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An ethoxyquin-inducible aldehyde reductase from rat liver that metabolizes aflatoxin B_1 defines a subfamily of aldo-keto reductases

(chemprotection/liver cancer/antioxidant)

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ABSTRACT **Protection of liver against the toxic and carcinogenic effects of aflatoxin B_1 (AFB_1) can be achieved through the induction of detoxification enzymes by chemoprotectors such as the phenolic antioxidant ethoxyquin. We have cloned and sequenced a cDNA encoding an aldehyde reductase (AFB_1-AR), which is expressed in rat liver in response to dietary ethoxyquin. Expression of the cDNA in *Escherichia coli* and purification of the recombinant enzyme reveals that the protein exhibits aldehyde reductase activity and is capable of converting the protein-binding dialdehyde form of AFB_1-dihydrodiol to the nonbinding dialcohol metabolite. We show that the mRNA encoding this enzyme is markedly elevated in the liver of rats fed an ethoxyquin-containing diet, correlating with acquisition of resistance to AFB_1. AFB_1-AR represents the only carcinogen-metabolizing aldehyde reductase identified to date that is induced by a chemoprotector. Alignment of the amino acid sequence of AFB_1-AR with other known and putative aldehyde reductases shows that it defines a subfamily within the aldo-keto reductase superfamily.**

The ability of certain compounds in the diet to inhibit the onset of chemically induced carcinogenesis has been heralded for some time as a means of cancer prevention in humans (for a review, see ref. 1). In the rat, antioxidants such as ethoxyquin, butylated hydroxyanisole, and oltipraz have been found to be particularly effective chemoprotectors (2–4). Evidence suggests that these compounds confer resistance by increasing the levels of enzymes involved in detoxification of carcinogenic substrates (5–7), in many cases by enhancing transcription of their genes (8). However, many of the studies in this area have used model substrates to measure the levels of detoxification enzymes and it is therefore unclear which particular enzymes are of functional importance in chemoprotection against specific carcinogens.

In an attempt to identify proteins that play a major role in the chemoprevention of liver cancer, we have studied the expression of enzymes that metabolize aflatoxin B_1 (AFB_1), a potent hepatocarcinogen produced by the mold *Aspergillus flavus* that is widely distributed in nature. Previously, we reported that an aldehyde reductase is expressed in the liver of rats fed on an ethoxyquin-containing diet and that expression correlates with resistance to AFB_1-induced carcinogenesis (9). This enzyme (AFB_1-AR) catalyzes the formation of a dialcohol from the cytotoxic dialdehyde form of AFB_1-8,9-dihydrodiol (10). In the present paper, we describe the isolation of a cDNA encoding the AFB_1-AR enzyme and show that it defines a previously unrecognized class of aldehyde reductase.§ Levels of AFB_1-AR mRNA are increased dramatically in response to dietary ethoxyquin, suggesting that the enzyme is regulated at the mRNA level.

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MATERIALS AND METHODS

Chemicals, Enzymes, Bacterial Strains, and Plasmids. Chemicals and enzymes were obtained from commercial sources. Male Fischer 344 rats were fed on a powdered MRC41B diet containing arachis oil. Ethoxyquin was administered to rats (150–175 g) at 0.5% for 5 days before sacrifice. *Escherichia coli* strain NM522 was used for propagation of plasmid DNA; XL1-Blue was used for screening the LambdaZAPII library; and BL21pLysS was used for the expression of AFB_1-AR. LambdaZAPII DNA was obtained from Stratagene. Plasmid pET15b was obtained from Novagen.

Enzyme Assays. Reductase activity toward 4-nitrobenzaldehyde was measured spectrophotometrically (9). AFB_1-AR activity toward aflatoxin was determined by the HPLC-based assay described by Judah *et al.* (10) that involves direct use of AFB_1-8,9-dihydrodiol.

Amino Acid Sequencing of AFB_1-AR. The purification of AFB_1-AR from rat liver has been described (9). After cleavage of the purified protein with either CNBr or *Staphylococcus aureus* V8 protease, fragments were isolated by reverse-phase HPLC and sequenced as described (9).

