



# **The AhR twist: ligand-dependent AhR signaling and pharmaco-toxicological implications**

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**The AhR twist{1. AU: Space added before twist. Is this what was intended?}:  
ligand-dependent AhR signaling and pharmaco-toxicological implications**

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**Keywords:** AhR, endogenous functions, SAhRM, ligands.

**Teaser:** The emerging concept of AhR ligands as potential pharmacological tools and the plasticity of the transcriptional responses of this receptor (depending on its ligands) are presented.

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The aryl hydrocarbon receptor (AhR) is a transcription factor which is activated by diverse compounds and regulates the expression of xenobiotic metabolism genes. Recent studies have unraveled unsuspected physiological roles and novel alternative ligand-specific pathways for this receptor. In this review, we discuss these novel aspects and focus on the different responses elicited by the diverse endogenous and/or exogenous AhR ligands. In addition to challenging the relevance of the 'agonist/antagonist' classification of ligands, we introduce the concept of AhR plasticity as a primordial factor in the generation of these pathways. Finally, we suggest several promising perspectives for the pharmacological modulation of these responses.

## 1 Endogenous functions and ligands of the AhR

2 The commonly accepted model of aryl hydrocarbon receptor (AhR) activation  
 3 has been described in several reviews (Figure 1) [1]. This model can be summarized,  
 4 briefly, as follows: in the cytosol AhR is associated with chaperones. When a ligand  
 5 binds to the receptor, the complex translocates into the nucleus and dissociates. In  
 6 the nucleus heterodimerization with its partner AhR nuclear translocator (ARNT)  
 7 occurs. This heterodimer binds to specific responsive elements called xenobiotic  
 8 responsive elements (XREs), located in target gene promoters – and it modulates the  
 9 transcription of these genes. The prototypical, and most studied, target gene is  
 10 cytochrome P450 1A1 (CYP1A1), a xenobiotic metabolizing enzyme.

11 Recent observations suggest that the AhR has physiological functions. This  
 12 hypothesis is supported by the marked degree of conservation among species and  
 13 the constitutive expression during development and in adult tissues of the AhR, as  
 14 well as, even more importantly, by the developmental aberrations observed in AhR  
 15 knockout animal models.

16 The AhR is expressed in invertebrates such as *Caenorhabditis elegans*  
 17 (roundworm) and *Drosophila melanogaster* (fruit fly) and in vertebrates. Interestingly,  
 18 in contrast to the vertebrate AhRs, there is no evidence that the fly and nematode  
 19 AhR orthologs can bind exogenous ligands such as 2,3,7,8-tetrachlorobenzo(*p*)dioxin  
 20 (TCDD) [2,3]. The reason for this lack of binding is probably because of the  
 21 divergence of the PAS-B{2. AU: Please define all acronyms if possible upon their first  
 22 usage} domains (essential for ligand binding in mammalian AhRs). Studies of  
 23 chimeras between murine and drosophila AhRs have identified subdomains, which  
 24 have crucial roles in the binding of ligands [4]. Although TCDD does not bind to the  
 25 AhR of invertebrates, this does not exclude the binding of other ligands, including

1 endogenous ones, by the AhR. Those ligands remain to be identified. Moreover, the  
 2 AhR signaling pathway in these invertebrates is highly conserved (i.e.  
 3 heterodimerization with ARNT orthologs and binding to XREs).

4 Studies using invertebrates and vertebrates in which the AhR has been  
 5 knocked out have unraveled some functions of the AhR that are not related to  
 6 xenobiotic metabolism. In the worm, AhR1{3. AU: I have changed AHR-1 to AhR1 to  
 7 tie in with style used below for the vertebrates. If AHR-1 is specific to the worm  
 8 please check this section and change back but also consider use of AhR1 and 2  
 9 below} is expressed in several neurons and blast cells during embryonic and larval  
 10 development. Worms that lack AhR1 display several neurological defects (e.g. social  
 11 feeding behavior), which affect neuronal differentiation, axon guidance and cell  
 12 migration. These functions appear to result from the indirect transcriptional control  
 13 that AhR1 exerts on a G-coupled{4. AU: Is this ok or should it be G-protein-coupled?}  
 14 neuropeptide receptor and on some members of the soluble guanylate cyclase family  
 15 [5]. In the fly, SPINELESS is expressed in several tissues during embryonic and  
 16 pupal development but it mainly regulates the leg development, the morphology of  
 17 the peripheral nervous system and the differentiation of photoreceptors that are  
 18 required for color vision [6].

