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Commentary

Regulating the regulator: Factors that control levels and activity of the aryl hydrocarbon receptor

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Abbreviations:
AHR, aryl hydrocarbon receptor
AHRE, AH response element (also known as DRE or XRE)
AHRR, aryl hydrocarbon receptor repressor
ARNT, aryl hydrocarbon receptor nuclear translocator
B[*a*]P, benzo[*a*]pyrene
E2, 17β-estradiol
ER, estrogen receptor
HNF, hepatic nuclear factor
IL-4, interleukin-4
MC, 3-methylcholanthrene
PAH, polycyclic aromatic hydrocarbon
PCB, polychlorinated biphenyl
TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene

ABSTRACT

The aryl hydrocarbon receptor (AHR) participates in a wide range of critical cellular events in response to endogenous signals or xenobiotic chemicals. Hence, it is important that AHR levels and activity themselves be well controlled in target tissues. The AHR is essentially ubiquitous in its distribution in mammalian tissues. However, levels of the receptor vary widely across different tissues and among different cell types. AHR levels and activity are modulated by exposure to the receptor's own ligands and are influenced by other xenobiotic chemicals. Many different factors impinge on AHR levels and AHR activity. These factors may alter responsiveness of downstream pathways, thereby affecting normal physiologic functions as well as responses to toxic environmental chemicals such as dioxins. Our commentary appraises the current literature on factors that regulate AHR levels/activity and attempts to identify fruitful strategies towards discovery of key pathways by which AHR levels are modulated in response to endogenous signals and in response to xenobiotic chemicals. An extraordinarily large number of agents alter the level or activity of the AHR. We have not yet entered an age of enlightenment sufficient to achieve true understanding of the interplay of mechanisms that regulate AHR expression in space and in time.

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1. Introduction

When first discovered, the aryl hydrocarbon receptor (AHR) was envisioned as a mechanism that regulates two conspicuous responses to xenobiotic chemicals: (1) induction of CYP1A1 and related enzymes and (2) toxicity of ''dioxin-like'' compounds [1–3]. Over the past two decades many laboratories showed that, in addition to its function as a receptor for xenobiotics, the AHR plays multiple roles in fundamental cell biology, organismic development and physiology [4–9]. Given the diverse and important regulatory roles played by the AHR in basic biology and in toxicology, it is to be expected that AHR levels and AHR activity are, themselves, subject to regulation or modulation by endogenous and exogenous influences.

2. Factors that influence AHR levels or AHR activity

The purpose of Tables 1 and 2 and Fig. 1 is to catalog the bewildering array of factors that alter AHR levels or AHR activity as a first approach to understanding regulation of this regulator.

Table 1 – AHR levels: agents and factors that alter levels in various cells or tissues

Agent or factor	Biological system	AHR property measured	Direction of effect on AHR level and fold change
Chemical agents			
In cell culture			
TCDD (hours)	Mouse hepatoma cells	Ligand binding	↓ 4
TCDD (hours)	Mouse and rat hepatoma cells	DNA binding	↓ 10+
TCDD (hours)	Mouse hepatoma cells	Protein	↓5
TCDD (hours)	Mouse hepatoma cells	mRNA	No change
TCDD (days)	Human keratinocytes	Protein	↓ 21
Benzo[*a*]pyrene	Mouse embryo fibroblasts	mRNA	↓ 3
α-Naphthoflavone	Trout hepatocytes	Protein	↑ 1.5
β-Naphthoflavone	Trout hepatocytes	Protein	↑ 4
Dimethylsulfoxide	Primary rat hepatocytes	mRNA	↑ 1.5
Dexamethasone	Rat hepatoma cells	Ligand binding	↑ 2-3
Dexamethasone	Rat mammary fibroblasts	Protein	↓ 3
17β-Estradiol	Human breast carcinoma cells	mRNA	↑ 4
In vivo			
TCDD (days)	Rat liver	Ligand binding	↑ 2–3
TCDD (days)	Rat liver	Ligand binding; mRNA; protein	↑ 2–3
TCDD (weeks)	Rat liver	Ligand binding; mRNA; protein	↑ 2–3
TCDD (h–days)	Rat liver, thymus, lung, spleen	Protein	↓ 2–9
TCDD (1 day)	Rat prostate	Protein	↓ 2–5
TCDD (high dose—weeks)	Mouse centrilobular hepatocytes	Protein	↓
TCDD (low dose—weeks)	Mouse centrilobular hepatocytes	Protein	↑
TCDD	Zebrafish heart (AHR2)	mRNA	↑ 2
Phenobarbital	Mouse and rat liver	Ligand binding	↑ 2
Non-coplanar PCBs	Rat liver	Ligand binding	↑ 2–3
TCPOBOP	Mouse liver	mRNA	↑ 2
trans-4-Acetylaminostilbene	Rat liver	Ligand binding	↑ 2
7,12-Dimethylbenz[*a*]anthracene	Rat liver	mRNA, protein	↑ 25–33
3-Methylcholanthrene (1 day)	Rat liver	mRNA	↑
Hormone replacement therapy	Human uterine endometrium	mRNA	↑ 2
Cigarette smoking	Human uterine endometrium	mRNA	↓
Hydrocortisone	Mouse embryonic palate	mRNA; protein	↑
Biological factors			
In cell culture			
Increased cell differentiation	Human keratinocytes	mRNA	↑ 8
Increased cell differentiation	Human keratinocytes	Protein	↑ 16
Increased cell differentiation	Mouse 3T3 preadipocyte cells	mRNA; protein	↓ strong
Cell senescence	Human keratinocytes	Protein	↑ 17
Extracellular matrix adhesion (Matrigel)	Rat mammary epithelial cells	Protein	↑ high[a]
Growth factor depletion	Rat mammary epithelial cells	Protein	↓ 2
Serum-free medium	Rat seminiferous tubule	mRNA	↑ 2–4[b]
Serum-free medium	Teleost hepatoma cells	Ligand binding	↓ 3
Serum-free medium	Mouse 3T3 fibroblasts	Protein	↓ 4–10
Insulin + dexamethasone + methylisobutylxanthine	Mouse C3H10T1/2 cells	mRNA; protein	↓ 10

