NOTES

Liver-Specific Expression of a Qa-Encoded Class I Gene Is Associated with DNA Hypomethylation

C. GARRETT MIYADA* AND R. BRUCE WALLACE

Department of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, California 91010

Received 8 August 1985/Accepted 7 October 1985

DNA methylation of two murine major histocompatibility complex (H-2) class I genes was examined in hybridizations to *MspI* and *HpaII* chromosomal DNA restriction digests. Q10, which exhibits liver-specific expression, and H-2K^b, a transplantation antigen gene, were examined in liver, spleen, thymus, and cell-line DNAs. Unmethylated Q10 gene sequences were detected only in the liver, whereas the H-2K^b gene was unmethylated in all tissues examined.

The class I genes of the murine major histocompatibility complex (H-2) constitute a multigene family whose protein products are involved in various immune responses (5). They are characterized by encoding 45,000-dalton glycoproteins found in association with β_2 -microglobulin. The classical transplantation antigens, encoded by the K, D, and L loci of the H-2 complex, are involved in cell-cell recognition events required for immune responses such as graft rejection and cell-mediated lympholysis (6). The transplantation antigens are expressed on virtually all somatic tissues; however, their level of expression varies from tissue to tissue (5, 9). Structurally related to the transplantation antigens are the class I genes encoded by the Qa and Tla regions of the H-2 complex. The function and regulation of these genes is less well understood, but they seem to exhibit tissue-specific expression (3). The Q10 gene is an example of a Qa-encoded class I gene (8). It is unusual because it encodes a secreted protein product (7). This gene is highly conserved among the different H-2 haplotypes of inbred mouse strains, and its transcription has been detected only in the liver (7, 9).

The large number of class I genes (over 20; 15, 20) presents a problem in studying an individual gene, because nick-translated probes normally cross-hybridize to other family members. Recently, we have shown that synthetic oligonucleotides can act as gene-specific hybridization probes to H-2 class I genes in chromosomal restriction digests (9). The K^b oligonucleotide hybridizes only to the H-2K^b gene, whereas the bm1 oligonucleotide is specific for the Q10 gene in restriction digests of C57Bl/6 mouse chromosomal DNA. Both oligonucleotides are complementary to the sequences encoding amino acids 150 to 157 in the C1 (α -2) domain of their respective protein product (Fig. 1). These two oligonucleotides were used as hybridization probes in comparing chromosomal restriction digests prepared with either *MspI* or its methylation-sensitive isoschizomer, *HpaII* (14, 18). The Q10 gene was undermethylated only in the liver, whereas the H-2K^b gene exhibited a lack of methylation in all of the tissues studied. These results suggest that DNA hypomethylation is associated with gene expression among H-2 class I genes. Our

H-2K^b gene results, however, contrast with an earlier study of that gene (16).

DNA isolations, agarose gel electrophoresis, oligonucleotide labeling, and hybridizations have been previously described (9). Restriction endonuclease digestions were done at a DNA concentration of 0.1 $\mu\text{g}/\mu\text{l}$ and 4 to 5 U of restriction enzyme per μg of DNA for 4 to 6 h at 37°C. *HpaII* restriction endonuclease digestions were assayed for completeness by mixing a small sample of the restriction digest with a cloned DNA control and assaying for complete digestion of the cloned DNA.

The digested DNA samples were subjected to electrophoresis on a 0.8% agarose gel, stained with ethidium bromide, denatured, neutralized, dried within the gel matrix (17), and hybridized with 5'-end-labeled bm1 oligonucleotide. The resulting autoradiogram (Fig. 2, left) showed that *HpaII* and *MspI* restriction digests produced a similar-sized hybridizing restriction fragment (2.1 kilobases [kb]) only in the liver DNA. In the spleen, thymus, and EL4 DNAs, a 2.1-kb hybridizing fragment in *MspI* restriction digests was detected, whereas a similar-sized restriction fragment was not seen in the corresponding *HpaII* digests. Hybridization to restriction fragments greater than 6.6 kb in the *HpaII* digestions was obscured by nonspecific binding of the oligonucleotide. The *HpaII* digests were then subjected to electrophoresis on a 0.4% agarose gel and treated and hybridized as in the previous experiment. In this case (Fig. 2, right), hybridization to restriction fragments greater than 4.4 kb was apparent in all of the samples. In liver DNA, most of the hybridization occurred to a 2.1-kb restriction fragment, although hybridization was also detected in restriction fragments 4.5 and 11 kb in size. In the spleen and thymus DNA samples, hybridization to 4.5-, 11-, and 18-kb restriction fragments was found. In EL4 (a thymoma cell line) DNA, most of the restriction fragments that hybridized to the bm1 probe were larger than 48.5 kb. Only the expressing tissue, the liver, exhibited an absence of methylation for the two *MspI* restriction sites surrounding the C1 domain (Fig. 1). However, not all of the liver DNA was unmethylated at these two restriction sites.

