Chromatin Structure of the Telomeric Region and 3’-Nontranscribed Spacer of *Tetrahymena* Ribosomal RNA Genes*

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The chromatin structure of the 3’-nontranscribed spacer of the linear rRNA gene molecules of *Tetrahymena thermophila* was examined. This region includes the transcription termination site, two sets of recently identified conserved spacer repeats (Type IV and V repeats (Challoner, P. B., Amin, A. A., Pearlman, R. E., and Blackburn, E. H. (1985) *Nucleic Acids Res.* 13, 2661–2680)), and the terminus of the molecule. Using sensitivity to nucleases as a probe, a unique chromatin structure was found in this rDNA region. Proceeding from the end of the rDNA molecule, the telomeric repeated sequence, (CCCTAA), was packaged in a non-nucleosomal complex adjacent to three phased nucleosomes. This nucleosomal structure was disrupted at the Type V repeat region, which, compared with the neighboring nucleosomal region, was more accessible to nucleases and, from both micrococcal nuclease and DNase I digestion results, was packaged in chromatin differently from the sequences flanking it on both sides. The region between the Type V repeats and adjacent to the transcription termination site was in yet another distinguishable chromatin structure as judged by its sensitivity to nucleases. It includes sites protected in chromatin and sites which were cleaved in chromatin but not detectably digested in DNA controls, suggesting that specific proteins are also associated with this region.

Telomeres are specialized structures at the ends of eukaryotic chromosomes which are required for stable chromosome maintenance. Part of the role of telomeres is likely to be in the completion of replication, since all known DNA polymerases require a primer and synthesize DNA only in the 5’ to 3’ direction (reviewed in Refs. 1 and 2). However, it is likely that telomeres also have other cellular functions. Experiments have shown that ends of broken chromosomes, in contrast to telomeres, are unstable, undergoing fusion, breakage, and other abnormal reactions (reviewed in Ref. 1). Studies on telomeres in lower eukaryotes have shown that the most terminal regions of the molecules consist of simple repeated sequences (reviewed in Ref. 2). Understanding how these repeats and their adjacent sequences function as telomeres will require investigation of how this region interacts with proteins.

Protozoans have been particularly amenable to the study of telomeres because many have nuclei which contain subchromosomal DNA molecules, effectively increasing the concentration of DNA ends. One such organism is the ciliated protozoan *Tetrahymena thermophila* which has two types of nuclei: a transcriptionally inactive micronucleus and a macronucleus, derived from the micronucleus, which is active during vegetative growth (reviewed in Ref. 3). The macronuclear DNA includes the amplified ribosomal RNA gene molecules (rDNA), which are linear 21-kilobase (kb) palindromic molecules present at 10^6 copies/cell (4, 5) (reviewed in Ref. 6). Initiation of transcription begins 1.8 kb from either side of the center of the palindrome and proceeds for 6.7 kb, terminating 2.1 kb from the end of the molecule (7) (Fig. 1). The 3’-nontranscribed spacer (3’-NTS) contains the site for termination as well as two recently identified sets of repeated sequence elements, designated Type IV and V repeats (7). These repeated elements are conserved in location and/or sequence in related species of ciliates (7), but because their functions are not known, we wished to examine their chromatin structure. A region of nonconserved spacer (7) separates the conserved repeats from the sequence at the very ends of the molecule, which consists of a simple hexanucleotide, CCCCTA, tandemly repeated over 50 times (8). There are several single strand breaks within the distal ~100 bp of the CCCCTA strand and at least one break on the GGGTTT strand (8, 9). This repeated sequence is at the ends of all macronuclear DNA molecules in the ciliates *Tetrahymena*, *Glaucocysta*, and *Paramecium* (10, 11).

