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Perspective

The Ah Receptor: Adaptive Metabolism, Ligand Diversity, and the Xenokine Model

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ABSTRACT: The Ah receptor (AHR) has been studied for almost five decades. Yet, we still have many important questions about its role in normal physiology and development. Moreover, we still do not fully understand how this protein mediates the adverse effects of a variety of environmental pollutants, such as the polycyclic aromatic hydrocarbons (PAHs), the chlorinated dibenzo-*p*-dioxins ("dioxins"), and many polyhalogenated biphenyls. To provide a platform for future research, we provide the historical underpinnings of our current state of knowledge about AHR signal transduction, identify a few areas of needed research, and then develop concepts such as adaptive metabolism, ligand structural diversity, and the importance of proligands in receptor activation. We finish with a discussion of the cognate physiological role of the AHR, our perspective on why this receptor is so highly conserved, and how we might think about its cognate ligands in the future.

CONTENTS

INTRODUCTION

The role of the Ah receptor (AHR) in human health and environmental toxicology continues to be an area of considerable interest. In this review, we provide a brief history of AHR research, our interpretation of recent discoveries, and our vision for the research path forward. Owing to the thousands of publications on this topic, we have attempted to provide our own perspective on the history of AHR discovery, current state of knowledge, and opportunities for further inquiry, rather than perform a comprehensive review. This approach was taken in an effort to provide a foundation for future research and present ideas designed to stimulate new

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scientific directions. In an effort toward clarity, we try to emphasize reviews and examples and have not attempted to generate an exhaustive review of the primary literature. Our rationale was that these citations will represent the complicated literature, alternative interpretations of the relevant science, and can serve as primary citations when further reading is of interest.

THE AH RECEPTOR

Historical Foundations. *Polycyclic Aromatic Hydrocarbons and Discovery of the Ah Locus.* Early indications for the existence of the AHR arose from studies designed to understand the metabolism of carcinogenic polycyclic aromatic hydrocarbons (PAHs), such as benzo[*a*]pyrene (BAP), 7,12-dimethylbenzanthracene (DMBA), and 3-methylcholanthrene (3MC) (Figure 1).[1,2] Compounds like these arise from

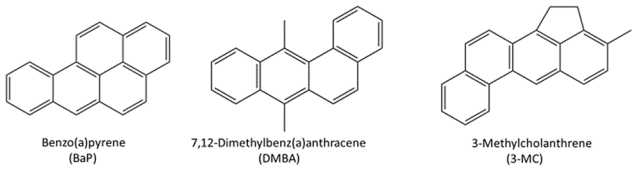

Figure 1. Investigations of carcinogenic PAHs led to discovery of the *Ah* locus. Studies using BAP, DMBA, and 3-MC provided early evidence for the existence of the AHR (see text for references).

combustion processes and are common contaminants in chimney soot, charbroiled foods, diesel exhaust, outdoor burns, cigarette smoke, and coal tar.[3−8]

In early carcinogen metabolism studies, the prominent enzymatic activity that oxidized PAHs to hydroxylated metabolites became known as "aryl hydrocarbon hydroxylase" or simply "AHH." From this work, three important observations arose.[9,10] First, AHH activity was the product of multiple cytochrome P450-dependent monooxygenases (P450s).[11] Second, AHH activity was significantly upregulated, or "induced", by prior exposure to a broad spectrum of these same PAHs.[12] Third, in mice, a single autosomal locus harbors significant control over induction sensitivity across inbred strains (i.e., some strains were more inducible than others).[13] The locus became commonly known as *Ah* for its role in regulating AHH activity and was formally renamed the "aryl hydrocarbon receptor" or *Ahr* locus in later years.[14,15] In addition to its importance as an early example of carcinogen metabolism regulation, the AHH system also became a widely studied model of mammalian enzyme induction and adaptive metabolism (i.e., exposure to a xenobiotic substrate inducing its metabolism).[16,17]

Dibenzo-p-dioxins and Discovery of the Ah Receptor (AHR). Additional evidence for the existence of an AHR arose from experiments designed to understand the mechanism of action of chlorinated dibenzo-*p*-dioxins and related environmental pollutants.[18] Chlorinated dioxins and the related chlorinated dibenzofurans have never seen commercial use but are commonly introduced into the environment as trace contaminants of many industrial processes, anthropogenic sources, and some natural processes (Figure 2 and Table 1). The structurally related coplanar polychlorinated biphenyls (PCBs) and coplanar polybrominated biphenyls (PBBs) have seen commercial production and are often introduced into the environment as the result of industrial accident or improper disposal. As a class, these compounds display similar environmental fates, are environmentally persistent, are lipophilic, bioaccumulate in the food chain, and they elicit similar biological responses dependent upon chlorination pattern.[19−21]

The dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is widely considered as the prototype for this class of environmental pollutant. Exposure to TCDD can lead to a broad spectrum of species-specific toxic effects, often referred to as the "dioxin toxic syndrome." This syndrome commonly includes epithelial hyperplasia/metaplasia, chloracne, porphyria, late-stage terata, lymphoid involution, intestinal damage, hepatocellular damage, and cancer.[18,22] Dioxins like TCDD are remarkably potent toxicants, with a median lethal dose (LD_{50}) that can be in the low μg/kg range in some animal models.[23,24] While dioxin toxicology has its research origins from an agricultural accident in poultry in the late 1950s,[25] subsequent human exposures resulting from numerous pollution sources and environmental accidents, as well as its presence in the Vietnam War defoliant known as "Agent Orange", sparked a modern effort to understand its mechanism of toxic action (Table 1).[26,27,2829] Despite the popular, regulatory, and scientific concern that has been in place for decades, halogenated dioxins, dibenzofurans, and biphenyls can still be found in human blood samples and in ecosystems around the globe.[19,30−33]

It was through investigations into the toxic action of TCDD that we learned that the *Ahr* locus encodes a receptor. This conclusion arose from four observations.[18,34−36] First, TCDD induced the same P450s and related enzymes as did BAP, DMBA, and 3MC but with much greater potency.[37] Second, radiolabeled dioxin analogues bound to a high affinity, low-capacity soluble protein site, designated as a receptor in target tissues.[35] Third, binding affinity for this "receptor" site segregated with the high and low responsiveness (inducibility) phenotype observed across the C57BL/6 ("responsive") and DBA/2 ("nonresponsive") mouse strains.[35,38] Fourth, the rank order potency for a given ligand's potency to induce AHH activity (or many aspects of toxicity) corresponds to its rank order potency for receptor binding affinity. In sum, the early

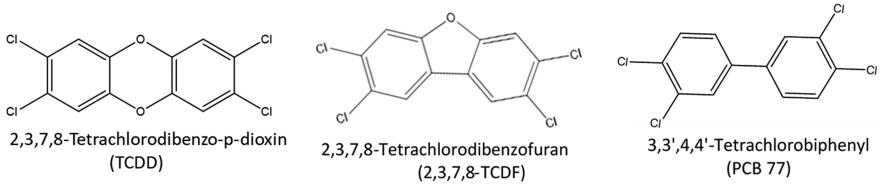

Figure 2. Further elucidation of the Ah locus arose from toxicity studies using the toxicant TCDD. TCDD, 2,3,7,8-TCDF (tetrachlorodibenzofuran), and PCB 77 (polychlorinated biphenyl) induce the same P450s and related enzymes as did BAP, DMBA, and 3-MC. TCDD has higher affinity for AHR and thus has greater potency, making TCDD a model inducer of AHR signaling (see text for details).

