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Molecular phylogenies and evolutionary behavior of AhR (aryl hydrocarbon receptor) pathway genes in aquatic animals: Implications for the toxicology mechanism of some persistent organic pollutants (POPs)

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ABSTRACT

Phylogenetic analysis of AhR pathway genes and their evolutionary rate variations were studied on aquatic animals. The gene sequences for the proteins involved in this pathway were obtained from four major phylogenetic groups, including bivalvia, amphibian, teleostei and mammalia. These genes were distributed under four major steps of toxicology regulation: formation of cytosolic complex, translocation of AhR, heterodimerization of AhR and induction of CYP1A. The NJ, MP, and ML algorithm were used on protein coding DNA sequences to deduce the evolutionary relationship for the respective AhR pathway gene among different aquatic animals. The rate of non-synonymous nucleotide substitutions per non-synonymous site (d_N) and synonymous nucleotide substitutions per synonymous site (d_S) were calculated for different clade of the respective phylogenetic tree for each AhR pathway gene. The phylogenetic analysis suggests that evolutionary pattern of AhR pathway genes in aquatic animals is characterized mainly through gene duplication events or alterative splicing. The d_N values indicate that all AhR pathway genes are well conserved in aquatic animals, except for CYP1A gene. Furthermore, compare with other aquatic animals, the d_N value indicates that AhR pathway genes of fish are less conserved, and these genes likely go through an adaptive evolution within aquatic animals.

1. Introduction

Persistent organic pollutants (POPs) are compounds that resist photolytic, chemical, and biological degradation. They are low water solubility and high lipid solubility, resulting in bioaccumulation in fatty tissues of living organisms (Cumanova et al., 2007). They are not only toxic, but also prone to long-range transport (Norstrom et al., 1988). Most of them can be classified into three categories: (1) industrial chemical product such as polychlorinated biphenyls (PCBs); (2) combustion and by-products such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); (3) and pesticides such as dichlorodiphenyltrichloroethane (DDT), dihedron, toxaphene. POPs can enter aquatic ecosystem through effluent, atmospheric deposition, runoff, and groundwater. Now, they have become ubiquitous in the biosphere (Swain, 1988) and they have seriously threatened the health of aquatic animals and human, so this problem has caught the worldwide attention in recent years.

A series of studies show that aryl hydrocarbon receptor (AhR) pathway plays a critical role in the mediation process of some POPs toxicology. AhR is a ligand-activated transcription factor that mediates many of the biological and toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polycyclic aromatic hydrocarbons (PAHs), and others. In the regulation pathway of AhR, the main target molecular is cytochrome P450 1 (CYP1). Cytochrome P450 1A1 (CYP1A1) is one of the xenobiotic metabolizing enzymes (XMEs), which is induced by TCDD, PAHs, etc. A large body of literature has revealed that the mechanism of the AhR-dependent CYP1A1 gene induction (see Fig. 1). AhR has a high binding affinity to TCDD. In the absence of a ligand AhR exists in a cytosolic complex with HSP90 (Perdew, 1988), co-chaperone p23 (Kazlauskas et al., 1999) and immunophilin-like protein XAP2 (Carver and Bradfield,

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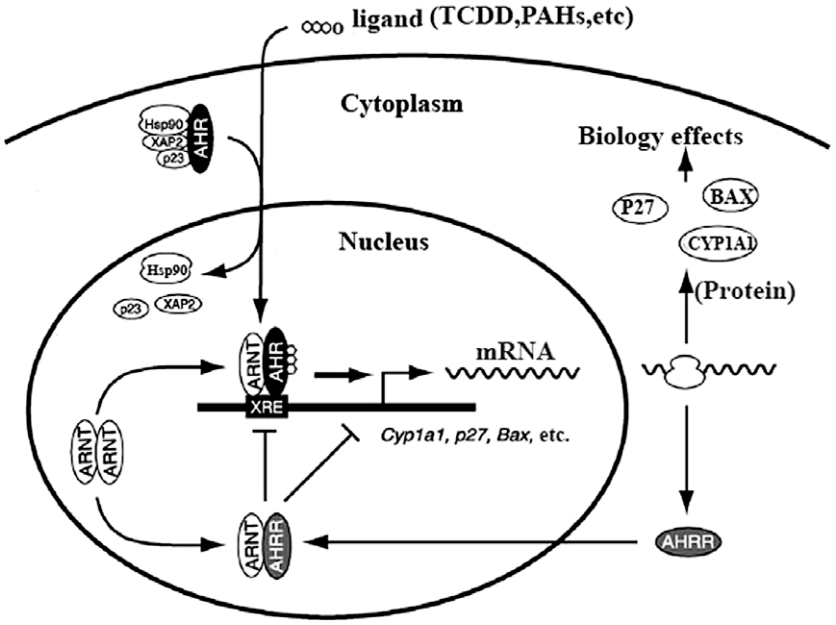

Fig. 1. Regulation Mechanism of AhR pathway gene. This figure adapted from Mimura and Fujii-Kuriyama (2003) with some modification.