Amplification of Regions of AFB_1-AR cDNA. Total RNA was isolated from rat livers essentially as described by Chomczynski and Sacchi (11) except that cells were broken by vortexing with glass beads. Total RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase. A region of the cDNA encoding part of AFB_1-AR was selectively amplified from the mixture by using pairs of degenerate oligonucleotide primers (5′-GARGARCAYTTYAAYGG-3′ and 5′-GCYTGRTCRAANGCRTC-3′; 5′-GAYGCNTTYGAYCARGC-3′ and 5′-CCRTTRAARTGYTCYTC-3′) for 30 cycles of successive denaturation, annealing, and extension. The 250-bp product from one pair of primers was excised from an agarose gel, and the ends of the fragment were phosphorylated and filled in before blunt-end ligation into the *Sma* I site of pTZ19r to give plasmid pEE56.

Library Construction, Screening, and Sequencing. Poly(A)$^+$ mRNA was purified on an oligo(dT)-cellulose column and cDNA with cohesive *Eco*RI ends was synthesized and ligated into the *Eco*RI site of LambdaZAPII. After packaging, a total of 2.4×10^8 plaque-forming units were obtained, of which 5×10^4 were screened in duplicate using the 250-bp *Eco*RI/*Hin*dIII fragment from pEE56 as a probe. The plasmid pEE60 was rescued from one positive plaque using M13KO7 helper phage. Subsequent subcloning was carried out in pTZ19r. Sequencing of both strands of the AFB_1-AR cDNA was carried out by the method of Sanger *et al.* (12) with Sequenase

Abbreviations: AFB_1, aflatoxin B_1; AFB_1-AR, AFB_1 aldehyde reductase; IPTG, isopropyl β-D-thiogalactoside.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. X74673).

(United States Biochemical) and specific oligonucleotide primers.

Expression in *E. coli*. Using site-directed mutagenesis, a unique *Nde* I site was introduced immediately 5′ to the ATG initiation codon of the AFB_1-AR cDNA sequence and a unique *Bam*HI site was introduced 3′ to the coding sequence (position 1025). The 1.1-kb *Nde* I/*Bam*HI fragment was ligated into the *Nde* I/*Bam*HI sites of pET15b to give plasmid pEE65, which would give rise to the fusion of a peptide, comprising six histidines and a thrombin cleavage site, with the N terminus of AFB_1-AR. *E. coli* cells transformed with pEE65 were grown to A_{600} = 0.5 before isopropyl β-D-thiogalactoside (IPTG) was added to 0.5 mM. Cultures were grown for a further 1.5 hr before harvesting. Cells were sonicated in 10 mM sodium phosphate buffer (pH 8.0) with 2 mM dithiothreitol and the fusion protein was purified from the soluble fraction with a nickel agarose column (Qiagen, Chatsworth, CA) by eluting with 200 mM imidazole. The polyhistidinyl N terminus was cleaved from the fusion protein by using human thrombin.

Northern Blots. Total RNA was denatured in formaldehyde/formamide and subjected to electrophoresis in formaldehyde-agarose gels (13) before transferring to Hybond-N membrane (Amersham) by capillary blotting. The entire AFB_1-AR cDNA was labeled with ^{32}P by random priming and used as a probe. Hybridization was carried out at 65°C in 6× standard saline citrate (SSC)/0.5% SDS, and the blot was washed at 65°C in 0.1× SSC before autoradiography. As a control, the blot was stripped and subsequently reprobed with [^{32}P]DNA encoding rat glyceraldehyde-3-phosphate dehydrogenase.

RESULTS

Cloning and Sequencing of AFB_1-AR cDNA. The purification from rat liver of an ethoxyquin-induced aldehyde reductase (AFB_1-AR) that metabolizes AFB_1-dialdehyde has been described (9). The amino acid sequence of several peptide fragments was determined after cleavage of the protein with either CNBr or *S. aureus* V8 protease. Sets of degenerate oligomers of 17 nucleotides were synthesized that corresponded to the least degenerate regions of two of the CNBr peptides. As the relative position of the peptide fragments in the protein was unknown, both alternatives were covered by using two pairs of degenerate primers. Total RNA isolated from a liver of an ethoxyquin-fed rat was reverse transcribed and used as a template to amplify the sequence between the primer pairs by the PCR. Only one pair of primers gave rise to a product, and subcloning and sequencing of this 250-bp fragment showed that it encoded two of the other peptide fragments in addition to the peptide sequences used to design the primers. The 250-bp fragment was used as a probe to screen a LambdaZAPII cDNA library made from liver mRNA of an ethoxyquin-fed rat. One positive clone was isolated and, after plasmid rescue using helper phage, this clone (pEE60) was found to contain a 1.2-kb insert.

