Ann. Rev. Pharmacol. Toxicol. 1986. 26:333–69

THE REGULATION OF CYTOCHROME P-450 GENE EXPRESSION

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INTRODUCTION

Scientific interest in cytochrome P-450 has increased dramatically since the unheralded discovery in 1958 of a hepatic microsomal carbon monoxide-binding pigment with an absorption maximum at 450 nm (1, 2). Omura & Sato subsequently showed that the pigment had the biochemical properties of a cytochrome and designated it "P-450" because of its unusual spectral characteristics (3–5). We now know that various forms of cytochrome P-450 exist in bacterial, plant, and animal organisms and that these hemoproteins catalyze the monoxygenation of a broad spectrum of lipophilic substrates. Biochemical analyses of cytochrome P-450 have revealed multiple isozymes, which often have broad and overlapping substrate specificities. We do not know how many forms of cytochrome P-450 exist; the data suggest that there may be between 30 and 100. In mammalian cells, cytochrome P-450-containing enzyme systems metabolize many different endogenous substances (e.g. steroids, fatty acids, prostaglandins), as well as exogenous compounds (e.g. drugs, dyes, pesticides, carcinogens).

The cytochrome P-450 apoproteins contain several regions of amino acid sequence homology. Presumably, these represent functionally similar domains among the different isozymes. Sequencing of cytochrome P-450 cDNAs and/or genes has revealed additional homology. Based on the degree of their relatedness in DNA sequence, the genes for cytochrome P-450 have been classified into different families, which together constitute a cytochrome P-450 multigene superfamily. These sequence analyses have generated a good deal of specula-

0362-1642/86/0415-0333$02.00

tion about the possible mechanisms involved in the evolution of the various cytochrome P-450 genes.

Specific chemicals induce one or more forms of cytochrome P-450. Inducibility may have evolved as a mechanism by which the cell can respond to specific chemical signals (e.g. hormonal or xenobiotic) by increasing the rate of specific cytochrome P-450-catalyzed reactions. In addition, the oxygenated product(s) of the cytochrome P-450-catalyzed reaction can be toxic, mutagenic, or carcinogenic. Therefore, some of the cytochromes P-450 also contribute to chemically induced toxicity or neoplasia. Enzyme induction also represents a potentially useful characteristic for studying changes in gene expression that occur in response to specific chemical signals, a topic that is the subject of this review. Because of space constraints, this chapter is limited to mammalian cytochrome P-450 gene expression. Other aspects of cytochrome P-450 biology have been reviewed in this series (6–9) and elsewhere (10–14). Additional reviews are cited in relevant areas of the text. Table 1 summarizes much of the data, which are discussed below.

GENES THAT RESPOND TO PHENOBARBITAL

About 25 years ago, three separate observations heightened scientific interest in cytochrome P-450. Studies of drug tolerance by Remmer revealed that chronic exposure to phenobarbital increased its rate of metabolism by the liver (15). Burns & Conney showed that barbiturates induced the hepatic metabolism of a carcinogenic azo dye (16). Cooper et al demonstrated the role of hepatic cytochrome P-450 in the metabolism of drugs and carcinogens (17). Because of the temporal association of these observations, phenobarbital has been known as an archetypal inducer of cytochrome P-450-catalyzed enzyme activities. The phenobarbital-inducible cytochromes P-450 oxygenate a number of lipophilic substrates, both exogenous (e.g. drugs) and endogenous (e.g. steroids) (18–21).

Early studies of cytochromes P-450 in rabbit (22) and rat (23) liver revealed three distinct forms (designated cytochrome P-450a, -b, and -c in the rat) and showed that phenobarbital induced the one designated cytochrome P-450b. Differences in the primary structures of the apoproteins indicated that each of the cytochromes P-450a, -b, and -c was encoded by a different gene (24). Later, Vlasuk et al (25) and Rampersaud & Walz (26) observed microheterogeneity within cytochrome P-450b; they identified at least six closely related forms of hepatic phenobarbital-inducible cytochrome P-450 polypeptides in different strains of rats. Analyses of the phenotypes of the F_1 progeny from inter-strain crosses suggested that the cytochrome P-450 apoproteins were encoded by two closely linked genetic loci, with (at least) four alleles at a locus designated *P-450b* and (at least) two alleles at a locus designated *P-450e* (26). By

Table 1 Summary of cytochrome P-450 gene expression[a]

Gene	Responds to[b]	Regulatory mechanism				Other properties[d]
		Direct vs. indirect[c]	↑ mRNA Accumulation	↑ Rate of transcription	Receptor	
P-450b,e	PB	Unknown	+	+	Unknown	1, 2
P-450b,e	DEX	Unknown	+	-	glucocorticoid?	
P-450c, P_1-450	PAHs	Direct	+	+	Ah	3
P-450d, P_3-450	ISF	Unknown	+	Unknown	Unknown	
P-450d, P_3-450	PAHs	Unknown	+	+	Ah	1
$P\text{-}450_{PCN}$	PCN	Unknown	+	Unknown	Unknown	4
$P\text{-}450_{scc}$	ACTH	Indirect	+	Unknown	ACTH?	1
$P\text{-}450_{11\beta}$	ACTH	Indirect	+	Unknown	ACTH?	1
$P\text{-}450_{17\alpha}$	ACTH	Unknown	+	Unknown	ACTH?	
$P\text{-}450_{C21}$	ACTH	Unknown	±	Unknown	ACTH?	
$P\text{-}450_{aromatase}$	ACTH	Unknown	Unknown	Unknown	ACTH?	

[a]See text for discussion of the regulatory mechanisms.

[b]Abbreviations used: PB, phenobarbital; DEX, dexamethasone; PAHs, polycyclic aromatic hydrocarbons; ISF, isosafrole; PCN, pregnenolone-16α-carbonitrile; ACTH, adrenocorticotrophic hormone.

[c]Direct: increases in mRNA accumulation and transcription rate do not require ongoing protein synthesis. Indirect: increases in mRNA accumulation and transcription rate require ongoing protein synthesis.

[d]1: Tissue-specific expression; 2: heme-dependent expression; 3: superinducible; 4: sex-dependent expression.

definition, forms b and e constitute the major phenobarbital-inducible species of cytochrome P-450 in the rat. An analogous situation apparently exists in other species, although the studies have been less extensive.

Microheterogeneity analogous to that observed for the proteins also occurs at the nucleic acid level. Analyses of hepatic mRNA from different rat strains reveal several phenobarbital-inducible mRNAs that direct the in vitro synthesis of different, but antigenically-related, cytochrome P-450 apoproteins (27). Analyses of cloned cDNAs imply the existence of (at least) two related mRNAs for phenobarbital-inducible cytochrome P-450 (28). Use of these cDNAs to analyze restricted rat liver genomic DNA reveal (at least) six distinct genomic segments with substantial sequence homology (29, 30). Similar observations have been made in mice (31, 32), rabbits (33), and humans (34). These restriction analyses suggest that additional DNA sequences that are homologous to phenobarbital-responsive cytochrome P-450 genes remain to be cloned and characterized. It is possible that some of the DNA sequences do not represent functional genes (29, 30).

The analyses of restricted genomic DNA imply that variation at the genomic level accounts for the heterogeneity observed among the phenobarbital-inducible cytochrome P-450 apoproteins; however, the exact number of genes that encode these proteins is not yet known. Because of their sequence homology, these genes define a cytochrome P-450 multigene family. The designation of the gene family as "phenobarbital-responsive," while logical from an historical standpoint, is now somewhat misleading, because the members are related by DNA sequence and not necessarily by responsiveness to phenobarbital. For example, although they exhibit substantial (i.e. 60–70%) sequence homology to phenobarbital-responsive genes, the genes for cytochrome P-450 form 1 and form 3b are constitutively expressed in rabbit liver and do not respond to phenobarbital (35–37). The mechanism(s) that control the constitutive expression are unknown. Analyses of the genomic DNA from mouse-hamster somatic cell hybrids indicate that, in mice, the members of this gene family are clustered on chromosome 7, at the coumarin hydroxylase *(Coh)* locus (32, 38). In humans, this gene family maps to chromosome 19 (39).

Phenobarbital-responsive cytochrome P-450 genes apparently have a typical mammalian gene structure. Analysis of a functional cytochrome P-450e gene from rat liver reveals a total length of about 14 kilobases (kb), with nine exons and eight introns. The putative transcription start site is located 30 base pairs (bp) upstream of the ATG initiation codon, and a TATA-like sequence is present 27 bp farther 5'-ward. The 3' end contains a putative polyadenylation signal 25–26 bp upstream of the poly(A) attachment site. The exon/intron boundaries contain the appropriate consensus sequences (40).

