*ON THE PARTICIPATION OF DNA IN RNA BIOSYNTHESIS**

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We have recently described an enzyme system in rat liver nuclei requiring four ribonucleoside triphosphates for the incorporation of ribonucleotides into RNA.[1] Further work with this system showed that the incorporation of ribonucleotides occurred throughout the entire polynucleotide molecule and that this activity disappeared after treatment of the mammalian particles with DNase.[2] Subsequent experiments with DNase-treated rat liver preparations indicated that a partial reactivation of the ribonucleotide-incorporating activity could be achieved with added DNA. In agreement with these observations, Stevens[3] and Hurwitz *et al.*[4] demonstrated the existence of a similar system in extracts from *E. coli*. In addition to requiring the presence of all four ribonucleoside triphosphates in the bacterial system, Hurwitz *et al.* first reported that the addition of DNA was also necessary.

More recently, Weiss and Nakamoto[5] reported the isolation of a similar enzyme system from extracts of the microorganism *M. lysodeikticus* and, using this preparation, demonstrated net synthesis of RNA. In order to obtain some insight into the role played by DNA in this reaction, nearest neighbor sequence analysis and base composition of the newly synthesized RNA were determined. Studies of this type were conducted for a number of different "primer" DNAs and are reported herein.

Using the partially purified *M. lysodeikticus* enzyme, RNA was isolated from the reaction mixture at different times of incubation. Calf thymus or rat liver DNA served as "primer" for this experiment. Analysis of the RNA so prepared suggested that polynucleotides with a similar statistical arrangement of bases were formed at these different reaction times (Table 1). In consequence, it was felt that the nearest neighbor sequence analysis and base composition studies of the RNA synthesized *in vitro* might be meaningful.

Although little information concerning the sequential arrangement of bases in nucleic acid is available, it is probable that the arrangement of deoxynucleotides in DNA differs in DNAs isolated from different sources. If the sequence of ribonucleotides in RNA is influenced by the DNA required for this reaction, then this influence should be reflected in the position of any one ribonucleotide in the RNA chain when various DNA "primers" are used. This relative position may be conveniently determined by preparing RNA enzymatically with one P^{32}-nucleotide, and hydrolyzing the P^{32}-RNA formed with alkali. Since the labeled phosphate is transferred to the adjacent nucleotide under alkaline hydrolysis, one may then examine the relative frequency with which the labeled nucleotide occurs next to a given neighbor.

Examination of the results obtained when calf thymus, rat liver, and different bacterial DNAs serve as "primers" in separate reactions suggests that the relative position of labeled cytidylate in the newly formed RNA is different for the various

"primers" used (Tables 1 and 2). Loss of the terminal nucleotide residue after hydrolysis, contamination of the enzyme or DNA with RNA, or the addition of carrier RNA should have no influence on the results obtained. A complete nearest neighbor study of this type for each labeled ribonucleotide, using a DNA which has

TABLE 1

RATIO OF LABEL IN ALKALINE HYDROLYZED PRODUCTS FROM CMP^{32}-RNA FORMED AT DIFFERENT INCUBATION TIMES

DNA Source	Reaction Time (Minutes)	Total Hydrolyzable Counts	Count Ratio of Isolated Nucleotides* 2′(3′)CMP	2′(3′)AMP	2′(3′)GMP	2′(3′)UMP
Calf thymus	4	120,000	1.00	1.15	1.01	1.44
Calf thymus	15	218,000	1.00	1.13	0.99	1.51
Rat liver	4	47,400	1.00	1.19	0.80	1.30
Rat liver	15	92,000	1.00	1.13	0.78	1.30