19 These observations demonstrate that AhR orthologs play a part in the  
 20 development of invertebrates. However, the mechanism by which AhR is activated in  
 21 these organisms remains elusive, particularly as to whether or how a ligand might be  
 22 involved. At present, there is a limited amount of evidence available to demonstrate  
 23 that the functions the receptor exerts in invertebrates, such as promoting neuronal  
 24 development, are conserved in vertebrates. One might speculate that, during

1 evolution, mutations in the PAS domains led to the acquisition of new functions (e.g.  
2 xenobiotic metabolism) and to a broader tissue distribution of the receptor.

3 In vertebrates, the AhR genes are divided into two distinct clade lineages,  
4 AhR1 and AhR2. During vertebrate evolution, these two clades underwent tandem  
5 gene duplication events. For example, in fish several AhR isoforms are present (i.e.  
6 2–6 per species), whereas mammals express only a single protein. In the zebrafish  
7 three forms have been identified [7]. The first form, AhR1a, does not bind exogenous  
8 ligands and its function is unknown. By contrast, the biological functions of AhR2  
9 appear to be similar to those of the mammalian AhR. Recently, a third form, AhR1b  
10 was identified which can also bind exogenous ligands [8]. The three isoforms are  
11 expressed differently in tissues. A number of questions remain unanswered. In  
12 particular, it is unclear whether AhR1a and AhR1b possess physiologic functions in  
13 the brain and in the other organs in which they are expressed and whether some or  
14 all of these functions are conserved in mammals.

15 The characterization of AhR orthologs and paralogs provides insights into the  
16 ancestral functions of the receptor and suggests that AhRs in mammals have roles in  
17 addition to that of regulating the expression of xenobiotic metabolizing enzymes.  
18 Indeed, three laboratories have observed phenotypic abnormalities in mice lacking  
19 the AhR [9]. These three mouse models share many phenotypic similarities such as  
20 resistance to dioxin toxicity, infertility, liver and cardiovascular abnormalities, and  
21 delayed growth. These models, however, also display significant differences, for  
22 example sensitivity to infection [10].

23 All these recent studies suggest that the AhR can be active in the absence of  
24 exogenous ligands. The discovery of endogenous ligands is, thus, a crucial aspect  
25 for understanding the biology of the AhR better. Heart and lung extracts [11] have

1 been shown to contain AhR activators and several molecules have been identified as  
 2 putative ligands: arachidonic acid and leukotrienes, heme metabolites and UV  
 3 photoproducts of tryptophan [12]. Among the most significant studies, 6-  
 4 formylindolo[3,2-b]carbazole (FICZ), a tryptophan derivative with a high affinity for the  
 5 AhR, has been identified as a ligand and activator of CYP1A1 transcription [13]. FICZ  
 6 action on cells is transient as a result of metabolism by CYP1. Another tryptophan  
 7 metabolite, kynurenine has been shown to bind to the AhR, and was recently  
 8 implicated in the development of brain tumors [14]. It is noteworthy that the AhR, as  
 9 well as several of its ligands, has been associated with the promotion or the  
 10 prevention of atherosclerosis [15,16]. The presence of AhR-activating compounds,  
 11 such as oxidized low-density lipoprotein (LDL), has also been demonstrated in  
 12 sheared human and bovine sera [17].

13 In parallel to the identification of putative endogenous ligands, new AhR  
 14 xenobiotic activators that are substantially different from polycyclic aromatic  
 15 hydrocarbons have been identified and characterized [12,18]. They have been  
 16 described as AhR antagonists because they usually antagonize CYP induction.  
 17 However, recent studies have demonstrated that these molecules activate the AhR,  
 18 differentially, through alternative transcriptional responses.

## 19 **Alternative AhR transcriptional responses (Figure 1)**

### 20 ***The XRE track***

21 The first relevant study in this area concerned the pro-apoptotic protein Bax, which is  
 22 induced by 7,12-dimethylbenz[ $\alpha$ ]anthracene (DMBA){5. AU: Is this definition ok?} – a  
 23 polycyclic aromatic hydrocarbon (PAH) – in mouse oocytes [19]. This regulation  
 24 largely explains oocyte death upon PAH treatment. The authors show that the AhR is  
 25 central to this induction, but surprisingly TCDD had no effect. Analysis of the

1 promoter led to the identification of a TCDD-unresponsive XRE, which was defined  
 2 as an unusual responsive element (Table 1). We also identified an unusual AhR  
 3 target, paraoxonase 1 (PON1), which is a high-density lipoprotein-associated  
 4 enzyme, involved in the elimination of organophosphorous xenobiotics and toxic  
 5 endogenous compounds. Several AhR ligands (e.g. polyphenolic compounds such  
 6 as quercetin or resveratrol but also 3-methylcholanthrene) have been shown to  
 7 induce PON1 in a human hepatoma cell line in an AhR-dependant manner [20].  
 8 However, TCDD was a poor inducer. Interestingly, promoter analysis has led to the  
 9 identification of a crucial sequence in the proximal part of the promoter, which is  
 10 slightly different from the consensus XRE (Table 1). Finally, another alternative  
 11 sequence similar to the one identified in the promoter of PON1 (named AhR-RE type  
 12 III; Table 1) was recently found in the tyrosine hydroxylase gene promoter [21].

