Biochemical Pharmacology, Vol. 33, No. 6, pp. 917-924, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00
Pergamon Press Ltd.

THE *Ah* RECEPTOR: BINDING SPECIFICITY ONLY FOR FOREIGN CHEMICALS?

DANIEL W. NEBERT* and HOWARD J. EISEN

Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205, U.S.A.

and

OLIVER HANKINSON

Laboratory of Biomedical and Environmental Sciences and the Department of Pathology, University of California at Los Angles, Los Angeles, CA 90024, U.S.A.

Abstract—The murine *Ah* locus controls the induction of at least four drug-metabolizing enzymes: cytochromes P_1-450, P_2-450, and P_3-450, and UDP-glucuronosyltransferase. The *Ah* gene codes for a cytosolic receptor. It is known that the induction response includes: (i) high-affinity binding of specific foreign chemicals to the *Ah* receptor; (ii) temperature-dependent translocation of the "activated" inducer-receptor complex into the nucleus; (iii) binding of the complex presumably to chromatin components; (iv) transcriptional activation of specific genes; (v) maximal increases in intranuclear high-molecular-weight precursor mRNA (pre-mRNA) that precede by several hours the maximal increases in cytoplasmic mRNA; (vi) translation of the mRNA principally on membrane-bound polysomes; and (vii) increases in the specific membrane-bound proteins (including architectural arrangement with other membrane-bound moieties) that reflect enhanced specific drug-metabolizing activities. It is not known how many of the other drug metabolism induction responses are also governed by receptors.

The *Ah* locus studies have been chiefly unraveled in the mouse, due to several inbred strains having a receptor defect. In addition to "classical" pharmacologic methods (such as structure–activity studies) and standard biochemical techniques, the newer methods of recombinant DNA technology and somatic-cell genetics in culture are shown to be important in understanding the *Ah* receptor and its induction response. It is possible that this receptor is required for endogenous functions critical to life processes, as well as its function in the induction of drug metabolism by certain polycyclic aromatic compounds.

If one examines all other chapters in this issue, the receptors described all have one property in common. Endogenous ligands—viz. growth factors, insulin, steroids, and neurotransmitters involving biogenic amines and neuropeptides—bind to these cell-surface or cytosolic receptors. In contrast, no endogenous ligand is presently known for the receptor characterized in this chapter.

Relatively planar polycyclic aromatic compounds such as benzo[a]pyrene and TCDD† cause the induction of certain drug-metabolizing enzymes and other proteins in virtually every mammalian tissue [reviewed in Ref. 1]. The induced enzymes, in turn, metabolize most efficiently those compounds that are structurally similar in size and shape to the inducing foreign chemicals. These inducers bind with high affinity (apparent $K_d \cong 1$ nM) to a cytosolic receptor protein encoded by the *Ah* locus. The inducer-receptor complex is known to activate other genes responsible for the drug-metabolizing enzyme proteins such as P_1-450 and P_3-450. We show in this review that, in addition to "classical" pharmacologic methods, the role of this receptor is being studied by methods involving somatic-cell genetics, recombinant DNA, and ancillary techniques.

* Correspondence should be addressed to: Dr. Daniel W. Nebert, Building 10, Room 6C-101, National Institutes of Health, Bethesda, MD 20205, U.S.A.

† Abbreviations include: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; B6, the inbred C57BL/6N mouse strain; D2, the inbred DBA/2N mouse strain; AHH, aryl hydrocarbon (benzo[a]pyrene) hydroxylase (EC 1.14.14.1); near-u.v., near-ultraviolet light. "P-450" designates any or all forms of membrane-bound cytochrome P-450 (multisubstrate monooxygenases). Mouse "P_1-450" and "P_3-450" are those forms of 3-methylcholanthrene-induced P-450 having the highest turnover number for AHH and acetanilide 4-hydroxylase activity, respectively. Mouse "P_2-450" is defined as that form of isosafrole-induced P-450 having the highest turnover number for isosafrole metabolism.

EXPERIMENTAL PROCEDURES

All materials, procedures described, and mice and cell cultures used have been detailed in the previously published articles cited [2–6]. One *unit* of AHH activity is defined [7] as that amount of enzyme catalyzing in 1 min at 37° the formation of hydroxylated products causing fluorescence equivalent to that of 1.0 pmole of the 3-hydroxybenzo[a]pyrene recrystallized standard. Specific activity denotes units per mg of liver microsomal protein or per mg of cell culture homogenate protein.

RESULTS AND DISCUSSION

"Classical" structure–activity relationship studies. Certain polycyclic aromatic foreign compounds induce AHH activity (cytochrome P_1-450) in liver and other tissues [8]. Particular strains of mice are much more responsive to TCDD or 3-methylcholanthrene than other strains. It was this fundamental difference in the P_1-450 induction response among inbred strains of mice [9–11] that has led to such thorough characterization of the *Ah* receptor in this species. The discovery of a "defective mutant" allows certain questions to be addressed that would otherwise be unapproachable by the molecular geneticist attempting to unravel the mysteries of this biochemical problem.

The difference in AHH induction sensitivity between B6 and D2 mice (Fig. 1) is about 10- to 20-fold. When B6 (Ah^b/Ah^b) are crossed with D2 (Ah^d/Ah^d), the B6D2F_1 heterozygote (Ah^b/Ah^d) exhibits AHH induction similar to the B6 parent; hence, the trait is inherited as autosomal dominant. This gene has come to be termed the "*Ah* locus", for *a*romatic *h*ydrocarbon "responsiveness." Of interest, although 3-methylcholanthrene cannot induce AHH activity in D2 liver at the highest experimental concentrations possible, a sufficiently high TCDD dose induces the activity in D2 mice (Fig. 1) to a level that is statistically the same as the maximally induced AHH activity in TCDD-treated B6 mice. In fact, TCDD-induced P_1-450 in D2 appears identical to TCDD-induced P_1-450 B6 mice; this finding is supported by such data as: (a) inhibition of catalytic activity by chemicals or antibody, (b) immunoprecipitation of the P_1-450 protein from microsomal membranes, and (c) characterization of P_1-450 mRNA by a cloned cDNA probe. These results are consistent with a "receptor-like" defect in D2 mice, in which the defect can be overcome by sufficiently large TCDD doses [2].

