MODE OF ACTION OF NON-HISTONE PROTEINS IN THE STIMULATION OF TRANSCRIPTION FROM DNA

N. C. Kostraba, R. A. Montagna and T. Y. Wang

Division of Cell and Molecular Biology
State University of New York at Buffalo
Buffalo, New York
14214

Received July 9,1976

SUMMARY: The non-histone chromosomal proteins of Ehrlich ascites tumor chromatin contain an active fraction which stimulates specific transcription from DNA *in vitro*. This non-histone protein fraction functions by binding selectively to the unique sequences in DNA and activating the initiation of transcription of these DNA sequences.

Teng, Teng and Allfrey (1) and Shea and Kleinsmith (2) first described a non-histone phosphoprotein fraction from rat liver nuclei that stimulates transcription from DNA *in vitro*. Subsequently, a similar fraction was isolated from the non-histone chromosomal proteins that are loosely bound in the chromatin of Ehrlich ascites tumor cells (3). The active non-histone protein fraction contains 0.9% phosphorus, binds selectively to homologous DNA and enhances transcription, preferentially in a eukaryotic RNA polymerase II system. Hence, it has been suggested (1, 3) that the non-histone proteins stimulate the initiation of transcription by interaction with specific DNA sequences. In this communication, we present evidence supporting this postulated mode of action for the non-histone phosphoproteins in transcription.

EXPERIMENTAL PROCEDURE

The activator non-histone protein fraction (NHP) was isolated from the 0.35 M NaCl-soluble proteins of Ehrlich ascites tumor chromatin by selective DNA-binding as described previously (3). Homologous DNA was prepared from Ehrlich ascites tumor nuclei (3) and sheared in a French press at 30,000 p.s.i. to approximately 500-600 nucleotides. The DNA fragments were fractionated into low C_ot (<0.2), middle C_ot (0.2-100) and high C_ot (>850) following the hydroxyapatite technique of Kohne and Britten (4). The DNA-binding assay of the NHP was essentially that of Riggs *et al.* (5) as modified by Johnson *et al.* (6) using B6 membrane filters (Schleicher and

Schuell). Purification of RNA polymerase II from Ehrlich ascites tumor cells and assay were reported elsewhere (7). Initiation of RNA synthesis in vitro was determined by following the incorporation of [γ-^{32}P]ATP and [γ-^{32}P]GTP into RNA as described previously (8).

RESULTS AND DISCUSSION

The DNA-dependent RNA polymerase II purified from Ehrlich ascites tumor cells catalyzes RNA synthesis initiated with ATP and GTP (7). When the NHP was added to this homologous RNA synthesizing system, it stimulated the incorporation of [γ-^{32}p]ATP into RNA, as shown in Fig. 1. It can also be seen that RNA synthesis initiated with [γ-^{32}P]GTP was not affected by the NHP. The average chain length of the synthesized RNA product was not changed as a result of the enhanced RNA initiation with [γ-^{32}P]ATP by the NHP. These data indicate that the NHP stimulates RNA synthesis initiated only with ATP, suggesting selective recognition of certain sites on DNA by the NHP.

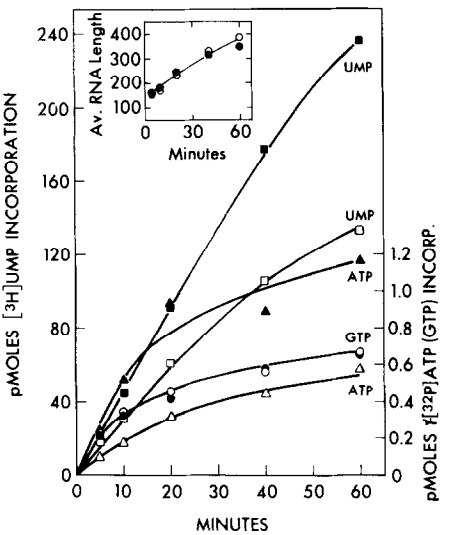

Fig. 1. Stimulation of RNA synthesis and chain initiation by NHP in Ehrlich ascites tumor RNA polymerase II reaction. The reaction mixture was in 0.25 ml and contained: Tris-HCl, pH 7.9, 10 µmoles; $MnCl_2$, 1.15 µmoles; $(NH_4)_2SO_4$, 12.5 µmoles; EDTA, 0.17 µmole; β-mercaptoethanol, 1.0 µmole; ATP, GTP, CTP and [^{3}H]UTP, 0.0625 µmoles each, the ATP and GTP used were either [γ-^{32}P]ATP or [γ-^{32}]GTP in two separate reaction mixtures; Ehrlich ascites tumor DNA, 10 µg; Ehrlich ascites tumor RNA polymerase II, 2 µg; and with (filled symbols) or without (open symbols) 10 µg of NHP as indicated. Isolation of the isotopically labeled RNA product and counting were described elsewhere (8).

The stimulatory effect of the NHP on DNA-directed RNA polymerase reaction was not the result of a prior interaction between the NHP and the enzyme. Repeated attempts failed to reveal the formation of a NHP-enzyme complex either by glycerol gradient centrifugation or by gel filtration. This result further supports a specificity for the NHP in its interaction with DNA.

