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METHYLATION OF SATELLITE DNA

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The lower amount of 5 methylcytosine in DNA from bull sperm relative to DNA of other bovine tissues is a result of the absence of this minor base from several of the satellite DNAs in sperm. This applies particularly to the 1.715, 1.711b and 1.709 satellites and less so to the 1.706 and 1.711a satellites. Mouse sperm DNA is also partially undermethylated.

DNA isolated from sperm of bulls, sheep and pigs has only about half the 5 methylcytosine content of DNA isolated from the corresponding somatic tissues (1, 2). Thus by analysing the composition of calf thymus DNA using base fractionation on Aminex A6 (ref.3) we find that 5.4% of cytosines are methylated compared with only 2.5% in bull sperm DNA. On the other hand a similar difference has not been found in sea urchin (4), fish, chick (5) or mouse where we find the same fraction of the cytosines are methylated in DNA from sperm, kidney and ascites cells. The major cause of the undermethylation of bovine sperm DNA may be the presence of methyl deficient satellite DNA. There are 8 major satellites in the bovine genome and these G+C rich DNA components make up about 23% of the bovine genome irrespective of cell type (6). In somatic cells satellite DNA is highly methylated (up to 20% of the cytosines are methylated compared with less than 3% of the cytosines in main band DNA) (7-11). In sperm DNA it is not established, however, whether the extent of this methylcytosine deficiency varies between different satellites of bovine sperm, nor whether this deficiency is typical for satellite DNA of mammalian sperm or whether only satellite DNA from the sperm of ungulates is undermethylated.

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METHODS

DNA was isolated from bovine sperm and mouse sperm as described by Borenfreund et al (12) and Shiurba and Nandi (13) respectively. Otherwise standard methods (3) were employed. Base analysis was performed on an Aminex A6 column (Bio Rad Labs) (3). Restriction enzymes were purchased from Bethesda Research Ltd. or New England Biolabs and used according to the suppliers instructions using several fold excess enzyme to ensure complete digestion. Electrophoresis was on 1.5% agarose gels as described by Tegtmeyer (14). Mouse satellite DNA was cut out and eluted from a gel similar to that shown in figure 2 and nick translated using ^{32}PdATP obtained from Amersham International for use as a probe.

RESULTS

Figure 1 shows the results of digesting DNA from several bovine tissues with restriction enzymes. In Fig.1 a5, b5 and c2 it can be seen that with EcoRI, satellite I (1.715 g/cm^3) gives the major band at 1400 bp and the related 1.711 b satellite gives bands at 970 bp and 1600 bp (11,15,16). The origin of a fourth band at 2200 bp is not yet established but it may arise from the 1.709 satellite (17). Higher molecular weight bands which are faintly visible may result from dimers or trimers of satellite I where the intervening EcoRI site has been lost. On double digestion with EcoRI and MspI (cuts CCGG) all the EcoRI bands are lost (see Figure 1 c5), but the major bands are not affected when skin or thymus DNA are digested with EcoRI and HpaII (cuts CCGG only if the internal cytosine is not methylated) (Figure 1 a4 and b4) showing these satellites to be methylated in skin and thymus DNA (The 2200 bp band does appear to be susceptible to Hpa II digestion). In contrast all the EcoRI bands disappear on digestion of bull sperm DNA with EcoRI and either MspI (Figure 1 c5) or HpaII (Figure 1 c6) showing satellites 1.715, 1.711b and 1.709(?) to be unmethylated at CCGG sites in bull sperm DNA.

HpaII digestion alone produces few bands from somatic cell DNA (Figure 1 a3 and d3). A faint band is visible at 1950 bp which further indicates only partial methylation of the 1.709 satellite (see below). MspI (and HpaII with sperm DNA) gives rise to a large number of bands (Figure 1 a2, b2, c3 and c4). The bands at 390 bp, 360 bp, 285 bp and

