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Eukaryotic gene transcription—studies on cytochrome P-450 as a model system

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Abstract

Studies on the transcriptional regulation of the CYP2B1/B2 subfamily of the cytochrome P-450 supergene family have yielded valuable information. Major promoters regulating this gene are located in the near upstream of the 5′ flanking region. Transcriptional regulation is modulated by both positive and negative *cis*-acting elements. It appears that phenobarbitone, the prototype inducer, may be regulating the phosphorylation status of the transcription factors, rather than acting through a specific phenobarbitone-responsive element on the gene. This concept is attractive in explaining the broad-based action of phenobarbitone and some other unrelated chemicals in activating a set of genes specifically as a short-term response and a global effect leading to hypertrophy as a long-term response.

Key words: Eukaryotic gene architecture, transcriptional regulation, cytochrome P-450.

1. Introduction

The last decade has witnessed striking advances in our understanding of the eukaryotic gene architecture. This has, however, to await the advent of recombinant DNA technology since genetic techniques applicable to prokaryotes are not often applicable to a variety of higher organisms. A detailed knowledge of the architecture of a typical eukaryotic gene has spurred intensive studies on the mechanisms involved in eukaryotic gene transcription.

This laboratory has been concerned with studies on the transcriptional regulation of the cytochrome P-450 gene superfamily in rat liver. This system offers several advantages to be used as a model to understand eukaryotic gene transcription. Many members of this family are inducible. They show developmental regulation and are widely distributed across species and tissues. The proteins are basically hydroxylases catalyzing crucial steps in a variety of metabolic pathways of endogenous chemicals[1], besides being involved in the biotransformation of a variety of foreign chemicals. This brief review summarizes the conceptual framework that is emerging to explain

the transcriptional regulation of CYP2B1/B2 subfamily, by the prototype inducer, phenobarbitone (PB), in rat liver and its implication for eukaryotic gene transcriptional regulation in general.

2. CYP1A1 *vs* CYP2B1/B2 families

The cytochrome P-450 superfamily is now divided into 27 families and subfamilies and the list is growing[2]. However, detailed studies on transcriptional regulation are mostly available only with the CYP1A1 subfamily, inducible by polycyclic aromatic hydrocarbons. Data from several groups of workers indicate that transcriptional regulation of the CYP1A1 subfamily by the prototype inducer, 3-methylcholanthrene, follows the 'steroid' pathway. A specific receptor for the hydrocarbon referred to as Ah receptor translocates the ligand from the cytoplasm to the nucleus. Specific *cis*-acting elements have been identified upstream of the CYP1A1 gene, functioning as xenobiotic response elements and interacting with the Ah receptor. Besides, a basal transcription element has also been identified[3-5].

The situation with the PB-inducible CYP2B1/B2 gene is far from clear. Although the B1 and B2 genes are distinct, they show 97% homology having 9 exons and at this stage are treated as a single unit, although differences do exist between the two genes in terms of expression. Efforts to identify a receptor for PB have not been successful. Unlike the polyaromatic hydrocarbons, PB is a broad-based inducer. It also induces other genes such as NADPH–cytochrome P-450 reductase, UDP–glucuronyl transferase, epoxide hydrolase, aldehyde dehydrogenase and is a hypertrophic agent. At the same time, the specific action of PB is indicated by the fact that CYP2B1/B2 is the major cytochrome P-450 species induced and the response in terms of transcriptional activation of the gene is seen within an hour of administration of the drug to the rat. Finally, CYP2B1/B2 is induced by apparent structurally diverse set of compounds, although the site of action is likely to be at the level of transcription[5].

In view of these interesting characteristics of the CYP2B1/B2 gene, this laboratory has focussed on the regulatory features of transcription of this gene. Since, earlier studies clearly indicated that transcriptional activation is the primary mechanism of induction of this gene[6,7], studies were initiated to identify *cis*-regulatory elements on the 5′-flanking region.