Alternatively, the same DNA samples were subjected to electrophoresis on a 2% agarose gel, stained with ethidium bromide, denatured, neutralized, and electrophoretically

* Corresponding author.

FIG. 1. *MspI* restriction sites in the H-2K^b and Q10 genes. The H-2K^b gene (19) and the Q10 gene (8) structures are diagrammed. Exons are shown as rectangles, with black representing translated regions and white representing untranslated regions. Introns are shown as thin-angled lines between the exons. At the top of the diagram is a scale measured in base pairs. Below each gene, the *MspI* restriction sites are shown as **I**, whereas unsequenced regions are shown as ----. Predicted restriction fragments and their respective lengths based on the known *MspI* sites that should hybridize to either the K^b probe (H-2K^b gene) or the bm1 probe (Q10 gene) are also shown below each gene diagram. The DNA sequence of the K^b oligonucleotide: 5'-CTGAGTCTCTGCTTCACCAGC-3'. The DNA sequence of the bm1 oligonucleotide: 5'-CTGTAATACTCTGCAGCACCAGC-3'.

transferred to a Gene Screen Plus hybridization membrane (New England Nuclear Corp.). The resulting membrane was hybridized with the K^b-specific oligonucleotide, and the resulting autoradiogram is shown in Fig. 3. In all of the samples, a restriction fragment of approximately 600 base pairs hybridized to the K^b oligonucleotide. (The hybridization to restriction fragments larger than 2 kb was nonspecific as determined in other hybridizations [data not shown].) The size of this restriction fragment agreed with that predicted

based on the available sequence data for the H-2K^b gene (19) (Fig. 1). Because all of the cells used for the DNA extractions produce the H-2K^b transplantation antigen, it appears that the expression of H-2K^b is correlated to the lack of methylation at the two *MspI* sites bordering the C1 domain coding exon (for the position of these sites, see Fig. 1).

Methylation of gene sequences may control gene expression (11, 12). Although a reduction in the methylation of certain genes does not activate them per se (4), a strong

FIG. 2. Autoradiograms of *MspI* and *HpaII* restriction endonuclease digestions of mouse tissue DNAs and EL4 DNA hybridized with the bm1 oligonucleotide. (Left) DNA samples (10 μ g) were digested with either restriction endonuclease *MspI* (M) or *HpaII* (H), subjected to electrophoresis on a 0.8% agarose gel, and hybridized directly in the gel matrix with ³²P 5'-end-labeled bm1 oligonucleotide. Lane C contains *PvuII*-digested R6E2, a positive control and a pBR322 derivative that contains the cloned H-2K^{bm1} gene. Also shown on the left are molecular size markers (in kilobase pairs) obtained from a *HindIII* digestion of phage λ DNA. (Right) *HpaII*-digested DNA samples (10 μ g) were subjected to electrophoresis on a 0.4% agarose gel and hybridized as above. Molecular size markers (in kilobases) are shown on the right. The hybridizations and washings were done as previously described (9). The washed gels were exposed to Kodak XAR-5 X-ray film between two Du Pont Cronex Lightning-Plus intensifying screens at -70°C for either 2.5 days (left) or 6 days (right).

FIG. 3. Autoradiogram of *MspI* and *HpaII* restriction endonuclease digestions of mouse tissue DNAs and EL4 DNA hybridized with the K^b oligonucleotide. DNA samples (10 μ g) were digested with either restriction endonuclease *MspI* (M) or *HpaII* (H), subjected to electrophoresis on a 2% agarose gel, and transferred by electrophoretic blotting to a nitrocellulose membrane before hybridization with 5'-end-labeled K^b oligonucleotide. Lane C contains *HpaII*-digested C1.4.1, a lambda clone that contains the H-2K^b gene (13). Shown at the left are molecular size markers (in kilobase pairs) obtained from a *HindIII* digestion of phage λ DNA and an *AluI* digestion of pBR322 DNA. The hybridization and washes were done as previously described for Northern blots, except that 0.5% sodium dodecyl sulfate was included in both procedures (9). Autoradiography was done as described in the legend to Fig. 2 for 7 days.

correlation between hypomethylation and transcriptional gene activity has been established (12). Our results with the Q10 and H-2K^b genes were consistent with this hypothesis; however, methylated sequences were also found in the liver tissue for the Q10 gene. This result was not too surprising, because the liver is made up of several cell types and only the hepatocytes are responsible for the secretion of plasma proteins (2). In the liver, hepatocytes compose about 78% of the parenchymal volume (2).

For the murine H-2K gene, it has been reported that transcriptional activity is correlated with an increase in DNA methylation (16). That study was performed with the teratocarcinoma cell line, F9, and utilized a probe that was complementary to the 3' untranslated region of the gene. Our results were obtained with a probe that is complementary to the C1 domain exon (Fig. 1). The apparent contradiction may be resolved by assuming that different methylation patterns exist for different domains of the expressed H-2K gene. A similar situation has been reported for the serum albumin gene in rat hepatoma cells (10).