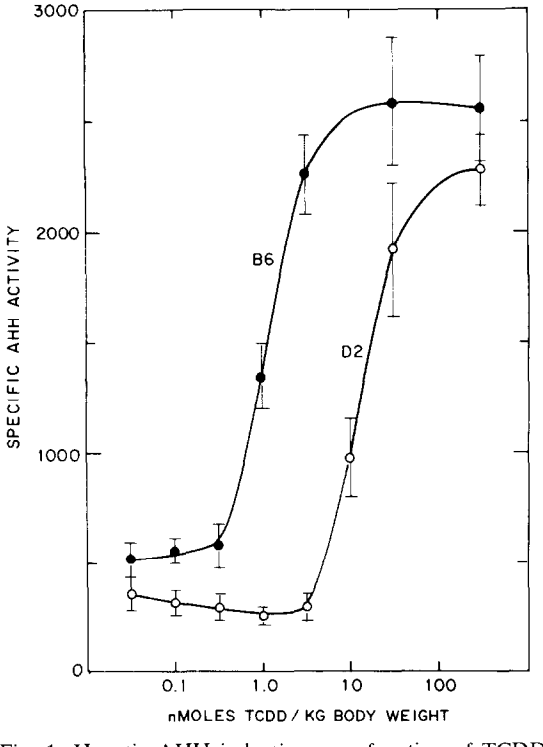


Fig. 1. Hepatic AHH induction as a function of TCDD dosage in B6 and D2 inbred mice [2]. The dose–response curves are given on a semilog plot. A single intraperitoneal injection of TCDD in *p*-dioxane (0.5 ml/kg) was given 72 hr before mice were killed. *Symbols* and *brackets* denote means ± standard deviations for groups of six mice.

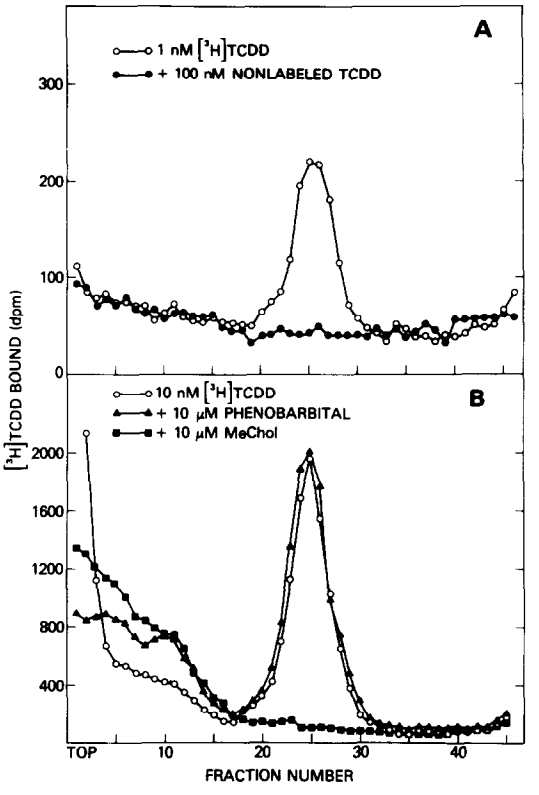


Fig. 2. Velocity sedimentation analysis of the *Ah* receptor in B6 liver cytosol [14]. Following incubation of the radioligand (in the absence or presence of 100- or 1000-fold excess of nonlabeled competitors) at 4° for 1 hr, the material was treated with dextran-charcoal and then centrifuged on a 5–20% sucrose density gradient. This peak, termed peak II, is negligible in D2 and other Ah^d/Ah^d mice.

The pioneering structure-activity experiments of Poland and coworkers [12, 13] have been confirmed and extended with use of a different receptor assay [14]. The results are unambiguous. An excellent correlation exists between the avidity with which various foreign chemicals bind to the *Ah* receptor and the potency of that chemical to induce AHH activity (P_1-450).

Physico-chemical properties of the Ah *receptor.* This protein has been well characterized by velocity sedimentation [14, 15] and gel permeation chromatography [3]. The sedimentation coefficient is ~9 S, $M_r \cong 200{,}000$. The saturable peak in Fig. 2 (which we refer to as "peak II") represents the *Ah* receptor,