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Table 1. Well-Known Accidents/Exposures to Dioxin and Dioxin-Like Compounds

location	contamination source	route of exposure	human health implications	example reference
Belgium	transformer oil in fat of animal feed	poultry, pork, beef, milk, eggs, and various fat-containing food items	50 kg of PCBs and 1 g of "dioxin" were contaminated in 500 tons of animal feed and distributed to farms in Belgium and on some nearby countries. 20−30% of these contaminants were estimated to have been consumed by the Belgian population.	[271]
Ireland	fuel from drying system used during animal feed production	cattle, pork meat, and pork products	Significant health concerns by public. Well-documented case of source and movement of dioxin and related compounds through commerce.	[272, 273]
Seveso, Italy	chemical factory accident: uncontrolled exothermic reaction during the manufacturing of trichlorophenol	air and soil	Immediate effects from cloud deposition: nausea, headache, skin lesions, and eye irritation. Long-term effects: Chloracne, increased incidence of diabetes, cancer, and mortality from cardiovascular and respiratory disease.	[274, 275]
Vietnam, Laos, and Cambodia	Agent Orange, Agent Pink, Agent Green, Agent Purple, Agent White, and Agent Blue: crude phenoxyacetic acid herbicides	air, soil, surrounding waters, and ingestion (legacy through hand-to-mouth contact)	Found association between dioxin exposure and soft tissue sarcoma, non-Hodgkin's Lymphoma, chronic lymphocytic leukemia, Hodgkin's lymphoma, chloracne, hypertension, and monoclonal gammopathy. Movement of dioxins into the human food chain and human tissues and biological fluids decades after use.	[276, 277]
Times Beach, Missouri	waste oil used for dust control	air and soil	Greater than 100 ppb in community. Human, wildlife, and livestock toxicity reported.	[278, 279]

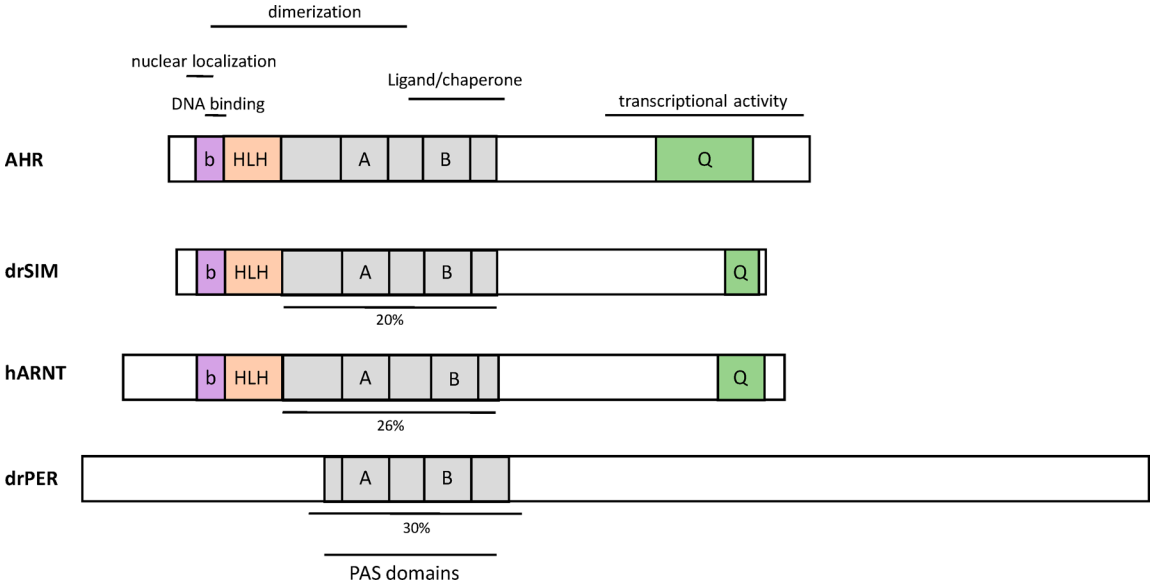

Figure 3. Mapping of AHR and ARNT functional domains and founding bHLH-PAS family members. The bHLH domain and N-terminus provide recognition of target DNA enhancers. The PAS domains control dimerization strength and selectivity, receptor repression, chaperone interactions, and ligand binding. Approximation of those domains for AHR are depicted as lines above. The C-terminus provides possible docking sites for coactivators.[53−60,63,64,66−69,71,73,74,76,78,79,90,130,280−282] Also see text for further details.

investigations into the genetics of PAH metabolism and the mechanism of dioxin's toxic mechanism converged to reveal a soluble receptor known as the AHR, encoded by the *Ahr* locus, and proved that this receptor mediates many of the biological effects of these environmentally important pollutants.

Biochemical and Molecular Characterization of the Ah Receptor (AHR). *Early Molecular and Biochemical Insights.* A better understanding of AHR signal transduction arose from a long history of pharmacologic and molecular studies of the regulatory elements of the genes encoding the induced enzymes that comprised AHH activity.[34,39−41] Although a bit more complex, we now know that AHH and PAH metabolism can be considered the composite activity of multiple genetic loci, including, *Cyp1a1*, *Cyp1a2*, and *Cyp1b1*. Each of the Cyp1 gene products encodes a member of the cytochrome P450-dependent monooxygenase family with metabolic activity toward PAH substrates.[9,11,42−45]

The genomic elements controlling the ligand-activated AHR-dependent induction were given multiple names over the years, including xenobiotic responsive elements (XREs), dioxin responsive elements (DREs), and AHR responsive elements (AHREs, which we will use here).[41,42,46] The discovery and characterization of these genomic enhancers were initially based mostly on studies of *Cyp1a1* regulation. Of particular importance were the observations that the enhancers controlling AHR-mediated upregulation of *Cyp1* genes commonly harbored consensus sequences of 5′-T/GNGCGTGA/C-3′. For *Cyp1a1* and many other inducible genes, these elements often existed in multiple copies proximal and 5′ to the transcriptional start site of the target promoter.[44,47−49]

Ah Receptor Nuclear Translocator. A significant step in developing a basic model of AHR signal transduction came from the molecular cloning of the AHR and its dimerization partner, the Ah Receptor Nuclear Translocator (ARNT).[50−52] These cloning experiments revealed that both the AHR and ARNT were structurally related, heterodimeric partners, harboring both basic helix−loop−helix (bHLH) and PER-

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ARNT-SIM (PAS) homology domains within their N-terminal halves[53,54] (Figure 3). The bHLH domain occurs in metazoan transcriptional regulators and commonly provides both a dimerization surface and an α-helix that interacts with specific sequences in the major and minor grooves of DNA.[53,55−59] The PAS homology domain was named based on the similarity between amino acid sequences within ARNT and the products of two regulatory loci found in *Drosophila melanogaster*, PER and SIM (products of the *per* and *sim* loci, respectively).[53] In addition to these two fruit fly gene products, PAS domains occur in a number of important mammalian regulatory proteins, including the "hypoxia-inducible factors" (HIFs) important in physiological adaptation to low oxygen and "clock" proteins central to the maintenance of circadian rhythms.[60] Importantly, PAS domains have evolutionary roots in prokaryotic and plant systems, where parallel domains also play a role in environmental adaptation to stimuli such as light and oxygen[61]

Functional Domain Maps. The importance of the bHLH-PAS region in dimerization and DNA binding is provided by numerous functional mapping studies in both the AHR and ARNT.[59,62−64] Like many bHLH proteins, the AHR and ARNT employ this domain as a dimerization surface and use the basic N-terminal helix to provide recognition of target DNA enhancers, with each basic region laying within a "half-site" of the AHRE (e.g., TNGC or GTG).[56−58,60,65−67] The functional role of the PAS domain can be thought of in the context of its two degenerate repeats or subdomains, referred to as PAS-A and PAS-B. The PAS-A domain plays a significant role in supporting the dimerization that drives DNA binding selectivity. In contrast, the PAS-B domain is important in dimerization but also harbors domains for receptor stabilization, receptor repression, chaperone interactions, and ligand binding.[60,62,68−72]

Although the C-terminal halves of the AHR and ARNT are highly divergent at the sequence level, this region appears to harbor domains of similar function in the two proteins. Studies employing fusions of this region with heterologous DNA binding domains reveal that potent transcriptional activation domains (TADs) reside within the C-terminal halves of these proteins, overlapping with glutamine-rich or highly acidic and disordered regions.[73−77] In more recent years, additional docking domains for some coactivators map to the bHLH-PAS domains of both the AHR and ARNT[78] (Table 2). While our understanding of coactivator associations is still nascent, the AHR−ARNT-dimer-mediated transcription of target genes such as *Cyp1a1* appears to involve, at least in part, classical chromatin modifications and recruitment of members of the transcription initiation complex.[78,79]

