Proc. Natl. Acad. Sci. USA
Vol. 76, No. 6, pp. 2541–2545, June 1979
Biochemistry

Polyamine-mediated phosphorylation of a nucleolar protein from *Physarum polycephalum* that stimulates rRNA synthesis

(nonhistone proteins/ribosomal DNA/chromatin/phosphoproteins/DNA-protein interaction)

GLENN D. KUEHN*, HANS-URS AFFOLTER†, VALERIE J. ATMAR*, THOMAS SEEBECK†, UELI GUBLER†, AND RICHARD BRAUN†

*Department of Chemistry, New Mexico State University, Las Cruces, New Mexico 88003; and †Institut für Allgemeine Mikrobiologie, der Universität Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

Communicated by Johannes Geiss, February 22, 1979

ABSTRACT **An acidic nucleolar phosphoprotein with a subunit M_r of 70,000 was purified as an apparent dimer of 139,000 from isolated nuclei of the slime mold *Physarum polycephalum*. The protein was purified without the aid of strong dissociating agents after its selective phosphorylation in isolated nuclei by a polyamine-mediated reaction. Its amino acid composition resembled that of a nucleolar phosphoprotein from Novikoff hepatoma ascites cells. The phosphoprotein stimulated rRNA synthesis 5-fold by RNA polymerase I within a nucleolar, ribosomal deoxyribonucleoprotein complex isolated from nucleoli of *P. polycephalum*. It was also identified as a component of the complex. It bound with high affinity and specificity to the palindromic ribosomal DNA of 38×10^6 M_r from *P. polycephalum*, which contained two coding sequences for 5.8S, 19S, and 26S rRNA. It also bound to three fragments of ribosomal DNA of M_r 21.2×10^6, 17.1×10^6, and 8.1×10^6, prepared by cleavage with restriction endonucleases *Hin*dIII, *Pst* I, and *Bam*HI, respectively. All of these fragments included the symmetry axis of the palindromic ribosomal DNA. The phosphoprotein that had been treated with alkaline phosphatase-agarose to hydrolyze the phosphate groups did not stimulate transcription and did not bind to ribosomal DNA or to the restriction fragments indicated. We have thus isolated a specific phosphoprotein with the capacity to stimulate transcription of a specific set of genes in a eukaryote. These findings suggest that this phosphoprotein may specifically regulate functions of ribosomal DNA in a manner dependent on its degree of phosphorylation.**

The polyamines, putrescine, spermidine, and spermine, have been implicated in the control and promotion of cell growth processes (1, 2). One favored proposal has linked the polyamines to the metabolism (1), structure (3), and function (3, 4) of the nucleic acids. Specifically, much correlative evidence implicates their involvement in stimulating rRNA synthesis although evidence for a causal relationship has not been conclusive (for reviews see refs. 1, 2, and 5).

Recently the polyamines were found to selectively stimulate the phosphorylation of two nonhistone acidic proteins in isolated nucleoli from the slime mold *Physarum polycephalum* (6). Numerous investigations support the general notion that genetic regulatory molecules of eukaryotic cells are to be found among the nonhistone chromosomal proteins (7). The two nucleolar phosphoproteins were of major interest for further investigation of putative regulation of rRNA synthesis because the genes that code for rRNA in *P. polycephalum* reside on a satellite DNA that has been extensively characterized. This ribosomal DNA (rDNA) is localized in the nucleolus in multiple copies. It is extrachromosomal, with a molecular weight of 38×10^6. Each rDNA molecule is a palindrome, or inverted repeat sequence, with two coding sequences for 5.8S, 19S, and 26S rRNA (8, 9). rDNA has recently been isolated in the form of a transcriptionally active deoxyribonucleoprotein (rDNP) complex of discrete size (10). This complex is composed of nucleolar RNA polymerase I, a complement of uncharacterized proteins and rDNA. It has no detectable nucleoplasmic RNA polymerase II associated with it.

In the present communication we report that one of the nonhistone acidic proteins that had been shown to be selectively phosphorylated in isolated nuclei and nucleoli in a polyamine-mediated reaction (6) is a component of the rDNP complex. This protein of 70,000 M_r has been purified to apparent homogeneity without the aid of strongly dissociating agents. Some of its chemical and apparent regulatory properties toward rDNA are described.

MATERIALS AND METHODS

Preparation of Nuclei and Nucleoli. Nuclei were isolated from 48-hr shake cultures of microplasmodia (6, 11). Slime-free nucleoli and nuclei were isolated by the Percoll (Pharmacia) gradient method (10) from synchronous surface cultures 18–21 hr after provision of nutrient medium (8).

