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Methylation of satellite sequences in mouse spermatogenic and somatic DNAs

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ABSTRACT

The distribution of 5-methyl cytosine (5-MeC) residues in a highly repetitive sequence, mouse major satellite, was examined in germinal versus somatic DNAs by digestion with the methylation sensitive isoschizomers Msp I and Hpa II and Southern blot analysis, using a cloned satellite probe. DNA from liver, brain, and a mouse fibroblast cell line, C3H 10T1/2, yielded a multimeric hybridization pattern after digestion with Msp I (and control Eco RI) but were resistant to digestion with Hpa II, reflecting a high level of methylation of the satellite sequences. In contrast, DNA from mature sperm was undermethylated at these same sequences as indicated by the ability of Hpa II to generate a multimeric pattern. DNAs from purified populations of testis cells in different stages of spermatogenesis were examined to determine when during germ cell differentiation the undermethylation was established. As early as in primitive type A, type A, and type B spermatogonia, an undermethylation of satellite sequences was observed. This suggests that this highly specific undermethylation of germ cell satellite DNA occurs very early in the germ cell lineage, prior to entry into meiosis.

INTRODUCTION

Changes in the methylation pattern of cytosine residues in specific DNA sequences have been correlated with transcriptional activity of genes during cellular differentiation and development (reviews 1-3,4). Comparisons are frequently made between DNA isolated from differentiated cells in which the genes are expressed and DNA from the totipotent germ line cells. For example, β-globin genes were shown to be undermethylated in DNA isolated from erythroid cells relative to DNA from mature sperm (5). Similarly, rRNA genes in sperm of Xenopus laevis are heavily methylated and a loss of rDNA methylation coincides with the onset of ribosomal gene transcription during early embryonic development (6). Although there are exceptions (e.g. α2(I) collagen gene, ref.7), the overwhelming trend is one of a high level of methylation in sperm relative to somatic cells in which the genes are expressed.

Less well documented are the methylation patterns of repetitive DNAs.

In a survey of several rodent and primate genomes, repetitive sequences in general were shown to be more methylated than moderately repetitive or single copy sequences (8). Among the repetitive sequences, the satellite DNAs of the murine and bovine genomes have been shown to be heavily methylated in various somatic tissues (9-11). There appear to be tissue specific differences in the methylation patterns of repetitive sequences in that an undermethylation of satellites was reported in bovine sperm DNA (11) and very recently, in mouse sperm DNA (12), relative to somatic DNAs.

Although considerable information has been obtained on the chromosomal localization, abundance, and sequence composition of satellite DNAs, their function is completely unknown. Satellite sequences are clustered at the heterochromatic centromeres and telomeres of most chromosomes, (e.g.13,14). This localization favors the hypothesis of an involvement in the assembly of the higher order structure of the eukaryotic chromosome. Since centromeres and telomeres are important in chromosomal recognition and pairing, satellite DNA could critically affect meiotic recombination (15). Large deletions of X heterochromatin, rich in satellite sequences, indeed result in abnormal meiosis, defective spermatogenesis, and reduced fertility in Drosophila (16). A critical role for satellite DNA in germ cells could thus be invoked. Whatever function satellite DNAs serve, it is probably mediated by interaction with specific proteins. This interaction can in turn be affected by variations in the extent of methylation of cytosine residues (2).

In the present study, the methylation pattern of mouse major satellite DNA in germ cells at various stages of spermatogenic differentiation was investigated, using the methylation sensitive isoschizomers Msp I and Hpa II and Southern blot analysis (17-19). Satellite sequences in DNA isolated from mature sperm were markedly undermethylated, relative to DNA from somatic tissues or a mouse fibroblast line, C3H 10T1/2. The undermethylation, as reflected by susceptibility of the DNA to Hpa II digestion, was less extensive than that observed with DNA from fibroblasts treated with 5-azacytidine (an inhibitor of DNA methylation, ref.20). In order to determine the developmental stage at which this undermethylation is established, populations of testis cells at various stages of spermiogenesis and meiosis were purified using a Celsep apparatus. The mouse major satellite sequences were undermethylated during spermiogenesis and throughout meiotic prophase. The earliest premeiotic spermatogenic populations obtainable were a mixture of premeiotic primitive type A and type B spermatogonia. The major satellite sequences were undermethylated in these

cells as well. Finally, the generality of this observation among repetitive sequences was examined, using as a probe a mouse "R-type" interspersed repetitive element (21,22). In contrast to the differential methylation of satellite sequences between germ line and somatic tissues,"R" element-associated sequences were not undermethylated in spermatogenic cells.

