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TISSUE-SPECIFIC METHYLATION IN THE 5' FLANKING REGION OF THE GAMMA-GLUTAMYL TRANSPEPTIDASE GENE

Julio Coloma* and Adelaida Garcia-Jimeno

Departamento de Bioquimica y Biologia Molecular, Facultad de Farmacia, Avda. Blasco Ibañez, 13, Valencia, 46010, Spain

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We have studied the relationship between the methylation and the expression of the gamma-glutamyl transpeptidase gene in adult rat liver and kidney. In the liver, where the level of expression is very low, the 5' flanking region of the gene appeared fully methylated, whereas in the kidney, where the gene is expressed at the highest level, it is undermethylated. In addition, kidney chromatin showed a DNase I hypersensitive site located near the origin of transcription. These results support a strong correlation between DNA undermethylation, DNase I sensitivity and tissue-specific gene expression.

Gamma-glutamyl transpeptidase(GGT) [E.C. 2.3.2.2] is a membrane bound enzyme, involved in the metabolism of glutathion and other gamma-glutamyl compounds (1). The enzyme is widely distributed and shows a tissue-dependent, ontogenic-dependent activity (2,3). GGT is frequently used as a marker for several hepatobiliary disorders (4) as well as in hepatocarcinogenesis (5). Although several GGT cDNAs have been isolated (6,7) and the structure of the rat gene has recently been reported (8,9), little is known about the regulation of GGT gene in different tissues. Methylation of specific bases in DNA has been considered a possible mechanism in the regulation of gene expression (10). DNA methylation can alter the local configuration of a gene and affect the binding of regulatory proteins (11). In the present study we have analyzed the level of methylation of the GGT gene in liver and kidney, two tissues with different levels of expression.

MATERIAL AND METHODS

DNA probes. Genomic DNA probes covering the 5' end and flanking region of the GGT gene (Figure 2) were a gift from Dr. D. Goodspeed, University of Wisconsin-Madison. Probes were ^{32}P-labeled by nick translation, using the Boehringer Mannheim kit.

* To whom correspondence should be addressed at Department of Molecular and Cell Biology, Pennsylvania State University, University Park, PA 16802.

Abbreviations used: GGT, gamma-glutamyl transpeptidase; Kb, kilobase; bp, base pair; SDS, sodium dodecyl sulfate.

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RNA isolation and Northern blot procedure. RNAs from rat liver and kidney were isolated by the guanidinium thiocyanate procedure (12). Fifty µg of liver and 10 µg of kidney total RNA were electrophoresed in a 1.2% agarose gel containing 2.2 M formaldehide (13). The RNAs were transferred onto a nylon membrane (Hybond-N, Amersham) and probed with a ^{32}P-labeled GGT cDNA clone (6). Prehybridizations and hybridizations were carried out at 42° C in a solution containing 50% formamide as described (13).

Nuclei isolation. Rat liver and kidney nuclei were isolated as described by Fritton et al. (14) with minor modifications. Isolated nuclei were used to prepare high molecular weight DNA and for DNase I digestions.

DNA isolation and Southern blot procedure. High molecular weight DNA was prepared by digesting the nuclei suspension with a volume of buffer S (20 mM Tris pH 7.6; 0.6 M NaCl; 10 mM EDTA;1% SDS and 0.5 mg/ml proteinase K) at 37°C overnight. The DNA was extracted with phenol, phenol/chloroform and precipitated with ethanol. The DNA was dissolved in TE (10 mM Tris pH 7.6, 1 mM EDTA) digested with RNase (1 µg/ml) for 1 hour at 37° C, extracted with phenol, ethanol precipitated and dissolved in TE. About 40 µg of DNA were digested with HindIII, MspI or HpaII (Boehringer Mannheim, 3 units/µg DNA) overnight at 37° C in the recommended manufacturer's buffers. DNA fragments were electrophoresed in 1% agarose gels and transferred onto a nylon membrane. Prehybridizations and hybridizations were carried out as described previously, using different genomic GGT probes.

DNase I digestion. Isolated nuclei (150 µl, 1 mg DNA/ml) were digested with different concentrations of DNase I (Sigma Chemical Co.) for 10 minutes at 0-4° C (14). Digestions were started by the addition of 5 mM $MgCl_2$ and increasing concentrations of DNase I. Digestions were stopped by the addition of a volume of buffer S and the DNA isolated as described above. The DNA was digested with Hind III, run on 1.2% agarose gels and transferred to a nylon membrane. DNA isolated from nuclei treated with $MgCl_2$ and no DNase I was used to check endogenous DNase I activity.
GGT activity and protein were measured as described (15,16).

