REPRODUCTION
REVIEW

Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction

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Abstract

The dioxin/aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor responsive to both natural and man-made environmental compounds. AhR and its nuclear partner ARNT are expressed in the female reproductive tract in a variety of species and several indications suggest that the AhR might play a pivotal role in the physiology of reproduction. Furthermore, it appears to be the mediator of most, if not all, the adverse effects on reproduction of a group of highly potent environmental pollutants collectively called aryl hydrocarbons (AHs), including the highly toxic compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Although a large body of recent literature has implicated AhR in multiple signal transduction pathways, the mechanisms of action resulting in a wide spectrum of effects on female reproduction are largely unknown. Here we summarize the major types of molecular cross-talks that have been identified for the AhR and linked cell signaling pathways and that are relevant for the understanding of the role of this transcription factor in female reproduction.
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Introduction

The aryl hydrocarbon receptor (AhR) is a member of the basic helix–loop–helix (bHLH)-Per-ARNT-Sim (PAS) family of transcriptional regulators that control a variety of developmental and physiological events, including neurogenesis, tracheal and salivary duct formation, toxin metabolism, circadian rhythms, response to hypoxia, and hormone receptor function. The unique feature of all bHLH-PAS proteins is the PAS domain, named after the first three proteins identified with this motif, the *Drosophila* Per, human ARNT and *Drosophila* Sim (Nambu *et al.* 1991). The PAS domain consists of 260–310 amino acids (Crews *et al.* 1988) and incorporates two well-conserved hydrophobic repeats, termed PAS-A and PAS-B, separated by a poorly conserved spacer. Overall, the PAS domain is not well conserved and, given its size and diversity in sequence, can mediate a number of diverse biochemical functions. Facilitating partner selection during formation of bHLH-PAS heterodimers (Huang *et al.* 1993), binding small molecules (Dolwick *et al.* 1993), and conferring target gene specificity of bHLH-PAS heterodimers are important functions of PAS-domains (Zelzer *et al.* 1997).

The AhR and its nuclear partner ARNT are two founding members of the bHLH-PAS family and their dimerization to form an active transcription factor complex has become a paradigm in studying mechanisms of bHLH-PAS protein function. Unliganded AhR is located in the cytoplasm associated with heat shock protein 90 (hsp90) (Denis *et al.* 1988, Perdew 1988) and a 38 kDa, immunophilin-related protein (XAP2) (Carver & Bradfield 1997, Ma & Whitlock 1997, Meyer *et al.* 1998). Ligand binding to the AhR is presumed to produce conformational changes in the AhR protein which result in the exposure of an AhR nuclear localization signal and the translocation of the whole complex into the nucleus (Pollenz *et al.* 1994). Within the nucleus, the AhR–ligand complex dissociates from associated proteins and dimerizes with ARNT (Reyes *et al.* 1992), to reconstitute an active transcription factor which binds defined DNA sequences with high affinity. Binding of the ligand-activated AhR–ARNT transcriptionally active complex to its specific DNA recognition site, the xenobiotic-responsive element (XRE), within the promoter region of AhR-regulated genes results in their increased transcription (Denison *et al.* 1988). Much of our understanding of AhR function derives from analyses of the mechanisms by which its prototypical ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces the transcription of CYP1A1. This gene encodes the microsomal enzyme cytochrome P4501A1 which oxygenates various xenobiotics as part of their stepwise detoxification (Conney 1982). Although the transcriptional activation of P450

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family members by AhR ligands is well known, additional routes of AhR-mediated actions have been proposed. For instance, TCDD causes a rise in Ca^{2+} uptake within 5 min in Hepa-1 cells which can not be explained on the basis of transcriptional changes (Puga *et al.* 1992). Furthermore, Enan and Matsumura (1995) demonstrated that TCDD induces changes in protein phosphorylation through the activation of protein tyrosine kinases within 10 min. This rapid effect is AhR dependent and occurs under cell-free conditions in the absence of a nucleus. On the basis of these observations a TCDD-induced protein phosphorylation pathway may be considered as a separate route of AhR signaling from the well-established nuclear translocation-dependent pathway. A scheme of the two different signaling pathways of the AhR upon ligand binding is shown in Fig. 1.

AhR ligands such as TCDD, coplanar polychlorinated biphenyls (PCBs) and dibenzo[*a*]antracene (DBMA), are widespread in the environment, potent toxicants and resistant to metabolic breakdown (examples of AhR ligands are shown in Fig. 2). The latter property is responsible for the accumulation of these compounds in the food chain and their sustained effects on animal and human health, including reproductive functions (Fischer 2000, Stapleton & Baker 2003). The induction of xenobiotic-metabolizing enzymes is considered an adaptive cellular response aimed at detoxifying lipophilic foreign compounds. Other responses to AhR ligands include alterations in endocrine homeostasis, cellular proliferation and tissue differentiation; these responses are associated with adverse health effects (Poland & Knutson 1982, Safe 1986, Peterson *et al.* 1993, Huff *et al.* 1994).