The bulk of the rDNA is packaged in nucleosomes (12–14). However, various functional regions of the rDNA behaved differently upon treatment with chemical agents and nucleases (15–19). In particular, the central nontranscribed spacer is highly organized, with seven phased nucleosomes at the very center of the palindrome flanked by nuclease-hypersensitive sites located in the origin of replication and in the region of transcription initiation (19). Additional DNase I-hypersensitive sites have been identified near the transcription termination site; all these rDNA spacer hypersensitive sites correlate with topoisomerase I-binding sites in the chromatin (20). The telomeric (CCCCCTA), sequences from total macronuclear DNA do not appear to be packaged as nucleosomes (15), and Blackburn and Chiou found that the telomeric region containing CCCCTA repeats from isolated rDNA chromatin behaved in an analogous manner. Recent work on the repeated telomeric sequence in macronuclear of the ciliate *Oxytricha* suggests that this sequence also has non-nucleosomal packaging and is flanked by phased nucleosomes (21).

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1 The abbreviations used are: kb, kilobase; bp, base pair; 3’-NTS, 3’-nontranscribed spacer.

1 E. H. Blackburn and S.-S. Chiou, unpublished results.
region is homogeneous in length (21), in contrast to the length heterogeneity of the ~300 bp telomeric repeats in Tetrahymena (8, 9, 15).

In this paper, we have examined the detailed chromatin structure of the entire recently sequenced (7) terminal region of the rDNA in isolated macronuclei using sensitivity to nucleases as a probe. The non-nucleosomal packaging of the telomeric CCCCAA repeats is confirmed. We show that adjacent to the CCCCAA repeats, the 3’-NTS is packaged into three specifically positioned nucleosomes. In addition, a uniquely non-nucleosomal chromatin structure is centered about the transcription termination site and the repeated spacer sequence elements downstream from it.

**Materials and Methods**

**Cell Culture and Macronuclei Isolation—**T. thermophila inbred strain B-1868 (IV), kindly provided by D. Namney, University of Illinois, was maintained in 1% proteose peptone (Difco), 0.1% yeast extract (Difco), and 0.003% Sequestrene (Ciba-Geigy) (1% PPYS) at room temperature. Aliquots of stock cultures were used to inoculate 2 liters of 2% PPYS, and cells were grown at 30 °C with constant shaking until they reached a density of 2–3.5 x 10^10 cells/ml. Macronuclei were isolated using a modified procedure of Gorovsky et al. (22, 23). The nuclei were washed once in buffer A (60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 2 mM EDTA, and 15 mM Tris HCl, pH 7.4) containing 0.34 M sucrose and resuspended in buffer A containing 2 mM CaCl2 and 2 mM MgCl2 at 2 x 10^10 macronuclei/ml (approximately 200 μg of DNA/ml). All before contained 1 mM phenylmethylsulfonyl fluoride.

Preparation and Enzyme Digestion of DNA—Digestions of macronuclei and DNA with micrococcal nuclease and DNase I (Worthington) were performed as described in the figure legends. Extents of digestions were determined from samples taken over a range of time points and estimating from Southern blots the fraction of intact DNA fragment. Several comparable points were used to compare nuclei and DNA samples. Reactions were stopped by the addition of an equal volume of NDS (0.5 x EDTA, 10 mM Tris-HCl, pH 9.5, and 2% sodium dodecyl sulfate) and then incubated for 10 min at 50 °C. One volume of Pronase (0.2 mg/ml) in 0.5 M EDTA, 10 mM Tris-HCl, pH 9.5, was added, and samples were incubated at 1 h at 55 °C. Samples were phenol/chloroform-extracted once and ethanol-precipitated twice. DNA was resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5, at a concentration of approximately 200 μg/ml. Restriction digest of samples were performed according to the directions of the manufacturers (New England Biolabs).

Plasmid DNA, used as hybridization probes, was prepared by the alkaline extraction method (25). Plasmids were digested with the appropriate restriction enzymes, fragments were separated by agarose gel electrophoresis, and the desired fragments were extracted using the glass bead method of Vogelstein and Gillespie (26).

