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Transferring DNA from electrophoretically resolved nucleosomes to diazobenzyloxymethyl cellulose: properties of nucleosomes along mouse satellite DNA

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ABSTRACT

Electrophoresis fractionates nucleosomes which possess different protein compositions. We report here a procedure for transferring the DNA components of electrophoretically resolved nucleosomes to diazobenzyloxymethyl cellulose (DBM-paper). Histones are first removed from nucleosome components by electrophoresis in the presence of cetyltrimethylammonium bromide (CTAB), leaving DNA fragments fixed within the original gel as the CTAB salts. The DNA is then converted to the sodium salt, denatured, and electrophoretically transferred to DBM-paper. The overall pattern of DNA on the resulting blot is visualized either by fluorography or by immunoautoradiography. This DNA pattern is then compared with autoradiograms obtained after hybridizing the same blot with specific ^{32}P-labeled probes. Using mouse satellite DNA as a hybridization probe, we illustrate the above techniques and demonstrate that nucleosomes carrying satellite sequences are compositionally heterogeneous. The procedures described here should also be useful in the analysis of the nucleic acid components associated with other nucleoprotein complexes.

INTRODUCTION

A number of studies have taken advantage of the ability of electrophoresis to fractionate nucleosomes into subclasses which differ in protein composition (1-27). Reconstitution experiments performed in our laboratory have defined the major subunit compositions of different electrophoretic forms of nucleosomes (12,17). Thus, the molecular basis of nucleosome fractionation by gel electrophoresis is largely understood. Nucleosome electrophoretic heterogeneity does not depend primarily on histone octamer compositions (5,10), degrees of post-translational histone modification (5), or differences in DNA lengths among mononucleosomes (3,11,12,17). Rather, the association of histone H1, nonhistone proteins, or both, results in the generation of specific electrophoretic forms of mononucleosomes (12,17).

Because the molecular basis of electrophoretic resolution of nucleosomes is well understood, the ability to hybridize the DNA components of

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electrophoretic displays of nucleosomes with specific probes would be particularly useful for a wide variety of studies. Recently, Levinger et al. (25) reported a two-dimensional method whereby the DNA components of electrophoretically resolved nucleosomes can be transferred to DBM-paper for purposes of hybridization. We report here a complementary technique which permits the transfer of DNA fragments from one-dimensional electrophoretic displays of nucleosomes. Our method takes advantage of: (i) the ability of CTAB both to precipitate nanogram quantities of DNA (28) and to displace the histones from nucleosomes (29); (ii) the high capacity of DBM-paper for covalent coupling of DNA (30,31); (iii) the use of electrophoresis to transfer DNA (25,32,33), and; (iv) the employment of either fluorography (34) or immunoautoradiography (35) to visualize the pattern of bulk DNA on the blot prior to, or after, hybridization with a ^{32}P-labeled probe. In the present report we illustrate these procedures by examining the properties of nucleosomes along mouse satellite DNA. In general, the experimental strategy presented here could also be employed to study specific sequences associated with other types of nucleoprotein complexes.

MATERIALS AND METHODS

Preparation of mono- and polynucleosomes from labeled mouse cells - Cultured mouse mastocytoma cells (P815) were uniformly labeled with either ^{3}H-thymidine or ^{3}H-lysine as described elsewhere (10). Labeling with 5,6-[^{3}H]uridine (50 Ci/mmol, ICN) was for 1 hr at 37°C using 75 μCi/ml and 10^{7} cells/ml. Mono- and polynucleosomes were prepared from isolated nuclei after digestion with micrococcal nuclease as described (5), except that all buffers contained 5 mM sodium butyrate and 1% thiodiglycol prior to EDTA treatment, and nuclei were not exposed to Triton X-100. Acid-soluble material was estimated as described previously (36).

