Rat Liver Glutathione S-Transferases

CONSTRUCTION OF A cDNA CLONE COMPLEMENTARY TO A Yc mRNA AND PREDICTION OF THE COMPLETE AMINO ACID SEQUENCE OF A Yc SUBUNIT*

(Received for publication, September 28, 1984)

Claudia A. Telakowski-Hopkins†, John A. Rodkey‡, Carl D. Bennett‡, Anthony Y. H. Lu§, and Cecil B. Pickett†

From the †Department of Molecular Pharmacology and Biochemistry, the ‡Department of Medicinal Chemistry, and the §Department of Animal Drug Metabolism, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065 and West Point, Pennsylvania 19486

Using polysomal immunoselected rat liver glutathione S-transferase mRNAs, we have constructed cDNA clones using DNA polymerase I, RNase H, and Escherichia coli ligase (NAD⁺)-mediated second strand cDNA synthesis as described by Gubler and Hoffman (Gubler, U., and Hoffman, B. S. (1983) Gene 25, 263–269). Recombinant clone, pGTB42, contained a cDNA insert of 900 base pairs whose 3' end showed specificity for the Yc mRNA in hybrid-select translation experiments. The nucleotide sequence of pGTB42 has been determined, and the complete amino acid sequence of a Yc subunit has been deduced. The cDNA clone contains an open reading frame of 663 nucleotides encoding a polypeptide comprising 221 amino acids with a molecular weight of 25,322. The NH₂-terminal sequence deduced from pGTB42 is in agreement with the first 39 amino acids determined for the amino-terminal sequence of a Ya-Yc heterodimer isolated from rat liver cytosol. Analysis of the nucleotide and amino acid sequences derived from the Ya (pGTB38) and Yc mRNA were utilized for the cDNA synthesis reactions. Both the first and second strands of the cDNA were synthesized as described by Gubler and Hoffman (6). The ds-cDNA was tailed with dCTP using terminal deoxynucleotidyltransferase as described previously (5). Approximately 200–200 ng of purified mRNA were utilized for the cDNA synthesis reactions. Both the first and second strands of the cDNA were synthesized as described by Gubler and Hoffman (6). The ds-cDNA was tailed with dCTP using terminal deoxynucleotidyltransferase as described previously (5).

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.

The cytosolic glutathione S-transferases represent a family of isoenzymes which catalyze the conjugation of glutathione to electrophilic ligands generated by the metabolism of various xenobiotics. In addition, these proteins bind with high affinity various exogenous hydrophobic compounds as well as potentially toxic endogenous compounds such as bilirubin and heme (1, 2). To date, at least seven rat liver glutathione S-transferases (B, AA, A, C, D, E, and M) have been purified and characterized to various extents (3). All isozymes appear to be heterodimers or homodimers comprised of subunits designated Ya, Yb, or Yc, which are electrophoretically distinguished on one-dimensional SDS-polyacrylamide gels (4).

Our laboratory has recently constructed cDNA clones complementary to the mRNAs specific for a Ya and Yb subunit of the rat liver glutathione S-transferases (5). One clone, pGTB33, hybrid selected mRNAs specific for both the Ya and Yc subunits as well as a third polypeptide which migrates faster than the Ya subunit on SDS-polyacrylamide gels. Another clone, pGTB33, contained a truncated cDNA insert corresponding to the internal region of pGTB38 and hybrid-selected mRNAs specific for the Ya subunit and the faster migrating polypeptide. However, no selection of the Yc mRNA was observed (5).

In order to identify cDNA clones complementary to the Yc mRNAs we have utilized the truncated cDNA clone, pGTB33, and the full length cDNA clone, pGTB38, to screen a cDNA library constructed from purified glutathione S-transferase Ya and Yc mRNAs. Those colonies which hybridized to pGTB38 (Ya and Yc mRNA positive) but did not hybridize to pGTB33 (Ya mRNA positive) were candidates for Yc clones. In this study we report the isolation and characterization of a nearly full length clone, pGTB42, which contains a cDNA insert complementary to a mRNA specific for a Yc subunit of the rat liver glutathione S-transferases. The entire DNA sequence of pGTB42 has been determined, and the complete amino acid sequence of the corresponding Yc subunit has been deduced. The amino-terminal sequence deduced from the DNA sequence of pGTB42 is in total agreement with the amino-terminal sequence determined by conventional protein sequence techniques of a Ya-Yc heterodimer isolated from rat liver cytosol. Analysis of the nucleotide and amino acid sequences derived from the Ya (pGTB38) and Yc (pGTB42) clones reveals that the two polypeptides are encoded for by two related yet distinct genes which have diverged significantly in their 5' and 3' untranslated regions.

