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Binding of E.coli RNA polymerase to chromatin subunits

Michael Bustin

Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014, USA

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ABSTRACT

Chromatin subunits were prepared from purified rat liver nuclei and the template properties of the nucleosome preparation studied. It was found that: 1) The fundamental template restriction of chromatin (as compared to deproteinized DNA) is retained in the isolated nucleosomes, 2) On the average one molecule of RNA polymerase is bound to one molecule of DNA purified from nucleosomes, 3) The number of RNA polymerase binding sites on chromatin subunits is 6 to 20 times lower than that of the DNA extracted from these subunits, 4) Transcription can proceed through nucleosomes resulting in RNA chains approximately 150 nucleotides long.

INTRODUCTION

It is well documented that the ability of DNA to serve as template for in vitro RNA synthesis is substantially restricted in chromatin [1,2]. The slower rate of RNA synthesis in chromatin, as compared to deproteinized DNA, is due to both a reduction in the number of RNA polymerase binding sites and to a slower rate of chain elongation [2].

The nontranscribed regions of chromatin consist of a string of covalently linked subunits called nucleosomes [3]. The exact structure of the transcribable portion of chromatin, however, is not fully known. Evidence has been forwarded suggesting that in a tissue, all genomic sequences, including those specifically transcribed, are in the nucleosome conformation [4-7]. However, Reeves and Jones [8] noted an inverse correlation between transcriptional activity and percent genome in the nucleosome conformation. DNAase I digestion also suggests that transcribable regions have an altered conformation [9]. In certain cases the transcribed portions do not reveal a beaded appearance when examined by electron microscopy [10].

Studies using nuclease and protease digestions suggest that some of the techniques used to isolate chromatin may alter the spacings between nucleosomes and cause some damage to the nucleosome structure [11]. Thus,

most of the studies on the ability of E. coli RNA polymerase to transcribe chromatin were done on preparations in which the nucleosome structure may have been altered. Thus, at present it is not known whether DNA in the nucleosome conformation can be transcribed.

The purpose of the experiments performed is to study the template properties of a preparation of chromatin subunits and thereby provide a comparison with previous studies using unfractionated chromatin. I find that 1) A nucleosome preparation retains the fundamental template restriction of chromatin. 2) On the average one molecule of RNA polymerase is bound per 185 base pairs of deproteinized DNA obtained from nucleosomes. 3) The number of RNA polymerase binding sites on chromatin subunits is 6 to 20 times lower than that of DNA of equivalent length and 4) Transcription can proceed through a nucleosome at very low rates resulting in RNA chains approximately 150 nucleotides long.

MATERIALS AND METHODS

Preparation and characterization of nucleosomes. Nucleosomes were prepared from rat liver nuclei by controlled digestion with micrococcal nuclease [12]. The nuclei, purified by washing with 0.35 M sucrose, 10 mM Tris-HCl, 1 mM $CaCl_2$, pH 8, were suspended at a concentration of 3 mg/ml DNA and incubated with 100 units micrococcal nuclease per ml at 37° for 2 min. The digestion was stopped by making the suspension 5 mM in EDTA, dialyzed to 0.25 mM EDTA, pH 7, homogenized with a teflon Potter-Elvejehm homogenizer and centrifuged in sucrose gradients isokinetic for a particle density of 1.51 with a meniscus concentration of 5% (w/w) sucrose [13]. Selected fractions were collected, dialyzed, and repurified by sedimentation through gradients as above. A nucleosome monomer and a nucleosome multimer fraction were obtained. The size of the DNA fragments, extracted with phenol from these preparations, was determined by electrophoresis in 3.5% acrylamide gels [14]. The monomer preparation was 95% pure with an average DNA size of 160 base pairs. The multimer fractions contained stretches of 5 to 8 nucleosomes long. The types of proteins present in the preparation were determined by electrophoresis in 18% discontinuous polyacrylamide gels run in the presence of 0.1% sodium dodecyl sulfate [15]. Each of the fractions contained histones H2A, H2B, H3 and H4 in approximately equal amounts. The monomer nucleosome fraction was markedly depleted in H1.

