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Different nucleosome spacing in transcribed and non-transcribed regions of the ribosomal RNA gene in *Tetrahymena thermophila*

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ABSTRACT

The chromatin structure of the palindromic macronuclear ribosomal RNA genes of Tetrahymena thermophila was probed with micrococcal nuclease. Independent of the state of transcriptional activity, the transcribed region had a shorter nucleosome repeat (184±3 base pairs) than the non-transcribed central spacer or bulk chromatin (both 200 base pairs). The transcribed region displayed an increased sensitivity to micrococcal nuclease in rapidly growing cells, which suggested an altered chromatin structure during transcription. At early stages of nuclease digestion, the central spacer appeared to be in a highly structured nucleosomal array. Based on the differences in nucleosome repeat distance and sensitivity to nuclease, we conclude that quite different chromatin structures are maintained in two adjacent regions of the Tetrahymena ribosomal RNA gene. The DNA of the non-transcribed terminal spacer was found to contain sequences which are highly susceptible to micrococcal nuclease, precluding any conclusions about nucleosome structure in this region.

INTRODUCTION

The nucleosome is the fundamental structural unit of chromatin.[1] Many transcriptionally active genes appear to be organized into nucleosome arrays similar to those of inactive chromatin, as judged by micrococcal nuclease digestion[2] or by electron microscopy (reviewed in ref. 3). Some active genes show a different pattern. When the heat shock genes of Drosophila are active, the pattern of fragments produced by micrococcal nuclease digestion changes from a regular nucleosome ladder to a much more diffuse smear.[4] The active rRNA genes of Physarum polycephalum are cleaved by micrococcal nuclease into a heterogeneous distribution of fragments that contrast with the 170-180 bp repeat characteristic of the bulk chromatin.[5]

The rDNA (rRNA gene) of Tetrahymena is a good system with which to further explore the structural differences between transcribed and non-transcribed chromatin. Each rDNA molecule is an extrachromosomal, palindromic dimer[6,7] containing transcribed regions adjacent to non-transcribed central and terminal spacers (Figure 1A).[8,9,10] The gene can be

obtained in a state of high transcriptional activity (~20 pre-rRNA transcripts/gene/min) or in a state of inactivity induced by starvation, where the amount of transcription is reduced 20 to 60-fold.[11,12,13]

In early micrococcal nuclease digestion studies with Tetrahymena pyriformis, the rDNA as a whole was found to have a nucleosomal repeat indistinguishable from that of bulk chromatin.[14,15] Subsequent studies revealed that different regions of the rDNA have different structures. The transcribed region of T. thermophila rDNA is much more accessible to psoralen crosslinking in vivo than are the adjacent non-transcribed spacers.[16] Neither the central spacer nor the gene region appear nucleosomal when probed by psoralen crosslinking.[16] The repeated C_4A_2 sequences at the very ends of the rDNA are packaged into a non-nucleosomal chromatin structure as judged by micrococcal nuclease digestion.[17] Recently Borchsenius et al. found that at low levels of micrococcal nuclease digestion the gene region of T. pyriformis rDNA appears non-nucleosomal, while the terminal spacer region has a regular nucleosomal pattern.[18]

In the work presented here we have investigated the chromatin structure of the central and terminal spacers and the gene region of T. thermophila rChromatin using micrococcal nuclease digestion. The structure of each region of the rChromatin in rapidly growing cells was compared to that present in starved cells to identify any changes in chromatin structure that are related to transcription. We find that the gene and spacer regions have different nucleosome repeat lengths, and that the gene region has a much different accessibility to the nuclease in the state of high transcriptional activity.

MATERIALS AND METHODS

Growth and Starvation of Cells

Tetrahymena thermophila strain B VII were grown as described by Zaug and Cech.[19] One liter cultures were grown at 30°C to a density of 0.7-1.2 x 10^5 cells/ml. The generation time was 2.3-2.5 hours. The cells were labeled by adding 150 µCi of [^{3}H]thymidine per liter for the entire growth period. Cells to be starved were grown to the density range stated above and then treated as described by Cech and Brehm,[20] with the following exceptions: cells from two 1 liter cultures were combined for the starvation in 1 liter of 50 mM Tris buffer (pH 7.5), and the temperature was 30°C. After 16 hours of starvation, the cells were pelleted and resuspended in fresh 50 mM Tris, then incubated for an additional hour.

Preparation of Nuclei

Nuclei from both log-phase and starved cells were isolated by a modification of the NP-40 detergent procedure of Higashinakagawa et al.[21] as described by Zaug and Cech,[19] except that aurintricarboxylic acid was omitted from the TMS [0.01 M Tris-HCl (pH 7.5), 0.01 M $MgCl_2$, 0.003 M $CaCl_2$, 0.25 M sucrose]. The nuclei from two 1 liter cultures were combined and resuspended in TMS to give a final concentration of 3-5 x 10^7 macronuclei per ml.

