Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 1332–1337, February 1997
Genetics

An unusual histone H3 specific for early macronuclear development in *Euplotes crassus*

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Communicated by David Prescott, University of Colorado, Boulder, CO, December 6, 1996 (received for review August 14, 1996)

ABSTRACT **Characterization of the histone H3 genes of the ciliated protozoan *Euplotes crassus* indicates that one gene functions only during the sexual phase of the life cycle. Maximum expression of this gene, as judged by transcript accumulation, correlates with DNA replications leading to polytenization of the micronuclear chromosomes before massive DNA elimination, which produces a transcriptionally active macronucleus. Transcripts of the other gene accumulate primarily during vegetative growth and in the sexual phase of the life cycle during replication phases not related to polytenization. Although both histone H3 genes encode proteins that are fairly divergent in sequence at the amino terminus, the meiotic/polytene-specific histone H3 contains two insertions in the amino terminus that increase the size of the protein by 15 amino acids. Analysis of micrococcal nuclease digests of chromatin using hybridization probes specific for micronuclear vs. macronuclear sequences indicates that a change in nucleosomal spacing correlates with the maximal expression of the meiotic/polytene-specific histone H3 gene. Thus, we surmise that this unusual histone H3 may play a key role in targeting DNA sequences for either transcriptional activation and retention in the macronucleus or heterochromatization and elimination.**

Ciliated protozoa possess two kinds of nuclei: (*i*) micronuclei that are transcriptionally repressed during most of the organism's life cycle, that divide mitotically and meiotically, and that participate in sexual exchanges that result in the generation of new zygotic micronuclei and (*ii*) macronuclei that are transcriptionally active, that divide by an "amitotic" mechanism, and that are generated from a copy of the zygotic micronucleus during the sexual phase of the life cycle (1). This nuclear duality has been long recognized as an ideal system for studying molecular differences in nuclear structure and function. This is epitomized by studies of *Tetrahymena* histones, in which many differences in histone proteins and their modifications have been correlated with the different functions of the micronucleus and macronucleus (summarized in refs. 2–4). Several of the histone modulations documented in *Tetrahymena* have been shown to be conserved in other organisms. For example, a histone H2A variant protein, hv1, that is specific for the transcriptionally active macronucleus (5) has homologs in other organisms that associate with nucleoli (6). Likewise, the details of histone acetylation during histone deposition (and as a function of transcriptional activation) have been characterized extensively in *Tetrahymena* (3) and have led to the realization that histone acetyltransferase plays a key role in transcriptional activation (7).

Hypotrichous ciliates such as *Oxytricha*, *Stylonychia*, and *Euplotes* have more dramatic differences between their macronuclei and micronuclei than the holotrichous ciliates, such as *Tetrahymena* and *Paramecium* (reviewed in refs. 8–10). For instance, (*i*) their micronuclear genomes contain vast amounts of DNA that are not represented in the transcriptionally active macronucleus and thus could be representative of completely inactive heterochromatic sequences in other organisms. Whereas *Tetrahymena* eliminates 10–15% of its micronuclear DNA sequences during formation of a macronucleus, the hypotrichs typically eliminate 90–95% of the sequences. Thus, the chromatin remodeling required to form a transcriptionally active macronucleus is likely to involve differentiation that targets a minority of sequences for activation and the majority of sequences for active elimination. (*ii*) The differentiation of a micronucleus into a macronucleus in hypotrichs involves the formation of polytene chromosomes of 64 times ploidy or higher. This may allow further differentiation of chromatin structure during a replicative phase. (*iii*) Macronuclei in hypotrichous ciliates contain short, "gene-sized," linear DNA molecules, in which the telomeres are frequently found within 100 bp of a gene coding region (reviewed in refs. 9 and 11). Thus, the condensation state and chromatin dynamics of these sequences could be quite different from *Tetrahymena*, in which macronuclear chromosomes average 600 kb in size (12, 13).

The hypotrichous ciliate *Euplotes crassus* is ideally suited for studies of developmental changes in chromatin structure because its defined mating types allow synchronization of the sexual phase of the life cycle, which includes numerous S phases and nuclear divisions (14, 15). The sexual phase initiates when starved cultures of two different mating types are mixed; hence, developmental timing is referred to in hours postmixing. Cells initiate conjugation, or pairing, in 2–4 h, followed by meiosis as well as further mitotic divisions of the resulting haploid products. Haploid nuclei are exchanged between paired cells and fuse with a resident nucleus to form a zygotic micronucleus that divides mitotically to produce four identical diploid nuclei, one of which differentiates into a macronucleus, or anlagen. Chromosomes in the anlagen are polytenized, after which they are fragmented, with concomitant telomere addition to the macronuclear-destined sequences and elimination of all non-macronuclear-destined DNA. At the end of development, the anlagen changes shape, forming the typical tubular structure seen in Euplotids, and further rounds of DNA replication lead to the highly amplified levels of macronuclear molecules.

