HEME BIOSYNTHESIS IN ERYTHROID CELLS:

Transcriptional Regulation of the

Human 5-Aminolevulinate Synthase 2 Gene

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for the degree of Doctor of Philosophy

by

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TABLE OF CONTENTS

CHAPTER TWO: MATERIALS AND METHODS

CHAPTER FIVE : IDENTIFICATION OF REGULATORY ELEMENTS AND CORRESPONDING TRANSCRIPTION FACTORS THAT DRIVE EXPRESSION OF THE ALAS2 PROMOTER

THESIS SUMMARY

The focus of research in our laboratory has involved the elucidation of the regulatory mechanisms that control heme biosynthesis in eukaryotic cells. 5-aminolevulinate synthase (ALAS) is a nuclear encoded mitochondrial matrix enzyme that catalyses the formation of 5-aminolevulinate from glycine and succinyl CoA, the first step in the heme biosynthetic pathway. This enzyme is of particular interest since it is the rate-controlling enzyme. There are two closely related isozymes of ALAS designated ALAS1 and ALAS2 which are encoded by separate genes located on different chromosomes. The housekeeping enzyme, ALAS1 is probably expressed in all tissues to provide heme for respiratory cytochromes and other heme containing proteins. In contrast, ALAS2, is an erythroid-specific enzyme, the synthesis of which is developmentally regulated and is markedly increased during erythropoiesis to accommodate the increased demand for heme during hemoglobin production. Previous studies have demonstrated that expression of the ALAS2 gene is regulated at both the transcriptional and post-transcriptional levels.

During erythropoiesis, transcription of the ALAS2 gene is markedly upregulated together with the genes for the other heme pathway enzymes and for globin. The human ALAS2 gene consisting of 11 exons spanning 22 kb, and extensive 5' (10 kb) and 3' flanking (3 kb) sequence was previously isolated and sequenced in our laboratory. Comparison of the genomic organisation of the human and murine ALAS2 genes exhibit remarkable similarity. The work presented in this thesis is concerned with the identification of regulatory regions responsible for the transcriptional control of the human ALAS2 gene in erythroid cells. A number of regions of the human ALAS2 gene which corresponded to DNase I hypersensitivity sites in the murine ALAS2 gene were examined in transient expression studies performed in erythroid cell lines.

Deletion analysis of the 5'-flanking region of the human ALAS2 gene demonstrated that the first 300 bp upstream of the transcription initiation site could function as a promoter in erythroid cells. Putative binding sites for the erythroid-enriched transcription factors, GATA-1, NF-E2 and the CACCC box binding protein, EKLF, and ubiquitous transcription factors were identified in the ALAS2 promoter. Gel shift assays were performed to determine the proteins binding to these sites and the functional contribution of these sites evaluated by site-directed mutagenesis and transient expression analysis. However, ALAS2

promoter constructs containing -293 bp to -10.3 kb of 5'- flanking sequence also expressed efficiently in non-erythroid COS-1 cells indicating that additional sequence or native chromatin structure may be required for the erythroid-specific expression of the human ALAS2 gene *in vivo*.

Examination of other regions of the human ALAS2 gene which corresponded to DNase I hypersensitivity sites in the murine ALAS2 gene, resulted in the identification of an erythroid-specific enhancer element within intron 8, located approximately 16 kb downstream from the transcription initiation site. This enhancer element was capable of transactivating both the human ALAS2 promoter and the heterologous thymidine kinase promoter in erythroid cells. Sequence analysis of intron 8 revealed putative GATA and CACCC box motifs which are highly conserved between the human, murine and canine ALAS2 genes. Mutational analysis established that a combination of two CACCC boxes and one GATA-1 binding site was essential for activity of the erythroid-specific enhancer.

The association of GATA-1 and CACCC box binding sites have previously been reported to play a critical role in the tissue-specific expression of many other erythroid-specific genes, including the enzymes of the heme biosynthetic pathway and the globin gene cluster. The identification of functional GATA-1 and CACCC box binding sites in the human ALAS2 promoter and in the erythroid-specific enhancer element located within intron 8, highlights the importance of these transcription factors in the erythroid-specific regulation of erythroid-cell expressed genes. Consequently, an interaction of a common group of transcription factors within the regulatory regions of the genes encoding the enzymes of the heme biosynthetic pathway and the globin gene cluster would ensure the coordinated synthesis of heme molecules and globin chains during erythropoiesis.