

CLONING AND NUCLEOTIDE SEQUENCE OF A FULL-LENGTH cDNA FOR HUMAN LIVER γ -GLUTAMYL-CYSTEINE SYNTHETASE¹

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SUMMARY: We have cloned and sequenced a full-length cDNA for human liver γ -glutamylcysteine synthetase (GCS), the rate-limiting enzyme in glutathione biosynthesis. The cDNA consists of 2634 bp containing an open reading frame encoding a protein of 367 amino acids and having a calculated $M_r = 72,773$. The nucleotide sequence of the cDNA for human liver GCS shares an 84% overall similarity with the composite rat GCS sequence deduced from three overlapping partial cDNAs (Yan and Meister, JBC 265: 1588-1593, 1990). The deduced amino acid sequences are 94% similar. Comparison of Northern blots of total RNA isolated from rat kidney or liver with that from human kidney revealed the GCS mRNA to be larger in the human tissue (~4.0 kb vs. ~3.7 kb). (The sequence for the human liver GCS cDNA has been assigned accession number M90656 in GenBank/EMBL databases.) © 1992 Academic

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Glutathione (L- γ -glutamyl-cysteinyl-glycine; GSH) is a ubiquitous non-protein thiol involved in cellular metabolism, maintenance of cellular redox state, protection against oxidative injury and the detoxification of xenobiotics (1). Elevation of intracellular GSH has also been implicated in the resistance of human tumor cells to certain chemotherapeutic agents (2-5). Although GSH elevations are commonly detected in drug-resistant tumor cells, the mechanism(s) responsible for the increase has not been determined. This is at least partially attributable to a lack of appropriate molecular reagents for examining the expression and regulation of key enzymes involved in GSH homeostasis; thus prompting the current investigation.

Glutathione is synthesized from its constituent amino acids in two sequential ATP-requiring reactions catalyzed by γ -glutamylcysteine synthetase (GCS) and glutathione synthase, respectively (1). Under normal conditions, the upper limit of intracellular GSH concentration is regulated by GSH inhibition of GCS, the rate-limiting step in *de novo* GSH biosynthesis (1,6).

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γ -Glutamylcysteine synthetase purified from rat kidney can be dissociated into two distinct subunits of M_r 73,000 and 27,700 (7). The larger subunit possesses all the catalytic activity of the isolated protein and can be inhibited by GSH (7). Recently, Yan and Meister (8) determined the nucleotide sequence of the M_r 73,000 subunit of rat kidney GCS following isolation and sequencing of three overlapping partial cDNA clones. The amino acid sequence derived from these cDNAs showed little overall (8%) similarity with the sequence for *E. coli* GCS deduced from sequencing of genomic DNA (9).

In this report, we describe the cloning and sequencing of a 2634 bp full-length cDNA for GCS isolated from an adult human liver cDNA library. The sequence of the human GCS cDNA and the deduced amino acid sequence of the corresponding enzyme are compared with those of the rat. Hybridization of total RNA isolated from rat kidney and liver and human liver with probes prepared from both the rat and human sequences was compared.

MATERIALS AND METHODS

Isolation of cDNA clones and DNA sequencing. A λ gt11 cDNA library was constructed using poly(A)⁺ RNA isolated from human kidney. A 1010 bp probe (rkGCS_{PCR}), corresponding to nucleotides 51-1061 of the rat GCS (8) sequence, was generated from rat kidney RNA by polymerase chain reaction using synthetic 20-mer oligonucleotide primers corresponding to nucleotides 51-70 and 1042-1061 of the rat sequence. The probe was radiolabeled with ³²P using the random primer technique and used to screen the human kidney library (10). cDNA was prepared from phage stock by a plate lysis method (10).

Following EcoR1 digestion of phage DNA, a partial (1679 bp) cDNA insert (hkGCS-1) was isolated from low melting agarose gels, purified by phenol/chloroform extraction and cloned into the EcoR1 site of the phagemid pSK-Bluescript. The nucleotide sequence was determined using Sequenase (U.S Biochemicals) according to the manufacturer's instructions, using T3, SK, T7 and KS primers as well as synthetic primers corresponding to internal GCS sequences. Nucleotide sequence was verified by bi-directional sequencing reactions. Sequence analysis was conducted using DNASTAR (Madison, WI) software.