Purification of the 70,000 M_r Phosphoprotein. The total nuclei isolated from 400 ml of wet-packed microplasmodia were incubated at 30°C in 200 ml of a phosphorylation mixture (6). In some experiments the mixture contained sodium [^{32}P]-phosphate (5.18×10^7 cpm/μmol) or [γ-^{32}P]ATP (3.18×10^6 cpm/μmol) (Amersham). After 1 hr the reaction mixture was chilled to 4°C and Na_2MoO_4 (Sigma) was added to 30 mM concentration to inhibit acid phosphatase activity.

All succeeding procedures were conducted near 4°C. Dialysis buffers were changed four times. The nuclei were collected by centrifugation at $12,000 \times g$ for 10 min, then were resuspended in 240 ml of 0.35 M NaCl/0.25 M Tris·HCl, pH 7.5/1 mM Na_2MoO_4/0.1 mM phenylmethylsulfonyl fluoride. Nuclei were ruptured by shaking 40-ml portions of the suspension with 20 ml of glass beads, 0.75–1.0 mm diameter, for 90 sec at high speed in a Braun homogenizer. Nuclear debris and the beads were removed by centrifugation for 10 min at $20,000 \times g$. The supernatant solution was then centrifuged for 1 hr at $100,000 \times g$. The resulting solution was dialyzed against 20 vol of 0.4 M NaCl/10 mM Tris·HCl, pH 7/1 mM Na_2MoO_4/0.1 mM phenylmethylsulfonyl fluoride. The dialyzed sample was applied to a column of Bio-Rex 70 (Na^+ form, Bio-Rad) as described (12). The acidic protein fraction that was eluted was dialyzed against 20 vol of 10 mM Tris·HCl, pH 7/10 mM NaCl/10 mM Na_2MoO_4/0.1 mM phenylmethylsulfonyl fluoride. The dialyzed protein fraction was applied to a 2 cm (inner diameter) × 30 cm column of AG3-X4A (200–400 mesh, Cl^- form, Bio-Rad) equilibrated in 10 mM Tris·HCl, pH 7/10 mM

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: rDNA, ribosomal DNA coding for rRNA; rDNP, deoxyribonucleoprotein complex containing rDNA associated with chromosomal proteins; $NaDodSO_4$, sodium dodecyl sulfate.

NaCl (buffer A). The column was washed with 300 ml of buffer A, then with 300 ml of 10 mM Tris·HCl, pH 7/2 M NaCl. The 70,000 M_r phosphoprotein was next eluted with 300 ml of 10 mM Tris·HCl/3 M NaCl. The effluent was collected as a single volume and was concentrated to 5 ml in an Amicon filtration cell equipped with PM10 membrane. This concentrate was next chromatographed on a 2.2 cm (inner diameter) × 82 cm column of Sephadex G-200 equilibrated with buffer A. Fractions containing the phosphoprotein were pooled and concentrated to 10 ml. The final yield was about 150 μg. Buffered solutions of the protein containing 10 mM K_2HPO_4 (pH 7) or buffer A were stored at 2°C or −20°C. Samples of the isolate gave a single stained protein band on electrophoresis in sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gels (6).

Isolation of rDNP Complex, DNAs, and rRNA. Where indicated, the rDNP complex was isolated by EDTA solubilization of chromatin from isolated nucleoli as described (10). Total nuclear DNA, [^{3}H]rRNA, and unlabeled rRNA were prepared from *P. polycephalum* as described (13). rDNA was extracted from isolated nucleoli and further purified according to Vogt and Braun (8).

Enzyme Assays. RNA polymerase I (EC 2.7.7.6) activity in the rDNP complex was assayed as described (10, 14).

Restriction Enzyme Digestion and Nick-Translation of Fragments. All digestions were performed at 37°C in 50 μl of buffer containing 1–2 μg of rDNA and 8 units of restriction endonuclease. The reaction components for digestion of rDNA with *Hin*dIII, *Bam*HI, and *Pst* I restriction endonucleases (Boehringer Mannheim) were those recommended by the vendor in product bulletins 220523, 220612, and 239313, respectively. Reactions were terminated by heating at 65°C for 3 min. DNA fragments were isolated on 0.8% agarose (Serva) slab gels as described (8, 15). Total rDNA, or fragments derived from restriction endonuclease hydrolyses, were labeled with [α-^{32}P]dGMP (Amersham) by the nick-translation method of Maniatis *et al.* (16).

