Transcriptional Activation of the UDP-Glucuronosyltransferase IA7 Gene in Rat Liver by Aryl Hydrocarbon Receptor Ligands and Oltipraz*

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UDP-glucuronosyltransferase UGT1A7 catalyzes the glucuronidation of benzo(a)pyrene metabolites and other bulky aromatic compounds. Both UGT1A7 mRNA and an associated enzyme activity (benzo(a)pyrene-7,8-dihydrodioltransferase activity) are markedly increased in livers of rats treated with β-naphthoflavone or 4-methyl-5-pyrazinyl-3H-1,2-dithiole-3-thione (oltipraz). Nuclear runoff assays show that the effects of both inducers are primarily due to transcriptional activation. A 27-kilobase region that included the UGT1A7 UGT1A6 promoter regions was cloned. Primer extension and RNase protection studies indicated ≥30 transcription start sites in five clusters between bases −85 and −40 respective to the translation start codon. There was no recognizable TATA box, but the promoter region is TA-rich. Sequence analysis revealed potential binding sites for CCAAT enhancer-binding protein, activator protein 1, and hepatic nuclear factors 1, 3, and 4, but no xenobiotic response elements or antioxidant response elements, implicated in the regulation of other genes by β-naphthoflavone or oltipraz, were found. A UGT1A7 gene reporter plasmid directed strong constitutive expression in transient transfection assays using primary rat hepatocytes. Treatment with 3-methylcholanthrene or oltipraz had no effect compared with similarly treated pGL3-Basic-transfected cells. These results suggest that the regulatory elements controlling xenobiotic inducibility of UGT1A7 transcription are located either 5′ or 3′ of bases −1600 to +54. One possibility is that the polycyclic aromatic-mediated regulation of UGT1A7 occurs via the xenobiotic response element flanking the UGT1A6 locus 7 kilobase pairs downstream.

UDP-Glucuronosyltransferases (UGTs)1 (EC 2.4.1.17) catalyze the reaction of UDP-glucuronic acid with substrates bearing a variety of different functional groups, including OH, NH2, SH2, and COOH. Because the glucurononides are usually less biologically active, more polar, and readily excreted from organisms, the UGTs are considered important for the general detoxification and elimination of a host of endogenous and exogenous substances, such as bilirubin, steroids, environmental pollutants, carcinogens, and dietary substances. Molecular cloning evidence indicates the existence of two large families of UGTs, UGT1 and UGT2 (1). UGT1 family members are encoded by a unique gene complex, UGT1, which utilizes exon sharing to generate ≥12 isozyme forms with homologous but unique amino-terminal (∼287 amino acids) and identical carboxy-terminal sequences (245 amino acids) (2, 3). UGT2 family members, in contrast, appear to be encoded by independent genes. Each UGT exhibits its own unique profile of substrate and tissue specificity and regulation by exposure to endogenous or xenobiotic compounds (1).

A recent focus of our studies has been the UGT isozymes responsible for the glucuronidating activity of rat liver microsomes toward benzo(a)pyrene metabolites. Benzo(a)pyrene is a prototype of the polycyclic aromatic hydrocarbons (PAHs), a class of carcinogens introduced into the environment from the partial combustion of organic material (e.g. tobacco). Although metabolism is a fundamental requirement for their carcinogenicity (reviewed in Ref. 4), their mutagenic properties are also subject to the activity of detoxification enzymes, such as the UGTs (5–7). Using benzo(a)pyrene 7,8-dihydrodiol as substrate, we observed a marked increase in UGT activity in liver microsomes from rats treated with β-naphthoflavone or oltipraz (4-methyl-5-pyrazinyl-3H-1,2-dithiole-3-thione (8)). A cDNA (clone LC14) was isolated from an adult rat hepatocyte-derived cell line encoding a UGT active in the glucuronidation of 14 different benzo(a)pyrene metabolites, including 7,8-dihydropyrene (9). Sequencing of LC14 revealed identity with the product encoded by the UGT1A7 locus (3). UGT1A7 mRNA is markedly increased in rat liver by PAHs and oltipraz. An identical pattern of mRNA regulation is observed for UGT1A6 (3, 9, 11, 12), the better known PAH-inducible UGT with activity toward 4-nitrophenol and other simple phenols but not benzo(a)pyrene phenols (13, 14). Because PAHs and oltipraz are known to induce transcription of other metabolizing enzymes by mechanisms involving the arylhydrocarbon receptor and/or oxidative stress (15–17), our findings suggest that UGT1A6 and UGT1A7 may each be under the control of cis elements implicated in these responses, i.e. the xenobiotoxic response element (XRE) for arylhydrocarbon receptor mediated effects and the antioxidant response element (ARE) for oxidative stress-associated effects.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession numbers AF059227.

