REGULATION OF THE EXPRESSION OF PHENOBARBITAL-INDUCIBLE P450 GENES

A THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE UNIVERSITY OF ADELAIDE

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DEPARTMENT OF BIOCHEMISTRY

MARCH 1989

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THESIS SUMMARY

The work embodied in this thesis is directed towards an understanding of the mechanism by which drug-inducible genes are controlled. The products of these genes, the cytochrome P450s (P450s), are found primarily in the liver and participate in the detoxification of numerous exogenous and endogenous substrates.

There is a great deal of scientific interest in how structurally unrelated molecules induce expression of cytochrome P450s. In addition, the mechanism by which cytochrome P450s and 5-aminolevulinate synthase (ALV-synthase), the rate-limiting enzyme in heme synthesis, are coordinately induced has yet to be conclusively defined. Experiments detailed in this thesis are aimed at dealing with these issues.

A chicken P450 cDNA clone, pCHP3, has been previously identified in this laboratory, and restriction mapping of chicken P450 genomic clones has identified two PB-inducible P450 genes, A and B. pCHP3 has been shown to be derived from a 3.5 kb P450 mRNA, which is transcribed from the chicken P450 A gene. In this thesis, the nucleotide sequence and characterisation of a second chicken P450 cDNA clone, pCHP7, are detailed. This cDNA sequence is full-length and is derived from a 2.2 kb P450 mRNA. The predicted amino acid sequence is found to be 92% similar to that derived from the nucleotide sequence of pCHP3. In addition, the 2.2 kb P450 mRNA is shown to originate from the P450 B gene. The relationship of these chicken P450s to other P450 genes, which together comprise a P450 gene superfamily, is discussed.

The drug-induced expression of the P450 and ALV-synthase genes are investigated. The proposal that drugs induce ALV-synthase as a consequence of the increased heme requirement for synthesis of the mature P450 protein implies that simultaneous expression of these genes following drug induction is obligatory. Results from an investigation into the tissue-specific expression of these genes in drug treated chickens indicate that P450 and ALV-synthase mRNAs increase in the liver, kidney and small intestine. are compatible with this proposal. Studies of the time courses of P450 and ALV-synthase mRNA accumulation also show this to be the case. Moreover, in this study, the induction response, in terms of both P450 and ALV-synthase mRNA accumulation, was quantitated. It was found that P450 mRNA levels increased up to 120-fold when the drug, 2-allyl-2-isopropylacetamide, was employed as inducer. ALV-synthase mRNA levels increased by up to 25-fold. By comparison with reported increases in P450 mRNA levels from mammalian studies, the chick embryo P450s are highly drug-inducible.

Nuclear transcription run-on assays using isolated nuclei from chick embryo hepatocytes demonstrated that the drug induction of P450 mRNA levels was due, in part, to transcriptional activation of the corresponding genes. As the increase in transcription of the P450 genes could not account for the observed increases in mRNA levels, then a post-transcriptional mechanism is indicated. This was not the case for ALV-synthase, where control of mRNA levels is primarily due to transcriptional activation of the ALV-synthase gene.

Control of P450 gene expression by heme was investigated in the livers of rats. It was found that, following administration of exogenous heme, both the basal and drug-induced expression of at least two P450 genes was repressed. These results imply that, in addition to the postulated positive role of heme in regulation of P450 synthesis, heme may also be involved in the repression of P450 synthesis.

Errata: The plural form of 'cytochrome P450' should read 'cytochromes P450'.