0026-895X/87/040471-08$02.00/0

MOLECULAR PHARMACOLOGY, 32:471-478

The Murine *Ah* Locus: A New Allele and Mapping to Chromosome 12

ALAN POLAND, EDWARD GLOVER, and BENJAMIN A. TAYLOR

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706 (A.P., E.G.) and The Jackson Laboratory Bar Harbor, Maine 04609 (B.A.T.)

Received May 27, 1987; Accepted July 21, 1987

SUMMARY

The *Ah* locus in mice, the presumed structural gene for the *Ah* receptor, is polymorphic in mice: some inbred strains carrying the Ah^b allele express a high affinity receptor and are sensitive to receptor agonists, while other strains carrying the Ah^d allele express a lower affinity receptor and are less sensitive to agonists. Using the photoaffinity ligand for the *Ah* receptor, [^{125}I]-2-azido-3-iodo-7,8-dibromodibenzo-*p*-dioxin, we have identified two allelic forms of the high affinity receptor (Ah^b). In mouse strains of the C57 and C58 family and MA/MyJ, the photoaffinity labeled peptide in hepatic cytosol has an apparent molecular mass of 95 kDa (Ah^{b-1} allele), whereas in other responsive strains (e.g., C3H/HeJ, BALB/cByJ, A/J) the labeled peptide has an apparent mass of 104 kDa (Ah^{b-2} allele). In genetic crosses and backcrosses between C57BL/6J and C3H/HeJ mice, the expression of these peptides is consistent with codominant inheritance of two alleles at a single locus. From Scatchard plots of the specific binding of the reversible ligand, [^{3}H]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin, to hepatic cytosol, most strains of mice with the Ah^{b-1} allele were found to have an *Ah* receptor with a binding affinity, K_D, of 0.4–0.7 nM, and a receptor concentration of 130–160 fmol/mg of protein, whereas most strains carrying the Ah^{b-2} allele have a slightly lower receptor affinity, K_D = 0.8–1.3 nM, and a slightly lower receptor concentration, 80–110 fmol/mg of protein. From analysis of the strain distribution pattern of the Ah^{b-1} and Ah^{b-2} alleles in recombinant inbred strains, the *Ah* locus was linked to several unmapped loci (*H-17*, *H-38*, *Ltw-2*, *Ly-18*, *D1Nyul*, *D1Nyu2*), and this entire group mapped to chromosome 12.

The *Ah* locus in mice was originally defined by a strain difference in response to the administration of polycyclic aromatic hydrocarbons (1–3): some inbred strains (e.g., C57BL/6) respond with the induction of hepatic cytochrome P_1-450 and associated monooxygenase activity, most commonly measured as AHH activity, whereas other strains (e.g., DBA/2) show no response. In appropriate genetic crosses, responsiveness (i.e., inducibility of AHH activity) segregates as a simple autosomal dominant trait. The locus determining inducibility was termed the *Ah* locus (for *a*romatic *h*ydrocarbon responsiveness) and the two alleles were designated Ah^b (responsive) and Ah^d (nonresponsive). Since many polycyclic aromatic hydrocarbons induce cytochrome P_1-450 and enhance their own metabolism, often to reactive intermediates, there is an extensive literature on the association of the carcinogenicity and toxicity of these compounds and the *Ah* locus (4).

TCDD and related halogenated aromatic hydrocarbons (halogenated dibenzo-*p*-dioxin, dibenzofuran, azo(xy)benzene, and biphenyl isomers) comprise another class of compounds which are potent inducers of cytochrome P_1-450 and AHH activity (5). TCDD induces this response in strains of mice carrying the Ah^b allele as well as those carrying the Ah^d allele (6). These observations led to the hypothesis that: 1) the *Ah* locus encodes a receptor, a macromolecule which specifically binds these agonists and mediates the enhanced expression of cytochrome P_1-450, and 2) mice with the Ah^d allele express a receptor with reduced affinity for these compounds, and hence display reduced sensitivity to induction. In the liver cytosol from C57BL/6J mice (Ah^b allele), a macromolecular species was identified which had high affinity, saturable binding for [^{3}H]TCDD, and for which the binding affinity of other agonists corresponded to their *in vivo* potency to induce AHH activity (7). Little or no specific binding of [^{3}H]TCDD was demonstrable in hepatic cytosol of DBA/2J mice (Ah^d allele).

Thus, the *Ah* locus appears to be the structural gene for the *Ah* receptor. The *Ah* receptor is a soluble protein present in a variety of vertebrate tissues that stereospecifically binds certain planar polyaromatic compounds and initiates the coordinate

This work was supported in part by National Institute of Environmental Health Sciences Grant ES-01884 and National Cancer Institute Core Grant CA-07175.

ABBREVIATIONS: AHH, aryl hydrocarbon hydroxylase; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDBF, 2,3,7,8-tetrachlorodibenzofuran; K_D, equilibrium dissociation constant; ED_{50}, effective dose that produces one-half the maximal response; SDP, strain distribution pattern; RI, recombinant inbred; EDTA, ethylenediaminetetraacetate.

expression of a number of genes. The mechanism of regulation has been most studied for the cytochrome P_1-450 gene, where sequences 5′ to the first exon have been shown to have enhancer activity, and which presumably bind the occupied receptor to initiate transcription (8–11). The *Ah* receptor has many structural and functional similarities with the steroid hormone receptors; however, no endogenous ligand has been identified for the former.

Characterization of the *Ah* receptor has depended on the reversible binding of radioligand and study of the physicochemical properties of the ligand-receptor complex under nondenaturing conditions (12–14). A photoaffinity ligand for the *Ah* receptor, [^{125}I]-2-azido-3-iodo-7,8-dibromodibenzo-*p*-dioxin, has been recently described which permits covalent labeling of the receptor and more direct analysis by denaturing gel electrophoresis (15). In the hepatic cytosol from C57BL/6J mice, the photoaffinity ligand labeled two peptides, one with an apparent mass of 95 kDa—the ligand-binding peptide of the *Ah* receptor,[1] and a 70-kDa peptide, which is a proteolytic fragment of the former, produced by a Ca^{2+}-dependent thiol proteinase (15a).

The strain distribution pattern of the Ah^b and Ah^d alleles in recombinant inbred mice has not permitted mapping of the *Ah* locus (16). Legraverend *et al.* (17), using inducibility of AHH activity in somatic cell hybrids formed from mouse spleen cells and Chinese hamster E36 cells, have reported mapping of the *Ah* locus to murine chromosome 17.