3. Major promoters are located in the near upstream of the CYP2B1/B2 gene

The DNA sequence up to –800nt of the upstream revealed several potential regulatory sites such as direct and inverted repeats, stretch of alternating purines and pyrimidines capable of assuming a Z conformation and putative NF1 and glucocorticoid-binding sites[8]. A novel whole nuclear transcription assay was developed to transcribe cloned minigene fragments using S1 nuclease protection assay. It was shown that a cloned DNA containing –178nt of the 5′-upstream and the first exon (pP450e178) can be transcribed in whole nuclei reflecting the transcription status of

the CYP2B1/B2 gene under different treatment conditions. Thus, the pP450e178 transcripts were hardly detectable in uninduced nuclei but significant amounts could be detected in nuclei isolated from PB-treated rats (PB-nuclei). Again, nuclei from PB+$CoCl_2$-treated rats gave a low level of P450e178 transcripts and this could be counteracted by adding heme *in vitro*[8]. These results are in conformity with the proposal that an optimal amount of heme is required for the transcription of the CYP2B1/B2 gene[7,9,10]. Thus, these results clearly indicate that the near upstream of the 5′-flanking region (–178nt) is able to confer the regulatory features of the gene manifested under a variety of treatment conditions *in vivo*. This does not, however, preclude the existence of further upstream and far upstream enhancers.

4. Identification of a positive *cis*-acting element in the near upstream of the 5′-flanking region

DNase-foot print analysis of pP450e178 DNA with nuclear extracts from PB-treated rats indicated protection of the region –54 to –89nt. Whole nuclear transcription analysis of Bal 31 mutants generated from pP450e178 revealed that the mutant pP450e116 (containing –116 of the upstream) is well transcribed, but the mutant pP450e75 (containing –75nt of the upstream) is poorly transcribed. Gel shift assays with the –178nt upstream and oligonucleotides spanning the region have revealed that oligonucleotide-1 (–69 to –98nt) gives a pattern similar to that of the –178nt fragment. Essentially, two major complexes are seen and the bottom complex intensifies with nuclear extracts from PB-treated rats. The transcription of pP450e178 DNA in cell-free extracts is inhibited by the addition of oligonucleotide-1 indicating that it competes for an essential transcription factor(s). All these results have indicated that the region –69 to –98nt acts as a positive *cis*-acting element in the transcription of the CYP2B1/B2 gene[11].

5. Identification of a negative *cis*-acting element in the near upstream of the 5′-flanking region

Preliminary unpublished results have indicated that the region –178 to –116nt may harbor a negative element. This is on the basis that the Bal 31 mutant pP450e116 is transcribed more efficiently than pP450e178 in whole nuclei, although pP450e75 is poorly transcribed. Besides, the gel retardation and DNase-1 foot analysis data indicate factor binding to this region in the uninduced state, that is inhibited under conditions of PB treatment. Further, the region also contains altered glucocorticoid response element, the core sequence TGTCCT being present twice. This region may mediate the repressive effect of dexamethasone on PB-dependent activation of CYP2B1/B2 gene transcription[12]. Thus, it appears likely that the region –178 to –116nt may harbor a negative element accounting for the repressed state of the gene in the uninduced animal and the repressive effect of the glucocorticoid when given along with PB.

6. Characterization of the *trans*-acting factors binding to the positive element

Crosslinking analysis of the gel-retarded complexes has revealed two proteins in the

range 38–42 kDa, which includes the size of the oligonucleotide present in the complex. Preliminary studies have yielded similar proteins, when the nuclear extract is fractionated on oligonucleotide-affinity columns. The purified preparations are capable of stimulating pP450e178 DNA transcription in cell-free extracts. Further studies are needed to characterize these factors in detail.

7. Phosphorylation of transcription factor(s) as a possible regulatory step

Recently, He and Fulco[13] have reported that a 17nt fragment within the positive element identified in this laboratory forms a strong complex with nuclear extracts from PB-treated rats. Interestingly, they have also shown that addition of PB *in vitro* to control extract (uninduced) has a similar effect. Preliminary studies now reveal that this effect of PB may be due to its interference with the phosphorylation machinery. It appears that the transcriptional regulation of the CYP2B1/B2 gene will be governed by the phosphorylation status of the transcription factors concerned.