The reaction system contained 9 μmoles of $MnCl_2$, 300 μmoles phosphate buffer of pH 7.5, 0.18 μmoles of CTP^{32} (3×10^7 c.p.m. per micromole), 2.4 μmoles each of ATP, UTP and GTP, 300 μgrams of calf thymus or rat liver DNA and 1.0 mg of *M. lysodeikticus* enzyme. The final volume was 3.0 ml and the vessels were incubated at 25° for the time periods indicated. The reaction was stopped by the addition of 0.60 ml of 3 *N* $HClO_4$ with 4 mg of yeast RNA added as carrier. The precipitate was collected by centrifugation, washed four times by solution in cold 0.10 *N* NaOH and reprecipitation with 0.50 *N* $HClO_4$. The washed residue was hydrolyzed in 0.2 *N* KOH for 18 hours at 37°, acidified with $HClO_4$ to below pH 1 and the acid-soluble fraction saved after centrifugation. This fraction was neutralized and subjected to paper electrophoresis as described previously.[2] The separated nucleotides were assayed for radioactivity. A minimum of 90 per cent of the radioactive material placed on paper was recovered in the nucleotides eluted.

* The total radioactivity found for each isolated mononucleotide was divided by the total counts found for 2′(3′)-cytidylic acid and are the value listed above. Hence, a values of 1.00 indicates a total number of counts equivalent to that found for 2′(3′)-cytidylic acid for that particular experiment.

TABLE 2

RATIO OF LABEL IN ALKALINE HYDROLYZED PRODUCTS FROM CMP^{32}-RNA PREPARED WITH VARIOUS DNAS

DNA Used in Reaction	Count Ratio of Isolated Nucleotides* 2′(3′)CMP	2′(3′)AMP	2′(3′)GMP	2′(3′)UMP
Pseudomonas	1.00	0.58	0.89	0.68
Serratia	1.00	0.66	1.55	0.80
E. coli	1.00	0.81	0.82	0.89

* The reaction system and the procedure used in these experiments were the same as described in Table 1 except that *Pseudomonas*, *Serratia*, and *E. coli* DNA were used.

been similarly characterized,[6] should give more detailed information as to the role played by DNA in RNA synthesis.

Further information on the participation of DNA in the synthesis of RNA was obtained by determining the base composition of the newly formed ribopolynucleotide. In a series of separate reactions using the microbial enzyme, RNA was prepared *in vitro* when a number of different purified microbial and salmon DNA preparations were present as "primers." The nucleic acid fraction isolated from the reaction mixture was hydrolyzed with alkali, and the products of hydrolysis were separated by paper electrophoresis. No carrier RNA was added at any time. In each of the experiments conducted, a net amount of ribonucleotide material corresponding to about 20 optical density units at 260 mμ was released into the acid-soluble fraction after alkaline hydrolysis. Control incubation vessels in which one nucleotide was omitted during the reaction, but re-added after the reaction had been stopped, gave only 10 per cent of the ultraviolet material obtained with the complete system. The 2′(3′)-nucleoside monophosphates, located on the paper under ultraviolet light after electrophoresis, were eluted and their concentrations determined in a Zeiss spectrophotometer at their respective absorption maxima. The results of these experiments are given in Table 3.

Examination of the base composition of the RNA formed shows a remarkable

TABLE 3

BASE COMPOSITION OF SYNTHESIZED RNA AS COMPARED TO PRIMER DNA
(BASE PROPORTIONS, MOLES PER CENT†)

Base*	*Pseudomonas* DNA	Synthesized RNA Expt. 1	Synthesized RNA Expt. 2	Salmon DNA	Synthesized RNA	*Serratia* DNA	Synthesized RNA	*E. coli* DNA	Synthesized RNA
G	33.0	28.2	35.0	20.8	21.0	29.0	32.6	24.1	23.4
A	18.2	18.3	16.5	29.7	29.0	21.1	18.6	25.4	23.4
C	30.0	32.8	29.5	20.4	23.0	29.0	28.0	25.7	24.8
T(U)	18.8	20.7	19.0	29.1	27.0	20.9	20.8	24.8	28.4
GC	63.0	61.0	64.5	41.2	44.0	58.0	60.6	49.8	48.2
$\frac{A + T(U)}{G + C}$	0.59	0.64	0.55	1.43	1.27	0.72	0.65	1.01	1.07