Several investigators have studied the mechanism(s) by which phenobarbital alters the cytochrome P-450 content of the cell. In intact animals, phenobarbital

stimulates the incorporation of amino acids into cytochrome P-450 protein (41). Phenobarbital produces an accumulation of cytochrome P-450-specific mRNA, measured either by in vitro translation (42–45, 48–51) or by hybridization to cDNA (46, 47, 50–52). The use of synthetic oligodeoxynucleotide probes specific for cytochrome P-450 b or e reveals that phenobarbital induces a marked accumulation of both mRNAs, from levels that are undetectable in livers of untreated rats (53). Nuclear transcription experiments indicate that, in rat liver, phenobarbital produces within three to four hours a 20- to 50-fold increase in the rate of transcription of the cytochrome P-450b (and, presumably, P-450e) gene (54–56). The transcription rate increases uniformly along the length of the cytochrome P-450 gene (M. Adesnik and E. Rivkin, unpublished observations). The increase in the rate of cytochrome P450 gene transcription is followed by the accumulation of polyadenylated cytoplasmic cytochrome P-450-specific mRNA (55). The increase in transcription rate is comparable to the 50-fold induction of cytochrome P-450b (and, presumably, P-450e) protein produced by phenobarbital under similar conditions (57). These data imply that phenobarbital induces the accumulation of cytochrome P-450 protein primarily by stimulating the rate of transcription of the corresponding cytochrome P-450 gene. Furthermore, phenobarbital apparently increases the rate of transcription initiation, because in uninduced liver there is no detectable transcription of the cytochrome P-450 gene in the region immediately downstream of the mRNA cap site (M. Adesnik and E. Rivkin, unpublished observations). The undetectable levels of mRNA for cytochrome P-450 b and e in uninduced animals (53) suggest that the presence of inducer may be absolutely required for the transcription of the cytochrome P-450 b and e genes.

The mechanism by which phenobarbital increases the rate of cytochrome P-450 gene transcription is unknown. The transcription rate begins to increase within an hour of phenobarbital administration (55), which suggests that induction does not involve many intermediary events. However, it is not known whether phenobarbital can stimulate the rate of cytochrome P-450 gene transcription directly (i.e. in the absence of ongoing protein synthesis).

The induction mechanism for polycyclic aromatic hydrocarbon-responsive forms of cytochrome P-450 involves the binding of the inducer to an intracellular protein receptor; the inducer-receptor complex then interacts with a genomic regulatory element to stimulate the transcription of the cytochrome P-450 gene (see next section). A similar mechanism governs the expression of steroid-responsive genes (58). By analogy, we might expect phenobarbital to act via a receptor-mediated mechanism. However, in contrast to compounds known to act at receptors, inducers of the phenobarbital-type are (*a*) effective only at high concentrations and (*b*) exhibit no obvious structural similarities (59); these properties are not suggestive of a receptor-mediated mechanism.

The development of more potent "phenobarbital-like" inducers has been modestly successful but has not yet helped to clarify the induction mechanism. For example, the compound TCPOBOP produces a phenobarbital type of response in mice, but not in rats; furthermore, TCPOBOP fails to antagonize the effects of phenobarbital in rats (60, 61). Thus, in rats, TCPOBOP does not seem to act at "phenobarbital receptors." The idea that phenobarbital-type inducers act via a nonreceptor mechanism and that induction reflects some physiochemical property that the compounds have in common (e.g. the ability to alter membrane structure) does not easily account for their ability to induce particular forms of cytochrome P-450 and not others. Thus, the data to date neither substantiate nor refute the idea that phenobarbital induces cytochrome P-450 gene expression by interacting with specific receptors. This is an area where additional research seems likely to generate useful information. For example, synthesis of more potent phenobarbital-type inducers may be useful in identifying specific receptors, if they exist. Mapping of nuclease-hypersensitive sites (62) in the vicinity of phenobarbital-responsive genes may help to identify genomic control elements that interact with regulatory proteins, including (hypothetical) inducer-receptor complexes. Construction of hybrid genes, combined with the use of gene transfer, may help to define the function of such genomic control elements (see next section for example). It is notable that genetic differences in responsiveness to phenobarbital exist among rats (63, 64) and mice (65) in the induction of hepatic aldehyde dehydrogenase. In mice, the genetic variation apparently occurs at a regulatory locus (65), which, therefore, might encode a phenobarbital-specific regulatory protein.

Heme may also regulate the level of cytochrome P-450 gene expression. Cobalt chloride and 3-amino-1,2,4-triazole, which are inhibitors of heme biosynthesis, also inhibit the synthesis of the apoprotein cytochrome P-450b (and, presumably, P-450e) (66). Using cDNA as a hybridization probe, Ravishankar & Padmanaban showed that both cobalt chloride and 3-amino-1,2,4-triazole inhibit the phenobarbital-induced accumulation of mRNA for cytochrome P-450b/e. Furthermore, nuclear transcription assays indicate that both compounds inhibit the phenobarbital-stimulated increase in the rate of cytochrome P-450b/e gene transcription (52). These results imply that the intracellular heme concentration influences cytochrome P-450 gene expression. Others have also observed a possible relationship between heme and cytochrome P-450 enzyme concentrations (67).

The mechanism(s) by which heme may regulate cytochrome P-450 gene transcription are unknown. However, an analogous system may function in *S. cerevisiae,* where heme regulates the transcription of the iso-1-cytochrome c *(CYC1)* gene. Heme-deficient cells transcribe the *CYC1* gene at a low rate and contain undetectable levels of iso-1-cytochrome c apoprotein. Heme increases the rate of *CYC1* gene transcription and acts at a genomic regulatory element

(termed an "upstream activation site") located several hundred nucleotides 5'-ward of the *CYC1* gene (68). Furthermore, a *trans*-acting, recessive mutation at the *HAP1* ("heme activator protein") locus renders the upstream activation site uninducible by heme (69). Thus, the *HAP1* locus probably encodes a regulatory protein that binds heme and activates *CYC1* gene transcription by binding to the upstream activation site. It will be interesting to determine if a similar mechanism controls the expression of some cytochrome P-450 genes.

Decreases in cytochrome P-450 protein concentration produced by some inducers may be related to decreased availability of heme. Using immunological techniques to quantitate various cytochrome P-450 apoproteins, several investigators (18, 70–72) have reported that inducers may increase the concentration of one form(s) of cytochrome P-450 while simultaneously decreasing the concentration of another form(s). For example, phenobarbital (and other inducers) produces a decrease in a form of cytochrome P-450 that is "constitutive". Likewise, β-naphthoflavone and 3-methylcholanthrene, which induce the PAH-responsive subset of proteins, produce decreases in the subset that is phenobarbital-inducible. Other workers have observed that the β-naphthoflavone-induced decrease in a phenobarbital-inducible form of cytochrome P-450 is associated with a corresponding decrease in its mRNA (48, 49). Thus, the lower cytochrome P-450 content probably reflects decreased transcription of the corresponding gene(s). The mechanism by which these decreases occur is unknown. However, in view of the apparent ability of heme to regulate the transcription of (at least some) cytochrome P-450 genes, one possibility is that, as a result of increased transcription of some cytochrome P-450 genes, the availability of heme may become rate-limiting for the transcription of others. An alternative possibility is that some inducers have more than one primary effect and may stimulate the expression of one cytochrome P-450 gene(s) while inhibiting the expression of another (72). This is an area that seems worthy of future study.

Responsiveness to phenobarbital is also a function of the tissue in which the cytochrome P-450 gene is expressed. For example, using three different cDNAs to measure mRNA concentrations, Leighton & Kemper detected all three mRNA species in rabbit liver, only one in kidney, and none in lung. Furthermore, in liver, two mRNA species were phenobarbital-inducible, and one was expressed constitutively (37). Thus, three closely related genomic sequences are differentially responsive to phenobarbital and exhibit tissue-specific patterns of expression. Others have made related observations at the protein level. For example, one form of cytochrome P-450, which is phenobarbital-inducible in rabbit liver, is constitutively expressed in the lung (73, 74). Presumably, these findings reflect tissue-specific differences in the transcription rate, because transcriptional control probably determines the tissue-specific pattern of gene expression (75). Furthermore, it seems likely

that *trans*-acting regulatory factors govern the tissue-specific transcription rate (76).