The nucleotide sequence of this insert was determined (Fig. 1). Despite the absence of a poly(A) tail, the cDNA sequence does contain a polyadenylylation signal (AATAAA) toward the 3′ end. An open reading frame of 983 bp starting at an ATG 68 bp from the 5′ end gives the predicted amino acid sequence and most of the peptide sequence previously determined can be located within this sequence (underlined in Fig. 1). The calculated M_r of this 327-residue polypeptide is 36,742, which is in close agreement with the M_r of 36,622 determined by desorption mass spectrometry of the purified AFB_1-AR protein (9).

AFB_1-AR cDNA Encodes an Aldehyde Reductase. To verify that the AFB_1-AR cDNA clone encodes the aldehyde reductase activity previously reported for the purified protein (9),

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   1 GAATTCCGCGGCCGCCAACGTCCTCTCTTACCCGCCACCTTCTTCTGCCACCTCTACCAC
       M S Q A R P A T V L G A M E M G R R
  61 GGTCACCATGTCGCAAGCCCGGCCTGCCACTGTGCTGGGTGCCATGGAGATGGGTCGCCG
     M D V T S S S A S V R A F L Q R G H T E
 121 CATGGATGTGACCTCCAGCTCCGCGTCGGTGCGCGCCTTCCTGCAGCGCGGCCACACGGA
     I D T A F V Y A N G Q S E T I L G D L G
 181 GATAGACACCGCCTTCGTGTATGCGAACGGTCAGTCTGAGACCATCCTAGGAGACCTGGG
     L G L G R S G C K V K I A T K A A P M F
 241 GCTCGGACTGGGCCGCAGCGGCTGCAAAGTAAAAATTGCCACCAAGGCTGCCCCAATGTT
     G K T L K P A D V R F Q L E T S L K R L
 301 TGGGAAGACACTGAAGCCAGCCGATGTTCGGTTCCAGCTGGAGACGTCACTGAAGAGGCT
     Q C P R V D L F Y L H F P D H G T P I E
 361 GCAGTGTCCCCGGGTGGACCTCTTCTATTTACACTTTCCAGACCACGGCACTCCTATAGA
     E T L Q A C H H V H Q E G K F V E L G L
 421 GGAGACCCTGCAGGCCTGCCACCACGTGCATCAGGAGGGCAAGTTTGTGGAGCTTGGTCT
     S N Y V S W E V A E I C T L C K K N G W
 481 GTCCAACTATGTCTCCTGGGAAGTGGCTGAGATTTGTACCCTCTGCAAGAAAAATGGCTG
     I M P T V Y Q G M Y N A I T R Q V E T E
 541 GATCATGCCAACTGTGTACCAGGGCATGTACAACGCCATCACCAGGCAGGTGGAGACTGA
     L F P C L R H F G L R F Y A F N P L A G
 601 GCTCTTCCCCTGCCTCAGACACTTCGGACTAAGGTTCTACGCCTTCAACCCTTTGGCTGG
     G L L T G R Y K Y Q D K D G K N P E S R
 661 GGGCCTGCTGACTGGCAGATATAAATACCAGGATAAGGATGGGAAGAATCCTGAGAGCCG
     F F G N P F S Q L Y M D R Y W K E E H F
 721 CTTCTTTGGGAATCCATTTTCTCAACTGTACATGGACCGCTACTGGAAGGAGGAACACTT
     N G I A L V E K A L K T T Y G P T A P S
 781 CAATGGCATCGCCTTGGTGGAGAAGGCTCTGAAGACTACCTATGGCCCCACTGCCCCCAG
     M I S A A V R W M Y H H S Q L K G T Q G
 841 TATGATCTCAGCTGCCGTACGGTGGATGTACCATCACTCACAGCTCAAGGGCACCCAAGG
     D A V I L G M S S L E Q L E Q N L A L V
 901 GGATGCAGTCATTCTGGGCATGTCCAGTCTGGAACAACTGGAGCAGAACTTGGCCTTGGT
     E E G P L E P A V V D A F D Q A W N L V
 961 CGAGGAAGGGCCTCTGGAGCCAGCTGTTGTGGATGCCTTTGACCAAGCCTGGAACCTAGT
     A H E C P N Y F R *
1021 TGCCCACGAGTGTCCCAACTATTTCCGCTAAGATACATCTGCCTTGGGGATGGCGCAGCT
1081 TACTGCCTGCCCCGCCTTGTCCTGGGCTCGATCTGATCTGGTTCTTTCCTTTTTAGACAG
1141 GTCACTGTCTTTTTCTTCCCTGCTTTCTATACAGCCAGTTGCTTTCAAAGTGAGAGCTGG
1201 CTGAGCCCCAATACCTCCTGCTGAATAAAACTGTTCCCTGTCACAGCCTGGGCTACAACT
1251 GGCGGCCGA
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FIG. 1. Sequence of AFB_1-AR cDNA and predicted amino acid sequence. The polyadenylylation signal AATAAA is underlined and a possible ATG initiating methionine is double underlined. The amino acid sequence that corresponds to that previously determined for peptide fragments of the purified AFB_1-AR protein is underlined.

