Molecular Mechanisms of Regulation of Human Cytochrome P4501A2 Gene Expression

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Abstract – Cytochrome P4501A2 (CYP1A2) is responsible for the metabolic activation of a number of aromatic amines and amides to mutagenic and carcinogenic moieties. Considerable variations in the level of *CYP1A2* expression in humans have been reported. Thus, the level of human *CYP1A2* may determine an individuals susceptibility to these chemicals. Given its importance, the molecular mechanisms of *CYP1A2* regulation have been studied by many groups. Direct interactions between transcription factors with the promoters of the gene represent one of the primary means by which the expression of *CYP1A2* is controlled. In this review, several important *cis* elements, transcription factors and the effects of deacetylation/methylation of promoter regions that play an important role in the induction by PAHs as well as constitutive expression of human CYP1A2 are discussed.

Keywords – Cytochrome P4501A2, CYP1A2, gene expression, promoter, transcription facror

Introduction

Human being are constantly exposed to foreign che micals in drugs, foods and the environment. It is well established that chemicals can adversely affect human health. Many cancers are believed to be due to exposure to chemical carcinogens found in tobacco, food, the air, and water. Since foreign chemicals are not soluble in water in general, most metabolism of foreign chemicals serve to detoxify or accelerate their elimination from the body. However, some chemicals that are inert can be converted into electrophilic metabolites by cytochromes P450. These electrophilic substrates covalently attack nucleophilic sites on macromolecules (Gonzalez and Gelboin, 1993).

Today, it is recognized that the cytochrome P450 group comprises a superfamily. Completion of both the mouse and human genome sequences in the private and public sectors has prompted comparison between the two species at multiple levels. There are 102 putatively functional genes and 88 pseudogenes in the mouse, and 57 putatively functional genes and 58 pseudogenes in the human (Nelson *et al.*, 2004). The cytochrome P450 family consists of constitutively expressed enzymes and those that require prior exposure of the animal to an inducer. Among them, the cytochrome P450 1 family includes three members, cytochrome P4501A1 (CYP1A1)[1], P4501A2 (CYP1A2) and P4501B1 (CYP1B1). In this presentation, attention will be paid to the human CYP1A2.

Enzyme Induction by Polycyclic Aromatic Hydrocarbons (PAHs)

Administration to animals to PAHs, such as 3-m ethylcholanthrane (3MC) and benzo(a)pyrene [B(a)P], and dioxins, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), leads to an induction mostly of 1A subfamily among cytochrome P450s, *i.e.*, CYP1A1 and CYP1A2 (Thomas *et al.*, 1983; Morville *et al.*, 1983). Previously in Dr. Bresnick's laboratory, Raval *et al.* (1991) showed that under basal conditions, there are less than 3 and 6 molecules of CYP1A1 and CYP1A2 mRNA, respectively, per rat liver cell. Induction by 3MC resulted in a 23-and 33-fold increase of CYP1A1 and CYP1A2 mRNA, respectively.

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[1]**Following abbreviations are used** – Ah, aromatic hy-drocarbon; B(a)P, benzo(a)pyrene; CYP1A1, cytochrome P4501A1 protein; *CYP1A1*, cytochrome P4501A1 gene; *CYP1A2*, cytochrome P4501A2 protein; CYP1A2, cytochrome P4501A2 gene; CYP1B1,cytochrome P4501B1; GNMT, glycine-N-meth yltransfe rase; HNF-1, hepatocyte nuclear factor-1; 3MC, 3-Methy-lcho lanthrane; PAHs, polycyclic aromatic hydrocarbons; RT-PCR, reverse transcription-polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; USF, upstream stimulatory factor.

Under basal and induced conditions in rat liver, more CYP1A2 molecules are found per cell than CYP1A1.