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1 The abbreviations used are: UGT, UDP-glucuronosyltransferase; PAH, polycyclic aromatic hydrocarbon; ARE, antioxidant response element; XRE, xenobiotic response element; PIPES, 1,4-piperazinediethanesulfonic acid; bp, base pair(s).

2 LC14 is encoded by a transcription unit beginning with the seventh unique amino-terminal-coding exon upstream of the shared carboxyl-terminal-coding exons in the UGT1 gene complex; it is therefore named UGT1A7. For an update of the current UGT nomenclature, see Ref. 10.
To characterize the mechanism of PAH inducibility of rat UGT1A6, Emi et al. (18) have cloned and characterized the UGT1A6 gene promoter. The structure of the transcription unit was unusual in that it was composed of six rather than the five exons that are typical of other UGT1 transcription units (2, 3). A putative XRE with the core sequence characteristic of all known XREs, 5′-GCCGTG-3′, was identified between −134 and −129 upstream from the transcriptional start site. This sequence was shown to be a functional XRE, mediating 9−15-fold inducibility of a chloramphenicol acetyltransferase reporter enzyme. These observations suggest that this particular sequence motif underlies the in vivo regulation of UGT1A6 by PAHs.

In this paper, we describe a study aimed at characterizing the mechanism of the increase in steady state UGT1A7 mRNA level by PAH and oltipraz. Nuclear runoff data indicate that increased gene transcription is the primary mechanism of mRNA accumulation in response to these agents. Characterization and sequencing of the UGT1A7 promoter and 5′-flanking region indicated several differences from UGT1A6, including its basic exon composition, the existence of >30 transcription start points, and the absence of any functional XRE or AREs in its immediate 5′ flanking sequence.

**EXPERIMENTAL PROCEDURES**

**Materials**—Rats used in this study were male Sprague-Dawley rats purchased from Harlan Laboratories (Indianapolis, IN). Animal handling and experimentation for this study were carried out with the approval of the Institutional Animal Care and Use Committee at Virginia Commonwealth University. β-Naphthoflavone and 3-methylcholanthrene were from Aldrich and Sigma, respectively. Oltipraz was from the Chemoprevention Branch, National Cancer Institute (Bethesda, MD). The rat genomic DNA library in (bacteriophage EMBL3 SP6-T7) was from CLONTECH (Palo Alto, CA). Custom oligonucleotides used in this study for primer extension and gene sequencing were synthesized by the Nucleic Acid Synthesis and Purification Core Resource of the Massey Cancer Center (Virginia Commonwealth University). Cultures of primary rat hepatocytes were prepared by the Hepatocyte Isolation and Preservation Core Facility of the Liver Center at the Medical College of Virginia (Virginia Commonwealth University). Lipofectin™ cationic liposomes and Luciferase Assay System™ were purchased from Lipofectamine, Inc. and Promega (Madison, WI), respectively. Lipofectin™ and Promega (Madison, WI) supplies (5×) were reconstituted and stored in aliquots of 200 μl.

