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Review

AhR acts as an E3 ubiquitin ligase to modulate steroid receptor functions

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ABSTRACT

The arylhydrocarbon receptor (AhR) mediates the adverse effects of dioxins, including modulation of sex steroid hormone signaling. The role of AhR as a transcription factor is well described. AhR regulates the expression of target genes such as CYP1A1; however, the mechanisms of AhR function through other target-selective systems remain elusive. Accumulating evidence suggests that AhR modulates the functions of other transcription factors. The ligand-activated AhR directly associates with estrogen or androgen receptors (ERα or AR) and modulates their function both positively and negatively. This may, in part explain the sex steroid hormone-related adverse effects of dioxins. AhR has recently been shown to promote the proteolysis of ERα/AR through assembling a ubiquitin ligase complex, CUL4-B^{AhR}. In the CUL4B^{AhR} complex, AhR acts as a substrate-recognition subunit to recruit ERα/AR. This action defines a novel role for AhR as a ligand-dependent E3 ubiquitin ligase. We propose that target-specific regulation of protein destruction, as well as gene expression, is modulated by environmental toxins through the E3 ubiquitin ligase activity of AhR.

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Contents

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1. Introduction

Dioxin-type environmental contaminants, such as tetra-chloro-dibenzo-*p*-dioxin (TCDD), exert toxic effects [1]. Some of these toxicities are estrogen- and androgen-related actions [2–7]. The arylhydrocarbon receptor (AhR) is a ligand-dependent transcription factor belonging to the basic helix-loop-helix/Per–Arnt–Sim (bHLH/PAS) family. AhR possesses a variety of biological and toxicological functions [8–11] (Figs. 1 and 2). AhR mediates the toxicological effects of dioxins. In addition, AhR plays a physiological role in various tissues such as the reproductive and immune systems. The transcriptional activity of AhR is regulated by direct binding of its ligands [12,13] (Figs. 1 and 2A). The unliganded AhR is sequestered in the cytosol by interacting with the Hsp90/XAP2 (also called as ARA9 or AIP) chaperon complex [8–11]. Ligand binding to the PAS-B region of AhR is thought to induce conformational changes and subsequent translocation of the AhR complex to the nucleus [8–10]. AhR then dimerizes with the AhR nuclear translocator (Arnt) in the nucleus after dissociating from the chaperon complex, recognizes the xenobiotic-responsive element (XRE), and recruits co-activators such as the histone acetyltransferase p300/CBP, chromatin remodeling factor Brg1, and the mediator (DRIP/TRAP) complex to activate transcription [8–10] (Fig. 1). The AhR/Arnt heterodimer induces the expression of target genes, such as CYP1A1, CYP1A2, and glutathione-S-transferase [1].

The actions of the direct target genes of AhR alone do not fully explain its toxicological and physiological effects. Accumulating evidence suggests that the AhR exhibits its regulatory functions by modulating the function of other transcription factors [2,11], including estrogen receptor (ERα and ERβ) [14–19] and androgen receptor (AR) [18,19] (Fig. 1). These cross-talk pathways are important mediators of the functions of endogenous and exogenous AhR ligands. The liganded AhR recently has been shown to promote the ubiquitination and proteasomal degradation of ERs and AR by assembling a ubiquitin ligase complex, CUL4B^{AhR} [18,19]. Thus, complexes of the AhR with ERs or AR appear to regulate transcription as functional units by multiple mechanisms. In this review, we will summarize a novel role for AhR as a component of an E3 ubiquitin ligase complex, which mediates cross-talk of AhR with sex steroid receptors through promotion of proteolysis.

2. Cross-talk of AhR with ERs or AR

2.1. *Transcriptional regulatory mechanism involving nuclear receptors*

ERs and AR belong to the nuclear receptor superfamily of transcription factors [20–22] (Fig. 2). Nuclear receptors, by acting as ligand-dependent transcription factors serve as
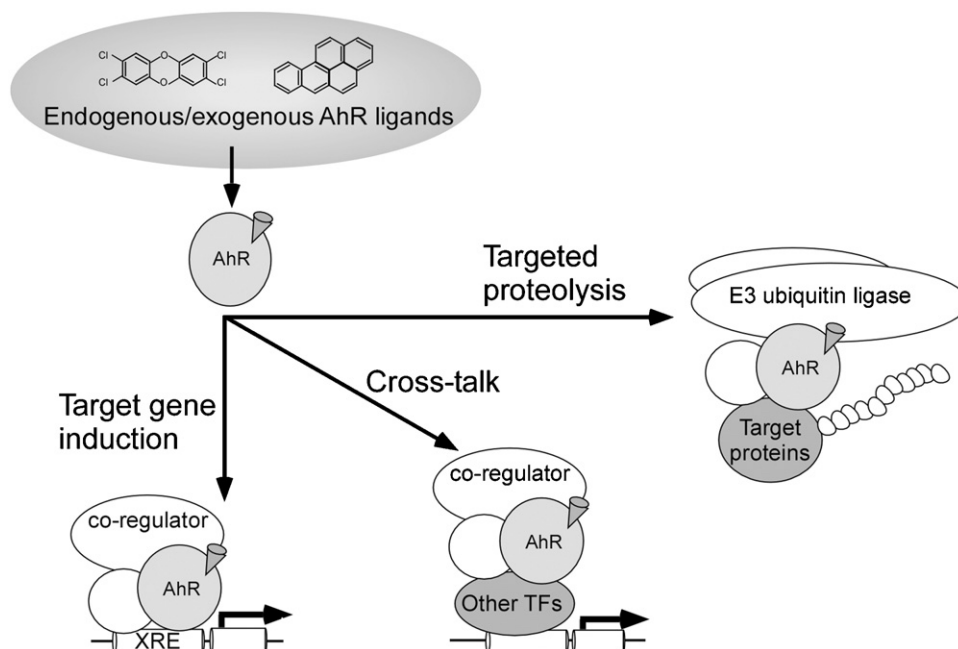
Fig. 1 – **Different modes of the AhR signaling pathways. Molecular pathways for AhR-mediated biological actions. AhR may exhibit its biological actions through different modes of pathways as illustrated. Typically, AhR directly binds to its target gene promoters and induces expression of these genes. In addition, cross-talk of AhR with other transcription factors, as well as the function of AhR as an E3 ubiquitin ligase, is considered important for AhR biology. XRE, xenobiotic-response element; TF, transcription factor.**