Digestion of Nuclei with Micrococcal Nuclease

The nuclei resuspended in TMS were split into six 250 µl aliquots (each containing 100-200 µg DNA) and digested with micrococcal nuclease (E.C. 3.1.4.7, Sigma #N-3744, 250 U/mg) at concentrations of 0, 0.25, 0.5, or 1.0 U/ml for 15 or 30 seconds at 37°C. To obtain a series of samples at more complete digestion, nuclei were incubated for 1, 2, 4, 8, or 16 min with 1.0 U/ml. The reactions were stopped by the addition of EDTA to 50 mM and SDS to 0.2%. Proteinase K was added to 0.30 mg/ml, and the lysed nuclei were incubated at 37°C for 2-4 hours. Quadruplicate 5% aliquots were removed from each sample to determine the amount of TCA solubility. The rest of each sample was diluted with an equal volume of TE buffer [0.01 M Tris (pH 8), 0.001 M EDTA] and then extracted once with phenol containing 0.1% 8-hydroxyquinoline and twice with chloroform-isoamyl alcohol (24:1). The nucleic acids were precipitated with 2 vol. of ice cold ethanol (overnight, -20°C), redissolved in TE buffer, adjusted to 0.1 M NaCl, and incubated at 37°C for 1-2 hours with RNase T_1 (0.2 U/ml) and RNase A (50 KU/ml). The solution was chilled on ice, extracted twice with chloroform-isoamyl alcohol, precipitated with 1.2 volumes of ethanol (overnight, -20°C), and the DNA redissolved in TE buffer.

Quantitation of Nuclease Digestion

The quadruplicate 5% aliquots from each sample were spotted on separate GF/C filters (Whatman). Two filters from each sample were washed sequentially with ~10 ml 10% TCA (trichloroacetic acid), ~30 ml 5% TCA, and ~2 ml ethanol, and then air dried. The other two filters from each sample were left unwashed. Filters were put into scintillation vials with 100 µl H_2O and 400 µl NCS (Amersham) and incubated overnight at 37°C. Following the addition of 50 µl acetic acid and 10 ml Triton X-100 fluor, the solution was thoroughly mixed and counted in a Beckman LS 7000 liquid scintillation counter. Percent TCA solubility was calculated as $\frac{U - W}{U} \times 100$, where U and W are the amounts of radioactivity on the unwashed and washed filters, respectively. It had been

determined previously that U = W for control nuclei that were not treated with micrococcal nuclease.

Preparation of DNA Probes

Plasmids pRP9 and pRP7, which respectively contained the 1.18 kbp and 2.2 kbp Hind III fragments of rDNA cloned into pBR322, were obtained from Ron Pearlman. The 2.6 kbp Hha I fragment was cleaved out of pTtr1. Plasmid pTtr1 contained the 4.3 kbp Hind III fragment from the center of the rDNA cloned into pBR322, and was obtained from Liz Blackburn's lab.

Plasmids were isolated essentially by the method of Birnboim & Doly.[22] Plasmid DNA was digested with appropriate restriction endonucleases (New England Biolabs) under conditions specified by the supplier. DNA fragments were separated by agarose gel electrophoresis and recovered from the gel by electrophoresis onto DE-81 paper[23] or by electroelution. The purified DNA fragments were then ^{32}P-labelled by nick translation.[24] All probes were specific for the region of the rDNA from which they were derived, as judged by hybridization to restriction endonuclease fragments of total cellular DNA from Tetrahymena (Figure 1B).

Gel Electrophoresis and Blotting of DNA

Micrococcal nuclease digests (5-10 µg of DNA) were electrophoresed in 1.5% agarose gels in 40 mM Tris-acetate (pH 7.9), 5 mM sodium acetate, 1 mM EDTA. Gels were 23-40 cm long and 3 mm thick. The sample wells were 9 mm wide. The well size, gel length, and voltage gradient (4-5 V/cm) were important for obtaining good resolution. Following ethidium bromide staining and photography, the entire gel was soaked in a denaturation buffer (1.5 M NaCl, 0.5 M NaOH) for 20 min. It was then rinsed in neutralization buffer [3.0 M NaCl, 0.5 M Tris-HCl (pH 7.0)] and soaked for 20 min in fresh buffer. The transfer "sandwich" was prepared: 1 filter paper (Whatman 3 MM) soaked in 20x SSC [3.0 M NaCl, 0.3 M sodium citrate (pH 7.0)] was the base of the sandwich, on top of which was placed the gel, drained of neutralization buffer. The nitrocellulose filter (Schleicher & Schuell BA83, 0.20 µm) pre-wet in 5x SSC was placed on the gel, followed by one filter paper soaked in 5x SSC. Atop this, 8 dry filter papers were placed. The sandwich was then wrapped in plastic wrap and weighted down with three electrophoresis plates. The transfer proceeded undisturbed for at least 3 hr. The nitrocellulose filter was separated from the gel and baked *in vacuo* at 80°-90°C for 2-6 hr.

