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Micrococcal nuclease digestion of nuclei reveals extended nucleosome ladders having anomalous DNA lengths for chromatin assembled on non-replicating plasmids in transfected cells

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ABSTRACT

The chromatin structures of a variety of plasmids and plasmid constructions, transiently transfected into mouse Ltk⁻ cells using the DEAE-dextran procedure, were studied by micrococcal nuclease digestion of nuclei and Southern hybridization. Although regularly arranged nucleosome-like particles clearly were formed on the transfected DNA, the nucleosome ladders, in some cases with 13 – 14 bands, were anomalous. Most often, a ladder of DNA fragments with lengths of approximately 300, 500, 700, 900 bp, etc. was generated. In contrast, typical 180 – 190 bp multiples were generated from bulk cellular or endogenous β-actin gene chromatin. Very similar results were obtained with all DNA's transfected, and in a variety of cell lines, provided that plasmid replication did not occur. Additionally, after digestion of nuclei, about 90% of the chromatin fragments that contained transfected DNA sequences could not be solubilized at low ionic strength, in contrast with bulk cellular chromatin, suggesting association with nuclear structures or nuclear matrix. The remaining 10% of transfected DNA sequences, arising from soluble chromatin fragments, generated a typical nucleosome ladder. These results are consistent with the idea that assembly of atypical chromatin structures might be induced by proximity to elements of the nuclear pore complex or by nuclear compartmentalization.

INTRODUCTION

Although transient transfection assays have been widely used to study gene regulation, little attention has been paid to the chromatin structure of the transfected DNA template. Because chromatin structure can influence gene expression (1–3), it seems important to know whether the transfected DNA is packaged into a typical chromatin structure upon its entry into the cell nucleus. It has been reported, for example, that calcium phosphate transfected DNA is sometimes assembled into non-nucleosomal material of an unknown nature (4, 5). It is known that this method leads to formation of high molecular weight concatamers (6), which apparently facilitates the incorporation into genomic DNA, generally required for stable transfection (6–8). On the other hand, DEAE-dextran transfected DNA remains episomal (9) and has been reported to be efficiently assembled into 'typical' chromatin, essentially the same as the bulk cellular chromatin (10). In the pioneering study (10) upon which the above conclusion is based, however, only 5 or 6 nucleosome oligomer bands at best were obtained from the plasmid chromatin after micrococcal nuclease (MNase) digestion of nuclei, making quantitative analysis of the data difficult. Moreover, repeat lengths were estimated by extrapolation to zero digestion time using only a few time points, a method that is not very reliable. When the more reliable slope method (11) is used to analyze the data that was reported, nucleosome repeat length values of about 170 bp are obtained (our analysis), significantly shorter than that of the bulk cellular chromatin (187 ± 5 bp).

We have transfected cells with a variety of non replicating plasmids using the DEAE-dextran procedure, and have probed MNase digests of nuclei specifically for the transfected DNA. In some cases ladders with 13–14 nucleosome oligomer bands could be resolved. Although regularly arranged nucleosome-like particles were formed on the transfected DNA, the MNase ladders obtained are shown to be anomalous and to arise largely from chromatin fragments that are associated with insoluble nuclear material. Both of these properties have been associated with active chromatin in studies of endogenous genes.

MATERIALS AND METHODS

Cells and transfection

Cells were grown in Dulbecco's modified Eagle's medium (D-MEM; GIBCO) supplemented with 10% fetal calf serum (Hyclone), penicillin and streptomycin (100 units/ml and 100 μg/ml, respectively; GIBCO) at 37°C in an atmosphere of 5% CO_2 saturated with water. Mouse Ltk⁻ (and Cos-1) cells were seeded at about 5×10^5 cells/10 cm culture dish (Falcon) 3 days before transfection. HeLa and CV-1 cells were seeded 2 days before transfection at cell densities of about 2×10^6 cells/dish.