Reproduction and developmental processes reflect an intricate and highly regulated chain of events and require the precise integration of a functional endocrine system. Exogenous environmental chemicals that mimic, inhibit or modulate endogenous endocrine messengers may inevitably disturb reproductive functions. TCDD and related compounds have been demonstrated to alter cell growth and differentiation, and to affect homeostasis and hormone balance by modulating the induction of enzymes, growth factors and hormones as well as their cognate receptors (DeVito & Birnbaum 1995). In addition, these xenobiotic compounds can act in an estrogenic or anti-estrogenic manner (Safe & Krishnan 1995), alter the levels of thyroxin (Van Birgelen *et al.* 1995) and several growth factors – including their cognate receptors (Abbott & Birnbaum 1990, Dohr *et al.* 1994) – or modulate the expression of glucocorticoids (Abbott 1995).

This review summarizes AhR-mediated cellular responses in the female reproductive tract, particularly focusing on the molecular cross-talk identified between AhR and other cell signaling pathways.

AhR cross-talk with steroid hormone receptors

Most AhR/steroid receptor interactions have been studied in human breast cancer and endometrial carcinoma cell lines. The liganded AhR targets specific genomic core inhibitory dioxin/xenobiotic responsive elements (iDRE/iXRE). Functional iXREs are present within the promoter

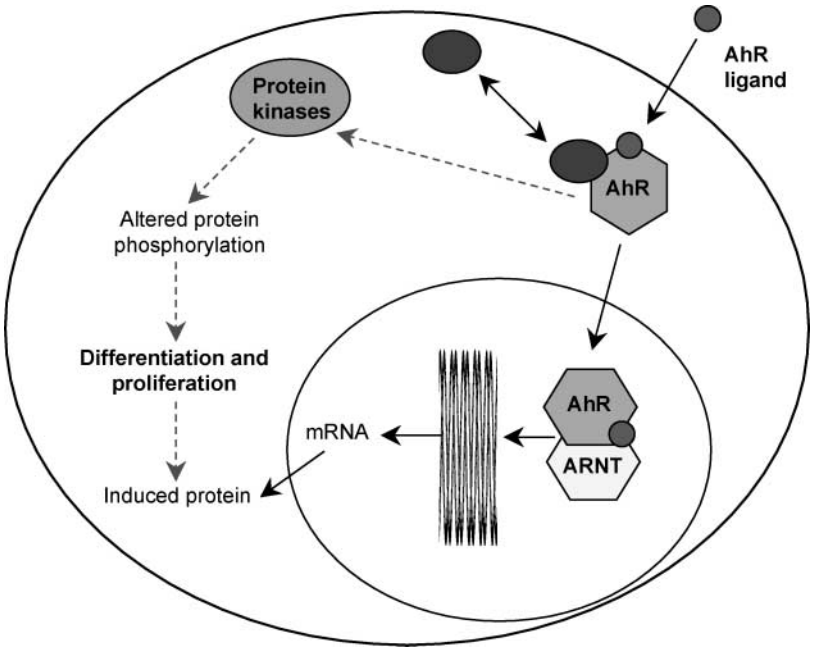

Figure 1 Binding of the ligand to the AhR results in the release of associated proteins and translocation to the nucleus followed by dimerization with ARNT. The AhR–ARNT complex binds the XRE promoting target gene transcription. Ligands can also exert their effects in the cytoplasm through AhR-associated protein kinases to alter the function of a variety of proteins through a cascade of protein phosphorylation.