An 0.8 kb Pst1 fragment isolated from hkGCS-1 (Figure 1) was labeled with ³²P and subsequently used to screen a human liver cDNA library (Stratgene).

Northern analysis. Total RNA was isolated from rat kidney and rat liver by the guanidinium method according to Chomczynski and Sacchi (11). Human kidney RNA was purchased from Clontech. Northern blots were prepared according to Sambrook, Fritsch and Maniatis (10).

RESULTS AND DISCUSSION

Initially, four clones were isolated from the human kidney cDNA library following screening with the radiolabeled rkGCS_{PCR} probe (solid box in rkGCS, Figure 1). However, of the four only one, the 1679 bp hkGCS-1, shared significant sequence homology with the published rat GCS sequence. Comparison with the rat sequence revealed that hkGCS-1 consisted of coding sequences for 387 amino acids on the carboxyl end of the protein and 398 nucleotides

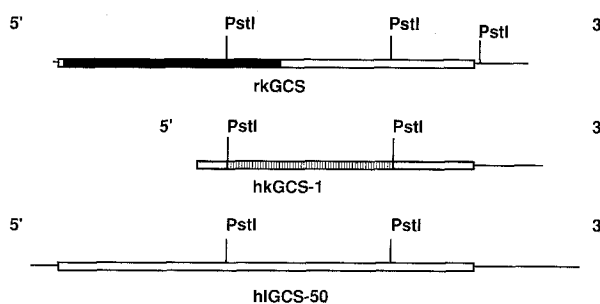


Figure 1. Restriction maps for rat (rkGCS) kidney cDNA, human kidney clone hkGCS-1 and the full-length GCS cDNA clone, hIGCS-50 isolated from human liver. Boxes represent open reading frame; the solid box in rkGCS corresponds to the PCR fragment (rkGCS_{PCR}) used to screen the human kidney cDNA library. The striped box in hkGCS-1 corresponds to the 0.8 kb PstI fragment utilized to screen the human liver library.

of non-translated sequence containing a putative polyadenylation signal (AATAAC) (8). Repeated attempts to clone a full-length cDNA from the human kidney library were unsuccessful so the cloning strategy was modified to include screening of a human liver library. An 0.8 kb PstI fragment (stripped box in hkGCS-1, Figure 1) corresponding to sequences within the open reading frame of hkGCS-1 were used to identify positive clones. A total of 50 clones were isolated from the liver cDNA library and subjected to restriction analysis. Of these positive clones, only one, hIGCS-50, contained a full length insert. A schematic representation of the rat GCS cDNA, hkGCS-1, and hIGCS-50 are shown in Figure 1.

The nucleotide sequence of the sense strand of hIGCS-50 and the deduced amino acid sequence are compared with the corresponding rat sequences in Figure 2. The human cDNA consists of 92 bp of untranslated 5' sequence, a 1911 bp open reading frame starting at the ATG codon at position 93 and 628 bp of 3' sequence. The two cDNAs share an 89% nucleotide sequence similarity within the open reading frame, and 84% overall. The sequence AATAAC, suggested to be the putative polyadenylation signal in the rat GCS cDNA (8), was located at nucleotides 2221-2226 in hIGCS-50. However, the polyadenylation consensus sequence, AATAAA, was identified at nucleotides 2493-2498 in the distal 3' region of hIGCS-50; a sequence extending beyond that included in the rat cDNA as reported by Yan and Meister (8).

