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REVIEW ARTICLE

Toxicology mechanism of the persistent organic pollutants (POPs) in fish through AhR pathway

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Abstract

With the development of industry and agriculture, the cases of cancer and tumor have been increasing gradually in the last 30 years, and quite a few cases are caused by persistent organic pollutants (POPs), some of them belonging to environmental endocrine disruptors, and they have become ubiquitous in the environment, especially in the aquatic ecosystem; so this issue has aroused the extensive attention of the world. The mechanism of POPs toxicology is very complicated, but it is mainly mediated by the aryl hydrocarbon receptor (AhR) pathway in fish. In order to gain a comprehensive understanding of the AhR pathway, the present paper focuses on reviewing it from four major steps, including formation of cytosolic complex, translocation of AhR, heterodimerization of AhR, and induction of CYP1A. This study summarized the isoform numbers of AhR pathway genes and the expression patterns in the regulation process of POPs toxicology in zebrafish.

Keywords: *Persistent organic pollutants; AhR pathway; toxicology mechanism; fish*

Introduction

Persistent organic pollutants (POPs) have become ubiquitous in the aquatic ecosystem, they can enter an aquatic ecosystem through effluent, atmospheric deposition, run-off, and groundwater, most of them belonging to AhR agonist, and their toxicity mainly mediated through AhR pathway, for example, 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), the coplanar polychlorinated biphenyl 126 (PCB126), 3-methylcholanthrene (MC), 3,3',4,4',5-pentachlorobiphenyl (PCB), β-Naphthoflavone, etc, Furthermore, they are not only very harmful for the health of aquatic animals but also for humans, they may impair most systems of humans and lead to the appearance of cancer and tumors; such as polychlorinated biphenyl (PCB) and OH-PCB which may damage the brain (Kimura-Kuroda et al. 2007). Polycyclic aromatic hydrocarbons (PAHs) can harm the immunity system (Davila et al. 1995) and the reproductive system (den Besten et al. 1990), and can cause DNA damage (Lemiere et al. 2005). PCB, polycyclic aromatic hydrocarbon (PAH), and other POPs are the most important risk factor for breast cancer (Gammon et al. 2004), lung cancer (Okona-Mensah et al. 2005), and prostate cancer (Ritchie et al. 2005). So the widespread occurrence of POPs has attracted considerable attention.

Lavine et al. (2005) reported that *Xenopus laevis* bind TCDD with 25–50-fold lower affinity than AhRs from more sensitive species, and different POPs have a broad affinity for specific species. So, we can find that the toxicology mechanism of POPs is very complicated, and it is widely accepted that aryl hydrocarbon receptor (AhR) pathway mediates most if not all of the toxicological effects of POPs. Additionally, the diversity of AhR pathway genes and the significant species differences in the spectrum of toxicity are observed within fish; furthermore, the AhR pathway genes were distributed under four major steps of toxicology regulation: formation of cytosolic complex, translocation of AhR, heterodimerization of AhR, and induction of Cyp1a. Thus, for better understanding the toxicology mechanism of POPs in fish, in the present work, we focus on elucidating

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the regulation mechanism of POPs toxicology in fish based on the above four steps.

AhR pathway

A series of experiments have revealed that the aryl hydrocarbon receptor (AhR) pathway plays a pivotal role in the mediation of POPs toxicology in fish. AhR pathway genes (e.g. AhR, AHRR) have been found in fish, and are detectable in many tissues. Their structure and function have been studied, including killfish, rainbow trout, zebrafish, medaka, and red seabream, etc. A large body of studies have revealed that the mechanisms of the AhR-dependent Cyp1a1 gene induction (see Figure 1). For better understanding the toxicology mechanism of POPs, we put forward that there are four major steps of toxicology regulation, including formation of cytosolic complex, translocation of AhR, heterodimerization of AhR, and induction of Cyp1a.

Formation of cytosolic complex

In the absence of ligands, AhR is associated with a cytoplasmic protein complex with two molecules of heat shock protein 90 (Hsp90) (Perdew 1988), immunophilin-like protein XAP2 (also known as ARA9 or AIP) (Carver and Bradfield 1997), and a 23-kDa co-chaperone protein (p23) (Kazlauskas et al. 1999). Hsp90 is an essential component of the AhR-signaling pathway, and loss of Hsp90 most likely results in an improperly folded or destabilized receptor protein, one sub-unit of the AhR complex, appears to direct proper folding and maintenance of the high affinity ligand binding conformation of the AhR in some species (Soshilov et al. 2006).