In this study, we have characterized histone H3 genes and their developmental pattern of transcript accumulation in *E. crassus.* The results reveal a modulation of histones not previously seen in *Tetrahymena*. We have identified two types

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. U65645 (*conZB1* cDNA), U65646 (*conZB1* macronuclear DNA molecule), and U75429 (vegetative histone H3 macronuclear DNA molecule)].
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of histone H3 genes with complementary transcription patterns. Transcripts of one gene accumulate specifically during the meiotic and early polytene stages of the sexual phase of the life cycle (to be referred to as a "polytene" histone H3) while transcripts of the other gene accumulate during vegetative growth and in the sexual phase at replication periods not including the polytene stage (to be referred to as a "vegetative" histone H3). The polytene histone H3 has an unusual structure that suggests it may play a major role in the chromatin remodelling that results in transcriptional activation of macronuclear genes and/or elimination of the majority of the micronuclear genomic sequences.

MATERIALS AND METHODS

Cell Culture, Nuclear Isolation, and Chromatin Digestion. The *E. crassus* strains, X1 and X2, used for the RNA preparations and nuclear digestions have been described (16, 17). Cells were cultured and mated as described (15, 18). Nuclear isolations were performed as described (15) except that cells were initially swollen in 10 mM Tris•HCl/1 mM EDTA, pH 8, followed by addition of Triton X-100 to 0.5% before homogenization. Pelleted nuclei were resuspended in 0.25 M sucrose/10 mM Tris•HCl, pH 7.5/15 mM NaCl/3 mM $MgCl_2$/0.5 mM $CaCl_2$ for digestion with micrococcal nuclease (Sigma). After incubation with the enzyme, reactions were stopped by the addition of EDTA to 10 mM. The nuclei were then pelleted, and DNA was isolated as described (18).

RNA Analysis. RNA was isolated by lysis of cells in guanidine, sedimentation through CsCl, and Qiagen (Chatsworth, CA) column purification (19). For Northern blot analysis, total RNA was electrophoresed on 1.5% agarose/2.2 M formaldehyde gels (20) and transferred and hybridized as described (19). Membranes were stripped by washing with boiling 0.1% SDS/2 mM EDTA, pH 8.0. Complete removal of probe was verified by autoradiography. Probes consisted of *in vitro* transcripts synthesized with T3 or T7 RNA polymerase (19). Primer extensions were performed essentially as described by Sambrook *et al.* (20), using the following primers and total RNA samples for H3(V) and H3(P), respectively: GB047, 5′-CTTTCTGGCGGATTTTGACCAATGTGCTTCC-3′, 72-h RNA; and GB056, 5′-TCTTTCTTGAAGCCTTCACTACACTTTTCC-3′, 20-h RNA.

Cloning and DNA Sequence Analysis. The vegetative histone H3 cDNA was obtained by rapid amplification of cDNA ends (21) using the degenerate oligonucleotide 5′-GARGAYACIAAYYTITGYGCIATYCA-3′ (Y = C/T, R = A/G, I = inosine), which corresponds to the conserved EDTNLCAI sequence near the carboxyl terminus of histone H3s (22). The PCR product was used as a hybridization probe to screen a macronuclear genomic library in phage λ gt10 (23). Isolation of the polytene H3 cDNA *conZB1* (clone LEDR-ZB1) has been described (24). The histone H3 insert of a plasmid derivative of this cDNA clone was labeled by the random hexamer procedure and was hybridized to a macronuclear genomic library (the LEMAC library described in ref. 25) to isolate clone LEMACZB1. The λ phage inserts were subcloned into pBluescript KS+ or SK+ (Stratagene), and dideoxy sequencing of the double-stranded templates was performed according to Kraft *et al.* (26) using Sequenase (United States Biochemical/Amersham). The complete sequences of the *conZB1* cDNA clone [*conZB1* (polytene histone H3) macronuclear DNA molecule and the vegetative histone H3 macronuclear DNA molecule] are available in the GenBank database under accession numbers U65645, U65646, and U75429, respectively.

