Nucleic Acids Research, 1995, Vol. 23, No. 6 ***1075–1082***

Deposition of histone H1 onto reconstituted nucleosome arrays inhibits both initiation and elongation of transcripts by T7 RNA polymerase

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Received June 13, 1994; Revised and Accepted December 16, 1994

ABSTRACT

The effect of histone H1 on transcription by bacteriophage T7 RNA polymerase was examined using reconstituted chromatin templates. A 3.8 kb linear DNA template consisting of a specific transcription promoter for T7 RNA polymerase placed upstream of 18 tandem repeats of a 207 bp nucleosome positioning sequence derived from the 5S rRNA gene of *Lytechinus variegatus* was used as a template for chromatin reconstitution. Regularly spaced arrays of nucleosome cores were assembled onto this DNA template from donor histone octamers by salt step dialysis. Histone H1 was incorporated onto free DNA or reconstituted chromatin templates and double label transcription assays were performed. The experiments indicated that histone H1 has a strong inhibitory effect on both transcription initiation and elongation. These effects are especially pronounced on chromatin templates, where both transcription initiation and elongation are virtually halted. The inhibition of transcription elongation appears to result from a dramatic increase in premature termination of transcripts. These experiments indicate that assembly of histone H1 into chromatin can result in structures which are completely repressed with respect to transcription.

INTRODUCTION

A thorough understanding of the mechanisms involved in eukaryotic gene expression must be predicated on a complete knowledge of the complex interplay between the transcription apparatus and chromatin structure. Virtually all of the DNA in the eukaryotic nucleus is packaged into nucleosomes, consisting of an octamer of core histones, a linker histone, such as histone H1, 168 bp of DNA and 10–50 bp of linker DNA. It has been established that histones are general repressors of transcription (reviewed in 1). Histone H1, which is necessary for the stability of condensed higher order chromatin structure (2,3), is strongly implicated in the repression of gene expression (for review see 4,5) and appears to be involved in the formation of repressed chromatin superstructures (6). Nearly stoichiometric amounts have been found in nucleosomes containing 5-methylcytosine, which is concentrated in transcriptionally quiescent chromatin (7). It has been shown that the DNA of inactive genes is arranged into canonical histone H1-containing nucleosome arrays (8). In contrast, there is evidence to suggest that histone H1 is depleted in active chromatin (9,10). Chemical cross-linking data indicate that histone H1 in repressed chromatin interacts through contacts with its globular regions and basic C- and N-terminal domains, whereas histone H1 in active chromatin interacts only through C- and N-terminal contacts (11). The implication of these observations is that alterations in the mode of histone H1 binding to chromatin may mediate changes in chromatin structure which lead to the more open structure characteristic of active chromatin. However, precise knowledge of the mechanistic roles of histone H1 in these structural and functional alterations is currently lacking.

In vitro transcription systems provide a useful approach to studies of the relationship between chromatin structure and transcription efficiency of reconstituted chromatin templates. The inhibitory effects of the core histones on *in vitro* transcription have been clearly demonstrated in a number of studies. Nucleosome cores inhibit transcription initiation by blocking access of promoter sites to RNA polymerases (12–16). It has been shown that bacteriophage RNA polymerases and eukaryotic RNA polymerases II and III can elongate through nucleosomes (13–15,17,18). However, the transcription elongation process is partially inhibited by nucleosome cores, which apparently enhance the use of transcription pause or termination sites intrinsic to the transcribed DNA template (18–20).

The effects of histone H1 on *in vitro* transcription have been much less extensively studied. Histone H1 is a general repressor of RNA polymerase II transcription *in vitro* (21). Laybourn and Kadonaga (22) have shown that this histone H1-specific inhibition is increased when histone H1 is incorporated into DNA templates containing reconstituted nucleosome cores. These studies have focused only on the effects of histone H1 on transcription initiation. To date, nothing is known concerning the effects of histone H1 on transcript elongation.

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In this study a linear DNA template derived from the construct pT207-18 (18) is used to examine quantitatively the effects of regularly spaced positioned histone H1-containing nucleosomes on transcript initiation and elongation by T7 RNA polymerase. The specific initiation of transcripts with a GTP residue by this RNA polymerase (23) allows the measurement of transcription initiation and elongation simultaneously in *in vitro* transcription assays. We demonstrate that incorporation of histone H1 onto free DNA templates results in relatively little inhibition of transcription elongation, whereas incorporation of histone H1 into nucleosomal arrays results in chromatin templates which are essentially inactive with respect to both transcription elongation and initiation.

MATERIALS AND METHODS

Preparation of DNA templates, core histones and histone $H1_{ab}$

A 3.8 kb DNA fragment containing 18 tandem repeats of a 207 bp nucleosome positioning sequence from *Lytechinus variegatus* downstream from a T7 transcription promoter was excised from pT207-18 (18) by digestion with *Hae*III and *Dde*I (New England Biolabs), and was separated from smaller vector fragments by anion exchange chromatography on a Mono Q fast protein liquid chromatography column (FPLC, Pharmacia).