Both the rat and human cDNAs contain an open reading frame 1911 bp in length, encoding proteins of 637 amino acid residues. The calculated molecular weight of the protein encoded by hIGCS-50 is 72,773 Daltons, in good agreement with the estimated molecular weight of the large subunit of rat GCS ($M_r=73,000$). The deduced amino acid sequences share 94% similarity with the deduced sequence for the rat enzyme. These results indicate that, unlike the situation with *E. coli* GCS (8), a high degree of conservation exists between the human and rat proteins.

hIGCS	1:	GGCACGAGGCTGAGTGCCGCTCTCGCGCCCGGAAGCGGGCGACCGCGCTCAGCCCGGAGGAGGAGGAG...GAGG...AGGAGGAGGAGGGGGCGGCC																									
rkGCS	1:	-.-.-.-.-C.-.-.-.-CT-C--CC-----C.,-----																									
hGCS	1:	Met	Gly	Leu	Leu	Ser	Gln	Gly	Ser	Pro	Leu	Ser	Trp	Glu	Glu	Thr	Lys	Arg	His	Ala	Asp	His	Val	Arg	Arg	His	
hIGCS	93:	ATG	GGG	CTG	CTG	TCC	CAG	GGC	TCG	CCG	CTG	AGC	TGG	GAG	GAA	ACC	AAG	CGC	CAT	GCC	GAC	CAC	GTG	CGG	CGG	CAC	
rkGCS	36:	-	-	-	-	-	-A	-	-	-	-	-	-	-A	-G	-	-	-	-	-	-	-	-	-	-	-	
rgCS	1:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Gln	-	-	-	-	-	-	-	-	-	
hGCS	26:	Gly	Ile	Leu	Gln	Phe	Leu	His	Ile	Tyr	His	Ala	Val	Lys	Asp	Arg	His	Lys	Asp	Val	Leu	Lys	Trp	Gly	Asp	Glu	
hIGCS	168:	GGG	ATC	CTC	CAG	TTC	CTG	CAC	ATC	TAC	CAC	GCC	GTC	AAG	GAC	CGG	CAC	AAG	GAC	GTT	CTC	AAG	TGG	GGC	GAT	GAG	
rkGCS	111:	-C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	26:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	51:	Val	Glu	Tyr	Met	Leu	Val	Ser	Phe	Asp	His	Glu	Asn	Lys	Val	Arg	His	Lys	Val	Leu	Val	Leu	Gly	Glu	Lys	Val	
hIGCS	243:	GTG	GAA	TAC	ATG	TTG	GTA	TCT	TTT	GAT	CAT	GAA	AAT	AAA	AAA	GTC	CGG	TTG	GTC	CTG	TCT	GGG	GAG	AAA	GTT	CTT	
rkGCS	186:	-	-G	-	-	-	-	-G	-C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	51:	-	-	-	-	-	-	-	-	-	-	-	-	Arg	-	-	Gln	-	Leu	-	Asn	-	Gly	Asp	-	-	
hGCS	76:	Glu	Thr	Leu	Gln	Glu	Lys	Gly	Glu	Arg	Thr	Asn	Pro	Asn	His	Pro	CCT	Thr	Leu	Trp	Arg	Pro	Glu	Tyr	Gly	Ser	
hIGCS	318:	GAA	ACT	CTG	CAA	GAG	AAG	GGG	GAA	AGG	ACA	AAC	CCA	AAC	CAT	CCT	ACC	CTT	TGG	AGA	CCA	GAG	TAT	GGG	AGT	TAC	
rkGCS	261:	-	-	-	-	-	-	-	-G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	76:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	101:	Met	Ile	Glu	Gly	Thr	Pro	Gly	Gln	Pro	Tyr	Gly	Gly	Thr	Met	Ser	Glu	Phe	Asn	Thr	Val	Glu	Ala	Asn	Met	Arg	
hIGCS	336:	ATG	ATT	GAA	GGG	ACA	CCA	GGA	CAG	CCC	TAC	GGA	GGA	ACA	ATG	TCC	GAG	TTC	AAT	ACA	GTT	GAG	GCC	AAC	ATG	CGA	
rkGCS	336:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	101:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	126:	Lys	Arg	Arg	Lys	Glu	Ala	Thr	Ser	Ile	Leu	Glu	GAA	Asn	Gln	Ala	Leu	Cys	Thr	Thr	Ser	Phe	Pro	Arg	Leu		
hIGCS	468:	AAA	CGC	CGG	AAG	GAG	GCT	ACT	TCT	TCT	ATA	TTA	GAA	GAA	AAT	CAG	GCT	CTT	TGC	ACA	ATA	ACT	TCA	TTT	CCC	AGA	
rkGCS	411:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	126:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	151:	Gly	Cys	Pro	Gly	Phe	Thr	Leu	Pro	Glu	Val	Lys	Pro	Asn	Pro	Val	Glu	Gly	Gly	Ala	Ser	Lys	Ser	Leu	Phe	Phe	