13 The participation of alternative XREs in the AhR transcriptional response  
 14 suggests that the binding of a particular ligand might adapt the structure of the AhR  
 15 to permit binding to a particular XRE sequence. The model hypothesizes that the  
 16 AhR-mediated transcriptional response is modulated by selective ligands of the  
 17 receptor, in accord with the selective AhR modulator (SAhRM) concept that was  
 18 initially described by Safe and McDougal [22]. The identification of selective  
 19 modulators of other receptors has strengthened the SAhRM concept, which is now  
 20 gathering additional support and more focus.

21

## 22 ***The SAhRM concept***

23 In some experimental systems, TCDD has been identified as an antiestrogenic  
 24 compound possibly having antitumorigenic effects. Safe and McDougal focused their  
 25 SAhRM research toward the identification of an AhR ligand (6-methyl-1,3,8-

trichlorodibenzofuran or methyl-substituted diindolylmethanes) with the same ability as TCDD to inhibit estradiol-induced mammary tumor growth but without any toxic effects associated with the antiestrogenic and antitumorigenic activities [22]. Since then, new discoveries that elucidate the interactions between the AhR and nuclear receptor signaling have been made. However, the impact the knowledge of these mechanisms has on the further development of SAhRMs is still unclear [23].

## **Crosstalk between AhR signaling and other transcription factors or pathways**

### ***Interaction with the estrogen receptor alpha pathway***

A number of AhR ligands have been shown to display endocrine-disrupting activities. One of the main interacting partners of the AhR is the estrogen receptor alpha (ER $\alpha$ ). Under certain experimental conditions, it has been shown that treatment with dioxin can decrease the incidence of mammary tumors in Sprague Dawley rats [24]. The AhR has been reported to inhibit ER $\alpha$  activity through a variety of mechanisms. Indeed, dioxin induces the expression of target genes such as CYP1A1 and CYP1B1, which are involved in the catabolism of steroid hormones including estrogens [25]. The heterodimer AhR/ARNT can also bind inhibitory XREs (iXREs) located in promoters of ER $\alpha$  target genes (i.e. pS2, Cathepsin D; Table 1), thus blocking their transcription [26]. Moreover, it has been demonstrated that the AhR can be part of a ubiquitin ligase complex that targets and degrades sex steroid receptors [27]. These studies suggest an antiestrogenic role for the AhR and appear to designate TCDD as an antagonist of ER $\alpha$  activation. However, recent studies have shown that the effect of TCDD is more complex. Indeed, in the absence of 17 $\beta$ -estradiol, [6]. AU: The symbols aren't showing properly in the version I'm working from. I have added alpha symbols above but I wasn't able to edit here.

Please confirm text here} interacts with ER $\alpha$  and this complex induces transcription of ER $\alpha$  target genes [28]. In conclusion, AhR has a dual role depending on the estrogen concentration.

Despite a large number of studies that have been conducted on this crosstalk, many questions remain unanswered. Indeed, it is tempting to hypothesize that diverse AhR ligands will act differently on the ER $\alpha$  pathway. Moreover, to date, the binding of the AhR to iXREs has not been investigated in the presence of these new AhR ligands.

### ***Interaction with the NF- $\kappa$ B pathway***

Clearly, the AhR is involved in inflammatory pathways. The interaction of the AhR with nuclear factor kappa B (NF- $\kappa$ B) could be a crucial feature of this effect. NF- $\kappa$ B is a family of ubiquitously expressed transcription factors, which involve the association of two subunits among RelA (p65), RelB (p50), c-Rel and p52.

Recent studies, attempting to unravel the mechanisms underlying the crosstalk between the AhR and NF- $\kappa$ B, have demonstrated a complex interplay between these transcription factors. First, the AhR physically interacts with different NF- $\kappa$ B subunits. A complex associating RelA and the AhR (but not ARNT) induces the proto-oncogene c-myc by binding to an NF- $\kappa$ B-responsive element in human breast cancer lines [29]. Conversely, it has been found that RelA represses AhR-controlled CYP1A1 expression [30]. Moreover, two signaling pathways [i.e. the classical AhR pathway and a novel pathway, which depends upon the activation of protein kinase A (PKA)] have been shown to induce the formation of a RelB–AhR heterodimer (without ARNT) in the human monocyte cell line U937 [31]. The existence of this latter pathway is supported by previous studies that indicate that cAMP and PKA can activate the AhR [32]. The authors have also demonstrated the

1 participation of a novel responsive element, RelB–AhRE (Table 1), localized in the  
2 promoter of the interleukin 8 gene [33,34].

