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Octamer displacement and redistribution in transcription of single nucleosomes

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ABSTRACT

Single nucleosomes were assembled on a 357bp DNA fragment containing a 5S RNA gene from sea urchin and a promoter for SP6 RNA polymerase, and were fractionated as a function of their positions by gel electrophoresis (1,2). Transcribed nucleosome positions were detected by observing band disappearance in gels, which in turn provided evidence for the displacement of the histone octamer upon transcription. Differential band disappearance showed that nucleosomes closer to the promoter were harder to transcribe, and transcription was blocked when the nucleosome proximal boundary was at the start site. Nucleosomes located at discrete positions were also eluted from the gel bands and transcribed. In this case, new bands appeared as a consequence of octamer redistribution. Such redistribution occurred over all untranscribed positions, as well as over transcribed positions close enough to the promoter. Similar conclusions were derived from another previously investigated fragment containing a *Xenopus* 5S RNA gene (3,4).

INTRODUCTION

The question of how RNA polymerases transcribe DNA when complexed with histones in chromatin has recently been readdressed and extensively reviewed (5–12). Early *in vitro* studies of chromatin transcription, using reconstituted chromatin and *E.coli* RNA polymerase holoenzyme, have allowed certain conclusions to be made which are still valid. Nucleosomes totally inhibit initiation but only partially inhibit elongation and do not lead to new pauses, with sequence-specific pauses only being accentuated. The polymerase could pass through nucleosomes without apparently destroying them, although a fast cycle of histone dissociation and reassociation could not be excluded (13–15). More recently, phage RNA polymerases were used to transcribe single nucleosomes. The block to initiation by a nucleosome on the promoter was confirmed (3,16), but conflicting results were obtained regarding nucleosome displacement. Recently, Felsenfeld's group (17) confirmed the nucleosome displacement upon transcription. However, the displaced octamer was not lost to solution but transferred to other positions of the same DNA molecule.

In this work, the fate of the histone octamer upon transcription was investigated through a new nucleosome fractionation approach (1,2). Single nucleosomes were reconstituted on short DNA fragments carrying an SP6 polymerase promoter linked to a 5S RNA gene. Upon gel electrophoresis, these nucleosomes migrated less when closer to the mid-position, and more when closer to either end of the fragment. Such fractionation is actually similar to that obtained with CAP protein in the circular permutation assay (18), and is expected to be the consequence of DNA bending on the histone core (1,2). The technique allowed the direct visualization of the transcribed nucleosome positions by monitoring the disappearance of bands in the gel. Moreover, when transcription was performed on nucleosomes located at discrete positions, the transfer of the displaced octamers to other positions of the fragment could be observed through the appearance of new bands.

MATERIALS AND METHODS

DNA

In the 357bp fragment (see its sequence in ref. 1), a promoter for phage SP6 RNA polymerase was linked to a 256bp EcoRI fragment containing a sea urchin 5S RNA gene (19,20). The fragment, obtained from a BamHI digest of plasmid pB357, was oligomerized using T4 DNA ligase, and the gel purified tetramer ligated to BamHI-cleaved pUC18. The construct was used to transform HB101 *E.coli* cells and generate plasmid pUC(357.4), allowing us to prepare circular permutations of the fragment using AvaI and RsaI (fig. 1). The 358bp fragment containing a *Xenopus* 5S RNA gene downstream of an SP6 promoter was obtained from a BglII–EcoRI digest of plasmid pXP14.4 (3,4). Dephosphorylation and 5′-end labeling with ^{32}P-ATP and T4 polynucleotide kinase were according to standard protocols.

Chromatin

Duck erythrocyte nuclei were isolated (21) and core histones prepared (22). Chromatin was reconstituted using the 'salt jump' procedure (23). Briefly, ^{32}P-labeled DNA fragments were added to the corresponding unlabeled fragments (or the supercoiled

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pXP14.4 plasmid DNA in the case of the 358bp fragment) and to core histones (histone/DNA weight ratios (r_w)=0.35−0.45; up to 1.0 with the 358bp fragment) to final DNA and NaCl concentrations of 200μg/ml and 2M. The mixture was diluted 4-fold and dialysed at 4°C against 10mM Tris−HCl (pH 7.5) and 0.25mM EDTA (22).

Chromatin was sedimented in a linear 5% to 20% (w/v) sucrose gradient in 10mM Tris−HCl (pH 7.5), 1mM EDTA, 10mM NaCl and 35μg of bovine serum albumin (BSA)/ml, in a Kontron TST55.5 rotor at 28,000 revs/min for 18h at 4°C. Fractions (140μl) were counted in Cerenkov, pooled and concentrated using a Centricon-30 microconcentrator (Amicon), which did not alter the stoichiometry of the bands nor the amount of contaminating naked DNA (not shown).

Gel electrophoresis and nucleosome elution

Chromatin was electrophoresed at room temperature in 4.5% polyacrylamide (acryl./bis.=29:1; w/w) slab gels (0.15×16×18cm) in TE buffer [10mM Tris−HCl (pH7.5) and 1mM EDTA]. When stated, 5% polyacrylamide (acryl./bis.=19:1; w/w) gels were used under similar conditions. Gels were preelectrophoresed for 2h and electrophoresed for 3.5h at 250V, with buffer recirculation. Gels were dried and autoradiographed at −80°C. Some gels were exposed to a photostimulatable storage phosphor imaging plate overnight, and the latent image was read and analysed as described (1).

For preparative purposes, gels (electrophoresed for 5h) were autoradiographed wet at 4°C. Thin gel slices were excised and nucleosomes were eluted by crushing with a teflon rod fitting in Eppendorf tubes. The slurry was resuspended with 4−5 volumes of extraction buffer (see below) and incubated for 10min on ice (room temperature for the glycerol-containing buffer). The supernatant was recovered by centrifugation for 5min at the elution temperature. One extraction buffer contained 10mM Tris−HCl (pH7.5), 0.25mM EDTA and 0.01% BSA, plus 50μg H1-depleted carrier chromatin/ml, prepared as described (1). The other two contained 40mM Tris−HCl (pH7.5), 0.25mM EDTA, 3mM $MgCl_2$, 0.01% BSA and either 50% glycerol or 100μg form I pUC18 DNA/ml.