Filter Binding Assay. Indicated amounts of the phosphoprotein and [^{32}P]rDNA or ^{32}P-labeled restriction fragment were incubated in 10 mM Tris·HCl pH 7.4/10 mM KCl/0.1 mM Na_2EDTA/5% (vol/vol) dimethyl sulfoxide (buffer B) at 25°C for 30 min in a reaction volume of 0.10 ml. Samples of 0.01 ml were filtered through nitrocellulose filters (Schleicher & Schuell, type BA85/1) in triplicate under conditions described (17), and each filter was then washed with 0.1 ml of cold buffer B. [^{32}P]DNA–protein complexes bound to the filters were analyzed on the dried filters in toluene/Omnifluor [4 g of Omnifluor (New England Nuclear) per liter of toluene].

Amino Acid Analyses. Analyses were performed with a Durram D-500 amino acid analyzer by the method of Spackman *et al.* (18). Half-cystine content was measured as cysteic acid after performic acid oxidation by the method of Moore (19). Tryptophan was determined after hydrolysis in 2% (vol/vol) mercaptoacetic acid (Pierce) (20).

Dephosphorylation of Phosphoprotein. Dephosphorylation reactions were conducted in 0.8 ml of 10 mM Tris·HCl, pH 8/5 mM $MgCl_2$ with 100 μg of phosphoprotein and 1.3 mg (10 units) of alkaline phosphatase-agarose (Sigma). The reaction tube was rotated gently at room temperature for 45 min, then centrifuged at 10,000 × *g* for 5 min. The upper supernatant solution containing the dephosphorylated protein was removed. The P_i content of this solution was determined by the method of Brunette *et al.* (21). When [^{32}P]phosphoprotein samples were dephosphorylated, the [^{32}P]P_i released was determined by measuring the radioactivity that passed through a dialysis membrane. Control reactions using agarose without covalently bound phosphatase did not hydrolyze detectable phosphate from the phosphoprotein.

Other Methods. Protein was determined by the method of Schaffner and Weissmann (22) or by estimation of the mass recovered from amino acid analyses. Purification of the phosphoprotein was monitored by analytical polyacrylamide gel electrophoresis in $NaDodSO_4$ (6, 23); the same method was used to determine the M_r of the phosphoprotein polypeptide subunits. The approximate native M_r of the purified phosphoprotein was estimated by Sephadex G-200 gel filtration (24). Total DNA from *Escherichia coli* and calf thymus DNA (Serva) were purified through CsCl gradients as described (13). DNA was determined by the modified diphenylamine reaction (13). Methods for competition experiments using RNA·DNA hybridizations have been described (10, 13). All buffers were adjusted to the indicated pH at room temperature.

RESULTS

Properties of the Phosphoprotein. The apparent native M_r of the phosphoprotein was 139,000, as determined by Sephadex G-200 chromatography with marker proteins of known molecular weight (data not shown). The subunit M_r of the phosphoprotein was approximately 70,000, as determined by gel electrophoresis in 0.1% $NaDodSO_4$ (6).

Table 1 shows the amino acid composition of the phosphoprotein. The amino acid composition of the phosphoprotein from *P. polycephalum* resembled that of protein C-14, derived from rat hepatoma cells by James *et al.* (25). The composition of protein C-14 is reproduced for comparison.

Association of Phosphoprotein with rDNP Complex. When isolated nucleoli from *P. polycephalum* were incubated in a phosphorylation mixture containing [γ-^{32}P]ATP and the polyamines, the rDNP complex isolated from these nucleoli had the ^{32}P-labeled 70,000 M_r phosphoprotein associated with it

Table 1. Amino acid analyses of the phosphoprotein from *P. polycephalum* and protein C-14 from Novikoff hepatoma ascites cells

Amino acid	*Physarum* phosphoprotein*		Novikoff hepatoma protein C-14†	
	Mol %	Residues/ 70,000 M_r	Mol %	Residues/ 70,000 M_r
Lys	8.3	55	7.8	50
His	3.2	21	1.2	8
Arg	4.2	28	4.5	29
Trp	0	0	0	0
Asx	11.0	73	11.9	76
Thr	4.5	30	6.9	44
Ser	7.2	48	7.8	50
Glx	16.2	107	13.9	89
Pro	4.1	27	4.2	27
Gly	12.2	81	9.2	59
Ala	7.7	51	7.8	50
½-Cys	0.2	1	1.4	9
Val	5.0	33	3.9	25
Met	0.8	5	2.2	14
Ile	3.5	23	4.4	28
Leu	7.4	49	7.5	48
Tyr	1.7	11	2.0	13
Phe	2.9	19	3.3	21
A/B‡		1.7		1.9

* Analyses were performed on samples hydrolyzed for 22, 24, 48, and 72 hr. Each datum is a composite of values for protein isolated from two different cultures. Appropriate extrapolations have been applied to correct for changes in amino acids recovered as a function of hydrolysis time. In general, variations in analysis of different isolates were within experimental error (±3–4%).