1997), but the results of Flaveny et al. (2009) show that p23 is dispensable for stable AhR protein levels, or efficient TCDD-mediated AhR activation of Cyp1A1 and Cyp1A2**.** On the other hand, in the presence of a ligand the AhR–ligand complex translocates from cytoplasm to nucleus, where it switches its partner molecule from HSP90 to AhR nuclear translocator (ARNT). The formed AhR/ARNT heterodimer binds a specific DNA sequence designated xenobiotic response element (XRE) in the promoter region of the target gene including CYP1A1, cell cycle regulation gene (p27) and others to enhance their expression. The AhR needs a ligand for nuclear translocation and heterodimerization with ARNT (Mimura et al., 1999), and Mimura and Fujii-Kuriyama (2003) found that aryl hydrocarbon receptor repressor (AHRR) form a regulatory feedback loop with AhR.

The diversity of AhR pathway genes and the species difference of the complicated regulation process of toxicology in different aquatic animals may throw light on the history of early molecular evolution. In order to obtain the details of the early evolution of toxicology regulation mechanism of AhR pathway, it was necessary to study the evolutionary behavior of the known AhR pathway genes in the major aquatic animals. The availability of protein and gene sequence information in public databases has provided an opportunity to analyze the evolutionary history of the ancient pathway.

The significant species difference in the spectrum of toxicity observed, for example, the LD_{50} for acute TCDD exposure varies from 1 μg kg^{-1} in the guinea pig, 20–40 μg kg^{-1} in the rat, 114 μg kg^{-1} in the mouse and rabbit, and 5000 μg kg^{-1} in the hamster (Poland and Knutson, 1982). In addition, the diversity of the AhR pathway genes in different aquatic animal suggests that the evolutionary history of this pathway may shed light on the early evolution. The current study investigates the molecular phylogeny of the AhR pathway, we have studied the evolutionary behavior of AhR pathway genes and the proteins constitute the pathway in the major aquatic animal. These phylogenies will contribute to the study of structural and sequence diversity and make it possible to characterize and infer the evolutional behavior of AhR pathway genes that constitute the diverse pathway in aquatic animal, and understanding of the functional evolution about these genes is essential to predict and interpret species difference in sensitivity to toxicity caused by POPs.

2. Methodology

2.1. DNA and protein sequences

The annotated and homologous sequences of the AhR pathway genes were retrieved from GenBank by using the PSI-BLAST (Altschul et al., 1997).

The AhR pathway genes dataset of complete coding sequences and protein sequences were obtained mainly from aquatic animals (including mollusc, amphibian, fish, and mammalian). These genes were distributed under four major steps of toxicology mediation (Detail information about the species and strains, gene isoform, nucleotide and corresponding protein length, and the genbank accession number used for this research is provided in Supplementary Table 1). These major steps are formation of cytosolic complex, translocation of AhR, dimerization of AhR, and induction of Cyp1A. The species and strain, taxonomy, and abbreviation used to indicate taxa in the trees of all the animal species used in the study are shown in Table 1.

2.2. Sequence alignment and phylogenetic analysis

These sequences were aligned using CLUSTAL X (version1.81, Thompson et al., 1997) with default options. In order to avoid a codon as one unit of sequence which was separated during the alignment; complete coding sequences were converted to amino acid sequences prior to the alignment and converted back afterwards. No additional manual adjustment by eye was made, but ambiguously aligned proportions were eliminated from the data set using Gblocks 0.91b (Castresana, 2000) with default parameters, and the filtered sequences were concatenated. In addition, all trees were rooted with C. elegans (Caenorhabditis elegans).

Phylogenetic trees were reconstructed using neighbor-joining (NJ) (Saitou and Nei, 1987) and maximum parsimony (MP) (Fitch, 1971) analyses of the concatenated datasets with default parame-

Table 1
Species and strains, abbreviations used to indicate taxa in the trees, taxonomy of all the aquatic animal species used in the study. These species and strains mainly come from aquatic animals, and distribute in four major phylogenetic groups: including bivalvia, amphibian, teleostei and mammalia.

