EFFECT OF NUCLEAR PROTEIN KINASES ON MAMMALIAN RNA SYNTHESIS

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SUMMARY

A protein kinase from rat liver nuclei stimulates RNA synthesis. The stimulatory effect is seen primarily with nucleolar RNA polymerase and only when rat liver DNA is the template. Phosphorylation of RNA polymerase appears to be another mechanism by which protein kinases regulate gene expression.

INTRODUCTION

The regulation of gene expression in mammalian cells is complex and poorly understood. Phosphorylation of histone with subsequent de-repression of gene activity appears to be an important regulatory mechanism in DNA transcription (1,2). Histone acetylation may have a similar role (3).

Recently, there has been renewed interest in the role of the non-histone chromatin proteins in RNA synthesis (4,5,6). It has been shown that phosphorylated proteins are a major component of these acidic nuclear proteins (7). Their phosphorylation is mediated by multiple nuclear protein kinases the properties of which have been outlined in several recent reports (8,9,10).

We have considered the possibility that nuclear protein kinases may control transcription through direct phosphorylation of RNA polymerase. In this communication we wish to report the effect of a nuclear protein kinase on RNA synthesis in rat liver.

METHODS

Isolation of nuclear polymerases and protein kinases: Rat liver nuclear polymerase I (nucleolar) and II (nucleoplasmic) were isolated as previously

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described (11) (Fig. 1). Nuclear protein kinases I and II were isolated simultaneously from the same DEAE-Sephadex column (Fig. 1). Further

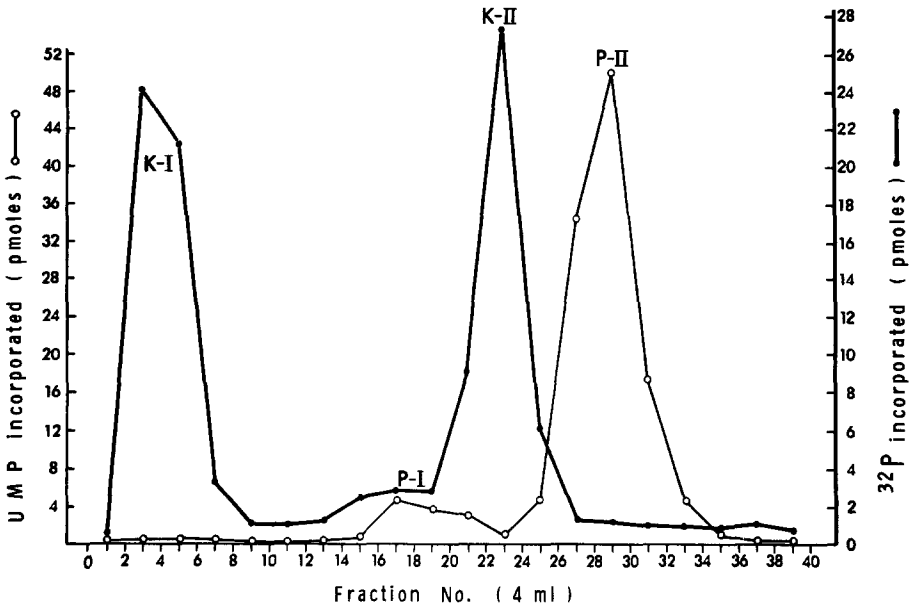

Figure 1. Rat liver nuclei were isolated and processed as previously described (11). Approximately 45mg of protein was loaded onto a DEAE-Sephadex column previously equilibrated with TGMED (0.05M Tris-HCl pH 7.9, 25% glycerol, 5mM $MgCl_2$, 0.1mM EDTA and 0.5mM dithiothreitol). The column was washed until a protein peak had been eluted and then further eluted with 2 bed-volumes of a linear gradient of 0.05 to 0.8M $(NH_4)_2SO_4$ in TGMED. Fractions were assayed for protein kinase and polymerase activity as described in METHODS.

purification of protein kinase I was carried out by chromatography on a phosphocellulose column as described previously (10). This step separates protein kinase from other acidic nuclear proteins.

Enzyme Assays: Reaction mixtures for RNA polymerase assay contained (0.2 ml): 50 mM Tris-HCl pH 7.9, 0.8mM ATP, GTP, and CTP, 2μM [^{3}H]-UTP (non-uniform label, sp. activity 20Ci/mmole), 5mM dithiothreitol, 5mM $MgCl_2$, 2mM Mn Cl_2, 20mg calf thymus or rat liver DNA and RNA polymerase. Reaction mixtures were incubated at 37° for 20 minutes. Samples were processed as described previously (12).

Protein kinase activity was determined by the incorporation of [^{32}P] from γ-[^{32}P]-ATP into CCl_3COOH precipitable material. The reaction mixture contained (0.2 ml); 50mM Tris-HCl pH 7.0, 0.2mM EDTA, 0.3mM EGTA, 1mM Mg acetate, 0.1mM ATP, γ-[^{32}P]-ATP (specific activity 5Ci/mmole) 400 mg casein and protein kinase. The reaction mixture was incubated at 37° for 20 minutes and samples processed as described previously (12,13).

