Biochemical Pharmacology, Vol. 59, pp. 65–85, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(99)00310-X

Role of the Aromatic Hydrocarbon Receptor and [*Ah*] Gene Battery in the Oxidative Stress Response, Cell Cycle Control, and Apoptosis

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ABSTRACT. The chronology and history of characterizing the aromatic hydrocarbon [*Ah*] battery is reviewed. This battery represents the Ah receptor (AHR)-mediated control of at least six, and probably many more, dioxin-inducible genes; two cytochrome P450 genes—P450 1A1 and 1A2 (*Cyp1a1*, *Cyp1a2*)—and four non-P450 genes, have experimentally been documented to be members of this battery. Metabolism of endogenous and exogenous substrates by perhaps every P450 enzyme, but certainly CYP1A1 and CYP1A2 (which are located, in part, in the mitochondrion), have been shown to cause reactive oxygenated metabolite (ROM)-mediated oxidative stress. Oxidative stress activates genes via the electrophile response element (EPRE) DNA motif, whereas dioxin (acutely) activates genes via the AHR-mediated aromatic hydrocarbon response element (AHRE) DNA motif. In contrast to dioxin, AHR ligands that are readily metabolized to ROMs (e.g. benzo[*a*]pyrene, β-naphthoflavone) activate genes via both AHREs and the EPRE. The importance of the AHR in cell cycle regulation and apoptosis has just begun to be realized. Current evidence suggests that the CYP1A1 and CYP1A2 enzymes might control the level of the putative endogenous ligand of the AHR, but that CYP1A1/1A2 metabolism generates ROM-mediated oxidative stress which can be ameliorated by the four non-P450 EPRE-driven genes in the [*Ah*] battery. Oxidative stress is a major signal in precipitating apoptosis; however, the precise mechanism, or molecule, which determines the cell's decision between apoptosis and continuation with the cell cycle, remains to be elucidated. The total action of AHR and the [*Ah*] battery genes therefore represents a pivotal upstream event in the apoptosis cascade, providing an intricate balance between promoting and preventing ROM-mediated oxidative stress. These proposed endogenous functions of the AHR and [*Ah*] enzymes are, of course, in addition to the frequently described functions of "metabolic potentiation" and "detoxification" of various foreign chemicals. BIOCHEM PHARMACOL **59**;1:65–85, 2000. © 1999 Elsevier Science Inc.

KEY WORDS. oxidative stress; [*Ah*] gene battery; mouse genetics; Ah receptor; endogenous ligand for Ah receptor; cytochrome P450 1A1; cytochrome P450 1A2; NAD(P)H:quinone oxidoreductase; UDP glucuronosyltransferase; aldehyde dehydrogenase; glutathione transferase; *14CoS* mouse line; human hereditary tyrosinemia type I; mouse hepatoma Hepa-1c1c7 wild-type and mutant cell culture lines; SV40-transformed cell culture lines; large T antigen; cell cycle; p53 (TRP53) protein; retinoblastoma (RB1) protein; apoptosis; dioxin

In prokaryotes a regulon comprises genes, generally in tandem and transcribed and translated together, that respond to a specific endogenous or exogenous signal. In eukaryotes, the "gene battery" closely resembles a bacterial regulon. A gene battery is defined as a "group of (generally, nonlinked) genes that exhibit cross-talk, having an intricate interrelationship with regard to up- and down-regulation, in response to a particular endogenous or exogenous signal; the battery's response is mediated by certain regulatory proteins whose effects may be combinatorial in nature" [1, 2]. The mouse aromatic hydrocarbon (*Ah*)-responsive†

Presented at Oxford, 10–12 June 1999.

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† *Abbreviations:* Ah, aromatic hydrocarbon; *Ahr* and AHR, mouse Ah receptor gene and protein; *Cyp1a1* and CYP1A1, mouse cytochrome P450 1A1 gene and protein; *Cyp1a2* and CYP1A2, mouse cytochrome P450 1A2 gene and protein; EL, putative endogenous ligand for the AHR; EP, putative endogenous product following metabolism of EL by CYP1A1/1A2; ARNT, Ah receptor nuclear transporter; *Nqo1* and NQO1, mouse NAD(P)H:quinone oxidoreductase [also called NMO1, quinone reductase, aminoazo reductase; DT-diaphorase] gene and enzyme; *Aldh3a1* and ALDH3A1, mouse cytosolic aldehyde dehydrogenase 3 gene and enzyme (formerly *Ahd4*, AHD4); *Ugt1a6* and UGT1A6, mouse UDP glucuronosyltransferase 1A6 gene and enzyme; *Gsta1* and GSTA1, mouse glutathione transferase (Ya; class α) gene and enzyme; Chr, chromosome; *gadd* and GADD, growth arrest and DNA damage-inducible genes and proteins; TCDD, dioxin or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AHREs, aromatic hydrocarbon response elements; EPRE, electrophile response element; ROMs, reactive oxygenated metabolites; RB1, retinoblastoma protein; p53, *TRP53* gene product; SV40, simian virus 40; G_1 and G_2, growth

gene battery is among the best characterized examples in eukaryotes [reviewed in Refs. 3–5]. The purpose of this review is to summarize and examine the latest experimental data concerning these dioxin-inducible genes. It now seems clear that this gene battery—which presumably exists in all vertebrates—plays an important role in the cell's defense against oxidative stress and in cell cycle control. [*Ah*] battery genes have the capacity not only to promote oxidative stress, and to prevent it by induced levels of oxidative stress-detoxifying enzymes, but the AHR appears to assist specific cell types in choosing between apoptosis and continuation with progression through the cell cycle.

HISTORY

The earliest genetic studies [6; reviewed in Ref. 3] began with the observation that, when mice are treated intraperitoneally with polycyclic aromatic hydrocarbons (e.g. benzo[*a*]anthracene, 3-methylcholanthrene, or benzo[*a*]pyrene), an enzyme activity (benzpyrene hydroxylase) is induced in response to this exogenous signal. The response was found to be striking in certain sensitive inbred strains, termed "*Ah*-responsive" (e.g. C57BL/6), and was negligible in other relatively resistant inbred strains, termed "*Ah*-nonresponsive" (e.g. DBA/2). The lack of induction was inherited as an autosomal recessive trait in crosses between the C57BL/6 × DBA/2 inbred lines, but the inheritance of *Ah*-nonresponsiveness was found to vary—depending upon which inbred strains were crossed [7].

Due to its extreme resistance to metabolic breakdown and high affinity for the AHR, dioxin was then discovered to be about 30,000 times more potent than benzo[*a*]pyrene in causing this enzyme induction response in rats [8; reviewed in Ref. 3]. Dioxin was found to induce benzpyrene hydroxylase (aryl hydrocarbon hydroxylase, now termed cytochrome P450 1A1 or CYP1A1) to the same extent in *Ah*-nonresponsive mice than in *Ah*-responsive mice; however, the dose–response curve in *Ah*-nonresponsive inbred mouse strains is shifted 15- to 20-fold to the right (Fig. 1). These data suggested at the time that the structural gene product (CYP1A1) is the same in both the C57BL/6 and DBA/2 mouse strains, but that a regulatory gene product—such as altered affinity for a dioxin-binding receptor—might differ between the two strains. This hypothesis was confirmed two decades later. Amino acid changes responsible for the AHR differences between these two strains have now been identified [12, 13].

Additional enzyme activities were found to parallel that seen for dioxin-inducible CYP1A1 activity in *Ah*-nonresponsive versus *Ah*-responsive mice [*reviewed in* Ref. 3]. The [*Ah*] battery is now known to contain at least six genes—and probably many more—that are coordinately induced by

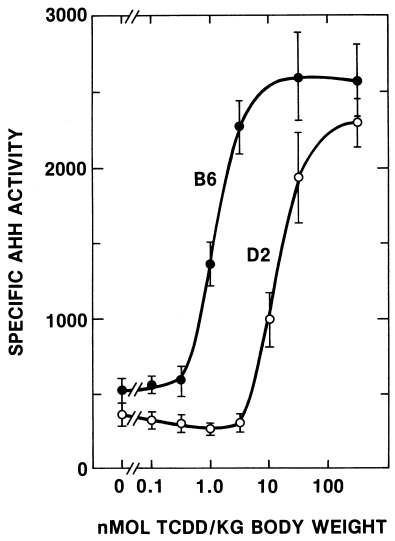


FIG. 1. Semilog plot of inducible hepatic aryl hydrocarbon hydroxylase (AHH; CYP1A1) activity as a function of dose of dioxin in the "sensitive" C57BL/6 (B6) and the "resistant" DBA/2 (D2) mouse. Intraperitoneal TCDD was given 24 hr before microsomes from individual livers were prepared. *Symbols* and *brackets* denote means and standard deviations around the mean (N = 6 mice at each TCDD dose). Allelic differences in the mouse *Ahr* gene were later found to be responsible for the sensitive and resistant phenotypes in these two inbred strains. The relative ease of induction in humans who have been exposed to identical amounts of cigarette pack-years of smoking history is expected to similarly vary due to allelic differences in the human *AHR* gene [9, 10] (Modified and redrawn from Ref. 11).

dioxin and polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene (Fig. 2). This transcriptional up-regulation requires: activation of the cytosolic AHR when bound to the above-mentioned foreign chemical inducers, or an EL as yet unknown [14–19]; and translocation of the ligand–receptor complex into the nucleus where it heterodimerizes with the ARNT before binding to AHREs upstream of dioxin-responsive genes [reviewed in Refs. 20–25].

In addition to two P450 genes—cytochrome P450 1A1 (*Cyp1a1*) and 1A2 (*Cyp1a2*)—this laboratory and others have used both mutant inbred mouse lines and cell culture variants to rigorously show [15, 26–28] that four non-P450 [*Ah*] genes include: NAD(P)H:quinone oxidoreductase (*Nqo1*); a cytosolic aldehyde dehydrogenase 3 (*Aldh3a1*); a UDP glucuronosyltransferase having 4-methylumbelliferone as substrate (*Ugt1a6*); and a glutathione transferase

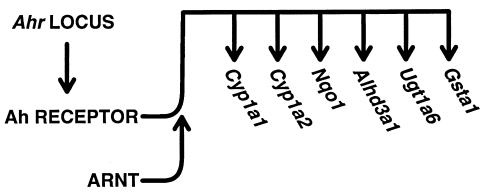


FIG. 2. The six members of the mouse [*Ah*] gene battery that are under discussion in this review. ARNT, the Ah receptor nuclear transporter protein.

phases of the cell cycle; S phase, DNA synthesis during the cell cycle; M phase, mitosis during the cell cycle; E2F, transcription factor that up-regulates genes in S phase; AP-1, activator protein-1; JNK, c-Jun NH(2)-terminal kinase; and *Fah* and FAH, fumarylacetoacetate hydrolase gene and enzyme.

having 2,4-dinitro-1-chlorobenzene as substrate (*Gsta1*, Ya). Pharmacologists and toxicologists for years have studied the "enzyme activities" of these gene products, using artificial substrates (Fig. 3). It seems quite clear, however, that there must exist one or more endogenous substrates for each of these enzymes that are involved in a cell type-specific response of this battery to distinct endogenous and exogenous signals and that the [*Ah*] gene battery has evolved in vertebrates over several hundred million years. Interestingly, the *AHR* and *ARNT* genes from *Caenorhabditis elegans* have been cloned, and the nematode AHR appears to exhibit no capacity for binding β-naphthoflavone in the manner that the vertebrate AHR does [29].