There are two types of Hsp90 genes within fish, namely Hsp90α and Hsp90β, and they encode two similar cytosolic isoforms, respectively. The synthesis of Hsp90 is triggered by stressful cellular conditions such as high temperature, anoxia, radiation, cancer, and environmental pollutant (Feder and Hofmann 1999). During heat shock, both Hsp90α and Hsp90β genes are upregulated in both mouse and human cells; in contrast, Hsp90β gene in zebrafish is weakly responsive or unresponsive to elevated temperature, whereas the Hsp90α gene is strongly upregulated (Krone and Sass 1994). Recently, Padmini and Usha Rani (2009) confirmed that environmental pollutant stress also can induce the Hsp90α expression in grey mullets. Thus, we can propose that the two isoforms genes have similarity function, but they have different expression patterns in fish under the environmental stress, such as POPs pollution.

In the AhR pathway, Hsp90 binding is thought to mask the AhR-NLS (nuclear localization signal) which is used for transport of AhR to the nucleus through the nuclear pore complex, and it is composed of two basic amino acid segments, AhR(13–16:RKRR) and AhR(37–39:KRH) in the N-terminal region of AhR (Ikuta et al. 2004), and this interaction is essential for the cytoplasmic retention of AhR (Kazlauskas et al. 2001). Furthermore, Hsp90 and the proteasome are playing a pivotal role in modulating AhR signaling and