MATERIALS AND METHODS

Preparation of cDNA and Construction of Recombinant Plasmids—In order to prepare ds-cDNA, glutathione S-transferase Ya and Yc mRNAs were purified by polyribosomal immunosorbent techniques as described previously (5). Approximately 200–200 ng of purified mRNA were utilized for the cDNA synthesis reactions. Both the first and second strands of the cDNA were synthesized as described by Gubler and Hoffman (6). The ds-cDNA was tailed with dCTP using terminal deoxynucleotidyltransferase as described previously (5).
Similarly pBR322 was cleaved with PstI and tailed with dGTP. An equimolar mixture of the dG-tailed pBR322 and the dC-tailed ds-cDNA was annealed in 0.15 M NaCl, 10 mM Tris-HCl, pH 7.8, and 1 mM EDTA at 70 °C for 10 min, 2 h at 48 °C, and 2 h at room temperature. The chimeric plasmids were used to transform Escherichia coli RR1 (7).

Hybrid-select Translation Assay—Hybrid-select translations were carried out according to the procedure described by Cleveland et al. (8). Approximately 10 µg of plasmid DNA were linearized with BamHI, adjusted to 0.2 N NaOH, and diluted 1:1 with 6 M SSC. The DNA was then pipetted on nitrocellulose filters. The filters were washed with 6 × SSC, blotted dry, and baked for 2 h at 80 °C in a vacuum oven. The dried filters were prehybridized for 24 h at 41 °C in 100 µl of 50% deionized formamide, 400 mM NaCl, 10 mM Pipes (pH 6.4), 4 mM EDTA, and 0.5 mg/ml yeast tRNA. After prehybridization, the solution was removed and replaced with 100 µl of the same solution containing 10 µg of total rat liver poly(A) mRNA isolated from 3-methylcholanthrene-treated rats. Hybridization proceeded for 16 h. After hybridization, the filters were washed as described by Cleveland et al. (8), and the hybrid-selected mRNA was eluted by boiling the filters for 90 s in 300 µl of sterile water. The eluted mRNA was precipitated by the addition of 30 µl of 2 M potassium acetate (pH 5.5) and 660 µl of absolute ethanol in the presence of 10 µg of calf liver tRNA carrier. The precipitated mRNA was collected by centrifugation, rinsed 1 time with 70% EtOH, dried with N₂, and resuspended in 10 µl of sterile water. The mRNA was translated in the rabbit reticulocyte lysate translation system as described previously (9). Aliquots of total translations were either run directly on 10% SDS-polyacrylamide gels or digested on 10% SDS-polyacrylamide gels subjected to immunoprecipitation (9). The radiolabeled polypeptides were identified by autoradiography.

In Vitro Labelling of cDNA—cDNAs were labeled in vitro either with [32P]dCTP by nick translation (10) or at the 5' end with [32P]dideoxy-ATP terminal deoxynucleotidyltransferase (11).

Restriction Mapping of cDNA Insert—A restriction map of the cDNA insert was constructed by the method of Smith and Birnstiel (12) using 5' end-labeled fragments. The sizes of the end-labeled fragments generated by partial restriction endonuclease digestion were determined on 5% polyacrylamide gels.

Nucleotide Sequence Analysis—The chemical method of Maxam and Gilbert was used for DNA sequence analysis (13). Appropriate restriction fragments were 5' or 3' end labeled and subjected to DNA sequence analysis.

