DIFFERENTIAL CONDENSATION OF MOUSE DNA FAMILIES IN CHROMATIN: ACCESSIBILITY TO NUCLEASE PROBES

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SUMMARY: A number of minor, previously unidentified GC-rich mouse DNA families have been observed in CsCl gradients. Since these DNA families are found in the DNA of mouse nuclei which is most resistant to both micrococcal nuclease and DNAase I, they must occur in highly condensed chromatin. Fractionation of high molecular weight nuclease digested DNA by sequential polyethylene glycol (PEG) precipitation demonstrates differential enrichment of these DNA families implying a differential condensation of these DNA fractions in chromatin.

INTRODUCTION

Much recent evidence indicates that a very large fraction of the DNA of higher organisms occurs in chromatin which is comprised of tandemly repeating nucleosomes (1-5). These DNA fractions include the simple sequence satellite DNAs (6-8), known to be located within regions of condensed heterochromatin defined by cytological techniques. Some of the most powerful experimental evidence for the nucleosome model of chromatin structure comes from studies of the digestion of intact nuclei or minimally sheared chromatin by DNAase I or micrococcal nuclease (1,2,6-9).

Nuclease digestion studies are capable of yielding valuable information concerning relative DNA accessibility at the nucleosome level of organization in chromatin. This study demonstrates qualitatively the differential condensation of a number of minor previously unidentified mouse DNA families in chromatin using both micrococcal nuclease and DNAase I.

MATERIALS AND METHODS

Nuclei were prepared from monolayer or spinner cultures of mouse L-cells labeled for 20 hours with 1 μCi/ml [^{3}H]-methylthymidine. The homogenization buffer was 10mM NaCl, 1.5mM $MgCl_2$, and 10mM Tris pH 7.0. The nuclei were

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purified by centrifugation through 70% w/v sucrose in homogenization buffer, washed, treated with DNAase I (EC 3.1.4.5.) of 2900 U/mg or micrococcal nuclease (EC 3.1.4.7.) of 19,000 U/mg at 37°C for three minutes and the reaction terminated with 20mM EDTA at 0°C. Prior to digestion with micrococcal nuclease the suspension of nuclei (4 x 10^6/cm^3) was made 1mM $CaCl_2$. SDS and NaCl were added to 1% and 1M respectively. Incubation of the suspension at 37°C for 30 minutes encouraged complete nuclear lysis. The suspension was made 20 µg/ml RNAase A (EC 2.7.7.16.), incubated for one hour at 37°C whereupon Proteinase K was added to 500 µg/ml; incubation was continued for four hours at 37°C. DNA was purified by phenol extraction and was subsequently dialyzed against electrophoresis buffer: 5mM Na acetate, 1mM EDTA and 40mM Tris pH 7.8.

Polyethylene glycol (PEG) fractionation was performed at the indicated PEG concentrations by incubation for 20 hours at 0°C in electrophoresis buffer (10). The precipitated DNA was pelleted by centrifugation at 4300 x g for 30 minutes. Sequential fractionation was performed by addition of the necessary weight of solid PEG to the previous supernatant to obtain the new PEG concentration.

DNA, in electrophoresis buffer, was electrophoresed at room temperature in 7 cm cylindrical 1% agarose gels (Seakem agarose) at 3 V/cm for the indicated times. Gels were stained uniformly during the electrophoresis due to the presence of 0.25 µg/ml ethidium bromide in the agarose gels and buffer trays. The gels were removed from the glass cylinders and the DNA visualized by ethidium bromide fluorescence. Excitation illumination was provided by a BLAK-RAY UVL-21 mineral lamp (Ultraviolet Products, Inc.). The gels were photographed with Polaroid Type 55 film through a Wratten 23A gelatin filter using a Polaroid MP-3 Land Camera.

DNA was recovered from gel slices dissolved at 50°C in an equal volume of 5M $NaClO_4$. Ethidium bromide was removed by two extractions with n-butanol. Hydroxyapatite (HAP) was added and the agarose was washed from the bound DNA at 60°C with 0.12M phosphate buffer pH 7.2. The DNA was eluted in 0.45M phosphate buffer.

Analytical ultracentrifugation of DNA in CsCl gradients was performed in an ANF rotor at 44,000 rpm 25°C for 24 hours in the Beckman Model E Analytical Ultracentrifuge. Ultraviolet photographs were taken and traced on a Joyce-Loebl Recording Microdensitometer.