MATERIALS AND METHODS

Sources of Enzymes, Reagents, and Recombinant Probes.

Eco RI was purchased from New England Biolabs and Msp I, Hpa II, and E. coli DNA Pol I were purchased from Boehringer Mannheim. ^{32}P-dXTPs were obtained from Amersham Radiochemicals. Oligo (dT) cellulose was purchased from Collaborative Research. Recombinant plasmids were obtained as follows: pMR 196 from N. Hastie (23); pMIA 7 from E. Kuff (unpublished); and pT 1 from D. Cleveland (24). Mature male Swiss Webster mice were purchased from Camm Research, Inc. and day 8 neonatal Swiss Webster mice from Blue Spruce, Inc.

Source of DNAs.

Somatic and germinal tissues: Liver, testes, and brain were dissected from mature male mice which had been sacrificed by cervical dislocation and used immediately for DNA isolation as described below.

Mature sperm: Sperm were isolated from the caudal epididymis and vas deferens of mature mice essentially according to O'Brien and Bellvé (25). The procedures maximize the homogeneity of the sperm with respect to state of nuclear maturation and minimize contamination by sperm mitochondria and by somatic cells (25, and Wood and Wolgemuth, unpublished observations). Sperm nuclei ($\sim 2 \times 10^8$/ml) were decondensed in 100 mM Tris-HCl pH 8.0, 0.2% SDS, 10 mM EDTA, 5 mM DTT. Proteinase K was added to 200 µg/ml for an overnight incubation at 37°C.

Separated testis cell populations - meiotic and post-meiotic stages: Testes of mature male mice were used for preparation of a cell suspension. The tissue disruption, enzymatic and washing procedures were essentially those of Romrell et al. (26) as modified by Bellvé et al. (27, 28). Cells in different stages of spermatogenesis, isolated from mature testes, were separated by sedimentation at unit gravity through a 2% to 4% gradient of bovine serum albumin using a Celsep apparatus (Wescor, Inc.). This apparatus is similar in principle of cell separation to the Staput apparatus used by others (26-28). Details of the use of the Celsep apparatus and its advantages and applications will be published elsewhere (29). Three sets of cell fractions were chosen for study: (a) fractions enriched for spermatocytes in the pachytene stage of meiotic prophase; (b) those enriched

for round spermatids in early spermiogenesis, stages 1-9 (classified according to ref. 30); and (c) those enriched for late spermatids, stages 14-17 (30). Pooled fractions were washed with Dulbecco's PBS and stored at -20°C or used immediately for DNA extraction.

Separated testis cell populations - premeiotic stages: In order to obtain sufficient numbers of pure pre-meiotic stage spermatogenic cells, it was necessary to use 20 to 30 prepuberal (day 8 post-partum) mice. Methods for preparation of the cell suspensions and isolation using the Celsep apparatus were similar to those used for tissues from mature animals (27,29). The fractions most enriched for pre-meiotic spermatogenic cells contained type A and type B spermatogonia, with very little contamination (<19%) of Sertoli cells or other somatic cells.

C3H 10T1/2 cells: DNA isolated from the C3H 10T1/2 fibroblast cell line grown in the presence or absence of 3 μM 5-azacytidine (5-Aza) for 24 hours was kindly supplied by S. Gattoni-Celli and W. Hsaio of the Institute for Cancer Research. Details on the propagation of the cells, 5-Aza treatment, and DNA isolation will be published elsewhere (31).

DNA Isolation.

