Structure and 5[']-flanking region of the *gene encoding* the mouse *it-glutamylcysteine* synthetase heavy subunit

(genomic structure, intron-exon organization, promoter elements, cDNA)

Joseph Osei-Frimpong [1], Jestina Mason [2], Russell M. Lcbovitzl,3
and
Michael W. Liebermanl*

Departments of Pathology!, Molecular Genetics [2] and Cell Biology3
Baylor College of Medicine
Houston, Tx. 77030

*Address all correspondence and reprint requests to:
Michael W. Lieberman
Department of Pathology
Baylor College of Medicine
One Baylor Plaza
Houston, Tx. 77030
Tel : (713) 798-3859
Fax : (713) 798-5838

Abbreviations: ARE-antioxidant responsive element., BAC-bacterial artificial chromosome: bp-base pair; eDNA- complimentary DNA; yGCS-7-glutamylcysteine synthetase: GSH-glutathione; kh-kilobasc; MRE-metal regulatory element; ORF- open reading frame

SUMMARY

The heavy subunit of the y-glutamylcysteine synthetase (yGCS) enzyme catalyzes the first and rate-limiting step in *de novo* synthesis of glutathione (GSH) and has been implicated in many GSH-related responses. We have cloned the mouse ?GCS gene and deduced its genomic structure. These efforts were facilitated by the cloning and sequencing of the mouse cDNA, which we also report here. The mousei'GCS gene is relatively large, spanning > 60 kb genomic DNA with an unusually big first intron (> 38 kb). The *1.9* kb coding region consists of 16 exons and 15 introns. We also describe here the isolation and characterization of the 5'-flanking region of the mouse GCS heavy subunit gene. Sequence analysis of this region shows similarities and differences to the 5'-flanking region of the human gene. Analysis for regulatory elements reveals the presence of a consensus putative TATAAAA box and several potential regulatory sites including several consensus putative SpI and potential AP1-like binding sites. There are also two identical potential antioxidant responsive elements (AREs) and a region consisting of several potential metal regulatory elements (MREs)-

AREs or MREs and therefore any duplications of these elements in the lower mammals were lost as the higher mammals evolved and developed consensus AREs or MREs as found in the human " ,GCS gene.

CONCLUSIONS.

We have cloned and characterized the genomic structure of the mouse GCS heavy subunit gene which was facilitated by our simultaneous cloning and sequencing of the mouse cDNA. We have isolated and characterized a 1.9 kb 5'-flanking region which reveals the presence of putative basal transcriptional **elements in** addition to a two potential antioxidant responsive elements and a region containing putative multiple metal response elements.

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