Southern Blotting and Hybridization—Following fractionation of samples by agarose gel electrophoresis, the DNA was bidirectionally transferred to nitrocellulose filters by the method of Smith and Summers (27). Hybridization of filters was as described by Botchan et al. (28). Labeling of hybridization probes was according to the procedure of Maniatis et al. (29).

**Results**

The structure of the 3’-NTS is shown diagramatically in Fig. 1b (7). The open box is the transcribed region. The regions marked IVa and IVb are 17-base pair repeated sequences near the previously mapped site of transcription termination (43). They are highly conserved in sequence and position not only in two _Tetrahymena_ species but also in the ciliate _Glaucoma_, suggesting that they have an important functional role, possibly in transcription termination (7). The Type V repeat sequences are located downstream; they are 132–134-base pair repeat units, each unit consisting of a 24-base pair variable sequence region embedded in a conserved region. The position and organization of the Type V repeats, but not their sequence, are also conserved between _T. thermophila_ and _Glaucoma chattoni_ (7). The very end of the 3’-NTS is also the telomere of the molecule and consists of tandem repeats of the hexanucleotide CCCCAA (wavy line in Fig. 1b). Restriction fragments containing the terminal sequence are heterogeneous in length (8), this heterogeneity being due to differences in the number of CCCCAA repeats.

In the experiments presented here, the chromatin fine structure of the macronuclear rDNA 3’-NTS was probed using sensitivity to three different nucleases. Macronuclei isolated from exponentially growing cells were digested with micrococcal nuclease, DNase I, or the restriction enzyme _AluI_. The macronuclear DNA was then extracted and where appropriate was further digested with another restriction enzyme for analysis. As controls in all experiments, DNA was first extracted from macronuclei and subsequently digested with the appropriate enzyme(s). Nuclease digestion patterns were analyzed by agarose gel electrophoresis and Southern hybridization. The DNA restriction fragments used as 32P-probes in the following experiments are shown in Fig. 1c.

**Mapping of Micrococcal Nuclease-sensitive Sites in the 3’-NTS Chromatin—**To determine whether nucleosomes were...
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**Fig. 2.** Mapping of the positions of micrococcal nuclease-sensitive sites in the 3'-NTS. a, macronuclei were isolated from logarithmically growing cells, resuspended in buffer A (at 2 x 10^7 nuclei/ml), and digested with micrococcal nuclease (3 units/ml) at 30°C for various lengths of time. Purified DNA (200 µg/ml) was digested in buffer A to comparable extents with micrococcal nuclease (1.4 units/ml) at 30°C. DNA was extracted from the sample and digested to completion with the restriction enzyme PstI. DNA was fractionated in a 1.5% agarose gel at 12 V/cm for 3 h (during electrophoresis, the gel was cooled to 14°C by a circulating water bath) and subsequently bidirectionally transferred to nitrocellulose. The filter was probed with a ^32^P-labeled PstI-HhaI fragment located 9.5-9.7 kb from the center of the palindrome (see Fig. 1). Lanes 1-6 are macronuclei digested with micrococcal nuclease for 0.5, 1, 2, 4, 8, and 12 min, respectively. Lane 7 is a macronuclei sample which was incubated for 0.5 min in the same way as lanes 1-6 except that it received no micrococcal nuclease. Lanes 8-12 are DNA controls treated with micrococcal nuclease for 0.5, 1, 2, 4, and 8 min, respectively. T7 DNA digested with KpnI-PvuII (sizes in kilobase are given to the left) or HaeIII (not shown) was used as fragment size standards. Lengths of bands A-H are 4.4, 1.1, 0.78, 0.59, 0.40, 0.26, 0.76, and 0.47 kb, respectively. b, the duplicate filter of the one in Fig. 3a was probed with the ^32^P-labeled HincII-HhaI fragment located 9.63-9.74 kb from the center of the palindrome (see Fig. 1).