CROSS-TALK BETWEEN [*Ah*] BATTERY GENES

The elegant genetic complementation studies of benzo[*a*]pyrene-resistant mutant lines derived from the mouse hepatoma Hepa 1c1c7 cell culture line [30] have clearly provided important tools for dissecting the [*Ah*] battery; these findings [15, 26–28, 31, 32] are summarized in Table 1. Complementation groups include "AHR-receptorless" such as the *c2* line, "ARNT-deficient" such as the *c4* line, and "CYP1A1 metabolism-defective" such as the *c37* line [20]. Whereas CYP1A1 is highly inducible by dioxin in the wild-type (*wt*) parent line, CYP1A1 mRNA is negligible in both the control and dioxin-treated "leaky" mutant *c2* line (which contains a functional AHR at levels ~5–10% of that found in the *wt* cells). On the other hand, CYP1A1 mRNA is undetectable in both the control and dioxin-treated "stone-cold" mutant *c4* line which is deficient in ARNT. These data confirm that CYP1A1 induction is dependent on both the AHR and the ARNT. Intriguingly, CYP1A1 mRNA was found to be as high in control *c37* cells as in dioxin-treated cells (Table 1). Why is CYP1A1 mRNA elevated in the absence of endogenous CYP1A1 metabolism in this mutant cell line? Introduction of a functional mouse CYP1A1 cDNA, or a human CYP1A2 cDNA, into the *c37* line caused a return to the wild-type phenotype: i.e. CYP1A1 mRNA becomes negligible in untreated cells and highly inducible in dioxin-treated cells.

Even more intriguingly, the same pattern found for CYP1A1 mRNA in all these cell lines is seen for NQO1 mRNA (Table 1), ALDH3A1 mRNA [27], UGT1A6 mRNA and GSTA1 mRNA [28]. These data can be explained by two possible models (Fig. 4). In Model 1, the CYP1A1 enzyme degrades the EL for the AHR; absence of CYP1A1 activity would lead to a build-up of EL, which would activate the *Cyp1a1* gene (and other [*Ah*] battery members). In Model 2, the CYP1A1 enzyme activates an endogenous substrate to become a repressor; absence of CYP1A1 activity would lead to no repressor activity and, hence, derepression of the *Cyp1a1* gene (and other [*Ah*] battery members). Currently, the experimental proof to date [19] would support Model 1. Hence, both the CYP1A1 and CYP1A2 enzymes are interchangeable and appear to be critical in controlling AHR-mediated functions (Table 1).

IDENTIFICATION OF THE AHRES AND ELECTROPHILE RESPONSE ELEMENT (EPRE)

The AHRE (also called XRE, DRE) was determined to be the DNA motif responsible for the up-regulation of genes induced by 3-methylcholanthrene, dioxin, or β-naphthoflavone; the initial studies examined the 5′ promoter region of the rat *CYP1A1* gene [33]. The EPRE (also termed ARE, antioxidant response element) was found to be the DNA motif responsible for the up-regulation of genes induced by electrophiles such as menadione and hydrogen peroxide, as well as metabolites of benzo[*a*]pyrene or β-naphthoflavone; the initial studies examined the 5′ promoter region of the rat *GSTA1* gene [34]. The invariant core sequences of the AHRE and EPRE, and the similarity of the DNA motif that binds FOS–JUN to cause AP-1 activity, are illustrated in Fig. 5. The 5 to 10 bases 5′-ward and 3′-ward flanking these core sequences of the AHRE have been shown to aid in determining AHR binding potency, cooperativity, and dioxin responsiveness [35]. NRF2, a member of the (CNC-basic leucine zipper (CNC-bZIP) family of transcription factors, and perhaps the small MAF protein are believed to participate in the EPRE complex [36; reviewed in Ref. 37].

INITIAL STUDIES IN THE c^{14CoS}/c^{14CoS} MOUSE

While Model 2 (de-repression, in Fig. 4) was still being considered in this laboratory, we were intrigued by two studies in the c^{14CoS} mouse line that reported abnormally high UGT1A6 and GSTA1 activities in untreated newborn liver [38, 39]. The c^{14CoS} mouse line has a radiation-induced 3800-kilobase (kb) deletion on Chr 7 that includes the *c* (tyrosinase; albino) locus (Fig. 6, A and B). This deleted region is estimated to contain between 70 and 120 genes. The untreated c^{14CoS}/c^{14CoS} homozygote dies during the first day postpartum for reasons not clear, while the c^{ch}/c^{ch} wild-type and the c^{ch}/c^{14CoS} heterozygote are completely fertile and viable [reviewed in Refs. 40–42]. Could these elevated UGT1A6 and GSTA1 activities in the *14CoS* mouse line [38, 39] be responding to the same signal as that in the *c37* cell line (Table 1)? Although we now know that the answer to this question is "no," the subsequent studies in the *14CoS* mouse and cell culture lines in this laboratory for more than the past decade have defined an interesting link between the [*Ah*] battery and the cell's oxidative stress response and apoptosis. In contrast to the *ch/14CoS* heterozygote and *ch/ch* wild-type, the untreated *14CoS/14CoS* exhibits decreases in numerous mRNAs, enzyme activities, and/or other proteins (Table 2). These findings suggest that one or more of the 70 to 120 genes in the deleted region might be involved in up-regulation of the genes listed in Table 2 or in stabilization of their gene products. In other words, in the untreated *14CoS/14CoS* mouse homozygous for the 3800-kb deletion, the gene(s) responsible for up-regulation is now absent, and these various mRNAs, enzyme activities, or other proteins are

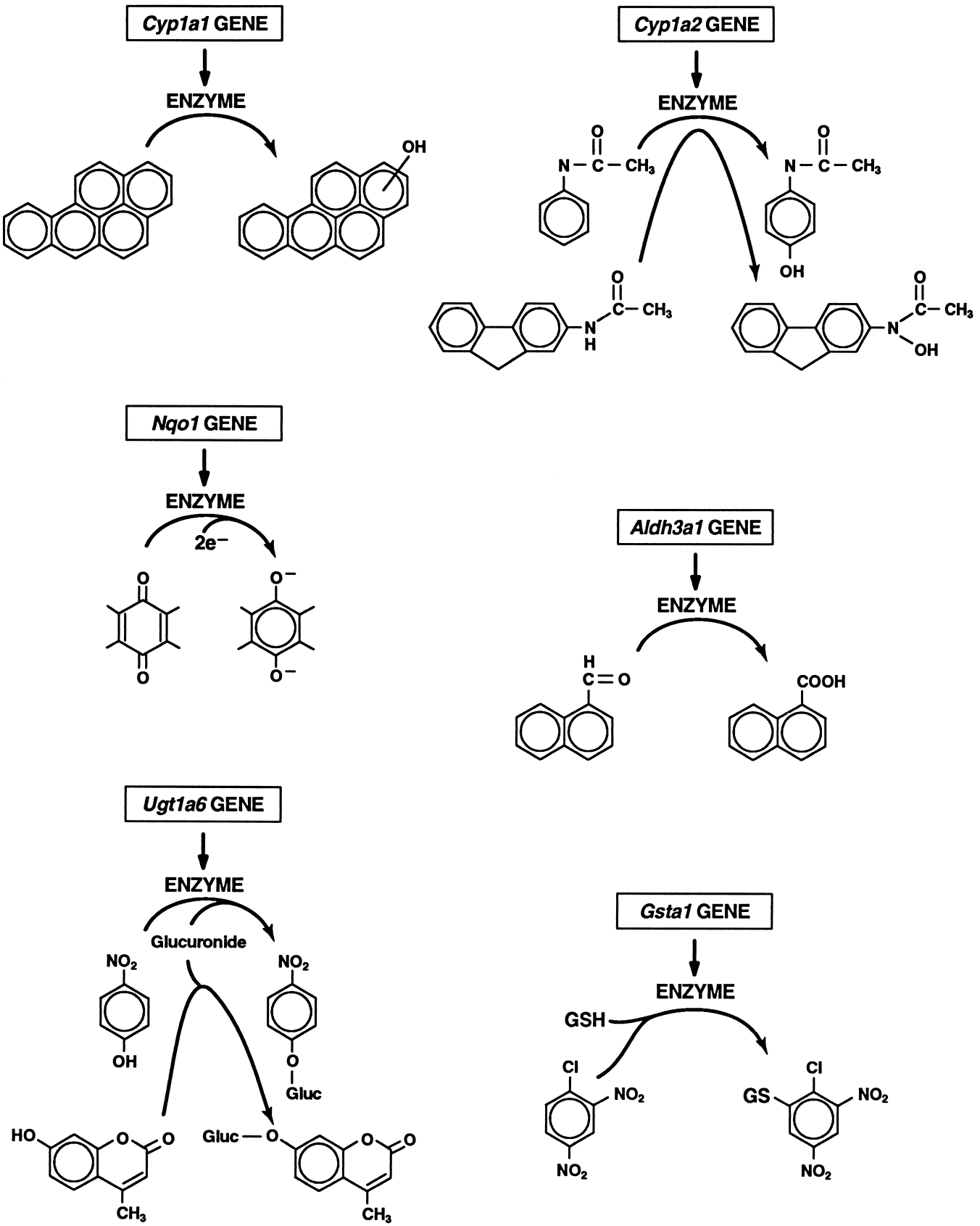


FIG. 3. The commonly performed enzyme assays for the six members of the [*Ah*] battery. CYP1A1 and CYP1A2 substrates include polycyclic hydrocarbons and arylamines, respectively. NQO1 substrates include a variety of quinones, including benzene *ortho-* or *para-*quinone. ALDH3A1 substrates include aldehydes such as naphthaldehyde. UGT1A6 substrates include *p*-nitrophenol and methylumbelliferone. GSTA1 substrates include 1-chloro-2,4-dinitrobenzene.

TABLE 1. CYP1A1 and NQO1 mRNA levels* in control and dioxin-treated cell lines

	Endogenous CYP1A1 mRNA		Endogenous NQO1 mRNA	
Cell line	Control	TCDD	Control	TCDD
wild-type (*wt*)	±	+++	±	++
c2	±	±	±	±
c4	O	O	O	O
c37	+++	+++	++	++
c37 [m1A1]†	±	+++	±	++
c37[h1A2]	±	+++	±	++

*+++, 25- to 100-fold increases, ++, 10- to 25-fold increases. ±, negligible amounts of mRNA but detected over prolonged exposure to the x-ray film. O, no mRNA detected even after prolonged exposure to x-ray film. It should be noted that the C*yp1a2* and G*sta1* genes are expressed at low levels, albeit detectable, in cell culture [3, 28].

†[m1A1] and [h1A2] denote the stable transfection of a functional mouse CYP1A1 cDNA and a functional human CYP1A2 cDNA, respectively, into the *c37* cell line [summarized in part from Refs. 14, 26].

