Membrane-bound dipeptidase (MBD) participates in the degradation of glutathione by cleaving the cysteinyl-glycine bond of cystinyl bisglycine (oxidized cysteinyl-glycine) following removal of a γ-glutamyl group by γ-glutamyl transpeptidase (GGT). In the mouse, MBD RNA is most abundant in small intestine, kidney, and lung and is represented by four distinct RNA species. These are generated by transcription from two promoters located 6 kilobases apart in the 5′ flanking region of the gene and by the use of two different poly(A) addition sites. Promoter I is used primarily in small intestine and kidney, whereas promoter II is most active in lung and kidney. We found a discordance in the expected co-expression of MBD and GGT; as expected, MBD and GGT are both expressed at high levels in the kidney and small intestine. However, in the lung, MBD is expressed at high levels, whereas GGT is almost undetectable. The reverse is true in the seminal vesicles and fetal liver. Thus, although both enzymes may function in concert to metabolize glutathione in kidney and small intestine, in other tissues they appear to act independently, suggesting that they have independent roles in other biological processes.

Membrane-bound dipeptidase (MBD; dehydropeptidase-I; renal dipeptidase; microsomal dipeptidase; EC 3.4.13.11) was initially identified for its β-lactamase activity and has been shown to catalyze the hydrolysis of a number of dipeptides, including antibiotics such as penem and carbapenem derivatives (1, 2); it is an ectoenzyme anchored to the plasma membrane by glycosylphosphatidylinositol (3). MBD has also been implicated in the degradation of glutathione. Following removal of a γ-glutamyl group by γ-glutamyl transpeptidase (GGT; EC 2.3.2.2), MBD is thought to cleave cystinyl bisglycine to its component amino acids. Thus, the sequential action of these two enzymes is believed to be essential in the recycling of GSH via the γ-glutamyl cycle (4, 5) and the formation of the mercapturic acid derivatives of toxins and xenobiotics (1, 4, 5).

The metabolism of peptidyl leukotrienes, prostaglandins, and the transport of cysteine and perhaps other amino acids are other biological processes in which MBD participates (5–8). Inactivation of these pathways by homologous recombination or inhibitors of enzyme function results in massive thiourea and reduced levels of plasma cysteine and intracellular GSH (4, 9–11).

The complete primary sequence of MBD has been elucidated for a variety of mammalian species by cDNA cloning (12–14). The human MBD gene has been cloned and found to span about 6 kb and consists of nine coding exons (15). Recently, Adachi et al. (16) demonstrated the importance of Glu-125 in the catalytic activity of human MBD, and this residue is also conserved in mouse MBD (12) and is present in coding exon 5 (this paper). Because of the potential therapeutic importance of β-lactam antibiotics, most of these efforts appear to have been directed toward understanding the structure-function relationship and the development of better inhibitors of MBD (16–18).

In contrast, other functions of MBD have been less extensively studied (19, 20). In view of the importance of MBD in the γ-glutamyl cycle and in the metabolism of leukotrienes and xenobiotics, more information about non-antibiotic aspects of MBD would be welcome. As a first step in the analysis of the regulation of MBD expression and its role in mammalian physiology, we have studied MBD expression in the mouse. We have examined tissue-specific steady-state MBD RNA levels on a comparative basis with GGT, identified four distinct MBD RNA species, and demonstrated that they are encoded by a single MBD gene.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases, T4 DNA ligase, and expand-long template PCR system were purchased from Boehringer Mannheim. T3 and T7 primers and PUC/M13 forward primer were obtained from Promega Corp. (Madison, WI). All other primers were synthesized using a Beckman oligo digo 1000 or oligo 1000 M DNA synthesizer. All radioisotopes were obtained from DuPont NEN.