present in the 3'-NTS, macronuclei were digested with micrococcal nuclease, which is known to cleave in the linker regions of nucleosomes. DNA from the micrococcal nuclease-treated nuclei was digested to completion with the restriction enzyme PstI, which cuts in the Type V repeats (at ~9.5 kb in the map shown in Fig. 1b). The method of indirect end labeling (30) was used to determine the positions of nucleosomes or other chromatin structures within the 3'-NTS. This method involves the use of a labeled probe which is homologous to a short region next to one end of a restriction fragment which covers the region of interest, effectively “end labeling” this fragment. Thus, any cleavages within the fragment can be accurately mapped from the indirectly labeled end. The PstI-HhaI fragment shown in Fig. 1c was used as a probe. The results are shown in Fig. 2a. The pattern of bands seen in the chromatin samples (lanes 1-7) is very different from the DNA controls (lanes 8-12). The full-length PstI fragment (band B) is 1.1 kb in length and extends from the 3' end of repeat Vc to the end of the rDNA molecule. The first band below band B in the chromatin samples is the 0.78-kb band C which corresponds to the loss of ~300 bp consisting of the entire block of telomeric CCCCAA repeats. Band C and the three bands below it (D-F) are spaced 190, 150, and 150 bp apart, indicating the presence of three specifically positioned nucleosomes. Bands G and H in lanes 8-12 (DNA controls) are different in size from bands C-F seen in the chromatin samples and result from sites which are prefered by micrococcal nuclease. The sharpness of bands C-F shows, first, that there is a narrowly defined inner boundary of the terminal CCCCAA repeats-protein complex in chromatin and second, that the adjacent nucleosomes in the great majority of the rDNA chromatin molecules are in a unique set of highly preferred positions in this region.

Band A above the 1.1-kb PstI fragment (band B) is the neighboring 4.4-kb PstI fragment which extends into the transcription unit and contains the Type Va-c repeats. The PstI-HhaI fragment used as a probe contains part of the Vc repeat and all of the Vd repeat and hence cross-hybridizes to the 4.4-kb band. To eliminate the possibility that some of the bands (C-H) were due to fragments derived from the 4.4-kb PstI fragment, the HincII-HhaI fragment probe shown in Fig. 1c, which contained as little of the Type V repeat sequence as possible (14 bp out of 114 bp instead of 72 bp out of 172 bp; Ref. 7), was used to probe the same blot (Fig. 2b). While hybridization to the 4.4-kb band decreased considerably in relative intensity, bands B–H remained unchanged, showing they are specific to the 1.1-kb fragment.

These results showed that there are three specifically positioned nucleosomes between the Type V repeats and the terminal CCCCAA repeats. Confirming results were obtained by using HhaI as the secondary restriction digestion enzyme instead of PstI (not shown). To determine whether similar nucleosomes extended into the Type V repeats as well, time courses of micrococcal nuclease digestions of chromatin and DNA were subsequently digested to completion with HaeIII. This enzyme produces a terminal 2.4-kb restriction fragment which extends from within the transcription unit to the end of the molecule (Fig. 1b). Southern blots were made and probed by the indirect end labeling technique using the HaeIII–HindIII fragment shown in Fig. 1c (Fig. 3a). In order
which can be seen most clearly at the early time points (Fig. 3a, lanes 1–3, and Fig. 3b, lanes B and C). In contrast to the chromatin, there appears to be little preference in the naked DNA for micrococcal nuclease cutting at these sites (lanes 10–12). These results provide evidence that the Type V repeats form a chromatin domain, in which this DNA region is packaged into a defined structure by proteins, bounded by two sites which are highly accessible to nuclease digestion. In the region mapping between the transcriptional unit and the Type V repeat regions (below band C in Fig. 3a), although the banding pattern and intensity of bands were generally similar between the chromatin and DNA, two exceptions were seen, in which a band occurred in the chromatin sample with no counterpart in DNA, and a band seen in the DNA sample was not seen in chromatin. These are marked with arrowheads in Fig. 3a, and they map respectively to positions 8.90 and 8.75 kb on the map in Fig. 1h, in the region between the Type V repeats and the Type IV repeats.