RESULTS

Isolation of Developmental Stage-Specific Histone Genes. The H3 histone specific to the meiotic/polytene stage of development was initially isolated in a screen for polytene stage cDNAs that did not hybridize to a cDNA probe derived from vegetative mRNA (24). Hence, it was designated as a conjugation-specific gene, *conZB1*. Subsequent sequence analysis of the cDNA indicated similarity to a wide range of histone H3s in the GenBank database. The vegetative histone H3 also was initially isolated as a cDNA. A degenerate oligonucleotide primer was used in the rapid amplification of cDNA ends procedure (21) to amplify products from RNA isolated at 75 h of development, which corresponds to the final stage of macronuclear development when the macronuclear linear DNA molecules are amplified to their >1000 copy number (timing of development for *E. crassus* strains X1 and X2 is described in refs. 16 and 17).

The above cDNAs were used as hybridization probes to screen macronuclear genomic DNA libraries, and the isolated macronuclear linear molecules bearing the respective genes were sequenced in their entirety. Restriction maps of the linear molecules are shown in Fig. 1, along with the location and size of the respective histone H3 gene coding regions. The sizes of the macronuclear molecules for these two genes are quite different. The vegetative gene is carried on a 1.7-kb molecule, and the polytene stage gene is on a 663-bp molecule. Despite significant similarity at both the DNA and amino acid sequence levels (see below), neither cDNA cross-hybridized to the other gene in the library screenings. Hybridization of the coding regions of the two genes to macronuclear DNA indicated that, although these genes will cross-hybridize, the degree of cross-hybridization is much less than self-hybridization (data not shown). At low stringency, the polytene-specific probe hybridizes to one size class of macronuclear molecule (0.95 kb) that the vegetative-specific gene probe does not detect. We have not determined whether this additional size class has features resembling a histone H3 gene, but we suspect it does not because it does not hybridize with coding region probes from the vegetative H3 histone even at low stringency. Thus, it seems likely that these are the only histone H3 genes for this organism.

Developmental Patterns of Transcript Accumulation. Previous characterization of the H3 polytene cDNA clone indicated that transcripts first appear early in conjugation and reach a peak during the periods of micronuclear meiosis and the beginning of macronuclear development (24). Transcript levels gradually decreased during the period of polytene chromosome formation until about the onset of the period of chromosome fragmentation/telomere addition, when transcripts were no longer detectable. In contrast, initial Northern blot analysis with the vegetative gene indicated that transcripts for this gene were surprisingly low or absent at the start of macronuclear development (20 h) even though high levels of

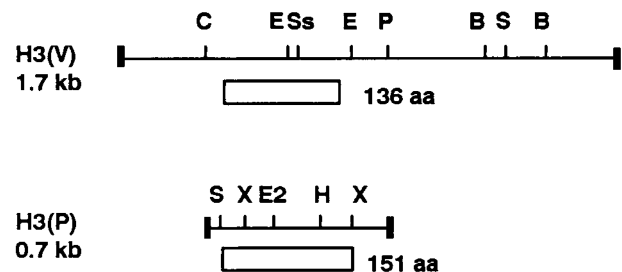

FIG. 1. Macronuclear molecules encoding *E. crassus* H3 histone genes. Restriction maps of the cloned H3 histone macronuclear linear DNA molecules are shown with the coding regions designated as boxes. B, *Bsm*I; C, *Cla*I; E, *Eco*RI; E2, *Eco*RII; H, *Hin*dIII; *P*, *Pst*I; S, *Ssp*I; Ss, *Sst*I; X, *Xho*II.

histone H4 mRNA were detectable. A direct comparison of the developmental patterns of transcript accumulation for these two genes is shown in Fig. 2 and confirms that the polytene gene transcripts are abundant at 20–30 h when low amounts of the vegetative H3 gene transcripts are detected. The histone H4 transcript accumulation appears to be the sum of the two histone H3 patterns (Fig. 2). All of the histone H3 transcripts decreased in abundance between 30 and 42 h as would be expected for the timing of the polytene replications (16, 17). The later time periods (55–60 h), when little or no histone message was detected, corresponded to the period of chromosome fragmentation and telomere addition and showed increased amounts of telomerase RNA. The vegetative histone transcripts accumulated again at 65–72 h postmixing, when the final replication phase begins. Thus, all of the histone transcripts detected for these genes corresponded to periods of DNA replication. In the RNA samples analyzed here, there was overlap in the timing of transcript accumulation for the two histone H3 genes during meiosis. However, this may be due to the lack of complete synchrony within the cell population and the fact that the "meiotic" RNA sample was pooled from samples of cells taken every 2 h over a 6-h interval.