Gel electrophoresis - Mono- and polynucleosomes were separated by electrophoresis using 0.3-cm thick gels composed of 3.5% acrylamide, 0.5% agarose, 30% glycerol (12). Sample loading buffer was 50% glycerol, 1 mM EDTA, pH 7.2. Purified DNA was separated electrophoretically in the presence of 0.1% sodium dodecyl sulfate (SDS) as described elsewhere (3), except that gels contained 5% acrylamide, 0.25% N,N'-methylenebisacrylamide. Ethidium bromide stained gels were illuminated with long wave UV light and photographed.

Dehistonization - Nucleoprotein gels were soaked with agitation in 10 vol of 5% acetic acid, 1% CTAB at 23°C for 2 hr. After rinsing gels in

dehistonization electrophoresis buffer [5% acetic acid, 0.01% CTAB (29)], each gel was placed between two moistened sheets of Whatman 3MM paper and sponge pads in an ElectroBlot (E-C Apparatus Corp.), and electrophoresis was performed at 3.25 volts/cm for 8 hr at 4°C with the 6 l of buffer recirculated toward the cathode. (Excess CTAB in the electrophoresis buffer progressively precipitates during this step and the electrophoresis buffer turns yellow.)

Preparation for DNA transfer - To remove CTAB after dehistonization, gels were soaked at 23°C with agitation in 10 vol. of the following solutions for the indicated times: 1M sodium acetate-50% methanol, 14-18 hr; 1M sodium acetate, 1 hr; 1M sodium acetate-50% methanol, 3 hr; 0.1M sodium acetate-50% methanol, 3 hr; and distilled water, 1 hr. DNA was solubilized and denatured by soaking gels for two 30 min. periods at 41°C in 5 vol of 98% formamide with agitation. Gels become fragile at this step but later regain their consistency. Finally, gels were soaked at 23°C for two 30 min periods in electrophoresis transfer buffer (25 mM sodium phosphate buffer, pH 6.3). If desired, gels can be stained with ethidium bromide at this step. (It should be noted that the extent of depurination which occurs during dehistonization is minimal as determined by DNA length measurements after alkali treatment.) Electrophoretically resolved purified DNA was transferred from 5% acrylamide gels after the following treatments. Gels were soaked for 30 min at 23°C in 10 mM HCl to partially depurinate bases. Following soaking in electrophoresis transfer buffer for 15 min at 23°C, gels were placed in boiling electrophoresis transfer buffer for 15 min to partially fragment and melt DNA. (In agreement with Levinger *et al.* (25) but in contrast to other reports (32,33), we emphasize that treatment of acrylamide gels with alkali to denature DNA is not acceptable because gels swell excessively during electrophoretic DNA transfer.)

Transfer of DNA to DBM-Paper - DBM-paper was prepared as described (31), but similar results were obtained using activated Transa-Bind™ (Schleicher and Schuell, Inc.). DNA was transferred to DBM-paper using the ElectroBlot apparatus (32). Electrophoresis was at 4°C for 4 hr at 5.85 volts/cm with the 6 l of buffer both recirculated and stirred to reduce pH gradients. After transfer, blots were incubated in electrophoresis buffer 6-18 hr to permit maximal covalent coupling of DNA. Efficient electrophoretic transfer of DNA could also be obtained using a Trans-Blot™ Cell (Bio-Rad Laboratories).

Removal of RNA from Blots - Blots were incubated for two 30 min periods at 37°C with 0.4 N NaOH to destroy RNA, washed with distilled water, and air dried.

Fluorography and Autoradiography - Fluorography was performed using preflashed film as described elsewhere (34), except that EN^{3}HANCE (NEN) liquid or spray was used as the fluor. Dried gels or blots were exposed at -70°C from one week to two months. Autoradiography was performed at -70°C using preflashed film in the presence of a DuPont Cronex Lightning-Plus AG intensifying screen. Exposure of film due to tritium radioactivity was alleviated by placing a sheet of black paper between the blot and the film. Fluorograms and autoradiograms were scanned and peak areas were estimated by weighing fitted components.