No.	Species and strains		Abbreviations used to indicate taxa in the trees	Taxonomy
	Common name	Name		
1	African clawed frog	*Xenopus laevis*	Afrog	Amphibia
2	American alligator	*Alligator mississippiensis*	Alligator	Archosauria
3	Asiatic toad	*Bufo gargarizans*	Toad	Amphibia
4	Atlantic croaker	*Micropogonias undulatus*	Croaker	Teleostei
5	Atlantic salmon	*Salmo salar*	Asalmon	Teleostei
6	Atlantic tomcod	*Microgadus tomcod*	Tomcod	Teleostei
7	Atlantic white-sided dolphin	*Lagenorhynchus acutus*	Dolphin	Mammalia
8	Baikal seal	*Phoca sibirica*	Bseal	Mammalia
9	Bastard halibut	*Paralichthys olivaceus*	Halibut	Teleostei
10	Bay scallop	*Argopecten irradians*	Scallop	Bivalvia
11	Beluga whale	*Delphinapterus leucas*	Bwhale	Mammalia
12	Brook trout	*Salvelinus fontinalis*	Btrout	Teleostei
13	Caenorhabditis elegans	*Caenorhabditis elegans*	C. elegans	Nematoda
14	Chimpanzee	*Pan troglodytes*	Chimpanzee	Mammalia
15	Chinese hamster	*Cricetulus griseus*	Chamster	Mammalia
16	Chinook salmon	*Oncorhynchus tshawytscha*	Csalmon	Teleostei
17	Chlamys farreri	*Chlamys farreri*	Chla	Bivalvia
18	Crab-eating macaque	*Macaca fascicularis*	Macaque	Mammalia
19	Daphnia magna	*Daphnia magna*	Daphnia	Crustacea
20	Domestic guinea pig	*Cavia porcellus*	guineapig	Mammalia
21	European eel	*Anguilla anguilla*	Eeel	Teleostei
22	European seabass	*Dicentrarchus labrax*	Seabass	Teleostei
23	Fugu rubripes	*Takifugu rubripes*	Fugu	Teleostei
24	Golden hamster	*Mesocricetus auratus*	Ghamster	Mammalia
25	Goldfish	*Carassius auratus*	Goldfish	Teleostei
26	Grass carp	*Ctenopharyngodon idella*	Gcarp	Teleostei
27	Gray seal	*Halichoerus grypus*	Gseal	Mammalia
28	Great cormorant	*Phalacrocorax carbo*	Cormorant	Aves
29	Haliotis asinine	*Haliotis asinine*	Hali	Gastropoda
30	Harbor seal	*Phoca vitulina*	Harborseal	Mammalia
31	Harp seal	*Phoca groenlandica*	Harpseal	Mammalia
32	House mouse	*Mus musculus*	Hmouse	Mammalia
33	Human	*Homo sapiens*	Human	Mammalia
34	Humpback whale	*Megaptera novaeangliae*	Hwhale	Mammalia
35	Japanese eel	*Anguilla japonica*	Jeel	Teleostei
36	Japanese medaka	*Oryzias latipes*	Medaka	Teleostei
37	Japanese wild mouse	*Musmusculus molossinus*	Jmouse	Mammalia
38	Killifish	*Fundulus heteroclitus*	Killifish	Teleostei
39	Lake trout	*Salvelinus namaycush*	Ltrout	Teleostei
40	Laternula elliptica	*Laternula elliptica*	Laternula	Bivalvia
41	Marbled flounder	*Pseudopleuronectes yokohamae*	MFlounder	Teleostei
42	Metapenaeus ensis	*Metapenaeus ensis*	Meta	Crustacea
43	Mexican tetra	*Astyanax mexicanus*	Tetra	Teleostei
44	North Atlantic right whale	*Eubalaena glacialis*	Nwhale	Mammalia
45	Norway rat	*Rattus norvegicus*	Rat	Mammalia
46	Oyster toadfish	*Ops an us tau*	Toadfish	Teleostei
47	Pacific oyster	*Crassostrea gigas*	Oyster	Bivalvia
48	Rainbow trout	*Oncorhynchus mykiss*	Rtrout	Teleostei
49	Red seabream	*Pagrus major*	Seabream	Teleostei
50	Ribbon seal	*Phoca fasciata*	Rseal	Mammalia
51	Sea otter	*Solea senegalensis*	Seaotter	Mammalia
52	Senegalese sole	*Enhydra lutris*	Sole	Teleostei
53	Soft-shell clam	*Mya arenaria*	Clam	Bivalvia
54	Southeastern Asian house mouse	*Mus musculus castaneus*	Smouse	Mammalia
55	Tigriopus japonicus	*Tigriopus japonicus*	Tigriopus	Crustacea
56	Turbot	*Psetta maxima*	Turbot	Teleostei
57	Western clawed frog	*Xenopus (Silurana) tropicalis*	Wfrog	Amphibia
58	Zebra mussel	*Dreissena polymorpha*	Zmussel	Bivalvia
59	Zebrafish	*Danio rerio*	Zebrafish	Teleostei

ters as implemented in MEGA 4.0 (Tamura et al., 2007), and maximum likelihood (ML) analyses of the concatenated datasets implemented in PhyML V2.4.4 (Guindon and Gascuel, 2003). Prior to the ML analysis, we used Modeltest 3.8 (Posada and Crandall, 1998) to select the best-fit model of nucleotide substitution for each dataset (HSP90, AhR + AHRR, ARNT and CYP1A), following the Akaike Information Criterion (AIC) (Posada and Buckley, 2004). The selected models were: GTR + I + G, for HSP90 concatenated data set; GTR + I + G, for AhR + AHRR combined concatenated data set; TrN + I + G, for ARNT concatenated data set; GTR + I + G, for CYP1A concatenated data set.

