Ann. Rev. Pharmacol. Toxicol. 1981. 21:431–62

GENETIC MECHANISMS CONTROLLING THE INDUCTION OF POLYSUBSTRATE MONOOXYGENASE (P-450) ACTIVITIES[1]

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INTRODUCTION

In the past several years a great deal of exciting information has become available with regard to the genetic regulation of certain drug-metabolizing enzymes. In this review, the present-day concepts concerning the multiple forms of these enzymes are first introduced. Second, the *Ah* locus and the *Coh* locus are reviewed as the two best-characterized genetic systems controlling induction of these enzymes. Third, the peroxidase induction by diethylstilbestrol is described. Fourth, from all that is presently known about these systems, the possible significance of receptors and overlapping

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substrate and inducer specificities is explored. Many of the new ideas and data presented are relatively recent and will require further corroboration and extension. We believe this research field has become extremely fascinating, and there remain many more questions than answers.

Phase I and Phase II Drug-Metabolizing Enzymes

At least 100,000 and possibly many more foreign chemicals exist in our environment. Many of these drugs and environmental pollutants, also called *xenobiotics,* are highly toxic, and a growing number of these chemicals are being shown to cause mutations, cancer, and birth defects.

How do living things respond to this chemical adversity? Most xenobiotics are so hydrophobic that they would remain in the organism indefinitely were it not for Phase I and Phase II drug-metabolizing enzymes (1). During Phase I metabolism, polar groups (such as alcohols) are introduced into the parent molecule, thereby presenting the Phase II conjugating enzymes with a substrate. The Phase II enzymes use the polar group as a "handle" for attaching other very water-soluble moieties such as glucuronide, sulfate, or glycine. The Phase I products (such as alcohols and quinones) and especially the Phase II conjugates are sufficiently polar to be easily excreted by the organism. Living things often respond to chemical adversity by the induction of both Phase I and Phase II enzymes.

Polysubstrate Monooxygenases

Monooxygenases are enzymes that insert one atom of atmospheric oxygen into their substrates (2, 3). The various forms of polysubstrate monooxygenases[2] represent a large subset of all monooxygenases. The mechanisms of monooxygenation, peroxidation, and other oxidative activities of P-450 (4, 5) are beyond the scope of this review.

Monooxygenase activities require the integrity of an electron flow (Figure 1) between the cofactor NADPH (in some cases, NADH) and the oxygenated form of P-450. More than three fourths of the liver microsomal reductase molecule is believed (7) to sit free of the lipid bilayer, whereas P-450 molecules are believed to be usually deeply embedded in the membrane, making solubilization of "pure" forms of P-450 extremely difficult. Detergent treatment involving micelle formation often interferes with normal function (rate of catalytic activity in intact microsomes or intact liver),

[2]At the recommendation of the Nomenclature Committee at the Third International Congress on the Biochemistry, Biophysics, and Regulation of Cytochrome P-450, Saltsjöbaden, Sweden, June, 1980, *polysubstrate monooxygenase* (trivial name, P-450) was chosen to describe the large group of NADPH- and NADH-dependent isozymes that metabolize thousands of xenobiotics and endogenous compounds with varying degrees of overlapping substrate specificity. Another abbreviation used in this review: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

so that "reconstituted" activity sometimes may differ from "intact" microsomal catalytic activity (8).

The functions of membrane-bound FAD- and FMN-containing flavoprotein (9), mitochondrial P-450 (10), cytochrome b_5 (11, 12), cyanide-sensitive fatty acid desaturase (13), UDP glucuronosyltransferases (14), sulfotransferases (15), and epoxide hydrolases (16) are beyond the scope of this review. There may exist some degree of coupling, or "assembly-line" process, among these membrane-bound moieties (Figure 2). In other words, a chemical may be metabolized sequentially by one or more Phase I enzymes and one or more Phase II enzymes—without the intermediate ever leaving the proximity of these membrane-bound moieties. Differences in benzo[a]pyrene metabolite ratios occur, for example, depending upon whether the nonmetabolized parent compound, or a hydroxylated metabolite, is introduced as the substrate to the intact microsomes (18, 19). Whether such differences also occur for xenobiotics that are more water-soluble than benzo[a]pyrene is presently not known.

P-450 molecules are believed to be arranged in heterogeneous clusters in the membranes (20, 21). It is not known whether the P-450 molecules are

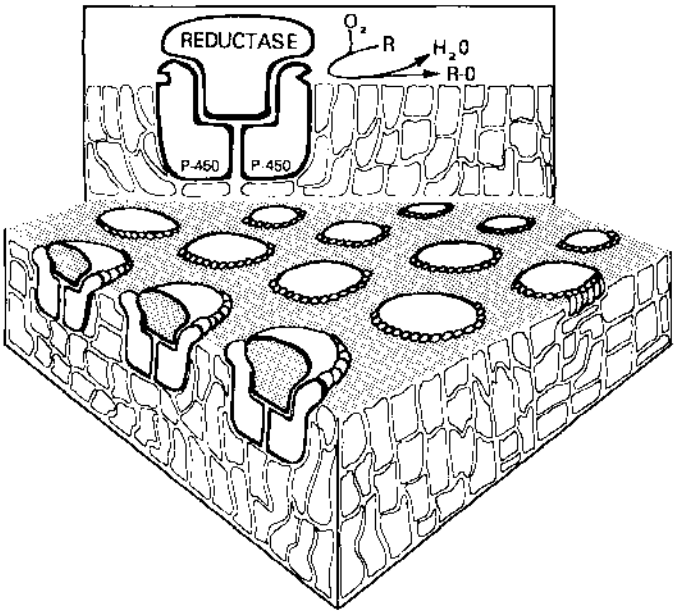

Figure 1 Hypothetical diagram of the relationship between flavoprotein reductases and forms of cytochrome P-450 embedded in cellular membranes (6). R, substrate (such as a drug); R-O, oxygenated intermediate or product. (Reproduced with permission from Dr. W. Junk Publishers.)