† From ref. 25.

‡ Ratio of acidic to basic amino acids.

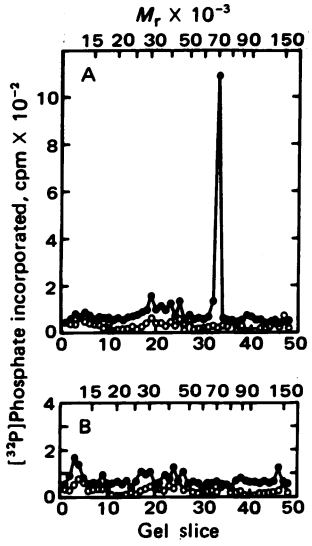

FIG. 1. Acidic phosphoproteins associated with the rDNP complex derived from nucleoli that had been phosphorylated with [γ-^{32}P]ATP in the presence (*A*) and absence (*B*) of the polyamines. Nucleoli were isolated in Percoll gradients from 50 surface cultures and were divided into two equal portions. (*A*) One portion of nucleoli was incubated for 60 min in a phosphorylation mixture containing [γ-^{32}P]ATP (3.18 X 10^6 cpm/μmol) and 0.33 mM each of putrescine, spermidine, and spermine (6). The nucleoli were collected by centrifugation at 1500 X *g* for 10 min and were solubilized in 0.5 ml of a buffered EDTA solution (10) containing 1 M urea/10 mM Na_2MoO_4. The soluble fraction and the insoluble nucleolar debris were separated by centrifugation at 3000 X *g* for 10 min. Total protein in the insoluble debris was solubilized by NaDodSO$_4$/phenol extraction (26), dialyzed (26), and fractionated by NaDodSO$_4$/polyacrylamide rod gel electrophoresis (○). The rDNP complex was isolated by sucrose gradient sedimentation from the nucleolar fraction that was soluble in buffered EDTA solution. The isolated rDNP complex was extracted with NaDodSO$_4$/phenol (26), dialyzed (26), and also fractionated on a polyacrylamide gel (●). (*B*) The second portion of nucleoli was similarly treated except that no polyamines were supplied to the phosphorylation mixture. Phosphoproteins associated with the rDNP complex (●) and the insoluble nucleolar debris (○) are shown. After electrophoresis, all gels were sectioned into 2-mm slices and solubilized in 30% H_2O_2, and radioactivity was measured.

(Fig. 1*A*). The rDNP complex derived from nucleoli similarly treated in a phosphorylation mixture lacking the polyamines did not contain any proteins that were appreciably labeled (Fig. 1*B*). Moreover, the insoluble nucleolar debris resulting from lysis of nucleoli to release the rDNP complex did not contain the [^{32}P]phosphoprotein in either of these two experiments (Fig. 1). Thus, the [^{32}P]phosphoprotein appeared to be associated with the rDNP complex in nucleoli and its phosphorylation was polyamine dependent in the phosphorylation reaction.

To ascertain whether the phosphoprotein could bind *in vitro* to the rDNP complex, we mixed purified [^{32}P]phosphoprotein (270 ng, 7440 cpm/μg) with the lysate from EDTA-solubilized nucleoli prepared from 25 surface cultures prior to purification of the rDNP complex by sucrose gradient centrifugation (10). Subsequent isolation of the rDNP complex in linear 15–40% (wt/vol) sucrose gradients showed that 55% of the radioactivity from the [^{32}P]phosphoprotein cosedimented with the rDNP complex (data not shown).