8. Regulation of transcription at the elongation step

Studies on run-on transcription of the CYP1A1 gene revealed that the transcripts quantified with a second exon probe were significantly higher than that obtained with the 7th exon probe, after accounting for the size of the probes used or the U residues present in the probes, since (α-32P) UTP was used to measure transcription[14]. This indicates that the rate of transcription across the gene is not uniform and is regulated. Preliminary studies with CYP2B1/B2 gene with a variety of exonic probes once again have indicated non-uniformity in transcription rates across the gene and antisense transcription has been detected as well. The significance of these observations needs to be investigated in depth.

9. Role of heme

Detailed studies with a variety of inhibitors of heme biosynthesis such as $CoCl_2$, 3-amino-1,2,4-triazole and thioacetamide[15] indicated that they all blocked the induction and transcriptional activation of the CYP2B1/B2 gene by PB. These effects were counteracted by administering low concentrations of hemin (25–50 μg/100g body weight) to the animal. But, it was found that succinylacetone, a highly specific and potent inhibitor of δ-aminolevulinate (ALA) dehydrase did not inhibit the PB-mediated induction of CYP2B1/B2 messenger RNA and, therefore, questions were raised on the involvement of heme in the transcriptional regulation of the gene[16]. However, detailed studies in this laboratory[17] with succinylacetone have revealed that it does inhibit PB-mediated induction of CYP2B1/B2 mRNA at short (3 h), but not at long, time intervals (16 h). This has been traced to the observation that succinylacetone is a weak and transient inhibitor of heme biosynthesis, although it is a powerful inhibitor of ALA dehydrase. This enzyme is present in large excess of ALA synthase and is not a rate-limiting enzyme in rat liver. Striking inhibition of the enzyme leads only to a small decrease in heme content, which is quickly compensated by the release of feedback regulation of ALA synthase, which is derepressed. This

leads to an overproduction of ALA that can now overcome the inhibition by the substrate analog, succinylacetone, on ALA dehydrase.

Although these results have clarified and established the involvement of nuclear heme in the transcriptional regulation of CYP2B1/B2 gene, its exact site of action is not clear. The results obtained in this laboratory have implicated its involvement in transcription factor binding to the 5'-upstream region as well as in the process of elongation of transcription[7,9]. One possibility appears to be the involvement of heme in modulating the phosphorylation status of transcription factors involved in the initiation and elongation steps of transcription of the CYP2B1/B2 gene. At least one kinase, namely, eIF-2α kinase, is known to be a heme-dependent enzyme.

10. Future perspectives

Although it is a little premature to extrapolate the CYP2B1/B2 gene transcriptional regulation as a general paradigm to explain features of eukaryotic gene transcription, certain newer insights can be pointed out. Although the failure to detect a receptor for PB does not rule out the existence of such a receptor, alternate modes of action can be considered. It is possible that the action of PB does not involve a PB-responsive *cis*-acting element. Instead, PB may be influencing the phosphorylation machinery. Since, several protein kinases and phosphatases have been reported, there is scope for some selectivity in this action of PB, which may involve a protein bound state of the drug. Thus, a receptor for PB, even if it were to exist, may be visualized to act at levels other than the PB-responsive element on the gene. It is also possible that the regulation of the phosphorylation machinery is degenerate and chemicals with apparent diverse structures may be influencing the activities of kinases and phosphatases. Thus, PB regulation of a set of genes can be visualized to be mediated by the phosphorylation status of the transcription factors regulating such genes, thus obviating the necessity to have specific PB-responsive elements in such genes. While these genes can be visualized as early responders, the ultimate global effect of an interference with the phosphorylation machinery may lead to hypertrophy, where several genes have to be activated so as to result in accelerated cell growth and increase in liver weight. Thus, PB action on liver genes can serve as a good model to enunciate a mechanism for the regulation of specific genes in a shorter timeframe and a global change leading to hypertrophy during longer and repeated treatment schedule. Since PB appears to have a direct effect on nuclei and nuclear extracts, the receptors/binding proteins, protein kinases and phosphatases in nuclei assume importance and have to be studied in detail.

Attempts are also being made in this laboratory to construct eukaryotic expression vectors using CYP2B1/B2 upstream sequences with a view to examine possible use in liver gene therapy.

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