Aromatic **H**ydrocarbon **R**esponse **E**lement (AHRE)
[also called "xenobiotic, dioxin response element" (XRE, DRE)]

5'-GCGTG-3'
3'-CGCAC-5'

Electro**p**hile **R**esponse **E**lement (EPRE)
[also called "antioxidant response element" (ARE)]

5'-TGACNNNGC-3'
3'ACTGNNNCG-5'

Binding to FOS-JUN (AP-1 activity)

5'-TGACTCA-3'
3'ACTGAGT-5'

FIG. 5. Illustration of the invariant core bases in the AHRE, EPRE, and AP-1-binding DNA motifs.

therefore decreased. Structural genes in the deleted region (Table 3) show levels of the mRNA or enzyme in the *ch*/*14CoS* heterozygote that are intermediate between that found in the *ch*/*ch* wild-type and the *14CoS*/*14CoS* homozygote. These data are expected for any structural gene product (i.e. a gene–dose effect for mice having two alleles, one allele, or no alleles of the deleted region on Chr 7).

Just as activities were found to be decreased in *14CoS*/*14CoS* mice (Table 2), this laboratory found that a number of activities are increased. In contrast to the *ch*/*14CoS* heterozygote and *ch*/*ch* wild-type (having the same levels), the *14CoS*/*14CoS* mouse or the SV40-immortalized hepatocyte cell line exhibits elevated mRNA, enzyme activities, and/or other parameters of the following: NQO1 but not CYP1A1 or CYP1A2 [44]; three *Gadd* genes [45]; superoxide dismutase, glutathione reductase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase activities [46]; UGT1A6 and ALDH3A1 mRNAs [47]; amount of protein binding to the EPRE DNA motif [47]; the Group II

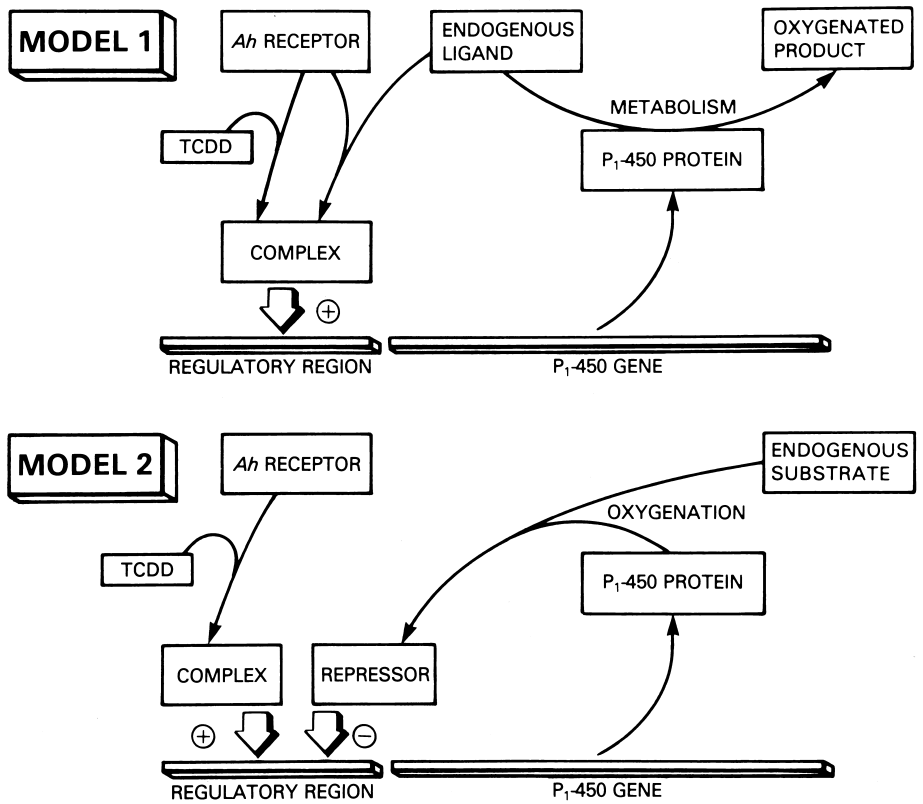


FIG. 4. Two models that could explain the data summarized in Table 1. The P_1-450 (*Cyp1a1*) gene product CYP1A1 might degrade the putative EL (*top*) in Model 1. The P_1-450 (*Cyp1a1*) gene product CYP1A1 might activate an endogenous substrate to a putative repressor (*bottom*) in Model 2 [14, 15].

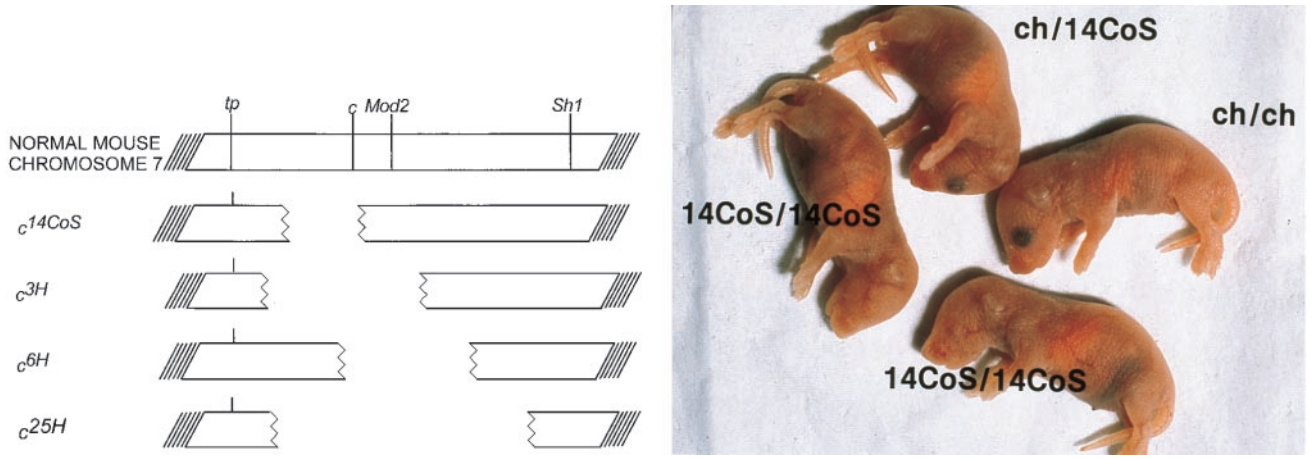


FIG. 6. (A) Complementation map of several mouse lines having radiation-induced albino deletions [modified from Refs. 40, 42]. Chr 7 loci include *tp* (taupe), *c* (albino), *Mod2* (mitochondrial malic enzyme), and *Sh1* (shaker-1). Of the more than 60 radiation-induced albino deletion lines [41], c^{25H} has the largest deleted region and c^{14CoS} has the smallest deleted region; the c^{25H}/c^{25H} homozygote dies at the 2-cell stage, whereas the c^{14CoS}/c^{14CoS} homozygote dies during the first 24 hr following birth. (B) Appearance of the *ch/ch* wild-type, *ch/14CoS* heterozygote, and *14CoS/14CoS* homozygote several hours following birth. Note that the *ch/14CoS* exhibits about half as much eye pigment as the *ch/ch* wild-type, and of course there is no pigment in the *14CoS/14CoS*; this pigment difference can be detected by gestational day 10 *in utero* at the time development of the eye has just begun.

Ca^{2+}-dependent secreted 14-kDa form of phospholipase A_2, increased arachidonic acid release, and elevated prostaglandins D_2, E_2, and $F_{2\alpha}$ [48]; and enhanced levels of NADPH and GSH concentrations [49]. All these findings are consistent with a striking oxidative stress response in the untreated *14CoS/14CoS* homozygote (Fig. 7). *Cyp1a1* and *Cyp1a2* are not activated in *14CoS/14CoS* mice [44]. These data are consistent with several studies which have shown that CYP1A1 and CYP1A2 are down-regulated by oxidative stress [51–53].

CURRENT UNDERSTANDING OF OXIDATIVE STRESS

"Oxidative stress" is defined as oxygen- or free radical-mediated damage in living organisms [reviewed in Ref. 37]. Atmospheric oxygen is dissolved in the cellular milieu and reduced to water in virtually all living organisms (Fig. 8). Diatomic oxygen, if reduced in three 1-electron steps, goes through several reactive and toxic intermediates before reaching water. Oxidases (e.g. mitochondrial cytochrome oxidase) can add 4 electrons quite efficiently during energy production, but (due to less-than-perfect "coupling") some of these reactive intermediates become inevitable byproducts. Monooxygenases (e.g. cytochromes P450) can efficiently "detoxify" diatomic oxygen to water, with little release of these reactive oxygen species or intermediates (ROS, ROIs) unless there is inefficient coupling of the P450–substrate complex with the NADPH–P450 oxidoreductase and/or cytochrome b_5 [54–56]. Superoxide dismutase reduces $\cdot O_2^-$ (formed as the result of redox cycling) to hydrogen peroxide in a 1-electron (Fig. 7), and peroxidases and catalase are able to reduce H_2O_2 to water in a 2-electron step. When these violently unstable and toxic intermediates (Fig. 8) react with intracellular macromolecules, damage can occur to cellular nucleic acids and proteins, including mutations and abnormal degradation of macromolecules. Small-molecular-weight molecules (M_r 250 ± 220) in metabolic pathways often include highly reactive electrophiles, or can become highly reactive electrophiles because of the above-mentioned ROS (Fig. 8), and these are termed ROMs. It has been demonstrated that Michael reaction acceptors, molecules having an electron-withdrawing heteroatom beta to, and in resonance, with a double bond where a nucleophilic attack can

TABLE 2. Expression differences due to putative regulatory gene(s) in deleted region of Chr 7

(*14CoS/14CoS* ≪ *ch/ch* = *ch/14CoS*)*

- Glucose-6-phosphatase
- Tyrosine aminotransferase
- Phosphoenolpyruvate carboxykinase
- Metallothionein
- Albumin
- α-Fetoprotein
- Transferrin
- CCAAT enhancer α-binding protein
- Hepatocyte nuclear factor 1α
- HNF-4α

*These activities or proteins are strikingly decreased in the *14CoS/14CoS* mouse, as compared with the *ch/14CoS* heterozygote or *ch/ch* wild-type that are not different from one another [40–42].

TABLE 3. Structural genes different between the *14CoS/14CoS*, *ch/14CoS* and *ch/ch* mouse*

- Tyrosinase (*c* locus)
- Glutamine synthetase (*Gs*)
- Serine dehydratase (*Sd*), corticosteroid-induced form(?)
- Fumarylacetoacetate hydrolase (*Fah*)

*The enzyme levels in the *ch/14CoS* heterozygote are intermediate between that in the *14CoS/14CoS* (where no activity is found) and the *ch/ch* wild-type [40, 43].