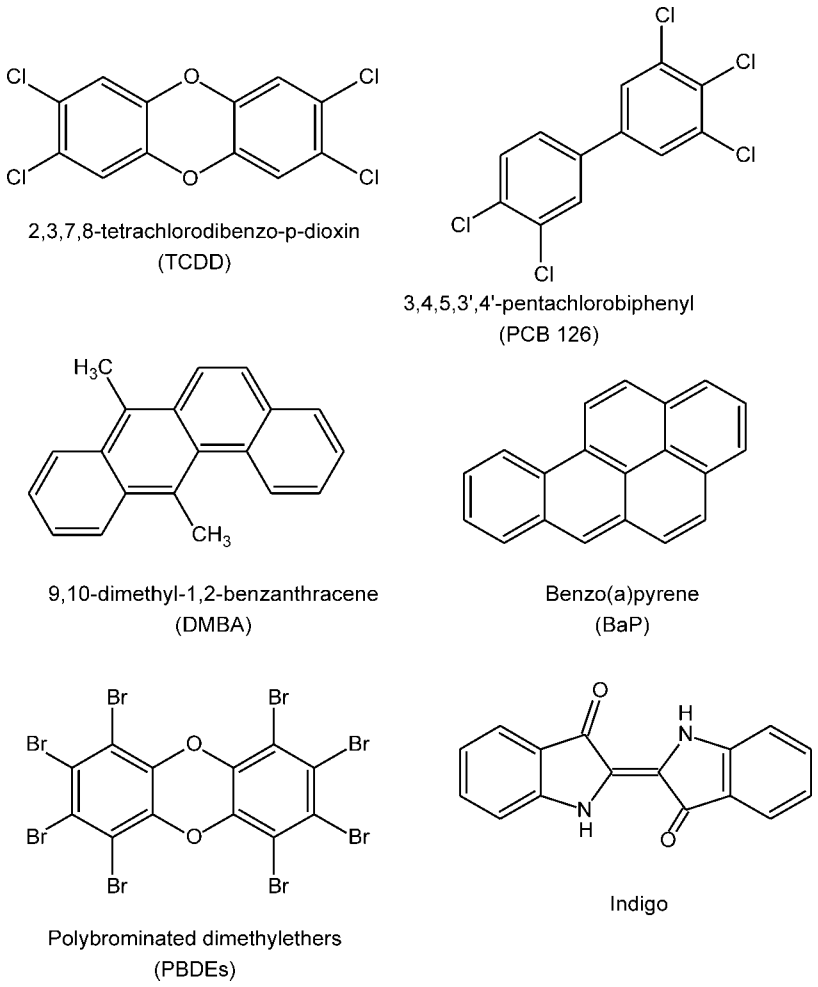

Figure 2 Molecular structure of some AhR ligands.

regions of the estrogen-inducible pS2, cathepsin D and c-fos genes (Safe *et al.* 2000). In HEC-1A human endometrial carcinoma cells, AhR–ARNT complexes competitively inhibit the binding of ER-alpha to imperfect Estrogen response element (ERE) sites adjacent to or overlapping with XRE sites (Klinge *et al.* 1999). TCDD-activated AhR inhibits estradiol (E2)-induced cathepsin D expression in MCF-7 breast cancer adenocarcinoma cells by binding to an iXRE within the promoter of the cathepsin D gene, thus preventing the formation of a transcriptionally active ER-SP1 complex (Duan *et al.* 1999, Wang *et al.* 2001). A similar AhR-mediated inhibitory AhR/ER cross-talk was demonstrated for the c-fos proto-oncogene promoter in MCF-7 (Safe *et al.* 2000). Liganded AhR–ARNT and ligand-activated ER-alpha do not directly interact but both ligand-activated transcription factor complexes can physically interact with and compete for SP1 protein (Kobayashi *et al.* 1996, Wang *et al.* 1998*a*). The liganded AhR was also shown to inhibit binding of ER-alpha to an ERE in the pS2 promoter by interaction with Activator protein-1 (AP-1) proteins (Gillesby *et al.* 1997). AP-1 consists of a heterodimer of c-jun and c-fos and promotes the binding of liganded ER-alpha to AP-1 DNA binding sites. Interaction of liganded AhR with AP-1 proteins promotes binding to a XRE/AP-1-like motif and, at the same time, prevents binding of ER-alpha to a neighboring ERE. Thus, AhR can function as a ligand-activated transcriptional repressor by binding to iXRE overlapping or neighboring ERE/AP-1 sites or ERE/SP1 sites respectively, quenching transcriptionally active ER/SP1 or ER/AP-1 complexes (Fig. 3A).

A rapid proteasome-mediated degradation of AhR and ER-alpha by activated AhR as another facette of an inhibitory AhR-ER-alpha cross-talk has been demonstrated in T47D human breast cancer cells (Fig. 3B). The extent of AhR-mediated activation of proteasomal degradation is ligand dependent with the classical AhR ligand TCDD displaying stronger effects as compared with AhR ligands such as benzo[*a*]pyrene (BaP) or 6-methyl-1,3,8-trichlorodibenzofuran (MCDF) (Wormke *et al.* 2000). In ECC-1 endometrial carcinoma cells exposed to TCDD, Ricci *et al.* (1999) also described an AhR-mediated reduction in ER-alpha protein and ER-alpha-mediated transcriptional activity. By contrast, TCDD-activated AhR did not reduce ER-alpha protein in the endometrial carcinoma cells KLE