Fig. 2 – Structure and molecular mechanism of AhR and nuclear receptors. A and B domain structures of AhR (A) and nuclear receptors (B). Domain architectures and cognate ligands for these receptors are illustrated. bHLH, basic helix-loop-helix; PAS, Per–Arnt–Sim domain; AD, activation domain; AF, activation function; DHT, dihydrotestosterone. (C) Mechanisms of gene regulation mediated by AhR and nuclear receptors. ERE, estrogen-response element.
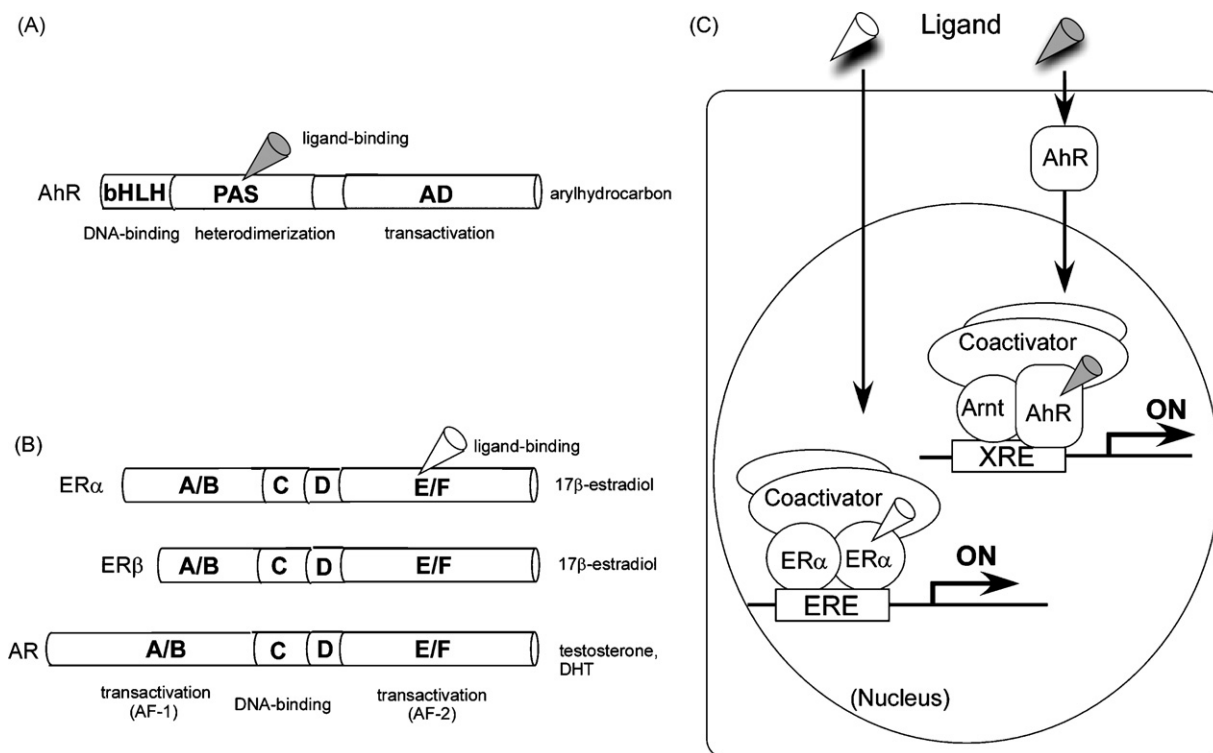
sensors for low molecular weight, fat-soluble ligands such as steroids/thyroid hormones, and vitamins A and D [20,21]. Members of the nuclear receptor gene superfamily share a common domain structure with distinct functional domains, designated A–E [21] (Fig. 2B). The ligand-binding domain is located in the C-terminal E domain. The most conserved C domain, located in the middle of the receptor, serves as the zinc finger-type DNA-binding domain. This domain specifically recognizes its cognate response elements in the target gene promoters. The N-terminal A/B domain and the C-terminal E domain are required for ligand-induced nuclear receptor transactivation functions. The autonomous activation function (AF-1) in the A/B domain is constitutively active but is presumably masked in the absence of ligand. The autonomous activation function (AF-2) in the ligand-binding E domain is, in contrast, dependent on ligand binding through the ligand-dependent conformational change of helix 12 and subsequent formation of a hydrophobic surface for the interaction with co-regulators [20] (Fig. 2).