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At the time of transfection, cells were about 70% confluent (except for Cos-1 cells which were about 50% confluent). DNA was introduced into cells by the DEAE-dextran protocol, as has been previously described (12, 13), with minor modifications. Briefly, the culture medium was removed and cells were washed once with warm medium (5 ml/dish) containing no serum. Cells were then overlaid with 4 ml of medium (without serum) containing 200 μg/ml DEAE-dextran (Pharmacia) and, typically, 5 μg DNA. After incubation for 4 hr, the cells were overlaid with 4 ml of 10% DMSO (Sigma) in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4). After 1 or 2 (for L and Cos cells) min at room temperature, cells were washed with 5 ml PBS/dish and overlaid with 10 ml D-MEM containing serum. Times post transfection were measured from the moment the DNA was added.

Nuclei isolation and digestion

Nuclei were isolated 4 days after transfection, at which time supercoiled, nicked, and linear forms of plasmids could be detected. At 2 days after transfection, a continuum of degraded DNA fragments was present (appearing as a background in MNase ladders), which degraded further to small fragments by 4 days. Culture dishes were washed twice with 5 ml each of ice cold buffer I (14), containing 0.34 M sucrose, 15 mM Tris−HCl, pH 7.4, 15 mM KCl, 0.5 mM spermidine-trihydrochloride, 0.15 mM spermine hydrochloride, 0.2 mM Na_2 EDTA, 0.2 mM EGTA, and 0.2 mM phenylmethylsulfonylfluoride, and then scraped into 1 ml of buffer/dish. After adding 1/3 volume of buffer I containing 1% Triton X-100, cells were disrupted in a Dounce homogenizer using 15 strokes of the loose-fitting pestle. Nuclei were sedimented at 3,000 rpm for 5 min using an SS-34 rotor (Sorvall) at 4°C, washed twice with buffer I without detergent, and suspended in 10 mM Tris−HCl, pH 8.0, 1 mM Na_2EDTA, 100 mM NaCl at a concentration of 5−20 A_{260} units (0.1 M NaOH)/ml. For MNase digestion, samples were equilibrated at 37°C for 10 min, adjusted to 2mM $CaCl_2$, 80 units enzyme (Worthington)/28 A_{260} units (0.1M NaOH), and incubated for the time stated with occasional gentle mixing. Reactions were stopped with addition of Na_2EDTA to 10 mM, samples were treated with RNase A and Proteinase K, and DNA was prepared for electrophoresis as previously described (15).