Preparative ultracentrifugation of ^{3}H-labeled DNA in CsCl gradients was performed with human or mouse marker DNA (ρ = 1.700 g/cm^3) and *M. lysodeikticus* marker DNA (ρ = 1.731 g/cm^3). The equilibrium gradients at 30,000 rpm, 25°C in either the 8 x 40 rotor (MSE) or Beckman 60 Ti (L2-65B) were fractionated from the top with a fraction collector and peristaltic pump. Absorbance readings of each fraction were determined either at 260 nm in a Beckman DB Spectrophotometer or by continuous flow recording through an ISCO U-4. Aliquots were counted in Aquasol. Background radioactivity (20 cpm) was subtracted from each fraction.

RESULTS

^{3}H-methyl-thymidine labeled mouse L-cell nuclei were digested with 500 U/ml micrococcal nuclease or 200 U/ml DNAase I and the purified DNAs electrophoresed on 1% agarose gels producing the ethidium bromide fluorescence patterns in Figures 1a and b. Most of the DNA in either gel is digested down to between the core and nucleosome monomer DNA size, about 170 base pairs (unpublished calibration), with some additional Figure 1a micrococcal nuclease

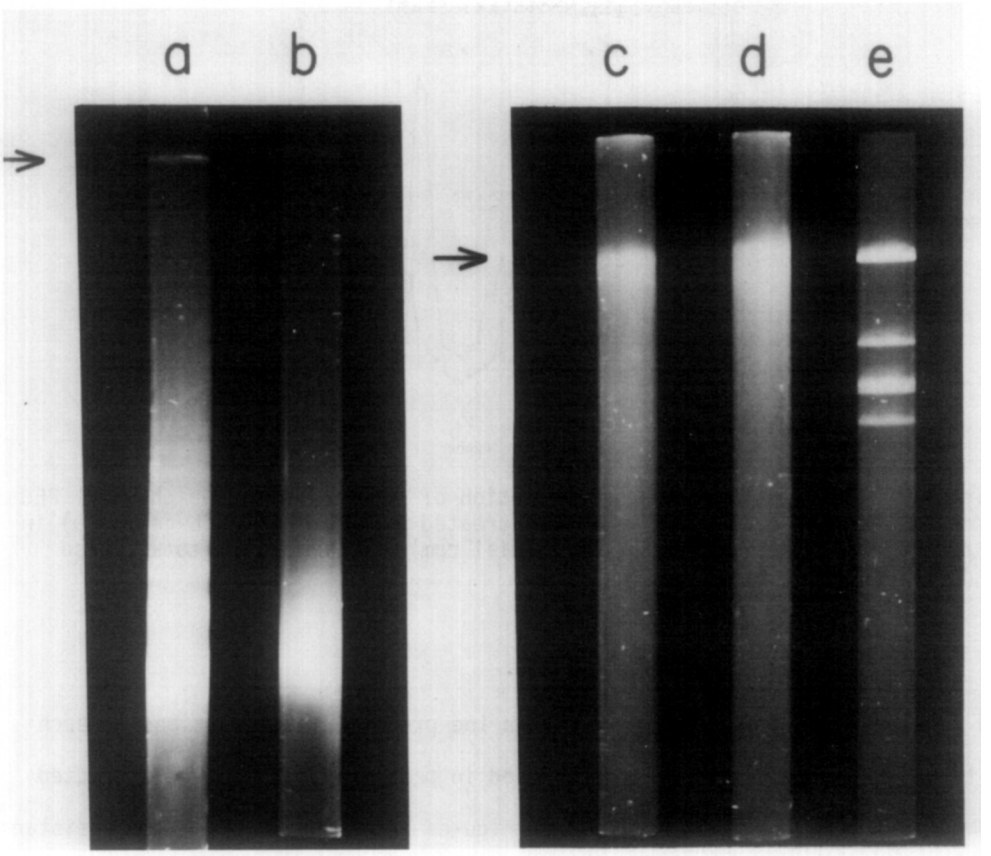

Figure 1. Mouse DNA electrophoresed for the indicated times in 1% agarose gels following nuclease digestion of mouse L-cell nuclei.
a) 500 U/ml micrococcal nuclease - 3 hour run.
b) 200 U/ml DNAase I - 3 hour run.
c) 5.0% PEG precipitated DNA (0.36% of initial cpm) from micrococcal nuclease treated nuclei - 7 hour run.
d) 5.1% PEG precipitated DNA (0.67% of initial cpm) from DNAase I treated nuclei - 7 hour run.
e) Eco RI restricted bacteriophage λ DNA - 7 hour run.

digested DNA at the higher integer nucleosome sizes. At the position of undigested DNA (arrows) in both Figures 1a and b is a small sharp DNA band which originates respectively from the chromatin most resistant to micrococcal nuclease and DNAase I.

