The H3/H4 Tetrmer Blocks Transcript Elongation by RNA Polymerase II in Vitro

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We have investigated transcript elongation efficiency by RNA polymerase II on chromatin templates in vitro. Circular plasmid DNAs bearing purified RNA polymerase II transcription complexes were assembled into nucleosomes using purified histones and transient exposure to high salt, followed by dilution and dialysis. This approach resulted in nucleosome assembly beginning immediately downstream of the transcription complex. RNA polymerases on these nucleosomal templates could extend their 15- or 35-nucleotide nascent RNAs by only about 10 nucleotides in 15 min, even in the presence of elongation factors TFII-F and SII. Efficient transcript elongation did occur upon dissociation of nucleosomes with 1% sarkosyl, indicating that the RNA polymerases were not damaged by the high salt reconstitution procedure. Since the elongation complexes were released by sarkosyl but not by SII, these complexes apparently did not enter the arrested conformation when they encountered nucleosomes. Surprisingly, elongation was no more efficient on nucleosomal templates reconstituted only with H3/H4 tetramers, even in the presence of elongation factors and/or competitor DNA at high concentration. Thus, in a purified system lacking nucleosome remodeling factors, not only the core histone octamer but also the H3/H4 tetramer provide an nearly absolute block to transcription elongation by RNA polymerase II, even in the presence of elongation factors.

The basic structural unit of the eukaryotic chromosome is the nucleosome, which consists of an octamer of the H2A, H2B, H3, and H4 histone proteins around which 145 bp of DNA are wrapped (recently reviewed in Ref. 1). Despite the apparently tight packaging of transcriptionally active DNA in nucleosomes (see Ref. 2 and references therein), RNA polymerase II achieves high rates (25 nucleotides/s) of RNA chain elongation in the cell (3, 4). However, attempts to elongate RNA polymerase II transcripts in vitro reconstituted chromatin templates have not reproduced efficient chain elongation (Refs. 5 and 6, but see also Ref. 7). A number of reasons can be envisioned for this failure. It is known, for example, that nucleosomes from transcriptionally active regions have highly acetylated histones (recently reviewed in Refs. 8 and 9); it has also been suggested that such nucleosomes might be deficient in histones H2A and H2B (10). Several molecular complexes have recently been described that can, in the presence of ATP, modify nucleosomes so that their DNA is more accessible for transcription factor binding (11–14). It is conceivable that these chromatin remodeling activities might act within transcribed regions, making DNA within the gene body more accessible to RNA polymerase (7). Numerous studies using single-subunit bacteriophage RNA polymerases have demonstrated that these enzymes can efficiently elongate nascent RNA on reconstituted chromatin templates (15–22). Interestingly, transcription of mononucleosomal templates by SP6 RNA polymerase has been shown to be accompanied by displacement of the nucleosome (17, 19). In this case, as the nucleosomal DNA is acquired by the leading edge of the advancing RNA polymerase, the octamer is exchanged onto a segment of free DNA, resulting in a transfer of the nucleosome away from its original location. Thus, efficient traversal of nucleosomal templates by RNA polymerase II in vitro could require free DNA molecules onto which nucleosomes could be exchanged.

Investigations of elongation on chromatin templates by RNA polymerase II are limited by the fact that assembly of nucleosomes on the promoter blocks initiation (23–26). Thus, either a nucleosome-free promoter must be ligated onto a preformed nucleosomal array (24) or elongation complexes must be obtained before loading nucleosomes on the DNA (25–27). We had previously studied transcript elongation on chromatin templates assembled using Xenopus oocyte extracts and RNA polymerase II ternary complexes paused early in elongation (i.e., within 20 bases of transcription start (5, 6)). This approach had the advantage of allowing chromatin assembly under transcription conditions, preserving the activity of the polymerase II elongation complex. However, substitution of other histones for those provided by the Xenopus extract is difficult, and the extended incubations required in the crude oocyte extract might allow the action of chromatin remodeling activities. Therefore, for the current work we decided to attempt chromatin reconstitution using only pure histones and purified transcript elongation complexes. We developed a modification of the standard high salt dialysis nucleosome reconstitution procedure that allowed us to assemble nucleosomes without cellular extracts while retaining activity of the transcription complex. We found that nucleosomes assembled in this way are completely inhibitory to transcript elongation, even in the presence of elongation factors and/or excess competitor DNA. Surprisingly, even H3/H4 tetramers serve as an essentially complete barrier to RNA polymerase under these conditions.