The molecular mechanism for induction of CYP1A2 is not well understood. The accumulation of CYP1A2 mRNA after administration of the PAHs occurs by an increase in the transcription rate in the livers of mice (Gonzalez *et al.*, 1984; Okino *et al.*, 1992) and rats (Pasco *et al.*, 1993). However, a posttranscriptional mechanism has also been implicated in mouse (Kimura *et al.*, 1986) and rat livers (Silver and Krauter, 1988, 1990). In particular, 3MC may induce CYP1A2 by increasing the processing of precursor RNA in the rat (Silver and Krauter, 1990).

Substrates for CYP1A2-Relation to Carcinogenesis

It has long been known that metabolic activation of a number of xenobiotics including the PAHs is required prior to eliciting mutagenic, teratogenic or carcinogenic properties. This activation is conducted in part by cy tochrome P450 mediated pathways.

CYP1A2 is critical enzyme that is responsible for the detoxification of aflatoxin B1. Aflatoxin B1 is metabolized to aflatoxin M1, P1 and Q1, all of which represent detoxification products. The formation of aflatoxin M1 is accomplished by CYP1A2 (Koser *et al.*, 1988).

CYP1A2 also catalyzes the activation for carcinogenesis of certain heterocyclic amine pyrolysis products including Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, 2-amino-1-methyl-6-phenylinidazo[4,5-f]pyridine (PhIP), and particularly, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (Aoyma *et al.*, 1989). While IQ has been known to induce liver cancer in monkeys (Adamson *et al.*, 1990), PhIP produces colon and mammary carcinomas in rats and lymphomas in mice. Thus, CYP1A2 can catalyze reactions that both contribute positively to the carcinogenic potential of certain chemicals and to the detoxification of other carcinogens.

Recent results from the studies using knockout mice has brought controversies in CYP1A2-related chemical carcinogenesis. First of all, *CYP1A2* gene knockout have not caused any deleterious effect so that knockout mice were overly normal. In addition, there was no statistical differences between the wild-type mice and the CYP1A2 knockout mice in PhIP and 4-aminobiphenyl (4-ABP)-induced carcinogenesis when examined in the neonatal bioassay, suggesting that other P450s could participate in the carcinogenesis of arylamines and heterocyclic amines (Kimura *et al.*, 2003; Gonzalez and Kimura, 2003). Furthermore, despite the fact that *in vitro*

studies using Cyp1a2-null and wild-type mouse liver microsomes revealed that CYP1A2 is the major metabolic activation enzyme required for PhIP N2-hy droxylation, higher incidences of hepatocellular ad enoma were observed in Cyp1a2-null mice when examined in the neonatal *in vivo* bioassay. Based on these results, Kimura *et al.* (2003) have suggested that CYP1A2 may even be protective against all transformation, especially in females. Therefore, the role of CYP1A2 in carcinogenesis of certain chemicals *in vivo* remains for further study.

Polymorphism

CYP1A2 exhibits considerable inter-individual variation in human liver in terms of the levels of enzyme activity, protein and mRNA (Wrightin *et al.*, 1986; Sesardic *et al.*, 1998, 1990; Butler *et al.*, 1989; Kalow and Tang, 1991; Schweikl *et al.*, 1993). Since CYP1A2 are involved in the biological activation of procarcinogens, the mechanism responsible for the inter-individual differences in the amounts of enzyme is of interest.

In the study of Nakajima *et al.* (1999), the existence of a point mutation from guanine (wild type) to adenine (mutated type) at position −2964 in the 5'-flanking region of *CYP1A2* was uncovered. The genetic CYP1A2/A polymorphism was associated with the significantly reduced CYP1A2 enzyme activity in Japanese smokers. Gel retardation analysis showed the existence of protein bound to the polymorphic locus suggesting that this polymorphism could be a causal factor of decreased CYP1A2 inducibility.

The mutation at −2964 bp among populations was also examined for the risk of prostate cancer. Interestingly, the proportion of individuals with the mutant genotype was higher in prostate cancer patient, although it was not statistically significant (Murata *et al.*, 2001). This interesting observation, *i.e.*, individuals with a lower CYP1A2 activity might have an increased risk of prostate cancer, could be explained by the suspected protective effect of CYP1A2 against all transformation as indicated in the study of Kimura *et al.* (2003).