Core histone octamers were prepared from HeLa nuclei as described previously (18). Linker histones were stripped from long chicken erythrocyte chromatin by sucrose gradient centrifugation in 600 mM NaCl, 0.2 mM EDTA, 10 mM Tris (pH 7.4). Top fractions of the gradient were further purified by loading onto a hydroxyapatite (HAP) FPLC column and eluting with a sodium phosphate gradient, according to the method of Breneman (24), yielding pure histone $H1_{ab}$.

Reconstitution procedures

Nucleosomes were reconstituted by salt step dialysis as described previously (25). Histone octamers and DNA substrate were mixed at a ratio of 0.9:1 (w/w) to a final A_{260} between 4 and 6 in 2 M NaCl, 0.2 mM EDTA, 10 mM Tris–HCl (pH 7.4), at 7–10°C. Small volumes were dialyzed to decreasing NaCl concentrations in 1–2 h steps [2, 1.5, 1, 0.75, 0.5 M NaCl, each including 0.2 mM EDTA, 0.1% NP-40, 10 mM Tris–HCl (pH 7.4)], and a final overnight step to 10 mM NaCl in the same buffer. For reconstitution of histone $H1_{ab}$ onto chromatin or DNA the step dialysis was interrupted at the 500 mM NaCl step, histone $H1_{ab}$ was added at the proper ratios, the samples were applied into the wells of a microdialyzer, and the procedure was continued as described above.

Micrococcal nuclease digestions

Reconstituted chromatin was digested at 37°C, at an A_{260} between 1.0 and 0.5, and with 3 and 0.3 U/ml enzyme, in 10 mM NaCl, 0.2 mM EDTA, 0.1% NP-40, 10 mM Tris–HCl (pH 7.4) and 0.5 mM $CaCl_2$. Nucleoproteins were deproteinized with phenol/chloroform, followed by extraction with ether and precipitation of the DNA with 0.1 vol of 4 M LiCl and 2 vol of ethanol at –20°C overnight. Micrococcal nuclease digested DNA was radiolabeled using phage T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (ICN), then analyzed by electrophoresis in 5% polyacrylamide gels in 1 × TBE, followed by autoradiography at room temperature without intensifying screens.

Transcription reactions

Transcription assays were performed in 25 µl reactions containing 200 ng of DNA or chromatin (final A_{260} of 0.16), 40 mM Tris–HCl, pH 7.5, 6 mM $MgCl_2$, 0.2 mM EDTA, 2 mM spermidine, 0.01% NP-40, 20–125 mM NaCl, 0.5 mM ATP, CTP, GTP and UTP, 1 U/µl RNasin (Promega), 10 µCi [γ-^{32}P]GTP and 1 µCi of [^{3}H]UTP (final concentrations). Reactions were pre-incubated at 37°C for 15 min, followed by addition of 200 U T7 RNA polymerase (New England Biolabs) and subsequent incubation for an additional 20 min. Reactions were terminated by addition of EDTA to 25 mM on ice and analyzed by DE-81 filter binding assays (26).

RESULTS

Reconstitution of nucleosome cores onto the sea urchin derived 207 bp tandem repeat sequences has been well characterized (25,27–29). Nucleosome cores form in a dominant translational position flanked by several minor positions within each 207 bp repeat, each of which are offset from the major position by multiples of 10 bp. Based on discrete positioning sites within the individual 207 bp repeats, nucleosome cores reconstituted onto these sequences form relatively homogeneous arrays. In the current study, a 3.8 kb linear DNA fragment containing 18 tandem repeats of a 207 bp nucleosome positioning sequence from the 5S rRNA gene of *Lytechinus variegatus* downstream from a T7 bacteriophage transcription promoter was reconstituted with HeLa histone octamers and chicken erythrocyte histone $H1_{ab}$ using a salt step dialysis method as described previously (25). Micrococcal nuclease digestion analysis of the reconstituted chromatin indicates that core nucleosomes are reconstituted onto the 207 bp tandem repeat sequence with a 207 bp periodicity (25), which appears as a faint ladder of bands under light digestion conditions (Fig. 1, 0H1). More extensive digestion results in the appearance of a core particle band with length of ~147 bp. Micrococcal nuclease digests of chromatin reconstituted with increasing amounts of histone H1 also show discrete bands with 207 bp periodicity under light digestion conditions. Extensive digestion of these samples also gives a core particle band of ~147 bp. Digestion of chromatin samples reconstituted with histone H1 also gives 168 bp bands, which are characteristic of the chromatosome stop of histone H1-containing chromatin. This 168 bp pause is quite weak in reconstitutions without histone H1, but shows a strong increase in intensity as the amount of histone H1 is increased in the reconstitutions. These results show that regularly spaced nucleosomes are reconstituted onto the DNA fragment used in this study. Further, the strong correlation between the amount of histone H1 included in reconstitutions and the strength of the 168 bp chromatosome pause strongly suggests the successful reconstitution of chromatosomes on these tandem repeat sequences. The linear 207 bp repeat DNA (207-18) construct used in transcription experiments is identical to that used in previous chromatin reconstitution studies (25,29), except for the addition of ~50 bp at the 5′ end containing the bacteriophage T7 transcription promoter. Nuclease digestion analyses of the reconstituted chromatin template (T7 207-18) in