We now report a third allele at the *Ah* locus in mice, i.e., two distinguishable forms of the high affinity *Ah* receptor (Ah^b), and from the strain distribution pattern of these alleles in recombinant inbred strains we have mapped the *Ah* locus to chromosome 12.

Experimental Procedures

Materials. Acrylamide, bisacrylamide, ammonium persulfate, sodium dodecyl sulfate, and *N,N,N′N′*-tetramethylethylenediamine were purchased from Bethesda Research Laboratories. MOPS and Na-MOPS, Coomassie brilliant blue, 2-mercapto-ethanol, and molecular weight standards were purchased from Sigma Chemical Co.

Animals. All inbred, recombinant inbred, and congenic mouse strains were purchased from The Jackson Laboratory.

Inbred strain. An inbred strain of mice is produced by 20 or more consecutive generations of brother-sister matings. This inbreeding produces a high degree of uniformity in the mice, i.e., homozygosity in the vast majority of loci.

Congenic strain. A congenic strain is an inbred strain that has a small segment of foreign chromosome introduced into its genome by repeated backcrossing and selection. This places a specified differential gene or genes of the segment into a homogeneous genetic background for controlled comparisons.

Recombinant inbred (RI) strains. A set of RI strains is produced from two existing inbred strains (called progenitor strains) by perpetuating for 20 or more generations by brother-sisters matings. This provides a new series of inbred strains (i.e., mice that are homozygous at the vast majority of loci), with each representing a reassortment of the alleles of the progenitor strains. The concordance or near concordance of the strain distribution pattern of the locus of interest and a marker locus is used to estimate linkage or map distance. The recombinational frequency, *r*, is a function of the fraction of RI strains, *R*, in which these two loci are recombinant or nonmatching: $r = R/(4 - 6R)$. The standard error of this estimate is:

$$SE = \sqrt{\frac{r(1 + 2r)(1 + 6r)^2}{4n}}$$

where n = the number of RI strains in a set (18).

Radiolabeled ligands. [^{3}H]TCDD (7) was purified on a reverse phase C-18 HPLC column to >95% purity (Specific activity of 27.5 Ci/mmol). [^{125}I]-2-Azido-3-iodo-7,8-dibromodibenzo-*p*-dioxin was synthesized and purified as described (14) (specific activity = 2176 Ci/mmol).

Buffers. The buffers used were: MβENG [25 mM MOPS, 1 mM 2-mercaptoethanol, 1 mM EDTA, 0.02% sodium azide, pH 7.5 (0°), plus 10% glycerol] and MEG [25 mM MOPS, 1 mM EDTA, pH 7.5 (0°), plus 10% glycerol].

Preparation of liver cytosol. The livers of two or three mice of the same strain (matched for sex and age) were pooled and homogenized in 9 volumes of MβENG buffer for use in reversible ligand binding, or individual livers were homogenized in 9 volumes of MEG buffer for use in photoaffinity labeling. The homogenates were centrifuged at 10,000 × *g* for 20 min at 4°, and the supernatant fractions were centrifuged at 100,000 × *g* for 1 hr at 4°. The cytosols were frozen at −80° until use.

[^{3}H]TCDD binding. One ml of hepatic cytosol diluted to 2–2.5 mg of protein/ml in MβENG buffer was incubated with [^{3}H]TCDD at nine concentrations from 0.33 to 2.1 nM in the presence or absence of a 200-fold molar excess of TCDBF. The ligands were added in *p*-dioxane (total solvent 10 μl per 1-ml incubation). Each sample was analyzed in duplicate. Following a 30-min incubation at 20°, 0.5 ml of a suspension of 3% charcoal/0.3% dextran was added, the mixture was gently agitated for 5 min, and the charcoal was removed by centrifugation. The radioactivity in fractions was quantified by liquid scintillation spectrometry: the radiolabel in the total incubation mixture without addition of charcoal/dextran = total ligand concentration; the radiolabel in [^{3}H]TCDD incubate after charcoal adsorption = total bound ligand; and the activity in the [^{3}H]TCDD + TCDBF tube after charcoal adsorption = nonspecifically bound ligand. Specific binding at each ligand concentration was determined as total bound minus nonspecifically bound; and the free ligand concentration was determined as total ligand concentration minus total bound ligand.

Binding parameters were estimated by a computer analysis of the Scatchard equation (19): $\frac{[B]}{[F]} = \frac{1}{K_D}[B_{max} - B]$, where $[B]$ = specifically bound, $[F]$ = free ligand concentration, B_{max} = maximum specifically bound, and K_D = equilibrium dissociation constant. The protein concentration was determined by the Lowry reaction (20) using bovine serum albumin as a standard, and binding was expressed per mg of protein. For each binding curve, three parameters were obtained, K_D, B_{max}, and the correlation coefficient *r*. Only curves with $r \geq 0.90$ for nine ligand concentrations were included. The data are reported as the mean ± standard error of multiple separate binding curves.

Photoaffinity labeling. For each inbred, recombinant, or congenic strain, a single mouse was killed, the liver was homogenized in 9 volumes of MEG buffer, and the cytosolic fraction was prepared and stored as described above. The liver cytosol was diluted to 1 mg of protein/ml with MEG buffer and incubated with 0.38 nM [^{125}I]-2-azido-3-iodo-7,8-dibromo-dibenzo-*p*-dioxin for 30 min at 20°, and then incubated for 5 min at 4°. One-tenth volume of a suspension of charcoal/dextran (10%:1%) was added for a 30-min incubation at 4° and removed by centrifugation. The supernatant was immediately irradiated with ultraviolet light as previously described (14). Four volumes of cold acetone were added and stored overnight at −20° for maximal precipitation of the protein. The precipitate was collected by centrifugation, washed with acetone/water (9:1), and dissolved in 1 ml of electrophoresis sample buffer (11) by heating in a boiling water bath for 2 min.

[1] From hydrodynamic measurements using nondenaturing conditions, the [^{3}H]TCDD-*Ah* receptor complex in mouse liver cytosol has an apparent mass of 100 kDa in high salt buffers, while a larger aggregate with a mass of 250–300 kDa is observed in low salt buffers (12, 13). The apparent mass of the peptide selectively labeled by the photoaffinity ligand is 95 kDa, estimated by denaturing gel electrophoresis. Since the composition of the large *Ah* receptor aggregate in low salt is unknown, and might include other peptides which aid in receptor function, we refer to the photoaffinity labeled peptide as the *ligand-binding peptide of the Ah receptor*, and not the *Ah* receptor.