To determine that the NHP binds to, and initiates transcription of specific sequences in DNA, DNA fragments of varying reiterated sequences

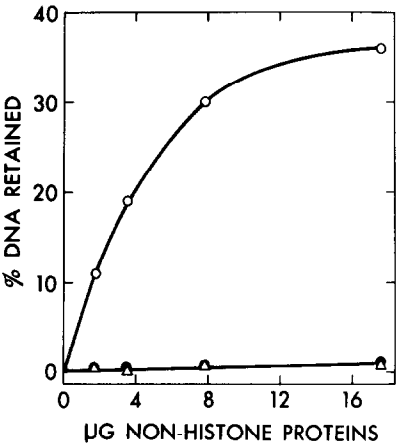

Fig. 2. Binding of the NHP to various C_0t DNAs. One µg of [^{125}I]-labeled low C_0t (<0.02) (●—●), or middle C_0t (0.02-100) (Δ—Δ), or high C_0t (>850) (O—O) DNA was interacted with various amounts of the NHP as indicated and assayed by the retention of the NHP-DNA complex on nitrocellulose filters as described by Johnson et al. (6).

were prepared and used for binding studies with the NHP. As shown in Fig. 2, the NHP binds only to unique DNA sequences of C_0t values >850. Highly reiterated (C_0t<0.02) and moderately reiterated (C_0t = 0.02-100) DNAs were ineffective in interacting with the NHP. To ascertain this binding result, binding assays of the NHP with high C_0t DNA were carried out in the presence of increasing amounts of low C_0t, middle C_0t and high C_0t DNAs. The result, shown in Fig. 3, indicates that the middle C_0t and low C_0t DNAs do not compete

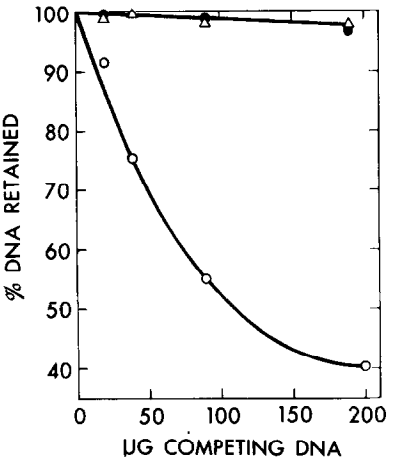

Fig. 3. Retention of NHP-high C_0t DNA complex nitrocellulose filters in the presence of low C_0t (●—●), or middle C_0t (△—△), or high C_0t (○—○) DNA. Two µg of the NHP were interacted with various amounts of the competing DNAs as shown, and subsequently reacted with 20 µg of [^{125}I]-labeled high C_0t DNA. The percentages of radioactive DNA retained on the filters after competition with various amounts of different C_0t DNAs are shown on the ordinate scale.

Table I

Effect of the NHP on the initiation and synthesis of RNA

in vitro templated by different C_0t DNAs

Template	Incorp. of radioactive nucleotides into RNA (pmoles)		
	[^{3}H]UMP	[γ-^{32}P]ATP	[γ-^{32}P]GTP
Low C_0t DNA	97.6	--	--
+ NHP	96.3	--	--
Middle C_0t DNA	83.8	--	--
+ NHP	81.1	--	--
High C_0t DNA	98.7	0.37	0.39
+ NHP	151.2	0.60	0.43

The reaction mixtures and assay conditions were the same as in Fig. 1 except that different C_0t DNAs were used as templates in place of intact DNA.

with the binding between the NHP and high $C_o t$ DNA, while high $C_o t$ DNA is an effective competitor. We conclude from these results that binding of the NHP to DNA is specific, involving only unique sequences in DNA.

If the selective binding of the NHP to unique DNA sequences reflects its specificity in the stimulation of transcription from DNA, one should expect that the NHP stimulates transcription of only high $C_o t$ DNA. Moreover, the stimulation in transcription should be manifested in the initiation of RNA chain, resulting in RNA synthesis initiated only with ATP. Such has been found to be the case, as shown in Table 1. It can be seen that the NHP has no effect on transcription of low $C_o t$ and middle $C_o t$ DNAs. The stimulated RNA synthesis templated by high $C_o t$ DNA is characterized by initiation with [γ-^{32}P]ATP, but not with [γ-^{32}P]GTP. These data thus indicate that the NHP binds specifically to the structural genes and activates these genes by promoting the initiation of their transcription.

ACKNOWLEDGEMENT

This work was supported in part by a research grant from the National Foundation-March of Dimes.

REFERENCES

1. Teng, C. S., Teng, C. T. and Allfrey, V. G. (1971) J. Biol. Chem., 246, 3597-3609.

2. Shea, M. and Kleinsmith, L. J. (1973) Biochem. Biophys. Res. Commun., 50, 473-477.

3. Kostraba, N. C., Montagna, R. A. and Wang, T. Y. (1975) J. Biol. Chem., 250, 1548-1555.

4. Kohne, D. E. and Britten, R. J. (1971) Procedures in Nucleic Acid Research, 2, 500-512.

5. Riggs, A. D., Bourgeois, S., Newby, R. N. and Cohn, M. (1968) J. Mol. Biol., 34, 365-368.

6. Johnson, E. M., Hadden, J. W., Inoue, A. and Allfrey, V. G. (1976) Biochemistry, 14, 3873-3884.

7. Chan, J. H. Y., Loor, R. M. and Wang, T. Y. (1976) Arch. Biochem. Biophys., 173, 564-576.

8. Kostraba, N. C. and Wang, T. Y. (1975) J. Biol. Chem. 250, 8938-8942.