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Formation of Stable Chromatin Structures on the Histone H4 Gene during Differentiation in *Tetrahymena thermophila*

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The relationship between chromatin structure and the transcriptional activity of the histone H4-I gene of *Tetrahymena thermophila* was explored. Indirect end-labeling studies demonstrated that major DNase I- and micrococcal nuclease-hypersensitive sites flank the active macronuclear genes but not the inactive micronuclear genes. Runon transcription experiments with isolated macronuclei indicated that histone gene transcription rates decreased when cells were starved. However, macronuclear nuclease-hypersensitive sites persisted upon starvation. Thus, one level of transcriptional control of the H4-I gene results in altered chromatin structure and is established during nuclear differentiation. The rate of transcription is also controlled, but not through hypersensitive site-associated structures.

Because histone mRNA synthesis is often regulated both during differentiation and periodically through the cell cycle, histone genes provide a good model for studying the control of transcription. For this reason, we have been isolating and characterizing histone genes in *Tetrahymena thermophila*, a ciliated unicellular protozoan (3, 4). One of these genes, H4-I, codes for the major histone (H4) of *T. thermophila*. Like most or all genes in *T. thermophila*, it appears to be transcriptionally silent in the germinal micronucleus and active in the macronucleus. Frequent DNA sequence rearrangements (chromosome fragmentation, interstitial deletions) accompany gene activation during macronuclear development in *T. thermophila* (1, 2, 6, 10, 16, 22, 29, 30). Although the role of these rearrangements, if any, in the expression of macronuclear genes has yet to be demonstrated (3, 22), they nonetheless offer the possibility that ciliates have unique mechanisms of gene activation. This possibility is heightened by reports that ciliates show peculiarities in the genetic code (11, 14, 15, 23), suggesting that they are evolutionarily ancient. For the H4-I gene region, restriction maps for macro- and micronuclei are indistinguishable, indicating that no major sequence alterations accompany the activation of this gene during development.

Unique sequence regions of the H4-I gene hybridize preferentially to one of two H4 cytoplasmic messages (both found on polysomes), indicating that it is not a pseudogene. It appears to be periodically active through the cell cycle (M. A. Gorovsky, unpublished observations) and, upon starvation, its abundance relative to other messages is diminished. This could reflect a shortened message half-life or a reduced rate of transcription during starvation. To distinguish between these possibilities, we labeled preinitiated transcripts in macronuclei from growing and starved cells by elongation in vitro. RNA from these reactions was hybridized to an excess of histone DNA on nitrocellulose filters, and the relative abundance of H4 gene transcripts was determined as previously described (21). The average rate of H4 message synthesis in seven separate determinations was about fourfold lower in starved than in growing cells (data not shown), indicating that the regulation of H4 mRNA levels with starvation occurs, at least in part, at the level of transcript initiation.

Because the H4-I gene is the first RNA polymerase II-transcribed gene isolated from *T. thermophila*, it was of interest to compare H4-I gene chromatin with the chromatin of specific genes in higher eucaryotes. The various transcriptional states in which the H4-I gene is found gives us the opportunity to compare its chromatin in active and inactive states (macro- versus micronuclei) and under conditions in which it is transcribed at either high or low rates (growing versus starved cell macronuclei). In higher eucaryotes, gene activation is usually accompanied by alterations in chromatin structure (see references 12, 21a, and 25 for reviews). In particular, DNase-I-hypersensitive sites are often associated with genes transcribed by RNA polymerase II only when the genes are activated. To look for similar hypersensitive sites, we lightly digested nuclei with DNase I and purified and cut the DNA with *Eco*RI by methods previously described (21). The DNA was then fractionated by electrophoresis, blotted to nitrocellulose, and hybridized with a single-copy probe to sequences abutting the *Eco*RI site. Fragments which hybridized were defined by DNase I cut on one end and by the *Eco*RI cut on the other end. DNase I cuts were then mapped relative to the restriction site (19, 26) (Fig. 1).