High molecular weight DNA from all sources was prepared essentially according to the methods of Wigler et al. (32) with minor modifications. Minced tissues or cells were disrupted in a glass-teflon Dounce homogenizer in 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 10 mM EDTA. Proteinase K (200 μg/ml) and SDS (0.2%) were added and digestion was carried out overnight at 37° C. Nucleic acids were extracted with equal volumes of phenol, phenol-isoamyl alcohol: chloroform (1:24), and isoamyl alcohol: chloroform, and the aqueous phase was treated with ether, followed by ethanol precipitation of total nucleic acids. DNA from mature sperm nuclei which had been decondensed and digested overnight, was purified in a similar manner. Following ethanol precipitation, the nucleic acids were dissolved in 1mM Tris-HCl, 0.1mM EDTA and approximate concentrations determined by reading at A_{260} or by calibration on agarose-ethidium bromide plates (33).

Restriction Enzyme Digestion and Southern Blot Analysis.

DNA digestions using Eco RI, Msp I, and Hpa II were carried out according to manufacturers' recommended conditions, using excess enzyme (2-5 units/μg) and incubation for 5 hrs at 37°C to ensure complete digestion. Digested DNAs were run on 1% agarose gels and transferred to nitrocellulose filters (34) according to Maniatis et al. (35). In the majority of experiments, hybridization was with the intact plasmid pMR 196, which

contains an insert of the mouse major satellite sequences (23), nick-translated with all four ^{32}P-dXTP's (36). Additional blot analysis utilized the plasmid pMIA 7 which contains an "R" element (21,22). The "R" sequence and some flanking unique sequence were cut out from the plasmid by a combined Eco RI and Kpn I digest and nick-translated. Autoradiographs were obtained following exposure to X-ray film. Careful attention was paid to load equal amounts of DNA from each different source in each of the sets of enzyme digests (Eco RI, Msp I, and Hpa II) in order that qualitative and rough quantitative comparisons could be made within a set by densitometric tracing, as discussed by Ellis et al. (37).

RNA Isolation and "Dot-Blot" Analysis.

RNA was isolated from freshly excised total testis from mature male mice essentially according to Chirgwin et al. (38) and poly(A)+ RNA was separated according to Maniatis et al. (35). RNA samples were either run on 1% agarose gels containing 2.2 M formaldehyde and transferred to nitrocellulose paper for Northern blot hybridization (35) or were loaded directly onto nitrocellulose filters for dot-blot analysis according to Chen et al. (33). Three series of samples were examined: Poly (A)+ RNA, poly (A)- RNA, and pBR322 DNA, as a control for DNase activity. Each sample was incubated at 37 C for 3 hours with DNase buffer alone (10mM Tris-HCl, 4mM $CaCl_2$, 5 mM $MgCl_2$, pH 7.5) or with increasing concentrations of RNase-free DNase (16, 80, 400 µg/ml). Hybridization procedures were carried out using intact pMR 196 or the insert of pT1, which contains chicken α-tubulin cDNA sequence (24).

RESULTS

In the initial experiments, a comparison was made between the methylation pattern of mouse major satellite sequences in DNAs isolated from germinal versus somatic tissues. The analysis involved digestion of DNA with the methylation sensitive isoschizomers Msp I and Hpa II, size-fractionation on agarose gels and transfer to nitrocellulose filters for Southern blot hybridization. The probe used was pMR 196, which contains an ∿200 bp insert of the mouse major satellite sequence (23). Digestion of DNAs from all sources with an enzyme insensitive to 5-MeC (Eco RI) revealed a multimeric hybridization pattern with a repeat unit of ∿110 bp (Figure 1, lanes 1,4,7), reflecting the tandem repetition of satellite DNA in the genome. Similarly, digestion with Msp I yielded a series of multimers of ∿230 bp in unit length (Figure 1, lanes 2,5,8). On the other hand, Hpa II digests of DNA from liver (Figure 1, lane 9) showed extensive methylation of the recognition sequence