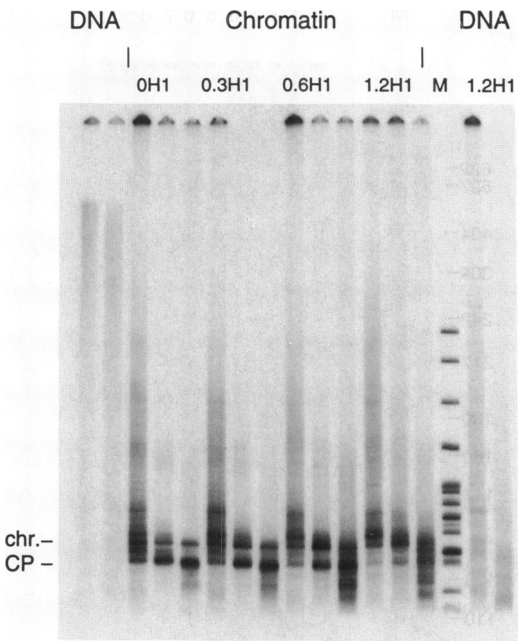

Figure 1. Micrococcal nuclease digestion of reconstituted linear T7-207-18 chromatin. Polyacrylamide gel (5%) showing core particle and chromatosome stops (autoradiogram). Lanes 1 and 2, naked DNA, digested for 1 and 2 min (0.3 U/ml); lanes 3–5, reconstituted chromatin without histone H1; lanes 5–7, reconstituted chromatin containing 0.3 H1/nucleosome; lanes 8–10, reconstituted chromatin containing 0.6 H1/nucleosome; lanes 11–13, reconstituted chromatin containing 1.2 H1/nucleosome, all digested for 1, 3 and 9 min (3 U/ml) and lanes 15 and 16, naked DNA containing 1.2 H1/207 bp repeat, digested for 3 and 9 min (0.3 U/ml). Marker (M) is an *Msp*I digest of pBR322 DNA.

the current study were indistinguishable from those those obtained previously with the similar 207-18 DNA template (25).

Transcription of reconstituted chromatin

We have previously determined the effects of arrays of reconstituted nucleosome cores on *in vitro* transcription of the plasmid construct pT207-18 by T7 RNA polymerase (18). It was demonstrated in this study that nucleosome cores inhibit both transcription initiation and elongation. However, nucleosome cores apparently exert only a partial inhibitory effect on transcription elongation, allowing passage of T7 RNA polymerase through continuous arrays of up to at least 10 nucleosome cores.

In the current study, we use a 3.8 kb fragment excised from the pT207-18 construct as a template for nucleosome reconstitution and transcription. T7 RNA polymerase transcript initiation begins with the specific incorporation of a GTP residue, resulting in a γ-phosphate at its 5′-end (23). Hence, *in vitro* transcription in the presence of [γ-^{32}P]GTP will result in transcripts with γ-^{32}P label incorporated only at their 5′-end and only once per transcript. This property can be exploited in double label transcription assays which include both [γ-^{32}P]GTP and a second labeled nucleotide, in this case [^{3}H]UTP, which will be incorporated throughout the length of the transcript. The level of incorporation of the ^{32}P label into transcripts is proportional to the number of transcripts synthesized in a given reaction, whereas the incorporation of the ^{3}H label is proportional to the total number of nucleotides incorporated into transcripts. The amount of ^{3}H incorporated divided by the amount of ^{32}P incorporated is proportional to the average length of the transcripts produced in a given reaction. Using this assay, we have examined the separate effects of reconstitution histone H1 onto free DNA or arrays of nucleosome cores on transcription initiation and elongation by bacteriophage T7 RNA polymerase. The effect of histone H1 on total nucleotide incorporation by T7 RNA polymerase is shown in Figure 2A. Total transcription from both free DNA and reconstituted templates decreases as increasing amounts of histone H1 are reconstituted onto transcription templates. However, the total nucleotide incorporation from reconstituted chromatin, which is initially about 35% of that obtained from an equal amount of free DNA, drops at a much greater rate than that obtained from free DNA as increasing amounts of histone H1 are reconstituted. Almost no change in nucleotide incorporation into transcripts is found for DNA templates reconstituted with up to 0.75 histone H1 molecules per 207 bp repeat. However, nucleotide incorporation from chromatin templates decreases 75–80% at this level of histone H1 reconstitution. An apparent 10-fold reduction in total nucleotide incorporation is obtained from chromatin templates at one molecule of histone H1 reconstituted per 207 bp repeat, whereas the corresponding DNA template shows a reduction of only about 20% at this ratio.