the coding region was inserted into the vector pET15b in such a way that expression of a polyhistidinyl-AFB_1-AR fusion protein could be directed from the T7 promoter in *E. coli* using an IPTG-inducible T7 polymerase. The fusion protein was purified by using a nickel agarose column, from bacteria containing the recombinant plasmid pEE65 grown in the presence of IPTG, and the polyhistidinyl N terminus was removed by using human thrombin. The protein comigrates during SDS/PAGE with that from rat liver and reacts with antisera raised against the rat liver protein (Fig. 2). The specific activity of this enzyme toward 4-nitrobenzaldehyde and AFB_1-dialdehyde is similar to that observed with AFB_1-AR purified from rat liver (Table 1) (9). These data suggest that the AFB_1-AR cDNA clone encodes an active aldehyde reductase and are consistent with it being identical to that previously purified from the liver of ethoxyquin-fed rats (9).

AFB_1-AR mRNA Is Elevated in the Liver of Ethoxyquin-Fed Rats. As the level of the AFB_1-AR protein is elevated in the liver of rats fed an ethoxyquin-containing diet (9), it was important to determine whether this may be related to an increase in AFB_1-AR mRNA. A Northern blot of total RNA from the liver of an ethoxyquin-fed rat and a control rat was probed with the 1.2-kb cDNA insert (Fig. 3). A 1.2-kb mRNA is present in the ethoxyquin-fed rat, which is undetectable in

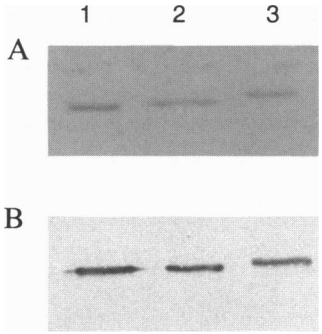

FIG. 2. AFB_1-AR protein purified from ethoxyquin-fed rat liver (lane 1) was compared with recombinant polyhistidinyl-AFB_1-AR fusion protein purified from *E. coli*, which was either cleaved with thrombin (lane 2) or uncleaved (lane 3). Protein was resolved by SDS/PAGE and stained with polyacrylamide gel blue 83 (*A*) or blotted onto nitrocellulose and probed with antiserum raised against rat liver AFB_1-AR (9) (*B*).

the control rat. This suggests that AFB_1-AR mRNA levels may determine the level of the AFB_1-AR enzyme.

Sequence Comparisons. Comparisons between the nucleotide and amino acid sequences of AFB_1-AR and other sequences present in data bases revealed several significant similarities. At the nucleotide level, the AFB_1-AR sequence is 80% identical to the coding region of a partial cDNA of unknown function from human brain (EST00883) (14). This similarity diminishes after the translational stop signal and the predicted amino acid sequence of EST00883 is 87% identical to that of the AFB_1-AR protein.