Ligand-bound nuclear receptors recruit a number of transcriptional co-regulators and co-regulator complexes to the target gene promoters to mediate ligand-dependent transcriptional control [21,22] (Fig. 2). These complexes can be classified into three categories according to their functions. The first class of co-regulator complexes modifies histone tails covalently [23]. The amino-terminal tails of histones are subjected to various covalent modifications such as acetylation, methylation, phosphorylation, and ubiquitination by specific histone-modifying enzymes. These post-translational

histone modifications are thought to serve as a 'histone code' that fine-tunes the transcriptional state through chromatin structure rearrangement [23]. The second class of complex mediates ATP-dependent dynamic remodeling of chromatin structure [22]. Chromatin remodeling complexes use ATP hydrolysis to rearrange nucleosomal arrays in a non-covalent manner. These chromatin remodeling complexes support the accessibility of co-regulator complexes and transcription factors to specific promoter regions. The last co-regulator complex class, the mediator complex, directly regulates transcriptional control by physically interacting with general transcription factors and RNA polymerase II. Recent evidence suggests that numerous co-regulators and nuclear receptors are recruited onto the promoters in an ordered manner, associating and dissociating transiently [24,25]. Nuclear receptors, as well as other transcription factors, serve as specific adaptors that connect co-regulator complexes and specific promoter regions.

The ligand-dependent nuclear receptor function is also regulated by other classes of signal transduction pathways. Such cross-talk pathways include at least two mechanisms: functional modulation through post-translational modification, and the association with other classes of transcription factors. MAPK, activated by EGF, phosphorylates ERα at serine 118 [26]. This in turn potentiates the ligand-dependent transactivation function of ERα [26] as well as its rapid turn-over. Phosphorylation-mediated functional modulation has been reported for a number of nuclear receptors to date.

Complex formation-based cross-talk mechanisms are also seen in several nuclear receptors including the glucocorticoid receptor (GR) [27]. GR ligands have an anti-inflammatory action, which is mediated through ligand-dependent repression of AP-1 activity through direct association. More recently, the exchange of different classes of co-regulator complexes has been reported to underlie the signal cross-talk pathway. Ligand-activated PPARγ typically assembles co-activator complexes on its cognate promoters. In the repression of NF-κB activity, PPARγ forms a complex with NF-κB, and this complex stably associates with an NCoR co-repressor complex by inhibiting the degradation of NcoR [28]. A current view of signal cross-talk at the transcription levels is that signal/ligand-dependent transcription factors associate with each other to assemble diverse types of co-regulator complexes. These exchange dynamically and regulate transcription in a manner specific for each cross-talk pathway [22].

2.2. Molecular mechanisms of cross-talk of AhR with estrogen or androgen receptors

Signal cross-talk pathways are important mediators of the functions of AhR ligands in various tissues. Dioxin-type environmental contaminants exert both estrogen- and androgen-related effects [1–3,5–7,29–32] (Fig. 3). Dioxins have well-described anti-estrogenic effects, such as the inhibition of estrogen-induced uterine enlargement, MCF-7 cell growth,

and target gene induction [3,7]. However, there is also evidence to the contrary as dioxins have also been shown to have estrogenic effects including the stimulation of uterine enlargement [29], induction of estrogen-responsive genes such as VEGF, c-fos, and TERT, and a similar pattern to estrogen of transcriptional regulation in a genome-wide study [6]. In addition, AhR-deficient mice exhibit impaired ovarian follicle maturation [33]. Using AhR-deficient cells, the importance of AhR in the proliferation of mammary cells has been confirmed [34]. These findings suggest that AhR, activated by its endogenous ligand, may modulate the estrogen signaling pathway. Similarly, dioxins exert both androgenic and anti-androgenic effects on prostate development in an age-specific manner [5]. As is true for other cross-talk pathways [22], the AhR appears to modulate estrogen/androgen signaling both positively and negatively depending on cellular context.