**Sensitivity of the 3’-NTS Chromatin to DNase I Digestion**—Chromatin and DNA control samples were prepared for DNase I digestion in the same manner as for the micrococcal nuclease experiments. Partial DNase I digests of chromatin and DNA were subsequently digested to completion with HaeIII, and the terminal 2.4-kb HaeIII fragment was probed by indirect end labeling using the radioactive HaeIII–HindIII fragment shown in Fig. 1c. This is the same probe and restriction enzyme used in the micrococcal nuclease digested samples shown in Fig. 3.

In the chromatin samples (Fig. 4, lanes 1–6), the region shown between the full-length fragment and the top arrowhead was protected from digestion. This corresponds to the region of the rDNA between the molecular terminus and the Type Vd repeat, which was shown by the micrococcal nuclease experiments described above to be packaged as a telomeric structure and three phased nucleosomes. The corresponding region in naked DNA had several preferred cleavage sites available (Fig. 4, lanes 8–10). In contrast, in the Type V repeat region (arrowheads between the 2.2- and 0.57-kb standards), sites in the chromatin were highly accessible to DNase I, whereas in the DNA controls this region appeared to be no more preferred than sites in the flanking regions (Fig. 4, lanes 8–11). This result provides strong evidence that there is a change in chromatin structure (31–33) extending in from repeat Vd. In addition, as indicated by the two lower arrowheads in Fig. 4, there were two DNase I sites in the chromatin which were not found in the DNA controls. These two bands, at 0.35 and 0.27 kb, map close to the Type IV repeats (at 8.65 and 8.55 kb in the map shown in Fig. 1), near the site of transcription termination.

**Accessibility of rDNA Chromatin to the Restriction Enzyme AluI**—To confirm independently the findings made with micrococcal nuclease and DNase I, we compared the digestion of the 3’-NTS of the rDNA in chromatin versus purified DNA by the restriction enzyme AluI. In order to help interpret results on the 3’-NTS region, we also tested the accessibility of the central rDNA spacer to this enzyme, as the chromatin structure in this region has been analyzed previously, using micrococcal nuclease and DNase I digestions (19). Palen and Cech (19) have shown that, at the very center of the palindromic rDNA, the 5′-nontranscribed spacer is packaged into seven phased nucleosomes. This region contains two AluI sites. The flanking sequences are much more accessible to

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**Fig. 3.** Mapping of the positions of micrococcal nuclease-sensitive sites relative to the HaeIII site in the pre-rRNA transcription unit. a, samples digested with micrococcal nuclease as described in the legend to Fig. 2 were subsequently digested to completion with the restriction enzyme HaeIII. Times of micrococcal nuclease digestion, gel electrophoresis conditions, and T7 DNA size standards were the same as for Fig. 2. The filter was probed with the 32P-labeled HaeIII-HindIII fragment located 8.25–8.36 kb from the center of the palindrome (see Fig. 1). Sizes of bands A–D are 2.4, 1.4, 0.80, and 0.43 kb, respectively. The size of the band in lane 6 marked by the arrowhead is 0.62 kb, and the band in lane 10, marked by a second arrowhead, is 0.49 kb. b, densitometric scans of lanes 4, 5, 10, and 11 of the autoradiogram shown in a.