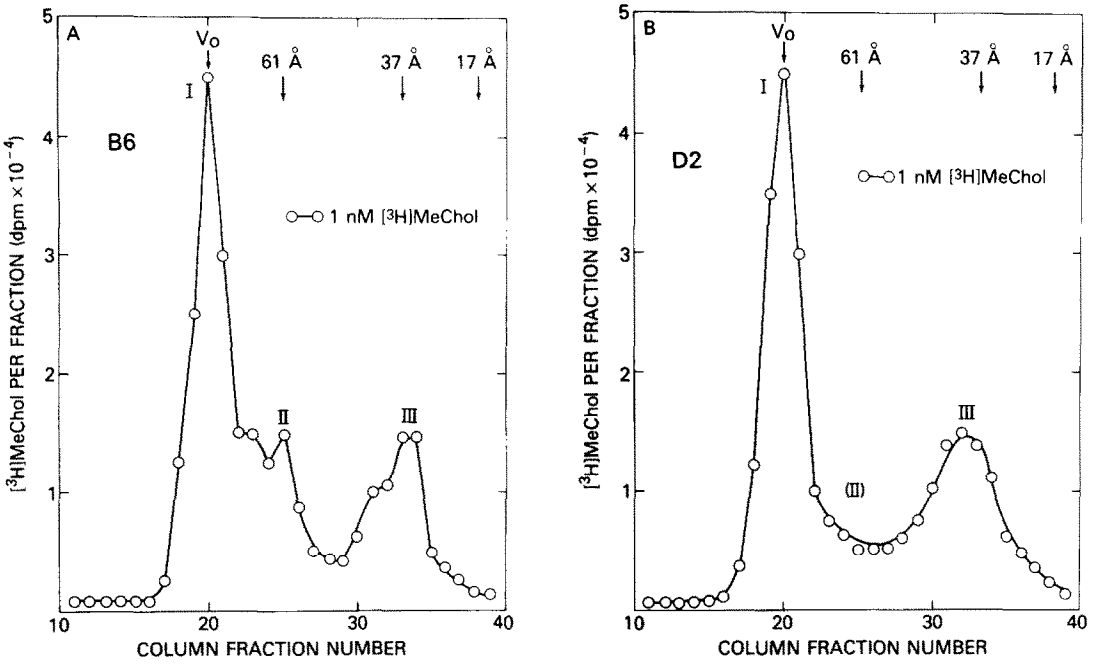


Fig. 3. Gel permeation chromatography of B6 (*left*) and D2 (*right*) liver cytosol, following exposure of liver cytosol to generally tritiated 3-methylcholanthrene ([^{3}H]MeChol). Incubation and dextran-charcoal adsorption conditions are identical to those in Fig. 2 [3].

being present in the liver cytosol of B6 but not D2 mice. Phenobarbital, a class of P-450 inducer distinct from TCDD or 3-methylcholanthrene, does not displace the radioligand from the *Ah* receptor. Peak III, a smaller protein ($M_r \cong 80{,}000$), is present in rodent livers at a concentration 10–20 times greater than that of the peak II protein [3] and binds polycyclic hydrocarbons such as benzo[a]pyrene or 3-methylcholanthrene (Fig. 3) much better than TCDD. Since peak III is clearly present in both B6 and D2 at approximately equal concentrations, we are certain that peak III is not associated with the *Ah* locus. Further, peak II (but not peak III) is saturable with a 100-fold excess of nonlabeled TCDD—additional evidence that peak II represents the high-affinity, saturable cytosolic *Ah* receptor protein.

The inducer-receptor complex undergoes translocation from the cytosol to the cell nucleus. In untreated mice and in tissue culture cell lines (such as the mouse hepatoma Hepa-1c1c7), the *Ah* receptor in the absence of inducer is detected only in cytosolic fractions. Following treatment *in vivo* with [^{3}H]TCDD, the [^{3}H]TCDD·*Ah* receptor complex is formed in the cytosolic compartment. In Hepa-1c1c7 cells, the translocation of the cytoplasmic inducer-receptor complex into the nucleus is a temperature-dependent process [15], occurring at 37° but not at 4°. The [^{3}H]TCDD·*Ah* receptor complex can be extracted from the nucleus with buffers containing 0.3 M NaCl. The intranuclear "acceptor site", however, has not been further identified. Although nuclear uptake of the [^{3}H]TCDD·*Ah* receptor complex occurs in the intact cell, this process cannot be duplicated *in vitro* with subcellular fractions [3]. Hence, the *Ah* receptor differs in this regard from steroid receptors.

The intranuclear inducer-receptor complex activates transcription of specific genes. AHH induction proceeds in D2 mice receiving sufficiently high TCDD doses, yet we are unable to detect a cytosolic *Ah* receptor in D2 liver. An apparent dilemma therefore exists. When B6 and D2 mice received [^{3}H]TCDD injections and liver nuclear extracts were subsequently examined by gel permeation chromatography (Fig. 4), the *Ah* receptor (peak II) was detectable for the first time in D2 mice [5]. A similar observation was noted by Mason and Okey [16]. In fact, if Ah^b/Ah^d and Ah^d/Ah^d progeny from the B6D2F$_1$ × D backcross were studied (Fig. 4), Ah^b/Ah^d exhibited *Ah* receptor levels intermediate between those in B6 and D2 mice. Ah^d/Ah^d offspring exhibited receptor levels about equal to those found in D2 liver nuclei. Hence, inheritance of *Ah* receptor concentrations is expressed as an additive trait [5, 12, 14].

As is also observed in Fig. 4, peak III is not found in the nucleus. This finding further counts against the notion that peak III is associated with the AHH induction response.

It is concluded that Ah^d/Ah^d mice contain a "defective" *Ah* receptor having decreased affinity for P$_1$-450 inducers. The Ah^b allele encodes the high-affinity form, the Ah^d allele, the putative poor-affinity form. It is now clear that sufficient doses of TCDD can overcome this receptor defect (which might be only a single amino-acid difference), resulting in high levels of AHH induction in Ah^d/Ah^d mice. The nuclear form of this inducer-receptor complex is apparently more stable than the cytosolic form, since

