. A strand of DNA containing this number of nucleotide residues would be expected to have a molecular weight of 13.1 × 10⁶ (sodium salt). This value is in agreement with that of 13.2 × 10⁶ calculated by Studier from the sedimentation coefficient of denatured T7 DNA (12). Burgi and Hershey estimated the molecular weight of native T7 DNA at 27 × 10⁶ from published S values (11). Thomas has calculated a molecular weight of 24.5 × 10⁶ from the measured length of T7 DNA (13).

IDENTIFICATION OF THE 5′-TERMINAL NUCLEOTIDES OF T7 DNA   The agreement between the molecular weight determined by end-group labeling and

TABLE VI

DETERMINATION OF THE MOLECULAR
WEIGHT OF THE SINGLE STRANDS OF T7 AND
λ-DNA BY END-GROUP LABELING

DNA	Nucleotides/strand × 10⁻⁴	Molecular weight (sodium salt) × 10⁻⁶	
		End-group labeling	Sedimentation analysis*
T7	4.0	13.1	13.2
λ	4.8	16.0	16.3

* From Studier (12).
Either native T7 or λ-DNA was incubated with alkaline phosphatase; the phosphatase was removed by phenol extraction, and the DNA was incubated with ³²P-ATP in the kinase reaction. After removal of unreacted ATP, the 5′-³²P-phosphoryl DNA was denatured and subjected to preparative zone sedimentation (see Figs. 4 and 5). The moles of ³²P and of phosphorus present in the major fractions of intact single strands were determined. The moles of nucleotide per strand represent the moles of nucleotide per mole of ³²P in the sample. The molecular weight of a single strand of DNA was calculated from the average molecular weight of a nucleotide residue in DNA (331 for sodium salt).

that found by sedimentation analysis provided additional evidence that all the 5′-termini of intact strands of T7 DNA had been labeled. It was possible therefore, to determine whether a specific nucleotide terminated each strand of the double-stranded molecule. T7 DNA strands, labeled at their 5′-termini with ³²P were isolated from the sucrose gradient and hydrolyzed to their constituent nucleoside 5′-monophosphates. Only the nucleotides present at the 5′-termini of the DNA strands would be expected to contain radioactive label. Chromatographic and electrophoretic analysis revealed that after complete hydrolysis 90% of the radioactivity was nearly equally divided between deoxyadenylate and deoxythymidylate (Table VII). This result could have been obtained if both strands of the helical DNA contained a random distribution of the two nucleotides at their 5′-termini. If the DNA molecules of T7 phages are homogeneous, however, then one strand must

always be terminated at the 5'-terminus by deoxyadenylate, and the complementary strand, of opposite polarity, by deoxythymidylate (see Fig. 4).

(b) Studies with λ-DNA[1]

The second DNA to which we have applied the technique of end-group labeling is phage λ-DNA, which, like T7 DNA, is a linear duplex molecule; the majority of its polynucleotide chains are continuous over the entire length of the molecule (11–13, 15).

PRESENCE OF FREE 5'-HYDROXYL GROUPS   In contrast to T7 DNA, λ-DNA could be phosphorylated in the kinase reaction without prior phosphatase treatment to approximately the extent expected from its estimated

TABLE VII

IDENTIFICATION OF THE 5'-TERMINAL
NUCLEOTIDES OF T7 DNA

Nucleotides	Per cent of total
dAMP	52
dTMP	42
dGMP	3
dCMP	3

From Richardson (8).

The intact single strands of T7 DNA, bearing ^{32}P at their 5'-termini, were isolated (see Fig. 5) and hydrolyzed to yield nucleoside 5'-monophosphates. The nucleotides were separated by both electrophoresis and chromatography; the radioactivity in each of the four deoxyribonucleotides was measured and is recorded as a percentage of the total.

molecular weight (16.3 × 10^6 for a single strand of λ-DNA, 12). Furthermore, the extent of phosphorylation was not significantly influenced by prior incubation of the DNA with phosphatase. Preparative zone sedimentation of the denatured 5'-^{32}P-phosphoryl λ-DNA confirmed that the intact strands of this DNA had been phosphorylated (Fig. 6). The ratio of ^{32}P to material with OD_{260} in the major component of unbroken strands was not altered by prior phosphatase treatment of the DNA. From this it can be concluded that the strands of DNA, isolated from phage λ, bear free 5'-hydroxyl groups. At present it is not known why the strands of λ-DNA bear free 5'-hydroxyl groups while those of T7 DNA bear phosphoryl groups. One explanation is that, due to differences in cell metabolism or in the procedure of λ-phage growth and isolation, the λ-DNA was exposed to phosphatase prior to isolation.[1] Another possibility is that hydrolysis of λ-DNA by a specific nuclease introduces 5'-hydroxyl end-groups.