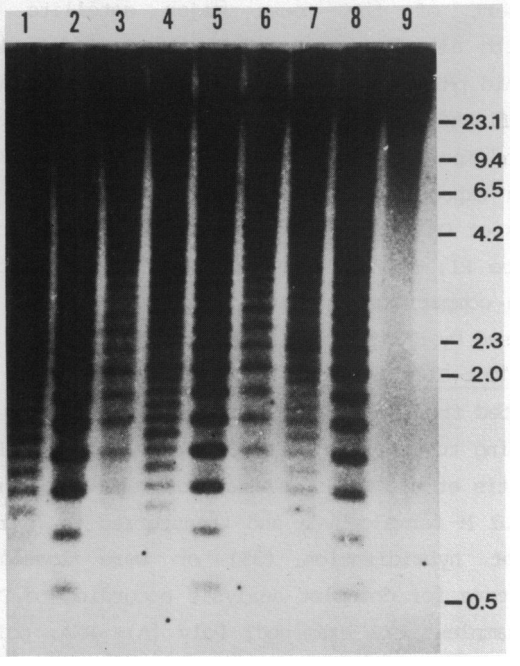

Figure 1. Southern blot of mouse somatic and germ cell DNA, hybridized with ^{32}P-labeled pMR 196. DNAs isolated from mouse testes (lanes 1,2,3) mouse sperm (lanes 4,5,6) and mouse liver (lanes 7,8,9) were digested with Eco RI (lanes 1,4,7), Msp I (lanes 2.5,8) and Hpa II (lanes 3,6,9) restriction endonucleases. In each set of digests (for example, lanes 1,2,3), equal amounts of DNA from a given source were used. Although the absolute amount of DNA digested varied between sets (for example, lanes 1,2,3 versus lanes 4,5,6), an effort was made to digest roughly comparable amounts of DNA from the different tissues. All autoradiographs of Southern blots hybridized with clone pMR 196 were purposefully overexposed in order to ensure detection of the multimeric pattern of bands. Molecular weight markers: λ DNA, digested with Hind III.

CCGG associated with satellite DNA. This was indicated by the absence of the multimeric pattern, reflecting the inability of Hpa II to cut when the internal cytosine in the recognition sequence is methylated (17). An identical result was obtained with DNA isolated from brain (data not shown).

In contrast, DNA isolated from mature sperm revealed the multimeric pattern following Hpa II digestion (Figure 1, lane 6), indicating an undermethylation of satellite sequences. Similarly, DNA from mature testis, which contains predominantly germinal cells, was also undermethylated at the major satellite sequences (Figure 1, lane 3) relative to DNA from liver and brain.

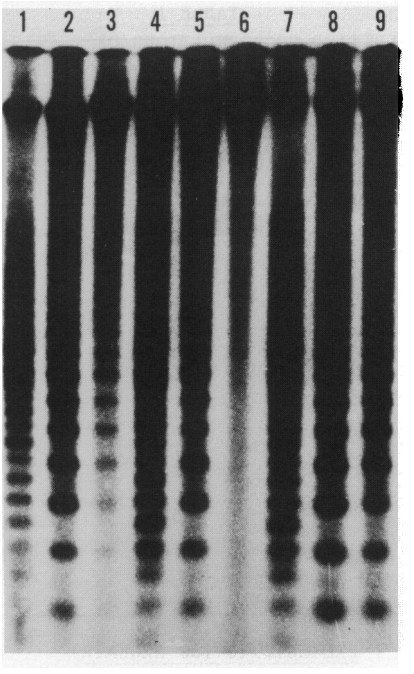

Figure 2. Southern blot of DNA isolated from mouse testes (lanes 1,2,3), C3H 10½ mouse fibroblasts untreated (lanes 4,5,6) and treated (lanes 7,8,9) with 5-azacytidine. The probe was clone pMR 196, labeled by nick translation with ^{32}P dXTPs. Lanes 1,4,7 - Eco RI digests; lanes 2,5,8 - Msp I digests; lanes 3,6,9 - Hpa II digests.