The effect of histone H1 reconstitution on transcript initiation in this system is shown in Figure 2B. The number of transcripts initiated from free DNA and reconstituted chromatin without histone H1 are quite similar. This probably reflects the absence of nucleosomes from the T7 transcription promoter region of this reconstituted DNA fragment, which contains only about a 50 bp extension upstream from the first 207 bp repeat, which is too short to form a complete nucleosome. We have observed greater inhibition of transcript initiation by nucleosome core reconstitution in transcription studies of the intact pT207-18 plasmid (18), presumably because the availability of vector DNA sequences adjacent to the T7 promoter region allows the reconstitution of a nucleosome which obstructs the binding of T7 RNA polymerase. Relatively little effect on transcript initiation is seen as histone H1 is reconstituted onto free DNA up to one histone H1 molecule per 207 bp repeat, whereas much greater inhibition is seen at the same level of histone H1 reconstitution on chromatin templates. Transcript initiation from chromatin templates is essentially shut down at 1.5 histone H1 molecules per 207 bp repeat.

The effect of histone H1 on transcription elongation is shown in Figure 2C. Transcripts from templates reconstituted with nucleosome cores are approximately one-third the length of those obtained from free DNA templates. This reflects the inhibitory effects of arrays of nucleosome cores observed in our previous study (18). As increasing amounts of histone H1 are reconstituted onto DNA or chromatin, transcript length steadily decreases. The rate of decrease of transcript length due to histone H1 appears to be the same for DNA and chromatin templates. However, transcript elongation is essentially abolished at a ratio of 1.5 histone H1 molecules per 207 bp. The corresponding free DNA template shows only about 15% inhibition of transcript initiation at this level of histone H1 reconstitution. Thus, the combined effects of nucleosome cores and histone H1 results in almost complete inhibition of transcription elongation and initiation.

Analysis of the transcripts produced from the DNA templates used in this study by urea–polyacrylamide gel electrophoresis are shown in Figure 3. The loading of approximately equal numbers

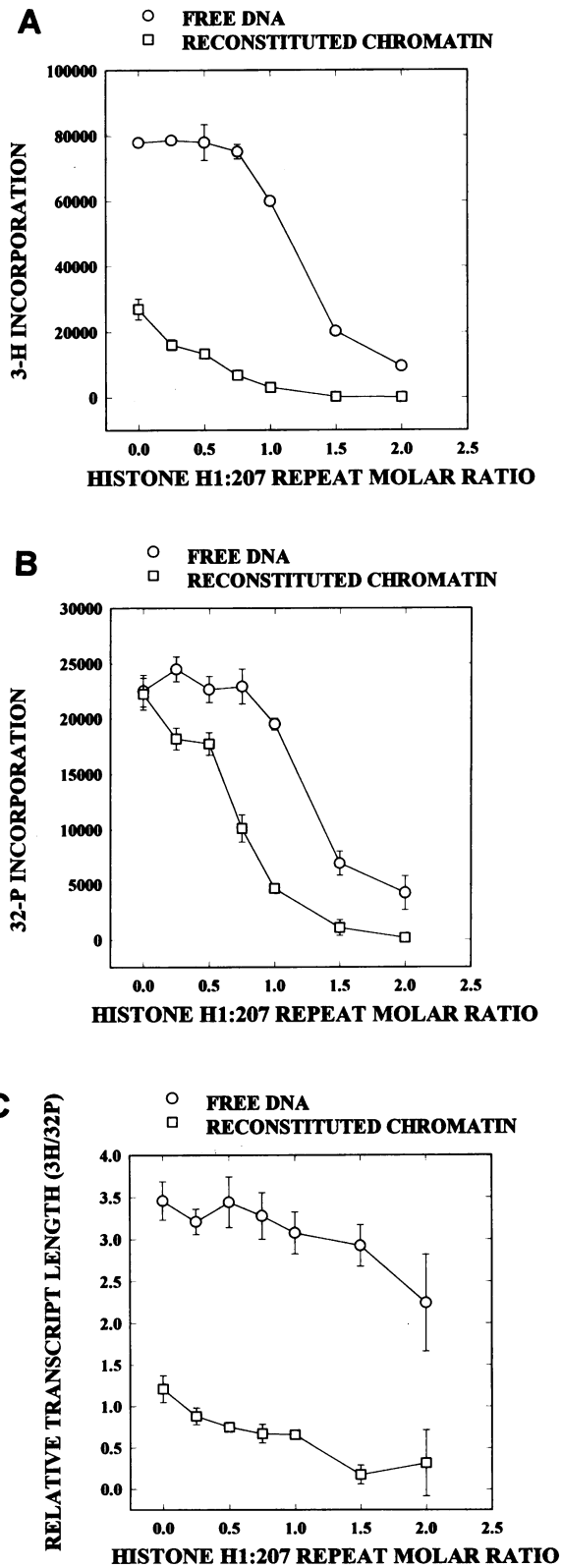

Figure 2. Effects of histone H1 on transcription of reconstituted chromatin by T7 RNA polymerase. In these assays, the incorporation of [^{3}H]UTP label was used to calculate the total nucleotide incorporation into transcripts (**A**), and ^{32}P incorporation into transcripts was used to calculate the number of transcripts produced per DNA template during the assay period (**B**). Transcript length was derived from the ratio of ^{3}H to ^{32}P incorporated into transcripts (**C**).