The reliability of these trees was estimated by the bootstrap procedure with 1000 replications, and these trees were analyzed and clades were marked alphabetically for the further analysis of synonymous and non-synonymous nucleotide substitutions within the major aquatic animal groups.

The frequency of synonymous nucleotide substitutions per synonymous site (silent; d_S) and non-synonymous nucleotide substi-

Table 2
Patterns of synonymous nucleotides substitution (d_S) in different AhR pathway genes. The frequency of synonymous nucleotide substitutions per synonymous site were calculated by the model of modified Nei–Gojobori method, applying the Juke–Cantor corrections with the transition/transversion ratio set to 2 and the multiple substitutions at the same site. The MEGA 4.0 software was used to compute the d_S value within different clade.

AhR pathway genes	Clade						
	A	B	C	D	E	F	G
Hsp90	1.021 ± 0.047[a]	0.700 ± 0.033	0.643 ± 0.046	0.332 ± 0.022	0.411 ± 0.020	0.584 ± 0.038	0.380 ± 0.025
AhR/AHRR	1.240 ± 0.215	0.709 ± 0.057	0.684 ± 0.040	0.197 ± 0.034	0.389 ± 0.034	1.087 ± 0.071	
ARNT	0.000 ± 0.000	0.246 ± 0.028	0.101 ± 0.022	0.126 ± 0..021	0.147 ± 0.019	0.076 ± 0.019	0.114 ± 0.020
CYP1A	0.199 ± 0.028	0.114 ± 0.019	0.137 ± 0.021				

[a] Standard error value.

Table 3
Patterns of non-synonymous nucleotides substitution (d_N) in different AhR pathway genes. The frequency of non-synonymous nucleotide substitutions per non-synonymous site were calculated by the model of modified Nei–Gojobori method, applying the Juke–Cantor corrections with the transition/transversion ratio set to 2 and the multiple substitutions at the same site. The MEGA 4.0 software was used to compute the d_N value within different clade.

AhR pathway genes	Clade						
	A	B	C	D	E	F	G
HSP90	0.094 ± 0.007[a]	0.043 ± 0.004	0.040 ± 0.004	0.004 ± 0.001	0.028 ± 0.003	0.022 ± 0.004	0.001 ± 0.001
AhR/AHRR	0.026 ± 0.008	0.056 ± 0.008	0.118 ± 0.012	0.028 ± 0.008	0.026 ± 0.006	0.250 ± 0.024	
ARNT	0.000 ± 0.000	0.171 ± 0.013	0.050 ± 0.009	0.076 ± 0.009	0.099 ± 0.009	0.038 ± 0.008	0.090 ± 0.010
CYP1A	0.246 ± 0.017	0.125 ± 0.015	0.158 ± 0.015				

[a] Standard error value.

tutions per non-synonymous site (amino acid-changing; d_N) (Tables 2 and 3) were calculated by the model of modified Nei–Gojobori method (Nei and Gojobori, 1986), applying the Juke–Cantor corrections with the transition/transversion ratio set to 2 and the multiple substitutions at the same site. The MEGA 4.0 software (Tamura et al., 2007) was used to compute the d_S and d_N value within different clade.

2.3. Protein domains

PFam database (version 23.0) (http://www.pfam.sanger.ac.uk/) (Finn et al., 2008) was used to identify putative domains present in the respective AhR pathway gene products.

3. Results and discussion

Phylogenies trees obtained from NJ, MP, and ML algorithms were found to be highly congruent. Therefore, in this article, we just discuss the results based on the NJ tree analysis.

3.1. Formation of cytosolic complex

In the absence of ligand, AhR is associated with a cytoplasmic protein complex with two molecules of heat shock protein 90 (HSP90), one X-associated protein 2 (XAP2) (also referred to as AIP or ARA9), and a 23-kDa co-chaperone protein (p23) (Denison et al., 2002). HSP90 is an essential component of the AhR-signaling pathway, and it is one subunit of the AhR complex appears to control proper folding and maintenance of the high affinity ligand binding conformation of the AhR in some species (Soshilov et al., 2006).