RNA synthesis. RNA polymerase (600-800 units per mg purchased from either Miles or Sigma) was incubated with template at 37° in 0.25 ml containing 10 mM Tris-HCl, pH 7.9, 2 mM $MnCl_2$, 0.15 mM dithiothreitol and

0.1 mM nucleotide triphosphate. Trace amounts of [α-^{32}P]ATP (25 Ci/mmol from ICN) were added to obtain a desired specific activity. [^{3}H]UTP (50 Ci/mmol) was obtained from Amersham. When [^{3}H] was used as tracer, the final UTP concentration was 0.05 mM. Incorporation of label into RNA was determined by removing an aliquot into 10% trichloroacetic acid, 0.01 M sodium pyrophosphate. The precipitated RNA was collected on Oxoid Nuflow membrane filters (Amersham) washed with 5% trichloroacetic acid, 0.01 M sodium pyrophosphate and with ethanol, dried, and counted in Aquasol (New England Nuclear). The size of the RNA synthesized was measured by removing aliquots, denaturing the RNA by boiling in 66% formamide, and subjecting the samples to electrophoresis in 12% polyacrylamide gels containing 7 M urea [16]. For autoradiography gels were wrapped in plastic wrap and placed on Kodak RP Royal film. Developed autoradiograms were scanned with an E-C Apparatus Co. densitometer.

Polymerase binding sites. The number of RNA binding sites was determined by several techniques as described in the text and in the legends to Figures.

RESULTS

The rate of incorporation of radioactive nucleotide into RNA, catalyzed by RNA polymerase from E. coli, in the presence of chromatin monomer, multimer (5-8 nucleosomes) and the DNA species extracted from these particles is presented in Fig. 1. To facilitate comparison with results obtained previously from other laboratories [1,2], the incorporation in the presence of high molecular weight commercial calf thymus DNA was also measured. The reaction was done under conditions where RNA polymerase was in excess. The production of trichloroacetic acid-precipitable counts is absolutely dependent on both template and enzyme and is NaOH labile.

After 3 minutes of synthesis, high molecular weight DNA seemed to serve as a more efficient template than DNA extracted from monomer or multimer nucleosomes. No significant difference was observed between DNA extracted from monomer and that extracted from multimer. In contrast, a given amount of chromatin monomer was about 60% more efficient in serving as a template for RNA synthesis than the same amount of chromatin composed of 5 to 8 nucleosomes. More significant, however, is the observation that the ability of chromatin subunits to serve as templates for RNA synthesis catalyzed by RNA polymerase is markedly reduced as compared to DNA extracted from the subunits (Fig. 1). The rate of RNA synthesis from chromatin monomer is about 8% to 15% of that obtained with the DNA extracted from the monomers.

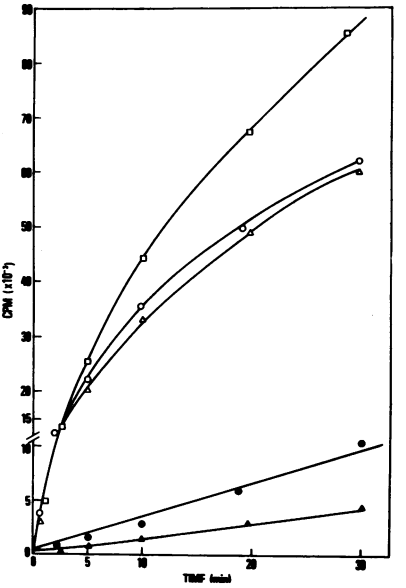

Fig. 1. Incorporation of [α-^{32}P]ATP into RNA at low ionic strength by various templates. RNA polymerase (10 units) incubated with 1.5 µg DNA under low ionic strength conditions described in the experimental procedure. □, calf thymus DNA; o, DNA extracted from monomer nucleosomes; △, DNA extracted from multimer (5-8) nucleosomes; ●, nucleosomes; ▲, multimer nucleosomes.