Effect of Phosphoprotein on Transcription by rDNP Complex. A marked stimulation of incorporation of [^{3}H]UMP into RNA synthesized by the rDNP complex was found when purified rDNP was preincubated with the phosphoprotein (Fig. 2*A*). The phosphoprotein itself had no RNA polymerase activity. Fig. 2*B* shows the percentage increase in transcription as a function of the amount of the phosphoprotein added. The

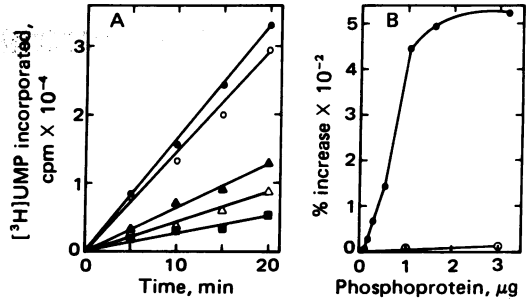

FIG. 2. Stimulation of *in vitro* transcription by the rDNP complex in the presence and absence of the phosphoprotein. Purified rDNP complex provided both RNA polymerase I activity and rDNA template for each assay. (*A*) Assay components included the "high UTP" assay mixture previously described (14) with the omission of calf thymus DNA template, [^{3}H]UTP (0.41 Ci/mmol) as the labeled nucleotide, approximately 0.4 μg of rDNA as the rDNP complex, and the following quantities of phosphoprotein: ■, control, no phosphoprotein; △, 0.26 μg; ▲, 0.53 μg; ○, 1.06 μg; ●, 3.18 μg. The reaction volume was 0.2 ml. (*B*) Stimulatory effect of increasing amounts of phosphoprotein (●) and no effect by the dephosphorylated acidic protein (○) after treatment of the phosphoprotein with alkaline phosphatase-agarose, which hydrolyzed 5.9 molecules of P_i per 70,000 M_r of protein.

dephosphorylated protein failed to stimulate transcription (Fig. 2*B*). Other proteins, including bovine serum albumin, β-galactosidase from *E. coli*, and casein, had no effect on the rate of transcription by the rDNP complex (not shown).

Characterization of [^{3}H]RNA Synthesized by rDNP When Stimulated by the Phosphoprotein. It was shown by competition hybridization experiments that more than 70% of the [^{3}H]RNA synthesized by the rDNP complex was complementary to those sequences of rDNA that coded for 19S and 26S rRNA (10). This result was corroborated by data shown in Fig. 3. To determine whether this fidelity of rRNA synthesis extended to the case of stimulated transcription in the presence of the phosphoprotein, similar hybridization studies were conducted. Fig. 3 shows that, indeed, 77% of the [^{3}H]RNA synthesized by the rDNP complex that was stimulated by the phosphoprotein could be competed for by unlabeled 19S and 26S rRNA from nitrocellulose filters containing bound rDNA.

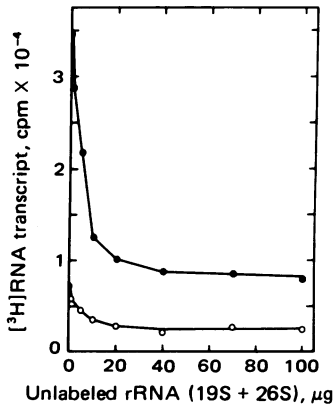

FIG. 3. Competition hybridization of [^{3}H]RNA synthesized *in vitro* by rDNP with unlabeled 26S and 19S rRNA. [^{3}H]RNA was synthesized *in vitro* (10) by equal amounts of the purified rDNP complex containing approximately 2 μg of rDNA in the absence (○) and presence (●) of 15 μg of the phosphoprotein. In each case, the synthesized [^{3}H]RNA was subsequently purified by phenol extraction after treatment with proteinase K (10). Nitrocellulose filters containing 10 μg of total nuclear DNA from *P. polycephalum* were hybridized in triplicate with 0.05 μg of the [^{3}H]RNA [6.80 X 10^5 cpm/μg (●) and 1.45 X 10^5 cpm/μg (○)] and the indicated amounts of an equimolar mixture of 19S and 26S rRNA.

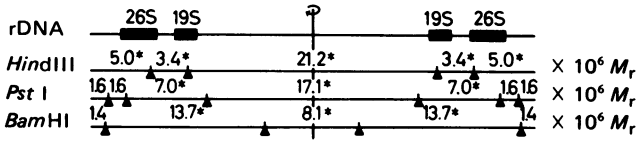

FIG. 4. Restriction endonuclease map of rDNA from *P. polycephalum*. The approximate M_r of DNA is 38×10^6. The top figure depicts the linear length of rDNA and the locations of the 19S and 26S rRNA coding sequences on the palindrome relative to the axis of symmetry (arrow and vertical line). The bottom three lines show the respective cleavage sites (▲) for restriction endonucleases *Hin*dIII, *Pst* I, and *Bam*HI. The number between cleavage sites above each fragment is the $M_r \times 10^{-6}$ of the respective fragment. Numbers followed by an asterisk represent the fragments that were tested in the protein binding assay.