Nucleosome positioning

Nucleosomes N1, N2 and N3 on the 357bp fragment were positioned previously (fig. 1). The procedure involved the elution of the fractionated nucleosomes, their digestion with exonuclease III, and the electrophoresis of the DNA products in sequencing gels. Positions of the exonuclease pauses were subsequently deduced from the fragment lengths measured by comparison with size markers (1). N1 and N2 were further mapped by DNaseI footprinting, leading to the same positions for the nucleosome dyad axes within ±2bp (1), which could be considered as the uncertainty in positioning by the exonuclease procedure. In this work, N0, N4, N5, N6, N'6, N7, N'7 and N8 (fig. 1) were positioned using the exonuclease procedure (not shown). N6 and N'6, as well as N7 and N'7, comigrate into unique N6 and N7 bands, respectively. As judged from the relative band intensities in the sequencing gels, N'6 overweighted N6 by approximately 2:1, while N7 and N'7 were about equal.

Eluted nucleosomes were sometimes circularized by incubation with T4 DNA ligase for 30min at 37°C in 10mM Tris−HCl (pH7.5), 50mM NaCl, 5mM $MgCl_2$, 5mM dithiothreitol (DTT) and 0.6mM rATP. Circular nucleosomes ([app]50% of the total) were electrophoresed, eluted using the chromatin-containing buffer, and relinearized by restriction digestion. These digestions, as well as secondary restriction digestions of similarly eluted linear nucleosomes, were performed with 5U of enzyme/0.2μg of total chromatin DNA for 30min at 37°C, as described (1). Prior to electrophoresis, digests were diluted 2-fold with TE buffer and 1μg of form I pUC18 DNA/slot was added.

Transcription

Total chromatin (25−40ng of DNA), or eluted nucleosomes (1−5ng of 357bp DNA, and occasionally 0.5 or 1μg of longer DNA in the form of carrier chromatin or DNA, respectively), were transcribed in 40mM Tris−HCl (pH7.5), 10mM NaCl, 3mM $MgCl_2$, 1mM spermidine, 5mM DTT and 0.01% BSA, with 0.5mM each of the four rNTPs (pH7.0) and 1U of RNasin (Promega Biotec)/μl. The 20μl mixtures were supplemented with SP6 RNA polymerase (Promega Biotec), and incubated at 40°C for 30min (total chromatin) or 20min (unique nucleosomes).

RNA transcripts

Chromatin was transcribed using the above conditions plus α^{32}P-UTP (Amersham), digested for 30min at 37°C with RNase-free DNaseI (Promega Biotec), extracted with phenol and ethanol precipitated. Pellets were resuspended in 90% deionized formamide, incubated at 60°C for 5min, and electrophoresed in 6% polyacrylamide (acryl./bis.=19:1; w/w) slab gels (0.02×16×40 cm) containing 8M urea in 89mM Tris, 89mM boric acid and 2mM EDTA, pH8.3, along with a size marker. Gels were electrophoresed at 1,500 volts for 2.5h, fixed, dried and autoradiographed at −80°C.

RESULTS

Mixed nucleosomes on the sea urchin 5S fragment

Single nucleosomes were reconstituted on the 357bp BamHI fragment (fig. 1), and fractionated by gel electrophoresis (fig. 2a, lane C). Upon incubation with increasing amounts of the polymerase (lanes 1−6), two major bands, N3 and N5, became weaker and eventually disappeared. This, together with the positions of these nucleosomes outside of the promoter (fig. 1), strongly suggests they were transcribed. However, it should be noted that different enzyme concentrations were required to provoke disappearance. N3 already disappeared in lane 5, while N5 disappeared only in lane 6. In contrast, the other two main nucleosomes, N1 and N2, invading the promoter by 44 and 2bp (fig. 1), respectively, were not displaced, suggesting they were not transcribed. N1 even appeared to be reinforced (compare lanes 1−6 with lane C). At the same time, naked DNA was released (Lin.). Mock transcriptions (lanes 7 and 8) showed both a retardation and a weakening of N3 and a reinforcement of N1 at the highest enzyme concentration (lane 7)(compare N3 and N1 in lanes 7 and 8), whie N5 was not affected.

Mononucleosomes purified in a sucrose gradient (fig. 2b) showed a band pattern (fig. 2c, lane C) quite similar to that of total chromatin, except for the smaller amount of residual naked DNA (Lin.). Upon transcription, N3 and N5 again disappeared and N1 became reinforced, while the release of the naked DNA was quite apparent (lane 3). A weakening of N3 and a reinforcement of N1, but without release of naked DNA, was also observed in mock transcription (lane 4). These mononucleosomes were also used to analyse the RNA transcripts. A strong band was visible in the gel (not shown) at the size expected for the run-off transcript (288 nucleotides). A weak band

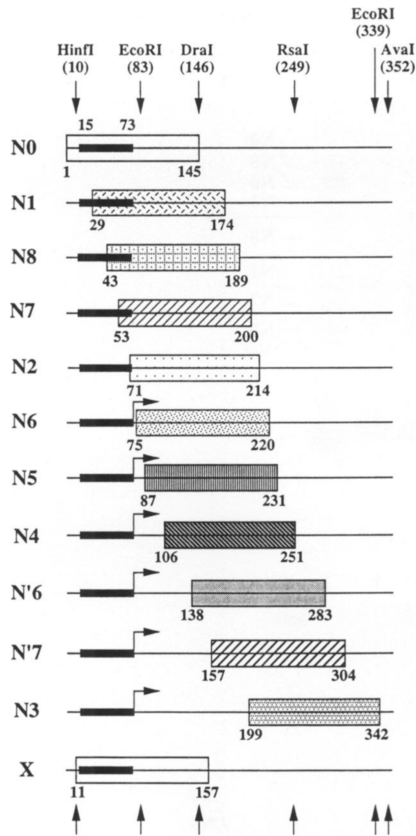

Figure 1. Nucleosome positions on the 357bp BamHI fragment. Numbers indicate nucleosome boundaries within ±2bp. The error is probably larger for X (see Results). Dark box: SP6 promoter. Horizontal and vertical arrows: start of transcription and restriction sites.

