Release of Nuclear DNA Template Restrictions by Specific Polyribonucleotides

Abstract. *Certain synthetic homo- and copolyribonucleotide polymers are capable of releasing the DNA template restriction of isolated nuclei of rat liver cells when assayed with an excess of or in the absence of exogenous DNA polymerase. The purine homopolyribonucleotides are far more effective than are the pyrimidine polymers.*

Isolated liver nuclei or soluble chromatin do not serve as an effective template for the synthesis of DNA in an assay system containing an excess of *Escherichia coli* DNA polymerase and the appropriate deoxyribonucleotides. However, these template restrictions on the nuclear DNA may be released in the presence of certain specific polyanions such as polyaspartic acid, polyglutamic acid, or heparin (*1*, *2*). Although this type of nuclear activation could not be observed with many other polyanions including yeast ribonucleic acid, additional attention was nevertheless drawn to the polyribonucleotides because the addition of polyadenylic acid or polycytidylic acid to suspensions of nondividing virulent pneumococci results in the production of an excess of DNA which was copied in a semiconservative manner (*3*). The newly synthesized DNA in the pneumococci was genetically competent with respect to its ability to transfer streptomycin resistance (*4*).

Naturally occurring homopolymers of polyadenylic and polyguanylic acids have been detected in mammalian tissues such as mouse liver and it appears that these purine polyribonucleotides are synthesized in the nucleus in concert with the ribosomal RNA (*5*).

In an effort to investigate the interaction of specific ribonucleic acids with nuclei, we have measured the template activity of isolated rat liver nuclei in the presence of an excess of *E. coli* DNA polymerase and the four essential deoxyribonucleotides.

The properties of this assay system have been described (*1*). In the present study, synthetic ribonucleic acids were tested as the homo- and copolymers, and a high degree of specificity was observed (Table 1). In general, the purine homopolymers were the most active in enhancing the DNA template activity of liver nuclei. None of the polyribonucleotides containing pyrimidines, either as the homopolymers or as mixed copolymers with the purine (single strands), were very effective. Furthermore, base pairing of the effective purine homopolymers with appropriate pyrimidine homopolymers, such as poly I·poly C (double strand), eliminated the ability of the poly I to activate the nuclei. Although these studies utilized exogenous bacterial DNA polymerase, it is important to note that the weak activity of endogenous liver DNA polymerase that was associated with the isolated nuclei was also markedly enhanced in the presence of certain of the polyribonucleotides.

In this study the incorporation of label into DNA was linear with time and dependent on the presence of magnesium ion and all four deoxyribonucleotides. The labeled reaction product could be solubilized by beef pancreatic deoxyribonuclease but not by ribonuclease. All of the polyribonucleotides, when tested alone in the absence of any added DNA template, incorporated less than 1 pmole of tritiated thymidine monophosphate per 30 minutes.

When the template for the *E. coli* DNA polymerase was purified calf thymus DNA, no further activation of DNA synthesis was observed when any of the above-mentioned polyribonucleotides were added. Indeed, in several cases the polyribonucleotides markedly inhibited the reaction (Table 1). The contrast between the activation of the nuclei by polyguanylic acid and the inhibition by the polyribonucleotide of purified calf thymus DNA template function was observed over a wide range of concentration of both DNA templates (Fig. 1). In addition, if the polyguanylic acid concentration is varied in the presence of a constant

Table 1. The effects (expressed as picomoles of [^{3}H]TMP incorporated per 30 minutes; 350 count/min = 100 pmole) of various polyribonucleotides on the template properties of isolated liver nuclei and purified DNA when assayed with *E. coli* DNA polymerase. A 0.1-ml reaction system contained: 10 μmole of tris-HCl, *p*H 7.4 at 37°C; 0.7 μmole of $MgCl_2$; 0.1 μmole of β-mercaptoethanol; 18.7 nmole each of dCTP, dGTP, dATP; 20.0 nmole of dTTP containing 1 μc of [methyl-^{3}H]dTTP; 0.1 unit of *E. coli* DNA polymerase (DNA deoxynucleotidyltransferase, E.C. 2.7.7.7). Specific activity 5000 unit/mg (Biopolymers, Fraction VII); 10 μg of the appropriate DNA template as indicated; 10 μg of neutralized polyribonucleotide where indicated; and an ATP-generating system containing 0.25 μmole of ATP, 0.50 μmole of sodium phospho(enol)pyruvate, and 0.4 μg of phospho(enol)pyruvate kinase (Sigma II). The system was incubated at 37°C for 30 minutes and assayed by the disk filter-paper procedure. After appropriate washing, the radioactivity on the disk was determined by scintillation counting. The synthetic polyribonucleotides were obtained from Miles Laboratories and exhibited a molecular weight by gel filtration > 100,000 and sedimentation coefficients (s_{20}) in 0.05*M* NaH_2PO_4, *p*H 7.0 in the range of 5.4*S* to 10.7*S*. Copolymers consisted of a base ratio of approximately 1 : 1. Liver nuclei were prepared by the procedure of Blobel and Potter (*12*), and their properties have been described (*1*). Abbreviations: X, xanthylic acid; G, guanylic acid; I, inosinic acid; A, adenylic acid; U, uridylic acid; dihydro U, dihydrouridylic acid; C, cytidylic acid.