3 Immunosuppressive effects of TCDD have also been described. A number of  
4 studies have shown that dioxin and the AhR can suppress NF- $\kappa$ B activity in immune  
5 murine cell lines [35,36]. Ruby and colleagues [35]{7. AU: Is this the correct citation  
6 that I have inserted here?} showed that this inhibition was caused by the formation of  
7 a RelA–AhR dimer, without DNA binding.

8 AhR ligands can be considered as potential modulators of NF- $\kappa$ B. In this  
9 respect, resveratrol and other stilbene derivatives also display anti-inflammatory  
10 properties [37]. Although several mechanisms probably play a part, it is conceivable  
11 that the AhR could be involved.

### 13 ***Signaling pathways controlling the AhR transcriptional responses (Figure 1)***

14 In addition to being ligand-mediated, the activity of the AhR is also controlled  
15 by phosphorylation via various kinases such as the c-Src kinase, mitogen-activated  
16 protein (MAP) kinases and protein kinase (PK)A or C. Although several MAP kinase  
17 (MAPK) phosphorylation sites of the AhR have been identified, their actual effects on  
18 ligand binding and signaling have not been studied yet. By contrast, several studies  
19 have focused on PKA and PKC. An increased intracellular cAMP concentration leads  
20 to the phosphorylation and nuclear translocation of AhR in a mouse hepatoma cell  
21 line via a PKA-dependent pathway [32]. Interestingly, ARNT does not co-precipitate  
22 with AhR under these conditions. Nuclear translocation of this phosphorylated form of  
23 AhR does not affect CYP1A1 transcription, suggesting that it could activate an  
24 unknown alternative response. Other studies have shown that PKC activity is  
25 required for ligand-dependent AhR signaling [38]. PKC-dependant phosphorylation of

the AhR and/or ARNT seems to be required but it is not sufficient for the transcriptional activity of this heterodimer. One can suspect that, as for nuclear receptors, this phosphorylation could also impact co-activator recruitment.

Calcium has also been shown to regulate the AhR pathway. Increased intracellular calcium in cells exposed to TCDD or PAH has been reported but  $\text{Ca}^{2+}$  also modulates the AhR response because it has been shown to be required for the complete induction of CYP1A1 [39]. Further investigations to characterize the calcium-related signaling pathway involved in the upregulation of AhR target genes by dioxin have identified CaMKI $\alpha$  in MCF-7 cells as being involved in TCDD-induced AhR activation [40].

The AhR can also modulate the activity of kinases: recent studies have shown that the AhR forms a cytosolic complex with Src. Binding of TCDD to the AhR triggers the release of Src and potentially affects the level of activation of this kinase which is crucial for several pathways involving cell proliferation and migration [41]. This is probably the best-characterized, non-genomic action of the AhR.

### ***Fine-tuning of the AhR response***

An important issue regarding the response of the AhR is whether its activation is transient or sustained. This might have an impact on the toxicity of the AhR pathway. Several fine-tuning mechanisms could contribute to the prevention of uncontrolled, sustained activation of the AhR. Several studies have shown that the amount of AhR protein is considerably decreased following activation by TCDD [42]. This decrease is caused by a stimulated, selective degradation by the proteasome and constitutes a simple feedback mechanism at the protein level. Another crucial feedback mechanism involving the AhR repressor (AHRR) was discovered several

years ago by the Evans *et al.* [43].{8. AU: Edit ok? DDT house style: citations in the manuscript should be the same as they appear in the reference list} The AhRR is induced by the AhR; it can form a heterodimer with ARNT, bind XRE and most probably acts as a dominant negative protein [43]. These mechanisms, however, have been challenged by several groups that have shown that the increased expression of ARNT or of a defective DNA-binding domain in the AhRR had no effect on the repressive action of the AhRR [44], [43]{9. AU: I did not want to affect your endnote numbering here. Please re-write as [43,44]}.

The distribution of AhR and ARNT, and their regulation during development, are also elements that are important for controlling their endogenous functions or their roles under stress. In C57Bl/6N mice embryos, in the early stages of development, the AhR is predominantly expressed in the neuroepithelium and in the heart. In these tissues, the level of AhR decreases at later stages. However, in other tissues (e.g. liver) it gradually increases during organogenesis [45,46]. These findings suggest a role for the AhR in development and indicate a possible switch in AhR function and control during development. ARNT also participates in additional signaling pathways such as the one stimulated by hypoxia, and it is essential for life because ARNT knockout mice are not viable [47]. Activation of these pathways could significantly reduce AhR signaling in tissues that express low levels of ARNT.