Probe preparation and nucleic acid hybridization - Mouse satellite DNA was purified from P815 nuclear DNA by 3 cycles of isopycnic centrifugation (37). Purified satellite DNA was labeled by nick-translation with [α-^{32}P]dATP (800 Ci/mmol, NEN) (38), yielding a specific activity of $\geq$ 1 x 10^8 cpm/µg. Fluorographed blots were washed twice with ethanol to remove fluor, and after washing with distilled water, blots were prehybridized and hybridized in the absence of dextran sulfate as described (31), except that E. coli DNA was used as the carrier and the hybridization reaction was for only 3 hr. The 3 hr period was chosen to maintain a low Cot value so as to reduce the possibility of the hybridization of less abundant contaminating sequences present in satellite DNA preparations. Hybridized blots were washed as reported elsewhere (39), and autoradiography was performed as described above. When repeated hybridization of blots was desired, probe was removed from hybridized blots either by incubation with 0.4 N NaOH for 45 min at 41°C or by several 15 min washes with 98% formamide at 80°C. After washing with distilled water, blots were prehybridized as described above.

Immunological detection of single-stranded DNA - The double antibody technique of Sager and coworkers (35) was employed with the following modifications. Probe was removed from hybridized blots by treatment with 0.4 N NaOH for 45 min at 41°C, followed by washing with distilled water and 20 X SSC. After decay of residual ^{32}P-radioactivity, blots were washed with Buffer A (10 mM potassium phosphate, 0.15 M NaCl, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.05% Nonidet-P40, 0.01% sodium azide, pH 7.1), and then incubated 16 hr at 4°C in Buffer A plus 1% human gamma globulin. After washing with Buffer A, blots were incubated at 37°C for 16 hr on a rotator with Buffer A plus 0.5% human gamma globulin plus 2 µg/ml of a monoclonal IgG specific for single-stranded DNA (H43SC1; see Ref. 40). Blots were washed with Buffer A and then incubated with Buffer A plus 3% horse serum for 2 hr at 37°C. Following a third wash, blots were incubated with 5 x 10^6 cpm/ml of

^{125}I-labeled (41), affinity purified rabbit anti-mouse IgG (1.3 x 10^7 cpm/µg) in Buffer A plus 0.1% human gamma globulin. Finally, blots were washed for two 90 min periods at 37°C with Buffer A plus 1% deoxycholate plus 1% Triton X-100. After rinsing with distilled water, autoradiography was performed as described above. Because only 80% of the labeled antibody could be removed by washing blots with 0.4 N NaOH at 41°C for 4 hr, nucleic acid hybridization preceded immunoautoradiographic analysis.

Salt Depletion and Reconstitution - Mono- and polynucleosomes prepared as described above were brought to either 0.35 M or 0.6 M Na^+ ion by the dropwise addition of equal volumes of 2X buffers with mixing at 4°C. The final mixtures consisted of either 0.55 M or 0.3 M NaCl, 50 mM $NaHSO_3$, 10 mM triethanolamine, 1 mM Na_2 EDTA, pH 6.5. The sample in 0.35 M Na^+ ion was subjected to gel filtration at 4°C using Sephacryl S-200, equilibrated with and eluted by the 0.35 M Na^+ ion buffer. Excluded material was dialyzed at 4°C against 2 mM EDTA, pH 7.2. The sample in 0.6 M Na^+ ion buffer was brought to low ionic strength according to the reconstitution dialysis procedure described elsewhere (12). Dialyzed samples were concentrated using an Amicon-B15 apparatus.

RESULTS

DNA transfer procedure - Figure 1 illustrates the procedure for transferring DNA from electrophoretic displays of nucleosomes. Mono- and polynucleosomes prepared from ^{3}H-thymidine labeled cultured cells are