hIGCS	543:	GGC	TGT	CCT	GGG	TTC	ACA	CTG	CCC	GAG	GTC	AAA	CCC	AAC	CCA	GTG	GAA	GGA	GGA	GCT	TCC	AAG	TCC	CTC	TTC	TTT	
rkGCS	486:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	151:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	176:	Pro	Asp	Glu	Ala	Ile	Asn	Lys	His	Pro	Arg	Phe	Ser	Thr	Leu	Thr	Arg	Asn	Ile	Arg	His	Arg	Arg	Gly	Glu	Lys	
hIGCS	618:	CCA	GAT	GAA	GCA	ATA	AAC	AAG	CAC	CCT	CGC	TTT	AGT	ACC	TTA	ACA	AGA	AAT	ATC	CGA	CAT	AGG	AGA	GGA	GAA	AAG	
rkGCS	561:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	176:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	201:	Val	Val	Ile	Asn	Val	Pro	Ile	Phe	Lys	Asp	Lys	Asn	Thr	Pro	Ser	Pro	Phe	Ile	Glu	Thr	Phe	Thr	Glu	Asp	Asp	
hIGCS	693:	GTT	GTC	ATC	AAT	GTA	CCA	ATA	TTT	AAG	GAC	AAG	AAT	ACA	CCA	TCT	CCA	TTT	ATA	GAA	ACA	TTT	ACT	GAG	GAT	GAT	
rkGCS	636:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	201:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	226:	Glu	Ala	Ser	Arg	Ala	Ser	Lys	Pro	Asp	His	Ile	Tyr	Met	Asp	Ala	Met	Gly	Phe	Gly	Met	Gly	Asn	Cys	Cys	Leu	
hIGCS	768:	GAA	GCT	TCA	AGG	GCT	TCT	AAG	CCG	GAT	CAT	ATT	TAC	ATG	GAT	GCC	ATG	GGA	TTT	GGA	ATG	GCG	AAT	TGC	TGT	CTC	
rkGCS	711:	-G	-A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	226:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	251:	Gln	Val	Thr	Phe	Gln	Ala	Cys	Ser	Ile	Ser	Glu	Ala	Arg	Tyr	Leu	Tyr	Asp	Gln	Leu	Ala	Thr	Ile	Cse	Pro	Ile	
hIGCS	843:	CAG	GTG	ACA	TTC	CAA	GCC	TGC	AGT	ATA	TCT	GAG	GCC	AGA	TAC	CTT	TAT	GAT	CAG	TTG	GCT	ACT	ATC	TGT	CCA	ATT	
rkGCS	786:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	251:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	276:	Val	Met	Ala	Leu	Ser	Ala	Ala	Ser	Pro	Phe	Tyr	Arg	Gly	Tyr	Val	Ser	Asp	Ile	Asp	Cys	Arg	Trp	Gly	Val	Ile	
hIGCS	918:	GTT	ATG	GCT	TTG	AGT	GCT	GCA	TCT	CCC	TTT	TAC	CGA	GGC	TAT	GTG	TCA	GAC	ATT	GAT	TGT	CGC	TGG	GGA	GTG	ATT	
rkGCS	861:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	276:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	301:	Ser	Ala	Ser	Val	Asp	Asp	Arg	Thr	Arg	Glu	Glu	Arg	Gly	Leu	Glu	Pro	Leu	Lys	Asn	Asn	Asn	Tyr	Arg	Ile	Ser	
hIGCS	993:	TCT	GCA	TCT	GTA	GAT	GAT	AGA	ACT	CGG	GAG	GAG	CGA	GGA	CTG	GAG	CCA	TTG	AAG	AAC	AAT	AAC	TAT	AGG	ATC	AGT	
rkGCS	936:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	301:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	326:	Lys	Ser	Arg	Tyr	Asp	Ser	Ile	Asp	Ser	Tyr	Leu	Ser	Lys	Cys	Gly	Glu	Lys	Tyr	Asn	Asp	Ile	Asp	Leu	Thr	Ile	
hIGCS	1068:	AAA	TCC	CGA	TAT	GAC	TCA	ATA	GAC	AGC	TAT	TTA	TCT	AAG	TGT	GGT	GAG	AAA	TAT	AAT	GAC	ATC	GAC	TTG	ACG	ATA	
rkGCS	1011:	-G	-T	-G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	326:	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hGCS	351:	Asp	Lys	Glu	Ile	Tyr	Glu	Gln	Leu	Leu	Glu	Glu	Gly	Ile	Asp	His	Leu	Leu	Ala	Gln	His	Val	Ala	His	Leu	Phe	
hIGCS	1143:	GAT	AAA	GAG	ATC	TAC	GAA	CAG	CTG	TTG	CAG	GAA	GGC	ATT	GAT	CAT	CTC	CTG	GCC	CAG	CAT	GTT	GCT	CAT	CTC	TTT	
rkGCS	1086:	-C	-CG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rgCS	351:	-	Thr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Figure 2. cDNA sequences and deduced amino acid sequences for rkGCS and hIGCS-50.

