electron density of about 4–5 residues after Lys-311. The fit of the sequence 317 to 348 appears plausible but it has not yet been assigned in detail. Likewise the region between 450 and 642 is in continuous density with the right number of residues but assignments have not yet been made. The interpretation of the course of the chain from residue 810 onwards differs markedly from that for phosphorylase *a* and assignments have still to be made in detail. The loop between residues 159–183 is in weak density, possibly due to some disorder caused by our platinum heavy atom derivative.

The assignments to date allow us to confirm the alignments of the following cyanogen bromide fragments: CB14–CB5 (Met-91), CB5–CB12 (Met-99), CB12–CB16 (Met-119), CB16–CB21 (Met-147), CB20–CB11 (Met-224), CB11–CB17 (Met-241), CB18–CB9 (Met-427), CB9–CB15 (Met-440), CB24–CB3 (Met-678), CB3–CB7 (Met-681), CB7–CB4 (Met-691), CB4–CB10 (Met-698), CB10–CB22 (Met-712), CB1–CB23 (Met-765), CB23–CB13 (Met-799). The overlap of CB21–CB20 (Met-176) is in weak density but since large regions of both fragments have been assigned there seems little reason to doubt the sequence. The overlap of CB17–CB18 is in an ambiguous region and it is hard to identify the C terminus of CB17. However the number of residues in this region is approximately in concordance with the sequence information. The density for Met-763 is weak for a methionine side chain but the alignment CB22–CB1 appears plausible in terms of the number of residues.

There are two regions where the electron density map appears to require fewer residues than those given in the sequence. The first is the region 360–364 (Trp-Asp-Lys-Ala-Trp). The two tryptophans are clearly visible and Trp-364 makes contact to glucose 1-phosphate bound at the active site (Weber et al., 1978). However, the density between the two tryptophans appears to indicate 2 rather than 3 residues. Secondly in the region 776–779 (Tyr-Glu-Glu-Tyr), the density suggests one rather than two glutamic acid residues.

In summary, at the present preliminary stage of our analysis, our results are consistent with the sequence data. In particular, binding studies with ATP have shown that Tyr-155 which can be covalently modified by an analogue by AMP (Anderson et al., 1973) is in the right position to form this link. At Lys-679 there is good density for the pyridoxal phosphate and this site is close (8 Å) to the site at which glucose 1-phosphate binds tightly (Weber et al., 1978). The two major mercury binding sites for the heavy atom derivative ethylmercury thiosalicylate are at Cys-782 and Cys-108, while the minor site which is also close to the major platinum site is at Cys-171.

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Transcription of Histone-Covered T7 DNA by *Escherichia coli* RNA Polymerase†

P. Williamson*,‡ and G. Felsenfeld

ABSTRACT: Purified core histones (H2A, H2B, H3, and H4) and bacteriophage T7 DNA have been reconstituted to form a nucleoprotein complex, and the properties of this complex as a template for transcription by *Escherichia coli* RNA polymerase have been studied. At low ionic strength, RNA chain elongation rates are slow, and the chains produced even after long incubation are short. At higher salt concentrations, chain-elongation rates approach those on naked DNA. Since the salt concentrations used are not in themselves sufficient to dissociate the histones from the DNA, some mechanism must exist that permits passage of the polymerase through histone-covered regions.

No understanding of transcription as it occurs in eukaryotes can be complete without some idea of the role played by the structure of the chromatin template in the various processes which finally give rise to specific RNA products. Thus, studies of the transcription of chromatin must eventually give a description of the effects of the nucleosomes on RNA chain initiation, propagation, termination, and processing. That these effects may be of some importance is indicated by evidence suggesting that transcriptionally active gene sequences are associated with some form (perhaps modified) of nucleosome (Foe et al., 1976; Lacy and Axel, 1975; Weintraub and Groudine, 1976; Camerini-Otero et al., 1978). In order to approach this problem, we have made use of a model system in which the effects of nucleosomes on chain elongation in transcription can be examined systematically. In this system, we have reconstituted the purified histones of the nucleosome core onto bacteriophage T7 DNA and have used conditions under which initiation and propagation by *E. coli* RNA polymerase can be measured separately. We find that both processes are affected. At low ionic strength, propagation is slowed, and eventually halted, by nucleosomes; these inhibitory

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effects are sensitive to higher salt concentrations and can be abolished at salt concentrations which are not normally sufficient to dissociate histones from the DNA. Under the higher salt conditions, the histones, though present, appear to have no effect upon the rate at which *E. coli* polymerase can transcribe the T7 DNA template.

Materials and Methods

DNA. T7 virus was grown and harvested as described by Yamamoto et al. (1970) and purified by CsCl density centrifugation. T7 DNA was isolated from the phage by gentle phenol extraction, dialyzed extensively against 0.1 M NaCl, 0.01 M Tris[1] (pH 8), 0.001 M EDTA (pH 7), and stored at 4 °C at a concentration of about 1 mg/mL.

Histones. A mixture of the four histones H2A, H2B, H3, and H4 was prepared by acid extraction of chicken erythrocyte chromatin which had been stripped of the lysine-rich histones H1 and H5 by treatment with 0.7 M NaCl (Camerini-Otero et al., 1976). Alternatively, mixtures of individual histones were used. These were purified to homogeneity by column chromatography (Van der Westhuyzen and Von Holt, 1974) and kindly provided by Drs. R. D. Camerini-Otero and R. Simon. Similar results were obtained with both kinds of histone preparations.

Reconstitution. Reconstitution of nucleosomes onto T7 DNA was carried out by gradient dialysis from 5 M urea, 2 M NaCl, as described earlier (Camerini-Otero et al., 1976).

RNA Polymerase. E. coli RNA polymerase purified through the DNA-cellulose chromatography stage by the method of Burgess and Jendrisak (1975) was kindly supplied by Dr. M. Zasloff and further purified by glycerol gradient (10–30%) centrifugation in 1.0 M KCl, 50 mM Tris-HCl (pH 8), 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM EDTA. The assay was that described by Burgess (1969), using as template high-molecular-weight duck DNA purified in our laboratory. One unit of activity is defined (Burgess, 1969) as the incorporation of 10 nmol of ATP in 10 min under standard conditions. To estimate the number of polymerase molecules from measured activity, a specific activity of 1025 units/mg of pure polymerase was assumed (Burgess and Jendrisak, 1975). This is based on a specific activity of 810 units/mg of protein (Burgess and Jendrisak, 1975) and a correction factor of 0.79 mg of polymerase/mg of protein as determined by Lowry assay (Mangel and Chamberlin, 1974), using bovine serum albumin as the standard. Our enzyme preparations had activities of about 500 units/mg of polymerase. The preparations were about 80% saturated with the σ subunit, as judged by gel electrophoresis.

Initiation Complex Formation. RNA polymerase and template were mixed in a buffer containing 10 mM Tris-HCl (pH 8), 1 mM $MnCl_2$, 0.4 mM DTT, and 80 μM each of ATP, UTP, and GTP, in final volumes ranging from 25 to 500 μL (Cedar and Felsenfeld, 1973). The RNA polymerase was diluted just before use into a solvent containing 50 mM Tris (pH 7.9), 0.125 mM EDTA, 1 mM DTT, 10% glycerol. The enzyme was added last, and the mixtures were incubated at 37 °C for 15 min or as described. On occasion, CTP was used in this reaction, and the UTP was added later with identical results.

Propagation in Ammonium Sulfate. To initiated complexes made as just described, 0.25 volume of 1.6 M $(NH_4)_2SO_4$, containing 21 mM $MgCl_2$ and 320 μM CTP labeled with ^3H or ^{32}P, was added. In some experiments (as indicated), rifampicin was also added.

Propagation in Rifampicin. To initiated complexes made as described above an equal volume of a solution containing 5 mM Tris (pH 8), 5 mM $MgCl_2$, 2 mM DTT, 80 μM each ATP, GTP, and UTP, 160 μM ^3H- or ^{32}P-labeled CTP, and 20 μg/mL rifampicin (Sigma) was added. Where indicated, KCl was added to a final concentration of 0.1 M. When a series of kinetic points was planned, each was made up in a separate tube to avoid possible difficulty in sampling in those cases where the chromatin might be partially precipitated.