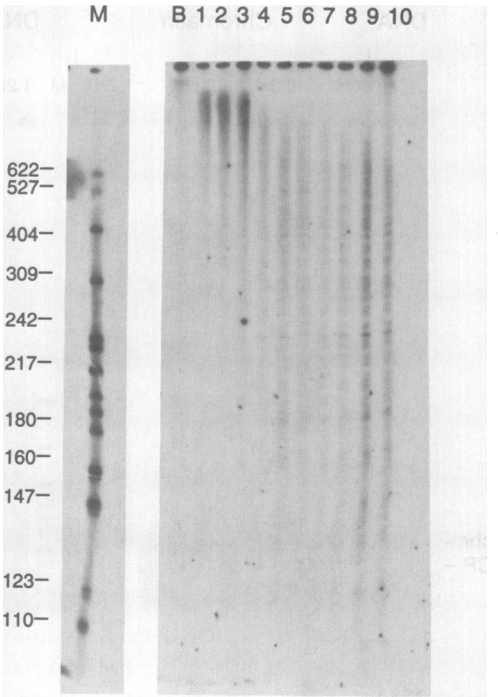

Figure 3. Electrophoretic analysis of transcripts produced from reconstituted chromatin templates. Reconstituted linear T7-207-18 DNA was used in transcription assays with T7 RNA polymerase and the transcripts were analyzed by electrophoresis in 8% polyacrylamide gels containing 8.3 M urea, followed by autoradiography. Lanes 1–3, naked DNA containing 0, 0.8 and 1.2 H1/207 bp repeat; lanes 4–10, chromatin reconstituted with 0.9 core histone–DNA (w/w) containing 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 histone H1/nucleosome. A blank reaction containing no DNA was loaded in lane B. An *Msp*I digest of pBR322 DNA was used as a marker.

of transcripts in each lane of this gel allows for a direct comparison of the relative lengths of the transcripts produced for each template. Transcripts produced from the free DNA templates approach the full length of the 3.8 kb DNA template, as judged by agarose gel electrophoresis (not shown). Addition of histone H1 to free DNA templates (lanes 2 and 3) results in shorter RNA bands, although the effect appears to be rather slight. The transcripts obtained from the templates reconstituted with nucleosome cores (lane 4) are markedly shorter than those obtained from the free DNA template, in agreement with the results of the double label transcription experiment shown in Figure 2C. Note the appearance of a number of bands of discrete lengths, which appear to result from specific transcription 'stops' along the DNA. As histone H1 is reconstituted on to the chromatin templates, (lanes 5–10) the resulting transcripts become shorter and the specific 'stops' become more pronounced. The banding pattern is consistent with the idea that transcripts become shorter through the use of specific transcription stops, which do not appear to be specifically induced by histone H1. The results clearly demonstrate that transcripts are dramatically shortened by core nucleosomes, and are further shortened by the presence of histone H1.

Time course of transcription

The rates of transcription from DNA and chromatin templates reconstituted with increasing amounts of histone H1 were examined in time course experiments (Fig. 4). The overall rate of

incorporation of nucleotides into transcripts is significantly greater from free DNA templates than from templates reconstituted with nucleosome cores (Fig. 4A). Reconstitution with histone H1 further decreases the rate of nucleotide incorporation for both free DNA and reconstituted chromatin templates. In Figure 4B, the rates of transcript initiation from free DNA and nucleosomal templates are quite similar. Reconstitution of histone H1 onto these templates reduces the apparent rate of transcript initiation for both free DNA and chromatin, but H1 has a somewhat larger effect on the chromatin templates. In the time course of transcript elongation shown in Figure 4C, the RNA transcript length reaches a peak in under 10 min for free DNA templates, after which the measured average transcript length apparently decreases. This decrease is probably due to nucleotide depletion effects and polymerase lability after prolonged exposure to transcription conditions (30). Histone H1 has a relatively small inhibitory effect on the rate of transcript elongation from free DNA templates. Transcription elongation from nucleosomal templates proceeds at a slower rate than from free DNA templates, reaching a stable plateau after ~10–20 min of transcription. Reconstitution of histone H1 onto nucleosomal templates further retards the transcript elongation rate. The data presented in Figure 4C indicate that the rate of elongation is reduced by the presence of nucleosome arrays, as observed previously (18). Histone H1 further retards the rate of transcription elongation from these templates. The plateau behavior indicates that nucleosomes affect the balance between rates of transcription elongation and termination (or polymerase stalling). In addition to a direct effect on the rate of transcription elongation, nucleosome cores may decrease transcript length by retarding the progress of the elongating T7 RNA polymerase, perhaps through premature termination of transcripts or enhanced pausing of the polymerase.

