



STUDIES ON THE 5-AMINOLEVULINATE SYNTHASE GENE

AND ITS REGULATION

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DECLARATION

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SUMMARY

5-Aminolevulinate synthase (ALV-S) is the first enzyme of the heme biosynthetic pathway. This thesis is concerned with control of hepatic ALV-S synthesis by negative feedback regulation by heme. Briefly, the chicken ALV-S gene has been isolated and characterised, and transcription run-on experiments in isolated rat liver nuclei have demonstrated that control of ALV-S synthesis in liver is exerted primarily at the level of transcription initiation.

1. A full-length cDNA clone (p105B1) for chick embryo liver 5-aminolevulinate synthase (ALV-S) was used as a hybridization probe to isolate 13 clones from a chicken genomic library. These clones were characterized by restriction enzyme analysis and Southern blot hybridization with p105B1. The clones overlapped, spanning a contiguous region of 40 kb of genomic DNA. One clone (designated λ cALA-S 1) which hybridized to DNA from both the 5' and 3' ends of p105B1 was chosen for further analysis.

2. Total chicken genomic DNA and λ cALA-S 1 were each digested with several different restriction enzymes and analysed by Southern blot hybridization with p105B1, at high and low stringency. Identical hybridization patterns were observed, indicating that λ cALA-S 1 contains the entire ALV-S gene. Also, this result provides strong evidence that ALV-S is coded for by a single gene, and that no other related sequences are present in the chicken genome.

3. Sequences hybridizing to p105B1 were contained within two **Bam**HI restriction fragments of 4.3 kb and 5.6 kb. These were cloned from λ cALA-S 1 into pUC19. DNA prepared from each of these subclones was used to generate a library of random overlapping clones in M13, and restriction fragments overlapping the 5' end of the gene and the internal **Bam**HI site were also subcloned into M13 for sequencing. The sequence of a 5.2 kb region of DNA including the 4.3 kb **Bam**HI fragment is presented in this thesis. This sequence extends 995 bp upstream of the mRNA transcription start site.

4. The gene spans 6.9 kb and is divided into 10 exons (156-280 bp), split by 9 introns (91-1100 bp). The exon-intron boundaries all conform to the GT-AG rule and the consensus sequences for eukaryotic splice junctions. Sequences with homology to the branch-point sequences implicated in the splicing mechanism are also present.

5. The ALV-S promoter contains many elements which have been shown to be important in the control of eukaryotic gene transcription. Two TATA boxes are present, at positions -30 and -71 relative to the transcription start site (+1) and CAAT boxes are located approximately 70 bp upstream of each TATA box.

Several features common to many eukaryotic housekeeping genes are also found. Five GC hexanucleotide boxes are present within 110 bp of the transcription start site. The promoter region has a high G+C content, averaging 62% G+C in a 1.5 kb region extending from 995 bp upstream of the transcription start site into the first intron. This high G+C content is also associated with clustered CpG dinucleotides.

An unusual tandemly repeated element is present at position -160 relative to the transcription start site (+1). The 10 bp sequence CCC(T/C)CATGG is reiterated 3 times in tandem and the sequence CCCCTCA also occurs twice, within a 50 bp segment of DNA. Another tandemly repeated element occurs at position -97. The 6 bp sequence CACGCC is repeated twice with a one nucleotide overlap and sequences similar to this occur in the yeast and bacterial ALV-S genes.

6. The transcriptional regulation of the rat ALV-S gene by heme and porphyrinogenic drugs was examined by transcription run-on experiments using nuclei isolated from the livers of normal rats and from animals treated with heme or its precursor ALV, or with the porphyrinogenic drug 2-allyl-2-isopropylacetamide (AIA), or with both. Treatment of normal animals with heme or ALV reduced ALV-S transcription to undetectable levels. Administration of AIA for 4 h increased ALV-S transcription 10 fold, and heme or ALV treatment completely prevented this induction. An unexpected finding was that transcription of a drug-inducible cytochrome P450 gene was also repressed by heme. Control experiments showed that the effects of heme and drugs were specific, since transcription of the serum albumin gene was unaffected. Heme and ALV had no effect on elongation of the ALV-S or cytochrome P450 transcripts *in vitro*, suggesting that administration of heme or drugs alters the rate of transcription initiation. The relative levels of ALV-S mRNA and enzyme activity correlated closely with the transcription rate of the ALV-S gene, and it is therefore likely that the major control of hepatic ALV-S synthesis is at the level of transcription initiation.