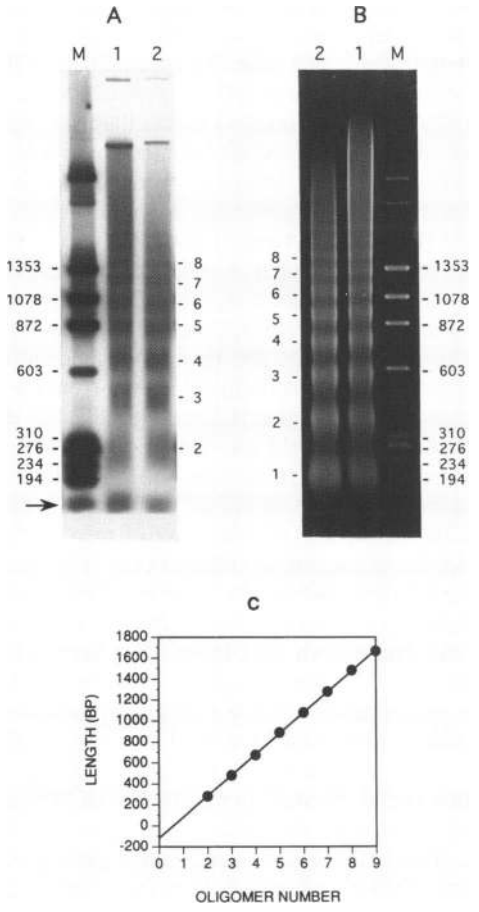

Figure 1. Nucleosome ladders generated by MNase digestion of nuclei from transfected mouse Ltk^- cells. The cells were transfected with pBR327 containing a 1.9 kbp chicken ovalbumin gene PvuII fragment (18). Nuclei were digested for 2 min (lane 1) or 4 min (lane 2). Lane M contained ^{32}P-labeled (plus unlabeled) φX174 RF HaeIII + AccI fragments as size markers; lengths in bp are indicated. (**A**) Southern blot specifically detecting transfected DNA; pBR327 DNA was used as the hybridization probe. The bands are numbered as described in the text. The arrow identifies heterogeneous length DNA < 100 bp in size, most of which ran off the gel. (**B**) Ethidium bromide stained gel, before Southern transfer, detecting bulk cellular DNA. Nucleosome oligomer bands are indicated. (**C**) Plot of DNA fragment length versus nucleosome oligomer number for the ladder in lane 1 (A). The slope of the least squares line through the points is 199 bp, and the y-intercept is −116 bp.

Gel electrophoresis and Southern blotting

Gel electrophoresis was performed as previously described (15). Approximately 10 μg of DNA per slot was loaded. After ethidium bromide staining, gels were photographed under UV illumination using a Kodak no. 9 Wratten filter. DNA was transferred to a nylon membrane (Gene Screen Plus) by the capillary blot procedure, following the manufacturers' recommendations, and hybridization was performed as previously described (15). Autoradiographic exposures (using Kodak XAR5 X-OMAT film) for detection of transfected DNA were at room temperature for a few hrs, without the use of an intensifying screen. To detect the endogenous β-actin gene, exposures were for 20 hr, using a screen.

Chromatin fractionation

Chromatin fractionation was performed by two slightly different methods. Following Rose and Garrard (16), MNase digested nuclei were chilled rapidly and, after 10 min, centrifuged 10 min in a microfuge; the supernatant provided fraction S1. The pellet was suspended (by pipeting) in 2 mM Na_2EDTA, pH 7.4. After 10 min at 0°C, the sample was centrifuged as above, providing supernatant S2, and the pellet P, which was re-suspended in the same volume. In the modified method, designed to increase the yield of soluble chromatin, digested nuclei were adjusted to 10 mM Na_2EDTA and chilled on ice for 10 min. After centrifugation for 3 min, the pellet was suspended using a Dounce homogenizer (pestle B), and incubated at 0°C for 4 hr with constant agitation. After centrifugation, the pellet was re-homogenized and agitated for an additional hr in the same buffer. After centrifugation, the combined supernatants, containing soluble chromatin, were layered onto 5% sucrose in the same buffer and centrifuged 12 hr at 44,000 rpm using an SW51 Ti rotor to concentrate the sample and remove any low molecular weight contaminants.

In vitro mixing experiments

To assess the effects of the presence of DEAE-dextran on MNase digestion of chromatin, high molecular weight chicken erythrocyte chromatin, soluble in 0.1 M NaCl, 10 mM Tris−HCl, pH 8.0, 1mM Na_2EDTA, was prepared as

previously described (17) and portions containing 5 μg DNA each were incubated with 0–100 μg DEAE-dextran for 1 hr at 37°C in the same buffer, $CaCl_2$ was then added to 2mM and samples were incubated 2 min at 37°C with 2 units each MNase. Reactions were stopped with Na_2EDTA to 10 mM, DNA was extracted, and MNase cutting patterns were examined by electrophoresis on a 1.5% agarose gel. At the highest DEAE-dextran concentration, substantial polymer co-purified with the DNA, causing most of the DNA to remain in the gel slot after electrophoresis.

To assess the effect of DEAE-dextran on DNA during electrophoresis, 0, 0.2, 2, or 20 μg of polymer was added to 3 μg portions of purified MNase ladder DNA (obtained from native chromatin) in TBE sample buffer, just before electrophoresis. At the highest polymer concentration, all of the DNA remained in the gel slot after electrophoresis.