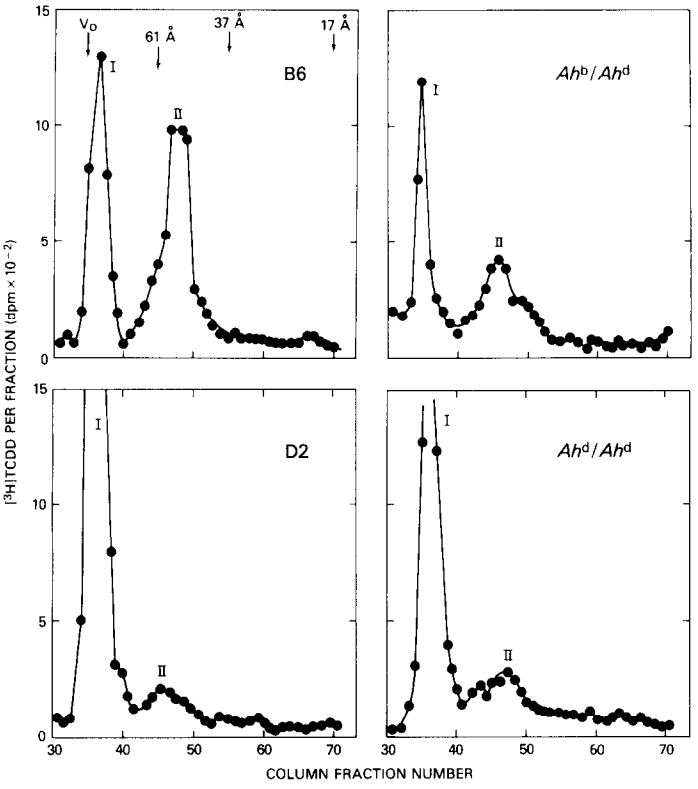


Fig. 4. Sephacryl S-300 analysis of hepatic nuclear extracts of B6, D2, and offspring from the B6D2F$_1$ × D2 backcross 4 hr after mice had received [^{3}H]TCDD (50 nmoles/kg) [5]. The *Ah*^{b}/*Ah*^{d} and *Ah*^{d}/*Ah*^{d} mice had been *Ah*-phenotyped 10 days beforehand by the zoxazolamine paralysis test [6]. Five mice per group were combined. [Reproduced with permission from MIT Press.]

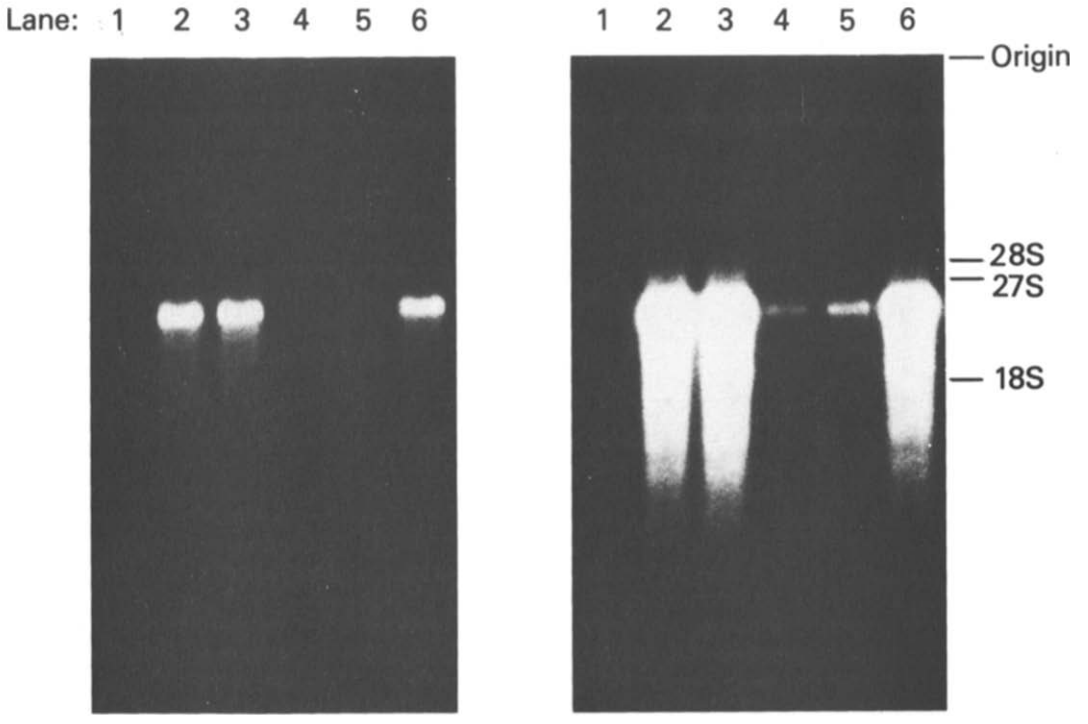
we have not been able to detect the receptor in D2 cytosol—at least with regard to our various technical procedures used to quantitate inducer-receptor complexes. Similar phenomena have been described for steroid hormone receptors, which can be stabilized by "activation" (receptor forms that bind to DNA) or by interaction with histones.

With the aid of a 3′-unique clone of P$_1$-450 cDNA [17, 18], it was determined that P$_1$-450 (23 S) mRNA levels are elevated any time AHH induction occurs in B6 or D2 mice (Fig. 5). Moreover, the induction of an intranuclear high-molecular-weight mRNA precursor [4] was found to precede by several hours the accumulation of cytoplasmic P$_1$-450 mRNA during the induction process, suggesting the likelihood of transcriptional control of P$_1$-450 induction by the inducer-receptor complex. An *in vitro* nuclear transcription assay was used recently to prove that the induction response is mediated principally by enhancing transcription rates of the P$_1$-450 and P$_3$-450 genes [19]. We also quantified P$_1$-450 mRNA content (via R$_o$t analysis with the 3′-unique P$_1$-450 cDNA

Table 1. *Ah* receptor levels and maximally inducible AHH activity in Hepa-1c1c7 parent line and six mutant clones

Cell culture line	Genetic characteristic	*Ah* receptor (fmoles/mg protein) Cytosol *in vitro* treatment	Cytosol, exposure in culture	Nuclei, exposure in culture	Maximal AHH activity (units/mg cellular protein) BzAnth as inducer	TCDD as inducer
Hepa-1c1c7	wild-type	20	12	6	210	520
c1	group A		7.6	2.0	<0.4	<0.4
c2	group B	2.1	1.7	0.5	42	110
c3	dominant		18	3.4	22	16
c4	group C		15	0.3	<0.4	<0.4
c5	group A		6.0	3.4	<0.4	<0.4
c6	group B	1.0	0.5	0.4	4	3

BzAnth, benzo[a]anthracene.