Binding of Phosphoprotein to [^{32}P]rDNA and to ^{32}P-Labeled Restriction Endonuclease Fragments. The association of the phosphoprotein with the rDNP complex suggested that it might demonstrate DNA-binding properties. Its capacity to bind to purified [^{32}P]rDNA and to ^{32}P-labeled restriction endonuclease fragments derived from rDNA was tested by use of the nitrocellulose filter binding assay (17). The specific restriction fragments that were prepared and labeled by nick-translation are shown in Fig. 4.

The binding results are summarized in Table 2. DNA binding by the phosphoprotein occurred with intact rDNA (trial 1a). This binding was not altered by 3 mM or 10 mM magnesium acetate, 0.1 mM dithiothreitol, bovine serum albumin at 50 μg/ml, yeast RNA at 1 mg/ml, single-stranded DNA prepared from total *P. polycephalum* DNA at 0.3 mg/ml, or NaCl up to 0.4 M (data not shown). Higher concentrations of NaCl inhibited binding. Total binding capacity was abolished by 0.8 M NaCl. Hydrolysis of 6.3 moles of phosphate per 70,000 M_r of the phosphoprotein with alkaline phosphatase-agarose diminished binding of the protein to rDNA by 94% (trial 1b). That the phosphoprotein was not a nonspecific DNA-binding protein

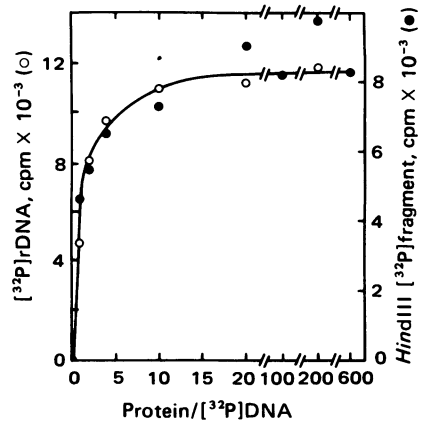

FIG. 5. Binding curves for variable additions of 70,000 M_r phosphoprotein to fixed amounts of ^{32}P-labeled rDNA (○) and 21.2×10^6 M_r *Hin*dIII restriction fragment (●). The molar ratio is shown on the abscissa. The DNA concentration for each type of DNA was 7.2×10^{-13} M. This value was determined by the [^{3}H]thymidine content in the rDNA introduced during growth of *P. polycephalum* plasmodia and the ratio of [^{3}H]thymidine to μg of DNA determined for nucleoplasmic DNA from the same plasmodia (13). The phosphoprotein concentration was determined by total amino acid mass recovered from amino acid analysis. Binding reactions were conducted in 0.10-ml volume at 25°C, and 0.01-ml samples were filtered in triplicate through nitrocellulose filters. Specific radioactivities for the ^{32}P-labeled DNAs are given in Table 2.

Table 2. Binding of phosphoprotein to [^{32}P]rDNA and restriction endonuclease fragments of [^{32}P]rDNA

Trial*	Triplicate filter set	Components in binding mixture†			Avg. net cpm per filter¶
		^{32}P-labeled rDNA or fragment‡	Unlabeled protein§		
			Phospho	Dephospho	
1	a	rDNA	+	−	11,682
	b	rDNA	−	+	689
	c	rDNA + 3 ng *E. coli* DNA	+	−	12,003
	d	rDNA + 3 ng calf thymus DNA	+	−	11,419
	e	rDNA + 3 ng total *Physarum* DNA	+	−	10,163
2	a	21.2×10^6 M_r fragment	+	−	7,240
	b	21.2×10^6 M_r fragment	−	+	566
3	a	17.1×10^6 M_r fragment	+	−	8,623
	b	17.1×10^6 M_r fragment	−	+	483
4	a	8.1×10^6 M_r fragment	+	−	9,491
	b	8.1×10^6 M_r fragment	−	+	788
5	a	3.4×10^6 M_r fragment	+	−	366
	b	3.4×10^6 M_r fragment	−	+	343
6	a	5.0×10^6 M_r fragment	+	−	305
	b	5.0×10^6 M_r fragment	−	+	330
7	a	7.0×10^6 M_r fragment	+	−	471
	b	7.0×10^6 M_r fragment	−	+	521
8	a	13.7×10^6 M_r fragment	+	−	433
	b	13.7×10^6 M_r fragment	−	+	412

* Nitrocellulose filters used in a given trial were prepared simultaneously prior to filtration (17).