There is also evidence for posttranscriptional control of the cytochrome P-450b gene. In rat liver, phenobarbital induces a 20-fold increase in the rate of cytochrome P-450b gene transcription, which is followed by a corresponding increase in mRNA accumulation. In contrast, dexamethasone has no effect on the transcription rate, yet it produces a 10-fold increase in mRNA concentration (C. B. Kasper, personal communication). These findings suggest that dexamethasone may stabilize cytochrome P-450b mRNA. The mechanism by which dexamethasone produces this effect is unknown. Possibly, it activates the transcription of a gene(s) whose product selectively inhibits the degradation of particular mRNAs.

The findings outlined above imply the existence of several distinct mechanisms that regulate the transcription of phenobarbital-responsive cytochrome P450 genes. A working hypothesis might envision a hierarchy of *cis*-acting genomic control elements, each of which influences the rate of gene transcription upon its interaction with a specific *trans*-acting regulatory factor. One element would respond to phenobarbital, a second to heme, and a third to a tissue-specific factor(s). Additional elements might also exist. At any given time, the rate of cytochrome P-450 gene transcription would reflect the contribution of each component of the regulatory hierarchy.

GENES THAT RESPOND TO POLYCYCLIC AROMATIC HYDROCARBONS

During studies of the hepatic metabolism of chemical carcinogens, the Millers observed that ingestion of polycyclic aromatic hydrocarbons (PAHs) increased the rate of carcinogen metabolism in rats and mice (77). Subsequent experiments implied that PAHs induced the synthesis of carcinogen-metabolizing enzyme molecules (78). Shortly thereafter, Cooper et al implicated cytochrome P-450 in carcinogen metabolism (17). By virtue of these findings, 3-methylcholanthrene became the prototype of a class of inducers of cytochrome P-450-catalyzed enzyme activities. The PAH-inducible cytochromes P-450 metabolize a spectrum of lipophilic compounds that overlaps those of other forms of cytochrome P-450 (18). Studies of the metabolism of carcinogenic PAHs, such as benzo(a)pyrene, have revealed the major role of the PAH-inducible cytochromes P-450 in chemically induced neoplasia and toxicity (79–81).

Studies in rat liver implied that 3-methylcholanthrene induced a single form of cytochrome P-450 (designated P-450c), which was distinctly different from the two other known forms of cytochrome P-450 (23). Subsequently, Ryan et al observed that isosafrole preferentially induces a fourth form of cytochrome

P-450 (designated P-450d), which is immunologically related to cytochrome P-450c, but which has different catalytic activities and a different primary structure (82–84). PAHs induce cytochrome P-450d to a somewhat lesser extent than they induce cytochrome P-450c (85). By definition, forms c and d constitute the major PAH-inducible species of cytochrome P-450 in the rat. Similarly, PAHs induce two forms of cytochrome P-450 in rabbit liver (86).

In mice, 3-methylcholanthrene (and other PAHs) also induces two hepatic forms of cytochrome P-450, which differ in their catalytic activities and primary structures; isosafrole preferentially induces one of the forms (designated cytochrome P_3-450) (87). The isolation, characterization, and comparison of cDNA and genomic clones (see below) for the PAH-inducible cytochromes P-450 in rats and mice indicate that cytochrome P-450c (rat) is equivalent to cytochrome P_1-450 (mouse) and that cytochrome P-450d (rat) is equivalent to cytochrome P_3-450 (mouse). Analogous isozymes (form 6 and form 4) exist in rabbits (88). In addition, in a mouse strain (DBA/2N), which is "nonresponsive" to PAHs (see below), isosafrole induces an hepatic form that has been designated cytochrome P_2-450 (89). However, the mRNAs for cytochrome P_2-450 and cytochrome P_3-450 are both 20S (90, 91), and anti-cytochrome P_2-450 antibodies precipitate the protein translated in vitro from cytochrome P_3-450 mRNA (91). These findings imply the existence of substantial similarities between cytochrome P_2-450 and cytochrome P_3-450; whether they represent the products of different genes remains to be established.

Several investigators have isolated cDNAs for PAH-inducible forms of cytochrome P-450 (91–102). Analyses of the cDNAs for rat cytochrome P-450c (96) and cytochrome P-450d (98), for mouse cytochrome P_1-450 and cytochrome P_3-450 (101), and for the human equivalent of cytochrome P_1-450 (102) reveal that they contain substantial sequence homologies. There is notably less sequence homology between PAH-responsive cytochrome P-450 genes and phenobarbital-responsive cytochrome P-450 genes. Thus, the PAH-responsive cytochrome P-450 genes represent a distinct family within the larger superfamily of cytochrome P-450 genes. Analyses of restricted genomic DNA imply that the PAH-responsive cytochrome P-450 gene family is considerably smaller than the phenobarbital-responsive cytochrome P-450 gene family (99, 101, 102).

Analyses of the PAH-responsive cytochrome P-450 genes in the rat (103–105) and mouse (99, 106) reveal similar exon-intron arrangements, which differ substantially from the exon-intron organization of the phenobarbital-responsive cytochrome P-450e gene. Sequence analyses reveal putative TATA structures and polyadenylation signals at appropriate locations. The 5'-flanking regions contain sequences capable of forming Z-DNA and sequences with some homology to viral enhancers. The functional significance of these

observations remains to be determined. It is notable that there is relatively little sequence homology between rat cytochromes P-450c and P-450d (and between mouse cytochromes P_1-450 and P_3-450) in the DNA flanking the 5' ends of the genes, despite the fact that each gene is PAH-responsive (103, 107). However, the genomic control element(s) that is recognized by the PAH-receptor complex may be located still farther 5'-ward of the regions that have been sequenced (see below).

Analyses of restricted genomic DNA from a panel of mouse × hamster somatic cell hybrids, using cytochrome P_1-450-specific and cytochrome P_3-450-specific DNA sequences as hybridization probes, reveal that both the cytochrome P_1-450 gene and the cytochrome P_3-450 gene are located on mouse chromosome 9 (107). Thus, the PAH-responsive cytochrome P-450 genes, the phenobarbital-responsive cytochrome P-450 genes, the PCN-responsive cytochrome P-450 genes, and the steroid 21-hydroxylase cytochrome P-450 genes (see following sections) are located on four different chromosomes in the mouse. Similar analyses of human × rodent somatic cell hybrids have localized the equivalent of the cytochrome P_1-450 gene to human chromosome 15 (108).

The induction mechanism for PAH-responsive cytochrome P-450 genes has been studied in some detail. PAHs induce the accumulation of mRNA specific for cytochrome P-450c and cytochrome P-450d in rat liver (109–114), for cytochrome P_1-450 and cytochrome P_3-450 in mouse liver (90, 91, 115–119), and for cytochrome P_1-450-specific mRNA in mouse hepatoma cells in culture (120). These findings suggest that PAHs may increase the rate of cytochrome P-450 gene transcription. However, rates of transcription were not measured in these studies. In rat liver, cytochrome P-450c mRNA and cytochrome P-450d mRNA accumulate at similar rates in response to 3-methylcholanthrene, and a measurable increase occurs relatively rapidly (within four hours). Furthermore, the induction mechanisms for both cytochrome P-450c and cytochrome P-450d exhibit similar sensitivities to PAH-type inducers. In contrast, in uninduced liver, the concentration of cytochrome P-450d mRNA is substantially higher than that of cytochrome P-450c mRNA. In addition, isosafrole preferentially induces the accumulation of cytochrome P-450d mRNA (113). Together, these findings imply that the regulatory mechanisms governing the expression of these two cytochrome P-450 genes may have a component (which controls PAH-responsiveness) that is similar, as well as components (which control "constitutive" expression and isosafrole-responsiveness) that are different.

In mouse liver, 3-methylcholanthrene increases the rate of synthesis of both cytochrome P_1-450 mRNA and cytochrome P_3-450 mRNA (119). Likewise, in mouse hepatoma cells, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, the most potent known PAH-type inducer) induces a 20-fold increase in the rate of cytochrome P_1-450 mRNA synthesis. In the cells in culture, the effect is maximal within 30 min and does not require ongoing protein synthesis; thus,

the increased cytochrome P_1-450 transcription rate is a primary response to the inducer (121). The simplest interpretation of all the data is that PAHs stimulate the rate of transcription of both PAH-responsive cytochrome P-450 genes. It remains to be determined whether (*a*) the elevated level of cytochrome P-450d (or cytochrome P_3-450) mRNA in uninduced cells and (*b*) the mRNA accumulation that occurs in response to isosafrole reflect corresponding increases in the rate of transcription of the cytochrome P-450d (or cytochrome P_3-450) gene. Furthermore, it is not known whether isosafrole acts via a receptor-mediated mechanism to induce the accumulation of cytochrome P-450 mRNA and protein (122). Thus, the mechanism(s) that governs the cellular response to isosafrole may be an area worthy of additional study.