To determine whether DEAE-dextran was removed from DNA after incubation at physiological ionic strength with an excess of competitor DNA, 0.5 μg pUC19 DNA was mixed with sufficient polymer (15 min incubation) to cause the complete disappearance of the supercoiled DNA band after electrophoresis. The sample, along with appropriate control samples, was then incubated several hrs at 37°C (in a warm room) in a small tube containing 5 μl of 0.2 M NaCl, 10 mM Tris–HCl, pH 8.0, 0.2 mM Na_2EDTA and a 20-fold excess of a larger plasmid (pBR322 containing a 4 kb insert). The NaCl concentration was then lowered by a 4-fold dilution with sample buffer, and samples were applied directly to a 1% agarose gel. The recovery of the small plasmid supercoiled DNA band intensity, relative to a control sample without DEAE-dextran, provided a measure of the removal of polymer from the small plasmid.

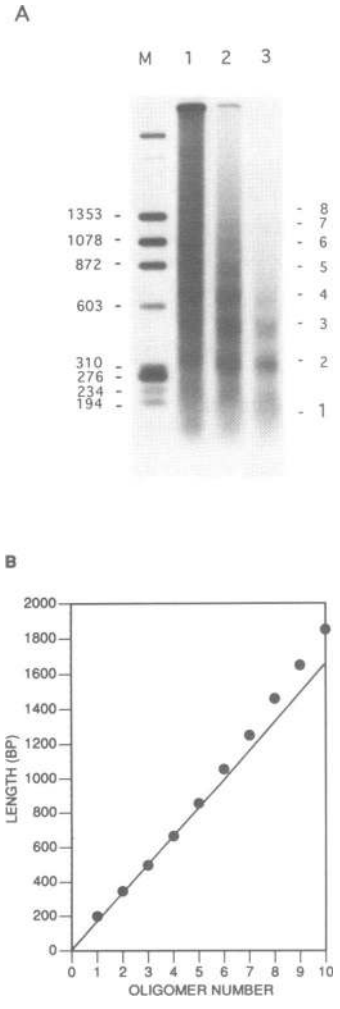

Figure 2. Alternative form of ladder occasionally generated by chromatin assembled from transfected DNA. A repetition of the experiment performed in Fig. 1 is shown. (A) Southern blot specifically detecting transfected DNA. Nuclei were digested with MNase for 2, 4, or 8 min in lanes 1–3, respectively. Lane M contained DNA size markers. Nucleosome oligomer-like bands are numbered. (B) Plot of DNA fragment length versus nucleosome oligomer number for lane 1. The straight line going through some of the points has a slope of 170 bp and a y-intercept of zero.

RESULTS

Nucleosome ladders from transfected DNA are anomalous

We have examined MNase digests from a variety of pBR322 derivatives in transfected mouse Ltk^- cells, probing either with bacterial plasmid sequences or with the eukaryotic DNA inserts. Generally, the same digestion pattern was obtained, shown in Figure 1 A, in a large number of independent experiments. The discrete DNA fragments excised from the plasmid chromatin differ in length from each other by approximately 200 bp, and the ladder extends from about 280 bp to over 2400 bp. The fastest migrating DNA detected (arrow) is less than 100 bp in length, and appears heterogeneous when examined on a polyacrylamide gel. This digestion pattern, although chromatin-like, is anomalous because the DNA lengths generated are not multiples of the unit repeat. The first discrete band at about 280 bp is far too long to be a mononucleosome and is assigned as an unusually short dimer. However, the ladder does not reflect an array of closely packed nucleosomes because the bands are spaced at physiologic 200 bp intervals, not at the 150 bp intervals indicative of close packing. A plot of the DNA fragment lengths from the anomalous ladder is presented in Figure 1C, showing that the data can be described well by a straight line with slope of 199 ± 5 bp and a y-intercept of -116 bp. In contrast, the bulk cellular chromatin ladder (Figure 1B) is well described in the same type of plot by a slope of 187 ± 5 bp and a y-intercept of zero (not shown), as is typical for the chromatin of cultured cells (19). Thus all of the nucleosome oligomer-like bands of the anomalous ladder appear to be shortened by about 100 bp from those of an ordinary ladder (such as the one shown in Figure 1B), accounting for the physiologic slope, but large negative intercept of the plot.