Effect of NaCl on transcription

Figure 5A shows the effect of NaCl concentration on transcription initiation from free DNA and chromatin templates reconstituted with histone H1. Increasing the NaCl concentration from 20–125 mM results in a dramatic decrease in the number of transcripts initiated from both free DNA and chromatin templates, in agreement with the observations of McAllister and Carter (31). NaCl concentration has a somewhat different effect on transcript elongation by this polymerase, shown in Figure 5B. Transcript length appears to reach an optimum at ~50 mM NaCl (the concentration used in the experiments presented in Figs 2 and 3), and then decreases somewhat as the NaCl concentration is increased to 125 mM. These limitations restrict the investigation of transcription under conditions which would significantly weaken histone–DNA interactions, i.e., 400–600 mM NaCl. Histone–DNA interactions apparently are not greatly altered under the conditions tested, as little variation in the transcription of reconstituted templates relative to free DNA templates can be observed.

DISCUSSION

Previous work has shown that bacteriophage SP6 polymerase can transcribe through one nucleosome core assembled on a plasmid DNA fragment (13) or on a 5S DNA fragment (14), with or without net displacement of the nucleosome core, respectively, depending on the DNA template (32). In addition, it has been demonstrated that SP6 RNA polymerase can elongate transcripts through at least two nucleosome cores with no apparent hindrance (15). However, we have shown that whereas the essentially similar bacteriophage T7 RNA polymerase can transcribe through arrays of at least 10 phased nucleosomes, the transcription elongation process is partially inhibited by nucleosomes (18). The work presented here constitutes the first transcription study of reconstituted arrays of histone H1-containing nucleosomes.

Reconstitution of nucleosome cores onto the sea urchin derived 207 bp tandem repeat sequences has been well characterized (25,27,28). Based on discrete positioning sites within the individual 207 bp repeats, nucleosome cores reconstituted onto these sequences form relatively homogeneous arrays. Studies in which chicken erythrocyte linker histones H1 or H5 were reconstituted onto these arrays of nucleosome cores indicated that the linker histones did not over-ride the underlying DNA signals which induce regular nucleosome spacing in these arrays but did affect the distribution of chromatosome positions between the observed sites (25). Hence, it was concluded that the chromatosome itself is a positioning entity. These studies have established methods for reconstitution of relatively homogeneous linear arrays of positioned chromatosomes on the 5S repeat sequences.

Micrococcal nuclease digestion studies of reconstituted linear chromatin templates in this study confirm the reconstitution of regular arrays of nucleosomes onto 5S 207 bp positioning sequences. As increasing amounts of histone H1 are included in reconstitutions a distinct 168 bp band appears and intensifies, a pattern essentially identical to that observed previously (25). This is the characteristic stop obtained from micrococcal nuclease digestion of histone H1 containing chromatin, the chromatosome stop. This is a positive indication that much if not all of the histone H1 is being incorporated into the reconstituted templates in the form of chromatosomes.

Transcription of reconstituted arrays of nucleosome cores on linear 5S 207-18 template with T7 RNA polymerase results in shorter transcripts than are obtained from the corresponding free DNA template. The data obtained here indicate that nucleosome cores inhibit transcription elongation in a manner and extent very similar to that observed on reconstituted circular DNA templates containing the 5S 207-18 repeat sequence (18). In the case of the linear 207-18 template used in this study, little or no inhibition of transcript initiation is seen as a result of nucleosome core reconstitution, in contrast to the significant inhibitory effects on transcript initiation seen when nucleosome cores are reconstituted onto the circular pT207-18 construct. This is probably because the linear template used in the current study contains only a very short stretch of DNA at its 5′ end, less than 50 bp including the T7 transcription promoter, which is too short to support the formation of a nucleosome core. Formation of nucleosome cores in the corresponding regions on the circular construct is the probable cause of the inhibition previously observed. This explanation is supported by the observation that the T7 transcription promoter sequence itself is a strong nucleosome positioning sequence (33). Hence, the linear DNA template is actually a better substrate for the study of the effects of nucleosome cores on transcription initiation than the intact circular construct, as the potentially confounding effect of the inhibition of transcript initiation by packaging of the T7 promoter sequence into a nucleosome is minimized through the use of the linear template.

Linker histones bind to free DNA in a cooperative manner, forming rod-like fibers with a density of one histone H1 molecule