Table 1 (*Continued*)

Agent or factor	Biological system	AHR property measured	Direction of effect on AHR level and fold change
Lipopolysaccharide	Mouse splenocytes and splenic B cells	mRNA; protein	↑ 5
Interleukin-4; interleukin-13	Mouse and human B cells	mRNA; protein	↑ 5–9
Human tumor cell lines			
Tumor vs. untransformed counterpart	Human T-cell leukemia	mRNA; protein	↑ high[a]
Increased tumor invasive potential	Human melanoma cells	mRNA; protein	↑ high[a]
TGFβ	Human lung carcinoma cells		↓
TGFβ	Human hepatoma cells		↑
Wnt/β-catenin	Human prostate cancer cells	mRNA; protein	↑ 2–4
In vivo			
Development from fetus to puberty	Rat liver and lung	Ligand binding	↑ 4–6
Aging of animal	Rat liver and lung	Ligand binding	↓ 3
Aging of animal	Rat liver	Ligand binding	↓ 3
Aging of animal	Rat prostate	mRNA; protein	↓ 3
Aging of animal	Mouse liver	mRNA	↓ 3
Increased cell differentiation	Mouse adipose tissue in vivo	mRNA	↓ strong
Increasing age of donor	Human buccal mucosal cells	mRNA	↑ r^2 0.3–0.4[c]
Hypophysectomy	Rat liver	Protein; ligand binding	↓ 1.5–3
Stress (immobilization)	Mouse liver	mRNA	↑ 1.5
Knockout of P450 reductase	Mouse liver	mRNA	↑ 1.5–2
Tumors/normal tissue	Human pancreatic cancers	mRNA	↑ 27
	Human lung carcinomas	Protein	↑
	Human desmoid tumors	mRNA	↑ 4

In cases where an arrow is present but no numerical value is attached, the original paper contained information on the direction of change but not the magnitude of change. See "supplementary data" for references to the original research papers which reported these effects.
[a] Fold-increase cannot be calculated because comparator cells have no detectable AHR mRNA or protein.
[b] Up-regulation can be blocked by follicle stimulating hormone.
[c] Magnitude not given; r^2 represents the correlation coefficient for relationship between age and AHR mRNA level in population studied.

2.1. *Chemical agents*

Exogenous chemicals can push AHR levels away from set-points that are characteristic for each cell or tissue. As with other receptor systems (e.g. G-protein-coupled membrane receptors) the question arose of whether the AHR's own ligands alter levels of this receptor in target tissues. In vivo treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) over a period of days can modestly increase levels of AHR mRNA and AHR protein in rodent liver [10,11]. However, TCDD also down-regulates AHR protein levels in several tissues in vivo (Table 1 and references in supplementary data). In most instances down-regulation in vivo manifests either after high doses or when the measurements are done soon after TCDD administration. This transient down-regulation in vivo is consistent with extensive findings in cell culture which show that AHR agonists such as TCDD or 3-methylcholanthrene (MC) provoke degradation of AHR protein (see Section 3.4). With persistent exposure to TCDD at low doses, AHR concentrations return to pre-exposure levels and even can be elevated two- or three-fold above the original basal level in liver [10,11].

Non-halogenated polycyclic aromatic hydrocarbon (PAH) ligands also affect AHR levels but in a manner that appears to depend upon the specific PAH and the particular cell-type or tissue. Benzo[*a*]pyrene (B[*a*]P) down-regulates AHR mRNA in mouse embryo fibroblasts in cell culture [12] whereas 7, 12-dimethylbenz[*a*]anthracene was reported to increase AHR mRNA and AHR protein more than 25-fold in rat liver in vivo [13]. The opposite effect in these two systems serves as a warning that there may not be a simple universal mechanism that explains all varieties of regulation of AHR levels by the receptor's own ligands.

Exposure to environmental contaminants in the real world never is to only a single chemical agent at a time. Several laboratories have tested the possibility that xenobiotic chemicals which are not necessarily ligands for the AHR might alter AHR levels. Phenobarbital has broad pleiotypic effects on gene expression in mammalian liver and one of phenobarbital's actions is to increase AHR levels [14]. Non-coplanar polychlorinated biphenyls (PCBs) and the synthetic phenobarbital-like compound, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), also elevate AHR mRNA [15] or AHR protein [16].

2.2. *Biological factors*

The history of the AH receptor began with studies in rodent liver and in rodent hepatoma cell lines [1,2,17]. Constitutive/basal AHR levels generally are high in liver but AHR also is abundant in diverse mammalian tissues such as placenta, thymus, lung, kidney, small intestine, heart and pancreas [18–20].

As described above, AHR levels can be modulated by several exogenous chemicals. However, AHR levels tend to be

Table 2 – AHR function: major cellular factors and processes that alter AHR activity

