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DNA Methylation and Demethylation Events during Meiotic Prophase in the Mouse Testis

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The genes encoding three different mammalian testis-specific nuclear chromatin proteins, mouse transition protein 1, mouse protamine 1, and mouse protamine 2, all of which are expressed postmeiotically, are marked by methylation early during spermatogenesis in the mouse. Analysis of DNA from the testes of prepubertal mice and isolated testicular cells revealed that transition protein 1 became progressively less methylated during spermatogenesis, while the two protamines became progressively more methylated; in contrast, the methylation of β-actin, a gene expressed throughout spermatogenesis, did not change. These findings provide evidence that both de novo methylation and demethylation events are occurring after the completion of DNA replication, during meiotic prophase in the mouse testis.

Mammalian DNA contains only one modified base, 5-methylcytosine (47); 5-methylcytosine is found primarily in the dinucleotide sequence 5′-CpG-3′ (11). Methylation changes in deoxycytosine residues of the dinucleotide sequence CpG in mammalian DNA have been implicated in the transcriptional activation of genes (for a review, see reference 6), changes in chromatin structure (18, 24), and parental imprinting (39, 43, 45). Most of the genes whose methylation patterns have been examined so far in mammalian spermatozoa are those that are expressed after fertilization. For many genes there are clear differences between CpG methylation in spermatozoa and that in somatic tissues. Many genes in spermatozoa are more highly methylated at CpG sites than in the somatic tissue where they are expressed; when these genes become methylated during germ cell development remains to be determined (22, 27, 28, 34, 36, 46, 48). Studies in the chicken indicate that de novo methylation events occur somewhere in the transition between spermatogonia and spermatocytes (18). In contrast, several DNA satellite sequences are less methylated in spermatozoa than in various somatic DNAs (9, 15, 16, 35, 42, 44); this undermethylation of satellite sequences occurs as early as primitive type A, type A, and type B spermatogonia (35).

Little is known about the methylation patterns of genes that are expressed exclusively in the testis; that is, whether the methylation of testis-specific genes changes during germ cell development in the testis and how such methylation changes relate to the transcription of these genes. We have used DNA from the testes of prepubertal mice of different ages and enriched populations of male germ cells to monitor the location and nature of methylation changes for three testis-specific genes during spermatogenesis.

The DNA methylation patterns for three postmeiotically expressed mouse genes encoding nuclear chromatin proteins, transition protein 1 (mTP1) (25), protamine 1 (mP1), and protamine 2 (mP2) (19, 20), were examined. mTP1 (26, 51), mP1, and mP2 (23, 50) are expressed at similar times during spermatogenesis, and their gene products serve a similar function in the testis (for a review, see references 19 and 20). We have compared the DNA methylation patterns of the three genes to gain an understanding of the role of methylation on the expression of testis-specific genes and to start to define the cellular localization of methylation and demethylation events in the testis.

Comparison of methylation patterns for mTP1, mP1, and mP2 in somatic and testicular tissues. Potential sites of CpG methylation in the coding and flanking regions for mTP1, mP1, and mP2 were monitored by digestion with restriction endonucleases; genes that are expressed throughout spermatogenesis—β-actin and the genes encoded by the mitochondrial genome—were included as controls.

The genes, probes, and restriction maps for mTP1, mP1, and mP2 are shown in Fig. 1. The localization and frequency of the dinucleotides CpG and GpC are shown for the sequenced areas of the three testis-specific genes. By using the criteria of Bird (5), the 5′ regions of mTP1, mP1, and mP2 were similar to those of other tissue-specific genes. The sequenced areas 5′ to the translation start site for mTP1 and the transcription start sites for mP1 and mP2 contained less than 50% G+C residues (mTP1, 44%; mP1, 47%; mP2, 49%) and were deficient in CpG dinucleotides, compared with GpC dinucleotides. There was an obvious increase in CpG residues in the coding regions of the three genes. Although a high CpG content is a common feature of tissue-specific genes (17), an alternative explanation could simply be that all three genes are arginine rich (10). Arginine is encoded by six different triplets; four of the triplets start with CpG. Sixty percent of the exon-containing CpGs in mTP1, 100% of those in mP1, and 81% of those in mP2 encode portions of arginine residues.

Since many of the correlations between methylation and transcriptional activity occur in the 5′ end of genes, we were interested in monitoring as many CpGs as possible. For mTP1, mP1, and mP2, adjacent 5′-flanking regions that have been sequenced contained very few CpG residues (Fig. 1). For the 5′ region of mTP1, three (43%) of seven of the CpGs were located within restriction sites suitable for monitoring methylation. For mP1, although the one site in the part of the 5′ area that has been sequenced could not be monitored, sites at −2.6 and −3.1 kilobases (kb) could be. For mP2, one (20%) of five of the CpGs occurring within 843 base pairs of the 5′ end of the gene could be monitored for methylation.