The photoaffinity labeled protein samples and molecular weight standards (β-galactosidase, 116 kDa; phosphorylase b, 97.5 kDa; bovine serum albumin, 68 kDa; ovalbumin, 45 kDa) were subjected to denaturing electrophoresis on discontinuous polyacrylamide slab gels by the method of Laemmli (21) as previously described (14). The separating gel was routinely 7.5% acrylamide, 11 cm long, but for mobility determinations, a 5–20% acrylamide gradient, approximately 25 cm long, was used. The gels were fixed and stained and autoradiographs prepared as described previously (14).

Phenotyping the *Ly-18* antigen. Monoclonal antiserum to the membrane antigen *Ly-18* (22) was kindly provided by Dr. Ulrich Hämmerling. Dr. Rex Risser used cytolysis of thymus cells to determine the *LY-18* phenotype using cells from C3H/HeJ as a negative control and cells from a B6·C-H-24^c/By congenic strain as a positive control.

Results

Examination of [^{3}H]TCDD binding to the *Ah* receptor in the hepatic cytosol from inbred strains of mice carrying the *Ah*^{b} allele (high affinity receptor) revealed modest but reproducible differences (Table 1). For strains in the C57 and C58 family, the *Ah* receptor affinity (K_D) was 0.4–0.7 nM, and the total number of binding sites was 130–160 fmol/mg of protein. Most of the other strains had slightly lower affinity binding (K_D = 0.8–1.3 nM) and a lower concentration of saturable binding sites (B_{max}[2] = 80–110 fmol/mg of protein). MA/MyJ, CE/J, and P/J mice yielded intermediate values or a dissociation of low K_D and high number of sites.

TABLE 1

Characterization of the Ah receptor in hepatic cytosol from inbred strains of mice: receptor affinity, concentration, and apparent molecular mass

The Ah receptor in the cytosol fraction of liver inbred strains of mice was characterized by a saturation binding curve with [^{3}H]TCDD and the apparent molecular mass of the photoaffinity labeled peptide as described in Experimental Procedures. The specific binding of [^{3}H]TCDD in the saturation binding curve was analyzed by linear regression analysis of the equation $\frac{[B]}{[F]} = \frac{1}{K_D}[B_{max} - B]$, to estimate the equilibrium dissociation constant, K_D, and concentration of binding sites B_{max}. Only binding curves with a correlation coefficient of $r \geq 0.90$ for all nine ligand concentrations are included.

	Reversible ligand binding				Photoaffinity labeled peptide (mass in kDa)
	n[a]	K_D[b]	B_{max}[c]	r[d]	
		nM	fmol/mg protein		
C57BL/6J	6	0.65 ± 0.15	151 ± 26	0.965	95
C57BL/6ByJ	3	0.47 ± 0.04	160 ± 4	0.980	95
C58/J	2	0.59	159	0.984	95
C57L/J	3	0.52 ± 0.08	131 ± 8	0.954	95
C57BR/cdJ	1	0.69	156	0.968	95
MA/MyJ	1	1.1	147	0.938	95
CE/J	1	0.69	108	0.966	104
P/J	3	1.1 ± 0.31	129 ± 31	0.952	104
BALB/cByJ	4	1.3 ± 0.37	82 ± 6	0.945	104
BUB/BnJ	3	0.96 ± 0.14	86 ± 9	0.965	104
SEC/1ReJ	3	1.1 ± 0.09	89 ± 30	0.935	104
A/J	4	0.81 ± 0.27	93 ± 5	0.969	104
C3H/HeJ	5	1.1 ± 0.17	86 ± 23	0.940	104
CBA/J	4	1.2 ± 0.33	84 ± 13	0.945	104
PL/J	4	1.3 ± 0.52	107 ± 17	0.951	104
SEA/GnJ	6	1.3 ± 0.51	93 ± 41	0.954	104
HRS/J	6	0.80 ± 0.41	90 ± 16	0.965	104
CXBD/By	2	0.41	168	0.982	95
CXBE/By	2	1.0	80	0.970	104
CXBG/By	2	0.83	81	0.980	104
CXBH/By	2	0.66	111	0.985	104
CXBI/By	2	0.92	120	0.958	104
CXBJ/By	2	0.55	162	0.965	95
CXBK/By	2	1.2	104	0.958	104
B6·C-*H-17*^{c}/By	1	1.2	95	0.962	104
B6·C-*H-34*^{c}/By	1	0.63	178	0.949	95
B6·C-*H-38*^{c}/By	2	1.0	73	0.962	104

[a] *n*, number of individual experiments.
[b] K_D, equilibrium dissociation constant; values are the mean ± SD of *n* determinations.
[c] B_{max}, receptor concentration, i.e., concentration of available binding sites/mg of cytosol protein; values are the mean ± SD of *n* determinations.
[d] *r*, mean correlation of *n* determinations.

We have previously characterized the *Ah* receptor in hepatic cytosol from C57BL/6J mice using the photoaffinity ligand [^{125}I]-2-azido-3-iodo-7,8-dibromodibenzo-*p*-dioxin (14). This ligand labels predominantly two peptides with apparent molecular masses of 95 kDa and 70 kDa, the labelings of which are both blocked by coincubation of unlabeled competing ligands. The 95-kDa peptide was shown to be the ligand-binding peptide of the *Ah* receptor, and the 70-kDa moiety was found to be a proteolytic fragment produced from the former by a Ca^{2+}-dependent thiol proteinase (15a). The major peptide labeled by the photoaffinity ligand in the C57 and C58 family of mice and in MA/MyJ has an apparent mass of 95 kDa, while in all other strains tested with the *Ah*^{b} allele, the major peptide has an apparent mass of 104 kDa (Fig. 1, Table 1).

The F_1 progeny of C57Bl/6J mice (95 kDa) and C3H/HeJ mice (104 kDa), express both peptides (Fig. 1). After analysis of further crosses and backcrosses by comparison of the observed and expected ratios in the χ^2 test, the inheritance was consistent with two alleles at a single locus with condominant inheritance: B6 × B6C3F_1 = 14 B6 phenotype: 11 F_1 phenotype, χ^2 = 0.36; C3H/He × B6C3F_1 = 40 C3H/He phenotype: 34 F_1 phenotype, χ^2 = 0.48; B6C3F_1 × B6C3F_1 = 24 B6 phenotype: 40 F_1 phenotype: 17 C3H/He phenotype, χ^2 = 1.05.