and RL95-2, suggesting that proteasomal degradation varies according to the specific cellular background (Sone & Yonemoto 2002). Reduced ER-alpha protein levels have been described for several human endometrial pathologies. For example, down-regulation of ER-alpha is an important finding in human endometriosis tissues (Brandenberger *et al.* 1999, Fujimoto *et al.* 2000, 2002), in human endometrial adenocarcinomas and also in poorly differentiated mixed mullerian tumors (Jazaeri *et al.* 2001). Reducing ER-alpha protein or ER-alpha transcriptional activity within the endometrium via inhibitory AhR/ER-alpha cross-talk might have a severe impact on endometrial physiology. It may result in decreased PR expression, an important E2-target molecule within the endometrium, diminishing the actions of progesterone (P4) on endometrial differentiation during the secretory phase of the cycle. TCDD has been shown to decrease P4-induced and transforming growth factor (TGF)-beta-mediated reduction of matrix metalloproteinase (MMP)-3 and MMP-7 during the secretory phase thus promoting experimental endometriosis (Bruner-Tran *et al.* 1999). TCDD causes increased expression of interleukin-1(IL-1)beta and plasminogen activator-inhibitor-2 (PAI-2) mRNA and a dose-dependent decrease in AhR gene activity in cultured human endometrial stromal cells (Yang 1999). Although the role of PAI-2 in promoting migration of tumor cells is well established (Morita *et al.* 1999), a definitive function of TCDD in the progression of endometrial carcinoma is unclear.

In human MCF-7 breast cancer cells, agonist-activated AhR–ARNT complexes have been shown to associate directly with ER-alpha and ER-beta in the absence of estrogen resulting in transcriptional activation of ERE-dependent genes (Ohtake *et al.* 2003). This ERE-dependent estrogenic effect of liganded AhR requires direct interaction of the nuclear AhR–ARNT complex with unliganded ER and the cofactor p300/CBP (Fig. 3C). By contrast, in the presence of estrogen, liganded AhR exhibits anti-estrogenic effects by suppressing estrogen-bound ER-mediated DNA binding. These results strongly indicate that the AhR-mediated regulation of estrogenic effects depends on the concentration of estrogens. This may partially explain the weak estrogenic effects of PCB 126 in the uterus of ovariectomized, as opposed to normal, rats (Lind *et al.* 1999).