Additions*	Template source (equivalent to 100 μg of DNA per milliliter)		
	Rat liver nuclei	Purified DNA (heat-denatured calf thymus)	Rat liver nuclei (minus *E. coli* DNA polymerase)
None	30	1012	8
	Homopolyribonucleotides (purines)		
Poly X	1530	768	39
Poly G	955	178	16
Poly I	690	875	27
Poly A	109	1040	9
	Homopolyribonucleotides (pyrimidines)		
Poly U	126	1040	11
Poly dihydro U	119	745	12
Poly C	41	1050	8
	Copolyribonucleotides		
Poly (G,I)	390	261	58
Poly (G,A)	318	718	29
Poly (I,A)	150	570	23
Poly (G,C)	82	965	12
Poly (I,U)	72	860	10
Poly (G,U)	42	884	7
Poly (A,U)	38	935	7
Poly (A,C)	32	950	8
Poly (I,C)	29	520	8
Poly (C,U)	43	820	7
Poly (I) · Poly (C)	31	830	6

* Other additions: sodium sulfate, sodium phosphate, sodium pyrophosphate, ribose, orotic acid, guanine, guanosine, GMP, GDP, GTP, adenine, adenosine, AMP, ADP, ATP, cytidine, CMP, CDP, thymidine, TMP, hypoxanthine, inosine, xanthine, xanthosine, 3′,5′-cyclic AMP, and base-hydrolyzed poly G all incorporated less than 40 pmole of tritiated TMP per 30 minutes when added singly to rat liver nuclei.

quantity of liver nuclei (expressed as the amount of nuclear DNA), then a biphasic curve is observed (Fig. 2). However, when purified calf thymus DNA is used as the template source, inhibition is observed at all concentrations of the polyribonucleotide. The specificity for these effects is dependent on the type of polyribonucleotide; for example, polycytidylic acid is virtually inactive in both systems (Fig. 2).

Heparin produces a similar type of activation and inhibition as is observed here with polyguanylic acid (*1*). The inhibition phenomenon was visualized as a direct competition between the polyanion and the purified calf thymus DNA for the DNA polymerase enzyme. Bach (*6*) reported that certain polyanions are competitive inhibitors with respect to DNA for the DNA polymerase enzyme. Therefore, the biphasic activation effect observed with nuclei may result from stoichiometric interaction of the polyanions with cationic elements of the nuclei thus releasing the constraints on the nuclear DNA template; an excess of polyanion added beyond the stoichiometric titration point would then be available to compete with the free DNA and inhibit the reaction, thus producing the biphasic curve.

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 nuclei and soluble chromatin preparations (*1*) and is therefore not simply explained on the entrance of certain polyanions into the nucleus. However, the specificity may be related to an optimum conformation of the polyanion which may permit an association or displacement of certain nuclear proteins and thus free the nuclear DNA template. Indeed, certain synthetic polyribonucleotides [poly (G,C) and poly (A,U)] have been reported to form associations with histones although these particular polyribonucleotides were incapable of displacing DNA from the reconstituted DNA-histone complex (*7*). However, in the present study these two copolymers were not effective in releasing nuclear template restrictions.

The mechanism for the stimulation of DNA synthesis in pneumococci by polyadenylic acid might be quite different from that observed here with polyribonucleotides and liver nuclei. Indeed, with respect to the release of nuclear template restrictions, polyadenylic acid was the least effective purine polyribonucleotide tested in our system (Table 1). Polyadenylic acid increases the DNA content of the DNA–cell membrane complex but does not affect the membrane content of RNA, protein, and phospholipid in pneumococci (*8*). Several enzymes in the DNA-membrane complex which were associated with the synthesis of DNA and its component deoxyribonucleotides were also increased severalfold in activity.