RNA Extraction—Total RNAs were prepared from FVB mice (adult albino Friend leukemia strain B) using the guanidinium thiocyanate procedure (21). Poly(A) RNA was isolated using poly(A) tract mRNA isolation system III from Promega Corp.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)—To generate MBD cDNA probes for screening, two pairs of oligonucleotide primers were synthesized based upon published MBD cDNA sequence (13): primer 1, 5′-GTGATCATCTGGTGGTTCTGG-3′ (sense); primer 2, 5′-CAGGTCAATCATGACCCAAGC-3′ (antisense); primer 3, 5′-GAACGCCTTTGGGTGTACCTGTA-3′ (sense); and primer 4, 5′-CAGAAAGTGAGAGACGGAGAGC-3′ (antisense). Approximately 2.5 μg of total RNA from mouse FVB kidney was reverse transcribed using the antisense primer by avian myeloblastosis virus reverse transcriptase. The resultant cDNAs were amplified by PCR using a Perkin-Elmer DNA
thermal cycle 480 under the following conditions: 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, 35 cycles. The PCR products corresponding to the 5'- and 3'-end of the cDNA were then gel purified and cloned into pT7 Blue (R)-T-Vector (Novagen, Madison, WI). The cloned cDNA were verified by sequencing.

RNA Analysis by Northern Blotting—Fifteen μg of total RNA from various mouse tissues were electrophoresed on 1% agarose gels and labeled mouse MBD cDNA fragments as described above. Appropriate the DNA was blotted onto nylon membranes as described under Southern's recommendations, and phage DNA was prepared essentially as described (23). Following digestion with different restriction endonucleases, separated on a 1% agarose gels, and the band corresponding to 200–600 bp was cut out, and com- pared to wild type. The probe used for hybridization in a mouse MBD 32P-labeled cDNA probe (for details, see "Materials and Methods"). A, steady state expression of GGT in the same tissues as described above. The probe used for hybridization is a 32P-labeled cDNA probe (see "Materials and Methods"). The lanes on the left (K, S, B, Al, and SI) were exposed for 16 h, while the lanes on the right (H, L, Sm, F, and Tv) were exposed for 1 week. C, analysis of MBD expression in the GGT-deficient mice as compared to wild type. The probe used for hybridization was a 32P-labeled Nhl-Sphl (351-bp) fragment from mouse MBD cDNA (32). Inspection of ethidium bromide-stained gels indicated approximately equal loading in all lanes (data not shown).

Restriction Mapping and DNA Sequence Analysis—Phage DNA samples were digested with various restriction enzymes under single and double digestion conditions and were run on a 1% agarose gels. Ethidium bromide-stained gels were photographed under UV light, and the DNA was blotted onto nylon membranes as described under Southern hybridizations. Hybridized cDNAs were cut out of the blotted nylon membranes as described above. Appropriate positive restriction fragments were isolated from 1% agarose gels and subcloned into the pBluescript SK phagemids were excised in vivo from plaque-purified phages, according to manufacturer's recommendations, and phage DNA was prepared essentially as described (23).

Restriction Mapping and DNA Sequence Analysis—Phage DNA samples were digested with various restriction enzymes under single and double digestion conditions and were run on a 1% agarose gels. Ethidium bromide-stained gels were photographed under UV light, and the DNA was blotted onto nylon membranes as described under Southern hybridizations. Hybridized cDNAs were cut out of the blotted nylon membranes as described above. Appropriate positive restriction fragments were isolated from 1% agarose gels and subcloned into the pBluescript SK (+/−) Vector (Stratagene).

Double-stranded plasmid DNA derived from genomic and cDNA subclones were sequenced using an automated Applied Biosystem 373 DNA sequencer using the Applied Biosystems dyeoxy terminator cycle sequencing kit (Perkin-Elmer Corp., Universal primers, such as T3 and T7 promoter primers, and synthetic primers designed from the mouse cDNA library in the Uni-Zap XR Vector and a mouse 129SvEv liver genomic library in the λ Fix II Vector from Stratagene Cloning Systems (La Jolla, CA) were screened using a 32P-labeled cDNA probe corresponding to 610 to 1230 of the mouse MBD cDNA, respectively. The cDNA library was plated out at ~50,000 plaques/dish, and the filter lifts in duplicate were hybridized to the probe under the following conditions. The hybridization was performed overnight at 65 °C in 6 × SSC, (1 × SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 0.25% Carnation nonfat dry milk, 100 μg/ml denatured salmon sperm DNA, and 10 6 cpm/ml α- [32P]dATP-labeled MBD cDNA and washed with 2 × SSC, 1% SDS, followed by 0.2 × SSC, 0.1% SDS at 65 °C. After three rounds of screening, pBluescript SK phagemids were excised in vivo from plaque-purified phages, according to manufacturer's recommendations, and phage DNA was prepared essentially as described above.