Amino Acid Sequence Determination—Ten nmol of rat liver glutathione S-transferase Yc-Ye heterodimer was placed in the cup of an unmodified Beckman 890C Sequencer containing 4 mg of Polybrene. The glutathione S-transferase Yc-Ye heterodimer (transferase B) was purified as described previously and consists of equal amounts of the Ya and Yc subunits as determined by one-dimensional SDS-polyacrylamide gel electrophoresis (5). The modified double cleavage program of Hunkapillar and Hood (14) was used for 44 cycles. High performance liquid chromatography was used to quantitate the phenylthiohydantoin derivatives produced at each step (15).

RESULTS

Construction and Identification of a cDNA Clone Complementary to Rat Liver Glutathione S-Transferase Yc mRNA—In a previous study, we reported the isolation, characterization, and complete nucleotide sequence of a cDNA clone, pGTB38, which hybridizes to the Ya and Yc mRNAs of the rat liver glutathione S-transferases (5). This cDNA clone also hybrid selected a third mRNA which is specific for a polypeptide with a molecular weight approximately 500–1000 less than the Ya subunit (5). The latter polypeptide most likely corresponds to the Ya subunit described by Tu et al. (16, 17).

Utilizing the same cDNA library, we also isolated a truncated cDNA clone, pGTB38, which cross-hybridized with pGTB38, shared identical restriction sites with an internal region of pGTB38, yet hybridized exclusively to the Ya mRNA and the mRNA specific for the faster migrating polypeptide. The inability of pGTB33 to hybridize to the Yc mRNA has allowed us to screen cDNA clones by differential colony hybridization using a 580-bp PstI fragment of pGTB38 which hybridizes to both the Ya and Yc mRNAs and the 300-bp truncated cDNA insert in pGTB33 which hybridizes only to the Ya mRNA. Those clones which hybridized to pGTB38 but did not hybridize to pGTB33 were putative Yc clones. One such cDNA clone, pGTB42, contained a cDNA insert of approximately 900 bp which is very similar to the size of the Yc mRNA determined previously by Northern blot analysis (5).

Restriction endonuclease mapping of the cDNA insert in pGTB42 confirmed that we had selected a unique cDNA clone. Although pGTB38 and pGTB42 do share a few common restriction sites (e.g. BglII, PstI, and HindIII), the map of pGTB42 presented in Fig. 1 is completely different from the map determined previously for pGTB38 (5). It should be pointed out that pGTB42 was constructed from polysomal immunopurified Ya and Yc mRNAs utilizing DNA polymerase I, RNase H, and E. coli ligase (NAD+)-mediated second strand synthesis (6). This cloning protocol eliminates the use of S-1 nuclease and facilitates the construction of full length pGTB 42

![Fig. 1. Restriction endonuclease map of pGTB42.](image)

In the restriction endonuclease map of pGTB42, the restriction endonuclease map of pGTB42 was determined by single and double digests as well as partial digestion of end-labeled fragments using the Smith and Birnstiel procedure (12). The dashed lines in the clone represents pBR322 sequences. The EcoRI site of pBR322 is located to the right of the cDNA insert. The 5' and 3' orientation in pGTB42 is from the BglII site toward the Ball site.

![Fig. 2. Hybrid-select translation analyses using the 5' and 3' regions of pGTB42.](image)

One-dimensional SDS-polyacrylamide gel (10%) of translation products directed by mRNAs hybrid selected by the 5' and 3' regions of pGTB42. Lane 1, endogenous translation products directed by the rabbit reticulocyte lysate in the absence of added mRNA; lane 2, total translation products directed by mRNAs hybrid selected by the 3' region of pGTB42; lane 3, immunoprecipitation of the translation products in lane 2 with antibody raised against the Ya and Yc subunits; lane 4, total translation products directed by mRNAs hybrid selected by the 5' region of pGTB42; lane 5, immunoprecipitation of the translation products in lane 4 with antiserum raised against the Ya and Yc subunits. In this experiment, aliquots of the immunoprecipitations applied to the gel (lanes 3 and 5) contained equal counts/min. The positions of the purified Ya and Yc subunits are indicated by arrows. Molecular weight markers are phosphorylase b (94,000), bovine serum albumin (68,000), ovalbumin (45,000), carbonic anhydrase (30,000), and soybean trypsin inhibitor (21,000).
cDNAs containing sequences complementary to the 5' end of the mRNA.