Control experiments (data not included) indicated that the atypical ladders reflect the (unusual) structure of the plasmid chromatin. First, mock-transfected cells did not produce a hybridization signal. Second, in the absence of MNase digestion no discrete bands that ran faster than supercoiled DNA were generated. Third, using a mouse β-actin gene probe, typical ladders were detected on Southern blots (see Figure 5), indicating that the results are not a consequence of the method of detection. Also, normal ladders were in all cases obtained for replicating plasmids in Cos-1 cells using the same transfection protocol (20). Additionally, we found that the same atypical ladders were generated under a wide range of experimental conditions, and for other cell lines. For example, the pattern was insensitive to variations in the cell density at the time of transfection, and over a 10-fold range in the amount of DNA transfected per dish. Also, the pattern was unaffected by the presence or absence of 0.1M NaCl in the MNase digestion buffer or to the temperature during the digestion 20°C instead of 37°C). Finally, essentially the same results were obtained with many different plasmid constructions

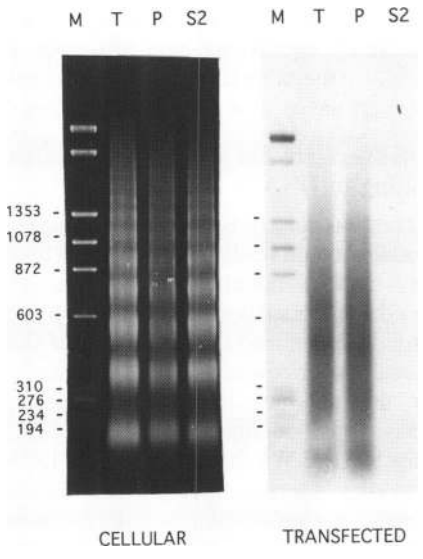

Figure 3. Fractionation of bulk cellular chromatin compared with chromatin assembled from transfected DNA. Mouse Ltk$^-$ cells were transfected with pHSV-106, a 7.76 kbp pBR322 construct containing the HSV thymidine kinase gene (24). Nuclei were digested with MNase for 2 min, and the chromatin was separated into a fraction that was soluble (S2) and a fraction that was insoluble (P) at low ionic strength, as described in the text. Fraction (T) denotes total (unfractionated) chromatin. Each lane contained DNA from an equal volume of sample. Lane M contained DNA size markers. On the left, a photograph of the ethidium bromide stained gel is shown detecting cellular DNA (CELLULAR). On the right, a Southern blot, obtained using pBR322 DNA as a hybridization probe, is shown detecting transfected DNA (TRANSFECTED).

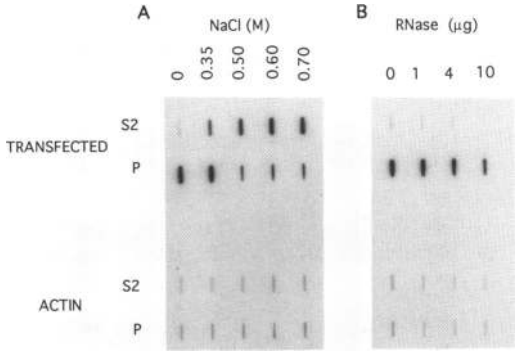

Figure 4. Effect of NaCl concentration or RNase A digestion on the chromatin fractionation. Mouse Ltk$^-$ cells were transfected with pBR327 containing the SV40 genome (5.2 kbp) in the EcoRV site (20). Nuclei were digested with MNase for 1.5 min, then chilled, centrifuged and re-suspended in 2 mM Na_2EDTA, as before. (**A**) Portions of equal volume of the nuclear suspension were adjusted to the NaCl concentrations indicated using small volumes of 5 M NaCl. After gentle agitation for 10 min at 0°C, samples were centrifuged, DNA was extracted from pellet (P) and supernatant (S2) fractions and dissolved in equal volumes of water. Equal sample volumes were applied to a membrane using a slot blot apparatus, and hybridization was performed using the plasmid that was transfected (TRANSFECTED) or using a mouse actin gene probe (ACTIN) as a control for sample recovery. (**B**) Portions (50 μl) of the nuclear suspension (without added NaCl) were incubated 10 min at room temperature with the amount of RNase A indicated, with gentle agitation. S2 and P fractions were blotted and hybridized as in (A).

of widely varying size (from 3 kb to 16 kb), for transfection of linear as well as supercoiled DNA, and for non-replicating plasmids in CV-1, HeLa, and Cos-1 cells, in addition to mouse L cells.