To enrich for the high molecular weight more resistant DNA fractions, polyethylene glycol (PEG) was used as a selective precipitant (10). When the Figure 1a and b DNA distributions were treated with 5% PEG, 0.36% and 0.67% of

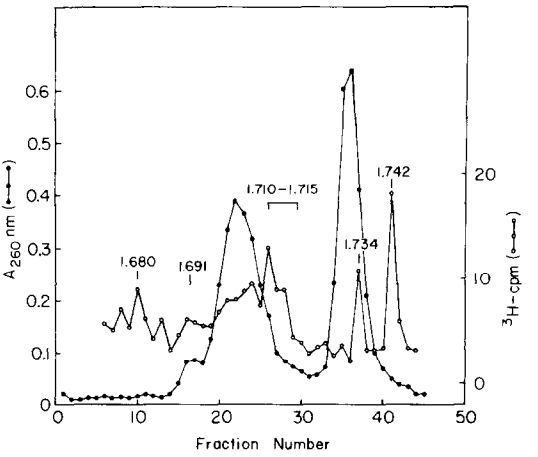

Figure 2. Preparative ultracentrifugation of high molecular weight 5.0% PEG precipitate from micrococcal nuclease treated nuclei of mouse L-cells (O) in a neutral CsCl gradient (0.36% of initial cpm); (●) unlabeled total mouse and M. lysodeikticus DNA.

the respective initial cpm were found in the precipitates. Portions of each of these PEG precipitates electrophoresed in parallel with Eco RI restricted λ DNA marker are shown respectively in Figures 1c, d and e. The very resistant DNA, almost 100-fold enriched, will be seen in both 1c and d to have a molecular weight well above the 13 x 10^6 d of the largest Eco RI restricted λ DNA fragment (11).

The PEG enriched micrococcal nuclease-resistant DNA banded in the Figure 2 preparative CsCl gradient is comprised of a major GC-rich DNA fraction in the 1.710-1.715 g/cm^3 buoyant density range. Two sharp DNA peaks appear reproducibly at densities of about 1.735 and 1.742 g/cm^3. In addition, the mouse satellite DNA region shows about a two-fold enrichment relative to unfractionated DNA in agreement with other studies (Marx and Jones, in preparation). The radioactivity at ρ = 1.680 g/cm^3 represents either a previously unreported mouse DNA fraction or an artifactual association of DNA with polyethylene glycol.

Differential condensation of various chromatin DNA families can be demonstrated by CsCl density gradient centrifugation of sequential PEG precipitated

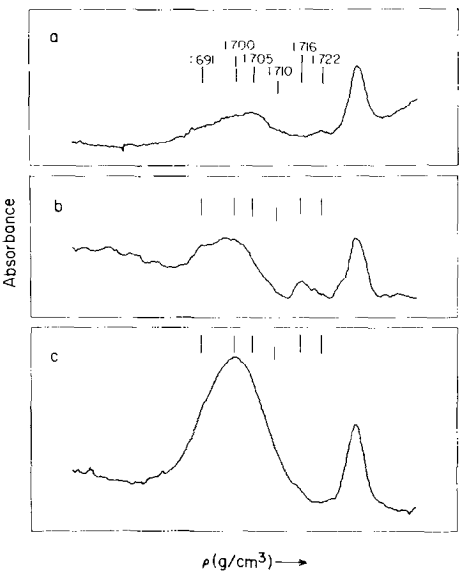

Figure 3. Analytical ultracentrifugation of sequential PEG precipitated mouse DNA from micrococcal nuclease treated nuclei of mouse L-cells in a neutral CsCl gradient. M. lysodeikticus marker DNA is at far right.
a) 5.0% PEG precipitated DNA (0.58% of initial cpm).
b) 5.15% PEG precipitated DNA (0.56% of initial cpm).
c) 5.3% PEG precipitated DNA (0.58% of initial cpm).

