THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 263, No. 20, Issue of July 15, pp. 9913-9916, 1988
Printed in U.S.A.
© 1988 by The American Society for Biochemistry and Molecular Biology, Inc.

γ-Glutamyl Transpeptidase: A Single Copy Gene in the Rat and a Multigene Family in the Human Genome*

(Received for publication, December 29, 1987)

André Pawlak‡§, Olivier Lahuna‡, Frédérique Bulle‡, Atsuo Suzuki‡¶, Nicolas Ferry‡, Sylvie Siegrist‡, Naima Chikhi‡, Marie Noëlle Chobert‡, Georges Guellaen‡, and Yannick Laperche‡‖

From the ‡Institut National de la Santé et de la Recherche Médicale Unité 99, Hôpital Henri Mondor, 94010 Créteil, France and the §Laboratoire de Chimie Pharmacologique, Centre du Médicament, 30 rue Lionnois, 54000 Nancy, France

γ-Glutamyl transpeptidase (GGT) genomic sequences were isolated from rat and human libraries using a rat GGT cDNA as a cross-species hybridization probe. Characterization of the human GGT clones by restriction mapping clearly establishes that at least four different GGT genes or pseudogenes are present in the human genome. All the rat genomic clones cover a 12.5-kilobase sequence and exhibit a unique restriction pattern. A precise quantitation of the rat GGT gene copy number by Southern blot analysis demonstrates that this sequence is present as a single copy/rat haploid genome. Therefore, the GGT gene organization is different between rat and human species; this raises the possibility of different regulatory mechanisms in the two species.

γ-Glutamyl transpeptidase (GGT)[1] ((5-glutamyl)-peptide:amino-acid 5-glutamyltransferase, EC 2.3.2.2) is a component of the γ-glutamyl cycle. It catalyzes the degradation of glutathione to glutamic acid and cysteinyl-glycine (1). The two GGT subunits, which are encoded by a common mRNA (2, 3), are located at the outer surface of the plasma membrane and play a key role in interorgan glutathione transport (4).

By far the highest GGT activity is found in the kidney (1), and most of the studies dealing with GGT structure and function have been performed on the kidney enzyme, but GGT of liver and liver-derived cells has attracted considerable attention. Indeed, in humans both hepatic and serum GGT activities are increased during chronic alcoholism (5) or in patients with hepatocellular carcinoma (6, 7). In the rat liver and in some rat liver-derived cell lines the GGT activity is also increased by alcohol (8), carcinogens (9), and glucocorticoids (10, 11); indirect evidence suggests that this effect requires RNA synthesis (8, 11). Regulation of GGT activity exhibits tissue and cell specificity since glucocorticoids and alcohol modulate the activity in the liver and in some hepatoma cell lines but not in the kidney or in HTC cells (8, 10).

Elucidation of the biochemical processes responsible for the regulation of GGT activity as well as its cell specificity clearly requires a detailed analysis of its genes. We recently isolated a nearly full-length cDNA sequence from rat kidney GGT mRNA which hybridizes to human GGT RNA under conditions that indicate a strong homology between the two species (12). Using this cDNA as a probe, we now report the isolation and characterization by restriction mapping of rat and human genomic DNA sequences. From the analysis of these genomic sequences we conclude that GGT is encoded by a single copy gene in the rat whereas a family of GGT genes or pseudogenes is present in the human genome.

EXPERIMENTAL PROCEDURES

Materials

Escherichia coli DNA polymerase I and the Klenow fragment were obtained from New England Biolabs (Beverly, MA). Restriction endonucleases were purchased from New England Biolabs, Boehringer Mannheim, or Genofit S. A. (Switzerland). Radiolabeled nucleotides and nylon membranes (Hybond™) used for DNA transfer were from Amersham Radiochemical Centre (United Kingdom). The human genomic library was a generous gift from Dr. A. Kahn, Institut National de la Santé et de la Recherche Médicale Unité 129, Paris, France. This library was constructed by insertion of partial *Sau*3A1 restriction digest (average size, 15 kb) of human fibroblast DNA into the *Bam*HI site of the bacteriophage EMBL4. The rat genomic DNA library purchased from Genofit S.A. was made by Clontech (Palo Alto, CA) from rat genomic DNA partially digested by *Eco*RI; the average size of the inserts was 9.5 kb. The GGT probes used to screen the two genomic libraries and to characterize the positive clones were obtained from the rat kidney cDNA sequence published previously (12) and described on Fig. 1.