We examined the SDP of these *Ah* alleles in seven CXB (BALB/cBy × C57BL/6By) recombinant inbred strain (Table 1, Fig. 2A). The SDP of the alleles was BCCCCBC, determined by photoaffinity labeling (Fig. 2A) and by reversible ligand binding, with the B phenotype having a higher affinity binding (K_D = 0.4–0.6 nM) and a larger number of binding sites (n = 160 fmol/mg) (Table 1). This same SDP has been observed previously for several other unmapped loci (23): 1) *Ly-18* (formerly *Ly-m18*), a lymphocyte surface alloantigen defined by a monoclonal antibody (22); 2) *Ltw-2*, a hepatic peptide polymorphism identified by two-dimensional gel electrophoresis (24); and 3) three minor histocompatibility loci, *H-17*, *H-34*, and *H-38* (25). *H-17*, *H-34*, and *H-38* are each defined by independently derived congenic strains in which BALB/cBy histocompatibility alleles were introduced onto the C57BL/6By strain background by continuous backcrossing. Linkage between *Ah* and *Ly-18* was confirmed by the observed complete concordance of these two loci in 17 AKXL and 12 BXH RI strains (Table 2). Recently, additional loci, including two DNA restriction fragment length variants related to endogenous xenotropic viral sequences (26) and the structural gene for apolipoprotein B-100 (27), have been added to this linkage group. As was noted previously (26, 27), these loci exhibit significant concordance with chromosome 12-specific, anonymous DNA restriction fragment length variants *D12Nyu1*, *D12Nyu2*, and

[2] B_{max}, the concentration of available ligand-binding sites, is equal to $n[R_T]$, where n is the number of ligand-binding sites per molecule of receptor and $[R_T]$ is the concentration of the receptor. Assuming n = 1, then B_{max1} is equivalent to $[R_T]$, the receptor concentration.

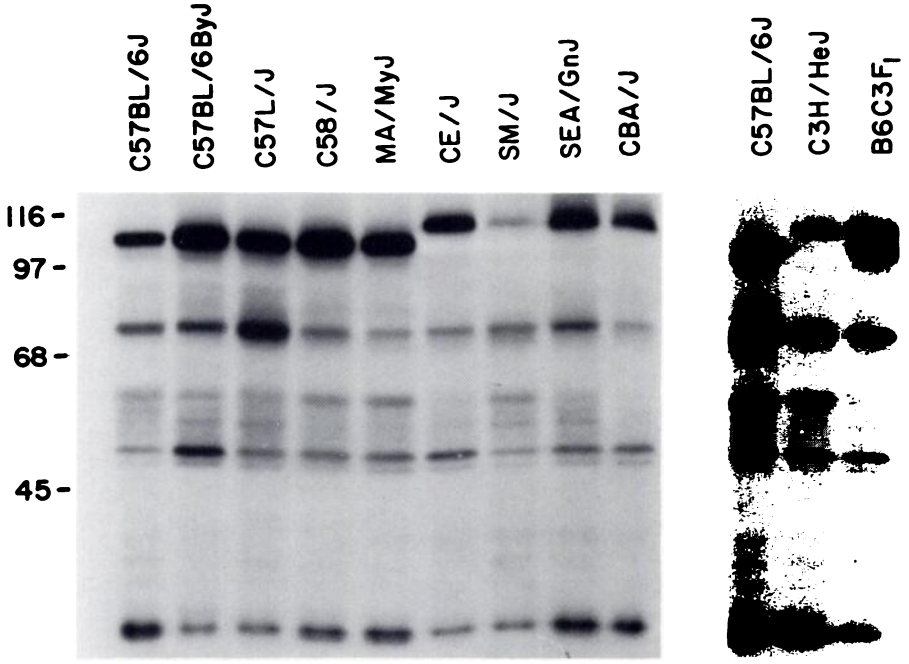

Fig. 1. Strain difference in the apparent m_r of the Ah receptor. The hepatic cytosol from inbred strains of mice was labeled with the ^{125}I-photoaffinity ligand; the products were analyzed by denaturing gel electrophoresis and visualized by autoradiography as described in Experimental Procedures. The autoradiograph on the *left* compares the *Ah* receptor in nine strains; the *right panel* compares the *Ah* receptor in C57BL/6J, C3H/HeJ, and B6C3F$_1$/J hybrid mice.

D12Nyu5 (formerly *D12-1*, *D12-2*, and *D12-5*) (28), indicating that this cluster of genes maps to chromosome 12.

The *Ah* genotypes of the three minor histocompatibility congenic strains, B6·C-*H-17*^{c}, B6·C-*H-34*^{c}, and B6·C-*H-38*^{c} were determined by photoaffinity labeling and electrophoresis (Fig. 2B). Both *H-17* and *H-38* congenics possess the *Ah* allele of the BALB/c (donor) strain, i.e., the 104-kDa peptide, indicating that the *Ah* allele had been cotransferred in the derivation of these strains. The results confirm that *H-17* and *H-38* are closely linked to the *Ah* locus and therefore on chromosome 12. In contrast, the *H-34* congenic has the *Ah* allele of the C57BL/6By strain, indicating that the *Ah* locus recombined with the *H-34* locus in the derivation of this strain. The latter result indicates that either: 1) *H-34* and *Ah* are unlinked; or 2) a crossover occurred between *H-34* and *Ah*. Monoclonal antibodies specific for *Ly-18* were used to show that the B6·C-*H-38*^{c} congenic also carried the BALB/c donor allele of *Ly-18*, indicating that the segment of chromosome 12 including *Ly-18*, *Ah*, and *H-38* had been cotransferred. Unfortunately, it was not feasible to test for *Ly-18* in the other two congenics. The fact that both B6·C-*H-17*^{c} and B6·C-*H-38*^{c} carry the BALB/c allele of *Ah* and also reject each other's skin grafts suggests that these minor histocompatibility loci flank the *Ah* locus but does not resolve the question of which is centromeric to *Ah*. Previous work has shown that the introduced chromosome 12 segments of B6·C-*H-17*^{c} do not extend as far proximally as the pol-7 DNA marker (26). The latter is apparently closely linked to the *D12Nyu2* marker.