RESULTS AND DISCUSSION

We have studied the relationship between the expression of the GGT gene and its methylation status in adult rat liver and kidney. GGT is an enzyme that shows variable levels of expression depending on the tissue and the developmental status of the animal. The enzyme is expressed at high level in fetal liver, but its activity decreases rapidly after birth, showing very low levels in adult liver (2). On the other hand, the kidney shows the opposite ontogenic pattern, low levels of expression during the fetal period and high levels in adulthood (3).

In our hands, both the activity (4.9 $\pm$ 0.23 U/mg protein) and the levels of specific GGT mRNA in the kidney (Figure 1, lane K), were significantly higher than those in liver (0.6 $\pm$ 0.14 mU/mg protein and Figure 1, lane L), suggesting that the regulation of this gene occurs at a transcriptional level. These results are in agreement with others (2,17).

Present evidence suggests that methylation of the cytosine in the CG dinucleotide plays a role in gene regulation (10) and embryonic development

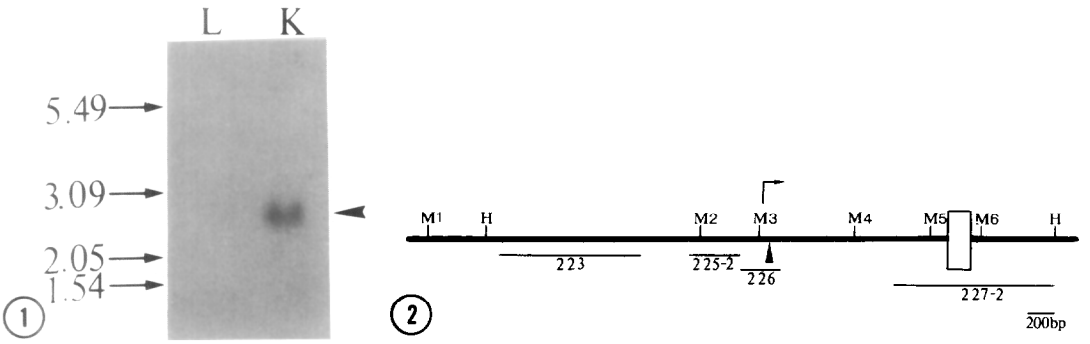

Figure 1. Northern blot analysis of liver (L) and kidney (K) RNAs. The RNAs were analyzed as described in Material and Methods. The arrow head indicates the 2.2 kb band corresponding to the GGT mRNA detected with a cDNA probe (6). The position and size of E.coli and mammalian rRNAs are indicated on the left.

Figure 2. GGT genomic probes used in Southern analysis. H, HindIII; M, MspI/HpaII. The open box represents the first exon. The arrow and arrow head indicate the transcription start site and the kidney DNase I hypersensitive site respectively. Redrawn from (9).

(18), even though the relation is far from clear. Some genes show an inverse correlation between methylation and expression, whereas others do not show any correlation (for a review, see ref. 19).

To assess the role of DNA methylation in GGT gene expression, we have used the methyl-sensitive restriction enzyme HpaII, that fails to cut the sequence CCGG when the internal C is methylated, in contrast to its isoschyzomer, MspI, that is not affected (20). Double digestions with HindIII were performed to futher map the MspI sites.

When digested with MspI, liver and kidney DNAs revealed a band of 1.9 kb when probed with clone 223 (Figure 3, lanes 6 and 8) corresponding to the fragment M1-M2 (Figure 2), a band of 420 bp when probed with clone 225-2 (Figure 4, lanes 6 and 8) corresponding to the fragment M2-M3, and a band of 660 bp when probe 226 was used (Figure 5, lanes 6 and 8) corresponding to the fragment M3-M4.

The HindIII/MspI digestions showed a band of 1.5 kb with the probe 223 (Figure 3, lanes 2 and 4) corresponding to the fragment H-M2 (Figure 2), and the same bands showed in MspI digestions when probes 225-2 and 226 were used (Figures 4 and 5, lanes 2 and 4).