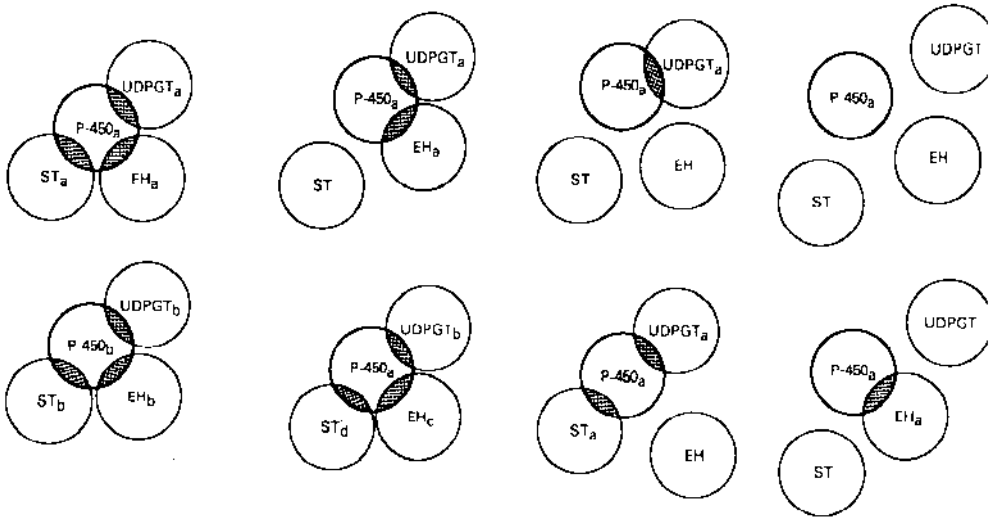

Figure 2 Possible coupling, or lack of coupling, of membrane-bound Phase I and Phase II drug-metabolizing enzymes. UDPGT$_a$ and UDPGT$_b$, distinct forms of UDP glucuronosyltransferase; P-450$_a$ and P-450$_b$, different forms of P-450; ST$_a$ and ST$_b$, different forms of sulfotransferase; EH$_a$ and EH$_b$, distinct forms of epoxide hydrolase. Several possible situations are depicted. *Far left:* A distinct form of P-450 might be coupled to distinct forms of UDPGT, ST, and EH, while a second distinct form of P-450 is coupled to other distinct forms of UDPGT, ST, and EH. *Middle left:* The ST might not be coupled to one form of P-450, while another ST is coupled to a second distinct form of P-450 (and UDPGT and EH are coupled to both forms of P-450). *Middle right:* Both forms of UDPGT and one form of ST are shown coupled to the two distinct forms of P-450, while the remaining Phase II enzymes are not coupled. *Far right:* Except for one form of EH coupled with one form of P-450, all other moieties are shown as not coupled. One such tight coupling between epoxide hydrolase and one form of induced P-450 has been demonstrated (17).

arranged in rosettes around a reductase molecule (as depicted in Figure 1) or the reductase moves among randomly located P-450 molecules, like a ship through a sea of rocks or a bee among clusters of flowers. The stoichiometry of P-450 molecules to reductase molecules ranges between 10:1 and 100:1 (22, 23). Not only is cytochrome b_5 able to reduce certain forms of P-450 (24), but it is also possible that some forms of P-450 are able to reduce other forms of P-450.

Polysubstrate monooxygenases therefore represent a large number of Phase I enzymes. The thousands of foreign chemicals and normal body substrates for these enzymes include polycyclic hydrocarbons such as benzo[a]pyrene (ubiquitous in the combustion of coal and in city smog, cigarette smoke, and charcoal-cooked foods), anthracenes, and biphenyl; halogenated hydrocarbons such as polychlorinated and polybrominated

biphenyls, defoliants, insecticides, and ingredients in soaps and deodorants; certain fungal toxins and antibiotics; many of the chemotherapeutic agents used to treat human cancer; ethanol; almost all drugs; almost all commonly used laboratory reagents; strong mutagens such as N-methyl-N'-nitro-N-nitrosoguanidine and nitrosamines; various chemicals found in cosmetics and perfumes; numerous aromatic amines, such as those found in hair dyes, nitro aromatics, aminoazo and diazo compounds, and heterocyclics; N-acetylarylamines and nitrofurans; most plant phytoalexins and wood terpenoid derivatives; epoxides; carbamates; alkyl halides; safrole derivatives; antioxidants, other food additives and many ingredients of foodstuffs and spices; both naturally occurring and synthetic steroids; prostaglandins; and other endogenous substrates such as biogenic amines, indoles, thyroxine, and fatty acids.

HOW MANY FORMS OF P-450 EXIST?

Endogenous P-450

Several control forms of P-450 have been purified to apparent homogeneity (Table 1). There are two adrenal cortex mitochondrial P-450 proteins (25): P-450$_{scc}$ that catalyzes the formation of pregnenolone from cholesterol, and P-450$_{11\beta}$ that catalyzes both the 11β- and 18-hydroxylation of a C_{21}-steroid and the 11β- and 19-hydroxylation of a C_{19}-steroid. Other apparently pure forms of endogenous P-450 from rabbit, rat, and pig liver are listed in Table 1. There is indirect evidence for a minimum of at least four forms (44) and seven forms (45) of endogenous P-450 from rat and mouse liver, respectively. In all of these cases, these forms of apparently constitutive P-450 are able to metabolize many xenobiotics as well as numerous normal body substrates—some compounds better than others, but always there appear to be varying degrees of overlapping substrate specificities.

It seems reasonable to assume that endogenous forms of P-450 might carry out more specific metabolic reactions than induced forms of P-450, because many biosynthetic and catabolic processes of steroids and biogenic amines, for example, might be critical to important life functions. The ability of constitutive forms of P-450 to metabolize xenobiotics might be viewed as fortuitous, yet also could be important for survival in a chemically adverse environment. It might be advantageous to the organism if endogenous P-450 could metabolize, to some extent, a toxic chemical suddenly added to the environment—until induced forms of P-450 are capable of metabolizing the toxic chemical more effectively.

How many forms of endogenous P-450 are there and how are they regulated? In view of the dozens of distinct catalytic activities described

Table 1 Forms of P-450 purified to apparent (electrophoretic) homogeneity

	Proposed name	Species	Tissue	Subcellular fraction	Inducer	M_r ($\times 10^{-3}$)	References
A. Endogenous P-450	$P\text{-}450_{scc}$	Cow	Adrenal cortex	Mitochondria		41	25
	$P\text{-}450_{11\beta}$	Cow	Adrenal cortex	Mitochondria		46	25
	Form 3	Rabbit	Liver	Microsomal		52.6	26
	LM_{3b}	Rabbit	Liver	Microsomal		53	27
	AU_1	Rabbit	Liver	Microsomal		49	28
	AU_2	Rabbit	Liver	Microsomal		46	28
	AU_3	Rabbit	Liver	Microsomal		52	28
	AU_4	Rabbit	Liver	Microsomal		54	28
	Cytochrome I	Rabbit	Lung	Microsomal		52[a]	29
	Cytochrome II	Rabbit	Lung	Microsomal		58[b]	29
	$P\text{-}450_{B_1}$	Rabbit	Liver	Microsomal		52	29a
	(Control P-450)	Rat	Liver	Microsomal		52.2	30
	(Control P-451)	Rat	Liver	Microsomal		52.4	30
	$P\text{-}450_a$	Rat	Liver	Microsomal		48	31
	(Control P-450)	Rat	Liver	Microsomal		50	32
	P450-Control	Pig	Liver	Microsomal		55	33
B. Induced P-450	LM_2	Rabbit	Liver	Microsomal	Phenobarbital	48	34
	Form 2	Rabbit	Liver	Microsomal	Phenobarbital	48.5	35
	$P\text{-}450_1$	Rabbit	Liver	Microsomal	Phenobarbital	49	28
	$P\text{-}450_2$	Rabbit	Liver	Microsomal	Phenobarbital	49	28
	$P\text{-}448_1$	Rabbit	Liver	Microsomal	Phenobarbital	54	28
	LM_4	Rabbit	Liver	Microsomal	β-Naphthoflavone	54	34, 36
	Form 4	Rabbit	Liver	Microsomal	TCDD	54.5	35
	Form 6	Rabbit	Liver	Microsomal	TCDD	57	35
	$P\text{-}448_2$	Rabbit	Liver	Microsomal	3-Methylcholanthrene		28