The phylogenetic tree of Hsp90 gene (Fig. 2A) demonstrates that the close evolutionary relationship of Hsp90 sequences among these aquatic animals with two defined clades of HSP90 protein. One consists of the protein from mollusc, which belong to invertebrates. The second clade includes amphibian, fish and mammalian. There are two types of Hsp90 genes within the amphibian, fish and mammalian clade, namely Hsp90a and Hsp90b, and they encode two similar cytosolic isoforms respectively. We can find that the most if not all vertebrates should have both paralogues, and this maybe arouse by a gene duplication event that took place very early in the evolution of eukaryotic cells. Krone and Sass (1994) confirmed it by zebrafish experiment. Despite the marked similarities between the two genes at molecular level, Hsp90α and Hsp90β exhibit different patterns of expression during embryonic development and cell differentiation, and also in response to environmental, physical and chemical stresses. (Csermely et al., 1998; Padmini and Usha Rani, 2009). During heat shock, both Hsp90α and Hsp90β genes are upregulated in both mouse and human cells, in contrast, Hspβ 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. So, we can conclude that the Hsp90β gene maybe have acquired the ability to become environmental stress-inducible late during vertebrate evolution. Thus, we propose that the two isoforms genes have similarity function, but they have different expression pattern in some lower vertebrate (for example, fish) under the environmental stress. Additionally, we can find that the Hsp90 gene isoform of European seabass and turbot should belong to the type of Hsp90b. On the contrary, the Hsp90 gene isoform of chinook salmon should belong to the type of Hsp90a, which received support from Palmisano et al. (1999). However, there is an exception that the Hsp90a and Hsp90b of bastard halibut cluster in one group, so, maybe they belong to one pair of alleles, and it needs further study.

The present study shows that all the HSP90 proteins have two well-conserved domains, namely HATPase_c and HSP90 (see Supplementary Table 2) through the Pfam analysis of HSP90 protein domains. HATPase_c interacts selectively with ATP, HSP90 binding is thought to mask the AhR-nuclear localization signals (NLS), and this interaction is essential for the cytoplasmic retention of AhR (Kazlauskas et al., 2001). Furthermore, HSP90 and the proteasome are playing a key role in modulating AhR signaling and Cyp1A responses in trout hepatocyte (Wiseman and Vijayan, 2007). In addition, we carry out the analysis of nucleotide substitutions of hsp90