Similarities of 21–33% identity between the AFB_1-AR protein and five other open reading frames in the data base were also identified (Fig. 4; Table 2). These are as follows: AUX115, an open-reading frame in an auxin-induced mRNA from *Nicotiana tabacum* (15); PORF, an open reading frame overlapping with the *igrA* gene (a gene conferring resistance to the herbicide glyphosphate) from *Pseudomonas* sp. (16); YCR107W, an open reading frame on *Saccharomyces cerevisiae* chromosome III (17); BBOAKR, a putative aldo-keto reductase from the hemoparasite *Babesia bovis* (18); and CHLR, human chlordecone reductase (19), a member of the aldo-keto reductase family (20). Comparisons between AFB_1-AR and human placental aldose reductase (20), another member of the aldo-keto reductase family, revealed 20% identity (43% similarity) (Table 2). Taken together, these similarities prove to be significant, as a multiple sequence alignment reveals regions of identity between all seven protein sequences (Fig. 4). These regions of similarity cover areas of the aldose reductase protein that are thought to be involved in cofactor binding (21, 22).

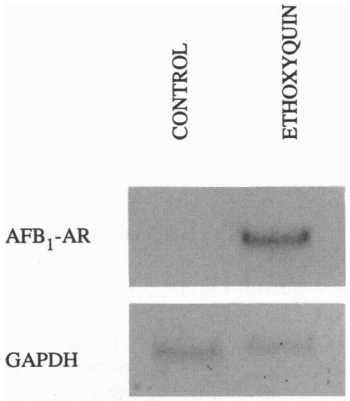

FIG. 3. Northern blot of total RNA isolated from the liver of a control rat and from the liver of a rat fed an ethoxyquin-containing diet. The blot was probed with ^{32}P-labeled full-length AFB_1-AR cDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA as control.

Table 1. Activity of AFB_1-AR expressed and purified from *E. coli*

Enzyme	Thrombin*	Specific activity† 4-NBA, μmol·min^{-1}·mg^{-1}	Specific activity† $AFB_1(CHO)_2$, nmol·min^{-1}·mg^{-1}
Rat liver AFB_1-AR	−	2.24	5.4
His-AFB_1-AR fusion	−	1.58	7.2
His-AFB_1-AR fusion	+	1.70	6.8

4-NBA, 4-nitrobenzaldehyde; $AFB_1(CHO)_2$, AFB_1 dialdehyde.
*The polyhistidinyl N terminus of the His-AFB_1-AR fusion protein was removed by cleavage with human thrombin.
†Data represent mean values from duplicate analyses. Coefficients of variation for the 4-NBA and $AFB_1(CHO)_2$ assays found are typically <5% and <8%, respectively.