**Plasmids**—prUGT1.6 contains a 783-bp fragment from the 5′ end (bases +28 to +810) of the UGT1A6 cDNA (8). The plasmid p1.XB contains a 1499-bp XhoI-BamHI fragment from the extreme 5′ end of the bacteriophage clone ARPT-6 inserted into the XhoI and BamHI sites of pSP7. The plasmid p2.XB contains the 5′ XhoI-BamHI fragment of ARPT-6 cloned into XhoI-BamHI cut pSP7. pCL14 (+9/+697) corresponds to a 690-bp fragment from the 5′ untranslated region (untranslated region) (8). The library was rescreened using the pLC14 into the 5′-flanking region of UGT1A6 and characterized by sequencing.

**RNA Isolation and Primer Extension Analysis**—Total RNA was isolated from liver (10) from livers of rats receiving either vehicle alone (1 ml/kg 30% polyethylene glycol-1450 (v/v) for 3 days), oltipraz (90 mg/ml, 300 mg/kg/day for 3 days), or β-naphthoflavone dissolved in corn oil (12 mg/ml, 80 mg/kg/day for 3 days). Poly(A)+ RNA was extracted by cellulose poly(dT) affinity chromatography (Boehringer Mannheim). Two non-overlapping UGT1A7 antisenes primers (6.375 and 6.376) were end-labeled with 32P using [γ-32P]ATP and T4 bacteriophage polynucleotide kinase to <1×10^6 dpm/μg and purified by centrifugation through Chroma Spin columns (CLONTECH). Primers (→1×10^6 dpm/μg) were hybridized to poly(A)+ RNA (10 μg) from each group for 16 h at 42 °C in 40 μl RNASE, 1 μl EDTA, 0.4 μg NaCl, and 40% formamide. The primer-anneled RNA was then precipitated and dissolved in 1 μl of deionized water prior to extension using AMV-reverse transcriptase (Promega). Primers from the plasmid contained 1× AMV-reverse transcriptase buffer (Promega) 0.2 μl 5′-terminal nucleotide (dNTP, RNasin (20 units, Promega), and 0.5 μg of actinomycin D. The products were analyzed on a 7 M urea, 8% polyacrylamide sequencing gel.

**RiNase Protection**—A 3′-labeled riboprobe corresponding to the antisenes strand of the sequence between the XhoI and PstI sites (positions −337 and +12 with respect to the UGT1A7 translation start) was synthesized in a reaction containing NotI-linearized p340XP (0.25 μg), 1× riboprobe synthesis buffer (supplied by Promega), 10 μg dithiothreitol, RNAsin (20 units), ATP/UTP/UTP (3 μM each), [α-32P]CTP (50 μCi, 12 μM), and T7 RNA polymerase (20 units, Promega) in a total volume of 20 μl for 60 min at 37 °C.

The reaction was treated with RNase (2 units, Promega) for 30 min, and the riboprobe was purified by centrifugation through a spin column (5′→3′, Inc., Boulder, CO). Riboprobe (5×10^6 cpm) was mixed with yeast tRNA, control rat liver RNA, or oltipraz-treated rat liver RNA (10 or 50 μg) in 50 μl containing 40 μl RNASE, 1 μl EDTA, 0.4 μg NaCl, and 40 or 80% formamide. The samples were treated with 300 μl of RNase (300 μl RNase, 10 μM Tris–HCl, pH 7.4, 5 μM EDTA, 2 μg/ml RNase T1, 40 μg/ml RNase A) for 30 min at 37 °C. Following proteinase K digestion, RNA was precipitated and dissolved in 1 μl of 0.9% saline and redissolved in 3-g samples were mixed into small pieces with scissors in 3 volumes of 0.3 M sucrose in Buffer A (15 μl Tris–HCl, pH 7.4, 15 μM NaCl, 60 μM KCl, 2 mM EDTA, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride). After a brief homogenization using a Polytron homogenizer (set on low speed), samples were completely homogenized in 50-ml Potter-Elvehjem glass homogenizing tubes with a drill-driven Teflon pestle (three passes), and nuclei were then isolated by sucrose gradient centrifugation as described. The final nuclear pellet was suspended in 50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, and 0.4% glycerol, and stored in aliquots of 200 μl at −70 °C. Nuclei concentrations were between 40 and 60% nuclei/200 μl. For nuclear runoff and nuclear runoff assays, nuclei were thawed, mixed with 50 μl of a 5× buffer containing 25 mM Tris–Cl, pH 7.5, 12.5 mM MgCl2, 0.75 mM KCl, 12.5 mM dithiothreitol, 2.5 mM each of ATP, CTP, and GTP, 100 μCi [32P]UTP (800 Ci/mmol), and incubated at room temperature for 15 min. RNase A (10 units) was added, and reactions were incubated for an additional 5 min. Yeast tRNA (50 μg/ml) and then added, and linear DNA was isolated by the method of Fedoroff and Drake (21). RNA (5 μg) was hybridized to Nytran membranes containing 5 μg of immobilized, XhoI-linearized pB-PA6, pHCYPIA1B5, pLC14 +9/+697, and pK7 +−7 in 10 μl of Tris-ethane sulfonic acid, pH 7.4, 10 mM EDTA, and 0.2% SDS for 48 h at 65 °C. Membranes were washed and exposed to film (7-day exposure at −80 °C with medium intensifying screen) and then to a Molecular Dynamics phosphorimaging cassette (1 day). Exposure was cut.
quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Northern Analyses—Northern analyses of total RNA isolated from liver tissue (0.3 g) or primary rat hepatocytes (8 × 10^6 cells/100-mm dish) was carried out as described (8). Probes were prepared from DNA inserts purified by low melting point agarose electrophoresis and labeled with [α-32P]dCTP by random priming (22).