The results demonstrate that a preparation of nucleosomes retains the fundamental template restriction previously observed with chromatin.

The reduction of the total amount of RNA synthesized by chromatin as compared to DNA, is due to a decrease in both the number of initiation sites for RNA polymerase and in the rate of chain propagation [2]. The question arises whether the same situation exists with isolated nucleosomes. Thus, it is possible that the synthesis of RNA on individual nucleosomes reflects a small fraction of nucleosomes supporting RNA synthesis at rates comparable to deproteinized DNA.

To answer this question the size of the RNA synthesized after various times was determined. It is known that under similar experimental conditions the rate of chain elongation is on the order of 100 nucleotides per min for DNA, and about 50 nucleotides per min with chromatin [2,17,18]. Thus, if a few nucleosomes would be transcribed at a relatively fast rate, the average size of the RNA transcript should not change after a few minutes of synthesis, while if the propagation is slow there should be an increase in the average size of the transcript. To clarify this point, RNA was

synthesized from either nucleosomes or DNA extracted from nucleosomes, using [α-^{32}P]ATP as the radioactive marker, and the product sized by gel electrophoresis and autoradiography. The scans of the autoradiograms, presented in Fig. 2, compare the size of RNA obtained after 11 min and 30 min transcription primed by nucleosomes to the size of RNA transcribed by DNA extracted from nucleosomes. In the latter case (curves C and D) no change in the size of RNA was observed, suggesting that the DNA molecules went through at least one cycle of transcription. Most of the RNA molecules were about 150 nucleotides long. In contrast, transcription through nucleosomes proceeds at a slower rate. Thus, after 11 min of transcription the average size of the transcribed RNA was about 35 nucleotides (curve A) while after 30 min a considerable amount of RNA 150 nucleotides long was detected (curve B). This result minimizes the possibility that a few nucleosomes are transcribed at a fast rate and suggests that the average nucleosome is transcribed at rates substantially smaller than that of deproteinized DNA. Furthermore, since a considerable amount of RNA was about 150 nucleotides long, the data presented in Fig. 2 suggest that transcription can go through the nucleosome, albeit very slowly.

The production of 150 nucleotide long RNA after relatively long incubation periods is not due to dissociation of the nucleosome during the assay conditions. This is obvious from the data presented in Fig. 1 where it can be seen that the rate of RNA synthesis from nucleosomes proceeds linearly with time at least up to 30 minutes. If dissociation would have occurred, the amount of histone-free DNA would increase with time, leading to an apparent time-dependent increase in the rate of RNA synthesis. Studies using SV40 nucleoprotein complexes also reveal that the transcription process does not lead to dissociation of DNA-histone complexes [19].

Initiation sites on nucleosomes. It has been shown by Cedar and Felsenfeld [2] that the number of RNA polymerase binding sites in chromatin was about one-tenth of the RNA polymerase binding sites in DNA. The finding that nucleosomes can serve as template for RNA synthesis suggests that polymerase binding sites occur in nucleosomes. The relative number of binding sites in nucleosomes and DNA was determined in two different ways, both following the techniques previously used for chromatin [2]. Fig. 3A presents the results of an experiment in which initiation occurred at low salt in the presence of ATP and GTP and propagation was started by adding CTP, [^{3}H]UTP and rifampicin (to prevent reinitiation). It can be seen that