Hybridization

DNA-DNA hybridizations were carried out in one solution, without prehybridization treatment. The hybridization solution contained 5x SSC, 50%

formamide, 50 mM sodium phosphate (pH 6.8), 500 µg/ml of denatured and degraded herring sperm DNA (Type IV; Sigma), and 1X Denhardt solution.[25] Hybridizations were carried out in sealable food bags (Dazey No. 6010) containing 1.5-5 x 10^6 cpm of heat-denatured ^{32}P-labelled probe and 60-100 ml of hybridization solution. Hybridization proceeded for 18-36 hours in a water bath at 40-42°C. The filter was washed with 100-200 ml of 5x SSC at room temperature for 20 min. The washing was then repeated and followed by two additional washings at 40-42°C with 5x SSC, 0.1% SDS, each lasting ~1 hr. The nitrocellulose filter was then blotted dry with Whatman 3MM paper and mounted for autoradiography (Kodak XAR or XRP-5 film with a Dupont Cronex Lightning Plus intensifying screen) for periods of 2 days-2 weeks. Densitometer tracings of the autoradiograms were done with the gel scanning accessory of a Cary 219 spectrophotometer.

RESULTS

Bulk Chromatin Structure

Nuclei from rapidly growing ("log") and from starved T. thermophila were digested to varying degrees with micrococcal nuclease. The DNA was then isolated and analyzed by gel electrophoresis (Figure 2A, C). Both log and starved cells had a nuclease digestion pattern characteristic of a nucleosomal chromatin structure. The fragment sizes at early times of digestion were multiples of 199±4 bp (Figure 3). Upon extensive nuclease digestion of log cell nuclei, an additional band appeared between the mononucleosome and dinucleosome bands.

Chromatin Structure of the rDNA Gene Region

The DNA fragments in Figure 2A, C were transferred to nitrocellulose by the technique of Southern[26] and hybridized with a probe for the transcribed region of the rDNA. The sequences represented by this probe are located ~2 kb from either nontranscribed spacer (Figure 1), so any fragments of chromatin $\leq$ 2 kb in size that hybridize with the probe must have been derived entirely from the gene region. The hybridization patterns are shown in Figure 2B and D. A very high background was present in the early digestion samples ($\leq$ 15% TCA solubility) of both log and starved cells, masking the nucleosome repeats (see also Figure 4A, B). The majority of the hybridization smear was centered around the 6th and 7th nucleosomes in starved cells, but around the 2nd nucleosome in log cells. At later digestion times ($\geq$ 18% TCA solubility), a clear nucleosomal repeat was seen for the gene region in both log and starved cells. Again, at an equivalent amount of digestion (judged by acid solubility

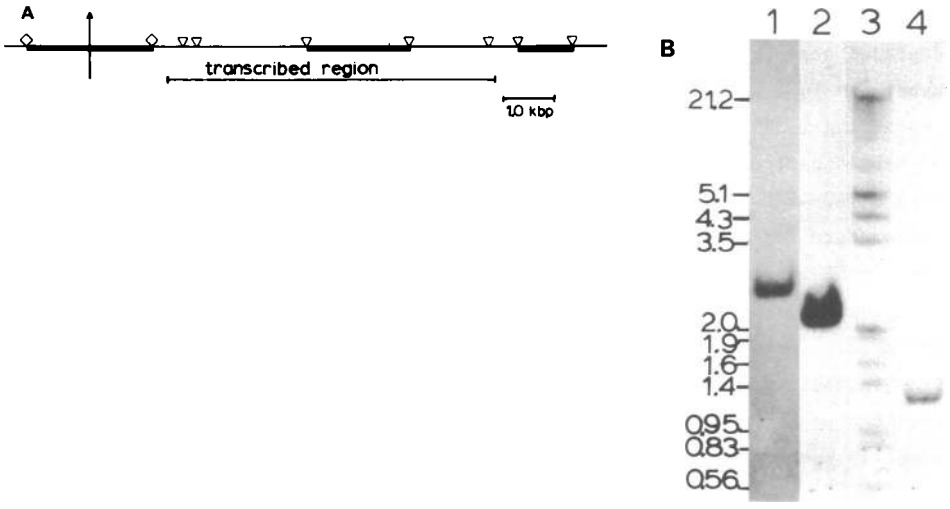

Figure 1. Location of probes and verification of their specificity.
(A) Schematic representation of half the rDNA molecule of Tetrahymena thermophila. The arrow represents the center of the molecule. All of the Hind III sites (▽) and the central-most Hha I site (◇) are represented in accord with Engberg et al.[10] The thicker regions represent areas of the DNA which were used as probes.
(B) Autoradiogram of nitrocellulose blot of total cellular DNA hybridized with the probes of interest. DNA had been digested to completion with restriction endonucleases prior to electrophoresis. (1) Digested with Hha I, hybridized with central spacer probe (2.6 kbp); (2) digested with Hind III, hybridized with gene region probe (2.2 kbp); (4) digested with Hind III, hybridized with terminal spacer probe (1.18 kbp). (3) λ DNA digested with both Eco RI and Hind III, then end-labelled with γ-^{32}P-ATP and polynucleotide kinase. The numbers to the left of the autoradiogram correspond to the size (in kbp) of the DNA fragments in (3). The sizes we determined for the probe DNA fragments were each about 10% larger than those previously published.[10] Additional verification of probe specificity was done with Hpa II, Bgl II, and Pst I digests (data not shown).

of the DNA) the rDNA gene region was digested to a lower molecular weight more quickly in log cells than in starved cells.