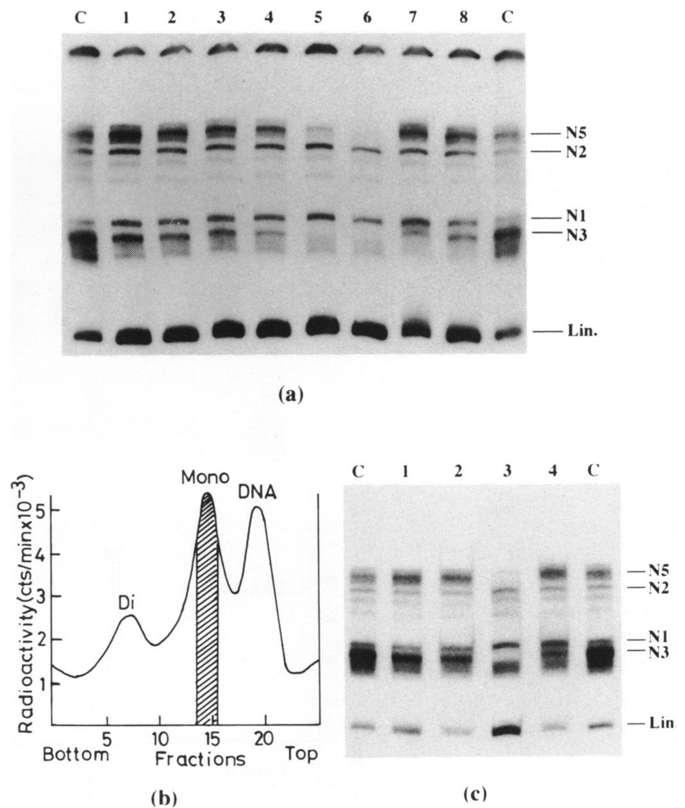

Figure 2. Transcription of mixed nucleosomes on the BamHI fragment. **(a)** 40ng (in total 357 bp DNA) of chromatin (r_w=0.4) (lane C) were transcribed with 0.1, 0.15, 0.2, 0.3, 0.6 and 1.5 U of SP6 RNA polymerase (lanes 1–6, respectively), and electrophoresed in a 5% polyacrylamide gel. Chromatin was also incubated with 15 (lane 7) and 6 U of polymerase (lane 8) without rNTPs. Aggregated material shows the start of the gel. Four main nucleosomes are indicated (fig. 1). Lin.: naked DNA. **(b)** Sedimentation profile of chromatin in lane C in (a) showing DNA, mono- (Mono) and dinucleosomes (Di). **(c)** Pooled mononucleosomes (hatched area in b) were concentrated (lane C) and 25ng (in DNA) aliquots incubated alone (lane 1) or with 3U of polymerase in the absence (lane 2) or presence of the 4 rNTPs (lane 3), or with 3U and 3rNTPs (A,U, C) (lane 4). Electrophoresis was as in (a).

was also observed at [app]225 nucleotides, corresponding to a pause of the polymerase in the middle region of a 28bp poly(purine)–poly(pyrimidine) track (see the fragment sequence in ref.1). This pause was also observed upon transcription of the naked fragment.

Unique nucleosomes

N3 on the BamHI fragment. The eluted nucleosome N3 (fig. 3a, lane 1) was slightly contaminated with its two neighbours, N0 and N1. Upon polymerase addition (lanes 2 and 3), N3 gradually disappeared and shifted into a faster band, X, virtually absent in total chromatin. At the same time, the two contaminating nucleosomes, N0, and especially N1, became reinforced, and several new bands appeared, which migrated like N5, N6, N2, N7 and N8. These bands, among which the latter three are the most visible, presumably correspond to authentic nucleosomes (see Discussion). Since they are not observed in mock transcriptions (not shown), they should arise from a redistribution onto the probe DNA of those histone octamers displaced from N3 by transcription.

Redistribution was accompanied by a release of naked DNA (Lin., lanes 2 and 3). To test whether displaced octamers may be in part captured by carrier chromatin, N3 was eluted in glycerol (fig. 3a, lane 4). Only a small release of free DNA was observed in lane 5, and most displaced octamers were redistributed. However, at the higher enzyme concentration (lane 6), all redistributed bands weakened and free DNA was again released. Surprisingly, N5 appeared as a redistributed nucleosome, despite its transcribability. Measurements of the peak areas in the radioactivity profiles (fig. 3b) indicated that the displaced octamer redistributed on average about 2 to 3 times more often onto N0 and N1 than on N2, N7 and N8, in both lanes 5 and 6. This calculation took into account the preexisting radioactivities in N0 and N1 as untranscribed contaminants of the starting N3 nucleosome in lane 4. Note that N0 and N1 were also stronger than N2, N7 and N8 in lane C (fig. 3b). Finally, N3 was also eluted in the presence of plasmid DNA (lane 7). Naked DNA was now released in large amounts at the lower enzyme concentration (lane 8), while upper redistributed bands became almost invisible (lanes 8 and 9). However, N1 and X remained in significant amounts, in contrast to N0 which appeared not to be enriched above its contaminating level.

N3 on the AvaI fragment. This fragment is circularly permuted by 5bp relative to the BamHI fragment (fig. 1). Lane C in fig. 3c shows the total chromatin band pattern, and lane 1 nucleosome