The identity of pGTB42 was confirmed by hybrid-select translation analysis using two fragments derived from the 5' and 3' regions of the cDNA insert. The fragments were derived by digesting pGTB42 with BglII and CiaI. Since there is only one CiaI site in pBR322 and a single BglII site in the cDNA insert (see Fig. 1), this digestion produces two fragments: 1) a 1490-bp fragment containing 717 bp of the cDNA insert corresponding to the 5' untranslated region and most of the coding region of the cDNA; and 2) a 3768-bp fragment containing 188 bp of the cDNA insert corresponding to the 3' end of the cDNA. As can be seen from Fig. 2, the 717-bp fragment hybrid selects the Ya and Yc mRNAs as well as a third mRNA specific for the faster migrating polypeptide. However, the Ya polypeptide is still the predominant translation product. In contrast, however, the 3' regions of the cDNA clone hybrid selects the Ya and Yc mRNAs along with the sequence of pGTB38 determined previously (5). The length of the cDNA insert is 865 bp excluding the dC tails. A comparison of the nucleotide sequence between the two cDNA inserts indicates that the 5'- and 3'-untranslated regions have diverged significantly whereas the coding regions are highly conserved. Over identical regions of both clones (nucleotides -39 to 780), there is a 66% nucleotide sequence homology. In the protein-coding region encompassing 1-663 nucleotides, there is a 75% nucleotide sequence homology between pGTB42 and pGTB38. Interestingly, the 5' region covering the first 221 nucleotides of the coding regions of the two cDNA inserts are more highly conserved (~83% homology) as compared to the middle region or 3' region of the cDNAs. These latter two regions are 73% and 70% homologous, respectively. The cDNA insert in pGTB42 contained an open reading frame of 663 nucleotides coding for a polypeptide of 221 amino acids with a molecular weight of 25,322. The complete amino acid sequence of the Ya subunit was determined previously by our laboratory (5). The amino acid sequences of the Ya and Yc subunits have an overall homology of 68% (Fig. 4); however, the first 74 NH2-terminal amino acids of the two subunits are 78% homologous, whereas the middle third and the last third of the two polypeptides which correspond to the carboxyl-terminal domain are only 68 and 60% homologous, respectively.

Amino Acid Sequence of a Ya-Yc Heterodimer—The amino acid sequence of the Ya-Yc heterodimer, glutathione S-trans-
we believe that the amino acid sequence of the Ya-Yc heterodimer deduced from the complete amino acid sequence of the Yc subunit differs, the sequence of the Ya-Yc heterodimer agrees with the amino acid sequence deduced previously from pGTB38 (5). Amino acids which are underlined represent divergence between the two sequences.

Fig. 4. Amino acid sequence of a Yc subunit deduced from nucleotide sequence analysis of pGTB42. The complete amino acid sequence of the Yc subunit deduced from nucleotide sequence analysis of pGTB42 is compared with the amino acid sequence deduced previously from pGTB38 (5). Amino acids which are underlined represent divergence between the two sequences.