The nucleotide and amino acid sequences are numbered on the left. The rkGCS residues that are identical to corresponding residues in hIGCS-50 are indicated by hyphens (-). Missing residues and gaps are shown as dots (.). * indicates the stop codon. The sequence for the human GCS cDNA has been assigned accession number M90656.

hGCS	376:	Ile Arg Asp Pro Leu Thr Leu Phe Glu Glu Lys Ile His Leu Asp Asp Ala Asn Glu Ser Asp His Phe Glu Asn
hIGCS	1218:	ATT AGA GAC CCA CTG ACA CTG TTT GAA GAG AAA ATA CAC CTG GAT GAT GCT AAT GAG TCT GAC CAT TTT GAG AAT
rkGCS	1161:	--- --- --- --- --- -C -T --- --- --- -T -T --- --- -C -C --- --- --- --- ---
rgCS	376:	- -
hGCS	401:	Ile Gln Ser Thr Asn Trp Gln Thr Met Arg Phe Lys Pro Pro Pro Pro Asn Ser Asp Ile Gly Trp Arg Val Glu
hIGCS	1293:	ATT CAG TCC ACA AAT TGG CAG ACA ATG AGA TTT AAG CCC CCT CCT CCA AAC TCA GAC ATT GGA TGG AGA GTA GAA
rkGCS	1236:	--- --- --- --- -C --- --- --- --- -G --- --- -T --- --- --- --- -T --- --- --- --- -G
rgCS	401:	- -
hGCS	426:	Phe Arg Pro Met Glu Val Gln Leu Thr Asp Phe Glu Asn Ser Ala Tyr Val Val Phe Val Val Leu Leu Thr Arg
hIGCS	1368:	TTT CGA CCC ATG GAG GTG CAA TTA ACA GAC TTT GAG AAC TCT GCC TAT GTG GTG TTT GTG GTA CTG CTC ACC AGA
rkGCS	1311:	--C --- --A --- --- --A --G --G --- --- --- --- --- --- --- --A --- --- --- --G --- --G
rgCS	426:	- -
hGCS	451:	Val Ile Leu Ser Tyr Lys Leu Asp Phe Leu Ile Pro Leu Ser Lys Val Asp Glu Asn Met Lys Val Ala Gln Lys
hIGCS	1443:	GTG ATC CTT TCC TAC AAA TTG GAT TTT CTC ATT CCA CTG TCA AAG GTT GAT GAG AAC ATG AAG GTA GCA CAG AAA
rkGCS	1386:	--- --- --C --A --- --- C-A --C --- --- --- --- --- --C --- --- --A --G --- --- G-G
rgCS	451:	- -
hGCS	476:	Arg Asp Ala Val Leu Gln Gly Met Phe Tyr Phe Arg Lys Asp Ile Cys Lys Gly Gly Asn Ala Val Val Asp Gly
hIGCS	1518:	AGA GAT GCT GTC TTG CAG GGA ATG TTT TAT TTC AGG AAA GAT ATT TGC AAA GGT GGC AAT GCA GTG GTG GAT GGT
rkGCS	1461:	C- - - -C - - - - -G - - - - -C - - - - -C - - - - -C - - - - -C - - - - -C - - - - -C - - - - -G
rgCS	476:	- -
hGCS	501:	Cys Gly Lys Ala Gln Asn Ser Thr Glu Leu Ala Ala Glu Glu Tyr Thr Leu Met Ser Ile Asp Thr Ile Ile Asn
hIGCS	1593:	TGT GGC AAG GCC CAG AAC AGC ACG GAG CTC GCT GCA GAG GAG TAC ACC CTC ATG AGC ATA GAC ACC ATC ATC AAT
rkGCS	1536:	--- A- - - -C --- --- -C --- --- T-C --- --- -CA T- - - - -G --- --- --- --- --- --- ---
rgCS	501:	- Ser - - - Thr - Ser - Pro Ser -