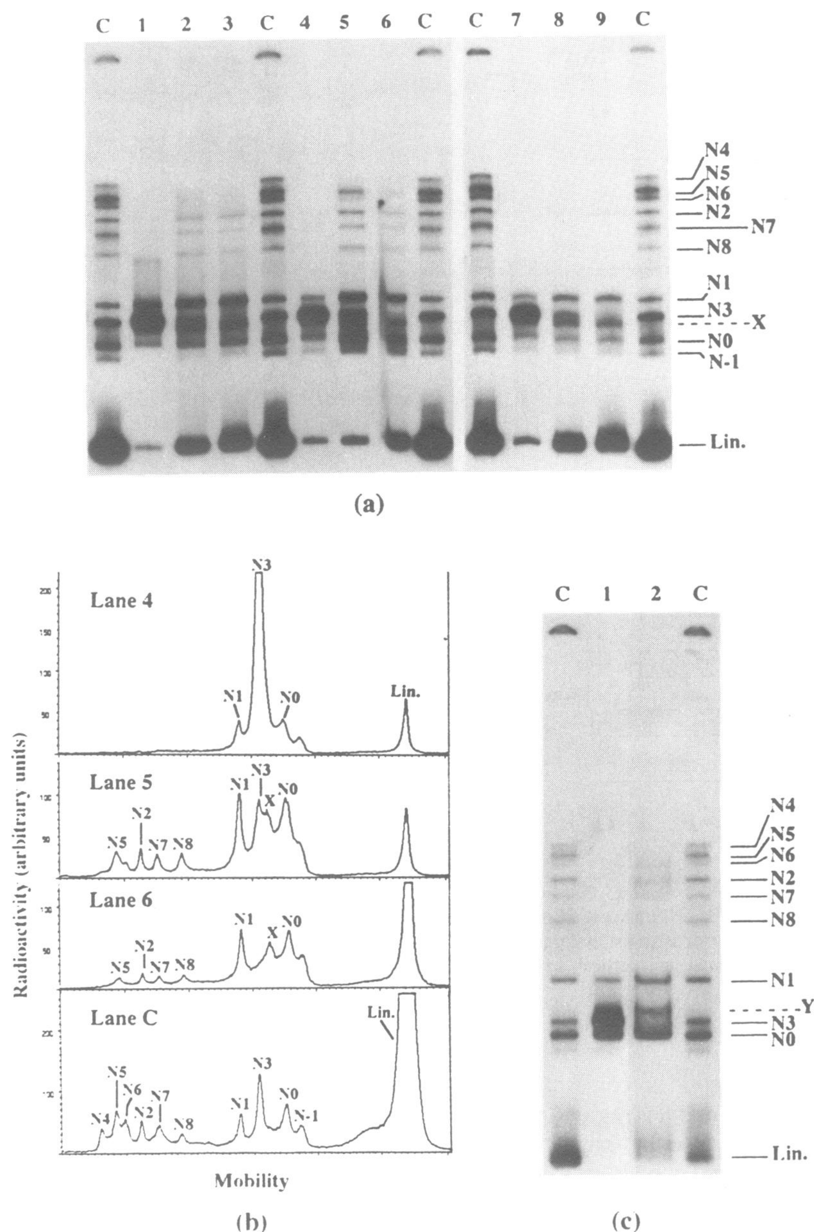

Figure 3. Transcription of N3. (**a**) N3 was purified from BamHI chromatin (lane C) by elution with native chromatin (lanes 1–3), glycerol (lanes 4–6), or plasmid DNA (lanes 7–9). 4.5ng (in 357bp DNA) aliquots were incubated alone (lanes 1,4,7), or with 0.2 (lanes 2,5,8) and 20 U of polymerase (lanes 3,6,9), and electrophoresed. N-1 was not positioned because it did not resist elution. X is generated by transcription. Lin.: naked DNA. (**b**) Radioactivity profiles of lanes 4–6 and C in (a). (**c**) N3 was purified from AvaI chromatin (lane C) using the chromatin-containing buffer, incubated alone (lane 1) or with 3U (for 2ng 357 bp DNA) of polymerase (lane 2), and electrophoresed. [Note that bands N6 and N7 in fig. 3a (lane C) should now split into their constituent nucleosomes, N6 and N'6 and N7 and N'7, respectively (fig.1). N'6 presumably comigrates with N2, while N'7 may comigrate with N8.] Y is identical to X in (a) (see Results).

N3 eluted with chromatin as carrier. The contamination with N0 was greater than the contamination with N1, in keeping with N0 higher relative intensity and closer proximity. Upon transcription (lane 2), N3 disappeared and higher redistributed bands appeared at the positions observed in total AvaI chromatin. Moreover, a new band, Y, without an equivalent in total chromatin and slower than N3, appeared (while X was faster than N3; see fig. 3a).

Y can be positioned using the mobility-*versus*-position curve constructed with total chromatin in lane C (see calibration curve in ref.1). The Y dyad was estimated to be at 92bp from the proximal end of the fragment. The X dyad was similarly located at 85bp from the proximal end of the BamHI fragment. The X dyad may therefore be either at 90 or 80bp from the proximal end of the AvaI fragment, respectively, depending on whether X is on the promoter or near the other side (fig. 1). Since 92 and 90bp are identical within experimental errors, one may conclude that X and Y represent the same nucleosome.

N5 on the BamHI fragment. Upon N5 transcription (fig. 4), X was present in the redistribution pattern (lanes 5 and 6), and N0 and N1 were again 2–3 fold stronger than N2, N7 and N8 (fig. 4b). The additional band (lanes 4 and 7) migrating to a position halfway between that of N0 and N3 could be a destabilization intermediate of N5, since it was not observed upon elution with