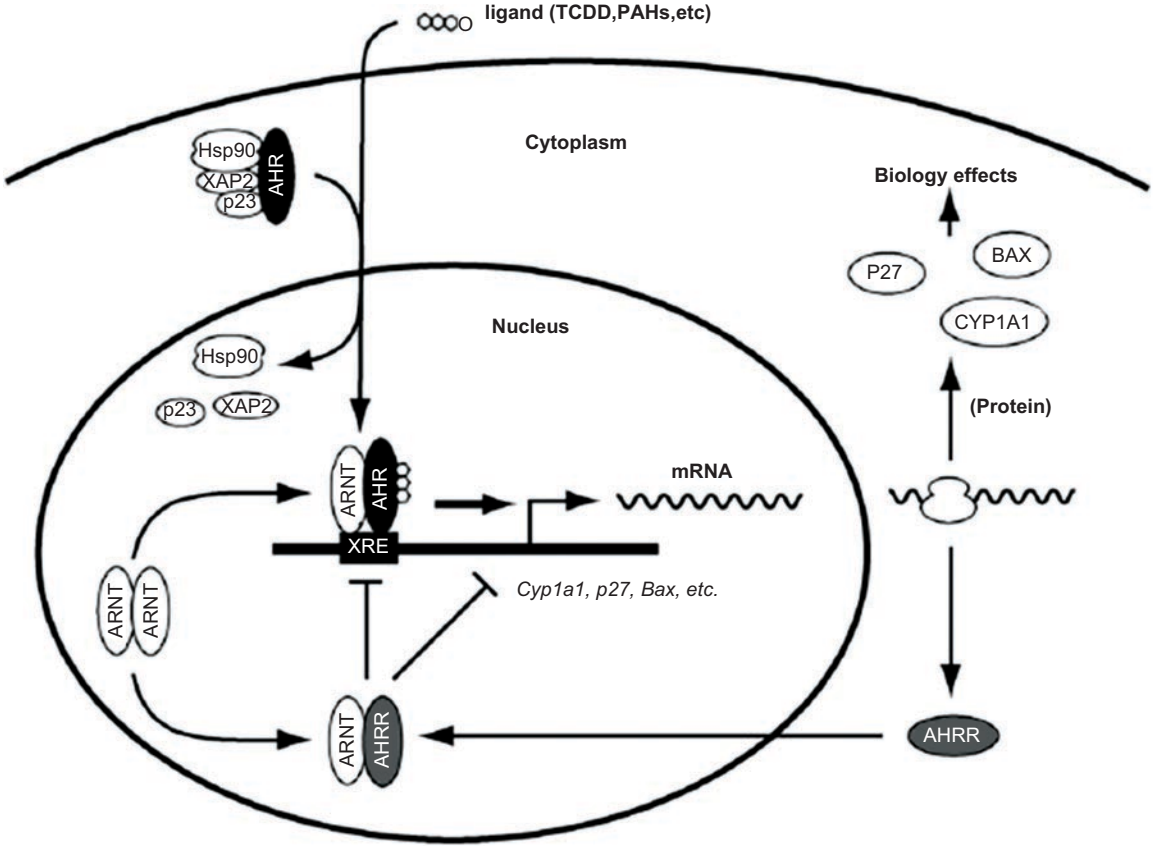

Figure 1. Regulation mechanism of AhR pathway gene. This figure adapted from Mimura and Fujii-Kuriyama (2003) with some modification.

Cyp1A response in trout hepatocytes (Wiseman and Vijayan 2007). Here, proteasome is an ATP-dependent protease and consists of ~ 40 polypeptides, and it can rapidly degrade the AhR protein (Tanaka 1998). In addition, the results of Kwak and Kensler (2006) indicate that expression of proteasome sub-unit PSMB5 is modulated by bifunctional enzyme inducers in a manner independent of the AhR/ARNT-XRE pathway but dependent upon the Nrf2 (transcription factor)-ARE(antioxidant response element) pathway.

Although the other two members (XAP2 and p23) of cytosolic complex are not essential for the AhR signalling, they play an important role to stabilize the cytosolic complex. XAP2, a 38-kDa protein that was initially identified as a protein binding to the hepatitis B virus X protein (Kuzhandaivelu et al. 1996), and also as a binding partner of AhR (Ma and Whitlock Jr 1997) to modulate AhR function has been studied extensively in cell culture systems; moreover, it is not a limiting component in AhR regulation (Hollingshead et al. 2006). It has been shown that XAP2 enhances the stability of the AhR, inhibits CRM-1-mediated nucleocytoplasmic shuttling of the AhR, and that cytoplasmic retention of the AhR is accomplished through the co-operative mechanisms of both XAP2 and chromosome region maintenance 1 (CRM1) (Petrulis et al. 2003; Pollenz et al. 2006). In addition, XAP2 competes with p23 for binding to the AhR/Hsp90 complex (Hollingshead et al. 2004), and protects AhR from being ubiquitinated, at least in vitro (Morales and Perdew 2007).

The phosphoprotein p23 is a small, acidic protein that is ubiquitously expressed in virtually all tissues highly conserved protein from yeast to humans, (Freeman et al. 2000). P23 appears to promote AhR/ARNT/DRE complex formation in an Hsp90-dependent manner by assisting with the heterodimerization of the AhR and ARNT. Further research of Cox and Miller Iii (2004) show that p23 can inhibit Hsp90 ATPase activity, thereby stabilizing ATP-Hsp90-client protein complexes, and does not interact directly with either the AhR or ARNT (Kazlauskas et al. 1999). However, recently, the results of Flaveny et al. (2009) show that p23 is dispensable for stable AhR protein levels, Taken together, the present results suggest that p23 acts as a stimulatory factor in regulating AhR activity.

Additionally, p23 was described to be up-regulated in rat brain ischemia, in human cancers, and metastatic tissue, and to be down-regulated in artherosclerotic plaques stimulated with aggregated low density lipoproteins, Further research of Mollerup and Berchtold (2005) suggests that apoptosis induced by extrinsic or intrinsic pathways led to caspase-mediated cleavage of p23 at its C-terminal tail. Moreover, treatment with endoplasmic reticulum (ER) stress-inducing agents also resulted in the cleavage of p23 accompanied by caspase processing (Bakhshi et al. 2008).

Translocation of AhR

The ligand-dependent nuclear import of AhR serves as the first step in the induction of target genes as a biological switch; thus, elucidation of the transloction mechanism underlying the import process is important for understanding the mechanism of POPs toxicology. The mechanisms of translocating AhR to the nucleus mainly include three pathways: three-step mechanism of prototypical ligand-dependent (direct), ligand-independent phosphorylation/dephosphorylation (indirect), as well as cell density-related pathways (Li and Wang 2010). At present, the latter two translocation mechanisms are still not very clear and the mediation process of POPs toxicology is mainly through the ligand-dependent pathway. Thus, in this article, we mainly focus on discussing the ligand-dependent pathway of the translocation mechanism of AhR.