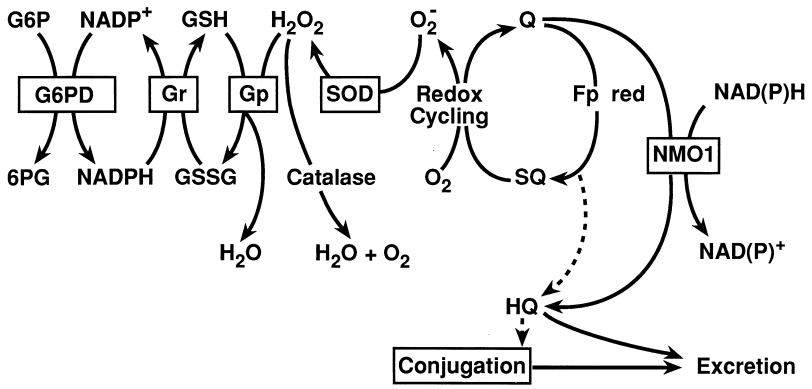


FIG. 7. Diagram of the major pathways by which the cell responds to reactive oxygenated metabolite (ROM)-mediated oxidative stress [modified from Refs. 46, 50]. G6P, glucose 6-phosphate. 6PG, 6-phosphogluconate. GSH and GSSG, reduced and oxidized glutathione, respectively. Q, quinones and related Michael reaction acceptors. SQ, semiquinones. Fp red, the 1-electron flavoprotein reductases. HQ, hydroquinones. G6PD, G6P dehydrogenase. Gr, GSSG reductase. Gp, glutathione peroxidase. SOD, Cu,Zn-superoxide dismutase. NQO1 (formerly called NMO1), quinone 2-electron reductase. "Conjugation" refers to UGT1A6 and GSTA1 activities. *Boxes* denote enzyme activities that were found to be elevated in liver of the untreated *14CoS/14CoS* newborn mouse.

easily occur (Fig. 9), induce EPRE-driven genes; moreover, the potency of electrophilicity of the Michael reaction acceptors is directly correlated with the potency of this induction process [57].

OXIDATIVE STRESS AND THE TYROSINE DEGRADATION PATHWAY

What gene is it, then, located in the deleted region of Chr 7 of the untreated *14CoS/14CoS* mouse, that leads to an oxidative stress response (Fig. 10)? This gene does not appear to act on *Cyp1a1* or *Cyp1a2*. A decade ago [42], we had considered three possibilities: 1) a repressor gene (which represses expression of all EPRE-regulated genes); 2) an activator gene (which activates the putative repression mechanism); or 3) a housekeeping gene (which, when absent, causes endogenous ROM-mediated oxidative stress). The last of these three possibilities has proven to be correct. It was found that oxidative stress in the *14CoS/14CoS* mouse was due to disruption in the *Fah* gene located at the proximal edge of the Chr 7 deleted region. Two lines of evidence support this conclusion. First, the *Fah*(−/−) knockout mouse line was shown to exhibit high levels (up-regulation) of *Nqo1* and *gadd153* [58]. Second, *14CoS/14CoS* mice, into which the *Fah* transgene had been inserted, were demonstrated to express *Nqo1* and *gadd153* at (low constitutive) wild-type levels [59].

How does absence of the *Fah* gene lead to oxidative stress? Disruption of this gene leads to the accumulation of potent electrophiles, which can cause ROM-mediated endogenous oxidative stress [47, 58]. Absence of the enzyme FAH, which participates in the tyrosine degradation pathway (Fig. 11), causes an accumulation of fumarylacetoacetate (FAA), maleylacetoacetate (MAA), succinylacetoacetate (SAA), and succinylacetone (SA), the first two of which are Michael reaction acceptors of varying potency, thereby leading to endogenous ROM-mediated oxidative stress in the untreated *14CoS/14CoS* newborn liver as well as the hepatocyte cell line.

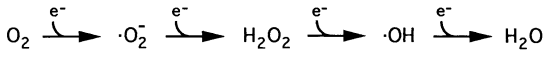


FIG. 8. Intermediates from normal metabolism involving diatomic oxygen from the atmosphere. The formation of $\cdot O_2^-$, H_2O_2, and ·OH and H_2O occurs by successive 1-electron reductions.

HEREDITARY TYROSINEMIA TYPE I (HT1)

In humans, FAH deficiency causes HT1, a severe autosomal recessive inborn error of metabolism in which children die at 1–2 years of age [60]. Given the evidence discussed in this review, we strongly believe that death in these children occurs, at least in part, due to pathologically elevated levels of ROM-mediated oxidative stress causing apoptosis. SA (Fig. 11) is the hallmark metabolite of HT1, usually measurable in plasma and urine but often not detected in human liver [61]. SA was looked for in *14CoS/14CoS* newborn liver [47, 62] and the *14CoS/14CoS* cell line [47], and highly elevated levels were not detected, presumably due to the reactive and labile properties of this metabolite. Many of the defects and the symptoms in HT1 and the *14CoS/14CoS* mouse are similar, while some differ [*reviewed in* Ref. 58]. For example, there is evidence for depletion of intracellular reducing compounds such as GSH in red cells of HT1 patients [63]; in contrast, GSH levels are approximately three times higher in the *14CoS/14CoS* than in the *ch/ch* cell culture line [49]. Accumulation of FAA and MAA, which cause endogenous ROM-mediated oxidative stress, triggers apoptosis, as demonstrated by the release of cytochrome *c* from mitochondria and prevention of apoptosis by caspase inhibitors [64, 65]. Of the four tyrosine degradation metabolites described above, FAA appears to be by far the most potent [65, 66].

IMPORTANCE OF REDUCED GLUTATHIONE GSH IN THE CELL'S PROTECTION AGAINST OXIDATIVE STRESS

It now appears clear that reactive foreign chemicals, physical agents such as irradiation, and endogenous ROMs (Fig. 12) are "detected" at or near the cell's outer membrane,

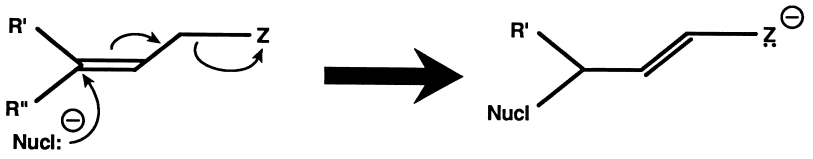


FIG. 9. Illustration of a Michael reaction acceptor. These molecules are olefins containing an electron-withdrawing heteroatom and therefore susceptible to attack by nucleophiles (Nucl:) such as reduced glutathione (GSH) [57].

thereby setting off an elaborate signal transduction cascade leading to the activation of transcription factors important in the cell's decisions about division, apoptosis, growth arrest, and differentiation. It is also clear that GSH can provide protection against all forms of oxidative stress in at least two ways (Fig. 12A): 1) upon the earliest moments of oxidative damage, GSH can act at the source of this impairment by scavenging free radicals and electrophiles (like a sponge, mopping up reactive chemicals); 2) once the oxidative stress signal transduction cascade is in full swing, GSH acts via the transcription factor redox factor-1 to reduce critical cysteine groups of transcription factors, thereby helping activate many genes that function to quench the oxidative damage. Although the FOS–JUN complex is a particularly good example (Fig. 12A) for combating oxidative stress, we anticipate that GSH will be shown to be critical in the reduction maintenance of numerous additional transcription complexes associated with the oxidative stress response [109].

GSH is the most abundant cellular thiol and plays an important role in many types of bioreduction and conjugation reactions [reviewed in Refs. 37, 110]. GSH is a tripepetide synthesized by sequential enzymic reactions (Fig. 13); first, glutamate and cysteine are ligated by glutamate–cysteine ligase (GCL) to form γ-glutamylcysteine and second, γ-glutamylcysteine is ligated to glycine by GSH synthase to form the final product. The biosynthetic step catalyzed by GCL is the rate-limiting step in glutathione biosynthesis. The GCL holoenzyme is actually a heterodimer composed of a structural subunit (GCLS) and a regulatory subunit (GCLR). Whereas GCLS itself is capable of synthesizing γ-glutamylcysteine, the presence of GCLR in the holoenzyme dramatically enhances the inhibition constant (K_i) of GSH more than 10-fold, rendering the enzyme less sensitive to feedback inhibition (Fig. 13) by GSH [111]. Hence, clarification of the regulation of *Gcls* and *Gclr* gene expression, and any posttranscriptional modifications and interactions between the GCLS and GCLR proteins that might occur, is pivotal to our understanding of the maintenance of intracellular GSH concentrations and the cell's response to oxidative insult. For these reasons, this laboratory has been developing conventional

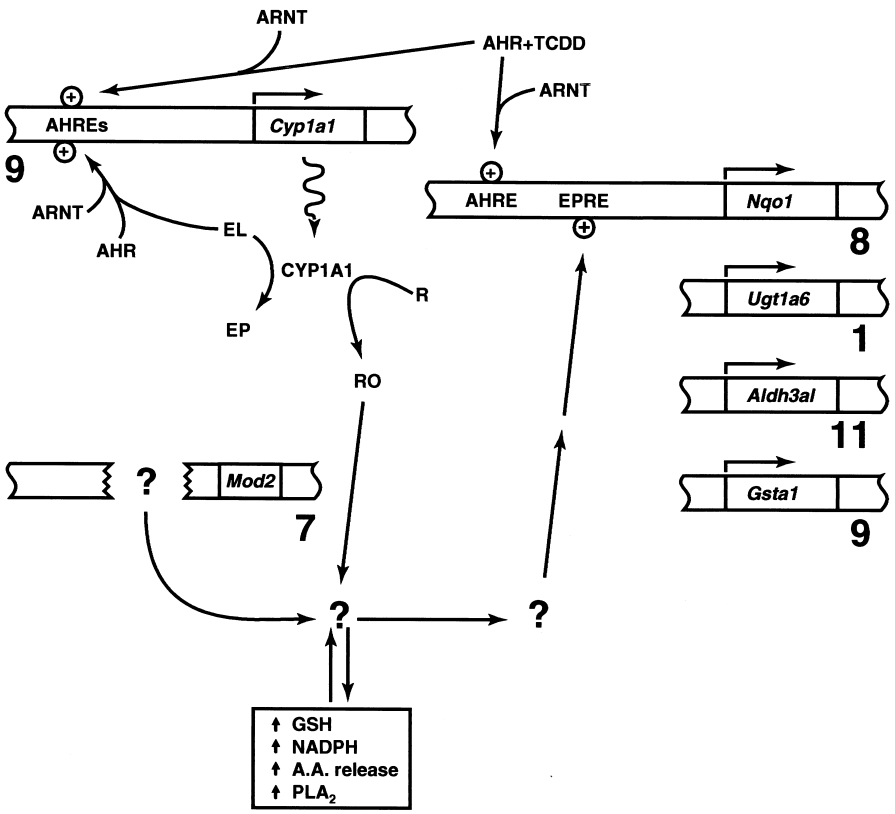


FIG. 10. Summary of the [*Ah*] battery to this point of discussion in the text. The EL or foreign chemical ligand dioxin (TCDD) activates the AHR by binding to it. A heterodimer with ARNT is then formed, which binds to the AHREs in regulatory regions of all dioxin-inducible genes. CYP1A1 (and CYP1A2, not shown) down-regulate AHR activation by degrading EL to an endogenous product (EP). A gene(s) missing because of the deletion of 3800 kb on Chr 7 in *14CoS/14CoS* mice, and the oxidative metabolism of endogenous and exogenous substrates (R) by CYP1A1/1A2 leading to the formation of reactive oxygenated metabolites (RO), both cause an oxidative stress signal (denoted by *question mark*). Part of the response is to increase intracellular GSH and NADPH levels, enhance arachidonic acid (A.A.) release, and induce phospholipase A_2(PLA_2). Another part of the cell's response is to increase EPRE-binding proteins and up-regulate transcription of the EPRE-driven genes such as the *Nqo1*, *Ugt1a6*, *Aldh3a1*, and *Gsta1* genes. The *large bold numbers* denote the mouse Chr on which each of these unlinked genes is found. *Mod2*, mitochondrial malic enzyme.