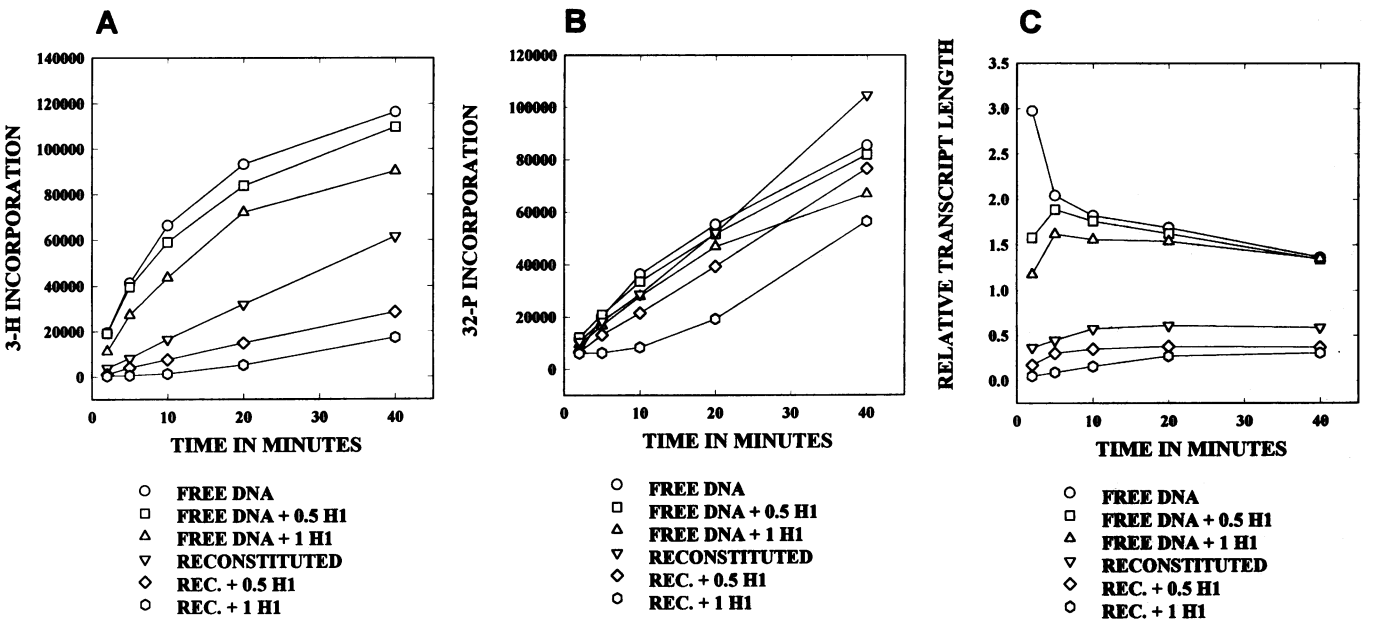

Figure 4. Time course of T7 RNA polymerase transcription elongation. Reconstituted pT207-18 minichromosomes (0.9 histone octamer to DNA weight ratio) containing 0, 0.5 or 1.0 histone H1 per nucleosome or 207 bp repeat were added to reaction mixes containing nucleotide triphosphates and spermidine, as described in Materials and Methods. After a 15 min preincubation at 37°C, T7 RNA polymerase was added. Aliquots were removed from the transcription reactions at various time intervals, EDTA was added to 25 mM and transcripts were analyzed by DE-81 filter binding assays. The incorporation of [^{3}H]UTP label was used to calculate the total nucleotide incorporation into transcripts (**A**), while ^{32}P incorporation into transcripts was used to calculate the number of transcripts produced per DNA template during the assay period (**B**). Transcript length was derived from the ratio of ^{3}H to ^{32}P incorporated into transcripts (**C**).

per 47 bp of DNA (34). It is expected therefore, that subsaturating levels of histone H1 would result in a mixture of H1–DNA fiber regions and free DNA regions. In contrast, the binding of histone H1 to nucleosomes occurs at intervals of 207 bp on the 207-18 constructs. Hence, direct comparisons between these two different kinds of complexes should be made cautiously. The reconstitution of histone H1 on to reconstituted arrays of nucleosome cores results in a sharp drop in total transcription. This is clearly due to a decrease in the level of transcription initiation, as outlined above. The effect probably results from two causes, the first of which is blockage of the promoter by histone H1. This could result from the direct binding of histone H1 to the T7 RNA promoter. The second cause probably arises indirectly through the histone H1-induced condensation of chromatin. Hansen and Wolffe (35) have observed that ionic conditions that cause compaction of reconstituted chromatin lacking histone H1 results in inhibition of transcription elongation by RNA polymerase II. It would be expected that such an effect would be amplified by the presence of histone H1, which is necessary for the formation of highly condensed chromatin structures. The ionic conditions used in the transcription reactions no doubt favor the formation of highly condensed structures (36,37), which tend to aggregate. Condensation and/or aggregation of chromatin would be expected to effectively reduce the number of T7 promoter containing templates available for transcription initiation. Aggregated chromatin was not separated from soluble chromatin for the transcription experiments presented here. However, this does not affect our conclusions because the double label assay used in this study measures the transcription of templates which do have active promoters. Thus, it is clear that the average transcript length is greatly reduced as histone H1 is reconstituted on to nucleosomal DNA templates. The added inhibitory effects of histone H1 on transcript elongation results in chromatin templates which are almost completely inactive with respect to transcription. Examination of the transcripts produced from reconstituted templates indicates a shortening of transcript length in templates reconstituted with nucleosome cores as compared to free DNA (18; present work, Fig. 3). This result is in good agreement with observations made previously by Kirov *et al.* (20) using T7 RNA polymerase and by Izban and Luse (19), who observed increased use of intrinsic transcription pause sites on nucleosomal templates by eukaryotic RNA polymerase II. Apparently, reconstitution of histone H1 further shortens the RNA transcripts obtained from transcription of reconstituted templates by T7 RNA polymerase *in vitro*. The inhibitory behavior is again consistent with the increased use of intrinsic pause or termination sites by the transcribing polymerase.