P–450$_b$	Rat	Liver	Microsomal	Phenobarbital	52	31
P–450$_c$	Rat	Liver	Microsomal	3-Methylcholanthrene	56	31
P–450–PB	Rat	Liver	Microsomal	Phenobarbital	48.5	37
P–450B	Rat	Liver	Microsomal	Phenobarbital	53.1	38
P–450D	Rat	Liver	Microsomal	Phenobarbital	54.3	38
P–450B (MC)	Rat	Liver	Microsomal	3-Methylcholanthrene	55.9	38
P–450$_d$	Rat	Liver	Microsomal	Isosafrole	52	39
50 mM phosphate eluate	Rat	Liver	Microsomal	Cholestyramine	50	40
80 mM phosphate eluate	Rat	Liver	Microsomal	Cholestyramine	50	40
(Induced P–450)	Rat	Liver	Microsomal	Pregnenolone–16α–carbonitrile	51	41
P–450A$_2$	Mouse	Liver	Microsomal	Phenobarbital	50	42
P–450C$_2$	Mouse	Liver	Microsomal	Phenobarbital	56	42
P$_1$–450	Mouse	Liver	Microsomal	3-Methylcholanthrene	55	43
P–448	Mouse	Liver	Microsomal	3-Methylcholanthrene	55	43
P450–PB–A	Pig	Liver	Microsomal	Phenobarbital	50	33
P450–PB–B	Pig	Liver	Microsomal	Phenobarbital	56	33
P450–PB–C	Pig	Liver	Microsomal	Phenobarbital	53	33
P450–PB–D	Pig	Liver	Microsomal	Phenobarbital	60	33
P450–β–NF	Pig	Liver	Microsomal	β-Naphthoflavone	55	33
P450–EtOH	Pig	Liver	Microsomal	Ethanol	55	33

[a] Lung cytochrome P–450–I cochromatographs with LM$_2$ and form 2 from rabbit liver microsomes (R. M. Philpot, personal communication).
[b] Lung cytochrome P–450–II chromatographs between form 4 and form 6 from rabbit liver microsomes (R. M. Philpot, personal communication).

for normal body substrates such as various steroids, indoles, biogenic amines, prostaglandins, and thyroxine, it seems reasonable to estimate that certainly more than 10 and probably fewer than 100 endogenous forms of P-450 (microsomal and mitochondrial) will ultimately be found. Differences in distribution of these forms among various tissues, and even among different cells in the same organ, might be expected. There are three studies suggestive of some sort of control of endogenous P-450: "feminizing factor" imprints irreversibly in the neonatal period a female sex steroid metabolism pattern in rat liver (46); ACTH induces a particular form of adrenal cortex microsomal P-450 (47); and LH induces a particular form of testis P-450 (48). Much more work on this subject is needed.

Induced Forms of P-450

There are several reports claiming to have purified an induced form of mammalian microsomal P-450 to apparent homogeneity (Table 1). The number of reports has grown extremely rapidly in recent months; we have tried to make Table 1 inclusive of all major different forms of P-450 and apologize to anyone who feels his data have been inadvertently overlooked. In sum, although the nomenclature is not standardized, there appear to be at least four forms of phenobarbital-inducible P-450, at least two forms of P-450 inducible by polycyclic aromatic compounds such as β-naphthoflavone, TCDD, or 3-methylcholanthrene, and a new form induced after isosafrole, cholestyramine, or pregnenolone-16α-carbonitrile. A single electrophoretic band and a single precipitin band by Ouchterlony immunodiffusion were regarded until recently as absolute criteria for homogeneous preparations of P-450; such criteria are no longer regarded as absolute, since different forms of P-450 (determined by dissimilar peptide maps and/or variance in the sequence of the first few amino acids at the NH_2-terminal end) have now been found to coelectrophorese and to produce single precipitin lines on Ouchterlony gels (28, 39).

How many forms of induced P-450 are there? Until now, the general consensus among most laboratories has been that two, or four, or certainly fewer than one dozen forms of induced P-450 exist and that overlapping substrate specificity accounts for all diversity seen when thousands of different chemicals are metabolized by the monooxygenase system. At the other extreme, it has been postulated (6) that organisms have the genetic capacity to induce as many distinct forms as there are inducers of P-450. Possessing the genetic capacity to form hundreds or thousands of different P-450 species does not mean that all of them would exist at any one time. At any given moment, an organism may be exposed to significant concentrations of perhaps only 10, or 20, important inducers, so that 10 or 20 forms of

induced P-450 would exist in quantities sufficient to be detected. At a later time, the same organism—because of changes in its environment—may develop a different profile of detectable forms of induced P-450. Such a hypothetical situation is similar to immunoglobulin formation in response to various antigens in the environment. Further work is needed to confirm or disprove this hypothesis about induced forms of P-450.

Multiple Inducers of P-450

More than 10 years ago (49), P-450 inducers were classified as being of two major types—"3-methylcholanthrene-like" and "phenobarbital-like"—although it was also well known that more than 200 drugs, carcinogens, other environmental pollutants, and steroids induce their own metabolism and/or that of other compounds via P-450 induction. This classification appears to have originated from research interest of cancer in the 1950s (50) and of barbiturate tolerance in the early 1960s (51). Polycyclic hydrocarbon carcinogens and barbiturates were each found empirically to induce a variety of drug-metabolizing enzyme activities, and these two "classes" of inducers appeared to be very different from each other and, in fact, appeared additive when administered together (49).

As more sophisticated techniques for separating forms of P-450 and for assaying an increased number of monooxygenase activities have become available these past several years, an increasing number of inducing compounds appear to be neither precisely 3-methylcholanthrene-like nor precisely phenobarbital-like. We have attempted to list most of these xenobiotic inducers in Table 2. Ellipticine, for example, induces its own metabolism better than 3-methylcholanthrene induces ellipticine metabolism (81). Isoniazid treatment induces the metabolism of such drugs as enflurane, whereas the rate of aminopyrine metabolism decreases; other inducers such as phenobarbital, 3-methylcholanthrene, β-naphthoflavone, and phenytoin do not enhance enflurane metabolism (98). Similar types of empirical observations have been made with the more than five dozen inducers listed in Table 2, suggesting that each may be inducing its own unique form of P-450. In other words, one or more of the 390 to 550 amino acids of the induced P-450 polypeptide may be different so that substrate specificity (and perhaps even molecular weight) may be unique.

What makes one chemical a better inducer than another? How is this induction process evoked, and what are the steps in the response? What is the significance of overlapping specificities of substrates and of inducers of P-450? Some aspects of these questions are considered following an analysis of several genetically controlled monooxygenase induction systems.

Table 2 Xenobiotics that stimulate their own metabolism and/or induce unique forms of P–450

Compound	References	Compound	References
Benzo[a]pyrene	50	Ethoxyquin	80
3-Methylcholanthrene	52	Ellipticine	81
Benzo[a]anthracene	52	Ethanol	33, 82
7,12-Dimethylbenzo[a]anthracene	53	Isosafrole	39, 83, 84
Phenobarbital	51	Piperonyl butoxide	84
Phenylbutazone	54	Ethinylestradiol	85
Aminopyrine	54	2-Acetylaminofluorene	86
Citrus red dye no. 2	55	Polybrominated biphenyls	87, 88
Chlorpromazine	56	Styrene	89, 90
Meprobamate	57, 58	*trans*-Stilbene oxide	91
Tolbutamide	59	Chlorinated antibacterials	92
Hexobarbital	60	TCDD	93
Pentobarbital	60	Rifampicin	94
Glutethimide	61	Terpenes and sesquiterpenes	95, 96
Chlorcyclizine	62	Lindane	97
Probenecide	62	Isoniazid	98
p,p'-DDT	63	α-Pinene	99
Chlordiazepoxide	64	Polychlorinated biphenyls	100–102
Benzene	65, 66	Theophylline	103
Methoxyflurane	67	Halogenated naphthalenes and terphenyls	104
Phenytoin	68	Synthetic pyrethroids	105
Caffeine	69	Acetone	106
Pregnenolone-16α-carbonitrile	41, 70	Cholestyramine	40
Alkanes	71	Carbamazepine 10,11-oxide	107
Volatile deodorants	72	Camphor	108
Kepone	73, 74	Cyclohexane	108
Mirex	73, 74	Dibromochloropropane	109
Methadone	75–77	Clofibrate	110
Griseofulvin	78	Toluene	111
Methylated benzenes and naphthalenes	79	Medroxyprogesterone	112
		Propanol–2	112a
		Pyrazole	Dr. Ritva P. Evarts, personal communication