Lane: 1 B6, Control
2 B6, MeChol
3 B6, TCDD
4 D2, Control
5 D2, MeChol
6 D2, TCDD

Fig. 5. Northern blot hybridization of total liver poly(A^+)-enriched RNA probed with the mouse P_1-450 cDNA clone, clone 46 [4]. B6 and D2 mice were treated intraperitoneally with 3-methylcholanthrene (MeChol) 200 mg/kg or TCDD (50 μg/kg) 24 hr before killing. Controls received corn oil (25 ml/kg) alone. Exposure of the diazobenzyloxymethyl paper to X-ray film was 3 hr (*left*) and 24 hr (*right*). RNA size markers (*far right*) included phage MS 2-RNA in addition to the 18 and 28 S mouse rRNA.

clone) as a function of the intranuclear appearance of the inducer-receptor complex (Fig. 6). These studies [5] thus demonstrate a "dose–response" relationship between the appearance of the intranuclear inducer-receptor complex and P_1-450 mRNA induction for Ah^b/Ah^b, Ah^b/Ah^d and Ah^d/Ah^d mice. These data provide the most direct proof yet for a close link between the *Ah* receptor and activation of the P_1-450 gene. These results represent an excellent example of examining a problem in molecular biology through the use of a genetically well-characterized mutant.

Somatic-cell genetics of AHH⁻ clones. In addition to the intact animal, genetically well-characterized mutants can be uncovered through the use of cultured cells. Mutant clones can be artificially generated, characterized for their relative abundance (or lack) of AHH induction and the *Ah* receptor, and then dissected by genetic and recombinant DNA techniques in order to explain the behavior of each mutation.

In many cell culture lines, AHH activity is inducible by polycyclic hydrocarbons and numerous other chemicals [reviewed in Ref. 20]. The mouse hepatoma line, hepa-1c1c7, has a particularly high induced AHH activity [21, 22]. When benzo[a]pyrene is added to the growth medium of Hepa-1c1c7, it causes induction of AHH, which then converts the chemical to cytotoxic metabolites [22, 23]. A single-step selection procedure (Fig. 7) for isolating clones resistant to 20 μM benzo[a]pyrene was developed, which capitalized on the extreme sensitivity of this cell line to the compound [22]. All benzo[a]pyrene-resistant clones have nondetectable or much decreased (i.e. less than 10%) levels of induced AHH activity, compared with the Hepa-1c1c7 parent (Table 1). The following observations strongly suggest that the variants are mutational in origin. They arise at the low rate of 2×10^{-7} events per cell generation [22]; their frequency is markedly increased by prior treatment with known mutagens; their phenotypes are stable over long-term culture [24]; and individual variants revert at a very low rate such that the frequency of revertants is usually 3×10^{-7} or less (J. R. Van Gurp and O. Hankinson, manuscript submitted for publication).

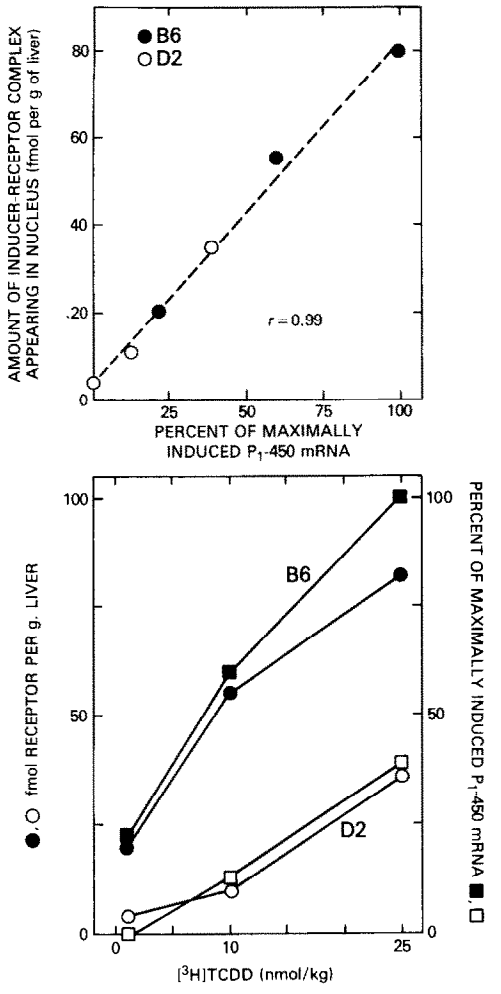


Fig. 6. Dose–response relationship in B6 and D2 liver between appearance of the [^{3}H]TCDD-receptor complex in the nucleus and the induction of P_1-450 mRNA [5]. Radioactivity in peak II of the Sephacryl S-300 column chromatogram from liver nuclear extracts was used to quantify the inducer-receptor complex. The percent of maximally induced P_1-450 mRNA was determined by $R_o t$ analysis of total liver poly(A$^+$)-enriched RNA probed with the P_1-450 cDNA clone. Each *point* represents ten mice combined and killed 18 hr after a single intraperitoneal dose of [^{3}H]TCDD. Both the inducer-receptor levels and P_1-450 concentrations were measured in the same ten mice. [Reproduced with permission from MIT Press.]