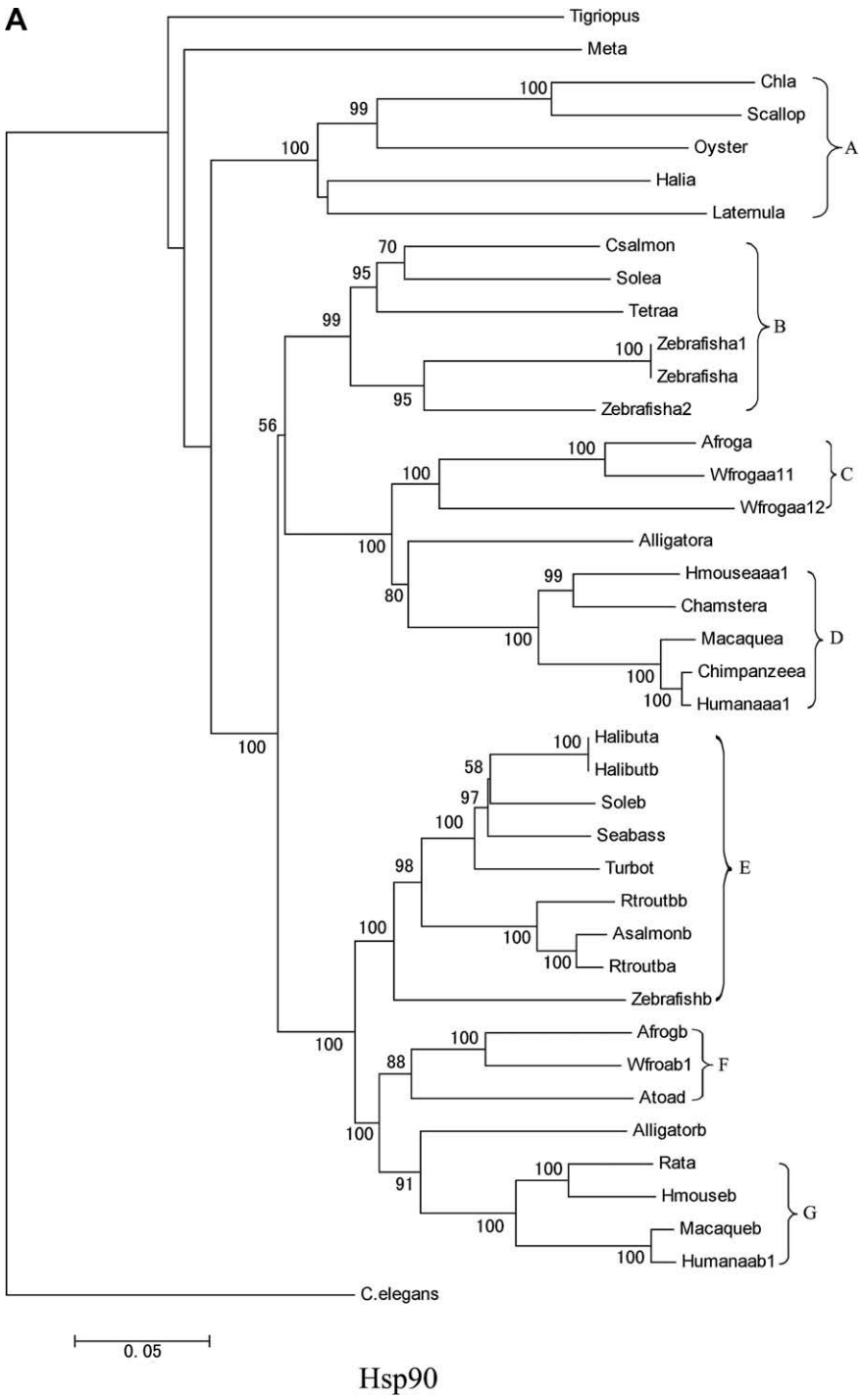

Fig. 2. NJ phylogenetic trees from AhR pathway genes (protein coding DNA sequences) from diverse aquatic animals. Numbers next to branch points are bootstrap values based on 1000 samplings. The corresponding gene sequences of C. elegans were used as outgroup of the four phylogenetic trees. Genes involved in (A) formation of cytosolic complex, (B) translocation of AhR, (C) dimerization of AhR, and (D) induction of CYP1A. Bootstrap values <50% are not shown. Tree A is based on Hsp90, B on AhR and AHRR, C on ARNT and D on CYP1A.

within different clade. Our results indicate that the Hsp90 gene of invertebrates (clade A) were observed to be less conserved than those of vertebrates (clades A–G) (d_N values for A = 0.094 ± 0.007, B = 0.043 ± 0.004, C = 0.040 ± 0.004, D = 0.004 ± 0.001, E = 0.028 ± 0.003, F = 0.022 ± 0.004, and G = 0.001 ± 0.001 respectively). All the values of $d_N/d_S < 1$, are calculated using the d_N and d_S values (Tables 2 and 3), which indicates a functional constraint on this gene product. As a whole, the isoform of Hsp90b is more conserved than Hsp90a (see Fig. 2A, compare clades B–D with E–G), and the higher vertebrate is more conserved than relative lower vertebrate within one isoform (compare within clades B–D and within E–G). So, we can conclude that each isoform of Hsp90 gene and each isoform in each phylogenetic group (mollusc, amphibian, fish, and mammalian) have different evolution rate within different clade. At present, though there is little similarity data can confirm it; these results can receive support from the amino acid or nucleic acid identity of Hsp90. The paralogs share about 85% amino acid identity within species in humans, mice, and chickens, but even greater identities are found comparing orthologous forms between species (Gupta, 1995). For example, human Hsp90a shares 95–99%