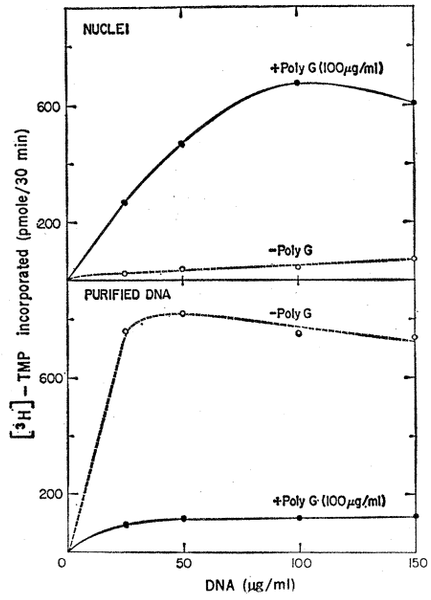

Fig. 1. The effect of varying the concentration of the DNA template (nuclei or purified calf thymus DNA, heat denatured) in the presence or absence of a constant amount (100 μg/ml) of polyguanylic acid. The ordinate represents the rate of DNA synthesis with DNA polymerase under conditions described in Table 1.

Certain specific synthetic polyribonucleotides are capable of releasing the template restrictions of liver nuclear DNA for DNA synthesis. This might suggest the possibility that other naturally occuring RNA's may also possess this property. Whether natural template-activating RNA's function in a regulating capacity within the cell remains to be determined.

In 1965, Frenster observed that the RNA polymerase activity of isolated condensed (hetero-) chromatin was enhanced in the presence of certain natural RNA's (*9*). He suggested that specific nuclear RNA's might function as derepressor agents in the interphase chromatin to unmask areas of the DNA genome for the synthesis of messenger RNA (*10*).

The process involved in the removal of template restrictions for DNA synthesis is quantitatively different from that involved during derepression for messenger RNA synthesis. During DNA replication, the entire component of the DNA in the chromatin must participate as an available template. Therefore, it is important to determine whether these template-activating RNA's are more closely associated with DNA replication or with derepression for the synthesis of messenger RNA's.

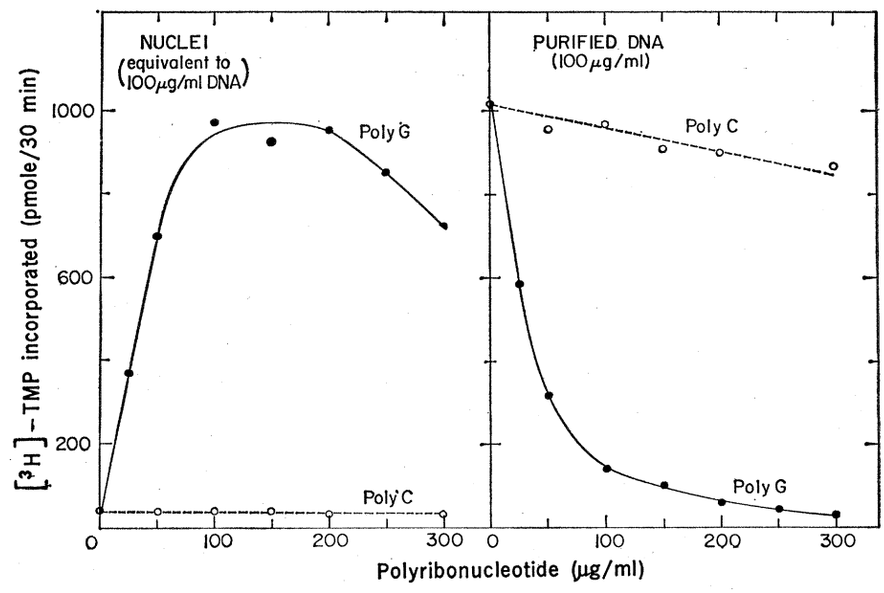

Fig. 2. A comparison of the effects of polyguanylic acid (solid circles) and polycytidylic acid (open circles) on the template properties of nuclei or purified heat denatured calf thymus DNA. The ordinate represents the rate of DNA synthesis with DNA polymerase under conditions described in Table 1.

In addition, certain synthetic homopolyribonucleotides are capable of inhibiting DNA synthesis in mammalian tissues and tumors (*11*). It would seem important to determine whether this inhibition is accomplished by competition of the exogenous homopolyribonucleotide with the templates of the cell in a similar manner as is observed here with DNA polymerase and purified calf thymus DNA.