The AhR protein has both a nuclear localization signal (NLS) and a nuclear export signal (NES), which play important roles in the AhR translocation. Ikuta et al. (2004) reported that the NLS comprised of amino acid residues 13–39 consists of two separate basic amino acid segments, one consisting of residues 13–16 (Arg-Lys-Arg-Arg) and the other spanning residues 37–39 (Lys-Arg-His). In addition, AhR has a leucine-rich NES that comprises amino acid residues 55–75 in helix 2, and the NES is necessary for the nuclear export of the AhR protein followed by proteasome degradation.

Based on a large number of previous researches, we can envisage a three-step mechanism of ligand-dependent nuclear import of the AhR, as shown in Figure 2. Without ligand, two molecules of Hsp90 mask the AhR-NLS, which is essential for cytoplasmic retention of the AhR. Thus, the first step is ligand binding to the AhR ligand binding domain which results in conformational changes that expose the nuclear localization sequence through alterations of XAP2 binding (Denison et al. 2002), which makes it necessary to facilitate interaction of the NLS with nuclear import components. At the same time, the AhR dissociates from Hsp90 complex. The second step is that importing α binds AhR through recognition of the NLS and it can joins importing β (Adam and Geracet 1991), while importing β interacts with the NPC (nuclear pore complex) (Moroianu et al. 1995), and then constituting the importing-NLS protein complex, this step doesn't require energy. Furthermore, this step can be regulated through phosphorylation or dephosphorylation. The third step is that NPC translocation of the importing-NLS protein complex, which requires two additional soluble proteins, the Ran GDP and p10 (Nigg 1997), this step is temperature dependent. Ran is a critical component of almost all known nucleocytoplasmic transport pathways. It has to interact with both a GTPase-activating protein and a small Ran-binding protein to achieve maximal GTPase activity (Mattaj and Englmeier 1998). Another soluble protein is p10/NTF2, which is required for efficient NLS-protein nuclear import in permeabilized cells (Melchior et al. 1995). And then AhR rapidly accumulates in the nucleus and is followed by heterodimerization with ARNT for transcription (Poland and Knutson 1982). Furthermore, other studies show that the inhibition of AhR degradation by proteasome inhibitors can increase the ligand-dependent or -independent nuclear translocation of AhR (Santiago-Josefat and Fernandez-Salguero 2003; Ohtake et al. 2007). On the