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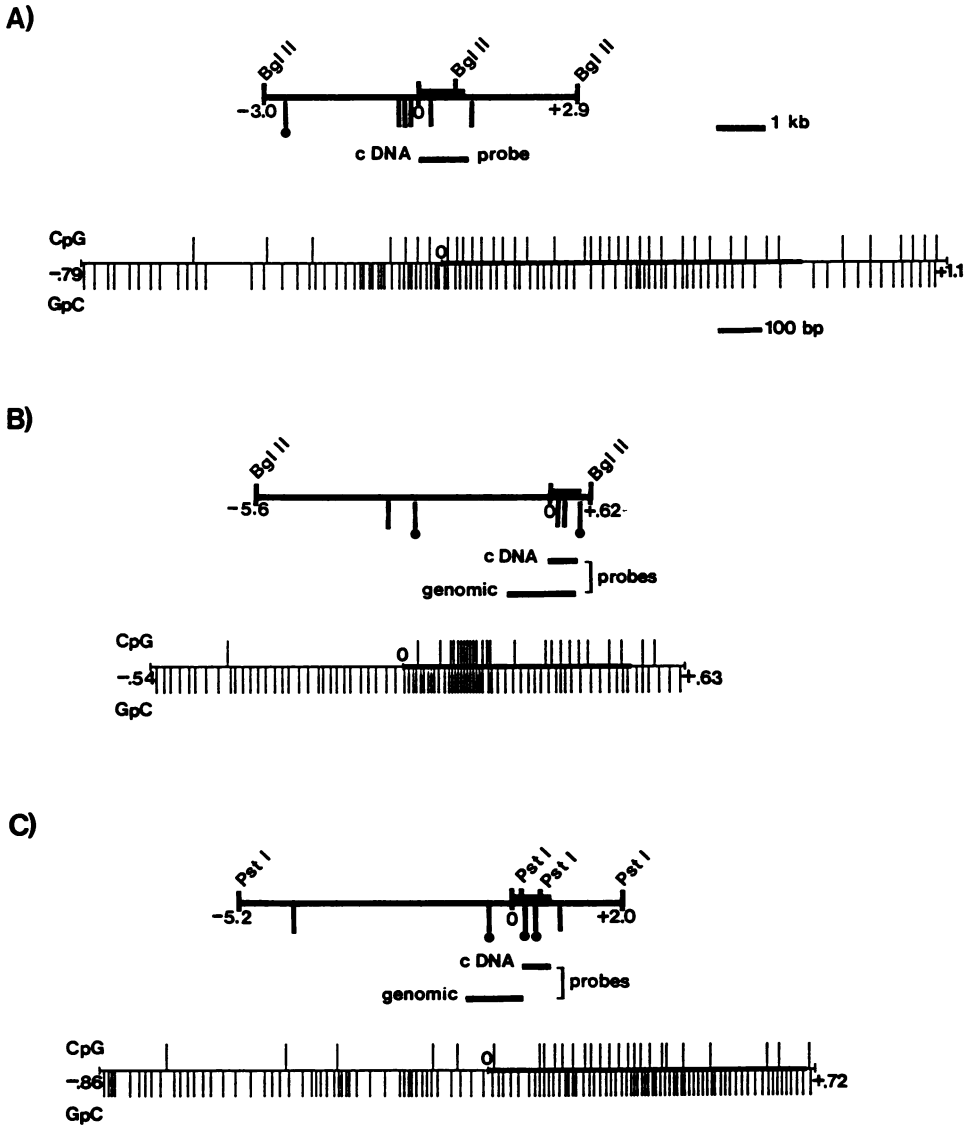

FIG. 1. Diagram of the gene maps, restriction enzyme sites, and probes for the analysis of mTP1 (A), mP1 (B), and mP2 (C). Map positions are shown with respect to the transcription start sites (position 0 on maps), except for mTP1, for which map positions are shown with respect to the translation start site (position 0). The bold-face lines represent the transcribed area of the genes. The locations of *Msp*I-*Hpa*II sites are indicated by vertical lines below the gene maps; *Hha*I sites are indicated by vertical lines with circles. The scale used for the gene maps is shown beside the map for mTP1; the same scale was used for the maps of mP1 and mP2. All CpG dinucleotides occurring within the sequenced area for mTP1, mP1, and mP2 are shown beneath the respective gene maps. The locations of CpG dinucleotides are marked by vertical lines above each gene map, whereas the locations of GpC dinucleotides are marked by vertical lines below each gene map. The scale used for the maps of CpG-GpC location is shown below the CpG-GpC map for mTP1; the same scale was used for the maps of mP1 and mP2.