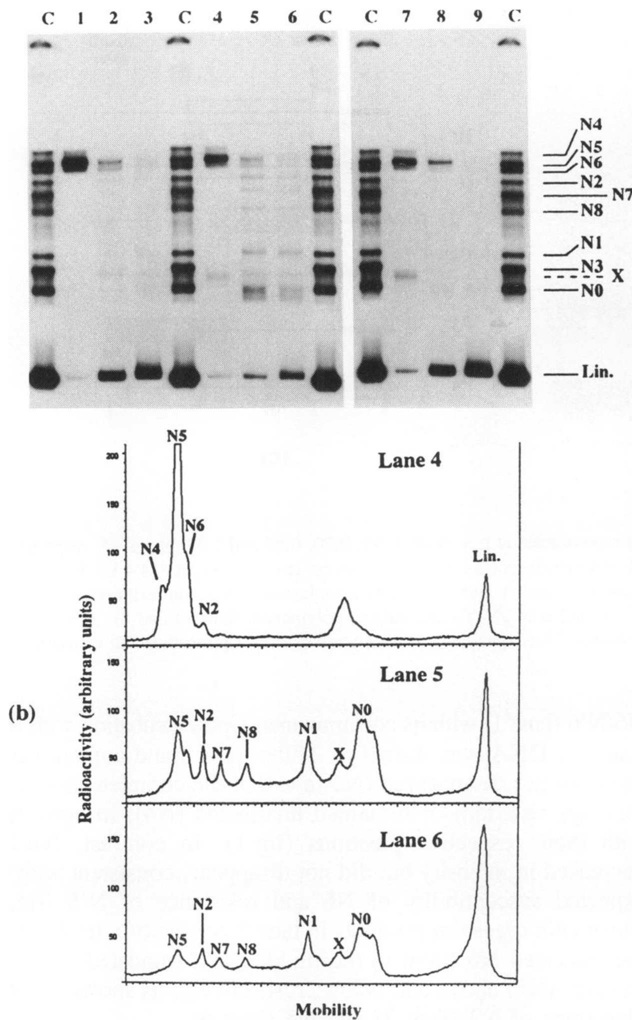

Figure 4. Transcription of N5 on the BamHI fragment. **(a)** N5 was purified from total chromatin (lane C) using the three buffers, and transcribed as described for N3 in fig. 3a, except that aliquots were 2.5ng in 357bp DNA. **(b)** Radioactivity profiles of lanes 4–6 in (a).

chromatin (lane 1) (i.e. under more stabilizing conditions). This band is unlikely to interfere with band X which is still produced in its absence (lane 2). Redistribution products were again faint in the presence of supercoiled DNA (lanes 8 and 9), if one excepts N0 (which may originate from N5 destabilization intermediate) and the higher bands representing N5 untranscribed contaminants.

A significant amount of N5 remained at the higher polymerase concentration (most pronounced in lane 3 in fig. 4a and lane 6 in fig. 4a,b). Interestingly, N5 was also observed in N3 transcription as a redistributed nucleosome (see lanes 5 and 6 in fig. 3b). Therefore, the incomplete disappearance of N5 in fig. 4 may not be due to an insufficient enzyme concentration, but to N5 redistribution on itself. Similarly, the absence of N3 as a redistributed nucleosome in N5 transcription (no such band is visible between N1 and X in lanes 5 and 6 in fig. 4b) is paralleled in N3 transcription by its ability to disappear completely (lane 6 in fig. 3b).

N5 on the RsaI fragment. This fragment is circularly permuted by 108bp relative to the BamHI fragment (fig.1), so that the promoter is located near the middle (fig. 5c). N5 and N1 were

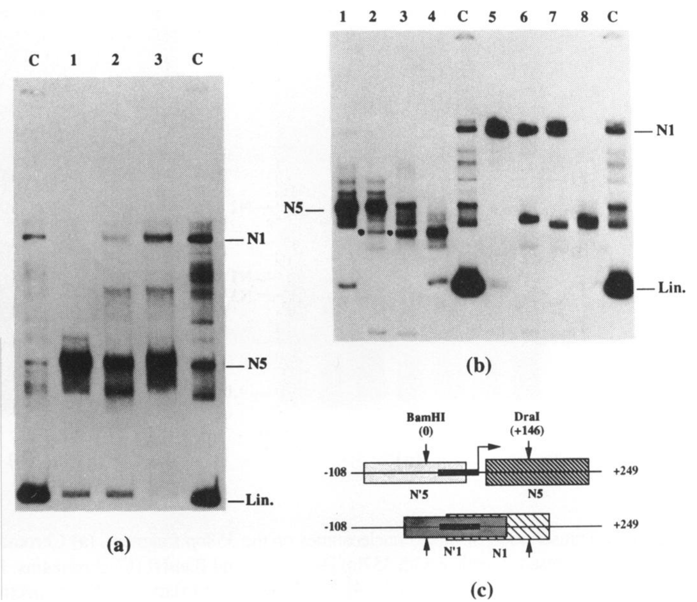

Figure 5. Transcription of N5 on the RsaI fragment. **(a)** N5 was purified from total chromatin (lane C) using the glycerol-containing buffer. 5 ng (in total 357 bp DNA) aliquots were incubated alone (lane 1), or with 0.2 and 20 U of polymerase (lanes 2 and 3, respectively), and electrophoresed. **(b)** N5 (lanes 1–4) and N1 (lanes 5–8), purified from total RsaI chromatin (lane C) in the chromatin-containing buffer, were incubated alone (lanes 1 and 5), or with DraI (lanes 2 and 6), BamHI (lanes 3 and 7) and DraI/BamHI (lanes 4 and 8), and electrophoresed. Dots indicate digestion-generated bands. **(c)** N5/N′5 and N1/N′1 symmetrical positions on the RsaI fragment. Estimated boundaries are −91/+55 (N′5) and −34/+112 (N′1). Dark box: SP6 promoter. Horizontal and vertical arrows: transcription start and restriction sites.

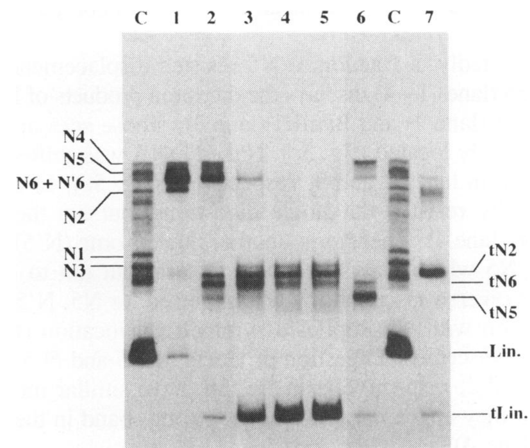

Figure 6. Transcription of N6 and N′6. N6/N′6 was purified from band N6 in total BamHI chromatin (lane C) using the chromatin-containing buffer (lane 1), and digested with RsaI (lane 2). The digest was diluted 4-fold with the transcription buffer, and 1ng (in probe DNA) aliquots were transcribed with 0.2, 20 and 40 U of polymerase (lanes 3–5, respectively), and electrophoresed along with unincubated N6/N′6 (lane 1) and RsaI digests of N5 (lane 6) and N2 (lane 7). RsaI-truncation is indicated by the prefix t.