EXPERIMENTAL PROCEDURES

Preparation and High Pressure Liquid Chromatography Fractionation of Histones—Nuclear pellets obtained during the preparation of nuclear extracts from HeLa cells (28, 29) were used as the source of histones. Crude histones were extracted with 4 M sulfuric acid as

The abbreviation used is: bp, base pair(s).
described by Workman et al. (30), dialyzed into storage buffer (0.5 mM NaCl, 1 mM EDTA, and 10 mM Tris, pH 8) and stored at −70 °C. These histones (10 mg, using an A280 of 4.2 as 1 mg/ml; see Ref. 31) were then applied to a Mono S column (Pharmacia) and eluted at 0.5 ml/min with a discontinuous NaCl gradient in 1 mM EDTA, 10 mM Tris, pH 8. The NaCl concentration was increased from 0.5 to 0.8 M over 20 min, followed by a wash with 0.8 M NaCl for another 20 min and a 1-h salt gradient from 0.88 to 2 M NaCl. Histone H1 eluted at 1.06 M NaCl, histones H2A/H2B at 1.15 M, and histones H3/H4 at 1.26 M NaCl, respectively. The H2A/H2B and H3/H4 fractions (200–500 ng/μl) were stored at −70 °C.

Preparation of Elongation Core Complexes and Chromatin Template Reconstitution—Elongation complexes were assembled and purified as described previously (5). Briefly, RNA polymerase II preinitiation complexes were obtained by incubation of HeLa nuclear extracts with circular plasmid DNA templates, which contain the adenovirus major late promoter. After gel filtration to remove residual NTPs, RNA polymerases were advanced to downstream locations (either +15 or +35) by incubation with an appropriate subset of NTPs. These NTPs were removed, and the transcription complexes were further purified by the addition of sarkosyl and a second round of gel filtration. The final transcription complex preparation typically contained 8 nM of template DNA.

For all of the studies reported here, the elongation complexes were assayed for transcriptional activity immediately after the second gel filtration step and were kept overnight at 4 °C until the assay was completed. Complexes that retained activity were assembled into chromatin at room temperature by mixing the elongation complexes with Mono S-purified histones (either H3/H4 tetramers and H2A/H2B dimers or H3/H4 tetramers alone) at histone:DNA ratios ranging from 0.5 to 2, for all four histones, or from 0.25 to 1 for H3/H4 alone (see the figure legends), along with sufficient NaCl to bring the final NaCl concentration to 1 M. The reconstitution mixture was immediately diluted to 666 mM with TE buffer (1 mM EDTA and 10 mM Tris, pH 7.9) containing 1 mM dithiothreitol. After 15 min, NaCl was diluted with TE to 466 mM, and after a further 15 min, the samples were dialyzed against 1 liter of MEM buffer (25 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, and 30 mM Tris, pH 7.9) for 30 min. Final DNA concentration in the dialyzed chromatin preparations was about 3 ng/μl. As controls, elongation complexes were processed through the reconstitution procedure with bovine serum albumin used in place of histones at a protein:DNA ratio of 1.

