THE DIRECTING ROLE OF DNA IN RNA SYNTHESIS

J.J. Furth*, Jerard Hurwitz** and Monika Goldmann

Department of Microbiology
New York University College of Medicine
New York, New York

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We have recently described enzyme preparations from Escherichia coli strain W which catalyze the incorporation of ribonucleotides into RNA (1). Incorporation was shown to require the presence of the four nucleoside triphosphates (UTP, ATP, GTP and CTP) all of which were incorporated. The reaction appeared to involve DNA since the addition of DNase was inhibitory and since DNA was shown to stimulate incorporation. Similar requirements for RNA synthesis have been reported by Steven (2) and Weiss (3). However, the precise role of DNA in the reaction was not clear.

With further purification of the enzyme from E. coli, we have now obtained virtually complete dependence upon the addition of DNA. The preparation catalyzes the net synthesis of RNA from the four nucleoside triphosphates. The base ratio in the RNA produced is determined by the base ratio in the DNA added. The following DNA preparations were found to be active: T2-DNA[1], Pneumococcal DNA (transforming DNA)[2], calf-thymus DNA, heated calf-thymus DNA (100° for 10 minutes

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* Postdoctoral Fellow of the National Institutes of Health.

** Senior Postdoctoral Fellow of the National Institutes of Health.

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1. T2 DNA was a gift from Dr. A. Hershey.
2. This sample was a gift from Dr. Harriet Bernheimer.

rapidly cooled), ϕX-174 DNA[3], E. coli DNA, Micrococcus lysodeikticus DNA and polydeoxythymidylate[4] (Poly T). From the nature of these DNA preparations it appears that the DNA need not be "double stranded" in order to direct RNA synthesis.

Enzymatic activity was followed by the incorporation of radioactive nucleotides (labeled with either C^{14}, or with P^{32} in the α-phosphate group) into an acid-insoluble product. Both the type of RNA formed and the requirements for its synthesis depended on the nature of the DNA added as inducer. With thymus DNA all four nucleoside triphosphates were required (Table 1) and all were incorporated into the product (Table 2). In contrast, with Poly T only ATP was required. Addition of the other three nucleoside triphosphates together with ATP was somewhat inhibitory (Table 1), and only ATP was utilized for RNA synthesis even when the other nucleoside triphosphates were present (Table 2).

The fact that DNA of varied base composition would meet the requirement for RNA synthesis by the enzyme preparations prompted a quantitative study of the base ratios of the ribonucleotides incorporated into the product. The base ratios (A + T)/(C + G) in the DNA preparations ranged from 0.40 (Micrococcus) to 1.86 (T2) and these ratios were closely reflected in the pattern of incorporation of ribonucleotides into the product (Table 3). In each case, the (A + G)/(U + C) ratio remained close to unity, reflecting the (A + G)/(T + C) ratio found in DNA. These results are consistent with the hypothesis that incorporation of ribonucleo-

3. This sample was a gift from Dr. R. L. Sinsheimer.
4. Poly T was a gift from Dr. H. G. Khorana. This material was eluted from DEAE-cellulose comumns with 1 M salt (4).

TABLE 1

Requirements for AMP Incorporation with Poly T and Thymus DNA as Primers

Additions	Thymus DNA	Poly T
	mμmoles incorporated	
1. Complete System	1.47	4.25
2. omit GTP, UTP and CTP	0.15	6.80
3. 1 + RNase (5 μg)	0.22	—
4. 2 + RNase (5 μg)	—	7.0
5. 1 + DNase (5 μg)	0.18	—
6. 2 + DNase (5 μg)	—	2.84

The complete system (0.5 ml) contained: C^{14}-ATP (50 mμmoles, 2 x 10^6 cpm per μmole), GTP, UTP and CTP (80 mμmoles each), Poly T (0.5 optical density units at 260 mμ) or thymus DNA (0.75 optical density units at 260 mμ), $MgCl_2$ (4 μmoles), $MnCl_2$ (2 μmoles) mercaptoethylamine (1 μmole), Tris buffer (25 μmoles, pH 7.5) and 12 μg of a 100-fold purified enzyme preparation obtained from E. coli W. After incubation for 20 minutes at 38° the reaction was stopped and the acid-insoluble material treated as previously described (1). The reaction rate was linear for at least 40 minutes under the conditions specified above. The RNase and DNase were added immediately before the E. coli enzyme. If DNA was pretreated with the DNAse, no detectable acid-insoluble radioactivity was formed.

tides is determined by the ability of the new polymer to form hydrogen bond pairs with the bases in the inducer DNA, as proposed by the Watson and Crick Model for DNA itself (5).