## **AhR ligands as potential pharmacological tools (Figure 2)**

Recent studies in several areas have suggested clearly that the pharmacological modulation of AhR activity will be important in the near future. For example, SAhRMs have been developed as new antitumorigenic agents, but have been shown to be relevant for application in fields other than cancer. Dioxins and the AhR have been linked to the control of inflammatory processes with interactions

1 between the AhR and NF- $\kappa$ B factors which seem to be key mechanisms [48–50].  
 2 Patel *et al.*{10. AU: Is this edit ok?} have demonstrated that several AhR ligands  
 3 repress genes involved in the acute inflammation response, through decrease in  
 4 RelA and C/EBP/ $\beta$ {11. AU: I have edited this symbol to a beta. Is this ok or did you  
 5 mean to use a sharp S? Please also define this acronym if possible} recruitment to  
 6 the gene promoter [51]. In addition, they synthesize a new AhR ligand (substituting a  
 7 hydroxy group for a methoxy one to a selective estrogen receptor modulator named  
 8 Way-169916), which does not exhibit ER binding [52] but still displays the anti-  
 9 inflammatory effect. Smith *et al.* {12. AU: Is this edit ok?} identified a unique set of  
 10 characteristics for a compound named GNF351, defined as a ‘pure antagonist’, which  
 11 displays a high affinity for the AhR and an anti-inflammatory potential [53].

12 In addition to its modulation of inflammation, the AhR has also been recently  
 13 associated with the regulation of other immune pathways [54,55]. Th17 cells are  
 14 suspected to have a role in several autoimmune diseases. By contrast, regulatory T  
 15 cells (Treg) are a specialized subpopulation of T cells that maintain tolerance to self-  
 16 antigens. The AhR specifically regulates the balance between both populations of  
 17 cells in mice [56,57]. Indeed, in a mouse model of autoimmune pathology (i.e.  
 18 experimental autoimmune encephalomyelitis) treatment with TCDD reduces the  
 19 pathological manifestations caused by an increased ratio of Treg:Th17. By contrast,  
 20 FICZ has the exact opposite effect to that of TCDD. The authors demonstrated the  
 21 crucial involvement of the AhR, which directs the differentiation of Th17 cells in the  
 22 presence of this ligand [58,59]. These observations indicate that two different ligands  
 23 of the AhR can trigger either positive or negative effects on health. Importantly, these  
 24 studies demonstrate that the AhR displays key immunological functions and indicate

1 that immunomodulation is a field in which the use of AhR ligands should be  
2 considered from a therapeutic perspective.

3 Finally, in a library of 100 000 molecules, an AhR ligand with antagonistic  
4 properties has been identified as a potent inducer of the proliferation of  
5 hematopoietic stem cells (HSC) *ex vivo* [60]. The latter discovery represents a major  
6 hope in the widespread use of HSC transplantation, even if safety studies remain to  
7 be carried out to confirm the potential of this molecule as a therapeutic option.

### 8 9 **Concluding remarks: the AhR(t) of plasticity**

10 Recent studies using multiple models have clearly suggested that the  
11 functions of the AhR are more diverse than previously thought. Interestingly, the  
12 effects of ligands on signaling pathways seem to be highly plastic with associated  
13 responses that cannot be classified using the simple agonist/antagonist  
14 pharmacological alternatives. The nature of the ligand and the way it binds to the  
15 receptor seem to determine the nature of the targeted XRE and the related  
16 transcriptional responses. For example, resveratrol must be considered not only as  
17 an antagonist of TCDD (which it actually is for the induction of CYP1A1) but also as  
18 an inducer and/or agonist of PON1. Nevertheless, this increased complexity of the  
19 AhR system is not bad news and has led to the concept of SAhRM and to the  
20 development of novel AhR ligands with interesting pharmacological activities.

21 Many mechanisms mediating the effects of AhR ligands remain elusive. Few  
22 large-scale studies have been performed that could be useful for studying the global  
23 transcriptional responses of the AhR bound to alternative ligands [61,62]. At the  
24 molecular level, there is still no structure for the AhR. The discovery by Zhao{13. AU:  
25 Is this edit ok?} and co-workers of an antagonist that selectively inhibits the effects of