To measure the extent of reconstitution, aliquots of the chromatin preparations were relaxed with topoisomerase I. One-fifth volume of 5 mM topoisomerase buffer (250 mM KCl, 7.5, 250 mM KCl, 50 mM MgCl2, 2.5 mM dithiothreitol, 0.5 mM EDTA, and 30 mM Tris, pH 7.9) for 30 min. Final DNA concentration in the dialyzed chromatin preparations was about 3 ng/μl. As controls, elongation complexes were processed through the reconstitution procedure with bovine serum albumin used in place of histones at a protein:DNA ratio of 1.

Elongation Factors and Elongation Assay—TFIIF was prepared from recombinant RAP30 and RAP74 subunits expressed in bacteria as described previously (32–34); note that the RAP74 construct was modified to match bacterial codon usage (33). Bacterial strains expressing RAP30 and RAP74 were the gift of Zachary Burton (Michigan State University). Recombinant human TFIIF was prepared from bacterial lysates using nickel-agarose chromatography. Superhelical templates may be accomplished by mixing histones and DNA at 1 mM NaCl, followed by gradual reduction of salt to less than 100 mM by dialysis (35). This approach was not useful in our case, however, since we wished to assemble nucleosomes on RNA polymerase II transcription complexes. Histones were prepared from HeLa cell nuclei by acid extraction followed by separation into H2A/H2B and H3/H4 fractions on a Mono S column, as described under “Experimental Procedures.” The essentially complete separation of each histone pair from the other core histones, histone H1, and other acid-extractable proteins is illustrated by the gel in Fig. 1A.

Modified Salt Reconstitution Method Allows Efficient Chromatin Template Assembly with either the Histone Octamer or the H3/H4 Tetramer—Reconstitution of chromatin with purified histones may be accomplished by mixing histones and DNA at 1 mM NaCl, followed by gradual reduction of salt to less than 100 mM by dialysis (35). This approach was not useful in our case, however, since we wished to assemble nucleosomes on RNA polymerase II transcription complexes. Polymerase II will elongate RNA chains in NaCl concentrations as high as 2 M (36), but RNA polymerase II complexes will not survive hours of exposure to high salt.3 We therefore developed a modified high salt assembly method, which minimizes the time in which components are exposed to NaCl concentrations greater than 0.5 M (see “Experimental Procedures”). This procedure, which requires only 1 h, results in assembly of nucleosomes using either all four core histones or only H3 and H4. The RNA polymerase remains fully active after this procedure (see Fig. 2). The short reconstitution time did require higher levels of input histones than in the extended dialysis procedure. To obtain complete reconstitution (i.e. about 1 nucleosome/200 bp

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FIG. 1. The purity of core histones used in this study and assembly of nucleosomes on plasmid pML5–4NR. Panel A shows an SDS-15% polyacrylamide gel, stained with Coomassie Blue, of histone proteins purified from HeLa cells. Lane 1 contains acid-extracted crude histones (8 μg) from HeLa nuclear pellets; lane 2 is the peak fraction of H2A/H2B (2 μg); and lane 3 is the peak fraction of H3/H4 (2 μg) from the Mono S column (see “Experimental Procedures”). Panel B, agarose gel (1.75%) of DNA topoisomer reactions after different levels of nucleosome assembly (see “Experimental Procedures”). The average number of nucleosomes assembled, which equals the average number of superhelical turns remaining in the DNA after topoisomerase I treatment and deproteinization (37), is given above each lane; also shown above each lane is the mass ratio of histones H3 and H4 to DNA in each reconstitution. Note that the octamer lanes also contained an amount of H2A and H2B equal to the amount of H3 and H4 used. Lane 7 contained plasmid DNA that had been partially relaxed with topoisomerase I.

RESULTS

Earlier work from this laboratory using RNA polymerase II and chromatin templates had relied on oocyte extracts as the source of histones and chromatin reconstitution factors (5, 6).

