PART I

HISTORICAL BACKGROUND
1

HISTORY OF RESEARCH ON THE AHR

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1.1 INTRODUCTION

Since Alan Poland's laboratory at the University of Rochester published the first report on the identification of the Ah receptor (AHR) in 1976 [1], there have been nearly 6000 additional publications (as determined by a PubMed search) detailing the characteristics of this protein, its function and regulation, and consequences of its activation in a variety of biological systems. As a graduate student at Rochester at the time of Alan’s discovery, I can still recall his dedication, excitement, and enthusiasm for what were particularly novel, but at the time somewhat controversial, findings. Little did we know that such a “cottage industry” of research activity (as Alan described it years later) would sprout from these initial studies. Over the years, many individuals contributed to the existing body of work on the AHR, and each one of these contributions has added some piece to our understanding of this transcription factor. Nevertheless, there have been major milestones along the way that have significantly transformed the field and that are likely to define the direction of future investigations. Many of these milestones have come about through a logical progression and focus in a particular research stream. Some were more serendipitous findings. Still other advances have been enabled by some major development in other areas of research that initially had little to do with the AHR. Finally, in some cases the importance of seemingly minor discoveries has yet to be appreciated.

The purpose of this chapter is to give an overview of the emergence and evolution of milestones in research on the AHR (Fig. 1.1), what these tell us about AHR biology in cells and tissues, and how this biology may relate to human disease. The involvement of the AHR in the toxicity of many xenobiotics is certainly one important part of this field. Much knowledge has been and will be gleaned about the possible normal function of this protein from our knowledge of the toxicity caused by these xenobiotics. However, we primarily touch on this vast toxicology literature only inasmuch as has contributed to our understanding of a biological function of this protein. Readers are referred to several excellent reviews that have discussed toxicity mediated by the AHR in both humans and a variety of animal models [2–6]. It is also worthwhile to point out that an earlier publication by Allan Okey [7] was very useful in providing references and reminders about some of the historical events in this area of research. It is hoped that this chapter complements and extends that provided by Dr. Okey.

1.2 A HYPOTHESIS IS CONCEIVED, AND GENETICALLY DEFINED GROWTH AND EXPANSION OF THE HYPOTHESIS LEADS TO THE IDENTIFICATION OF THE Ah LOCUS

In the early 1950s while working as a graduate student in the lab of James and Elizabeth Miller at the University of Wisconsin, a young Allan Conney became intrigued by the findings of H. L. Richardson and colleagues at the University of Oregon Medical School that treatment of rats with the carcinogen 3-methylcholanthrene (3-MC) inhibited the hepatocarcinogenic activity of 3'-methyl-4-dimethylaminoazobenzene [8]. From this grew a hypothesis that treatment with 3-MC or other polycyclic aromatic hydrocarbons (PAHs) would alter the metabolism of the carcinogenic aminoazo dyes and thus protect from subsequent carcinogenic activity. Allan went on to find that indeed a single injection of 3-MC
could induce the activity of not only aminoazo dye N-de- 
methylase and azo-link reductase [9], but benzo(a)pyrene 
(BP) hydroxylase as well [10]. Further work by Dr. Conney 
while at the National Institutes of Health (NIH) in Bethesda 
showed that there was a selective stimulation of the metab-
olism of some drugs and xenobiotics but not others [11], 
suggesting that the induction of these enzymes was under 
separate regulatory control. This was the first bit of evidence 
consistent with the notion that there may be a selective 
signaling pathway that mediates this enzyme induction.

A major step in the pathway leading eventually to the 
identification of the AHR came when it was recognized by 
Dan Nebert at the NIH in the late 1960s that aryl hydrocar-
bon hydroxylase (AHH; previously called BP hydroxylase) 
was inducible by a number of PAHs in several inbred mouse 
strains but not others [12, 13]. This was consistent with an 
earlier observation that some inbred strains differed in 
sensitivity to skin ulcerations in response to 7,12-dimethyl-
benzanthracene [14]. Nebert’s group further found that 
the inheritance of this inducibility among mouse strains segre-
gated primarily as an autosomal dominant trait [15, 16]. 
Based on this responsiveness to aromatic hydrocarbons, a 
new genetic locus, the Ah locus, was defined. Later it was 
found that the inheritance of this trait in mice is actually 
more complex when crosses in other strains were exam-
ing [17]. Nevertheless, it was clear that in some mouse 
strains 3-MC and other PAHs induced AHH activity, while in 
other strains no such induction was observed; the responsive 
Ah<sup>b</sup> allele was typified by C57BL/6 mice, whereas the 
“nonresponsive” Ah<sup>a</sup> allele was typified by DBA/2 mice. 
Additional work by Dr. Nebert and a number of other groups 
demonstrated that besides AHH inducibility, the Ah locus 
also regulates carcinogenic, mutagenic, teratogenic, and 
toxic responses to PAHs that are closely related to the ability 
of these compounds to be metabolized by the induced 
enzymes [17, 18].

### 1.3 A FOCUS ON PUBLIC HEALTH LEADS TO 
THE IDENTIFICATION OF THE Ah RECEPTOR

In the early 1970s, Alan Poland was a physician interested in 
the mechanisms by which workers in factories producing 
2,4,5-trichlorophenol (2,4,5-T) succumbed to industrially 
acquired acne (chloracne) and porphyria cutanea tarda [19]. 
While 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was 
known to be the active acnegenic contaminant in 2,4,5-
T [20, 21], the cause of the porphyria was still unclear at 
the time. To test whether TCDD was also responsible for the 
porphyria, Dr. Poland examined the effects on ALA synthet-
ase, the rate-limiting step in the heme biosynthetic pathway, 
using a chick embryo model. TCDD was extremely potent in 
inducing ALA synthetase in this model [22]. Since it was 
found that many compounds that induced ALA synthetase 
also induced microsomal mixed-function oxidase activity, 
Dr. Poland’s lab did additional work to show that TCDD was 
also a potent inducer of AHH activity [23]. Notably, they 
observed that the structure–activity relationship (SAR) for 
the ability of different dioxin congeners to induce AHH 
was the same as that for ALA synthetase, strongly indicating 
that these responses were mediated by the same initial step 
or binding site [22, 23]. Importantly, these were the first SAR 
studies of these compounds, predating the identification of 
the AHR, detailing a structural basis for their toxicity. Since 
that time, these structural requirements have been further 
refined and are still widely used to show that a particular 
response is mediated by the AHR. This principle also served 
as a basis for the many screening assays developed later to 
assess the presence of dioxin-like chemicals in the environ-
ment, as well as for risk assessment of these chemicals.