In macronuclei, two regions flanking the gene are hypersensitive to DNase I. Each region appears to consist of two closely spaced sites. Such multiple cuts in a nuclease-hypersensitive region could reflect cutting to either side of a DNA-bound transcription factor (27). We found no obvious sequence homologies between these two regions, suggesting that factors responsible for these two hypersensitive regions are different. Cuts in the upstream hypersensitive region were centered about 102 and 279 base pairs 5′ to the translation start site and just 5′ to the presumptive transcription start site (preliminary transcript mapping [J. Bowen, S. Horowitz, and M. A. Gorovsky, unpublished observations] placed the 5′ end of the message about 50 base pairs upstream of the ATG). Cuts in the downstream hypersensitive region lay about 286 and 418 base pairs from the last

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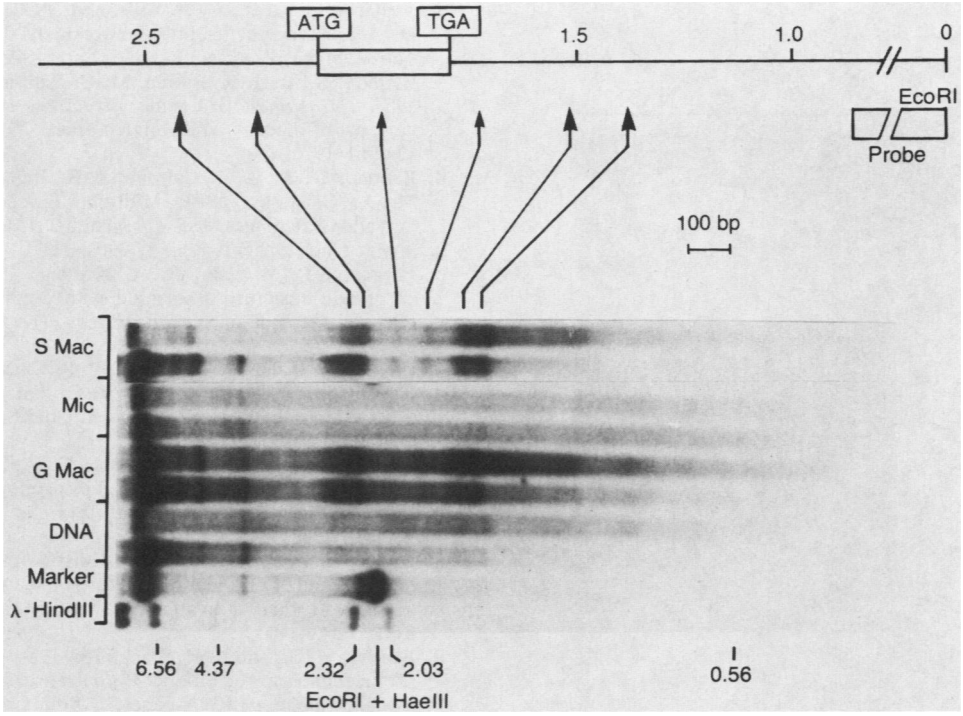

FIG. 1. DNase I cuts in the H4-I gene region of *T. thermophila*. *T. thermophila* B7 was grown and DNA and nuclei were isolated and digested with DNase I as previously described (21). DNA was purified, cut with *Eco*RI, electrophoretically fractionated, partially hydrolyzed, denatured, blotted to nitrocellulose, and hybridized with a nick-translated probe (homologous to the region shown) by previously described methods (22). Lanes labeled S Mac, Mic, G Mac, and DNA represent, respectively, digests of macronuclei from starved cells, micronuclei from growing cells, macronuclei from growing cells, and purified DNA. Fragments were sized by using standards consisting of λ DNA cut with *Hin*dIII and φX174 replicative-form DNA cut with *Hae*III. The first two codons of the H4 gene are specified by the sequence 5′-ATGGCC-3′. Since this sequence contains a *Hae*III site (5′-GGCC-3′), DNA cut with *Eco*RI and *Hae*III served as a reference point to relate cutting sites to the translation start site in the gene. Major cuts made by DNase I in macronuclei were mapped onto a drawing of the H4-I gene region. In the drawing, distances from the reference *Eco*RI site are indicated in kilobase pairs, and the locations of translation start and stop sites are indicated, respectively, by an ATG and a TGA. bp, Base pairs.

base of the translation stop site, near the end of the message, which has been provisionally mapped at about 450 base pairs from the TGA. Two relatively minor DNase I-sensitive sites lay between these hypersensitive regions at roughly nucleosome-sized intervals (≅ 220 base pairs).

None of the DNase I-hypersensitive sites were prominent in digests of purified DNA or in transcriptionally inactive micronuclei (Fig. 1). The minor cuts in the free DNA substrate at the sites cut in macronuclei may have been due to an endogenous nuclease that digests free DNA prior to its isolation from macronuclei. Similar faint bands in micronuclei may have been derived from low levels of macronuclear contamination in micronuclear preparations. The correlation between nuclease hypersensitivity and transcriptional activity seen here is like that observed during the activation of histone genes during sea urchin development (8, 28).