[1] The DNA used in these experiments was isolated from λc$_{26}$ bacteriophage, a c_1 mutant ot λ obtained from Dr. M. Meselson.
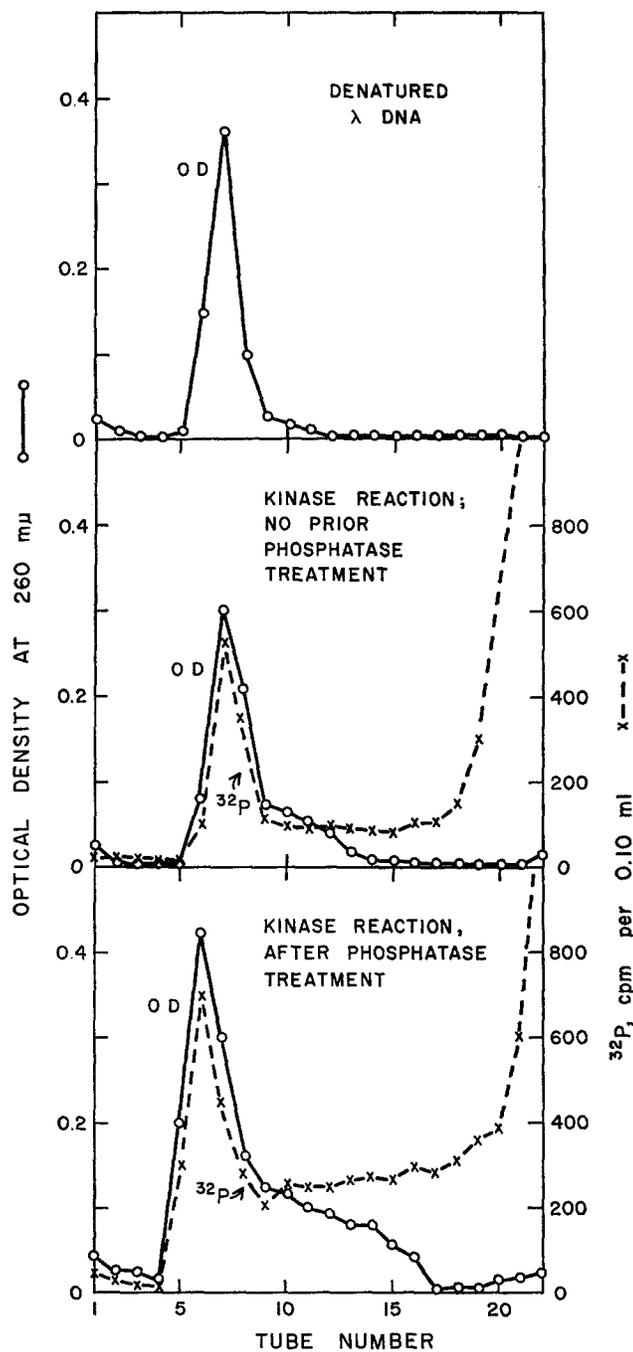
FIGURE 6. Analysis of phosphorylated λc-DNA by preparative zone sedimentation. The enzymatic incubations, procedure for isolation of DNA from the incubation mixtures, and sedimentation analysis are those previously described for T7 DNA (8).
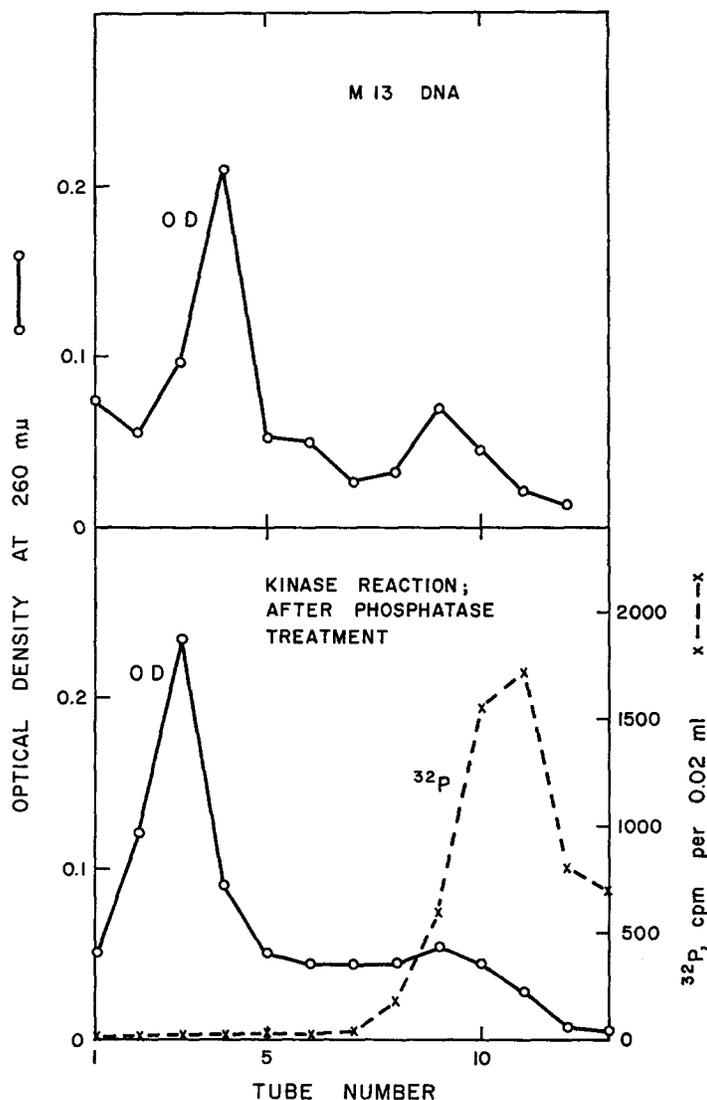
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FIGURE 7. Analysis of M13 DNA by preparative zone sedimentation. The enzymatic incubations and isolation procedures for the phosphorylated DNA are those previously described for T7 DNA (8). Zone sedimentation was carried out in linear concentration gradients of 5 to 20% (w/v) sucrose in 0.1 M NaOH, 0.9 M NaCl, 0.001 M EDTA. Centrifugation was for 7 hr at 38,000 RPM at 4° in the swinging bucket rotor SW 39 of the Spinco model L centrifuge.