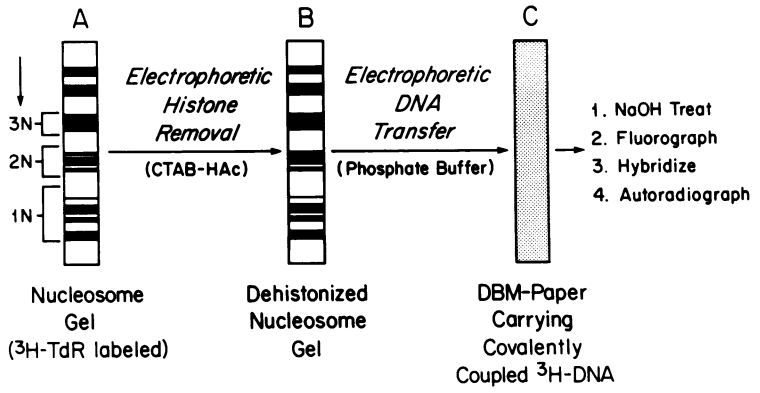

Figure 1: Experimental strategy for transferring DNA from electrophoretically resolved nucleosomes to DBM-paper (see text for details).

separated by gel electrophoresis into various subfractions. Histones are removed from nucleosome components by electrophoresis in the presence of CTAB, leaving DNA fragments fixed within the original gel as the CTAB salts. By subsequent soaking of the gel in various solvents, CTAB is removed and DNA fragments are both solubilized and denatured. The DNA fragments are then electrophoretically transferred to DBM-paper, and after covalent coupling occurs, traces of RNA are removed by alkaline hydrolysis. The total pattern of transferred DNA is visualized by fluorography, and the pattern of a specific DNA sequence is examined by nucleic acid hybridization and autoradiography. Alternatively, the pattern of transferred DNA can be visualized after hybridization and probe removal by immunoautoradiography using an antibody specific for single-stranded DNA.

Figure 2 shows the results of control experiments which demonstrate the efficiency of each step of the procedure by following the fates of labeled basic proteins, RNA, and DNA. From densitometric analyses of fluorograms exposed under conditions where a linear relationship existed between sample radioactivity and absorbance of the film image (34), the following conclusions can be reached. Approximately 93% of ^{3}H-lysine labeled protein is removed by the dehistonization step under conditions which lead to no detectable loss of DNA. The efficiency of transfer of mononucleosomal DNA to DBM-paper is

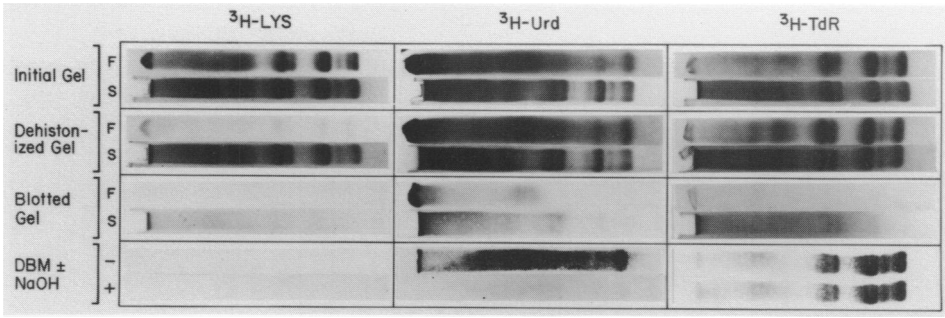

Figure 2: Fates of basic proteins, RNA, and DNA during each step of the transfer procedure. Mono- and polynucleosomes prepared from labeled mouse cells were separated by gel electrophoresis (left to right). Identical samples were then processed through the indicated steps of the transfer procedure, and either fluorographed (F) or stained with ethidium bromide (S). Fluorograms of the blots before (-) and after (+) NaOH treatment are shown at the bottom.

greater than 95% as judged from the amount of material present in gels before and after electrophoretic transfer. However, lower transfer efficiencies are noted for larger DNA fragments. It is noteworthy that if histones are not removed prior to attempting transfer, no detectable DNA transfer occurs under these same conditions (data not shown). Furthermore, the presence of histones on DBM-paper would be expected to increase non-specific binding of labeled hybridization probes. In other experiments using ^{32}P-labeled DNA standards (data not shown), the efficiency of covalent coupling and the capacity of DBM-paper were found to be similar to published reports (30-33). Finally, the patterns of transferred DNA carried covalently by DBM-paper resemble the DNA patterns of nucleosome components in the initial gel, and alkali treatment of blots removes greater than 99% of the RNA but less than 7% of the DNA (thus eliminating the possibility that subsequent hybridization patterns of blots could be attributed to RNA contamination).