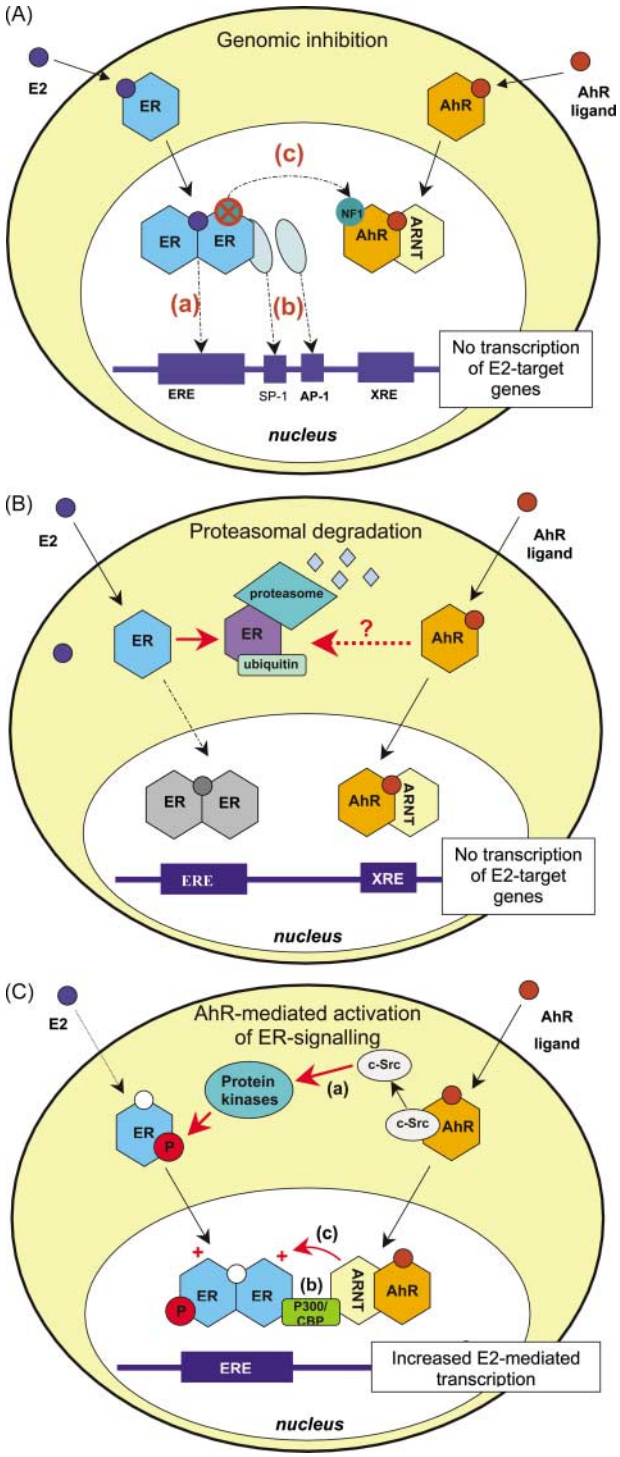

Figure 3 (A) Mechanisms of genomic inhibition of steroid-mediated transcription by ligand-activated AhR. Transcriptional inhibition depends on iXREs within promoter regions of estrogen-regulated genes which prevent ER transcriptional activity by: (a) being located close by to neighboring EREs; (b) disrupting the formation of ER–SP-1 or ER–AP-1 DNA complexes; and (c) competing for limited cofactors (e.g. NF-1). These mechanisms apply to interactions of the AhR with all sex steroid hormone receptors interactions and are shown here for the estrogen receptor (ER). (B) Activation of AhR by exogenous ligands can rapidly reduce the levels of ER protein by proteasomal degradation. The exact mechanism by which liganded AhR initiates that degradation is presently unknown. (C) AhR-mediated mechanisms leading to the activation of sex steroid hormone signaling in the absence of estrogen. (a) Upon binding of the ligand, a conformational change causes the release of active c-Src from the cytoplasmic AhR–protein complex. c-Src activates several protein kinases leading to phosphorylation and activation of ER. (b) A direct association of AhR–ARNT complexes with nuclear ER and the cofactor p300/CBP in the absence of E2. (c) Activation of steroid hormone receptor by direct protein interaction with the AhR–ARNT complex in the absence of hormone has so far only been observed for the ER.

Apart from its transcriptional activity and independent of its nuclear localization, ligand-bound AhR appears to act via a second pathway which is located in the cytoplasm. Unliganded AhR in the cytoplasm is associated with HSP90, XAP2, p23 and the tyrosine kinase c-Src ($pp60^{src}$). Upon ligand binding, active c-Src appears to be released from this aggregate resulting in the stimulation of other protein kinases (Perdew 1988, Hutchison *et al.* 1992, Matsumura 1994, Vogel & Matsumura 2003). Active c-Src may phosphorylate and activate steroid receptors such as ER resulting in estrogenic effects in the absence of estrogens. Thus, by triggering protein kinases ligand-activated AhR may elicit multiple and unpredicted cellular responses (see reviews of Matsumura 1994, and Carlson and Perdew 2002).

Apart from the studies mentioned above, to date there have been only a few reports on the cross-talk between AhR and ER in reproductive-related cells. In porcine follicular cells, recent studies indicate a TCDD-induced decrease in estrogen synthesis and that the exposure to the action of AhR or ER blockers (alpha naphthoflavone and 4-OH-tamoxifen respectively) is able to completely reverse the inhibitory effect (Gregoraszczuk 2002). A further report indicates a potentiation of TCDD activity through contemporary exposure to estradiol in mouse ovarian cells, and that this effect can be reversed through exposure to specific ER blockers (Son *et al.* 2002). Despite the fact that the precise molecular mechanisms involved in these phenomena have not been yet further investigated, these studies strongly suggest that a positive cross-talk between the two signaling pathways exists in ovarian cells.

Investigations on the interaction of AhR with other steroid hormone receptors have revealed a bi-directional cross-talk. Recently, an anti-androgenic effect of ligand-activated AhR was described in LNCaP prostate cancer cells. Interaction of the AhR ligand complex with AP-1 proteins resulted in diminished induction of prostate-specific-antigen (PSA) by testosterone. However, this was not caused by a decrease in intracellular levels of the androgen receptor (AR) or concentrations of intracellular dihydrotestosterone (DHT) (Kizu *et al.* 2003). Although this interaction was shown in LNCaP human prostate cancer cells, the presence of AR within the ovary (Pelletier *et al.* 2000) and endometrium (Slayden *et al.* 2001, Apparao *et al.* 2002, Brenner *et al.* 2002) would suggest the presence of a potential AhR/AR cross-talk to also be effective within female reproductive organs.