Restriction Mapping and DNA Sequence Analysis—Phage DNA samples were digested with various restriction enzymes under single and double digestion conditions and were run on a 1% agarose gels. Ethidium bromide-stained gels were photographed under UV light, and the DNA was blotted onto nylon membranes as described under Southern hybridizations. Hybridized cDNAs were cut out of the blotted nylon membranes as described above. Appropriate positive restriction fragments were isolated from 1% agarose gels and subcloned into the pBluescript SK (+/−) Vector (Stratagene).

Construction of Mouse Kidney MBD cDNA Clones by RACE—Mouse kidney poly(A)+ RNA (2.5 μg) was used to generate 5'-ends of MBD cDNAs using the Marathon cDNA amplification kit (Clontech Laboratories, Inc., Palo Alto, CA). The synthesis of double-stranded cDNA and anchor ligation were done essentially according to the manufacturer's instructions. The anchor-ligated cDNA was then amplified using anchor primer 1 and MBD-specific complementary primer 5, 5'-CGCGTGTG- GGTCTCATGATAG-3'. The type II MBD probe consists of 65 bases of the 5'-end of the coding region encompassing the translation initiation site, 52 bases of the common untranslated region, and 51 bases of the unique type I sequence (Fig. 6). The type II MBD probe consists of 65 bases of the 5'-end of the coding region (see above), 52 bases of the common untranslated region, and 133 bases of the unique translated region. One hundred μg of kidney, small intestine, lung, or spleen RNA was used for the assay. After 18 h of hybridization of 1.2 × 106 cpn of probe to the RNA, the hybrids were digested as described previously (24) using Ambion ribonuclease protection assay kit (Ambion, Inc., Austin, TX). The protected fragments were then separated on a 7% urea, 6% polyacrylamide gel and quantitated by either an AMBUS radioanalytic imaging system and an AMBUS Quantiprobe software version 4.01 (AMBIS, Inc., San Diego, CA) or by a Betagen Betascope 603 blot analyzer.

In Situ Hybridization—Sequences representing 52 bp of the 5'-common untranslated region and 95 bp of the coding region were amplified by RT-PCR using mouse kidney RNA as the template using primer 5 (see under “Construction of Mouse Kidney MBD cDNA Clones by RACE”) as the antisense primer and primer 7, 5'-ACCGAGTGGC- CAAAAGCCGCTG-3', as the sense primer. This fragment was then cloned into the pT7 Blue (R)-T-Vector in both orientations.

Riboprobes were generated by transcribing the cassettes in both orientations from BamHI-linearized templates using T7 RNA polymerase in the presence of digoxigenin-labeled UTP (labeling mixture, Boehringer Mannheim). Typically, 1 μg of template yielded 4–6 μg of riboprobe. No specific labeling was seen using a prehybridization RNase wash. Frozen sections were mounted on RNase-free coated slides, fixed in fresh 4%
paraformaldehyde, and prehybridized, hybridized, and washed as reported earlier (25).

GGT Enzyme Histochemistry—GGT enzyme histochemistry was performed as described by Rutenberg et al. (26).

RESULTS

Analysis of MBD Steady State RNA Levels in Mouse Tissues—We used Northern analysis to evaluate MBD expression in a variety of tissues and found that RNA levels were highest in the small intestine and about 2-fold lower in kidney (Fig. 1A). Appreciable levels of expression were also observed in lung, and much lower levels were seen in heart and skeletal muscle. We could not detect MBD RNA in spleen, brain, adult or fetal liver, or seminal vesicles by this method. We identified two distinct RNA species of ~1.6 and ~2.2 kb in all tissues that expressed MBD. The ratio of expression of the two species (smaller:larger) was ~1.5:1.0 in small intestine and kidney and the reverse in lung, heart, and skeletal muscle.

Comparison of MBD and GGT Expression—Because MBD and GGT are both ectoenzymes and function in concert to metabolize GSH, we evaluated the tissue distribution of steady state RNA levels of these two enzymes on a comparative basis (Fig. 1A and B). As expected, kidney and small intestine both expressed high levels of MBD and GGT. In contrast, lung expressed high levels of MBD RNA, whereas GGT RNA was undetectable by Northern analysis. Seminal vesicles and fetal liver displayed the reverse pattern, with undetectable levels of MBD RNA and detectable expression of GGT RNA.