More is known about the mechanism by which PAHs stimulate the expression of the mouse cytochrome P_1-450 gene. This knowledge stems in part from observations that, in certain inbred strains of mice, a single autosomal dominant gene apparently controls several responses to PAHs (123–128). For example, the induction of aryl hydrocarbon hydroxylase (AHH), a cytochrome P_1-450-catalyzed enzyme activity, is expressed as an autosomal dominant trait in crosses between the C57BL/6 and DBA/2 mouse strains (127, 128). In addition, other responses to PAHs segregate in the same pattern as AHH inducibility (129). Therefore, the genetic locus controlling the expression of these responses is thought to be regulatory, and has been designated *Ah*, for aromatic hydrocarbon responsiveness. The observations (*a*) that TCDD can induce AHH activity in mouse strains (e.g. DBA/2) that do not respond to other PAHs (130), (*b*) that the AHH induction mechanism in responsive mouse strains (e.g. C57BL/6) is 10- to 20-fold more sensitive to TCDD than the induction mechanism in nonresponsive strains (131), and (*c*) that the livers of responsive strains contain a protein that binds TCDD with high affinity and low capacity (132) led to the concept that the *Ah* locus encodes an intracellular "receptor" protein that is required for the induction response. Nonresponsive mouse strains contain a receptor that binds TCDD poorly (and less potent PAHs not at all) (133). The F_1 progeny of crosses between C57BL/6 (responsive) and DBA/2 (nonresponsive) mice exhibit the responsive phenotype for AHH induction, yet contain about one-half the number of *Ah* receptors as the C57BL/6 parent. This observation implies that, in the C57BL/6 mouse, the number of receptors is not rate limiting for the induction of AHH by PAHs. This and other aspects of *Ah* receptor biology have been reviewed recently (134).

The induction of AHH activity involves the formation of inducer-receptor complexes and the temperature-dependent accumulation of these complexes in the nucleus (135, 136). It is not yet clear whether, in the intact cell, unoccupied receptors are cytoplasmic (137) or nuclear (138). Furthermore, the related issue of whether the temperature-dependent step in enzyme induction is a "translocation" of the inducer-receptor complex from the cytoplasm to the nucleus (137)

or some other event (e.g. enzymatic modification), which takes place in the nucleus (138), remains to be resolved. There is general agreement, however, that the inducer-receptor complex acts in the nucleus to stimulate the transcription of the cytochrome P_1-450 gene. In mouse hepatoma cells, the accumulation of inducer-receptor complexes in the nucleus correlates temporally with increases in the rate of cytochrome P_1-450 gene transcription (D. I. Israel and J. P. Whitlock, Jr., unpublished observations).

Studies of AHH induction in cells in culture have revealed additional aspects of the control of cytochrome P_1-450 gene expression. Hankinson devised a selection protocol, which takes advantage of the metabolic activation of PAHs by the AHH system (139), to isolate mutant mouse hepatoma cells defective in AHH activity (140). Using the same (Hepa 1c1c7) cells, Miller & Whitlock employed the fluorescence-activated cell sorter to isolate variants that either underproduce or overproduce AHH in response to PAHs (141). Both groups identified two complementation groups of receptor-defective variants with altered AHH induction mechanisms (142, 143). Variants that form few inducer-receptor complexes accumulate little cytochrome P_1-450 mRNA and AHH activity in response to TCDD (120, 144), and variants in which the inducer-receptor complex binds weakly to the nucleus (138) fail to transcribe the cytochrome P_1-450 gene in response to TCDD (121) and contain no detectable cytochrome P_1-450 mRNA or AHH activity (120, 144). These findings indicate that transcription of the cytochrome P_1-450 gene requires functional inducer-receptor complexes. Hankinson and co-workers have isolated additional classes of AHH-defective mutants; one class apparently contains a lesion(s) in the cytochrome P_1-450 structural gene (145); another class exhibits a dominant phenotype (146).

The existence of dominant, AHH-defective mutants (146), which also fail to accumulate cytochrome P_1-450 mRNA in response to TCDD (144), implies that a *trans*-acting inhibitory factor(s) may regulate cytochrome P_1-450 gene transcription. Studies of the "superinduction" of AHH activity support this hypothesis. In cells in culture, temporary exposure to cycloheximide (an inhibitor of protein synthesis) plus a PAH inducer is followed by an increase in AHH activity that is greater than the maximal increase produced by the PAH alone (147). Superinduction of enzyme activity is associated with the (super) accumulation of cytochrome P_1-450 mRNA (120), which is due to a (super) increased rate of cytochrome P_1-450 gene transcription (148). Cycloheximide produces no detectable effect on the properties of *Ah* receptors. The superinduction response indicates that functional inducer-receptor complexes are not sufficient for maximal transcription of the cytochrome P_1-450 gene. The observations suggest the existence of a labile repressor protein that inhibits the transcription of the cytochrome P_1-450 gene (148). The possible relationship between the negative regulatory factor implied by the dominant mutants and the labile repressor implied by the superinduction response remains to be de-

termined. Together, the data imply that (at least) two *trans*-acting factors control cytochrome P_1-450 gene expression: the inducer-receptor complex stimulates, and a labile repressor inhibits, the transcription of the cytochrome P_1-450 gene. Therefore, expression of this gene reflects a balance between positive and negative control of transcription.

These *trans*-acting regulatory factors act via *cis*-acting genomic control elements located upstream (5'-ward) of the cytochrome P_1-450 gene. Jones et al have analyzed variant mouse hepatoma cells (high activity variant [HAV] cells) that overexpress AHH activity in response to TCDD. The HAV cells overtranscribe the cytochrome P_1-450 gene in response to TCDD, contain no detectable alteration in their TCDD receptors, and exhibit a phenotype that is co-dominant with wild type, suggesting that they contain a *cis*-acting regulatory alteration (149). Jones et al isolated a 2.6-kb DNA fragment located 5'-ward of the cytochrome P_1-450 gene in the HAV cells (150). In order to analyze the function of this DNA, they constructed a recombinant molecule that contained the 2.6-kb fragment situated immediately upstream of the bacterial chloramphenicol acetyltransferase (CAT) gene. Transfection experiments showed that, in this construct, the CAT gene was inducible by PAHs. Furthermore, deletion analyses revealed that the 2.6-kb DNA fragment contained three functional domains: a promoter, located immediately upstream of the transcription start site; an inhibitory domain (which presumably interacts with the labile repressor), located 700–1000 bp upstream of the promoter; and a PAH-responsive domain (which presumably interacts with the inducer-receptor complex), located 1300–1600 bp upstream of the promoter. The regulatory model is depicted schematically in Figure 1.

The data imply that two different regulatory proteins act via two distinct genomic elements to control cytochrome P_1-450 (and, by analogy, cytochrome P-450c) gene transcription. Whether similar components control the transcription of the cytochrome P_3-450 (and cytochrome P-450d) gene remains to be determined. The existence of tissue-specific differences (90, 151–153) and developmental differences (90, 154) in the expression of PAH-responsive cytochrome P-450 genes suggest that additional regulatory proteins and their affiliated genomic control elements remain to be characterized.

