TISSUE-SPECIFICITY OF THE NONHISTONE PROTEIN THAT INHIBITS RNA SYNTHESIS *IN VITRO*

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SUMMARY

The nonhistone protein which inhibits RNA synthesis *in vitro* has been isolated from calf thymus chromatin. The calf thymus protein preferentially inhibits transcription of homologous DNA and acts at the initiation step of RNA synthesis, similar to the previously reported Ehrlich ascites tumor inhibitor protein. However, the electrophoretic mobility and subunit composition of the calf thymus inhibitor nonhistone protein differ from those of the tumor protein. These results indicate that the inhibitor nonhistone chromosomal protein is tissue-specific, thus supporting a regulatory role for the protein in gene control.

We reported previously (1) the isolation of a nonhistone protein from Ehrlich ascites tumor chromatin that inhibits transcription of DNA *in vitro*. The tumor inhibitor nonhistone protein (I-NHP) is a phosphoprotein, consisting of a single polypeptide of molecular weight of 11,000. It acts on DNA by inhibiting the initiation of its transcription. The mode of action of the I-NHP is such that it binds only to reiterated sequences in DNA and inhibits transcription of these sequences (2). These observations suggest a regulatory role for the I-NHP in the control of gene activity and led us to inquire the possible tissue specificity of the I-NHP. In the work reported here, we isolated I-NHP from calf thymus chromatin and compared some of its properties with the tumor I-NHP. The results thereof show that the I-NHP isolated from calf thymus differs from the tumor protein described earlier (1,2), indicating tissue specificity for the I-NHP.

EXPERIMENTAL PROCEDURE

Calf thymus nuclei were isolated by the procedure of Allfrey et al (3) and further purified by centrifugation through 2.2 M sucrose (4). Chromatin

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was prepared from the purified nuclei by the procedure of Seligy and Miyagi (5). In subsequent steps, phenylmethylsulfonyl fluoride was supplemented to all the solutions to 0.1 mM. The chromatin was extracted with 2.0 M NaCl-0.02 M Tris-HCl, pH 8.0 by stirring overnight. The suspension was centrifuged at 105,000 g for 2 hr and supernatant from this centrifugation was dialyzed against 13 volumes of 0.01 M Tris-HCl, pH 8.0. The precipitated DNA-protein complex was collected and extracted with 0.4 N H_2SO_4 to remove histones. I-NHP was isolated from the de-histonized DNA-protein complex by phenol extraction according to the procedure of Teng et al. (6). Details of the complete procedure are given elsewhere (1).

RNA polymerase II was prepared from calf thymus nuclei by the method of Kedinger and Chambon (7) through the phosphocellulose step, followed by purification on a BioGel A-15m column. The assay reaction mixture, in a total volume of 0.25 ml, contained: Tris-HCl, pH 7.9, 10 μmole; $MgCl_2$, 1.15 μmole; $(NH_4)_2SO_4$, 12.5 μmoles; EDTA, 0.017 μmole; β-mercaptoethanol, 1.0 μmole; ATP, CTP and GTP, 62.5 nmoles each; [^{3}H]UTP, 6.25 nmoles (1.0 μCi); native calf thymus DNA, 5 μg; and the enzyme. Incubation was at 37° for 60 min. Processing of acid-insoluble precipitate and radioactivity counting were as described previously (1).

Initiation of RNA chain was determined by incorporation of [γ-^{32}P]ATP and [γ-^{32}P]GTP into RNA using the RNA polymerase assay mixture except that all the ribonucleoside triphosphates were 62.5 nmoles each and DNA, 10 μg. The radioactive RNA product was isolated by phenol extraction and counted. Details of the procedure have been reported previously (1).