Illustration of the transfer procedure - Mouse satellite DNA was chosen as a hybridization probe to illustrate the efficacy of the present technique. Nuclei prepared from ^{3}H-thymidine labeled cultured mouse cells were digested to different extents with micrococcal nuclease. The resulting mono- and polynucleosome preparations were separated by electrophoresis and the gel was subjected to the transfer procedure described above. After fluorography, the blot was hybridized with ^{32}P-labeled mouse satellite DNA and autoradiographed. Finally, the hybridized probe was removed and the blot was immunologically probed using a monoclonal antibody specific for single-stranded DNA. Figure 3 shows the resulting patterns of ethidium bromide staining, fluorography, and autoradiography.

The fidelity of the DNA transfer procedure can be evaluated by comparing the pattern of ethidium bromide staining of the initial gel with the fluorogram profile of DNA on the resulting blot. As shown in panels A and B of Figure 3, these patterns are similar in the regions where mono- and dinucleosomes migrate. This attests to the general maintenance of resolution throughout the procedure for DNA fragments derived from nucleosome components with short chain lengths. (While one would also like to show that bulk DNA sequences on the blot are uniformly hybridizable, such a control experiment is not possible; hybridization with nick-translated whole genomal DNA would lead to the preferential detection of repetitive DNA sequences.)

The properties of nucleosomes along satellite DNA can be evaluated by comparing the fluorogram pattern of transferred DNA with the autoradiogram obtained after hybridizing the same blot with ^{32}P-labeled satellite probe. As

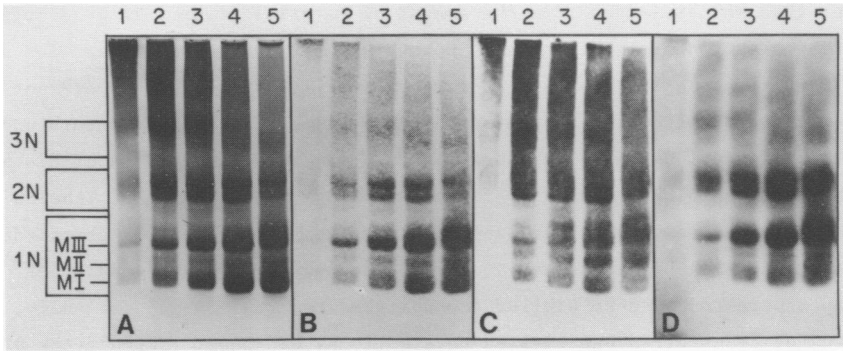

Figure 3: Fidelity of DNA transfer and location of satellite sequences. Mono- and polynucleosomes were prepared from ^{3}H-thymidine labeled mouse cells after digestion of nuclear DNA to acid-solubilities of 1, 6, 12, 15, and 19% with micrococcal nuclease (samples 1-5, respectively). Samples were separated by electrophoresis (top to bottom) and the gel was processed through the transfer procedure. (A), ethidium bromide stained initial gel; (B), fluorogram of the resulting blot; (C), autoradiogram of the same blot after hybridization with ^{32}P-labeled satellite DNA probe; (D), autoradiogram of the same blot after probe removal, reaction with a monoclonal antibody against single-stranded DNA, followed by reaction with ^{125}I-labeled rabbit anti-mouse IgG. 1N, 2N, and 3N depict the positions where mono-, di-, and trinucleosomes migrate, while MI, MII, and MIII depict different electrophoretic forms of mononucleosomes.