The CpG content in the coding areas of these three genes was high; consequently, a smaller percentage of the available CpGs in the coding region versus the flanking regions could be monitored (Fig. 1).

The methylation patterns for mTP1 and mP1 for somatic and testicular DNA are shown in Fig. 2A and B, respectively. Testes and spleens from prepubertal (6, 8, 10, 12, 14, 16, and 22 days of age) and adult (60 to 74 days of age) CD-1 mice were collected for DNA extraction. Preliminary studies revealed that DNA methylation patterns did not differ between individual mice (J. M. Trasler and N. B. Hecht, unpublished results). Unless otherwise indicated, each sample was obtained from tissues of four to six mice. DNA was isolated by using previously published procedures (1, 12). Samples (20 μg) of each DNA preparation were digested first with the restriction endonucleases *Bgl*II (mTP1 and mP1), *Pst*I (mP2), or *Eco*RI (β-actin) by using 8 to 10 U of enzyme per μg of DNA. For the analysis of methylation patterns, a second enzyme digestion was performed with the methylation-sensitive restriction enzymes *Hpa*II or *Hha*I. Both *Hpa*II and *Msp*I recognize the sequence 5′-CCGG-3′; *Hpa*II only cuts if neither cytosine residue is methylated, whereas *Msp*I cuts regardless of the state of methylation of the internal cytosine residue. *Hha*I recognizes the sequence 5′-GCGC-3′ but will cut only if neither of the cytosine residues is methylated. Methylation studies for each gene and type of sample were repeated two to four times with different samples. The methylation patterns shown in Fig. 2

