THE EFFECT OF A REPORTER MOLECULE ON CHROMATIN TEMPLATE ACTIVITY

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SUMMARY: A reporter molecule, which is said to bind exclusively to the minor groove of DNA, does not interfere with the transcription of chromatin by an exogenous E. coli RNA polymerase. This is in contrast to the marked inhibition of chromatin template activity by actinomycin D. Taken together with previous observations, these results support the hypothesis that the chromatin proteins regulating transcription by RNA polymerase are located in the major groove of DNA.

INTRODUCTION

In eukaryotic organisms DNA is associated with various basic and acidic proteins. This complex is referred to as chromatin and is active as a template for RNA synthesis (1). Earlier studies have, in fact, shown that the chromatin-associated DNA available as template for transcription by RNA polymerase constitutes only 5 to 20% of the total DNA and that the RNA synthesized *in vitro* under these conditions is identical to the RNA synthesized *in vivo* (2-10). It has been generally assumed that the proteins of chromatin play a role in regulating the transcription of DNA and, therefore, in the mechanism of cellular differentiation in eukaryotic organisms (2-12).

However, the detailed molecular architecture of chromatin is not known. Information on the structure of chromatin is necessary in order to understand the regulation of transcription in eukaryotic organisms. In particular, the nature of the interaction between nucleoproteins and DNA and the relationship of this interaction to the action of RNA polymerase has yet to be elucidated.

Recently, the structural properties of chromatin have been studied by examining the binding of a reporter molecule. The reporter, N,N,N-trimethyl-N'-N'-dimethyl-N'-(β-2,4-dinitroanilinoethyl)-1,3-diammoniumpropane dibromide, was shown by Gabbay (13-17) to bind exclusively to the minor groove of DNA. In addition, Simpson (18) has shown that the number of reporter binding sites is identical for free DNA and for DNA complexed in chromatin. This suggested that the proteins of chromatin occupy only the major groove of the DNA double helix. In the present paper we show that binding of the reporter to chromatin does not inhibit transcription of chromatin by an exogenous RNA polymerase.

METHODS

S_3 HeLa cells were grown in suspension culture as previously described (19). Chromatin was prepared from log phase cells by a modification of the method of Marushige and Bonner (4). The cells were harvested by centrifugation and washed three times in Eagle's Spinner salts solution. The cells were resuspended in 20 ml 0.075 M NaCl-0.025 M EDTA, pH 8.0, and sonicated for 30 sec in three 10-sec bursts at 50 watts in a Branson WI25 sonifier. The sonicate was centrifuged at 10,000 x g for 30 min and washed twice with 20 cc of 0.075 MaCl-0.025 M EDTA, pH 8.0, each wash followed by centrifuga-

tion at 10,000 x g. The sediment was then washed twice in 0.05 M Tris-Cl, pH 8.0 (centrifugation again at 10,000 g for 30 min) and resuspended in 15 ml 0.01 M Tris-HCl, pH 8.0. Five ml aliquots were layered over 25 ml 1.7 M sucrose. The upper two-thirds were mixed and the chromatin sedimented by centrifugation at 25,000 rpm in a SW 25.1 rotor for 3 hr. The chromatin pellet was resuspended in 2.0 ml 0.01 Tris-Cl, pH 8.0, and dialyzed overnight against 4 liters of the same buffer.

Preparation from early log phase E. coli of RNA polymerase and the assay of chromatin template activity were as previously described (20).

Binding of the reporter to DNA was measured by a hypochromic shift at 345 mμ. Details are given in the legend to Table 1.

RESULTS

It was first necessary to show binding of the reporter to DNA under the conditions used to assay chromatin template activity with E. coli RNA polymerase. Table 1 shows the binding of the reporter to DNA first in 0.02 M Tris-Cl buffer, pH 8.0, and then in the same buffer with the additional constituents of the RNA synthesis assay, $MgCl_2$, $MnCl_2$, β-mercaptoethanol and the four nucleotide triphosphates. This shows the effect of the buffer separately from the effect of the constituents of the RNA polymerase reaction. The concentration of the reporter was 5×10^{-5} M and DNA 50 μg/ml. There is binding of the reporter to DNA in Tris buffer at pH 8.0 and this binding is only partially reduced by the constituents of the RNA polymerase assay. We concluded that the reporter is binding to DNA under the conditions used to

Table 1

Binding of the Reporter to DNA

	O.D. 345
A. Minus DNA	0.750
B. Plus DNA	0.608
C. DNA plus incubation mixture for chromatin template assay	0.680