On the other hand, steroid hormone receptors can also inhibit AhR signal transduction and this inhibitory nuclear receptor cross-talk is caused by competition of AhR and ER for the rate-limiting co-regulators ERAP140 and SMRT (Nguyen *et al.* 1999). In the human endometrial carcinoma cell line ECC-1, competition was described for nuclear factor-1 (NF-1), a transcription factor capable of binding both ER-alpha and AhR. Competitive binding of NF-1 by estrogen-activated ER resulted in diminished TCDD-mediated CYP1A1 transcriptional activation (Ricci *et al.* 1999). A unidirectional inhibitory progesterone receptor (PR)/AhR cross-talk involves both PR isoforms, PR-A and PR-B, and repression of AhR–ARNT transcriptional activity requires the active progesterone responsive element (PRE)-binding form of PR-B, but not PR-A (Kuil *et al.* 1998).

In conclusion, AhR activation can result in the inhibition or promotion of steroid hormone signaling in reproductive tissues and this may, in part, explain the contradictory results of estrogenic or anti-estrogenic effects mediated by AhR ligands. Environmental AhR ligands have been implicated in promoting endometriosis and endometrial cancer in various species (Cummings *et al.* 1996, Johnson *et al.* 1997, Mann 1997, Mayani *et al.* 1997, Rier 2002). Yet, epidemiological data from the accidental exposure to TCDD in Seveso, Italy (1976), have revealed a decrease in the incidence of endometrial carcinoma in TCDD-exposed women (Bertazzi *et al.* 1997). Similarly, TCDD appears to have a breast cancer protective function (Greenlee *et al.* 2001). The molecular mechanisms by which TCDD exerts these anti-tumorigenic effects are unknown.

Cell cycle and apoptosis

It is now clear that the AhR plays a pivotal role in cell cycle regulation (Ma & Whitlock 1996, Vaziri & Faller 1997, Puga *et al.* 2002) and apoptosis (Zaher *et al.* 1998, Reiners & Clift 1999). The inhibition of estrogen-mediated induction of cell cycle activators such as cyclin D1 and the activation by liganded AhR of CDK2, CDK4 and CDK7 (Wang *et al.* 1998*b*) suggest a supportive role for AhR in E2-dependent tumor growth in the female reproductive tract (Bertazzi *et al.* 1997). However, the AhR also has a direct influence on the cell cycle by induction of the cyclin/cdk inhibitor p27 (Kip1) as demonstrated in rat 5L hepatoma cells (Ge & Elferink 1998). Upon nuclear translocation, liganded AhR engages in a protein–protein interaction with retinoblastoma protein (pRb) via two binding motifs: a high-affinity LXCXE motif located within the N-terminal 364 amino acids and a low-affinity binding site located within the glutamine-rich transactivation domain of the AhR. Ligand-activated AhR binds preferentially to hypo-phosphorylated Rb (pRb) which represents the active form of Rb leading to G1 arrest in rat L5 hepatoma cells (Ge & Elferink 1998). AhR synergizes with pRb in potentiating the repression of E2F-dependent transcription inducing cell cycle arrest (Puga *et al.* 2000). As hypophosphorylated Rb is limited to G0 and G1 phases of the cell cycle, so is pRb-dependent AhR action (Elferink *et al.* 2001). In MCF-7 cells, the direct interaction of liganded AhR with hypo-phosphorylated Rb was shown to be independent of ARNT and therefore does not require XRE-mediated transcriptional activity. This non-genomic function of AhR deserves further investigation, since

alterations in cell cycle progression in exposed tissues by potential endogenous (Carlson & Perdew 2002) and environmental AhR ligands may contribute significantly to the physiological roles of this bHLH transcription factor.