Several important additional steps were taken when 
Dr. Poland’s lab demonstrated that TCDD is about 30,000 
times more potent than 3-MC at inducing AHH in rat liver 
[24], and then, teaming up with Dr. Nebert’s group, showed
that TCDD could overcome the “nonresponsiveness” to 3-MC in a number of mouse strains [25]. These studies clearly indicated that the “nonresponsive” mice possess the regulatory and structural genes for the expression of AHH activity. However, the question remained about exactly what was the defect in these mice that made them “nonresponsive” to 3-MC but responsive to TCDD. The additional finding that responsive and “nonresponsive” mouse strains differed in their sensitivity to TCDD as well [26] was clearly consistent with the hypothesis that the product of the Ah locus is a binding site for both 3-MC and TCDD and initiates events leading to the expression of the gene encoding AHH. Furthermore, these data indicated that the mutation in the Ah locus in “nonresponsive” mice is responsible for the production of a “receptor” with reduced affinity for 3-MC, TCDD, and other inducing chemicals [27].

Synthesis of radiolabeled TCDD by Andrew Kende at the University of Rochester was the important technological advancement that ultimately led to the identification of the AHR protein by Dr. Poland’s lab [1]. This landmark study utilizing detailed cell fractionation studies, SAR for both binding and enzyme induction, basic protein biochemistry, and taking advantage of the genetic differences between mouse strains identified a high-affinity and saturable TCDD binding protein that exhibited the same SAR for binding other dioxins and PAHs as their ability to induce AHH activity. This investigation still stands (at least in our opinion) as the single most important contribution to this field.

1.4 THE 1980s: GROWTH AND MATURATION OF THE PARADIGM

Dr. Poland had postulated that the binding of TCDD or other PAH ligands initiates a series of events whereby the AHR–ligand complex translocates to the nucleus and, in a manner analogous to that described for steroid hormone receptors, alters transcription and translation of “induction-specific” RNA [1]. In the years subsequent to the initial identification of the AHR and the hypothesis regarding its mechanism of action, there was a flurry of activity from Dr. Poland’s lab and many others to further characterize the properties of this protein, examine its presence in a variety of tissues and different species, and detail the events leading to its ability to modulate gene expression. Work by Bill Greenlee while a graduate student in Dr. Poland’s lab [28], and by Allan Okey while on sabbatical in the Nebert lab [29], demonstrated ligand-dependent nuclear translocation of the AHR. This was later confirmed by Rick Pollenz, working in Dr. Poland’s lab (then at the University of Wisconsin), who used immunofluorescent approaches and recently developed antibodies against the AHR [30, 31]. Studies in a number of other labs, including that of Mike Denison (while at Cornell University and then with Dr. Okey), Tom Gasiewicz at Rochester, Jan-Ake Gustafsson at the Karolinska Institute, Allan Okey in Toronto, and Lorenz Poellinger (originally with Dr. Gustafsson), identified and confirmed properties of the AHR in a variety of tissues and species. These investigations noted similarities and differences in both physical–chemical characteristics and relative binding affinities for TCDD in a variety of species including humans [32–42]. A striking and consistent finding from these studies was that the apparent characteristics of the AHR complex changed following ligand binding and translocation into the nucleus [36, 43]. These data suggested that other proteins were associated with the AHR under physiological conditions and that these associations may be necessary for AHR function as a gene regulatory protein.

From these studies, an overall model of action for the AHR was emerging that, as Dr. Poland had predicted, was similar to that for the steroid hormone receptors. But it was still unclear whether the AHR directly bound to DNA or indirectly modified gene expression, and, if the former, whether binding to DNA was assisted by another protein or proteins. Utilizing a series of mutant cell lines and examining differences in their ability to induce AHH in response to TCDD, Oliver Hankinson and his group at the University of California at Los Angeles and others collaborating with Dr. Hankinson obtained results implying that AHH induction requires formation of the TCDD–AHR complex as well as the interaction of this complex with some other component(s) of the cell nucleus [44–46]. The same conclusions were reached by Jim Whitlock and his group at Stanford examining differences in the ability of mutant cells to metabolize benzo[a]pyrene [47, 48]. Although it became recognized that the TCDD–receptor complex is a DNA binding protein [36, 49], the mechanisms that designated specificity were still not known.

Through a series of extensive and detailed investigations of the mechanisms whereby the cytochrome P1-450 gene (Cyp1a1) (the product responsible for AHH activity) was induced by TCDD, the Whitlock lab identified the specific nucleotide sequence to which the nuclear AHR complex binds within the 5’-flanking region of the gene [50–52]. The discovery immediately had broader implications for other genes and species, including humans. One important aspect was that the dioxin-responsive element (DRE), as it was called, to which the AHR bound, could activate transcription from a heterologous promoter and even within a heterologous species, that is, not just mice, but rats as well as humans. The discovery was confirmed by the Fujii-Kuriyama laboratory at Tohoku University who named the enhancer element the “xenobiotic-responsive element” (XRE) in recognition of the fact that other xenobiotics, in addition to TCDD, could activate transcription through this AHR-dependent pathway [53]. The identification of the core recognition sequence (5’-TNGCGTG-3’), more recently termed AHR-responsive element (AHRE), was arguably the second most important
landmark in the history of AHR research. Many groups have subsequently identified AHREs in the upstream regions of numerous genes in addition to Cyp1a1 [54–57] and refined our understanding of variations that likely account, at least in part, for noted differences in the regulation of a variety of AHR-responsive genes within a given species as well as between species. For example, differences in the nucleotides flanking the core AHRE have been shown to affect both binding of the AHR complex as well as transcription [58, 59]. While data showing that the AHR complexes from different species are able to bind the mouse AHRE suggested some conservation of this interaction [60], more recent data suggest that the set of target genes regulated by the AHR may not be well conserved across species [61]. In addition, several investigators discovered an AHRE-II enhancer element (CATG[N6]C[T/A]TG) that is conserved across human, mouse, and rat orthologs and that appears to regulate some sets of genes for ion channels and transporters [62, 63]. Furthermore, as first demonstrated by the work in Steve Safe’s lab at Texas A&M, it has become clear that inhibitory effects on certain genes can be produced by the positioning of the AHREs relative to other transcription factor binding sites [64–67]. Similarly, the presence of negative regulatory elements within AHR-responsive genes has been shown to inhibit AHR-dependent transcription without affecting AHRE binding [68, 69].