A C/A polymorphism in intron 1 of the *CYP1A2* gene at position 734 downstream of the first transcribed nucleotide was identified. A/A genotype was associated with increased CYP1A2 activity or high inducibility as measured by caffeine metabolism in Caucasian smokers (Sachse *et al.*, 1999).

Additional three polymorphisms in the 5' flanking region of *CYP1A2* was identified: a T-3591G substitution,

a G-3595T substitution, and a T-3605 insertion. The frequency of the T-3591G substitution was found to be significantly higher in Taiwanese compared to Caucasians or African Americans, although polymorphisms at these sites appeared to be functionally insignificant (Aitchison et al., 2000).

Regulation of *CYP1A2* Gene Expression

Tissue specificity – While CYP1A1 has generally been reported to be low or undetectable in non-induced human liver, it is induced in extrahepatic tissues of smokers. In contrast, expression of *CYP1A2* appears to be restricted to the liver where it is expressed constitutively in the human (Song et al., 1985; Sesardic et al., 1990; Pasanen et al., 1990; McKinnon et al., 1991).

In animals, similar liver-specific expression of *CYP1A2* was also reported by many groups. Tuteja et al. (1985) found that induction of CYP1A1 by 3MC readily occurred in liver, kidney, lung, and intestine of C57BL/6N mice, whereas CYP1A2 induction was substantial only in liver. Later, Limura et al. (1986) reported the elevation by TCDD of both CYP1A1 and CYP1A2 mRNA to some extent in mouse liver, kidney, lung, spleen, small intestine and large intestine; however, CYP1A2 mRNA was much more responsive than CYP1A1 mRNA to induction by low doses of TCDD in liver, kidney and lung.

Similar studies in the rat by Pasco et al. (1998) indicated that mRNA for CYP1A1 was readily induced by PAHs in liver, lung, kidney and heart, whereas mRNA for CYP1A2 was detected only in liver. The CYP1A2 gene in kidney was transcribed at a high rate, but mature mRNA did not accumulate because of some tissue-specific alteration in post-transcriptional processing. Although CYP1A2 is characterized by its restriction to hepatic tissue, several reports have suggested its occurrence in the rodent olfactory mucosa (Ding et al., 1986; Ding and Coon, 1990; Gillner et al., 1987) and human lung (Wei et al., 2001) as well.

Developmental regulation – Altered induction and constitutive expression of the *CYP1A2* has been reported as associated with development in rabbit (Pineau et al., 1991) and rat (Horbach et al., 1990). Similar de-velopmental changes were demonstrated in human liver by Ratanasavanh et al. (1991). CYP1A2 was very low in fetal liver and its presence was detected only several weeks or months after birth as judged by immuno-histochemical and immunoblot analyses. Hakkola et al. (1996) have reported the expression of CYP1A2 as detected by reverse transcription-polymerase chain reaction (RT-PCR) in human placenta as

early as in first trimester of pregnancy. However, neither enzyme activity nor protein was detected.

Tobacco smoking – Smoking has been reported to induce CYP1A2 mRNA and enzyme activity in human liver (Sesardic et al., 1990; Sherson et al., 1992; Vistisen et al., 1992; Ilett et al., 1993). Moreover, epidemiological evidence exists that cigarette smoking reduces the risk of primary hepatoma in individuals from an aflatoxin-endemic region of China, indicating that induction of CYP1A2 might decrease the car cinogenic potential of aflatoxin B1, as observed pr eviously in animal studies (Lin et al., 1991). However, other laboratories have reported considerable variation in protein level and enzyme activity among smokers and nonsmokers (Wrighton et al., 1986; Kalow and Tang, 1991; Schweikl et al., 1993). For example, Schweikl et al. (1993) reported that although the liver of one smoking subject co ntained by far the highest amounts of both CYP1A1 and CYP1A2 mRNA, the mRNA levels in two other smokers were in the range of the nonsmokers. Therefore, smoking history did not completely account for the observed variability.

