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Review

Aryl hydrocarbon receptor (AHR): "pioneer member" of the basic-helix/loop/helix *per-Arnt-sim* (bHLH/PAS) family of "sensors" of foreign and endogenous signals☆

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ABSTRACT

The basic-helix/loop/helix *per-Arnt-sim* (bHLH/PAS) family comprises many transcription factors, found throughout all three kingdoms of life; bHLH/PAS members "sense" innumerable intracellular and extracellular "signals" — including endogenous compounds, foreign chemicals, gas molecules, redox potential, photons (light), gravity, heat, and osmotic pressure. These signals then initiate downstream signaling pathways involved in responding to that signal. The term "PAS", abbreviation for "*per-Arnt-sim*" was first coined in 1991. Although the mouse *Arnt* gene was not identified until 1991, evidence of its co-transcriptional binding partner, aryl hydrocarbon receptor (AHR), was first reported in 1974 as a "sensor" of foreign chemicals, up-regulating cytochrome P450 family 1 (CYP1) and other enzyme activities that usually metabolize the signaling chemical. Within a few years, AHR was proposed also to participate in inflammation. The mouse [*Ah*] locus was shown (1973–1989) to be relevant to chemical carcinogenesis, mutagenesis, toxicity and teratogenesis, the mouse *Ahr* gene was cloned in 1992, and the first *Ahr*(−/−) knockout mouse line was reported in 1995. After thousands of studies from the early 1970s to present day, we now realize that AHR participates in dozens of signaling pathways involved in critical-life processes, affecting virtually every organ and cell-type in the animal, including many invertebrates.

"During the oral defense of my thesis (Spring, 1964), one of the examining professors commented, 'Everyone knows that genes in the DNA are transcribed into RNA which is translated into protein. You're proposing that protein might control DNA? Why, that's heresy!' After a long and awkward silence — my mentor Professor Howard S. Mason spoke up, 'And what's wrong with a little heresy?'"
Daniel W. Nebert

1. Introduction

The first evidence for existence of aryl hydrocarbon receptor (AHR) occurred more than four decades ago. What do we know today about the AHR transcription factor, and in what critical-life processes does AHR participate?

To address these questions, we begin by describing the history of enzyme induction by foreign chemicals and inducible cytochrome P450 (CYP) monooxygenases; earliest studies were carried out in rat liver. Inbred mouse strains, unlike rats, were found to differ quite dramatically in degree of inducibility of certain P450 enzyme activities; this led to comparison of "potency" of inducers such as polycyclic aromatic hydrocarbons (PAHs) *vs* 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD; called "dioxin" in lay terms). Astonishingly, TCDD was found to be ~36,000 times more potent than PAHs.

A landmark study followed, comparing dose-response curves between TCDD-treated C57BL/6 (B6) and DBA/2 (D2) mice, showing that "inducible-resistant" D2 mice could be "forced" by TCDD to "turn on" their enzyme activity; due to the shape of the dose-response curve, it

Abbreviations: AA, arachidonic acid; AHH, aryl hydrocarbon hydoxylase; AHR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor translocator; B6, C57BL/6J mice; bHLH, basic-helix/loop/helix domain in proteins; Chr, chromosome; CNS, central nervous system; CYP, cytochrome P450; CYP1 family of enzymes, CYP1A1, CYP1A2, and CYP1B1; D2, DBA/2J mice; DHA, docosahexaenoic acid; DHETEs, dihydroxyeicosatrienoic acids; EETs, epoxyeicosatrienoic acids; EPA, eicosapentaenoic acid; GI, gastrointestinal; HETEs, hydroxyeicosatetraenoic acids; HIF, hypoxia-inducible factor; HpETEs, hydroperoxyeicosatetraenoic acids; LM, lipid mediator; LPS, lipopolysaccharide; LTB_4, leukotriene B_4; NQO1, NAD(P)H:quinone oxidoreductase-1; PAH, polycyclic aromatic hydrocarbon; PAS, per-Arnt-sim; *Per*, periodic locus (*Drosophila*); PUFA, polyunsaturated fatty acid; *Sim*, single-minded locus (*Drosophila*); TCDD, 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin

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was concluded that a "receptor must exist that recognizes TCDD and regulates AHH activity." The manuscript was first rejected in 1973 by reviewers, with comments such as "heresy" and "implausible to think that a foreign chemical would bind to an intracellular receptor." Eventually, after rebuttal letters, the manuscript was accepted for publication; it appeared in 1974.

Next, AHR not only recognized foreign chemicals but was also found to be associated with inflammation — again, a hypothesis rejected by many colleagues. After the mouse *Ahr* and human *AHR* genes had been cloned and sequenced, AHR was finally identified as "a member of the PAS domain family of signal sensors." Shortly thereafter, *Ahr*(−/−) knockout mouse lines provided strong evidence of the vast importance of AHR in numerous critical-life processes independent of foreign chemical treatment. AHR is now appreciated to function during the cell cycle, cell migration, cell adhesion, and other embryonic stem (ES) cell functions; these findings are consistent with early studies that had shown AHR-dependent birth defects in PAH- and especially TCDD-treated laboratory animals. Finally, it became appreciated that AHR is involved in many signaling pathways that affect various critical-life functions in most organs, tissues and/or cell types — in both vertebrates and invertebrates.