Adaptive Metabolism Pathway. *Model for Adaptive Metabolism.* As the result of the first 50 years of investigation into the AHR, we have a working model of the functional domains and signaling steps that regulate the expression of the xenobiotic-metabolizing enzymes such as CYP1A1 (Figure 4).[12,36,46,47,57,72,80] Through the use of molecular reagents from cloned AHR and ARNT, mutant hepatoma cell lines, immunochemical tools for localization and precipitation, and high-affinity radioligands, the importance of subcellular localization, chaperones, and the ordering of signaling steps for upregulation of genes is becoming clearer.[34,36] The most common description of AHR signaling as it relates to *CYP1A1* gene induction is as follows: In the absence of an inducing ligand, the AHR protein resides predominantly in the cell's cytoplasm in a complex with a number of chaperones, including a dimer of the 90 kDa heat shock protein (Hsp90) and smaller chaperones known as the AHR interacting protein (AIP, also known as ARA9 or XAP2) and the P23 protein.[81−88] Upon the binding of ligand to the AHR, a conformational change in the receptor leads to a reorganization of chaperones and allows presentation of the NLS in the AHR's N-terminus. Translocation of the AHR to the nuclear compartment then allows dimerization with its nuclear partner, ARNT.[57,83,86] The AHR−ARNT dimer produces a competent DNA binding dimer with specificity for AHREs within chromatin and activation of nearby target promoters such as that for *Cyp1a1*. While much is still to be learned about this final transcriptional step, the AHR−ARNT dimer has been shown to increase promoter accessibility and alter chromatin structure through association with numerous known coactivators[89−97] (see examples in Table 2).

Pathway Feedback Inhibition. One intriguing observation about AHR signal transduction is that several mechanisms exist to downregulate this signaling. Primary evidence for the

Table 2. Examples of Some Coactivators That Have Been Shown to Associate with AHR or Its Complex[a]

coactivator	reference	notes
BRCA-1	[283, 284]	interaction with both AHR and ARNT
BRG-1	[285, 286]	interaction with AHR, enhances complex activity
CARM-1	[287]	interaction with AHR
CoCoA	[288]	interaction with both AHR and ARNT
COUP-TF1	[289]	interaction with AHR and not ARNT
ERα	[289]	interaction with AHR and not ARNT
ERRα	[289]	interaction with AHR and not ARNT
ERAP140	[290]	interaction with AHR−ARNT complex
GAC63 (GRIP1)	[291]	interaction with AHR
Mediator	[96]	interaction with AHR−ARNT complex
P160 (NcoA-1-3)	[116]	interaction with both AHR and ARNT
NcoA-4	[92]	interaction with both AHR and ARNT
P300	[116, 292, 293]	interaction with both AHR and ARNT
PGC-1	[287]	interaction with AHR
RB	[294]	interaction with AHR
RIP140	[295, 296]	interaction with AHR, cross talk with ERα
SHP	[297]	interaction with ARNT and not AHR
SMRT	[290, 298]	interaction with AHR−ARNT complex and AHR
SRC1 (NcoA-1)	[282, 287]	interaction with AHR Q-rich region
SRC2 (NcoA-2)	[287]	interaction with AHR
SRC3 (NcoA-3)	[287]	interaction with AHR
TAF4	[282]	interaction with AHR Q-rich region
TAF6	[282]	interaction with AHR Q-rich region
TBP	[282]	interaction with AHR Q-rich region
TIF2	[282]	interaction with AHR Q-rich region
TRAP220	[287]	interaction with AHR
TRIP230	[299]	interaction with ARNT
SIN3A	[300]	enhances complex activity

[a]This table of examples was generated by a cross reference of the topics, "Ah receptor" and "Coactivator" in the "Web of Science" search engine of scientific publications, apps.webofknowledge.com (8/1/2019). It was then supplemented with information found in two reports on the topic.[78,116] Clear, alternative names of coactivators are given in parentheses. The table is meant to represent the diversity of known AHR−coactivator interactions and is not intended to be an exhaustive list.

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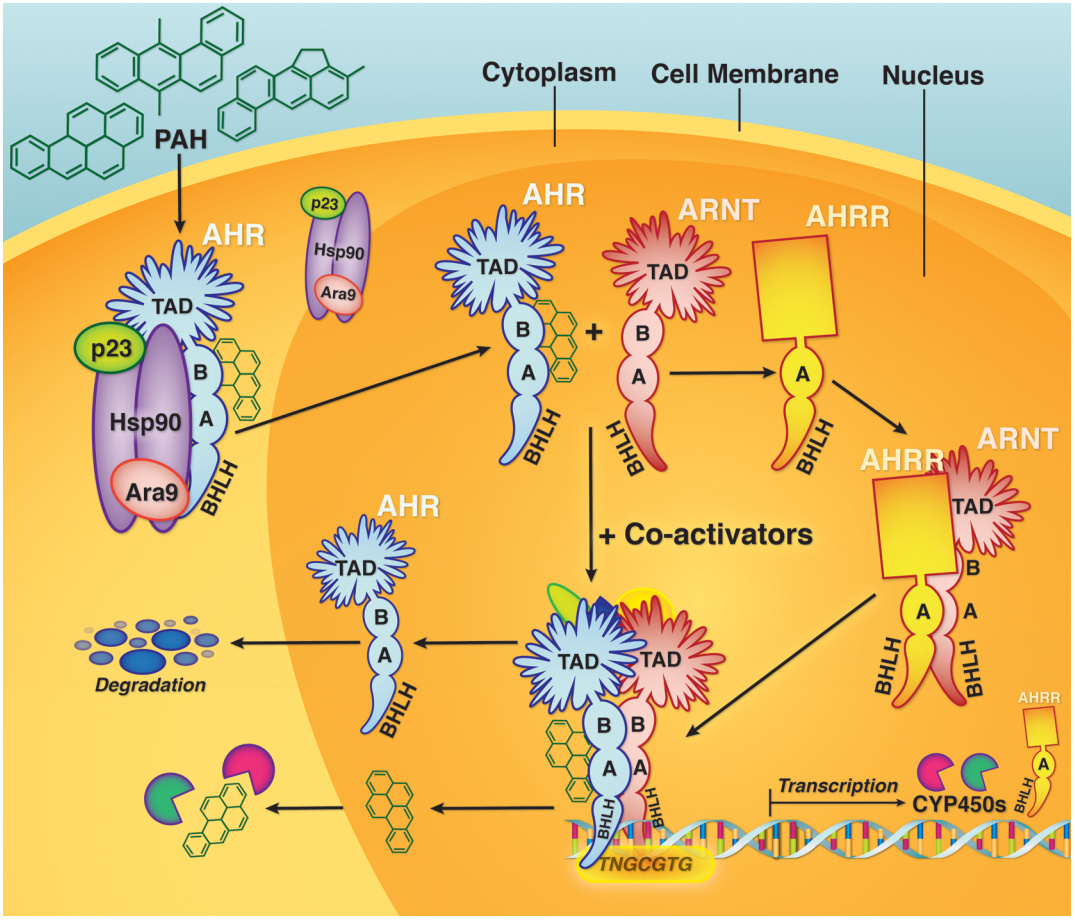

Figure 4. Classic AHR signaling pathway. Prior to ligand binding, AHR remains in cytosol bound to HSP90, P23, and ARA9. When a ligand binds, a conformational change occurs, exposing the nuclear localization sequence (NLS) in AHR's N-terminus. Presentation of NLS permits the translocation of AHR to the nucleus and subsequent dimerization with ARNT. The AHR−ARNT heterodimer recognizes and binds to AHREs in the genome and initiates transcription of select genes. This interaction can be inhibited by AHRR, CYP1 metabolism of ligands, and post-translational modification of the receptor.[12,34,36,46,47,57,72,80−88,90−92,95,96,290,293,296,301]

importance of feedback inhibition comes from one of the more recently discovered targets of the ligand-activated AHR−ARNT complex, an additional bHLH-PAS protein known as the Ah receptor repressor (AHRR).[98,99] The AHRR not only dimerizes with ARNT and competes for AHRE occupancy but also inhibits AHRE-mediated transcription by influencing the chromatin structure around the promoters of *CYP1A1* and presumably related AHRE-driven genes.[100,101] In addition to this upregulated repressor activity, the AHR also appears to be the target of multiple additional downregulators.[102] Not only has the ligand-activated AHR been shown to be constitutively degraded by ubiquitination and proteasomal degradation,[103−105] one of its AHRE-driven target genes, *Tiparp*, may ADP-ribosylate the AHR, reducing its activity and half-life.[106,107]

The existence of AHRE regulated genes such as AHRR and TIPARP provides support for an additional perspective on AHR signaling. While we classically think of this system as a pathway to adapt to PAH molecules generated exogenously or endogenously, it is also interesting to think of the *CYP1A1/CYP1A2/CYP1B1* gene targets as additional participants in a negative feedback loop. That is, activation of the AHR by PAHs (and other ligands described below) leads to the upregulation of CYP1 monooxygenases and their consequent metabolic degradation and excretion of inducing ligands. This would appear to represent a classic substrate inducing its own metabolism in a feedback loop.[12,46,47] These observations lead to the question: why is so much biology directed toward AHR downregulation and attenuation of signaling? Possible answers are that the AHR is part of a biological response that must be rapidly attenuated to avoid pathological consequences or that it is part of a chronic response that must be precisely modulated over time (see below).