DNA Sequence Analysis of the Macronuclear Molecules. The clones of the macronuclear DNA molecules encoding the vegetative and polytene histone H3s were completely sequenced. The vegetative gene encoded a normal sized histone H3 protein of 136 amino acids, including the initiating ATG methionine. In contrast, the sequence of the polytene gene revealed an unusually large open reading frame capable of encoding a 151-amino acid histone H3-like protein. Alignment of the predicted protein with other histone H3 protein sequences indicated that the C terminus was very similar to other histone H3 proteins, but the N terminus contained two blocks of 12 and 3 amino acids that were not present in other histone H3 proteins (Fig. 3). To verify that these interruptions were not due to introns, the *conZB1* cDNA clone was sequenced. The 5′ end of the cDNA began with the codon encoding the threonine at position 11 (Fig. 3) and contained sequences

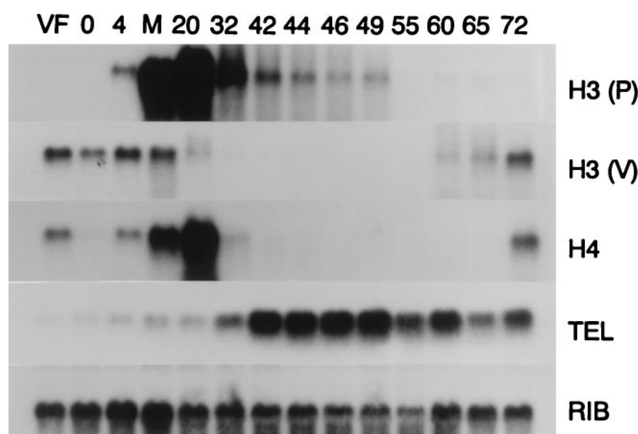

FIG. 2. Northern blot analysis of H3 histone transcripts. Total RNA (10 μg/lane) prepared from vegetative or mated *E. crassus* at various times of development was electrophoresed, blotted, and probed sequentially with the following gene sequences: H3(P), polytene-specific H3 histone; H3(V), vegetative H3 histone; H4 (23), H4 histone; Tel (27), telomerase RNA; and RIB (M. Erbeznik and C.L.J., unpublished observation), a probe for the small subunit rRNA. Developmental times of RNA samples are designated as follows: VF, vegetative fed cells (i.e., some cells are actively replicating DNA); 0, starved cells (unmated); and 4, 20, 32, 42, 44, 46, 49, 55, 60, 65, and 72, RNA prepared at X hours postmixing, where X is the number shown and M is the meiotic stage of development, consisting of total RNA prepared from pooled cells at 6, 8, 10, and 12 h postmixing of cells. Developmental times are specific to the X1 and X2 strains described (16, 17).

coding for the two insertions; thus, no evidence of introns was detected. The cDNA clone did contain three base differences relative to the macronuclear gene: two in the 3′-untranslated region and one in the coding region that would change an aspartic acid residue at position 97 to glutamic acid. We tentatively identified this as a second allele of *conZB1* although we cannot rule out that the base changes were the results of errors during cDNA synthesis. In addition, primer extension to map the 5′ end of the polytene histone H3 transcript used a primer that included 15 bp of the larger of the two insertions at the 3′ end of the primer. Significant levels of the primer extension products were observed, which indicates that these sequences are present in the mature messages.

Alignment of the predicted *E. crassus* H3 proteins with the sequences from *Tetrahymena* (28) and an H3 histone consensus sequence (22) are shown in Fig. 3. Both proteins are unusual in that they possess amino acid changes at residues that are highly conserved in other organisms. The amino acid differences in both sequences are especially prevalent in the amino-terminal region where the polytene gene contains the insertions. The H3s from *E. crassus* are 76% similar over their entire lengths (including the gapped regions due to the insertions). Comparison of the C-terminal 104-amino acid residues to the insertions shows 83% similarity. The entire encoded protein for the vegetative gene is 82%, 77%, and 86% similar to *Tetrahymena* HHT1, *Tetrahymena* HHT3, and the human replication-dependent H3, respectively (28, 29) whereas the entire polytene protein is 77%, 75%, and 75% similar to the same three sequences, respectively. Comparison of the C-terminal 104 residues of the polytene H3 yielded similarities of 83%, 81%, and 82% with these three sequences, respectively. Thus, in general, the comparisons of predicted proteins indicated that the two *E. crassus* proteins are about as similar to each other as they are to histone H3 proteins from other organisms and that both proteins are among the most divergent histone H3 proteins described.