When the DNAs were digested with HpaII or HindIII/HpaII, the liver and kidney digestion patterns were completely different. In all cases the kidney

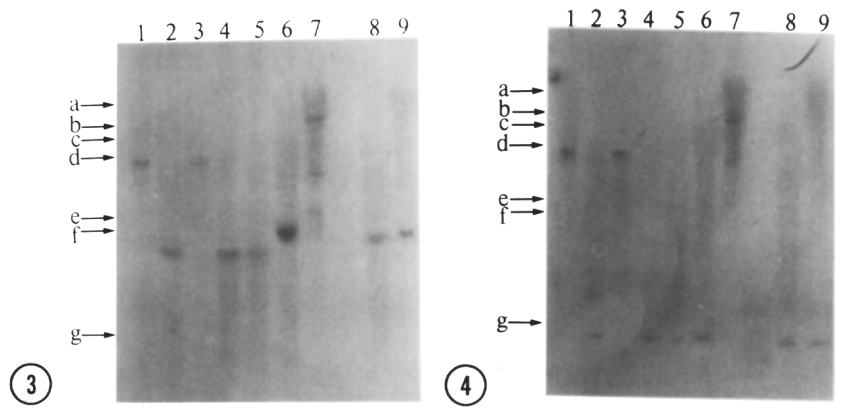

Figure 3. Southern blot analysis of digested liver and kidney DNAs probed with clone 223. Lanes: 1. liver HindIII; 2. liver HindIII/MspI; 3. liver HindIII/Hpa II; 4. kidney HindIII/MspI; 5. kidney HindIII/HpaII; 6. liver MspI; 7. liver HpaII; 8. kidney MspI; 9. kidney HpaII. Arrows at the left denote the mobility of HindIII-digested lambda DNA fragments:a, 23.13 kb; b, 9.416 kb; c, 6.557 kb; d, 4.361 kb; e, 2.322 kb; f, 2.027 kb; g, 0.564 kb.

Figure 4. Southern blot analysis of digested liver and kidney DNAs probed with clone 225-2. Lanes and size markers as in Figure 3.

showed the same bands present in MspI or HindIII/MspI digestions (Figures 3, 4, and 5, lanes 5 and 9), indicating that those sites were not methylated.

In contrast, liver DNA showed two bands of 6.5 and 3.6 kb (Figures 3, 4, and 5, lane 7) when digested with HpaII and a band of 4 kb when digested with HindIII/HpaII (Figures 3, 4, and 5, lane 5). This band corresponded to the HindIII-HindIII fragment containing the 5' end of the gene (Figure 2).

A problem we found in using these probes was a high background in the Southern blots, that forced us to use in some cases, astringent washing conditions and long time exposures to improve the signal/noise ratio. This problem was acute when using clone 227-2. In spite of the high background, we were able to detect the fragments M4-M5 and M6-H in kidney HindIII/HpaII digestions (data not shown).

Our data did not show a MspI site located between M2 and M3 (8,9) (Figure 4). That could be due either to a polimorphism or to a methylation of the 5' C in the recognition sequence CCGG (21).

Another feature that characterizes transcriptionally active chromatin is a hypersensitivity to digestion to DNase I and other nucleases (22). This preferential nuclease sensitivity has been interpreted as a reflection of the

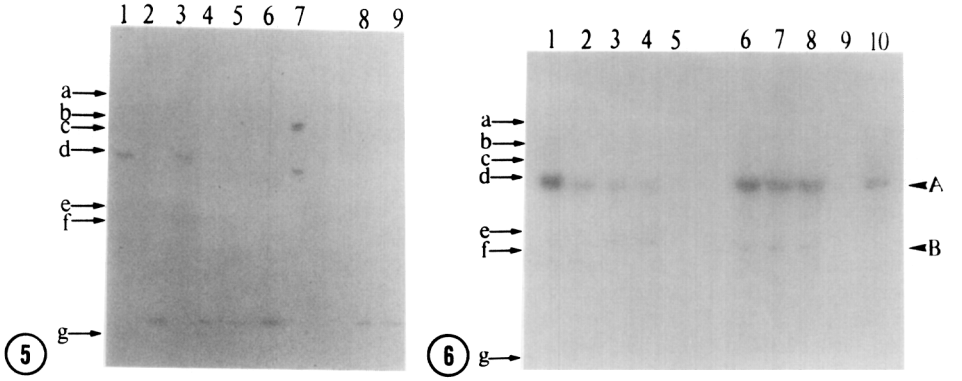

Figure 5. Southern blot analysis of digested liver and kidney DNAs probed with clone 226. Lanes and size markers as in figure 3.