That this difference was indeed due to an alteration in methylation of satellite sequences was confirmed by analyzing DNA isolated from C3H 10T1/2 cells, a mouse fibroblast line, treated with 5-Aza, an inhibitor of DNA methylation (20). Satellite sequences in DNA from the untreated fibroblast cells were resistant to Hpa II digestion (Figure 2, lane 6), similar to satellite in DNA from somatic tissues (Figure 1, lane 9). The cells treated with 5-Aza yielded two identical multimeric patterns with Msp I and Hpa II (Figure 2, lanes 8 and 9), suggesting virtually complete demethylation of the internal cytosine of CCGG sequences in satellite DNA. The pattern obtained was similar to that observed with DNA from total testis (Figure 2, lanes 2 and 3) and mature sperm (Figure 1, lanes 5 and 6), except that in sperm and testis, the bands obtained digesting with Hpa II are of lower intensity with respect to the Msp I bands. It should be noted that equal amounts of DNA were loaded in each set of Eco RI, Msp I and Hpa II digests. It is therefore possible to estimate roughly the extent of undermethylation of satellite

TABLE 1

TYPICAL PURITIES OF POPULATIONS OF TESTIS CELLS SEPARATED ON A CELSEP APPARATUS

MEIOTIC (ADULT TESTIS)			
Fraction #	Spermatogenic Cell Type	Purity	Contaminating Cell Type
25-34	Pachytene Spermatocytes	82%	Spermatogonia (3%) Sertoli Cells (4%) Other (11%)
58-67	Early Spermatids	76%	Spermatogonia (16%) Sertoli Cells (1%) Other (7%)
76-82	Late Spermatids	96%	<4% Contamination with Nucleated Cells
85-88	Residual Bodies and Cytoplasmic Fragments	87%	Late Spermatids (12%) Other (1%)

PRE-MEIOTIC (d8 NEONATE)			
Fraction #	Spermatogenic Cell Type	Purity	Contaminating Cell Type
38-45	Primitive Type A Spermatogonia	18%	Sertoli Cells (12%)
	Type A Spermatogonia	35%	Other (7%)
	Type B Spermatogonia	28%	Meiotic (0%)
	Total Pre-meiotic Cells	81%	

sequences in sperm DNA by comparing the intensity of the bands obtained with parallel Msp I and Hpa I digests (37), using densitometric scanning of autoradiographs such as those depicted in Figure 1. An estimate of an approximate one-third increase in Hpa II digestible sites in sperm versus somatic satellite DNAs was made, reflecting a corresponding decrease in methylated cytosine at the recognition site.

We wished to determine when during spermatogenesis this undermethylation of satellite sequences in the male germ cells was established. This was accomplished by isolating DNAs from purified populations of cells from the mature testis which had been separated on a Celsep apparatus (29). Representative distributions of cell types in the samples from which DNAs were isolated for use in the present study are shown