Other factors – Many other factors also affect the expression and activity of CYP1A2. Pregnancy and oral contraceptives reduce CYP1A2 activity in the human as measured by a caffeine metabolite ratio (Kalow and Tang, 1991; Vistisen et al., 1992). In the same study, vigorous exercise and broccoli ingestion resulted in an increased CYP1A2 activity with minimal gender-related diffe-rences. A study by Relling et al. (1992) showed reduced activities in females and in blacks compared with males and whites, respectively, indicating gender and racial differences in CYP1A2 activity. Yet, no variation in activity was associated with race in a study comparing Chinese and Europeans, nor was there a variation associated with gender in the European group (Kalow and Tang, 1991).

One representative drug that alters CYP1A2 in the human is omeprazole, an inhibitor of gastric acid secretion. In primary cultures of human hepatocytes and liver microsomes from patients, omeprazole produced a time- and concentration-dependent increase in CYP1A2 protein accumulation, CYP1A2-related enzyme activities, CYP1A2 de novo synthesis (Diaz et al., 1990).

Malaria infection and fever reduced CYP1A2 activity in the rat (Kokwaro et al., 1993). Insulinoma-bearing rats displayed high CYP1A2 activity, and the administration of insulin to normal rats enhanced this enzyme activity (Barnett et al., 1992). Imaoka et al. (1990) has also shown a reduction in CYP1A2 by star vation in rat.

Molecular Mechanisms of Regulation of CYP1A2 Gene Expression

In contrast to CYP1A1, the detailed molecular mechanism underlying the induction of CYP1A2 is less understood. Part of this problem had been related to the absence of stable cell culture model system in which induction of this gene by PAH or dioxin can be demonstrated, although some success has been achieved in inducing CYP1A2 mRNA and protein in primary rodent hepatocytes (Pasco et al., 1988; Silver and Krauter, 1988; Sinclair et al., 19990; Padgham et al., 1992; Nemoto and Sakurai, 1993).

The feasibility of using HepG2 human hepatoma cells to study the regulation of CYP1A2 expression was suggested by Chung and Bresnick (1994). The RT-PCR assay revealed that HepG2 cells constitutively express *CYP1A2* and are able to respond to 3MC by an induction of CYP1A2 mRNA. The CYP1A2 steady-state mRNA level increased to a maximum of 12-fold at 24 h after exposure of the cells to 1 µM 3MC. Both a reduction in basal expression as well as an increased accumulation of CYP1A2 mRNA appeared responsible for the overall induction at 24 and 48 h. The transient changes in basal expression appear to be related to enrichment with fresh medium containing 10% fetal bovine serum.

Receptor-mediated induction by polycyclic hydrocarbons : Ah receptor vs 4s PAH-binding protein – A receptor-mediated pathway for CYP1A1 induction has been demonstrated as a proceeding through the Ah (or dioxin) receptor or the 4S PAH-binding protein. A model for the induction of CYP1A by PAHs and dioxins is depicted in Fig. 1.
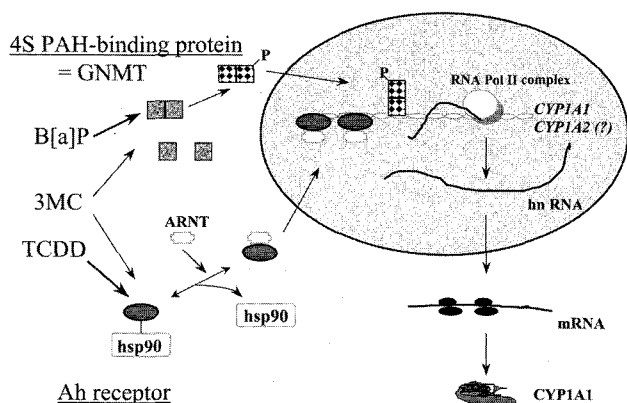

Fig. 1. Model for the induction of CYP1A1 and CYP1A2 by polycyclic hydrocarbons and dioxins. PAHBP, polycyclic aromatic hydrocarbon-binding protein; ARNT, Ah receptor nuclear translocator; hnRNA, heteronuclear RNA; GNMT, glycine-N-methyltransferase.