At low and intermediate extents of nuclease digestion, the repeat between successive multimers in the rDNA gene region is significantly smaller than that of the total nuclear DNA in both log and starved cells. The difference is readily seen by comparing the third nucleosome band (15% starved) with the 603 bp marker DNA fragment (lane M): the two have very similar mobilities in the ethidium bromide stained gel (Figure 2C), whereas the band hybridizing to the gene region probe is significantly smaller than 603 bp (Figure 2D). Measurement of the fragment sizes (Figure 3B) gave a nucleosome repeat of 184

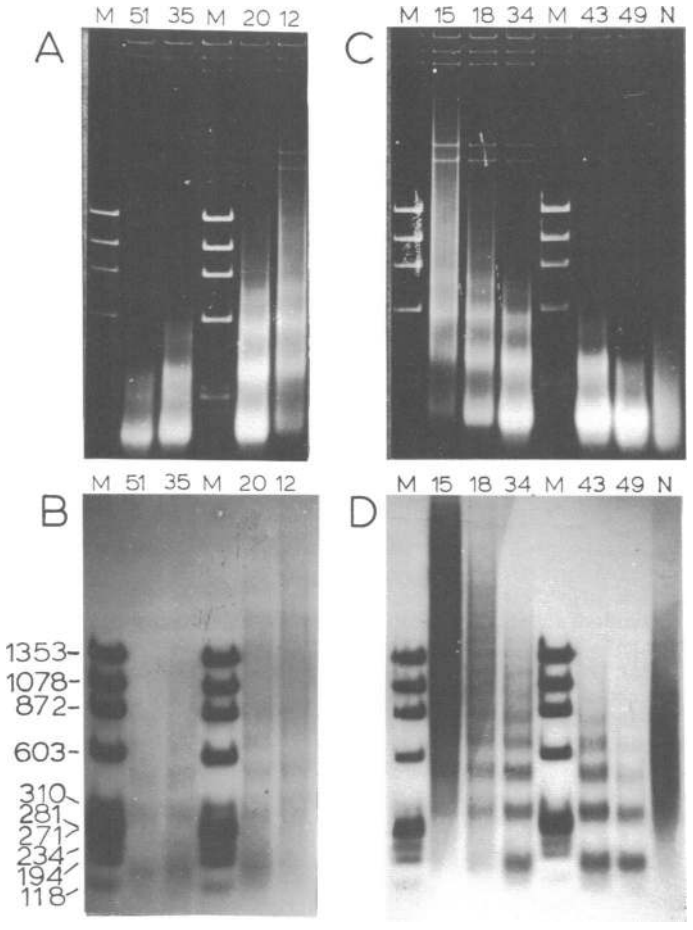

Figure 2. The gene region of the rDNA is organized in a repeated nucleosome structure.
Cells were grown under log-phase or starved conditions. DNA was isolated from nuclei after micrococcal nuclease treatment. All the DNA samples were electrophoresed on the same 1.5% agarose gel and transferred to the same nitrocellulose filter. Equivalent amounts of DNA were applied to each lane.
(A) Photograph of the ethidium bromide stained gel containing log-phase cell DNA. λ-Hind III markers were added to each DNA sample to check for aberrant migration. Numbers above lanes correspond to percent TCA solubility. Marker lanes (M) contained Hae III fragments of ϕX174 DNA which had been end-labelled with γ-^{32}P-ATP and polynucleotide kinase.
(B) Autoradiogram of nitrocellulose blot of A hybridized with gene region probe (see Figure 1). Numbers adjacent to B correspond to the size in bp of the fragments in the marker lanes.
(C) Ethidium bromide stained gel containing starved cell DNA. (N) deproteinized total cellular DNA (naked DNA) which had been treated to a limited extent with micrococcal nuclease.
(D) Autoradiogram of nitrocellulose blot of C hybridized with gene region probe.