More to Be Learned about Functional Domains and Signaling. It is important to note that this current description of the AHR domain map and signal transduction is almost certainly an oversimplification, with issues such as the importance of receptor phosphorylation and the events dictating receptor transformation still unclear.[108−111] Similarly, while we have learned a great deal about the bHLH domain, much is still to be learned about the N-terminal half of the AHR. While the bHLH tail is thought to harbor both DNA recognition and nuclear localization sequences (NLS), this region also appears to play additional roles in receptor signaling. In one example of this idea, this same region harbors a nuclear export sequence (NES), which influences receptor subcellular localization, and also a motif for cellular chaperone interaction, which can influence receptor concentration and transformation.[71,112−114] Finally, our understanding of how this domain interacts with the genome is probably also

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incomplete. This conclusion is supported by the identification of noncanonical enhancer target sites that are in addition to classical AHREs as defined above.[115]

Similarly, our understanding of the C-terminal halves of these proteins and the transactivation events they mediate is also limited, with the role of specific coactivators in distinct cellular responses still to be determined and the importance of this domain in receptor transformation still unclear. An improved understanding of the multiple coactivator interactions and insights into their combinatorial and dynamic nature will be important if we are to explain the wide variety of cell-, species-, and ligand-specific responses induced by AHR agonists and antagonists.[90,95,115,106,117] In this regard, many of the species-, tissue-, and ligand-dependent effects of AHR agonists may be due to unique consequences of specific coactivator recruitment within a given cellular environment (in addition to differences in ligand-binding affinity/specificity). Moreover, ligand-dependent recruitment of specific coactivators could underlie the unique pharmacology of distinct agonist classes.

Dioxin-Like Compound Concept. An early observation that has greatly influenced our thinking is that while most AHR agonists can achieve similar efficacy with respect to upregulation of AHH activity (i.e., CYP1A1, CYP1A2, and/or CYP1B1), only the most potent and metabolically recalcitrant (i.e., long $T_{1/2}$) ligands induce the "dioxin toxic syndrome." Weaker agonists, such as the PAHs and variety of natural ligands, which are rapidly metabolized and have lower potency, appear to upregulate the CYP 1s but do not induce chloracne and so on. The model proposed to explain this phenomena is that there is a "restricted pleiotropic response", in addition to the upregulation of genes such as *CYP1A1*, which is induced by longer-lived, pharmacologically unique agonists, and this response is required for the dioxin toxic syndrome.[18] In fact, certain end points like CYP1A1 induction (i.e., adaptive metabolism) can occur in response to a broad spectrum of ligands, whereas end points of the dioxin toxic syndrome appear to require receptor activation by compounds with "dioxin-like" pharmacological properties. As a heuristic, chlorinated dioxins, dibenzofurans, and biphenyls with halogens in lateral positions show the greatest potential to induce the dioxin toxic syndrome and are therefore often designated as "dioxin-like compounds" (DLCs) (Figure 5).[20]

The idea that DLCs elicit effects that are distinct from other classes of receptor ligands (like the PAHs) has regulatory implications. Agencies such as the World Health Organization (WHO) that direct global health efforts and agencies like the Environmental Protection Agency (EPA) that govern chemical releases within the United States employ the principle that compounds of environmental concern that are structurally and pharmacologically related to TCDD and that elicit toxicity through a common mechanism are formally designated as DLCs, and their exposures and release are regulated concordantly.[20] The principle used is that these compounds are assessed for their relative effect potencies (REP) from dose–response assessments for a pathological end point associated with AHR activation and toxicity. This information is used to generate toxic equivalency factors (TEFs), which are weighted measures that reflect the relative potencies of a pollutant of concern as compared to TCDD (Figure 5).[23,118–123] Currently, TEFs are applied to 29 compounds of environmental concern, 7 polychlorinated dibenzo-*p*-dioxins, 10 polychlorinated dibenzofurans, and 12 polychlorinated biphenyls. The advantage of this approach is that it provides a measure of toxicity from complex mixtures of chlorinated dioxins, dibenzofurans, and biphenyls, which are common in human exposure scenarios.

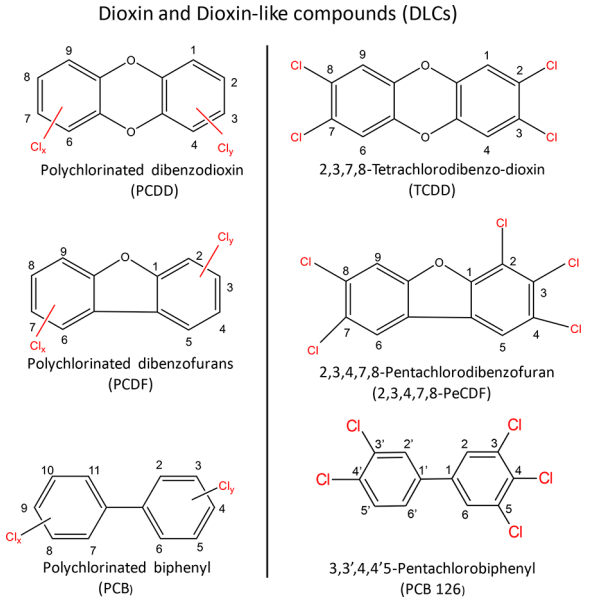

Figure 5. Dioxin-like compound concept and approach to measuring human exposure to mixtures. Toxic equivalency factors (TEFs) are weighted measures that reflect the relative potencies of pollutants of concern as compared to TCDD. Toxic equivalents (TEQs) are reported values used for risk characterization and management (see text for details). Left: Structures of the three classes of chlorinated DLCs. Right: Examples of three formally designated DLCs. To calculate TEQ, the mass of each chemical in a mixture is multiplied by its TEF and summed.[23,118,119121,123,208,302,303]

Insights from Naturally Occurring Structural Diversity in the Ah Receptor. *Genetic Variation/Polymorphism.* Early evidence indicates that the AHR was functionally and structurally variable both within and across species. Support for this idea arose from the observation that murine *Ahr* polymorphisms lead to differential induction of P450s across strains.[35,42] Further, examination of additional animal species, including hamster, guinea pig, rat, dog, and human, revealed significant differences in sensitivity and response to dioxins.[22,124,125] This idea of receptor diversity gained further support with the development of antibodies and photoaffinity radioligands that revealed biochemical differences in AHR both across and within model species.[126–129] The molecular cloning of the AHR cDNAs from multiple animal species revealed important codon polymorphisms in the *Ahr* gene that, when paired with radioligand-binding experiments and immunochemistry, led to the identification of codons that influence receptor size and ligand-binding affinity (see below).[51,52,130,131]

Molecular Insights from the Structural Gene. The AHR structural gene, *Ahr*, resides on mouse chromosome 12 or on a highly syntenic region on human chromosome 7.[132–135] Comparison of the structural genes and cDNAs from mouse and human indicates that the open reading frame is encoded by 11 exons with highly conserved intron–exon boundaries across species. A comparison of these genes reveals that alternate termination codons for the open reading frame in exon 11 explain much of the receptor size differences observed within and across species. This molecular information indicates

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that the receptor open reading frame extends further in some species (e.g., human and rat) and much less in others (e.g., the C57BL/6J mouse). This leaves some proteins with longer C-termini than others and explains how the AHR can be as small as 97 kDa in the C57 mouse and as large as 105 kDa in the human or 124 kDa in the hamster.[127,129,131,134,136]

