THE SUBUNIT STRUCTURE OF MOUSE SATELLITE CHROMATIN

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SUMMARY: Brief digestion of mouse liver nuclei with micrococcal nuclease cuts both the satellite DNA and the bulk DNA into a series of fragments which are multiples of approximately 170 base pairs. Similar results are obtained whether the nuclei are fixed with formaldehyde prior to digestion or not. When deproteinized, radioactive satellite DNA is mixed with a large excess of unlabeled nuclei, micrococcal nuclease rapidly degrades the labeled DNA with no formation of discrete fragment sizes. These results suggest that mouse satellite, a heterochromatic simple sequence DNA, is complexed with histones in the same subunit structure as is the bulk of the DNA.

In eukaryotic nuclei the bulk of the DNA is associated with histones to form a characteristic subunit structure resembling beads on a string (1,2). A major support for this conclusion is the observation that brief digestion of nuclei with micrococcal nuclease can convert up to 85% of the DNA into an oligomeric series of fragments (3). It is of interest to know whether there are any specialized DNA sequences which are exempt from this general histone-generated subunit structure or whether all DNA is organized in this manner regardless of its function. In this report we show that the heterochromatic, nontranscribed satellite DNA of mouse liver is organized in subunit structure.

METHODS

Isolation and digestion of nuclei - Nuclei were isolated from the livers of fasted mice by the procedure of Blobel and Potter (4). Isolated nuclei were suspended in 0.32 M sucrose, 10 mM NaCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Tris.HCl, pH 8 at a DNA concentration of 1 mg/ml to which micrococcal nuclease were added at 8 μg/ml. Digestion was at 37°. At various time intervals reactions were stopped by addition of EDTA, and DNA was purified by pronase digestion in the presence of 0.5% Na dodecyl sulfate followed by phenol extraction. DNA was concentrated by ethanol precipitation prior to electrophoresis.

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Abbreviation: EDTA, ethylenediaminetetraacetate.

In some experiments freshly isolated nuclei were immediately fixed with 2% formaldehyde for 30 min in the cold as described by Chalkley and Hunter (5). The fixed nuclei were then centrifuged through a cushion of 2 M sucrose to remove excess formaldehyde and immediately digested with micrococcal nuclease under conditions identical to those used for unfixed nuclei. Digested nuclei were then subjected to overnight treatment at 37° with 1 mg/ml pronase in 0.5% sodium dodecyl sulfate followed by an overnight phenol extraction. In other experiments with labeled nuclei this procedure yields at least a 90% recovery of DNA into the aqueuous layer. The DNA fragments were then electrophoresed and hybridized as usual.

Electrophoresis of DNA and RNA-DNA hybridization - For analytical purposes DNA fragments were run on 4% acrylamide slab gels cross-linked with bisacrylamide and the DNA was stained with ethidium bromide. For hybridization purposes DNA was run on 6% acrylamide tube gels cross-linked with ethylene diacrylate, an alkali-labile cross-linker (6). Tube gels also contained 10% glycerol to allow freezing in ethanol-dry ice. Frozen gels were sliced transversely and each slice was hydrolyzed with alkali to liberate and denature the DNA. Additions were then made to each tube to neutralize the solution, raise the salt to 0.6 M NaCl, 0.2 M Tris pH 8 and 20 mM EDTA and introduce radioactive RNA in a final volume of 200 µl.

After incubation at 70° for 10 min reactions were stopped by chilling on ice and adding 0.8 ml of RNase (50 µg/ml pancreatic RNase plus 50 units/ml T_1 RNase in water). Following RNase digestion for 10 min at 37° reactions were chilled and 2 ml of high salt (3 M NaCl, 10 mM $MgCl_2$, 0.5 M Tris pH 8) was added. Hybrids were trapped on nitrocellulose filters (Millipore HAWP 0.45 µm pore size, 2.2 cm diameter) by slow filtration followed by a 2 ml rinse with the high salt mixture.

This procedure for assaying hybrids yields lower backgrounds than does acid precipitation but results in the partial loss of some low molecular weight material. From control experiments (not shown) we estimate that about 50% of hybrids in the 170 base pair range are lost while larger hybrids are trapped with efficiencies increasing with fragment size to nearly 100%.