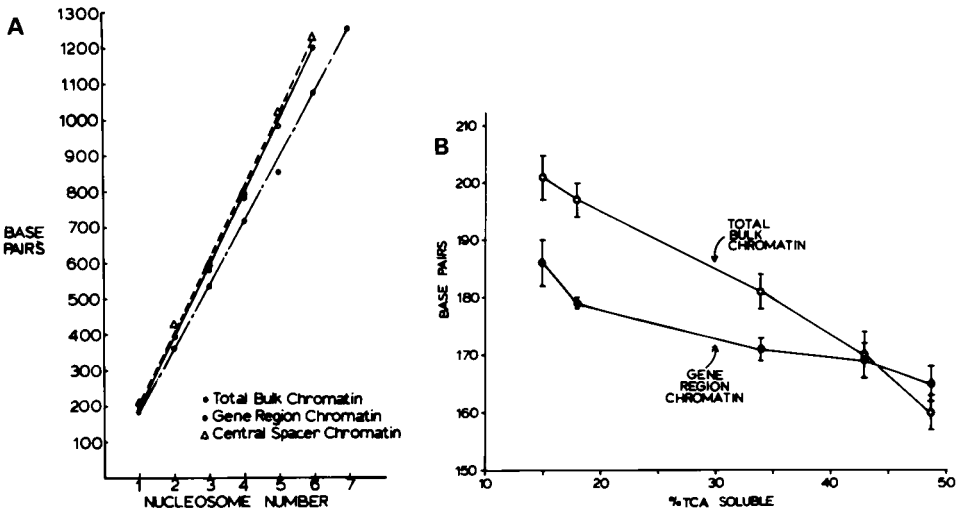

Figure 3. The nucleosomal repeat size in the gene region is shorter than that in the central spacer or bulk chromatin.
(A) A sample of a plot for the determination of the nucleosome repeat size, obtained by measurement of the data in Figures 2C,D and 5 (15% TCA soluble starved cell sample lanes). The slope of the line corresponds to the nucleosome repeat size.
(B) Nucleosome repeat size as a function of the extent of nuclease digestion. Bulk chromatin data were obtained from Figure 2C, and gene region chromatin data from Figure 2D. Each point was determined from the slope of a plot similar to that in A. The error in the nucleosome repeat size was determined as the standard deviation of the repeat sizes calculated for the individual nucleosome multimers in each distribution.

± 3 bp for the gene region in both log and starved cells at early extents of digestion (15% TCA solubility). With increased digestion, both the gene region and the total chromatin repeat sizes decreased, and converged at ~165 bp. Hind III restriction fragments of λ DNA were added to the nuclease digestion samples to test for any aberrant migration of DNA. As seen in the ethidium bromide stained gels, the electrophoretic mobilities of these fragments were not altered by the presence of the Tetrahymena nuclear DNA samples.

Although the gene regions of rDNA of log and starved cells produced the same size micrococcal nuclease fragments for equivalent amounts of digestion, the degree of hybridization was much greater in the case of the starved cell DNA (Figure 4).

Total nuclear DNA which had been deproteinized prior to limited micrococcal nuclease treatment gave a broad smear of hybridization with the

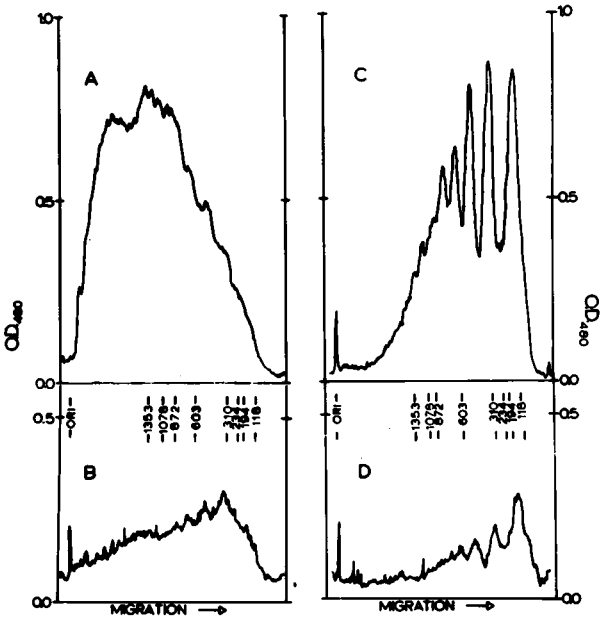

Figure 4. A distinctive nucleosome pattern in the gene region is revealed only after extended nuclease digestion, and more of the DNA is organized in this pattern in starved than in log-phase cells.
Densitometric scans of 4 lanes of the autoradiogram shown in Figure 2B,D:
(A) starved, 15% TCA solubility
(B) log, 12% TCA solubility
(C) starved, 34% TCA solubility
(D) log, 35% TCA solubility
The numbers correspond to the size in bp of the ^{32}P-labelled ϕX174-Hae III DNA markers. (ORI) origin. The area of the peak corresponding to each nucleosome repeat was measured. The areas of the peaks in C averaged ∿5 times greater than those in D.

gene region probe (Figure 2D, lane N). Thus, the nucleotide sequence preference of this nuclease is not contributing detectably to the chromatin digestion patterns.