Molecular Insights from the Mouse Model. The mouse is an important animal model for the study of the AHR and its signaling pathways. The initial mouse "responsiveness" polymorphism was explained through the comparison of the AHR cDNAs derived from responsive (Ahr^b allele) and the less responsive strains (Ahr^d allele). These experiments revealed that there were numerous polymorphisms between the Ahr^b and Ahr^d alleles.[137−139] Among these is a polymorphism in the stop codon, resulting in an additional 43 amino acids in the carboxyl terminus of the AHR^d receptor as compared to the AHR^{b1} (identical to the cause of the cross-species differences described above). Interestingly, two additional responsive alleles were characterized (named Ahr^{b2} and Ahr^{b3}),[140] one of which (Ahr^{b2}) closely resembles the Ahr^d allele in all but three amino acids and includes an identical elongated c-terminal tail. Using ligand binding of expressed polymorphic proteins, it was concluded that a primary driver of the ligand-binding affinity was residue 375, where an alanine (A) confers higher affinity ligand binding and greater responsiveness in mice harboring the b1, b2, and b3 alleles. In contrast, in Ahr^d mice, a valine (V) at this position confers lower affinity binding and decreased responsiveness.[138,139] Through investigations into other residues, it was also observed that the elongated C-terminal tail found in Ahr^{b2} and Ahr^d mice may reduce ligand binding slightly as compared to Ahr^{b1}.[139] It remains unclear the extent the C-terminal half plays in ligand binding, as this region also alters receptor stability and thus perhaps cellular concentration.

Predictions of AHR structure have been modeled using receptor homology data from other PAS family proteins, such as HIF-2α. These analyses support the importance of residue 375 in ligand binding as well as the influence of alanine and valine at this position.[68,141−144] That is, the valine at 375 encoded by the Ahr^d allele is bulkier and hypothesized to have repulsive properties toward the ligand while also altering the adjacent hydrogen bond network. Interestingly, the human harbors a valine residue at this position, and this may be better modeled by "humanized" or the AHR^d models.[145]

Molecular Insights from the Rat Model. The rat has also served as a powerful early model of AHR biology. This utility arose from the classical use of this model as a tool in toxicology, its sensitivity to TCDD induced carcinogenicity,[146] and the existence of an informative polymorphism in the receptor that influences a strain's responsiveness to agonist.[124] Similar to the mouse, some rat strains are resistant (Han−Wistar, HW, 98 kDa), while others are sensitive (e.g., Long−Evans, LE, or Sprague−Dawley, SD, 106 kDa) to the toxic and inductive effects of ligands like TCDD. Through molecular analysis of the cDNAs and structural genes of these AHR open reading frames, it is now known that the explanation for reduced signaling by the HW receptor is due to a variation at the splice junction at exon−intron 10. While multiple consequences of this altered splice junction can occur, this polymorphism commonly leads to a truncation of the C-terminal end of the HW-AHR, yielding as many as two novel protein products possible.[147] Physicochemical studies indicate that this truncation reduces receptor concentration, possibly due to influences on receptor stability or the potency of the nearby transcriptionally active domains.[147,148] This naturally occurring receptor polymorphism in the rat provides considerable evidence for the role of the receptor's C-terminus in AHR signaling and dioxin toxicity. An additional note is the observation that while HW rats are resistant to many of the acute toxic effects of high-dose dioxin exposure, they display similar dose−response curve for end points such as CYP1A1 induction. Such a result would seem to be an indication that classes of AHR-mediated biological/toxicological responses exist, some of which require less receptor activation than others.[149,150] Such an observation is in keeping with the restricted pleiotropic model described above.[18]

What Is the Normal Physiological Role of the Ah Receptor? While the toxicology of PAHs and dioxins led to the discovery of the AHR as well as the discovery of the AHR's roles in regulating xenobiotic metabolism, many significant questions remain regarding the role of this receptor in normal physiology. Perhaps one of the most important questions is why this receptor exists in such a wide range of animal species and in such a broad array of tissues and cell types? Early research focused on the concept that the receptor was part of a system that evolved to allow metabolic adaptation to xenobiotics, especially PAHs, which have existed on the earth for millennia due to natural processes such as fires and volcanic activity.[151] Parallel thinking suggests that the AHR evolved as an allelopathic defense system, similar to those systems reducing exposures to lipophilic natural products that display toxicity when levels rise in an organism.[152,153] While these ideas are all probably correct in some form, it is also probable that this is not the only physiological role of the AHR nor are they the primary reason for its evolutionary conservation (see below).

Lessons from Tissue and Cellular Expression. One common approach used to deduce the physiological role of a gene product is to determine where and when the protein is expressed in an organism. This method relies on the premise that tissue-specific or developmental expression will highlight the relevant biological system. This approach has been used to understand AHR biology and includes studies based upon ligand binding, antibodies, and RNA analysis to report receptor expression at the organ and tissue level.[127,130,154−156] These early studies are now complemented by high-throughput gene expression resources such as BioGPS, ENCODE, and The Human Protein Atlas.[157−159]

While the interpretation of the collective data from the above sources is complex, a few important observations are noteworthy. At the organ level, the AHR is expressed at many sites, with the placenta expressing the highest levels of the AHR mRNA in the human.[130] The human lung is also a highly expressing tissue in almost all reported studies and databases, with levels in liver and bladder/urinary tract also reproducibly high. In contrast to humans, in the mouse and rat, the lung is typically the highest expressing organ and the placenta is much lower. While issues such as gestation day may play a role in this reported cross-species difference, it is notable that the human placenta is physiologically distinct from rodent placentas.[160] We draw two conclusions from these observations. The first is that the AHR is most highly expressed at tissues that represent important oxygen interfaces (lung and placenta). The second is that if the AHR is important in human placental biology, current animal models may significantly misrepresent this important physiology.

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Predictions about endogenous function based on higher resolution and temporal expression data (i.e., immunohistochemistry and *in situ* hybridization techniques) are difficult to simplify, because these studies describe AHR expression in a remarkable array of cellular compartments and developmental times. For example, in the E13.5 day embryo, the AHR is highly expressed in the primitive pituitary, nasal septal cartilage, dorsal surface of the tongue, developing thymus lung parenchyma, liver, mucosa of the developing gut, urogenital sinus, and genital tubercle.[161] A parallel analysis of the CNS indicates that AHR and ARNT are coexpressed in regions of the hypothalamus and brainstem associated with appetite and circadian regulation, and it is also highly expressed in cardiac and skeletal muscle and epithelial regions associated with epithelial to mesenchymal transitions.[162−164] Adding to this diversity, are reports of AHR expression in rabbit morula and blastocysts, human pancreatic ductal and acinar cells, immune cells of the intestinal stroma and ovarian granulosa cells.[165−168] Given that this is only a small list of unique sites of AHR expression, it seems likely that this receptor will be shown to have more than one significant role in normal animal physiology and development.

Lessons from Ahr Null Rodents. Another method used to identify putative physiological roles for the AHR is to create mammalian models that are null for the *Ahr* gene product and assess the consequences of that null allele on the host's biology. Generation of the null allele has been performed by at least three independent laboratories for the mouse model and at least once in the rat model.[169,170,170,171,172] In rodent models, the *Ahr* null allele has again provided evidence that the AHR regulates multiple developmental and physiological processes. In this regard, *Ahr* null mice have been reported to display a number of phenotypes, including patent *ductus venosus*, hepatic atrophy, altered immunity, vascular defects, decreased barrier integrity of the skin and gut, and reduced reproductive capacity.[12,161,165,173−182] While initial reports of variously generated mouse null alleles appear to display some discordance, there is little evidence to indicate that any differences are allelic; it is more likely they are due to genetic background issues, unique pathogen loads, and different dietary regimens.[175]

Interestingly, the rat null model displays a phenotype that is distinct from the mouse, with pathological alterations primarily in the urinary tract and kidney and no reported hepatovascular pathology (i.e., patent *ductus venosus*) which is a hallmark mouse phenotype studied in our laboratory.[172] Moreover, while immune effects in the mouse have predominantly been studied for adaptive immunity and T-lymphocyte biology, the effects in the rat have been reported primarily for B-lymphocyte function.[183−185] Taken in sum, these data strongly support a role for the AHR in normal biology, with initial indications of an important role for this receptor in barrier integrity, immunity, reproduction, vascular development, as well as hepatic and renal biology.[165,183,186−188] Additionally, these cross-laboratory and cross-species studies indicate that experimental environment and genetic background are likely to have a marked influence on AHR null phenotypes and AHR biology writ large.