RESULTS

The selective inhibition of polymerase II but not of polymerase I by the mushroom toxin α-amanitin was used to identify the polymerases (11) (not shown). At a concentration of .025µg/ml, α-amanitin completely inhibits polymerase II whereas polymerase I is unaffected.

At a concentration of 1mM, dibutyryl-cyclic AMP (DBC) greatly stimulates RNA synthesis (Table 1). This suggested that cyclic AMP directly or indirectly stimulates transcription. Similar stimulation was seen using rat liver chromatin as a source of RNA polymerase (unpublished observations).

Kinase I stimulated polymerase I predominantly (3-fold) at a concentration of 60µg per assay tube. Polymerase II is stimulated by about 50%. The stimu-

TABLE 1. EFFECT OF DBC ON ENDOGENOUS NUCLEAR RNA SYNTHESIS

ADDITIONS	UMP INCORPORATED (pmoles)
None	0.23
Nuclei	19.7
Nuclei + DBC (1mM)	28.8

The reaction mixture contained (0.2ml): 50mM Tris-HCl pH 7.9, 2mM mercaptoethanol, 5 x 10^{-4} M ATP, GTP, CTP, 1µM [^{3}H]-UTP (200µCi/ml), 0.4mM KHP04 Buffer, pH 7.4, 8mM KCl, 2mM MnCl2, 2mM $MgCl_2$, 1mM DBC and 30 mg rat liver nuclei. After incubation, samples were proced as described in METHODS.

lation of polymerase I and II using rat liver and calf thymus DNA is shown in Table 2. Kinase I, with no endogenous polymerase activity, stimulated the incorporation of UMP into RNA by polymerase I. The stimulation was seen only when rat liver DNA was the template.

As shown in Fig. 2, maximal stimulation of polymerase I is observed

TABLE 2. EFFECT OF KINASE I ON RNA SYNTHESIS

	UMP INCORPORATED (pmoles)			
	RAT LIVER DNA		CALF THYMUS DNA	
ADDITIONS	NATIVE	DENATURED	NATIVE	DENATURED
Polymerase I	1.4	5.0	3.2	7.0
Polymerase I + Kinase I	5.6	7.0	3.0	7.9
Polymerase I + Kinase II	2.7	5.3	3.0	6.6
Polymerase II	14	60	21	52
Polymerase II + Kinase I	20	66	20	63
Polymerase II + Kinase II	14	57	18	63

The reaction mixture for RNA polymerase assay was identical to that described in METHODS except as follows (0.2ml): 60µg kinase I, II, 20µg polymerase I and II and 20 mg calf thymus and rat liver DNA.

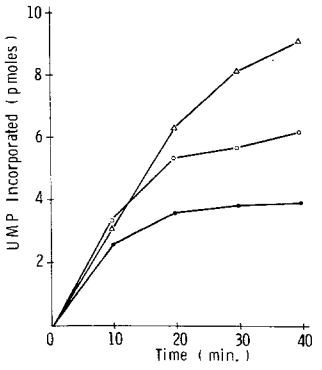

Figure 2. The reaction mixture for RNA polymerase assay was identical to that described in METHODS except as follows: Rat liver DNA was used as template, RNA polymerase activity alone (•——•) and 60µg of protein kinase was added at 0 time (Δ----Δ) and 10 minutes after initiation (O----O).

when kinase I is added before incubation (Δ-Δ), suggesting an effect on initiation of RNA synthesis.

The stimulation of RNA synthesis by Kinase I suggests that a phosphorylation of RNA polymerase may be occurring. This possibility was tested employing γ-[^{32}P]-ATP as substrate. The incorporation of [^{32}P] into acid-precipitable material using the partially purified polymerase I and II as acceptors is

TABLE 3. PHOSPHORYLATION OF POLYMERASES I and II by KINASE I

ADDITIONS	^{32}P INCORPORATED (pmoles)
None	0.15
Kinase I	0.30
Polymerase I	3.2
Polymerase II	0.0
Polymerase I + Kinase I	6.1
Polymerase I + Kinase I + Cyclic AMP	9.0
Polymerase II + Kinase I	3.0
Polymerase II + Kinase I + Cyclic AMP	6.5
Kinase I + Casein	66.0
Kinase I + Histone	1.2
Boiled Kinase I + Casein	2.7

The reaction mixture for kinase assay was identical to that described in METHODS except as follows (0.2ml): 60µg kinase I, 80mg casein, 80mg histone (lysine-rich), 20mg RNA polymerase I and II and 2µM cyclic AMP.

shown in Table 3. Kinase I phosphorylates casein but not histone. It also phosphorylates polymerases I and II. Kinase II does not phosphorylate either polymerase by phosphorylates casein. As assayed here, these nuclear kinases are partially cyclic AMP-dependent.