To study templates with modified histones or subsets of the core histones, as well as to avoid the possible introduction of chromatin remodeling factors from extracts, we decided to assemble transcriptionally active chromatin templates using only pure core histones and purified transcription complexes. Histones were prepared from HeLa cell nuclei by acid extraction followed by separation into H2A/H2B and H3/H4 fractions on a Mono S column, as described under “Experimental Procedures.” The essentially complete separation of each histone pair from the other core histones, histone H1, and other acid-extractable proteins is illustrated by the gel in Fig. 1A.

Modified Salt Reconstitution Method Allows Efficient Chromatin Template Assembly with either the Histone Octamer or the H3/H4 Tetramer—Reconstitution of chromatin with purified histones may be accomplished by mixing histones and DNA at 1 mM NaCl, followed by gradual reduction of salt to less than 100 mM by dialysis (35). This approach was not useful in our case, however, since we wished to assemble nucleosomes on RNA polymerase II transcription complexes. Polymerase II will elongate RNA chains in NaCl concentrations as high as 2 M (36), but RNA polymerase II complexes will not survive hours of exposure to high salt.3 We therefore developed a modified high salt assembly method, which minimizes the time in which components are exposed to NaCl concentrations greater than 0.5 M (see “Experimental Procedures”). This procedure, which requires only 1 h, results in assembly of nucleosomes using either all four core histones or only H3 and H4. The RNA polymerase remains fully active after this procedure (see Fig. 2). The short reconstitution time did require higher levels of input histones than in the extended dialysis procedure. To obtain complete reconstitution (i.e. about 1 nucleosome/200 bp

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of DNA), we needed either 2 μg of core histones (Fig. 1B, lane 1) or 1 μg of histones H3 and H4 (Fig. 1B, lane 4) per μg of DNA, about twice the amount of histone needed with extended dialysis times. Correspondingly lower reconstitution levels were obtained with 0.75 or 0.5 times the amounts of core histones or histones H3 and H4 needed for full reconstitution (Fig. 1B, lanes 2 and 3 and lanes 5 and 6). It is important to note that the DNA topology assay reveals the number of nucleosomes on the DNA but not their location. We will return below to the question of the spacing of the assembled nucleosomes.

**Ternary Complexes Cannot Continue Elongation on Nucleosomal Templates, but They Do Not Become Arrested**—RNA polymerase II elongation complexes stalled at +15 or +35 downstream of the adenovirus major late promoter were prepared with gel-filtered preinitiation complexes and appropriate subsets of the nucleoside triphosphates. These complexes were then purified by transient exposure to sarkosyl as described previously (5, 6). The purified transcription complexes were then assembled into chromatin using the cross-link method discussed above. Control complexes were subjected to the reconstitution procedure but without the addition of histones. The 15- and 35-base RNAs in the initial stalled complexes are shown in lane 1, left and right panels, of Fig. 2. RNA polymerase II remains active after exposure to the reconstitution procedure in the absence of histones, as shown in lane 8 in both panels. On this template, there are four prominent arrest sites located approximately 195, 380, 565, and 750 nucleotides downstream of transcription start (5); these are indicated by dots to the right of each panel. When saturating levels of SII and TFIIF were added to controls of the mock reconstitutions, arrest was essentially abolished, and the expected elongation rate of 20–25 nucleotides/s (6) was achieved (lane 9 of both panels). However, on the chromatin templates reconstituted with one nucleosome/200 bp, RNA polymerase II was unable to extend nascent RNAs by more than a few bases in a 15-min reaction, even at saturating concentrations of TFIIF and SII (lanes 2 and 3 of each panel). Somewhat surprisingly, the use of underreconstituted chromatin templates did not result in a substantial improvement in the elongation efficiency (lanes 5 and 6 of each panel). Since the results in panels A and B were the same, failure of transcription was not dependent on choice of stalling site for the ternary transcription complex.