Glutathione S-Transferase Yc mRNA

| 9 | Yc MET Pro Gly Lys Pro Val Leu His Tyr Phe Asp Gly Arg Gly Arg Met Glu Pro |
| 18 | Ya MET Ser Gly Lys Pro Val Leu His Tyr Phe Asn Ala Arg Gly Arg Met Glu Cys |
| 27 | Ile Arg Trp Leu Ala Ala Ala Gly Val Glu Phe Glu Glu Gln Phe Leu Lys |
| 36 | Ile Arg Trp Leu Ala Ala Ala Gly Val Glu Phe Glu Glu Lys Leu Ile Gin |
| 45 | Thr Arg Asp Asp Leu Ala Arg Leu Arg Asn Asp Gly Ser Met Phe Gin Glu |
| 54 | Ser Pro Gly Asp Leu Glu Lys Leu Lys Asp Gin Asn Leu Met Phe Gin Glu |
| 63 | Val Pro Met Val Glu Ile Asp Gly Met Lys Leu Val Gin Thr Arg Ala Ile Leu |
| 72 | Val Pro Met Val Glu Ile Asp Gly Met Lys Leu Ala Gin Thr Arg Ala Ile Leu |
| 81 | Asn Tyr Ile Ala Thr Lys Tyr Asn Leu Tyr Gly Lys Asp Met Lys Glu Arg Ala |
| 90 | Asn Tyr Ile Ala Thr Lys Tyr Asn Leu Tyr Gly Lys Asp Met Lys Glu Arg Ala |
| 99 | Leu Ile Asp Met Tyr Ala Gly Val Ala Asp Leu Asp Ala Ile Leu His |
| 108 | Leu Ile Asp Met Tyr Ser Gly Leu Asp Thr Glu Met Ile Ile Gin |
| 117 | Tyr Pro Tyr Ile Pro Pro Gly Glu Lys Glu Ala Ser Leu Ala Lys Ile Lys Asp |
| 126 | Leu Val Ile Cys Pro Pro Asp Gin Arg Glu Ala Thr Ala Leu Ala Lys Asp |
| 135 | Lys Ala Arg Asn Arg Tyr Phe Pro Ala Phe Glu Val Leu Ser His Gly |
| 144 | Arg Thr Lys Asn Arg Tyr Leu Pro Ala Phe Glu Val Leu Ser His Gly |
| 153 | Gin Asp Tyr Leu Val Gly Asn Arg Leu Ser Arg Ala Asp Val Tyr Leu Val Gin |
| 162 | Gin Asp Tyr Leu Val Gly Asn Arg Leu Thr Arg Val Ile His Leu Leu Glu |
| 171 | Val Leu Tyr His Val Glu Leu Asp Pro Ser Ala Leu Ala Asp Pro Leu |
| 180 | Val Leu Tyr His Val Glu Leu Asp Pro Ser Ala Leu Ala Asp Pro Leu |
| 189 | Leu Lys Ala Leu Arg Thr Arg Val Ser Asn Leu Pro Thr Val Lys Phe Leu |
| 198 | Leu Lys Ala Leu Arg Thr Val Ser Ile Ser Ser Leu Pro Val Asn Val Lys Phe Leu |
| 207 | Gin Pro Gly Ser Gin Arg Lys Pro Leu Asp Gin Lys Val Glu Ser Ala |
| 216 | Gin Pro gly Ser Gin Arg Lys Pro Leu Asp Gin Lys Val Glu Ser Ala |
| 225 | Val Lys Ile Phe Ser Arg Lys Val Phe Lys Phe |

Discussion

In this study we have identified a cDNA clone, pGTB42, which is complementary to a mRNA specific for a Yc subunit of rat liver glutathione S-transferase. The specificity of this cDNA clone was determined by using the 5' and 3' regions in hybrid-select translation experiments. The 3' region of the clone hybrid selected significantly more Yc mRNA as compared to Ya mRNA, whereas the 5' region hybrid selected slightly more Ya mRNA as compared to Yc mRNA. A mRNA specific for a third polypeptide which migrates faster than the Ya subunit was also hybrid selected. This polypeptide has an apparent M, which is 500–1000 less than the Ya subunit and most likely corresponds to the Ya polypeptide described by Tu and co-workers (16, 17). Based upon the specificity of the 3' end of pGTB42 in hybrid-select translation experiments as well as the differential colony hybridization experiments described under “Results,” we have concluded pGTB42 is complementary to a Yc mRNA.

The complete nucleotide sequence of pGTB42 has been determined, and the entire amino acid sequence of a Yc subunit has been deduced. Although the Yc subunit migrates in general agreement with the sequence determined for the Yc subunit. The only difference is at position 10 where Frey et al. (18) reports an asparagine and we have found aspartic acid. Thus, we believe that the amino acid sequence of the Ya-Yc heterodimer determined in our study represents the sequence of a Yc subunit.

