Animals and humans are exposed each day to a multitude of chemicals in the air, water and food. They have developed a battery of enzymes and transporters that facilitate the biotransformation and elimination of these compounds. Moreover, a majority of these enzymes and transporters are inducible due to the activation of xenobiotic receptors which act as transcription factors for the regulation of their target genes (such as xenobiotic metabolizing enzymes, see below §4 for the AhR). These receptors include several members of the nuclear/steroid receptor family (CAR for Constitutive Androstane Receptor, PXR for Pregnane X Receptor) but also the Aryl hydrocarbon Receptor or AhR, a member of the bHLH-PAS family (basic Helix-Loop-Helix - Period/ARNT/Single minded). In addition to the regulation of xenobiotic metabolism, numerous alternative functions have been characterized for the AhR since its discovery. These alternative functions will be described in this review along with its endogenous functions as revealed by experiments performed on knock-out animals.

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1. Introduction

Animals and humans are exposed each day to a multitude of chemicals in the air, water and food. They have developed a battery of enzymes and transporters that facilitate the biotransformation and elimination of these compounds [1,2]. Moreover, a majority of these enzymes and transporters are inducible due to the activation of xenobiotic receptors which act as transcription factors for the regulation of their target genes (such as xenobiotic metabolizing enzymes, see below §4 for the AhR) [3]. These receptors include several members of the nuclear/steroid receptor family (CAR for Constitutive Androstane Receptor, PXR for Pregnane X Receptor) [4] but also the Aryl hydrocarbon Receptor or AhR, a member of the bHLH-PAS family (basic Helix-Loop-Helix — Period/ARNT/Single minded) (Fig. 1). In addition to the regulation of xenobiotic metabolism, numerous alternative functions have been characterized for the AhR since its discovery. These alternative functions will be described in this review along with its endogenous functions as revealed by experiments performed on knock-out animals [5].

2. The AhR ligands

Numerous ligands (Fig. 2) for the AhR have been described. Xenobiotics, which are mostly aromatic hydrocarbons (including dioxins or PCBs “polychlorinated biphenyls”) were the first ligands discovered. The main source of human exposure (>90%) to aromatic hydrocarbons is through contaminated food. Acute exposure to high doses of dioxins in the workplace or due to industrial accidents can cause skin lesions such as chloracne. Long-term environmental exposure results in more extensive toxic
effects among which are immunotoxicity, neurodevelopmental abnormalities, thyroid dysfunction, disruption of steroid hormones and reproductive functions. Experiments in animals have demonstrated carcinogenicity, with multiple cancer sites, in a large number of species (recent epidemiological studies on occupationally exposed persons are in agreement with these findings). The International Agency for Research on Cancer (IARC) has classified TCDD in group 1 (carcinogenic to humans) whereas PCBs are classified in an intermediate group, 2A (probably carcinogenic to humans). Recently, natural compounds which are found in food have been characterized as AhR ligands. Flavonoids such as quercetin and resveratrol, the most abundant class of polyphenols, are found in fruits and vegetables. Indoles such as indole-3-carbinol (I3C) are derived from cruciferous vegetables such as broccoli or Brussels sprouts. Finally, molecules in the body which are formed by endogenous metabolism, such as FICZ (formylindolo [3,2-b] carbazole), indirubin, indigo, metabolites of arachidonic acid or kynurenine pathway metabolites, also have been described as AhR ligands. In the central nervous system, the catabolism of tryptophan leads to the production of NAD⁺, neuroactive metabolites such as kynurenic acid, glutamatergic agonists (NMDA) or neurotransmitters (quinolinic acid). In mammals, three enzymes catalyze the first limiting step of catabolism of tryptophan to N-formyl-kynurenine: TDO2 ("tryptophan-2,3-dioxygenase") and IDO1 and 2 ("indoleamine-2,3-dioxygenases") [6].

3. The AhR complex

The non-activated form of the AhR is cytoplasmic and it forms a complex with several chaperones [7] among which are two HSP90 (Heat Shock Protein 90), a co-chaperone p23, a XAP-molecule 2 (hepatitis B Virus X-associated protein 2). Some studies suggest that the Src tyrosine kinase also is a member of the complex. These proteins maintain the correct folding of the AhR, allow a proper recognition of the ligand by the receptor and, subsequently, ensure indirectly an efficient transcriptional effect [8].

