THE RELEASE OF CHROMATIN TEMPLATE RESTRICTIONS BY NATURAL POLYRIBONUCLEOTIDES*

David G. Brown and Donald S. Coffey**

Department of Pharmacology and Experimental Therapeutics
The Johns Hopkins University School of Medicine and
The James Buchanan Brady Urological Institute,
The Johns Hopkins Hospital, Baltimore, Maryland 21205

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SUMMARY. Certain natural RNAs are capable of releasing the DNA template restrictions on soluble rat liver chromatin, when assayed with an excess of exogenous DNA polymerase. A marked difference in the capacity for DNA template activation was observed among the RNAs isolated from yeast, Escherichia coli, rat liver and MS-2 and Q_β bacteriophages. The effects observed with the natural RNAs were compared with several synthetic polyribonucleotides.

INTRODUCTION. Soluble chromatin and intact liver nuclei do not serve as an effective DNA template for the synthesis of DNA in an assay system containing an excess of E. coli DNA polymerase and the appropriate deoxyribonucleotides. These DNA template restrictions may be released in the presence of certain polyanions, such as synthetic polyamino acids (1) and polyribonucleotides (2). Among the polyribonucleotides, the synthetic purine homopolymers were more effective than the pyrimidine homopolymers.

Naturally occurring homopolymers of adenylic and guanylic acids have been detected in mammalian tissues (3,4) and it appears that these purine polyribonucleotides may be synthesized in the nucleus in concert with the ribosomal RNA (3,5).

The present study reveals that natural ribosomal and bacteriophage RNAs are capable of releasing the DNA template restrictions of soluble chromatin.

METHODS. Liver nuclei were purified from adult, male, Sprague-Dawley rats (250-300g) by the method of Blobel and Potter (6). Cytological and chemical analysis showed a highly homogenous nuclear preparation (DNA:RNA:Protein = 1.0:0.1:3.2) with insignificant cytoplasmic contamination. Soluble

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chromatin was prepared from the nuclei by a modification of the method of Marushige and Bonner (7). The Waring blender homogenization was eliminated to reduce shearing of the chromatin. The DNA to RNA to protein ratios of the chromatin preparation were 1.0:0.05:1.6.

Nucleic acids were extracted from the various preparations by the methods of Hutchinson et al. (8). DNA was measured by Burton's modification of the diphenylamine method (9) using calf thymus DNA as a standard, and RNA was measured by the orcinol reaction (10) with purified yeast RNA as the standard. Protein was determined by the Folin-Ciocalteau method (11) using purified bovine serum albumin as the standard.

DNA template activity was measured by the DNA-dependent incorporation of radioactivity from ^{3}H-TTP into acid insoluble material (1,2). The constituents and properties of the reaction are described in Tables I and II. MATERIALS. Highly polymerized calf thymus DNA (Sigma) was dissolved in 0.015M NaCl-0.0015M Na-citrate at concentrations of approximately 1mg of DNA per ml and was denatured by heating to 100° for 10 minutes followed by rapid cooling in an ice bath.

^{3}H-methyl-TTP (Sp. act. 11.1Ci/mmole) was purchased from Schwarz-Mann, Orangeburg, New York. E. coli DNA Polymerase Fraction VII (12), 5000 units/mg, was purchased from Biopolymers, Dover, New Jersey. All other components of the assay system were purchased from Sigma Chemical Company, St. Louis, Missouri.

Rat Liver ribosomal RNA was isolated by the method of Marmur (13) and purified by the method of Petrovic et al. (14) (Schwarz-Mann). E. coli 23S + 16S ribosomal RNA mixture and the separated 23S and 16S RNAs were purchased from Miles Laboratories, Elkhart, Indiana. Isolated MS-2 bacteriophage RNA was a generous gift of Dr. Daniel Nathans of the Department of Microbiology, The Johns Hopkins University School of Medicine. Q_{β} bacteriophage RNA was purchased from Miles Laboratories. In addition, all of the synthetic polyribonucleotides were purchased from Miles Laboratories.

Yeast RNA (15), yeast soluble RNA (16) and yeast "core" RNA (17) were purchased from Sigma, and E. coli soluble RNA and E. coli methionyl-t-RNA were gifts from Dr. Herbert Dickerman of the Department of Medicine, The Johns Hopkins Hospital. Bovine pancreatic deoxyribonuclease (ribonuclease free) and ribonuclease T1 were obtained from Sigma.