The molecular mechanisms of AhR modulation of ERα have been extensively studied, and both direct and indirect regulatory mechanisms have been proposed. First, TCDD/AhR either increases or decreases estrogen levels through an indirect mechanism [2,35]. TCDD promotes the clearance of estrogen, thereby repressing ER transcriptional activity [35]. AhR-deficient mice have decreased estrogen production due to impaired induction of aromatase (CYP19) gene expression [33]. Another indirect mechanism involves competitive DNA binding of AhR and ER on the responsive promoters [2]. AhR and ER, each bound to its own target promoter recruits transcriptional co-regulators
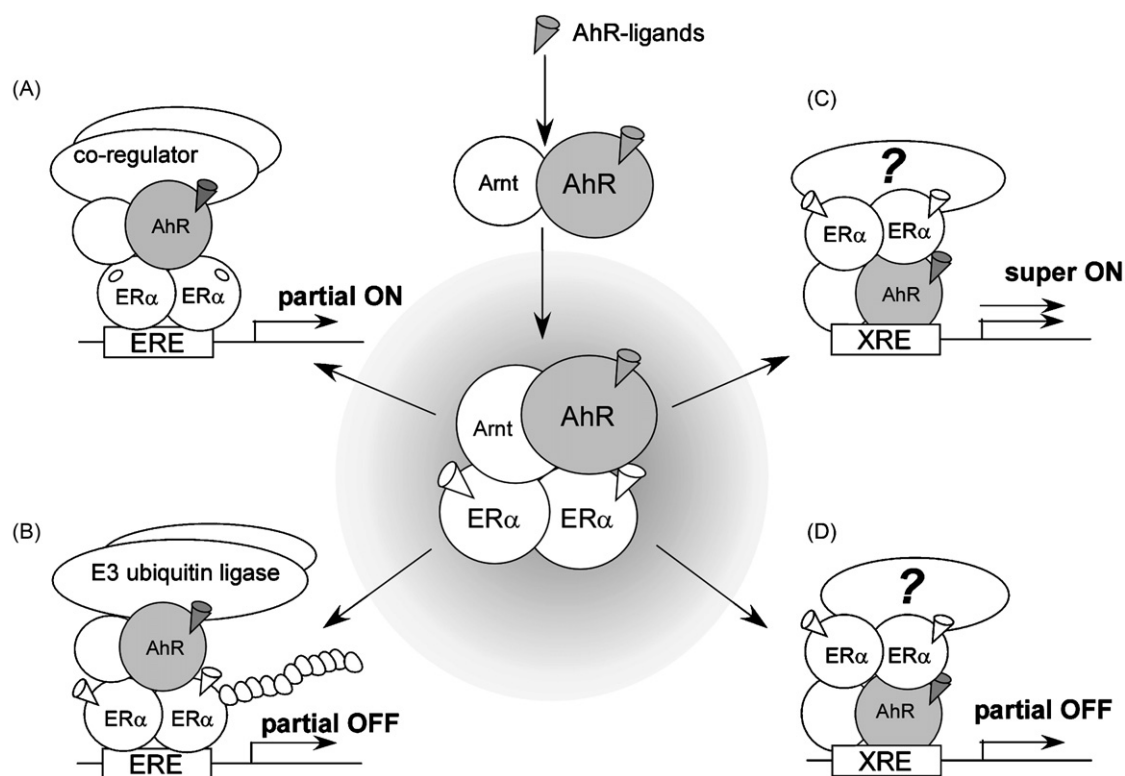
Fig. 3 – Cross-talk of AhR with ERα through direct association. Ligand-bound AhR directly associates with estrogen or androgen receptors (ERα, ERβ, or AR) in the nucleus. This association leads to different types of cross-talk between AhR and ERs/AR, as illustrated (see text for details). (A) Ligand-bound AhR associates with unliganded ERs upon ERE and recruits transcriptional co-activators. (B) Ligand-bound AhR forms E3 ubiquitin ligase complex and recognizes ERs for proteolysis. (C) Ligand-bound ERα associates with AhR and activates transcription through XRE. (D) Association of ERα with AhR results in repression through XRE.

in a competitive manner. This mechanism may be limited to specific genes and conditions since not all of the estrogen-responsive promoters contain XRE.

More recently, direct association of AhR with ERs has been independently reported. Ligand-activated AhR/Arnt associates with ERα and ERβ through the N-terminal A/B region within ERs [14–18] (Fig. 3). By means of this association, the liganded AhR potentiates the transactivation function of 17β-estradiol (E_2)-unbound ERα (Fig. 3A), while it represses E_2-bound ERα-mediated transcription upon the estrogen-responsive element (ERE) [14] (Fig. 3B). The interaction of AhR/ER is induced by different AhR ligands, such as TCDD, 3-methylcholanthrene (3MC), and β-naphthoflavone (βNF). The activation of AhR is thought to be sufficient for the interaction with ERα, as a constitutively active form of AhR [12] modulates ERα function in the absence of AhR ligand [19]. These results suggest that the cross-talk of AhR with ER is initiated primarily through stimulation of AhR. Supporting this, ERα is predominantly located in the nucleus, whereas AhR translocates to the nucleus upon ligand stimulation. The association of AhR/ERα has been shown by several independent approaches, including in vitro [36], in vivo, and biochemical methods [18]. Moreover, AhR/ERα cross-talk in the transcriptional regulation of ERα-responsive genes is abolished in AhR-deficient mice [10,33], confirming the specificity of the molecular pathway in vivo [14]. Reciprocally, E_2-bound ERα associates with XRE-bound AhR to either potentiate [15] (Fig. 3C) or repress [16] (Fig. 3D) AhR-mediated transcription. Considered together, the AhR/ERα complex may be able to bind to either XRE or ERE through the attachment functions of AhR or ERα, respectively. Alternatively, different complex subtypes that contain AhR/ERα may control promoter selectivity (Fig. 3). Reflecting this functional cross-talk, Arnt also acts as a co-regulator for both ERα and ERβ [37].

The proposed mechanism of AhR/ER association is a reasonable explanation for dioxin/estrogen cross-talk. First, this mechanism explains the functional AhR/ER cross-talk irrespective of differences in target gene promoters. Second, ligand-dependent AhR/ER association may result in a rapid cellular response to dioxins in terms of ER activity. The responses of ER transcriptional activity to AhR ligands are observed within a few hours in cultured cells as well as in mice, which supports the existence of direct cross-talk mechanisms. Third, variations in the AhR/ER containing co-regulator complexes may result in the complex, bi-phasic consequences of AhR/ER cross-talk. Given that complexes containing different classes of transcription factors can recruit co-regulator complexes distinct from their cognate associating complexes [22], it is possible that the AhR/ER complex, acting as a functional unit, may recruit different types of complexes depending on the cellular context. A current area of interest is the identification of the molecular determinants by which the activity of the AhR/ER complex is controlled.