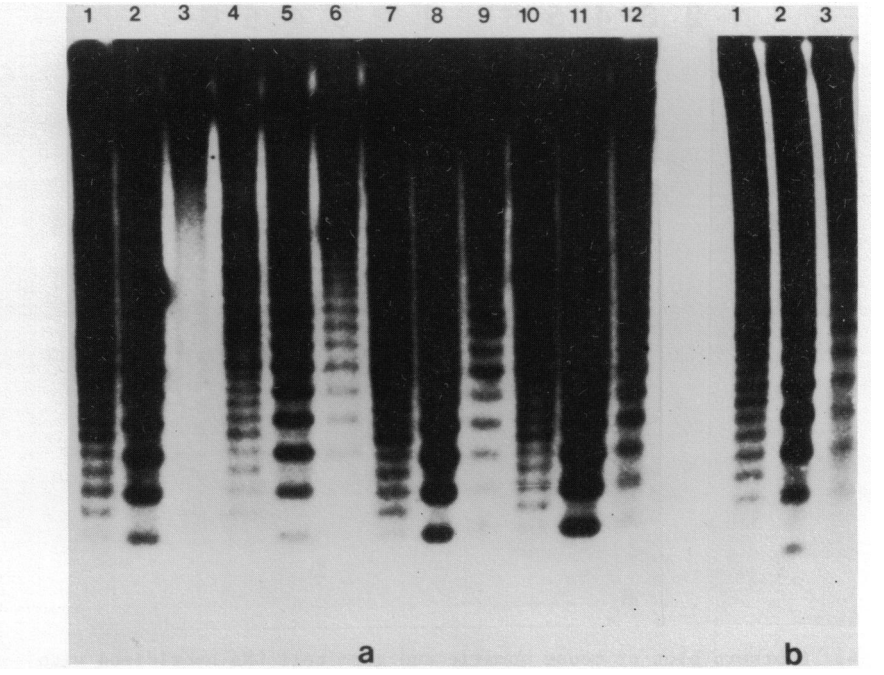

Figure 3. Southern blot of DNA isolated from mouse liver and separated populations of mouse spermatogenic cells. The probe was ^{32}P-nick translated clone pMR 196. (a) Lanes 1,2,3 - mouse liver; lanes 4,5,6 - mouse epididymal sperm; lanes 7,8,9 - pachytene spermatocytes; lanes 10,11,12 - early spermatids; lanes 1,4,7,10 - Eco RI digests; lanes 2,5,8,11 - Msp I digests; lanes 3,6,9,12 - Hpa II digests. (b) Lanes 1,2,3 - pre-meiotic spermatogonia; lane 1 - Eco RI digest; lane 2 - Msp I digest; lane 3 - Hpa II digest.

in Table 1. Southern blot analysis of DNA from cells in early (Figure 3a, lanes 10,11,12) and late (not shown) spermiogenesis as well as in pachytene of meiotic prophase (Figure 3a, lanes 7,8,9) indicated that the satellite sequences were undermethylated at these stages and presumably at stages in between.

The possibility that the undermethylation of satellite sequences occurred concomitant with entry into meiosis was considered. Populations of purified pre-meiotic spermatogonia were therefore isolated from immature testes, using animals at day 8 of neonatal life to minimize meiotic cell contamination (27). DNA from these cells, consisting of a highly enriched population of primitive type A, type A, and type B spermatogonia (Table 1), was isolated and used for Southern blot analysis with the cloned major

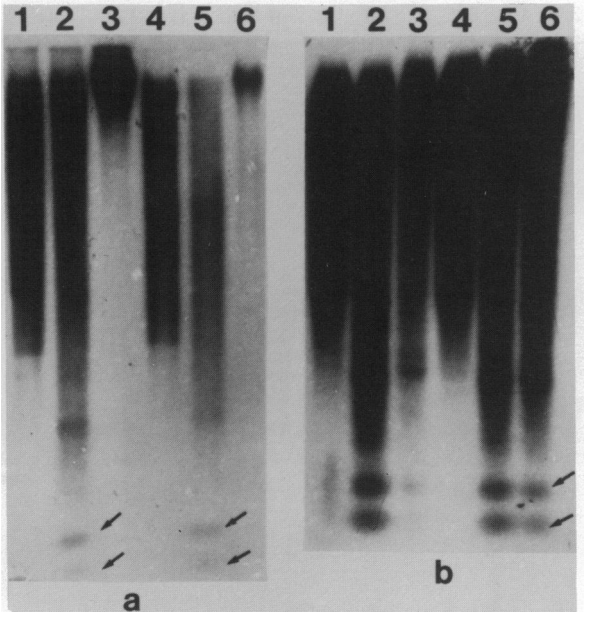

Figure 4. Southern blot of mouse somatic and germ cell DNA hybridized with ^{32}P-labeled insert from clone pMIA 7. Sources of DNA were liver (4a - lanes 1,2,3); sperm (4a - lanes 4,5,6); C3H 10½ fibroblasts untreated (4b - lanes 1,2,3) and treated (4b - lanes 4,5,6) with 5-azacytidine. Arrows indicate discrete bands observed after Msp I and Hpa II digestion and hybridization with "R" element probe. Lanes 1 and 4 on both panels - Eco RI digests; lanes 2 and 5 on both panels - Msp I digest; lanes 3 and 6 on both panels - Hpa II digest.

satellite probe. As can be seen in Figure 3b, lanes 2 and 3, pre-meiotic male germ cells were also undermethylated in the major satellite sequences.