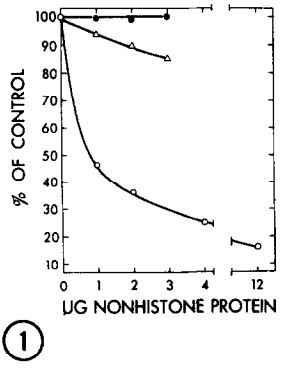
Velocity sedimentation was performed in a Spinco model E analytical ultracentrifuge and equilibrium ultracentrifugation was carried out in a 12 mm 6-channel external loading cell with sapphire windows according to Roark and Yphantis (8).

Polyacrylamide gel electrophoresis of I-NHP in the presence of sodium dodecylsulfate was performed by the method of Laemmli (9), and that in the absence of the detergent, by the procedure of Krakow (10).

RESULTS AND DISCUSSION

Fig. 1 shows the effect of the calf thymus I-NHP on RNA synthesis in the RNA polymerase II reaction templated by DNAs prepared from calf thymus, Ehrlich ascites tumor and Øe phage. Comparison of the three DNA templates for the effect of calf thymus I-NHP indicates that, with heterologous DNAs, calf thymus I-NHP either moderately inhibits transcription (Ehrlich ascites tumor DNA) or had no effect at all (Øe DNA). Transcription of homologous calf thymus DNA, however, was strongly inhibited by calf thymus I-NHP in proportion to protein concentration.

Like the tumor I-NHP, calf thymus I-NHP inhibits RNA synthesis also at the initiation step. This is shown by results of the time course experiment in Fig. 2. It can be seen that incorporation of both [γ-^{32}P]ATP and



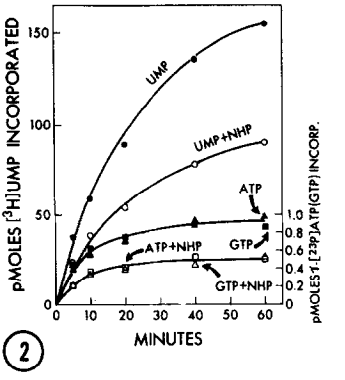
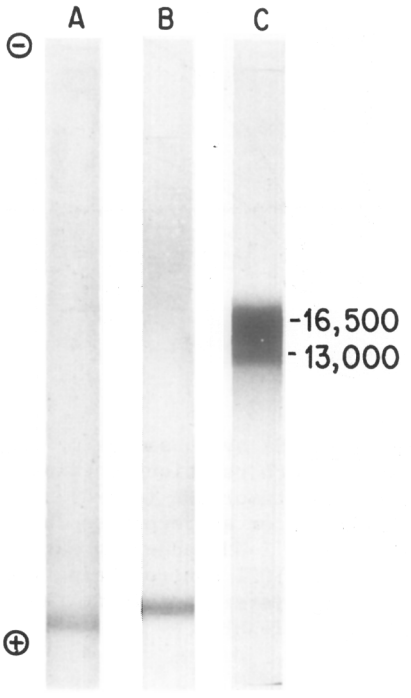


Fig. 1. Effect of calf thymus I-NHP on in vitro RNA synthesis using calf thymus RNA polymerase II and various DNA templates. Each reaction contained 5 μg of either calf thymus DNA (o-o-o), Ehrlich ascites tumor DNA (Δ-Δ-Δ), or Øe phage DNA (●-●-●) with standard RNA synthesizing system as described in Methods. Abscissa indicates the amounts of calf thymus I-NHP added to each reaction. The ordinate represents percent of control, 100% control being the amount of [^{3}H]UMP incorporated in an RNA synthesizing system containing the respective DNA without the addition of the calf thymus I-NHP.