The failure of transcript elongation by RNA polymerase II on the chromatin templates was not simply the result of inactivation, for example by a contaminant in the histone preparation, because the addition of 1% sarkosyl to the chromatin transcription reactions (which will strip nucleosomes from the DNA template) allowed transcription to proceed (Fig. 2, lanes 4 and 7, left and right panels). In the presence of sarkosyl, transcription occurred with equal levels of pausing at each arrest site, at the rate expected from earlier work on transcription of this template as pure DNA (5, 6). It is important to note that resumption of transcription on the chromatin templates is not facilitated by SII but is essentially dependent on sarkosyl. This indicates that the RNA polymerases did not fall into a state of transcriptional arrest when they encountered the initial nucleosome on the chromatin template.

**H3/H4 Tetramer Can Block Elongation by RNA Polymerase II (Fig. 3)—**We repeated the experiment in Fig. 2 using either H3/H4 tetramers or the complete histone octamer as control. In this case, all transcription complexes were stalled at +15 prior to chromatin assembly. As in Fig. 2, the complexes were fully active after mock reconstitution (Fig. 3, lane 20) but completely blocked on templates fully (lanes 2 and 3) or partially (lanes 5, 6, 8, and 9) assembled with histone octamers. Very similar results were obtained with the H3/H4 tetramers. Transcription elongation could not continue on templates fully (lane 11) or partially (lane 14) assembled with tetramers; a very low level of elongation was seen in 15 min on the least reconstituted samples (lane 17). There was essentially no effect of elongation factors in these reactions (compare lanes 12, 15, and 18 with lanes 11, 14, and 17). The blocked RNA polymerases were released by sarkosyl (lanes 13, 16, and 19), again indicating that polymerases on the assembled templates were active but not in an arrested state.

**The Addition of DNA Acceptors for Nucleosome Transfer Does Not Release Nucleosomal Blockade on Chromatin Template**—We next challenged fully reconstituted templates bearing either histone octamers or H3/H4 tetramers with free DNA to test the possibility that providing an acceptor for nucleosome transfer might facilitate elongation on the chromatin templates. A 5-fold excess of either superhelical pUC18 or pUC18 restriction fragments was used as potential acceptor. The elongation activity of RNA polymerase II on mock-reconstituted templates, with or without elongation factors, was not influenced by the large excess of DNA (Fig. 4, lanes 18–21). Elongation on templates assembled with histone octamers was apparently identical with or without the addition of pUC18 DNA (lanes 2–7). The addition of pUC18 DNA, particularly in the circular form, had a very slight effect on templates with the H3/H4 tetramer (lanes 11–14); however, the resulting elongation activity was very low compared with either the sarkosyl
control (lane 15) or the 1-min elongation on the naked, mock-reconstituted template (lanes 18–21). The stronger effect of the superhelical pUC18 fragments (lanes 11 and 12 versus lanes 13 and 14) may have resulted from the higher binding preference of nucleosome to superhelical DNA, compared with the relaxed form (37, 38). We did not detect any bulk loss of nucleosomes from the template plasmid as a result of the incubation with competitor DNA (data not shown). Thus, it is possible that the very modest stimulation of elongation on the tetramer templates did result from transcription-driven nucleosome displacement.

On the Reconstituted Templates, Nucleosomes Are Located Adjacent to RNA Polymerase II in a Close Packed Pattern—We were somewhat surprised that even partially reconstituted templates, using either the histone octamer or the H3/H4 tetramer, provided nearly a complete block to elongation. This could be explained if nucleosome assembly begins directly adjacent to the transcription complex; in this case, there would always be an extensive nucleosome array downstream of the RNA polymerase, even if relatively few nucleosomes are reconstituted. This idea is based on the known tendency of nucleosomes to assemble in close packed groups when the decreasing salt assembly method is used (39). To investigate the location of nucleosomes on our reconstituted templates, we used the accessibility of restriction sites as a probe. Four HindIII sites are located at +235, +420, +605, and +790 relative to transcription start site on the pML5–4NR plasmid. We could thus assay for the presence of a nucleosome directly adjacent to the stalled RNA polymerases by cleaving the assembled templates with HindIII, releasing the blocked polymerases with sarkosyl, and observing the level of 235 base run-off RNA produced. The absence of any run-off RNA would indicate that the HindIII site was always protected; i.e., a nucleosome was always present on the site to block cleavage. Unfortunately, such a negative result could also occur for trivial reasons, such as the inability of the HindIII to cut in the linker between nucleosomes. In this context, it is important to recall that only a small percentage of templates are expected to be transcriptionally active in our experiments (40). Thus, if the distribution of nucleosomes on the bulk DNA were random, HindIII would cleave at +235 on a portion of the reconstituted plasmids. This fraction can be predicted from the known number of nucleosomes assembled.