The linkage of *Ly-18* and *Ah* was demonstrated in RI strains (Table 2) bearing three *Ah* variants (*Ah*^{b}, 95-kDa peptide in C57BL/6 and C57L; *Ah*^{b}, 104-kDa peptide in BALB/cBy and C3H/He; and *Ah*^{d} in AKR or DBA/2), indicating that these variants are linked, if not in fact allelic, as assumed here. The lack of recombination between *Ah* and *Ly-18* among 36 CXB, AKXL, and BXH RI strains establishes a 95% upper confidence limit of 2.3 cM for the map distance separating these loci. There are a total of 15 recombinants between *Ah* and *D12Nyu1* among 68 informative RI strains for an estimated map distance of 8.2 ± 2.8 cM. The most probable gene order *D12Nyu2*, (*Ah*, *Ly-18*) and *D12Nyu2*, in the 19 BXH and CXB RI strains makes it unnecessary to postulate any double crossovers, whereas alternative gene orders required three or more double crossovers. The estimated distance between (*Ah*, *LY-18*) and *D12Nyu2*, 5.2 ± 3.6 cM, is subject to large sampling error and must be considered tentative.

Discussion

In this report, we have identified a third allele at the murine *Ah* locus, which subdivides the previously designated *Ah*^{b} (responsive) allele into two types: those with the *Ah*$^{b-1}$ allele, expressing a 95-kDa peptide, and those with the *Ah*$^{b-2}$ allele, expressing a 104-kDa peptide. From the strain distribution pattern of these alleles in RI strains, the *Ah* locus has been linked to several unmapped loci, and all of these loci mapped to chromosome 12.

The *Ah* locus/*Ah* receptor. The *Ah* locus was originally defined by a functional polymorphism (1–3). Inbred strains carrying *Ah*^{b} alleles (e.g., C57BL/6) respond to the administration of 3-methylcholanthrene with the induction of hepatic cytochrome P$_1$-450 and AHH activity, whereas strains carrying the *Ah*^{d} allele (e.g., DBA/2) fail to respond. In genetic crosses responsiveness is inherited as a simple autosomal dominant trait. Subsequent biochemical, genetic and pharmacologic investigations have supported the hypotheses that: 1) the *Ah* locus encodes a soluble protein, the *Ah* receptor, which reversibly binds 3-methylcholanthrene (and other agonists), and initiates the transcriptional regulation of cytochrome P$_1$-450; and 2) the responsive mice express a receptor with a high affinity for agonists, whereas nonresponsive strains (*Ah*^{d} allele) express an *Ah* receptor with substantially reduced affinity for inducing compounds. We believe that all the available evidence is compatible with the hypothesis that the *Ah* locus is a single gene that encodes the *Ah* receptor (i.e., the ligand-binding peptide of the *Ah* receptor). Although other loci may affect the induction of AHH activity, (see below), it is confusing to refer to these loci as part of the *Ah* locus or *Ah* complex.

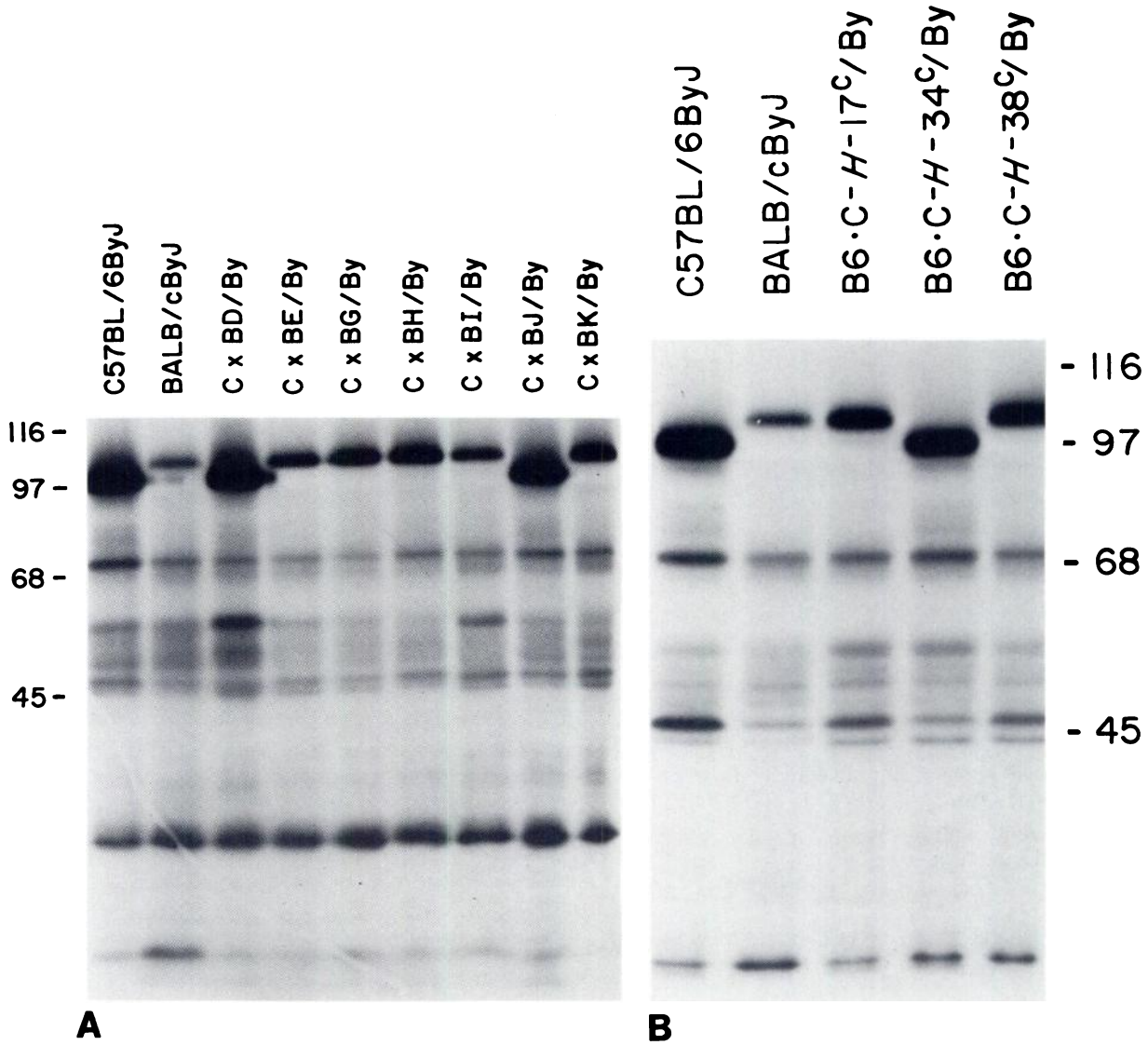

Fig. 2. Allelic forms of the *Ah* receptor: strain distribution pattern in CXB/By RI mice and B6·C/By congenic strains. The hepatic cytosols from individual mice were photoaffinity labeled and the products analyzed by gel electrophoresis and autoradiography as described in Experimental Procedures. A. C57BL/6ByJ, BALB/cByJ, and CXB/By RI strains. B. Parental C57BL/6ByJ and BALB/cByJ strains and congenic B6·C-*H-17*^{c}/By, B6·C-*H-34*^{c}/*By*, and B6·C-*H-38*^{c}/By strains.