identified in total RsaI chromatin (fig. 5a, lane C) from their mobility using the mobility-*versus*-position curve. To verify these identifications, N5 and N1 on the BamHI fragment were circularized and subsequently relinearized with either RsaI or BamHI (see Materials and Methods). The products were found

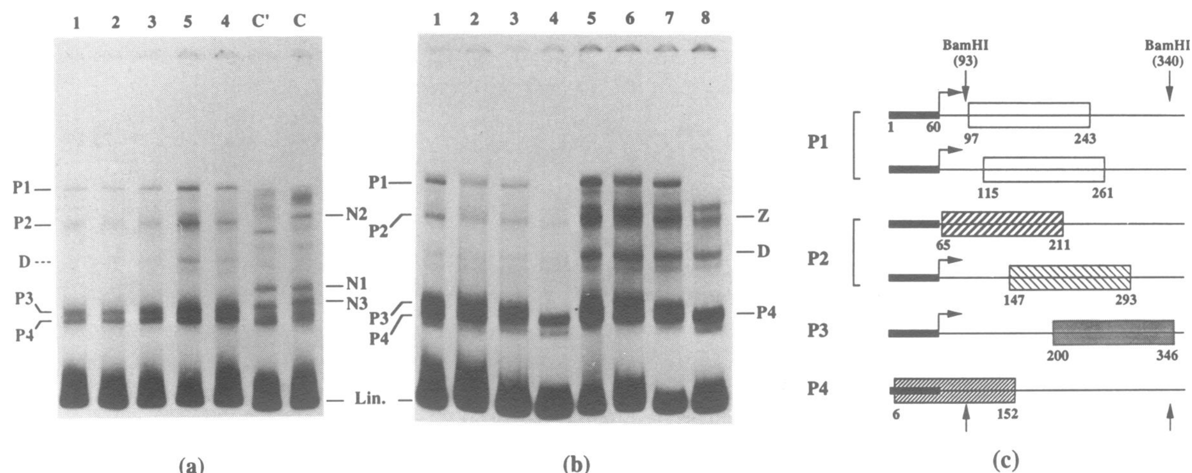

Figure 7. Transcription of mixed nucleosomes on the 358bp fragment. **(a)** Chromatin was reconstituted at r_w = 0.40, 0.55, 0.70, 0.85 and 1.0 (lanes 1–5, respectively), and electrophoresed, together with 357bp HinfI (C') and BamHI (C) chromatins. P1–P4: mononucleosomes. D: dinucleosome. **(b)** 32ng (in pXP14.4 DNA) chromatin aliquots from lane 1 in (a) (lanes 1–4) and lane 4 in (a) (lanes 5–8), respectively shown in lanes 1 and 5, were electrophoresed after transcription with 20U of polymerase but no rNTPs (lanes 2 and 6); with rNTPs but no polymerase (lanes 3 and 7); and with rNTPs and 20U of polymerase (lanes 4 and 8). Z is generated by transcription. **(c)** Nucleosome potential positions on the fragment. Dark box: SP6 promoter. Horizontal and vertical arrows: transcription start and restriction site.

to comigrate with putative N5 and N1 in the RsaI pattern when digested with RsaI, while they recovered their original mobility when digested with BamHI (not shown).

N5 was eluted (fig. 5a, lane 1) and assayed for transcription (lanes 2,3). N1 was again a major nucleosome in redistribution, as it was in total chromatin (lane C). X, which is located approximately 18bp upstream of N1 (fig.1) and is therefore close to the centre of the RsaI fragment, should be visible above N1 in fig. 5a. Instead, a broad band exists at this position, which has not been identified, but its amount is small compared to that of N1.

Unexpectedly, a fraction of N5 resisted displacement in lane 3. Fig. 5b (lanes 1–4) displays the digestion products of N5 (lane 1) by DraI (lane 2) and BamHI (lane 3), whose sites are nearly symmetrically located (fig. 5c). Naked DNA was shifted to 254 and 249bp in lanes 2 and 3, respectively (fig. 5c). In contrast, N5 partially resisted the single digestions, but not the double digestion (lane 4). Therefore, another nucleosome (N'5) existed in band N5 which was susceptible to DraI but not to BamHI, since the reverse susceptibility was expected for N5. N'5, whose comigration with N5 implies a symmetrical location (fig. 5c), was not transcribed. Digestion products of N5 and N'5 (dots in lanes 3 and 2, respectively, in fig. 5b) have similar mobilities, such that they appeared as a unique, strong, band in the double digest (lane 4).

N1 also contained another symmetrically located nucleosome, N'1. Digestion products of N1/N'1 (fig. 5b, lane 5) with DraI (lane 6) and BamHI (lane 7) have slightly different mobilities, and a doublet appeared in the double digest (lane 8). N'1 is located on the promoter (fig. 5c) and is not expected to be transcribed. Therefore, N'1 may be a redistribution product of N5, and contribute to N1 intensity in fig. 5a, lane 3.

N6 on the BamHI–RsaI fragment. N6 in BamHI chromatin contains two comigrating, symmetrically located nucleosomes, N6 and N'6 (fig. 1). N6 location in between a transcribed (N5) and an untranscribed nucleosome (N2) makes it interesting to study. Fig. 6 shows total BamHI chromatin (lane C) and eluted N6/N'6 (lane 1) with its contaminants. Upon incubation with RsaI (lane 2), DNA was shifted to 249bp (tLin.) and contaminating nucleosomes disappeared (N2)(a test of the completeness of the cleavage reaction) or remained unaffected (N4), in agreement with their respective positions (fig.1). In contrast, N6/N'6 decreased in intensity but did not disappear, consistent with the expected susceptibility of N6 and resistance of N'6 (fig.1). Chromatin digestion products in lane 2 consist of a triplet, with the truncated N6 (tN6) in the middle, and truncated N2 (tN2) and N5 (tN5) above and below, respectively, as shown by RsaI digestions of N2 (lane 7) and N5 (lane 6).