Evaluation of MBD and GGT Expression in Individual Cells—In situ hybridization for MBD was performed on kidney, lung, and seminal vesicles using an antisense MBD probe (Figs. 2, A, C, and E). In kidney proximal convoluted tubules, the staining pattern was similar to that seen for GGT, predominantly basolateral and in a brush border pattern; in addition, some cells in the cortical collecting ducts were positive. In fact, these cells stained more strongly than the proximal tubular cells. Glomeruli, vessels, interstitium, and other epithelial cells did not hybridize to the probe. Hybridizations with a sense probe were uniformly negative. The lung was positive in the epithelial cells of the conducting airways and to a lesser extent in other epithelial cells of the alveolar ducts. Both ciliated bronchiolar epithelial cells and non-ciliated bronchiolar cells (Clara cells) were positive. A few interstitial cells, which were difficult to characterize without specific markers, were also positive. Blood vessels and pleura were negative. The seminal vesicle epithelium and stroma were negative.

We used enzyme histochemistry to characterize GGT expres-
sion (Fig. 2, B, D, and E). As anticipated, in kidney, proximal tubular cells were positive, whereas all other cells were negative. In lung, we were not able to identify positive staining for GGT. Seminal vesicles showed staining of epithelial cells, which was more prominent on the luminal side. Stroma were negative.

MBD Expression in GGT-deficient Mice—We have recently developed mice with total GGT deficiency. Because these mice lack GGT, cysteinylglycine and its oxidized derivative, cystinylbisglycine, are not formed in appreciable quantities in these mice. We have used these mice to determine if the absence of GGT alters MBD RNA levels (Fig. 1C). We have found similar levels of expression of MBD in kidney, small intestine, and lung in wild-type mice and in mice homozygous for GGT deficiency, indicating that MBD RNA levels are insensitive to GGT expression and MBD substrate accumulation.

Alternative Processing at the 3'-End of MBD Transcripts—The presence of two distinct bands by Northern analysis with an MBD probe suggested the following possibilities (Fig. 1A). The MBD transcripts are alternatively processed: (a) at the 3'-end of the mRNA; or (b) at the 5'-end of the mRNA; or (c) both. Other possibilities, such as alternative splicing and "exon" skipping, also exist. We screened a mouse kidney oligo(dT)-primed cDNA library with a MBD 3'-coding probe. We identified 21 cDNAs that fell into two unique groups. One group (13 clones, type a) had a shorter 3'-untranslated region (189 bases) and its own polyadenylation signal; a poly(A) tail was present 40 bases downstream. The second group of cDNA clones (8 clones, type b) had a longer 3'-untranslated region (758 bases), which included type A and its poly(A) signal but extended further 3'; it had a second poly(A) signal, followed by a poly(A) tail, 24 bases downstream. The diagrammatic representation of the organization of the 3'-end of MBD mRNA is shown in Fig. 3A, and the sequences are shown in Fig. 3B.

Determination of Variations at the MBD 5'-End by RACE—Having defined the 3'-end of the mRNA, we explored the possibility of heterogeneity at the 5'-end of the MBD RNA. We constructed 5'-anchored cDNA library from mouse kidney and obtained 34 clones specific for MBD cDNA. We sequenced and characterized them and classified them into two distinct groups. Type I cDNAs (22 clones) contained a short unique 5'-untranslated region (51 bases), a common region of 52 bases, followed by the coding region at 3'-end of these sequences. In contrast, type II cDNAs (12 clones) consisted of a longer 5'-untranslated region (255 bases), which splices to a site with a common noncoding region (51 bases) and a coding region identical to type I. The coding region contains an identical stretch of cDNA with the same ATG region. The schematic representation of the 5' organization of MBD RNA types I and II and sequences obtained from the longest cDNAs are presented in Fig. 4, A and B, respectively.

Quantitation of MBD RNAs using 5'-Specific Probes—We used nuclease protection and antisense riboprobes to determine the relative abundance of these two RNAs in kidney, small intestine, and lung. RNA from spleen was used as the negative control. For type I RNA, the riboprobe that was used is the longest cDNA that we obtained from the kidney RACE library (Fig. 4A). If the MBD RNA in these tissues corresponds to type I, the protected species will be 168 bases in length. If not, it will be cleaved and will be represented by a band corresponding to a region of 117 bases common to all MBD RNAs. In kidney, we found that about 60% of the MBD RNA can be accounted for by type I, whereas the remainder (40%) repre-
sented MBD RNA species other than type I. The double bands marked S (Fig. 4C) probably represent other minor transcription start sites for MBD type I RNA. This supposition is supported by the few cDNA clones obtained from the RACE library that extended only that far 5' on the RNA (Fig. 4C). In the small intestine, almost 96% of MBD RNA is type I, whereas in lung, 95% of it was protected by the common region, suggesting that almost all of the lung MBD RNAs are types other than type I (see below). In spleen, there was no clear band representing either type I or the common region, concurring with our Northern analysis that spleen makes very little MBD (Fig. 4C).