Transient Transfection of Primary Rat Hepatocytes—Hepatocytes (8 × 10^6) isolated from collagenase-perfused livers of male Sprague-Dawley rats were plated on gelatin-coated plastic tissue culture dishes (35 mm), and 6 h later, the medium was removed and cells were washed once with serum-free medium (Williams E containing 0.6 μg/ml insulin, 100 μg/ml transferrin, 1 μg/ml dexamethasone, fungizone, and gentamycin). Lipofectin™ (7.5 μg/dish) mixed with reporter plasmid (1.5 μg) in 1.5 ml of serum-free medium was then added to the plate. After overnight (≥12 h) incubation in a humidified, 5% CO2 atmosphere, the medium was removed and replaced with fresh serum-free medium containing the inducing agents at the indicated concentration. Twenty-four h later, the medium was again removed and replaced with fresh inducer-containing medium. Cells were harvested at 48 h following the start of drug treatment. Luciferase activity was measured using the Luciferase Assay System (Promega) and a Berthold Luma LB9501 luminometer.

RESULTS

Effect of Oltipraz and β-Naphthoflavone on the Rate of UGT1A7 Transcription—To investigate the mechanism of the inducer-associated increases in liver UGT1A7 mRNA level, nuclear runoff assays were carried out using nuclei isolated from rats 12 h after treatment with vehicle (30% polyethylene glycol), 250 mg/kg oltipraz or 80 mg/kg β-napthoflavone. The Northern analysis of β-actin, cytchrome P4501A, and UGT1A7 transcripts at this time point is shown in Fig. 1A. Quantitation by phosphorimaging showed that oltipraz and β-naphthoflavone elevated the CYP1A mRNA 10- and 173-fold, respectively, and the UGT1A7 mRNA 19- and 43-fold. β-actin RNA levels were only slightly affected (2.5- and 2.8-fold for oltipraz and β-naphthoflavone, respectively).

A representative autoradiograph from a nuclear runoff assay (using nuclei from animals 2, 6, and 8) is shown in Fig. 1B. In the vehicle control, the rate of β-actin mRNA synthesis was the highest of the three mRNAs assayed and was not affected by either oltipraz or β-naphthoflavone treatment. In contrast, transcriptional rates of CYP1A and UGT1A7 were significantly increased by each agent. When signal intensities were corrected for background binding to vector alone, the oltipraz and β-naphthoflavone treatments produced increases of 4.4- and 29-fold for CYP1A1 and 3.7- and 8.4-fold for UGT1A7, respectively (Fig. 1C). Similar results were obtained in a second nuclear runoff analysis using nuclei from animals 3, 5, and 9. These results indicate that the increases in mRNA levels are due primarily to increased rates of mRNA synthesis.

