OPPOSING KINETIC EFFECTS OF AN ACIDIC NUCLEOLAR PHOSPHOPROTEIN FROM *PHYSARUM POLYCEPHALUM* ON HOMOLOGOUS AND HETEROLOGOUS TRANSCRIPTION SYSTEMS

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1. Introduction

Considerable information is now available indicating that the acidic non-histone chromosomal proteins may exert a vital role in the control of gene expression [1,2]. The phosphorylated non-histone chromosomal proteins in particular demonstrate characteristics that support their putative participation in gene modulation, such as a capacity to bind to DNA with resultant stimulation of transcription.

We reported on the isolation of a specific ribosomal DNA (rDNA)-binding phosphoprotein from nucleoli of *Physarum polycephalum*, which stimulated transcription of rRNA gene regions in a deoxyribonucleoprotein complex (rDNP), or minichromosome, containing the rRNA genes [3]. Many properties of this phosphoprotein suggest that it may exert a regulatory role in rRNA gene expression. Phosphorylation of the protein was demonstrated in nucleoli to be polyamine-dependent. Dephosphorylation of the phosphoprotein abolished its capacity to bind a specific region of the palindromic rDNA and to stimulate transcription [3].

In order to determine the generality of the mechanism by which this phosphoprotein stimulated transcription of the rRNA genes in the homologous rDNP complex, its capacity to modulate transcription by a heterologous RNA polymerase and other DNA templates was tested. Many investigations of chromatin transcription in vitro have used heterologous bacterial RNA polymerase as a probe of chromatin structure and component function ([4] and references therein). More specifically, bacterial RNA polymerase has been used to test potential regulatory properties of acidic phosphoproteins of the nucleus [5–8]. Here we show that the phosphoprotein from *P. polycephalum* demonstrated marked stimulatory capacity only within the homologous rDNP complex. It was a potent inhibitor of heterologous transcription systems. In both cases, the observed effect was dictated by the phosphorylated form of the protein. Neither the phosphorylated nor the dephosphorylated non-histone protein altered the transcriptive capacity of partially purified RNA polymerase I from *P. polycephalum*.

2. Materials and methods

The 70 000 M_r phosphoprotein was purified from *P. polycephalum* plasmodia and stored as in [3]. The [^{32}P]phosphoprotein was prepared by initial phosphorylation in intact isolated nuclei in a polyamine-dependent reaction [9] prior to isolation. RNA polymerase I activity in the rDNP complex and *Escherichia coli* RNA polymerase (EC 2.7.76) (Boehringer) were assayed using the 'high UTP' assay in [10]. [^{3}H]UTP (41 Ci/mmol) was from Amersham. The rDNP complex was isolated from nucleoli of *P. polycephalum* by EDTA solubilization of nucleolar chromatin as in [11]. RNA polymerase I from nucleoli of *P. polycephalum* was purified through the phosphocellulose column chromatography step [12]. Total DNAs from *E. coli* (Serva), calf thymus (Serva) and *P. polycephalum* were prepared and purified by CsCl density gradient centrifugation [13]. The 70 000 M_r phos-

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phoprotein was dephosphorylated by incubation with alkaline phosphatase–agarose (Sigma) [3]. When [^{32}P]phosphoprotein samples were dephosphorylated, the $^{32}P_i$ released was determined by measuring the radioactivity that passed through a dialysis membrane. Protein concentrations were determined as in [14].

3. Results and discussion

We have reported that 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 purified phosphoprotein. Moreover, 77% of the [^{3}H]RNA synthesized by the rDNP complex that was stimulated by the phosphoprotein could be competed for by unlabeled 19 S and 26 S rRNA from nitrocellulose filters containing bound rDNA [3]. Fig.1. confirms [3] in that the phosphoprotein used here also stimulated RNA synthesis by the rDNP complex to a maximum of 5.7-fold above the control experiment. Dephosphorylation of the phosphoprotein with alkaline phosphatase–agarose, which removed 5.4 molecules P_i/70 000 M_r of protein, abolished its capacity to stimulate transcription.

The mechanism by which the phosphoprotein interacted with other elements of rDNP complex to enhance the transcription of the rRNA gene regions is entirely unknown. The phosphoprotein could enhance transcription by:

(i) Direct interaction with RNA polymerase I;
(ii) Altering the nucleosome structure [15] through complexation with histones [16] and their removal from the rDNP complex;
(iii) Changing the chromatin organization after recognition of a specific rDNA-binding site.

That the phosphoprotein indeed recognized a relatively specific region of rDNA has been shown from binding experiments with nick-translated restriction endonuclease fragments of rDNA [3]. It was thought that possibilities (ii) and (iii) might be reflected in a detectable change in the sedimentation properties of the minichromosome upon addition of the purified phosphoprotein to the rDNP complex. Fig.2. shows that the rDNP complex did not undergo any detectable change in sedimentation rate when the minichromosome was saturated with excess purified [^{32}P]phosphoprotein prior to sedimentation in a sucrose gradient. Thus it can be concluded that if enhanced rRNA gene transcription by the phosphoprotein involved complexation with and removal of histones from the minichromosome, the amount of histone removed was not extensive. Similarly, it would appear that there was no accompanying, gross alteration of the chromatin structure that could be detected by the method in fig.2.