The longest type II cDNA clone (Fig. 4A) was cut with AvaI to generate an antisense probe of 303 bases. About 40% of the MBD RNA from kidney was protected by the probe, giving an expected band size of 244 bases (Fig. 4D). In small intestine, very little (5%) of MBD RNA was protected by type II probe, indicating that this RNA is a very minor species in this tissue. In contrast, in lung, about one-half of the MBD RNA was protected by the probe. Since lung makes little type I MBD RNA (see above) and only 50–55% of the MBD RNA is type II,
it follows that there are other MBD RNAs that we have not yet identified. RNA from spleen did not hybridize.

DNA Blot Analysis—Southern blot analysis of mouse kidney DNA digested with EcoRI, HindIII, PstI, KpnI, PvuII, and BglII was performed under high stringency conditions using the fragment corresponding to bases +610 to +1230 of the mouse MBD mRNA as the probe to determine if there are multiple MBD or MBD-related genes (Fig. 5). As shown in Fig. 5, EcoRI and HindIII digestions gave single DNA fragments of about 18 and 9 kb, respectively, suggesting the presence of a single gene. PstI, PvuII, BglII, and KpnI cutting yielded several bands that are known to reflect the presence of restriction sites in the introns or exons present in genomic DNA (see below). Thus, in the mouse, MBD is a single copy gene.

Isolation and Characterization of Mouse MBD Gene—We screened ~1 x 10^6 recombinant phages from a mouse 129Sv/Ev genomic library using a mouse MBD cDNA probe (corresponding to bases +4 to +636 of the mouse MBD mRNA) and obtained nine positive clones. Restriction mapping and Southern blotting of these clones using cDNA probes indicated that some were identical and were grouped into five independent phages. The intron-exon organization of the gene was constructed by subcloning and sequencing phage fragments containing the different exons. All of the intron-exon boundaries were sequenced, and the lengths of the introns were determined by sequencing, PCR, or restriction mapping. From this information, we constructed a restriction map of the 19-kb genomic region encompassing the 11 exons and 10 introns of the mouse MBD gene (Fig. 6).

Expression of Different MBD mRNAs by Kidney—In theory, the use of two promoters and two poly(A) addition sites should allow for four distinct MBD RNAs. We used RT-PCR to determine how many of these are actually present in mouse kidney (Fig. 7A). If these are all synthesized in kidney, then one would expect four products: 1525 bp (type 1a), 2094 bp (type 1b), 1721 bp (type 1a), and 2293 bp (type 1b). PCR amplification yielded all four products, indicating that kidney makes all four possible MBD RNAs (Fig. 7B).

**DISCUSSION**

We have investigated the expression of MBD in the mouse and found that steady state RNA levels are highest in the small intestine, kidney, and lung. At least four different types of RNA are expressed, and these are generated by a combination of the use of two different promoters and two different poly(A) addition sites. Because we have found that a significant portion (~40–50%) of lung MBD RNA is not protected in nuclease protection assays by probes specific for type I or type II MBD RNA (Fig. 4C), additional MBD RNAs must exist. It is not clear at present whether these are generated by the use of other promoters or alternative splicing or both. All tissues examined use both poly(A) addition sites but with different frequencies (Fig. 1A), and in kidney at least, all four types of MBD RNA have been identified (Fig. 7). It seems likely that the MBD like GGT and other genes may utilize transcription from different promoters as a strategy to achieve unique patterns of tissue-specific expression (5, 27–29). It appears that the MBD gene uses these different 5′- and 3′-ends to achieve fine adjustments in mRNA levels, depending on the physiological requirements of each tissue. Whether this heterogeneity of expression has biological importance is not clear.