shown in panels B and C of Fig. 3, the general features of these patterns are largely similar. However, it is noteworthy that the distribution of satellite sequences differs in at least two respects from the distribution of bulk DNA. First, relative to the distribution of bulk DNA on the blot (Fig. 3B), the intensity of satellite hybridization is disproportionately represented in DNA fragments derived from nucleosomes with longer chain lengths (Fig. 3C). Thus, polynucleosomes containing satellite sequences appear to be processed more slowly to mononucleosomes by micrococcal nuclease digestion than are polynucleosomes of bulk DNA. Second, although satellite sequences are represented in the same multiple electrophoretic forms of mononucleosomes found in bulk chromatin, the distribution of satellite sequences within different mononucleosome classes exhibits quantitative differences from the distribution exhibited by bulk DNA. The magnitude of these quantitative differences depends on the extent of nuclease digestion. At intermediate periods of micrococcal nuclease cleavage, mononucleosome class MII is enriched in satellite sequences (Fig. 3C; see below). In contrast, after prolonged digestion mononucleosome class MI is depleted in satellite sequences (Fig. 3C). This depletion probably results from the production of insoluble

subnucleosomal particles by micrococcal nuclease cleavage within the nucleosome core.

Finally, after removal of hybridized probe, the distribution of bulk DNA on the blot can also be monitored by immunoautoradiography using a monoclonal antibody specific for single-stranded DNA. As shown in Figure 3D, the pattern of antibody binding to DNA on the blot resembles the fluorogram of transferred DNA depicted in Figure 3B and significantly differs from the pattern of satellite hybridization shown in Figure 3C. (It should be noted that quantitation of the reaction of the monoclonal antibody reveals that certain nucleosome classes are underrepresented. This observation is presently under investigation and may be related to a nucleotide sequence specificity possessed by this antibody.)

Several additional hybridization experiments have been performed to validate the results described above. First, no detectable hybridization occurs when ^{32}P-labeled pBR322 is used as the probe (data not shown). Second, when electrophoretically separated Eco RII cut mouse DNA is transferred to DBM-paper and probed with labeled satellite DNA, hybridization is observed only in the characteristic fragments that correspond to the unit repeats and subrepeats of mouse satellite sequences which have been described by Southern (42) (Fig. 4, lane 3). Finally, when DNA purified from micrococcal nuclease digested mouse nuclei is electrophoretically separated, transferred to DBM-paper, and probed with labeled satellite DNA, hybridization is observed in multiple bands which correspond in length to nucleosomal DNA fragments (Fig. 4, lanes 2 and 4). The results of this experiment confirm the observation described above that satellite chromatin is relatively resistant to digestion by micrococcal nuclease; satellite sequences are enriched in DNA fragments originating from nucleosomes with longer chain lengths (Fig. 4, lane 4).

Nature of the Heterogeneity of Mononucleosomes Carrying Satellite Sequences - We have previously demonstrated that accessory proteins are the primary determinants of the electrophoretic heterogeneity of mononucleosomes derived from bulk chromatin (11,12,17). Therefore, salt depletion and reconstitution experiments were performed to determine if the observed electrophoretic heterogeneity of mononucleosomes carrying satellite sequences is also caused by the association of accessory proteins. Figure 5 shows the electrophoretic profiles of native mononucleosomes (lane 1), of mononucleosomes depleted of accessory proteins by 0.35 M Na^+ ion extraction (lane 2), and of mononucleosomes reconstituted after exposure to 0.6 M Na^+ ion by dialysis to low ionic strength without the removal of released accessory

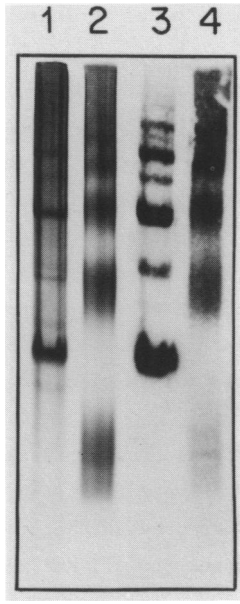

Figure 4. Fidelity of hybridization. (lanes 1 and 2): ethidium bromide stained pattern of Eco RII digested purified mouse nuclear DNA (lane 1) and DNA purified after digestion of mouse cell nuclei with micrococcal nuclease to 11% acid-solubility (lane 2). Samples were separated by electrophoresis (top to bottom) in adjacent lanes of a 5% acrylamide - 0.1% SDS slab gel. (lanes 3 and 4): autoradiogram obtained after electrophoretically transferring denatured DNA from lanes 1 and 2 to DBM-paper and hybridizing the blot with ^{32}P-labeled satellite DNA.