Isolation and Sequencing of the LC14 Unique Exon and Identification as UGT1A7—To learn more about the mechanism(s), we isolated four genomic DNA clones from the UGT1A7/UGT1A6 locus using 5’ fragments of the UGT1A6 and/or LC14 cDNAs as probes as described under “Experimental Procedures.” The overlap relationships of these clones (designated ARPT6, ARPT12, ARPT19, and AVIP14) was determined by restriction site mapping ofHXol, BamHI, and EcoRI sites (Fig. 2A) together with Southern analysis. Two of the clones, ARPT6 and AVIP14, hybridized with both the UGT1A6 and LC14 probes. Together the cloned inserts form a contig of ~27 kb. A detailed restriction map is included in Fig. 2B to establish the identity of the DNA investigated in this study. In agreement with the report of Emi et al. (3), the UGT1A6-amino-terminal coding exon was localized to a 5.1-kbp internal EcoRI fragment of ARPT6 immediately 5’ of two small EcoRI fragments (0.5 and 0.9 kb). The UGT1A6* leader exon was localized by the presence of HindIII, PstI, and HindIII sites 2 kb 5’ of 0.1 kb 5’, and 0.3 kb 3’ of the EcoRI site in the exon, respectively (Fig. 2B).

The UGT1A7 exon was localized in clones ARPT6 and AVIP14 by Southern analysis with pLC14 (+9/+697) insert, which revealed an exon with an internal BamHI site mapping 1.4 and 2.0 kb from the termini of ARPT6 and AVIP14, respectively. Consistent with its identity as the LC14 origin, the exon also contained a PstI and a PacI site 0.26 and 0.44 kb, respectively, upstream of the BamHI site. To establish this conclusively, the 1.4-kb XhoI-BamHI fragment at the terminus of ARPT6 was cloned (p1.4X) and sequenced (Fig. 3; 1499 bp total). The 3’-486-bp sequence extending from the BamHI site were identical to the LC14 5’ end.

Determination of UGT1A7 Transcription Start Point—To define the UGT1A7 transcription unit, we carried out primer extension and RNase protection experiments. For primer ex-
tension, two antisense primers, 6.375 and 6.376, with an offset of 32 bases, were utilized for analysis of poly(A) RNA from the livers of vehicle-, oltipraz-, and β-naphthoflavone-treated rats. The results are presented in Fig. 4. As expected, the products were only visible in samples from inducer-treated animals, especially with β-naphthoflavone. In these samples, similar ladders of extension products with the expected length differential of 32 bases were seen. In both reactions, the longest...
product maps to a guanosine residue at position -85 from the
translation start codon. This residue is designated as base 1
in Fig. 3 and maps 11 bases upstream of the first base in one
of the cDNA clones isolated from RALA255–10G LCS-3 cDNA
library (clone LC11).

To determine whether the multiple bands corresponded to
multiple transcription start points or were due to artifacts in
the extension reaction, we carried out RNase protection anal-
ysis using a radiolabeled riboprobe corresponding to the region
between the XbaI and PstI sites at -337 and +12 with respect
to the start of translation. The results are shown in Fig. 5A. A
ladder of bands corresponding to more than 30 different prod-
ucts was observed in all the lanes with oltipraz-treated RNA
samples, whereas relatively few bands were detected in the
vehicle sample. Using a T3-primed pSK+ sequencing reaction
to estimate the 5' site of initiation, we observed a good correla-
tion between the data generated by primer extension analyses
and RNase protection (Fig. 5B) including bases where
transcription is initiated and their relative intensity (abundance).

FIG. 4. Primer extension analysis of UGT1A7 mRNA. Two
coding antisense primers (6.375 and 6.376) were end-
labeled with [γ-32P]ATP and used for primer extension analysis
of poly(A)+ RNA from control (lanes 1 and 4), oltipraz-treated (lanes 2 and
5), and β-naphthoflavone-treated (lanes 3 and 6) rats as described
under “Experimental Procedures.” The sequencing ladders shown
were generated using the indicated primers and p1.4XB as template.
The groups of products corresponding to each other and to the RNase
protection products in Fig. 5 are indicated (A–E).