TABLE I
Comparison of the Template Capacities of Purified DNA and Soluble Chromatin in the Presence of Various Synthetic and Natural Polyribonucleotides

Additions (100μg/ml)	SOURCE OF DNA TEMPLATE equivalent to (50μg DNA/ml)		
	Soluble Chromatin (Rat Liver)	Purified DNA (calf thymus heat-denatured)	None
	(pmoles incorporated per 30 minutes)		
None	70	650	0
	Synthetic Polyribonucleotides		
Poly I	590	470	0
Poly G	430	80	0
Poly U	235	670	0
Poly C	75	660	0
Poly (A,C,U,G)	100	670	0
	Natural Polyribonucleotides		
Ribosomal RNA, rat liver (28S + 18S mixture)	350	480	18
Ribosomal RNA, E. coli (23S + 16S mixture)	360	550	0
23S RNA, E. coli	320	570	0
16S RNA, E. coli	316	700	9
MS-2 RNA, bacteriophage	335	640	0
Q_β RNA, bacteriophage	350	550	1
Soluble RNA, yeast	75	410	0
'Core' RNA, yeast	80	441	0
RNA, yeast (Crestfield)	87	524	39
Soluble RNA, E. coli	65	600	0
Methyionyl-tRNA, E. coli	65	660	0

A 0.1ml reaction system contained 10μmoles tris-HCl, pH7.4 at 37°C; 0.7μmole $MgCl_2$; 0.1μmole β-mercaptoethanol; 18.7 mμmoles each of dCTP, dGTP, dATP; 20.0mμmoles of TTP containing 1μCi of ^{3}H-TTP; 0.1 unit of E. coli DNA polymerase; 5μg of the appropriate DNA template as indicated; 10μg of neutralized polyribonucleotide where indicated; and an ATP generating system containing 0.25μmole ATP, 0.50μmole sodium phospho(enol)pyruvate and 0.4μg pyruvate kinase. The system was incubated at 37°C for 30 minutes and assayed by the filter paper disk procedure (18). After appropriate washing, the radioactivity on the disk was determined by the scintillation counting (350cpm = 100pmoles). The synthetic polyribonucleotides exhibited a molecular weight by gel filtration > 100,000 and sedimentation coefficients (S20) in 0.05M NaH_2PO_4, pH7.0 in the range of S5.4 to S10.7. Poly (ACUG) consisted of a base ratio of 1.1:1.2:1.3:1.0. Abbreviations: I, inosinic acid; G, guanylic acid; U, uridylic acid; C, cytidylic acid; A, adenylic acid; X, xanthylic acid.

RESULTS. Specific natural and synthetic polyribonucleotides are capable of releasing the template restrictions on soluble rat liver chromatin when assayed with E. coli DNA polymerase (Table 1). Among the natural RNAs, ribosomal and bacteriophage RNA were the most active in enhancing the DNA template activity of soluble chromatin, while among synthetic polyribonucleotides, the purine homopolymers were the most active (see ref. 2). Qualitatively similar results were obtained when isolated rat liver nuclei were used as the DNA template source.

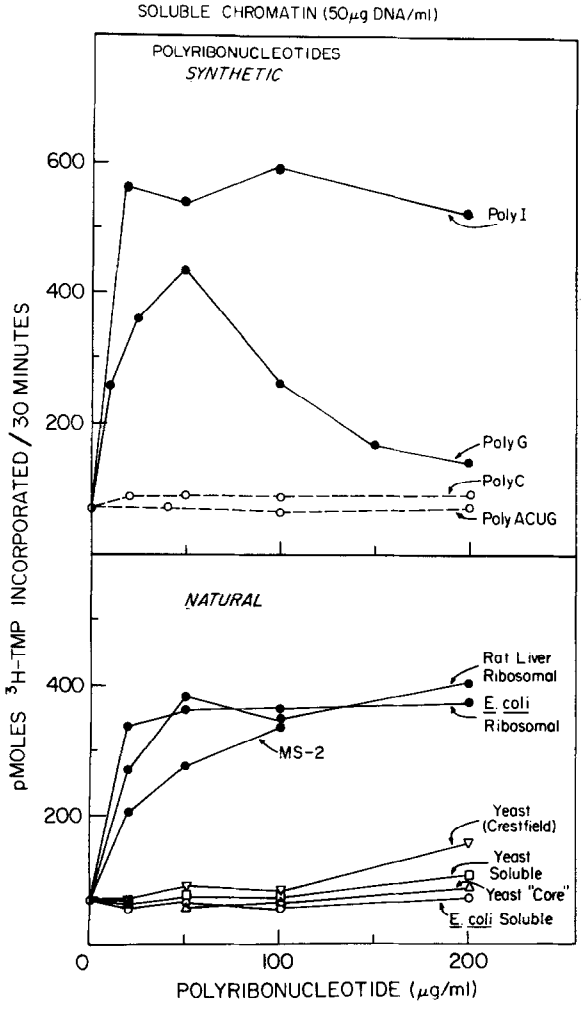

Figure 1. The effect of various synthetic and natural polyribonucleotides on the template properties of soluble rat liver chromatin when assayed with E. coli DNA polymerase. The ordinate represents the rate of DNA synthesis at different concentrations of polyribonucleotides. The conditions for the reaction are described in Table 1.