**Fig. 4.** Mapping of DNase I-sensitive sites in the 3’-NTS. Macronuclei were isolated from logarithmically growing cells, resuspended in buffer A (at 2 × 10^7 nuclei/ml), and digested with DNase I (0.6 unit/ml) at 30°C for various lengths of time. Purified DNA (200 μg/ml) was digested in buffer A to comparable extents with DNase I (0.15 unit/ml) at 30°C and digested to completion with the restriction enzyme HaeIII. Electrophoresis conditions and T7 DNA standards were the same as described for Fig. 2. Lanes 1–5 are macronuclei digested for 0.5, 1, 2, 4, and 8 min with DNase I, respectively. The DNA in lane 6 was incubated under the same conditions as lanes 1–5 except that no DNase I was added. Lanes 7–11 are DNA control samples digested for 0.5, 1, 2, 4, and 8 min, respectively. The filter was probed with the 32P-labeled HaeIII-HindIII fragment located 8.25–8.36 kb from the center of the palindrome (see Fig. 1). The arrowheads to the left correspond to rDNA fragments of estimated sizes (top to bottom) 1.4, 1.2, 1.1, 0.78, 0.45, and 0.27 kb.
micrococcal nuclease and DNase I, with several hypersensitive sites (19). There are four more AluI sites in this DNase I- and micrococcal nuclease-accessible region adjoining and within the transcription unit (19). We found that the AluI sites located in the core regions of the nucleosomes (19) were not accessible to AluI, strongly suggesting that the specifically positioned nucleosomes in this region can completely block AluI digestion of DNA in nuclei (data not shown). In contrast, the AluI sites located in the DNase I- and micrococcal nuclease-accessible region closer to the transcription unit were digests were probed using a DdeI fragment from the 3'-NTS the AluI sites located in the DNase I- and micrococcal nuclease-accessible region closer to the transcription unit were highly accessible to AluI (data not shown).

Macronuclei, or purified macronuclear DNA as a control, were digested with AluI for increasing times, and the AluI digests were probed using a DdeI fragment from the 3'-NTS region between the Type V and CCCCAAA repeats (see Fig. 1c). There are several AluI sites in the 3'-NTS (see Fig. 1b); three close to the CCCCAAA repeats, five between the Type V and IV repeats, and three in the portion of the transcription unit shown. In the DNA control samples partially digested with AluI (Fig. 5, lanes 4–8), several bands were seen as expected. Band C (1.4 kb) is the most prominent band at later digestion times and extends from the AluI sites just 5' of the repeat Va to the AluI site nearest the 10-kb position (see Fig. 1b). However, in the chromatin samples, this band was barely detectable. Instead, a very prominent broad band at 1.7 kb (band B) and another at 2.3 kb (band A) were seen. These bands are fragments which extend respectively from the AluI sites between the Type IV and V repeats (band B) and from the AluI sites in the transcription unit (band A) to the end of the molecule. Therefore, in contrast to the naked DNA, all three AluI sites between the Type V repeats and the terminal CCCCAAA repeats are very resistant to AluI digestion when packaged into chromatin. These AluI sites all fall in the core regions of the three phased nucleosomes mapped by the micrococcal nuclease results described above. By analogy with the findings for the central spacer region, these results further support the conclusion that there are uniquely positioned nucleosomes in this region of the 3'-NTS and a different chromatin structure in the region between the Type IV and V repeats.

DISCUSSION

The results presented here show that the telomere-associated 3'-NTS of *Tetrahymena* rDNA has a highly specific chromatin structure in the macronucleus. Using three different nuclease digestions, we have shown that there are sites in the 3'-NTS which are differentially accessible when whole macronuclei are digested, compared with purified DNA. These sites have been mapped relative to known sequence and functional features of this region of the rDNA. Fig. 6 shows diagrammatically the nuclease cutting patterns of the rDNA 3'-NTS in macronuclei, superimposed on the map of this rDNA region (7), and the chromatin structure proposed on the basis of these findings.

