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NOTES

Methylation Site within a Facultatively Persistent Sequence in the Macronucleus of *Tetrahymena thermophila*

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DNA methylation occurs at the adenines in the somatic macronucleus of *Tetrahymena thermophila*. We report on a methylation site within a DNA segment showing facultative persistence in the macronucleus. When the site is present, methylation occurs on both strands, although only 50% of the DNA molecules are methylated.

DNA methylation in the ciliates is unusual compared with that in most eucaryotes. In *Tetrahymena thermophila*, 0.6 to 0.8% of the adenines are methylated (5), methylation occurring at the sequence 5′ AT 3′ (3). Methyladenines are only present in the pseudopolyploid somatic macronucleus, which is transcriptionally active, and not in the diploid germinal micronucleus, which is transcriptionally silent (5, 7). As a new macronucleus develops after conjugation, the chromosomes are fragmented and micronuclear DNA sequences are eliminated (reviewed in reference 4). At the same time, DNA becomes methylated (7). Within the ribosomal DNA of the macronucleus, all identified GATC sites are methylated on both strands; however, any particular rDNA molecule is only methylated at one or a few of the possible methylation sites (2). Uniformly methylated GATC sites are found near the genes coding for a histone H4 gene and a heat-shock-induced protein, hsp73 (6). These sites are methylated de novo during the development of the macronucleus, and the state of methylation does not change under physiological conditions expected to change gene activity (6). Adenine methylation is also seen at a specific *Eco*RI site on both DNA strands of the macronuclear copy of the *cnjC* gene (8).

We recently studied a DNA region that includes a sequence which shows facultative persistence in the macronucleus (11). In some cell lines the sequence is eliminated, and in other cell lines it is retained in the macronucleus (10). In this report, we investigated a double-stranded methylation site which is found within this persistent sequence. All cell lines and methods used in this study have been described previously (11).

We have reported on a repetitive family of eliminated sequences, the C-B-A1 family (12), which is defined by DNA subclones C, B, and A1 derived from a micronuclear DNA clone, pT1008. One member of this repetitive family persists in the macronucleus of some cell lines. Half of this persistent sequence, along with adjacent sequences, is present in the macronuclear DNA clone pMS1. A subclone of flanking sequence, pMS1-a, was obtained. This sequence is found in a single copy in the macronucleus (11). The restriction maps of pT1008, pMS1, and pMS1-a and that of the macronuclear genomic DNA containing the persistent sequence are shown in Fig. 1.

The single-copy pMS1-a was used as a probe against genomic blots of macronuclear DNA digested with *Mbo*I. Four cell lines whose macronuclear contains the persistent sequence were used. Two bands (2.6 and 3.0 kilobases [kb]) of equal intensity were detected in the macronuclear DNA of all four cell lines (Fig. 2). They were also found in the macronuclear DNA of all other cell lines that contained the persistent sequence (data not shown). The restriction enzyme *Mbo*I cuts the sequence 5′ GATC 3′ only when it is not methylated (9). *Sau*3A is an isoschizomer of *Mbo*I which recognizes the same site but will cut at that site even if the