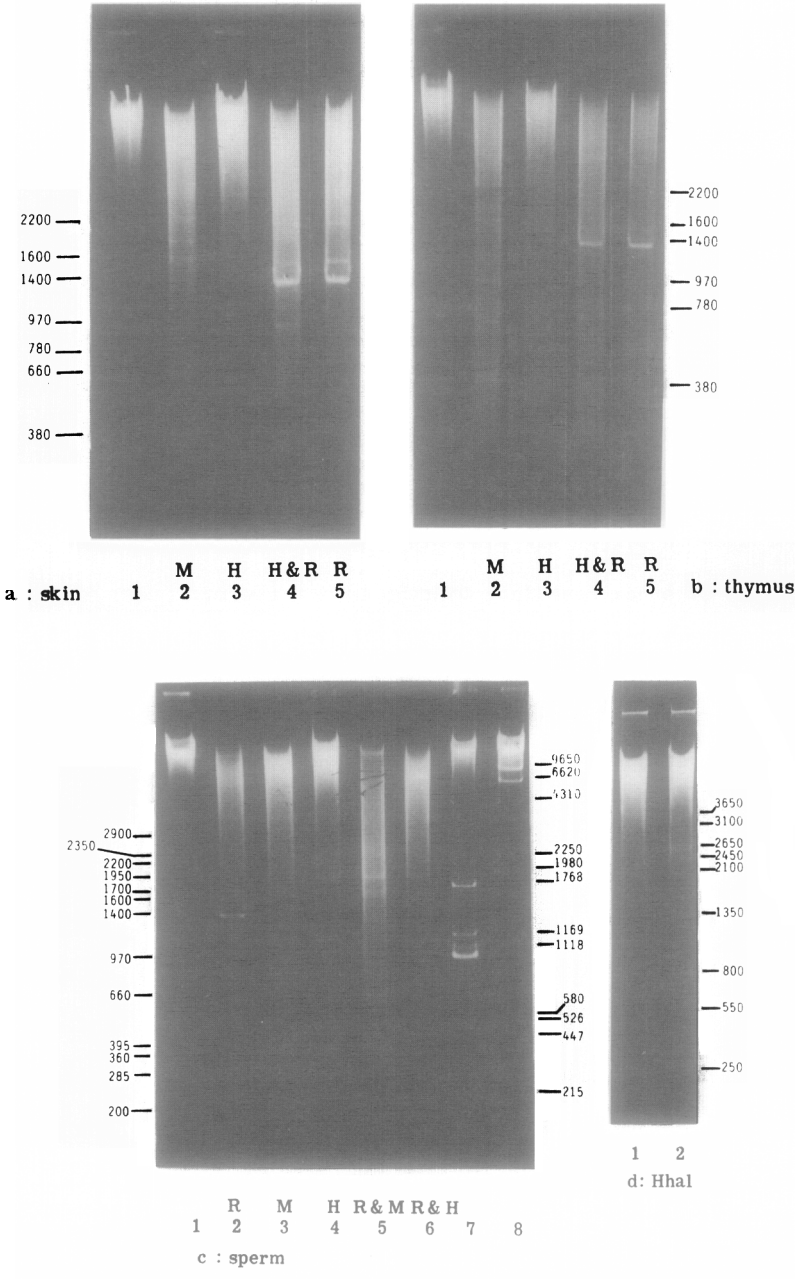

Figure 1 Restriction enzyme digestion of bovine DNA

DNA was prepared from bovine somatic tissues (skin lymphosarcoma (a) or thymus lymphosarcoma (b)) or sperm (c) and restricted with EcoRI (a5, b5, c2); MspI (a2, b2, c3); HpaII (a3, b3, c4) or EcoRI plus MspI (c5) or EcoRI plus HpaII (a4, b4, c6). The figures indicate the size of bovine satellites cleaved with EcoRI and EcoRI plus AvaII (a and b) or phage λ DNA and SV40 DNA cleaved with HindIII (c right). The numbers to the left of c indicate the size of the fragments produced by MspI or MspI plus EcoRI.

HhaI was also used to digest skin (d1) or sperm (d2) DNA; the size of the fragments is indicated. Thanks are due to Professor W. Jarrett for supply of the bovine lymphosarcomas and Professor T. Douglas for supply of the bull sperm.

smaller arise from satellite I (11, 15), (the 360 bp band is cut by EcoRI) but higher molecular weight bands at 660, 780, 1100 and 1400 probably also arise from satellite I indicating some sequence heterogeneity. The strong band at 1950 bp may arise from the 1.709 satellite which is present in bovine DNA to a similar extent as the 1.715 satellite (6) though again no definite evidence for this is yet available. The bands at 1700 bp and about 660 bp arise from the 1.706 satellite (9) and the former constitutes the only difference between an MspI and HpaII digest of sperm DNA being much fainter with HpaII indicating only partial methylation of this satellite in sperm. Other bands of unknown origin are also present indicating incomplete methylation of other satellite DNAs in bovine sperm.

Following HhaI (cuts GCGC only if the internal cytosine is unmethylated) digestion 6 major bands are produced from sperm DNA, but DNA from thymus (not shown) and skin does not produce any bands (Figure 1d). The bands at 550 bp and 800 bp arise from the 1.715 satellite as may most of the higher molecular weight bands (8, 20) as a result either of partial methylation of one or both of the two HhaI sties in a repeat; or, more likely, as a result of the loss by mutation of these sites in some repeats. The 1.706 and 1.711a satellites are made up of two regions known as Pvu and Sau segments (8,15). The Pvu segments of 1.706 and 1.711a satellites lack HhaI sites, but such sites occur on the Sau segments (indeed are frequent in the 1.711a satellite, (8, 15)). However, no band could be identified which would result from cleavage of HhaI sites and it is possible that the GCGC sites are methylated, at least in part, in these satellites in all the tissues investigated including sperm. HhaI digestion does however, result in a band of 2450 bp which does not arise from the 1.715 satellite and this may indicate that a single GCGC site in the 1.706 satellite is unmethylated.