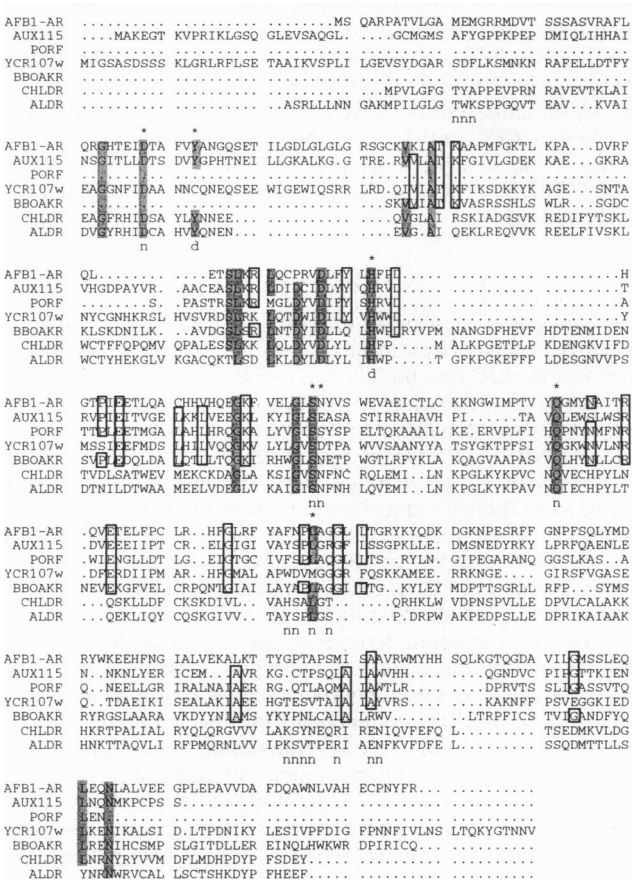

FIG. 4. Multiple sequence alignment of AFB_1-AR with six related open reading frames and protein sequences. AUX115, open reading frame from an auxin-induced mRNA from *N. tabacum* (15); PORF, open reading frame overlapping the *igrA* gene from *Pseudomonas* sp. (16); YCR107w, open reading frame from *S. cerevisiae* chromosome III (17); BBOAKR, putative aldo-keto reductase from *B. bovis* (18); CHLDR, human chlordecone reductase (19); ALDR, human placental aldose reductase (20); n, residues of ALDR thought to be involved in cofactor binding (21, 22); d, potential hydrogen donor sites of ALDR (21, 22); *, residues of AFB_1-AR that are identical to cofactor binding or potential hydrogen donor sites of ALDR. Shaded residues indicate identity between all sequences (allowing for one mismatch). Boxed residues indicate identity between the subfamily members (AFB_1-AR, AUX115, PORF, YCR107w, and BBOAKR) allowing for one mismatch.

Table 2. Relationship between AFB_1-AR amino acid sequence and other known and putative aldo-keto reductases

	% identity (% similarity)					
	AFB_1-AR	PORF	AUX115	BBOAKR	YCR107w	CHLDR
PORF	32.6 (54.3)					
AUX115	31.4 (52.7)	30.9 (58.6)				
BBOAKR	30.6 (55.8)	28.9 (49.7)	29.7 (54.8)			
YCR107w	21.6 (49.7)	27.0 (54.6)	25.0 (51.3)	23.4 (48.4)		
CHLDR	24.1 (48.7)	23.7 (46.8)	21.8 (44.4)	23.6 (51.9)	20.7 (42.8)	
ALDR	20.1 (44.2)	24.4 (51.9)	28.8 (51.2)	24.5 (49.8)	19.6 (47.1)	49.0 (68.4)

PORF, open reading frame overlapping the *igrA* gene from *Pseudomonas* sp. (16); AUX115, open reading frame from an auxin-induced mRNA from *N. tabacum* (15); BBOAKR, putative aldo-keto reductase from *B. bovis* (18); YCR107w, open reading frame from *S. cerevisiae* chromosome III (17); CHLDR, human chlordecone reductase (19); ALDR, human placental aldose reductase (20).

DISCUSSION

In the present paper, we describe the cloning of a cDNA encoding an aldehyde reductase that appears to play a critical role in resistance to AFB_1. This enzyme helps prevent the cytotoxic effects of AFB_1 by catalyzing the reduction of the AFB_1-dialdehyde phenolate form of AFB_1-dihydrodiol to its corresponding dialcohol (9, 10). The reductase activity toward this dialdehyde metabolite of AFB_1 is increased by dietary ethoxyquin, and the Northern blot shown in Fig. 3 demonstrates that the level of AFB_1-AR mRNA is markedly increased in response to ethoxyquin, suggesting that the enzyme is either transcriptionally regulated or that mRNA stability is involved.

Ethoxyquin has also been shown to increase the activities of other liver detoxification enzymes [for example, UDPglucuronosyl transferase (23), epoxide hydrolase (24), NADPH:quinone reductase (25), glutathione *S*-transferase (5, 26), and cytochrome P450 (27)], but of these, only glutathione *S*-transferase and cytochrome P450 have been reported to metabolize AFB_1. Aflatoxin metabolites, such as AFP_1, can form glucuronides but it is not known whether the UDPglucuronosyl transferase(s) responsible for catalyzing this conjugation reaction is induced by ethoxyquin. It is probable that ethoxyquin-induced resistance to AFB_1 involves the coordinated regulation of several detoxification enzyme systems, and perhaps also P-glycoprotein, metallothionein, and DNA repair enzymes (for a review, see ref. 28). It is not known how ethoxyquin regulates any of these enzymes, but the molecular mechanism(s) may resemble that of other chemical inducers such as *tert*-butyl hydroquinone (BHQ) or 3-methylcholanthrene, which can transcriptionally activate genes encoding detoxification enzymes (29, 30). If regulation of AFB_1-AR also occurs at the transcriptional level, it would be of interest to identify cis- and trans-acting factors involved and to compare the mechanism of induction to those of other genes. The involvement of regulatory elements such as the antioxidant response element/electrophile response element (29, 31) and the xenobiotic response element (32, 33), of regulatory proteins such as AP-1 (Jun/Fos) (34), and the Ah receptor (35) remains to be established.