Factor/process	Functional consequence
(A) Core components of the cytosolic or nuclear AHR complex	
hsp90	Maintains AHR in ligand-binding conformation and prevents AHR·ARNT dimerization
AIP/ARA9/XAP2	Stabilizes AHR and enhances cytoplasmic localization
p23	Ligand-mediated release of AHR from hsp90
ARNT	Dimerizes with AHR to form AHRE-binding complex
(B) Factors involved in repression or termination of AHR signaling	
AHRR	Negatively regulates AHR function by dimerizing with ARNT and binding to AHRE
AINT	Binds to ARNT and decreases ARNT nuclear localization
ADPF	Incompletely characterized labile factor involved in the ubiquitin-dependent proteasomal degradation of AHR
(C) Coactivators, coreppressors, chromatin remodeling factors and general transcription factors	
SRC-1, NCoA-2, p300/CBP, p/CIP	Coactivators with histone acetylation activity that interact with AHR and/or ARNT and facilitate gene activation
RIP 140	Binds to AHR and enhances AHRE-driven transcription
SMRT, SHP	Inhibit transcriptional activity of AHR·ARNT complex
Brg-1	ATPase-dependent histone modifier that enhances transcription mediated by AHR·ARNT complex
Med220, CDK8	Subunits of the mediator complex involved in transcriptional activation by AHR·ARNT
PolII, TBP, TFIIB, TFIIF	Key components of the general transcriptional machinery that are directly involved in gene activation by AHR·ARNT
(D) Phosphorylation	
PKC	Increases transcriptional activation by AHR·ARNT, but mechanisms remain controversial
c-Src	An AHR-associated tyrosine kinase that is activated by AHR agonists leading to rapid and diverse effects on cellular phosphorylation cascades
cAMP	Triggers nuclear accumulation of AHR in a form that does not interact productively with ARNT
(E) Cross-talk with other transcription factors and signaling molecules	
NF-1	A general transcription factor involved in AHR-dependent induction of *CYP1A1* via interaction with CCAAT box in proximal promoter region
Sp1	Cooperatively up-regulates expression of genes under AHR·ARNT control
ERα	Recruited to dioxin-responsive promoters, resulting in transactivation or transrepression of AHR-dependent gene expression
NF-κB	Direct interaction between p65 subunit of NF-κB and AHR, such that NF-κB activation suppresses *CYP1A1* expression by inhibiting histone acetylation at the promoter
Rb	Direct interaction between Rb and AHR required for maximum *CYP1A1* induction, suggesting a coactivator role for Rb
HIF-1α	Hypoxia inhibits AHR transcriptional activity independent of competition for ARNT
Mybbp1a	Associates with AHR and enhances its transactivation
Nedd8	Interacts with AHR leading to increased nuclear accumulation or retention and enhanced transcriptional activity

Abbreviations: hsp90, 90 kDa heat shock protein; AIP, AHR interacting protein; ARA9, AHR-associated protein 9; XAP2, hepatitis B virus X-associated protein 2; AINT, ARNT interacting protein; ADPF, AHR degradation promoting factor; SRC-1, steroid receptor coactivator 1; NCoA-2, nuclear coactivator 2; CBP, cAMP-responsive element-binding protein (CREB)-binding protein; p/CIP, p300/CBP cointegrator protein; RIP 140, receptor-interacting protein 140; SMRT, silencing mediator of retinoic acid and thyroid hormone receptors; SHP, small heterodimer partner; Brg-1, Brahma/SWI2-related gene 1; Med220, mediator subunit of 220 kDa; CDK8, cyclin-dependent kinase 8; PolII, RNA polymerase II; TBP, TATA-binding protein; TFIIB, general transcription factor IIB; TFIIF, general transcription factor IIF; PKC, protein kinase C; NF-1, nuclear factor 1; ERα, estrogen receptor-α; NF-κB, nuclear factor-κB; Rb, retinoblastoma protein; HIF-1α, hypoxia-inducible factor-1α; Mybbp1a, Myb-binding protein 1a; Nedd8, neurally expressed developmentally down-regulated protein 8. See ''supplementary data'' for references to research papers and reviews which reported these effects.

perturbed more dramatically by biological factors than by xenobiotic chemicals and the interactions among biological factors can be very complex. An example of this complexity is the interaction between transforming growth factor-beta (TGFβ) and the AHR. The phenotype associated with the *Ahr*-null mouse suggests that loss of AHR activity results in altered TGFβ activity. Both TGFβ and latent TGFβ-binding protein-1 are markedly increased in *Ahr*-null mice [21,22], indicating that the AHR is a negative regulator of TGFβ levels. Conversely, it has been shown that TGFβ regulates AHR expression in a cell-specific manner by promoting Smad protein interaction with a TGFβ-responsive element approximately 2500 nt upstream of the human AHR promoter [23]. Down-regulation of AHR expression by TGFβ may explain the mechanism by which the presence of TGFβ prevents TCDD-mediated cleft palate [24].

The impact of biological factors is most apparent in experiments conducted in cell culture. Several laboratories found that the state of cell differentiation exerts a strong effect on AHR levels as do manipulations such as serum-deprivation, growth factor depletion or alteration of the hormonal milieu (Table 1). It is apparent from data in Table 1 that the effects on AHR levels of cell differentiation status or conditions of the growth medium are highly dependent upon the cell type being

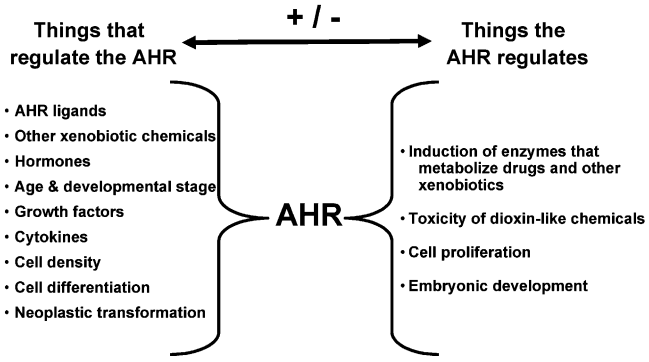

Fig. 1 – Schematic summary of the many factors that regulate the AHR and the general biological events that the AHR regulates. The arrow indicates that regulation is likely to flow in both directions, i.e. some events regulated by the AHR may, in turn, regulate levels or expression of the receptor itself in either a positive or negative direction.

studied. For example, dexamethasone increases AHR protein in rat hepatoma cells but decreases AHR in rat mammary fibroblasts (Table 1).

3. Mechanisms that regulate AHR levels or AHR activity

Are there unifying principles that can adequately describe how AHR expression is regulated by the diverse chemical and biological agents listed in Table 1? A simple, unified scheme would be of great value in helping to understand the interplay of AHR with other cellular components and extracellular agents in normal biological regulation and in response to toxic environmental chemicals. Regrettably, at present the individualistic nature of the responses shown by different cell types and different tissues precludes creation of a universal scheme to explain regulation of AHR levels or activity.