proteins (lane 3). Panels A and B of Figure 5 depict the patterns of bulk DNA for these samples, present in the initial gel and on the resulting blot, respectively. Again, the fluorogram of the blot closely resembles the pattern of ethidium bromide staining of the initial gel, thus attesting to the fidelity of the transfer technique (Fig. 5A,B). In agreement with previous studies (11,12,17), selective depletion of the majority of histone H1 and nonhistone proteins from mononucleosomes results in the conversion of nucleosome classes MIII and MII to MI (Fig. 5A,B, lane 2). The release and subsequent reassociation of these proteins leads to the reconstitution of the electrophoretic pattern exhibited by native nucleosomes, but with a reduction in the relative amounts of nucleosome classes MI and MII (Fig 5A,B, lane 3). Figure 5C shows the distribution of satellite sequences present in these samples. In general, satellite sequences are found in the same mononucleosome classes that carry bulk DNA, except that component MII is enriched in satellite sequences in native nucleosomes (Fig. 5C, lane 1), and this

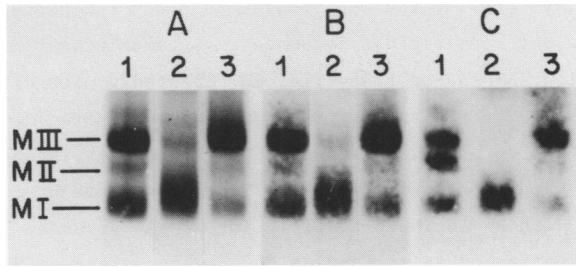

Figure 5. Accessory proteins determine the electrophoretic heterogeneity of nucleosomes carrying satellite sequences. Mono- and polynucleosomes were prepared from ^{3}H-thymidine labeled mouse cells after digestion of nuclear DNA to 12% acid-solubility. A portion of this material was depleted of 0.35 M Na^{+} ion extractable proteins. Another portion of this material was brought to 0.6 M Na^{+} ion and then dialyzed to low ionic strength. Resulting samples were separated by electrophoresis (top to bottom) on the same slab gel and the gel was processed through the transfer procedure. (lanes 1-3): native-, depleted-, and reconstituted mononucleosomes, respectively. (panels A-C): ethidium bromide stained initial gel, fluorogram of the resulting blot, and autoradiogram of the same blot after hybridization with ^{32}P-labeled satellite DNA. Only the mononucleosome region is shown, with MI, MII, and MIII depicting different electrophoretic forms of these nucleoprotein species.

enrichment is lost upon the release and subsequent reassociation of accessory proteins (Fig. 5C, lane 3). In conclusion, the observed electrophoretic heterogeneity of nucleosomes which carry satellite sequences can be explained by the association of accessory proteins with histone octamer:DNA complexes, but cannot be reconstituted by the method used here.

DISCUSSION

The procedure introduced here for transferring DNA from one-dimensional electrophoretic displays of nucleosomes provides the opportunity to analyze the distributions of specific DNA sequences in many different samples simultaneously. Electrophoretic mobility comparisons can be performed and data can be quantitated by densitometry. One-dimensional transfers can also be used as a screening procedure to select samples which exhibit interesting hybridization patterns for two-dimensional electrophoretic analysis. In this way the DNA lengths of specific sequences which are possessed by different electrophoretic forms of nucleosomes may be determined (25).