THE *Ah* COMPLEX

General Description: The Pleiotypic Response

The murine *Ah* locus regulates the induction by polycyclic aromatic compounds (such as 3-methylcholanthrene, β-naphthoflavone, benzo[a]pyrene, and TCDD) of numerous drug-metabolizing enzyme activities in virtually all tissues (113, 114). The *Ah* complex is viewed as a combination of regulatory, structural, and probably temporal genes (Figure 3) which may or may not be linked.

The first events during the induction process are believed to be the passive transfer of xenobiotic inducer across the cell membrane (116) and the highly

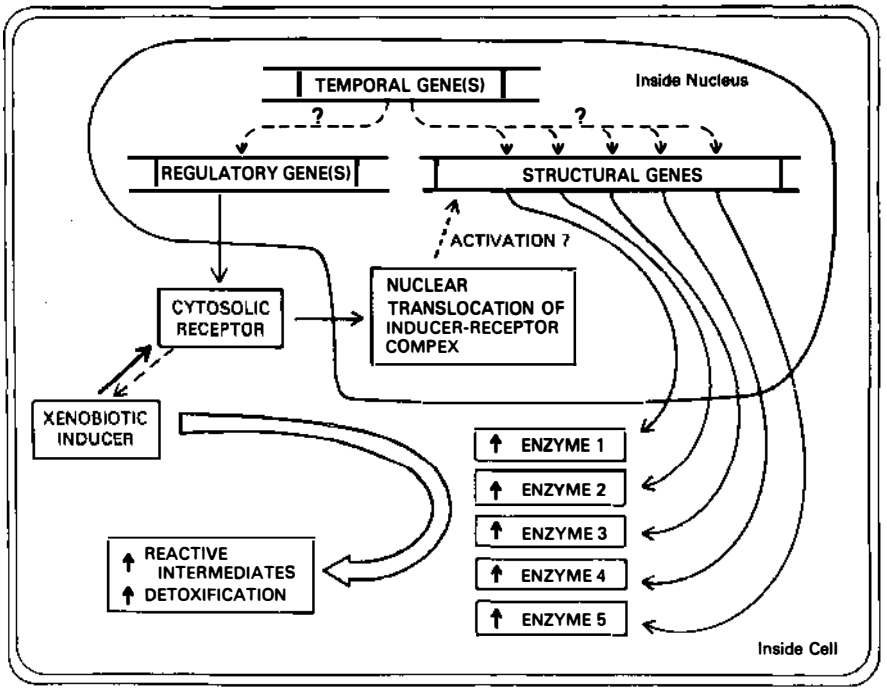

Figure 3 Diagram of a cell depicting *Ah* complex (115). Major product of the regulatory gene is the cytosolic receptor. Various induced enzymes represent products of different structural genes of the *Ah* complex. Although a temporal gene or genes are postulated within the *Ah* complex, it is not presently clear whether this temporal control affects regulatory or structural genes. Most inducers are metabolized by various induced enzymes to reactive intermediates and innocuous products. Depending on half-life of the intermediate, rate of formation of reactive intermediates, and rate of conjugation and other means to detoxify the intermediate, important covalent binding may occur in the same cell in which metabolism occurred or in some distant cell and may be associated with initiation of cancer or drug toxicity. (Reproduced with permission from US Government Printing Office.)

specific binding to the cytosolic *Ah* receptor (117, 118), regarded as the major regulatory gene product. Following the apparent translocation of the inducer-receptor complex into the nucleus, the pleiotypic response includes the activation of numerous structural genes. The induction of several drug-metabolizing enzymes (structural gene products) leads to a delicate balance between increased formation of reactive intermediates and detoxication. Discussion of the formation of reactive intermediates—which bind covalently to protein and nucleic acids and which have been shown to be associated with genetic differences in birth defects, drug toxicity, mutations, and cancer, even among individual siblings (113)—is beyond the scope of this review.

Responsiveness to *a*romatic *h*ydrocarbons was first characterized in the C57BL/6 inbred strain (B6, responsive, Ah^b), and DBA/2 was the first nonresponsive mutant inbred strain characterized (D2, nonresponsive, Ah^d). The Ah^b/Ah^d heterozygote is responsive, indicating a Mendelian autosomal dominant trait. The new monooxygenase activities can be induced in nonresponsive mice by a dose of TCDD 12 to 18 times larger than that needed in responsive inbred strains (119), suggesting that the nonresponsive mice have a defective *Ah* receptor but intact structural genes. This difference in sensitivity for any *Ah*-locus-associated inducer has been found for numerous monooxygenase activities in virtually every tissue of the mouse (113).

It should be noted that the *Ah* locus does not reflect a single-gene difference but rather is much more complicated (113, 114). Regulation alone must involve a minimum of six alleles and two loci, and structural gene mutants appear to exist as well.

Regulatory Gene Product: The Ah Receptor

The cytosolic receptor is regarded as the major product of the *Ah* regulatory genes. Sucrose density gradient analysis, following dextran-charcoal treatment, is among the most reliable methods for characterizing the receptor (118). The apparent K_d for TCDD binding is about 0.7 nM, and approximately 5500 binding sites per cell (60 fmol/mg cytosolic protein) are found in C57BL/6N mouse liver. Representative inducers that bind with greatest avidity (Figure 4) are polycyclic and are, in general, planar. A size of 6S for both the cytosolic and nuclear receptor is estimated on sucrose density gradients in the presence of 0.4 M KCl (118, 120). All nonresponsive strains so far examined have no detectable *Ah* receptor. It remains possible that these nonresponsive mice have as many as 100 "normal" receptor molecules per cell, because this number is not detectable in our assay. Under the conditions of our assays for receptor (requiring between 6 and 18 hr), TCDD "off-rates" may become important because these conditions do not reflect an equilibrium; nonresponsive mice may therefore have more *Ah* receptor that is just not being detected. Alternatively, these strains may have larger numbers of receptor molecules per cell but poorer affinity toward TCDD.