Somatic-cell hybridization experiments were also performed [24, 25]. When the majority of mutants were fused with the wild-type, Hepa-1c1c7, the resultant hybrids possessed AHH activities that were 50% or more of that of the wild-type. (The activities being referred to in these experiments were in induced cells). For the sake of simplicity, we refer to such mutants as being recessive, although strictly speaking, AHH activity was inherited additively in each case. A few mutants were dominant. Hybrids between these mutants and the wild-type possessed less than 5% of the wild-type AHH activity. The recessive mutants were then fused in various pairwise combinations in order to investigate whether complementation occurred between individual mutants. Hybrids between c2 and c4 (Table 1), for example, have wild-type levels of induced AHH activity, although both parents have none or very low activities. It can therefore be concluded that c2 and c4 belong to different complementation groups and have mutations in different genes. Three different complementation groups were identified in such experiments.

c2 and c6, which are both mutants in complementation group B, have markedly decreased levels of the *Ah* receptor (Table 1). Cytosolic and nuclear receptor levels are both diminished by the same degree in these mutants. Group B mutants thus are not defective in nuclear translocation. The group B mutants also retain low levels of inducible AHH activity, which may be explained by the fact that they are not totally devoid of the *Ah* receptor. c4, a group C mutant, contains normal cytosolic receptor levels but has very low levels in the nucleus [6]. Recently Miller and Whitlock [26] isolated benzo[a]pyrene-resistant mutants of Hepa-1c1c7 and have characterized several clones that are similar to our group B and group C mutants. The c1 and c3 clones (Group A mutants) appear to exhibit normal receptor levels and nuclear uptake of the inducer-receptor complex (Table 1).

The above results suggest that the mutated genes in the group B and group C complementation groups may code for structural components of the receptor. This finding therefore opens up the intriguing possibility that the *Ah* receptor is a heterodimer or heteropolymer. A word of caution is necessary, however, in this regard. It remains possible that the group B and group C clones do not contain mutations in the *Ah* receptor gene. The group B mutants, for example, could be defective in a protein necessary for post-translational modification of the receptor; group C mutants could be defective in a protein required for high-affinity binding of the inducer-receptor complex to chromatin.

The P_1-450 cDNA probe has been used to measure the amount of P_1-450 mRNA in the wild-type, Hepa-1c1c7, and representative mutants (O. Hankinson, R. D. Anderson, B. W. Birren, N. Negishi and D.

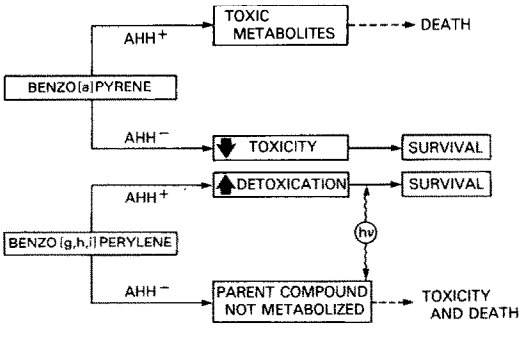


Fig. 7. Diagrammatic representation of selection for AHH-deficient (*top*) and AHH-positive (*bottom*) clones from mouse Hepa-1c1c7 cultures. AHH$^+$, cells having high AHH activity; AHH$^-$, cells having negligible AHH activity; hν, irradiation of cells with near-u.v. light (340- to 400-nm wavelength) at a total flux of 10 kJ/m^2.

W. Nebert, unpublished data). TCDD treatment causes about a 50-fold increase in the amount of P_1-450 mRNA in the wild-type. As expected, the group C mutant c4 has nondetectable P_1-450 mRNA after induction, while the group B mutants have much reduced levels that are decreased by the same degree as their AHH activities. The dominant mutant (c3) also has very low levels of P_1-450 mRNA. These data indicate that the dominant class of mutants is defective in the transcriptional regulation of P_1-450 or, less likely, have an enhanced rate of degradation of the mRNA. The most interesting results are those obtained with the group A mutants: these mutants are very heterogeneous in their behavior. Even when grown without TCDD, some have P_1-450 mRNA levels as great as that possessed by the fully induced wild-type. TCDD treatment does not enhance P_1-450 mRNA levels further in these mutants. Other group A mutants have low levels of the mRNA that are not increased by TCDD treatment, whereas still others have low levels that are inducible by TCDD. We are currently investigating the basis for the aberrant behavior of the group A mutants. One possibility, which is compatible with many of the properties of these mutants, is that the group A clones exhibit mutations in the P_1-450 structural gene.

Selection of AHH^+ *cells.* Benzo[a]pyrene toxicity can be used to select for AHH-deficient cells. For many studies, however, it would be useful to select for cells possessing high levels of AHH activity in the presence of a vast excess of AHH^- cells. We have recently succeeded (Fig. 7) in developing such a single-step "reverse selection" procedure [27].

The selection takes advantage of the following facts. The compound benzo[g,h,i]perylene is metabolized by AHH, but, unlike benzo[a]pyrene, the products of its metabolism are not cytotoxic. In addition, benzo[g,h,i]perylene itself is rendered highly cytotoxic by illumination with near-u.v. light. In the procedure 5×10^5 AHH^- cells are inoculated per dish in medium containing TCDD. (TCDD will induce AHH in any AHH^+ cell.) The next day the medium is removed and replaced with medium containing 0.5 μM benzo[g,h,i]perylene. Two hours later the latter culture medium is removed, replaced with medium containing no benzo[g,h,i]perylene, and the cells incubated for an additional four hours. During this period, the compound is metabolized and excreted by any AHH^+ cells that are present in the culture, whereas benzo[g,h,i]perylene concentrations remain high in AHH^- cells. At the end of the 4-hr incubation period, the cells are illuminated with near-u.v. light. AHH^- cells are rapidly killed by this treatment. Fresh medium is added back, the dishes are incubated for a further 12 days, after which clones of AHH^+ cells appear against a lawn of dead cells.