3.1. *Regulatory motifs in AHR genes*

There are a few things that we do know about mechanisms which regulate AHR levels. As with any gene, understanding its expression is facilitated if we know the gene structure. Genomic cloning of the mouse *Ahr* gene [25,26] and the human *AHR* gene [27] revealed that both genes lack a TATA box in the promoter region. Although TATA boxes tend to be most abundant in promoters of genes that are expressed in a limited tissue-specific fashion [28], both the mouse and human *AHR* genes contain multiple GC boxes and Sp1 transcription factor binding sites that are typical of promoters found in housekeeping genes.

Garrison and Denison [29] extensively mapped the 5′-flanking region of the mouse *Ahr* gene by deletion analysis and found that full constitutive expression required regulatory elements that lie between −184 and +380. A region further upstream (−721 to −1431) appears to repress constitutive activity of the mouse *Ahr* promoter in a cell-specific and species-specific fashion. Their experiments also revealed that histone acetylation has a strong influence on *Ahr* promoter activity.

With regard to possible ‘‘autoregulation’’ of AHR expression by the AHR’s own agonists, there are potential AHR:ARNT binding sites (AHRE-I) clustered around the human *AHR* promoter (two sites between 0 and +750 nt) or mouse *Ahr* promoter (four sites between −100 and +400 nt). In rat there are three putative AHRE-I motifs upstream of the *AHR* promoter (−300 to −5000 nt). However, no functional studies have been done to demonstrate that any of these sites is responsible for up-regulation of AHR expression by AHR ligands.

Regions upstream of the proximal promoter in rodent and human *AHR* genes contain other regulatory motifs that might account for high expression in certain tissues. For example, numerous binding sites for the hepatic nuclear factor family of transcription factors exist in human, mouse and rat *AHR* genes (Fig. 2) and some of these may function to maintain the high constitutive expression levels that typically are found in mammalian livers. The *AHR* genes in all three species also contain multiple copies of the BRN3-like binding motif and the PIT1-like binding motif that may support basal expression in brain and pituitary. AHR, ARNT and related bHLH/PAS proteins are constitutively expressed in rat hypothalamus [30] but pituitary has not yet been examined for AHR expression.

In mouse 3T3-L1 cells, AHR levels are substantially reduced when the cells differentiate into adipocytes. Depletion occurs at the level of transcription and has been attributed to a loss of a protein factor during differentiation. This factor appears to bind to a sequence in the −378 to −359 region of the *Ahr* 5′-flank and stimulate promoter activity in preadipocytes [31]. The stimulatory factor has not been identified but has a molecular mass of about 20 kDa [31].

IL-4 up-regulates AHR levels in a human Burkitt lymphoma cell line and in the human hepatoma cell line, HepG2. The authors [32] propose that a STAT6-binding motif at about −1100 (Fig. 2) mediates up-regulation of AHR by IL-4 and IL-13 in human cells. Our examination of the 5′-flanking region reveals a similar STAT6 site at about −850 in mouse but no equivalent site close to the promoter of the rat *AHR* gene (Fig. 2).

3.2. *Endocrine control of AHR expression and function*

Dioxins and other AHR ligands are known to act as endocrine disruptors. This is best exemplified by the anti-estrogenic actions of these chemicals [33]. However, much less is known about how hormonal factors modulate AHR expression and function.

The pituitary gland represents a master control center for the endocrine system. Hypophysectomy attenuates the hepatic CYP1A induction response to aromatic hydrocarbons in rats [34]. However, it remains unclear whether this response is related to alterations in hepatic AHR expression. An early study [35] showed that hypophysectomy has no effect on hepatic AHR ligand binding as assessed using isoelectric focusing. However, a more recent investigation found that hepatic AHR protein levels in hypophysectomized rats were decreased about 40% compared to sham-operated rats and hypophysectomy caused a 70% decrease in AHR ligand binding

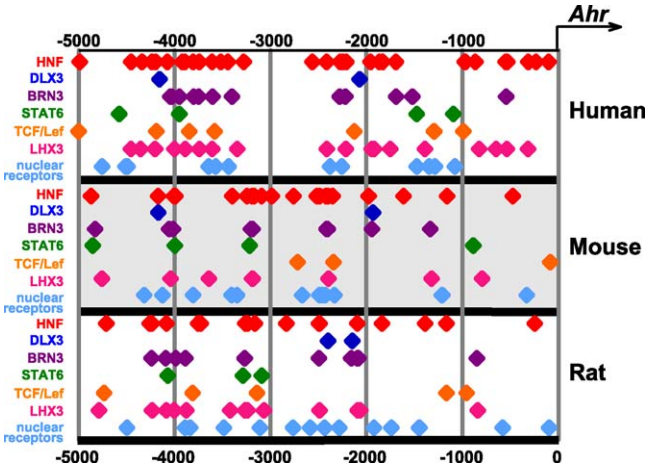

Fig. 2 – Examples of regulatory motifs in the 5′-flanking regions of *AHR* genes. We searched up to 5000 nucleotides upstream in AHR promoters in human (May 2004), mouse (March 2005) or rat (June 2003). AHR promoter sequences were transferred from http://www.genome.ucsc.edu to MatInspector Professional Version 7.4; January 2005 (http://www.genomatix.de) and searched for potential transcription factor binding sites for all vertebrate families (Matrix Family Library Version 5.0; February 2005) [99]. In total there were 1322 matches in the human sequence, 1145 matches in the mouse sequence and 753 matches in the rat sequence. Representative potential transcription factor binding sites are shown. Hepatic nuclear factor (HNF) sites include HNF-1, HNF-4 and HNF-6. Nuclear receptors include potential binding sites for the progesterone receptor (PR), androgen receptor (AR), glucocorticoid receptor (GR), peroxisome proliferator-activated receptor (PPAR), farnesoid X receptor (FXR) and Vitamin D receptor (VDR).