Propagation experiments designed to study the effects of NaCl concentration (e.g., Figures 11 and 12) were performed using a modification of this procedure. Initiation was carried out in a solution containing 180 μM ATP, 180 μM GTP, 16 μM [^3H]UTP, 1 mM $MnCl_2$, 10 mM Tris buffer (pH 8), and 0.4 mM DTT. Typically, 0.3 unit of enzyme and 8 μg of DNA as chromatin template were added to 0.4 mL of reaction mixture and incubated for 15 min at 37 °C. Initiation was stopped by adding 25 μg of rifampicin. To begin propagation, 1.2 mL of a solution was added containing 7 mM $MgCl_2$, 160 μM CTP, 40 μM ATP, 40 μM GTP, 8 μM UTP, and a variable concentration of NaCl. The use of a 3:1 volume ratio for the propagation solvent relative to the initiation solvent assures that the local concentration of NaCl during addition is never greater than 1.33 times the final concentration. Since the propagation solvent was added dropwise, with gentle mixing, the actual local concentration of NaCl was even closer to the final concentration so that subsequent effects on propagation reflect the final concentration of salt and not the transient effects of addition.

Formaldehyde Fixation. Chromatin or DNA samples were dialyzed at 4 °C into 5 mM sodium phosphate (pH 7.4). They were made 1% in formaldehyde and stored overnight at 4 °C and then again dialyzed against phosphate buffer, followed by 1 mM Tris (pH 8), 0.1 mM EDTA. These fixed complexes were used in the transcription experiments described in Figures 9 and 10.

To measure the amount of histone bound to DNA under typical high-salt concentration propagation conditions, T7 DNA–histone complexes in 0.45 M NaCl, 0.02 M sodium phosphate (pH 7.0) were brought to 37 °C, and 0.1 volume of 15% formaldehyde was added. This mixture (350 μL) was incubated for 1 h at 37 °C, 284 mg of CsCl was then added, and the solution was centrifuged to equilibrium in the Model E ultracentrifuge at 44 000 rpm. The distribution of DNA was then determined using the absorption optics and UV scanner. Bacteriophage λ treated similarly was used as a density standard in a separate cell.

Gel Electrophoresis. Electrophoresis of RNA transcripts was carried out in 1.5% agarose slab gels containing methyl mercury hydroxide, as described by Bailey and Davidson (1976). The gels were calibrated for RNA size using internal markers of 28S, 18S, and tRNA. Transcript RNA, labeled with [^{32}P]UTP, was detected by slicing the gel into sections 4 mm in length, dissolving in 5 M sodium perchlorate at 60 °C, and counting after addition of Hydromix liquid scintillation medium.

Results

Reconstitution. We have prepared nucleosome-covered T7 DNA using a mixture of the four "core" histones and employing the method of gradient dialysis from high salt and urea concentrations used extensively in this laboratory. To examine the integrity of the protein component of the reconstitute, NaDodSO$_4$–gel electrophoretic analysis was used. In

[1] Abbreviations used: Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EDTA, (ethylenedinitrilo)tetraacetic acid; DTT, dithiothreitol.

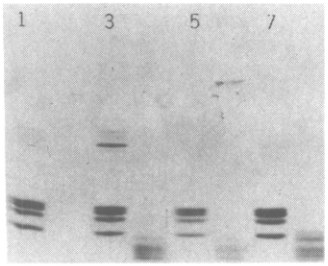

FIGURE 1: $NaDodSO_4$–gel analysis of histones and reconstitutes. Samples were prepared with 5 μg of acid-extracted histones from salt-stripped chromatin (lanes 1 and 2), 6 μg of duck chromatin (lanes 3 and 4), 3.5 μg of salt-stripped chromatin (lanes 5 and 6), and 8 μg of reconstituted T7 chromatin (lanes 7 and 8), in 20 μL of 10 mM Tris-HCl (pH 8), 1 mM EDTA. One of each pair of samples was left on ice, while to the other was added 0.5 μg of trypsin freshly diluted into the same buffer. After 30 min of incubation at 37 °C, 10 μL of a solution containing 3% $NaDodSO_4$, 10% 2-mercaptoethanol, 0.1% phenol red, and 50% glycerol was added to all samples. After a 2-min incubation at 100 °C, the samples were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis as described by Weintraub and van Lente (1974).

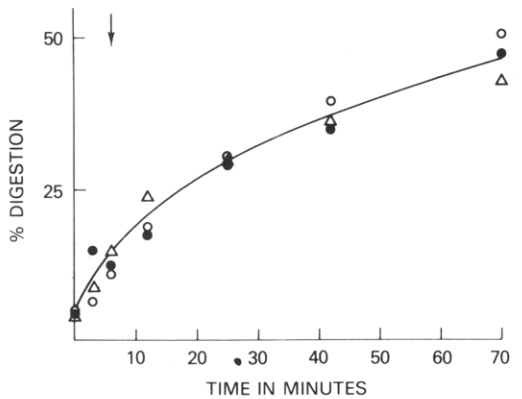

FIGURE 2: DNase digestion kinetics of T7-histone reconstitutes. Chromatin samples containing 200 μg of DNA in 1.5 mL of a buffer containing 10 mM Tris-HCl (pH 8), 10 mM NaCl, and 3 mM $MgCl_2$ were digested with 0.5 μg of DNase I (Worthington). At the times indicated, aliquots were removed and the extent of digestion was monitored by precipitation with perchloric acid: (○) T7 DNA reconstituted with histones acid extracted from salt-stripped chromatin; (●) T7 DNA reconstituted with histones individually purified (see Materials and Methods); (△) salt-stripped duck erythrocyte chromatin. The arrow indicates the point at which radioactive naked DNA, mixed with similar amounts of salt-stripped chromatin, was digested to completion, as measured in a separate experiment.

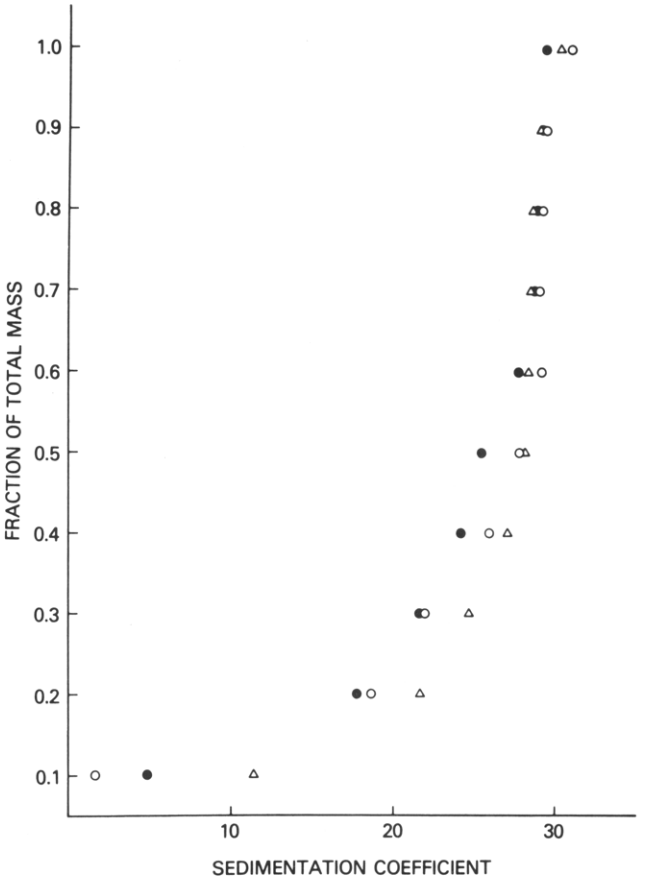

FIGURE 3: Sedimentation velocity analysis of T7 DNA before and after reconstitution. Samples at a concentration of about 200 μg of DNA/mL were made 0.25% sarkosyl, 1 M NaCl, 100 μg/mL proteinase K and incubated at 37 °C for 1 h. These were then diluted tenfold in 0.89 M NaCl, 0.11 M NaOH, 0.02 M EDTA, 0.2% sarkosyl and centrifuged in a Beckman Model E ultracentrifuge: (△) starting DNA; (○) DNA extracted from a reconstitute with individual purified histones; (●) DNA extracted from a reconstitute with histones acid extracted from NaCl-stripped chromatin. The graph shows the integral distribution of the sedimentation coefficient, expressed in Svedberg units.

lane 7 of Figure 1 is shown histone recovered from reconstitution with T7 DNA. Comparison with lane 1, in which are shown the purified histones used as starting material, indicates none of the histones is selectively lost or detectably degraded during the reconstruction procedure. In addition, other experiments indicate that all of the histone is recovered bound to the DNA, as measured by the method of Camerini-Otero et al. (1976) (data not shown).