**FIG. 3.** The H3/H4 tetramer can block elongation by RNA polymerase II. RNA polymerases were stalled at +15 on pML5–4NR and assembled into chromatin using all four histones or only H3 and H4. The histone:DNA ratio and any additions to the chase reactions are indicated (see also “Experimental Procedures”). RNA from the initial +15 complex is shown in lane 1, and DNA size markers are shown in lane M. The locations of the four arrest sites are indicated by the four dots to the right.
and the assumption that cleavage can take place anywhere except within the roughly 150 bp of DNA in each nucleosome core particle. The ability of HindIII to cleave at +235, as opposed to its other sites, can be determined by purifying the HindIII-cleaved DNA and cutting it to completion with EcoRI, which has a unique cleavage site in pML-4NR. EcoRI cleavage gives a 416-bp DNA in the HindIII-EcoRI double digest only if HindIII cleavage occurred at +235.

Fig. 5A shows an agarose gel, stained with ethidium bromide, containing total DNA from three reconstitution experiments (lanes 1–3) as well as pure pML5–4NR DNA (lane C). All of the DNAs were digested with HindIII and EcoRI; for the chromatin samples, digestion with HindIII took place on intact chromatin, and EcoRI cleavage was done on the purified DNA. All samples showed the expected three fragments; also as expected, the yield of 416-bp fragment indicative of HindIII cleavage at +235 in chromatin decreased as the level of chromatin reconstitution increased. When the intensity of the 416-bp fragment was quantified, the values for the bands in lanes 1 and 2 were 20 and 50%, respectively, of the intensity of the band in lane 3. The expected values, assuming that 17 and 11 nucleosomes were assembled in lanes 1 and 2 (see Fig. 1B) and that each nucleosome protects 150 bp of DNA, would be 29 and 54%.

Thus, there is reasonably good agreement with the assumption that the overall distribution of nucleosomes on the DNA is random. However, when we checked for the ability of HindIII-cleaved chromatin templates to produce a 235-base run-off RNA in a sarkosyl chase, no such RNA was obtained (Fig. 5B, lanes 4 and 7). Note that the expected run-off RNA was obtained when mock-reconstituted templates were chased with sarkosyl after HindIII cleavage (lanes 8 and 9).

**DISCUSSION**

Despite significant advances in our understanding of both nucleosome structure and the mechanisms of transcript elongation by RNA polymerase II, it has not been possible (at least in the absence of activators; see Ref. 7) to reproduce efficient transcript elongation *in vitro* using reconstituted chromatin
templates. Our laboratory had explored this question previously using nucleosomes assembled with Xenopus oocyte extracts (5, 6). In those experiments, we found that the transcript elongation rate by polymerase II was very low in the absence of elongation factors and at best $1/10$ of the rate seen on pure DNA templates when saturating amounts of TFIIF and SII were supplied (6). To extend those studies with better characterized components, we have employed pure histone proteins and a modification of the decreasing salt reconstitution method, which does not inactivate the RNA polymerase II elongation complex. We report here that nucleosomal templates assembled in this way form an essentially absolute barrier to transcript elongation by polymerase II. This barrier is not relieved by the addition of TFIIF and SII, nor is it substantially reduced by providing a considerable excess of naked DNA onto which the nucleosomes might be displaced. The H3/H4 tetramer alone proved nearly as effective as the intact histone octamer in blocking passage of the RNA polymerase.