1 dioxin and other related HAHs{14. AU: Please define} (but not those of PAHs,  
 2 flavonoids and indirubin) suggests that the promiscuous PAS-B domain could either  
 3 have different binding sites or a highly plastic one [63]. The consequences of the  
 4 activation of AhR by alternative ligands extend beyond the binding to XREs alone.  
 5 The recruitment of co-activators or co-repressors has not been investigated  
 6 extensively with the exception of a study by Hestermann and Brown that  
 7 demonstrated 3,3'-diindolylmethane and  $\beta$ -naphthoflavone ( $\beta$ -NF) enabled the  
 8 recruitment of p160 family co-activators (NCoA1-3) and p300 acetyltransferase.  
 9 Interestingly, those recruitments (including the AhR) display a sinusoidal variation  
 10 after  $\beta$ -NF treatment suggesting that this process is highly dynamic. However, only  $\beta$ -  
 11 NF triggered the binding of RNA polymerase II [64]. Finally, several studies have  
 12 established that AhR ligands can also trigger AhR-independent processes at the cell  
 13 membrane. These effects need to be characterized better to determine the  
 14 responses regulated by the AhR. Several approaches can be proposed to solve  
 15 these problems. For example, AhR knockout models will be suitable tools to decipher  
 16 the effects of alternative ligands such as resveratrol and large-scale, high-throughput  
 17 studies will be relevant in this experimental picture. Moreover, development of  
 18 reporter genes sensitive to each pathway modulated by the AhR as well as the  
 19 elucidation of its 3D structure will be breakthroughs in the field.

20 The AhR has been, directly or indirectly, linked to pathophysiological  
 21 processes including atherosclerosis, inflammation, immunomodulation and cancer.  
 22 Interestingly, even if many aspects of the AhR are insufficiently developed in terms of  
 23 drug development, many drugs approved by the FDA are AhR ligands or at least  
 24 activators of this pathway, including omeprazole, leflunomide, flutamide and  
 25 nimodipine [65]. Toxicological and pharmacological consequences should be

1 considered. This is, therefore, a stimulating time for all AhR experts and discoveries  
2 concerning this receptor are certainly forthcoming, which could expand the interest in  
3 the AhR well beyond traditional toxicological considerations.

4

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7

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## Figure legends

### **Figure 1. The aryl hydrocarbon receptor (AhR) is activated by various ligands,**

**leading to alternative transcriptional responses.** The AhR is a cytoplasmic protein that is complexed to heat shock proteins (HSPs). Upon binding to a ligand, the AhR translocates into the nucleus and forms a heterodimer with its partner, AhR nuclear translocator (ARNT). The release of the AhR from the complex is also associated with the cytoplasmic activation of Src kinase. The binding to aromatic hydrocarbons (dioxins, polycyclic aromatic hydrocarbons) leads to adaptative responses including the upregulation of xenobiotic metabolism enzymes (XMEs; indicated to the right of the figure). Alternatively, several polyphenols have been shown to activate the AhR but activate the adaptative responses associated with xenobiotic metabolism poorly. Quercetin or resveratrol, two polyphenols, activate the AhR and the upregulation of paraoxonase 1 (PON1) through binding to alternative xenobiotic responsive elements (XREs). Finally, the AhR displays an endogenous function that might be regulated by endogenous ligands or other kinds of stimuli (e.g. phosphorylation) and might involve the binding of the receptor on responsive elements{15. AU: Is this ok or should this be response elements?} (REs). The use of AhR knockout models has shown that this function (which also depends on nuclear translocation, heterodimerization and DNA binding) is associated to the regulation of liver, immune system, cardiovascular and neuronal functions. In this model, the binding of exogenous ligands to the AhR might lead to disruption of the endogenous AhR regulatory process.

**{16. AU: I am unable to edit the figure file without impacting resolution. Please make the following edit to Fig.1 for consistency with your manuscript text: change SRC to Src. Please make the following edits for DDT house style:**

1 remove hyphen to close up cardiovascular; use a lower case p for  
2 polyphenols}

3 **Figure 2. The AhR regulates multiple signaling pathways.** The AhR regulates the  
4 activity of NF- $\kappa$ B members, estrogen receptors and recent studies demonstrate that it  
5 modulates immune and stem cell functions. According to the type of ligand that binds  
6 the AhR, selective AhR modulation can be obtained that might be of significant  
7 impact for the pharmaceutical field. Moreover, the AhR is connected to several  
8 kinases through multiple interactions (e.g. modulation of the AhR activity by  
9 phosphorylation) and calcium signaling. The AhR repressor (AhRR) is an important  
10 AhR-responsive gene with activity that balances{17. AU: In this context the word  
11 balances is ambiguous. Please state exactly what is meant here: regulates,  
12 counters?} the activity of the AhR.