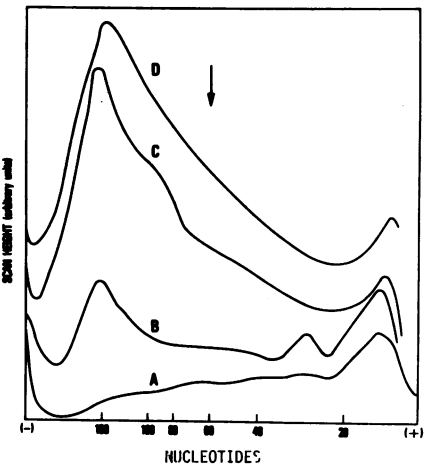

Fig. 2. Densitometer scan of autoradiographs of polyacrylamide gels. [α-^{32}P]-labeled RNA was synthesized under low salt conditions. After 11 min and 30 min of propagation, aliquots were removed, added to 2 volumes of formamide and boiled for 2 min. The boiled samples were immediately ice cooled and electrophoresed in 12% polyacrylamide gels containing 7 M urea as described in Methods. Electrophoresis proceeded until the bromophenol blue marker reached the end of the gel. The wet gels were subjected to autoradiography. Arrow points out position of the xylene-cyanol FF tracking dye. The mobility of a polynucleotide of a defined length in this gel can be ascertained from the calibration chart of Maniatis et al. [16]. (A) Nucleosome, 11 min propagation, (B) Nucleosome, 30 min propagation, (C) DNA extracted from nucleosomes, 11 min propagation, (D) DNA extracted from nucleosomes, 30 min propagation.

saturation of 11 units of RNA polymerase was achieved with 3 µg of DNA extracted from nucleosomes. The same level of RNA synthesis was reached with 15 µg of nucleosome. Using the Millipore titration technique (Fig. 3B), it was found that 0.55 units of RNA polymerase were saturated by 0.15 µg DNA as compared to about 3.0 µg of intact monomer.

DISCUSSION

The ability of chromatin isolated by various techniques to serve as template for RNA polymerase has been the subject of several investigations [1,2]. Evidence that DNA from transcribable regions is in the nucleosome conformation has been obtained [4-7]. So far nucleosome preparations have not been directly transcribed and direct evidence that RNA transcription can proceed through nucleosomes has not been presented. Here, evidence is presented that the template restriction observed in nucleosomes is of the same magnitude as that observed with chromatin isolated from rat liver by "classical" techniques [1,2]. E. coli RNA polymerase can bind to isolated

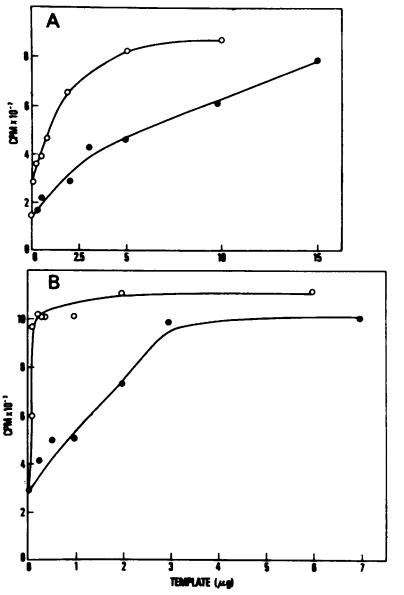

Fig. 3A. Titration of RNA polymerase (11 units) with nucleosomes (●) and DNA extracted from nucleosomes (o). To test tubes containing various amounts of template in a constant volume of 0.25 mM EDTA, 1 mM Tris-HCl, pH 8, a constant amount of premix containing ATP, GTP, Tris-HCl, $MnCl_2$ and RNA polymerase was added. After incubation at 37° for 15 min, a constant amount of premix containing rifampicin (20 μg/ml), CTP and [^{3}H]UTP was added and propagation continued for 45 min. o, DNA; ●, Nucleosomes.

Fig. 3B. Titration of RNA polymerase with various templates using Millipore filtration. To test tubes containing various amounts of either nucleosomes (●) or DNA extracted from nucleosomes (o) in a constant volume of 0.25 mM EDTA, 1 mM Tris-Cl, pH 8, a premix containing RNA polymerase (0.55 units per tube) Tris-Cl (0.01 M, pH 7.9, final conc.), $MnCl_2$ (1 mM final conc.) and [α-^{32}P]ATP (0.02 mM final conc.) was added. The mixture was incubated for 45 min at 37°. At this time the mixture was made 0.4 M in $(NH_4)_2SO_4$. After an additional 10 min incubation at 37° the mixture was passed over a prewashed Millipore filter (type HA) and washed with 10 mM Tris-Cl, pH 7.9, 4 mM $MgCl_2$, 0.4 M $(NH_4)_2SO_4$, as recommended by Cedar and Felsenfeld [2].