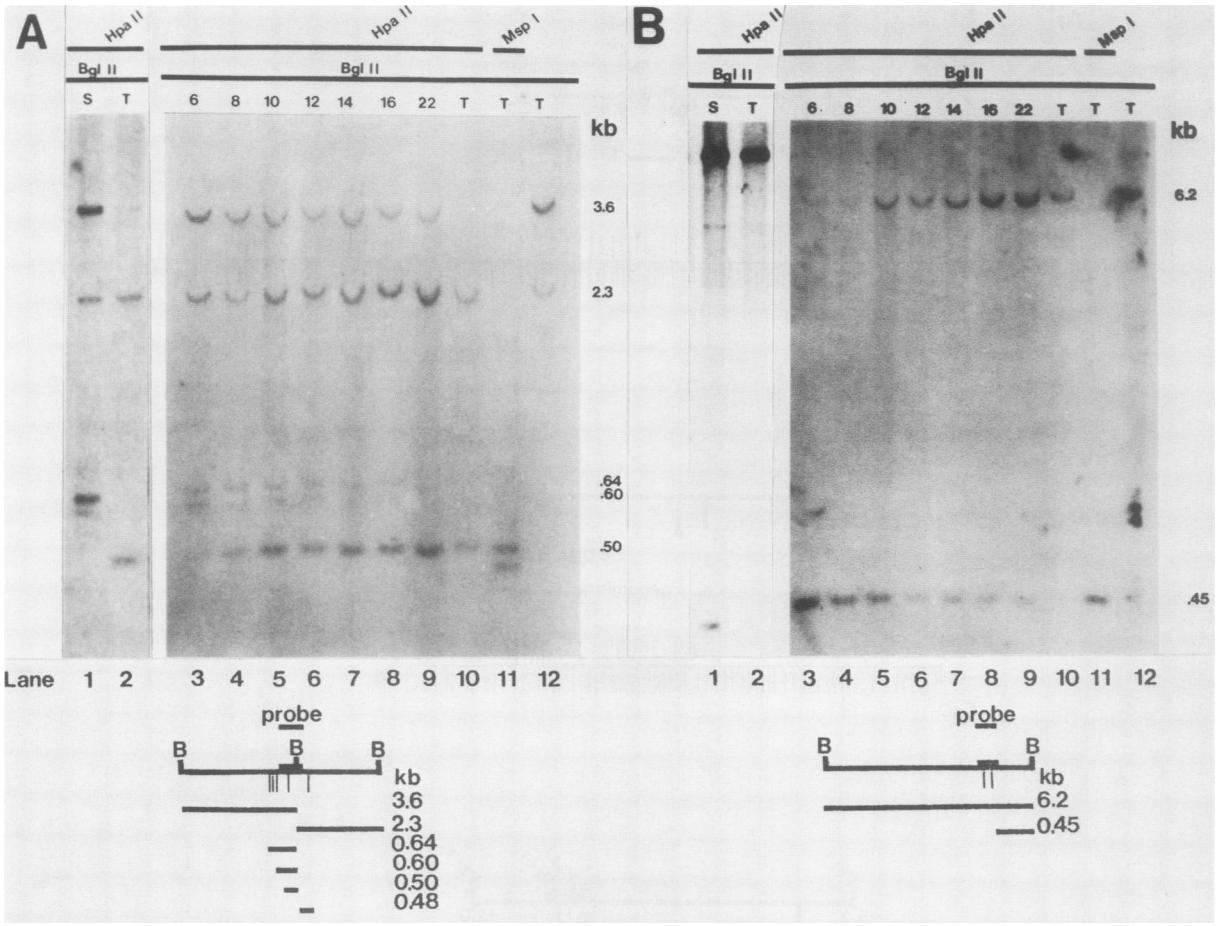

FIG. 2. Southern blots of somatic and testicular DNAs. Spleen (S) and testis (T) DNA from adult mice and testis DNA from prepubertal mice 6, 8, 10, 12, 14, 16, and 22 days of age (age matches numbers above each lane) were digested with *Bgl*II alone, *Bgl*II and *Hpa*II, or *Bgl*II and *Msp*I for mTP1 (A) and mP1 (B). The mTP1 and mP1 cDNA probes were used in A and B, respectively. The sizes of the bands are marked to the right of each blot. Beneath each autoradiogram are diagrams for the interpretation of the restriction fragment sizes. The positions of the probes are shown above each map. B, *Bgl*II. *Hpa*II sites are indicated by the vertical lines below the maps.

and 3 are representative of the results obtained after repeated sampling. DNA was electrophoresed in agarose gels (1) and transferred to Zetabind nylon membranes (Cuno, Meriden, Conn.) by the alkaline Southern blotting procedure (38). Southern blots were hybridized with cDNA or genomic probes; the probes were labeled, by the random priming method of Feinberg and Vogelstein (14), to specific activities of 5×10^8 to 1×10^9 cpm/μg of DNA. The membranes were prehybridized and hybridized under conditions suggested by the supplier.

Somatic and testicular DNA methylation patterns were compared first. When mouse genomic DNA was digested with *Bgl*II and probed with a 404-base-pair mTP1 cDNA (51) (Fig. 2A), a 3.6-kb 5′ band and a 2.3-kb 3′ band were seen (lane 12); digestion with *Bgl*II and *Msp*I (methylation insensitive) resulted in two small bands of 0.48 and 0.50 kb (lane 11), whereas digestion with *Bgl*II and *Hpa*II (methylation sensitive) resulted in an intermediate pattern (lanes 1–10). The decreased intensity of the 3.6-kb band and the appearance of the 0.50-kb band in testis DNA that was digested with *Bgl*II and *Hpa*II (Fig. 2A, lane 2) indicates that 5′-CCGG-3′ sites in the 5′ and coding regions of mTP1 in testis DNA were less methylated than those in spleen DNA (Fig. 2A, lane 1). The fact that the 2.3-kb band was not digested by *Bgl*II and *Hpa*II in either testis or spleen DNA to produce the smaller 0.48-kb band found in *Bgl*II-*Msp*I digests shows that the 5′-CCGG-3′ site in the 3′ region of mTP1 was methylated in both spleen and testis DNA. Similarly, for mP1 and mP2, the methylation patterns in the spleen differed from those in the testis. The 5′-CCGG-3′ sites examined in mP1 and mP2 were more methylated in testis DNA than in spleen DNA; the differences in methylation patterns between spleen and testis DNA for two such sites in mP1 are shown in lanes 1 and 2 of Fig. 2B. In contrast, methylation patterns for β-actin and mitochondrially encoded genes were identical in spleen and testis DNA (data not shown).

For the three testis-specific genes examined here, there were clear differences between the methylation patterns for CpG dinucleotides in spleen DNA compared with the same sites in testis DNA. The results suggest that methylation of mTP1, mP1, and mP2 is occurring during spermatogenesis. Since the testis contains somatic cells as well as mitotic, meiotic, and postmeiotic germ cells, testis DNA from prepubertal mice was examined to determine whether methylation changes were occurring during germ cell differentiation in the mouse testis.