There is a good structure-activity relationship between biologic response and the chemical's capacity to displace [^{3}H]TCDD from the *Ah* receptor (117, 118). Dozens of endogenous compounds, including glucocorticoids and sex steroids, do not displace the radioligand from its receptor—even at 1000-fold excess concentrations. Presence of the Ah^b allele correlates well with the apparent temperature-dependent nuclear translocation of the inducer-receptor complex (118, 120). Recent data indicate that some chemi-

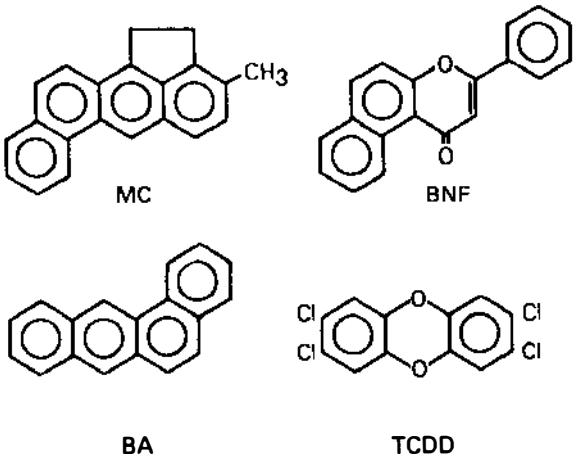

Figure 4 Molecules that interact with the *Ah* receptor. MC, 3-methylcholanthrene; BA, benzo[a]anthracene; BNF, β-naphthoflavone; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

cals displace the radioligand from the *Ah* receptor very well but do not produce the biologic response (121). Certain chemicals may therefore be competitive antagonists, may bind to other sites on this molecule (noncompetitive antagonists), or may interact with other proteins present in this same sucrose density peak. Among mouse C3H/10T½CL8 fibroblast cultures, a clone has lost its aryl hydrocarbon hydroxylase inducibility by 3-methylcholanthrene while retaining its inducibility by benzo[a]anthracene (122). Differences between 3-methylcholanthrene- and β-naphthoflavone-induced rat liver microsomes have also been observed (123). Such data support the possibility of heterogeneity in the *Ah* receptor. Much more work needs to be done.

Evidently the *Ah* receptor is present in fetal tissues from very early in gestation (124, 125) and has been associated with genetic differences in birth defects (125–127). The *Ah* receptor is suppressed during pregnancy (124) and varies between about 60 fmol/mg cytosolic protein in August to 20–25 fmol/mg cytosolic protein in February (118). Seasonal changes in the "A" subunit of the progesterone-receptor complex and its capacity for binding to certain protein-DNA complexes have also recently been reported (128, 129). For the chick oviduct progesterone receptor, it was suggested (129) that these circannual changes are important in seasonal reproduction and molting; why these circannual changes in the *Ah* receptor exist in mouse liver is not understood.

Multiple Structural Gene Products: Induced Forms of P-450

The induction of at least two dozen monooxygenase activities appears to be under the control of the same *Ah* receptor. These activities include C-hydroxylations of benzo[a]pyrene and several other polycyclic hydrocarbon carcinogens, biphenyl, zoxazolamine, acetanilide, naphthalene, aflatoxin B_1, and theophylline; O-deethylations of 7-ethoxycoumarin, phenacetin, and ethoxyresorufin; O-demethylation of *p*-nitroanisole; N-demethylations of 3-methyl-4-methylaminoazobenzene and theophylline; and as yet undetermined oxygenations of β-naphthoflavone, α-naphthoflavone, ellipticine, lindane, niridazole, caffeine, and theobromine (113). These substrates are highly variable in size and shape, and the question was therefore raised long ago (130) whether these induced activities represent the relatively nonspecific metabolism by a single form of polycyclic aromatic-induced P-450 or more specific metabolism by a whole family of individual enzymes.

Most laboratories have been hesitant to accept the possibility of the existence of more than a single form of "induced cytochrome P-448" in a 3-methylcholanthrene-treated animal—in spite of evidence to the contrary for more than 5 years (Table 3). With the use of two detergents and two column chromatographic steps, P-450 from 3-methylcholanthrene-treated B6 mouse liver microsomes was recently separated into 16 fractions, and many reconstituted monooxygenase activities were studied (45). Practically every 3-methylcholanthrene-induced activity was dissociable from every other 3-methylcholanthrene-induced activity. Hence, biphenyl 2-hydroxylase activity is not synonymous with P-448, nor is aryl hydrocarbon hydroxylase activity or ethoxyresorufin O-deethylase activity synonymous with P-448. By two-factor analysis of variance, it was concluded that the data can be explained statistically be a minimum of 19 different groups of P-450:12 induced by 3-methylcholanthrene (and associated with the Ah^b allele) and 7 endogenous forms. In fact, the data suggest more than one 3-methylcholanthrene-induced P-448 and more than one endogenous form of P-448 (45).

P-450-mediated monooxygenase activities known *not* to be associated with the *Ah* locus include the induced and control metabolism of aminopyrine, *d*-benzphetamine, phenytoin, hexobarbital, aniline, benzenesulfonanilide, chlorcyclizine, ethylmorphine, pentobarbital, estrogen, and testosterone. Also *not* associated with the *Ah* locus is the induction of NADPH cytochrome *c* reductase and NADPH-P-450 reductase, epoxide hydrolase, and glutathione transferase. Other inducible enzymes that are not monooxygenases but which appear to be metabolically coordinated (Figure 5) and associated with the Ah^b allele include microsomal UDP glucurono-

Table 3 Distinctive differences between hepatic cytochrome P_1–450 and P–448 among four species

Species	P_1–450 Definition: Inducible form of P–450 that most efficiently metabolizes benzo[a]pyrene to benzo[a]pyrene 7,8-oxide	P–448 Definition: Inducible form of P–450 that has maximal blue shift of Soret peak when hemoprotein·CO complex is reduced	References
Rabbit	Termed $LM_{1,7}$	Termed LM_4	34
		Glycoprotein; 75% or more ferric iron in high spin state	36
	57,000-dalton subunit present in neonate, not in adult	54,000-dalton subunit, present in adult, not in neonate	131
		More closely associated with 2-acetylaminofluorene N-hydroxylation than P_1–450	131
	Termed P–450b, 57,000-dalton subunit, 448.0 nm Soret peak	Termed P–450c, 54,500-dalton subunit, 447.0 nm Soret peak	35
	Termed form 6	Termed form 4	35
	Form 6 is only form to metabolize benzo[a]pyrene to a mutagen	Form 4 is only form to metabolize 2-aminoanthracene to a mutagen	132
Rat	No detectable 3-methylcholanthrene-induced aryl hydrocarbon hydroxylase activity in Morris hepatoma 7777	"Blue spectral shift" to 448-nm induced by 3-methylcholanthrene in Morris hepatoma 7777	133
	56,000-dalton subunit, developmentally appears 2 to 4 days earlier than P–448	54,000-dalton subunit, developmentally appears 2 to 4 days later than P_1–450	134
	2,3,3′,4,4′-Pentachlorobiphenyl is poor inducer of aryl hydrocarbon hydroxylase activity	2,3,3′,4,4′-Pentachlorobiphenyl is good inducer of P–448	102
Mouse	Aryl hydrocarbon hydroxylase induction earlier than "blue spectral shift" during induction time study	"Blue spectral shift" later than aryl hydrocarbon hydroxylase induction during induction time study	135
	55,000-dalton subunit, developmentally appears 2 to 4 days earlier than P–448	54,500-dalton subunit, developmentally appears 2 to 4 days later than P_1–450	134
		More closely associated with acetanilide 4-hydroxylation than P_1–450	134
	Soret peak 449.3 nm	Soret peak 448.0 nm	43
	Not glycoprotein; slower rate of turnover (synthesis/degradation) than P–448	Glycoprotein; more rapid rate of turnover than P_1–450	136
Fish	Highly inducible aryl hydrocarbon hydroxylase activity	No detectable "blue spectral shift"	137–139

syltransferase and cytosolic reduced NAD(P):menadione oxidoreductase (113). Cytosolic ornithine decarboxylase becomes markedly enhanced early during the induction process and is also associated with the presence of the *Ah* receptor (143).