Methods

Genomic DNA Southern Blot Analysis—High molecular weight DNA was extracted from Wistar rat kidney by powdering frozen samples (5 g) in liquid nitrogen (13). The powder was lyophilized and resuspended in 300 ml of 7 M urea, 2% SDS, 10 mM Tris, pH 8, 1 mM EDTA, 0.2 M NaCl and the volume brought to 900 ml with H_2O. The solution was extracted three times in phenol/chloroform (3:1), once in chloroform, and dialyzed against 10 mM Tris-HCl, pH 8, 1 mM EDTA, 10 mM NaCl. After the SDS concentration was raised to 2%, the DNA extract was digested by RNase A (900 μg) for 3 h at 37 °C and incubated for 1 h at 37 °C with 900 μg of proteinase K. The mixture was phenol-extracted and ethanol-precipitated and the DNA resuspended in water. DNA was extracted from human lymphocytes by the same protocol except that the sample was dissolved directly in the urea buffer. Genomic DNA, digested with several restriction enzymes, was fractionated on an 0.8% agarose gel in 90 mM Tris-borate, pH 8, 2 mM EDTA and transferred to a nylon membrane filter as described by Southern (14). The blots were hybridized to nick-translated cDNA probes (10^8 cpm/μg) in 50% formamide, 5 × SSC (0.15 M NaCl, 15 mM sodium citrate), 1 × Denhardt's solution (0.02 g/liter Ficoll, 0.2 g/liter polyvinylpyrrolidone, 0.2 g/liter bovine serum albumin (Fraction V), 50 mM NaH_2PO_4, pH 6.5, containing 200 μg/ml denatured salmon sperm DNA at 68 °C and washed twice

* This work was supported by Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, and the University Paris-Val-de-Marne. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Recipient of a grant from the Fondation pour la Recherche Médicale. Present address: Kinki University, School of Medicine, Sayama-Cho, Minamikawachi-Gun, Osaka, Japan.

‖ To whom correspondence should be addressed.

[1] The abbreviations used are: GGT, γ-glutamyl transpeptidase; SDS, sodium dodecyl sulfate; kb, kilobase pair(s).

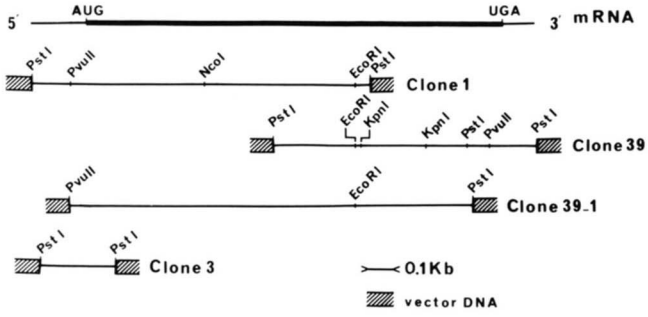

FIG. 1. **Restriction map of the rat cDNA clones.** Rat cDNA sequences were inserted in the *Pst*I site of PBR 322 (clones 1, 3, and 39) (Ref. 12) or at the *Pvu*II-*Pst*I sites in the polylinker of the pSP64 vector (clone 39.1) (Ref. 17). All the restriction sites used to prepare the different cDNA probes are shown.

for 1 h in 0.5 × SSC, 0.1% SDS at 55 °C for 1 h.