Sanford et al. (39) determined that a mouse minor satellite was also undermethylated in the male germ line. It was thus of interest to determine if undermethylation was characteristic of repetitive sequences in general or if the undermethylation was unique to satellite sequences. To this purpose we hybridized filter-bound DNA from liver, sperm and fibroblasts treated and untreated with 5-Aza, to a cloned probe, pMIA 7, comprising ∿400 bp of an "R"-type repetitive sequence (21,22). The "R"-type elements are present in about 100,000 copies per haploid genome and appear to constitute the conserved 3' end of a larger (∿7 kb) highly repetitive element (L1Md) interspersed throughout the genome in numerous variant forms resulting from random truncation at the 5' end of the element (40,41). Hybridization with this probe produced a predominant smeared pattern with all restriction

enzymes used (Figure 4), as was expected given the interspersion and high abundance of "R" elements in the genome and the apparent lack of Eco RI or Msp I recognition sites within the cloned "R" element sequence (E. Kuff, unpublished observations). Sequences associated with "R" elements were highly resistant to Hpa-II digestion in DNA from both liver (Figure 4a, lane 3) and sperm (Figure 4a, lane 6), as compared with DNA from 5-Aza treated fibroblasts (Figure 4b, lane 6). This suggested that such "R"-neighboring sequences were similarly methylated in both germinal and somatic DNAs. In addition, a minor portion of the mouse genome did hybridize to the "R" element probe as discrete bands after Msp I digestion (arrows in Figure 4a, lanes 2,5; and 4b, lanes 2,5,6). The CCGG recognition site (s) which generated these bands was clearly and similarly methylated in liver and sperm DNA (Figure 2, lanes 3 and 6) as revealed both by the lack of the bands after Hpa II digestion, as well as by the presence of the bands after Hpa II digestion of 5-Aza treated DNAs (Figure 4b, lane 6).

Since undermethylation of specific sequences has been shown to correlate with transcriptional activity, it was of interest to determine if the undermethylated satellite sequences were expressed in the germ line. RNA was isolated from total testis and used in Northern (35) and "dot-blot" (33) analysis. In the first set of experiments, consisting of Northern analysis, both poly(A)+ and poly(A)- RNA samples produced a strong smeared pattern of hybridization wih the satellite probe. The same poly(A)+ RNA preparation yielded a single ∿1900 bp band, when hybridized with the α-tubulin probe (data not shown). Given the very high abundance of satellite DNA in the mouse genome, even a minor DNA contamination of our RNA samples could have been responsible for the strong positive signal observed. In order to rule out this possibility, subsequent experiments (RNA dot-blot) included RNA samples digested with RNase-free DNase. The results strongly indicated that virtually all hybridization indeed resulted from DNA contamination (data not shown).

DISCUSSION

Nearest neighbor analysis has shown that satellite DNAs are enriched in the dinucleotide CpG relative to main band DNA (42). A high proportion of the cytosines in this dimer are methylated in satellite DNAs from somatic tissues as determined by chromatography (9,43) cytogenetic localization of antibodies to 5-MeC (44), and restriction endonuclease digestion with methylation- sensitive isoschizomers Msp I and Hpa II followed by Southern

blot hybridization with total satellite DNA (12,45). The results of the present study, using a cloned major satellite sequence for hybridization after Msp I-Hpa II digestion, confirm and extend the observation of a high level of methylation of satellite DNAs in somatic tissues. Liver and brain satellite sequences were highly resistant to cutting with Hpa II, reflecting methylation of the internal cytosine at the recognition sequence CCGG. In contrast, digestion of DNAs isolated from total testis or mature sperm revealed that these same sequences are markedly undermethylated in male germ cells. Sanford et al. (39) have noted a similar undermethylation of both major and minor satellite sequences in male and female germ cells.

As a positive control for differential Hpa II digestion of satellite sequences being due to undermethylation, we made use of DNA isolated from fibroblasts grown in the presence of the inhibitor of DNA methylation 5-Aza. Digestion with Hpa II of DNAs isolated from these cells revealed a pattern identical to that obtained with Msp I, reflecting virtually complete demethylation. This result confirmed that the analogous pattern of Hpa II digestion obtained with sperm of mature testis DNA was indeed due to undermethylation of the internal cytosines within the recognition sequence CCGG. In addition, the results suggested that methylation of the external cytosine was undetectable. Msp I is known to be sensitive to the presence of methyl groups on the external cytosine in the recognition sequence CCGG (46). Since there was apparently no change in the pattern of Msp I with digestion with and without 5-Aza treatment, the external cytosines of CCGG sequences detected by our satellite probe were presumably unmethylated.