Shortly after the discovery of the AHRE, it was observed that the AHRE binding form of AHR has different physicochemical properties from the cytosolic form [70–72], indicating that there was an additional, as yet unidentified, component that helped to designate specificity of binding. This was further consistent with Dr. Hankinson’s data, indicating that certain mutants of a mouse hepatoma cell line have a functionally defective ligand–AHR complex that fails to accumulate in the nucleus [44, 46]. In 1991, Hankinson’s group reported the cloning of a human gene capable of restoring AHR nuclear uptake and function in these cells and named it the AHR nuclear translocator, or ARNT [73]. Shortly thereafter, they recognized that ARNT heterodimerizes with the AHR to generate the active AHRE binding transcription factor [74]. Later, it was determined that in most cells ARNT resides predominantly, if not exclusively, in the nucleus and does not appear to be directly involved in translocating the AHR [31]. The discovery of ARNT was an exciting and significant breakthrough for a number of reasons. First, it solidified a mechanism whereby AHR could directly act as a transcription factor and selectively regulate gene expression. The AHR–ARNT complex became the first example of a heterodimer within the basic helix–loop–helix–Per/Arnt/Sim (bHLH–PAS) family of proteins [75]. Furthermore, this provided one of the first insights that the AHR signaling pathway could interact with other intracellular signaling pathways making the biology of the AHR much more complex—and indeed more intriguing.

Several years after ARNT was cloned and the amino acid sequence defined, it was recognized that the hypoxia-inducible factor (HIF) protein was composed of two subunits, HIF1α and HIF1β, and that in fact HIF1β was identical to ARNT [76]. There has been much subsequent work on the interactions between the hypoxia and AHR signaling pathways [56, 77–79].

About the same time that the AHRE was discovered and defined, Dr. Poland’s lab, then at the University of Wisconsin, was working vigorously to purify and sequence the AHR. The development of the radiolabeled photoaffinity ligand 2-azido-3-[125]iodo-7,8-dibromodibenzo-p-dioxin by Dr. Kende provided the essential tool leading to this purification and the preparation of the first AHR-specific antibodies [80–83]. Several years later, but a year after ARNT was identified, two labs, Dr. Bradfield’s in Wisconsin and Dr. Fujii-Kuriyama’s, reported the cloning of the receptor’s cDNA [84, 85].

### 1.5 DETAILS, DETAILS

The cloning and sequencing of both the AHR and ARNT were very significant developments that enabled rapid progress on several fronts. First of all, it became clear that the AHR was not similar in sequence and structure to the steroid hormone receptors as many had believed. In fact, AHR and ARNT contained domains with high sequence homology to two recently identified proteins in Drosophila, PER and SIM [86–89]. Since that time, the bHLH–PAS family of transcription factors has expanded rapidly to include a number of other proteins such as HIF, CLOCK, and MOP that are involved in physiological responses to cellular and tissue environmental signals including circadian rhythms, oxygen tension, and oxidation–reduction status [75, 90]. At about the same time that the AHR and ARNT were being purified, cloned, and sequenced, additional data were accumulating from many investigators, including Chris Bradfield, Gary Perdew, and Lorenz Poellinger, to indicate that several other proteins — hsp90, p23, and XAP2 (also called ARA9 or AIP) — interact with the AHR to regulate its intracellular stability, ligand binding ability, and cytoplasmic– nuclear trafficking [91–101]. Mapping of specific domains within the AHR and ARNT that were coupling sites for these proteins provided confirmation of the interactions and a further understanding of the mechanisms by which ligand binding regulated AHR function. The mapping of these domains also provided a greater mechanistic understanding of the specificity for ligand binding, AHRE recognition, and transactivation of gene expression [102–110].

Second, the cloning and sequencing of the AHR have expanded our understanding of the evolution of this protein and its possible function in a variety of species. Early studies demonstrated some similarity in TCDD binding ability of the AHR contained in different lab animals [37, 111]. However,
more detailed physicochemical analyses demonstrated clear differences in molecular mass of AHR proteins from a variety of species and among rodent strains [112–114]. Subsequent cloning of the AHR genes from a number of animals, mainly by Mark Hahn’s lab at the Woods Hole Oceanographic Institution [115–121], revealed some interesting features: (1) Mammals appear to have a single AHR gene, while other vertebrates such as fish and birds may have up to five. Additional investigation of these divergent forms may provide interesting clues to functions of the AHR. (2) The N-terminal region of the AHR, containing the DNA binding domain, nuclear localization sequence, and dimerization domain within the bHLH–PAS region, is, for the most part, highly conserved. The C-terminal region, containing the transactivation domain, is more variable. Although yet to be determined, these variations may eventually help explain the differences in the spectra of AHR-responsive genes among species, as well as the differences in sensitivity of these species to the toxic effects of various xenobiotics (see Section 1.6). (3) Invertebrates possess single AHRs that do not recognize the typical xenobiotics that bind and activate vertebrate AHRs. Based on these findings, Dr. Hahn proposed that the ability of the AHR to bind to dioxins, PAHs, and other xenobiotics, and thus also the ability to regulate xenobiotic metabolizing enzymes, occurred through gene duplications and gene loss during the evolution of vertebrates [118]. (4) Differences in AHR binding affinities and sensitivities to AHH induction among mouse strains were verified and found attributable to a mutation of alanine to valine within the ligand binding domain (LBD) [115]. (5) The latter finding was of particular interest because cloning of a human receptor (hAHR) [122] indicated a valine at position 381 and a proline at position 480 that are also present in the human receptor (hAHR) [122] indicated a valine at position 381 and a proline at position 480 that are also present in the human receptor (hAHR) [122]. This knowledge may prove to be important in estimating the relative risk to humans of toxic AHR ligands such as TCDD (see below). On the other hand, development of these approaches will also facilitate the assessment of human AHR polymorphisms to determine whether some humans may possess high-affinity receptors or other AHR mutations that predispose (or protect) these individuals to (from) certain diseases (see below).

1.6 LET US NOT FORGET THE TOXICOLOGY

1.6.1 New Questions

Although much research during the 1970s, 1980s, and early 1990s focused on the properties of the AHR and the mechanisms whereby this protein modulates gene expression, the toxicology of xenobiotic AHR ligands continued to be a focal point. In fact, the discovery of the AHR greatly expanded the research efforts on dioxins and dioxin-related chemicals as well as the number of toxicologists and individuals in regulatory agencies that became involved in these efforts. New questions about the toxicity of these chemicals and possible relationships to the AHR needed to be addressed. Does the AHR mediate the toxicity of these chemicals? If so, is the induction of AHH or other drug metabolizing enzymes responsible for this toxicity? If not, then what other genes might be responsible? Do the different properties of the AHR among species have anything to do with the varying sensitivities of these species to toxic AHR ligands? Can the properties of the hAHR give us clues as to the relative sensitivity of people to these chemicals? How can our understanding of the properties of the AHR and the mechanisms by which it mediates toxicity and regulates intracellular processes be used to improve our risk assessment of these chemicals? Also, based on this knowledge, what might be the most sensitive toxic end points in people and thus the potentially most sensitive subpopulations?