Genetic studies using inbred mice imply that the *Ah* regulatory system contains additional complexity. In crosses between the C3H/He (responsive) and DBA/2 (nonresponsive) strains, AHH inducibility segregates in co-dominant fashion (155, 156). This may indicate the existence of a third allele at the *Ah* locus. In crosses between the C57BL/6N (responsive) and AKR/N (nonresponsive) strains, the nonresponsive phenotype segregates in autosomal dominant fashion (156). As yet, there has been no published model that can account for all of these observations. It would seem worthwhile to follow-up these provocative findings (in particular, the unusual reversal of dominance) with more detailed studies of cytochrome P-450 gene expression in these

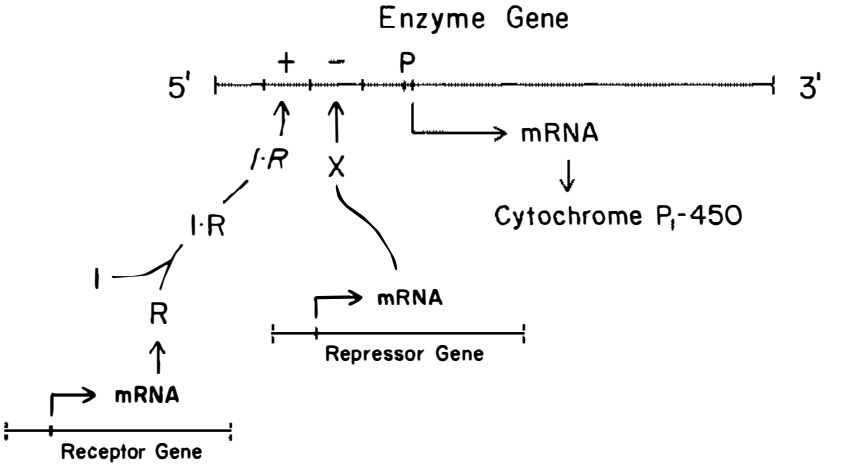

Figure 1 Regulation of cytochrome P_1-450 gene expression in HAV mouse hepatoma cells. The inducer (I) forms a complex (I·R) with an intracellular receptor (R), which (presumably) is encoded by a gene at the *Ah* locus. The inducer-receptor complex undergoes a temperature-dependent change to a form (*I·R*), which binds tightly to chromatin. The binding of the inducer-receptor complex to a genomic domain (+) upstream of the promoter (P) stimulates cytochrome P_1-450 gene transcription. A hypothetical, cycloheximide-sensitive labile repressor (×) binds to a second upstream genomic domain (−) and inhibits transcription. Therefore, cytochrome P_1-450 gene transcription represents a balance between positive and negative control. See Ref. 150 for experimental data. It is possible that additional regulatory factors and genomic control elements also exist for the cytochrome P_1-450 gene (see text).

animals. Likewise, the existence of a mouse fibroblast cell line in which some, but not all, PAHs induce AHH activity (157, 157a) raises the question of heterogeneity of the *Ah* receptor. This observation also seems worthy of additional study.

The chromosomal location of the *Ah* locus is unknown. Although studies in somatic cell hybrids suggest that a gene that regulates AHH inducibility is located on mouse chromosome 17, there is no direct evidence that it encodes the receptor protein (158). Furthermore, the organization and structure of the *Ah* locus is unknown. To describe it as the "*Ah* complex" is misleading, in that there is no evidence that the locus encodes more than one protein. Similarly, designation of the cytochrome P_1-450 and cytochrome P_3-450 apoproteins as "structural products of the *Ah* locus" is not justified, in the absence of any evidence that the gene(s) that encodes the receptor is linked to the genes that encode the enzymes. Although these issues may seem semantic, the failure to use precise terminology adds needless confusion to an area of study that is already complicated.

Several investigators have described other proteins that saturably bind 3-

methylcholanthrene, benzo(a)pyrene, and/or other PAHs with high affinity (159--162). These proteins differ from the receptor encoded by the *Ah* locus with respect to their PAH binding and/or physical characteristics. Although their properties are consistent with those expected for PAH "receptors", their function(s) remains unknown.

In summary, the data imply that PAHs induce cytochrome P-450-catalyzed enzyme activity by activating the rate of transcription of the corresponding gene. The inducer forms a complex with an intracellular regulatory protein (encoded by the *Ah* locus in mice); the complex binds to a *cis*-acting genomic control element upstream of the cytochrome P-450 gene. The ability of the genomic control element to activate transcription from a distance suggests that it may be a PAH-responsive enhancer (163). (At least) one additional regulatory component (an inhibitory one) modulates the response of the cytochrome P_1-450 gene to PAHs. Additional control elements may also exist: those mediating tissue-specific expression, temporal (developmental) expression, and, possibly, responsiveness to heme. The instantaneous rate of cytochrome P-450 gene transcription presumably reflects the contribution of each component of this putative regulatory hierarchy.

GENES THAT RESPOND TO STEROIDS

Inducers of cytochrome P-450 were originally classified as either "phenobarbital-type" or "3-methylcholanthrene-type," based upon the profile of microsomal enzyme activities that each compound induced. In the early 1970s, Selye (164) studied in rats a class of "catatoxic" steroids that conferred protection against toxic chemicals by increasing their rate of metabolism. The most potent, pregnenolone-16α-carbonitrile (PCN), caused proliferation of hepatic endoplasmic reticulum and an increase in hepatic cytochrome P-450 content. In addition, PCN induced a profile of cytochrome P-450-catalyzed enzyme activities different from those induced by either phenobarbital or 3-methylcholanthrene (165). The latter observation suggested that PCN might induce a unique form(s) of cytochrome P-450 and, thus, would represent a third class of inducer. Subsequently, Elshourbagy & Guzelian purified from rat liver a PCN-inducible form of cytochrome P-450. The enzyme had distinctive biochemical, immunologic, and catalytic properties, implying that it was encoded by a unique cytochrome P-450 gene (166). The physiological substrate(s) for this form of cytochrome P-450 is unknown. However, its high constitutive expression in male rats suggests its possible involvement in androgen metabolism.

Hardwick et al constructed a cDNA library using immunoenriched mRNA from male rats and identified a cDNA for cytochrome $P\text{-}450_{PCN}$ (167). Analysis of restricted genomic DNA using the cDNA as a hybridization probe reveals

multiple bands containing 50 to 60 kb of DNA (167, 168). This finding implies either that the cytochrome P-450$_{PCN}$ gene is large and contains much more intron DNA than exon DNA or, more likely, that the rat genome contains additional elements with sequence homology to the cytochrome P-450$_{PCN}$ gene. Together, the elements may constitute a distinct cytochrome P-450 gene family (169). It is unknown whether these additional DNA elements are either functional or PCN-responsive.

Simmons et al used the rat cytochrome P-450$_{PCN}$ cDNA to analyze restricted genomic DNA isolated from a panel of mouse-hamster somatic cell hybrids, containing different combinations of mouse chromosomes. All the mouse sequences hybridizable to the cDNA mapped to chromosome 6, implying that the cytochrome P-450$_{PCN}$ gene(s) is located on this chromosome in the mouse, at a locus tentatively termed *Pcn* (168).

Because PCN was ineffective as an inducer in several established cell lines, Guzelian et al utilized primary cultures of female rat hepatocytes to study the mechanism of cytochrome P-450$_{PCN}$ induction. Immunoprecipitation experiments indicate that PCN increases both the rate of synthesis of the cytochrome P-450$_{PCN}$ protein and the accumulation of translatable cytochrome P-450$_{PCN}$ mRNA (170). These findings imply the existence of a transcriptional step in the induction mechanism.

In male rats, PCN induces within 12 hours a 6-fold increase in the hepatic content of cytochrome P-450$_{PCN}$-specific mRNA (167). Phenobarbital also induces a 4-fold increase in cytochrome P-450$_{PCN}$ mRNA accumulation. However, the response to phenobarbital is considerably slower than the response to PCN, suggesting that, in this system, the two inducers might act via different mechanisms. Experiments to determine whether phenobarbital and PCN have an additive (or synergistic) effect on cytochrome P-450$_{PCN}$ mRNA accumulation have not been reported.

The mechanism by which PCN induces the accumulation of cytochrome P-450$_{PCN}$ mRNA is not known. It is unknown whether PCN-induced mRNA accumulates in the absence of ongoing protein synthesis. Furthermore, we do not know if PCN acts to increase the rate of transcription of the cytochrome P-450$_{PCN}$ gene, although this seems likely, given what we know about the mechanism of cytochrome P-450 enzyme induction by phenobarbital and PAHs.