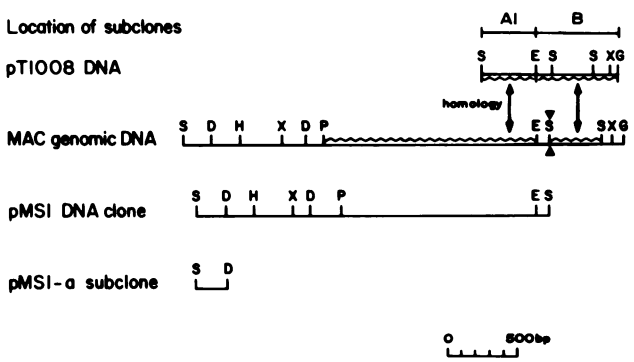

FIG. 1. Restriction maps of the macronuclear DNA (MAC) fragment, pMS1, flanking subclone PMS1-a, and pT1008 subclones A1 and B. The restriction map of the macronuclear DNA fragment from cell line 14C containing the persistent sequence was constructed by using genomic blots (11). The restriction maps of pMS1 and pT1008 were constructed by direct analysis of the plasmids and are more accurate than the genomic map. The restriction sites are designated as follows: D, *Hin*dIII; E, *Eco*RI; G, *Bgl*II; H, *Hpa*I; P, *Pvu*II; S, *Sau*3A or *Mbo*I; and X, *Xba*I. The regions of homology between the macronuclear DNA fragment and the subclones of pT1008 are shown as wavy lines. The solid triangles represent the site of methylation. bp, Base pairs.

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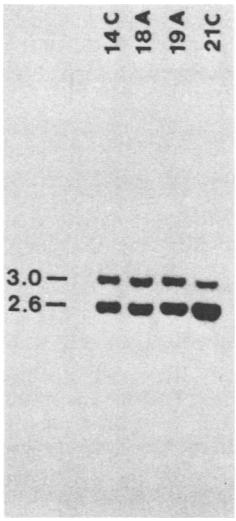

FIG. 2. Genomic blots of macronuclear DNAs from four cell lines that contain the persistent sequence were probed with pMS1-a. Each lane contains 5 μg of macronuclear DNA digested with *Mbo*I. The two bands of interest are labeled, with their sizes in kilobases.

adenine is methylated (9). To determine whether these two bands were the result of methylation, macronuclear DNA from the four cell lines was digested with *Mbo*I or *Sau*3A and the genomic blots were probed with A1, one of the subclones of pT1008 which recognizes the persistent sequence. The results with macronuclear DNA from one cell line are shown in Fig. 3; identical results were obtained with the other three cell lines. Both the 3.0- and 2.6-kb fragments were present in *Mbo*I digests of macronuclear DNA; however, only the 2.6-kb fragment was present in the *Sau*3A digests. Subclone B of pT1008 is adjacent to subclone A1 in pT1008 and also recognizes the persistent sequence. Subclone B also hybridized to the 3.0-kb fragment in *Mbo*I digests of macronuclear DNA, although it did not hybridize to the 2.6-kb fragment (Fig. 3). In addition, subclone B hybridized to several small fragments, including a 0.37-kb fragment. The small fragments other than the 0.37-kb fragment have already been characterized (10) and are not involved with the persistent sequence. When genomic blots of macronuclear DNA digested with *Sau*3A were probed with subclone B (Fig. 3), the 3.0-kb fragment was not detected, whereas the 0.37-kb fragment increased in intensity. This suggests that DNA subclone B hybridizes to the 0.37-kb fragment immediately adjacent to the 2.6-kb fragment. From the restriction maps in Fig. 1, we can predict that the site of methylation lies within the persistent sequence. The A1-B region of pT1008 is so similar to the persistent sequence of macronuclear DNA that we would expect a specific *Mbo*I site within subclone B of pT1008 to be analogous to the methylated *Mbo*I site in the persistent sequence (Fig. 1).

*Dpn*I is an isoschizomer of *Mbo*I and *Sau*3A and cuts at the restriction site only if the adenines on both strands are methylated (9). To determine whether methylation occurred on both strands of DNA, pT1008 was used as a probe of genomic blots of macronuclear DNA digested with *Mbo*I, *Sau*3A, *Mbo*I plus *Dpn*I, or *Sau*3A plus *Dpn*I. The genomic blots (Fig. 4) show that the 3.0-kb *Mbo*I band was resolved into the 2.6- and 0.37-kb bands when *Dpn*I was added. Thus, methylation occurs on both strands of the DNA at this methylation site. Similar observations were made for other GATC sites (2, 6). The 3.0-kb fragment was not completely digested, indicating that this site may have been hemimethylated, possibly during replication of the macronuclear DNA in cells in logarithmic growth.