The similarity of the nucleoprotein complex to chromatin was checked by comparing the behavior of the two with respect to a variety of enzymatic probes. Although histones in solution are digested by trypsin (lane 2, Figure 1), they acquire partial resistance when complexed in nucleosomes (Weintraub and Van Lente, 1974), so that they are degraded to a series of discrete fragments. These resistant fragments, which are observed both with total chromatin (lane 4, Figure 1) and chromatin stripped of histones H1 and H5 (lane 6, Figure 1), are also obtained when the T7 DNA–histone reconstitute (with a histone/DNA ratio of 1 g/g) is digested with trypsin (lane 8, Figure 1). Nuclease digestion studies have also been used to demonstrate the fidelity of the nucleosomes produced by this reconstitution procedure (Axel et al., 1974; Camerini-Otero et al., 1976; Sollner-Webb et al., 1976). Of particular interest for the transcription studies below is the observation that the kinetics of digestion of T7 DNA–histone reconstitutes with pancreatic DNase show no rapidly digested component (Figure 2) as might be expected if substantial regions of the DNA remained naked after the reconstitution. As previously described for reconstitutes with other DNAs (Axel et al., 1973; Camerini-Otero et al., 1976; Sollner-Webb et al., 1976), digestion of the T7 DNA–histone reconstitutes with pancreatic DNase or staphylococcal nuclease gives the single- or double-stranded DNA fragment patterns, respectively, associated with nucleosome structure (data not shown).

In view of the lengthy procedures and extensive handling undergone by the DNA during the reconstitution, we examined the integrity of the T7 DNA molecules by alkaline sedimentation velocity analysis. In our hands, as isolated from the virus particles, about 20–30% of the DNA mass shows a reduced sedimentation coefficient (Figure 3) in the single-stranded form. Neutral sedimentation velocity analysis indicates that these are nicks rather than double-strand breaks. When DNA is reisolated from a reconstituted complex, an additional

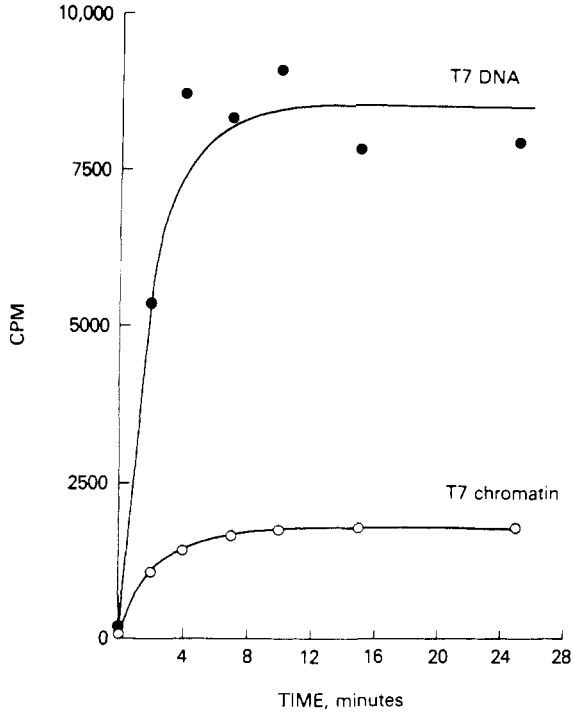

FIGURE 4: Kinetics of formation of initiation complexes on T7 DNA and T7 chromatin. Initiations were carried out as described under Materials and Methods with about 0.008 unit of RNA polymerase and either 4 μg of T7 DNA or 3 μg of T7 chromatin. At various times during the initiation period, the propagation mixture containing $(NH_4)_2SO_4$, rifampicin, radioactive UTP (final specific activity 1600 cpm/pmol), and the fourth triphosphate was added and the initiated polymerases were allowed to propagate for 10 min as described for propagation in $(NH_4)_2SO_4$: (●) DNA; (○) chromatin.

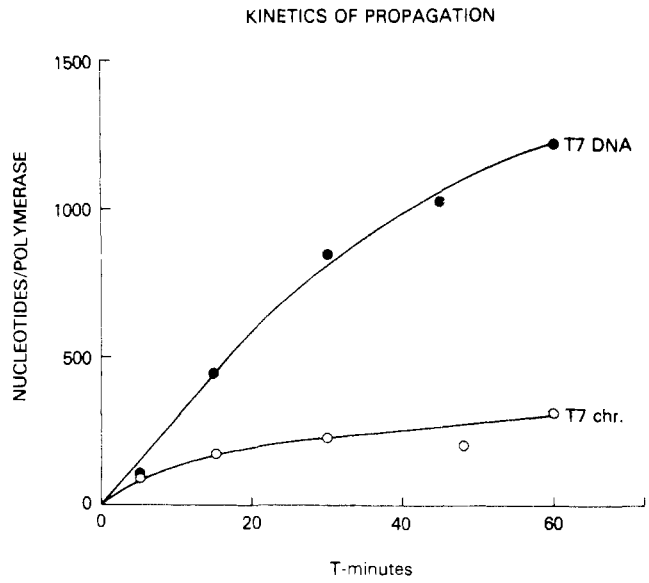

FIGURE 5: Kinetics of polymerase propagation on T7 DNA and T7 chromatin at low ionic strength. Propagation reactions were carried out as described under Materials and Methods using 2.4×10^{-2} units of RNA polymerase and 1.65 μg of DNA (●) or 1.5 μg of chromatin (○). The final mixture contained 50 mM KCl. The specific activity of UTP in the propagation reaction was 1000 cpm/pmol. The number-average length of the chains (in nucleotides) is equal to the total number of nucleosides incorporated divided by the number of bound polymerase molecules. Since in this experiment substrate is in excess of enzyme, all the added polymerase molecules are bound (Cedar and Felsenfeld, 1973).

10-20% of the strands has undergone scission (Figure 3), resulting finally in somewhat less than one nick per duplex molecule. Sedimentation velocity analysis of the reconstituted T7 chromatin in 5 mM Tris-HCl (pH 8) showed a single hypersharp boundary with a sedimentation coefficient roughly three times that of the DNA analyzed under similar conditions (data not shown).

Kinetics of Initiation. In order to determine the effect of nucleosomes in the path of propagating RNA polymerase molecules, an assay was used which measures propagation independently of initiation. The assay method involves an initial incubation of the test template with RNA polymerase at low ionic strength, using only three of the four triphosphates. Under these conditions (Hyman and Davidson, 1970; Cedar and Felsenfeld, 1973), only a limited number of initiations occurs on each molecule of template. In the first set of experiments, the template/enzyme ratio was such that enzyme concentration was limiting. In this way, all polymerase molecules propagate during the assay, and various templates could be compared directly for propagation efficiency.

In order to use this method to study propagation, the initiation step must be allowed to proceed to completion, i.e., until all accessible sites are saturated. To find proper conditions, the initiation was carried out as described above, and at various times after the start of the reaction rifampicin was added to a final concentration of 10 μg/mL along with the fourth nucleoside triphosphate. As shown in Figure 4, rifampicin-resistant complex formation under the initiation conditions is complete in about the same time (within a factor of two) on naked DNA and on the nucleoprotein complex; thus, the presence of the histones on the DNA does not appreciably alter the time required to saturate the template.

These results confirm earlier experiments by Cedar and Felsenfeld (1973) for formation of salt-resistant complexes under conditions of RNA polymerase excess. The initiation period used routinely was 15 min, a time sufficient to assure that all potential complexes were formed. In the experiments to be described below, complexes initiated in this way are used to measure propagation rates in a variety of solvents. Alternatively, propagation on different templates can be allowed to proceed under "standard" conditions, so that the rate of incorporation becomes a function of the number of bound polymerase molecules (Cedar and Felsenfeld, 1973). The effects of ionic conditions on propagation rates are discussed first. It will then be shown that, under the initiation conditions described here, the number of RNA polymerase molecules bound to the T7 DNA–histone complex is much smaller than the number bound to protein-free T7 DNA.

Propagation. The kinetics of polymerase propagation on the T7 nucleosome complex at low ionic strength are compared in Figure 5 with propagation under the same conditions on naked DNA. Early in the reaction, propagation on the nucleohistone proceeds at a rate about half that found on DNA. However, the rate of incorporation gradually slows, so that by 60 min an average polymerase has traveled only 20% as far on the chromatin template as on the naked DNA.

