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# RNA chain elongation on a chromatin template

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## ABSTRACT

The rate of RNA chain elongation has been measured with DNA and chromatin as template. RNA propagation on chromatin is about 50% of the rate found with DNA. Kinetic experiments demonstrate that the inhibition is not due to interference with the addition of the nucleoside triphosphates. Analysis of the dependence of propagation on the $T_m$ of DNA shows that the inhibition cannot be due to an effect on DNA unwinding. We conclude that chromatin proteins interfere with the translocation of the RNA polymerase along the DNA template.

## INTRODUCTION

It is generally believed that the proteins of chromatin play a major role *in vivo* in limiting the template-activity of the DNA to which they are bound and that this limitation is related to cellular differentiation. The work of Bonner and his collaborators (1) and of other workers (2, 3) has shown that the rate of *in vitro* synthesis of RNA by DNA-dependent RNA polymerase is markedly slower when chromatin is used as a template than when the template is protein free DNA. The inhibition of RNA synthesis on a chromatin template is of two types. Cedar and Felsenfeld (4, 5) using RNA polymerase from bacterial and eukaryotic sources have shown that the major effect of chromatin proteins is to restrict the number of RNA polymerase initiation sites. This result is consistent with the biological role proposed for chromatin.

In addition to limiting the number of available initiation sites on chromatin, the nucleoproteins also inhibit the elongation of RNA chains *in vitro* (2, 4). Using *E. coli* RNA polymerase the rate of RNA propagation on chromatin is about 40% that found with DNA. In this paper we attempt to uncover the



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mechanism of this inhibition. We show that the chromatin proteins inhibit RNA chain elongation by interfering with the translocation of the enzyme along the DNA.

## MATERIALS AND METHODS

Nucleoside triphosphates were purchased from Calbiochem. [$^3$H] UTP (40 Ci/mmole) was obtained from New England Nuclear. Poly-d-lysine (PDL) (70,000 average molecular weight) was from Sigma.

Chicken erythrocyte DNA and chromatin were prepared as described previously (5). *E. coli* DNA was the gift of Dr. U. Bachrach. *E. coli* RNA polymerase, Fraction V was prepared according to Berg *et al.* (6). Poly-d-lysine DNA complex was prepared by the slow addition of a dilute solution of PDL in 10 mM Tris-HCl, pH 7.9 to DNA in the same buffer (7). The complex used in this paper had a PDL to DNA ratio of 0.6 moles of lysine per mole of DNA phosphate. The complexes of histone f2b with *E. coli* DNA or chicken DNA were prepared in the same way. Both complexes had a ratio of 0.9 moles of lysine and arginine per mole of DNA phosphate.

The rate of chain elongation per RNA molecule was measured by a modification of the procedure described by Cedar and Felsenfeld (4). In this method RNA polymerase is incubated with template at 37°C in 0.1 ml containing 10 mM Tris-HCl (pH 7.9), 1 mM $MnCl_2$ and 40 $\mu$M each of ATP, GTP and UTP. Under these limited conditions, RNA polymerase initiates synthesis but cannot elongate due to the lack of CTP. After 10 min this initiation reaction is complete (4). Chain elongation is then started by the addition of CTP in a volume of 0.5 ml. This mixture also contained [$^3$H] UTP (10 $\mu$Ci), ATP, GTP, CTP and KCl as indicated. Propagation was determined by following the incorporation of radioactive UTP into TCA precipitable material after 1 min, during which time the rate of propagation is linear (8). To determine the propagation rate per RNA chain we have measured the number of RNA molecules initiated using a method described previously (4). When the above method is employed to measure chain elongation, initiation of RNA molecules occurs only during the preincubation period, and not during the measurement of propagation.

## RESULTS

a) Kinetics of nucleoside triphosphate addition – Chain elongation of RNA polymerase on a DNA template can be thought of as taking place in three steps (8)

DNA unwinding ⟶ Translocation ⟶ Nucleotide addition (1)

Since the rate of RNA propagation using chromatin is about 50% of that with DNA, it is logical to assume that the proteins on chromatin inhibit the rate of RNA chain elongation. We have attempted to analyze the mechanism of this inhibition. Hyman and Davidson (9) have demonstrated that actinomycin D inhibits propagation by interfering with the nucleotide addition step of Mechanism (1). By studying the effect of actinomycin at various concentrations of nucleoside triphosphates they were able to show that this drug interfered with the addition of GTP and CTP, but not UTP or ATP to the growing RNA chain.