Upon transcription (lanes 3–5), N'6 weakened already in lane 3, while tN6 was hardly affected. Only in lanes 4 and 5 did tN6 weaken significantly. In contrast, tN2 remained undisturbed, as expected. These features suggest that N6 is transcribable, although much less than N'6. Lanes 2–4 also showed the progressive disappearance of tN5, in agreement with its transcribability. The residual amount of tN6 in lane 5, in contrast with N'6 complete disappearance, reflected its redistribution on itself, or its relative resistance to transcription. Note the release of 249bp DNA (tLin.) in lanes 3–5, and the absence of 357bp DNA (Lin.) expected to be released from N'6, which shows that RsaI remained active during transcription.

Mixed nucleosomes on the *Xenopus* 5S fragment

Upon reconstitution on the 358bp fragment (fig. 7a, lanes 1–5), four mononucleosomes, P1–P4, can be seen, while D becomes significant at large r_w ratios and presumably corresponds to dinucleosomes. Chromatin was assayed for transcription at lower (fig. 7b, lanes 1–4) and higher r_w ratios (lanes 5–8). Under mock transcription, P1–P3 became fuzzy and decreased their mobility slightly (lanes 2 and 6), suggesting they are transcribable. Under effective transcription (lanes 4 and 8), P1 and P3 disappeared while P2 shifted to a slightly faster band, Z, virtually absent in control chromatin (compare lanes 7 and 8). Z may therefore be a redistributed position. The reinforcement of P4 (compare lanes 3 and 4, and 7 and 8) indicates it is another stronger, redistributed position.

Fig. 7c shows nucleosome positions on the 358bp fragment estimated from the mobility-*versus*-position curve constructed with the 357bp fragment (lanes C and C′). Two potential, symmetrical positions are shown for P1 and P2. One could be eliminated in P3, which is transcribed, and P4 which is not. In contrast, both P1 positions are transcribable. In the case of P2, the promoter-proximal nucleosome may also be transcribed, as N6 on the 357bp fragment (see above). Z, as a potential redistributed nucleosome, is either poorly transcribed or not transcribed at all. Its migration, slightly ahead of P2, suggests that Z is a few bp closer to the promoter than the promoter-proximal nucleosome in P2.

DISCUSSION

Octamer displacement and redistribution

A new method involving the position-dependent fractionation of single nucleosomes on short DNA fragments by gel electrophoresis (1,2) was a powerful tool in the present analysis. Transcribable nucleosome positions could be detected from the disappearance of the bands in the gel (fig. 2), providing evidence for the displacement of the histone octamer upon transcription. Moreover, band disappearance with unique nucleosomes was associated with release of naked DNA and formation of new bands. As a rule, these bands have the same mobility as nucleosomes in total chromatin. This was observed using both BamHI (figs 3a and 4a) and AvaI chromatins (fig. 3c), although any given nucleosome has a different mobility in the two chromatins, a consequence of the 5bp permutation (fig. 1). This suggests the new bands represent genuine, redistributed nucleosomes, which originate through octamer displacement from their own DNA molecules (5). Therefore, new bands are unlikely to be complexes of the polymerase with naked DNA or nucleosomes. In fact, faint bands were sometimes observed near the top of the gels (not shown), which could correspond to such complexes. These complexes may actually be labile and not resist penetration into the gel matrix. Redistributed nucleosomes should also have a correct histone complement, and not be depleted of one or two (H2A, H2B) dimers for example. Indeed, in our hands, reconstitution of the 357bp fragment with a 1:1 mixture of H3 and H4 did not lead to any band pattern but rather to a smear (not shown).

The balance between DNA release and nucleosome redistribution was found to depend on the presence and nature of the histone acceptor. With supercoiled DNA, release was maximum and redistribution minimum. Without an acceptor, in contrast, release was minimum and redistribution maximum. An intermediate situation was observed in the presence of H1-depleted native chromatin, in agreement with chromatin reported affinity for extra-histones (23,24).

Redistributed nucleosomes. The case of nucleosome X

The stoichiometry of redistributed nucleosomes may be very similar to that of nucleosomes in total chromatin (compare lanes 5 and 6 with lane C in fig. 3b). Consistently, N3 and N5 redistribution patterns are virtually identical (compare lanes 6 in figs 3b and 4b), except for the lower bands N0 and N1 introduced as untranscribed contaminants of N3 but not of N5. In contrast, X, which appears upon N3 (fig. 3a,b) and N5 transcription (fig. 4), is only occasionally observed as a faint band in total chromatin (not shown). X may be suspected to be a rare example of a stable DNA− or nucleosome−polymerase complex. To check for this possibility, N3 was transcribed on the AvaI fragment. If X was an authentic nucleosome, it would be slower than N3 instead of faster as on the BamHI fragment, and this was indeed observed (Y in fig. 3c).

In contrast, X was virtually absent in the redistribution pattern of N5 on the RsaI fragment (fig. 5) (i.e. when X was forced to occupy a more central position). This discrepancy suggests that X may be enhanced on BamHI and AvaI fragments through end-effects occurring during the reassociation step of the redistribution process. DNA ends also influence distal nucleosomes in total chromatin, although not necessarily in the same way. This is apparent when total BamHI and AvaI chromatins are compared (lane C in fig. 3a,c). Intensities of N0 and N3 relative to N1 and upper nucleosomes are quite different between the two fragments, although they are permuted by only 5bp. In contrast, the relative stoichiometry of N1 and upper nucleosomes appears stable.

Transcriptional properties as a function of nucleosome distance to the promoter

Transcription of total chromatin (fig. 2a) already demonstrated the different transcriptional abilities of N5 and N3, located respectively 14 and 126bp downstream of the transcription start site (fig.1). While N3 was significantly stronger than N5 in control chromatin (lane C), N3 became weaker than N5 already in lane 2 and disappeared in lane 5, whereas N5 disappeared only in lane 6. Differences between N6 and N′6, located 2 and 65bp respectively downstream of the start, were also apparent in fig. 6, where the early disappearance of N′6 was demonstrated together with the relative persistance of N6.