4. Activation and modulation of the AhR

Several signaling pathways can be activated by the AhR. The first pathway to be described was the genomic pathway (Fig. 3) and it is now well-characterized. After a ligand is bound, the AhR translocates into the nucleus and it binds to ARNT to form an active heterodimer. This heterodimer modulates the expression of targets by binding to xenobiotic responsive elements (XRE) and coregulators. The amount of protein expressed from targeted genes is reduced by 80–95% in many cell culture models within 4 h of treatment by a ligand [9–11]. After being exported out of the nucleus, the AhR is rapidly degraded in the cytoplasmic compartment by the proteasome [12]. Proteasomal degradation of the AhR involves its binding of ubiquitin covalently. Other post-translational modifications of the AhR have been observed. SUMOylation enhances AhR stability through inhibition of its ubiquitinylation. However, this may suppresses its transactivating activity [13, 14]. The different ligands of the AhR may activate the receptor differentially. We have shown that resveratrol does not strongly activate the expression of CYP1A1 in a human hepatocellular cell line. However, resveratrol does activate the expression of paraoxonase 1.
Endogenous ligands
- Physiological ligands:
  - Kynurenine pathway metabolites
  - Arachidonic acid metabolites
  - Tetrapyrroles

Exogenous ligands
- Synthetic and natural ligands:
  - Halogenated aromatic hydrocarbons
  - Polycyclic aromatic hydrocarbons
  - Polyphehols

AhR activation
- Cell-cell contact
- Ligand binding
- Constitutive active form

AhR mechanisms
- Transcriptional regulation of genes
- Cell cycle regulation
- Protein interactions
- Epigenetic mechanisms
- Other signalling pathway cross-talk

Physiopathological effects
- Toxic effects of TCDD
- Cancer promotion
- Cell migration
- Stem cell renewal inability
- Liver fibrosis
- Dermatological lesion
- Inflammation

Physiological effects
- Organism detoxification
- Anti-proliferative activity
- Cell adhesion
- Reproduction
- Vascular development
- Hematopoietic development
- Nervous development

Fig. 2. The functional relationship between the AhR ligands and the regulatory roles of this receptor in physiology and pathophysiology. Synthetic ligands such as Halogenated Aromatic Hydrocarbons (HAHs) such as PCBs (“Polychlorobiphenyls”) and PCDD (polychlorinated dibeno-pa-ras-dioxins) or PAHs (Polycyclic Aromatic Hydrocarbons) which include benzo (α) pyrene (B(a)P) or 3-methylcholanthrene (3-MC) were among the first molecules to be identified as AhR ligands. These molecules are present in the air or in foods as complex mixtures, they are very stable; some may accumulate in the body (TCDD has a half-life of about seven years in humans) and they are powerful inducers of AhR [80]. Dioxins or PCBs are highly soluble in fats and can, therefore, reach high concentrations in fatty foods such as dairy products, fishes, meats and seafood. More recently, ligands of natural origins (food and endogenous ligands) such as flavonoids or indole derivatives also have been characterized as AhR ligands. Flavonoids are found in fruits and vegetables and represent the most abundant class of polyphenols. Among them, quercetin and resveratrol activate the AhR [8,81] and exert both agonist and antagonistic effects. Indoles such as indole-3-carbinol (I3C), which are derived from cruciferous plants such as broccoli or Brussels sprouts, are reported to have anti-cancer properties. Part of the effects of I3C occurs via activation of the AhR [82]. In addition, physiological endogenous ligands of the AhR such as indole amino acid metabolites (tryptophan, tryptamine, indole acetic acid) recently have been characterized. A photoprodut of tryptophan also has been identified through structural and chromatographic studies [83]: FICZ (6-formylindenolo [3,2-b] carbazole) [84]. Indurbin and indigo represent another group of indoles [85] which are detected in human urine under normal physiological conditions and, therefore, are present in our organisms, and are strong inducers of the AhR [85,86]. Physiological ligands also include metabolites of arachidonic acid (lipoxin A4, some prostaglandins (PGG2)) [87,88], tetrapyrroles (bilirubin, a degradation product), heme and biliverdin [89]).
the subsequent production of arachidonic acid. The parallel
activation of MAP kinases by Src leads to the transcription of
cyclooxygenase 2 (COX2) which uses arachidonic acid to
produce prostaglandins that can cause inflammation. Thus,
these two signaling pathways, which were initially activated by
TCDD, converge towards the stimulation of inflammation [24].

Moreover, the AhR interacts with Wnt/β-catenin, ER-alpha or
NF-kB and strongly modulates their actions [25–28]. On the
other hand, these transcription factors also impact AhR
signaling. For example, β-catenin is now described as a co-
activator of this receptor [29].