Figure 6. Southern blot of DNA isolated fron DNase I digested nuclei. 1-5, liver nuclei; 6-10, kidney nuclei. Nuclei were digested as described in Material and Methods with none (1,10), 400 U/ml (2,6), 800 U/ml (3,7), 1,600 U/ml (4,8) and 2,400 U/ml (5,9) of DNase I. The DNA was isolated, cut with HindIII, separated on agarose gel and transferred to nylon. The blot was probed with clone 223. A, HindIII-HindIII 4 kb fragment; B, 2 kb fragment generated by DNase I digestion. Size markers as in Figure 3.

accessibility of the DNA within those regions to the transcription machinery, due to a more relaxed or open conformation of the chromatin (23).

Using and indirect labeling technique (24), Figure 6 shows that kidney chromatin presented a DNase I hypersensitive site located 2 kb from the 5' HindIII site. This site, that is absent in liver, mapped near the origin of transcription (Figure 2).

Our results show that in the adult liver, where the expression of the GGT gene is very low, the 5' region of the gene is fully methylated, whereas in kidney, where the expression is much higher, the gene is undermethylated. In addition, a DNase I hypersensitive site present specifically in kidney mapped near the origin of transcription. These data support a strong correlation between DNA undermethylation, DNase I sensitivity and tissue-specific expression of the GGT gene.

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REFERENCES

1. Tate, S.S., and Meister, A. (1981) Mol. Cell. Biochem. 39, 357-368.
2. Sulakhe, S.J., and Lautt, W.W. (1987) Int. J. Biochem. 19, 23-32.

3. Wapnir, R.A., Mancusi, V.J., and Goldstein, L.A. (1982) Experientia 38, 647-648.
4. Rosalki, S.B. (1975) Adv. Clin. Chem. 17, 53-107.
5. Hanigan, M.H., and Pitot, H.C. (1985) Carcinogenesis 6 165-172.
6. Coloma, J., and Pitot, H.C. (1986) Nucleic Acids Res. 14, 1393-1413.
7. Goodspeed, D.C., Dunn, T.J., Miller, C.D., and Pitot, H.C. (1989) Gene 76, 1-9.
8. Rajagopalan, S., Park, J.H., Patel, P.D., Lebovitz, R.M., and Lieberman, M.W. (1990) J. Biol. Chem. 265, 11721-11725.
9. Kurauchi,O., Lahuna, O., Darbouy, M., Aggerbeck, M., Chobert, M-N., and Laperche, Y. (1991) Biochemistry 30, 1618-1623.
10. Doerfler, W. (1983) Ann. Rev. Biochem. 52, 93-124.
11. Cedar, H. (1988) Cell 53, 3-4.
12. Chirgwin, J.M., Przybyla, A.E., McDonals, R.J., and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
13. Maniatis, T., Frisch, E.F., and Sembrook, J. (1982) Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.
14. Fritton, H.P., Sippel, A.E., and Igo-Kemenes, T. (1983) Nucleic Acids Res. 11, 3467-3484.
15. Sirica, A.E., Richards, W., Tsukada, J., Sattler, G., and Pitot, H.C. (1979) Proc. Natl. Acad. Sci. USA 76, 283-287.
16. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
17. Chobert, M.N., Lahuna, O., Lebargg, F., Kurauchi, O., Darbony, M., Bernaudin, J.F., Guellaen, G., Barouki,R., and Laperche, Y. (1990). J. Biol. Chem. 265, 2352-23557.
18. Jahner, D., and Jaenisch, R. (1984) In DNA methylation: Biochemistry and biological significance (A. Razin, H. Cedar, and A.D. Riggs Ed.) pp.189-219. Springer-Verlag, NY.
19. Adams, R.L.P., and Burdon, R.H. (1985) Molecular biology of DNA methylation. pp. 115-161. Springer-Verlag. NY.
20. Bird, A.P. (1978) J. Mol. Biol. 118, 49-60.
21. Jentsch, S., Gunthert, U., and Trautner, T.A. (1981) Nucleic Acids Res. 9, 2753-2759.
22. Reeves, R. (1984) Biochem. Biophys. Acta 782, 343-393.
23. Conklin, K.F., and Groudine, M. (1984) In DNA methylation: Biochemistry and biological significance (A. Razin. H. Cedar, and A.D. Riggs Eds) pp. 293-351. Springer-Verlag, NY.
24. Wu, C. (1980). Nature 286, 854-860.