**Packaging of the Telomeric CCCCAA Repeats of the rDNA**—Previous work has shown that the (CCCATG), sequences from total macronuclei do not appear to be packaged as nucleosomes (15). Instead, the block of repeated sequence in macronuclei was digested by micrococcal nuclease to a limit size of DNA fragments forming a broad band of average length of approximately 300 base pairs, a finding confirmed in the present study. This study also shows that the inner boundary of the telomeric CCCCAA repeats is clearly demarcated by an adjacent specifically positioned nucleosome. The terminal heterogeneity of the rDNA therefore must account for the previously seen size heterogeneity of the protected telomeric repeats in chromatin (15). Recent work on the telomeric sequence of the macronuclear DNA of the ciliate *Oxytricha*, which consists of <50 base pairs of tandemly repeated CCCCATG, suggests that this sequence also has non-nucleosomal packaging (21). Gottschling and Cech (21) have suggested the possibility that the CCCCATG repeats of *Oxytricha* macronuclear telomeres are protected by a half-nucleosome. However, the limit size of the block of CCCCAA repeats (~300 base pairs) protected in *Tetrahymena* macronuclei precludes this interpretation in this organism. The CCCCAA-containing complex also behaved differently from macronuclear nucleosomes under various ionic conditions, providing further evidence for non-nucleosomal packaging (15). We have previously examined the telomeric region of rDNA for covalently or tightly associated proteins of the type found on linear viral DNA molecules. However, none were detected even under conditions where a low amount of tightly bound polypeptide was detected in internal regions of the rDNA (9). The specialized roles proposed for telomeres in their replication and stabilization of chromosome ends make it likely that they are packaged with proteins involved in one or both of these functions.

**Occurrence of Specifically Positioned Nucleosomes Adjacent to Telomeric Repeats**—Our results provide strong evidence that there are three specifically positioned nucleosomes immediately adjacent to the terminal CCCCAA repeats of the rDNA. The micrococcal nuclease digestion results indicate that these nucleosomes are spaced 190, 190, and 150 nucleo-

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**Fig. 5. Accessibility of rDNA in macronuclei to AluI digestion.** Macronuclei were isolated from logarithmically growing cells, resuspended in buffer A (2.7 × 10⁷ nuclei/ml), and digested with AluI (1 unit/µl) for 1, 3, and 10 min (lanes 1–3, respectively) at 30°C. Purified DNA (250 µg/ml) was digested in buffer A with AluI (1 unit/µl) for 1.5, 10, 20, and 30 min (lanes 4–8, respectively) at 30°C. DNA was extracted from the samples, fractionated on a 1.2% agarose gel at 2.3 V/cm for 14 h, and bidirectionally transferred to nitrocellulose filters. The filter shown was probed with the 32P-labeled DdeI fragment located 9.8–10.1 kb from the center of the palindromic rDNA (see Fig. 1). Sizes of bands A–C are 2.5, 1.7, and 1.2 kb, respectively.
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**Fig. 6. Schematic representation of the chromatin structure in the telomeric region of T. thermophila rDNA.** The positions of nuclease cutting sites used to infer the chromatin structure are shown. $\ddagger$, telomeric CCCCAA repeats; $\mathbf{\ddagger}$, 3'-NTS; open bar, transcription unit; two solid rectangles, Type IV repeats; downward arrowheads and white arrow, micrococcal nuclease cutting sites; upward arrowheads, DNase I sites; upward triangles = AluI cutting sites; arrows marked T, positions of topoisomerase I-binding sites identified in chromatin by Bonven et al. (20). Nuclease sites unique to, or enhanced in, the chromatin compared with naked DNA are shown by filled symbols; sites whose accessibility is blocked in the chromatin are shown by open symbols. The hatched region indicates the chromatin structure encompassing the Type V repeats; the three phased nucleosomes are cross-hatched. Bar on lower right = 0.2 kb. Open bar over the DNA map in the transcription unit and downstream region indicates a chromatin region relatively accessible to nucleases.

tides apart. This spacing is similar to that of the seven phased nucleosomes previously found in the rDNA central spacer region (19). In addition, our results with AluI strongly suggest that DNA packaged in a nucleosomal core can be well protected from digestion with this restriction enzyme.