[36]. The identity of the pituitary factors that regulate hepatic AHR expression and the functional consequences remain unknown and form the basis for important avenues of ongoing research.

Adrenal glucocorticoids are strong candidates as endocrine factors with the ability to regulate AHR expression and function. Glucocorticoids potentiate induction of rat CYP1A1 by AHR agonists, a response that is at least partially attributed to glucocorticoid-responsive elements located in the first intron of the *CYP1A1* gene [37]. Stress, characterized by a marked increase in glucocorticoid levels, potentiates induction of hepatic CYP1A catalytic activity caused by aromatic hydrocarbons [38] but there is conflicting evidence regarding regulation of AHR levels by glucocorticoids. Dexamethasone increases AHR ligand binding in H4IIE rat hepatoma cells [39] and treatment of pregnant mice with cortisol results in offspring with elevated AHR mRNA and protein in craniofacial tissue [40]. On the other hand, dexamethasone decreases AHR protein levels in rat mammary fibroblasts [41].

Thyroidectomy protects rats from some TCCD toxicities [42]. Interestingly, removal of the thyroid gland does not reduce hepatic AHR ligand binding [43] and does not appear to alter induction of hepatic P450 nor UDP-glucuronosyltransferase activities [43,44]. It may be productive to explore further the roles modulation of AHR expression and AHR function play in the protection afforded by thyroidectomy.

A final hormonal factor of potential importance is 17β-estradiol (E2). Female rats are more sensitive than male rats to some aspects of TCDD toxicity and certain TCDD toxic responses can be enhanced by estrogens [45]. Although there is some evidence that estrogens can interfere with AHR signaling [46], the balance of evidence suggests that E2 exerts a positive influence on AHR signaling [47–49]. The AHR and estrogen receptor-α (ERα) pathways appear to cross-talk in multiple ways and with complex consequences. In contrast to extensively studied anti-estrogenic effects of AHR ligands [33], it appears that the activated AHR can interact with ERα in the absence of bound E2 to form a functional transcriptional complex in the regulatory region of ERα-responsive genes [50]. Very recent work demonstrates that the activated AHR can recruit ERα to dioxin-responsive promoters, resulting in either ERα-mediated transactivation [51] or transrepression [52] of AHR-dependent gene expression. In addition, there is evidence that E2 may modulate expression and activity of AHR-regulated genes by increasing levels of the AHR itself, at least in target cells such as breast cancer [53]. Much remains to be learned about the complex interactions between the AHR and ERα signaling pathways.

3.3. *Developmental regulation of AHR expression*

AHR and ARNT mRNA are detectable as early as the preimplantation stage of developing mouse embryo [54]. AHR protein levels in rodents are highest early in life, then progressively decline with age [35,55–58]. The mechanisms responsible for high expression during early development and the decrease with age are not known.

Studies in non-vertebrate organisms may provide clues to mechanisms that regulate AHR expression during development. The AHR is phylogenetically ancient [4,59]. Homologous genes exist in invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster*, model organisms that are widely used in developmental studies. In *C. elegans*, AHR-1 is the homolog to mammalian AHR. *C. elegans* also possesses a partner protein, AHA-1, that is a homolog of ARNT [60]. POU-domain transcription factors represent candidate regulators of the *C. elegans AHR-1* gene. *AHR-1* in *C. elegans* is regulated by *unc-86* a founder member of the POU domain family of transcription factors. The nucleotide sequence of the preferred DNA recognition site of the POU-IV subclass of transcription factors (such as unc-86) [61] strongly resembles BRN3 motifs which are found in multiple copies in the 5′-flanking regions of rat and human *AHR* genes (Fig. 2).

Clues to potential AHR regulators also may emerge from work done with the AHR homolog in *D. melanogaster*. Spineless is the *Drosophila* homolog of mammalian AHR. Tango, the partner protein to spineless, is an ARNT homolog [62,63]. Expression of spineless in *Drosophila* is primarily under control of a homeodomain transcription factor, distal-less (*Dll*) [62]. In mammals, Distal-less 3 (DLX3) is a homeobox factor that functions as a placental-specific transcriptional regulator [64]. Dlx3-null mice have impaired placental development and do not survive in utero past embryonic day 9.5 [64]. Our

examination of the 5′-flanking regions of mouse, rat and human *AHR* genes (Fig. 2) shows that there is a positionally conserved DLX3 binding site at about −2000 upstream in all three mammalian species, suggesting that in mammals as well as in invertebrates, this motif may be involved in regulating AHR expression in developmentally important times and tissues.

In addition to DLX3 we found a putative binding site for another homeodomain-containing transcription factor, NKX3, in the 5′-flank of the *AHR* gene. NKX3 is expressed in a prostate-specific manner and is necessary for normal development of both the dorsolateral and anterior prostate in mice [65]. The AHR mediates teratogenic and toxic effects of TCDD on prostate following exposure in utero or lactational exposure [66]; thus, factors which may regulate early prostate-specific expression of the AHR are important to normal development as well as to reproductive toxicology.

The transcription factor, LHX3, is our final example of a regulatory factor that potentially is important in relation to AHR and development. LHX3 is required for pituitary development. We found potential DNA-binding sites for the LHX3 transcription factor in the 5′-flanking region of the *AHR* gene (Fig. 2) as well as binding sites for an additional pituitary-specific transcription factor, PIT-1 (not shown). Many studies have been done on the impact of AHR ligands on diverse endocrine functions but relatively little is known about expression of the AHR in the ''master gland'', the pituitary.

The regulatory motifs briefly described above (BRN3, DLX3, NKX3, LHX3 and PIT-1) are promising sites to explain certain aspects of AHR expression during mammalian development but none of these motifs has yet been tested for its actual function in AHR expression during development; this may be a fruitful avenue for investigation, particularly given the importance of the AHR to normal development.