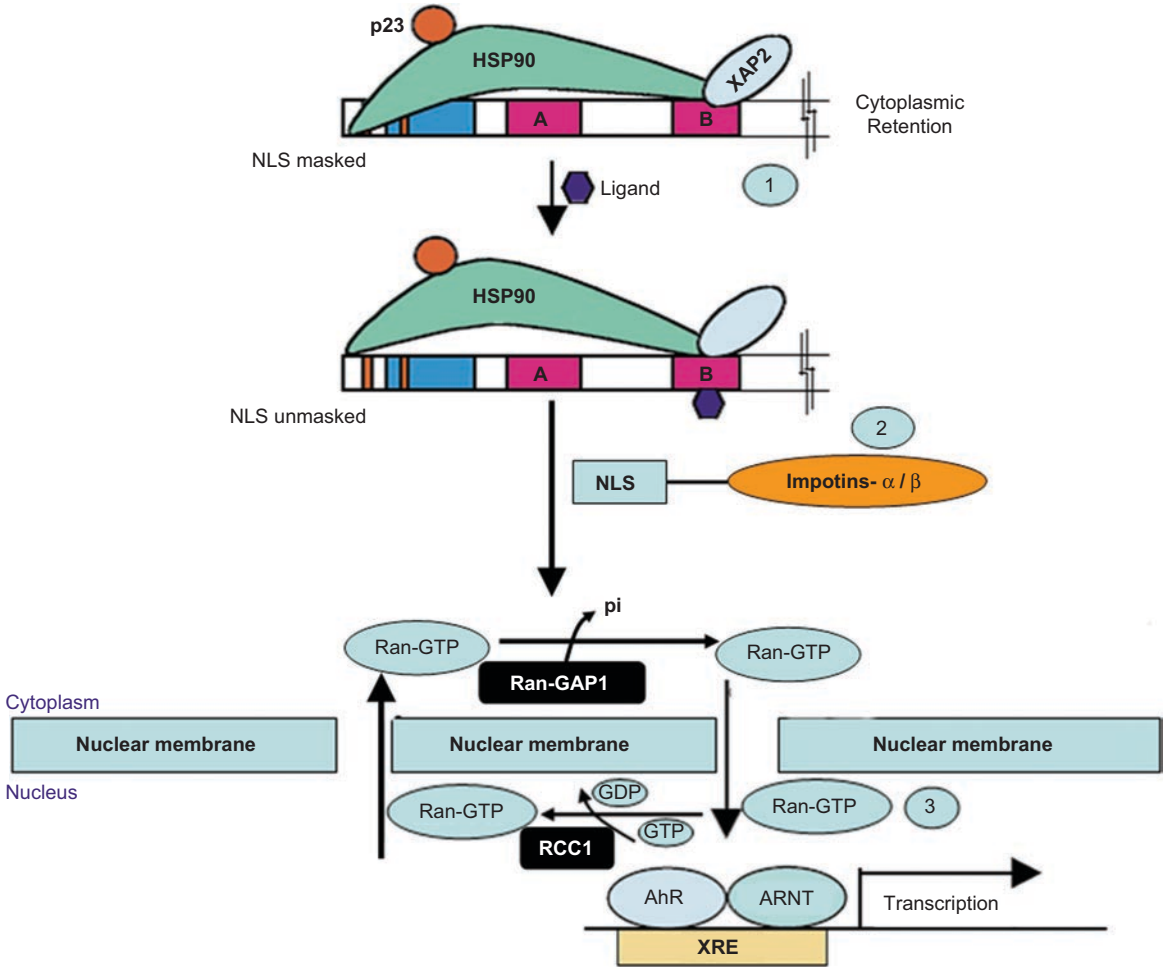

Figure 2. Three-step mechanism of the ligand-dependent nuclear import of the AhR. The figure adapted from Kawajiri and Ikuta (2004) with some modification. (1) ligand binding to the AhR ligand binding domain, (2) importing α binds AhR through recognition of the NLS, (3) NPC translocation of the importing-NLS protein complex. NLS: nuclear localization signal, NP: Nuclear pore, NPC: Nuclear pore complexs, pi: phosphoryl group, RCC1/Ran-GAP1: Ran regulators. (See colour version of this figure online at www.informahealthcare.com/txm)

other hand, NES-dependent nuclear export is mediated by chromosome region maintenance 1 (CRM1) in concert with Ran-GTP (Nigg 1997).

Heterodimerization of AhR

When AhR binds to ligand, it is translocated to the nucleus and dissociates from the Hsp90 complex to form a heterodimer with ARNT. The AhR/ARNT heterodimer binds to the xenobiotic response elements (XRE) sequence in the promoter regions of target genes encoding drug-metabolizing enzymes, including CYP1A1, quinone reductase, etc., and alters their expression (Kikuchi et al., 2003). Furthermore, the results of Davarinos and Pollenz (1999) show that blockage of AhR degradation results in an increase in the concentration of AhR/ARNT complexes associated with DNA and extends the duration that the complex resides in the nucleus. Thus, we can find that the degradation of AhR protein is a critical component of the AhR-mediated signal transduction pathway. Moreover, the release and degradation of AhR/ARNT heterodimer from the XRE sequence of target genes is very important for the regulation of AhR activity; however, the precise mechanism still remains elusive. Up to date, there are several mechanisms by which AhR signaling may be down-regulated. One mechanism involves ligand-dependent degradation of AhR protein through a proteasomal pathway (Wentworth et al. 2004); namely, the activated AhR is quickly exported to the cytosol where it is degraded by the 26S proteasome (Pollenz 2002). However, the results of Roberts and Whitelaw (1999) indicate that AhR can also be degraded within the nucleus by a 26S proteasome-dependent manner. Other mechanisms by which AhR-dependent signaling can be reduced involve transcriptional repression of AhR target genes. AHRR may play a role in releasing the AhR/ARNT heterodimer from the XRE sequence, facilitating its degradation. Additionally, AHRR expression is induced by a variety of AhR agonists, such as benzo[a]pyrene (BaP), 3,3′ ,4,4′ ,5-pentachlorobiphenyl (PCB-126), and benzo[k]fluoranthene (BkF) (Evans et al. 2005). Collectively, we can find that the release and degradation of AhR/ARNT heterodimer accompanied with the AhR agonists may enter into the nucleus, and this may be an important defence mechanism of the organism for adapting the environmental pollution.