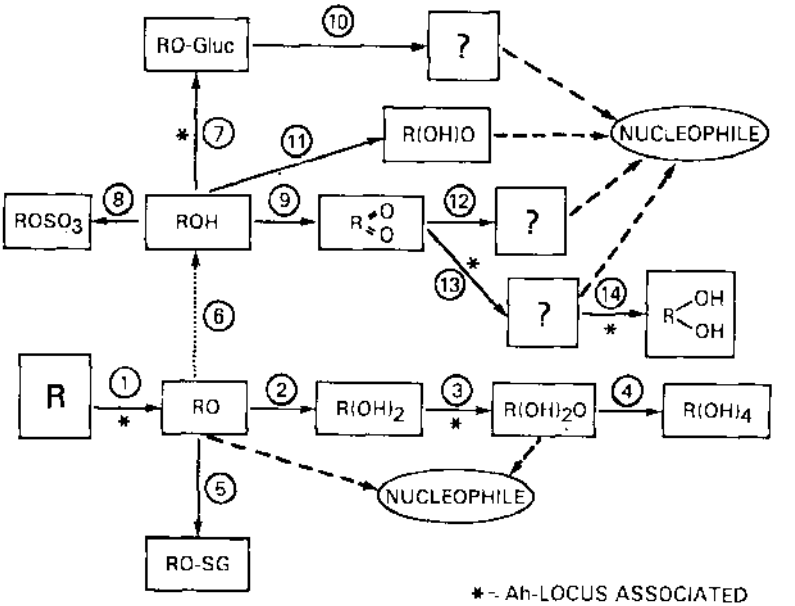

Figure 5 Most of the possible metabolic pathways for a chemical (R) such as a polycyclic hydrocarbon. Formation of an arene oxide, diol-epoxide, glucuronide, and reduced quinone involve enzymes whose inducibility is regulated—at least in part—by the *Ah* receptor. Electrophiles known to interact with nucleophilic cellular macromolecules include arene oxides and diol-epoxides (140), the glucuronide following β-glucuronidase action (141), and quinones metabolized further (142).

Suggestive Evidence for Temporal Genes: Developmental Differences in P-450 Induction

Developmental data in rabbit (131), rat (134), and mouse (43, 134) liver have demonstrated differences in P_1-450 and P-448 induction by polycyclic aromatic compounds—in the apparent presence of sufficient *Ah* receptor. The rabbit is most interesting, because the *Ah* receptor is detectable early in gestation and throughout adulthood (124)—yet P_1-450 but not P-448 is inducible in the newborn, and P-448 but not P_1-450 is inducible in the adult liver. Some type of temporal control therefore must be operational, in order to explain these developmental findings. Whether temporal gene(s) exist within the *Ah* complex is not yet known. In view of the apparent presence of adequate *Ah* receptor, temporal control most likely affects the expression of structural gene products (i.e. transcription of mRNA for these enzyme proteins) rather than regulatory gene products. Further studies, however, are necessary in order to understand this interesting developmental system.

OTHER GENETICALLY REGULATED DRUG-METABOLIZING ENZYMES

No other genetically regulated induction of drug-metabolizing enzymes has been studied to anywhere near the same extent as the *Ah* locus-associated induction process. Several interesting systems have been uncovered and should be exploited further.

The Coh Locus

Coumarin 7-hydroxylase activity reflects one or more of the polysubstrate monooxygenases. The basal and phenobarbital-induced levels of this activity are 5- to 10-fold higher in DBA/2J than AKR/J mice (144, 145). Nine other basal and phenobarbital-induced monooxygenase activities show only minor differences between these two strains. Aniline in vitro preferentially inhibits the phenobarbital-induced activity from DBA/2J more than from AKR/J, and metyrapone in vitro preferentially inhibits the phenobarbital-induced activity from AKR/J more than from DBA/2J. These data suggest that different structural gene products (different forms of phenobarbital-induced P-450) are responsible for coumarin 7-hydroxylation in these two strains (145). The *Coh* locus has been recently mapped on chromosome 7 on the centromeric side of *Gpi-1* (146). Many further interesting studies should be done. Are the induced forms different from the basal forms? Do the induced forms exist at all in the control, untreated mouse? Does a receptor exist for the phenobarbital induction process?

Peroxidase Induction by Diethylstilbestrol

To our knowledge, there is only one other known example besides the *Ah* complex in which a xenobiotic binds to a cytosolic receptor with great avidity and an enzyme, in turn, is induced to metabolize the xenobiotic. A peroxidase (of unknown function) has been known to exist in target tissues and to be highly inducible by estrogen and its analogues (147–150). Diethylstilbestrol appears to use the estrogen receptor (or subclass thereof) and induces this peroxidase only in estrogen-sensitive tissues. In the past, diethylstilbestrol was regarded as metabolically inert. As more sophisticated techniques have developed for the detection of metabolites, however, it has become clear that diethylstilbestrol is metabolized to numerous oxygen-containing intermediates and products (151) and that the induced peroxidase in turn metabolizes the inducer diethylstilbestrol (151, 152). The enzyme is a microsomal hemoprotein (153), easily dissociable from the microsomal membrane by calcium salt treatment (150). Recent data indicate that there are two induced peroxidases with apparent molecular weights of 92,000 and 40,000 (153).

Other Miscellaneous Systems

There are numerous other reports indicating potentially interesting genetic differences in the regulation of P-450 and other related enzymes. Mouse kidney β-glucuronidase induction by testosterone, elegantly studied in detail by Paigen and co-workers (154), might be considered as the induction of a drug-metabolizing enzyme. Other systems that show promise but have not yet been well exploited include apparent induction of cholesterol 7α-hydroxylase activity by phenobarbital in one rat strain but not in another (155); defective adrenal cortex 18- and 11β-steroid hydroxylation in sensitive (S) rats (who respond to high salt intake with a marked increase in blood pressure) but not in R rats (resistant to high salt intake) (156); genetic differences in 1,2-dimethylhydrazine-induced colon cancer (157); apparent induction of certain plasma esterase activities by several carcinogens (158); the association of aflatoxin B_1 4-hydroxylase induction and predicted decreases in hepatocarcinogenesis with the Ah^b allele (159); genetic variation in N-acetylation of carcinogenic arylamines (160–162); and genetic differences in phenytoin metabolism (163) and in theophylline metabolism and toxicity (103).

POTENTIAL SIGNIFICANCE OF THE *Ah* COMPLEX

The Cytosolic Receptor

What is the *Ah* receptor? There are interesting similarities between the potent enzyme inducers TCDD and diethylstilbestrol (Table 4). In the case of diethylstilbestrol, an endogenous receptor (normally for estrogen) is clearly used by this xenobiotic. In the case of TCDD, if an endogenous ligand for the *Ah* receptor exists, it remains unknown. We could have an experimental situation similar to that with the opiate receptor, studied for years before the endorphins became recognized as the endogenous ligand (Figure 6). This possibility has been suggested (127) to explain the extreme

Table 4 Similarities between TCDD and diethylstilbestrol

1. Response elicited by small doses (μg/kg range).
2. Originally believed not to be metabolized; more recently shown to be metabolized, through greater than 90% believed to be excreted as nonmetabolized parent compound.
3. Cytosolic receptor believed to be necessary in order to elicit the response.
4. Both cytosolic receptors estimated to be 6S in size (high ionic strength).
5. Both inducer-receptor complexes believed to translocate into the nucleus via a temperature-dependent step.
6. Believed to be potent teratogen (in tissues having specific receptor).
7. Believed to be highly toxic (in tissues having specific receptor).
8. Believed to be weak carcinogen and mutagen.