Mouse MBD is a single copy gene consisting of 11 exons and is similar in structure to the human gene (15). Using the nomenclature established for human MBD RNA (15), type I RNA is derived from exon 1. Type II RNA has its origin ~6 kb 5′ of this region in an exon that we have designated −1 (Fig. 6). Type II transcripts are spliced at a site 3′ of the origin of type I RNA and common to both types I and II RNA (Fig. 6).

Because MBD acts in concert with GGT to cleave GSH into its component amino acids, we evaluated the coexpression of these two genes. In tissues like kidney and small intestine, where significant extracellular GSH degradation occurs, not surprisingly, we have found high expression of both genes (Fig. 1). However, the cellular distribution of MBD and GGT are somewhat different in the kidney (Fig. 2, A and B); while both are expressed in the proximal convoluted tubules, MBD is also expressed in some cortical collecting duct epithelial cells. Al-
though the reason for expression there is unclear, it implies that MBD and GGT function are not always tightly coupled. Additional evidence for this formulation comes from evaluation of expression in lung and seminal vesicles. Small intestine and kidney have high steady state levels of both MBD and GGT RNAs; in contrast, we could not detect GGT expression in mouse lung by Northern analysis or in histochemistry. We know that lung expresses low levels of GGT (~0.3% of kidney levels) from other studies. However, lung expresses low levels of GGT (~0.3% of kidney levels) from other studies. Thus, in lung, MBD appears to function independently of GGT, but its role in lung physiology is at present unclear. Its expression in bronchiolar epithelial cells and Clara cells may indicate its involvement in the detoxification of xenobiotics or leukotriene metabolism in the lung.

The reverse situation occurs in seminal vesicle, where GGT is clearly expressed in luminal epithelial cells (Fig. 2, E and F), but MBD expression is not detectable. This observation raises the question of whether seminal fluid has high levels of cysteinyl glycine (generated by the action of GGT on GSH) or some other dipeptidase cleaves this dipeptide there. Similarly, fetal liver expresses GGT (Fig. 1; Ref. 24); however, we were unable to detect MBD in this tissue. These observations underscore the discordance of MBD and GGT expression.

It is likely that MBD plays multiple roles in biological processes. Its main function in kidney may be the recycling of GSH in conjunction with GGT, while in the intestine it may participate in similar recycling and also play a role in the catabolism of dietary proteins. It is likely that MBD may be involved in the metabolism of eicosanoids, including leukotriene D4. Thus, relative tissue levels of leukotrienes C4, D4, and E4, and the renal and gastrointestinal clearance of these compounds, may be regulated by the relative activities of MBD, GGT, and a leukotriene synthetase that we have recently described. In this regard, the absence of MBD in tissues in which GSH metabolism and/or eicosanoid activity is high may tell us as much about the function of this enzyme as its presence.

Acknowledgments—We thank Amy L. Wiseman for technical assistance with the GGT-deficient mice and Shirley Baker for typing the manuscript.

REFERENCES
1. Kozak, E. M., and Tate, S. S. (1982) J. Biol. Chem, 257, 6322–6327
2. Kropp, H., Sundelof, J. G., Hajdu, R., and Kahan, F. M. (1982) Antimicrob. Agents Chemother. 22, 62–70
3. Kenny, A. J., and Turner, A. J. (1987) in Mammalian Ectoenzymes (Kenny, A. J., and Turner, A. J., eds) pp. 329–351, Elsevier Science Publishing Co., Inc., New York
4. Meister, A., and Larsson, A. (1995) in The Metabolic and Molecular Basis of Inherited Diseases (Scriber, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 1461–1477, Elsevier Science Publishing Co., Inc., New York
5. Lieberman, M. W., Barrios, R., Carter, B. Z., Habib, G. M., Lebovitz, R. M., Rajagopal, S., Sepulveda, A. R., Shi, Z.-Z., and Wan, D. F. (1995) Am. J. Pathol. 147, 1175–1185
6. Hannigan, M. H., and Ricketts, W. A. (1993) Biochemistry 32, 6302–6306
7. Keppler, D. (1992) Rev. Physiol. Biochem. Pharmacol. 121, 1–30
8. Gonzalez, J., and Esteller, A. (1990) in Glutathione Metabolism and Physio-

---

**Fig. 7.** Schematic representation of MBD mRNAs synthesized by mouse kidney. A, expression of all four types of MBD transcripts by mouse kidney. Types Ia, Ib, Ia, and Iib represent the various combinations of MBD mRNAs expressed in kidney. Open boxes denote the 5’-noncoding ends of the mRNAs (I and II). Solid boxes show the coding region with ATG as indicated. Hatched boxes indicate the 3’-untranslated regions (a and b). B, detection of the four types of mRNA by PCR amplification in kidney. The anchored cDNA obtained from the 5’-3’ RACE library was used as a template, and amplification was done using different primer combinations. The location of the primers used in amplification are indicated in A above (see "Materials and Methods" for details). The faint extra band that is seen in the lane corresponding to type Ia could be an artifact of PCR amplification. M1 and M2, λ BstEII and φ HaeIII markers, respectively.