Aryl hydrocarbon receptor repressor (AHRR) is an AhR-related protein, and represses the transcription activity of AhR

by competing with AhR for heterodimer formation with ARNT and subsequently for binding to the XRE sequence (Mimura et al. 1999). These results indicate that AhR and AHRR form a regulatory feedback loop (Mimura and Fujii-Kuriyama 2003). Recently, Evans et al. (2008) proposed a mechanism of AHRR action involving 'transrepression' of AhR signaling through protein–protein interactions rather than by inhibition of the formation or DNA binding of the AhR-ARNT complex. In the future, targeted knock-down of one or both AHRR proteins by application of morpholino oligonucleotides can be used to further characterize these duplicate zebrafish AHRRs and to elucidate their potential roles in development and in the developmental toxicity of chemicals such as TCDD.

ARNT and AHRR proteins show HLH and PAS as two conserved domains. PAS domains can also govern target gene specificity of different heterodimers (Zelzer et al. 1997). Dimers of individual PAS proteins bind specific DNA target sequences in interactions that involve the basic region (Bacsi and Hankinson 1996) and possibly additional distinct regions of a protein (Pongratz et al. 1998), enabling transcriptional activation or repression.

Now, although it remains to be studied how AhR and AHRR are involved in the other TCDD-induced biological effects such as teratogenesis and immunosuppression. It is well known that these adverse biological effects are caused by untimely activation of gene expression by ligand-activated AhR and AHRR in the biological processes.

Induction of Cyp1a

The ligand–AhR–ARNT heterodimer interacts with AhR response elements (AhREs; also known as XREs or DREs) to activate or repress gene expression from target genes (Hahn et al. 2005; 2006). The best characterized targets of the AhR pathway are Cytochrome P4501a (Cyp1a) genes, which are strongly induced by TCDD and PAHs (Whitlock 1999). They have a broad affinity for polycyclic, aromatic hydrocarbons, as well as aromatic amines, and some endogenous substrates (Gonzalez and Kimura 2003; Teraoka et al. 2003). Also, they play a central role in biotransformation, detoxification, and elimination of various structurally diverse xenobiotics (Monostory and Pascussi 2008). The induction of Cyp1a family member expression is regulated by a heterodimer composed of the AhR and ARNT (Fujii-Kuriyama and Mimura 2005). In contrast, the expression of Cyp2, 3, and 4 family members is regulated by the nuclear receptors CAR (Constitutive Androstane Receptor), PXR (pregnenolone X receptor), and PPAR (Peroxisome proliferator activated receptor), respectively (Waxman 1999).

The induction of Cyp1a is an important step in the response to POPs, some researchers have identified several consensus response elements; there are eight potential xenobiotic response elements (XREs) in the promoter region of the European flounder Cyp1a gene, but not all of these sequences are necessarily for activation, just only four out of eight different XREs are functional in the regulation of Cyp1a. The activity of these response elements enhances the evidence for considerable diversity in vertebrate Cyp1a regulation (Lewis et al. 2004). In a word, we can conclude that the AhR pathway plays a pivotal role in the regulation process of POPs toxicology in fish.

The expression pattern of AhR pathway genes in zebrafish

There are several gene types of each AhR pathway gene in zebrafish. For better understanding of the toxicology mechanism of POPs in fish, we summarized and discussed the isoform numbers and expression pattern of AhR pathway genes in zebrafish (refer to Table 1).

Expression pattern of Hsp90

Hsp90 protein is well conserved within aquatic animals, there are two types of Hsp90 genes within the fishes, namely Hsp90a and HSP90b, which encode two similar cytosolic isoforms (Moore et al. 1987; Krone and Sass 1994). Despite marked similarities between the two genes at a molecular level, Hsp90α and Hsp90β exhibit different patterns of expression during embryonic development and cell differentiation, and also in response to environmental stress (Csermely et al. 1998). As far as the zebrafish is concerned Hsp90α gene is strongly expressed following heat shock, whereas the Hsp90β is only weakly up-regulated under similar stress conditions (Krone and Sass 1994). These results reveal both functional similarities and key functional differences in the individual members of this protein family (Taherian et al. 2008).