Isolation of mouse satellite DNA and synthesis of RNA - Unlabeled mouse DNA was isolated from mouse liver. Radioactive mouse DNA was prepared by labeling a mouse lymphoma cell line (provided by Dr. K. Ozato) with ^3H thymidine. In both cases the DNA was purified by standard pronase-phenol methods. The AT-rich mouse satellite was isolated in preparative Ag^{++}-Cs_2SO_4 density gradients and the L-strand was isolated in alkaline CsCl gradients (7). Complementary RNA was transcribed from the L-strand using Escherichia coli RNA polymerase (8).

Analytical CsCl gradients showed that the satellite was 92% pure after the Ag^{++}-Cs_2SO_4 density gradient step. Analaytical centrifugation of the isolated L-strand showed no detectable contamination (less than 5%). Since E. coli RNA polymerase preferentially transcribes the AT-rich satellite (8) any contamination of the RNA with sequences complimentary to the bulk DNA should be negligible.

RESULTS

Nuclei were isolated from mouse liver under isotonic conditions and then digested with micrococcal nuclease for various lengths of time. The DNA fragments were purified, concentrated by ethanol precipitation, and aliquots were electrophoresed on 4% acrylamide gels. The results, shown in Figure 1, are in general agreement with those published for nuclei

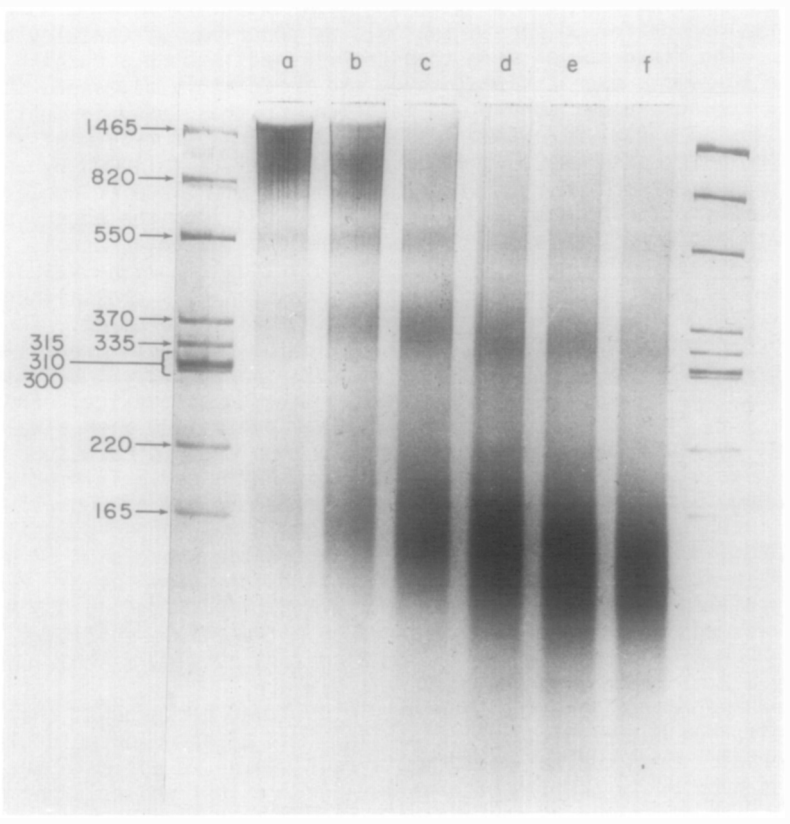

Fig. 1. Digestion of Mouse Liver Nuclei with Micrococcal Nuclease. Mouse liver nuclei (DNA concentration/mg/ml) were digested with micrococcal nuclease (8 μg/ml) at 37° for varying lengths of time. DNA was purified and electrophoresed on a 4% acrylamide gel as described in Methods. Restriction endonuclease HaeIII fragments of Simian Virus 40 DNA were run on either side of the slab as markers (13). DNA sizes are given in base pairs. Nuclei were digested for a) 0.5, b) 2.5, c) 5, d) 10, e) 15, f) 20 minutes.

from a variety of sources. There is an apparent monomer band with a size of 170 base pairs followed by successive bands of dimers, trimers, tetramers, etc. Even with the most brief digestion we have never obtained a monomer larger than about 170 base pairs. This was true for hypotonically isolated nuclei from Xenopus cultured cells (Reeder and Higashinakagawa, unpublished data) as well as the isotonically isolated mouse liver nuclei shown in Figure 1.