Screening of Rat and Human Libraries—Approximately 3 haploid genome eq of the rat and human libraries were plated using *E. coli* strain LE392 at a density of about $5 \cdot 10^4$ plaques/plate. The bacteriophage DNA was transferred to nitrocellulose filters according to Benton and Davis (15). The filters were processed as described by Grunstein and Hogness (16). The rat genomic library was screened using the rat kidney cDNA sequence 39.1 (1600 base pairs) extending from the most 5′ *Pvu*II site to the most 5′ *Pst* site of the rat kidney cDNA and subcloned in the plasmid pSP 64 (17) (Fig. 1). The human genomic library was screened using the rat kidney cDNA insert from clone 1 (Fig. 1). These two probes were purified by electroelution from agarose gels and radiolabeled by nick translation to a specific activity of 10^8 cpm/μg. Filters were prehybridized in 6 × SSC, 1 × Denhardt's solution containing 200 μg/ml of denatured salmon sperm DNA for 5 h at 68 °C; hybridization was performed overnight at 68 °C in 2 × SSC, 1 × Denhardt's solution, 0.05% SDS, 2 mM EDTA, 25 mM NaH_2PO_4, pH 7.2, containing 200 μg of salmon sperm DNA and 50 ng of the probe ($10–30 \cdot 10^6$ cpm). The filters were washed in 0.1 × SSC, 0.1% SDS at 58 °C. Positive signals were taken through an additional five rounds of screening at lower plaque density. Plate stocks were made from each positive clone. Recombinant bacteriophages, amplified in liquid medium (18), were purified by polyethylene glycol precipitation and through two cesium chloride gradient centrifugations (19).

Restriction Endonuclease Mapping—Single or double restriction digestion was carried out according to the recommendations of the enzyme suppliers. Digestions were followed by DNA size fractionation on a 0.8% agarose gel. DNA was transferred to a nylon membrane and hybridized to a nick-translated cDNA probe as described for genomic DNA. The data obtained from the different digestions were used to construct the restriction map.

Evaluation of Rat GGT Gene Copy Number—This evaluation was carried out by Southern blot analysis according to Zehner and Paterson (20). Total rat genomic DNA and genomic GGT sequences cloned in the bacteriophage Charon 4A were digested by *Eco*RI. Known amounts of rat genomic DNA were loaded on a 1% agarose gel along with increasing amounts of *Eco*RI-digested genomic clone corresponding to a known number of copies of the GGT gene. Salmon sperm DNA, digested by *Eco*RI, was added to the cloned DNA in order to load the same amount of material in each lane. The nitrocellulose blot was hybridized to a nick-translated 39.1 rat cDNA probe as described above. Following autoradiography, the radioactivity that hybridized to the *Eco*RI fragments was counted. The number of GGT gene copies was directly estimated by comparing the counts in the two *Eco*RI genomic DNA fragments to standard curves where the radioactivity found in cloned genomic DNA sequences had been plotted against the number of gene copy equivalents/haploid genome.

RESULTS

Southern Blot Analysis of Total Rat and Human Genomic DNA—Total rat and human genomic DNA were analyzed on Southern blot to assess the complexity of the GGT sequences. Total DNA was digested with different enzymes, electrophoresed, and blotted on a nylon membrane probed to a nick-translated 500-base pair *Eco*RI-*Pvu*II cDNA fragment from the clone 39. The very simple hybridization pattern observed with the rat samples suggests that there is only one gene coding for the GGT in the rat (Fig. 2*A*). Following digestion by *Xba*I, *Hin*dIII, *Bam*HI, *Eco*RI, and *Bgl*II, a single restriction fragment hybridized to the cDNA probes, while two fragments were detected following digestion by *Kpn*I. Under the same experimental conditions, the pattern obtained with the human genomic DNA prepared from lymphocytes is more complex (Fig. 2*B*). In the human DNA samples digested by the different restriction enzymes, three to seven fragments were found to hybridize to the rat cDNA probe. This complexity indicates that there are probably several GGT sequences in the human genome.

Isolation and Characterization of the Human GGT Genomic Clones—Screening of approximately $6 \cdot 10^5$ clones from the human genomic library, using the rat cDNA clone 1 (Fig. 1) as a probe, resulted in the purification of 14 positive clones containing inserts ranging from 10 to 15 kb. A restriction map of the DNA inserts was established by digesting the 14 phages with the *Eco*RI, *Kpn*I, *Hin*dIII, and *Bam*HI endonucleases separately and with all the possible double combinations. The maps, as well as the hybridization experiments described below, revealed that all the different clones share an identical pattern for only some *Eco*RI, *Hin*dIII, *Kpn*I, and *Bam*HI sites (Fig. 3*A*), but divergences occur for some other sites distributed along the sequence. Differences in the restriction map allow the identification of at least four different gene types in the human genome (F_{30}, F_{15}, F_{19}, F_{11}).