DNA fractions from micrococcal nuclease treated mouse L-cell nuclei (Figure 3). That the sequential PEG precipitated DNA fractions decrease in molecular weight can be shown by gel electrophoresis with marker DNA (Marx and Jones, in preparation). The mouse satellite DNA (ρ = 1.691 g/cm^3) in Figure 3b is enriched about 2-3 fold over that in total mouse DNA. However, in Figure 3a, it does not appear to be similarly enriched due to its dilution by other DNA families occurring in more condensed chromatin.* Figure 3a suggests that the 1.722 g/cm^3 DNA fraction occurs in more condensed chromatin than either the mouse satellite DNA or 1.716 g/cm^3 DNA enriched in Figure 3b. The 1.735 g/cm^3 and 1.742 g/cm^3 DNAs of Figure 2 are not seen in Figure 3, perhaps obscured by

*This interpretation is supported by the fluorographic distribution of mouse satellite cRNA hybridized to millipore strip immobilized DNA, from nuclease digested mouse liver nuclei, electrophoresed into 1% agarose gels before being transferred (Marx and Jones, in preparation).

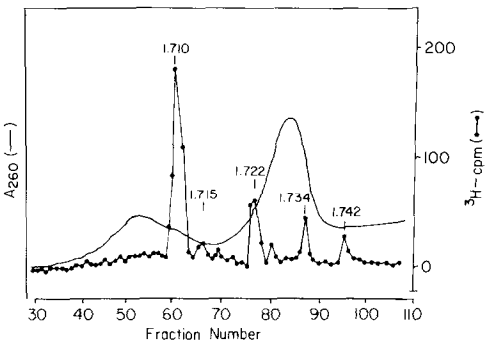

Figure 4. Preparative ultracentrifugation of the high molecular weight fraction of 5.1% PEG precipitated DNA from DNAase I treated nuclei of mouse L-cells (●) in a neutral CsCl gradient (0.05% of initial cpm); (—) unlabeled total human and M. lysodeikticus DNA.

the M. lysodeikticus marker DNA. The 1.680 g/cm^3 band is absent as well, further suggesting its possibly artifactual origin. The main band DNA peaks at 1.705 g/cm^3, which is greater than the 1.700 g/cm^3 unfractionated mouse DNA density and consistent with the known AT base digestion preference of this enzyme (Marx and Jones, in preparation; 12).

To avoid ambiguity associated with the known base digestion preference of micrococcal nuclease, DNAase I treated chromatin DNA was examined. PEG enriched DNAase I treated chromatin DNA was electrophoresed in a 1% agarose gel, the very high molecular weight edge of the resistant band region was sliced from the gel and the DNA isolated by $NaClO_4$-HAP chromatography. This very high molecular weight DNA was then centrifuged to equilibrium in a neutral CsCl gradient in Figure 4. The major component of this nuclease-resistant chromatin DNA, some 60%, is the 1.710 g/cm^3 DNA. The remainder occurs in discrete peaks at: 1.715 g/cm^3 (5%), 1.722 g/cm^3 (20%), 1.735 g/cm^3 (8%), and 1.742 g/cm^3 (6%). All of these DNAase I-resistant chromatin DNAs were found enriched as well in the Figures 2 and 3 PEG enriched micrococcal nuclease-resistant chromatin DNA. Therefore, the nuclease-resistance of these GC-rich DNA fractions is primarily a consequence of their condensed nucleoprotein organization, rather than their particular sequence properties.

DISCUSSION

Following digestion of mouse chromatin DNA in intact nuclei by both micrococcal nuclease and DNAase I, a number of minor primarily GC-rich mouse DNA fractions are found enriched in the high molecular weight DNA, representing presumptive, highly condensed chromatin fractions. These mouse DNA fractions, with the exception of mouse satellite DNA, have heretofore not been described in the literature. The 1.722 g/cm^3 DNA may be mouse ribosomal DNA since mammalian ribosomal DNAs are known to band in this density range (13) and to be located in regions of cytologically defined constitutive heterochromatin (14,15). Based upon the differential enrichment of the minor mouse DNA fractions in sequential PEG precipitates from total DNA of nuclease digested nuclei (Figures 2-4), the following order has been assigned to qualitatively describe the relative degree of condensation of these chromatin DNA families: 1.710 > 1.722; 1.735; 1.742 > 1.716; 1.691 (mouse satellite) g/cm^3.

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