It should be noted that the published consensus sequence for mouse major satellite DNA does not contain an Msp I site (47). Clearly , however, a ladder of repeats of an $\sim$230 bp unit was obtained with Msp I digestion and hybridization with the major satellite probe in DNAs from all tissue sources. Similar results were obtained by Sanford et al. (39), using the same major satellite probe, and by Reilly et al.(45), with Msp I digestion of DNA followed by hybridization with total satellite. This multimeric repeat pattern has been termed a "type B" pattern by Horz and Altenberger (47). Cleavage sites that yielded a type B pattern were present on only a few percent of the repeat units. The variant repeats were clustered, however, generating the characteristic tandem array. For example, a mutation at position 33 from an A to C in the satellite consensus sequence would produce an Msp I site which could then, as suggested by Horz and Altenberger, be spread to neighboring repeat units.

The observation of a differential methylation in these satellite sequences between somatic and germ line tissues led to the question of when during spermatogenesis the undermethylation was established. Undermethylation of satellite sequences could possibly be involved with such differentiative events as entry into meiosis, genetic recombination, or nuclear protein turnover. Each event is well defined in mammalian spermatogenesis and cells in different stages can be separated by characteristic changes in size and shape. DNA from fractions enriched in early spermatids, late spermatids, pachytene spermatocytes and premeiotic spermatogonia always produced a Hpa II pattern similar to that from mature sperm, indicating an undermethylation of the satellite sequences in all these cell types. This suggested that as early in spermatogenesis as the primitive type A spermatogonial stage, the major satellite sequences detected by our probe were undermethylated. This is similar to the observation of Sanford et al. (39) on a minor satellite component in spermatogenic cells. Their observations have been extended to the female germ line. Although the cell populations from which DNA was isolated were not as pure as the male germ cell populations, an analogous undermethylation of major and minor satellites was observed.

On the other end of the developmental spectrum, Chapman et al. (48) have recently shown that the minor satellite sequences remain undermethylated in extra-embryonic lineages but are methylated early in the embryonic cell lineage. Our combined observations suggest two equally intriguing possibilities regarding changes in methylation during germ cell differentiation. First, it is possible that at some point in mid embryogenesis, between early embryogenesis and the stage at which germ cells enter meiotic differentiation (which would be day 12 of gestation for the female mouse and post-natally for the male mouse), a specific demethylation event occurs which is then inherited in subsequent mitotic and then meiotic stages. Alternatively, it is possible that there are a few cells in the early embryonic lineage that serve as progenitors to primordial germ cells, which in turn give rise to spermatogonia or oogonia . These progenitor cells could escape the de novo methylation that apparently occurs in the rest of the embryo (48) and would thus transmit a non-methylated pattern to their daughter cells. Either possibility is interesting in that the former hypothesis requires a specific demethylation event of a subset of genomic sequences at a specific developmental stage, while the latter raises the possibility of sequestration of the germ line during mammalian development at stages which have not formerly been identified.

Finally, the question of whether or not satellite sequences are transcribed is of interest in that a possible regulatory function for repetitive sequences during development has been proposed (49). Although attempts have been made to detect satellite transcripts in various tissues (e.g. 50,51), the only unambiguous demonstration has been in the oocytes of newts (52), where it is apparently the result of read-through transcription (53). We therefore considered it possible that satellite sequences would be transcribed in the mouse male germ cells. To date, we have not been able to detect satellite transcripts in RNA from mouse testis. However, it should be noted that a low level of transcription may not have been detected above background, and even a moderate level of transcription by a small subset of testicular cells might also have been missed in analysis of RNA from total testis. Experiments to resolve this latter possibility are in progress. Irrespective of whether satellite sequences are transcribed, the observation of an undermethylation of specific satellite sequences during spermatogenesis suggests a differential role of satellite DNA in the germ line as compared with somatic tissues.

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