There are several possible explanations of this phenomenon. The polymerase molecules might be inactivated irreversibly during incubation with the chromatin template; they might terminate transcription, fall off, and be unable to continue synthesis because they cannot reinitiate; or they might remain bound but with continued propagation blocked by bound histones. To test these possibilities, propagation reactions which had nearly halted under low salt concentration conditions were made 0.4 M in $(NH_4)_2SO_4$ to alleviate inhibition by bound histone (Cedar and Felsenfeld, 1973). As shown in Figure 6, upon addition of the salt, propagation immediately recom-

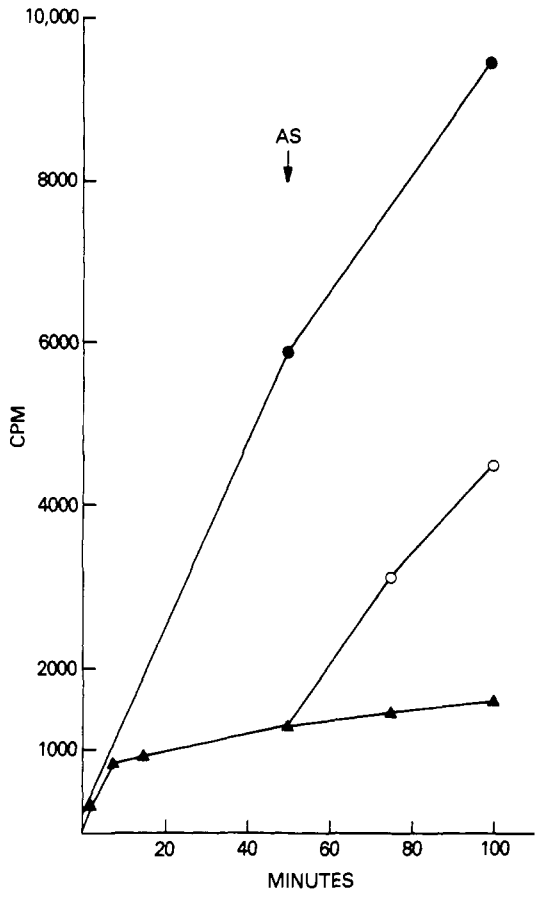

FIGURE 6: Estimation of active polymerases on T7 chromatin after extensive propagation. The experiment was similar to that shown in Figure 5, except that at 45 min ammonium sulfate was added to the propagating system with T7 DNA as template and to one of two samples with T7 chromatin as template. The final concentration of $(NH_4)_2SO_4$ was 0.4 M: (●) propagation on T7 DNA, with last half in 0.4 M $(NH_4)_2SO_4$; (▲) propagation on T7 chromatin; (○) propagation on T7 chromatin after the addition of ammonium sulfate.

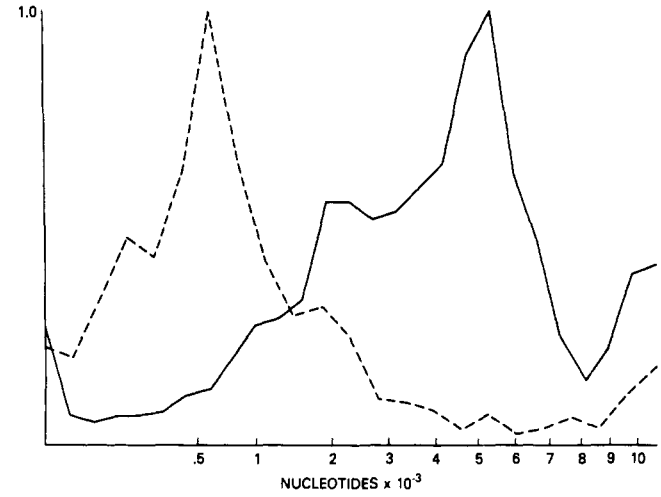

FIGURE 7: Sucrose gradient analyses of RNA from propagation reactions on T7 DNA and chromatin. After 30 min of propagation in 25 mM KCl (see Materials and Methods), aliquots were removed and further reaction was terminated by addition of $NaDodSO_4$ to 0.5% and EDTA to 10 mM. The reaction mixture was extracted with phenol, layered on a 5–20% sucrose gradient prepared over a 0.2-mL shelf of saturated CsCl, and centrifuged for 100 min at 20 °C at 56 000 rpm in the Beckman SW60 rotor. Fractions were collected from the bottom and Cl_3AcOH precipitated. Sedimentation coefficients were determined from the position of internal markers, 18S and 28S RNA, the positions of which were determined by their optical absorbance. Sedimentation coefficients were converted to nucleotide chain lengths using the relationship $\log n = 2.10 s_{20,w} + 0.644$ (Hyman and Davidson, 1970), and the profiles were normalized by computer to the counts per minute in the highest fraction. This fraction contained 111 cpm over background in the case of the chromatin and 375 cpm over background in the case of the DNA: (—) DNA template; (- - -) chromatin template.

mences at a rate very similar to the propagation rate on DNA under the same conditions. Since no reinitiation is possible under these conditions, the polymerases must neither have fallen off nor been inactivated; the great majority must remain bound to the template, capable of propagating but halted by a salt-sensitive obstacle.

The simplest explanation for this inhibition of propagation on the nucleohistone complex is that the polymerase stops when it reaches a nucleosome. Several additional experiments suggest that this might not be the case. A first consideration is the distance traveled by the polymerase. At a histone/DNA ratio of 1, the average interbead spacing is about 50 nucleotides (Sollner-Webb and Felsenfeld, 1975). Since the number of propagating enzyme molecules and the amount of synthesized RNA are known in these reactions, the number-average size of the RNA made can be calculated, as shown along the ordinate of Figure 5, to be 400–500 nucleotides, about the equivalent of two nucleosomes. To verify independently this estimate of the distance traveled by the enzyme, the transcribed RNA was isolated from propagation reaction mixtures and analyzed by sucrose gradient sedimentation, as shown in Figure 7. The RNA transcribed from the chromatin shows a peak of radioactivity at about 600 nucleotides and includes molecules greater than a thousand nucleotides long. When the molecular weight of individual fractions is used to calculate the number-average molecular weight of the RNA as described by Cedar and Felsenfeld (1973), the result is in substantial agreement with that calculated from the amount of synthesis measured at the time that the aliquot is taken (about 350 nucleotides for the sample shown in Figure 7). Because of the possibility of RNase degradation during the incubation, these averages may represent an underestimate. In any case, the RNA product is much shorter than that transcribed under similar conditions from protein-free T7 DNA (Figure 7), implying that propagation is, in fact, inhibited in the presence of histones.

Although the average distance traveled by the enzyme during the propagation reaction on the nucleoprotein template is greater than the average expected distance between nucleosomes, these experiments do not rule out the possibility that the enzyme is preferentially initiating on relatively protein-deficient DNA molecules, where the average internucleosomal distance is much greater. To investigate this issue, we undertook an examination of the buoyant density of the molecules upon which propagation actually takes place. In order to increase the resolution of these experiments, we sheared the T7 DNA, before reconstitution, to fragments about 20% of the length of the intact molecule. Control experiments indicated that both the DNA and the DNA–histone complex made from it retained the same number of initiation sites as intact materials and that the propagation kinetics were identical (data not shown). Initiated enzyme molecules were allowed to propagate for a short distance to label those molecules on which extension was actually occurring, and then the complexes were fixed with formaldehyde and centrifuged to equilibrium in a CsCl gradient containing 4 M guanidinium chloride to reduce intermolecular aggregation (Jackson and Chalkley, 1974). As shown in Figure 8A, when transcription complexes on protein-free DNA are analyzed in this fashion, they are found at

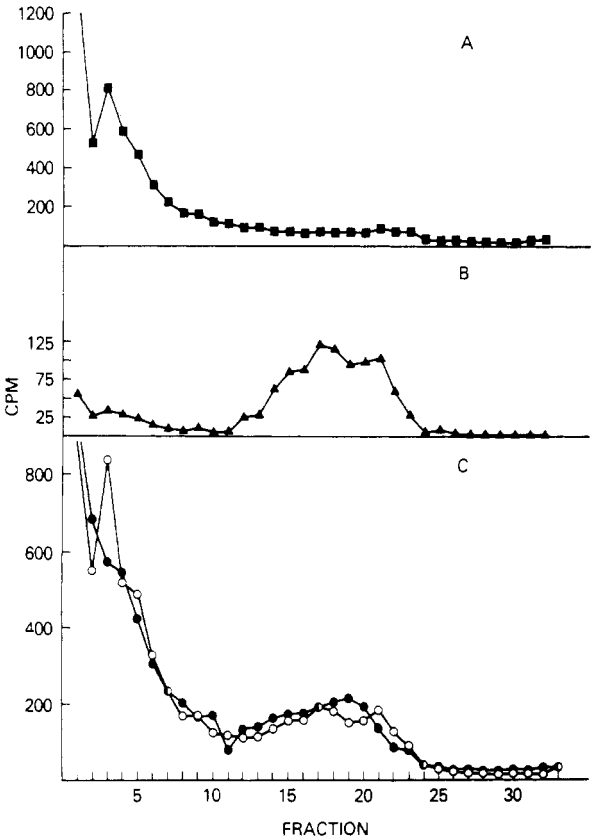

FIGURE 8: CsCl density gradient analyses of active templates in propagation reactions on T7 DNA and chromatin. Propagation reactions were carried out in 100 mM KCl under standard conditions (see Materials and Methods) using [^{3}H]UTP. Propagation reaction mixtures were chilled, fixed with formaldehyde, and centrifuged to equilibrium in CsCl-guanidinium chloride as described in the text. Tubes contained 3.05 g of guanidine hydrochloride and 2.38 g of cesium chloride made up to a total volume of 8 mL with 5 mM Tris-HCl (pH 8), 0.1 mM EDTA. Centrifugation was at 4 °C, 40 000 rpm in a Beckman SW65 rotor. The [^{3}H]UTP incorporated into acid-precipitable material was measured for each fraction: (A) naked DNA; (B) chromatin; (C) duplicates of A and B mixed before the addition of the formaldehyde where ● is observed distribution of acid-precipitable radioactivity and ○ is the sum of A and B.