3.4. *Degradation of AHR protein*

As described in the preceding, many chemical agents and biological factors alter AHR expression at the transcriptional level. Cellular AHR content also can be profoundly changed by multiple chemical agents, particularly the receptor's own ligands which provoke degradation of the AHR protein via the ubiquitin-proteasome pathway [67–69] without altering levels of AHR mRNA [70]. Please see the extensive review by Pollenz [69] for details on proteolytic pathways.

Not all agents that activate the AHR lead to the receptor's subsequent degradation. For example, IL-4 provokes nuclear localization of AHR in a human Burkitt lymphoma cell line and in a human hepatoma cell line (HepG2) where IL-4 also up-regulates AHR levels [32]. IL-4 induces CYP1A1 mRNA in these cells but, unlike conventional AHR ligands, does not trigger degradation of AHR protein [32]. The authors speculate that IL-4 is able to activate the AHR without putting the AHR protein into a conformation that is susceptible to ubiquitination and proteasomal degradation.

Very recently Pollenz et al. proposed that two distinct systems may be at work to degrade AHR protein: the first is a mechanism to remove protein that is misfolded or abnormally localized within the cells; the second is a mechanism that regulates ''physiological degradation'' after the receptor has been recruited to DNA as a component in the transcriptional apparatus [71].

3.5. *Cellular factors and processes that modulate AHR activity or AHR function*

The central focus of this Commentary is on factors and processes that potentially impact AHR function by modulating the level of AHR protein. However, biological functions of the AHR can be regulated by numerous factors and processes that do not necessarily alter receptor levels per se. Space does not permit a detailed examination of these processes. The major cellular factors and processes that are known to modulate AHR functional activity are summarized in Table 2. Most of these modulators are familiar from their similar roles in regulating activity of other receptors in the nuclear receptor superfamily.

One factor, the AHR-repressor (AHRR), deserves particular attention as a novel method to potentially restrain AHR-dependent gene transcription. The N-terminal region of the AHRR protein is similar in sequence to the AHR [72,73] but the AHRR does not bind ligands [72]. AHRR is induced by AHR agonists such as TCDD or MC [30,72,74] via binding of the AHR to AH response elements (AHRE-I), similar to the mechanism of CYP1A1 induction [30,72,74]. AHRR can quench AHR-dependent up-regulation of transcription by dimerizing with the ARNT protein and competing with the AHR–ARNT complex for binding to AHRE-I sites [72]. Thus, a gene product (AHRR) induced by AHR agonists serves to impede the ability of the AHR to transactivate gene expression. Induction of the AHRR and its subsequent feedback inhibition may prevent over-expression of particular genes when the cell is exposed to potent AHR agonists.

As was seen with other factors that regulate AHR levels or AHR activity, the effect of AHRR varies widely across different tissues because AHRR itself is not equally expressed nor equally inducible in various cells and tissues. In untreated rodents, AHRR levels are low in virtually all tissues [73]. In untreated mice, constitutive AHRR expression is highest in heart and brain whereas after treatment with B[*a*]P the AHRR is induced in liver, spleen, lung and ovary but there is no significant induction in heart or brain [75]. The tissue patterns of AHRR expression in mice do not seem to explain why induction of AHR target genes such as CYP1A1 varies across different tissues [75].

In contrast, in rats the highest constitutive expression of AHRR is in testis. Induction of AHRR by TCDD is highest in rat kidney, spleen and heart but the induction of CYP1A1 was not clearly related to AHRR induction across these tissues [73].

Like rat, the highest constitutive expression of AHRR in adult human tissues appears in testis [76]. Constitutive AHRR expression also is observed in adult human lung, ovary, spleen and pancreas [76]. Among these tissues, CYP1A1 expression is highest in lung [76]; therefore, since both AHRR expression and CYP1A1 expression are high in human lung, high constitutive expression of AHRR does not always successfully quench expression of AHR-regulated genes. A comparative study in several human tumor cell lines indicated that induction of AHRR by PAHs and nitro-PAHs generally paralleled induction of CYP1A1 in the same cells; in other words, cell lines that were

most responsive for AHRR induction also were most responsive for CYP1A1 induction [77]. This indicates that, at least in these tumor cell lines, CYP1A1 can be highly induced despite the concomitant increase in the repressor protein. Nevertheless, it is possible that constitutive AHRR expression or induction of AHRR and its subsequent quenching effect may be much higher in some individuals than in others. For example, it would be useful to know if lungs of some smokers that show high AHRR induction do exhibit lowered CYP1A1 expression. Such data might be relevant in assessing individual differences in cancer risk but are not yet available.

3.6. *AHR polymorphisms in relation to AHR function*

No polymorphisms are known that have a significant effect on AHR *expression* in animal models. However, rodent *AHR* genes contain some polymorphic sites that have a major impact on *function* of the AHR protein. For example, in mice the Ahr^{b1} allele (that encodes alanine at codon 375) produces an AHR protein that has about a 10-fold higher affinity for TCDD than does the receptor encoded by the Ahr^{d} allele (which encodes valine); as a consequence, mice with the Ahr^{b1} allele are about 10-fold more sensitive than mice with the Ahr^{d} allele to a wide range of biochemical and toxic effects when exposed to TCDD (reviewed in [78–80]). The Han/Wistar (*Kuopio*) rat strain expresses a variant AHR in which a polymorphism at an intron/exon boundary leads to deletion of a large segment from the transactivation domain of the AHR protein; these animals are extraordinarily resistant to the lethal effects of TCDD [80].

In humans, the *AHR* gene contains relatively few single nucleotide polymorphisms. Recently Koyano et al. [81] described two rare polymorphisms in the Japanese population. When constructs containing the variant alleles are transfected into HeLa cells they give rise to 50% lower AHR protein (but not AHR mRNA) than wildtype, resulting in decreased AHR-mediated gene expression. None of the other known human *AHR* polymorphisms confers a strong phenotypic effect. The paucity of polymorphisms in the human *AHR* gene suggests that selective pressures foster structural conservation in key functional domains of the human AHR [78,79].