In the mouse, the timing of the different stages of the spermatogenic cycle is precise and well known (31, 32), as is

the temporal appearance of successive cell types during the first spermatogenic wave following birth. For example, in addition to interstitial cells, the testes of 6-day-old mice contain Sertoli cells and type A spermatogonia, the testes of 16-day-old mice contain Sertoli cells and germ cells up to and including meiotic cell types, and the testes of 22-day-old mice include early haploid cell types (2, 30).

When genomic DNA isolated from the testes of prepubertal mice was digested with *Bgl*II and *Hpa*II and probed with mTP1 cDNA (Fig. 2A, lanes 3 through 9), the 3.6-, 0.64-, and 0.60-kb bands gradually decreased in intensity with increasing mouse age, whereas the 0.50-kb band increased in intensity. The *Bgl*II-*Hpa*II digestion did not result in a change in the intensity of the 3′ 2.3-kb band or in the appearance of the 3′ 0.48-kb band from *Bgl*II-*Msp*I digestion (lane 11). The findings indicate that 5′-CCGG-3′ sites in the 5′ and coding regions of mTP1 became progressively less methylated as spermatogenesis proceeded; in contrast, the methylation status of sites in the 3′ region did not change as the testis developed.

Mouse genomic DNA that was digested with *Bgl*II and probed with the 437-base-pair mP1 cDNA (50) (Fig. 2B) produced a 6.2-kb band (lane 12); digestion with *Bgl*II and *Msp*I resulted in a 0.45-kb band (lane 11), whereas digestion with *Bgl*II and *Hpa*II resulted in the presence of both the 6.2- and 0.45-kb bands (lanes 1 through 9). In DNA from the testes of prepubertal mice (lanes 3 through 9), the 6.2-kb mP1 band gradually increased in intensity relative to the 0.45-kb band. The findings indicate that specific 5′-CCGG-3′ sites in the coding region of mP1 become progressively more methylated as the germ cells differentiate. When the 1.4-kb 5′ genomic mP1 probe (33) was used to monitor any 5′ *Msp*I-*Hpa*II sites, no difference in methylation patterns was seen with testis development; the 5′ site at −3.1 kb remained methylated (data not shown).

Similar studies with mP2 indicated that the 5′-CCGG-3′ site in the 5′ region of mP2 (Fig. 1C) became progressively more methylated as spermatogenesis proceeded; in contrast, the methylation of 3′ *Hpa*II-*Msp*I sites (Fig. 1C) did not change (data not shown).

Mouse testis DNA was also digested with *Hha*I to detect methylation differences at 5′-GCGC-3′ sites (data not shown). The results were similar to those found for the *Hpa*II-*Msp*I digestions. The *Hha*I site at −2.6 kb in the 5′ region of mTP1 became less methylated as spermatogenesis proceeded. The *Hha*I site at −2.6 kb in the 5′ region of mP1 and the three *Hha*I sites in the 5′ and coding regions of mP2 became more methylated with increasing testis development. The methylation of the *Hha*I site at +0.80 kb in the 3′ region of mP2 was not altered.

Interestingly, analysis of prepubertal testis DNA revealed that all three postmeiotically expressed genes showed marked alterations in DNA methylation patterns between days 6 and 10 after birth. The differences between 6- and 10-day-old mice could be accounted for by decreases in the percentage of Sertoli cells in the seminiferous tubules at day 10 (84% at day 6, compared with 52% at day 10) and/or by the presence at day 10 but the absence at day 6 of preleptotene and leptotene spermatocytes (2, 30). A further change in methylation patterns occurred in DNA from the testes of mice between 22 days of age and adulthood. The methylation differences between 22-day-old and adult mice could be due to the increased number of haploid cell types—10% of the seminiferous tubule cells at 22 days of age, compared with approximately 70% in adult mice (3).