Occasionally, somewhat more typical looking ladders were obtained such as the one shown in Figure 2A. Here, ordinary mono- and dinucleosome bands are present. However, analysis of such ladders (Figure 2B) indicates that they are still anomalous. Whereas, the first 4 or 5 bands can be described reasonably well by a straight line with slope 170 bp and y-intercept of zero, the higher bands systematically deviate from the straight line to a significant extent. Moreover, comparison with Figure 1A (or C) shows that the positions of nucleosome oligomer bands greater than 3-mers are essentially the same in the two experiments. We have not been able to find conditions that reproducibly generate this type of anomalous ladder; usually ladders appearing as in Figure 1 were obtained.

Most of the chromatin assembled from transfected DNA is associated with insoluble nuclear material

In order to investigate the possibility that the atypical chromatin of transfected DNA might be associated with subnuclear compartmentalization, we employed the chromatin fractionation procedure of Garrard and co-workers (16, 21, 22) to MNase-digested nuclei. This technique yields three fractions termed S1, S2, and P, which represent material that is directly solubilized during the digestion, material that is solubilized at low ionic strength in the absence of divalent ions, and material that is insoluble, respectively. It is assumed that the insoluble fraction contains material that is associated with nuclear structures (16, 23).

Figure 3 shows the results of such a fractionation procedure for nuclei from transfected cells. From the ethidium bromide staining of total (cellular) DNA, it can be seen that most of the cellular chromatin was solubilized (compare fraction S2 with P). The exact fraction solubilized was dependent upon the extent of digestion, time of incubation, and the extent of sample agitation. The S1 fraction, which contained only nucleosome monomer and dimer length DNA is not shown. Analysis of these fractions by Southern hybridization using transfected DNA as a probe (transfected) revealed that the vast majority of the chromatin assembled from transfected DNA was insoluble (fraction P), in contrast with the partitioning of bulk cellular chromatin. The higher background signal over the ladder in lane T and, particularly, lane P, compared with Fig. 1, apparently resulted from the manipulations performed here before the digestion reaction was stopped completely. These results suggest that transfected DNA is associated with insoluble nuclear structures.

To investigate how this chromatin might be associated with insoluble nuclear material, we washed the insoluble material with different NaCl concentrations to determine at which salt concentration transfected DNA sequences could be eluted. The insoluble nuclear material remains insoluble at NaCl concentrations in excess of 2.0 M (25). Also,it is known that almost all nonhistone proteins are released from chromatin fragments by 0.35 M NaCl, histone H1 dissociates between 0.50 and 0.60 M NaCl, histones H2A+H2B between 0.60 M and 1.0 M NaCl, and histones H3+H4 at greater than 1.0 M (26). Only very unusual or covalent interactions with DNA would be maintained at 2.0 M NaCl. The results, shown in Figure 4A (transfected), indicate that transfected DNA sequences are released from the insoluble nuclear material between 0.35 and 0.60 M NaCl, the salt concentrations required for the dissociation of tightly bound nonhistone proteins and histone H1 from chromatin. The endogenous β-actin genes which partition roughly