Chromatin Structure of the Central Spacer of rDNA

The deproteinized micrococcal nuclease digests were cleaved with HhaI restriction endonuclease prior to electrophoresis. This procedure insured that all fragments of chromatin that hybridized with the central spacer probe were derived entirely from that region of the gene. Some of the Southern hybridization data are presented in Figure 5. The central spacer region has a nucleosome repeat pattern which mimicks that of the bulk chromatin. The pattern was prominent only at early times of digestion for both log and

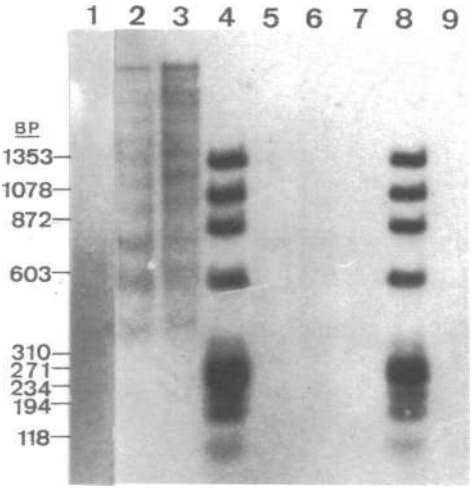

Figure 5. The central spacer is organized in a repeated nucleosome structure with the same repeat size as bulk chromatin.
Autoradiogram of nitrocellulose blot containing nuclease digests of starved and log-phase cell DNA hybridized with the central spacer probe (see Fig. 1). DNA was digested to completion with Hha I prior to electrophoresis. (1) naked DNA digested with micrococcal nuclease; (2), (5) and (7) log, 12%, 20% and 35% TCA soluble, respectively; (3), (6) and (9), starved, 15%, 18% and 34% TCA soluble, respectively; (4) and (8) molecular weight markers, ^{32}P-labelled ΦX174-Hae III fragments.

starved cell nuclei, and became very faint at later times. The repeat distance was 198 ± 6 bp for log and 205 ± 5 bp for starved cell rDNA. There was greater hybridization to some fragment sizes, which we attribute to prominent sites in the chromatin which were highly accessible to nuclease attack. The distributions terminated at 2.6 kbp, the size of the Hha I restriction fragment. "Naked" total DNA partially digested with micrococcal nuclease gave a broad smear of hybridization (Figure 5, lane 1). Thus, the hybridization patterns obtained with the chromatin digests are not simply due to DNA sequence specificity of micrococcal nuclease.

Structure of the Terminal Spacer of rDNA

DNA derived from micrococcal nuclease digests of starved or log cell nuclei was blotted and hybridized with the cloned 1.05 kb Hind III fragment of rDNA. [The probe encompasses a major portion of the terminal spacer, starting 256 bp downstream from the termination point of transcription and ending ~200 bp upstream from the repeated C_4A_2 sequence at the rDNA termini.[27]] In some cases, the DNA was cleaved with Hind III to limit hybridization to fragments