When purified calf thymus DNA was used as the template source, no activation of DNA synthesis was observed with any of the RNAs or polyribonucleotides. In fact, Poly G markedly inhibited the DNA polymerase activity with purified DNA (Table I, also ref. 2). In addition, when the Poly G concentration was varied, in the presence of a constant amount of chromatin (expressed as chromatin DNA), a biphasic curve was obtained (Figure 1). The inhibition phenomenon has been visualized as a direct competition between

TABLE II

Characteristics of E. coli DNA Polymerase Activity with Purified DNA and Soluble Rat Liver Chromatin in the Presence of Rat Liver Ribosomal RNA

System	Template Source (equivalent to 50μg DNA/ml)	
	Soluble Chromatin + Rat Liver Ribosomal RNA (100μg/ml)	Purified DNA Calf Thymus (heat denatured)
	(pmoles ^{3}H-TMP incorporated per 30 minutes)	
Complete	340	650
Omit DNA or Chromatin	18	0
Omit Mg^{2+}	15	5
Omit dCTP, dGTP, dATP	18	3
Omit DNA Polymerase	5	0
Deoxyribonuclease[a]	7	6
Ribonuclease[b]	334	588
Reaction Products Base Hydrolysed[c]	332	645
Omit Ribosomal RNA	70	—
Hydrolysed Ribosomal RNA[d]	98	—

a) Reaction mixture treated during last 10 min. with 50μg pancreatic DNase (RNase free), 2,240 Units/mg.
b) Reaction mixture treated during last 10 min. with 200 units RNase T_1.
c) Reaction incubated for an additional 1 hour at 37° in the presence of 0.3M KOH.
d) Rat liver ribosomal RNA (200μg) was hydolysed with 200 units of RNase T_1 for 15 min. at 37° before the RNA was added to the assay system.

The reaction system of 0.1ml final volume was prepared as described in Table I and contained 0.1 unit E. coli DNA polymerase Fraction VII. The system was incubated for 30 minutes at 37°.

certain polyanions and the purified calf thymus DNA for the DNA polymerase enzyme (1,2). The biphasic effect, therefore, may result from stoichiometric interaction of the polyanions with cationic elements of the chromatin thus releasing the constraints on the DNA template; an excess of polyanion added beyond the stoichiometric titration point would then compete with the free DNA and inhibit the reaction. In contrast, high concentrations of Poly I or the naturally occurring template-activating RNAs do not inhibit the DNA polymerase reaction. The specificity for these effects depends on the nature and characteristics of the polyribonucleotide since Poly C, Poly (A,C,U,G) and several natural RNAs neither activate the template properties of chromatin DNA nor inhibit those of purified DNA.

In all of the reactions reported in this study, the incorporation of radioactivity into DNA was linear with time and was dependent on the presence of magnesium ion, all four deoxyribonucleotides and exogenous E. coli DNA polymerase (Table II). The labeled reaction products could be solubilized by beef pancreatic DNase but not by RNase T_1 or by hydrolysis in 0.3M KOH for 1 hour at 37°C.

DISCUSSION. The basic mechanism producing the specificity of certain polyanions in the release of nuclear template restrictions is not known. The specificity has been observed with both soluble chromatin and intact nuclear preparations (1,2) and is therefore, not simply explained on the entrance of certain polyanions into the nucleus. However, the specificity may be related to an optimal size or conformation of the polyanion which may permit an association or displacement of certain chromosomal proteins and soluble chromatin is dependent on the molecular size of the template-activating polyanion (1,19).

The amount of secondary structure in these types of synthetic homopolyribonucleotides has been determined (20). It appears that the order of stability of these polymers may correspond to their capacity to release DNA template restrictions [e.g., Poly X > Poly G > Poly I > Poly U > Poly A > Poly C (2)]. Since purified animal (21) and bacterial ribosomal RNAs and

MS-2 bacteriophage RNA (23) have been shown to possess considerable secondary structure in neutral solution, it is important to determine whether or not the specificity for DNA template activation is dependent on the structure of the polyribonucleotides. In addition, a specific sequence of nucleotides within the polymer may be required to produce these effects.

Frenster observed (24) that the RNA polymerase activity of isolated, condensed chromatin was enhanced in the presence of certain natural RNAs. He suggested that specific nuclear RNAs might function as de-repressor agents in the interphase chromatin to unmask areas of the genome for the synthesis of messenger RNA (25). However, the process involved in the removal of template restrictions for DNA synthesis is quantitatively different from that involved during de-repression for messenger RNA synthesis. During DNA replication, the entire component of the DNA in the chromatin must participate as an available template. Therefore, these RNAs might be associated with either DNA replication or with de-repression for the synthesis of messenger RNAs.

Whether these natural template-activating RNAs function in a regulating capacity within the cell remains to be determined. At present it is difficult to visualize how ribosomal RNA could serve in this capacity. However, considerable insight into this problem may be obtained from the recent work in Amoeba proteus by Goldstein and Trescott (26) who showed that specific RNAs with sedimentation constants of 30S, 19S and 4-6S can migrate from the cytoplasm into the nucleus.

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