The 5′ to 3′ orientation of the different genomic clones, with respect to the rat kidney mRNA, was determined. The different genomic phages digested by *Bam*HI and *Hin*dIII were hybridized to *Pst*I-*Kpn*I and *Kpn*I-*Kpn*I fragments from clone 39 (Fig. 1). The probe *Pst*I-*Kpn*I recognizes the same *Hin*dIII-*Bam*HI fragment in the four different phages. This fragment is located upstream from the *Hin*dIII-*Hin*dIII or *Bam*HI-*Bam*HI sequences which hybridized to the *Kpn*I-*Kpn*I probe (Fig. 3*A*).

Isolation and Characterization of the Rat GGT Genomic Clones—Ten recombinant GGT genomic clones were isolated by screening the rat genomic library under stringent hybrid-

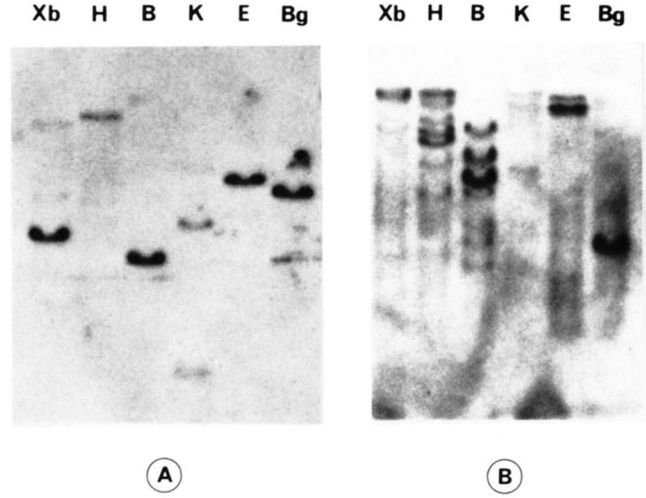

FIG. 2. **Southern blot of rat (*A*) and human (*B*) genomic DNA.** Genomic DNA (15 μg) was digested with restriction enzymes (*Xb*, *Xba*I; *H*, *Hin*dIII; *B*, *Bam*HI; *K*, *Kpn*I; *E*, *Eco*RI; *Bg*, *Bgl*II and electrophoresed on a 0.8% agarose gel. After transfer to nylon membrane, the DNA fragments were hybridized to the nick-translated *Eco*RI-*Pvu*II sequence from clone 39 as described under "Methods." The nylon membrane was autoradiographed at −80 °C for 48 h with an intensifying screen.

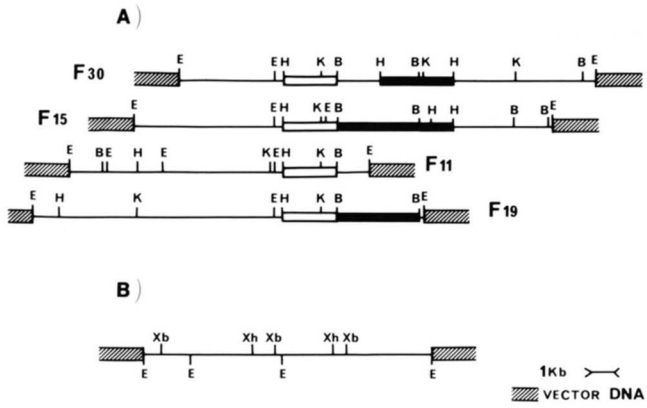

FIG. 3. **Restriction map of human (*A*) and rat (*B*) GGT genes.** Four different human GGT genomic sequences (F_{30}, F_{15}, F_{11}, F_{19}) were distinguished in the 14 positive phages after digestion with restriction enzymes (*E*, *Eco*RI; *H*, *Hin*dIII; *K*, *Kpn*I; *B*, *Bam*HI). The genomic sequences, which hybridize to the *Pst*I-*Kpn*I (□) and *Kpn*I-*Kpn*I (■) cDNA fragments from clone 39 are *boxed*; they give the 5′ to 3′ orientation of the genes. A single rat genomic sequence was characterized by restriction mapping of the 10 GGT recombinant phages by *Eco*RI, *Hin*dIII, *Xho*I, and *Xba*I. The 5′ to 3′ orientation was determined as indicated under "Results."

ization conditions. All the clones were shown to be identical by restriction mapping analysis. Digestion of the 12.4-kb insert of these clones by *Eco*RI gives three fragments of 6.4, 3.9, and 2.1 kb (Fig. 3*B*).