Other steroid hormones, such as dexamethasone, act via intracellular protein receptors to activate the transcription of specific genes (58). By analogy, we might expect PCN action to be receptor-mediated. In intact rat liver and in primary rat hepatocyte cultures, dexamethasone induces cytochrome P-450$_{PCN}$; in fact, some glucocorticoids are more effective inducers than PCN itself (171–173). However, the mechanism by which dexamethasone induces cytochrome P-450$_{PCN}$ does not exhibit characteristics typical of other glucocor-

ticoid-induced responses. For example, in cultured hepatocytes, (*a*) maximal induction of cytochrome P-450$_{PCN}$ requires a dexamethasone concentration that is at least two orders of magnitude higher than that required to maximally induce tyrosine aminotransferase (TAT); (*b*) PCN and spironolactone induce cytochrome P-450$_{PCN}$ but do not induce TAT; (*c*) the kinetics of induction of cytochrome P-450$_{PCN}$ and TAT by dexamethasone are different; (*d*) the rank order of potency of various steroids as inducers of TAT is different from their rank order as inducers of cytochrome P-450$_{PCN}$; (*e*) glucocorticoid antagonists inhibit induction of TAT but enhance induction of cytochrome P-450$_{PCN}$ (173). Thus, the induction of cytochrome P-450$_{PCN}$ by steroids apparently involves a mechanism distinct from those described for other systems in which classical glucocorticoid receptors mediate the induction response (58). Whether the response to PCN is receptor-mediated remains to be determined. The identification of more potent inducers of the PCN-type, the identification of PCN antagonists, and the synthesis of PCN-type inducers that can be used as photoaffinity reagents should facilitate the study of this system in the future.

In addition to its control by steroids, the cytochrome P-450$_{PCN}$ gene also exhibits sex-dependent expression. Uninduced male rats contain a substantial amount of hepatic cytochrome P-450$_{PCN}$ mRNA and protein (18, 167, 174). In fact, in untreated male rats, cytochrome P-450$_{PCN}$ constitutes about 15% of the total cytochrome P-450. In contrast, untreated females contain very little cytochrome P-450$_{PCN}$. Presumably, these sex-dependent differences reflect an increased "constitutive" rate of cytochrome P-450$_{PCN}$ gene transcription in the male. The mechanism that governs the increased expression in the male is not well understood. It might be similar to the mechanism(s) that controls the sex-dependent expression of other cytochrome P-450 genes (see section on sexual dimorphism).

Thus, the cytochrome P-450$_{PCN}$ gene apparently exhibits (at least) two distinct control mechanisms: one is steroid-dependent, the other is sex-dependent. Both presumably operate at the transcriptional level. Each control mechanism apparently functions relatively independently, because even in males the gene responds to PCN.

GENES THAT RESPOND TO PITUITARY HORMONES

Studies of steroid biosynthesis in the adrenal cortex led to the discovery of the physiological function of cytochrome P-450. Using Warburg's photochemical action spectrum technique, Estabrook et al demonstrated that the microsomal hemoprotein functioned as an oxygenase in the C_{21}-hydroxylation of steroids (175). Subsequently, several additional steroidogenic reactions were found to be catalyzed by various forms of cytochrome P-450. Two forms (cytochrome P-450$_{scc}$ and cytochrome P-450$_{11\beta}$) are integral components of the inner

mitochondrial membrane and are synthesized as larger precursor molecules. The mitochondrial electron transport system consists of both a flavoprotein (NADPH-adrenodoxin reductase) and an iron-sulfur protein (adrenodoxin), analogous to the situation in bacteria. Other forms (cytochrome P-450$_{17\alpha}$, cytochrome P-450$_{C21}$, and cytochrome P-450$_{aromatase}$) are microsomal, are synthesized as the mature protein, and utilize an electron transport system consisting of only a flavoprotein (NADPH-cytochrome P-450 reductase). [For review, see (176, 177).]

Cytochrome P-450$_{scc}$

This enzyme catalyzes the conversion of cholesterol to pregnenolone (a reaction termed cholesterol side-chain cleavage), which is the rate-limiting step in the biosynthetic pathway leading from cholesterol to steroid hormones. Bovine adrenal cytochrome P-450$_{scc}$ cDNA has been cloned by several groups (178–180). Analyses of restricted bovine adrenocortical genomic DNA using a partial-length cDNA as a hybridization probe reveal a relatively simple pattern, suggesting that the genome does not contain multiple elements homologous to the cytochrome P-450$_{scc}$ gene (179). This observation awaits confirmation in studies using full-length cDNA. The cytochrome P-450$_{scc}$ cDNA hybridizes to a 2-kb polyadenylated RNA species, which is present in bovine adrenal cortex and corpus luteum but not in liver, heart, or kidney. Thus, the cytochrome P-450$_{scc}$ gene exhibits tissue-specific expression appropriate to its function.

In the intact rat, endocrine ablation and hormone replacement experiments suggest that ACTH contributes to the maintenance of cytochrome P-450-catalyzed enzyme activities in the adrenal cortex (181). Using a bovine adrenocortical cell culture system, DuBois et al found that ACTH produces, over 36 hr, an increased rate of synthesis of cytochrome P-450$_{scc}$, which is accompanied by a comparable increase in the amount of translatable cytochrome P-450$_{scc}$ mRNA (182). In this system, ACTH stimulates the formation of cyclic AMP, which apparently is responsible for producing the accumulation of cytochrome P-450$_{scc}$ protein and translatable mRNA (183). The rate of ACTH-induced cytochrome P-450$_{scc}$ mRNA accumulation is relatively slow (179). Furthermore, ACTH does not induce cytochrome P-450$_{scc}$ mRNA accumulation in the presence of cycloheximide, indicating that mRNA accumulation requires ongoing protein synthesis [unpublished observations, cited in (184)].

One interpretation of these findings is that ACTH (presumably via membrane receptors) increases the intracellular concentration of cAMP. Cyclic AMP then may induce the synthesis of a "steroid hydroxylase-inducing protein" (184), which subsequently induces the accumulation of cytochrome P-450$_{scc}$ mRNA, either by increasing its rate of synthesis or by performing a processing or stabilizing function at posttranscriptional level [see, for example, (185)].

Measurements of nuclear transcription rates would help to distinguish between these possibilities.

In primary cultures of bovine granulosa cells, follicle-stimulating hormone (FSH) and analogs of cyclic AMP increase the rate of synthesis of cytochrome P-450_{scc} about 4-fold (186, 187). By analogy with the findings for ACTH-stimulated adrenocortical cells, the interaction of FSH with its membrane receptor presumably stimulates the intracellular production of cyclic AMP, which subsequently leads to the accumulation of cytochrome P-450_{scc} mRNA. However, studies of mRNA synthesis and accumulation have not yet been described in this system. In addition, low density lipoprotein stimulates the rate of synthesis of cytochrome P-450_{scc} in granulosa cells, although the effect is less than 2-fold. This increase may reflect the increased availability of the substrate, cholesterol, in the presence of LDL; however, the mechanism by which cholesterol would increase the rate of enzyme synthesis is not clear.

Both cytochrome P-450_{scc} protein (in adrenocortical cells and in granulosa cells) and P-450_{scc} mRNA (in adrenocortical cells) are detectable in uninduced cells (179, 183, 187). This could reflect residual hormonal stimulation of the primary cultures; however, the lack of detectable cytochrome P-$450_{17\alpha}$ under similar conditions argues against this possibility. On the other hand, these findings may reflect the constitutive expression of the cytochrome P-450_{scc} gene. If so, the control mechanism(s) that permits constitutive expression remains to be determined. The data also indicate that, in (at least some) nonsteroidogenic tissues, P-450_{scc} mRNA is undetectable. Thus, a tissue-specific mechanism for the control of cytochrome P-450_{scc} gene expression presumably also exists.

Cytochrome P-$450_{11\beta}$

This enzyme catalyzes the 11β hydroxylation of 11-deoxycortisol to form cortisol. The enzyme is present in the adrenal cortex but not in the testis, ovary, or placenta (176, 177). John et al have isolated two overlapping partial-length adrenocortical cDNA clones for cytochrome P-$450_{11\beta}$ (184). Filter hybridization analyses of adrenocortical RNA reveal three distinct species of polyadenylated RNA, each of which directs the in vitro synthesis of immunoprecipitable cytochrome P-$450_{11\beta}$. These RNA species are detectable in the adrenal cortex but not in the corpus luteum, liver, heart, or kidney. It is not yet clear whether the presence of multiple mRNAs reflects the expression of more than one cytochrome P-$450_{11\beta}$ gene or whether the mRNAs are generated by the differential processing of a single transcript. Filter hybridization analyses reveal that, over a 24 hr period, ACTH and dibutyryl cyclic AMP each induce in adrenocortical cell cultures a 4- to 8-fold accumulation of P-$450_{11\beta}$ RNA; the data do not reveal whether each of the three RNA species is induced equally. Cyclohex-

imide inhibits the ACTH or dibutyryl cyclic AMP-induced RNA accumulation; thus, mRNA induction requires ongoing protein synthesis. Uninduced cells contain a substantial amount of cytochrome P-450$_{11\beta}$ mRNA. Again, this could reflect residual hormonal stimulation or constitutive expression of the cytochrome P-450$_{11\beta}$ gene.