1.6.2 Agonists Versus Antagonists—Or Somewhere In Between

In the early 1980s, Dr. Poland’s laboratory reported that the toxicity of TCDD in various mouse strains segregated with the Ah locus and that the potency of various dioxins, halogenated biphenyls, and PAHs to bind to the AHR correlated with their ability to produce a variety of toxic end points and was structure dependent [123, 124]. In the subsequent 15 years, these data were extended by a number of laboratories. In particular, work by Dr. Steve Safe and his colleagues (first at the University of Guelph and later at Texas A&M University) increased the tools available by synthesizing a number of chlorinated dibenzofurans, dibenzo-α-p-dioxins, dibenzofurans, and biphenyls (PCBs) that were previously unavailable for research purposes. They and others demonstrated that, for the most part (except for those congeners more susceptible to metabolism), the SAR for AHR binding was closely related to the ability of the chemicals to produce a number of toxic end points in different laboratory animals [125–130]. These studies also extended our understanding of the structural requirements for ligand binding to the AHR (see further discussion of AHR ligands below).

The interest in SAR also led to the search for compounds that could bind to the AHR but act as AHR antagonists to block toxicity. In the 1980s, Dr. Safe and colleagues recognized that some of the chlorinated dibenzofurans and biphenyls had relatively high affinity for the AHR but were poor inducers of AHH activity, and furthermore could partially antagonize the response elicited by TCDD [131]. This finding led to the synthesis of additional compounds by the Safe lab that proved to be effective antagonists [132–134]. These compounds, as well as those later identified and/or...
synthesized by the labs of Drs. Safe, Gasiewicz, Perdew, and others [135–142], provided additional evidence for the role of the AHR in mediating toxicity, as well as valuable tools for exploring mechanisms by which the AHR acted to modulate cell functions. The potential of such compounds to block the toxicity caused by the inadvertent exposure of animals and humans to TCDD was also being explored. However, as research progressed on these compounds, it became clear that many factors determine their relative ability to block toxic effects mediated by the AHR. While most of the identified AHR antagonists appear to compete with TCDD for the same binding site, others may act as antagonists by several different mechanisms [143–148]. Furthermore, those that bind to the AHR may affect its activity in a very species-specific manner such that a compound may act more like an antagonist in one species but more like an agonist in another species [149, 150]. Importantly, the agonist versus antagonist activity of a particular compound may depend on relative affinity as well as intrinsic efficacy [151], coincident with an ability to produce a particular conformational change that may be dependent on the species-specific amino acid sequence within the ligand binding site [106, 150, 152]. Thus, while the development of antagonists provided valuable tools for research, their more practical applications to incidents of poisoning were hindered by the lack of understanding of the factors that regulated their ability to turn on or turn off AHR activity, the specificity of the chemicals for these actions, and the growing knowledge that the AHR may have some normal functions (see below).

1.6.3 The AHR Model as a Means to Approach Risk Assessment

It is important to note that evidence to date based on SAR and genetics supports the contention that the toxicity of TCDD and related chemicals is mediated through the AHR. Although over the years there have been some reports suggesting that certain toxic effects, particularly some immune system alterations, may be AHR independent [153–158], there is a general consensus that the AHR has an obligatory role in TCDD-induced toxicity, and this is an important factor in risk assessment. However, since TCDD is just one of a large number of structurally related contaminants that are widely distributed in the environment, generally in complex mixtures, the toxicity testing of all of them individually would be cost prohibitive. Recognition that many of them likely act through this same AHR-dependent mechanism led the international community in the late 1980s and early 1990s to devise an approach to risk assessment for these congeners based on a principle of “toxic equivalency factors” (TEFs) [159]. In 1990, Dr. Safe published a summary of the existing information on the relative potency of these chemicals and suggested TEF values for those chemicals that had dioxin-like activity [160]. Based on additional data over the years, these values have undergone several reevaluations [161]. Despite the general acceptance of the use of TEFs and validation of the approach by several labs [162–166], considerable controversy remains [167–172]. Today, it is widely recognized that the assigned TEF values are of necessity approximations based on incomplete data and may not accurately reflect additive or synergistic interactions or the actual metabolism of individual compounds within different tissues, considerations of different types of exposures (e.g., acute versus chronic), different toxic end points in different animal species, and the possible species-specific agonist versus antagonist activity of different ligands (as discussed above; see also Ref. 173). Although it is clear that knowledge gaps in the differences in biology among these chemicals need to be addressed to more accurately approach the risk assessment for xenobiotic AHR ligands, the concept that their toxicity is mediated by the AHR has nevertheless played, and will continue to play, a key role in driving the directions for this process.

1.6.4 More Evidence to Support the Model: Knockouts, Lots of Genes, and More Questions

As the evidence mounted that the toxicity of TCDD and related xenobiotics was mediated through the AHR and that this protein clearly functioned as a ligand-activated transcription factor, much research in the late 1980s became directed at determining the genes that were dysregulated by TCDD and associated with particular toxic responses. Early studies were focused on the “AH gene battery” that was initially identified and included those genes encoding CYP1A1, CYP1A2, NAD(P)H:quinone oxidoreductase, aldehyde dehydrogenase 3, UDP-glucuronosyltransferase 1A6, and glutathione S-transferase Ya [192]. Altered CYP expression may contribute to tissue damage and tumorigenesis, for example, through induced metabolism of some xenobiotics and DNA adduct formation, as well as oxidative DNA damage via the production of reactive oxygen species [3, 174–177]. The clearest association between TCDD-elicited induction of a Cyp gene and induced toxicity was made though the collaboration of the Andrew Smith lab at Leicester University, Dan Nebert (then at the University of Cincinnati), and Peter Sinclair at Vermont, when it was found that knocking out the expression of the Cyp1a2 gene protects against uroporphyria and hepatic injury following treatment with TCDD [178]. In most cases, however, a clear association between induction of these enzymes and the species- and tissue-specific toxicity of TCDD has not been shown; indeed, it has been demonstrated, for example, that some tissues and species show a substantial induction of these without observable toxicity [179], and elimination of CYP1A1 expression does not ablate induced toxicity [180].

The 1990s brought new technology to the search for differentially expressed genes. Drs. Sutter and Greenlee,
working at what was then called the Chemical Industry Institute of Toxicology (now the Hamner Institute for Health Sciences), first used differential hybridization to identify in human keratinocytes several genes regulated by the AHR that may play a role in TCDD-elicted alterations in inflammatory and differentiation processes [181]. Since that time the invention and use of such technologies as gene expression arrays has resulted in the identification of hundreds of TCDD-modified genes and signaling pathways in a variety of tissues [182–191]. Advances in cloning, sequencing, bioinformatics, biostatistics, transfection techniques, and chromatin analyses and the use of specific gene reporters have further allowed researchers from the labs of Dr. Puga at Cincinnati, Dr. Zacharewski at Michigan, and others to determine the presence of functional AHREs in regulatory regions with the objective to define which modified genes are actually directly responsive to the AHR and the interactions between the signaling pathways regulated by these genes [54, 56, 57, 61, 189, 192, 193].