At or near the G_1/S boundary lays the point of divergence between continuation of the cell cycle and apoptosis. Apoptosis plays a critical role in reproduction, during development and in the maintenance of tissue and organ homeostasis (Scott *et al.* 1996, Jacobson *et al.* 1997) and many toxicants exert their cytotoxic effects by virtue of apoptosis. Several studies implicate the fine-tuned balance between the levels of AhR battery enzymes and the AhR as an important factor in aiding the cell to choose between apoptosis and continued cell proliferation. Immunohistochemical analysis of embryonic tissues showed that AhR expression is developmentally regulated and occurs in regions undergoing tissue remodeling processes (Abbott *et al.* 1995). Up-regulation of AhR and ARNT expression was observed during early outgrowth and elevation of palatal shelves. In addition, altered relative expression of these two bHLH transcription factor genes was observed after exposure to TCDD and this correlated with a higher incidence of cleft palate in developing mice (Abbott *et al.* 1999). Furthermore, stimulation of resting T cells with mitogens resulted in a marked increase of AhR expression paralleled by an increase in apoptosis (Crawford *et al.* 1997). Comparisons of the sensitivities of AhR + /+ and AhR − /− mice to the exposure of TCDD revealed thymic atrophy as a result of T-cell apoptosis in wild-type mice, but not in TCDD-resistant AhR − /− mice (Fernandez-Salguero *et al.* 1996, Kamath *et al.* 1997). Also, reduced liver size in AhR null mice was associated with incidence of apoptosis (Zaher *et al.* 1998). Several other studies have demonstrated the ability of a variety of AhR ligands – such as DMBA, BaP and TCDD – to induce apoptosis in various cell types of non-reproductive tissues, including pre-B cells (Jyonouchi *et al.* 1999), Hepa1c1c7 murine hepatoma cells (Lei *et al.* 1998) and mouse epidermis (Miller *et al.* 1996). However, the molecular mechanisms by which these chemical compounds induce programmed cell death remain unclear.

In the ovary, apoptosis is the principal mechanism by which oocyte depletion is mediated under both normal and pathologic conditions (Perez & Tilly 1997, Morita & Tilly 1999, Pru & Tilly 2001). It has been known for over two decades now that exposure of female mice to AhR ligands causes a rapid depletion of primordial and primary oocytes (Mattison *et al.* 1989) and these ovotoxic effects can be prevented by selective AhR antagonists (Shiromizu & Mattison 1985). Thus, it is tempting to suggest that in the ovary genes involved in the regulation of cell death are prime targets for the transcriptional regulation by the activated AhR. This hypothesis is supported by Heimler *et al.* 1998 who demonstrated that TCDD not only disrupts ovarian steroid production but, at the same time, is able to induce apoptosis in human follicular granulosa cells. Moreover, Matikainen and co-workers (2001) have recently reported two XRE binding sites for the AhR–ARNT complex in the promoter of the pro-apoptotic gene, Bax. Production of Bax protein and subsequent Bax-dependent increase in apoptosis are increased in murine oocytes upon exposure to the AhR ligand DMBA, but not with TCDD (Matikainen *et al.* 2001). Further analyses have shown that substitution of the existing guanine or cytosine to an adenine three bases downstream of the core XRE sequence renders the Bax promoter inducible by TCDD. These data elegantly demonstrate the ligand-dependent discrimination of DNA response elements (Matikainen *et al.* 2001).

There are only a few studies investigating the impact of AhR-mediated apoptosis in female reproductive tissues other than the ovary. Flaws *et al.* (1997) demonstrated that *in utero* exposure to TCDD induces cleft clitoris and vaginal threads of mesenchymal tissue in female rat offspring, indicating a disturbed balance between proliferation and apoptosis during the development of female genitalia.

AhR and the regulation of the hypothalamo-pituitary–gonadal (HPG) axis

AhR-mediated actions can affect the regulation of the HPG axis by altering the secretion pattern of preovulatory follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion in primed female rats exposed to TCDD or related AhR ligands (Li *et al.* 1995, Gao *et al.* 1999). Exposure to environmentally relevant concentrations of TCDD induces a significant reduction of FSH and LH during the preovulatory period in rats (Gao *et al.* 1999), strongly suggesting that the reproductive toxicity of TCDD can in part be related to a dysregulation of the hypothalamo-hypophyseal axis by mechanism(s) not yet completely understood. The observation that treatment with exogenous gonadotropin-releasing hormone (GnRH) partially overcomes the blockage of preovulatory surges of LH and FSH after TCDD exposure (Gao *et al.* 2000) may indicate insufficient production in and/or release of GnRH from the hypothalamus as a result of TCDD action to the central nervous system (CNS). This TCDD-induced inhibition of gonadotropin surges has been explained by a decreased responsiveness of the hypothalamus to the positive feedback of estrogens, without affecting preovulatory serum estrogen levels (Gao *et al.* 2001). This hypothesis is confirmed by the observation that tenfold higher than physiological serum concentrations of estrogens completely reverse the TCDD effects on gonadotropin secretion (Gao *et al.* 2001). This latter effect can be blocked by the partial estrogen antagonist tamoxifen providing further evidence for a functional relationship between both the aryl hydrocarbon- and estrogen-mediated signaling pathways.