13 **{18. AU: I am unable to edit the figure file without impacting resolution. Please**  
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17 **case r for receptors (in Estrogen receptors – this is because we use sentence**  
18 **case in all headings/figs, etc.)**

Figure 1

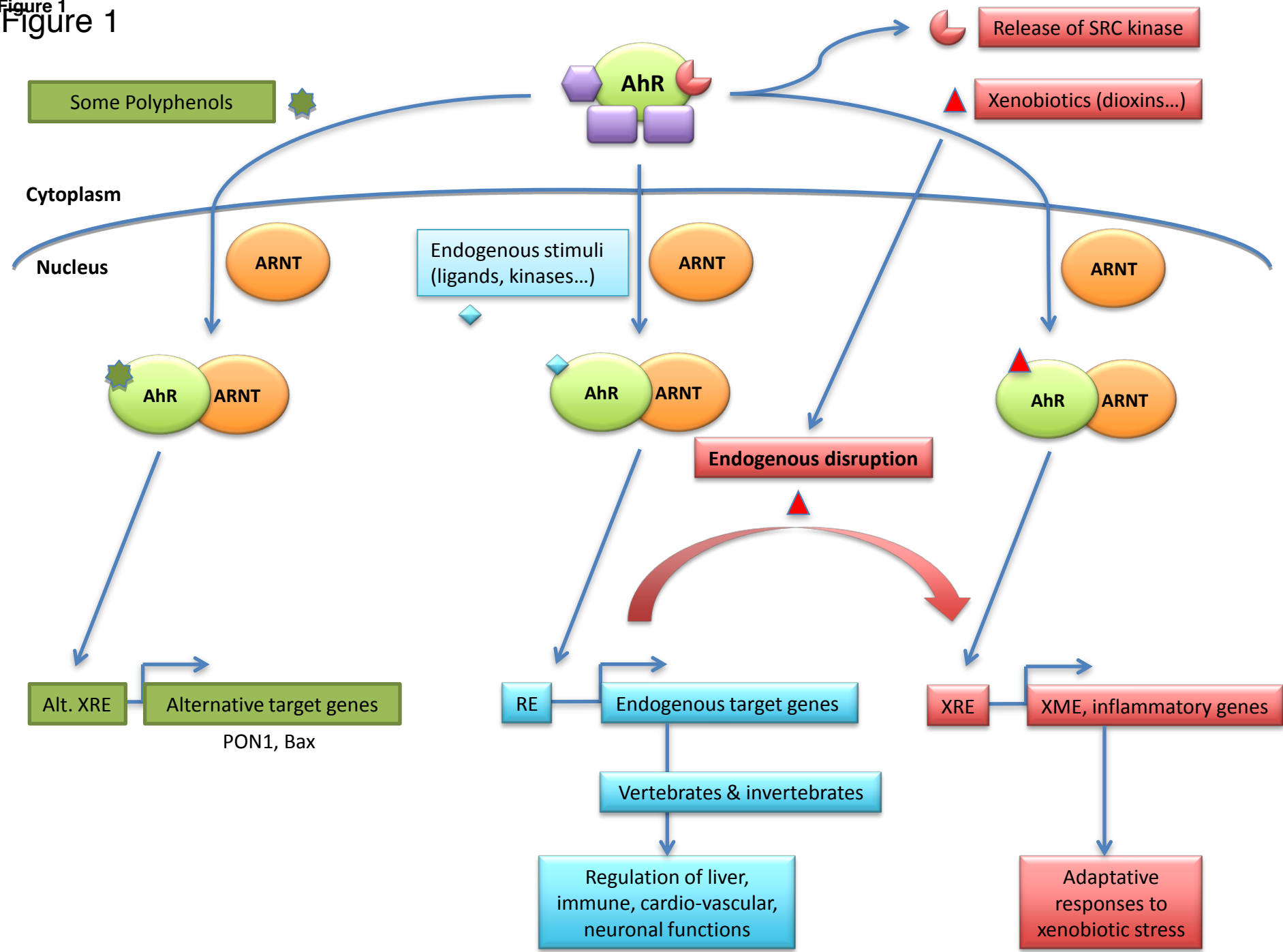
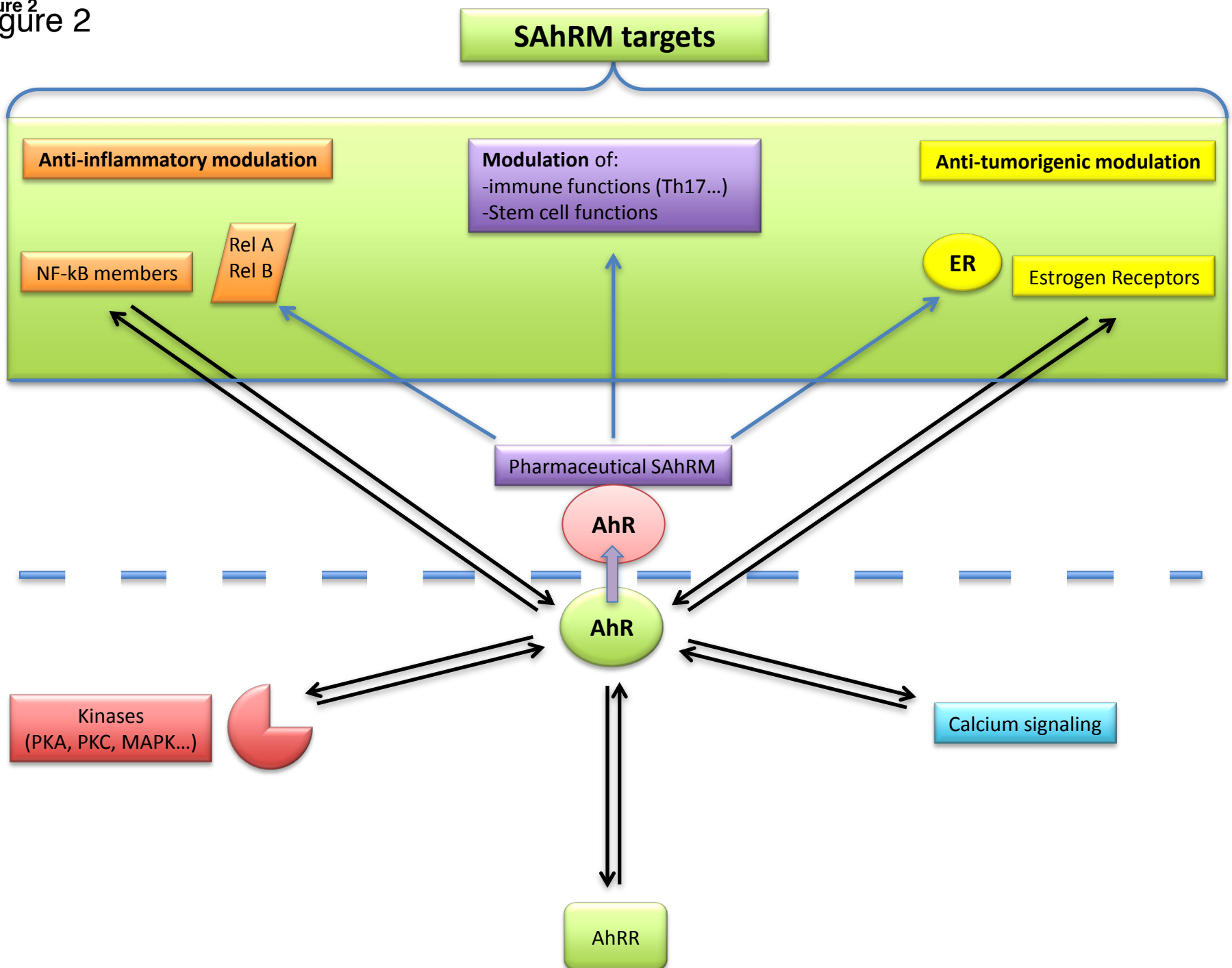