† Binding reactions were conducted in buffer B. Binding mixtures containing [^{32}P]DNA without added protein served as the control for each trial. The reaction volume was 0.10 ml; 0.01-ml samples were filtered in triplicate. The amount of input DNA retained on the filters for different trials varied from 47 to 78%. However, within a given trial the variability among filters was <5%.

‡ Concentration, 7.2×10^{-13} M. Specific radioactivities as cpm/μg were: [^{32}P]rDNA, 8.4×10^7; 21.2×10^6 M_r fragment, 6.0×10^7; 17.1×10^6 M_r fragment, 1.1×10^7; 8.1×10^6 M_r fragment, 2.6×10^7; 3.4×10^6 M_r fragment, 5.0×10^7; 5.0×10^6 M_r fragment, 5.0×10^7; 7.0×10^6 M_r fragment, 1.2×10^7; and 13.7×10^6 M_r fragment, 1.2×10^7. See Fig. 4 for identification and location of fragments on the linear rDNA molecule.

§ Concentration, 7.2×10^{-11} M.

¶ Each value represents the average cpm from three different filters, calculated by subtracting the control of each trial that did not contain binding protein.

was indicated by the failure of *E. coli* DNA (trial 1c) or calf thymus DNA (trial 1d) to successfully compete against the binding of the phosphoprotein to [^{32}P]rDNA. Specificity toward rDNA, however, was not absolute. Total DNA from *P. polycephalum* competed 13% for the binding capacity of the phosphoprotein (trial 1e). This occurred when [^{32}P]rDNA and total unlabeled DNA, which is composed of 1–2% rDNA, were supplied at equal masses in the binding assay. Thus, total DNA from *P. polycephalum* appeared to contain significant regions of binding recognition by the phosphoprotein that could not be attributed to contaminating unlabeled rDNA present in the total DNA preparation.

Additional binding trials were conducted with restriction endonuclease fragments of rDNA (see Fig. 4) in order to determine the region of specificity for interaction between rDNA and the phosphoprotein. The phosphoprotein also bound to the large 21.2×10^6 M_r *Hin*dIII fragment (trial 2a), the 17.1×10^6 M_r *Pst* I fragment (trial 3a), and the 8.1×10^6 M_r *Bam*HI fragment (trial 4a). Dephosphorylation of the phosphoprotein reduced the binding capacity of the acidic protein to each of these fragments by more than 90%. The phosphoprotein or the dephosphorylated protein failed to exhibit any specific binding to the ^{32}P-labeled *Hin*dIII fragments of 3.4×10^6 M_r (trials 5a and 5b) and 5.0×10^6 M_r (trials 6a and 6b), to the *Pst* I fragment of 7.0×10^6 M_r (trials 7a and 7b), or to the *Bam*HI fragment of 13.7×10^6 M_r (trials 8a and 8b).

Only a small number of phosphoprotein molecules were required to bind to rDNA or the large *Hin*dIII restriction fragment in order to retain them on nitrocellulose filters (Fig. 5). More than 90% of either type of DNA was trapped on the filters at a ratio of 8 moles of 70,000 M_r phosphoprotein per mole of DNA.

DISCUSSION

Interest in the phosphoprotein described in this communication was initially provided by the observation that its phosphorylation in isolated nuclei and nucleoli was stimulated by the polyamines (6). The mechanisms that account for this apparent polyamine-dependent phosphorylation are not evident from our work. The amino acid composition of the phosphoprotein from *P. polycephalum* closely resembles that of the nucleolar protein C-14 isolated from Novikoff hepatoma acites cells (25). Within an experimental error of ±3–4%, the two proteins have identical compositions for 10 amino acids.

The phosphoprotein stimulated transcription of the ribosomal genes within the nucleolar rDNP complex. It bound with high affinity and specificity to a defined region of rDNA. Neither property was retained after dephosphorylation of the phosphoprotein. These findings argue in favor of a regulatory function for this protein. However, it is not known at this time whether binding of the phosphoprotein to rDNA was a prerequisite for stimulating transcription within the rDNP complex. Phosphorylation of the acidic protein in nuclei and nucleoli by a seemingly polyamine-dependent reaction is of interest in view of the proposal that the synthesis of the polyamines stimulates proliferating cell cultures to synthesize rRNA (for reviews see refs. 1, 2, and 5). There have been criticisms of this proposal (1, 2, 5). Whether the polyamine-mediated phosphorylation of specific nonhistone acidic proteins represents a mechanism in intact cells leading to a stimulation of rRNA gene transcription is uncertain. However, specific temporal scheduling of rRNA synthesis has been well defined in the cell cycle of synchronized plasmodia of *P. polycephalum* (27). An analysis of intervals during the cell cycle in which the 70,000 M_r protein is phosphorylated may provide valuable correlations to the study of this problem.