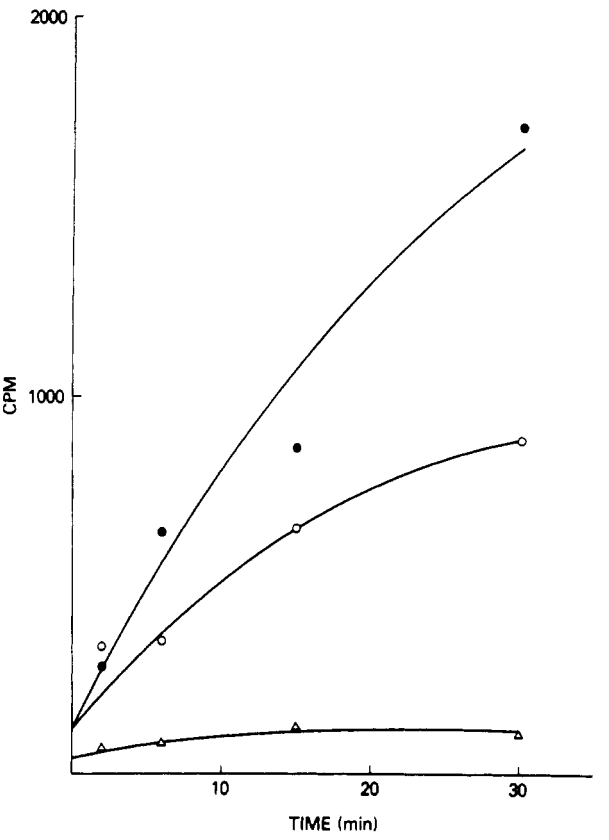

FIGURE 9: Propagation on formaldehyde-fixed chromatin: Propagation reaction on (●) T7 DNA; (○) T7 chromatin; (△) T7 chromatin fixed with formaldehyde as described under Materials and Methods. Reactions were carried out as described in Figure 5. The various reaction mixtures all contained about 4 μg of DNA. The T7 DNA reaction contained 0.01 unit of RNA polymerase. The other reactions contained 0.02 unit.

or near the bottom of the tube. Transcription complexes on the nucleoprotein complexes contain very little such material, as shown in Figure 8B; almost all material bands in the upper half of the gradient with the unlabeled chromatin, suggesting that the molecules active as template are histone covered. It seemed possible that complexes on protein-deficient DNA molecules were not detected because they had been cross-linked by the formaldehyde to fully covered DNA molecules. This possibility was eliminated by mixing samples in which propagation was proceeding separately on DNA and nucleohistone templates and then fixing. As shown in Figure 8C, the observed distribution of complexes is just the sum of the two individual mixtures, implying that no intermolecular cross-linking occurs under the conditions used.

Although these experiments demonstrate that the propagation is not occurring on chromatin template molecules that are greatly deficient in histones, they leave open the possibility that the polymerase has selected regions on the chromatin where the DNA is locally deficient in nucleosomes. However, if this is true and if the enzyme stops because it is unable to pass nucleosomes during the propagation reaction, it should not matter whether the nucleosomes are immobilized by fixation. In that case, propagation on a chromatin template which has been fixed with formaldehyde should resemble that seen normally. To determine whether this is so, propagation was carried out on T7 chromatin fixed with formaldehyde. The data in Figure 9 show that propagation is severely inhibited on the fixed complex. Sucrose gradient analysis of the product indicates that this inhibition results from a drastic reduction (all product <100 nucleotides long) in the size of the transcript (data not shown).

Several control experiments were carried out in order to be sure that this inhibition of propagation was not due to effects of fixation on the DNA itself. As shown in Figure 10, propagation is inhibited on the fixed chromatin even after addition of ammonium sulfate to 0.4 M, conditions which eliminate the inhibitory effects of unfixed histones. In contrast, naked DNA carried through the same fixation procedures remains a vigorous template for enzyme propagation, indistinguishable from untreated DNA. Finally, the data in Figure 10 show that propagation is observed easily on a fixed chromatin template reconstituted at a lower protein/DNA ratio to increase the average interbead distance. These data, therefore, imply that, as expected, the polymerase encounters an obstacle at fixed nucleosomes. If the fixation has not altered the placement of the nucleosomes, these data then suggest that when the polymerase encounters these same nucleosomes on the unfixed template the obstacle is not impassible, and some propagation is, therefore, observed. It should be noted, however, that if the fixation procedure transfers histones onto regions which are naked in the unfixed template similar results might be obtained. Although we have no evidence that this process occurs, we cannot rule out this possibility.

The gradual inhibition of propagation by nucleosomes in the

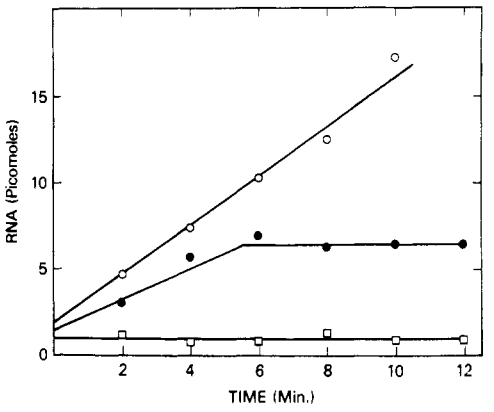

FIGURE 10: Propagation on formaldehyde-fixed templates. Propagation was carried out in 0.4 M ammonium sulfate, after initiation under standard conditions (see Materials and Methods). Samples were formaldehyde treated as described under Materials and Methods: (○) 5.7 μg of T7 DNA; (●) 7.1 μg of T7 DNA-histone complex, protein/DNA ratio 0.2; (□) 6.8 μg of T7 DNA-histone complex, protein/DNA ratio 1.0. Each reaction mixture contained 1.2 units of RNA polymerase.

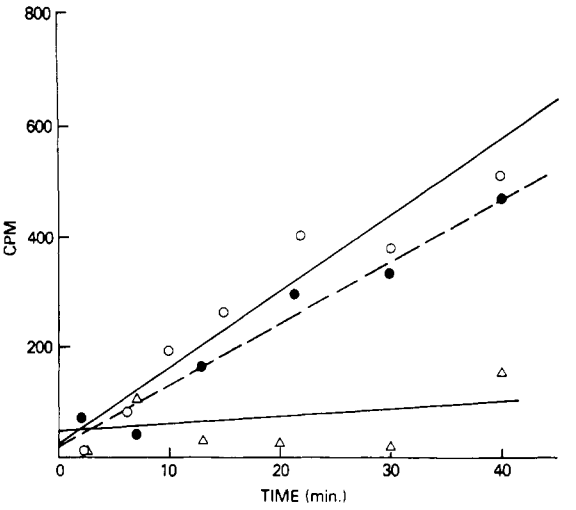

FIGURE 11: Propagation reaction on T7 chromatin at different ionic strengths. Each reaction mixture contained 8.4 μg of T7 chromatin, three of the four triphosphates, and 0.3 unit of enzyme in a volume of 0.4 mL (see Materials and Methods). After incubation at 37 °C for 14 min, rifampicin was added followed by 1.2 mL of solvent containing the fourth triphosphate and sufficient salt to give the desired final concentration (see Materials and Methods). 0.2-mL aliquots were taken as a function of time; incorporation during the initiation period has been subtracted: (△) 1 mM Tris (pH 8); (●) 0.4 M $(NH_4)_2SO_4$; (○) 0.45 M NaCl.

previous experiments is observed in low ionic strength buffers (10–50 mM monovalent ions) where the salt linkages between histones and DNA would be expected to be most effective. We have, therefore, investigated the effect of salt concentration on the rate of propagation over long time periods. Initiation of transcription was carried out as before, using three of the four nucleoside triphosphates in low salt concentrations; the fourth triphosphate, rifampicin, and sufficient salt solution to give the desired final concentration were added to begin propagation (see Materials and Methods). In these experiments, the polymerase concentration is sufficient to saturate the template (Figure 4). The number of initiated polymerases per unit template is, thus, held constant, and reinitiation is prevented by the presence of rifampicin (as well as by the presence, in some cases, of high salt concentrations). The rate of incorporation during the propagation step is, therefore, a direct reflection of the rate of chain elongation. Typical results are shown in Figure 11, where the propagation rates are measured at the low salt concentrations used above, as well as in 0.4 M ammonium sulfate and in 0.45 M NaCl. It is evident that the elongation rates in the latter two solvents are about the same. Furthermore, propagation in 0.4 M ammonium sulfate is as fast on chromatin templates as on DNA (Cedar and Felsenfeld, 1973). Thus, in 0.45 M NaCl, the propagation rate on T7 chromatin is similar to that on naked T7 DNA.