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Hydrocortisone-Mediated Increase of Norepinephrine Uptake by Brain Slices

Abstract. *Slices of cerebral cortex from the adult rat were incubated with and without hydrocortisone succinate, and their subsequent uptake of isotopically labeled norepinephrine was measured. Preincubation with hydrocortisone resulted in a statistically significant increase in the amount of exogenous norepinephrine taken up by the slices. Preincubation with corresponding concentrations of sodium succinate was without effect. The principal effect of the hydrocortisone-mediated increase in norepinephrine concentration is apparently on an active transport mechanism.*

Catecholamine and steroid hormones have both been implicated as regulators of mood and behavior in humans and experimental animals, and, although their mode of interaction is unclear, there is much work that suggests that cortisol has an important "permissive" role in mediating the physiological actions of the catecholamines (*1–3*). A significant number of patients with severe depressions, in which a central nervous system deficiency of functional norepinephrine (NE) may exist, have elevated concentrations of plasma hydrocortisone, excrete greater than normal amounts of cortisol metabolites, and exhibit dexamethasone resistance (*4*). Since tissues such as heart and brain are able to accumulate exogenous NE against a gradient by an energy-dependent transport mechanism (*5, 6*), such preparations offer the possibility for examining the presently ill-defined relation between hydrocortisone and the disposition of the catecholamines. We present here data indicating that, in the presence of cortisol, the uptake of exogenous NE by slices of cerebral cortex is significantly increased and that the principal effect of the steroid hormone is on an active transport process or "pump" mechanism.

Adult rat cerebral cortex "first" slices (50 to 60 mg) were prepared as described by McIlwain (*7*) and then incubated at 37°C in 2.5 ml of a 10 m*M* solution of glucose and Krebs-Ringer bicarbonate (Fisher Scientific) in an oxygen atmosphere for 30 minutes with and without the indicated amounts of hydrocortisone succinate (Upjohn) or sodium succinate (Calbiochem). Various concentrations of *dl*-[7-^{3}H]norepinephrine (10 c/mmole; New England Nuclear) were then added, and the incubation was continued for another 15 minutes at which time the reaction was terminated by placing the mixture in an ice bath. The incubation medium was removed by suction. The slices were washed twice with 2.5 ml of cold medium and homogenized in 0.2*N* HCl. The radioactivity of a portion of the homogenate was measured, and another portion was adsorbed onto Al_2O_3 (M. Woelm Co.) by a modification of the method of Anton and Sayre (*8*). The catechols containing no amines were extracted from the acid eluate with ethyl acetate (*8–10*). A portion of the aqueous phase was dissolved in 3.0 ml of ethanol and was added to 10 ml of scintillator solution containing 2,5-diphenyloxazole (5 g/liter) and 1,4-*bis*-2-(4-methyl-5-phenyloxazoyl)-benzene (2.5 g/liter). The radioactivity was measured in a Packard liquid scintillation spectrometer. Control slices were included in each experiment, and corrections were made for recovery losses on alumina adsorption. When the effect of the inhibitor ouabain was tested, the incubations with hydrocortisone and the appropriate controls were conducted at 37°C for 30 minutes, at which time the [^{3}H]NE was added, and the incubation mixture was made 1×10^{-4}*M* with respect to ouabain. As before, the subsequent incubation was carried out for 15 minutes at 37°C. For the determination of uptake of NE at 0° to 4°C the initial incubations, with and

Table 1. Uptake of NE by slices of rat cerebral cortex. The NE concentration was 29 ng/ml in a 10 m*M* solution of dextrose and Krebs-Ringer bicarbonate incubation medium. Samples were preincubated with the indicated additives for 30 minutes at 37°C, [^{3}H]NE was added, and the incubation was carried out for another 15 minutes. The incubation mixture was then chilled in ice to stop the reaction. In some experiments, as indicated, the 15-minute incubation period was done at 0° to 4°C (the preincubation in all cases was done at 37°C). Results are averages of 3 to 10 experiments.

Incubation medium additive			Temp (°C)	[^{3}H]NE incorporation		In/Ex
Sodium succinate (*M*)	Hydrocortisone succinate (*M*)	Ouabain (*M*)		Amount per 15 minutes (ng/g)	[^{3}H]NE / [^{3}H]Total	
			37	67.15 ± 8.01	0.79 ± 0.05	2.32
5×10^{-5}			37	66.00 ± 6.44	0.84 ± 0.09	2.28
	4.2×10^{-5}		37	86.42 ± 12.30	0.86 ± 0.05	2.98
	8.4×10^{-5}		37	102.60 ± 12.36	0.85 ± 0.03	3.54
1.0×10^{-4}		1×10^{-4}	37	37.23 ± 9.39	0.68 ± 0.08	1.28
	8.4×10^{-5}	1×10^{-4}	37	27.05 ± 5.86	0.78 ± 0.13	0.93
1.0×10^{-4}			0–4	16.32 ± 3.28	1.16 ± 0.05	0.56
	8.4×10^{-5}		0–4	17.70 ± 3.78	1.03 ± 0.23	0.61