The electrophoretic mobilities and component DNA fragment sizes are now known for mononucleosome species which possess, in addition to the histone octamer, one H1 molecule, or one or two molecules of either uH2A or HMG-14 and HMG-17 (12,17,19,25). Therefore, DNA transfers from one- and two-dimensional

nucleosome displays provide the opportunity to study the protein compositions of nucleosomes along specific DNA sequences. Because transferred DNA is covalently coupled, blots can be hybridized repeatedly with different radioactive probes. Hybridization patterns can be compared directly to the pattern of bulk DNA on blots, as revealed by fluorography when experiments are performed using samples containing ^{3}H-TdR labeled DNA, or by immuno-autoradiography with an antibody specific for single-stranded DNA when experiments are performed using samples containing unlabeled DNA. In addition, with the appropriate modifications, similar methods could be applied to study the nucleic acid sequences associated with other nucleoprotein complexes, such as the RNA species of ribonucleoprotein particles.

We have illustrated the present technique by using mouse satellite DNA as a hybridization probe. Although this highly repetitive DNA sequence family has been shown previously to be packaged into nucleosomes (43-45), little was known with regard to the properties of the nucleosomes along this sequence. Our results show that satellite chromatin is relatively resistant to micrococcal nuclease digestion (Figs. 3,4). This is in spite of the known dA+dT rich digestion preference of this enzyme on chromatin (36), and the fact that mouse satellite DNA is dA+dT rich (46). The observed resistance of satellite chromatin to nuclease digestion may be due to steric inaccessibility resulting from the higher order packaging of satellite sequences into blocks of heterochromatin in interphase nuclei (47). Another possible explanation is that the specific nucleotide sequences of satellite DNA which have recently been identified to be hypersensitive to cleavage by micrococcal nuclease may not be accessible, even at the level of the primary structure of satellite chromatin, because of the positioning of nucleosomes along these sequences (48).

Our results indicate that mouse satellite sequences are present in the same multiple electrophoretic forms of mononucleosomes found in bulk chromatin. Although the distributions of satellite sequences are quantitatively different from those of bulk DNA in various mononucleosome classes, we have previously shown that certain electrophoretic forms of nucleosomes can be interconverted in precise manners by continued nuclease digestion (11,17). Thus, the mass proportions of mononucleosomes that carry a specific sequence will be determined not only by the protein compositions of the nucleosomes along that sequence, but also by the cleavage preference of micrococcal nuclease for the accessible segments of that sequence.

The results of salt extraction and reconstitution experiments strongly

suggest that the observed electrophoretic heterogeneity of nucleosomes carrying satellite sequences is due to the association of several types of accessory protein molecules (Fig. 5). Heterogeneity in the protein composition of satellite chromatin might be expected since these sequences are present on all autosomic mouse chromosomes (49), and as many as 10^6 nucleosomes per haploid genome could be associated with this highly repetitive DNA sequence family. More specifically, satellite sequences are localized in nucleosome classes which possess as major protein components, histone octamers alone (MI), or histone octamers plus HMG-14 or HMG-17 (MII), or histone octamers plus histone H1 (MIIIA) (Figs. 3,5; see Ref. 17). The finding that satellite sequences are localized in a nucleosome class which possesses HMG-14 or HMG-17 as major accessory proteins is unexpected because these proteins are believed to be associated preferentially with expressed chromatin domains (50), and mouse satellite DNA is thought to be transcriptionally inert (45,51). Levinger et al. (25) have also found that a highly repetitive human DNA sequence is partially localized in a nucleosome class containing HMG-14 or HMG-17. This observation was attributed to non-specific binding of these proteins to nucleosomes in nontranscribed chromatin domains. However, we wish to point out that nucleosomes which package satellite DNA need not possess these HMG proteins because the association of other proteins of similar size with nucleosomes could cause such nucleosomes to migrate coincidently with nucleosomes containing the HMG proteins (17).

In conclusion, the initial application of the procedure reported here has revealed that nucleosomes along mouse satellite DNA are chemically heterogeneous. Clearly, the extent of nucleosome heterogeneity along single copy sequences remains to be determined.

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