In contrast to satellite DNA the bulk of the DNA of sperm is somewhat less affected by digestion with HpaII or HhaI than is the DNA of

somatic tissues indicating a high level of methylation of non satellite sperm DNA.

It is clear that in sperm DNA satellites 1.715, 1.711b, and tentatively 1.709 are unmethylated at CCGG and GCGC sites whereas satellites 1.706 and 1.711a (and possibly the related 1.720b) (18) are incompletely methylated. All the satellites are extensively methylated in DNA from skin and thymus with the possible partial exception of the 1.709 satellite. These results extend those of Kaput and Sneider (19), Sturm and Taylor (2) and Pages and Roizes (20). However, the fact that HpaII will only act if both strands of DNA are unmethylated rules out the hemimethylated model of Kaput and Sneider (19).

Treatment of mouse DNA with AvaII, EcoRII or Sau 961 gives a ladder of bands resulting from cleavage of the satellite DNA (21, 22). Sequence divergence has led to loss of some of the sites for these restriction enzymes but the monomer (234 bp) band is the strongest (Figure 2.3). There is no MspI/HpaII site in mouse satellite DNA but the MnlI site at position 75-78 bp is blocked by methylation of the adjacent cytosine (21). Double digestion of mouse DNA from L929 or Krebs II ascites cells with AvaII and MnlI does not affect the satellite DNA banding pattern and MnlI alone does not produce a banding pattern visible on stained gels. However, mouse sperm DNA treated with MnlI produces a faint banding pattern with the higher order fragments producing the most prominent bands (Figure 2.2). This is most clearly seen when a Southern transfer of the gel is probed with nick translated satellite DNA (Figure 2.7) when a less intense pattern is also seen with mouse L929 cell DNA (Figure 2.5). Double digestion of mouse sperm DNA with AvaII and MnlI produces a similar banding pattern to AvaII alone although the intensity of the monomer band is reduced. These results show that in mouse sperm satellite DNA most, but not all of the MnlI sites are methylated. It is unusual, however, to find two adjacent

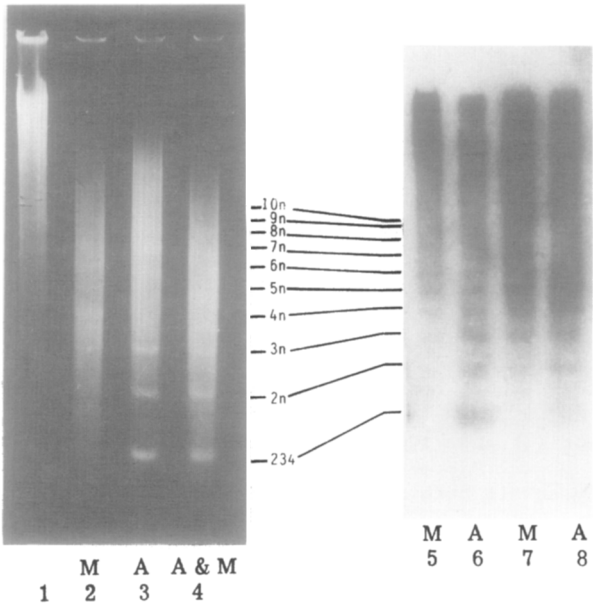

Figure 2 Restriction enzyme digestion of mouse DNA

Mouse sperm (1-4, 7, 8) or L929 cell (5, 6) DNA was digested with MnlI (2, 5, 7), AvaII (3, 6, 8) or AvaII plus MnlI (4). Slots 5-8 represent the autoradiograph of a Southern transfer probed with ^{32}P labelled nick translated satellite DNA cut out and extracted from a gel similar to 3. Thanks are due to N. Bansal and T. Davis for help with the Southern transfer experiment.

repeats which are unmethylated showing that undermethylation does not occur in blocks.

DISCUSSION

Most satellite DNAs appear to be largely or slightly undermethylated in DNA from sperm relative to DNA from somatic tissues of cattle and mouse respectively. In particular bovine satellites 1.715, 1.711b and 1.709 (and possibly some sheep and pig satellites) are completely unmethylated at CCGG and GCGC sites in sperm DNA. We found little evidence of unmethylated satellite DNA in skin or thymus DNA. An explanation for this diversity must be sought in the function or location of the various satellite DNAs and should take account of the propensity of short sequences containing the dinucleotide mCG to become amplified resulting in formation of satellite DNAs (whether GC or AT rich) with high levels of methylcytosine (7, 9, 10, 23-26).