The above mechanism for RNA synthesis does not appear to require RNA as a primer. RNase addition did not affect AMP incorporation when Poly T was used as a primer (Table 1), while DNase addition was inhibitory. Further evidence was obtained by studying end group

TABLE 2

Specificity of Poly T and Thymus DNA in Governing Nucleotide Incorporation

	Labeled Precursor Incorporated			
	C^{14}-AMP	P^{32}-GMP	P^{32}-UMP	P^{32}-CMP
	mμmoles incorporated			
Thymus DNA + complete system	1.95	1.40	2.06	1.20
Poly T + complete system	4.4	0.05	0.11	< 0.02
Poly T + labeled nucleotide only	6.15	0.09	< 0.02	< 0.02

The complete system contained 40 mμmoles each of all 4 nucleoside triphosphates with one labeled as indicated in each case. The specific activities (cpm per μmole) of the labeled nucleotides were: C^{14}-ATP, 1.95×10^6; GTP^{32}, 1.78×10^6; UTP^{32}, 0.64×10^6; CTP^{32}, 1.66×10^6. All other additions were as described in Table 1.

formation in the polyriboadenylate (Poly A) product formed from C^{14}-ATP with Poly T as inducer. The quantities of end products after alkaline degradation were: pAp (518 cpm), Ap (21,500 cpm) and adenosine (852 cpm). The presence of radioactivity in the P-terminal end of the chain suggests that the synthesis of Poly A, at least in part, began with the added labeled nucleotide[5]. Moreover, the average chain length of the polyriboadenylate appears to be between 25 and 40 nucleotide units.

With natural DNA preparations the reaction product, freed of nucleotides by dialysis, appeared to consist in part of an RNA-DNA complex. At least 35% of the labeled product was not rendered acid-soluble by RNase or RNase plus DNase. Treatment with DNase alone

5. The action of a ribonuclease which would lead to 5'-phosphate ended oligonucleotides would also yield C^{14}-labeled pAp after alkaline degradation. This type of enzymatic activity has not been detected in the purified enzyme fractions.

TABLE 3

Influence of Different DNA Preparations on Incorporation of Nucletides

DNA Added	$\frac{A+T}{C+G}$ reported	Ref.	Nucleotide Incorporation in mμmoles AMP	UMP	GMP	CMP	$\frac{A+U}{C+G}$ observed	$\frac{A+G}{U+G}$
T2-DNA	1.86	(6)	0.54	0.59	0.31	0.30	1.85	0.96
Thymus-DNA	1.35	(7)	3.10	3.30	2.0	2.2	1.52	0.93
E. coli DNA	1.0	(8)	2.70	2.74	2.90	2.94	0.93	0.98
Micrococcus DNA	0.40	(9)	0.55	0.52	1.10	1.12	0.48	1.01

All additions were as described in Table 1 with the following exceptions; with T2-DNA, 0.56 optical density units and Mn^{++} was omitted; with thymus DNA, 25 μg of protein were added; with E. coli DNA, 1 optical density and 24 μg or protein were added; with Micrococcal DNA, 1 optical density unit was added.

was without effect. The radioactivity in this complex after acid precipitation or heating at 100° for 3 minutes was rendered acid-soluble by treatment with RNase but not by treatment with DNase. Recent observations of Rich (10), Hall and Spiegelman (11) and Schildkraut et al.(12) have provided evidence for the existence of such mixed RNA-DNA complexes.

The observations reported here serve to define in greater detail the role of DNA in the enzymatic synthesis of RNA. Ribonucleotide incorporation into RNA shows base ratios which reflect the base composition of the DNA, and with Poly T as inducer a Poly A polymer is formed even when the other RNA precursors are present. The enzyme preparations under investigation appear to meet the requirement for the DNA-directed synthesis of RNA.

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