Targeted gene disruption is an additional technology developed in the 1990s that has enabled another milestone in our understanding of the AHR’s roles in both toxicity and normal physiology. In 1995, Frank Gonzalez and his group at NIH published the first report describing the successful creation of an Ahr-null allele (AHR-KO) mouse [194]. This was closely followed by publications from the Bradfield and Fuji-Kuriyama labs, also demonstrating the creation of KO mice based on targeting a different exon of the Ahr gene [195–197]. Studies using these mice verified that the presence of the AHR is necessary for susceptibility to a number of TCDD-induced effects, including lethality, hepatotoxicity, immunotoxicity, teratogenesis, effects on developing prostate and uterus, and carcinogenicity [194, 196, 198–206]. These mice are also resistant to BP-induced carcinogenesis, UV radiation-induced inflammatory responses in the skin, and benzene-induced hematotoxicity [207–209].

These data coupled with our growing knowledge of the details of AHR structure and characteristics have given us a wealth of information. However, both despite and because of this knowledge, it has become clear that determining what specific molecular pathways designate a particular toxic outcome in response to TCDD is likely to be much more complex than what most of us working in this area might have believed. By the end of the 1990s, several features had become apparent. For example, although the presence of the AHR appears to be necessary for toxicity, it is not sufficient. Accumulating data show that the toxic and genomic responses to TCDD and related chemicals are clearly dose, cell, tissue, and species specific. These different molecular and biological responses may reflect differences in the developmental stage, state of cell cycling and differentiation, and the tissue environment surrounding the particular cell type responding to TCDD. In most cases, the embryo and fetus are much more susceptible to the toxicity of dioxin-like chemicals than adult animals (see Ref. 210 and Section 1.9.1). We also know that species and strain differences in responses likely reflect differences in AHR structure. As indicated above, certain mouse strains possess a mutation in the ligand binding domain that alters their affinity and thus sensitivity to certain dioxin-like xenobiotics [115]. Other data resulting from collaborations between Dr. Pohjanvirta’s lab in Finland and that of Dr. Okey indicate striking differences in the transactivation domain of the AHR among species and strains that likely alter the AHR’s ability to differentially interact with other transcription factors, coregulators, or corepressors to produce gene selectivity [120, 121, 211–213]. In addition, it is becoming more apparent that in most, if not all, cases, TCDD-induced toxicity results from the dysregulation of multiple genes and pathways rather than one specific gene. To date, only a few specific target genes and signaling pathways have been associated with particular end points of toxicity [214, 215]. As such, tissue-, species-, and developmental stage-specific differences in the regulation of one or several of these pathways are likely to make a huge difference in the ability of TCDD to elicit a biological response. Furthermore, it is becoming recognized that developmental, long-lasting, and even transgeneration effects of TCDD and related xenobiotics may be mediated by epigenetic mechanisms. As the 1990s progressed and ended, more and more researchers in this area have been focusing their attention on the mechanisms of very specific and sensitive in vivo responses to TCDD, recognizing that many in vitro systems may not accurately represent the in vivo situation and that defined mechanisms in one tissue may not be relevant in another tissue or even at a different stage of development. Many of the chapters in this book are a reflection of these efforts (see Fig. 1.2).

1.7 RECOGNIZING THE IMPORTANCE OF REGULATING AHR PRESENCE AND ACTIVITY

Since the discovery of the AHR, many publications have documented differences in its expression among a variety of tissues and cells and changes in its expression or activity in response to a number of agents and conditions (reviewed in Ref. 216). Although it was well recognized that these changes could potentially alter a tissue’s toxic response to xenobiotic ligands, throughout most of the 1990s there was little understanding of the mechanisms that regulated AHR levels in tissues or its activity. In the past 15 years, several important discoveries have added much to our understanding of these mechanisms.

Several labs reported that, in many but not all cases, exposure to AHR agonists elicited a rapid depletion of AHR protein without an effect on AHR mRNA [217–220]. In 1999 and 2000, the labs of Drs. Pollenz, Whitelaw, and Ma
independently demonstrated that this loss of AHR protein required nuclear uptake of the ligand-bound AHR, ubiquitination, and proteasome-mediated degradation [221–223]. Several subsequent studies have shown that this degradation depends on other cell-specific conditions and factors and may also occur independently of ligand [224–227]. Furthermore, most of these investigations were carried out using cultured cells, and it is not clear whether this induced degradation would occur in most tissues within an intact animal. Although, in theory, blocking receptor degradation may make a cell hyperresponsive to its own ligand [228], and indeed inhibiting proteosomal degradation has been shown to cause a “superinduction” of CYP1A1 by TCDD [229], it does not necessarily follow that all responses to any AHR ligand

FIGURE 1.2 Overview of the multiple roles of AHR as covered in this book. Parts (a), (b), and (c) correspond to the sections of the book. Numbers in diamonds, for example, indicate the chapter in which the indicated pathway, process, or feature is discussed.
would be heightened. The importance of this pathway to control AHR protein levels and particular toxic or physiological responses to AHR ligands clearly needs to be examined in more detail.

In addition, the level of AHR expression has been found to change with cell density, state of cell differentiation, presence or absence of growth factors and hormones, and neoplastic transformation in the absence of known AHR ligands [216]. It seems likely that in some cases this may reflect differential regulation of the Ahr gene. The cloning of the Ahr gene from human, rat, and mouse in the early 1990s revealed the presence of many potential transcription factor binding sites [197, 230–232]. The presence of these sites could explain the observations made by many labs of tissue- and differentiation state-dependent expression, as well as the up- and downregulation of AHR expression by several factors such as IL-4, TGFβ, and pituitary hormones [216, 233–236]. In 2001, it was subsequently shown by Dr. Abel’s group that TGFβ regulates Ahr expression through Smad binding sites within the 5′-flanking region [236]. However, for the most part, the functionality of other potential transcription factor binding sites in the Ahr gene remains largely unexplored. It would seem that this would be extremely fertile ground for further important investigations.