DNA methylation patterns for DNA from isolated testicular cells. Data from the prepubertal mouse testis DNA indicated that DNA methylation changes were occurring in early phases of spermatogenesis. It was unclear, however, whether the changes were occurring in germ cells or in somatic cells, such as the interstitial or Sertoli cells. To resolve this question, DNA from isolated testicular cells was analyzed. Type A spermatogonia (average purity, 84%; n = 3 cell separations), type B spermatogonia (average purity, 81%; n = 3), and interstitial cells (30 to 50% Leydig cells; 5 to 10% germ cells; 5 to 10% erythrocytes; n = 2) were isolated from 240 8-day-old mice (2). Preleptotene spermatocytes (average purity, 86%; n = 2), leptotene-zygotene spermatocytes (average purity, 81%; n = 2), and prepubertal pachytene spermatocytes (average purity, 82%, n = 2) were isolated from 100 17-day-old mice by the unit gravity sedimentation procedure (2). Pachytene spermatocytes (average purity, 82%; n = 3) and round spermatids (average purity, 90%; n = 3) were isolated from the testes of 10 sexually mature mice by the unit gravity sedimentation procedure (3, 40). Purified spermatozoa were obtained from the cauda epididymides of 10 adult mice (1). A blot of genomic DNA prepared from isolated testicular cells, digested with *Bgl*II and *Hpa*II, and probed with mTP1 cDNA is shown in Fig. 3A. The 3.6-kb band decreased in intensity, whereas the 0.50-kb band increased in intensity between type B spermatogonia and pachytene spermatocytes, i.e., during meiotic prophase. Interstitial cells from 8-day-old mice (Fig. 3A, lane I) showed the somatic pattern seen in spleen (Fig. 2A, lane 1). The results indicate that, at a premeiotic stage, 5′-CCGG-3′ sites in germ cell DNA in the mouse testis became less methylated in the 5′ and coding regions of mTP1. In addition, there were also indications of changes occurring between type A and type B spermatogonia. The DNA from type A spermatogonia appeared to be less methylated than that of type B spermatogonia. The same changes between type A and type B spermatogonia were found for DNA samples prepared from testicular cells isolated from two other cell separations performed on 8-day-old mice.

Similarly, for mP1 and mP2, methylation patterns were found to change between type B spermatogonia and pachytene spermatocytes. Results for mP2 are shown in Fig. 3B. Mouse genomic DNA was digested with *Pst*I and probed with a 940-base-pair *Bam*HI-*Pst*I fragment from an mP2 genomic clone (23). The 4.3- and 5.3-kb bands resulting from the digestion with *Pst*I-*Msp*I and *Pst*I, respectively, are shown in the last two lanes; digestion with *Pst*I and *Hpa*II yielded both the 5.3- and 4.3-kb bands. When DNA from isolated testicular cells was digested with *Pst*I and *Hpa*II, the 4.3-kb band of mP2 gradually decreased in intensity in relation to the 5.3-kb band between type B spermatogonia and pachytene spermatocytes, i.e., during meiotic prophase (Fig. 3B). The findings indicate that certain 5′-CCGG-3′ sites in the 5′ region of mP2 became progressively more methylated as the germ cells developed. The fact that the 4.3-kb band was present in pachytene, round spermatid, and spermatozoal DNA indicates either that a small portion (<10%) of the DNA was unmethylated or, more likely, that the purity of the isolated cells was less than 100%. Interstitial cell DNA was predominantly unmethylated. Methylation changes for mP2 were also evident between type A and type B spermatogonia; the DNA from type A spermatogonia was more methylated than the DNA from type B spermatogonia. Again, when the methylation studies were repeated on samples from two other 8-day cell separations, the results were similar.