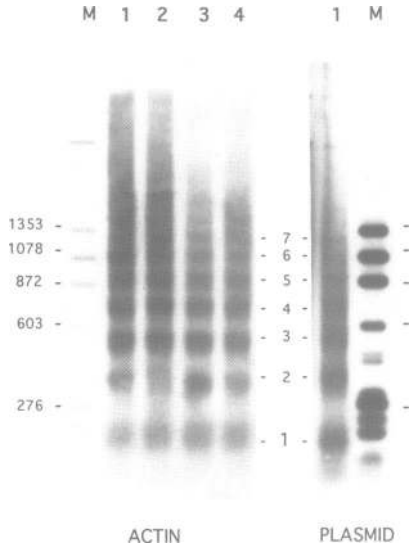

Figure 5. Typical MNase ladders from the chromatin of an endogenous cellular gene and the soluble fraction of chromatin from a transfected plasmid. The small fraction of soluble chromatin fragments was isolated from MNase digested nuclei of mouse Ltk$^-$ cells transfected with pBR327, as described in the text. The Southern blot was probed with plasmid DNA (PLASMID). In a separate experiment (ACTIN) nuclei from transfected cells were digested with MNase for 2 min (lanes 1 and 2) or 4 min (lanes 3 and 4) at 37°C (odd lanes) or 20°C (using 7-fold more enzyme, even lanes). The Southern blot was probed with a 400 bp mouse β-actin gene fragment. Lanes labeled M contained the same ^{32}P-labeled DNA size markers; sizes are indicated on the left.

equally in the S2 and P fractions served as a control for sample recovery in each fraction, and are seen to be present in roughly equal amounts in all fractions (actin).

We next investigated the effect of RNase treatment of the insoluble nuclear material. Nuclear RNA is present in the insoluble material in the form of ribonucleoprotein fibrils, and RNase A digestion has been found to alter nuclear matrix morphology (25). Precipitated ribonucleoprotein could conceivably entrap chromatin fragments, or RNA could be involved in linking chromatin to nuclear structures. We found, however, that RNase A digestion did not result in the release of tansfected DNA sequences (Figure 4B) The decreased hybridization signals in both S2 and P for the highest RNase concentration is also reflected in the actin control, indicating that less nuclei were present in this pipetted volume of the suspension. These results suggest that the insoluble chromatin is not linked through or entrapped by RNA.

The small fraction of soluble chromatin assembled from transfected DNA generates a typical ladder

Using a slight modification of the chromatin fractionation scheme described above (see Materials and Methods), it was possible to obtain enough soluble chromatin fragments containing transfected DNA for analysis. Figure 5 (plasmid) shows that this chromatin generates a typical MNase ladder, which is well described by a 177 ± 5 bp repeat, and is essentially identical to the ladder detected using a β-actin gene probe (actin). This result suggests that the relatively small ($< 10\%$) fraction of chromatin assembled on transfected DNA that is not associated with nuclear structures is packaged in an ordinary fashion. In other experiments, both anomalous and ordinary ladders from transfected DNA in the same cells could be detected (by the same probe) in adjacent lanes of a gel (data not shown).

Anomalous ladders are not a direct consequence of DEAE-dextran binding to DNA

We have performed control experiments to assess whether the anomalous ladders could be directly explained by the binding of the positively charged polymer to DNA or chromatin. For example, could the association of the polymer with chromatin during MNase digestion lead to the excision of DNA fragments with the anomalous lengths observed? Alternatively, could the presence of the polymer in DNA samples prepared for electrophoresis cause a uniform decrease in mobility by approximately 100 bp? We found that adding DEAE-dextran to chromatin in vitro severely inhibited MNase digestion (not shown). In contrast, similar extents of digestion were observed for transfected and cellular DNA from nuclei of transfected cells (Figure 1). Additionally, adding DEAE-dextran to DNA before electrophoresis caused dramatic and non-uniform decreases in electrophoretic mobility, and at relatively low levels of polymer (compared to what is used in a transfection experiment) the DNA failed to enter the gel at all (data not shown). Polymer-coated DNA does not appear to aggregate, however, because it remains completely in the supernatant after a 2 min microfuge spin, even in a buffer containing 0.1 M NaCl. Based upon these experiments, it is very unlikely that the anomalous ladders observed are direct consequences of DEAE-dextran binding to normal chromatin or to the presence of the polymer in samples prepared for electrophoresis.

By a simple gel assay (see Materials and Methods), we have also found that DEAE-dextran is efficiently removed from DNA at physiological ionic conditions (0.2 M NaCl, 37°C) by incubation with a relatively small excess (20-fold by weight) of competitor DNA (data not included). It has been estimated that histones do not become associated with transfected DNA until more than 15 hr after transfection (10). Thus, it is likely that during transfection the polymer is competed off of the transfected DNA by the tremendous excess of concentrated DNA (chromatin) in the cell nucleus before chromatin assembly ensues.