Finally, after exposure to a ligand, the level of the AhR protein
has been found, both in vitro and in vivo, to decrease rapidly
without the level of the messenger RNA being altered [9].
5. Regulation of cellular functions by the AhR

The best-characterized AhR function to date is the establishment of a protective adaptive response to xenobiotics through induction of the synthesis of xenobiotic metabolism enzymes. Aromatic hydrocarbons activate the AhR which induces family 1-P450 cytochromes (1A1, 1A2, 1B1) and the functions of which deal mostly with hydrocarbon detoxification. This elegant regulatory loop protects xenobiotic-exposed animals by detecting and then metabolizing these substances. However, the high degree of conservation of this receptor among species [21], its pattern of expression during development and in adult tissues [30] and the phenotypic alterations observed in AhR-deficient mice [31–33] suggest a strong involvement of the AhR in cell physiology which is independent of the metabolism of xenobiotics. The detoxification function of the AhR may have been acquired late in evolution.

5.1. Cell proliferation

One of the most intriguing and exciting aspects of AhR biology is its ability to promote or inhibit cell proliferation. For example, AhR KO mouse embryonic fibroblasts exhibit slow growth and accumulation in the G2/M phase of the cell cycle [34]. In human hepatoma cells (HepG2), AhR-siRNAs block the G1/S transition of the cell cycle and decrease cyclins D1 and E as well as CDK2/4-dependent cyclin kinases. This supports a pro-proliferative role for the receptor [35]. TCDD also can affect the expression of genes involved in cell proliferation (TGF-β, IL-1β and PAI-2), regulation of the cell cycle (JunB and JunD [36]) and inflammation [37–39]. In human breast cancer cells (MCF-7), NF-kB, via its RelA subunit, physically interacts with AhR [25] which results in transactivation of the c-myc proto-oncogene. With respect to the cell cycle, the expression of JunD and subsequently cyclin A, which blocks cell contact inhibition and favors proliferation [36], is triggered by TCDD-activated AhR via a novel ARNT-independent pathway. The role of AhR as a cancer promoter has been demonstrated in murine models which overexpress a constitutively active form of the AhR [40,41]. All these results suggest that the AhR favors cell proliferation. However, other studies have revealed an anti-proliferative activity of the AhR. AhR stimulates the transcription of the tumor suppressor gene, p27Kip1, in non-proliferative hepatoma cells or in the fetal thymus [42,43]. The AhR also regulates the function of the pro-proliferative factor E2F (E2F factors can be inhibited by direct interaction with retinoblastoma protein, pRb, and their function also depends on the presence of co-activators p300) in 3 different ways: 1) TCDD activates the physical interaction between the AhR and pRb which promotes its binding to E2F and stops the cell cycle [44], 2) in addition, TCDD stimulates the interaction between AhR and p300, which leads to displacement of p300 from E2F sites [45], 3) finally, a direct inhibitory interaction was detected between the AhR and E2F with potential implications for stem cell renewal [46,47].

Overall, the activity of the AhR on cell proliferation is probably dependent on the cell type, the timing of the cell cycle (and the expression of interacting partners such as RelA or pRb), the developmental period (if considering an animal model). Therefore, any specific action which would include the use of an AhR ligand to control the cell cycle, needs to be carefully evaluated in regard to these parameters.

5.2. Adhesion and cell migration

The contribution of the AhR in adhesion processes, which involve cell-cell and cell-extracellular matrix interactions, has recently emerged. Cell density also influences the compartmentalization of the AhR and low densities lead to a nuclear localization of the AhR [48]. These interactions are also very important for metastatic processes.

Knock-out models seem to confirm this involvement of the AhR in cell migration. Immortalized mammary fibroblasts derived from AhR KO mice, display decreased migration which is associated with an increased formation of cytoskeleton stress fibers and a reduction in the formation of lamellipods [49]. Signaling pathways which regulate cell migration are also inhibited in AhR-deficient cells which exhibit weaker activation of focal adhesion kinase (FAK), PKB/Akt (protein kinase B), ERK1 (extracellular signal-regulated kinase 1) and Rac-1 (Ras-related C3 botulinum toxin substrate 1). In addition, these fibroblasts induce fewer tumors in vivo in immunodeficient NOD-SCID mice (non-obese diabetic/severe combined immunodeficiency) than in wild-type mice [49].