The 3.0- and the 2.6-kb fragments were both present in *Mbo*I digests of macronuclear DNA that contains the persistent sequence (Fig. 2). Since both fragments were present, methylation did not occur on all the DNA molecules that contained the site of methylation. The fact that the bands were roughly equivalent in intensity suggests that only half of the molecules were methylated at this site. Methylation of 50% of the DNA molecules at a site is unusual. It cannot be due to allelic differences because the two methylation states have never shown phenotypic assortment, a behavior characteristic of alleles which occurs in all macronuclei (reviewed in reference 1).

The identification of a specific methylation site within a persistent sequence is a novel finding, as is the observation that only 50% of the molecules were methylated at this site. Since the state of methylation at GATC sites is resistant to changes in the physiological state, it has been suggested that methylation may not be involved in the activation of gene transcription but, instead, may function in DNA rearrangement (2, 6). Perhaps methylation occurs as a result of the

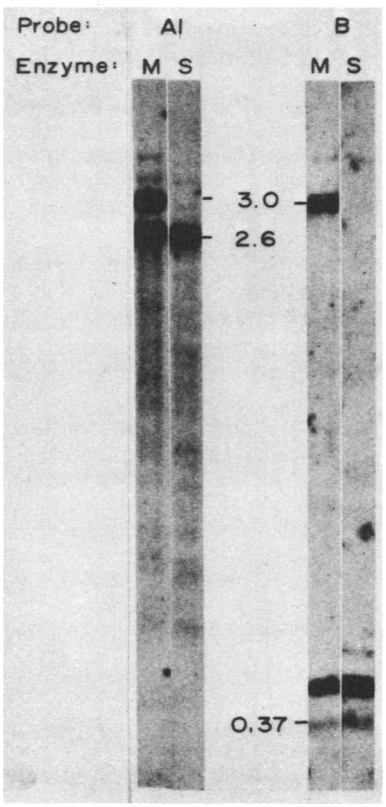

FIG. 3. Genomic blots of macronuclear DNA probed with subclones A1 and B derived from pT1008. Each lane contains 5 μg of macronuclear DNA from cell line 14C digested with either *Mbo*I (M) or *Sau*3A (S). The three bands of interest are labeled, with their sizes in kilobases. Other bands have been described previously and are not associated with this persistent sequence (10).

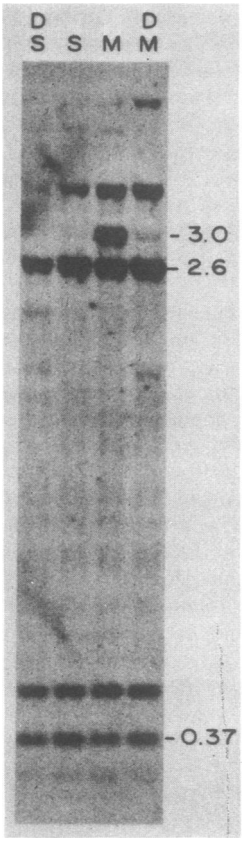

FIG. 4. Genomic blots of macronuclear DNA digested with one or more of the three enzymes *Mbo*I (M), *Sau*3A (S), and *Dpn*I (D). Each lane contains 5 μg of macronuclear DNA from cell line DI which contains the persistent sequence. The genomic blots were probed with pT1008. Bands of interest are labeled, with their sizes in kilobases. Other bands have previously been characterized (10) and are not associated with this persistent sequence.

occasional retention of this sequence during macronuclear development. Indeed, three other sites of methylation were detected in association with facultatively persistent sequences (data not shown). However, further analysis is not possible because macronuclear DNA clones that contain flanking sequences are not available for these persistent sequences, all of which belong to families of repeated sequences. Whatever the relationship between methylation and DNA rearrangement, both processes appear to act together to construct a region of functional DNA in the macronucleus.

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