3. Ubiquitin ligase activity of AhR

3.1. The ubiquitin–proteasome system

The transcriptional regulatory system and the ubiquitin–proteasome system are two major target-selective systems that control intracellular protein levels in response to various cellular contexts in metazoans (Fig. 4A). Whereas the transcriptional regulatory system is targeted by environmental fat-soluble ligands, the involvement of the ubiquitin–proteasome system in the adverse effects of these environmental toxins remains largely unknown. The target selectivity of these systems depends on the recognition of specific DNA elements by sequence-specific transcription factors [20–22] and recognition of degradation substrates by E3 ubiquitin ligases [38–41] (Fig. 4B). These transcription factors and E3 ubiquitin ligases primarily serve as specific adapters to subsequently recruit enzymes such as transcriptional co-
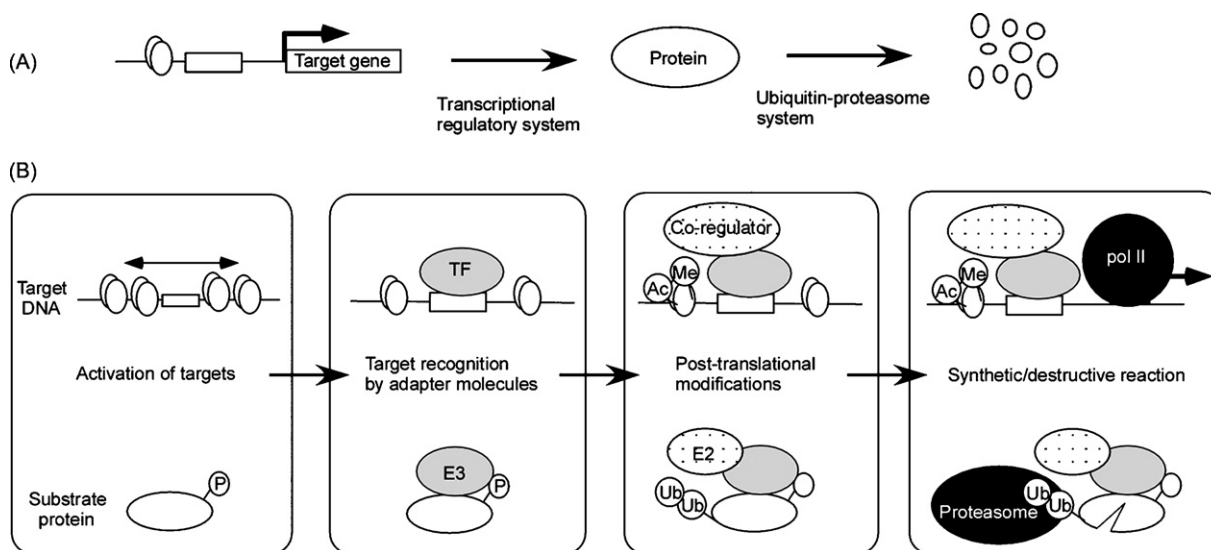
Fig. 4 – The ubiquitin–proteasome system. (A) The transcriptional regulatory system and the ubiquitin–proteasome system are two major target-selective systems that control intracellular protein levels. (B) The transcription factors and E3 ubiquitin ligases primarily serve as target-specifying adapters in these systems. Ub, ubiquitin; P, phosphorylated serine/threonine; Ac, acetylated lysine; Me, methylated lysine; Pol-II, RNA polymerase II.

regulators and E2 ubiquitin-conjugating enzymes, respectively, to appropriate targets. Considering the functional analogy of E3 ubiquitin ligase and transcription factors, it is possible that E3 ubiquitin ligase also serves as a target of environmental toxins.

The ubiquitin–proteasome system, which regulates cellular protein degradation, plays a pivotal role in cellular homeostasis [38–41]. Ubiquitin is a 76 amino acid polypeptide that is highly conserved among eukaryotes. Ubiquitin is covalently attached to lysine (Lys) residues of substrate proteins. Ubiquitination of proteins is catalyzed by sequential reactions involving ubiquitin activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin protein ligase (E3). Ubiquitin is conjugated either as one molecule (mono-ubiquitination) or as a tandem polymer (poly-ubiquitination). Poly-ubiquitination can occur at any of seven lysine residues in the ubiquitin molecule. The Lys48-linked poly-ubiquitin chain is then recognized by the 26S proteasome for subsequent proteolysis (Fig. 4B).

Among E1, E2, and E3 enzymes, the E3 ubiquitin ligases are most diverse and therefore possess substrate specificity. E3 acts as a bridge between E2 and the substrate, maintaining the appropriate distance. E2 then conjugates ubiquitin to the substrate [38–41]. Of the RING-type E3s, the largest class is comprised of the cullin–RING ubiquitin ligases (CRLs) [40–44]. CRLs are multisubunit complexes that include a cullin (CUL1, 2, 3, 4A, 4B, or 5) subunit, a RING finger protein Rbx1/Roc1 or Rbx2/Roc2, and a substrate-recognition subunit. Cullin serves as a scaffold protein, binding to the substrate-recognition subunit or adapter protein at its N-terminus while binding to Rbx1 at its C-terminus [41]. Rbx1 binds to E2 enzymes through RING finger to support efficient conjugation of ubiquitin to the substrates. Their diverse substrate-recognition subunits enable CRLs to target numerous substrates. The best characterized CRLs are the SCF (Skp1–CUL1–F-box) complexes. In SCF complexes, F-box proteins function as a substrate-recognition subunit by binding to Skp1, which is bound to the N-terminal region of CUL1. F-box proteins and other types of substrate-recognition subunits serve as adapters for target-specific substrates. Therefore, any protein binding to E3 core components can potentially act in a manner similar to substrate-recognition subunits. More interestingly, F-box proteins and other types of substrate-recognition subunits are rapidly degraded through an auto-catalytic mechanism once they are integrated into the CRL core complexes [42]. In this way, CRLs can efficiently ubiquitinate different substrates by associating with different substrate-recognition subunits. This raises the possibility that F-box and F-box 'equivalent' proteins act either as substrates or as adapter components, as in the case of DDB2 in the CUL4-based CRL complex [45–50].