In 1999, a report from the Fuji-Kuriyama lab identified a novel protein that appeared to bind to the AHR and restrict AHR-dependent gene transcription [237]. Two years later, this group published details on the structure and expression of the gene that encoded for this bHLH–PAS protein, called the Ah receptor repressor (AHRR) [238]. The N-terminal region of the AHRR is similar to the AHR, but lacks the PAS-B domain that functions in ligand binding and hsp90 interaction. It is hypothesized that AHRR blocks AHR activity by dimerizing with ARNT and competing with the AHR–ARNT complexes for binding to AHRE sites. Notably, the Ahr gene is directly upregulated by activated AHR [238], and the levels, as one might expect, are considerably lower in AHR-null allele mice [239]. However, the pattern of AHRR expression does not appear to always correlate directly with the ability to block the response to AHR ligands [239–241]. As such, it is possible that the AHRR may have additional functions, may repress AHR function by additional mechanisms, or may be very tissue specific and have some selectivity for particular AHR-responsive genes [242]. Recent reports suggest that AHRR may directly interact with ERα and suppress its transcriptional activity [243] and may act as a tumor suppressor for several types of human cancers [244]. Clearly, much more work is needed to understand the multiple functions of AHRR, especially since several studies suggest a linkage between some AHRR gene variants and reproductive disorders in humans (reviewed in Ref. 244).

Much evidence gathered over the past 30 years shows a clear tissue-specific developmental regulation of AHR expression [38, 245–250]. Our increased knowledge of gene regulatory processes also indicates that developmental gene expression is regulated, in part, by epigenetic mechanisms [251, 252]. A recent report from Dr. Klaassen’s lab at the University of Kansas suggests that an enrichment of specific methylation patterns may regulate the ontogenic expression of AHR mRNA in mouse liver [253]. These data, along with the report that silencing of the Ahr gene may be associated with the progression of certain cancers [254–256], suggest that the epigenetic regulation of AHR expression is also an important area that needs to be explored.

These findings, and others summarized elsewhere [216], indicate that normal cellular processes regulate the level of AHR protein and its activity. It is safe to say that these are important for the organism’s responses to a variety of environmental chemicals. Furthermore, it is increasingly apparent that endogenous AHR regulatory processes play important roles in cell growth, differentiation, and susceptibility to disease (see below). That this represents an area rich for further investigation is surely an understatement.

1.8 AHRE-DEPENDENT VERSUS AHRE-INDEPENDENT PATHWAYS

As the twenty-first century started, most evidence was consistent with the notion that the ligand-activated AHR modulates gene expression and intracellular signaling pathways through its ability to interact with ARNT and bind AHREs in responsive genes. However, it was (and still is) not unreasonable to hypothesize that a ligand-induced change in AHR conformation could also alter its ability to interact with other proteins to influence signaling pathways in a manner not dependent on the AHR interacting with DNA. Indeed, during the past 10–15 years, there have been several such reports suggesting AHRE-independent actions. These include interactions with c-Src kinase [257–260], NF-κB subunits [261–266], retinoblastoma protein [267–269], estrogen receptor [66, 270, 271], Nedd8 [272], Myb binding protein [273], Nrf2 [274, 275], p38 MAPK [276], β-catenin [277], E2F1 [278], and CD4K [279]. Furthermore, it is reasonable to speculate that the known interactions of the AHR with chaperone proteins in the AHR core complex [100] as well as basal transcription factors [280] could indirectly affect other signaling pathways. Since the middle 1980s, Fumio Matsumura at the University of California at Davis has proposed that nongenomic pathways, in particular those involved in inflammatory processes, may be mediated by the ligand-activated AHR [281]. This has been supported, in part, by a recent finding by the Perdew lab that the AHR can repress acute phase response gene expression without AHRE binding [282]. The finding published in 2007 that the AHR is a ligand-dependent E3 ubiquitin ligase [270] also has implications for affecting many signaling pathways.
Within the past 8 years, the Bradfield lab developed two mouse models that have mutations at the nuclear localization sequence or the DNA binding domain of the AHR, and the use of these suggests that many toxic responses to TCDD are absolutely dependent on the ability of the AHR to translocate to the nucleus and bind to AHREs [283, 284]. On the other hand, it is possible that some toxic end points not yet assessed in these models are mediated through mechanisms not dependent on AHRE binding. Furthermore, the AHR may affect a number of signaling pathways involved in normal physiological functions of the AHR, that is, not related to toxic end points, through non-AHRE binding mechanisms (see below). Relevant to this, comparison of the transcriptome in AHR-KO and wild-type mice implies that the AHR regulates distinct TCDD-dependent and TCDD-independent gene batteries [285], although it is not yet clear whether these differences are due to AHRE-dependent or AHRE-independent mechanisms. Certainly, future efforts should be directed at determining the role of non-AHRE-mediated mechanisms in AHR-dependent toxicity and in regulating normal cellular processes.

1.9 THE PHYSIOLOGICAL ROLE IS STILL ELUSIVE

Since discovery of the Ah locus and the AHR, researchers in the field have been struggling with questions surrounding the normal function of this protein. Is it just a xenobiotic sensor? What are its endogenous activators? Does it function to monitor changes in the tissue environment, including those brought about by xenobiotic exposures, and act to produce adaptive responses? Does the AHR have more fundamental roles in normal development and tissue differentiation?

As indicated above, much effort has been devoted to determining mechanisms of xenobiotic toxicity and risks to exposed human populations. However, there has always been an underlying interest in the role of the AHR in normal cellular processes and how this may lead us to a greater knowledge of events leading to human disease that may not necessarily be mediated by xenobiotic exposure. In fact, many of us have used TCDD as a “molecular probe” [27] to gather such information. As far back as the middle 1980s, Dan Nebert noted significant differences in the health, fertility, and life span of mouse strains differing at the Ah locus. For example, strains possessing a “high-affinity” receptor (for TCDD) have a longer life span than those possessing a “low-affinity” receptor [286, 287]. However, it has only been within the past 6–8 years that questions regarding the physiological function of the AHR have become the major focus of much of the research in the AHR field. This has been driven by the search for physiological AHR ligands and ligands that could have potential therapeutic uses and by the accumulating evidence of the importance of AHR in many cellular and tissue processes.

1.9.1 Are the Toxic Responses to Xenobiotic AHR Ligands Adaptive or a Reflection of Out-of-Control Physiological Responses That Normally Maintain Homeostasis?