4. Consequences of altered AHR levels or AHR activity

What are the consequences of having too much or too little AHR protein or AHR activity? AHR expression in vertebrate cells is essentially ubiquitous; this implies important and widespread biological roles. The bHLH/PAS protein superfamily to which the AHR belongs has an ancient evolutionary history [59]. Strong links of bHLH/PAS proteins to morphogenesis have been demonstrated in animals from *Drosophila* to mouse. The AHR may be particularly critical during embryonic/fetal development and in early postnatal stages. AHR levels decrease substantially as mammals age (Table 1 and references therein), suggesting a lesser role in mature or senescent animals.

The importance of AHR levels in vivo is perhaps best illustrated by extreme cases. If AHR expression is completely obliterated by gene knockout in mice, the animals are viable but there are multiple adverse effects on development [4–9]. Developmental deficits also can be produced in mice by using gene manipulation to reduce AHR levels (''AHR hypomorphic mice'') [8]. Although AHR knockout or hypomorphic reduction of AHR creates developmental problems for the animals, both of these genetically manipulated reductions in AHR levels have the benefit of making the mice highly resistant to toxicity from dioxin-like chemicals, as does being hypomorphic for the AHR's dimerization partner, ARNT [8,82].

From general pharmacologic theory, receptor levels are a major determinant of a tissue's responsiveness to ligands. In regard to the AHR, Andersen and Barton [83] showed by modeling that ''autoinduction'' of AHR by its own ligands may place the cell in a ''highly ligand-responsive state''. If cells are treated with agents that block proteasomal degradation of the AHR, TCDD causes ''superinduction'' of CYP1A1 [84]. Conversely, if cells are treated with siRNA to decrease AHR levels, the inducibility of CYP1A1 by TCDD is decreased substantially [85].

However, the answer to the question of whether altered AHR levels/activity are ''good'' or ''bad'' for the cell or organism is not straightforward. Bradfield and coworkers [4,82] provided a highly useful conceptual approach to AHR roles by classifying AHR-regulated events into ''adaptive'' (enhanced metabolism of xenobiotics), ''toxic'' (adverse effects of dioxin-like compounds) or ''developmental'' (physiologic functions in the absence of xenobiotic chemicals). Similarly, it may be rational to attempt to classify the various alterations of AHR levels/AHR activity into categories of ''adaptive'' or ''maladaptive''.

4.1. *Adaptive changes in AHR levels or AHR activity*

The variations in AHR levels that occur physiologically during development are, almost by definition, an adaptive change. Ligand-induced down-regulation due to receptor proteolysis also can be viewed as an adaptive change that provides short-term desensitization of the cell and may ameliorate potential adverse effects that could occur if AHR pathways were threatened with ''over-stimulation'', particularly by highly potent xenobiotic agonists.

The modest increase in AHR levels that is observed in some tissues after persistent exposure to TCDD also can be viewed as an adaptive response given that, on balance, induction of CYP1 enzymes and phase-II enzymes mediated by the AHR generally protects the animal from toxicity of xenobiotic chemicals [86,87].

4.2. *Maladaptive changes in AHR levels or AHR activity*

AHR expression has been reported to be up-regulated in several types of human tumors (Table 1). It is not known whether up-regulation of AHR is central to the pathogenesis of these cancers or whether increased expression is an epiphenomenon that accompanies neoplastic transformation in some cell types.

It is known, however, that higher cellular AHR levels generally favor cell proliferation. Moreover, tumor promoting agents such as TCDD act via the AHR to inhibit apoptosis of

malignantly transformed cells. The potential for high AHR levels to simultaneously support proliferation of tumor cells and to block their apoptotic destruction clearly is advantageous to the tumor cell but detrimental to the animal hosting the tumor.

The impact of AHR levels in tumor cells depends on factors other than the AHR alone, most notably, possible exposure to exogenous ligands. In the absence of xenobiotic AHR agonists, cell clones selected to be AHR-deficient (relative to their parental line) exhibit a decreased proliferation rate in culture [6,88]. Fibroblasts from *Ahr*-null mice also have a low proliferation rate [5]. Thus, from the perspective of the cell in culture, loss of the AHR or a decrease in AHR levels is an impediment to growth. On the other hand, in circumstances where cells are exposed to potent xenobiotic AHR-agonists such as TCDD, the effect of the agonist usually is to inhibit cell proliferation. Even though TCDD can inhibit cell proliferation by acting on the AHR, TCDD is a potent tumor promoter. Like virtually all toxic effects of TCDD, TCDD's tumor promoting activity occurs via AHR activation which, through uncertain pathways, prevents apoptotic removal of cells that have been malignantly transformed by other agents [89].

Even if elevated AHR levels in tumor cells are maladaptive by fostering their survival and proliferation, the AHR in some tumors also presents a new therapeutic target for cancer chemotherapy. Safe [90] has advocated development of selective AHR modulators (''SAhRMs'') for treatment of breast or prostate cancers to exploit the ability of the AHR to interfere with steroid signaling pathways in these tumors. In another recent development, novel anti-neoplastic drugs have been designed whose cytotoxic activity depends on activation of the drug by CYP1 enzymes. These agents may be particularly well targeted to cells that express high levels of AHR; they are not effective in cells that are AHR-deficient [91,92].

4.3. *Changes in AHR levels or AHR activity whose consequences are uncertain*

From the viewpoint of the cell or organism, some changes in AHR expression may be of little consequence. For example, the decrease in AHR levels that occurs in older animals hypothetically could be one of the factors that contribute to the aging process. Human keratinocytes in culture exhibit a very large increase in AHR levels as they stop proliferating and undergo senescence [93]. It is possible, in teleological terms, that this increase in AHR represents a futile ''attempt'' by the cell to restore proliferation and avoid senescence. As with many initiatives to identify ''aging'' factors, it would not be a trivial matter to determine whether the decline in AHR plays an active role in mammalian aging or whether the decrease in AHR simply is an indifferent companion to the aging process.