Results given below (Figure 14) show that there are about 10% as many sites available on the histone-covered template as on protein-free DNA under the conditions of initiation used here. By direct titration with polymerase (Hyman and Davidson, 1970; Cedar and Felsenfeld, 1973), it can be shown that each T7 DNA molecule will bind about one polymerase molecule under the particular initiation conditions used in this set of experiments. From this information it can be estimated that the propagation rate on T7 chromatin in 0.45 M NaCl shown in Figure 11 corresponds to an elongation rate of about 30 nucleotides per minute per chain.

Over a range of intermediate NaCl concentrations, intermediate rates are observed for propagation on chromatin, as shown in Figure 12. Over the same range of conditions, propagation on naked DNA varies only slowly (data not shown). To prove further that these increases are, in fact, due to an increase in propagation rate, a gel electrophoretic analysis was performed on RNA made during propagation reactions in 0.45 M NaCl, as shown in Figure 13. After 20 min of propagation, the distribution of RNA chain lengths now peaks at about 1500 nucleotides. We have also determined the size of this RNA by sucrose gradient sedimentation, with similar results (data not shown). The RNA chain lengths obtained after propagation in 0.45 M NaCl are considerably larger than those obtained by propagation at low ionic strength for comparable times (Figure 7), and transcripts on nucleoprotein and DNA templates are similar in size. All of the data are consistent with the conclusion that as salt concentrations are increased, RNA polymerase propagation rates through histone-covered DNA are progressively increased.

Although the histone species present in the T7-histone template are normally bound to DNA at NaCl concentrations as high as 0.45 M, it seemed desirable to ascertain that this was true for the particular samples used here. We, therefore, fixed the histones to the DNA with formaldehyde in 0.45 M NaCl at 37 °C and centrifuged the product to equilibrium in CsCl (see Materials and Methods). Between 85 and 90% of the DNA, as measured by ultraviolet absorbance in the analytical ultracentrifuge, banded as a unimodal distribution in the range of densities from 1.44 to 1.56, with a weight average density of 1.497 g/cm^3. The latter is the expected value for a 1:1 protein-DNA complex. We conclude that the histones remain bound to DNA at the highest ionic strength used in the transcription experiments.

Just as the effect of histones on RNA chain propagation rates can be determined if the number of initiation sites is known, the number of growing chains can be determined if the propagation rate is known. The initiation step is carried out as described earlier, in the presence of only three triphosphates and an excess of enzyme. By making the reaction mixture 0.4 M in ammonium sulfate simultaneously with the addition of the fourth nucleoside triphosphate, reinitiation is prevented. Furthermore, the propagation rate in ammonium sulfate is the same on histone-containing templates as it is on naked DNA (Cedar and Felsenfeld, 1973). When limiting template is used, therefore, the propagation rate in ammonium sulfate is a measure of the relative number of initiation sites available to

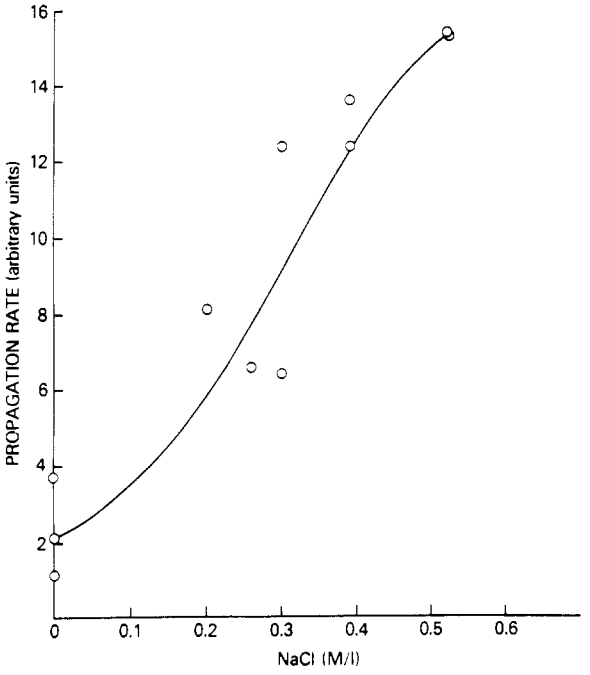

FIGURE 12: Propagation rates on T7 chromatin as a function of ionic strength. Initiation and propagation were carried out as described under Materials and Methods. In each experiment, the initiation step was performed under identical conditions using low ionic strength and three nucleoside triphosphates. At the end of the initiation period, rifampicin was added, followed by the fourth nucleoside triphosphate and sufficient NaCl to give the final concentration shown. The propagation rate is calculated from the slope of a graph of [^{3}H]UTP incorporation vs. time for a 0–30-min period of propagation. The mean propagation rate in 0.4 M $(NH_4)_2SO_4$ on this template is 12.3 in the arbitrary units used on this graph.

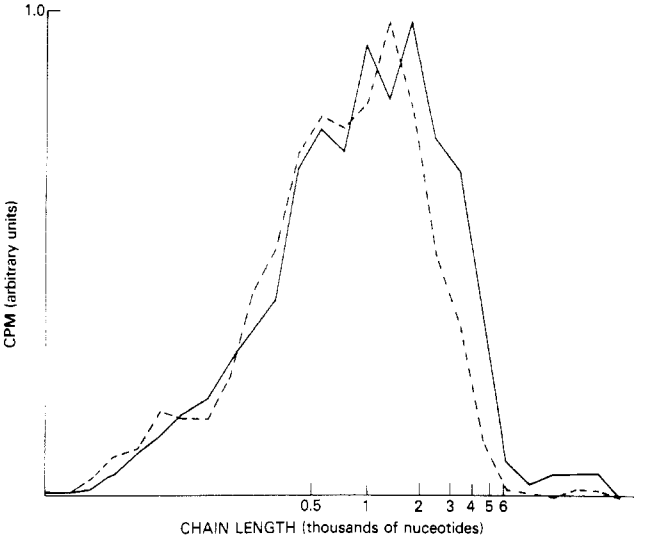

FIGURE 13: Electrophoretic analysis, carried out in 1.5% agarose slab gels containing methyl mercury hydroxide, of RNA chains from high salt propagation reactions on T7 chromatin and DNA. Propagation reactions were carried out as described under Materials and Methods and Figure 12, at a final NaCl concentration of 0.45 M, but [^{32}P]UTP was substituted for [^{3}H]UTP. After 20 min of incubation at 37 °C, the reaction was stopped by making the solution 90 μg/mL in actinomycin D. The solution was made 0.34 M in NaCl, 34 mM in magnesium acetate, and 2.8 mM in $CaCl_2$. Pancreatic DNase, previously iodoacetate treated, was added to a final concentration of 7 μg/mL, and incubation was continued for 30 min. The solution was extracted with phenol and with chloroform–isoamyl alcohol (24:1), and the RNA was precipitated with 2 volumes of ethanol at −20 °C. The RNA was centrifuged, redissolved in 0.25 mL of a solution containing 0.2% sodium dodecyl sulfate, 1 mM Tris buffer, and 1 mM EDTA (pH 6.5) and passed over a 0.75 × 28 cm Sephadex G-50 column in the same solvent to remove nucleotides. After ethanol precipitation of the material eluting at the front, it was redissolved in water and buffer to a final concentration of 0.025 M boric acid, 2.5 mM sodium borate, 0.5 mM EDTA, 5 mM sodium sulfate, 5 mM methyl mercury hydroxide. The sample was electrophoresed and analyzed by the method of Bailey and Davidson (1976) (see Materials and Methods): (—) Transcript from T7 DNA–histone complex; (- - -) transcript from T7 DNA. Totals of 13 100 and 15 300 cpm were recovered from the chromatin and DNA gel channels, respectively, representing in each case about 60% of the total acid-precipitable counts incorporated in the original reaction mixtures.

the polymerase under the conditions we have used (see Discussion).