There were structural features common to all of the restriction endonuclease fragments of rDNA that bound to the phosphoprotein. All contained the symmetry axis for the rDNA palindrome plus regions rich in localized inverted-repeat sequences (8). These structures are all distally removed from the known coding regions for 19S and 26S rRNA. If the binding of the phosphoprotein to rDNA is indeed required for stimulating transcription of these gene sequences, there are the interesting questions of how this is achieved over such a large intranucleotide distance and what role the palindromes may have in this process.

This work was supported by Grants GM18538 from National Institutes of Health to G.D.K., 3.501.75 from the Swiss National Science Foundation to R.B., and National Research Service Award 1-F32-CA05199 from the National Cancer Institute to V.J.A. Partial salary and travel support were provided to G.D.K. by the Roche Research Foundation during a sabbatical leave at Bern.

1. Karpetsky, T. P., Hieter, P. A., Frank, J. J. & Levy, C. C. (1977) *Mol. Cell. Biochem.* **17**, 89–99.
2. Jänne, J., Pösö, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293.
3. Quigley, G. J., Teeter, M. M. & Rich, A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 64–68.
4. Sakai, T. T. & Cohen, S. S. (1976) *Prog. Nucleic Acids Res.* **17**, 15–42.
5. Tabor, C. W. & Tabor, H. (1976) *Ann. Rev. Biochem.* **45**, 285–306.
6. Atmar, V. J., Daniels, G. R. & Kuehn, G. D. (1978) *Eur. J. Biochem.* **90**, 29–37.
7. Stein, G. S. & Kleinsmith, L. J., eds. (1975) *Chromosomal Proteins and Their Role in the Regulation of Gene Expression* (Academic, New York).
8. Vogt, V. M. & Braun, R. (1976) *J. Mol. Biol.* **106**, 567–587.
9. Molgaard, H. V., Matthews, H. R. & Bradbury, E. M. (1976) *Eur. J. Biochem.* **68**, 541–549.
10. Seebeck, T., Stalder, J. & Braun, R. (1979) *Biochemistry* **18**, 484–490.
11. Mohberg, J. & Rusch, H. P. (1971) *Exp. Cell Res.* **66**, 305–316.
12. Kostraba, N. C., Montagna, R. A. & Wang, T. Y. (1975) *J. Biol. Chem.* **250**, 1548–1555.
13. Hall, L. & Braun, R. (1977) *Eur. J. Biochem.* **76**, 165–174.
14. Smith, S. & Braun, R. (1978) *Eur. J. Biochem.* **82**, 309–320.
15. Tabak, H. F. & Flavell, R. A. (1978) *Nucleic Acids Res.* **5**, 2321–2332.
16. Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1184–1188.
17. Riggs, A. D., Suzuki, H. & Bourgeois, S. (1970) *J. Mol. Biol.* **48**, 67–83.
18. Spackman, D. H., Stein, W. H. & Moore, S. (1958) *Anal. Chem.* **30**, 1190–1206.
19. Moore, S. (1963) *J. Biol. Chem.* **238**, 235–237.
20. Penke, B., Ferenczi, R. & Kovacs, K. (1974) *Anal. Biochem.* **60**, 45–50.
21. Brunette, M. G., Vigneault, N., Danan, G. & Bertrand, F. (1978) *Anal. Biochem.* **86**, 229–237.
22. Schaffner, W. & Weissman, C. (1973) *Anal. Biochem.* **56**, 502–514.
23. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
24. Andrews, P. (1964) *Biochem. J.* **91**, 222–233.
25. James, G. T., Yeoman, L. C., Matsui, S.-i., Goldberg, A. H. & Busch, H. (1977) *Biochemistry* **16**, 2384–2389.
26. LeStourgeon, W. M. & Beyer, A. L. (1977) in *Methods in Cell Biology*, eds. Stein, G., Stein, J. & Kleinsmith, L. J. (Academic, New York), Vol. 16, pp. 387–406.
27. Hall, L. & Turnock, G. (1976) *Eur. J. Biochem.* **62**, 471–477.