The effect of varying nucleoside triphosphate concentrations on the propagation of RNA polymerase using DNA and chromatin is shown in Figure 1. In these experiments the concentrations of three of the nucleoside triphosphates are kept at a fixed concentration while the concentration of the fourth, $\beta$, is varied. When $1/v$ is plotted against $1/S_\beta$ one expects to obtain a straight line (8, 9). An inhibitor of nucleoside triphosphate addition should change the slope of this line, and the relative slope should be proportional to the amount of inhibition (9).

The propagation rate using chromatin is about 50% of the rate using a DNA template (Fig. 1). For all nucleoside triphosphates (i. e., $\beta$ = ATP, CTP, GTP or UTP), there is no effect of chromatin on the slope of the Lineweaver-Burke plot. In other words, the inhibition observed with chromatin is independent of the concentrations of the nucleoside triphosphates. The data shown in Figure 1 were obtained using fixed nucleoside triphosphate concentrations of 40 $\mu$M. Similar results have also been obtained at concentrations of 100 $\mu$M (data not shown).

b) Effect of $T_m$ on RNA chain elongation – We have previously demonstrated that DNA unwinding is a necessary, though not rate limiting step in RNA synthesis (8). Since chromatin proteins can raise the $T_m$ of the DNA to

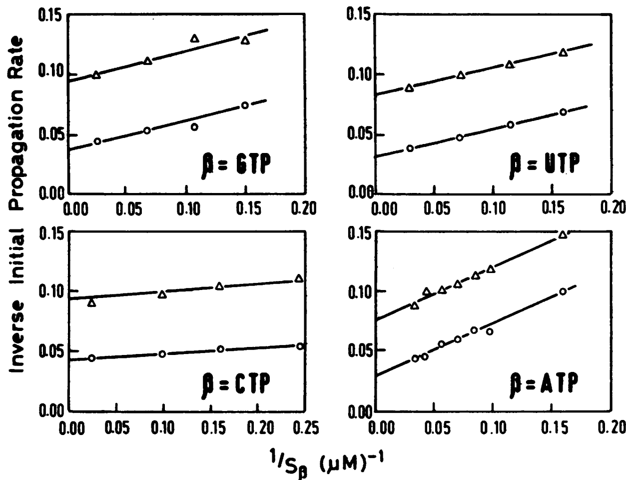



Figure 1. Dependence of RNA propagation on the concentration of nucleoside triphosphates. RNA synthesis was initiated as described in Materials and Methods and propagation then measured under varying conditions of nucleoside triphosphate concentrations. The concentrations of the fixed nucleoside triphosphates in each experiment was 40 μM. Each tube contained 2.5 units of E. coli RNA polymerase and either 1.7 μg DNA or 75 μg chromatin. Activity was measured as pmoles UTP incorporated per min and the activity in the absence of triphosphate was 2 pmoles UTP/min. DNA (o); chromatin (Δ).

which they are bound it was of interest to see if the inhibition of RNA synthesis might be explained by the effect of these proteins on the ability of the DNA to unwind.

When DNA is put in a high salt environment, the $T_m$ is increased according to the equation developed by Schildkraut and Lifson (10).

$$T_m(^\circ C) = GC/2.44 + 81.5 + 16.6 \log M \qquad (2)$$

At effective $T_m$ above 92°C, the rate of RNA propagation (v) becomes rate limiting and is dependent on the $T_m$ as described by Equation (3)(8).

$$\ln (v) \sim \frac{1}{T_m} \qquad (3)$$

If chromatin proteins inhibit RNA synthesis by preventing DNA unwinding

one would expect that the inhibition would depend on the $T_m$ of the chromatin. Under the salt conditions of the assay mix the $T_m$ of chromatin is 88°C (Fig. 2). As can be seen in Table 1 chicken DNA has a $T_m$ of 88°C at a salt concentration of 0.2 M. At this $T_m$, however, RNA propagation on DNA is not inhibited. 50% inhibition of RNA synthesis on DNA is obtained only when the $T_m$ reaches 96°C. Thus, the inhibition observed with chromatin cannot be attributed to the effect of chromatin proteins on $T_m$.

c) Propagation rate on artificial DNA-protein complexes – When DNA is mixed with histones or poly-d-lysine (PDL) a DNA-protein complex is formed which has some properties similar to those of chromatin. These complexes, for example, have a decreased number of initiation sites and a