Ah receptor - The aromatic hydrocarbon (Ah) receptor binds a number of environmental pollutants, *i.e.*, some of PAHs, heterocyclic amines and polychlorinated aromatic compounds. The most potent ligand for this receptor is TCDD.

Once the ligand binds the Ah receptor that is complexed with hsp90, the hsp90 dissociates from the complex to allow the binding of the ARNT to the ligand-bound Ah receptor. The ligand-bound Ah receptor/ARNT complex undergoes a "transformation" into the nucleus. This heterodimer, in turn, acts as a transcription factor for the xenobiotic response element (XRE), resulting in activation of CYP1A1 transcription (Whitlock, 1990; Landers and Bunce, 1991; Hoffman et al., 1991; Reyes et al., 1992; Burbach et al., 1992)

4S PAH-binding protein – 4S PAH-Binding protein, that is distinct from the Ah receptor has also been implicated as a receptor that is responsible for PAH-mediated CYP1A1 induction. The 4S protein was reported to interact with high affinity and in a saturable manner with certain PAHs such as 3MC, B(a)P and B(e)P. The binding of radiolabeled B(a)P to the 4S protein was competed of by the addition of unlabeled B(a)P, B(e)P, and 3MC but not by TCDD, indicating that TCDD is not a ligand for the 4S protein. In additional studies, 3MC partially competed against the 8S Ah receptor-TCDD interaction suggesting that 3MC is a ligand for both receptors. The 4S protein has been purified to homogeneity and a partial sequence of the 33KD protein revealed its identity as glycine-N-methyltransferase (GNMT). Introduction of polyclonal antibodies to GNMT and antisense mRNA against GNMT into rat hepatoma H4IIE cells caused an inhibition of the PAH-madiated induction, but not of the TCDD-mediated induction of EROD, a CYP1A1-mediated enzyme activity, suggesting two in dependent mechanisms for CYP1A1 induction (Raha et al., 1990, 1994, 1995; Sterling et al., 1994).

Induction of CYP1A2 by PHs – While HepG2 cells have been reported to contain the necessary machinery that mediates the induction of CYP1A1 by PHs, e.g., the Ah receptor and 4S PAH-binding protein, it has not been clear whether such receptors are responsible for the induction of CYP1A2. A DNA sequence approximating a XRE, CACGC, a region to which the Ah receptor binds, is found in the 5'-flanking region of the human and rabbit CYP1A2.

The results of transient expression experiments revealed that the 5'-flanking region from −3203 to +58 bp of human CYP1A2 is only weakly responsive to 1 µM of 3MC regardless of the presence of one XRE at −2903 bp

(Chung and Bresnick, 1995; Chung, 2000). Similar observations, *i.e.*, the XRE of nonfunctional as determined by transfection experiments with promoter-reporter gene fusion constructs in HepG2 cells, were also made with the rabbit and human *CYP1A2* promoter regions (Postlind *et al.*, 1993).

3MC at higher concentration, *e.g.* 10 µM, appeared to increase the CYP1A2 transcripts in part through the 5'-flanking regulatory region, probably mediated by the sequences termed X1 and X2, *i.e.*, from −2505 to −2487 bp and from −2160 to −2142 bp, respectively, as reported by Quattrochi *et al.* (1994). These observations indicated that 3MC at higher concentration can induce CYP1A2 *via* transcriptional initiation as shown for CYP1A1. However, in same study, transfection experiments showed only 2 to 3 fold induction of human CYP1A2 promoter activity in HepG2 cells by 1 µM 3MC (Quattrochi *et al.*, 1994). This small enhancement of promoter activity cannot explain the observations made in HepG2 cells (Chung and Bresnick, 1994), *i.e.*, 12 to 14 fold-enhanced CYP1A2 mRNA from 1 µM 3MC treated HepG2 cells compared with vehicle treated cells. Additional studies are needed to fully establish the mechanisms by which 3MC upregulates CYP1A2 expression.