This behavior could be explained by the model of von Hippel and Yager (38). These authors propose that a dynamic balance exists between transcription termination and elongation. In their model, this balance changes as the DNA sequence underlying the transcribing polymerase changes, so that sequences which favor particular DNA or RNA structures could affect the stability of the transcription elongation complex. Because nucleosomal structures stabilize the DNA duplex against denaturation (reviewed in 39) the energetic barrier to DNA strand separation in transcription elongation would be much higher in nucleosomes than in free DNA. Hence, the balance between elongation and termination could be skewed toward termination, particularly when the polymerase passes through DNA sequences which already favor termination.

Strong evidence exists for the presence of histone H1 in regions of transcriptionally active chromatin (40). However, histone H1 appears to be somewhat depleted in these regions (9,10).

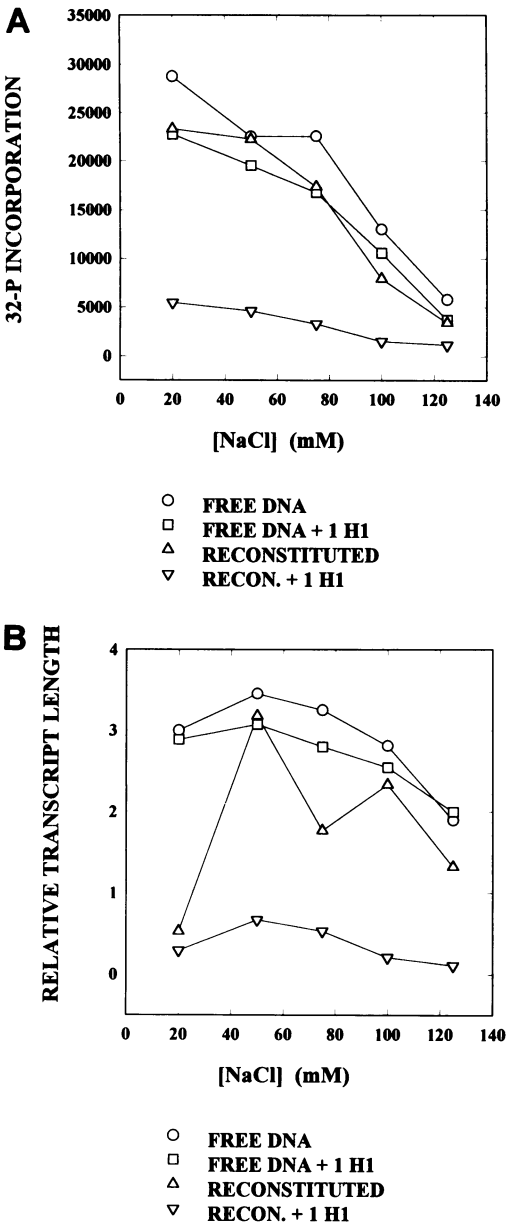

Figure 5. Effect of NaCl on transcription of histone H1-containing chromatin. Naked DNA or reconstituted chromatin (0.9 core histone–DNA, w/w) each containing either 0 or 1.0 histone H1 per nucleosome (or 207 bp repeat) were transcribed with T7 RNA polymerase in *in vitro* reactions containing 20–125 mM NaCl. (**A**) Effect of NaCl on transcript initiation; (**B**) Effect of NaCl on transcript length.

Selective depletion of histone H1 from chromatin can lead to transcriptionally competent structures, whereas addition of histone H1 can lead to repression of transcription *in vitro* (41,42). Other evidence suggests that the interaction of histone H1 within the chromatin of active genes is somewhat altered (11). Histone modifications are expected to alter the interaction of histone H1 with chromatin, thereby mediating its transcriptional competence. Support for this idea is provided by the observation that histone acetylation reduces the ability of histone H1 to condense active chromatin (43).

The inhibitory effects of H1 observed in the present study are in strong accordance with the large body of structural and functional work done previously. It is clear that histone H1 containing chromatin can exist in a nearly static, inert structure, and it has been shown here that a type of transcriptionally static structure can be produced through *in vitro* reconstitution. Further studies are necessary to ascertain the relationship between the static structures produced in the current study and those found *in vivo*, and the mechanism which governs the transition from static to active chromatin structures. Of particular importance in this regard are the further development and applications of chromatin transcription systems using eukaryotic RNA polymerases, which may utilize special structural adaptations to overcome the problems inherent in the transcription of chromatin templates.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr R. T. Simpson for his kind gift of p5S-207-18, Dr Peter M. Yau for technical assistance and helpful suggestions and Dr Janet G. Smith for critical reading and assistance in preparation of the manuscript. Support for this work was provided by grant PHS GM 26901 from the National Institutes of Health.

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