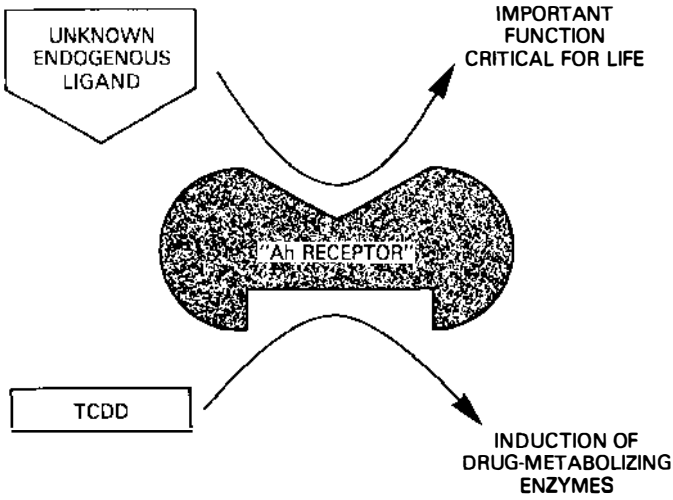

Figure 6 Hypothetical scheme in which the *Ah* receptor has one (agonistic) site for an unknown chemical whose function is critical for life and a second (antagonistic) site for TCDD.

toxicity and teratogenicity of TCDD. If TCDD binds to the receptor—and remains bound for days or weeks because of its extremely slow metabolism —this binding might prevent the interaction of the receptor with an endogenous ligand whose binding is important for some critical life function. The prolonged prevention of such binding by TCDD might lead to thymic atrophy, liver damage, and birth defects. Chlordecone (Kepone) (164, 165) and *p,p'*-DDT (166) are interesting agonists with estrogenic activity, though neither molecule bears much similarity to estrogen in terms of chemical shape and structure.

It has been proposed that receptor proteins, although hydrophobic in their overall nature (167), have an amphiphilic character, shifting toward a more hydrophilic or hydrophobic state by agonists and antagonists, respectively (168). This view is most likely overly simplistic but will become testable with the development of antibodies to the various receptors. When a receptor has no ligand or binds an agonist or antagonist, will there be changes in the protein's hydrophilicity or hydrophobicity? By studying material eluted from ion-exchange columns and complexed with antibody, one can approach this hypothesis experimentally. Obviously, membrane-bound receptors will be more difficult to study than cytosolic receptors.

Another possibility (114) is the organism's need for such a receptor in order to ensure survival in a chemically adverse environment. Benzo[a]pyrene, cholanthrenes, anthracenes, and halogenated dibenzo-*p*-dioxins are

all ligands for the *Ah* receptor and are all found in naturally occurring ubiquitous combustion processes (forest fires, burning of bituminous coal, and petroleum, etc) (169, 170). From early in evolution at least some prokaryotes and lower eukaryotes have used polycyclic hydrocarbons as an energy source; later there might have evolved enzymes to detoxify these chemicals. P-450-mediated metabolism of camphor in *Pseudomonas* and benzo[a]pyrene in yeast and insects has been well characterized (23, 137, 171–175). Phylogenetically, plant terpenoids and other alkaloids occurred much later than bacterial or yeast P-450, and the sophisticated mammalian steroid hormones developed even much later than terpenoids. The similarities between the pleiotypic response elicited by inducers binding to the *Ah* receptor and the pleiotypic response elicited by steroids or their analogues binding to the glucocorticoid receptor (Figure 7) are striking. The glucocorticoid receptor and the induction of gene expression (176) are involved in the anti-inflammatory action of dexamethasone. A large number of monooxygenase inducers have been shown to cause immunosuppression [discussed in refs. (6, 177)]. The biosynthesis and degradation of plant and

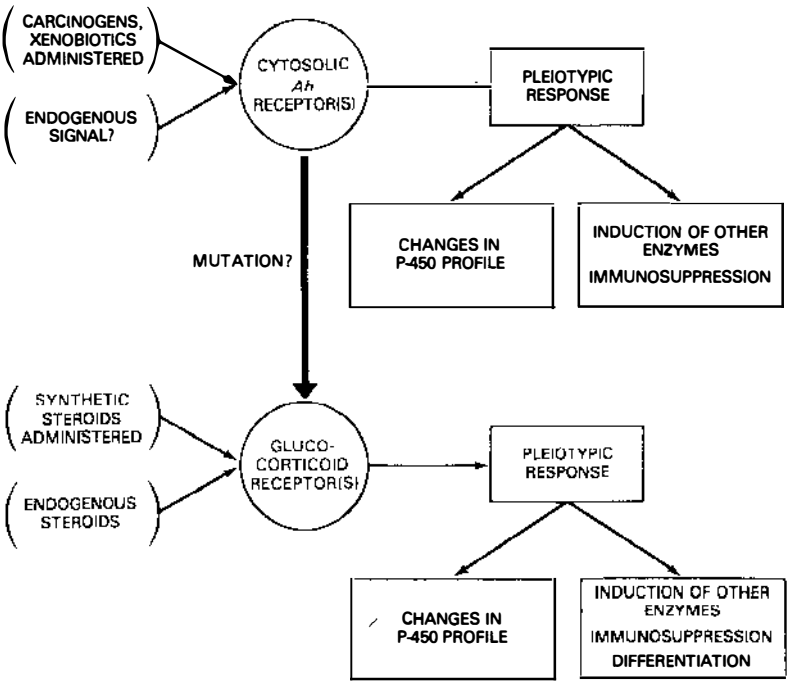

Figure 7 Scheme of the stimulus (foreign chemical or steroid) interacting with its particular cytosolic receptor and a list of responses included under "pleiotypic response."

animal hormones require P-450 in many instances, and the presence of steroids and related synthetic foreign chemicals are known to cause changes in P-450 levels. Could it be that during evolution the glucocorticoid receptor developed as a mutated *Ah* receptor? Do plants possess alkaloid receptors or drug receptors? Does *Pseudomonas* possess a camphor receptor? Much more work is needed before we understand this fascinating subject.

Overlapping Specificities of P-450 Substrates and Inducers

There are many distinct differences between the P-450 system and the immune system, yet a number of interesting similarities also exist (6, 114). Although one antigen binds to a specific antibody with high affinity (e.g. an apparent K_a of 0.1 μM), other antigens will also bind but with one to three orders of magnitude poorer affinity (Table 5). Similarly, one antigen may stimulate principally the synthesis of one specific immunoglobulin but also will stimulate several dozen other antibodies with lesser degrees of specificity (178). The meaning of this weak "biologic damper" is unknown. It might be viewed as advantageous to the organism, however, if some existing forms of antibody immediately interacted with a challenging antigen to *some* degree, until a more specific antibody became available in large amounts.