Lessons from Evolution. Another strategy to elucidate the physiological role of the AHR is to study its evolution. Such an approach anticipates that certain correlates might explain the selective pressures that led to the receptor's emergence and maintenance in biological systems. The AHR, ARNT, and AHRR are members of the bHLH-PAS family of transcription factors, which arose early in evolution, with PAS domains having been found in plants, animals, and bacteria.[61,189] Domains reminiscent of PAS domains are found in prokaryotes, where they play roles in phototropism and oxygen sensing, and in plants, where they are involved in photoreception and phototransduction.[190]

Diversification of the PAS gene family occurred early in evolution. All of the major bHLH-PAS gene subfamilies (e.g., AHR, ARNT, HIF, SIM, CLOCK, TRH, BMAL, NCOA, NPAS4) are shared by protostomes and deuterostomes and thus must have been present already in the ancestral bilaterian animal, which lived ∼570 million years ago.[191,192] Metazoan PAS domain-containing proteins play roles in a variety of signal transduction pathways, many of which are involved in developmental processes and environmental adaptation.[193−195] Additional diversification of bHLH-PAS genes occurred early in the vertebrate lineage as a result of two whole-genome duplications,[196] leading to the multiple paralogues (ohnologues) within each subfamily that exist in most vertebrates, including mammals (e.g., three HIF genes, two CLOCK genes).

The AHR genes have undergone duplication and diversification like other bHLH-PAS genes, involving both whole-genome duplications as well as a tandem duplication event. The presence of multiple AHR genes in both bony and cartilaginous fishes suggests that the AHR gene duplications occurred early in vertebrate evolution, well before the emergence of mammals.[197,198] A tandem duplication produced the genes now known as AHR1 and AHR2, which occur in fish, birds, reptiles, and some early diverging mammals but have been lost from most later mammalian groups. Another duplication event produced AHRR, which evolved as a transcriptional repressor of AHR function.[99,199] In mammals, AHRR may exhibit additional regulatory interactions besides repressing AHR activity, consistent with data demonstrating that human AHRR can have multiple effects on cell growth and differentiation.[199] Overall, phylogenetic and comparative genomic analyses suggest that there are five groups (clades) in the AHR subfamily: AHR, AHR1, AHR2, AHR3, and AHRR, which exhibit gene- and taxon-specific functional specialization.[198]

Functional analyses of AHRs from extant vertebrate and invertebrate species suggest that the ability to bind to planar aromatic compounds such as PAHs and dioxins evolved in early vertebrates. It has been hypothesized that one selective force may have been the need to detoxify halogenated aromatic natural products, which are prominent in the marine environment, where early vertebrates arose.[200,201] Although AHR homologues from invertebrate species appear to lack the ability to bind PAHs and dioxins, it is unknown if they can be activated by other types of ligands.

While evolutionary information does not point us toward a clear physiological role for the AHR, some intriguing observations stand out from this analytical approach. First, PAS domains have a propensity to exist in sensor proteins of environmental stimuli such as light and oxygen tension.[60,190,202] This role seems to have evolved early (prokaryotes and plants) and been maintained throughout millions of years of evolution. The AHR's role as a chemical sensor is consistent with this idea. Second, the AHR, as defined by phylogenetic analysis (orthology) within the bHLH-PAS family, has been found in almost all eumetazoan groups.[198]

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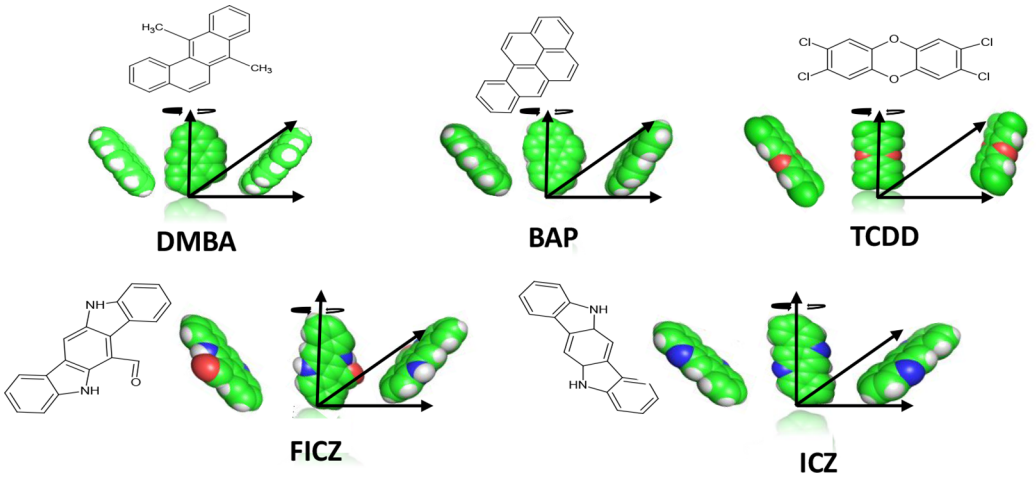

Figure 6. The flat hydrophobic rectangle model of AHR ligands. Molecular and ball and stick models of some AHR ligands discussed in this review that conform to the FHR concept of ligand structure. The three-dimensional structures are only provided as approximations, as some subtle bending and puckering of structure may occur that is not predicted by common algorithms. Figure was generated with ChemDraw software.

This suggests that whatever evolutionary pressures have led to the maintenance of this gene, they have existed for millennia. Third, there may be a common thread that unites AHR function across the metazoan: such as a role in controlling cell fate during the development of neural systems and, in particular, sensory structures. For example, in the cnidarian *Nematostella*, AHR is expressed in the apical tuft (a sensory structure).[192] In arthropods (e.g., *Drosophila*), AHR controls the development of the distal segment of the antenna (a chemosensory structure), mechanosensory bristles, and photoreceptors.[203,204] In nematodes (e.g., *C. elegans*), AHR controls the development of touch receptor neurons and sensory neurons that contact the pseudocoelomic fluid.[205] Emerging evidence for a role of AHR in neural development in mammals suggests this could be one possible conserved role shared by all animals.[198,206] Despite these intriguing findings, it will remain a challenge to identify conserved physiological roles of AHRs and to distinguish them from novel functions that evolved in specific taxonomic groups.

Diversity of AHR Ligands. *Structure–Activity Relationships.* Early structure–activity relationship (SAR) analysis based on various halogenated aromatic hydrocarbons (HAHs), PAHs, and related compounds, suggested that the AHR ligand-binding pocket binds near-planar ligands with dimensions that approximated a 3 × 10 Å (Å) rectangle.[18,207] More recent analyses based, in part, on structure–activity studies and on structural similarity to crystallized domains of other PAS proteins, support the idea that absolute planarity is not a requirement for receptor binding and that maximal dimensions of the ligand-binding pocket may be more closely approximated by a pocket of 14 × 12 × 5 Å (reviewed in ref 153). It has also been observed that both the hydrophobicity and the polarizability of a compound's substituents add an additional layer of complexity in regards to affinity for AHR.[153,208−212] While current structural models are useful, a solved binding pocket structure through X-ray crystallography or NMR is needed if we are to confidently predict chemical binding to the AHR and anticipate the biological effects that emerging environmental pollutants and therapeutics will induce. In the meantime, those of us with limited expertise in physical chemistry are left with a preliminary "flat hydrophobic rectangle" (FHR) model as a predictor of AHR ligand-binding activity (Figure 6).