FIG. 5. RNase protection analysis of UGT1A7 mRNA. A, RNase
protection analysis using an antisense riboprobe spanning from -337
(XbaI) to +12 (PstI) with respect to the start of translation was per-
formed as described under “Experimental Procedures.” The sequencing
ladder corresponds to the pSK+ multiple cloning region using T3 as
primer. B, correspondence between initiation sites predicted by primer
extension and RNase protection. Initiation sites predicted from primer
extension with primers 6.375 or 6.376 (data from lanes 3 and 6 of Fig.
4) and RNase protection were determined and assigned relative signal
intensities of 0 (not detectable), 1 (weak but detectable), 2 (intermedi-
ate), or 3 (strong). The plot shows the additive scores for each nucleotide
position (maximum score = 9).

primers -375 and 288 from the translation start codon. This
residue is designated as base 1 in Fig. 3 and maps 11 bases
upstream of the first base in one of the cDNA clones isolated
from RALA255–10G LCS-3 cDNA library (clone LC11).

To determine whether the multiple bands corresponded to
multiple transcription start points or were due to artifacts in
the extension reaction, we carried out RNase protection anal-
ysis using a radiolabeled riboprobe corresponding to the region
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to estimate the 5' site of initiation, we observed a good correla-
tion between the data generated by
UGT1A6 and UGT1A7 amino-terminal sequences, indicating a fairly distant evolutionary relationship. Similar to UGT1A6, however, UGT1A7 contains sequences closely matching known CCAAT enhancer-binding protein and hepatic nuclear factor 3-binding sites (Fig. 3).

**Induction of UGT1A7 and CYP1A mRNA in Primary Cultures of Rat Hepatocytes—** Analysis of the mechanism(s) of the inducer-mediated increases in UGT1A7 transcription requires an appropriate cellular model. Previous studies carried out with an immortalized SV40-transformed adult rat hepatocyte cell line (RALA LCS) indicated high constitutive UGT1A7 mRNA expression compared with in vivo expression. Fig. 6 compares the in vivo and in vitro responses to Ah receptor agonists and oltipraz at the level of mRNA. The low constitutive expression of UGT1A7 mRNA observed in vivo in livers of untreated rats (Fig. 6, lane 4) is maintained in untreated primary rat hepatocytes (lane 1). Treatment with aryl hydrocarbon receptor agonists and with oltipraz leads to a strong stimulation of UGT1A7 mRNA both in vivo (Fig. 6, lanes 6 and 5, respectively) and in primary hepatocytes (lanes 2 and 3, respectively). β-Actin mRNA was highly expressed both in vivo and in hepatocytes in vitro but was not affected by either treatment. Some qualitative differences were observed in the constitutive expression and inducibility of the CYP1A RNAs as detected using the human CYP1A probe. Compared with in vivo, oltipraz was not as effective at inducing the CYP1A1 and CYP1A2 mRNAs in primary hepatocytes in vitro. Overall, the data lend support for the usefulness of primary rat hepatocytes as a cellular model for investigating mechanisms of UGT1A7 transcriptional control.

**Effect of 3-Methylcholanthrene and Oltipraz on Luciferase Expression Controlled by UGT1A7**—The above studies predicted that the DNA flanking the UGT1A7 exon should act as an orientation-dependent promoter with cis regulatory elements controlling UGT1A7 transcription. To investigate this, an XhoI-PvuII fragment of ARPT-6 corresponding to bases −965 to +56 was cloned in either orientation upstream of the luciferase reporter gene of pGL3-Basic, and the resulting constructs were transfected into primary rat hepatocytes. Lysates of cells transfected with the promoter fragment in the correct orientation exhibited more than 50-fold higher luciferase activity than cells transfected with the reverse construct. The activity was not affected by oltipraz treatment, but it was increased 2–3 fold by 3-methylcholanthrene (Fig. 7A). Similar results were observed for a reporter plasmid containing a 660-base 5′ extension (p-1600/+56 1A7-Luc). However, the base luciferase vector itself (pGL3-Basic) yielded ~3-fold inducibility by 3-methylcholanthrene (Fig. 7B). Similar “inducibility” was observed with pGL3-Promoter plasmid (data not shown), suggesting that the 3-methylcholanthrene responsiveness is not dependent on the UGT1A7 sequence but rather on a sequence present in the vector itself. In contrast, experiments with hepatocytes transfected with an analogous pGL3-based UGT1A6 luciferase reporter plasmid (pSTM11) showed much greater responsiveness to 3-methylcholanthrene; unlike p-965/+56 1A7 Luc, pSTM11 was responsive to oltipraz.