nucleosomes and bring about the synthesis of RNA chains approximately 150 nucleotides long. Solage and Cedar [17,18] have calculated that the rate of RNA propagation in chromatin is about 50 nucleotides per minute. The fact that after 11 minutes of transcription the average size of the transcript obtained from nucleosome did not reach a steady state suggests that transcription through nucleosomes proceeds at a very slow rate.

Depending on the experimental conditions, the number of RNA binding sites in nucleosomes is 6-20 times lower than that of deproteinized DNA of the same size. This is comparable to the 10-fold difference between

unfractionated chromatin and large molecular weight DNA [2]. The amount of deproteinized DNA extracted from nucleosomes necessary to saturate 1 unit of RNA polymerase is 0.30 μg as measured by propagation in the presence of rifampicin and 0.36 μg as measured by the Millipore titration experiment. Thus, from the average specific activity of RNA polymerase (720 units/mg protein) and from the known molecular weight of the enzyme (490.000 for the β', β, γ, 2α, 2ω complex) it can be calculated that 5.0×10^{-10} moles of base pairs saturated 2.6×10^{-12} moles of enzyme. Thus, there is an average of one molecule of enzyme per 185 base pairs of DNA, suggesting that each molecule of DNA bound a polymerase molecule. It is not presently clear whether the transcription process involving isolated, purified nucleosomes is directly comparable to the processes involving a strain of nucleosomes.

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REFERENCES

1 Bonner, J., Dahmus, M.E., Fambrough, D., Huang, R.C., Marushige, K. and Tuan, D.Y.H. (1968) Science 159, 47-56.
2 Cedar, H. and Felsenfeld, G. (1973) J. Mol. Biol. 77, 237-254.
3 Kornberg, R.D. (1977) Ann. Rev. Biochem. 46, 931-954.
4 Lacy, E. and Axel, R. (1975) Proc. Natl. Acad. Sci. 72, 3978-3982.
5 Mathis, D. and Gorovsky, M.A. (1976) Biochemistry 15, 750-755.
6 Piper, W.P., Celis, J., Kaltoft, K., Leer, J.C., Nielsen, O.F. and Westergaard, O. (1976) Nucleic Acids Res. 3, 493-505.
7 Kuo, M.T., Sahasrabuddhe, C.G. and Saunders, G.F. (1976) Proc. Natl. Acad. Sci. 73, 1572-1575.
8 Reeves, R. and Jones, A. (1976) Nature 260, 495-500.
9 Weintraub, H. and Groudine, M. (1976) Science 193, 848-856.
10 Franke, W.W., Scheer, U., Trendelenburg, M.F., Spring, H. and Zentgraf, H. (1976) Cytobiologie 13, 401-434.
11 Noll, M., Thomas, J.O. and Kornberg, R.D. (1975) Science 187, 1203-1206.
12 Noll, M. (1974) Nature 251, 249-251.
13 McCarty, K.S., Jr., Vollmer, R.T. and McCarty, K. (1974) Anal. Biochem. 61, 165-183.
14 Peacock, A. and Dingman, C. (1967) Biochemistry 6, 1818-1827.
15 LeStourgeon, W.M. and Rusch, H.P. (1973) Arch. Biochem. Biophys. 155, 144-158.
16 Maniatis, T., Jeffrey, A. and van de Sande, H. (1975) Biochemistry 14, 3787-3794.
17 Solage, A. and Cedar, H. (1976) Nucleic Acids Res. 3, 2207-2222.
18 Solage, A. and Cedar, H. (1976) Nucleic Acids Res. 3, 2223-2241.
19 Cremisi, C., Chestier, A., Dauguet, C. and Yaniv, M. (1977) Biochem. Biophys. Res. Commun. 78, 74-82.