Further studies of [^{32}P] labeled polymerase were made by polyacrylamide gel electrophoresis of the radioactive polymerase I. Unstained gels were sliced and soaked in buffer for polymerase activity and [^{32}P] elution as described in Table 4. Slice 12 contains the phosphorylated polymerase I.

RNA polymerase phosphorylation was further investigated by intraperitoneal

TABLE 4. ELUTION OF ^{32}P-POLYMERASE FROM POLYACRYLAMIDE GEL

SLICE #	UMP INCORPORATED(cpm)	^{32}P(cpm)
1	158	400
2	150	462
3	180	790
4	160	478
5	175	431
6	180	469
7	160	472
8	250	490
9	320	490
10	200	486
11	160	370
12	550	1680
13	178	387
14	201	456
15	223	382
16	205	287
17	280	406
18	159	301
19	150	236
20	150	240

Unstained sliced (1mm) polyacrylamide gels were soaked at 4°C overnight in 50mM Tris-HCl pH 7.9, 25% glycerol, 5mM $MgCl_2$, 0.1mM EDTA and 0.5mM Dithiothreitol (0.1ml). The eluate (50µl) was assayed for polymerase activity and counted for [^{32}P].

injection of rats with [^{32}P] and DBC as described in the legend to Fig. 3, followed by isolation of RNA polymerase. The results are shown in Fig. 3. The [^{32}P] radioactivity corresponds to polymerase I but not exactly with polymerase II. A phosphorylation of one of the two forms of polymerase II (11) could explain these results.

DISCUSSION

The results reported here show that the nucleolar RNA polymerase of rat liver is stimulated by a homologous nuclear protein kinase. The phosphorylation of a contaminating protein in the partially purified polymerase pre-

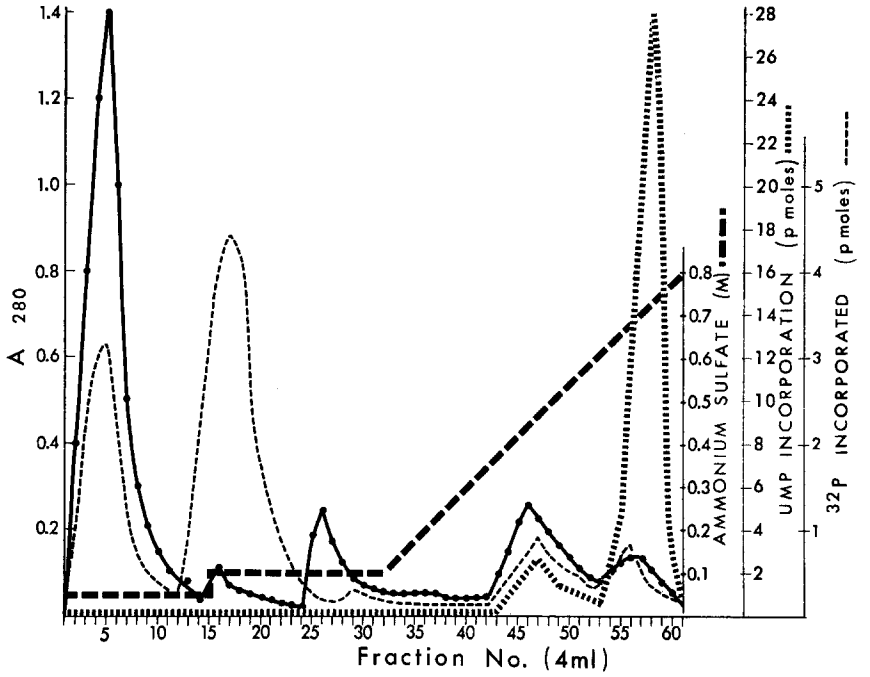

Figure 3. Rats (80gms) were injected intraperitoneally with 1mCi of [^{32}P] (pH 7.0) in 0.14 M NaCl containing 0.1mM carrier phosphate and 10mg of DBC. The rats were sacrificed 90 minutes later followed by RNA polymerase isolation as described in METHODS. Fractions were assayed for polymerase activity (▬▬) and counted for [^{32}P] (----).

paration cannot be ruled out. However, the stimulation of RNA synthesis by kinase I does suggest that a phosphorylating mechanism is directly or indirectly involved in transcription in mammalian cells. Furthermore, the stimulatory effect seen only with rat liver DNA as template suggests that protein kinases play an important role in DNA transcription in mammalian cells. In E. coli cells, we have previously shown that phosphorylation of the σ-factor may have a role in transcription in micro-organisms (12,14).

The possibility that RNA polymerase in mammalian cells may be under control of cyclic AMP and protein kinase is of great interest since many hormones elevate cyclic AMP in various organs with the subsequent increase in RNA synthesis and protein (15,16).

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