The involvement of AhR in mobility and cellular plasticity also has been demonstrated by studies based on xenobiotic treatments. Exposure of human MCF-7 or HepG2 cells to TCDD causes morphological changes such as the appearance of lamellipodia, which cause greater cell adhesion and motility. This is associated with a reorganization of the cytoskeleton mainly due to a redistribution of actin and vinculin and to the activation of the FAK and Src kinases (Fig. 4) [23]. These cellular effects are accompanied by changes in the expression of certain genes such as E-cadherin and by the activation of JNKs [23]. E-Cadherin downregulation is a hallmark of epithelial-mesenchymal transition which is triggered by transcription factors such as Slug, a direct AhR target gene [50,51].

6. Physiological roles of the Ah receptor

AhR KO mice display developmental abnormalities which highlight the roles of the receptor in female fertility [52], perinatal growth [18,31], regulation of blood pressure, production of peripheral lymphocyte counts [31,32,53] and increased susceptibility to colitis [54]. Recently, it has been shown in a mouse model of induced-colitis, that FICZ (a high-affinity endogenous AhR ligand) prevents intestinal barrier function via AhR activation by suppressing IL-6 and claudin-2 expression [55].

Depending upon the model, AhR KO mice develop cardiac hypertrophy [56], dermatological lesions, portal vascular hypertrophy [32] and pyloric hyperplasia of the gastrointestinal tract [56]. One of the most common phenotypes to all AhR KO
models is vascular. The mice exhibit a systematic persistence of ductus venosus [57], a porto-fetal shunt of the developing liver, which normally closes immediately after birth [58,59]. These abnormalities result in reduced liver size associated with portal fibrosis and early lipid accumulation [60,61]. A candidate gene that could explain this liver phenotype is Transforming Growth Factor β (TGF-β). The AhR KO mouse livers have increased levels of TGF-β in the portal space [62] which could contribute to the development of fibrosis and to a low proliferative capacity as a result of the pro-fibrogenic and anti-proliferative activities of this cytokine. Additional studies have shown that this elevation of TGF-β is related to the accumulation of retinoic acid and to a reduction in retinoic acid metabolism, which lead to a decrease in CYP2C39 [63]. Other vascular abnormalities have been observed in these mice, such as the persistence of the hyaloid artery and an impairment of limbic vascularization in the developing eye [57]. The AhR KO mice also develop an ocular pathology which consists of a horizontal pendular nystagmus which is associated with myelin defects of the optic nerve and a local inflammation [64]. Similar myelin defects also have been identified recently in the peripheral nervous system [65]. Moreover, the AhR is involved in neuroendocrine pathways such as those that control the brain-pituitary-interrenal and gonadal axes. Treatment of rainbow trout with β-naphthoflavone and resveratrol, agonist and antagonist of AhR, respectively, has elucidated the role of the receptor in the disruption of steroid production after PCB exposure [66].

Studies in KO models suggest that AhR ligands activate AhR-independent pathways but these results require thoughtful interpretation as to the mechanisms involved. For example, in AhR KO rats, treatment with alpha-naphthoflavone (an AhR antagonist) causes an increase in the rate of ovulation and in follicular growth [67]. Abnormalities in the immune system also have been explored. After administration of TCDD, AhR KO rats displayed changes in immune phenotypes such as a decrease in CD8+ T cells and CD11+ but an increase in NKT cells [68].

Although similar consequences frequently are found in different species (such as the insensitivity to the effects of TCDD in AhR-deficient animals as compared to the wild-type), some AhR KO phenotypes are species-specific, such as the differences that occur between mice and rats [69,70]. For example, vascular phenotypes that are found in AhR KO mice, such as the persistent ductus venosus of the liver or hyaloid artery in the eye, are not observed in AhR KO rats. Or, alterations of the urinary tract (renal dilatation or degenerative changes and ureter dilatation) have been identified in AhR KO rats, but not in AhR KO mice.

7. Conclusion

In recent years, new functions of the AhR have been identified both in vertebrate and invertebrate models. In vertebrates, the AhR regulates the functions of transposable elements (including retrotransposons) which are suspected to regulate a large number of gene expression patterns [71,72], chromatin functions (insulators) and also epigenetic mechanisms through the regulation of SIRT1 activity or miR expression [5]. This could have potential impacts in terms of evolution. The AhR is also a protein whose functions have been modified throughout evolution. In invertebrates, no ligand for the AhR has been identified to present [73–75]. This suggests that the protein has acquired detoxification functions over time.

Conflict of interest

There is no conflict of interest for all authors.

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