Frey et al. (18) published the first 19 NH2-terminal amino acids reported at step 1, it is unclear whether the serine is derived from the Ya subunit. If the serine residue is in fact derived from a Ya subunit, we would have expected two amino acids at positions where the Ya and Yc subunits diverged in sequence. However, only one sequence was obtained. Recently Frey et al. (18) published the first 19 NH2-terminal amino acids derived from a Ya-Yc heterodimer isolated from rat liver. Only one sequence was obtained which is in general agreement with the sequence determined for the Yc subunit. The only difference is at position 10 where Frey et al. (18) reports an asparagine and we have found aspartic acid. Thus, we believe that the amino acid sequence of the Ya-Yc heterodimer determined in our study represents the sequence of a Yc subunit.
5824

Glutathione S-Transferase Yc mRNA

Table I

Comparison of NH₂-terminal sequence of a purified Ya-Yc heterodimer with NH₂-terminal sequences deduced from the Yc and Ya cDNA clones

| Step | Amino acid | Yield | NH₂-terminal sequence of a Ya subunit deduced from pGTB42 | NH₂-terminal sequence of a Ya subunit deduced from pGTB38 |
|------|------------|-------|----------------------------------------------------------|----------------------------------------------------------|
| 1    | Pro        | 0.69  | Pro                                                      | Ser⁸                                                     |
|      | (Ser)      | (0.87)|                                                          |                                                          |
| 2    | Gly        | 0.81  | Gly                                                      | Gly                                                      |
| 3    | Lys        | 1.99  | Lys                                                      | Lys                                                      |
| 4    | Pro        | 0.42  | Pro                                                      | Pro                                                      |
| 5    | Val        | 2.46  | Val                                                      | Val                                                      |
| 6    | Leu        | 2.28  | Leu                                                      | Leu                                                      |
| 7    | His        | 1.01  | His                                                      | His                                                      |
| 8    | Tyr        | 1.81  | Tyr                                                      | Tyr                                                      |
| 9    | Phe        | 1.85  | Phe                                                      | Phe                                                      |
| 10   | Asp        | 1.59  | Asp                                                      | Asn*                                                     |
| 11   | Gly        | 0.69  | Gly                                                      | Ala*                                                     |
| 12   | Arg        | 1.18  | Arg                                                      | Arg                                                      |
| 13   | Gly        | 0.93  | Gly                                                      | Gly                                                      |
| 14   | Arg        | 1.24  | Arg                                                      | Arg                                                      |
| 15   | Met        | 1.30  | Met                                                      | Met                                                      |
| 16   | Glu        | 1.18  | Glu                                                      | Glu                                                      |
| 17   | Pro        | 0.27  | Pro                                                      | Cys*                                                     |
| 18   | Ile        | 1.16  | Ile                                                      | Ile                                                      |
| 19   | Arg        | 0.84  | Arg                                                      | Arg                                                      |
| 20   | Trp        | 0.35  | Trp                                                      | Trp                                                      |
| 21   | Leu        | 0.87  | Leu                                                      | Leu                                                      |
| 22   | Leu        | 1.01  | Leu                                                      | Leu                                                      |
| 23   | Ala        | 0.86  | Ala                                                      | Ala                                                      |
| 24   | Ala        | 0.91  | Ala                                                      | Ala                                                      |
| 25   | Ala        | 0.99  | Ala                                                      | Ala                                                      |
| 26   | Gly        | 0.32  | Gly                                                      | Gly                                                      |
| 27   | Val        | 0.67  | Val                                                      | Val                                                      |
| 28   | Glu        | 0.56  | Glu                                                      | Glu                                                      |
| 29   | Phe        | 0.48  | Phe                                                      | Phe                                                      |
| 30   | Glu        | 0.52  | Glu                                                      | Glu                                                      |
| 31   | Glu        | 0.57  | Glu                                                      | Glu                                                      |
| 32   | Gin        | 0.20  | Gin                                                      | Lys*                                                     |
| 33   | Phe        | 0.41  | Phe                                                      | Leu*                                                     |
| 34   | Leu        | 0.50  | Leu                                                      | Ile                                                      |
| 35   | Lys        | 0.25  | Lys                                                      | Gin*                                                     |
| 36   | Thr        | 0.11  | Thr                                                      | Ser*                                                     |
| 37   | Arg        | 0.22  | Arg                                                      | Pro*                                                     |
| 38   | Asp        | 0.40  | Asp                                                      | Glu*                                                     |
| 39   | Asp        | 0.35  | Asp                                                      | Asp                                                      |

a Assuming 2 subunits of 25,000 daltons, one of which is blocked, the initial yield was 65%. The repetitive yield was 95%. (Ten nmol of the Ya-Yc heterodimer was applied in the cup of the sequenator; 3.2 nmol was the initial yield.)

b See text for explanation of asterisk.

amount of SDS bound to the polypeptides rather than any real difference in their molecular weights.