The refractory responsiveness of the *CYP1A2* promoter to 3MC treatment on HepG2 cells may have been explained by Fisher *et al.* (1990) who suggested that differences in the number of Ah receptor binding sites and the distance between these sites would determine the level of induction of a gene by polycyclic hydrocarbons. In contrast to *CYP1A2*, *CYP1A1* 5'-flanking regions contain the clustered Ah receptor recognition sites (Nebert and Jones, 1989). Furthermore, the mouse *CYP1A1* promoter contains an additional GC box between two XREs that can augment the enhancer function once the Ah receptor binds to XREs (Fisher *et al.*, 1990).

Additional putative XRE sites of CYP1A cluster were identified by sequence analysis after isolation a human genomic clone (Corchero *et al.*, 2001). The *CYP1A1* and *CYP1A2* genes are separated by a 23 kb segments that contains no other reading frames. The *CYP1A1* and *CYP1A2* are in opposite orientation, indicating that the 5' flanking region is in common between the two genes. It further suggest that some of the regulatory elements known to control *CYP1A1* expression, could also control *CYP1A2* expression (Corchero *et al.*, 2001). However, the 5' upstream region of human *CYP1A1* contains at least seven XREs within the first 1300 bp, whereas only one XRE is located at −2903 of the *CYP1A2* 5' flanking region. Therefore putative XREs beyond −3000 bp of 5'

flanking region of *CYP1A2* remain to be proved as functional sites.

HNF-1 (hepatocyte nuclear factor-1) – HNF-1 is a transcription factor that contributes to the liver-specific expression of several genes. HNF-1 binds to the palindromic consensus sequence GTTAATNATTAAC (Mendel and Crabtree, 1991) as a dimer, although one moiety of the palindrome appears more conserved than the other. The difference in the two regions of the palindrome may contribute to altering the affinity of HNF-1 for its binding sites or allowing HNF-1 to bind as a heterodimer (Tronche and Yaniv, 1992).

An enhancer-like positive regulatory element within a 259-bp sequence (−2352 to −2094 bp) of the human *CYP1A2* was identified. Three protein binding sites were identified by DNase I footprinting analyses within the 259 bp sequence: protected region A, PRA (−2283 to −2243 bp), PRB (−2218 to −2187 bp), and PRC (−2124 to −2098 bp) (Chung and Bresnick, 1995). Transfection experiments defined the PRB and PRC as containing positive and negative regulatory elements, respectively. HNF-1, which contributes to the liver specificity of genes, enhanced reporter gene activity in a PRC sequence-dependent manner in human breast carcinoma MCF-7 cells. The results showed considerable complexity of CYP1A2 gene regulation. PRC could exist bound to a repressor which was displaceable by other transcription factors such as HNF-1 (Chung and Bresnick, 1997).

Cheung *et al.* (2003) examined a potential role for HNF-1 in the regulation of major CYP genes in the livers of mice lacking HNF-1. The expression of Cyp1a2 was expressed at markedly lower levels in the livers of HNF-1-deficient mice as shown by Northern blot analysis. There was also a corresponding decrease in the level of CYP1A2 protein. A decrease in CYP1A2 mRNA level is consistent with the results of other study (Chung and Bresnick, 1997) showing that HNF-1 may act as a positive regulator of *CYP1A2* expression through specific HNF-1 binding sites contained in its promoters.