Mapping of the transcription start sites for the two genes indicated that the vegetative gene has extensive 5′ nontranscribed sequences compared with the polytene stage gene, where the start site of transcription is only 3 bases internal to the telomere (Fig. 3*B*). This is the shortest distance between a telomere and transcription start site seen for an *E. crassus* macronuclear molecule to date (9, 11). We cannot rule out the possibility that transcripts originate from the micronuclear copy of the gene. However, we have demonstrated that at least one other conjugation-specific gene is transcribed from the macronucleus (24).

Chromatin Structure Changes in the Developing Macronucleus. The timing of transcript accumulation for the polytene vs. vegetative histone H3 genes suggested that the polytene histone H3 protein might accumulate in chromatin before the polytene stage. To date, we do not have clear data on the timing of accumulation of the protein because of the difficulties in separating micronuclei from old macronuclei at this stage and because we do not yet have antibodies specific to the polytene H3 histone. However, we have examined the periodicity of nucleosomes on micronuclear-specific sequences to determine whether changes in spacing correlate with the transcript accumulation for the polytene histone H3 relative to the vegetative histone H3.

We used micrococcal nuclease digestion of nuclei to examine chromatin structure in the micronucleus and developing macronucleus (anlagen) as well as the vegetative macronucleus. Digestions used preparations of total nuclei (micronucleus and macronucleus) or partially purified nuclei containing a combination of the anlagen and old macronuclei. The specific nucleosomal spacing patterns for the micronucleus or anlagen vs. the macronucleus were distinguished by sequentially hybridizing blots with probes specific to the two classes of nuclei. The micronucleus/anlagen-specific probe (Fig. 4*A*)

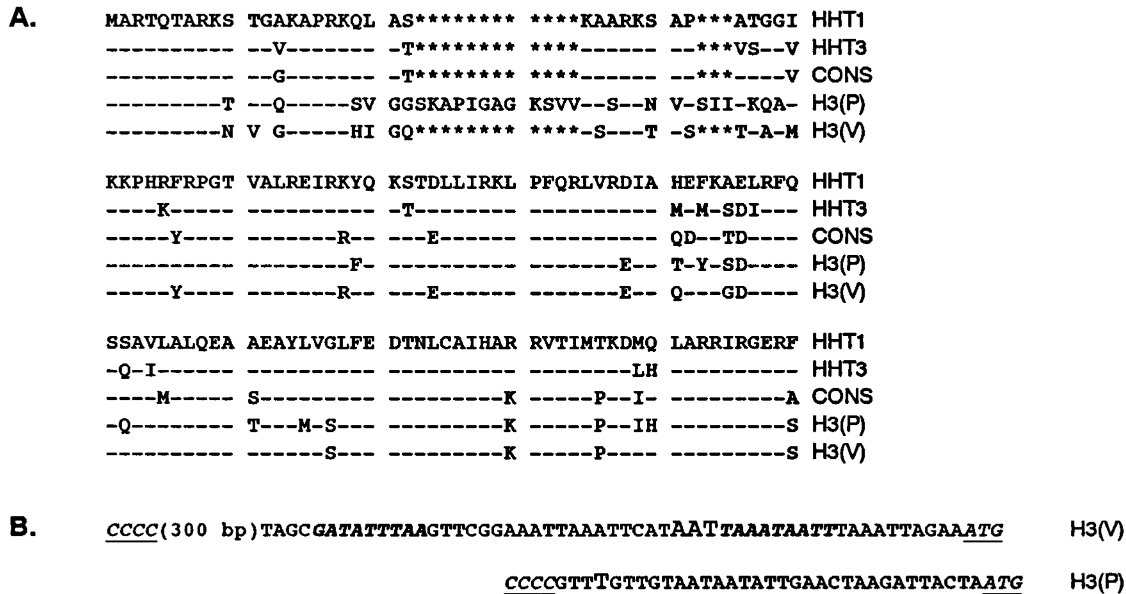

FIG. 3. (*A*) Alignment of predicted amino acid sequences of the *E. crassus* histone H3 genes with the *T. thermophila* histone H3 sequences. The sequences of two of the three *T. thermophila* H3 histones (HHT1, -3) (28) and a histone consensus sequence (22) were aligned with the deduced amino acid sequences of the *E. crassus* polytene-specific (P) and vegetative (V) H3 histones using the MEGALIGN program (DNAstar, Madison, WI). The third *Tetrahymena* gene, HHT2, encodes a protein that is identical to HHT1. Stars indicate the positions where the two insertions occur in the polytene-specific H3 histone. Positions where the amino acid sequences are identical to the *T. thermophila* HHT1 sequence are designated as dashes. *(B)* The DNA sequence of the 5′ noncoding regions of the two histone H3 genes is shown with the start sites of transcription indicated in a larger font. The ATG start codon and end of the telomeric repeats (CCCC) are italicized and underlined. Boldface italics in the H3(V) sequence indicate two 9-bp stretches of sequence identity with sequences surrounding the transcriptional start site of the H4 histone gene described previously (11).