The earlier results of Cedar and Felsenfeld (1973) and Cedar (1975) indicated that the presence of histones on eukaryote DNA reduces the total number of available RNA polymerase initiation sites under these same conditions. We have carried out similar studies of the effect of nucleosomes on the availability of initiation sites for RNA polymerase on T7 DNA. As shown in Figure 14, reconstitution of histones onto the DNA reduces the number of available sites; the level of inhibition depends on the protein/DNA ratio. By this assay, the lysine-rich histones were not significantly more efficient inhibitors on a weight basis than the four core histones. Additional experiments (not shown) indicate that the avian erythroid histone H5 behaves like histone H1.

Although previous studies indicated that nucleosomes reduced the number of available sites, they could yield no interpretable information as to whether the presence of nucleosomes affects the specificity of initiation. This problem can be addressed in this system because of the low sequence complexity of the T7 DNA (molecular weight 2.5×10^7). As a first step in addressing this problem, we separated the two strands of T7 DNA by CsCl gradient centrifugation in the presence of poly(U,G) (Summers and Szybalski, 1968). These separate strands were then used in excess in a solution hybridization assay to test for the strand specificity of the transcription products.

When RNA transcribed from naked T7 DNA was annealed with these probes, roughly 75% of the transcript hybridized to the "heavy" strand of T7 DNA (Table I). Surprisingly, however, RNA transcribed from nucleosome-covered T7 DNA did not display this asymmetry. Both strands are represented equally in the transcript. To see whether the DNA had acquired new sites, perhaps because of the slight increase in the number of DNA nicks found in reconstitutes (Figure 2), T7 DNA was reisolated from the nucleosome complex and retested for transcription asymmetry. As shown in the last column of Table I, this template is as asymmetrically transcribed as DNA isolated from the bacteriophage, implying that only when the histones are present on the DNA can the alteration in initiation specificity be observed.

Because the separated strands of DNA were present in excess, one possible explanation for the extensive hybridization to the "light" strand might be contamination of this DNA with small amounts of the "heavy" DNA strand. If so, however, the hybridization reaction should be slower than would be expected from the apparent DNA concentration, which is mostly light strand. When the rates of the hybridization reactions with heavy and light strands were compared, the light-strand reaction was found to occur as rapidly as the heavy-strand reaction at similar DNA concentrations (Figure 15), indicating that the former was not being driven by a small contaminant of the test DNA. The observed shift in hybridization specificity, therefore, represents a true change in strand specificity in the transcription reaction induced by the presence of the nucleosomes.

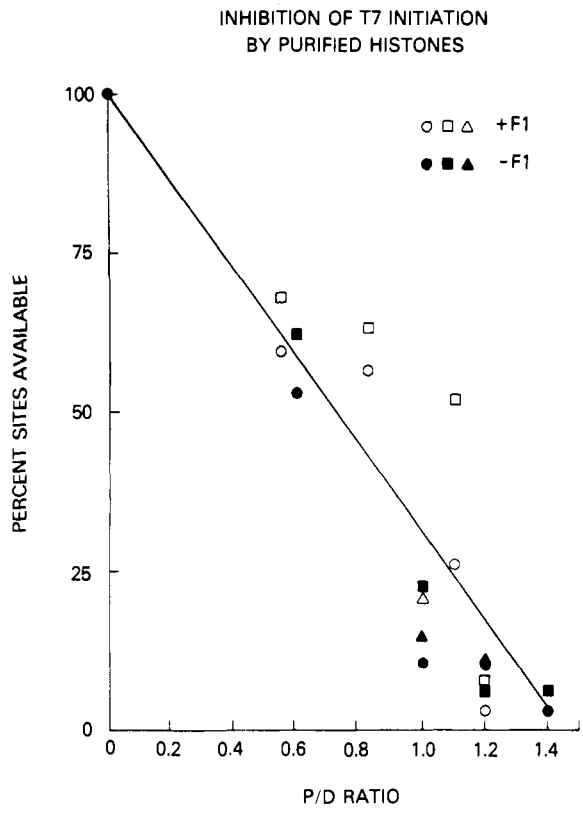

FIGURE 14: The number of initiation sites available on templates reconstituted at the protein/DNA ratios shown was determined from the slope of the kinetics of propagation in ammonium sulfate as described under Materials and Methods.

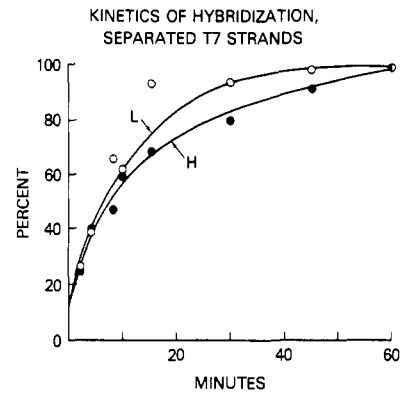

FIGURE 15: Kinetics of hybridization to separated strands of DNA. T7 DNA transcripts were prepared, purified, and hybridized to separated strands of T7 DNA as described in Table I. Aliquots were treated with S1 nuclease at the indicated times: H, hybridization to the H strand; L, hybridization to the L strand.

TABLE I: Hybridization Analysis of Transcripts.[a]

strand	DNA (%)	chromatin (%)	chromatin DNA (%)
H	70 (±4)	49 (±3)	74 (±11)
L	30	51	26

[a] Propagation reactions were carried out as described under Materials and Methods, except that Hg-UTP was added to a level of 25% of the total UTP level. Initiation and propagation were normal with this level of substitution. After 60 min of incubation, the reactions were terminated by the addition of $NaDodSO_4$ to a final concentration of 0.5% and EDTA to 10 mM. These mixtures were then passed over a 15 × 0.5 cm Sephadex G-50 column equilibrated in 0.1 M NaCl, 0.05 M Tris-HCl (pH 7.5), 2% $NaDodSO_4$. The peak emerging at the excluded volume was collected and stirred with 0.25 mL of thiol-agarose for 2 h at room temperature. The agarose beads were then loaded into a 1-mL syringe and washed, and the RNA was eluted by washing with the same buffer containing 0.1 M mercaptoethanol. After the addition of NaCl to a final volume of 0.3 M, followed by ethanol precipitation, the sample was redissolved in water and hybridized in 0.2 M sodium phosphate (pH 6.8), 1 mM EDTA, and 0.15% $NaDodSO_4$ to 180 ng of separated T7 DNA strands in a final volume of 10 μL. The extent of hydridization was determined by S1 nuclease treatment as described elsewhere (Williamson and Felsenfeld, in preparation). The results are expressed as the extent of hybridization to one strand divided by the sum of the extents of hybridization to the two strands. This latter amount was generally about 90% of the input radioactivity. Over the incubation periods used in these reactions, there is no self-annealing of the RNA observed in the absence of added DNA. Results are the average of four, six, and two determinations, respectively, for DNA, chromatin, and chromatin DNA transcript.

The experimental design used here does not have sufficient resolution to identify unambiguously the initiation sites used under these conditions. The data of Table I indicate that the strand specificity of transcription on naked T7 DNA resembles that observed under other initiation conditions (Chamberlin, 1974). However, the number of initiation sites measured per T7 DNA molecule varies depending on the method of initiation. The method used here, involving preinitiation in the presence of three triphosphates, gives about one initiation per DNA molecule, while an RNA polymerase binding assay gives six to eight initiation sites per molecule (Hinkle and Chamberlin, 1970). In considering initiations on the DNA–histone template, this ambiguity must be taken into account. It is also possible that new initiation sites on the nucleoprotein template occur at nicks or through the action of core enzyme. Much additional work will be required to characterize the mechanism of RNA polymerase initiation on chromatin templates.

Discussion

Evidence has accumulated that some form of nucleosome may be associated with actively transcribed gene sequences (Axel et al., 1973; Lacy and Axel, 1975; Foe et al., 1976; Camerini-Otero et al., 1978). If this is the case in vivo, then it is of considerable interest to understand the details of the mechanism of transcription as it occurs on DNA associated with histones. Although a large body of experimental information exists concerning transcription of chromatin by exogenously added RNA polymerases, much of this information suffers from the disadvantages that (1) effects on various aspects of the transcription reaction were not separated, (2) the nature of the base line activity of the polymerase on the naked DNA was not well understood, and (3) the nucleohistone templates used were very complex, both with respect to underlying DNA sequence and overall protein composition. In an attempt to address these problems, we have turned to a relatively simple transcription system involving *E. coli* RNA polymerase and a complex of bacteriophage T7 DNA with the core histones. We have used reconstitution procedures developed earlier to allow us to cover T7 DNA with defined amounts and species of purified histones, so that they alone can contribute to the effects observed. We have also made use of methods previously developed for limiting the number of polymerase molecules that can initiate on the template, so that propagation rates can be measured independently of the initiation step. The analysis of early promoters on T7 DNA is

complex and not yet completely resolved (Chamberlin, 1974). In most of the experiments reported here, the precise identity of the initiation sites is irrelevant. However, further work in this area may eventually allow study of the effect of histones on the details of the initiation process.