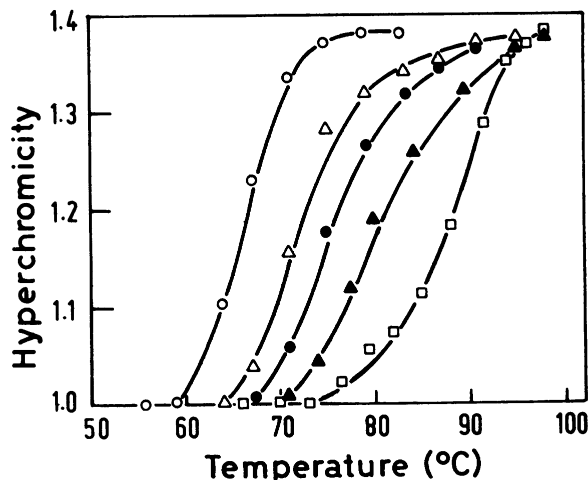



Figure 2. Melting temperature of DNA, chromatin and DNA-protein complexes. Hyperchromicity of each template was measured in a Zeiss spectrophotometer using a heated water bath. The temperature shown was that recorded in the water bath after the system was allowed to equilibrate for 15 min at each point. Each sample was at a concentration of about 50 μg/ml and was extensively dialyzed against 10 mM Tris-HCl (pH 7.9) before use. Degassing was accomplished by bubbling He gas for 5 min, and the cuvettes were sealed against evaporation by layering purified mineral oil on each sample. The templates were as follows: DNA (o); PDL-DNA complex (Δ); f2b-chicken DNA complex (●); f2b-E. coli DNA complex (▲); and chromatin (□).

TABLE 1. Dependence of propagation on KCl

| Salt concentration (M) | $T_m$ (°C) | Relative propagation rate |
|---|---|---|
| 0.01 | 66.4 | 1.0 |
| 0.21 | 88.2 | 1.0 |
| 0.41 | 91.7 | 1.0 |
| 0.71 | 95.7 | 0.5 |

RNA synthesis was initiated using 25 μg DNA and 2.5 units E. coli RNA polymerase as described in Materials and Methods and propagation was started by the addition of 0.5 ml containing all of the nucleoside triphosphates (40 μM) and various concentrations of KCl. The final concentration of salt (including 10 mM Tris-HCl pH 7.9) in the assay mix are shown in the table. $T_m$ was calculated from Equation (2). The relative propagation rate is based on the rate obtained in 10 mM Tris-HCl (pH 7.9), without added KCl. This rate was 28 pmoles UTP/min.

slower RNA propagation rate as compared to DNA (Solage, Zuruki and Cedar, unpublished experiments). When RNA chain elongation was measured as a function of the concentration of GTP for a DNA-PDL complex or a DNA-f2b complex, the kinetics were similar to that observed with chromatin (Fig. 3).

In order to clarify the relationship between propagation rate and $T_m$ we have determined the $T_m$'s of the complexes used for the experiments shown in Figure 3 (see Fig. 2). The DNA-PDL complex was found to inhibit RNA propagation about 40%. Although this is similar to the inhibition observed with chromatin, the $T_m$ of this complex is 16°C below the $T_m$ of chromatin. The DNA-f2b complex had the same extent of inhibition as chromatin, yet it too had a $T_m$ far below that of chromatin. In parallel to the DNA-f2b complex made with chicken DNA, we have made a similar DNA-f2b complex using E. coli DNA. Both complexes have the same f2b/DNA ratio. Since the GC content of E. coli DNA is higher than that of chicken DNA, the complex using E. coli DNA had a higher $T_m$. Despite this difference in $T_m$, both complexes had the same degree of inhibition of RNA propagation (Fig. 3).

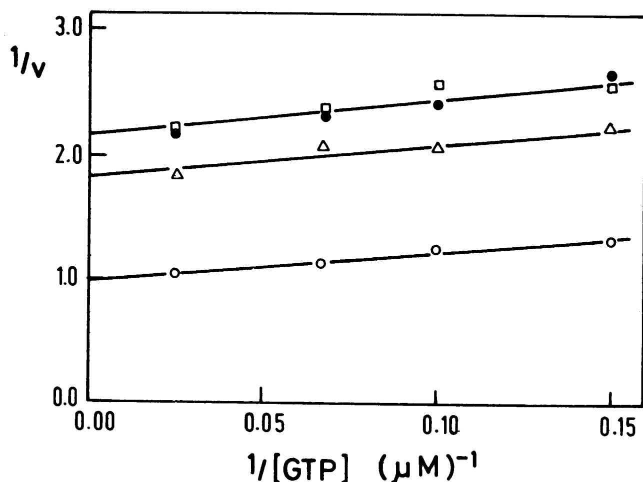
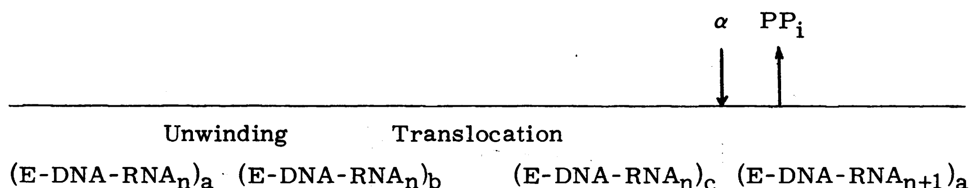