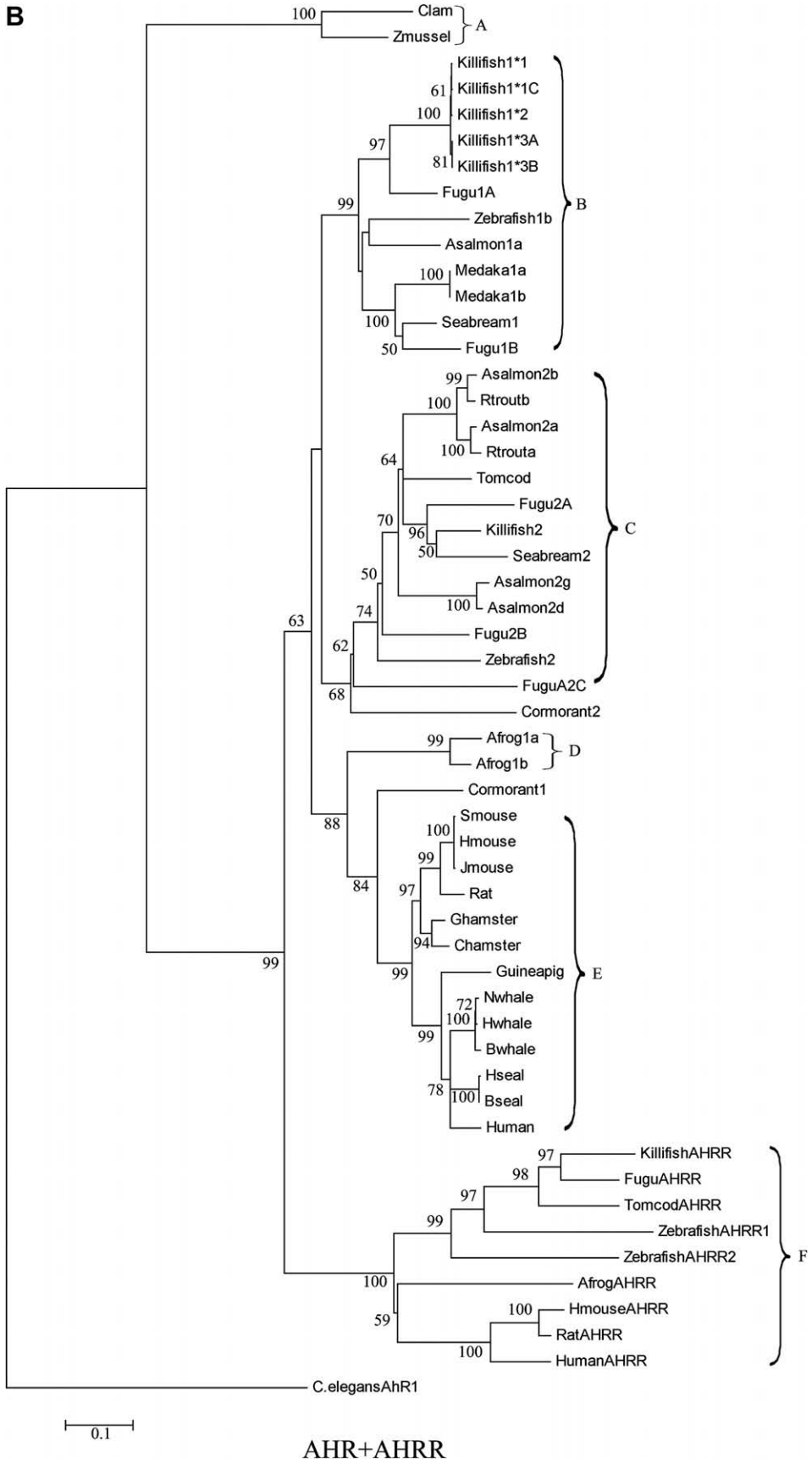

Fig. 2 (*continued*)