A question of great interest immediately posed by the possibility that active genes are covered by histones is whether propagating polymerases can simply read through histone-covered DNA, perhaps by some unfolding of the nucleosome (Weintraub et al., 1976). When this question is investigated using the T7 nucleohistone under conditions of low ionic strength, when ionic bonds should be relatively stabilized, initiated *E. coli* polymerase molecules are found capable of propagating for only a short time (Figure 5). The immediate resumption of propagation upon addition of ammonium sulfate suggests that the polymerase molecule has stopped while remaining bound to the template; the nucleosomes at low ionic strength prove to be an obstacle to RNA chain propagation, at least as catalyzed by the bacterial polymerase. Interestingly enough, however, the inhibition of propagation by nucleosomes does not appear to be exerted immediately but rather occurs only after the polymerase has propagated over distances corresponding to the DNA complement of several nucleosomes. Because only a small fraction of the DNA in a propagation reaction is actually copied by the polymerase, it is extremely difficult to prove conclusively that the enzyme has not sought out for transcription some special region of the chromatin where nucleosomes are not found. However, the controls presented all strongly suggest that this is not the case. For example, the distance mentioned is a *number-average* distance, so that it cannot be argued that only a few of the transcribing enzymes give rise to RNA molecules longer than an average internucleosome space. Moreover, the bulk of the DNA molecules that are actually being transcribed are not grossly depleted in protein, as indicated by their density (Figure 8). Finally, the nucleosomes can easily be converted to immediate obstacles by fixing them in place with formaldehyde, under conditions which leave naked DNA, even if surrounded by nucleosomes, an active template for propagation (Figure 10).

Taken together, therefore, these data suggest that even at low ionic strength propagating RNA polymerase molecules can transcribe some nucleosome-covered DNA. The question then arises why, if polymerases can pass one nucleosome, they cannot pass an indefinite number at low ionic strength. Two classes of models can be presented to explain these results. The first suggests that there is more than one kind of nucleosome and that 15–30% of those reconstituted onto the T7 DNA template have the ability to stop propagation. A second model suggests that the polymerase molecules can transcribe nucleosome-covered DNA because the nucleosomes slide along the DNA; the increasing inhibition would result from the nucleosomes piling up in front of the polymerase. If this were the case, it might be predicted that as histone–DNA bonds were weakened, this sliding might become more efficient or nucleosome exchange induced at higher salt concentrations could prevent sliding nucleosomes from piling up. The ionic-strength dependence for polymerase propagation on T7 chromatin (Figure 12) is certainly consistent with this model. Whatever the mechanism, it is clear that as the ionic strength is raised the bound histones have a decreasing effect on the propagation rate. In 0.45 M NaCl, the propagation rate on the nucleoprotein template is similar to that on protein-free T7 DNA, even though the core histones are still bound to DNA in that solvent. Our observations, thus, differ from those of Cremisi et al. (1977), who studied transcription of SV40 nucleoprotein complexes by *E. coli* polymerase. They found that these complexes were poor templates, requiring enzyme/DNA ratios two orders of magnitude larger than those we have used in the present studies. On the other hand, Meneguzzi et al. (1978) find that the minichromosome of BK virus can be transcribed by *E. coli* RNA polymerase in 0.15 M NaCl, although more slowly than in the case of a naked DNA template. The RNA product is much longer than the longest observed internucleosome spacing, and the authors suggest, as we do, that the polymerase is capable of passing through histone-covered regions. Similar results have been reported recently for endogenous RNA polymerase II active on SV40 chromatin templates (Brooks and Green, 1977).

It should be evident that caution must be used in extending the results we have reported with *E. coli* polymerase to the transcription of eukaryotic chromatin in vivo. Furthermore, there is reason to suspect that the histones of transcriptionally active chromatin in vivo may be chemically modified, perhaps with the effect of rendering them more mobile or structurally unstable. Our experiments do show however that in 0.45 M NaCl the unmodified histones of the nucleosomes core offer very little resistance to the passage of *E. coli* RNA polymerase as it transcribes T7 DNA. Even at much lower ionic strength, there is evidence suggesting that the polymerase can traverse histone-covered regions, though in this case the polymerase is eventually impeded. The mechanism by which histone complexes can accommodate the passage of the polymerase is not revealed by these experiments. There are several possibilities: nucleosomes can unfold, slide, or exchange. The T7 DNA–histone complex described here should provide a valuable tool for distinguishing among these possibilities.

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Histone–Histone Interactions in a Lower Eukaryote, *Tetrahymena thermophila*†

Claiborne V. C. Glover and Martin A. Gorovsky*

ABSTRACT: The six pairwise interactions of *Tetrahymena* macronuclear histones H2A (formerly HX), H2B, H3, and H4 have been studied using the techniques of circular dichroism and continuous variation. Parallel experiments have been performed with calf thymus histones as controls, and the 12 possible interspecies pairs have also been examined. The behavior of three of the ciliate histones, H2A, H2B, and H3, is virtually identical with that of their vertebrate counterparts. *Tetrahymena* H4 exhibits a pattern of interactions identical with that of calf thymus H4, but displays significant quantitative differences in the strength of those interactions. Although we have not entirely eliminated the possibility that these differences may be artifactual, we suggest that they reflect the unique primary structure of *Tetrahymena* H4. These results extend the pattern of histone–histone interactions defined for plants, animals, and fungi to include the protists as well and imply the strong evolutionary conservatism of this pattern in spite of the electrophoretic and compositional differences which exist between homologous histones of different organisms. In addition, they demonstrate conclusively that histone HX should be redefined as *Tetrahymena* H2A.

Understanding of the subunit structure of chromatin has become increasingly more refined since the concept was first proposed (Kornberg, 1974). Genomes of higher eukaryotes appear to be organized in particulate structures approximately 100 Å in diameter containing 140 base pairs of DNA complexed with a protein octamer consisting of two each of the four inner histones H2A, H2B, H3, and H4 (for review, see Kornberg, 1977). An H3–H4 tetramer (the arginine-rich histone kernel) appears capable of organizing DNA in much the same manner as it is organized in the intact nucleosome (Sollner-Webb et al., 1976; Camerini-Otero & Felsenfeld, 1977) and presumably is of central importance to nucleosome structure. This hypothesis is supported by the profound evolutionary conservatism of these two proteins (DeLange et al., 1969; Patthy et al., 1973). Histone–histone interactions within the nucleosome have been explored using a variety of techniques, and it has been found that the pattern of interactions (for review, see Isenberg, 1977, 1978), like the histones themselves, has been highly conserved over evolutionary distances as great as those which separate animals (D'Anna & Isenberg, 1974b), plants (Spiker & Isenberg, 1977), and fungi (Mardian & Isenberg, 1978).

The ciliated protozoan, *Tetrahymena thermophila,* is a lower eukaryotic organism possessing two types of nuclei, a transcriptionally active macronucleus and a transcriptionally inactive micronucleus, the latter being responsible for genetic continuity of the species (Gorovsky, 1973). The chromatin of both nuclei exhibits typical periodic as well as particulate structure (Gorovsky et al., 1978), and the properties of *Tetrahymena* histones have been studied in some detail. Histones H1, H2B, H3, and H4 share extensive biochemical similarity with their vertebrate homologues, and only HX cannot be equated unambiguously with a vertebrate histone (Johmann & Gorovsky, 1976a).[1] Although all five histones are found in the macronucleus, we have been unable to demonstrate the presence of either H1 or H3 in the micronucleus (Gorovsky & Keevert, 1975).

The pattern of interactions exhibited by *Tetrahymena* histones has been investigated by electron microscopic analysis of nucleosomes reconstituted from three calf and one *Tetrahymena* histone (Gorovsky et al., 1978). By this assay, *Tetrahymena* H2B and H3 substitute efficiently for their vertebrate counterparts, while HX and H4 do not. These and other observations have led us to speculate on the possible existence of unique interactions among *Tetrahymena* histones, particularly between HX and H4, which might explain the formation of micronuclear nucleosomes in the absence of H3.

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[1] We have previously shown that a fraction formerly referred to as *Tetrahymena* HY is monoacetylated H2B (Johmann & Gorovsky, 1976a).

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