THE NUMBER OF NUCLEOTIDE RESIDUES IN A SINGLE STRAND OF λ-DNA   The number of nucleotides in a single strand of λ-DNA can also be determined by the procedure described for T7 DNA. With either preparation of phosphorylated DNA (untreated or phosphatase-treated) an average of one mole of ^{32}P was found per 4.8 × 10^4 moles of nucleotide in the isolated intact single

strands; a strand of DNA of this length would have a molecular weight of 16.0 × 10⁶ (Table V). Studier, from sedimentation analysis, has calculated a molecular weight of 16.3 × 10⁶ for a single strand of λ-DNA (12). Burgi and Hershey estimated the molecular weight of native λ-DNA to be 30 to 34 × 10⁶ from their calibration of S against molecular weight (11). Thomas has calculated a molecular weight of 33.7 × 10⁶ from the measured length of λc++ DNA (13).

(c) Studies with M13 DNA

M13 coliphages (17) contain DNA molecules which consist of circular single-stranded polynucleotide chains (18, 19). The low molecular weight (approximately 2 × 10⁶) of M13 DNA makes it possible to detect easily the presence of 5'-hydroxyl termini in a preparation by end-group labeling. Incubation of M13 DNA in the kinase reaction, before or after phosphatase treatment, did not result in any detectable phosphorylation of the intact molecules (Fig. 7). Apparently, M13 DNA does not have 5'-termini available for phosphorylation. This finding is consistent with the proposed circular structure of M13 DNA; all 5'-hydroxyl groups would be present in phosphodiester linkage.

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Discussion

Dr. Harvey Cohen: Do you get the same quantitative results using RNA as you do with DNA?

Dr. Richardson: We have carried out a few preliminary experiments with TMV RNA and R17 bacteriophage RNA. With either preparation the molecular weight determined by end-group labeling did not agree with the molecular weight calculated by other methods. This could be due to the presence of ribonuclease activity in the enzyme preparation. We are now attempting to purify the enzyme further.