AP-1 – The AP-1 transcription factors are composed of Jun and Fos proteins that function as transcriptional regulators in a heterodimeric complex (Lee *et al.*, 1987; Angel and Karin, 1991). The AP-1 consensus sequence, TGA(C/G)TCA, was originally identified as an activator element which binds transcription factor AP-1, a homodimer of product of protooncogene c-jun or a heterodimer of the products of protooncogenes c-jun and c-fos (Lamph *et al.*, 1988). The activation of protein kinase C by phorbol ester such as TPA increases the DNA-binding activity of AP-1 and induces the transcription of AP-1

responsive genes (Imbra and Karin, 1987).

Chung and Bresnick (1997) have previously reported on the importance of a *cis* element named PRB in the regulation of human *CYP1A2*. It appeared that this element acts as a positive regulatory element. Close examination of the PRB sequence (−2218 to −2187 bp) revealed a putative AP-1 binding site, TGACTAA, at −2212 bp. Cotransfection of c-Jun and c-Fos expression vectors induced transactivation by five to six fold from CYP1A2 promoter constructs. However, deletion of PRB element did not affect the degree of activation by c-Jun and c-Fos. Thus, it is not likely that c-Jun and c-Fos activates the CYP1A2 promoter through this AP-1 consensus-like sequence in PRB region (Chung and Jung, 2002).

Quattrochi *et al.* (1998) reported on two AP-1 sites which were named as 5'AP-1 and 3'AP-1. 5'AP-1 and 3'AP-1 sites reside at −2212 bp and −2029 bp of human CYP1A2 promoter, respectively. 5'AP-1 was the same site as the AP-1 sequence within PRB. It appeared that members of the AP-1 family of transcription factors bind to both AP-1 sites. TPA treatment increases the binding of c-Jun, JunD, and c-Fos to theses sites.

First of all, reporter gene assay with 3'AP-1 containing construct displayed an six fold induction of reporter gene activity by TPA treatment, which indicates 3'AP-1 as functional site.

In regard to the putative AP-1 site at −2212 bp in PRB or 5'AP-1, few observations has been made as follows; (1) reduction of constitutive promoter activity by mutation (Chung and Bresnick, 1997), (2) unresponsiveness to the c-Jun and c-Fos as determined by reporter gene assay (Chung and Jung, 2002), (3) reduced transactivation (from 5.9 fold to 2.8 fold) by TPA when this sequence was included in reporter gene assay (Quattrochi *et al.*, 1998) and (4) abundant binding of other nuclear factors in addition of AP-1 family of proteins to this site (Quattrochi *et al.*, 1998). These results suggest that further characterization of the transcription factors for this *cis* element is required for a proper understanding of human CYP1A2 regulation.

The candidate of another AP-1 site would be TGCCTGA at −2333 bp of 3'strand of CYP1A2, which remains to be analyzed for its functionality.

USF (upstream stimulatory factor) − The E-box consensus sequence, CANNTG, is recognized by members of the basic helix-loo-helix (bHLH) family of tran-scription factors such as EsA, E2-2, HEB, MyoD, Myc, USF and ARNT (Massari *et al.*, 2000). The E-box at −2202 bp of human *CYP1A2* 5' regulatory region could interact with USF-1 and USF-2 transcription factor. Site-directed

mutagenesis of this site resulted in a 60% reduction in constitutive expression of reporter gene activity. Cotransfection of USF-1 or USF-2 expression vector activated *CYP1A2* promoter activity. These results support the role of USF as a constitutive transcriptional activator of human *CYP1A2* (Pickwell *et al.*, 2003).

Methylation − DNA methylation is involved in the control of gene expression. This epigenetic modification occurs largely in the dinucleotide sequence CpG that is often located in the 5'-flaking region of the gene. Methylation of critical cytosines can decrease the affinity between tran scriptional factors and DNA leading to the suppression of gene expression (Siegfried and Cedar, 1997).

Hammons *et al.* (2001) assessed the methylation status of the CCGG site (bp −2759) located in the 5'-flanking region of the *CYP1A2* in liver samples from a pool of 55 human donors. Results showed that the methylation state can vary among individuals and that hypermethylation at this site appears to be associated with reduced CYP1A2 expression.