The 5′ to 3′ orientation of the cloned genomic sequences, with respect to GGT mRNA, was determined by hybridization to two probes which correspond to two different regions of the mRNA. The cDNA 39.1 probe hybridizes to the 3.9- and 6.4-kb *Eco*RI fragments, whereas the *Eco*RI-*Pvu*II fragment from clone 39, a 3′-specific probe, recognizes only the 6.4-kb fragment; this result shows that the 6.4-kb fragment contains the most 3′ sequences and that the unique *Eco*RI site found in the cDNA separates the 3.9- and 6.4-kb genomic fragments. Within the 6.4-kb fragment, the *Eco*RI-*Pvu*II cDNA probe hybridizes only to the 2.1-kb *Eco*RI-*Xho*I sequences, thus indicating that at least 98% of the mRNA coding region maps on the 5′ side of the most 3′ *Xho*I site (Fig. 3*B*).

The rat GGT genomic clones hybridize to the *Pvu*II-*Nco*I cDNA fragment but not to the cDNA insert from clone 3 (Fig. 1) (data not shown). This means that the cloned genomic sequence extends beyond the *Nco*I site of the rat cDNA. Therefore we isolated a 12.5-kb genomic sequence which contains the 3′ end of the rat gene and approximately 72% of the sequence coding for the polypeptide chain. Hybridization of kidney genomic DNA digested by *Eco*RI reveals a 15-kb fragment which was not cloned. This fragment hybridizes also to the cDNA from clone 3 and therefore contains sequences corresponding to the 5′ end of the gene. We are now attempting to clone the 5′ end of the rat GGT gene from another genomic library.

Copy Number of Rat Genomic GGT Sequences—Evaluation of the gene copy number of the GGT sequences in the rat genome was carried out by Southern blot analysis. Known amounts of total genomic DNA and of cloned genomic sequences were digested by *Eco*RI, electrophoresed, and transferred to nitrocellulose. After hybridization to the cDNA insert 39.1 (Fig. 1), two *Eco*RI fragments (3.9 and 6.4 kb) were detected in the standard lanes and three (3.9, 6.4, and 15 kb) in the lane containing the total rat genomic DNA (Fig. 4*A*). A precise quantitation of the gene copy number was done by comparing radioactivity counted in the two 3.9- and 6.4-kb *Eco*RI genomic fragments to the standard curves (Fig. 4*B*);