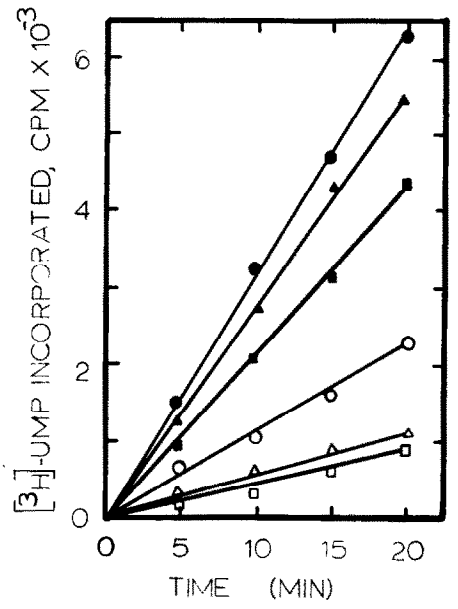

Fig.1. Stimulation of in vitro transcription by the rDNP complex in the presence and absence of the phosphoprotein. Purified rDNP complex provided both RNA polymerase I activity and rDNA template for each assay. Assay components included the 'high UTP' assay mixture [10] with the omission of calf thymus DNA template, [^{3}H]UTP (0.41 Ci/mmol) as the labeled nucleotide, ~0.4 μg rDNA as the rDNP complex, and the following quantities of phosphoprotein: (△) control, no phosphoprotein; (○) 0.50 μg; (■) 0.75 μg; (▲) 1.0 μg; (●) 3.0 μg. The time course plot (□) contained 3.0 μg dephosphorylated phosphoprotein prepared by treatment of the phosphoprotein with alkaline phosphatase–agarose that hydrolyzed 5.4 molecules P_i/70 000 M_r protein.

To ascertain whether the phosphoprotein might interact with a heterologous RNA polymerase to yield enhanced transcription generally, its effect on *E. coli* RNA polymerase was examined. Fig.3. shows that regardless of the origin of the DNA template provided to an in vitro transcription assay, the phosphoprotein was a potent inhibitor. Dephosphorylation of the phosphoprotein diminished the inhibitory effect by ⩾75%. Other proteins such as bovine serum

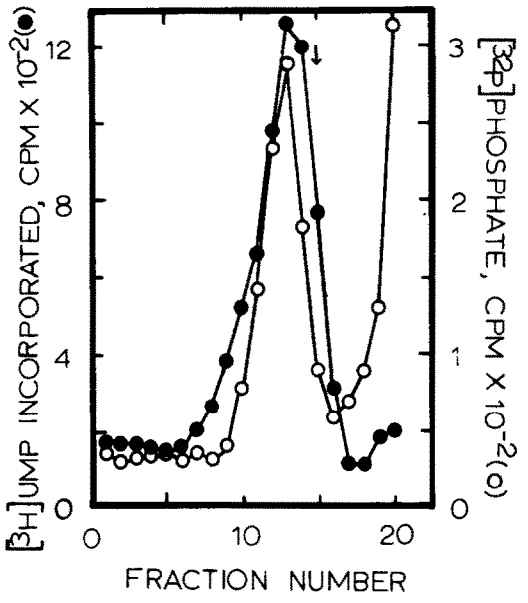

Fig.2. Binding of [^{32}P]phosphoprotein by the rDNP complex in a sucrose gradient. Purified [^{32}P]phosphoprotein (270 ng, 7,440 cpm/μg) was mixed with the lysate from EDTA-solubilized nucleoli prepared from 25 surface cultures prior to purification of the rDNP complex by sucrose gradient centrifugation [11]. Subsequent isolation of the rDNP complex (•) in a linear 15–40% (w/v) sucrose gradient showed that 55% of the radioactivity from the [^{32}P]phosphoprotein (○) cosedimented with the rDNP complex. The rDNP complex was located by assaying 10 μl samples from each fraction for RNA polymerase I activity in the presence of 1 μg/ml of α-amanitin. The [^{32}P]phosphoprotein was located by counting 0.20 ml samples of each gradient fraction in 10 ml scintillation cocktail [9]. Simultaneous isolation of the rDNP complex without the addition of [^{32}P]phosphoprotein yielded profiles virtually identical to that shown. The arrow indicates the position of Herpes simplex viral DNA as a marker [11].