If a single passage of the polymerase suffices for displacement (see below), then our results suggest that more promoter-distal (D) nucleosomes (N3 or N′6) than promoter-proximal (P) nucleosomes (N5 or N6) have undergone this single passage. If displacement requires several passages, then more passages have occurred on D than on P nucleosomes. Alternatively, the number of passages could be the same on both types, but P nucleosomes would require more passages to be displaced. This would imply that P nucleosomes are more stable than D nucleosomes against displacement, a remote possibility since this would correlate stability to promoter distance. Therefore, it is most likely that P nucleosomes were transcribed at lower rates than D nucleosomes. This may be due to an hindrance in the formation of the polymerase initiation complex for nucleosomes close to the promoter, in keeping with the notion that the formation of this complex is the rate limiting step in transcription (25). Consistently, N2, only 4 bp upstream of N6 and 2 bp upstream of the transcription start site (fig. 1), blocks transcription completely.

No attempt was made in the present work to measure transcription rates directly through quantitation of the transcripts, since such measurements may be technically difficult to carry out. Contaminating nucleosomes indeed make the actual amount of the nucleosome of interest uncertain. Moreover, naked DNA, presumably a much better substrate for the polymerase, is present in variable amounts and its release upon transcription is also variable.

N5 is a redistributed nucleosome in N3 transcription (fig. 3a,b), but N3 is not in N5 transcription (fig. 4). This is presumably the consequence of the lower transcriptional ability of N5 relative to N3. A displaced octamer may indeed have a chance to reassociate at all positions, and this chance may be proportional

to the initial strength of the positions in total chromatin. Nucleosomes at non transcribed positions will remain stable, while those at transcribable positions will again be transcribed differentially. This latter effect appears to dominate here, so that N5 is favoured over N3 in the redistribution pattern, despite its lower chance to be formed in the first place (compare N5 and N3 strengths in total chromatin; fig. 3b, lane C).

Transcription on the *Xenopus* 5S fragment

Among the four main nucleosomes on this 358bp fragment, P1−P4 (fig. 7), the first three are transcribed, while P4 is not and is a strong redistributed position. Since P4 is close to the end of the fragment (fig. 7c), its strength in total chromatin and in redistribution may result from end-effects. An additional nucleosome, Z, appeared upon transcription, which migrates very close to P2, and may preexist in minor amounts in total chromatin, unresolved from P2. The disappearance of P2 upon transcription, together with the enrichment of Z by redistribution, would then result in the apparent shift observed (fig. 7b, lanes 4 and 8).

In contrast to these results, Brown's and Kornberg's groups (3,4) showed a single band in the gels which was believed, on the basis of DNaseI footprints, to reflect a unique nucleosome. This band, as suggested from its relative mobility in ref.4, is presumably the unresolved P3/P4 doublet. P3 and P4 have approximately equal intensities in the doublet, and are by far the major reconstituted product. Upon incubation with the polymerase, the doublet was either not affected (3), or decreased in intensity by up to 50% (4). In fact, only P3 in the doublet was transcribed and displaced. Depending on the extent of its redistribution onto P4, the decrease in the whole band intensity could vary as observed.

Kornberg's group (16) had previously reported a complete nucleosome displacement upon transcription on other fragments, suggesting that the nucleosome on the *Xenopus* 5S fragment was exceptionally stable (4). This stability has subsequently been confirmed (26), and the relatively small release of naked DNA observed here upon transcription, together with the significant redistribution on Z and P4 (compare lanes 3 and 4, and 7 and 8 in fig. 7b), may reflect this stability. However, present data demonstrate that displacement still occurs unabated, showing that all (single) nucleosomes may be equally displaced, regardless of their stability.

Other work and further questions

Felsenfeld's group (17), also using a single nucleosome but on a plasmid size DNA, did not observe any nucleosome loss. Instead, all nucleosomes were redistributed. This is in contrast to the release of DNA occurring here (figs 3,4). Such lower transcriptional stability presumably results from a lower chromatin concentration : 0.05 to 0.25μg DNA/ml, against 5 μg/ml (17). Similarly, the failure of extra ΦX174 form I DNA to trap the displaced octamers (17) may be explained by its low relative amount: extra DNA over probe DNA weight ratio was equal to $\sim$1.5, against 100 to 1000 ratios in this work.

Felsenfeld's group (17) also discussed models for the fate of the histone octamer during transcription. They excluded that the octamer could be pushed and slide ahead of the polymerase because redistribution remained the same after linearization of their circular DNA template. This argument applies here since in this case a nucleosome would only redistribute downstream of its initial position and not upstream, as observed. Instead, they suggested that the octamer could diffuse freely and be recaptured by the same DNA molecule, or transferred by intramolecular DNA−DNA collisions. Such collisions are restricted in our short fragment. However, if SP6 polymerase bends the DNA (27), they would be facilitated. Our data are therefore consistent with these models.

How many polymerase passages are necessary for displacement? The transcribed-through nucleosome may unfold, and have a chance to diffuse or be transferred only during the brief time interval before it refolds. The chance to be displaced would then be greater if the number of passages is larger. The time interval separating two successive passages (i.e. the transcription rate) may also be important. If this interval is short enough, the unfolded octamer may not have the time to refold before the next passage, and these passages could have an additive effect in destabilization. It has been argued, however, that a single passage is sufficient for octamer displacement (4).

E.coli and T7 RNA polymerases can transcribe through polynucleosomes with cross-linked octamers (28,29). This implies that histone partial release is not necessary, and that the octamer may be displaced (even transiently) as a whole. However, a cross-linked octamer may still unfold upon polymerase passage. Cross-linking, since it involves the NH_2 of basic residues, may even facilitate polymerase passage by loosening DNA−histone interactions. This is illustrated by exonuclease III, another DNA processing enzyme which can invade the nucleosome. Indeed, the first two pauses at 140 and 120 nucleotide positions were virtually lost after cross-linking with dithiobis (30).

Recent experiments have shown that phage RNA polymerases can transcribe through nucleosome oligomers (31−34) *in vitro* without net nucleosome displacement. The difference with other data (17) and those of these present experiments is that free DNA is no longer available on the same molecule, so that the octamer may have no choice other than refolding at the same position. Alternatively, the transcription rate may be sufficiently lower, compared to mononucleosomes, to allow the octamer to refold between successive passages of the polymerase (see above discussion).

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