We have used the reverse selection to isolate revertents from representatives of each of our three complementation groups of AHH-deficient mutants and from a dominant mutant (J. R. Van Gurp and O. Hankinson, manuscript submitted for publication). One revertant of the group C mutants is very interesting in that it possesses much higher basal AHH activities than those of the wild-type (equal to 9% of the activity in the fully induced wild-type). TCDD treatment does not further increase AHH activity in this strain. The properties of this and other strains having high "constitutive" AHH promise to provide considerable insight into the mechanism of AHH induction.

Is there a limit to the level of constitutive and inducible AHH activity in a cell? If there exist excessive levels of functional P_1-450 mRNA, can its rate of translation into P_1-450 protein and AHH activity become rate-limiting? In fact, there are some observations suggestive of translation becoming rate-limiting in the fully induced B6 mouse [28]. It may be possible to address these questions in Hepa-1c1c7 cells by using the reverse selection procedure. By this procedure it might also be possible to select cells that contain amplified P_1-450 genes. One could then ask whether, under optimal inducing conditions, such "amplification mutants" possess elevated levels of P_1-450 mRNA, P_1-450 protein, and AHH activity, and, furthermore, whether the levels of the protein and catalytic activity increase in proportion to the level of P_1-450 mRNA.

Research directions in the near future. The possibility of *Ah* receptor heterogeneity has been discussed [29] and presently has not been rigorously ruled out. Presence of the AHH induction response (and presumably adequate levels of *Ah* receptor) in the preimplantation mouse embryo [30, 31] and greater levels of AHH activity in the human fetal adrenal than in human fetal liver [32–34] are puzzling observations. Such data suggest, for P_1-450 and perhaps the receptor, that there exist other roles than merely the metabolism of polycyclic hydrocarbons.

Recent findings that prostaglandin biosynthesis and arachidonic acid metabolism [35–38] and leukotriene B_4 inactivation [39] can occur via P-450 and the reverse, that non-P-450-dependent [40] "prostaglandin synthetase" (cyclooxygenase activity plus hydroperoxidase activity) can metabolize polycyclic hydrocarbons and their metabolites [41–45], are intriguing. Furthermore, inhibitors of prostaglandin or leukotriene synthesis have been found to block the dietary fat enhancement of 7,12-dimethylbenzo[a]anthracene-induced mammary tumorigenesis [46] and polychlorinated biphenyl-induced toxicity in the chicken egg [47]. The *Ah* receptor has recently been shown also to have an association with fertility, fitness and longevity [48] and resistance to intraperitoneal ethanol toxicity (S. W. Bigelow, A. C. Collins and D. W. Nebert, unpublished data). Understanding any endogenous functions of the *Ah* receptor thus appears to be forthcoming.

Several methods exist for introducing DNA into mammalian cells. If appropriate selection procedures exist, then colonies derived from rare cells that have taken up, and express, a particular gene of interest can also be isolated. The gene in question can then be rescued from cells of the colony, and cloned. In this way several mammalian genes (e.g. thymidine kinase), and indeed several cellular oncogenes, have been isolated. In an analogous fashion we are attempting to isolate the genes corresponding to each of our three AHH-deficient complementation groups of Hepa-1c1c7. Briefly, the basis of these experiments is as follows. Representative mutants are

treated with a calcium phosphate precipitate of human DNA, and AHH$^+$ cells are selected for by means of our benzo[g,h,i]pyrene plus near-u.v. reverse selection procedure. It should be possible to distinguish true transfectants from revertants, because the former should contain human DNA sequences detectable by means of a probe to human middle-repetitive sequencies. A λ-phage or cosmid library would then be made from the DNA of a transfectant, and bacterial colonies containing human DNA (and therefore the whole or part of the "AHH induction process gene" in question) could be identified by means of the above human probe. In fact, we believe that such "gene rescue" methods may provide more information about the *Ah* receptor at the DNA level sooner than information will be obtained about the purified *Ah* receptor protein.

Acknowledgements—Valuable discussions with Drs. Allan C. Collins, Sanford W. Bigelow, Catherine Legraverend, Sirpa O. Kärenlampi, and John R. Van Gurp are appreciated. We thank Ms. Ingrid E. Jordan and Linda A. Hamilton for expert secretarial assistance.