Figure 3. Kinetics of nucleoside triphosphates with DNA-protein complexes. Propagation was measured as described in Materials and Methods using 2.5 units E. coli RNA polymerase with a concentration of ATP, CTP and UTP of 40 μM. The template was either 2.5 μg DNA (o); 5 μg PDL-DNA complex (Δ); 8 μg f2b-chicken DNA complex (□); or 8 μg f2b-E. coli DNA complex (●). The results are expressed as propagation rate per chain normalized to the rate as measured on DNA. The number of chains initiated on each template was determined as previously described (4).

## DISCUSSION

Although it is known that chromatin proteins inhibit RNA propagation as well as initiation, the mechanism of this inhibition is not well understood. We have attempted to clarify this mechanism using an assay which specifically measures the rate of RNA chain elongation per RNA molecule. This assay is linear with time and gives kinetics which are consistent with the equations derived for this reaction (8).

RNA polymerase propagation can be thought to operate in three steps (Scheme 1): DNA unwinding, translocation and nucleoside triphosphate addition. Using the nomenclature of Cleland (11) this can be represented as follows:




where $\alpha$ is a nucleoside triphosphate; E-DNA-$RNA_m$ represents the enzyme-DNA-RNA complex with RNA chain length m; and a, b and c designate different forms of the complex. The chromatin proteins could cause inhibition by operating at any of these steps.

In order to determine if chromatin interfered with the addition of nucleoside triphosphates, we studied the rate of propagation for DNA and chromatin at varying concentrations of the nucleoside triphosphates. Unlike actinomycin D inhibition, which operates by preventing the addition of GTP and CTP to the growing RNA chain, the slower rate of propagation on chromatin could not be overcome by the addition of higher concentrations of nucleoside triphosphates. In fact, using a Lineweaver-Burke plot we find that the slope of the line is the same for DNA and chromatin. This type of parallel kinetics is indicative of uncompetitive inhibition. This inhibition is obtained when the inhibitor interferes with a reaction step which is independent of the step involving the variable substrate. Thus the chromatin proteins affect RNA propagation either by preventing DNA unwinding or translocation.

The experiments reported in this paper demonstrate that the inhibition of propagation cannot be due to the effect of chromatin proteins on the $T_m$ of the DNA. Although high salt concentrations can be used to raise the $T_m$ of DNA and thus cause inhibition of RNA chain elongation on this template, the $T_m$ necessary to cause inhibition is much higher than the $T_m$ of chromatin (Table 1). In addition, several artificial DNA protein complexes exhibited inhibition of RNA synthesis similar to that observed with chromatin, despite the fact that the $T_m$ of these complexes were below that of chromatin.

We conclude that chromatin proteins inhibit RNA chain elongation by interfering with the RNA polymerase movement from one nucleotide on the

DNA to the next. This seems to be a non-specific function of chromatin proteins which can be simulated by other DNA-protein complexes. Although there is no direct evidence, this effect might be one of the functions of chromatin proteins in vivo.

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## REFERENCES

1. Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R. C. C., Marushige, K. and Tuan, D. Y. H. (1968) Science 159, 47-56.
2. Koslov, Yu. V. and Georgiev, G. P. (1970) Nature 228, 245-247.
3. Kurashina, Y., Ohba, Y. and Mizuno, D. (1970) J. Biochemistry 67, 661-665.
4. Cedar, H. and Felsenfeld, G. (1973) J. Mol. Biol. 77, 255-277.
5. Cedar, H. (1975) J. Mol. Biol. 95, 257-269.
6. Berg, D., Barrett, K. and Chamberlin, M. (1971) Methods in Enzymol. 21, 506-519.
7. Axel, R., Cedar, H. and Felsenfeld, G. (1975) Biochemistry 14, 2489-2495.
8. Solage, A. and Cedar, H. (1976)
9. Hyman, R. W. and Davidson, W. (1970) J. Mol. Biol. 50, 421-438.
10. Schildkraut, C. and Lifson, S. (1965) Biopolymers 3, 195-208.
11. Cleland, W. W. (1963) Biochim. Biophys. Acta 67, 188-196.