It should be noted that the pleiotropic response regulated by the *Ah* receptor is far more extensive than the induction of cytochrome P_1-450 (29). TCDD and related halogenated aromatic hydrocarbons produce a spectrum of biological responses (e.g., proliferation and/or differentiation of certain epithelial tissue, immune suppression, altered lipid metabolism, hepatic damage, and porphyria, tumor promotion). Two lines of evidence suggest these responses are mediated through the *Ah* receptor: a) several of these agonist-induced responses have been shown to segregate with the *Ah* locus in mice, and b) there is a correlation between the binding affinity of agonists and their potency to produce various biologic responses.

***Ah*^{b} alleles.** In this report we have found that among strains responsive to polycyclic aromatic hydrocarbons, there are two allelic forms of the *Ah* receptor: *Ah*$^{b\text{-}1}$, which encodes a peptide with an apparent mass of 95 kDa, and *Ah*$^{b\text{-}2}$ which encodes a peptide of an apparent mass of 104 kDa. In most *Ah*$^{b\text{-}1}$ strains, the *Ah* receptor in hepatic cytosol has a slightly higher binding affinity for TCDD and higher receptor concentration (K_D = 0.4–0.7 nM, B_{max} = 130–160 fmol/mg of protein) than in strains carrying the *Ah*$^{b\text{-}2}$ allele (K_D = 0.8–1.3 nM, B_{max} = 8–110 fmol/mg of protein). The qualitative marker of apparent receptor mass is a more accurate phenotype than the quantitative estimate of reversible binding parameters.

***Ah*^{d} allele.** Although mice with the *Ah*^{d} allele fail to respond to 3-methylcholanthrene, administration of the more potent agonist, TCDD, induces hepatic AHH activity to a comparable extent as that observed in strains with either *Ah*^{b} allele, but an approximately 10-fold greater dose is required (7). It has not been possible to demonstrate appreciable specific ligand binding in the hepatic cytosol from *Ah*^{d} mice, but following *in vivo* administration of [^{3}H]TCDD, a macromolecular-radioligand complex has been extracted from hepatic nuclei which has sedimentation properties similar to those of the *Ah*-receptor-[^{3}H]TCDD in mice with *Ah*^{b} alleles (30). These observations suggest the *Ah*^{d} allele encodes an *Ah* receptor with a lesser affinity for agonists resulting in diminished sensitivity. We do not know if all *Ah*^{d} strains express the same allele, because it is difficult to characterize this low affinity receptor.

TABLE 2

Strain distribution pattern of *Ah*, Ly-*18*, D12Nyu*1*, and D12Nyu*2* alleles in recombinant inbred strains

The abbreviations for the recombinant inbred strains are: AKXL, (AKR/J × C57L/J); CXB, (BALB/cBy × C57BL/6By); SWXL, (SWR/J × C57L/J); BXD, (C57BL/6J × DBA/2J); and BXH, (C57BL/6J × C3H/HeJ). The symbols A, L, B, C, S, D, and H are used as generic symbols for alleles inherited from the progenitor strains AKR/J, C57L/J, C57BL/6J (J or By), BALB/cBy, SWR/J, DBA/2J, and C3H/HeJ. "x" means disordant; –, not determined. The strain distribution patterns for *Ly-18*, *D12Nyu1*, and *D12Nyu2* are published in Refs. 22 and 23. The strain distribution patterns for the *Ah* locus have been published previously (17, 23) or were determined in this report for CXB and BXH RI strains by photoaffinity labeling.

AKXL

	5	6	7	8	9	12	13	14	16	17	19	21	24	25	28	29	37	38
Ly-18	A	A	L	L	A	–	L	A	A	A	L	L	A	A	L	A	L	A
Ah	A	A	L	L	A	A	L	A	A	A	L	L	A	A	L	A	L	A
													x					
D12Nyu1	A	A	L	L	A	A	L	A	–	A	L	L	L	A	L	A	L	A

CXB

	D	E	G	H	I	J	K
D12Nyu2	B	C	C	C	C	B	B
							x
Ah,Ly-18	B	C	C	C	C	B	C
	x						
D12Nyu1	C	C	C	C	C	B	C

SWXL

	4	7	12	14	15	16	17
Ah	S	S	L	L	S	L	L
			x			x	
D12Nyu1	S	S	S	L	S	S	L

BxD

	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
Ah	B	D	B	B	B	D	B	B	B	B	D	B	B	D	D	D	D	D	D	B	D	B	B	B	D	D
	x	x			x				x											x		x	x			
D12Nyu1	D	B	B	B	D	D	B	B	D	B	D	B	B	D	–	–	D	D	D	D	D	D	D	B	D	D

BXH

	2	3	4	6	7	8	9	10	11	12	14	19
D12Nyu2	B	B	B	H	H	B	H	B	H	B	H	H
				x						x		
Ah, Ly-18	B	B	B	B	H	B	H	B	H	H	H	H
	x					x		x				
D12Nyu1	H	B	B	B	H	H	H	H	H	H	H	H

Evidence for other alleles at the *Ah* locus or other loci affecting the induction of AHH activity. The strain difference in response to induction of AHH activity by polycyclic aromatic hydrocarbons, which was used to identify the *Ah* locus, is a complex phenotype and could be affected by other loci. Robinson *et al.* (31) presented some surprising results in crosses between NIH substrains, including the observation that nonresponsiveness (noninducibility) in some crosses was dominant. The authors suggested that these results were not fully explainable even with a model of two loci and three alleles at each locus. One is at a loss to interpret these observations, since they have not been pursued or confirmed by other laboratories in the 13 years since the original report.