Despite differences in H4-I gene transcription rates, no major differences were observed between the DNase I-hypersensitive sites in macronuclei of growing and starved cells (Fig. 1). Therefore, these sites probably reflect a transcriptionally competent state rather than transcriptional activity per se. Further, modulation of the rate of transcription appeared not to occur through changes at the hypersensitive sites. The absence of a change in nuclease hypersensitivity in response to a physiologically induced change in transcription rates is in contrast to that seen for the 5S ribosomal genes of *T. thermophila* (21), for the PHO5 gene of *Saccharomyces cerevisiae* (5), or for the heat shock genes of *Drosophila melanogaster* (13, 17, 24, 25, 26).

The spacing between the DNase I-sensitive sites and between each of these and the adjacent hypersensitive regions (≈210 and 270 base pairs) suggests the presence of three discretely positioned nucleosomes between these sensitive sites. To gain further information about nucleosome positioning, we lightly digested nuclei and purified DNA with micrococcal nuclease and analyzed them by the methods used for mapping DNase I cuts (Fig. 2). The pattern and relative intensity of the cuts in the H4-I region of macronuclei were similar to those seen with DNase I digestion. However, cuts at or near the macronuclear sites were also evident in micronuclei and in free DNA, indicating that they are made at micrococcal nuclease-preferred sequences. Because the cuts occurred at nucleosome-sized intervals, the pattern could be interpreted as reflecting either randomly positioned nucleosomes or discretely positioned nucleosomes in which the sites preferred by micrococcal nuclease are exposed in linker regions. Nonrandom micrococcal nuclease cutting of free eucaryotic DNA at nucleosome-sized intervals was recently reported but was restricted largely to noncoding DNA (18).

With this study, genes transcribed by all three eucaryotic polymerases have been shown to contain nuclease-hypersensitive sites in the macronuclei of *T. thermophila*. Although the chromatin of the large rRNA gene (7, 9, 20) has not yet been studied in micronuclei, the hypersensitive cuts

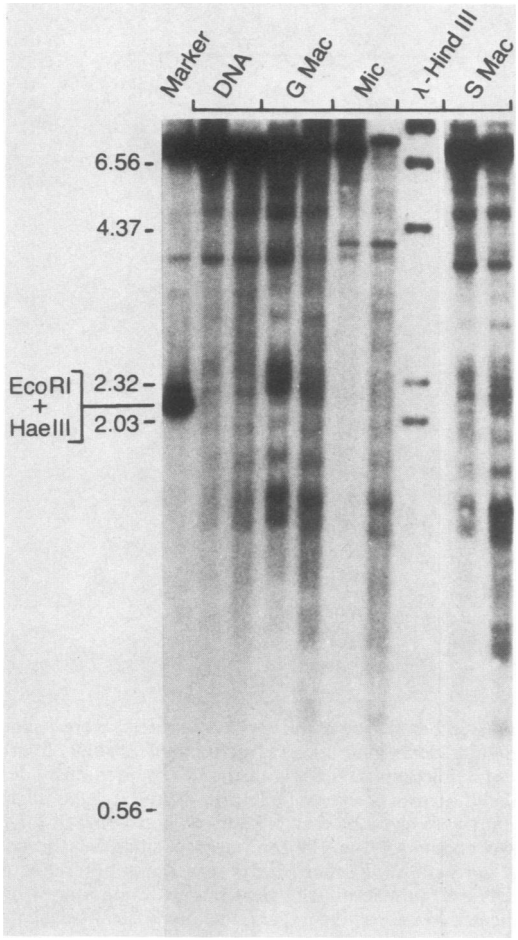

FIG. 2. Micrococcal nuclease cuts in the H4-I gene region of *T. thermophila*. Macronuclei from growing or starved cells, micronuclei, and purified DNA were digested with micrococcal nuclease as previously described (21) and analyzed as described in the legend to Fig. 1.

in a cluster of 5S genes (21) and in the H4-I gene (this study) have been shown to be macronucleus specific. The structural features of *T. thermophila* chromatin described here lend support to the use of macro- versus micronuclear comparisons as a model for nuclear differentiation in muticellular organisms. Since macro- and micronuclei reside in a single cytoplasm and are closely juxtaposed for much of the cell cycle, these studies also suggest that the hypersensitive sites reflect stable intranuclear structures rather than responses to a particular cytoplasmic milieu. The mechanisms by which alternative structural states of chromatin are established (presumably during their nonoverlapping S periods) and maintained in each nucleus are currently under investigation.

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