In order to locate mouse satellite sequences amont the bulk DNA fragments, ^{3}H-labeled RNA complementary to purified mouse satellite

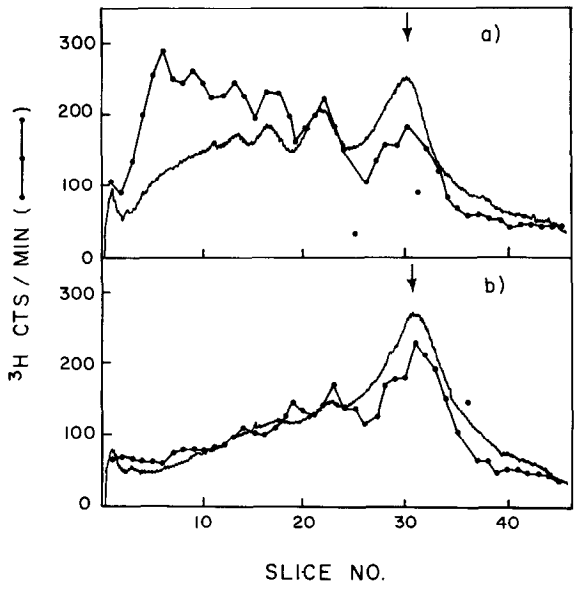

Fig. 2. Hybridization of Mouse Satellite RNA to DNA Fragments from Unfixed Nuclei. Approximately 0.3 μg of DNA fragments from the experiment shown in Figure 1 were electrophoresed on a 6% acrylamide tube gel, the gel was sliced and the DNA in each slice was hybridized with ^{3}H-labeled RNA transcribed from mouse satellite L-strand (5 pg, 4500 cts/min). The distribution of bulk DNA fragments was visualized by running 10 μg of digest in a parallel gel and staining with ethidium bromide followed by tracing a photographic negative with a densitometer. a) Fragments from nuclei digested for 5 min, b) Fragments from nuclei digested for 20 min. The arrow indicates the position of the presumed monomer at approximately 170 base pairs.

L-strand was hybridized to DNA fragments which had been electrophoretically fractionated. The results of such an experiment are shown in Figure 2. At two different levels of digestion the hybridization of satellite L-strand follows the distribution of the bulk DNA. This indicates that both satellite and bulk DNA sequences have the same general subunit structure.

Since the nuclease digestions were done under conditions where histone migration along the DNA might possibly occur, we repeated the experiment shown in Figure 2 using nuclei which had been previously fixed with formaldehyde to prevent any protein rearrangement. The results, shown in Figure 3, are essentially identical with those obtained with unfixed nuclei. Therefore, we conclude that the observed subunit organization of mouse satellite DNA

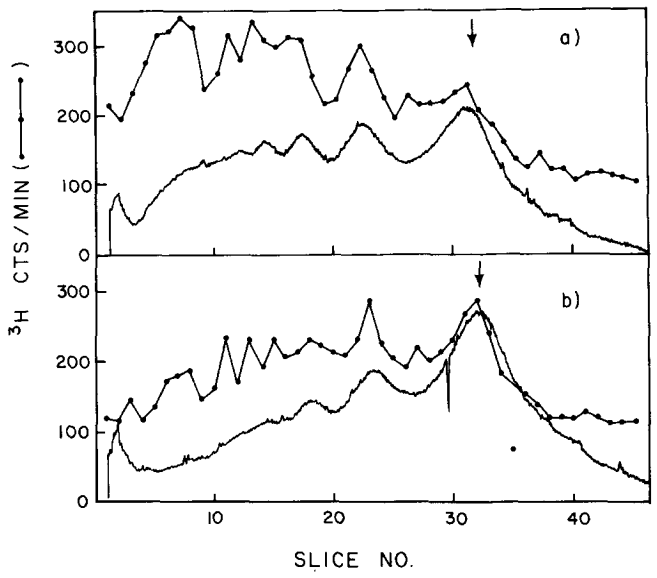

Fig. 3. Hybridization of Mouse Satellite RNA to DNA Fragments from Formaldehyde-Fixed Nuclei. The experiment shown in Figure 2 was repeated exactly except that the nuclei were fixed with formaldehyde prior to nuclease digestion. a) Digestion for 5 min, b) Digestion for 20 min. The arrow indicates the position of the presumed monomer at approximately 170 base pairs.

probably reflects the true *in vivo* situation and is not a result of artifactual rearrangement during nuclease digestion. As a further control radioactively labeled mouse satellite DNA was mixed with a large excess of unlabeled nuclei and the mixture was digested with micrococcal nuclease exactly as described in the legends to Figures 2 and 3. As shown in Figure 4, under these conditions the labeled DNA is rapidly degraded into small pieces while the bulk unlabeled DNA appears in the usual series of distinct peaks. We conclude that satellite DNA by itself is not digested into subunit fragments nor can histones exchange onto it to afford such protection under the conditions of our experiment.