Figure 2  
Figure 2



Target gene Responsive element	Sequence	Model	AhR ligand
CYP1A1 Canonical XRE	TnGCGTG A/G G/C A	Hepatic cell lines, hepatocytes and many models	Halogenated or polycyclic aromatic hydrocarbons
CYP1A2 XRE II	CATG{N6}C [T/A]TG	Hepatic cell lines, hepatocytes	3-Methylcholanthrene (3MC), TCDD (dioxin)
Bax TCDD-unresponsive, BaP-responsive XRE	AcaagcctggGCGTGggc tatattg	Mice oocytes	Benzo(a)pyrene (BaP)
PON1 TCDD-unresponsive, polyphenol-responsive XRE	TgccgacccgGCGGGgaggggcggg	HuH7 cell line	3MC, BaP Resveratrol, quercetin
Tyrosine hydroxylase AhRE III	tgtcttcatgtcgtgtctaggcg	Neuro2a cell line	TCDD
Cathepsin D, c-fos, pS2 iXRE	CnG GCGTG A/G G/C C	MCF-7 cell line	TCDD
RelB-AhRE	AGATGAGGGTGCATAAGTTC	U937 cell line	TCDD and forskolin

**Table 1. The various AhR-responsive elements, the corresponding genes in which they are located, the model used to characterize them and the type of ligand that leads to recruitment of the AhR**

- The AhR is a transcription factor activated by diverse exogenous compounds
- The AhR regulates the expression of xenobiotic metabolism genes.
- KO models have revealed that the AhR also regulates endogenous processes
- AhR modulators might be used as anti-tumorigenic or anti-inflammatory drugs
- The AhR also regulates the proliferation of hematopoietic stem cells.

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