Similar observation has been reported by Nakajima *et al.* (2003). The treatment of a human breast MCF7 cells with DNA methylase inhibitor, AzaC, increased the constitutive expression of *CYP1A2*. However, the same treatment did not affect the levels of induction by TCDD. In a human cervical carcinoma HeLa cells, suppression of DNA methylation by AzaC increased the constitutive expression as well as induction of CYP1A2 by TCDD.

Histon acetylation − Histone acetylation and deace-tylation constitute a carefully controlled and highly specific regulatory process that leads to the activation and repression of some genes (Wu, 1997).

The degree of histone acetylation in the promoter regions appears to affect the promoter activity by mediating changes in the nucleosomal and chromatin structure of these regions, presumably affecting the accessibility of transcription factors to their *cis* regulatory elements (Wu, 1997).

According to Nakajima *et al.* (2003), while the treatment of a human breast MCF7 cells with trichostatin (TSA), an inhibitor of histon deacetylase, caused the increasement of constitutive expression of *CYP1A2*, the same treatment rather decreased the level of induction of *CYP1A2* by TCDD. In HeLa cells, the inhibition of histon deacetylation increased the constitutive expression as well as induction of *CYP1A2* by TCDD.

Summary

Fig. 2 shows a possible model by which human *CYP1A2* gene expression might be regulated based on the
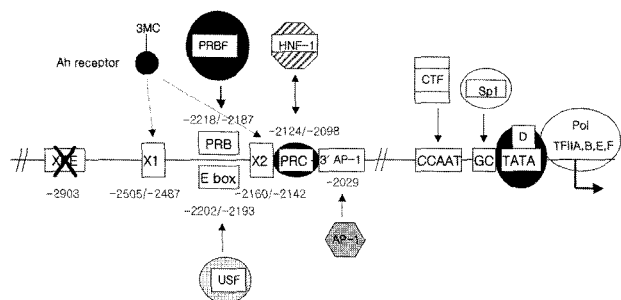
Fig. 2. Proposed model of DNA-regulatory protein interactions in the human *CYP1A2* promoter (see text for detail).

reported results. The human *CYP1A2* contains several functionally important *cis* elements in the 5'-flanking region, *i.e.*, the proximal 42 bp sequence that has GC and CAAT boxes near the TF II D binding site, a TATA box as in many other eukaryotic promoters. The well-characterized Sp1 and CTF/NF1 transcription factors would bind to the GC and CAAT boxes, respectively, and stimulate transcription by stabilizing the basic transcriptional assembly of RNA polymerase II and initiation factors (grouped together in Fig. 2) via coactivators that are associated with TBP as a part of the TF II D complex. The coactivators which function as adaptors are different from the basic transcription initiating factors.

An enhancer-like element termed PRB at −2218 to −2178 bp, and a composite sequence termed PRC at −2124 to −2094 bp would be closely involved in the regulation of *CYP1A2*. The transcription factors for these elements need to be further defined. PRB would be a recognition site for PRB-binding factor(s) (PRBF). PRBFs appears not to be c-Jun/c-Fos. PRC shows the complexity of transcriptional regulation. PRC would be occupied by a repressor until positive-acting factors such as HNF-1 would replace this suppression. As with most tran-scription factors, the concentrations of available negative and positive acting factors in the cells would determine the role of PRC. At least a functional site for AP-1 transcription factor resides at −2029 bp. TPA would increase CYP1A2 expression *via* AP-1 site. USF could bind to the E-box at −2202 to −2193 bp and increase the transcription of *CYP1A2*. In regard to 3MC-mediated induction, the XRE located at −2903 bp appears to be nonfunctional at least in HepG2 cells. 3MC at higher concentration, e.g. 10 µM, would in crease the CYP1A2 transcripts in part through the 5'-flanking regulatory region, probably mediated by the sequences termed XRE-like sequence X1 and X2, *i.e.*, from −2505 to −2487 bp and from −2160 to −2142 bp, respectively.

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