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June 5, 1995.

Dear Dr. Migliore:

Thanks for your letter dated May 3, 1995. Please find enclosed a summary of the progress report on my Moran Foundation project entitled 'Cloning and sequence analysis of the mouse γglutamylcysteine synthetase (γGCS) gene'. During the past year my efforts has been directed towards characterization of the genomic structure of the mouse γGCS gene. I am happy to inform you that I have completed sequencing and characterizing about 90% of the mouse γGCS gene and will start preparing a manuscript for publication in the very near future.

I am also in the process of writing a new but related research proposal entitled "Disruption of the γglutamylcysteine synthetase (γGCS) locus in embryo-derived stem (ES) cells" which I will submit to The Moran Foundation for financial consideration for the fiscal year 1995-1996.

I would like to thank the Scientific Advisory Committee and the Moran Foundation for supporting this research.

Sincerely yours,
Joseph Osei-Frimpong, PhD
Joseph Osei-Frimpong, Ph.D.

Progress Report - Cloning and sequence analysis of the of the mouse γglutamylcysteine synthetase (γGCS) gene - [3-93-0067]

Characterization of the Genomic Structure of the Mouse γGCS Gene

A. Cloning of mouse γGCS cDNA

The γGCS λ-phage clones characterized previously (see last year's Progress Report) were originally isolated using a rat γGCS cDNA probe. Identification of mouse γGCS intron-exon borders and 5'-flanking regions would be greatly facilitated by access to mouse γGCS cDNA clones, since cDNAs from different species are most likely to diverge at the 5' and 3' non-coding regions. Using a modification of the rapid amplification of 5'-cDNA ends (5'-RACE) procedure, I have cloned a partial mouse γGCS cDNA (the first 300 bp from the ATG) from mouse kidney. This clone was sequenced and found to have a 94% sequence similarity index with the same region of the rat cDNA.

B. Exonic Structure of the mouse γGCS Gene

Using Southern blotting with mouse γGCS cDNA probes, high speed sequencing and PCR (for introns > 300 bp) I have deduced the exonic structure of the mouse gene at the 5'-most coding region from my γGCS λ clones. Also based on published rat cDNA sequence (pending the cloning of a full length mouse cDNA), I have also tentatively deduced the rest of the exonic structure of the mouse gene, with the exception of exons one and thirteen, which are yet to be precisely localized and sequenced. All exons are contained on the tentative map deduced from alignment of the γGCS clones (see Progress Report of last year). This tentative genomic structure of the mouse γGCS gene (Osei-Frimpong, Lebovitz, Lieberman, unpublished) is shown in Figure 1 below. The mouse γGCS heavy subunit gene most likely consists of 16 exons and spans at least 23 kb. The biggest exon is the 16 th exon (465 bp), which consists of both coding and non-coding regions. The smallest exon is the 5 th exon (59 bp).

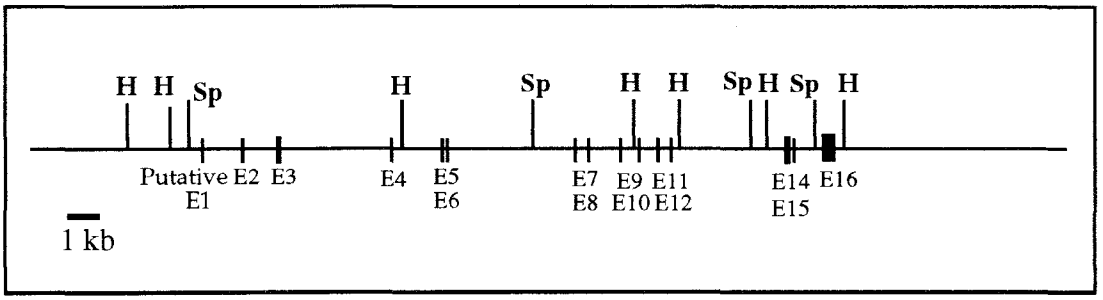

Figure 1. Genomic structure of the mouse γGCS heavy subunit gene (unpublished); exons are represented by black boxes. All exon and intron lengths are drawn to scale. H-HindIII, Sp-SpeI, E-exon, kb-kilobase