Several reviews have provided a comprehensive description of the structural diversity of AHR ligands and sources.[24,153,208,213] While structural classification of AHR ligands based upon chemical backbone is useful (dioxins, biphenyls, PAHs, flavonoids, etc.), it is also useful to think of these compounds based upon nonstructural properties, such as source, risk for human exposure, receptor binding affinity, and biological half-life. In this regard, ligands coming from anthropogenic sources such as diesel exhaust, commercial production, or industrial contamination (PAHs, PCBs, and dioxins), are produced as natural products, or they are generated endogenously in human tissues (indigoids, indolocarbazoles, etc.). For many of these source classes, member ligands display EC_{50} values or binding affinities for the AHR that differ by multiple orders of magnitude. Ligands from these source classes can also harbor markedly different biological half-lives that span from hours to months.[214−216]

Importance of Proligands. An important concept to consider is that many compounds that are thought to activate the AHR are not actual ligands of the AHR but are proligands. Proligands are precursors that are chemically transformed to the ultimate ligand, which strongly binds to the AHR pocket. Proligands typically form the ultimate ligands via condensation reactions of precursor molecules into larger planar, more stable, polycyclic aromatics.[217,218] Such reactions can often be spontaneous or nonenzymatic. The first discovered and perhaps clearest example of a proligand is indole 3-carbinol (I3C) produced in broccoli, Brussels sprouts, and kale. This naturally occurring 3-substituted indole is produced from enzymatic breakdown in the plant tissue from a glucosinolate known as glucobrassicin.[218−220] Indole-3-carbinol was originally studied as an anticarcinogenic substance by virtue of its activity as an inducer of carcinogen metabolism.[221,222] The premise was that dietary I3C protected against coadministered carcinogens such as BAP and DMBA by "blocking" their action through a reduction in their relative metabolic flux to ultimate electrophiles that damage DNA.[223] Interestingly, we now know that I3C itself is not a ligand of the AHR, but when I3C is ingested, it hits the low pH environment of the stomach and spontaneously undergoes an acid-catalyzed condensation

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reaction, converting it to a variety of AHR ligands including the potent agonist indolo[3,2-*b*]carbazole (ICZ).[218,224,225] Condensation products of I3C, such as ICZ, are high-affinity binders of the AHR and can be found in the bloodstream after exposure to I3C in the diet.[218]

One important lesson to be learned from the proligand idea is that when a compound does not fit the FHR model described above, some caution should be ascribed to any inclusion of the compound into a list of *bona fide* endobiotic or xenobiotic ligands. A list of "nonclassical" compounds that activate CYP1A1 expression but that do not obviously fit the FHR model is included in a recent review and includes SKF71739, thiabendazole, omeprazole, and 1,5-diaminonaphthalene.[153] We propose that, often, such ligands may actually be proligands. In addition to I3C described above, a number of other examples support the concept that proligands are a common source of receptor activation, including the identification of alanine serine aminotransferase (AST) and D-amino acid oxidase (DAO) as enzymes capable of activating the AHR in cell culture.[226,227] The biochemical explanation for receptor activation by these enzymes is the generation of indole-3-pyruvic acid (I3P) from tryptophan (TRP) through deamination.[226,228] Like I3C, I3P is a reactive indole, and this α-keto acid spontaneously condenses to a number of di-indol structures and possibly related backbones that are the ultimate AHR ligands or a more proximal precursor to them.[228] Similarly, in recent studies of the immune system, numerous laboratories made the observation that small relatively polar immunomodulators produced from TRP by the enzyme indoleamine 2,3-dioxygenase (IDO) can activate the AHR.[229] Given the lack of fit of many of these IDO products to the FHR model, it was again shown that IDO products such as kynurenine (KYN) or 3-hydroxyanthranilic acid (3HAA) are also proligands that are converted to a series of "trace extended aromatic condensation products" (TEACOPs).[230,231] It is probable that molecules such as these are high-affinity ligands and potent AHR agonists *in vivo*.

The idea that proligands may be more common than is currently appreciated may explain how AHR ligands display so much reported structural diversity. In our simplistic view, it may be that all AHR ligands must fit the FHR model, and when a structure does not fit, it is more likely a proligand rather than a true ligand. Either it is being converted to a TEACOP or the TEACOP is a trace contaminant of the material being used in the experiment. In this regard, if one examines a potential ligand with a high EC_{50} for induction of an AHRE-mediated response, some consideration of the possibility that the ligand is contaminated with, or is generating TEACOPs, should be considered. In this regard, I3C has an EC_{50} that is approximately 5 orders of magnitude higher than ICZ for competition with TCDD for AHR occupancy (i.e., 5 orders of magnitude lower affinity).[218] In the absence of acid condensation conditions, the I3C response may be explained by the contamination of ICZ equivalents at 1 part in 100 000 (0.001%). We argue that many compounds that are activators of the AHR at high concentrations may be contaminated with or generate a series of TEACOPs, thereby confusing structure−activity relationships.

Classifying Ligands: Xenobiotic, Endobiotic, and Cognate. We often think of AHR ligands as existing in two physiologic classes: "xenobiotic" and "endobiotic." We employ the term xenobiotic for those compounds found in an organism that are not produced within that organism. Their presence in the organism is "foreign" or from a foreign source ("xeno").[232] Common sources of xenobiotic ligands include diesel exhaust (e.g., PAHs), chlorophenol manufacturing (e.g., dioxins), or pharmaceutics (e.g., omeprazole). Xenobiotic ligands can also be "natural" and include normal constituents of fruits and vegetables (e.g., chrysin, quercetin, and galanin).[233−235] In contrast, we reserve the term endobiotic ligand to denote any AHR ligand that is produced readily in a given biological system, including within the gastrointestinal tract. A few widely studied endogenous ligands are 6-formylindolo[3,2-*b*]carbazoles (FICZ), 2-(1′*H*-indole-3-carbonyl)-thiazole-carboxylic acid methyl ester (ITE), indigo, indirubin, and bilirubin.[236−239]

A final definition that may also be useful going forward is the term "cognate." We use this term to refer to those ligands that have provided the selective pressure for the evolutionary conservation of the AHR. This class of ligand has also been referred to as "the endogenous ligand", "the physiological ligand", or even "the ancient ligand." We define cognate ligands as those ligands that correspond to the evolutionary pressure that has led to the emergence and maintenance of this receptor through evolution. Put another way, these are the ligands that have the most important consequences on normal physiology. In the absence of these ligands, the organism cannot thrive under all developmental and physiological stresses. This concept of the cognate ligand is important, because it implies that the AHR has evolved in parallel with a ligand (or set of ligands) as its evolutionary pressure. In turn, this implies that the AHR has a physiological role that is separate from and in addition to the adaptive metabolism of xenobiotics.

Xenokine Model of Ah Receptor Signaling. In a previous review, rudimentary models of AHR signaling have been put forth that might explain how potent DLCs lead to the dioxin toxic syndrome.[18,36] Given that many of those models are still untested or unrefuted, we have turned our attention toward an understanding of the AHR's cognate signaling with the underlying idea that such insight will explain many of the pathological consequences of DLCs. Based upon this history, the ideas presented above, and the models of toxicity described previously, we close this manuscript with an attempt to provide testable models of AHR cognate signaling. While our initial plan was to summarize all the ideas that have been put forth in the peer-reviewed literature, we found that there were so many ideas and so much intriguing evidence, that we could not objectively integrate them all. In this regard, the remarkable breadth of phenotypes influenced by AHR biology is provided in a recent review and reflects a potential role for this receptor in almost every major organ system and an influence on processes as diverse as cell cycle progression, immunity, DNA biochemistry, reproduction, and circadian rhythmicity.[12] Therefore, we chose to articulate a parsimonious model that employs ideas borrowed from the works of many AHR scientists but that is interpreted through the prism of our own laboratory's research experience over the last 30 years.[12,18,169,186,240−245]

This integrated model of cognate signaling has its roots in the adaptive metabolism of PAHs, dioxin toxicology, and recent results from AHR null mouse models.[12,18,169,186,241−245] We refer to this way of thinking about AHR biology as the "xenokine model." The xenokine model is based on the idea that there is parallelism between AHR's role in the adaptive metabolism of xenobiotic environmental ligands and a similar class of endobiotic ligands that we collectively think of as

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"xenokines." These xenokines are generated within the organism but commonly outside of cells, such as in interstitial spaces, the lumens of organs, or regions of cellular disruption. We propose that these xenokines are generated by endogenous chemical reactions that generate agonists in a manner similar to the generation of agonists found in the environment. In turn, these xenokines are sensed by the AHR, which stimulates a transcriptional response that is linked to a new physiological state better adapted to the new challenge they represent. Like the adaptive response to xenobiotics, it follows that the pathway is under feedback regulation and xenokine action is rapidly attenuated through CYP1 induction, AHRR upregulation, and so on.