AFB_1-AR is not the only inducible aldehyde reductase, although its level of induction by xenobiotics (≈20-fold) is substantially greater than reported for other members of the aldo-keto reductase superfamily. Recently, Ciaccio *et al.* (36) reported that the levels of a 37.5-kDa protein, which is related to prostaglandin F synthase, is increased between 2.0- and 3.5-fold in a human colon cell line after treatment with ethacrynic acid and other Michael acceptors. Also, in gerbil liver, chlordecone reductase is induced 1.4-fold by exposure to chlordecone (37). It should be noted, however, that neither the human colon 37.5-kDa protein nor chlordecone reductase has been shown to metabolize carcinogens and therefore their involvement in the prevention of carcinogenesis is unclear.

In addition to protection against AFB_1, ethoxyquin protects against the toxic and carcinogenic effects of other chemicals and tumor promoters (pyrrolizidine alkaloids, sesquiterpene lactones, ciprofibrate, polycyclic hydrocarbons) (2, 38–41). Whether resistance against any of these compounds is mediated by AFB_1-AR is not known, but it will now be possible to assess the contribution of this aldehyde reductase to protection against chemical insult by expressing the AFB_1-AR cDNA in cultured cells exposed to these agents. Apart from its protective role, it is possible that AFB_1-AR has an additional role in the normal metabolism of an endogenous substrate or substrates in liver or other tissues. Our finding that AFB_1-AR is 87% identical to the protein encoded by a partial cDNA from human brain (EST00883) (14) may provide a clue about the endogenous role of a similar enzyme in humans (e.g., metabolism of aldehydes derived from neurotransmitters); interestingly, the AFB_1-AR enzyme is not detectable in rat brain (9).

It is surprising that the enzyme we have characterized is only distantly related to the aldo-keto reductase family of enzymes (20). Comparison of the deduced amino acid sequence of AFB_1-AR with those of members of the aldo-keto reductase superfamily indicates that it is most closely related (24% identity) to chlordecone reductase, a human liver enzyme involved in the detoxification of the organochlorine pesticide chlordecone (19). By contrast, other members of the aldo-keto reductase superfamily possess between 45% and 70% identity with each other (20); these enzymes catalyze the reduction of aldehyde and ketone groups on a wide variety of substrates including sugars (20), steroid hormones (42, 43), and toxic aldehydes (44), as well as the oxidation of *trans*-dihydrodiols of polycyclic aromatic hydrocarbons and steroid hormones (43, 45). However, AFB_1-AR is only 18–21% identical to the rest of the superfamily and shows particular variation at the C terminus, suggesting a divergence of function. Among the other aldo-keto reductases, the crystal structure of aldose reductase has shown that this enzyme consists of an α/β barrel and possesses an unusual NADPH-binding site (21, 22). Some of the residues that may be important for cofactor binding are conserved in AFB_1-AR; in addition, amino acids that represent putative hydrogen donors are also conserved (Fig. 4).

Comparison of the amino acid sequence of AFB_1-AR with sequences in data bases revealed that rat AFB_1-AR is between 21% and 33% identical to four other open reading frames encoding proteins of unknown function from a wide range of nonmammalian organisms (YCR107w, AUX115, BBOAKR, and PORF) (Fig. 4; Table 2) (15–18). Despite the diversity of species from which they were obtained, these sequences are more similar to each other than to other members of the aldo-keto reductase family. We therefore propose that they form a subfamily of enzymes within the aldo-keto reductase superfamily. The rat AFB_1-AR enzyme is the only member of this subfamily for which an aldehyde

reductase activity has been demonstrated and for which a role in chemoprotection has been ascribed.

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