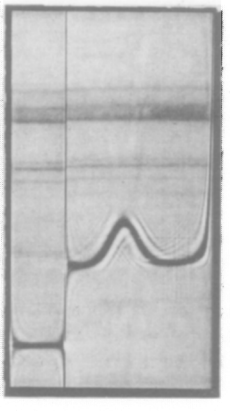
Fig. 2. Effect of the calf thymus I-NHP on RNA synthesis and chain initiation in calf thymus RNA polymerase II reaction. The reaction mixtures included 10 μg of calf thymus DNA with (o-o-o) and without (●-●-●) the addition of 10 μg of the calf thymus I-NHP using [^{3}H]UTP, and either [γ-^{32}P]ATP or [γ-^{32}P]GTP with (ΔΔΔ, □□□) or without (▲▲▲, ■■■) the addition of the calf thymus I-NHP.

[γ-^{32}P]GTP into RNA are inhibited by the I-NHP. These and the above results thus indicate that the calf thymus I-NHP inhibits preferentially the initiation of transcription from homologous DNA, similar to those observed with I-NHP isolated from Ehrlich ascites tumor.

The calf thymus I-NHP appeared as a single band when subjected to non-detergent polyacrylamide gel electrophoresis (Fig. 3B). Analytical ultracentrifugation of this protein also revealed one apparently symmetric peak of 3.0S (Fig. 4). The molecular weight of the calf thymus I-NHP, determined by high speed equilibrium sedimentation method, was 30,800 ± 2,400. However, when the calf thymus I-NHP was subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis, two bands were observed, with estimated molecular weights of 16,500 and 13,000, as shown in Fig. 3C. This is in contrast to the tumor I-NHP



(3)



(4)

Fig. 3. Polyacrylamide gel electrophoresis of calf thymus I-NHP. (A) Ehrlich ascites tumor I-NHP and (B) calf thymus I-NHP, 4 μg each, were subjected to electrophoresis in 5% acrylamide under non-denaturing conditions at 0.4 mA for 5.5 hr as described elsewhere (10). (C) Electrophoresis of calf thymus I-NHP (15 μg) in 20% acrylamide in the presence of sodium dodecyl sulfate for 12 hr according to Laemmli (9).

Fig. 4. Centrifugation of calf thymus I-NHP at 52,640 rpm. Temperature, 8.7°; time, 142 min. after reaching speed.

which shows only one subunit of molecular weight of 11,000 (1). The calf thymus I-NHP also migrated with a different mobility than the tumor I-NHP (Fig. 3A).

Both the calf thymus I-NHP, as described in this study, and the previously reported I-NHP isolated from Ehrlich ascites tumor (2) manifest their inhibitory effect on RNA synthesis in preference to DNA template from homologous tissue. Such preferential effect probably reflects specificity of the I-NHP

in recognition of and binding to selected DNA sequences. The protein should then be tissue-variable. In this work, the calf thymus I-NHP is shown to have a different electrophoretic mobility as well as subunits than the tumor I-NHP. These data, taken together, strongly suggest a regulatory role for the I-NHP in the control of gene expression.

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REFEREMCES

1. Kostraba, N.C. and Wang, T.Y. (1975) J. Biol. Chem. 250, 8938-8942.
2. Kostraba, N.C., Newman, R.S. and Wang, T.Y. (1977) Arch. Biochem. Biophys. 179, 100-105.
3. Allfrey, V.G., Mirsky, A.R. and Osawa, S. (1957) J. Gen. Physiol. 40, 451-462.
4. Chauveau, J., Moulé, Y. and Roueller, C. (1956) Exp. Cell Res. 11, 317-321.
5. Seligy, V. and Miyagi, M. (1969) Exp. Cell Res. 58, 27-34.
6. Teng, C.S., Teng, C.T. and Allfrey, V.G. (1971) J. Biol. Chem. 246, 3597-3609.
7. Kedinger, C. and Chambon, P. (1972) Eur. J. Biochem. 28, 283-290.
8. Roark, D.E. and Yphantis, D.A. (1969) Ann. N.Y. Acad. Sci. 164, 245-278.
9. Laemmli, U.K. (1970) Nature 227, 680-685.
10. Krakow, J.S. (1971) Methods Enzymol. 21D, 520-528.