hGCS	526:	Gly Lys Glu Gly Val Phe Pro Gly Leu Ile Pro Ile Leu Asn Ser Tyr Leu Glu Asn Met Glu Val Asp Val Asp
hIGCS	1668:	GGG AAG GAA GGT GTG TTT CCT GGA CTG ATC CCA ATT CTG AAC TCT TAC CTT GAA AAC ATG GAA GTG GAT GTG GAC
rkGCS	1611:	--- --- --- -C --- --- --- --- -C --- --- -C --- --- --- --- --- --- --- --- --- --- ---
rgCS	526:	- -
hGCS	551:	Thr Arg Cys Ser Ile Leu Asn Tyr Leu Lys Leu Ile Lys Lys Arg Ala Ser Gly Glu Leu Met Thr Val Ala Arg
hIGCS	1743:	ACC AGA TGT AGT ATT CTG AAC TAC CTA AAG CTA ATT AAG AAG AGA GCA TCT GGA GAA CTA ATG ACA GTT GCC AGA
rkGCS	1686:	--- C- - -C - - - - -G -T - - - - -G
rgCS	551:	- - - -C -
hGCS	576:	Trp Met Arg Glu Phe Ile Ala Asn His Pro Asp Tyr Lys Gln Asp Ser Val Ile Thr Asp Glu Met Asn Tyr Ser
hIGCS	1818:	TGG ATG AGG GAG TTT ATC GCA AAC CAT CCT GAC TAC AAG CAA GAC AGT GTC ATA ACT GAT GAA ATG AAT TAT AGC
rkGCS	1761:	--- --- -A - - - -T -G - -C - -C - - - - -
rgCS	576:	- - - -A - - - -T -
hGCS	601:	Leu Ile Leu Lys Cys Asn Gln Ile Ala Asn Glu Leu Cys Glu Cys Pro Glu Leu Leu Gly Ser Ala Phe Arg Lys
hIGCS	1893:	CTT ATT TTG AAG TGT AAC CAA ATT GCA AAT GAA TTA TGT GAA TGC CCA GAG TTA CTT GGA TCA GCA TTT AGG AAA
rkGCS	1836:	--C --- --- --A --- -C --- --- --- --- -G --- --- -T --- --- --- --- -G --- -GC --- --A ---
rgCS	601:	- -
hGCS	626:	Val Lys Tyr Ser Gly Ser Lys Thr Asp Ser Ser Asn
hIGCS	1968:	GTA AAA TAT AGT GGA AGT AAA ACT GAC TCA TCC AAC TAG'ACATTCTACAG.AAAGAAAAATGCATTATTGACGAAGTGGCTACAGT
rkGCS	1911:	-CG --G --C --- --- G- - - -GC --- -C-T --A G- - TAG'-----C-----G--G.....-G-----C
rgCS	626:	Ala - - - - Gly - Ser - Pro - Asp
hIGCS	2053:	ACCATG.CCTCTC.AGCCCGTGTA.T.A.A...TATGAAGACCAATGATAGAAGTGTACTGTTTTCTGGGCCAGTGAGCCAGA.AATTGATTAAG.
rkGCS	1980:	...-A-...-G-----C-C-G-CGC-G-----G-C...-A-G-----T-----C-C-G--C-...-A
hIGCS	2142:	.GCTTTCTTTGGTAGGTAAATCTAGAGTTTATACAGTGTACATGTACATAGTAAAGTATTTT..G...ATTAACAATGATTTTAAATAACA.T..ATC
rkGCS	2056:	A---C---CA-----G-----G--T-----T-----AT-ATT-----C-GT---
hIGCS	2232:	TAAAGTCATCATGAAGTGGCTTGTACATTTTAAATCTTACTCTGGAGCAACCTACTGTCTAAGCAGTTTGTAAATGTACTGGTAATTGTACAATAC
rkGCS	2147:	--C-----GG-----
hIGCS	2331:	TTGCATTCCAGAGTTAAATGTTTACTGTAAATTTTGTCTTTTAAAGACTACCTGGGACCTGATTATTGAAATTTTCTCTTTAAAAACATTTTCT
hIGCS	2430:	CTCGTTAATTTTCTTTGTCTATTTCTTTGTGTCTACATTAAATCACTTGAATCCATTGAAAGTGCTTCAAGGGTAATCTTGGGTTCTAGCACCTTA
hIGCS	2529:	TCTATGATGTTTCTTTTGAATTTGAATAATCACTTGGTCACCTTGCCCCAAGCTTCCCTCTGAATAAATACCCATTGAAGTCTGAAAAA
hIGCS	2628:	AAAAAA