REFERENCES

1. H. J. Eisen, R. R. Hannah, C. Legraverend, A. B. Okey and D. W. Nebert, in *Biochemical Actions of Hormones* (Ed. G. Litwack), p. 227. Academic Press, New York (1983).
2. A. P. Poland, E. Glover, J. R. Robinson and D. W. Nebert, *J. biol. Chem.* **249**, 5599 (1974).
3. R. R. Hannah, D. W. Nebert and H. J. Eisen, *J. biol. Chem.* **256**, 4584 (1981).
4. R. H. Tukey, D. W. Nebert and M. Negishi, *J. biol. Chem.* **256**, 6969 (1981).
5. R. H. Tukey, R. R. Hannah, M. Negishi, D. W. Nebert and H. J. Eisen, *Cell* **31**, 275 (1982).
6. C. Legraverend, R. R. Hannah, H. J. Eisen, I. S. Owens, D. W. Nebert and O. Hankinson, *J. biol. Chem.* **257**, 6402 (1982).
7. D. W. Nebert, *Meth. Enzym.* **LII**, 226 (1978).
8. D. W. Nebert, R. H. Tukey, P. I. Mackenzie, M. Negishi and H. J. Eisen, in *Extrahepatic Drug Metabolism and Chemical Carcinogenesis* (Ed. J. Rydström), p. 379. Elsevier/North-Holland Biomedical Press, Amsterdam (1983).
9. D. W. Nebert and H. V. Gelboin, *Archs Biochem. Biophys.* **134**, 76 (1969).
10. D. W. Nebert and L. L. Bausserman, *J. biol. Chem.* **245**, 6373 (1970).
11. D. W. Nebert, F. M. Goujon and J. E. Gielen, in *Fonds de la Recherche Scientifique Medicale, Groups de Contact*, p. 240. 11, ru d'Egmont, Bruxelles 5, Belgium (1971).
12. A. P. Poland, E. Glover and A. S. Kende, *J. biol. Chem.* **251**, 4936 (1976).
13. A. Poland and E. Glover, *Molec. Pharmac.* **13**, 924 (1977).
14. A. B. Okey, G. P. Bondy, M. E. Mason, F. G. Kahl, H. J. Eisen, T. M. Guenthner and D. W. Nebert, *J. biol. Chem.* **254**, 11636 (1969).
15. A. B. Okey, G. P. Bondy, M. E. Mason, D. W. Nebert, C. Forster-Gibson, J. Muncan and M. J. Dufresne, *J. biol. Chem.* **255**, 11415 (1980).
16. M. E. Mason and A. B. Okey, *Eur. J. Biochem.* **123**, 209 (1982).
17. M. Negishi, D. C. Swan, L. W. Enquist and D. W. Nebert, *Proc. natn. Acad. Sci. U.S.A.* **78**, 800 (1981).
18. M. Nakamura, M. Negishi, M. Altieri, Y.-T. Chen, T. Ikeda, R. H. Tukey and D. W. Nebert, *Eur. J. Biochem.* **134**, 19 (1983).
19. F. J. Gonzalez, R. H. Tukey and D. W. Nebert, *J. biol. Chem.* manuscript submitted for publication.
20. I. S. Owens, A. Niwa and D. W. Nebert, in *Gene Expression and Carcinogenesis in Cultured Liver* (Eds. L. E. Gerschenson and E. B. Thompson), p. 378. Academic Press, New York (1975).
21. A. Niwa, K. Kumaki, and D. W. Nebert, *Molec. Pharmac.* **11**, 399 (1975).
22. O. Hankinson, *Proc. natn. Acad, Sci. U.S.A.* **76**, 373 (1979).
23. S. O. Kärenlampi, H. J. Eisen, O. Hankinson and D. W. Nebert, *J. biol. Chem.* **258**, 10378 (1983).
24. O. Hankinson, *Somat. Cell Genet.* **7**, 373 (1981).
25. O. Hankinson, *Somat. Cell Genet.* **9**, 497 (1983).
26. A. G. Miller, D. Israel and J. P. Whitlock, Jr., *J. biol. Chem.* **258**, 3523 (1983).
27. J. R. Van Gurp and O. Hankinson, *Cancer Res.* **43**, 6031 (1983).
28. R. H. Tukey, M. Negishi and D. W. Nebert, *Molec. Pharmac.* **22**, 779 (1982).
29. S. W. Bigelow and D. W. Nebert, *Toxicol. Lett.* **10**, 109 (1982).
30. S. M. Galloway, P. E. Perry, J. Meneses, D. W. Nebert and R. A. Pedersen, *Proc. natn. Acad. Sci. U.S.A.* **77**, 3524 (1980).
31. R. Filler and K. J. Lew, *Proc. natn. Acad. Sci. U.S.A.* **78**, 6991 (1981).
32. M. R. Juchau and M. G. Pedersen, *Life Sci.* **12**, 193 (1973).
33. A. B. Rifkind, S. Bennett, E. S. Forster and M. I. New, *Biochem. Pharmac.* **24**, 839 (1975).
34. O. Pelkonen, *Acta pharmac. toxicol.* **541**, 306 (1977).
35. J. Capdevila, L. Parkhill, C. Nicholas, R. Okita, B. S. S. Masters and R. W. Estabrook, *Biochem. biophys. Res. Commun.* **101**, 1356 (1981).
36. J. Capdevila, L. J. Marnett, N. Chacos, R. A. Prough and R. W. Estabrook, *Proc. natn. Acad. Sci. U.S.A.* **79**, 767 (1982).
37. E. H. Oliw, F. P. Guengerich and J. A. Oates, *J. biol. Chem.* **257**, 3771 (1982).
38. V. Ullrich, L. Castle and P. Weber, *Biochem. Pharmac.* **30**, 2033 (1981).
39. B. Bösterling and J. R. Trudell, *Biochem. biophys. Res. Commun.* **114**, 850 (1983).
40. R. W. Egan, P. H. Gale, E. M. Baptista, K. L. Kennicott, W. J. A. VandenHeuvel, R. W. Walker, P. E. Fagerness and F. A. Kuehl, Jr., *J. biol. Chem.* **256**, 7352 (1981).
41. K. Sivarajah, H. Mukhtar and T. Eling, *FEBS Lett.* **106**, 17 (1979).
42. J. Guthrie, I. G. C. Robertson, E. Zeiger, J. A. Boyd and T. E. Eling, *Cancer Res.* **42**, 1620 (1982).
43. G. A. Reed and L. J. Marnett, *J. biol. Chem.* **257**, 11368 (1982).
44. J. A. Boyd, J. C. Barrett and T. E. Eling, *Cancer Res.* **42**, 2628 (1982).
45. K. Sivarajah, K. G. Jones, J. R. Fouts, T. Devereux, J. E. Shirley and T. E. Eling, *Cancer Res.* **43**, 2632 (1983).
46. C. A. Carter, R. J. Milholland, W. Shea and M. M. Ip, *Cancer Res.* **43**, 3559 (1983).
47. A. B. Rifkind and H. Muschick, *Nature* **303**, 524 (1983).
48. D. W. Nebert, D. D. Brown, D. W. Towne and H. J. Eisen, *Biol. Reprod.* in press (1984).