A comparison of the nucleotide sequence of the Ya clone, pGTB38, determined previously (5) with the Yc clone, pGTB42, indicates an overall nucleotide sequence homology of 66%. However, the 5'- and 3'-untranslated regions of the two mRNAs share little sequence homology. Based upon the nucleotide differences in the coding region of the Ya and Yc mRNAs along with divergent 5'- and 3'-untranslated regions, we feel the Ya and Yc subunits of the rat liver glutathione S-transferases are derived from different genes rather than by post-transcriptional processing of a single gene. This conclusion is supported by previous experiments from our laboratory which have demonstrated that the Ya and Yc mRNAs are regulated independently by phenobarbital and 3-methylcholanthrene (5, 19, 20). The hypothesis that the Ya subunit is generated from the Yc subunit by post-translational proteolysis (21, 22) can also be ruled out by our present findings. These polypeptides are clearly products of distinct mRNA species.

When the deduced amino acid sequence of the Yc subunit is compared with the sequence of the Ya subunit deduced from pGTB38, there exists significant amino acid sequence homology. Interestingly the first 74 amino acids corresponding to the NH₂-terminal domain of both polypeptides are more highly conserved than the amino acids corresponding to the middle or carboxyl-terminal domains of the polypeptides. The functional significance of a highly conserved NH₂-terminal domain as compared to other regions of the protein may be related to a common property shared by the two polypeptides (e.g. glutathione-binding site). Since the two subunits have different enzymatic activities toward various substrates, it is also not surprising to find amino acid sequence divergence. For example, the Ya homodimer has high activity toward cumene hydroperoxide whereas the Ya homodimer has high steroid isomerase activity (23). At this time it is not possible to correlate the differences in the primary sequence with the catalytic or binding properties of each subunit.

Recently Frey et al. (18) published an NH₂-terminal sequence for a glutathione S-transferase Ya-Yc heterodimer. With the exception of position 10, where we have found aspartic acid as compared to asparagine in the sequence reported by Frey et al. (18), this sequence agrees with the amino acid sequence deduced from DNA sequence analysis of pGTB42. In addition, in this study we have determined the sequence of the first 39 amino acids of a Ya-Yc heterodimer, and the amino acid sequence is in total agreement with the amino acid sequence deduced from pGTB42. Although only a single amino acid sequence is obtained from the Ya-Yc heterodimer, we believe the sequence generated from the heterodimer represents a Ya subunit and that the Ya subunit is most likely blocked. As mentioned previously, the Ya subunit has a serine following the initiation methionine residue, and this amino acid represents a common blocked amino acid in vivo (24). This interpretation differs from Frey et al. (18) who suggested that the Ya and Yc subunits have identical NH₂-terminal amino acid sequences. Both our laboratory (5) and Tu's laboratory (25) have sequenced nearly full length Ya cDNA clones, and the NH₂-terminal sequences deduced from both clones diverge at positions 1, 10, 11, and 17 from the sequence reported by Frey et al. (18).

Tu et al. (25) have reported recently the construction and characterization of a cDNA clone, pGTR262, which contains a partial sequence for a Ya subunit. The nucleotide sequence of pGTR262 corresponds to nucleotides 223–821 of pGTB42 and is 93 nucleotides longer in the 3'-untranslated region. Although the two sequences are virtually identical over this common region, it is impossible to make meaningful comparisons without having full length cDNA clones to compare. For example, the two nearly full length Ya clones described to date, pGTB38 and pGTR261, have only 15 nucleotide differences in their coding sequence which contains 666 nucleotides (5, 25). These two sequences only diverge in their 3'-untranslated regions.