Expression pattern of AhR

Fishes have more AhR genes than other vertebrates because they have retained AhR2 genes and because of a fish-specific whole-genome duplication event in their early evolutionary past (Hahn et al. 2006). The structural and functional diversity of AhR proteins may confer species- and strain-specific differences in the sensitivity to toxic AhR ligands (Hahn et al. 2005), and it is possible that numerous, possibly diverse, physiological roles are partitioned among multiple AhRs and AHRRs.

AhR is an ancient protein, which is well conserved in vertebrates and invertebrates, indicating its pivotal function throughout evolution (Karchner et al. 2002). Although in mammals the single AhR (AhR1 ortholog) is required for TCDD

Table 1. Isoform numbers of AhR pathway genes and the expression patterns of zebrafish. This table mainly summarized the expression patterns of the four genes (Hsp90, AhR +, ARNT, and CYP1A) which distribute in four steps in zebrafish: including formation of cytosolic complex, translocation of AhR, dimerization of AhR, and induction of Cyp1A, and the isoform numbers of AhR pathway genes within different phylogeny group (mainly comprised of mollusc, amphibian, fish, and aquatic mammal).

AhR pathway genes	Class				Expression patterns of zebrafish
	Bivalvia	Amphibia	Euteleostomi	Mammalia	
HSP90	1	2	2	2	Hsp90α
AhR	–	2	2–6	1	AhR2
ARNT	–	2	2	2	ARNT1
CYP1A	–	1	1–2	2	CYP1A

'–' indicates that there is no data at present.

toxicity during development (Mimura et al. 1997), however, it is the AhR paralog (AhR2) that plays this role in zebrafish (Carney et al. 2006). Whether this is specific to zebrafish or is true generally in fish remains to be determined.

Expression pattern of ARNT

Zebrafish possess two ARNT genes—ARNT1 and ARNT2—and in both cases ARNT1 appears to be the toxicologically most relevant partner for AhR2 (Prasch et al. 2004; Walisser et al. 2004). Additionally, low levels of ARNT could decrease the sensitivity of a particular tissue to agonist, despite high AHR levels (Schmidt and Bradfield 1996).

Expression pattern of CYP1A

Fishes, including zebrafish, generally possess a single CYP1A gene (Morrison et al. 1995; 1998); eels and salmonids are notable exceptions (Rabergh et al. 2000; Mahata et al. 2003). Mammalians, in contrast, generally possess two paralogous CYP1A genes, CYP1A1 and CYP1A 2 (Kimura et al. 1984; Quattrochi et al. 1985). Fish CYP1A s share significant sequence similarity with both CYP1A 1s and CYP1A 2s (Morrison et al. 1995) and display a combination of catalytic functions characteristic of the mammalian isoforms (Gorman et al. 1998). However, fish CYP1As are considered more CYP1A 1-like on the basis of slightly higher levels of pairwise sequence identity and similarities in patterns of gene expression.

Discussion

Fish species vary widely in their sensitivity to POPs, the number, type, and expression pattern of AhR pathway genes may contribute to inter-species differences in aryl hydrocarbon toxicity, possibly through distinct interactions with additional PAS-family proteins. Veldhoen et al.'s (2008) results show that the AhR gene involves the autoimmune. Therefore, it may help fishes to adapt to the various stimuli of environmental pollutants.

At present, we can find that most of the aquatic animals all conformed to the same toxicology mechanism; in contrast, there are some other species that are insensitive to the TCDD toxicity, especially at the early life stage, for example, African clawed frog. What causes the difference between different animal species remains for further study.

In addition, previous researchers just focus on one or a few genes in the AhR pathway. Actually, it is universally acknowledged that there are a series of related genes involved in the regulation of POPs toxicology, and then, which and how many genes are involved in the regulation, and what's the relationship between them? We think that we should consider this issue from the whole pathway of AhR, and this provides a novel insight into the research of POPs toxicology mechanism in aquatic animals.

Declaration of interest

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