**Discussion**

Work in two different laboratories now conclusively supports the existence of two different PAH- and oltipraz-inducible forms of UDP-glucuronosyltransferase in rat liver (3, 9, 18, 23). The two forms are encoded by the UGT1A6 and UGT1A7 loci and have distinct substrate specificities toward planar phenols. Given the close physical proximity of the two loci and the many

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**Fig. 6. Comparison of in vivo and in vitro responses of CYP and UGT1A7 mRNAs to oltipraz and PAH-type inducing agents.**

Northern analyses of β-actin, CYP1A, and UGT1A7 were carried out as described. Lanes 1–3 are RNA samples from cultures of primary rat hepatocytes exposed for 48 h to vehicle (0.1% Me2SO; lane 1), 3-methylcholanthrene (2.5 μM; lane 2) or oltipraz (50 μM; lane 3). Lanes 4–6 are from rats treated in vivo with vehicle (lane 4), oltipraz (lane 5), or β-naphthoflavone (lane 6).

**Fig. 7. Effect of 3-methylcholanthrene and oltipraz on expression of luciferase in primary rat hepatocytes transfected with various luciferase reporter plasmids.** Primary rat hepatocytes (three dishes/group) were transfected with the indicated luciferase reporter plasmid and exposed to vehicle, oltipraz, or 3-methylcholanthrene as described in the text. Cells were harvested 48 h after the start of drug treatment, and luciferase activity was determined. The data represent the mean ± S. D. of three independent determinations. A, data presented in absolute terms. B, data presented in relative terms (percentage of the mean of corresponding vehicle).
parallels in their patterns of mRNA expression (e.g. repressed constitutive expression and high inducibility by both PAHs and oltipraz), we expected that the genes would share certain characteristics involving exon structure and organization and transcriptional initiation. Instead, key differences between the two PAH-regulated UGT genes stand out.

One difference involves the total number of exons composing the complete transcription unit of each gene: six exons for UGT1A6 and five for UGT1A7. The difference lies in the number of exons coding for the unique 5' sequence of each respective mRNA. UGT1A6 uses two exons for this purpose, including a short 142-bp non-coding leader exon (labeled UGT1A6b in Fig. 2) and a longer exon containing the first 845 bp of the UGT1A6 coding region (labeled UGT1A6a in Fig. 2). Our primer extension and RNase protection data demonstrate that UGT1A7 differs from UGT1A6 in utilizing only a single exon to code for its entire unique 5' terminal sequence. This arrangement predicts that the DNA flanking the long UGT1A7 unique amino-terminal-coding exon functions as a gene promoter. In accordance with this, a fragment representing bases −965 to +56 of UGT1A7 promoter drove high expression of a downstream luciferase reporter when inserted in the correct (5'-3') but not the reverse (3'-5') orientation. UGT1A7, therefore, possesses the 5' exon structure that is more typical of UGT1 genes such as UGT1A1, UGT1A4, and UGT1A6 (2).