Figure 2-Continued

The 1.0 kb rkGCS_{PCR} fragment and hGCS-1 cDNA were used to probe Northern blots of total RNA isolated from rat kidney, rat liver and human kidney. Both probes identified similar RNA species (Figure 3). GCS mRNA was more abundant in rat kidney than in rat liver, consistent with previous observations (8). Both probes hybridized with a single 4.0 kb band in

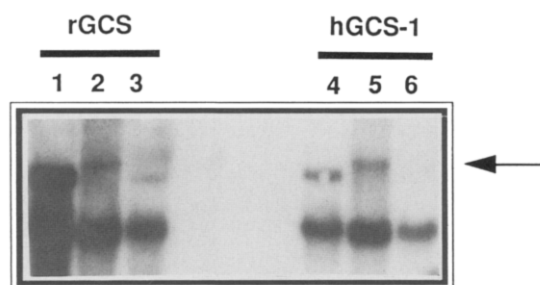


Figure 3. Northern blot analysis of total RNA isolated from rat kidney (lanes 1 and 4), rat liver (lanes 2 and 5) and human kidney (lanes 3 and 6). Ten μ g of total RNA was loaded per lane. Lanes 1-3 were probed with the 1.0 kb rkGCS_{PCR} fragment while lanes 4-6 were probed with hkGCS-1. The arrow indicates the position of the human kidney GCS message. The lower band in each of the lanes corresponds to β -actin message.

RNA isolated from human kidney. This RNA was approximately 300 base pairs larger than the species identified in the RNA isolated from the rat tissues. This species-specific size differential is similar to that reported for γ -glutamyl transpeptidase, another GSH metabolizing enzyme (12).

Glutathione plays a prominent role in cellular homeostasis and is particularly important in the response of cells to various types of injurious agents. The ability of cells to survive exposure to xenobiotics capable of inducing oxidative damage, ionizing radiation, heavy metals or alkylating agents has been correlated with intracellular levels of GSH. While the regulation of GSH levels in response to these insults has not been defined, there is ample evidence in the toxicological literature to support the role of GCS in many GSH-related adaptive responses (13-16). Defects in GSH synthesis and/or metabolism, particularly in red blood cells, have also been associated with various disease states (17). The availability of a full-length cDNA clone for human GCS will permit the investigation of GSH regulation at the molecular level; an approach which should provide insights to alternative means of manipulating GSH levels to experimental and therapeutic advantage.

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