The complete system contained 30 μmoles of $MnCl_2$, 1.0 mmole of phosphate buffer of pH 7.5, 10 μmoles each of CTP, ATP and GTP, 1.0 mg. of bacterial or salmon DNA and 8 to 10 mg of enzyme (absorbency ratio of 280/260 mμ = 1.54). The reaction was incubated for 1 hour at 25° in a final volume of 10 ml. After incubation, the reaction was treated as described in Table 1, except for the following modifications. No carrier RNA was added. An extraction of the washed acid-insoluble material was made with 10 per cent NaCl, pH 8, at 90° for 30 minutes. The nucleic acid was precipitated twice from the salt extract with two volumes of ethanol. This modification considerably lowered the optical density units released, after alkaline hydrolysis, in control vessels where one nucleotide had been omitted from the reaction mixture.

* Base composition of DNA as reported by Belozersky and Spirin.[11]

† The moles per cent of the bases found in RNA were calculated by assuming that the nucleotides eluted from paper, after electrophoresis, represented 100 per cent recovery. The loss of terminal nucleotide residues by this procedure, as well as endogenous RNA, account in part for the experimental error observed.

similarity to the base composition reported for the respective DNA "primers." *Pseudomonas* and *Serratia* DNA have a relatively high GC mole per cent content, while Salmon DNA has a low GC mole per cent content. Similarly, it can be seen that the GC base content of the RNA formed in the presence of *Pseudomonas* and *Serratia* DNA is high, while the GC content for the RNA found when salmon DNA was used is correspondingly low. When *E. coli* DNA was present in the reaction mixture, the RNA formed had nearly equivalent amounts of each ribonucleotide, which also reflects the base composition of this particular DNA. In each of these experiments, experimental error does not allow for exact comparison between DNA and RNA bases. Examination of experiments 1 and 2 with *Pseudomonas* DNA shows the limit of error incurred. However, the DNAs used here are sufficiently different in composition to exclude experimental error in the interpretation of the results obtained.

Examination of P^{32}-RNA formed after coli infection with phage led Volkin and Astrachan[7] to conclude that the RNA synthesized in the infected cells resembled phage DNA in base ratio. Belozersky and Spirin,[8] after studying DNA and RNA composition in a number of different bacterial species, speculated that a small portion of the cellular RNA might fully correlate in its composition with that of DNA. Yčas and Vincent[9] presented evidence that a metabolically active RNA fraction in yeast had a base composition resembling yeast DNA. More recently, Hall and Spiegelman[10] reported on complex formation between denatured phage DNA and specific RNA. These authors concluded that only T2-DNA complexes with T2-specific RNA since they possess complementary nucleotide sequences. The work described in the present paper agrees with and supports the concept put forth by the above authors.

The *M. lysodeikticus* enzyme used in our experiments will form ribopolynucleotides when mammalian, salmon, sea urchin, bacterial and phage DNA serve as "primers." It is quite possible that in the presence of a specific DNA this enzyme will assemble complementary ribopolynucleotides. However, it should be em-

phasized that we have not yet proved with certainty that the RNA thus synthesized is an exact replica of the "primer" DNA.

Summary.—Experiments are described in which the nearest neighbor to cytidylate and base composition was determined for RNA synthesized by a partially purified bacterial enzyme which requires DNA. The results show that different DNAs alter the position of cytidylate in the newly assembled RNA chain and that the ribopolynucleotide has an average base composition similar to the "primer" DNA used.

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FURAZAN OXIDES, I. THE STRUCTURE OF BENZOFURAZAN OXIDE

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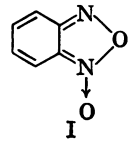
The structure of the compound $C_6H_4N_2O_2$, which may be obtained by treatment of *o*-nitroaniline with alkaline sodium hypochlorite or by pyrolysis of *o*-nitrophenyl azide, has been the subject of controversy since the compound was first recognized in 1894.[1] There is still no general agreement on which of the variety of structures, I–VI, that have been proposed[2] by different workers since that time is correct.



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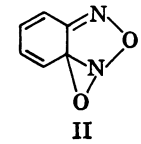

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