Binding is measured by a hypochromic shift at 345 mμ. Each reaction mixture contained 5 x 10^{-5}M reporter in a final volume of 1.0 ml. A. 0.02 M Tris-Cl, pH 8.0; B. 0.02 M Tris-Cl, pH 8.0; 50 μg calf thymus DNA (Sigma); C. 0.02 M Tris-Cl, pH 8.0; 50 μg DNA; 0.004 M $MgCl_2$; 0.001 M $MnCl_2$; 0.4 mM ATP, UTP, CTP and GTP; 0.012 M β-mercaptoethanol. Results are averages of duplicates ± 0.002.

measure chromatin template activity.

The effect of the binding of the reporter on the activity of the chromatin as a template for RNA synthesis, using an exogenous E. coli RNA polymerase, is shown in Fig. 1, which compares the rate of RNA synthesis as percentage of controls at different concentrations of the reporter from 10^{-5}M to 10^{-2}M. It can be seen that there is no effect on the rate of RNA synthesis at a reporter concentration of 5 x 10^{-5}M and until concentrations greater than 10^{-4}. At 10^{-2}M (5 mg reporter/ml) there is only 60% inhibition of RNA synthesis. This is such a high dose that the inhibition of RNA synthesis probably is not a specific effect.

Table 2 compares the effect of the binding of actinomycin D or the reporter on chromatin template activity. At 5 μg/ml (4 x 10^{-7}M), actinomycin causes a 96% inhibition of RNA syn-

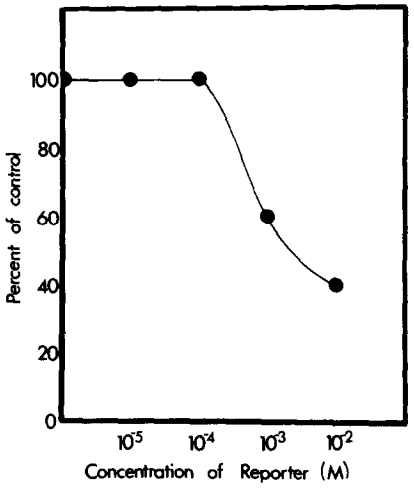

Fig. 1. Effect of a reporter on the transcription of HeLa cells chromatin by E. coli RNA polymerase. Each reaction mixture contained 12 µg HeLa cell DNA as chromatin and 20 µg E. coli RNA polymerase in a final volume of 0.250 ml. Template activity was measured as previously described (20). Results are the averages of duplicates ± 5%. 100% represents 200 pmoles (^{14}C) AMP incorporated into RNA in 10 min.

Table 2

Comparison of the Effect of the Reporter and Actinomycin D on Chromatin Template Activity

	pmol {^{14}C}AMP incorporated
A. Complete system	200
B. 10^{-4}M reporter	200
C. 4 x 10^{-7}M actinomycin D	8

Chromatin template activity was measured as previously described (20). Each reaction mixture also contained 12 µg HeLa cell DNA as chromatin and 20 µg E. coli RNA polymerase in a final volume of 0.250 ml. Results are the averages of duplicates ± 5%.

thesis. At a concentration 250 times greater (10^{-4}) there is no inhibition of RNA synthesis by the reporter.

DISCUSSION

We have shown that the binding of a reporter molecule to chromatin does not inhibit transcription of chromatin by an exogenous RNA polymerase. This is in striking contrast to the effect of actinomycin D, which also binds to DNA (21). Actinomycin D inhibits transcription almost completely at a concentration of 4×10^{-7}M in agreement with previous observations of other investigators (22).

Gabbay and coworkers (13-17) have shown that the reporter binds exclusively to the minor groove of DNA, and Simpson (18) found that the number of reporter binding sites is identical for free DNA and for DNA complexed in chromatin. The number of actinomycin D binding sites in chromatin, on the other hand, is only 25% of the number in free DNA (23). Under conditions leading to binding of the reporter to chromatin, there is no inhibition of the template activity of chromatin for RNA synthesis. There is, therefore, a correlation between the binding to free DNA and to chromatin and the inhibition of RNA synthesis.

These observations and those of Simpson (18) support the hypothesis that the chromosomal proteins are bound only to the major groove of DNA where they regulate the transcription of DNA by RNA polymerase. Furthermore, this interaction of the chromosomal proteins with DNA presumably blocks the binding of actinomycin to DNA complexed in chromatin.

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