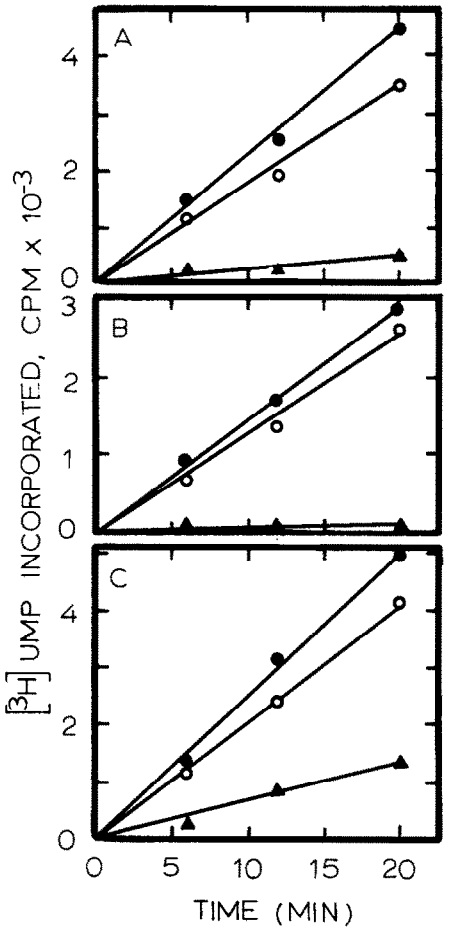

Fig.3. Effect of the 70 000 M_r phosphoprotein and the dephosphorylated phosphoprotein from *Physarum polycephalum* nucleoli on *Escherichia coli* RNA polymerase provided with template DNA from: (A) *P. polycephalum*; (B) calf thymus; (C) *E. coli*. The assay components required for in vitro transcription were the same as those in fig.1 with the modifications that each reaction contained: 4.7 μg *E. coli* RNA polymerase; 5 μg DNA template; no rDNP complex added. Symbols denote the additions of: (•) control, no phosphoprotein or dephosphoprotein; (○) 1.0 μg dephosphoprotein; (▲) 1.0 μg phosphoprotein.

albumin, ovalbumin, and β-galactosidase did not affect this transcription assay.

Neither the phosphoprotein nor its dephosphorylated form altered the capacity of partially purified RNA polymerase I from *P. polycephalum* to transcribe heterologous DNA templates (fig.4). In [3] we observed that *E. coli* DNA, calf thymus DNA, and total DNA from *P. polycephalum* did not physically combine with the phosphoprotein. Thus, it can be concluded indirectly, that the phosphoprotein interacted with *E. coli* RNA polymerase to exert its inhibitory property, but that the phosphoprotein has no such effect on purified RNA polymerase I which is not incorporated in the rDNP complex. This report provides no definite indication of how the phosphoprotein promotes transcription within the rDNP complex. The results here and those in [3], however, do suggest that the enhancement requires interaction between the phosphoprotein and a binding region near the symmetry axis of palindromic rDNA. Moreover, the opposing kinetic effects observed for this phosphoprotein in homologous versus heterologous transcription assays, demonstrate the need

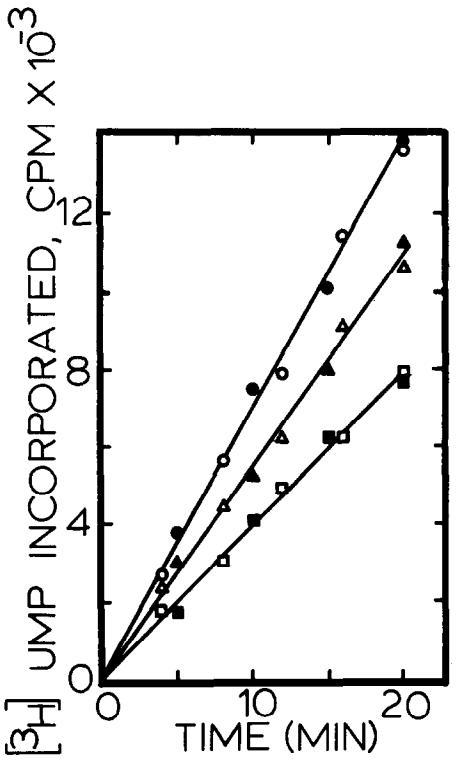

Fig.4. Effect of the 70 000 M_r phosphoprotein from *P. polycephalum* nucleoli on partially purified RNA polymerase I provided with template DNA from: (○, ●) *P. polycephalum* nuclei; (△, ▲) calf thymus; (□, ■) *E. coli*. The assay components were the same as those in fig.3, except that 10 μg RNA polymerase I preparation was added to each assay instead of the enzyme from *E. coli*. Symbols denote the additions of: (○, △, □) 1 μg phosphoprotein; (●, ▲, ■) controls, no additions of phosphoprotein.

for future caution. This example clearly illustrates the ambiguities that will arise by continued use of heterologous transcription systems in attempts to identify potential regulatory proteins among the acidic phosphoproteins of the nucleus and nucleolus.

Acknowledgements

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