Members of the AhR signaling pathway are expressed in the preoptic area of the brain (POA), a region known to control reproductive functions (Petersen *et al.* 2000). The distribution pattern of AhR gene expression closely

overlaps with that of glutamic acid decarboxylase (GAD) 67, the enzyme necessary for gamma-aminobutyric acid (GABA) synthesis. Interestingly, the GAD 67 gene contains multiple canonical XRE sequences (Erlander *et al.* 1991, Pinal *et al.* 1997). GAD 67 mRNA levels in the rostral POA/anteroventral periventricular nucleus (rPOA/AVPV) and in the rostral portion of the medial preoptic nucleus (MPN) are higher in females than in males (Hays *et al.* 2002). GABAergic neurons in the AVPV play a role in onset of puberty, the E2-dependent gonadotropin surge and in ovulation. Developmental exposure to TCDD can specifically down-regulate GAD 67 expression in the rPOA/AVPV in female rats resulting in abolished sexual differentiation of this area. *In utero* exposure of female rats to TCDD induces delayed onset of puberty and increases the time required to achieve pregnancy in a continuous mating situation (Gray & Ostby 1995, Gray *et al.* 1997). Thus, sex- and region-specific suppression of GABA synthesis in the CNS adds to the multiple cellular actions by which TCDD can disrupt female reproductive functions.

Evidence for a physiological role of AhR in reproduction

Many attempts have been made to identify endogenous ligands that could trigger AhR-dependent signaling under physiological conditions. However, with the exception of the tryptophane analogs indirubin and indigo, which have been recently identified in human urine (Adachi *et al.* 2001), and 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester in the porcine lung (Song *et al.* 2002), the nature of endogenous AhR ligands remains elusive. The attempt to assess the role of the AhR in the absence of activation by xenobiotic compounds led to the production of AhR-deficient mice from three independent research groups (Fernandez-Salguero *et al.* 1995, Schmidt *et al.* 1996, Mimura *et al.* 1997). Those AhR-knockout animal models demonstrated a range of complex reproductive deficiencies in female mice in the absence of AhR gene expression. These adverse reproductive parameters included deaths of the −/− females during pregnancy and lactation, small litter size at birth, poor survival of pups during the first 2 weeks after birth, and death of −/− pups after weaning. The poor reproductive success in these AhR-null animals possibly reflects the disruption of multiple biological processes. A more extensive analysis of older AhR −/− animals showed hypertrophic uteri displaying thrombosis and mineralization of the serosal vessels coinciding with a reduction of the litter size (Fernandez-Salguero *et al.* 1997). In addition, increased numbers of primordial follicles were detected in 2- to 3- or 4-day-old AhR −/− females (Benedict *et al.* 2000, Robles *et al.* 2000). Female AhR-null mice at 53 days of age displayed a decreased number of antral follicles (Benedict *et al.* 2000) and a resistance of germ cells to undergo apoptosis (Robles *et al.* 2000). These results indicate that the decreased number of antral follicles observed in the AhR −/− mice may be insufficient to support the hormone synthesis needed during pregnancy and lactation. Furthermore, a recent study by Benedict *et al.* (2003) suggested that AhR deletion impairs follicular growth and, concomitantly, reduces the number of follicles that ovulate and become corpora lutea. This would implicate AhR as a novel regulator of ovulation. In agreement with this hypothesis, the ovulatory gonadotropin surge has been shown to induce the expression of AhR gene activity in the macaque ovary (Chaffin *et al.* 1999) and we have recently demonstrated the AhR-mediated induction of CYP1A1 in the absence of exogenous ligands in *in-vitro* matured bovine oocytes, suggesting a direct role of the AhR signaling pathway in the resumption of meiosis in mammalian oocytes (Pocar *et al.* 2004).

Thus, the bHLH transcription factor AhR appears to have a prominent functional role in female reproduction that deserves further attention.

Conclusions

It is clear now that the many complex reproductive effects of dioxins and related compounds observed in mammals reflect four important findings:

1. Although the biological effects of dioxin-like compounds require the AhR, the role of this bHLH transcription factor extends beyond the activation of the AhR gene battery and includes also non-genomic pathways.
2. There exists an intricate network of interactions, direct and indirect, between sex steroid receptors and the AhR–ARNT system which can modify reproductive processes.
3. AhR-mediated actions affect all levels of a reproductive system, from the HPG axis to the reproductive organs themselves, causing adaptive short-term and irreversible long-term effects on the reproductive system.
4. Developmental and transgenic mouse studies have clearly demonstrated that the AhR transcription factor is more than just a xenobiotic sensor but potentially an integral key component of normal reproductive physiology.

The complex role of the AhR in female reproduction is still largely elusive. A multidisciplinary approach with areas of expertise in toxicology, pathology, endocrinology and molecular/developmental biology will be required to further unveil the secrets of the role of AhR in reproduction of the female.

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