Although it appears that the Ya and Yc subunits are derived from related yet different genes, these genes most likely arose from a common ancestral gene which during the course of evolution duplicated and diverged. The divergent 5'-untranslated regions found between the Ya and Yc clones may play an important role in the differential regulation of the Ya and Yc mRNAs by xenobiotics (5, 9, 19, 20) as well as their tissue-specific regulation reported by Tu et al. (25). The construction and characterization of the nearly full length Ya clone,
Glutathione S-Transferase Yc mRNA

pGTB38, and Yc clone, pGTB42, should facilitate the isolation and characterization of the structural genes as well as elucidate the mechanisms by which these genes are regulated by xenobiotics.

Acknowledgment—We would like to thank Joan Kiliyanski for assistance in the preparation of this manuscript.

REFERENCES

1. Arias, I. M., Fleischner, G., Kirsch, R., Mishkin, S., and Gatmai- tan, Z. (1976) in Glutathione: Metabolism and Function (Arias, I. M., and Jakoby, W. B., eds) pp. 175-188, Raven Press, New York
2. Litwack, G., Ketterer, B., and Arias, I. M. (1971) Nature (Lond.) 234, 466-467
3. Jakoby, W. B., and Habig, W. H. (1980) in Enzymatic Basis of Detoxification (Jakoby, W. B., ed) Vol. 2, pp. 63-94, Academic Press, New York
4. Bass, N. M., Kirsch, R. E., Tuff, S. A., Marks, I., and Saunders, S. J. (1977) Biochim. Biophys. Acta 492, 163-175
5. Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G. J.-F., Argenbright, L., and Lu, A. Y. H. (1984) J. Biol. Chem. 259, 5182-5188
6. Gubler, U., and Hoffman, B. S. (1983) Gene 25, 263-269
7. Peacock, S. L., Melver, C. M., and Monahan, J. J. (1981) Biochim. Biophys. Acta 655, 243-250
8. Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J., and Kirschner, M. W. (1980) Cell 20, 95-105
9. Pickett, C. B., Donohue, A. M., Lu, A. Y. H., and Hales, B. F. (1982) Arch. Biochem. Biophys. 215, 539-543
10. Rigby, P. W. J., Dieckmann, M., Rhodes, C., and Berg, P. (1977) J. Mol. Biol. 113, 237-251
11. Yousaf, S. I., Carroll, A. R., and Clarke, B. E. (1984) Gene 27, 309-313
12. Smith, H. O., and Birnstiel, M. L. (1976) Nucleic Acids Res. 3, 2387-2398
13. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499-560
14. Hunkapiller, M. W., and Hood, L. E. (1978) Biochemistry 17, 2124-2133
15. Spiess, J., Rivier, J. E., Rodkey, J. A., Bennett, C. D., and Vale, W. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2974-2978
16. Tu, C. P. D., Weiss, M. J., Li, N., and Reddy, C. C. (1983) J. Biol. Chem. 258, 4659-4662
17. Reddy, C. C., Li, N. Q., and Tu, C. P. D. (1984) Biochem. Biophys. Res. Commun. 121, 1014-1020
18. Frey, A. B., Friedberg, T., Oesch, F., and Kreibich, G. (1983) J. Biol. Chem. 258, 11231-11235
19. Pickett, C. B., Wells, W., Lu, A. Y. H., and Hales, B. F. (1981) Biochem. Biophys. Res. Commun. 99, 1002-1010
20. Pickett, C. B., Telakowski-Hopkins, C. A., Donohue, A. M., Lu, A. Y. H., and Hales, B. F. (1982) Biochem. Biophys. Res. Commun. 104, 611-619
21. Bhargava, M. M., Ohmi, N., Listowsky, I., and Arias, I. M. (1980) J. Biol. Chem. 255, 718-723
22. Scully, N. C., and Mantle, T. J. (1981) Biochem. J. 193, 367-370
23. Mannervik, B., and Jenson, H. (1982) J. Biol. Chem. 257, 9909-9912
24. Jornvall, H. (1975) J. Theor. Biol. 55, 1-12
25. Lai, H.-C., Li, N., Weiss, M. J., Reddy, C. C., and Tu, C. P. D. (1984) J. Biol. Chem. 259, 5536-5542
26. Tu, C.-P. D., Lai, H.-C. J., Li, N-Q., Weiss, M. J., and Reddy, C. C. (1984) J. Biol. Chem. 259, 9434-9439