ACTH and dibutyryl cyclic AMP induce the accumulation of mRNA for both cytochrome P-450$_{11\beta}$ and cytochrome P-450$_{scc}$. For each enzyme, the kinetics of mRNA accumulation are similar, and cycloheximide inhibits mRNA accumulation. These observations suggest that, in the adrenal cortex, the mitochondrial steroidogenic cytochromes P-450 may be regulated coordinately at the transcriptional level (176). On the other hand, the tissue-specific expression of the two enzymes is different, which implies that the control mechanisms governing the expression of these two genes are not identical.

Cytochrome P-450$_{17\alpha}$

This enzyme catalyzes the hydroxylation of progestins at the 17α position, a step in the pathway of androgen biosynthesis. The enzyme is present in the adrenal cortex, the gonads, and the placenta (176, 177). Experiments in intact animals suggest that the enzyme in the adrenal cortex is under pituitary control (181). In cultured bovine adrenocortical cells, ACTH induces a marked increase both in the rate of synthesis of cytochrome P-450$_{17\alpha}$ protein and in the accumulation of translatable cytochrome P-450$_{17\alpha}$ mRNA. Dibutyryl cyclic AMP produces similar effects (188). Likewise, luteinizing hormone or 8-bromo-cyclic AMP induce cytochrome P-450$_{17\alpha}$ enzyme activity in primary cultures of mouse Leydig cells (189). The time course of cytochrome P-450$_{17\alpha}$ enzyme induction is relatively slow and resembles those for cytochrome P-450$_{scc}$ and cytochrome P-450$_{11\beta}$. This similarity in induction kinetics plus the similarity in hormone responsiveness suggests that a "steroid hydroxylase-inducing protein" (184) may also regulate the expression of the cytochrome P-450$_{17\alpha}$ gene. On the other hand, uninduced cells synthesize no detectable translatable cytochrome P-450$_{17\alpha}$ RNA, a finding that differs from those for the two mitochondrial cytochromes. This apparent lack of constitutive expression may reflect a regulatory difference between cytochrome P-450$_{17\alpha}$, on the one hand, and cytochrome P-450$_{scc}$ and cytochrome P-450$_{11\beta}$ on the other. A cDNA clone for cytochrome P-450$_{17\alpha}$ has recently been isolated (M. X. Zuber, personal communication). This will allow more detailed studies of cytochrome P-450$_{17\alpha}$ gene regulation in the future.

Cytochrome P-450$_{C21}$

This enzyme catalyzes the 21-hydroxylation of progestins, a step in the biosynthesis of glucocorticoids and mineralocorticoids. The enzyme is present in the adrenal cortex, but not in the gonads or placenta (176, 177). Hereditary

deficiency of the enzyme is the most common cause of the syndrome of congenital adrenal hyperplasia (190). Studies in intact animals suggest that the enzyme is under pituitary control (181).

In the bovine adrenocortical cell system, ACTH induces after 24 hr about a 15-fold increase in the rate of cytochrome P-450$_{C21}$ synthesis, but only a 2- to 3-fold increase in the amount of translatable cytochrome P-450$_{C21}$ RNA (191). The reason(s) for this apparent discrepancy is not known. Cyclic AMP may mediate the cytochrome P-450$_{C21}$ induction response (192).

Two groups have isolated partial-length cDNA clones specific for bovine adrenal cytochrome P-450$_{C21}$ (193, 194). Using cDNA as a hybridization probe, White et al identified two cytochrome P-450$_{C21}$ genes in the vicinity of the H-2 major histocompatibility complex on chromosome 17 in the mouse (195). Similarly, two groups have found that the human cytochrome P-450$_{C21}$ gene maps within the HLA complex, adjacent to the locus encoding the C4A component of complement (196, 197). Two copies of the cytochrome P-450$_{C21}$ gene are present in the human genome. However, it is possible that only one copy is functional, since one form of congenital adrenal hyperplasia apparently results from the deletion of only one of the two genes (196). Furthermore, individuals who do not carry the other copy of the gene are, nevertheless, hormonally normal (197a).

Cytochrome P-450$_{aromatase}$

This enzyme catalyzes the aromatization of androgens in the pathway of estrogen biosynthesis. The biochemical properties of the enzyme have been studied primarily in the placenta; it has not yet been purified, and antibodies are not available. Therefore, conclusions about the regulation of the cytochrome P-450$_{aromatase}$ gene(s) must be inferred from measurements of enzyme activity. In rat granulosa cells, FSH (via formation of cyclic AMP) induces aromatase activity; the time course of induction is relatively long (176, 177, 198). By analogy with the regulation of other steroidogenic cytochromes P-450, the increased enzyme activity presumably reflects increased transcription of the cytochrome P-450$_{aromatase}$ gene.

Cytochrome P-450$_{aromatase}$ is present in many tissues including skin, muscle, fat, and nerve, where it may contribute to sex-specific differences in cellular metabolism (177, 198, 199). In particular, cytochrome P-450$_{aromatase}$ in certain regions of the brain may play an important role in the irreversible neonatal "imprinting" of sex-specific differences in cytochrome P-450-catalyzed enzyme activities in adult rat liver (199, 200) (see next section).

In summary, the data imply that each gene encoding a steroidogenic form of cytochrome P-450 is responsive to ACTH or FSH, depending upon the cell type. The hormonal signal is transduced from the plasma membrane to the nucleus via cAMP, which, in other systems, is known to activate the transcrip-

tion of specific genes (201, 202). Because the accumulation of cytochrome P-450-specific mRNA requires ongoing protein synthesis, cAMP apparently does not stimulate cytochrome P-450 gene transcription directly; instead, it (presumably) activates the transcription of a gene(s) that encodes a hypothetical steroid hydroxylase-inducing protein (SHIP). The SHIP would then regulate transcription by binding to a specific *cis*-acting genomic control element for the appropriate cytochrome P-450 gene(s). In addition, (at least some of) the steroidogenic cytochrome P-450 genes exhibit tissue-specific expression. Because this phenomenon probably reflects control at the level of transcription (75, 76), each steroidogenic cytochrome P-450 gene presumably has a second regulatory component responsible for tissue-specific control.

SEXUAL DIMORPHISM IN CYTOCHROME P-450 GENE EXPRESSION

A variety of biochemical reactions in the liver exhibit sexual dimorphism (203). For example, gender influences the pattern of cytochrome P-450-catalyzed hepatic metabolism of some steroids and drugs (204, 205). The sex-related differences are substantial in the rat; however, in other species the differences may be less marked. The clinical importance of such differences in humans is not known. Studies of sex-dependent differences in the hepatic metabolism of steroids have revealed an interesting neuro-hepatic-endocrine axis in rats (206).

Testosterone 16α-hydroxylase of rats has been the most thoroughly studied hepatic cytochrome P-450-dependent enzyme activity that exhibits sex-dependent expression. Enzyme activity is high in males and is virtually absent in females (207). The same rat enzyme apparently has been purified by several groups, although each has given the enzyme a different name (71, 208–212). Harada & Negishi have purified an analogous cytochrome P-450 from mice (213) and have used a cDNA clone to show that the high enzyme activity in males is associated with a high hepatic content of the corresponding mRNA (214). Thus, the sex-related difference in testosterone 16α-hydroxylase activity presumably reflects different rates of transcription of the corresponding cytochrome P-450 gene. Cytochrome $P\text{-}450_{PCN}$ also exhibits high constitutive activity in male rats. These animals contain a high hepatic concentration of translatable cytochrome $P\text{-}450_{PCN}$ mRNA (174). However, the mechanism of the sex-dependent expression of the cytochrome $P\text{-}450_{PCN}$ gene has not been studied in detail.

MacGeoch et al (215) have studied the regulation of a cytochrome P-450 steroid 15β-hydroxylase that is present at a high level in the livers of female rats and is undetectable in males (210, 211). The evidence implies that the mechanisms governing the expression of this female-specific enzyme are similar to those regulating the male-specific testosterone 16α-hydroxylase, described below.