3.2. AhR is an E3 ubiquitin ligase

As discussed above, dioxins, through activating the AhR, have well-described effects on the transcriptional regulatory system. TCDD is also reported to decrease the uterine ERα protein level in the rat [51], suggesting that AhR may also be involved in the control of protein stability. Somewhat unexpectedly, our own study has shown that in a ChIP analysis, the ligand-bound AhR does not block co-activator

recruitment of liganded ERα. In addition, repression of ERα transcriptional activity by AhR is not observed when ERα is over-expressed in transient reporter assays (Ohtake et al., unpublished data). These observations imply that the ligand-activated AhR has an additional molecular role beyond transcriptional regulation, at least in the modulation of sex hormone signaling.

Exploring the functions of AhR in sex hormone signaling, we found that upon activation of AhR by binding of AhR ligands such as 3MC and βNF, as well as by expression of constitutively active AhR, protein levels of endogenous ERα, ERβ, and AR, were drastically decreased without alteration in mRNA levels [19] (Fig. 5). Since ligand-bound AhR and ERα proteins are ubiquitinated for proteasome-mediated degradation [52–57], we tested whether the functional modulation of ERs and AR by activated AhR is related to this degradation system. 3MC-enhanced degradation of sex steroid receptors is attenuated in the presence of a proteasome inhibitor MG132, and 3MC-enhanced poly-ubiquitination of ERα is consistently observed irrespective of E2 binding. MG132 treatment abrogates the transcriptional modulation of liganded sex steroid receptor function by activated AhR. This indicates that the ubiquitin–proteasome system mediates the repressive AhR–ER cross-talk pathway.

These experiments provide evidence that AhR acts as an E3 ubiquitin ligase component. First, FLAG–AhR immunoprecipitated complexes exert a self-ubiquitination activity in an E1/E2 enzyme-dependent manner in vitro. Second, 3MC-dependent recognition of ER and AR by AhR [14] appears to induce ubiquitination of ER/AR. Third, degradation of AhR itself is accelerated upon activation of degradation of sex steroid receptors, which is a typical sign of self-ubiquitination of the E3 component [42]. Taken together, these properties of AhR resemble that of classical adapter components of the E3 ubiquitin ligase complex such as F-box proteins in the SCF complex [39,42], DDB2/CSA in the CUL4A complex [45–49], and VHL in the CUL2 complex [58]. Therefore, we reasoned that activated AhR might serve as an E3 ubiquitin ligase component.

Supporting this idea, an AhR associating ubiquitin ligase complex has been biochemically purified [59] from HeLa cells. This complex includes cullin 4B (CUL4B) [39,60], damaged-DNA-binding protein 1 (DDB1) [61,62], and Rbx1 [39] together with subunits of the 19S regulatory particle (19S RP) of 26S proteasome as well as Arnt and transducin-beta-like 3 (TBL3) (Fig. 5). The core complex appears to constitute a CRL-type E3 ligase, and therefore is referred to as CUL4B^{AhR}. Although the typical CUL4B-type CRL complex contains substrate-recognition components having a WDXR/DWD motif [45–49], no such component has been identified in this complex. AhR directly interacts with the N-terminal region of CUL4B in GST pull-down assays. Together with the direct interaction of AhR with ER, it appears that AhR may act as a substrate-recognition component in the CUL4B^{AhR} complex. Using an in vitro reconstituted ubiquitination assay, the E3 ubiquitin ligase activity of CUL4B^{AhR} for ERα is dependent only on 3MC, and not on E$_2$. This suggests that CUL4B^{AhR} has the unique property of being able to respond to ligand signals by complex assembly and ubiquitin ligase activity (Fig. 5). The importance of the CUL4B^{AhR} components for the promotion of ERα ubiquitina-
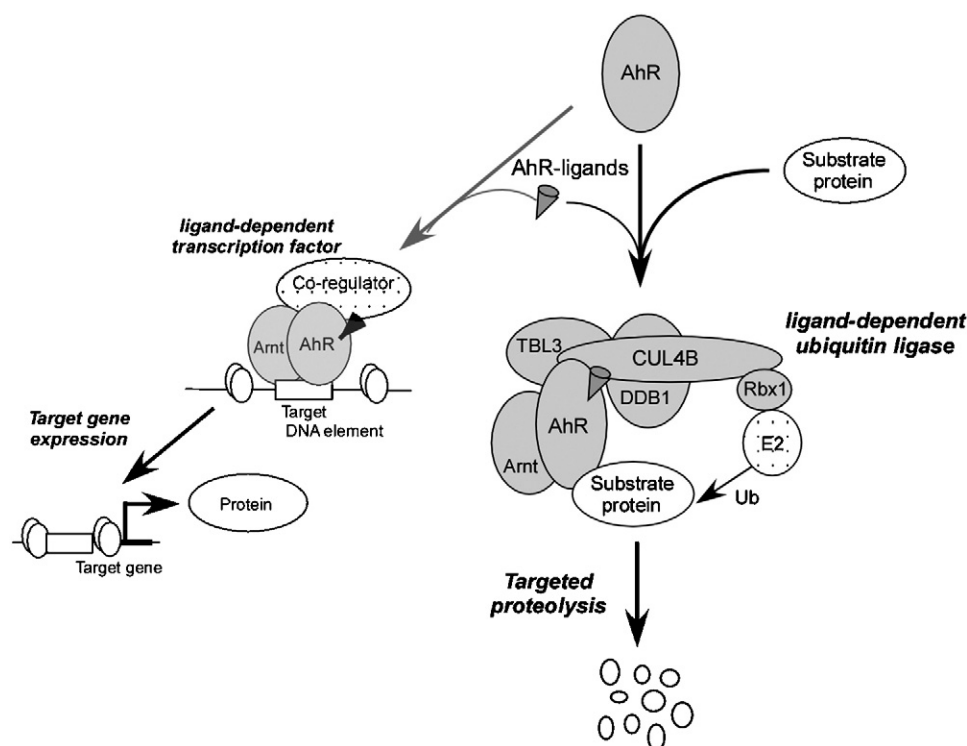
Fig. 5 – An E3 ubiquitin ligase activity of AhR. Ligand-bound AhR assembles a CUL4B-based atypical E3 ubiquitin ligase complex, CUL4B^{AhR}, to mediate a non-genomic signaling pathway of fat-soluble ligands. AhR serves as a ligand-dependent ubiquitin ligase, as well as a transcription factor (see text for details). DDB1, damaged-DNA-binding protein 1; TBL3, transducin-beta-like 3.