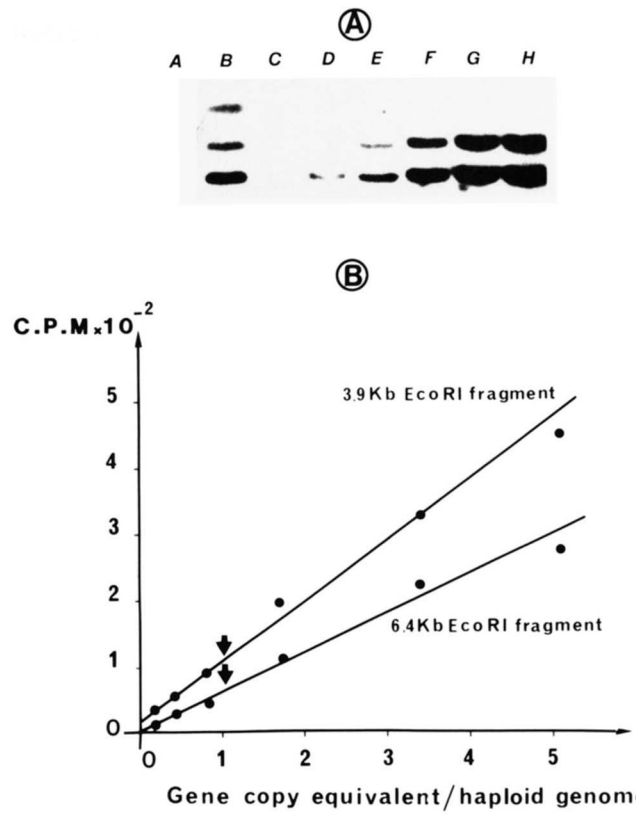

FIG. 4. **Determination of rat GGT gene number.** *A*, carrier salmon sperm DNA (15 μg) (*lane A*), rat genomic DNA (*lane B*), and increasing amounts of phage DNA equivalent to 0.17, 0.42, 0.85, 1.7, 3.4, and 5.1 gene copies/haploid genome (*lanes C–H*) were digested by *Eco*RI and loaded on a 1% agarose gel. The DNA was transferred to nitrocellulose filter and hybridized to cDNA insert 39.1 as described under "Methods." The sizes of the detected fragments were determined by running size DNA markers on the same gel. The blot was autoradiographed for 48 h at −80 °C using an intensifying screen. *B*, the radioactivity counted for each spot was plotted against the number of gene copy equivalents loaded in the standard tracks. With increasing gene dosage, there is a linear increase in the hybridization signal in the standard tracks for both the 3.9 and the 6.4 *Eco*RI fragments. The *arrows* mark the radioactivity values for the same *Eco*RI fragments in the total genomic DNA.

this quantitation clearly established that the two fragments, and therefore that the GGT gene, are represented only once per rat haploid genome.

DISCUSSION

In this paper we report the isolation and restriction maps of genomic clones corresponding to one rat GGT gene and several human GGT genes or pseudogenes. Characterization, by restriction map analysis of the different phages from the human library, establishes the existence of a multigene GGT family in humans which includes at least four different types of genes. Hybridization experiments to rat cDNA, as well as preliminary sequencing data, allow us to estimate the minimal length of the GGT gene to be 15 kb; comparison of the size of the gene to the 2.2-kb GGT sequence detected in human fetal liver mRNA (12) demonstrates an intron-exon structure. The GGT gene hybridization pattern of genomic DNA on Southern blots is in agreement with the presence of several GGT sequences in the human genome. The extensive cross-hybridization with the rat cDNA under stringent conditions indicates that the four human GGT genes that we identified are highly homologous. One cannot exclude the possibility that among these four types of genes, two of them represent

different alleles. However, Southern blot analysis of genomic DNA from unrelated individuals does not reveal any polymorphism (data not shown).

Several lines of evidence indicate that GGT coding sequences are present only once in the rat genome. 1) All the positive clones have a unique restriction map; 2) direct evaluation of the gene copy number by Southern blot DNA analysis indicates a value of 1, this value having been obtained for the two *Eco*RI genomic fragments which were quantitated independently; and 3) the very simple hybridization pattern of the rat genomic DNA on Southern blots is perfectly compatible with a single copy gene.

In humans, the different genes arise most probably by duplication of a unique gene which occurred after species diversion between rat and human. Integration of cDNA copies into the genome-like processed pseudogenes can be excluded since all the genes have an intron-exon structure, and most of them, if not all, are clustered in 22q 111-112 on the same chromosome (17).

Few examples of gene duplication after the evolutionary divergence of rat from that of human have been reported. Insulin is encoded by a single copy gene in birds and most mammals including human and the Syrian hamster and by two genes in the rat (21, 22). The two insulin gene sequences are closely related since they code for two preproinsulins which differ by only seven amino acids; the divergence reaches 22% in the DNA silent sites. Another example of recent gene duplication is given by the relaxin gene which is represented once in the rat genome (23) and twice in the human genome (24).

The physiological significance of GGT gene duplication in the human genome remains to be established. The human GGT genes could be expressed independently in different tissues at different times during development and/or under different stimuli, as occurs in several multigene families (25, 26). A multigene family could also facilitate the production of isozymic forms which become specialized for particular roles. Alternatively, among the duplicated sequences, one of them can emerge as a dominant member, thus allowing the other members to drift to pseudogenes. Further analysis of the GGT gene transcripts in different tissues will allow us to determine whether all the GGT genes are transcribed into translatable GGT mRNA.

For the time being, we observed that comparable levels of GGT activity are found in human and rat tissues and no difference in GGT regulation has been reported between the two species. Multiple GGT forms have been described in human (27) and also in rat tissues (28), but they arise from differences in the post-translational processing of the protein. However, it was recently reported that in HepG2 cells, a human hepatoma cell line, the GGT enzyme is a single chain species; this might be due to a different protein backbone of the GGT precursor (29).