In the ciliate *Oxytricha*, the macronuclear telomeres also were shown to be adjacent to phased nucleosomes (21). In addition, analysis of the rDNA chromatin structure in *Dicytostelium* suggests a similar situation (34), although the positions of the telomere-associated nucleosomes appear to be less clearly defined than those seen adjacent to the ciliate macronuclear telomeres. Other examples of phased nucleosomes have also been seen in regions of chromatin positioned on at least one side by sites for sequence-specific binding of proteins. The regions flanking the centromeres of yeast chromosomes or plasmids are packaged in phased nucleosomes (35), and nucleosome phasing has been reported in the repeated 5 S RNA gene regions of *Xenopus* and *Tetrahymena* (36, 37) and in untranscribed tRNA genes (38). Some data suggest that in these situations nucleosome phasing is imposed by the binding of specific protein(s) to neighboring DNA sequence(s), which results in exclusion of nucleosomes from that region. However, the specific positioning of nucleosomes adjacent to yeast centromeres is also consistent with this positioning being influenced by preferred DNA-histone interactions (35). In the case of the rDNA telomere-associated regions, one boundary of the region of DNA available for nucleosome formation most likely is set by the telomeric repeated sequence and its associated non-nucleosomal protein(s). From the results reported in this paper, the other boundary in the *T. thermophila* rDNA 3'-NTS is very likely set by the domain consisting of the Type V repeats, which are packaged differently from the region found as the three phased nucleosomes.

Chromatin Structure of the Transcription Termination Region and the Type IV and V Repeats—Conservation in *Tetrahymena* and *Glaucoma* of the recently identified Type IV and V repeats in the 3'-NTS suggested that they are of functional importance (7). The results reported here support this hypothesis, since we find they are packaged in a distinctive chromatin structure, different from the three phased nucleosomes in the adjoining spacer region. A domain spanning virtually the entire Type V repeat region was demonstrated by micrococcal nuclease- and DNase I-sensitive sites at both ends (Fig. 6). In striking contrast to the neighboring region of three phased nucleosomes, this chromatin domain was accessible to DNase I cutting. Such relative sensitivity to DNase I is similar to that reported previously for specific regions in the 5'-NTS region of *T. thermophila* rDNA (19). Although the role of the Type V repeats is not known, it is noteworthy that in *Tetrahymena*, *Glaucoma*, and *Physarum* rRNA genes, Type V repeats with conserved unit lengths all occur at a fixed distance (~470 bp) downstream from the transcription termination site (7). Thus, they may be involved in transcription-related functions.

In addition to a micrococcal nuclease-sensitive site near the Type V repeats in rDNA chromatin, there were clear DNase I-accessible sites very close to the Type IV repeats in rDNA chromatin (Figs. 4 and 6). The accuracy of the mapping of these sites in this study allows them to be placed very near, but not coincident with, two phylogenetically conserved topoisomerase I-binding sites in this region (20). DNase I-sensitive sites have been shown to be the sites of DNA sequence-specific binding proteins in other systems (39, 40), and the proximity of the highly conserved Type IV repeats to the transcription termination site (7) suggests that this region may be involved in transcription termination.

The macronuclei used in these experiments were prepared from exponentially growing *Tetrahymena* cells, in which the rDNA and the separate 5 S RNA genes are transcribed at high rates. The nuclease-sensitive sites in the rDNA 3'-NTS are therefore likely to be those of actively transcribed rDNA molecules. Although the nuclease cutting patterns of the 5'-NTS region of the rDNA are not significantly altered in starved cells (19), in which rDNA and 5 S gene transcription are arrested, an intragenic putative promoter region of the 5 S RNA gene loses DNase I hypersensitivity in starved *Tetrahymena* cells (37). Likewise, in yeast a 3' hypersensitive site downstream of a MAT locus transcription unit loses hypersensitivity in the nonexpressed locus (41). The nuclease accessibility of the *Tetrahymena* rDNA 3'-NTS might therefore be altered in starved cells. It will be of interest to determine whether nuclease-hypersensitive sites 3' to transcription units reflect a state of ongoing transcriptional activity while 5' sites may be involved in setting up a transcriptionally competent state.

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