5. Unknowns and challenges

As summarized in this Commentary, an astonishing number of factors intertwine in regulation *by* the AHR and regulation *of* the AHR. We do not yet know the full breadth of biological events that are regulated by the AHR nor do we have a comprehensive understanding of the spectrum of endogenous and exogenous factors that regulate levels and activity of the receptor itself.

5.1. *Unknowns*

The key unknowns regarding ''what regulates the regulator'' with respect to the AHR can be summarized under three ''Ts'':

Tissue-specific factors (spatial distribution). We have only rudimentary knowledge of the mechanisms that account for the wide variation in AHR expression levels across different tissues and across different vertebrate species. HNFs are undoubtedly important to the high expression seen in most mammalian livers but we do not know what accounts for high levels of expression in such tissues as human placenta. Relatively few studies have been done to map functional regulatory motifs in non-coding regions of vertebrate *AHR* genes in order to better understand the endogenous and exogenous factors that govern this gene's transcription. In silico comparisons of conserved regulatory motifs (''phylogenetic footprinting'') and laboratory-based detailed functional analyses of the promoter and 5′-flanking region of the *AHR* gene in multiple animal species should be a productive avenue for identifying and mapping the key regulatory elements that are responsible for tissue-specific differences in constitutive AHR expression as well as elements that permit modulation of AHR expression by xenobiotic chemicals.

Temporal regulation (developmental expression at specific times). Currently the information that we have on AHR expression during development is mainly descriptive. For example, TCDD is able to affect closure of the ductus venosus in mouse embryos as early as day 12.5 [8] but we lack insight into what governs appearance of the AHR in different cells and tissues during embryogenesis and early post-natal development.

Tumor dysregulation. Up-regulation of AHR in human cancers may be important both in pathogenesis of the disease and in relation to potential chemotherapy. For prostate cancer cells, up-regulation of AHR levels is via WNT/β-catenin pathways [94]. For other tumor sites such as pancreas (where AHR overexpression is dramatic [95], Table 1) we have no mechanistic explanation.

5.2. *Puzzles and challenges*

Related to pancreas, a recent clinical study found that the AHR's dimerization partner, ARNT, is dramatically down-regulated in pancreatic beta cells in patients with type-2 diabetes and that this loss is functionally important in the disease [96]. This significant clinical finding indicates that altered levels of components in the AHR signaling pathway can have substantial impacts on health. It is not yet known whether down-regulation of ARNT in pancreas might also affect functions of the AHR. However, mice that are hypomorphic for ARNT (ARNT expression reduced to about 10% of wildtype) are highly resistant to dioxin toxicity but still display essentially normal CYP1A1 induction in response to AHR agonists [82]. This reminds us that the consequences of altered

levels of components in the AHR pathway may not be the same for all downstream events controlled by the receptor.

One curiosity that arises from consideration of multiple components and multiple endpoints in AHR pathways is the efficacy of the AHRR protein. AHRR does not seem to be all that successful at repressing expression of the prototypical AHR-regulated gene, *CYP1A1*, as evidenced by the fact that in many tissues CYP1A1 levels and AHRR levels seem to rise in parallel after treatment with an AHR agonist. Is AHRR better at preventing overexpression of *other* genes than it is at suppressing induction of CYP1A1? Is AHRR primarily a mechanism to dampen the influence of xenobiotic chemicals rather than being a key regulator of normal physiologic functions of the AHR? The fact that constitutive expression of AHRR (in the absence of xenobiotic chemicals) is very low in most tissues suggests that AHRR ''rises to the occasion'' mostly after the cell has been challenged by a foreign chemical. Perhaps gene expression array studies in cells transfected with AHRR constructs expressed at different levels can be used to test the ability of AHRR to suppress induction of genes other than *CYP1A1* to gain some appreciation of the broader functions of this repressor.

Another mystery is whether our understanding of factors that regulate AHR levels and AHR activity is unduly colored by the fact that most studies in this field have been conducted with toxic exogenous ligands such as TCDD. For example, degradation of the AHR protein and induction of AHRR both are responses to exposure to exogenous ligands. Is there, in fact, an equivalent mechanism that down-regulates receptor levels or receptor function in response to *endogenous* signals as suggested by Pollenz et al. [71]?

Fig. 1 portrays the AHR as a landmark at the crossroads of multiple regulatory pathways. Regulation *by* the AHR and regulation *of* the AHR are likely to constitute a web of interactions with complex levels of crosstalk, feedback and feed-forward. This predicted complexity arises inevitably from the vast number of factors that lie on both the input and output sides around the AHR fulcrum. By thoroughly mapping the biological responses regulated by the AHR and determining its normal physiologic roles we will be in a better position to understand how modulation of AHR levels and activities affects the overall function of the organism. In teleological terms, the better we grasp the functions of the AHR the more able we will be to understand ''*why*'' the AHR needs to be regulated as springboard to determining *how* it is regulated.

Modeling of complex genetic networks is a formidable task but recent developments suggest that it may be possible to achieve ''coarse-grained'' models that provide utility for understanding regulation, even in the absence of extensive kinetic details about interactions among the components [97]. A systems approach that successfully integrates current information and fills in critical knowledge gaps about relationships among components in AHR pathways is not on the immediate horizon; however, essential data continue to accrue. For example, recently we used gene expression arrays in *Ahr*-null mice contrasted with wildtype mice to identify batteries of genes that are regulated by the AHR in dioxin-dependent or dioxin-independent fashions [98]. Many AHR-regulated genes that emerged from this study were the ''usual suspects'' encoding CYP1 enzymes or Phase II enzymes. However, this array study also revealed numerous AHR-regulated genes whose products carry out diverse functions not hitherto suspected of being susceptible to AHR control.

We still are largely in the dark ages in our attempt to understand the factors and forces that regulate expression and activity of the AHR. There are many observations and phenomena, but no simple unifying model to integrate these multiple potential mechanisms. Younger investigators need not worry that they will run out of puzzles to solve in this field.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2006.01.007.

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