A number of studies using single-subunit bacteriophage RNA polymerases have shown that these enzymes can elongate transcripts efficiently on reconstituted chromatin templates (15–22). Felsenfeld and co-workers (19, 21) demonstrated that transcription of mononucleosomal templates with SP6 RNA polymerase leads to relocation of the nucleosome. This occurs when free DNA upstream of the transcription complex invades the nucleosome, occupying binding sites that were made available by the removal of the original DNA for transcription. The nucleosome is thereby spooled from its original position to an upstream location. The ability of the phage polymerase to pass through the nucleosome barrier depends on the formation of the intranucleosomal DNA loop, where the rotation of polymerase around the DNA duplex is sterically limited (21). The much larger size of RNA polymerase II may completely prevent such motion, which could explain the inability of excess naked DNA fragments to facilitate transcript elongation in our system.

In considering our results, it is important to note that the RNA polymerase II was not inactivated by the reconstitution procedure. Complexes that paused on chromatin templates always resumed efficient elongation upon the addition of sarkosyl to the reaction mixtures (see, for example, lanes 2 and 4 of Fig. 2). It might be argued that the salt assembly procedure left basic catalytic functions intact but somehow interfered with the ability of the RNA polymerase to respond to elongation factors. We addressed this point by performing a second round of sarkosyl rinsing on transcription complexes that had been fully assembled into nucleosomes. The response of these complexes to TFIIF and SII was indistinguishable from that of complexes that had never been exposed to the reconstitution procedure (data not shown).

A potentially significant difficulty with our experimental approach is the presence of histones that have not assembled
into nucleosomes. We employed about a 2-fold excess of histones over DNA to achieve the assembly of one nucleosome/200 bp of DNA. Since these histones were not removed before transcript elongation, one could argue that they associated with the DNA in some nonnucleosomal form and interfered with transcript elongation. In this context, it should be noted that our chromatin templates were cleaved by HindIII at essentially the level predicted for the number of nucleosomes assembled on the DNA (see Fig. 5), indicating that access to the template was not generally blocked by nonnucleosomal histones. Reines and Mote (44) have shown that the blockade imposed on RNA polymerase II transcript elongation by lac repressor is relieved by SII. If our transcriptional block were imposed by some nonnucleosomal histone-DNA interaction, it seems reasonable to expect from the results with lac repressor that SII would have stimulated efficient transcript elongation in our system as well. However, SII had no effect on elongation in the current study (Figs. 2–5).

The HindIII cleavage assay (Fig. 5) failed to find evidence for nonnucleosomal histone-DNA interactions. However, we thought it would be useful to test for such interactions by another method as well. It has been shown (45) that during decreasing ionic strength assembly of the nucleosome, the H3/H4 tetramer is bound to DNA at 1 mM salt, while the H2A/H2B dimers associate with the tetramer as salt is further lowered, assembly being complete by 0.6 mM. We reasoned that treatment of our chromatin transcription complexes with salt concentrations just above 0.6 mM would impose the harshest possible conditions consistent with retaining nucleosome structure. This approach should disrupt nonnucleosomal histone-DNA interactions. Our experimental design paralleled the sarkosyl-rinsing procedure (5), except that 650 mM NaCl was substituted for sarkosyl. Thus, the transcription complexes were exposed to high salt during part of their residence time in the gel filtration column, but they eluted in the lower salt buffer normally used for transcription. We found (data not shown) that the transcriptional blockade was retained after rinsing with 650 mM NaCl, both for complexes assembled with all four histones and complexes assembled with only H3 and H4. This observation provides strong evidence that nonnucleosomal histone-DNA interactions are not responsible for the block to elongation that we see in our experiments.