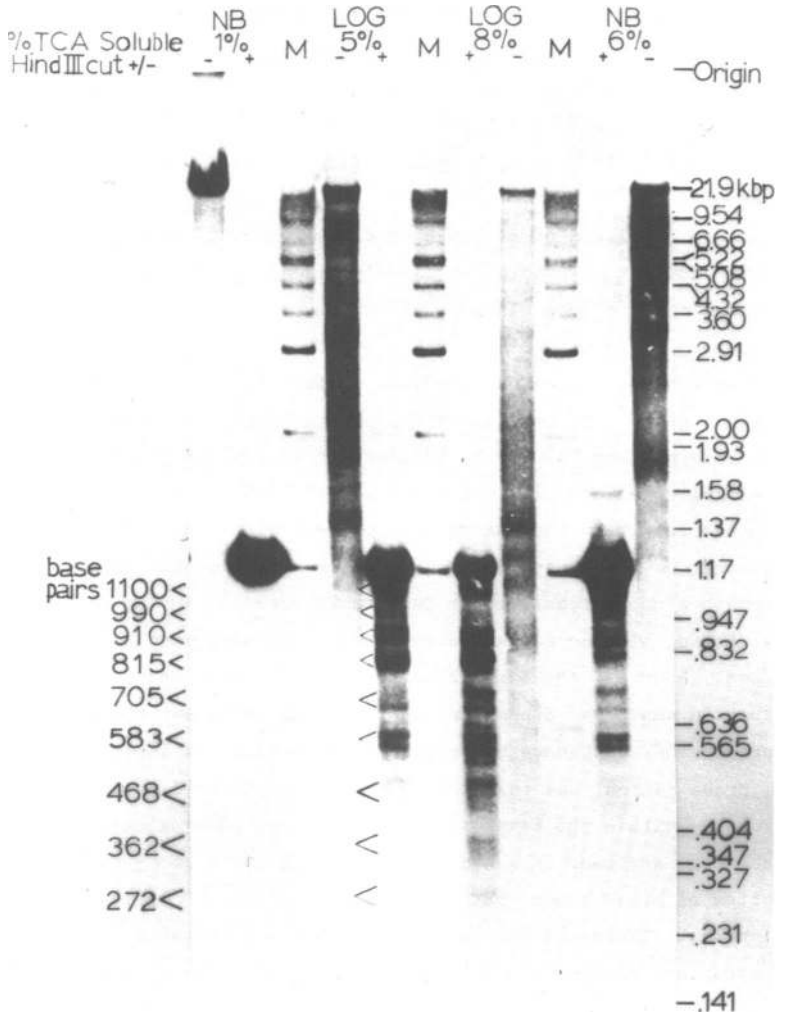

Figure 6. The cleavage pattern of the terminal spacer is dominated by the sequence specificity of the nuclease.
DNA derived from micrococcal nuclease digests of log-phase nuclei (log) or of naked bulk DNA (NB) was cut with Hind III (+) or left uncut (-). DNA was then subjected to electrophoresis on a 1.4% agarose gel, transferred to nitrocellulose, and hybridized with the terminal spacer probe. Marker lanes (M) contained an Eco RI-Hind III double digest of λ DNA and a Taq I digest of ϕX174 DNA. (<) designates the doublet or triplet bands making up a 100 base pair ladder of nuclease sensitive sites.

derived entirely from the terminal spacer. In these hybridizations, a very complicated pattern of micrococcal nuclease cleavages was revealed. Except for some band intensity differences, however, the sites were mimicked in the nuclease digestion pattern of naked DNA (Figure 6). The same pattern was present in log and starved nuclei, and in isolated ribosomal gene chromatin (data not shown).

The pattern consisted of a series of eight groups of bands, each group comprised of a doublet or triplet of closely spaced bands. Each group of bands is separated by a mean distance of 100 bp (see Figure 6). This pattern persisted in samples taken at later times of digestion (18-30% TCA solubility, data not shown), where a clear nucleosomal pattern was evident in the ethidium bromide stained gels. The corresponding, more extensively digested naked DNA controls also maintained the 100 bp banding pattern, while the ethidium bromide staining revealed a low molecular weight smear.

In the samples where the DNA was not cleaved with Hind III, broad, dark bands were observed. The bands did not produce a nucleosomal repeat (Figure 6), even though a nucleosomal repeat pattern was present in the bulk chromatin. Broad, high molecular weight bands were also seen in the naked DNA control lanes. However, the band patterns were different than those observed in the chromatin samples. Since many of the bands represent fragments larger than the probe, they must have one end at a micrococcal nuclease cleavage site within the probe region, and the other end at a site outside the probe region, probably within the transcribed region. The more extensively digested samples from log, starved, or naked DNA revealed a smear of hybridization with a distribution of broad bands that roughly matched the 100 bp banding pattern (data not shown). Therefore not much information was revealed about the chromatin structure of the terminal spacer region with any of these samples.

DISCUSSION

We have shown that in isolated nuclei, the transcribed region of the rDNA is cleaved by micrococcal nuclease in a periodic manner indicative of nucleosomes. However, at early times of digestion ($\leq$ 15% TCA solubility), a heterogeneous smear of DNA fragments was superimposed on the nucleosomal repeat pattern. This indicates that a fraction of the transcribed regions may not be nucleosomal. The non-transcribed central spacer of the rDNA appeared to be nucleosomal as judged by micrococcal nuclease. The pattern was observable only at early stages of digestion and vanished when more than 15% of the bulk DNA had been solubilized. This observation may be explained if

the central spacer is packaged in a higher order, nucleosomal structure which is rather nuclease insensitive. After nicking by the nuclease, the structure unfolds into a rapidly degraded form. We were unable to determine whether the non-transcribed terminal spacer was nucleosomal, because the sequence specificity of micrococcal nuclease[28-30] dominated the cleavage pattern in this region.

Starved vs. Log Cells

We compared actively transcribing log cells with starved cells, which have only 4% of the rDNA transcriptional activity of log cells.[12,13] It is not known whether 4% transcriptional activity corresponds to 4% of the genes being completely active, all the genes being 4% active, or the genes existing in an intermediate state.

In the central spacer, we found similar nucleosomal repeats for both transcriptional states. There was, however, a difference in the most prominent sites of nuclease attack within the central spacer. Preliminary mapping experiments (T. Palen, unpublished data) have shown that some of these prominent sites correspond to the sites of initiation of replication[20,31] and transcription.[32]

In the gene region we found ~5-fold less hybridization of the probe to log cell DNA when compared to an equivalent amount of starved cell DNA. The gene region DNA in log cells was also more quickly degraded to smaller fragment sizes. These observations are in good agreement with earlier work done in Xenopus[33] and Physarum.[34] In both these studies, rRNA gene sequences had a greater sensitivity to micrococcal nuclease when they were active in transcription, than when they were inactive.