Satellite DNAs are known to be associated with amplified genes (27) and are also associated with the heterochromatin at the centromeres of chromosomes (28). It is probable that satellite DNA in germ cells is involved in the pairing of chromosomes and in recombination events which normally occur at meiosis (19, 29). The extent of recombination is extremely high in meiosis and if similar recombination reactions were to occur in somatic cells considerable disruption of chromosomal organisation may well occur.

Such recombination events may be blocked in somatic cells by methylation which could affect the binding of enzymes that bring about recombination (30, 31). That is, it would be imperative to methylate (and thereby inactivate) the recognition site of such enzymes soon after meiosis. The time at which this inactivation occurs may vary with different satellite DNAs but would depend on the presence of a de novo methylase which may be active only in germ cells and early embryos (32, 33).

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REFERENCES

1. Vanyushin, B.F., Tkacheva, S.G. and Belozersky, A.N. (1970) Nature 225, 948-949.
2. Sturm, K.S. and Taylor, J.H. (1981) Nucl. Acids Res. 9, 4537-46.
3. Adams, R.L.P., McKay, E.L., Craig, L.H. and Burdon, R.H. (1979) Biochim. Biophys. Acta 563, 72-81.
4. Pollock, J.M., Swihart, M. and Taylor J.H. (1978) Nucl. Acids Res. 5, 4855-63.
5. Vanyushin, B.F., Mazin, A.L., Vasilyev, V.K. and Belozersky, A.N. (1973) Biochim. Biophys. Acta 299, 397-403.
6. Macaya, G., Cortedas, J. and Bernardi, G. (1978) Eur. J. Biochem. 84, 179-188.
7. Gautier, F., Bunemann, H. and Grotjahn, L. (1977) Eur. J. Biochem. 80, 175-183.
8. Kopecka, H., Macaya, G., Cortadas, J., Thiery, J.P. and Bernardi, G. (1978) Eur. J. Biochem. 84, 189-195.
9. Pech, M., Streeck, R.E. and Zachau, H.G. (1979) Cell 18, 883-893.
10. Poschl, E. and Streeck, R.E. (1980) J. Mol. Biol. 143, 147-154.
11. Gaillard, C., Doly, J., Cortadas, J. and Bernardi, G. (1981) Nucl. Acids Res. 9, 6069-6082.
12. Borenfreund, E., Fitt, E. and Bendich, A. (1961) Nature, 191, 1375-1377.

13. Shiurba, R. and Nandi, S. (1979) Proc. Natl. Acad. Sci. U.S.A., 76, 3947-3951.
14. Tegtmeyer, P. (1972) J. Virol. 10, 599-604.
15. Taparowsky, E.J. and Gerbi, S.A. (1982) Nucl. Acids Res. 10, 1271-1281.
16. Streeck, R.E. (1981) Science 213, 443-445.
17. Philippsen, P., Streeck, R.E., Zachau, H.G. and Muller W. (1975) Eur. J. Biochem. 57, 55-68.
18. Taparowsky, E.J. and Gerbi, S.A. (1982) Nucl. Acids Res. 10, 5503-5515.
19. Kaput, J. and Sneider, T.J. (1979) Nucl. Acids Res. 7, 2303-2322.
20. Pages, M. and Roizes, G. (1982) Nucl. Acids Res. 10, 565-576.
21. Horz, W. and Altenburger, W. (1981) Nucl. Acids Res. 9, 683-696.
22. Manuelidis, L. (1981) FEBS Letts. 129, 25-28.
23. Salomon, R., Kaye, A.M. and Herzberg, M. (1969) J. Mol. Biol. 43, 581-592.
24. Harbers, K., Harbers, B. and Spencer, J.H. (1975) Biochem. Biophys. Res. Comm. 66, 738-746.
25. Heller, R. and Arnheim, N. (1980) Nucl. Acids Res. 8, 5031-5042.
26. Deumling, B. (1981) Proc. Natl. Acad. Sci. 78, 338-342.
27. Bostock, C.J. and Clark, E.M. (1980) Cell 19, 709-715.
28. Kurnit, D.M., Shafit, B.R. and Maio, J.J. (1973) J. Mol. Biol. 81, 273-284.
29. Bostock, C. (1980) Trends in Biochem. Sci. 5, 117-119.
30. Korba, B.E., Hays, J.B. (1982) Cell 28, 531-541.
31. Hsieh, T-S. and Brutlag, D.L. (1979) Proc. Natl. Acadi. Sci. 76, 726-730.
32. Stewart, C.L., Stuhlmann, H., Jahner, D. and Jaenisch, R. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 4098-4102.
33. Jahner, D. Stuhlmann, H. Stewart, C.L., Harbers, K. Lohler, J., Simon, I. and Jaenisch, R. (1982) Nature, 298, 623-628.