Recently, a study was published on the state of methylation of the Q10 gene transformed in tissue culture cell lines (1). That study found no direct correlation between DNA methylation and Q10 expression; a mixture of methylated and unmethylated sequences was found in both expressing and nonexpressing cell lines. The authors suggested that *trans*-acting factors may also be responsible for Q10 expression, a result that our study neither confirmed nor rejected.

We thank A. D. Riggs for critically reading this manuscript and A. Mellor and R. A. Flavell for sending the cloned Q10 gene.

This work was supported by Public Health Service grant GM 31261 from the National Institutes of Health to R.B.W. R.B.W. is a member of the Cancer Center at the City of Hope (Public Health Service grant CA 33572, also from the National Institutes of Health).

LITERATURE CITED

- Barra, Y., K. Tanaka, K. J. Isselbacher, G. Khoury, and G. Jay. 1985. Stable transfer and restricted expression of a cloned class I gene encoding a secreted transplantation-like antigen. *Mol. Cell. Biol.* 5:1295-1300.
- Feldmann, G. 1979. Morphologic aspects of hepatic synthesis and secretion of plasma proteins. *Prog. Liver Dis.* 6:23-41.
- Hood, L., M. Steinmetz, and B. Malissen. 1983. Genes of the major histocompatibility complex of the mouse. *Annu. Rev. Immunol.* 1:529-568.
- Hsiao, W.-L. W., S. Gattoni-Celli, P. Kirschmeier, and I. B. Weinstein. 1984. Effects of 5-azacytidine on methylation and expression of specific DNA sequences in C3H 10T1/2 cells. *Mol. Cell. Biol.* 4:634-641.
- Klein, J. 1975. *Biology of the mouse major histocompatibility-2 complex*. Springer-Verlag, New York.
- Klein, J., A. Juretic, C. N. Baxevanis, and Z. A. Nagy. 1981. The traditional and a new version of the mouse H-2 complex. *Nature (London)* 291:455-460.
- Kress, M., D. Cosman, G. Khoury, and G. Jay. 1983. Secretion of a transplantation-related antigen. *Cell* 34:189-196.
- Mellor, A. L., E. H. Weiss, M. Kress, G. Jay, and R. Flavell. 1984. A nonpolymorphic class I gene in the murine histocompatibility complex. *Cell* 36:139-144.
- Miyada, C. G., C. Kiofelt, A. A. Reyes, E. McLaughlin-Taylor, and R. B. Wallace. 1985. Evidence that polymorphism in the murine major histocompatibility complex may be generated by the assortment of subgene sequences. *Proc. Natl. Acad. Sci. USA* 82:2890-2894.
- Orlowsky, A., and L. A. Chasin. 1985. A domain of methylation change at the albumin locus in rat hepatoma cell variants. *Mol. Cell. Biol.* 5:214-225.
- Razin, A., and A. D. Riggs. 1980. DNA methylation and gene function. *Science* 210:604-610.
- Riggs, A. D., and P. A. Jones. 1983. 5-Methylcytosine, gene regulation and cancer. *Adv. Cancer Res.* 40:1-30.
- Schulze, D. H., L. R. Pease, Y. Obata, S. G. Nathenson, A. A. Reyes, S. Ikuta, and R. B. Wallace. 1983. Identification of the cloned gene for the murine transplantation antigen H-2K^b by hybridization with synthetic oligonucleotides. *Mol. Cell. Biol.* 3:750-755.
- Singer, J., J. Roberts-Em, and A. D. Riggs. 1979. Methylation of mouse liver DNA studied by means of the restriction enzymes *MspI* and *HpaII*. *Science* 203:1019-1021.
- Steinmetz, M., A. Winoto, K. Minard, and L. Hood. 1982. Clusters of genes encoding mouse transplantation antigens. *Cell* 28:489-498.
- Tanaka, K., E. Appella, and G. Jay. 1983. Developmental activation of the H-2K gene is correlated with an increase in DNA methylation. *Cell* 35:457-465.
- Tsao, S. G. S., C. F. Brunk, and R. E. Perlman. 1983. Hybridization of nucleic acids directly in agarose gels. *Anal. Biochem.* 131:365-372.
- Waalwijk, C., and R. A. Flavell. 1978. *MspI*, an isoschizomer of *HpaII* which cleaves both unmethylated and methylated *HpaII* sites. *Nucleic Acids Res.* 5:3231-3236.
- Weiss, E., L. Golden, R. Zakut, A. Mellor, K. Fahrner, S. Kvist, and R. A. Flavell. 1983. The DNA sequence of the H-2K^b gene: evidence for gene conversion as a mechanism for the generation of polymorphism in histocompatibility antigens. *EMBO J.* 2:453-462.
- Weiss, E. H., L. Golden, K. Fahrner, A. L. Mellor, J. Devlin, H. Bullman, H. Tiddens, H. Bud, and R. A. Flavell. 1984. Organization and evolution of the class I gene family in the major histocompatibility complex of the C57Bl/10 mouse. *Nature (London)* 310:650-655.