We predict that xenokines may be as structurally varied as the spectrum of known environmental ligands but will fit the FHR model described above. We anticipate that the identification of all cognate ligands may be difficult to achieve due to the possibility that each tissue or organ system may have its own unique variety of xenokines that arise from the distinctive chemistries of each specific tissue and environmental stimuli. While the exact identity of the cognate ligands are still to be elucidated, evidence from the literature suggests they could arise from products of polyunsaturated fatty acids or heme metabolites, or they could be produced from aromatic amino acids like TRP through enzymatic reactions, nonenzymatic condensation reactions, free radical reactions, microbial metabolism, inflammation, and UV irradiation.[12,153,208,246−251]

Aromatic amino acids such as TRP and phenylalanine are potentially important proligands and sources of AHR cognate ligands.[226,252−254] In fact, the molecule that has the most experimental support for this definition of xenokine is the TRP photoproduct and TEACOP known as FICZ.[246] Evidence that this indolocarbazole is an important cognate ligand includes the observations that FICZ harbors an AHR binding affinity among the highest ever observed, is produced endogenously at epithelial barriers in response to UV irradiation, and appears to play a role in AHR-mediated immune and epithelial response to environmental stressors including bacterial invasion, oxygen stress, and UV damage.[186,246] Moreover, FICZ is rapidly metabolized by the CYP1 monooxygenases, implying its levels are tightly regulated by the feedback loop described above.[214] Lesser but provocative evidence exists for the physiological importance of additional TRP-related xenokines at other tissues. For the intestinal barrier, evidence supports the idea that TRP metabolites arising from gut microflora play important roles in activating AHR signaling to influence gut barrier integrity through influence on intestinal lymphocyte populations (e.g. refs 255−257). Even more speculative is the idea that products of the enzyme DAO, which harbors metabolic activity toward D-amino acids such as D-TRP found in bacteria, or AST, which harbors metabolic activity toward TRP, can both generate the AHR proligand I3P.[226−277,228,252] Thus, oxidases, deaminases, and transaminases like these have potential to generate proligands and ultimately xenokines *in vivo*. Enzymatic mechanisms such as these also have the potential to generate xenokines not only at environmental interfaces but also internally under conditions of tissue damage, inflammation, or remodeling.

In its simplest form, the above model can be summarized as follows. Tissues experience alterations in their external environment or their neighboring cellular environment, through inflammation, tissue damage, UV exposure, developmental remodeling, and changes in microbial populations or oxygen concentration. Each of these changes yields a unique chemistry that produces xenokines through reactive proligand intermediates that activate an AHR-mediated physiological response at the tissue level. In the gut and skin, the response is exemplified by increased barrier integrity, possibly through an influence on resident lymphocyte populations. In the lungs, hyperoxia may be eliciting its own unique chemistry and subsequent xenokine production to adapt to a new higher oxygen tension or microbiological challenges presented by the ambient air at parturition.[248,258] In the vascular system, tone and vascular remodeling may respond to systemic release of xenokines or through internal production consequent to cellular remodeling, changes in oxygen tension, or shear stress.[259,260]

Outputs of Xenokine Signaling. This perspective has emphasized the AHR signaling pathway as an adaptive metabolic system with the plan to dedicate a future perspective on the identities of those target or output genes that might facilitate the physiological and toxicological consequences of receptor activation. While evidence for the significance of this pathway in adaptive metabolic ligand clearance is recounted above, two pieces of evidence show how tightly this adaptive response must be regulated *in vivo*. In one example, competitive inhibition of CYP1 activity by ligands was shown to influence the signaling of the putative cognate ligand FICZ, presumably by reducing its clearance and increasing its steady state.[214] In another example, the global/constitutive expression of the *Cyp1a1* gene in the mouse induced a partial phenocopy of the AHR null phenotype, presumably by reducing levels of an essential cognate ligand and/or CYP1A1 substrate.[261]

It is also important to note here that there is evidence both for and against the centrality of CYP 1s as outputs essential for the physiological or toxicological effects of this receptor. Arguing for their importance as outputs is evidence that such monooxygenases influence the levels of lipid mediators (LMs) derived from polyunsaturated fatty acids or arachidonic acid.[12,249] Such LMs could have broad vaso- and immune activities that may ultimately explain aspects of DLC toxicology or phenotypes observed in AHR null models. A separate idea is that some of DLC toxicity or cognate physiology may be mediated through AHR's role as a sensor of reactive oxygen species or even mediator of an oxidative stress response.[262−265] While many related ideas have been proposed, one of the longest-standing is that the upregulation of CYP1-dependent monooxygenases leads to an increase in reactive oxygen species, which in turn can influence cellular physiology.[266−268] Arguing against the importance of CYP 1s as important output genes are observations from our own laboratory, in which the CYP1A1/CYP1A2 upregulation can be genetically dissociated from hallmark phenotypes of the AHR null model (e.g., patent *ductus venosus*) or classical toxic end points from TCDD exposure.[269,270]

Finally, the AHR field has been heavily focused on the idea that a cognate ligand exists. While we have argued for the importance of xenokine ligands as well as the adaptive response for xenobiotic and xenokine ligands, the AHR may also function constitutively in some situations. Such a possibility is supported by the expansive evolutionary data described above, where AHR orthologues exist that do not appear to recognize any ligand and appear to signal constitutively and the observation that ligand recognition of PAHs seems to be a

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vertebrate receptor characteristic. Despite all the data on the hundreds of xenobiotic ligands and the preliminary data related to cognate ligands like FICZ, we must be accepting of the formal possibility that the AHR is a bifunctional transcription factor, a transcription factor with both intrinsic activity and ligand-inducible transactivating properties.

In Closing. The AHR field is abundant with evidence for its role in biological processes as disparate as immunity, vascular biology, stemness, neurosensory signaling, reproduction, cell cycle regulation, and nucleic acid biochemistry. We began this perspective with the objective of developing a comprehensive review of modern thought related to the role of the AHR in normal human physiology. The review evolved into a discussion of the adaptive metabolism paradigm and the promotion of the xenokine model. We conclude recognizing that we have only touched the surface, only having discussed a small portion of the provocative ideas that have been put forth over the past 50 years. As we move forward into the next half-century of AHR research we must continue asking: How can all these AHR-mediated biological processes be true? How can dioxins cause so many distinctive effects? As this effort unfolds, we suspect that the simpler answer will be the correct one and look forward to the development of an understanding of AHR signal transduction that unifies the many scientific disciplines that have been touched by this enigmatic signaling molecule.

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Notes

The authors declare no competing financial interest.

Biographies

Mele Avilla holds a bachelor's degree in biology and is currently completing her Ph.D. in Molecular and Environmental Toxicology at the University of Wisconsin at Madison. Early on, both in her academic and scientific career, Mele has participated in mammalian signal transduction research related to her interests in human health and disease prevention. Mele is currently employing molecular genetic approaches to understand the role of the Ah receptor (AHR) in murine fertility. Her research focuses on the idea that the AHR is more important to human physiology than just its role in pollutant toxicology and is currently testing the idea that the AHR has an endogenous role in many stages of female reproductive physiology.

Kristen Malecki, PhD, MPH is trained in environmental epidemiology and health policy at Johns Hopkins Bloomberg School of Public Health. The goal of her research is to discover and explain persistent health disparities and their biological underpinnings using multiomic biomarkers of exposure and response (epigenomic, transcriptomic, and microbiomic). As a member of the Molecular Environmental Toxicology Center, she conducts translational research around Ah receptor signaling and immune response to explain environmental and host susceptibility to chronic diseases including cancer. She is also Director for the Survey of the Health of Wisconsin program.

Rachel Wilson graduated from the University of Colorado-Boulder where she majored in Molecular, Cellular, and Developmental Biology. She is currently enrolled in the Molecular and Environmental Toxicology Program at the University of Wisconsin-Madison in the lab of Dr. Christopher Bradfield. Her research interests include genetic mouse model development, endogenous Ah receptor signaling, and PAS protein biology.

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Chris Bradfield received his BSc. in Environmental Toxicology from the University of California at Davis and his Ph.D. in Nutrition from the University of California at Berkeley. After a postdoctoral fellowship in the Laboratory of Alan Poland, he joined Northwestern University and then the University of Wisconsin where he is currently Professor of Oncology and Director of the Biotechnology Center. His awards include a RIVER and a MERIT award from the NIEHS, a Pew Scholar Award in the Biomedical Sciences, a Burroughs Wellcome Foundation Scholar Award in Toxicology, and The Achievement Award from The Society of Toxicology.

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