Another key difference between the UGT1A6 and 1A7 loci is the utilization by UGT1A7 of a complex of transcription start sites, clustered in a 45-bp region between 85 and 40 bases upstream of the translation start codon. Regardless of the site of initiation within this cluster, all transscripts are predicted to encode the full-length UGT1A7 protein, with the first AUG codon being the normal translation start site of UGT1A7. The basis for this heterogeneous initiation appears to be related to the lack of an efficient TATA box to direct precise transcriptional initiation. The region flanking the complex of transcription sites is enriched in TA residues, which are postulated to serve as weak TATA factor binding sites. The UGT1A7 promoter does not appear to be TATA-independent (housekeeping type) because there are no Sp1-binding sites and the promoter region overall is low in GC dinucleotide content. Furthermore, similar to other TATA-dependent genes, UGT1A7 possesses a sequence in its near upstream region closely matching a functional CCAAT enhancer-binding protein in the rat apolipoprotein B gene (24). The lack of a strong transcriptional initiating sequence may account for the lower relative abundance of UGT1A7 mRNA compared with UGT1A6 in livers of PAH- and oltipraz-treated rats (9) and the low or undetectable expression of UGT1A7 mRNA in human liver samples (25).

One major goal of research is to identify transcription factors regulating the expression of rat UGT1A7, because variation in such factors may underlie variability in UGT1A7 expression and thereby alter individual susceptibility to PAH-associated toxicities. The UGT1A7 promoter contains sequences closely matching (≤2 mismatches) known functional or consensus binding sites for hepatic nuclear factor 1 (rat glutathione S-transferase Ya gene) (26), hepatic nuclear factor 3, hepatic nuclear factor 4 (27), and activator protein 1. Further analysis is necessary to determine whether these sites are functionally involved in the control of UGT1A7 gene expression. Computer-aided inspection of the sequence failed to uncover any close matching ARE or XRE sequences; these elements were previously implicated in the regulation of other phase 1 and/or phase 2 metabolizing enzymes by PAHs (15, 18) and oltipraz (16).

Consistent with these negative findings, no PAH or oltipraz inducibility (above the 2.5-fold level observed with the control luciferase vector) was conferred by the UGT1A7 promoter and 5' flanking sequence. Clearly, UGT1A7 lacks an XRE in an analogous position to that of UGT1A6.

Because the nuclear runoff assays indicate that the effects of PAHs and oltipraz on UGT1A7 mRNA are due primarily to transcriptional activation, the data suggest that the cis elements mediating this activation are located either 5' or 3' of the region studied in this report. One possibility is that the inducibility of UGT1A7 is controlled by the XRE located 7 kb downstream in the UGT1A6 promoter region. This model is attractive because it could explain the concordant patterns of UGT1A6/UGT1A7 expression in various species. In mice and rabbits (and apparently humans), expression of both UGT1A6 and UGT1A7 is high in the constitutive state, and each is relatively refractory to inducing agents (28, 29). In rats, both UGT1A6/1A7 have low constitutive expression and are highly responsive to the inducing agents. The loss of transcriptional inducibility of two genes, UGT1A6 and 1A7, is most simply explained by a mutation involving only a single regulatory element. Another possibility, however, is that their inducibility by PAHs or oltipraz does in fact occur through distinct regulatory elements. We are currently testing the intronic region of the UGT1A7 gene for evidence of inducer-responsive enhancers or repressors.

In contrast to PAHs, the sequence mediating the responsiveness of UGT1A6/1A7 to oltipraz remains unknown. Our transient transfection analysis with the UGT1A6 luciferase reporter provides the first evidence that the UGT1A6 promoter flanking sequence contains oltipraz-responsive transcriptional enhancer elements. It is not clear whether this element corresponds to the XRE element previously identified by Emi et al. (3) or some other sequence motif (i.e. ARE). Evidence is appearing that oltipraz or perhaps one of its metabolites may be a weak activator of the arylhydrocarbon receptor (30). These findings are further supported by our study, showing transcriptional stimulation of CYP1A genes and increased CYP1A1/A2 mRNA levels following oltipraz treatment in vivo and in rat hepatocytes in vitro.

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A species difference exists in the number of exons for the UGT1A6 gene (six for rat, five for human).
Transcriptional Activation of the UDP-Glucuronosyltransferase IA7 Gene in Rat Liver by Aryl Hydrocarbon Receptor Ligands and Oltipraz
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