DISCUSSION

Although the mechanism whereby DEAE-dextran facilitates nuclear uptake of DNA, and allows subsequent gene expression, is unknown, the following scenario seems plausible. The positively charged polymer probably coats the DNA, neutralizing much of its charge, but does not cause DNA aggregation. The polymer coating apparently facilitates endocytosis and may also protect nucleic acids from degradation in the cytoplasm (27, 8). The coated DNA probably enters the nucleus through nuclear pores by passive diffusion, the polymer competed off of the transfected DNA by the presence of a vast excess of cellular DNA, and then the DNA becomes associated with histones. This mechanism is consistent with previous ideas and with the experiments reported here.

By carefully examining the chromatin structures of transfected DNA in the nucleus, we have found two unusual and unexpected properties: (i) a chromatin structure that generates an anomalous MNase ladder and (ii) association with nuclear structures. It may

be of significance that both atypical ladders (16, 22, 28, 29) and nuclear matrix (loosely defined) association (16, 22, 30, 31) have been associated with active or potentially active chromatin. These results thus suggest that most of the transfected DNA molecules that enter the nucleus might be transcriptionally competent. The anomalous ladders shown in Figure 1A are, in fact, strikingly similar to those reported for active β-globin chromatin in chicken red blood cells (29). In that study, it was suggested that active β-globin chromatin contained a subset of nucleosomes which were unusually susceptible to exonucleolytic trimming by MNase. Thus, typical nucleosome oligomers would be initially excised by MNase, but then readily processed by removal of about 50 bp from each end, generating the anomalous ladder. The partially anomalous ladders sometimes observed (Figure 2), resemble those previously reported by Cereghini and Yaniv (10) over the range that they were able to resolve discrete bands. Such ladders could reflect the existence of occasional normal nucleosomes interspersed between atypical (active) nucleosomes. The large fraction of possibly transcriptionally competent chromatin generated by transfection cannot be attributed to the presence of *cis*-acting elements in the DNA. Equivalent results were obtained with plasmids that contained or lacked eukaryotic promoters.

Why should both the generation of anomalous (active chromatin-like) ladders and the association with nuclear structures be associated with transfected DNA? An interesting possibility is that these properties are conferred upon chromatin assembled from transfected DNA by its proximity to elements of the nuclear pore complex (NPC), part of the insoluble nuclear material, consistent with the gene gating hypothesis of Blobel (32, 33). The gene gating hypothesis (32) proposes that a subset of active chromatin is associated with (and its structure possibly influenced by) elements of NPCs, in a tissue-specific fashion, such that the active chromatin extends toward the interior of the nucleus into a subjacent nuclear compartment (see also 34, 35). In contrast with the cellular DNA for which only a subset of active genes might be associated with NPCs, all of the transfected DNA is proximal to elements of the NPC as it enters the nucleus through nuclear pores. This mechanism could therefore explain how chromatin assembled from transfected DNA (irrespective of base sequence) might resemble 'nuclear matrix' associated active chromatin. Additionally, our finding that linkage between insoluble nuclear structures and chromatin fragments is disrupted by NaCl concentrations of 0.35−0.60 M, suggests that proteins resembling those of the high mobility group (36), or possibly histone H1, might be associated with the NPC, leading to the properties observed.

Finally, we emphasize that anomalous ladders from DEAE-dextran transfected DNA were observed only for non-replicating plasmids. We have also examined in some detail the chromatin resulting from plasmids that contained the SV40 replication origin and replicated efficiently in Cos-1 cells, using the same transfection protocol. In all cases normal ladders were obtained (20). It is possible, however, that this difference may not depend on replication *per se*, but on a change in nuclear compartmentalization. We find that replicating plasmids partition during chromatin fractionation in the same way as bulk cellular chromatin (data not shown), in contrast with non-replicating plasmids. The solubilization of replicating transfected DNA may just reflect its abundance in the nucleus, being present well in excess of the number of attachment sites on nuclear structures, consistent with the above hypothesis.

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