With regard to the P-450 system, this same type of phenomenon would also be advantageous to the organism. If existing forms of endogenous (or induced) P-450 immediately began to metabolize, to some degree, any challenging toxic chemical until a more specific P-450 became induced,

Table 5 Hypothetical scheme showing overlapping specificities of drugs for different forms of P–450 and antigens for different antibodies

Form of P–450	Apparent K_m for Benzo(a)pyrene	Acetanilide	Ethoxyresorufin
A	10^{-3}	10^{-2}	10^{-3}
B	10^{-4}	10^{-2}	10^{-4}
C	10^{-5}	10^{-4}	10^{-7}
D	10^{-6}	10^{-5}	10^{-6}
E	10^{-7}	10^{-3}	10^{-5}

Form of antibody	Apparent K_a for Dinitrochlorobenzene	Honey bee venom	Eucalyptus tree pollen
I	10^{5}	$10^{4.5}$	10^{5}
II	$10^{5.5}$	10^{5}	10^{6}
III	10^{6}	$10^{5.5}$	10^{7}
IV	$10^{6.5}$	10^{7}	10^{6}
V	10^{7}	10^{6}	10^{4}

such a mechanism would aid in survival. Numerous substrates for P-450 are metabolized most efficiently by one form of P-450 but are metabolized by numerous other forms of P-450 (Table 5) at a rate of 2 to 1000 times less (179, 180). Polycyclic aromatic compounds bound to the *Ah* receptor appear to induce at least 12 new forms of P-450 (45) and at least two dozen monooxygenase activities (113).

Though there is an excellent structure-activity relationship between the shape of the molecule and the capacity to induce aryl hydrocarbon hydroxylase activity (181, 182), there is also a 100- to 1000-fold difference in inducer concentrations that are able to displace the most avid inducer from the cytosolic binding site(s) (117, 118). Overlapping specificities therefore exist, both in terms of inducers interacting with one or more receptors and substrates interacting with numerous forms of P-450.

Hypothetical Genetic Model

Knowledge about the *Ah* complex has given us the clearest picture so far about possible mechanisms for the genetic regulation of P-450 induction. Modifications of the Britten-Davidson model (183) illustrate our ideas best (Figure 8). The effectors α, β, and γ represent inducer-receptor complexes entering the nucleus in order to evoke the response; consider that these represent TCDD, isosafrole, and phenobarbital, respectively—although no data yet exist demonstrating the presence of any specific receptor for these

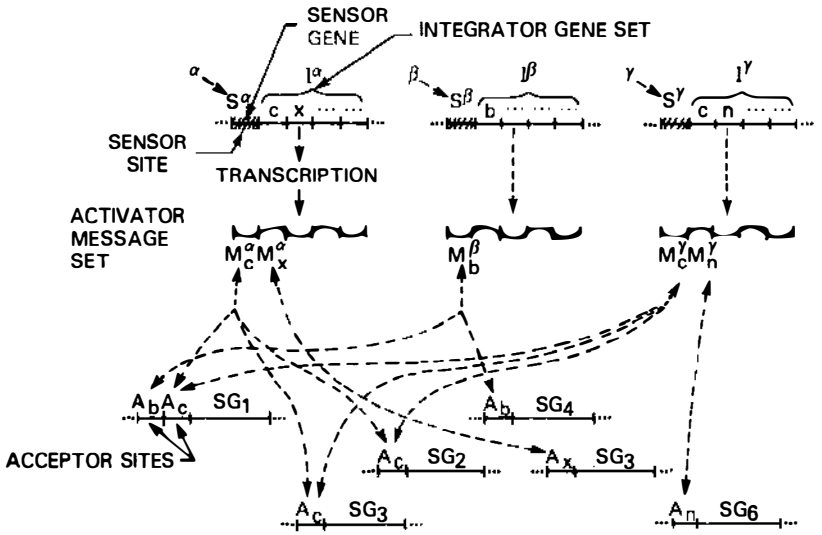

Figure 8 Modified Britten-Davidson scheme. α, β, and γ are effectors (such as inducer-receptor complexes); S, sensor site and gene; M, activator message RNA; A, acceptor sites to the 5' end of SG, structural genes.

latter two inducers. The effector may bind to a sensor gene, a promoter region to the 5' end of an integrator gene set. The resultant transcription produces activator message, large-molecular-weight RNA capable of turning on structural genes not only in the proximity of the integrator gene set (*cis* regulation) but also in distal sites of the same chromosome or on other chromosomes (*trans* regulation). The activator message RNA does this by binding to acceptor sites, promoter regions to the 5' end of structural genes. Activator message RNA is purely hypothetical. What happens during transcription of these structural genes may involve gene amplification, DNA insertion sequences, DNA and/or RNA intervening sequences, or intragenic recombination (6, 114). Definitive studies to prove or disprove this hypothesis almost certainly will require isolation of the genomic DNA (with its nearby 5' and 3' nontranslated regions) associated with the various forms of P-450.

If a particular acceptor site is blocked at certain times during development, this could be the result of temporal control. In other words, in the presence of sufficient amounts of receptor, one form of P-450 but not another form may become induced at a given developmental age (124, 131, 134). The expression of inducibility of multiple forms of P-450 can thus vary with age, as a function of acceptor sites being available or being blocked by temporal gene products.

Differences in affinity between activator message and acceptor sites could lead to differential expression of induced enzymes. Thus, a given dose of inducer may induce one enzyme more than another; a higher dose of inducer may induce both enzymes fully. This type of phenomenon has been seen, comparing polycyclic aromatic-induced aryl hydrocarbon hydroxylase activity with UDP glucuronosyltransferase induction (184) and the induction of aflatoxin B_1 and B_2 metabolism (185).

Such a model (Figure 8) could also explain the existence of structural gene mutants. In other words, in the presence of adequate receptor in a particular 3-methylcholanthrene-treated mouse, aryl hydrocarbon hydroxylase but not zoxazolamine 6-hydroxylase activity might be induced. We have found evidence for such mutants.[3]

Such a model could also explain the induction of apparently the same form of P-450 by both a polycyclic aromatic compound and phenobarbital (28, 36). In the integrator gene set for both the *Ah* complex (I^{α}) and the phenobarbital induction process (I^{γ}), the gene *c* is illustrated in Figure 8. Both activator messages (M_c and M_c) would bind to the promoter region (A_c) of structural gene$_2$ (SG_2), thereby leading to formation of the same mRNA and of the same form of P-450 protein. The cloning of these genes and an understanding of the nucleotide sequences (6, 114) will be necessary

[3]D. W. Nebert, N. M. Jensen, S. W. Bigelow, and H. J. Eisen, manuscript in preparation.

to test this hypothesis. Much work remains to be done in this very exciting research field.

CONCLUSIONS

All organisms most likely possess several genetically regulated mechanisms for coping rapidly with adverse changes in the environment. Studies of the *Ah* complex have greatly aided our understanding of the induction of drug-metabolizing enzymes. Other genetic systems involving the regulation of P-450 induction have also been discussed. Similarities between the P-450 induction process by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and the peroxidase induction process by diethylstilbestrol have been described.

It is now clear that the various forms of P-450 represent a multigene family. The number of highly purified forms of well-characterized P-450 and the number of "unique" P-450 inducers that differ distinctly from either 3-methylcholanthrene or phenobarbital are increasing rapidly each year. If the regulation of P-450 induction resembles in any way the other methods by which prokaryotes and eukaryotes cope genetically with the many forms of environmental selective pressures, it is very likely that most organisms have the genetic capacity to produce not only hundreds, but probably thousands, of inducible forms of P-450.

ACKNOWLEDGMENT

The expert secretarial assistance of Ms. Ingrid E. Jordan is gratefully acknowledged.

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