---

3 B. Z. Carter, A. L. Wiseman, R. Orkiszewski, K. Ballard, J. E. Shields, Y. Will, D. J. Reed, C.-N. Ou, and M. W. Lieberman, submitted for publication.
4 M. W. Lieberman and A. L. Wiseman, unpublished results.
logical Functions (Vina, J., ed) pp. 295–316, CRC Press, Inc., Boca Raton, FL
9. Griffith, O. W., and Meister, A. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 268–272
10. Anderson, M. E., Bridges, R. J., and Meister, A. (1980) Biochem. Biophys. Res. Commun. 96, 848–853
11. Huber, M., and Keppler, D. (1988) in Glutathione Conjugation (Sies, H., and Ketterer, B., eds) pp. 449–470, Academic Press, New York
12. Satoh, S., Keida, Y., Konta, Y., Maeda, M., Matsumoto, Y., Niwa, M., and Kohsaka, M. (1993) Biochim. Biophys. Acta 1163, 234–242
13. Rached, E., Hooper, N. M., Janes, P., Semenza, G., and Turner, A. J. (1990) Biochem. J. 271, 755–760
14. An, S., Schmidt, F. J., and Campbell, B. J. (1994) Biochim. Biophys. Acta 1226, 337–340
15. Satoh, S., Kusunoki, C., Konta, Y., Niwa, M., and Kohsaka, M. (1993) Biochim. Biophys. Acta 1172, 181–183
16. Adachi, H., Katayama, T., Nakazato, H., and Tsujimoto, M. (1993) Biochim. Biophys. Acta 1163, 42–48
17. Hikida, M., Kawasaki, K., Nishiki, K., Furukawa, Y., Nishizawa, K., Saito, I., and Kuwao, S. (1992) Antimicrob. Agents Chemother. 36, 481–483
18. Keynan, S., Hooper, N. M., and Turner, A. J. (1994) FEBS Lett. 349, 50–54
19. Curthy, N. P. (1990) in Glutathione Metabolism and Physiological Functions (Vina, J., ed) pp. 217–225, CRC Press, Inc., Boca Raton, FL
20. Turner, A. J. (1990) in Molecular and Cell Biology of Membrane Proteins (Turner, A. J., ed) pp. 129–150, Ellis Harwood, New York
21. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
22. Laperche, Y., Bulle, F., Aissani, T., Chobert, M. N., Aggerbeck, M., Hanoune, J., and Guellaen, G. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 937–941
23. Chisholm, D. (1989) BioTechniques 7, 21–23
24. Habib, G. M., Carter, B. Z., Sepulveda, A. R., Shi, Z-Z., Wan, D-F., Lebovitz, R. M., and Lieberman, M. W. (1995) J. Biol. Chem. 270, 13711–13715
25. Schaffner, D. L., Barrios, R., Massey, C., Baner, E. I., Ou, C. N., Raggiopalan, S., Aguilar-Cordova, E., Lebovitz, R. M., and Lieberman, M. W. (1993) Am. J. Pathol. 142, 1051–1060
26. Rutenberg, A. M., Kim, H., Fischbein, J. W., Haner, J. S., Wasserkrug, H. L., and Seligman, A. M. (1969) J. Histochem. Cytochem. 17, 517–526
27. Sepulveda, A. R., Carter, B. Z., Habib, G. M., Lebovitz, R. M., and Lieberman, M. W. (1994) J. Biol. Chem. 269, 10699–10705
28. Toda, K., Simpson, E. R., Mendelson, C. R., Shizula, Y., and Kilgore, M. W. (1994) Mol. Endocrinol. 8, 210–217
29. Kengaku, M., Misawa, H., and Deguchi, T. (1993) Mol. Brain Res. 18, 71–76

Mouse Membrane-bound Dipeptidase