DISCUSSION

In the hybridization reactions shown here the conditions of DNA and RNA concentration and of time were adjusted so that doubling DNA amount caused a doubling of the hybrid formed. This assured a more accurate

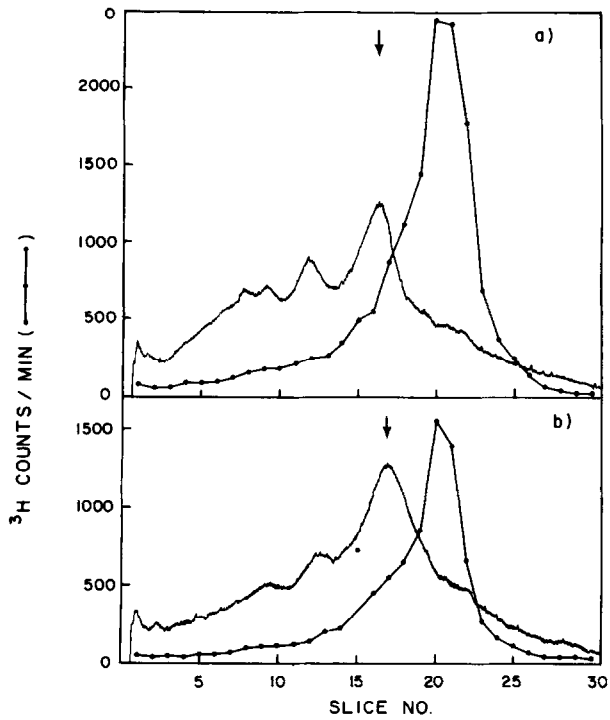

Fig. 4. Co-digestion of Purified ^{3}H-labeled Mouse Satellite DNA and Unlabeled Nuclei. Deproteinized ^{3}H-labeled mouse satellite DNA (14 µg, 250,000 cts/min) was mixed with unlabeled mouse liver nuclei (1 mg DNA) in 1 ml and the mixture was digested with micrococcal nuclease (8 µg/ml) for various periods of time. The DNA was then purified and run on 6% acrylamide tube gels. Each gel was stained with ethidium bromide and photographed to locate the bulk DNA. The gel was then sliced. Each slice was hydrolysed with alkali and the acid precipitable radioactivity in each slice was determined. a) 5 min of digestion, b) 20 min of digestion.

assessment of the distribution of satellite sequences across a gel. However, under such conditions only about 5-10% of the input RNA ended up in hybrid. Due to the purity of the RNA (see Methods) and the fact that mouse satellite comprises 10% of the genome and is the most rapidly reannealing fraction (7,9) we feel confident that the hybrids we detect are due to mouse satellite sequences and not some other part of the genome.

In mouse liver the major AT-rich satellite DNA is located in condensed, centrometric heterochromatin and by all tests applied so far is non-transcribed (8,10,11). The results in this paper demonstrate that this hetero-

chromatic material appears to have the same subunit structure as does the bulk of the DNA. Presumably this means that it is complexed with at least four major histones (F2a2, F3, F2b, and F2a1) (2).

Lacey and Axel (12) have recently shown that DNA from isolated nucleosome monomers contains sequences complementary to the entire genome including transcribed sequences. Mathis and Gorovsky (personal communication) have found that the ribosomal genes of Tetrahymena are arranged in subunit structure. This agrees with our own data (Reeder and Higashinakagawa, unpublished) that both the transcribed gene and the non-transcribed spacer regions of Xenopus ribosomal genes yields subunit fragments upon nuclease digestion. These results together with the present report raise the possibility that at least the four major histones are associated with all DNA sequences in the eukaryotic nucleus whether transcriptionally active or not.

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