consisted of a fragment from the highly repetitive Tec1 transposon, which is dispersed throughout the micronuclear genome in ≈7000 copies per haploid genome and is absent from the macronucleus. A fragment from the rDNA molecule (Fig. 4*A*) was used as a probe to examine macronuclear nucleosomal spacing because the rDNA is present in $>10^5$ copies per macronucleus and $<10^2$ copies per micronucleus or anlagen. Both of these probes encompass close to 1 kb of DNA; thus, in partial digests with micrococcal nuclease analyzed on agarose gels, they would hybridize to an array of DNA fragments produced from a minimum of five nucleosomes from within the probe as well as regions up to ≈1 kb on either side. Therefore, we assumed that the average nucleosome spacing detected with these probes would be representative of the micronuclear or macronuclear genomes. To minimize error due to gel-to-gel and lane-to-lane variability, blots of nucleosome-derived DNAs were first hybridized with the micronuclear/anlagen-specific probe, then stripped and rehybridized with the macronuclear-specific probe.

Micrococcal nuclease digestion experiments on nuclei derived from vegetative cells indicated that the amount of DNA per nucleosome (core plus linker) is greater in the macronucleus than in the micronucleus (Fig. 4*B*). Based on measuring the differences in sizes between DNA fragments derived from multimers of nucleosomes in eight different samples analyzed on four different gels (13 measurements), the mic-specific (Tec1 1.0 *Eco*RI fragment) probe gave a nucleosome spacing of 146 ± 15 bp (mean ± SD) whereas the mac-specific (rDNA 0.8 *Eco*RI fragment) probe revealed a spacing of 181 ± 29 bp. This micronuclear vs. macronuclear difference is similar to that reported for *Tetrahymena thermophila* and *Stylonychia lemnae* (30, 31) although our sizes appeared smaller (probably because of the low ionic strength and lack of spermidine in our nuclear isolation and digestions).

In micrococcal nuclease digestions carried out on nuclei (anlagen plus old macronuclei) derived from cells at 20, 25, and 28 h of macronuclear development (8 samples, 4 gels, 12 measurements), the mic-specific probe detected a spacing of 187 ± 27 bp, which was similar to the nucleosome spacing revealed by the mac-specific probe (175 ± 27 bp) on the identical blots (data not shown). The results for the analysis of 28-h samples with the mic-specific probe are shown in Fig. 4*B* for direct comparison with the vegetative samples. These results demonstrate a change in nucleosome spacing in the developing macronucleus compared with its micronuclear progenitor. To determine when this change occurred, additional analyses were carried out on nuclei from developing cells at 2-h intervals; the results of the 18- and 20-h analyses are shown in Fig. 4*C*. At 18 h, the nucleosome spacing observed with the mic-specific probe was identical to that observed for the vegetative micronucleus, but, by 20 h, the spacing shifted to a size similar to that of the vegetative macronucleus. Analyses of the same blot with the mac-specific probe revealed no change in spacing, as expected. The time of the observed change in spacing corresponded to the start of macronuclear development during the first of two periods of DNA replication involved in the formation of polytene chromosomes (16).

DISCUSSION

A surprising outcome of our characterization of histone H3 genes and their transcript accumulation in *E. crassus* was the unusual structure of the polytene H3 histone with the two insertions in the amino terminus. Although histone H3s from other protozoans are similarly divergent (32, 33), they have typically been identical or smaller in size to histone H3s from higher eukaryotes, with decreases in the length of the amino terminus rather than the increase in size seen for the *E. crassus* polytene histone H3. To date, the only two H3-like histone proteins identified that are this unusual at their amino termini are centromeric proteins, CenpA from humans (34) and the CSE4 protein from yeast (35). Both of these proteins have been implicated in the modification of nucleosome and higher order chromatin structure at centromeres. We expect that the extra