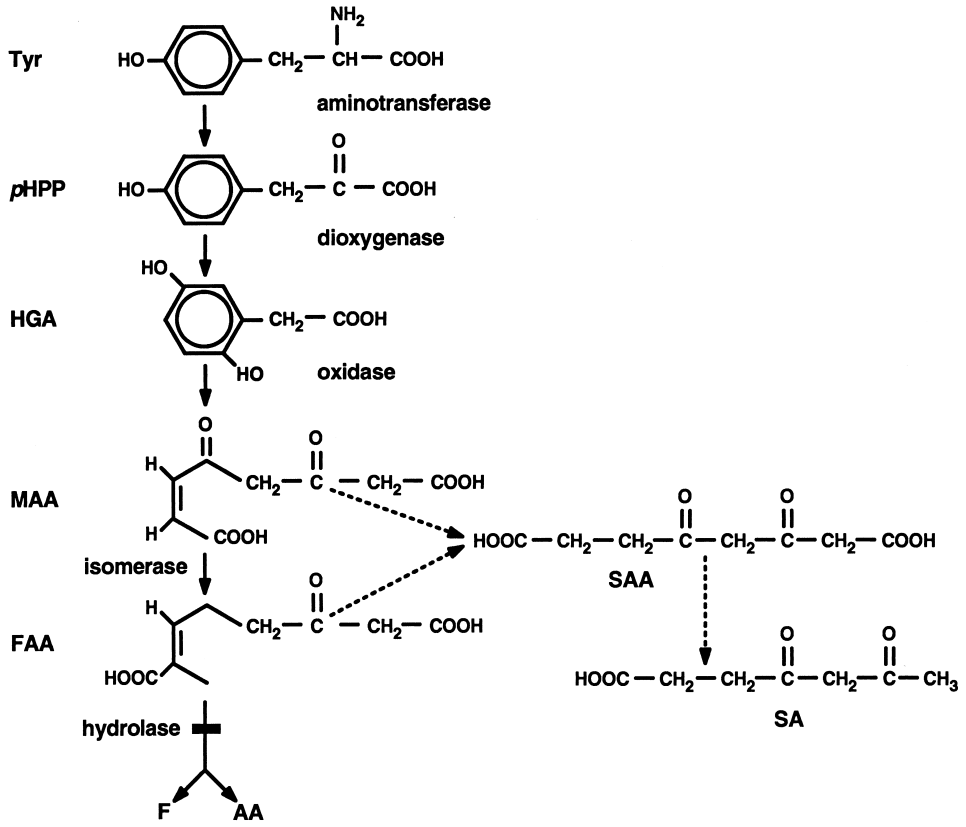


FIG. 11. Tyrosine degradation pathway. Tyr, tyrosine. *p*HPP, *p*-hydroxyphenylpyruvate. HGA, homogentisic acid. MAA, maleylacetoacetate. FAA, fumarylacetoacetate. F, fumarate. AA, acetoacetate. SAA, succinylacetoacetate. SA, succinylacetone. *Dashed lines* denote metabolic steps in which the exact enzymes and mechanisms involved are not known. FAA hydrolase, encoded by the *Fah* gene, has been shown to be absent in the *14CoS/14CoS* mouse [43] and is defective in human hereditary tyrosinemia type I [60].

and conditional *Gcls*(−/−) and *Gclr*(−/−) knockout mouse lines; because of the critical importance of GCLS and GCLR to the entire redox signal transduction pathway, we would predict that the conventional *Gcls*(−/−) and *Gclr*(−/−) knockout mouse lines might be lethal *in utero*.

ROLE OF TRP53 IN OXIDATIVE STRESS, THE CELL CYCLE, AND APOPTOSIS

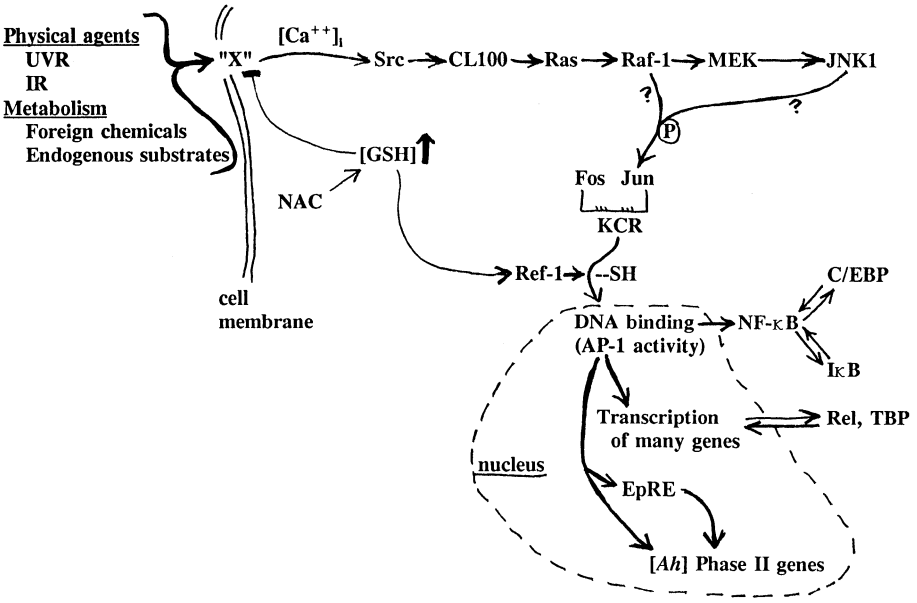
The cell cycle is reviewed in Fig. 14. At or near the G_1/S boundary and at or near the G_2/M boundary, the cell appears to choose between apoptosis and continuation with the cell cycle, due to the cell's response to local signals that are not yet clear. The p53 protein TRP53 plays a major role in cell cycle control. TRP53 exists in four distinct forms (Fig. 15A). DNA damage and intracellular oxidative stress will change the complexed form to the antiproliferative form and can change the antiproliferative form to the mutant form [112]. It is the antiproliferative form that functions in G_1/S (and probably also G_2/M) arrest, and viral proteins that bind to the antiproliferative form (Fig. 15B) promote formation of the complexed form of TRP53 wherein cell division rather than apoptosis is encouraged. TRP53-dependent apoptosis has been suggested to occur via a 3-step process [116]: the transcriptional up-regulation of *Nqo1*, *Gsta1*, and other redox-related genes; the formation of ROM-mediated oxidative stress; and the oxidative degradation of mitochondrial components (see below).

DISCREPANCIES BETWEEN NEWBORN LIVER AND SV40-TRANSFORMED CELL LINES

Interestingly, some of the parameters listed above [28, 44–49]—which increased in *14CoS/14CoS* but not in the *ch/14CoS* heterozygote or *ch/ch* wild-type—occur in both newborn liver and the SV40 transformed hepatocyte cultures [117], whereas other parameters occur in either newborn liver or the hepatocyte cell line. A likely explanation for these discrepancies concerns the SV40 large T antigen, known to interact with cell cycle proteins such as the p53 protein (Fig. 15B) and the RB1, which is expressed in the SV40-transformed *14CoS/14CoS* and *ch/ch* cell lines, but not of course in newborn liver of the corresponding mouse lines [28]. Further evidence in support of this possibility is presented below.

INTRINSIC INTERACTION BETWEEN THE [*Ah*] BATTERY GENES AND AHR IN OXIDATIVE STRESS

Figure 16 summarizes our latest understanding of the [*Ah*] gene battery. Both foreign chemicals that are readily oxygenated (e.g. benzo[*a*]pyrene) and the putative endogenous ligand are degraded by CYP1A1 (and CYP1A2, not shown). CYP1A2 is expressed in fewer tissues than the ubiquitously expressed CYP1A1 [118]. Beyond the scope of this review, CYP1B1, which metabolizes polycyclic aromatic hydrocarbons and has its own unique cell-specific


Physical agents
UVR
IR
Metabolism
Foreign chemicals
Endogenous substrates
"X"
[Ca++]i
Src
CL100
Ras
Raf-1
MEK
JNK1
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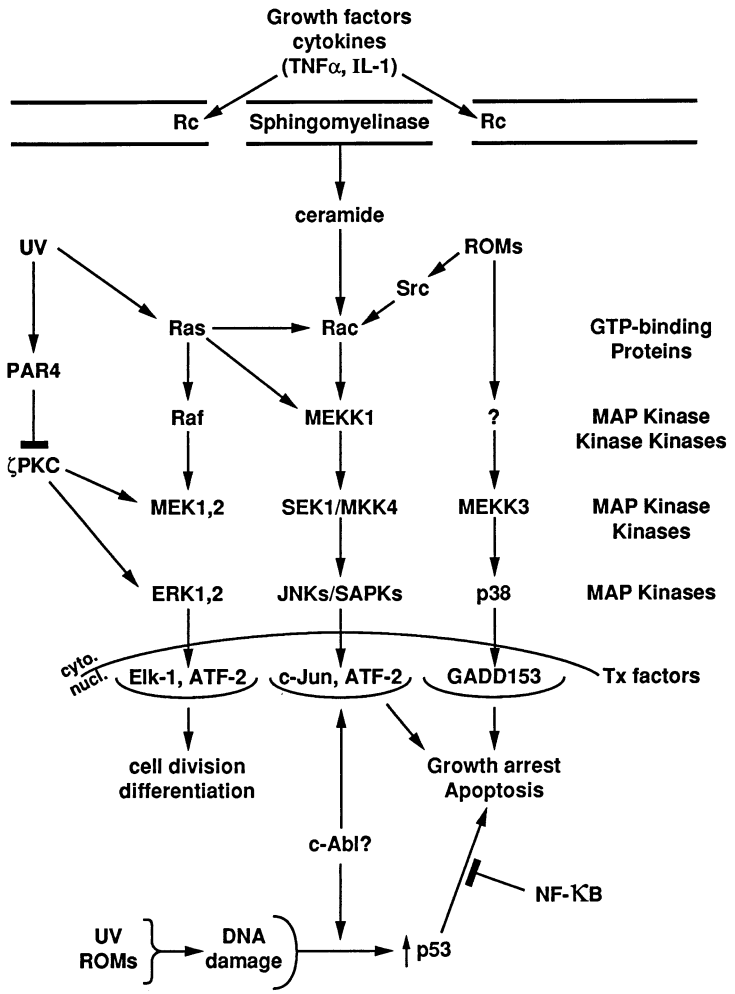
P
[GSH]
NAC
Fos Jun
KCR
Ref-1
--SH
cell membrane
C/EBP
DNA binding (AP-1 activity)
NF-κB
IκB
Transcription of many genes
Rel, TBP
nucleus
EpRE
[Ah] Phase II genes


Growth factors
cytokines
(TNFα, IL-1)
Rc
Sphingomyelinase
Rc
ceramide
UV
ROMs
Src
Ras
Rac
GTP-binding Proteins
PAR4
Raf
MEKK1
?
MAP Kinase Kinase Kinases
ζPKC
MEK1,2
SEK1/MKK4
MEKK3
MAP Kinase Kinases
ERK1,2
JNKs/SAPKs
p38
MAP Kinases
cyto.
nucl.
Elk-1, ATF-2
c-Jun, ATF-2
GADD153
Tx factors
cell division
differentiation
Growth arrest
Apoptosis
c-Abl?
NF-κB
UV
ROMs
DNA damage
p53

expression, is presumably a "member" of the [*Ah*] battery [119].