tion and degradation has been demonstrated in knock-down experiments. Degradation of ERα or AR in the uterus and prostate is inducible by treatment with AhR ligands. Such degradation of ERα or AR is not seen in AhR-deficient mice [10,33]. This confirms that the AhR has E3 ubiquitin ligase activity *in vivo*. The anti-estrogenic effects of AhR ligands on estrogen-dependent uterine cell proliferation [14] appear to be mediated by the E3 ubiquitin ligase activity of AhR.

3.3. Perspectives on the E3 ubiquitin ligase activity of AhR in cross-talk pathways

Although it is well established that AhR is a key factor in mediating the adverse effects of dioxin-type compounds [8–10], the underlying mechanisms for this remain elusive. The putative functions of the previously identified target genes for AhR appear unlikely to fully explain the diverse range of biological actions of AhR ligands [11] (Fig. 1). The discovery of CUL4B^{AhR} suggests that the adverse effects of AhR ligands in sex hormone signaling are, at least in part, attributable to the enhanced degradation of sex steroid receptors through E3 ubiquitin ligase activity of AhR [18,19] (Fig. 5). Target selectivity of the transcriptional regulatory system and the ubiquitin–proteasome system depends on specificity conferred by sequence-specific transcription factors and E3 ubiquitin ligases. To date, however, no single factor has been shown to function as a specificity factor in both target selection systems. Therefore, AhR is the first sequence-specific transcription factor identified that acts as an E3 ubiquitin ligase

that also targets substrates for accelerated protein degradation. It is possible that other transcription factors, such as nuclear receptors, also function as E3 ubiquitin ligase components in some cellular contexts. Fat-soluble ligands for nuclear receptors are reported to have 'non-genomic' actions independent of transcriptional regulation-mediated effects. Considered together, ubiquitin ligase-based signaling mechanisms may possibly be involved in these non-genomic actions of various fat-soluble ligands.

