THE DIRECTING ROLE OF DNA IN RNA SYNTHESIS SPECIFICITY OF THE DEOXYADENYLATE DEOXYTHYMIDYLATE COPOLYMER AS A PRIMER

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The RNA-synthesizing enzyme (RNA polymerase), partially purified from Escherichia coli W, catalyzes the formation of RNA when the triphosphates of the four ribonucleosides commonly found in RNA are incubated with primer DNA. The base ratios of ribonucleotides incorporated into RNA reflect the base ratio of the DNA added, and if polydeoxythymidylate is used as a primer, only ATP is utilized for the formation of the acid-insoluble product (Furth, Hurwitz and Goldmann, 1961). In order to examine the specificity of the reaction in greater detail, the copolymer of deoxyadenylate and deoxythymidylate (d-AT) was used as the "priming" DNA.[1]

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1. The d-AT copolymer was a generous gift of Drs. C.M. Radding and A. Kornberg. This material was prepared with DNA polymerase in the absence of primer.

Table 1

Specificity of the d-AT Copolymer as Primer

Labeled precursor as substrate	mμmoles incorporated
C^{14}-ATP	0.97
C^{14}-UTP	1.10
P^{32}-GTP	<.03
P^{32}-CTP	<.03
C^{14}-UTP (omit CTP and GTP)	1.06

The reaction vessels contained (in 0.5 ml): $MgCl_2$ (4 μmoles), $MnCl_2$ (2 μmoles), mercaptoethanol (1 μmole), Tris buffer pH 7.5 (25 μmoles), d-AT copolymer (.22 optical density units at 262 mμ), 12 μg of a 100-fold purified enzyme preparation obtained from E. coli W, and all four ribonucleoside triphosphates. One of the nucleoside triphosphates was labeled with either C^{14} or P^{32} in the α phosphate group. The amounts and specific activity of the labeled nucleotides were: C^{14}-ATP, 50 mμmoles (1950 cpm/mμmole), C^{14}-UTP, 21 mμmoles (637 cpm/mμmole), P^{32}-GTP, 41 mμmoles (780 cpm/mμmole), and P^{32}-CTP, 35 mμmoles (728 cpm/mμmole). 80 mμmoles of the three other ribonucleotides were added in each experiment except where otherwise indicated. After incubation of the mixture for 20 minutes at 38°, the reaction was stopped and the acid insoluble material treated as previously described (Hurwitz, Bresler and Diringer, 1960).

With d-AT as primer only AMP and UMP are incorporated and when both substrates are in excess, the rates of their incorporation are approximately equal (Table 1). GMP and CMP are not incorporated and their addition or omission has no appreciable effect. On the other hand, AMP incorporation is dependent on the presence of UTP, and UMP incorporation is dependent on the presence of ATP (Table 2).

Since d-AT is composed of alternating units of deoxyadenylate

Table 2

Requirements for AMP and UMP Incorporation with the d-AT copolymer as Primer

Additions	mμmoles incorporated
C^{14}-ATP + UTP	1.0
C^{14}-ATP, omit UTP	0.17
C^{14}-UTP + ATP	1.03
C^{14}-UTP, omit ATP	0.04

The additions were as in Table 1 except that the nucleoside triphosphates were added as indicated, CTP and GTP were omitted, and 0.11 optical density units of d-AT copolymer were added. The reaction was terminated after 20 minutes.

and deoxythymidylate (Schachman, et al. 1960) it seemed desirable to examine the product of the d-AT primed reaction. Alkaline hydrolysis of RNA results in transfer of P^{32} from the ribonucleotide originally esterified at the 5'-carbon of the ribose to the 2 or 3'-carbon of the adjacent ribonucleotide. The data in Table 3 show that nearly every P^{32}-adenylate was adjacent to uridylate and that every P^{32}-uridylate was adjacent to adenylate.[2] Thus d-AT copolymer primes the synthesis of a polymer made up of AMP and UMP units in alternating sequence.

2. The incorporation of 0.17 mμmoles of ATP in the absence of UTP (Table 2) and the 6% of P^{32} isolated as AP^{32} following alkaline hydrolysis (Table 3) may be due to the contamination of the enzyme preparation with an enzyme which forms polyriboadenylate from ATP (August, Ortiz and Hurwitz, unpublished observations).

Table 3

Distribution of P^{32} Following Alkaline Degradation of the Product Prepared Using the d-AT Copolymer as Primer

Substrates	mμmoles Incorporated	Distribution of Radioactivity after alkaline hydrolysis (%)			
		AMP	CMP	GMP	UMP
$AP^{32}PP$ + UTP	16.3	6	<0.5	<0.5	94
ATP + $UP^{32}PP$	19.5	100	<0.5	<0.5	<0.5

The reaction vessels contained (in 5.0 ml) either 400 mμmoles of $AP^{32}PP$ (specific activity 765 cpm/mμmole) plus 800 mμmoles of UTP, or 472 mμmoles of $UP^{32}PP$ (specific activity 242 cpm/mμmole) plus 800 mμmoles of ATP. Both contained d-AT copolymer (0.44 optical density units), $MgCl_2$ (40 μmoles), $MnCl_2$ (20μmoles), Tris buffer pH 7.5 (250 μmoles) mercaptoethanol (10 μmoles) and 120 μg of enzyme protein.

After 30 minutes at 38°, the reaction was stopped with perchloric acid and 1.2 mg of bovine serum albumin were added. The acid-insoluble material was washed with 1% perchloric acid, dissolved in 1.5 ml of 1N NaOH and incubated for 16 hours at 38°. The hydrolysates were then adsorbed to Norit, washed with water, and the nucleotides eluted with ethanolic-NH_3. The nucleotides plus carrier amounts of each of the 2',3'-nucleotides were then fractionated by paper electrophoresis, located by ultraviolet examination, eluted with water and their radioactivity determined.

Approximately 75% of the radioactivity in the alkaline hydrolysate was eluted from Norit. The elution of the material after paper electrophoresis was quantitative. The amounts of the alkaline hydrolysate used for electrophoresis were 2.53 mμmoles and 3.94 mμmoles for the experiments with $AP^{32}PP$ and $UP^{32}PP$, respectively.

The observations reported here indicate that not only do the deoxynucleotides in the primer determine the ribonucleotides in the RNA produced, but the sequence of the deoxynucleotides in the primer determines the sequence of the ribonucleotides in the product.

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