It is clear from the above discussion (see Section 1.6), as well as the publication by Bock and Kühle [210], and discussion by a number of other investigators in this book and elsewhere that TCDD disrupts, directly or indirectly, a large number of tissue and cellular processes. These include, but certainly are not limited to, T-cell development, inflammatory responses [57, 288–296], cardiovascular development and function [190, 297–299], growth and regulation of reproductive tissues [300], pathways of cell differentiation [301–303], signals in cell cycle that regulate the balance between cell proliferation, death, senescence, and differentiation [268, 304–306], circadian rhythms [307–309], mesenchymal–epithelial interactions during development of tissues such as the palate [310] and prostate [311], cell trafficking and invasive tumor growth [312–315], neurological development and function [316–319], and bone development and maintenance [320–323]. Certainly, there is much overlap among several of these processes. However, in terms of the cell biology, some of the most consistent effects of TCDD-induced AHR activation are altered cell cycle, altered patterns of cellular proliferation and differentiation, and altered cell–cell communication. Based on this, it is reasonable to predict that the most sensitive tissues to TCDD would be those undergoing differentiation and proliferation, such as in the fetus or in the immune system even in adults. Furthermore, if the toxicity of xenobiotic AHR ligands is a reflection of dysregulated physiological functions, then the AHR is likely to have a normal role in those processes. Both of these generalizations appear to be appropriate. As indicated in Section 1.6, despite the challenging research path to decipher the exact molecular targets of TCDD–AHR and pathways leading to specific toxic phenotypes, a few have been identified, as recently reviewed [215]. Clearly, the dysregulated pathways are likely to be tissue and developmental stage specific and may involve multiple interacting pathways. Indeed, much of the available data on induced gene alterations indicates that this is the case [54, 56, 57, 189, 192, 212, 216, 324]. Many tools are now available to apply to this quest, so rapid expansion of our understanding of the molecular mechanisms of AHR-mediated toxicity is anticipated.

1.9.2 Step by Step, Chemical by Chemical, the Search for Endogenous AHR Ligands Continues

In 1984, a publication by Drs. Nebert, Eisen, and Hankinson asked the question of whether the AHR has “... specificity
only for foreign chemicals” [325]. They concluded that the receptor may be “required for endogenous functions critical to life processes, as well as its function in the induction of drug metabolism.” However, during the 1980s much of the effort was still directed to the toxicology associated with the AHR and the identification of xenobiotic AHR ligands (see Section 1.6.2). It really was not until the 1990s that many labs began an earnest search for other compounds, both dietary and physiological, that may serve as AHR agonists or antagonists. Many of those identified are dietary components as well as products and intermediates of normal metabolic pathways (reviewed in Refs 326 and 327). Of these, the flavonoids are the largest group of dietary ligands that have both agonist and antagonist activities [139, 326, 328]. However, the majority of these have AHR binding affinities that are orders of magnitude less than TCDD and potencies lower than might be expected for a physiological ligand.

About this same time, several indole-based compounds were identified that are the most promising of the putative physiological ligands. These include indigo and several photooxidation and/or metabolite products of tryptophan [327, 329–332]. The tryptophan derivatives are of particular interest. It was recognized as early as 1987 that several photooxidation products of tryptophan such as 6-formylindolo[3,2-b]carbazole (FICZ) bind to the AHR with very high affinity [333]. Subsequently, indolo[3,2-b] carbazole has been found in certain plants of the Brassica genus [334], and a putative secondary metabolite of tryptophan, 2-(1H-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester, has been isolated from porcine lung [335]. Both have high affinity for the AHR [334, 336], although it is not clear whether the latter compound is actually produced in tissues or was a purification by-product. Several sulfoconjugates of FICZ have been found in human urine [337], strongly indicating the endogenous presence of tryptophan derivatives that could be AHR ligands. Although ultraviolet light and/or circadian rhythms may play a role in mediating AHR signaling by creating products of tryptophan, for better or worse, especially in the skin [338, 339], the Bradfield lab has demonstrated that both aspartate aminotransferase and d-amino acid oxidase can also generate AHR ligands from tryptophan [340–342]. Interestingly, indoleamine-2,3-dioxygenase (a major pathway of tryptophan metabolism notably in inflammation and in dendritic cells) is activated by TCDD, and its products, kynurenine and kynurenic acid, can activate AHR [343, 344]. Furthermore, AHR activation by kynurenine mediates FoxP3+ Treg cell generation [344, 345].

Arachidonic acid derivatives are another class of endogenous substances that was discovered in the late 1990s to bind the AHR [346–349]. Tissue concentrations of these compounds can be as high as the μM range. Furthermore, the fact that many of these compounds are metabolized by cytochrome P450 isozymes regulated by the AHR, and are involved in inflammatory responses observed in TCDD-treated or AHR-KO mice, makes them attractive as bona fide endogenous AHR ligands [347, 350]. The ability of modified low-density lipoprotein to activate the AHR [351] could be mediated by products of arachidonic acid metabolism.

Although it is likely that one or more of these compounds, especially the tryptophan and arachidonic acid derivatives, will prove to be physiological ligands for the AHR, the definitive studies have yet to be performed. However, it is important to mention that the years of research on the AHR have provided convincing evidence of the existence, though not yet the identity, of endogenous ligands. For example, in the absence of known exogenous ligands, AHR-dependent regulation of the cell cycle [352, 353], activation of the AHR and induction of responsive genes under different conditions of cell culture [354–357], and cytoplasmic–nuclear shuttling of the AHR [358, 359] have been observed. There may be certain conditions during cell cycle, differentiation, or interactions with other cells in which endogenous ligands are produced. It is also possible that endogenous ligands may be generated by changes in the tissue environment that occur during tissue development, remodeling, repair, or during processes such as angiogenesis. This is particularly intriguing given that several members of the bHLH–PAS family are involved in sensing of changes in tissue environments [75]. However, although in most cases the transcriptional activity of the AHR is clearly ligand dependent, it is not yet known whether there are other pathways or intracellular signals, for example, posttranslational modifications such as phosphorylation, that may lead to AHR activation in the absence of ligand.

Given the ever-increasing knowledge of AHR physiology and ligands, there is also recent interest in whether any AHR ligands might have therapeutic uses or whether some therapeutic compounds already in use or under development may, in part, by modulating AHR activity. In particular, several anti-inflammatory and antiallergic drugs have been found to have AHR agonist activity [360–363]. In support of the anti-inflammatory properties of some AHR ligands, the Perdew lab has shown that WAY-169916, a selective estrogen receptor modulator (SERM) with anti-inflammatory properties in models of rheumatoid arthritis, is also an AHR ligand. Furthermore, the suppression of acute phase response activity by this compound is dependent on AHR binding but not on ER- or AHR-mediated gene expression [364]. The group went on to synthesize a derivative of WAY-169916 that lacked ER binding but retained high-affinity AHR binding as well as anti-inflammatory properties [365]. Very recently, groups at the Scripps Research Institute and Novartis Research Foundation, in collaboration with Gary Perdew and Mike Denison, found selective human AHR antagonists that were able to promote the expansion of human hematopoietic stem cells [366] and further suggested that this may facilitate the use of stem cell transplants. These observations are consistent with data published by the Gasiewicz and Kanno...
groups, indicating substantial effects of TCDD on hematopoietic stem cell characteristics and function [307, 367–370], as well as altered cycling of these cells from Ahr-null allele mice [368]. While together these data strongly suggest an important function of the AHR in the immune system, and particularly in hematopoietic stem cells, the exact signaling pathways involved have not yet been determined.