From a mechanical point of view, AhR appears to be a unique and atypical type of substrate-specific component in cullin-based E3 complexes. AhR does not bear the reported signature motifs such as F-box [39], but directly associates with CUL4B. Substrate recognition by the other substrate-specific components in ubiquitin ligase complexes is usually evoked by substrate modifications such as phosphorylation [38–41] and hydroxylation [43,44,58]. However, recognition and subsequent ubiquitination of sex steroid receptors by AhR requires dioxin-type ligands, and does not occur following normal modifications of sex steroid receptors. Thus, it is plausible that activation of atypical E3 complexes may be a strategy of sensors for environmental stresses to respond to these stresses (Fig. 6). Supporting this, Hsp70 acts as an atypical substrate-specific adapter within the CHIP E3 complex in response to heat shock stress [63]. Hsp70 interacts with misfolded proteins and promotes their degradation. It later undergoes auto-catalytic degradation through CHIP [63]. In response to DNA damage, an atypical E3 complex alters the stability of TIP60, which in turn regulates ataxia-telangiectasia
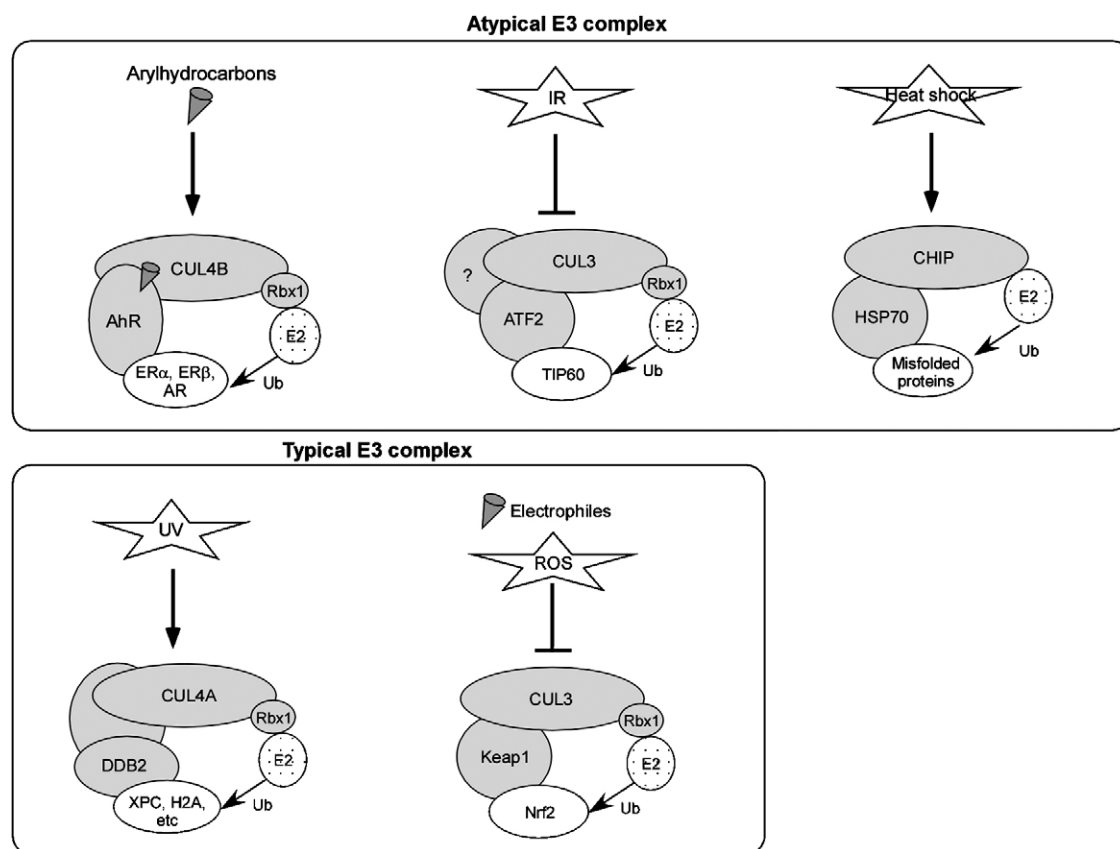
Fig. 6 – Atypical E3 complexes as sensors for environmental stresses. Several examples of E3 ubiquitin ligase-based perception of environmental stresses are illustrated. In the top panel, signal-responsive factors serve as atypical components of E3 complexes. In the bottom panel, canonical E3 components with conserved signature motif act as signal-responsive factors. ATF2, activating transcription factor-2; TIP60, tat interactive protein 60; CHIP, C-terminus of Hsp70 interacting protein; Hsp70, heat shock protein 70; XPC, xeroderma pigmentosum group C; H2A, histone H2A; Keap1, Kelch-like ECH-associated protein 1; Nrf2, NF-E2-related factor 2; IR, ionizing radiation; ROS, reactive oxygen species.

mutated (ATM) activation in DNA repair [64]. Activating transcription factor-2 (ATF2) promotes the degradation of TIP60 by assembling a CUL3-based complex under non-stressed conditions. ATF2 dissociates from TIP60 in response to ionizing radiation (IR), resulting in enhanced TIP60 stability and activity [64]. Functional regulation of E3 components is also seen with the CUL3-based component Keap1 in the oxidative stress response [65], and CUL4A-based components DDB2 and CSA in the DNA damage response [61]. Considered together, E3 components that respond to environmental stress may be more diverse than initially believed (Fig. 6). It is possible that CUL4B^{AhR} may cross-talk with these stress-responsive E3 ligases to modulate their functions. As WDXR/DWD motif containing components, including DDB2 and CSA, also bind to CUL4B [46], it is possible that AhR may associate or interfere with these CRL subunits.

The E3 ubiquitin ligase activity of AhR and the transcriptional activity of AhR appear to be responsible for a distinct set of biological events induced by AhR ligands (Fig. 5). As substrate-specific adapters of ubiquitin ligase complexes are capable of recognizing a number of proteins, identification of other CUL4B^{AhR} substrate proteins may reveal new molecular links between AhR-mediated signaling and other signaling pathways

and cellular events. In this regard, it is of interest that AhR interacts with various transcription factors [11], such as Rb/E2F1 [66], SF1/Ad4BP [33], and NF-κB [67], to modulate their functions. AhR has recently been shown to regulate the differentiation of Th17 and T_{reg} cells [68–70]. This may be mediated by a functional interaction with STAT1 [70]. In addition, although the underlying mechanisms remain unknown, AhR also modulates the function of transcription factors [71] such as GR and RAR [72,73]. Considering the evolutionary conservation of AhR, it is likely that the intrinsic function of AhR is to mediate the signal transduction of endogenous ligands in cross-talk pathways. A current area of interest is the identification of candidate degradation substrates for AhR which are abnormally stabilized in AhR-deficient mice. In summary, several lines of recent evidence define a novel role for AhR as a ligand-dependent E3 ubiquitin ligase to regulate target-specific protein destruction. The ubiquitin ligase activity of AhR, together with the cross-talk of AhR with nuclear receptors through direct association, provides an additional layer of complexity for AhR biology. Characterization of these new molecular aspects of AhR function may lead to a greater understanding of the diverse biological actions induced by endogenous and exogenous AhR ligands.

Conflict of interest

The authors declare no competing financial interests.

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