Acknowledgments—We gratefully thank Drs. J. Hanoune, R. Barouki, D. Stengel, and D. Ojcius for their helpful comments during the preparation of this manuscript and L. Rosario for expert secretarial assistance.

REFERENCES

1. Tate, S., and Meister, A. (1981) *Mol. Cell. Biochem.* **39,** 357–368
2. Finidori, J., Laperche, Y., Haguenauer-Tsapis, R., Barouki, R., Guellaen, G., and Hanoune, J. (1984) *J. Biol. Chem.* **259,** 4687–4690
3. Nash, B., and Tate, S. S. (1984) *J. Biol. Chem.* **259,** 678–685
4. Curthoys, N. P. (1983) *Miner. Electrolyte Metab.* **9,** 236–245
5. Rosalki, S. B., and Rau, D. (1972) *Clin. Chim. Acta* **39,** 41–47
6. Gerber, M. A., and Thung, S. N. (1980) *Am. J. Pathol.* **98,** 395–400
7. Tsuji, A., Matsuda, Y., and Katunuma, N. (1980) *Clin. Chim. Acta* **104,** 361–366
8. Barouki, R., Chobert, M. N., Finidori, J., Aggerbeck, M., Nalpas, B., and Hanoune, J. (1983) *Hepatology* **3,** 323–329
9. Cameron, R., Kellen, J., Kolin, A., Malkin, A., and Farber, E. (1978) *Cancer Res.* **38,** 823–829
10. Billon, M. C., Dupre, G., and Hanoune, J. (1980) *Mol. Cell. Endocr.* **18,** 99–108
11. Barouki, R., Chobert, M. N., Billon, M. C., Finidori, J., Tsapis, R., and Hanoune, J. (1982) *Biochim. Biophys. Acta* **721,** 11–21
12. Laperche, Y., Bulle, F., Aissani, T., Chobert, M. N., Aggerbeck, M., Hanoune, J., and Guellaën, G. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 937–941
13. Blin, N., and Stafford, D. W. (1976) *Nucleic Acids Res.* **3,** 2303–2308
14. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517
15. Benton, W. D., and Davis, R. W. (1977) *Science* **196,** 180–182
16. Grunstein, M., and Hogness, D. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72,** 3961–3966
17. Bulle, F., Mattei, M. G., Siegrist, S., Pawlak, A., Passage, E., Chobert, M. N., Laperche, Y., and Guellaën, G. (1987) *Hum. Genet.* **76,** 283–286
18. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K., and Efstratiadis, A. (1978) *Cell* **15,** 687–701
19. Yamamoto, K. R., Alberts, B. M., Benzinger, R., Lawhorne, L., and Treiber, G. (1970) *Virology* **40,** 734–744
20. Zehner, Z. E., and Paterson, B. M. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 911–915
21. Perler, F., Efstratiadis, A., Lomedico, P., Gilbert, W., Kolodner, R., and Dodgson, J. (1980) *Cell* **20,** 555–566
22. Bell, G. I., Pictet, R. L., Rutter, W. J., Cordell, B., Tischer, E., and Goodman, H. M. (1980) *Nature* **284,** 26–32
23. Haley, J., Crawford, R., Hudson, P., Scanlon, D., Tregear, G., Shine, J., and Niall, H. (1987) *J. Biol. Chem.* **262,** 11940–11946
24. Crawford, R. J., Hudson, P., Shine, J., Niall, H. D., Eddy, R. L., and Shows, T. B. (1984) *EMBO J.* **3,** 2341–2345
25. Weydert, A., Daubas, P., Caravatti, M., Minty, A., Bugaisky, G., Cohen, A., Robert, B., and Buckingam, M. (1983) *J. Biol. Chem.* **258,** 13867–13874
26. Shaw, P. H., Held, W. A., and Hastie, N. D. (1983) *Cell* **32,** 755–761
27. Echetebu, Z. O., and Moss, D. W. (1982) *Enzyme* **27,** 1–8
28. Matsuda, Y., Tsuji, A., and Katunuma, N. (1980) *J. Biochem. (Tokyo)* **87,** 1243–1248
29. Tate, S. S., and Galbraith, R. A. (1987) *J. Biol. Chem.* **262,** 11403–11406