In rats, sex-related differences in drug and steroid metabolism do not appear until puberty. Furthermore, castration of post-pubertal males reduces some steroid-metabolizing enzyme activities to levels near those of females, an effect that is reversible by administration of testosterone (216, 217). These findings implicate circulating androgens in the maintenance of male-female differences in some cytochrome P-450-catalyzed enzyme activities. However, androgen administration fails to induce a male pattern of steroid metabolism in females that are castrated after puberty (216). Furthermore, the failure of females to respond to androgens apparently is not due to a lack of androgen receptors (218). These findings suggest that the mechanism by which androgens control sex-related differences in hepatic drug and steroid metabolism does not operate primarily at the level of the liver.

The male pattern of metabolism never develops in adult rats that are castrated at birth. However, a single administration of testosterone during the neonatal period reverses the effect of castration and allows the male pattern to develop at puberty (207, 217, 219–221). Similarly, a single dose of testosterone administered during the neonatal period to ovariectomized females establishes a male pattern of steroid metabolism (222). These observations imply that the presence of testosterone (or a metabolite) during a critical neonatal period irreversibly programs the animal to develop the male pattern of hepatic metabolism as an adult.

The mechanism by which this irreversible "imprinting" occurs apparently involves the pituitary and hypothalamus. Hypophysectomy, in females, establishes a male pattern of hepatic metabolism (223, 224) and, in males, abolishes the response to androgens (225). Implantation of a pituitary gland ectopically into hypophysectomized males or females produces a female pattern of metabolism (223, 225). In males, lesions in particular regions of the hypothalamus generate a female pattern of metabolism (226). These observations imply (*a*) that an intact pituitary is required to establish male-female differences in metabolism, (*b*) that the pituitary secretes a factor that produces a feminine pattern of metabolism, and (*c*) that, in males, the hypothalamus inhibits the pituitary-controlled development of a female pattern of metabolism. Presumably, an area in the supraoptic region of the hypothalamus (227) undergoes neonatal imprinting and programs the adult male pattern of metabolism. In cases of other imprinted sexual phenotypes, the evidence suggests that, during the critical neonatal period, cytochrome $P\text{-}450_{aromatase}$ in the hypothalamus converts testosterone (secreted by the developing testis) to estradiol, which mediates the imprinting action via an estrogen receptor-mediated mechanism (200). This may involve the altered growth and/or differentiation of specific neurons in the hypothalamus (228). However, the mechanism that governs the imprinting of hepatic cytochrome P-450-catalyzed enzyme activities may not be identical (229).

Imprinting in the hypothalamus apparently produces a sex-specific differ-

ence in the temporal pattern of growth hormone (GH) release by the pituitary. Males exhibit a more episodic pattern of GH secretion than do females (230). Continuous (as opposed to intermittent) infusion of GH into male rats markedly decreases the level of testosterone 16α-hydroxylase activity (231). Furthermore, GH is apparently the feminizing factor that is responsible for establishing the female pattern of hepatic steroid metabolism (232). Because two other hormones, growth hormone releasing factor and somatostatin, control the secretion of GH, imprinting may alter the development of cells that synthesize these hypothalamic hormones. Indeed, antibodies to somatostatin partially feminize the pattern of hepatic steroid metabolism in male rats (233).

The mechanism by which the episodic vs continuous release of GH may differentially alter the expression of specific genes is unknown. It is possible that some of the actions of GH are direct, whereas others are mediated secondarily by other effectors; in addition, the secretory rhythm may influence the sensitivity of the effector cell as a result of the up or down regulation of membrane receptors (234). The possible changes in chromatin structure and/or DNA modification (e.g. methylation) that might accompany the imprinting of cytochrome P-450 gene expression are unknown.

The data to date imply that a neuro-hepatic-endocrine axis programs the sexual dimorphism observed in the expression of (at least some of) the cytochrome P-450 genes that encode steroid-metabolizing enzymes. It is not yet clear whether the same regulatory axis controls the expression of other cytochrome P-450 genes, which encode drug-metabolizing enzymes. There is evidence for the gonadal and pituitary control of drug-metabolizing enzyme activities (reviewed in Ref. 206). However, at least some of these activities (eg. ethylmorphine N-demethylation) may be catalyzed by a steroid-metabolizing form(s) of cytochrome P-450 (18) that is already known to exhibit sexual dimorphism.

Finally, Faris & Campbell have made the provocative observation that neonatal exposure to phenobarbital, an inducer of some forms of cytochrome P-450, may irreversibly alter the pattern of cytochrome P-450-catalyzed enzyme activities in adult male rats (235, 236). The mechanism by which this apparent imprinting occurs, and whether it influences the susceptibility of the host to chemically induced toxicity or neoplasia, remains to be determined.

FUTURE PROSPECTS

Control of the transcription rate plays a major role in the regulation of phenobarbital-responsive and PAH-responsive cytochrome P-450 genes. Presumably, future experiments will show that the other cytochrome P-450 genes are also primarily under transcriptional control. Thus, studies of the selective modulation of cytochrome P-450 gene transcription in response to specific chemical

signals should continue to be an interesting area of research in the future. The cytochromes P-450 offer a variety of experimental systems that should be useful in studying the mechanisms by which mammalian cells transduce extracellular chemical signals into alterations in the expression of specific genes. Experiments in yeast and *Drosophila* also may be productive, because these systems are particularly amenable to genetic manipulation.

The development of techniques that permit cells in culture to retain a differentiated phenotype (e.g. 237, 238) could make an important contribution to a better understanding of cytochrome P-450 gene expression (239–241). For example, many established cell lines respond poorly to phenobarbital. The availability of a phenobarbital-responsive cell line would simplify the functional analyses of putative phenobarbital-responsive genomic control elements. In addition, the selection of variant cells from such a phenobarbital-responsive cell line would allow genetic experiments to complement the biochemical studies of cytochrome P-450 gene regulation. Similarly, the availability of appropriately responsive cell lines should facilitate analyses of the mechanisms by which other cytochrome P-450 genes are regulated.

The contribution that chromatin structure makes to the control of cytochrome P-450 gene expression is virtually unknown; this may be a potentially fruitful area for future research. Changes in chromatin structure may be particularly important in the imprinting and tissue-specific expression of cytochrome P-450 genes. Differences in nuclease sensitivity may be useful in identifying genomic regions potentially involved in transcriptional regulation. Isolation of cytochrome P-450 genes in minichromosome form may facilitate the study of protein-DNA interactions in the cytochrome P-450 genes and might be a prerequisite for establishing studies of regulated transcription in vitro.

The techniques of mutagenesis and gene transfer have the potential to reveal functional aspects of *cis*-acting genomic elements involved in the control of cytochrome P-450 gene transcription. The structural and functional analyses of such elements should provide insights into the mechanisms of signal transduction from the extracellular environment to the genome. Presumably, both stimulatory and inhibitory genomic control elements exist. Whether they can function relatively independently of each other, whether their effects are synergistic, additive, or antagonistic when several elements are linked, whether its position in a hierarchy influences the function of an element, and whether other position or orientation effects occur are interesting problems for the future. Similarly, the structures of the binding sites for *trans*-acting regulatory factors remain to be determined.

The properties of the *trans*-acting regulators that interact with the genomic control elements remain largely unknown. First of all, a number of hypothetical regulatory factors have not been characterized at the biochemical level at all (e.g. tissue-specific regulators, phenobarbital receptors, cycloheximide-

sensitive repressors, etc). Even for *Ah* receptors, little is known of possible functional domains (e.g. inducer binding, chromatin binding), subunit structure, and overall receptor heterogeneity. In addition, the receptor-mediated, temperature-dependent step in enzyme induction remains to be analyzed. The isolation and characterization of additional regulatory variants, and attempts to complement defective variants using gene transfer methodology, may be productive experimental approaches for studying these regulatory factors in the future. The general question of how the binding of a regulatory factor to its cognate control element results in the modulation of gene transcription will not be easy to answer. However, the various cytochrome P-450 genes, with their selective responsiveness to different chemical stimuli, comprise an experimental system with unusual potential for addressing this question in the future.

Finally, the chaotic cytochrome P-450 nomenclature that has evolved over the past 20 years is unusually complicated and confusing. Future agreement on a coherent terminology (if possible) would increase everyone's understanding and appreciation of this interesting and important class of enzymes.

ACKNOWLEDGMENTS

I thank numerous colleagues for the sharing of information, fruitful discussion, and constructive criticism. I thank Karen Benight for expert secretarial assistance. The work in my laboratory has been supported by grants from the American Cancer Society and the National Institutes of Health.

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