More convincing evidence that genes other than the *Ah* locus influence the induction of AHH activity comes from the studies of Hankinson and colleagues (32–34) and Whitlock and coworkers (35, 36) on Hepa 1 cells, a cloned cell line derived from a hepatoma in C57L/J mice (37). From the wild-type cell, which is highly inducible for AHH activity, at least four classes of stable variants have been identified with absent or poorly inducible AHH activity. Fusion experiments have demonstrated these four classes are complementary and hence represent different genetic loci. Group A variants (Hankinson's classification) have *Ah* receptor binding and nuclear translocation[3] of the receptor comparable to wildtype Hepa 1 cells, with minimal induction of AHH activity, and appear to represent mutations in the structural gene for cytochrome P_1-450. Group B variants have reduced *Ah* receptor binding (10% of normal), normal nuclear uptake of the receptor, and the maximal induced AHH activity is 1–20% of the wild type, with a similar ED_{50}. Miller *et al.* (35) isolated clones of similar phenotype, with diminished sensitivity to induction, suggesting an altered receptor affinity. It is likely that this complementation group is the *Ah* locus. Group D variants have normal *Ah* receptor binding and translocation, but noninducibility is a dominant trait in fusion experiments. It is proposed that these variants produce a *trans*-acting dominant regulator. Group C variants have normal *Ah* receptor binding but the receptor does

[3] *Nuclear translocation.* Upon incubation of Hepa 1 cells with [^{3}H]TCDD at 4°, the radioligand-*Ah* receptor complex is found in the cytosol fraction (high speed supernatant fraction), whereas, following incubation at 37°, much of the [^{3}H]TCDD-Ah receptor complex is associated with the nuclear pellet (38). This suggests that occupied receptor undergoes a temperature-sensitive cytoplasm to nuclear translocation. However, Whitlock and Galeazzi (39) have presented evidence that the unoccupied *Ah* receptor resides in the nucleus, and its presence in the cytosol is an artifact of cell disruption. Thus, it is not established whether, upon ligand binding, the *Ah* receptor undergoes nuclear translocation or activation from a loose binding to a more tightly binding nuclear form.

not translocate to the nucleus. Thus, a complementation group distinct from that of the *Ah* locus controls nuclear uptake. This result is in contrast to glucocorticoid receptor mutants in lymphoblastoid cells, where mutations affecting receptor binding and nuclear translocation are in the same complementation group (40, 41).

Linkage and mapping the *Ah* receptor. We have found that the *Ah* locus is linked to two histocompatibility loci, *H-17* and *H-34*, a soluble liver protein, *Ltw-2*, and the lymphocyte surface antigen, *Ly-18*. The *Ah* locus previously has been linked to audiogenic seizure locus in BXD RI strains (42). All of these loci are now mapped to chromosome 12 by linkage to the *D12Nyu1* and *D12Nyu2* restriction fragment length polymorphism described by D'Eustachio (28). Lusis *et al.* (27) have recently mapped the apolipoprotein B structural gene to the proximal region of chromosome 12 and shown linkage to the *Ah* locus.

The *Ah* locus should serve as a very useful marker for the centromeric end of chromosome 12, because of the appreciable frequency of each of the three alleles among inbred and RI strains, the expression of the receptor in a wide variety of tissues and cell types in cultures, and the ease of phenotyping.

Structural variation in the *Ah* receptor among mammalian species. In contrast to the analogous steroid hormone receptors which show remarkable structural conservation among mammalian species, the *Ah* receptor (i.e., the photoaffinity labeled ligand-binding peptide of the receptor) shows remarkable variation. The apparent mass of the labeled peptide from eight vertebrate species varied from 95 kDa to 124 kDa, and two allelic forms of the receptor were identified in the rat (43). The Ah^d allele in mice clearly affects receptor function. In mammals, or possibly all vertebrate species, there does not appear to be selection pressure to conserve the *Ah* receptor structure, and this may indicate the absence of an endogenous (or physiologic) agonist for the receptor.

Acknowledgments

We wish to thank Dr. Ulrich Hämmerling for his gracious gift of the monoclonal antibody to *Ly-18*, and Dr. Rex Risser for phenotyping the *Ly-18* antigen. We wish to thank Dr. Aldons Lusis for providing a preprint of his manuscript.