CYP1A1/1A2 metabolism of exogenous and endogenous substrates invariably leads to ROM-mediated oxidative stress (Fig. 16). Dioxin is extremely potent because of its negligible metabolism by CYP1A1/1A2 or any other enzyme. Dioxin has been shown to cause chronic, sustained oxidative stress in the whole animal [120]. Depending upon the concentration of any foreign chemical AHR ligand or the EL, the AHR is activated, leading to the AHR–ARNT heterodimerization and up-regulation of all AHRE-mediated genes. Interestingly, a recently characterized AHR repressor (*Ahrr*) gene is also governed by the AHR–ARNT heterodimer [121], leading to negative feedback control.

The *Nqo1*, *Aldh3a1*, *Ugt1a6*, and *Gsta1* genes (and probably numerous others) are up-regulated by AHRE- as well as EPRE-mediated pathways as part of the cell's oxidative stress response (Fig. 16). NQO1 offers a 2-electron reduction of quinones [122], which is much safer for the cell than the 1-electron redox cycling (Fig. 7) that promotes the formation of reactive oxygen species and ROMs. ALDH3A1 oxidizes aldehydes to ketones and carboxylic acids, thereby detoxifying damaging electrophilic aldehydes such as 4-hydroxy-2-nonenal, an end-product of intracellular lipid peroxidation, which also induces H_2O_2 production [123]. Peptide aldehydes inhibit the degradation of I-κBα and prevent nuclear factor-kappaB (NF-κB) activation [124]; activation of NF-κB would be another potential mechanism by which ALDH3A1 might participate in blocking the oxidative stress response and in affecting the cell cycle. UGT1A6 and GSTA1 both can conjugate (i.e. adding glucuronide or GSH, respectively) toxic phenols and quinones, thereby decreasing the amount of oxidative damage to the cell. Conjugation of endogenous substrates by these enzymes is likely (see below).

Finally, cytokines such as interleukin-2 and tumor necrosis factor-α [99, 125] are another source of oxidative stress (Fig. 16). We postulate that the EL for AHR is affected by certain cytokines; for example, it is possible that the EL participates in the arachidonic acid cascade [48; reviewed in Ref. 126]. Cytokines, as well as other causes of oxidative stress, have also been found to down-regulate *Cyp1a1* gene expression through an NF1 (nuclear factor-1) C/CTF domain [53]. It is possible that *Cyp1a2* gene expression might be affected similarly.

Dioxin appears to cause the sustained activation of NF-κB and AP-1 by a signal that can be blocked by antioxidants and which is dependent upon both CYP1A1 and the AHR–ARNT complex [127]. Could this "signal" represent the EP?

From Fig. 16, it becomes obvious that CYP1A1/1A2 and

⩽

FIG. 12. (A) The oxidative stress signal transduction pathway, from an unknown molecule ("X") at the cell surface, to the nucleus. The moieties inside the *dashed line* illustrate those events occurring in the nucleus. It is likely that every P450 enzyme can metabolize foreign as well as endogenous chemicals to form ROMs, e.g. CYP1A1/1A2 [67] and CYP2E1 [68]. UVR, UV irradiation. IR, ionizing irradiation. $[Ca^{2+}]_i$, intracellular calcium ion. NAC, *N*-acetylcysteine. (P), phosphorylation. KCR, Lys-Cys-Arg region critical for DNA binding to AP-1 sites. "X" denotes the as-yet-unknown "receiving molecule," believed to be localized at or near the plasma membrane [69]. Sufficient levels of GSH, which can be enhanced by NAC treatment, can quench the incoming oxidative stress signal [70, 71]; other forms of reducing agents and free radical scavengers probably would also be involved in quenching this incoming signal [72]. Following increases in the flux of intracellular calcium, the subsequent steps in the pathway and their approximate order include a series of phosphorylations and dephosphorylations: *Src* tyrosine kinase(s) [70]; *CL100*, a Tyr/Ser phosphatase [73]; *Ras* and *Raf-1* [74–76]; nitrogen-activated protein kinase kinase and JNK1, a distant relative of the MAP (mitogen-activated protein) kinase group [75, 77]. This cascade results in the phosphorylation of the Jun protein, heterodimerization of Fos and Jun, and binding of the Fos–Jun heterodimer to DNA at AP-1 binding sites, causing increases in AP-1 activity [75, 78–81]. Sufficient levels of GSH are needed in order for the redox factor (Ref-1 protein) to maintain the cysteinyl sulfhydryl group in the critical KCR domains of the Fos and Jun proteins, thereby enabling the heterodimer to bind to AP-1 binding sites [82–84]. Increases in AP-1 activity lead to activation of NF-κB [85, 86], the levels of which associate with C/EBP family members [87, 88] and I-κB [89, 90]. Additional transcription factors that become activated include members of the *Rel* superfamily and TATA box-binding protein (TBP) [91]. Quite remarkably, most of this cascade, comprising more than 15 components as illustrated here, can occur in cells without DNA or without a nucleus [92]. It must be emphasized that this pathway is overly simplified; numerous other steps could be added. For example: NF-κB might be directly activated by UV light without going through all the illustrated steps [85]; the response to UVR appears to involve several growth factor receptors [69]; I-rel [93] and the proto-oncogene *Bcl-3* [94] can act as specific inhibitors of NF-κB binding to DNA; casein kinase II is a negative regulator of Jun binding to DNA and therefore of AP-1 activity [95]; c-*fos* expression can depend on protein kinase C activity [96]; tumor necrosis factor-alpha and interleukin-1 can phosphorylate IκB, thereby turning on NF-κB [97]; AP-1 activity is also activated via extracellular signal-regulated kinases (ERKs) by phorbol 12-myristate 13-acetate and serum, as well as by Ha-*ras* [98]; interleukin-1β and reactive oxygen species mediate JNKs by two independent pathways [99]; prostaglandin A_1 can increase I-κBα expression [100]; the role of NF-κB in the oxidative stress has been recently reviewed [101]. (B) The "fundamental" oxidative stress signal transduction pathway, showing the circuitry and cross-talk among members in at least three more-or-less parallel pathways. Basically, the cascade progresses from GTP-binding proteins to mitosis-associated protein (MAP) kinase kinase kinases to MAP kinase kinases to MAP kinases to the activation of transcription (Tx) factors; depending upon the ratios of Tx factor concentrations in the nucleus, the cell appears to choose between continuation with the cell cycle (i.e. division), differentiation, growth arrest, and apoptosis. UV damage activates PAR4 and Ras, ROMs activate both Src and MEKK3, while both UV and ROMs via DNA damage can cause p53-dependent apoptosis. This pathway is also overly simplified, and numerous other steps could be added. For example: ceramide functions as a transducer of signals in a generalized stress–response pathway in which apoptosis is only one potential outcome [102]; a family of stress-inducible GADD45-like proteins mediate the activation of MEKK4 [103]; transcriptional cross-talk between NF-κB [104] and p53 is involved in the cell's decision as to whether or not to proceed with apoptosis [105]; and there might be five or more parallel pathways with crossovers [106], instead of the three shown here [modified from Refs. 107, 108].

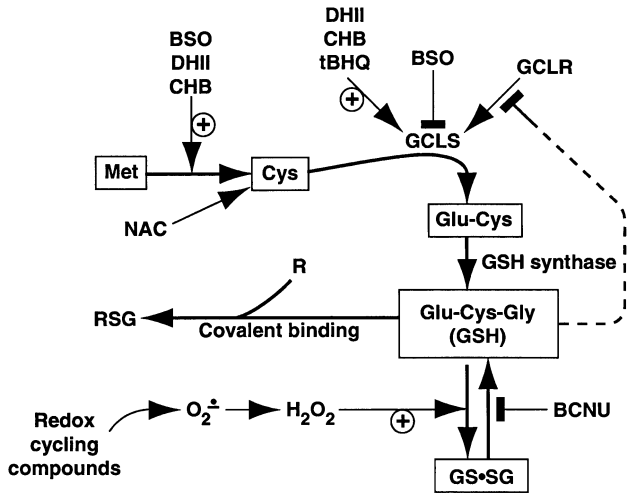


FIG. 13. Pathway of GSH production and degradation. BSO, L-buthionine-(*S,R*)-sulfoximine. DHII, 5,10-dihydroindeno[1,2-*b*]indole. CHB, cyanohydroxybutene. tBHQ, *tert*-butylhydroquinone. GCLS, glutamate-cysteine ligase structural unit. GCLR, glutamate-cysteine ligase regulatory unit. Met, methionine. Cys, cysteine. Glu, glutamic acid. Gly, glycine. NAC, *N*-acetylcysteine. BCNU, *bis*-chloronitrosourea. GS · SG, oxidized glutathione. GSH itself is an allosteric feedback inhibitor of GCLS, whereas BSO inhibits, DHII and CHB (or their metabolites?) enhance, and tBHQ itself directly stimulates, GCLS activity. Redox cycling compounds lead to GSH oxidation to GS · SG. Physiologic events and chemicals that cause redox cycling include reperfusion injury, quinones (e.g. menadione), quinoneimines (e.g. acetaminophen), bipyridinium compounds (e.g. paraquat), and metals (e.g. iron). Classes of compounds (R) that bind covalently with GSH (RSG) include epoxides (e.g. styrene epoxide), halogenated compounds (e.g. phosgene, bromobenzene), free radicals (e.g. carbon tetrachloride), and Michael reaction acceptors (e.g. phorone, diethylmaleate, quinones, quinoneimines).

the AHR must have an intricate and continuous interaction, even in the absence of foreign chemical inducers. Anything causing a build-up in EL concentrations will activate the AHR, leading to induction of the *Cyp1a1/1a2* genes and oxidative stress, but also degradation of excess EL. Excessively activated AHR will also be quenched by the AHR repressor. Treatment of the cell with an exogenous inducer (e.g. dioxin, benzo[*a*]pyrene) would simply overwhelm the action of the EL but act as an agonist on *Cyp1a1/1a2* induction and cause much larger increases in oxidative stress. In competition with this are the non-P450 [*Ah*] battery enzymes, which then combat the growing levels of oxidative stress in the cell.

SIGNALS FROM THE NON-P450 [*Ah*] BATTERY GENES THAT AFFECT THE P450 [*Ah*] BATTERY GENES

Metabolism of the EL or foreign chemicals by CYP1A1/1A2 affects expression of the non-P450 [*Ah*] battery genes. There are a number of intriguing reports suggesting cross-talk in the reverse direction. For example, in the untreated *UGT1*-null Gunn rat (i.e. having no UGT1A6 activity), CYP1A1 and CYP1A2 are markedly elevated during the first month of postnatal life [128]. Absence of the *GSTM1* (glutathione *S*-transferase M1) gene is associated with high inducibility of CYP1A1 transcription [129]. Mouse cells lacking the *Gstp1* gene exhibit elevated JNK activity, and expression of GSTP1 returns JNK to basal levels [130]. Could it be that the EP formed by CYP1A1/1A2 (Fig. 16) is further degraded by the non-P450 [*Ah*] battery enzymes? Absence of these downstream activities might lead to a build-up of the EP, and then the EL, thereby activating the P450 [*Ah*] genes in untreated animals. Dioxin, known to cause sustained oxidative stress [120], lowers gluconeogenesis, which in turn leads to hypoglycemia [131]. The oxidatively stressed *14CoS/14CoS* mice (Fig. 7) display hypoglycemia, although the low blood sugar is not the cause of death in the neonatal period [40]. These observations appear to be somehow consistent and might be related to one another. It should also be noted that each [*Ah*] gene is activated at distinctly different levels of foreign chemical inducer—and presumably the same would hold true for the EL. For example, mouse *Cyp1a2* is about 10-fold more sensitive than *Cyp1a1* to induction by 3-methylcholanthrene [132], whereas rat *UGT1A6* is about 1000 times less sensitive than *CYP1A1* to induction by dioxin [133]. Differential sensitivity in the promoter region of each [*Ah*] gene will, of course, be important as to how much oxidative stress might occur at a particular intracellular concentration of EL or foreign chemical ligand of the AHR.