The interaction of some ligands with both the ER and AHR is of particular interest, especially since a number of studies, even from the 1970s, noted crosstalk between the AHR and ER signaling pathways. In 1978, a group led by Richard Kociba reported that the chronic dietary treatment of female rats with TCDD inhibited spontaneous mammary and uterine tumor formation [371]. Since that time, a number of investigators, mainly Steve Safe at Texas A&M, have observed crosstalk between these pathways at a number of different levels (reviewed in Ref. 372; see also Section 1.8). These studies eventually led Dr. Safe and his colleagues to develop several compounds that were selective aryl hydrocarbon receptor modulators (SARMs) inhibiting hormone-induced growth of ER-positive tumors, particularly breast cancer [373–376]. Recently, Richard Peterson, at the University of Wisconsin, showed that one of these compounds, 6-methyl-1,3,8-trichlorodibenzo-p-dioxin, inhibited prostate tumor metastasis in a mouse model (TRAMP) that spontaneously develops prostate cancer [255]. In a variety of human breast cancer cell lines (ER-dependent and ER-independent) and in breast cancer stem-like cells, AHR activation inhibited invasive and metastatic activities [377, 378]. On the ER side, a recent publication from the McDonnell lab noted that 4-hydroxytamoxifen, an active metabolite of tamoxifen, directly binds to and modulates the transcriptional activity of the AHR [379]. Their studies further suggest that some of the effects originally attributed to the antiestrogenic action of tamoxifen may in fact be mediated through the AHR.

For purposes of defining AHR ligands that are toxic xenobiotics, endogenous mediators, or effective therapeutics, much information is likely to emerge from an analysis of the ligand binding domain. Although there is currently no information on three-dimensional structure for the AHR, in the past 10 years several groups have used mutational analysis and comparisons with structural data from homologous PAS-containing proteins to develop working models of the AHR LBD [380–382]. However, some of this modeling is based on the binding of xenobiotics such as TCDD, and it is not clear whether the parameters are also relevant for endogenous ligands. Furthermore, genes regulated by endogenous ligand-activated AHR may or may not overlap those regulated by xenobiotic ligand–AHR interactions. Indeed, some work suggests that the AHR regulates distinct TCDD-dependent and TCDD-independent gene batteries [285]. Recent work indicates that there may be some diversity in ligand selectivity designated by the human and mouse AHR [383, 384]. As such, modeling a rodent AHR may not accurately represent ligand activity toward the human AHR.

1.9.3 Knockout and Knock-In Show Glimpses of Physiological Functions

The development in the middle 1990s of several lines of mice lacking AHR [194, 196, 197] has proven to be another milestone in the quest to understand physiological functions of the AHR. Since that time, an extensive literature has developed in this area, complemented by the development of mice expressing a constitutively active [385, 386] or hypomorphic AHR [387], or with AHR that can be conditionally expressed [388]. Use of these models has suggested physiological roles of the AHR in angiogenesis and cardiovascular development and function [389–396], hematopoiesis and development and function of the immune system [57, 194, 263, 293, 294, 368, 370, 387, 397–402], melanogenesis [403], development of female reproductive tissues [404–409], mammary gland development [410], prostate development [411], maintenance of pregnancy [404], wound healing [412], tumorigenicity [413], and aging [414, 415]. However, use of Ahr-null mice, in which the AHR is lacking in all tissues, may lead to misinterpretations of primary versus secondary changes as a result of AHR loss. Using cell type-specific AHR deletion, for example, the Bradfield lab demonstrated that AHR within endothelial cells is necessary for normal development of liver vasculature, whereas AHR-mediated hepatotoxicity depends on expression of AHR in hepatocytes [388]. This group has likewise developed mice with analogous modifications of expression of the AHR chaperone protein, AIP [416, 417]. Cell type-specific effects of AIP deletion on vascular development and on TCDD-induced hepatotoxicity were consistent with the role of AIP in maintenance of AHR levels. Clearly, further study of the consequences of AHR loss in specific cell types will provide important new understanding of the physiology of the AHR.

1.10 SUMMARY AND FUTURE OF AHR RESEARCH

Less than 40 years ago, the AHR was a newly discovered binding protein associated with toxicity of a specific class of structurally related pollutants. Now it is additionally recognized as a ubiquitous transcription factor within a large family of related proteins that have important roles in development and cell signaling. Among the many significant milestones (Fig. 1.1) in the relatively short history of research focused on this protein, the following are surely highlights:

- discovery and initial characterization of the AHR protein (Fig. 1.2a);
- the understanding that the toxicity of a large class of xenobiotics is mediated by their binding to the AHR (Fig. 1.2b) provided an important tool for risk assessment of these chemicals;
• advances in biomedical research technology enabled elucidation of its activity as a ligand-activated transcription factor and revealed AHR to be a member of a large family of bHLH–PAS proteins that crosstalk with numerous other signaling pathways (Fig. 1.2a);
• further development of tools in molecular biology simplified the identification of AHR-mediated changes in gene expression and made possible the manipulation of the Ahr gene in vitro and in vivo.

From the extensive and detailed work reviewed throughout this book, it has become clear that not only is AHR a necessary mediator of essentially all toxic end points of TCDD, but it is also an important physiological signaling factor in many cells and tissues, notably during development (Fig. 1.2c).

Research interest in this protein and its functions will doubtless continue, and the spectrum of pathways that are discovered to have AHR involvement will doubtless further expand. Clearly the importance of AHR in toxicology will not wane, as identifying the key target genes and signaling pathways responsible for the diverse manifestations of toxicity of its xenobiotic ligands will be another major milestone in this history. However, possibly the most fruitful and intriguing directions for further study of AHR are expected to be in its basic biology. Determination of its endogenous ligand(s) and the regulators of its expression (temporally and spatially) will be important milestones not only for AHR research and toxicology, but also in our understanding of basic physiology and cell biology for which TCDD is an invaluable tool rather than just a toxic contaminant. Particular goals for further research include

• unraveling of the role(s) of AHR in normal development and how they may change during differentiation of various cell types;
• understanding in more detail the biology of AHR, including the critical target pathways impacted by its activation by toxic ligands, which may lead to development of therapeutic ligands or other means of perturbing pathways impacted by AHR-mediated toxicity or disease;
• investigating potential epigenetic mechanisms of AHR-mediated toxicity and endogenous signaling, especially in fetal development and differentiation;
• defining whether there are non-AHRE-dependent pathways of AHR response to either xenobiotic or endogenous signals;
• understanding which human diseases may have AHR-dependent etiology (besides or in addition to toxicity from xenobiotic ligands) or may be impacted by AHR-dependent pathways.

Perhaps in the not so distant future, another retrospective on AHR research will include milestones on the pathway toward such goals, as well as new insights from research directions that are currently unpredictable.

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