References

1. Nebert, D. W., J. E. Goujon, and J. E. Gielen. Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons: simple autosomal dominant trait in the mouse. *Nature New Biol.* **235**:107–110 (1972).
2. Thomas, P. E., R. E. Kouri, and J. J. Hutton. The genetics of aryl hydrocarbon hydroxylase induction in mice: a single gene difference between C57BL/6J and DBA/2J. *Biochem. Genet.* **6**:157–168 (1972).
3. Nebert, D. W., and J. E. Gielen. Genetic regulation of aryl hydrocarbon hydroxylase induction in the mouse. *Fed. Proc.* **31**:1315–1325 (1972).
4. Nebert, D. W., and N. M. Jensen. The Ah locus: genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochromes P-450 mediated monooxygenases. *CRC Crit. Rev. Biochem.* **6**:401–437 (1979).
5. Poland, A., and E. Glover. Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol. Pharmacol.* **10**:349–359 (1974).
6. Poland, A., and F. Glover. Genetic expression of aryl hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: evidence for a receptor mutation in genetically nonresponsive mice. *Mol. Pharmacol.* **11**:389–398 (1975).
7. Poland, A., E. Glover, and A. S. Kende. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol. *J. Biol. Chem.* **251**:4936–4945 (1976).
8. Whitlock, J. P., Jr. The regulation of cytochrome P-450 gene expression. *Annu. Rev. Pharmacol. Toxicol.* **26**:333–369 (1986).
9. Jones, P., D. Galeazzi, J. Fisher, and J. P. Whitlock, Jr. Control of cytochrome P_1-gene expression by dioxin. *Science (Wash. D. C.)* **227**:1499–1502 (1985).
10. Jones, P., L. K. Durrin, P. Galeazzi, and J. P. Whitlock, Jr. Control of cytochrome P_1-450 gene expression: analysis of a dioxin-responsive enhancer system. *Proc. Natl. Acad. Sci. USA* **83**:2802–2806 (1986).
11. Sogawa, K., A. Fujisawa-Sohara, M. Yamame, and Y. Fuijii-Kuriyama. Location of regulatory elements responsible for drug induction in the rat cytochrome P-450c gene. *Proc. Natl. Acad. Sci. USA* **83**:8044–8048 (1986).
12. Denison, M. S., L. M. Vella, and A. B. Okey. Structure and function of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.* **261**:3987–3995 (1986).
13. Poellinger, L., J. Lund, M. Gillner, L.-A. Hansson, and J.-A. Gustafsson. Physicochemical characterization of specific and nonspecific polyaromatic hydrocarbon binders in rat and mouse liver cytosol. *J. Biol. Chem.* **258**:13535–13542 (1983).
14. Hannah, R. R., D. W. Nebert, and H. J. Eisen. Regulatory gene product of the Ah complex. *J. Biol. Chem.* **256**:4584–4590 (1981).
15. Poland, A., E. Glover, F. H. Ebetino, and A. S. Kende. Photoaffinity labeling of the Ah receptor. *J. Biol. Chem.* **261**:6352–6365 (1986).
15a. Poland, A., and E. Glover. Ca^{2+}-dependent proteolysis of the Ah receptor. *Arch. Biochem. Biophys.*, in press.
16. Taylor, B. A. The aryl hydrocarbon hydroxylase inducibility locus (Ah) of the mouse, a genetic perspective, in *Biological Mechanisms of Dioxin Action. Banbury Report 18* A. Poland and R. Kimbrough, eds.). Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 97–108 (1984).
17. Legraverend, C., S. O. Kärenlampi, S. W. Bigelow, P. A. Lalley, C. A. Kozak, J. E. Womack, and D. W. Nebert. Aryl hydrocarbon hydroxylase induction by benzo(*a*)anthracene: regulatory gene localized to the distal portion of mouse chromosome 17. *Genetics* **107**:447–461 (1984).
18. Bailey D. W. Recombinant inbred strains and bilineal cogenic strains, in *The Mouse in Biomedical Research* (H. L. Fox, J. D. Small, and J. F. Fox, eds.), vol. 1. Academic Press, New York, 223–239 (1981).
19. Scatchard, G., I. H. Scheinberg, and S. H. Armstrong, Jr. Physical chemistry of protein solutions. IV. The combination of human serum albumin with chloride ion. *J. Am. Chem. Soc.* **72**:535–540 (1950).
20. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
21. Laemmli, U. K. Cleavage of structural proteins during assembly of the head of bacteriophage T4. (*Nature Lond.*) **227**:680–685 (1970).
22. Kimura, S., N. Tada, Y. Liu, and U. Hämmerling. A new mouse cell-surface antigen. *Immunogenetics* **13**:547–555 (1981).
23. Taylor, B. Recombinant inbred strains, in *Genetic Variants and Strains of the Laboratory Mouse* (M. Green, ed.). Gustav Fischer Verlag, New York, 394–407 (1981).
24. Elliot, R. W. The use of two-dimensional electrophoresis to identify and map new mouse genes. *Genetics* **91**:295–308 (1979).
25. Bailey, D. W. Genetics of histocompatibility in mice immunogenetics. **2**:249–256 (1975).
26. Rossomondo, A., and D. Meruelo. Viral sequences associated with many histocompatibility genes. *Immunogenetics* **23**:233–245 (1986).
27. Lusis, A. J., B. A. Taylor, D. Quon, S. Zollman, and R. C. LeBoeuf. Genetic factors controlling structure and expression of apolipoproteins B and E in mice. *J. Biol. Chem.* **262**:7594–7604 (1987).
28. D'Eustachio, P. A genetic map of mouse chromosome 12 composed of polymorphic DNA fragments. *J. Exp. Med.* **160**:827–838 (1984).
29. Poland, A., and J. Knutson. 2,3,7,8-Tetrachlorodizenzo-*p*-dioxin and related halogenated aromatic hydroxcarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**:517–554 (1982).
30. Mason, M. E., and A. B. Okey. Cytosolic and nuclear binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to the Ah receptor in extrahepatic tissues of rat and mice. *Eur. J. Biochem.* **123**:209–215 (1982).
31. Robinson, J. R., N. Considine, and D. W. Nebert. Genetic expression of aryl hydrocarbon hydroxylase induction. *J. Biol. Chem.* **249**:5851–5859 (1974).
32. Hankinson, O. Single-step selection of clones of a mouse hepatoma line deficient in aryl hydrocarbon hydroxylase. *Proc. Natl. Acad. Sci. USA* **76**:373–376 (1979).
33. Legraverend, C., R. R. Hannah, H. J. Eisen, I. S. Owens, D. W. Nebert, and O. Hankinson. Regulatory gene product of the Ah locus. *J. Biol. Chem.* **257**:6402–6407 (1982).
34. Hankinson, O., R. D. Andersen, B. W. Birren, F. Sander, M. Negishi, and D. W. Nebert. Mutations affecting the regulation of transcription of cytochrome P_1-450 gene in the mouse Hepa-1 cell line. *J. Biol. Chem.* **260**:1790–1795 (1985).
35. Miller, A. G., D. Israel, and J. P. Whitlock, Jr. Biochemical and genetic analysis of variant mouse hepatoma cells defective in the induction of benzo(*a*)pyrene-metabolizing enzyme activity. *J. Biol. Chem.* **258**:3523–3527 (1983).
36. Israel, D. I., and J. P. Whitlock, Jr. Induction of mRNA specific cytochrome P_1-450 in wild type and variant mouse hepatoma cells. *J. Biol. Chem.* **258**:10390–10394 (1983).
37. Bernhard, H. P., C. J. Darlington, and F. H. Ruddle. Expression of liver phenotypes in cultural mouse hepatoma cells: synthesis and secretion of serum albumin. *Dev. Biol.* **35**:83–96 (1974).
38. Okey, A. B., G. P. Bondy, M. E. Mason, D. W. Nebert, C. J. Foster-Gibson, J., Muncan, and M. J. Dufresne. Temperature-dependent cytosol-to-nucleus translocation of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in continuous cell culture lines. *J. Biol. Chem.* **255**:11415–11422 (1980).
39. Whitlock, J. P., Jr., and D. R. Galeazzi. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

receptors in wild type and variant mouse hepatoma cells. *J. Biol. Chem.* **259**:980–985 (1984).
40. Gehring, U., K. R. Yamamoto, and G. M. Tomkins. Complementation analysis of steroid hormone action. *Res. Steroids* **7**:43–48 (1977).
41. Pfahl, M., and S. Burgeois. Analysis of steroid resistance in lymphoid cell hybrids. *Somat. Cell Genet.* **6**:63–74 (1980).
42. Seyfried, T. N., R. K. Yu, and G. H. Glaser. Genetic analysis of audiogenic seizure susceptibility in C57BL/6J × DBA/2J recombinant inbred strains of mice. *Genetics* **94**:701–718 (1980).
43. Poland, A. and E. Glover. Variation in the molecular mass of the Ah receptor among vertebrate species and strains of rats. *Biochem. Biophys. Res. Commun.* **146**:1439–1449 (1987).

Send reprint requests to: Dr. Alan Poland, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706.