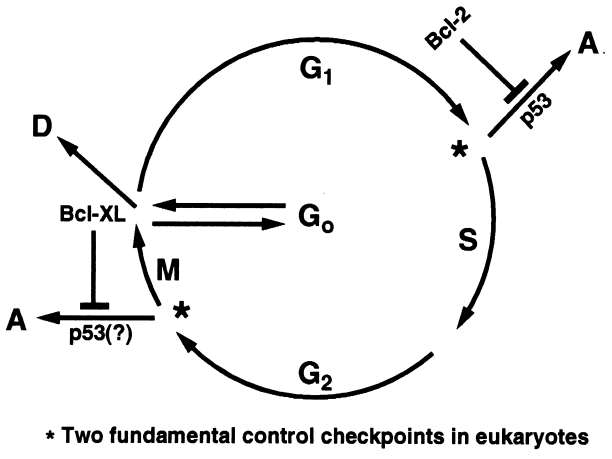


FIG. 14. Diagram of the cell cycle, illustrating the two checkpoints in eukaryotes. G_1 and G_2, two growth phases. Following the G_1 phase, the cell can choose between DNA synthesis during the S phase or apoptosis (A)—part of which is TRP53-dependent and all of which can be prevented by BCL2. Following the G_2 phase, the cell can choose between mitosis during the M phase or apoptosis—part of which appears to be TRP53-dependent and all of which can be prevented by BCL-XL. G_0, growth-arrested cells. D, differentiation.

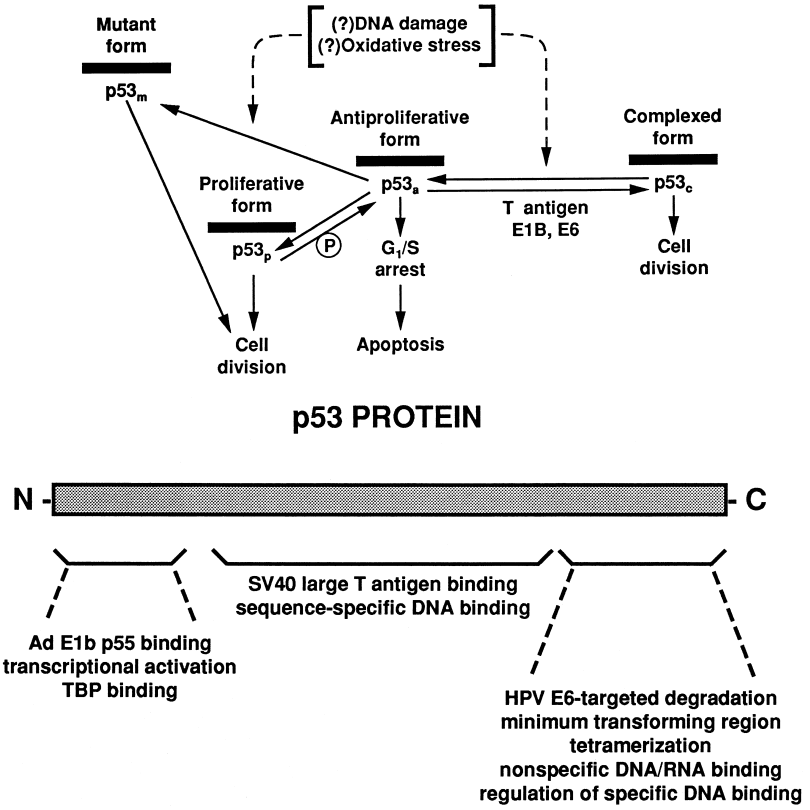


FIG. 15. (A) Regulatory roles of the TRP53 (p53) protein in apoptosis and cell growth. TRP53 is activated by phosphorylation to the antiproliferative form and, under normal circumstances, this form is involved in apoptosis. The mutant form and the proliferative form of TRP53 both lead to cellular proliferation. Ⓟ, phosphorylation. The antiproliferative form of TRP53 can be rendered non-functional by interactions with several viral proteins, which can lead not only to cell survival but proliferation. (B) Diagram of the TRP53 (p53) protein. N, amino terminus. C, carboxy terminus. The various proteins that bind to TRP53 and the functions carried out by such binding are shown below [112, 113]. Ad, adenovirus. TBP, *trans*-activation binding domain. HPV, human polyoma virus. Interestingly, p53 inhibits cyclooxygenase-2 gene expression [114]. We realize that p73 and p63, members of the p53 gene family, also play roles in the decision between apoptosis and cell division but do so more during differentiation [115].

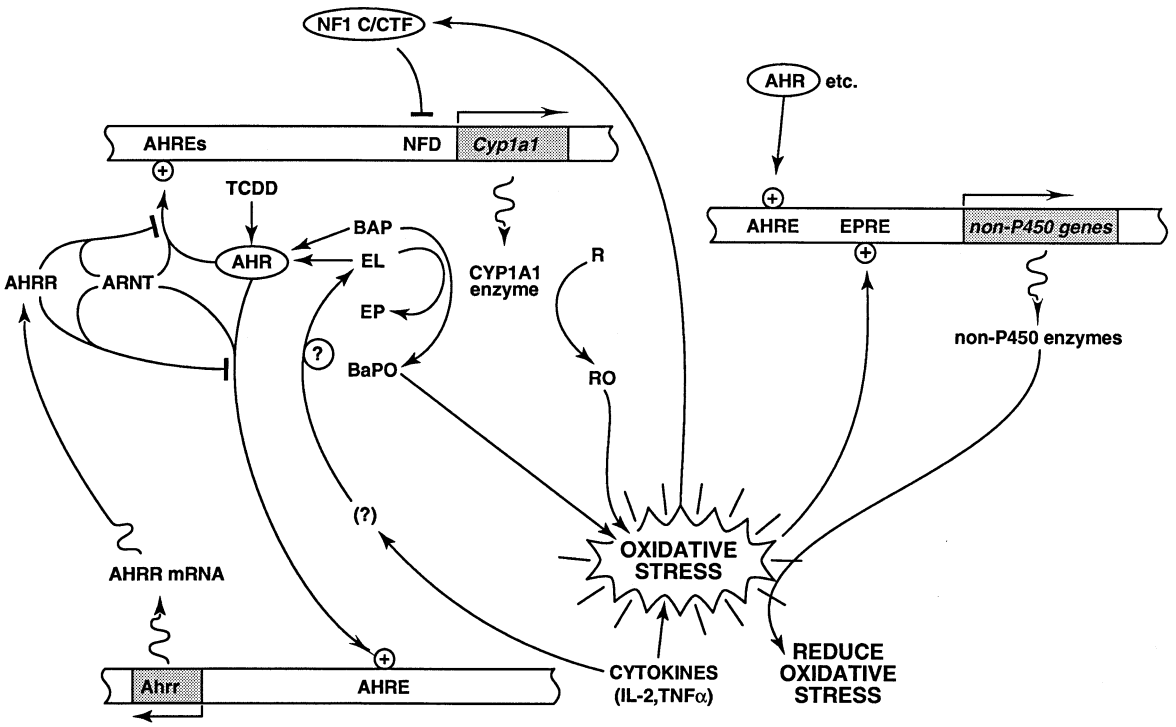


FIG. 16. Latest update of the [*Ah*] gene battery. *Ahrr* and AHRR, AHR repressor gene and protein. BaP, BaP-O, benzo[*a*]pyrene and oxygenated metabolites of BaP. EP, endogenous product. R, all endogenous and exogenous substrates for CYP1A1. IL-2, interleukin-2. TNFα, tumor necrosis factor-α. NF1 C/CTF, nuclear factor-1 transcription protein. NFD, NF1-binding domain. See the Fig. 10 legend and text for further details.

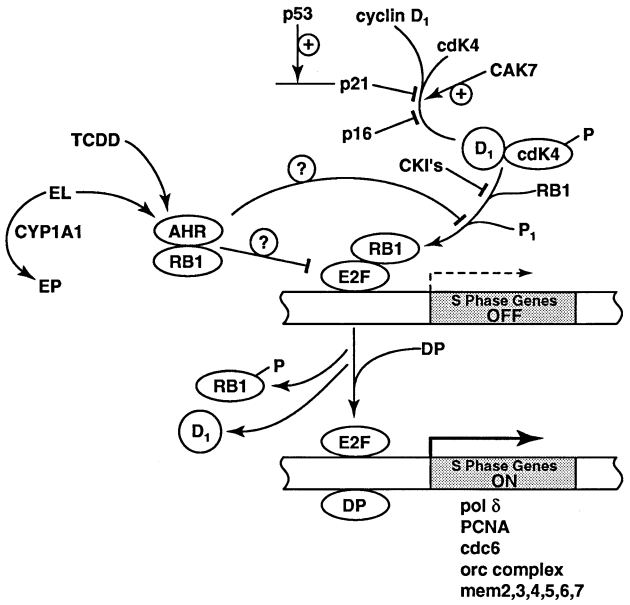


FIG. 17. Scheme of our current understanding of the steps between cyclin D_1 and E2F-mediated transcription of S phase genes [142, 143]. TRP53 (p53) up-regulates TRP21 (p21), which along with p16 can block the phosphorylation of cyclin D_1 by cyclin D_1 kinase-4 (cdK4). Cyclin-dependent kinase inhibitors (CKI's) [144] can block formation of the RB1–E2F complex which binds upstream of S phase genes. Removal of the D_1 phosphate group (P) by D_1 phosphatase (DP) leads to release of both the phosphorylated RB1 and cyclin D_1, following which E2F-mediated transcription of S phase genes (listed at *bottom*) takes place [143]. The precise mechanism by which the AHR–RB1 complex prevents the S phase genes from becoming activated [145] is not fully understood. CAK7, cdk-activating kinase; PCNA, proliferating cell nuclear antigen.

INTERACTION BETWEEN THE [*Ah*] BATTERY GENES AND AHR IN THE CELL CYCLE

It is now clear that the AHR plays one or more pivotal roles in cell cycle regulation [127, 134–138] and apoptosis [139, 140]. The AHR interacts with RB1 [141], and this AHR–RB1 complex appears to prevent the normal progression of G_1 to S phase (Fig. 17) by blocking E2F-mediated transcription of the S phase genes [145]. Therefore, in the presence of sufficient amounts of EL or a foreign chemical ligand such as dioxin or benzo[*a*]pyrene, the activated AHR apparently participates in arrest at the G_1/S boundary. The levels of CYP1A1, and of CYP1A2 in those cell types in which it is expressed, would thus be critical (because they would degrade the EL and foreign chemical ligands of the AHR, other than dioxin) in preventing the AHR from blocking progression from G_1 into the S phase.