The decreasing salt reconstitution procedure appeared to result in the production of close packed arrays of nucleosomes, beginning immediately downstream of the transcription complex (Fig. 5). This suggested an additional question: would inhibition of elongation be as severe if only a single nucleosome were located downstream of the transcription complex? To answer this question, we repeated our reconstitution/elongation experiment using templates that had been cleaved before nucleosome reconstitution with HindIII. In this case, since the DNA extended only 235 bp downstream of transcription start, only a single nucleosome could be assembled ahead of the RNA polymerase. We found (data not shown) that these templates behaved in all respects like the circular templates shown in Figs. 2–4; i.e., transcript elongation was essentially completely blocked by assembly of either the full octamer or only the H3/H4 tetramer on the template. This blockade was not relieved by elongation factors TFIIF and SII. It was also unaffected by an excess of pure DNA, either linear or circular.

Our current results might seem to contradict our previous work (6), in which some transcript elongation by RNA polymerase II was observed on nucleosomal templates, particularly in the presence of elongation factors. It is important to note that chromatin reconstitution in the earlier study was accomplished with unfraccionated oocyte extracts. Thus, it is reasonable to speculate that these extracts contain factors that specifically facilitate transcription of chromatin templates. Such components could be elongation factors for RNA polymerase II, although our results from the present study would argue against TFIIF or SII. Furthermore, the recently described elongin (46) and ELL (47) factors would not be the likely sources of the elongation activity, since these factors are functional analogs of TFIIF. It is possible that the oocyte factor is an analog of P-TEFb (48, 49), which increases the processivity of transcript elongation on pure DNA templates through phosphorylation of the C-terminal domain of the largest subunit of RNA polymerase II. Another candidate would be TFIIH, which has been shown to affect the efficiency of transcript elongation in oocytes (50); however, it has not been possible in highly purified transcription systems to demonstrate an association of TFIIH with the transcription complex beyond about 30 bases into the transcription unit (51).

An alternative view would suggest that the oocyte factor is not a transcription component but a modifier of the chromatin structure. Chromatin remodeling complexes (such as the SWI/SNF complex (52, 53), RSC (14), or a Drosophila nucleosome remodeling factor, NURF (13)) might have been present in the oocyte extracts. These factors could have acted once ATP was supplied for transcription. Since these activities increase the accessibility of transcription factor binding sites within nucleosomes (see, for example, Refs. 11 and 12), it is reasonable to suppose that they might also allow better access of the RNA polymerase to the DNA template. Modifications of the Xenopus histones, such as acetylation, might have facilitated the passage of the RNA polymerase. However, earlier work with oocyte extracts suggested that the assembly of histones into chromatin is accompanied by deacetylation, at least of histone H4 (54). HMG-14 has been shown to increase the rate of transcript elongation by RNA polymerase II on SV40 minichromosomes (55); thus, the oocyte factor could have been a high mobility group protein.

Lorch et al. (24) showed that purified RNA polymerase II could cross one nucleosome after initiation from a 3′ single strand extension of the template (dC tail). This finding suggests that chromatin remodeling might not be necessary for transcript elongation on chromatin templates by RNA polymerase II. However, it is important to note that most dC-tailed template transcription results in strand displacement (56); i.e., as transcription proceeds, a long RNA-DNA hybrid is made, and the non-template strand is released as a single strand. Such an event could destabilize a nucleosome and facilitate transcript elongation in a nonphysiological manner.

In summary, the work presented here demonstrates that RNA polymerase II elongation complexes cannot traverse nucleosomes assembled in vitro in a system consisting only of purified polymerase II elongation complexes and highly purified histone proteins. These results set the stage for evaluating the possible role of histone and chromatin modifications in the transcription process. The system also provides an assay for novel transcription factors necessary for efficient transcript elongation on chromatin templates.

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