*Msp*I-*Hpa*II sites in DNA from isolated populations of

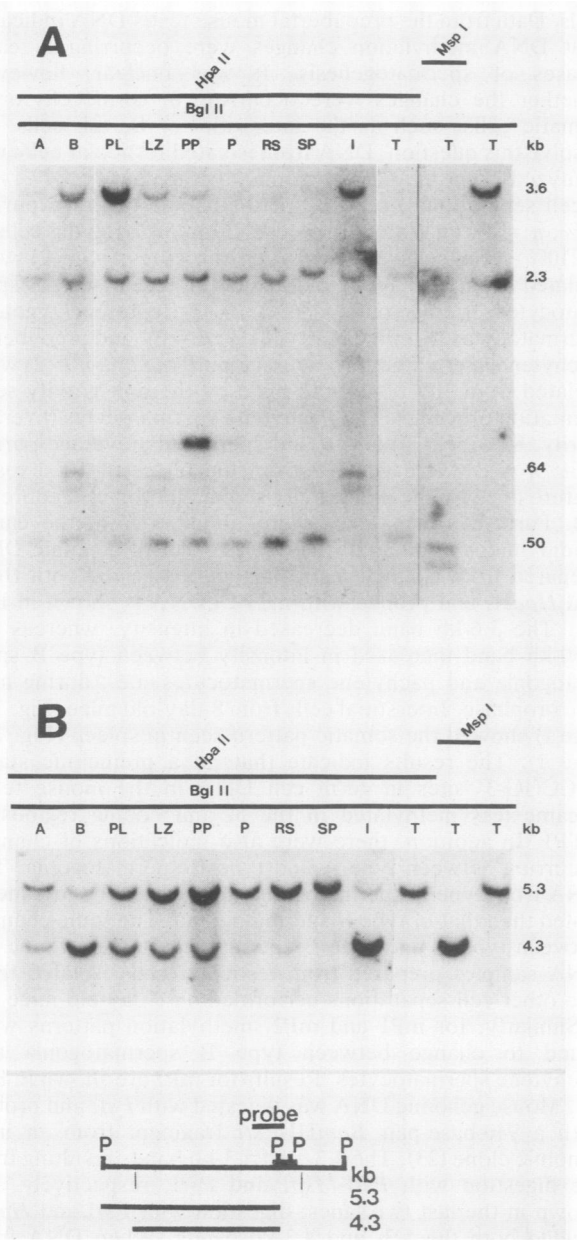

FIG. 3. Southern blots of DNA from isolated testicular cells analyzing methylation patterns for the mTP1 and mP2 genes. The DNAs were digested with *Bgl*II alone, *Bgl*II and *Hpa*II, or *Bgl*II and *Msp*I for mTP1 and were digested with *Pst*I alone, *Pst*I and *Hpa*II, or *Pst*I and *Msp*I for mP2. The mTP1 cDNA probe (A) and the mP2 5′ genomic probe (B) were used to detect bands. A, Type A spermatogonia; B, type B spermatogonia; PL, preleptotene spermatocytes; LZ, leptotene-zygotene spermatocytes; PP, prepubertal pachytene spermatocytes; P, pachytene spermatocytes; RS, round spermatids; SP, epididymal spermatozoa; I, interstitial cells from 8-day-old mice; T, adult testis. The sizes of the bands are marked to the right of each blot. An anomalous band just above the .64-kb marker is seen in lane PP in Fig. 4A. A diagram for the interpretation of the restriction fragment sizes for mP2 is shown below panel B. The position of the probe is shown above the map. P, *Pst*I. *Hpa*II sites are indicated by the vertical lines below the map.

cells, digested with *Bgl*II and *Hpa*II and probed with mP1, showed increases in methylation between type B spermatogonia and pachytene spermatocytes (data not shown). These findings indicate that 5′-CCGG-3′ sites in germ cell DNA in the mouse testis became more methylated in the coding region of mP1 and the 5′ region of mP2 during meiotic prophase.

DNA methylation patterns for genes expressed throughout spermatogenesis were included as controls. Unlike the testis-specific genes which showed variable methylation patterns depending on the tissue tested, both the 5′ region of β-actin (13) and the genes encoded by the mitochondrial genome (4) were unmethylated throughout spermatogenesis. Our results on the methylation of mitochondrially encoded genes during spermatogenesis confirm and extend those of earlier observations (21). Results from this study indicated that 5′-CCGG-3′ sites in mitochondrial DNA were unmethylated as early as day 6 in mice. Since genes encoded by mitochondrial DNA are expressed throughout spermatogenesis (1), it is perhaps not surprising that they remained unmethylated. It is also possible that the sequestration of mitochondrial DNA within the mitochondrion protects it from DNA methyltransferases present in the nuclei of cells. β-Actin is also expressed throughout spermatogenesis but it is not sequestered; there must therefore be some mechanism, e.g., protein binding, that prevents it from becoming methylated in the testis (5).

The results from the prepubertal testis and isolated testicular cells demonstrate that methylation changes for mTP1, mP1, and mP2 occur during germ cell development in the mouse testis. For the isolated germ cells, the most marked DNA methylation changes occurred between type B spermatogonia and pachytene spermatocytes. The fact that methylation changes occurred premeiotically before the expression of these three genes in haploid cells suggests that if methylation and transcription are linked as has been found for other genes, the link is not temporally tight. The timing of the methylation changes may be due to some of the unique characteristics of spermatogenesis. For instance, since no demethylating enzymes have been found to date, decreases in methylation in somatic cells are believed to occur solely during DNA replication (37). If demethylation were to occur only during replication in the testis, the only time the mTP1 gene could become demethylated would be premeiotically, since the last DNA replication of spermatogenesis occurs in the preleptotene spermatocyte. Interestingly, mTP1 is in fact further demethylated in leptotene-zygotene spermatocytes and early pachytene spermatocytes (from 17-day-old mice). These results argue that demethylation occurs after DNA replication in the testis; perhaps demethylation occurs during repair synthesis in pachytene or more likely through the action of a demethylase enzyme in the early stages of meiotic prophase.