Constitutive CYP1A1 mRNA is detectable in the mature oocyte [146], as well as in liver and many other mouse tissues. Twelve hours after fertilization of the ovum, there is a more than a 100-fold increase in constitutive CYP1A1 mRNA levels (Table 4); this dramatic increase completely disappears by the 2-cell stage at gestational day 1.5 (GD 1.5), as well as in the blastocyst at GD 3.5. Because one function of the CYP1A1 enzyme is to remove EL for the AHR (Fig. 16), and the AHR appears to play a role in cell cycle regulation and apoptosis, it seems plausible that this striking abundance of constitutive CYP1A1 mRNA in the fertilized ovum at GD 0.5 might be important for maintaining sufficient amounts of the CYP1A1 enzyme during the transition from maternal to zygotic control (GD 0.5 to GD 1.5 embryo). Availability of catalytically active CYP1A1 would ensure that any (exogenous or endogenous) AHR ligand be rapidly degraded, so that any chance of an AHR–RB1 block in E2F-mediated gene transcription (Fig. 17) would be eliminated during these very critical moments of early mammalian embryogenesis such as the first cell division [146]. If an endogenous AHR ligand is a substrate for the CYP1A1 enzyme, one would expect that expression of the *Ahr* and *Cyp1a1* genes might go hand in hand during early embryogenesis. Indeed, there is evidence that *Ahr* gene expression occurs in the mouse preimplantation embryo [147], and that AHR-mediated *Cyp1a1* promoter activity [148], AHR expression [149], ARNT expression [150], and the combination of AHR–ARNT–CYP1A1 expression [151, 152] occur in many cell types during differentiation. Perhaps not unexpectedly, normal rates of fecundity [153] and dioxin-induced teratogenesis [154] have been shown to require a functional AHR.

TABLE 4. Semi-quantitation by reverse transcription-polymerase chain reaction (RT-PCR) analysis of constitutive CYP1A1 mRNA in oocytes, early embryos, and adult liver

	Liver, Control	Oocyte	Ovum, GD0.5	2-cell, GD1.5	Blastocyst GD3.5
DNA	370	1400	<10	1600	9600
RNA	23	57	200	<10	63
RNA/DNA ratio	**0.06**	**.04**	**>20**	**<0.007**	**<0.007**

Although mRNA was detected by RT-PCR, the CYP1A1 protein or enzyme activity was not examined in this study. The CYP1A1 mRNA (cDNA)-to-DNA ratios for 3-methylcholanthrene-treated liver were >15,000, compared with the other values listed [summarized in part from Ref. 146].

INTERACTION BETWEEN THE [*Ah*] BATTERY GENES AND AHR IN OXIDATIVE STRESS AND APOPTOSIS

As discussed above, it is at or near the G_1/S boundary when the cell decides between apoptosis and continuation with the cell cycle. Exogenous and endogenous signals that cause apoptosis (Fig. 18) include activation of the "death" receptors, decreases in growth factor expression, and oxidative stress [164, 165]. The tumor necrosis factor (TNF-α) receptor-associated protein (TRADD) can signal apoptosis. Even viral infection leads to oxidative stress, lowered GSH levels, and decreases in intracellular pH; activation of heat shock factor-1 (HSF-1) then results in preventing nuclear factor-kappaB activation, leading to down-regulation of inflammatory and viral genes [166]. Oxidative stress can cause DNA damage and, thus, TRP53-dependent apoptosis. Oxidative stress can also perturb ion efflux, cell volume,

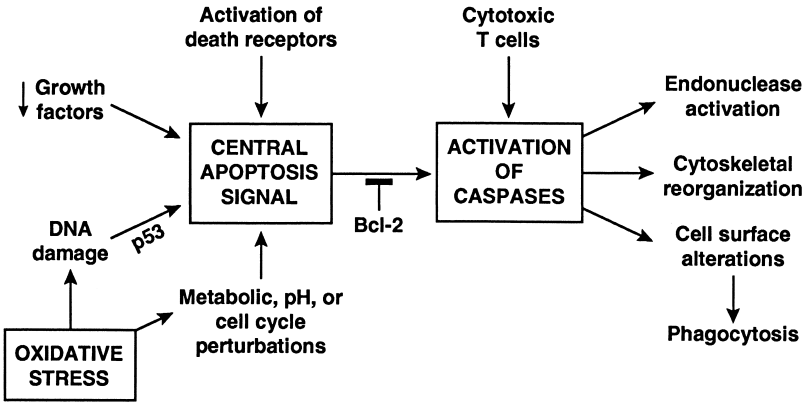


FIG. 18. Simplified diagram of the initial events which trigger a "central apoptosis signal," followed by downstream events such as activation of the caspase cascade, and, finally, endonuclease action, cytoskeletal reorganization, cell surface blebbing, and phagocytosis by neighboring cells [155–158]. Cytochrome *c* release from mitochondria might be the "central signal" to initiate apoptosis [159, 160], and caspase activation then becomes central [161, 162]. Of interest, by activating the MEK/ERK (mitogen-activated protein kinase kinase/extracellular signal-regulated kinase) pathway (Fig. 12B), B-Raf appears to block apoptosis downstream of cytochrome *c* release from mitochondria [163].

and intracellular pH [167–169], as well as the cell cycle, leading to TRP53-independent apoptosis. The fine-tuning between the [*Ah*] battery enzyme levels and the AHR is therefore intimately connected with promoting or preventing oxidative stress and is involved in aiding the cell to choose between apoptosis and continuation with the cell cycle.

BCL2 is downstream of the "central apoptosis signal," thereby preventing apoptosis from proceeding (Fig. 18). Proposed functions of BCL2 include mediation of ROM levels and redox status of the cell, prevention of nucleocytoplasmic trafficking of TRP53 and other cell cycle regulatory factors, neutralization of BAX, BAD and other proteins that heterodimerize and promote apoptosis, and deterrence of mitochondrial cytochrome *c* release [170–173]. The *Caenorhabditis elegans* cell death-9 (*ced-9*) gene is the homologue of the mammalian *BCL2* gene, and *ced-9* is part of a polycistronic locus in *C. elegans* that includes the *cyt-1* gene encoding a protein similar to cytochrome b_{560} [174]. Cytochrome b_{560} is an inner mitochondrial membrane protein involved in system II (succinate dehydrogenase), which transfers an electron from succinate to ubiquinone. Interestingly, this dehydrogenase is a member of the *Nqo1* gene superfamily. Even more fascinating is the recent discovery that CYP1A1 and CYP1A2 are, in significant amounts, localized between the inner and outer mitochondrial membrane [175–177]. Mitochondrial phospholipid hydroperoxide glutathione peroxidase prevents oxidative injury to cells [178]; is this enzyme another member of the [*Ah*] battery, and does it participate early in the cell's decision between apoptosis and continuation with the cell cycle?

There are reports that oxidative stress can inhibit apoptosis [e.g. Ref. 179]. As described above, it is likely that too little or too much oxidative stress will not result in the apoptosis cascade.

NEGATIVE REGULATION OF [*Ah*] BATTERY GENES

Several studies have described a ***n***egative ***r***esponse ***e***lement (NRE) upstream of [*Ah*] battery genes. The NRE has been proposed on the basis of several lines of experimental evidence, primarily promoter-bashing studies [180; reviewed in Ref. 4]. A number of analyses of the human *CYP1A1* gene have suggested that a 21-base pair inverted repeat between 750 and 800 bases from the transcription start site is involved in negative regulation, and similar homologous regions have been identified upstream of the rat and mouse *CYP1A1* genes [181–184]. A putative NRE has been described upstream of the rat [185] and mouse [186] *ALDH3A1* genes. It is clear that the AHRE(s) can participate in negative as well as positive transcriptional regulation of dioxin-inducible genes [35]. The AHR and ARNT are also known to participate as basal transcription factors in the constitutive expression of [*Ah*] genes [187]. The octamer-binding transcription factor-1 has been shown to bind to the rat *CYP1A1* promoter and down-regulate this gene [188, 189]. Upstream stimulatory factor-1 (USF-1) competes with the AHR–ARNT complex in the rabbit [190] and human [191] *CYP1A1* promoter. Transforming growth factor-β1, which is inducible by dioxin, causes down-regulation of human [*Ah*] battery genes in lung cancer A549 cells [192]. Down-regulation of Chinese hamster cell *CYP1A1* promoter by the gut-enriched Krüppel-like factor (GKLF) is Sp1-dependent [193]. The exact role of the negative response element and other DNA motifs, relative to the AHREs and AHR and [*Ah*] gene expression, remains to be determined.

AHR-INDEPENDENT INDUCTION OF [*Ah*] BATTERY GENES

Finally, it should be noted that dioxin causes certain intracellular effects that are AHR-independent [194], and these even include up-regulation of [*Ah*] battery genes. For example, there are reports that AHR is not involved in dioxin-mediated apoptosis [e.g. Ref. 195]. Induction of mouse *Cyp1a2* and *Cyp1b1* by piperonyl butoxide and acenaphthylene [196], human *CYP1A1* by benzimidazole [197], mouse *Cyp1a1* by myristicin [198], and human *CYP1A1* by primaquine [199] are further examples of the activation of [*Ah*] battery genes that appear to be AHR-

independent. Further studies on this type(s) of up-regulation are needed.

CONCLUSIONS

In summary, the [*Ah*] battery represents the AHR-mediated control of at least six, and probably dozens, of dioxin-inducible genes; two P450 genes—*Cyp1a1* and *Cyp1a2*—and four non-P450 genes (*Nqo1*, *Aldh3a1*, *Ugt1a6*, and *Gsta1*) have been experimentally documented to be members of this battery. Metabolism of endogenous and exogenous substrates by many, if not all, cytochromes P450 can result in ROM-mediated oxidative stress. Metabolism of endogenous and exogenous substrates by CYP1A1 and CYP1A2, because they are located in part within the mitochondrion, is especially intriguing because the release of cytochrome *c* from mitochondria is among the earliest signals in the apoptosis cascade. Oxidative stress is a major signal in precipitating apoptosis. All [*Ah*] genes are activated by AHR ligands via the AHREs. The non-P450 [*Ah*] genes, in addition, are activated by oxidative stress via the EPRE, and this would include AHR ligands that are metabolized to ROMs (e.g. benzo[*a*]pyrene, β-naphthoflavone). It is clear that the AHR plays a role in cell cycle regulation; an AHR–RB1 complex has been shown to block cells in G_1 from progressing into S phase. The CYP1A1 and CYP1A2 enzymes appear to control the level of the EL of the AHR, whereas there is a balance between CYP1A1/1A2 metabolism (which enhances ROM-mediated oxidative stress) and the four non-P450 EPRE-driven genes in the [*Ah*] battery (which protect against oxidative stress). Specific spatial and temporal generation of ROMs might occur at critical subcellular sites or compartments. The sum action of the AHR and [*Ah*] battery genes thus appears to embody a control upstream in the apoptosis cascade, providing a delicate rheostat between promoting and preventing ROM-mediated oxidative stress. The precise mechanism, or molecule(s), which determines the cell's decision between apoptosis and continuation with DNA synthesis and mitosis, however, still remains to be elucidated. These postulated actions on putative endogenous substrates and ligand are in addition to the commonly accepted and frequently described functions of the P450, and non-P450, [*Ah*] enzymes in foreign chemical metabolic potentiation and detoxification, respectively.

We thank our colleagues—especially Alvaro Puga, Mike Carvan, and Howard Shertzer—for valuable discussions and their critical review of this manuscript. We also appreciate Mike Waterman for valuable discussion. Supported in part by NIH Grants R01 AG09235, R01 ES06321, R01 ES08147, and P30 ES06096.

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