2. History and background

2.1. Inbred mouse strain differences in enzyme induction

The earliest studies of enzyme induction by foreign chemicals — in liver of PAH-treated rats — were conducted by Allan Conney, a graduate student in the Millers' laboratory [23,24]; as a postdoctoral fellow in the laboratory of Jim Gillette, Conney continued those studies [22]. Subsequently, induced "benzpyrene hydroxylase" throughout the rat gastrointestinal (GI) tract was described, following oral benzpyrene treatment; highest induced enzyme levels were found in duodenum [208]. Thus, here was an exciting concept: a novel "signal" is introduced to the animal, or cell; the "response" is to increase enzyme(s) to metabolize that signal. This model was reminiscent of earlier studies in *E. coli*: the "signal" (addition of tryptophan to tryptophan-deficient culture medium) led to a bacterial "response" of dramatic increases in enzymes in the tryptophan-metabolizing pathway [142].

Following these studies by Conney and Wattenberg, the original "benzpyrene hydroxylase" name was changed to the broader term "aryl hydrocarbon hydroxylase" (AHH), because the substrate was shown to include any of several PAHs, and several PAHs were shown to be inducers having varying potency; formation of hydroxylated benzo[*a*]pyrene (nmol/min/mg *protein*) became the standard AHH assay, which was developed and applied to PAH-treated cultures of fetal hamster cells [129,130]. Induced AHH activity in cell culture was shown to involve both transcription of DNA into mRNA and translation of mRNA into protein [132]. Subsequently, substantial differences in AHH inducibility between PAH-treated B6 and D2 mice were reported [131]; lack of AHH inducibility was then shown to behave usually as an autosomal recessive trait [51,174].

These genetic differences led to a model system far superior to that of PAH-treated *vs* untreated rats, *i.e.* an identical dose of the same chemical in genetically different mice results in striking differences, apparently based predominantly on a single gene. This single gene was subsequently found to be largely responsible for PAH-induced cancer of multiple types, mutagenesis, toxicity and birth defects [*reviewed in* [122]]. In fact, PAH treatment of a pregnant mouse with a particular genotype, and then observing differences in toxicity and/or teratogenesis *in utero* among her offspring having different genotypes — became an especially powerful tool for studies in developmental embryology [[133,196] *& reviewed in* [122]].

With regard to clinical relevance, human AHH activity in placenta — comparing cigarette smokers with nonsmokers during pregnancy — revealed that cigarette smoke induces AHH activity [138,209]. This finding has important implications for the health of newborns from cigarette-smoking mothers.

2.2. Proof that AHH activity is a P450 monooxygenase

"Cytochrome P-450" was first detected as a "colored pigment in the cell [199] which — when reduced with NADPH and bound to CO — shows a spectrophotometric Soret peak wavelength at 450 nm" [146,147]. Soon thereafter at the same symposium, three independent laboratories reported that "microsomal mixed-function oxidase" named for electron spin resonance properties of "microsomal Fe_x" [113], enzymatic functions of microsomal cytochrome P-450 [148], and particular steroid hydroxylases [41] all appeared to be one and the same enzyme or enzyme family.

The enzyme active-site comprises a heme-iron center — with tetrahedral iron tethered to the four nitrogen atoms of the porphyrin ring, cysteine-thiolate in fifth position, and binding of H_2O or substrate (hydroxyl group, nitrogen atom, or molecular O_2) in the sixth position. The O atom transferred to the substrate is derived from atmospheric diatomic O_2 rather than H_2O [63,111,112]; hence, the name "monooxygenase" is more suitable for these enzymes.

Because of spectral properties similar to those of mitochondrial cytochromes, P-450 was misnamed a "cytochrome" [146], an inaccurate label that unfortunately has persisted to this day. A more appropriate term would have been "heme-thiolate monoogenase" [27]; however, the name "cytochrome P450" had become thoroughly entrenched — long before details of the enzyme proteins and functions had been recognized.

After PAH treatment of rats, a second form of liver microsomal cytochrome P-450, called "P-448" could be detected spectrophotometrically [52,106]; another lab termed the PAH-inducible enzyme "P_1-450" [151]. It was thus postulated that "AHH activity" was "P-448" or "P_1-450." Therefore, a spectrophotometric assay — to study the height and location of the Soret peak — was carried out in PAH-treated fetal hamster cell cultures; indeed, upon treatment of the cell homogenate with NADPH and CO, a peak developed and was associated with increasing AHH activity as a function of time, during which the Soret peak shifted from 450 to 446 nm [121]. In later studies, it became clear that PAH-inducible AHH activity is associated with two distinct enzymes, "P_1-450" and "P-448" [4,5]; ultimately, these were named "CYP1A1" and "CYP1A2," respectively. The latter represents high-spin iron Fe^{3+} that causes a hypsochromic shift in the Soret peak of reduced CO-bound heme.

2.3. Genetic differences in mouse AHH induction by TCDD

In clinical studies spearheaded by Ray Suskind before 1970, workers exposed to TCDD in trichlorophenol-processing factories were shown to be at extremely high risk for chloracne and porphyria cutea tarda [*reviewed in* [218]]. This led Alan Poland, using chick egg liver [156], to show that TCDD was ~36,000 times more potent than any PAH in the induction of δ-aminolevulinic acid synthetase — a key enzyme in porphyrin synthesis.

Then came the first "**Aha!**" moment: If δ-aminolevulinic acid synthetase activity is strikingly induced by the highly potent TCDD, and heme is a product of porphyrin synthesis, and AHH represents a P450 hemoprotein, would TCDD be superior to PAHs in causing AHH induction? In particular — would the "lack of AHH induction," seen in PAH-treated D2 mice [51,131], be overcome by TCDD treatment? Further, would TCDD be able to increase inducible AHH activity in B6 mice to even higher levels? Following a telephone call, these questions were answered by Alan Poland visiting the Nebert laboratory, where the conclusive experiments were performed together [159].

Fig. 1 illustrates dose-response curves of B6 *vs* D2 hepatic AHH activity as a function of TCDD dosage. Earlier studies, with PAH inducer 3-methylcholanthrene (80 mg/kg; 24 h), had shown 5- to 10-fold