Results from this study demonstrate increases in methylation during meiotic prophase. Rahe et al. (36) compared testicular DNA methylation patterns from 18-day-old prepubertal mice with those from adult mice and spermatozoa for three somatic tissue-specific genes, β-globin, pancreatic amylase, a type 1 histocompatibility gene, and one testis-specific gene, lactate dehydrogenase C. All of these genes were methylated in the testis as early as day 18 after birth. These investigators did not look at earlier time points or use cell separations; their results, however, like ours, suggest that methylation changes occur early during spermatogenesis. Furthermore, our results argue that in addition to demethylation, de novo methylation must occur after DNA replication in the testis.

In contrast to our results, the testis-specific trout protamine 3 gene, which is expressed early during spermatogenesis, had the same DNA methylation pattern throughout

spermatogenesis (10). Species differences between trout and mouse or the fact that we were examining postmeiotically expressed protamines in the mouse could account for the difference in results between our study and that of Delcuve and Davie (10).

The fact that CpGs in the 5′ and coding regions of mTP1 are less methylated in the testis where mTP1 is expressed is consistent with the results from other studies that link demethylation and the transcriptional activation of genes (for a review, see reference 6). However, distinct but opposite methylation changes occur for mP1 and mP2 during germ cell development in the testis. There are few examples in the literature of an association between increases in DNA methylation and transcription (29, 37). The fact that the increases in CpG methylation for mP1 and mP2 occur in the 5′ and coding regions but not in the 3′ region suggests that methylation could play a role in their activation. Bird (5) has argued that the 5′ regions of tissue-specific genes may be unmethylated in the tissue of expression because of DNA-protein interactions; Bird postulates that DNA-protein interactions may make the CpGs inaccessible to methylases. This may be true for mTP1. Conversely, the fact that the CpGs in the 5′ regions of mP1 and mP2 become methylated prior to transcription suggests that certain sites may become accessible to methylases, perhaps because of the dissociation of protein factors from DNA or changes in chromatin conformation. It is also possible that methylation of the protamine genes is not related to transcription but to other processes, e.g., changes in chromatin structure, nuclear condensation events, or association with the nuclear matrix. We can speculate that although mTP1, mP1, and mP2 are expressed at approximately the same time during spermatogenesis and serve similar functions in the testis, mTP1 may be regulated differently than the protamines. We wish to emphasize that we were only looking at a subset of CpG dinucleotides and that the methylation of other CpG sites not accessible by the restriction enzyme analysis employed here needs to be analyzed. Future studies using a combination of genomic sequencing and in vivo footprinting could clarify the role of CpG methylation or demethylation on DNA-protein interactions in the promoter regions of mTP1, mP1, and mP2 (7, 41).

The methylation changes occurring between type A and type B spermatogonia merit consideration. They could reflect (i) distinct differences between these two cell populations, (ii) events associated with genome reorganization during the transition from proliferation (spermatogonia) to differentiation (meiotic prophase cells), or (iii) more somatic cell contamination in the type B spermatogonia population than in the type A spermatogonia. The latter is unlikely, because (i) the same changes were seen in DNA prepared from three different cell separations of 8-day-old mice, (ii) cell counts performed by microscopy indicated that both type A and type B spermatogonia were contaminated with somatic cells to approximately the same degree, and (iii) when RNA prepared from type A and type B spermatogonia was probed with a cDNA for sulfated glycoprotein-2 (8), a Sertoli cell-specific probe, minimal contamination was seen (A. A. Alcivar, personal communication).

It is likely that there are substantial molecular differences between type A and type B spermatogonia. Type B spermatogonia are involved in the last mitotic division prior to the entry of cells into meiotic prophase. A recent study reported that certain nuclear proto-oncogenes were specifically expressed at high levels in type B spermatogonia; the researchers suggest that nuclear proto-oncogenes could play a role in altering programs of gene expression at this particular phase of spermatogenesis (49).

The studies reported here constitute, to our knowledge, the first demonstration in mammals of a clear-cut switching of methylation patterns during germ cell development in the testis. The evidence to date that some genes, i.e., tissue-specific genes, are methylated throughout spermatogenesis (18, 36) and that other DNA sequences, i.e., satellite sequences (15, 35, 42) and genes like β-actin and mitochondrially encoded genes, are undermethylated throughout spermatogenesis while testis-specific genes switch methylation patterns during spermatogenesis suggests that CpG methylation must be a tightly regulated process in the testis. Furthermore, the results indicate that demethylation and de novo methylation of postmeiotically expressed testis-specific genes occur at a time during meiotic prophase when DNA is not replicating.

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