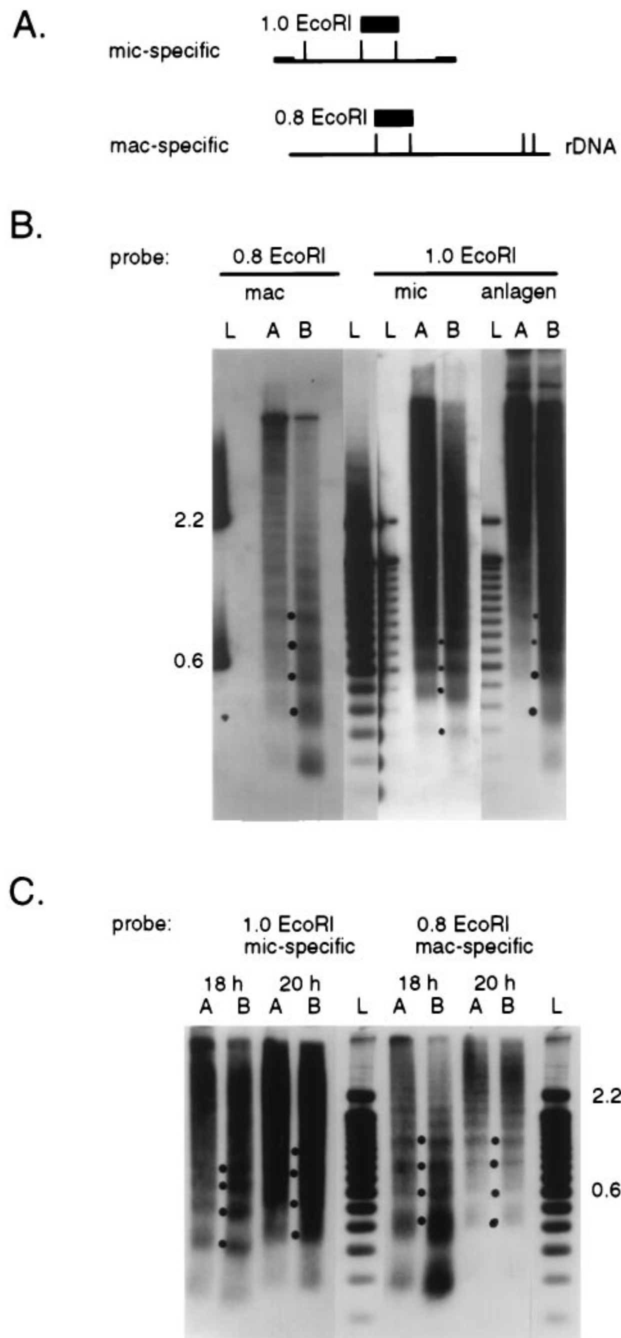

FIG. 4. Micrococcal nuclease digestions of chromatin. *(A)* Locations of the restriction fragments used as hybridization probes within the 5.3-kb Tec1 element or the 7.5-kb macronuclear rDNA molecule are shown as boxes; the Tec element inverted repeats are shown as small rectangles. *(B)* Autoradiographs of Southern blot analyses of DNAs derived from micrococcal nuclease-digested chromatin hybridized with either the mic-specific Tec element or mac-specific rDNA probes. Nuclei from vegetative cells (mac and mic) and from mated cells at 28-h postmixing (anlagen) were incubated with micrococcal nuclease for 2 (A lanes) or 10 min (B lanes), and the DNA was extracted and electrophoresed side-by-side with marker DNA fragments (L lanes; the 100-bp ladder from GIBCO/BRL). After Southern blotting, the filter was hybridized with the mic-specific (1.0 *Eco*RI) probe, stripped, and then hybridized with the mac-specific (0.8 *Eco*RI) probe. Fragments in the marker lanes were visualized by hybridization with nick-translated 100-bp ladder or, in the case of the rDNA 0.8 *Eco*RI probe, with the associated plasmid sequences, which hybridized only to the 600-bp and 2.2-kb ladder fragments (labeled to the left). Dots between the A and B lanes indicate the positions of bands corresponding to the dimer, trimer, tetramer, and pentamer nucleosome repeats used to determine spacing. *(C)* Autoradiographs of Southern blot analyses containing DNA isolated from micrococcal nuclease-digested chromatin from developing cells at either 18 or 20 h of development. Methods and labeling of the figure are as described above.

amino acids in the *E. crassus* polytene histone H3 may allow a similar modification of chromatin structure in the developing macronucleus.