The increased sensitivity we observe in log-phase cells does not appear to be occurring within a normal nucleosome array. Instead, it appears that the majority of the gene region chromatin has an altered DNA-histone interaction which makes the DNA very accessible to micrococcal nuclease cleavage, producing a smear rather than discrete digestion products. Such altered DNA-histone interaction may be responsible for the non-beaded "rho" chromatin observed in the ribosomal genes of Oncopeltus fasciatus immediately prior to transcriptional activation.[43] An alternative explanation for our nuclease cleavage data -- that the actively transcribing gene region contains no nucleosomes -- appears unlikely in light of a recent study of Tetrahymena pyriformis nucleolar chromatin. Colavito-Shepanski and Gorovsky report that actively transcribed rDNA chromatin has the same histone to DNA ratio as bulk chromatin.[35] It may be that the nucleosomal bands observed in the log cells

are caused by a small population of inactive genes. In starved cells, where there are a greater number of inactive genes, more of the DNA is packaged into unaltered nucleosomes.

Transcribed vs. Non-transcribed Regions

The micrococcal nuclease repeat size in the central spacer was found to be similar to that of bulk chromatin (198±6 bp for log, 205±5 bp for starved). A smaller repeat size was found in the gene region (184±3 bp). Though this difference is small, we are confident of its significance because ^{32}P-labelled size markers were included on each gel and unlabelled fragments were included within each lane to check for aberrant migration. To our knowledge, this is the first report of different nucleosome repeats in transcribed and non-transcribed regions of the same molecule. We have interpreted this difference to be due to nucleosome spacing, because at higher amounts of nuclease digestion the gene region and bulk chromatin repeat sizes converge to the same size core (~165 bp). The shorter gene region repeat is maintained in starved cells, suggesting that it may be a property of inactive but readily activatable chromatin. Upon activation further alteration of the chromatin occurs.

The transcribed gene region of rDNA in Physarum has been shown to have greater micrococcal nuclease sensitivity than adjacent non-transcribed spacers or bulk DNA.[34,35,36] Pruitt and Grainger[37] have examined the rDNA chromatin in Xenopus by electron microscopy using conditions which more closely approximated physiological conditions than previous studies.[38] They found that the adjacent non-transcribed spacer regions were compacted into a more highly ordered structure. These studies, in conjunction with our report, support the idea that the structure of transcribed rChromatin and adjacent non-transcribed rChromatin are different. The DNA which is transcribed (or potentially transcribed) must be in a chromatin state conducive for the transcriptional process.

Comparison with Other Studies

Mathis and Gorovsky[14] and Piper et al.[15] found Tetrahymena rDNA as a whole to be nucleosomal. From our work, we would add that both the central spacer and gene region do indeed contribute to a nucleosomal pattern. We find it more difficult to reconcile our data with that of Borchsenius et al.,[18] who examined the chromatin structure of the gene region and terminal spacer of the rDNA in T. pyriformis. They reported a non-nucleosomal smear of DNA fragments in the gene region after digestion with micrococcal nuclease. We observed this smearing effect only at low extents of nuclease digestion. Since we feel

that the nucleosome pattern may be due to inactive genes, we suggest that the cells studied by Borchsenius et al.[18] may have had a higher percentage of active genes. Different levels of gene activity could be intrinsic to the different species, or would be caused by differences in cell growth conditions. For the terminal spacer, they report a 200 bp nucleosome repeat.[18] We also see a repeat, but it is ~100 bp and is not due to chromatin structure, but to DNA sequences that are highly susceptible to micrococcal nuclease cleavage. Again, species differences may explain the discrepancy.

The structure of the rDNA chromatin in rapidly growing T. thermophila has also been probed by trimethylpsoralen crosslinking.[16] The central spacer chromatin was highly resistant to psoralen crosslinking, and those crosslinks that were introduced in the DNA had no discernible regular spacing. In contrast, we now find that micrococcal nuclease produces a clear 200 bp repeat pattern in this region. This apparent discrepancy may be explained by a higher order structure for the central spacer chromatin. Such a structure might make it difficult to unwind the DNA to intercalate the psoralen, but micrococeal nuclease cleavage might relax the structure to reveal an underlying 200 bp repeat. In the terminal spacer chromatin, psoralen crosslinked the DNA at 200 bp intervals.[16,39] Psoralen crosslinking and micrococcal nuclease have different sequence specificities. The sequence specificity of the former probe did not interfere with the detection of the nucleosome repeat in the terminal spacer. In the gene region, psoralen crosslinks were found to occur with a sub-nucleosomal repeat (~110 bp) that was interpreted most easily as reflecting an altered nucleosome structure.[16] Such an altered structure might also give increased accessibility to micrococcal nuclease, allowing cleavage of the DNA at sites that would be protected within the core of the nucleosome in its standard conformation.

Histone variants specific to the transcriptionally active macronucleus of Tetrahymena[40] and histone modifications such as phosphorylation[41] and acetylation[42] may be the key to the chromatin structure differences along the rChromatin. It would be very interesting to see whether these modified and/or variant histones are unique to the gene region of isolated rChromatin.

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