Structural studies of nucleosomes indicate that the histone H3 amino termini project outwards into the spacer region between nucleosomes and are not involved in the nucleosome core structure (36). Thus, we would not expect the insertions in the polytene histone H3 to affect the nucleosomal core structure. However, recent experiments in yeast indicate that the amino termini of histones H3 and H4 play a role in nucleosome spacing during chromatin assembly (37). Thus, an unusually large H3 histone, such as the *E. crassus* polytene H3, might lead to an altered spacing such as that seen for the Tec element sequences. On the other hand, the spacing may be a function of what other proteins interact with the nucleosomes that contain this unusual histone. For instance, changes in linker histones or the lack of linker histones could alter the spacing.

Our analysis of transcript accumulation for the two histone H3 genes from *E. crassus* suggests that the polytene H3 is the only histone H3 incorporated into the developing macronucleus during polytene chromosome formation. Although some overlap occurs in the timing of transcript accumulation for these two genes, only the polytene gene shows the extremely high amounts of transcripts that are seen for histone H4 (Fig. 2) and histone H2B (C.M.T. and C.L.J., unpublished observations). Amplification in the polytene stage involves replication of more DNA (20 times the micronucleus) (38) than any S phases associated with micronuclear divisions earlier in the sexual phase (2- or 4-time increases), so it seems likely that the meiotic increase in the transcripts of the polytene H3 gene results in a later (polytene), specific incorporation of the protein. This scenario is supported by the observed timing of changes in nucleosome spacing. The chromatin structure detected with the Tec element probe (Fig. 4) changes spacing at the midpoint of the first replication period in the polytene stage (16). This suggests that a modified nucleosome structure has been incorporated during this replication.

The specificity of this gene for the polytene stage suggests that this unusual histone may play a role that is specific to the polytene chromosome structure. For instance, this protein may allow interactions between daughter chromatids in the polytene state. In addition, the timing of the change in chromatin structure (18–20 h) suggests a role for this protein in DNA elimination because the change in Tec element chromatin structure immediately precedes en masse excision (21–23 h) (16, 17). It is possible that nucleosomes containing the polytene histone H3 are specifically targeted to sequences that are either eliminated or retained in a manner analogous to the human CENP-A histone H3 protein that localizes to the centromere (34). However, the relatively high levels of expression of the polytene H3 gene relative to the vegetative H3 gene suggest that the histone protein derived from the former may be globally incorporated into replicating DNA during the polytene chromosome stage. Even if this is the case, it is still possible that the polytene histone H3 protein could be involved in defining specialized chromatin domains through the differential modification of its unusual N-terminal region. Studies of yeast histone H3 and H4 have illustrated the importance of the amino termini in defining alternate states of chromatin despite their global incorporation. Genetic and biochemical studies indicate that the amino termini interact with the SIR3 and SIR4 proteins that are involved in forming a repressed chromatin structure at the silent mating type loci (*HML* and *HMR*) and at telomeres (39). Transcriptional repression and activation at many other loci also require the amino-terminal tails

(40, 41). Some of the activating effects of the amino termini of H3 and H4 are likely to be mediated by acetylation (7) with repression requiring deacetylation (42). By analogy, protein interactions with the unusual *E. crassus* polytene histone H3 may be critical for targeting sequences as heterochromatic domains that are to be eliminated, and acetylation of this histone could specify sequences as transcriptionally active and macronuclear-destined. Although the larger of the two insertions in the *E. crassus* polytene histone H3 alters the spacing of two of the acetylation sites that are conserved in other organisms, differential acetylation is possible. The insertion includes lysine residues that may be acetylated, thus this histone may have an altered, and possibly increased, number of acetylation sites.

A candidate for a protein that could specify heterochromatization and elimination of DNA has recently been identified in *Tetrahymena.* The Pdd1p protein colocalizes with eliminated DNA sequences in condensed chromatin structures near the periphery of the developing macronucleus (43). The protein also is found in old macronuclei that are undergoing an apoptotic-like degeneration. Sequencing of the gene encoding this protein indicates that it is related to chromodomain proteins known to be associated with heterochromatin in *Drosophila* and fission yeast. Thus, in *Tetrahymena*, there appears to be an association between heterochromatin and DNA sequence elimination. Chromodomain proteins do not bind DNA directly but instead appear to affect chromatin via protein–protein interactions (44), so it seems likely that histones or other DNA-binding proteins may interact with the chromodomain protein to produce heterochromatin. This could be analogous to the interactions of histones with SIR3 or SIR4 in yeast (39). In *E. crassus*, the insertions in the amino terminus of the polytene H3 histone may mediate interactions with other proteins, such as the machinery that eliminates DNA from the developing macronucleus.

This work was supported by grants from the National Science Foundation to C.L.J. (MCB-9319009) and L.A.K. (MCB-9414416).

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