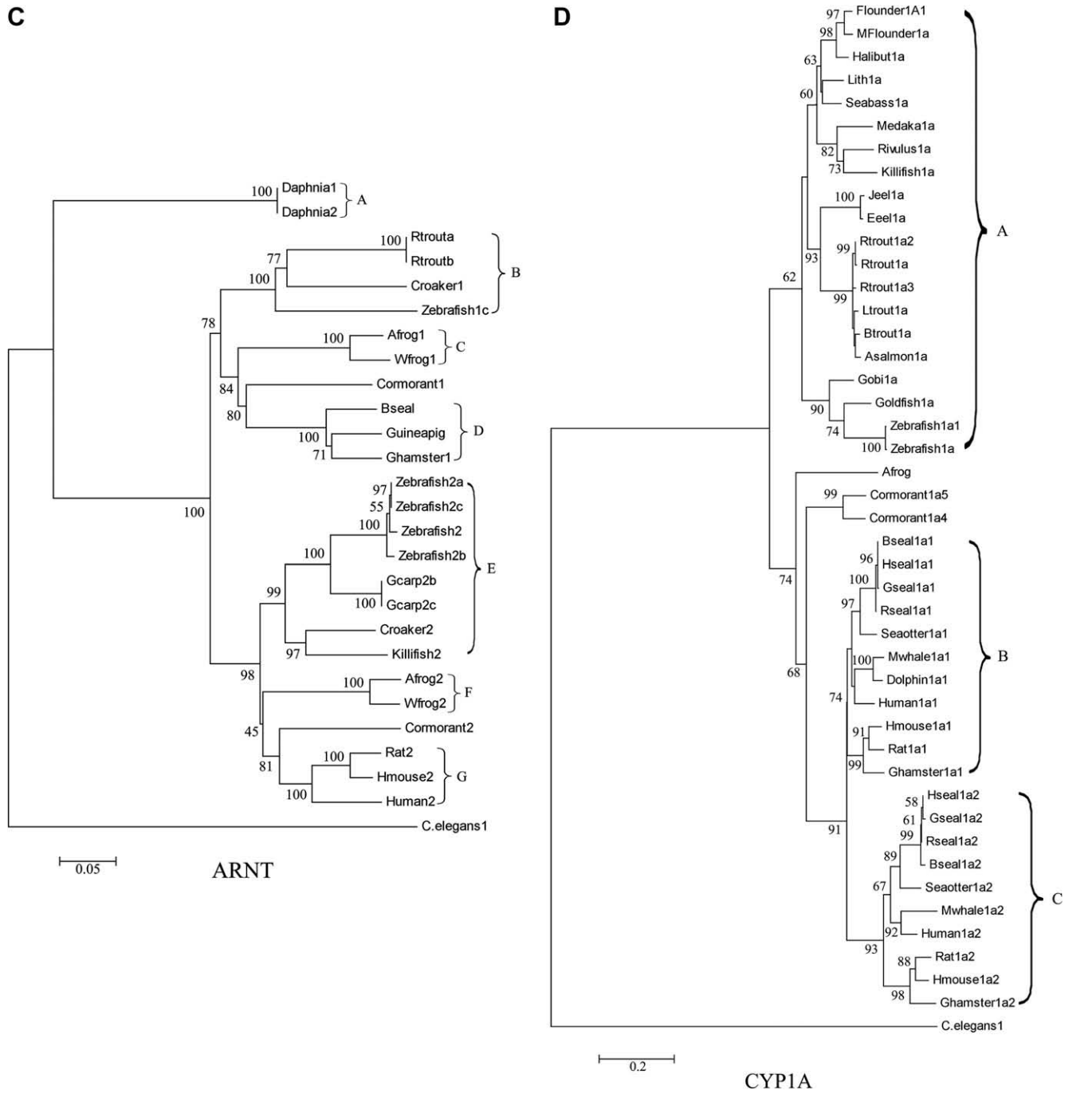

Fig. 2 (*continued*)

identity with a form from chick, mouse, hamster, and pig; and human Hsp90b is 94–99% identical to its orthologous b forms in chick, rat, and mouse. Furthermore, Rutherford et al. (2007) found that Hsp90 as a homodimer, which associates with co-chaperone in an ATP-dependent manner to facilitate proper maturation and maintain the activity of over 150 signal transduction proteins in many different regulatory pathways. So the functional constraint of Hsp90 gene production could probably because the multiple role of Hsp90 in the cell, and it is these in aggregate.

3.2. Translocation of AhR

Upon binding to a ligand (TCDD or others), the AhR complex translocates into the nucleus and AhR dissociates from HSP90 complex to form a heterodimer with its partner molecule, ARNT (Poland and Knutson, 1982). The transcription factor AhR plays an important role in response to environmental pollutants. It has been extensively studied as a mediator of toxicity of a diverse group of xenobiotics, including polychlorinated dioxins and dibenzofurans, PCBs, PAHs, etc. (Ma, 2001). Interestingly, a large number of studies have demonstrated that AhR1 is a key regulatory protein contributing to differential sensitivity to dioxin-like compounds in several animal models (Okey, 2005). For example, in Atlantic killifish, AhR1 variants or their interaction with other killifish loci (AhR2, AHRR) that may contribute to differences in dioxin sensitivity (Hahn et al., 2004). As well as in avian has the same scenarios, the AhR1 has a high affinity for dioxin-like compounds, but AhR2 below AhR1 (Yasui et al., 2007). The molecular basis for differential

sensitivity to dioxin-like compounds in aquatic animal is not well understood. However, A recent study suggests that the amino acid residues corresponding to Ile324 and Ser380 in the chicken aryl hydrocarbon receptor 1 (AhR1) are important determinants of differential biochemical responses to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in chickens and common terns (Karchner et al., 2006). Furthermore, the results of Goryo et al. (2007) suggested that Phe318, Ile319, and His320 these three amino acids play an important role for ligand binding in HeLa cells. Notably, there have many works need to be done for better understanding the detail molecular mechanism for differential sensitivity to POPs in aquatic animal.

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 negative feedback loop (Mimura and Fujii-Kuriyama, 2003); expression of AHRR is regulated by the AhR, and AHRR acts as a transcriptional repressor of AhR function. Like the AhR, AHRR can dimerize with the ARNT, and the AHRR–ARNT complex also can bind to AhR-responsive enhancer elements (AHREs). Repression occurs through competition between AhR and AHRR for binding to AHREs (Mimura et al., 1999) as well as through additional mechanisms that do not involve competition for ARNT and are independent of AHRE binding by AHRR (Evans et al., 2008). Recently, studies of Karchner et al. (2009) revealed that AHRR△8 (a novel human AHRR cDNA) is the active form of human AHRR and reveal novel aspects of its function and specificity as a repressor. Without doubt, many issues have not been answered; so, targeted knockdown of one or both AHRR proteins by application of morpholino oligonucleotides can be used to further characterize the AHRRs and to elucidate their potential roles in development and in the developmental toxicity of chemicals such as TCDD in the future.

The phylogenetic tree (as seen in Fig. 2B) of AhR genes from different aquatic animals, shows that the vertebrate AhR genes are divided into two distinct evolutionary lineages, AhR1 and AhR2, which is consistent with the results of Hahn et al. (1997, 2002). In addition, avian AhR1 and AhR2 are orthologous to mammalian AhR1 and fish AhR2, respectively (Lee et al., 2009); these results suggest that an ancestral AhR gene underwent tandem duplication prior to the divergence of fish and tetrapod lineages. Basing on our result, we can find that frog have AhR1a and AhR1b two isoform genes, this is somewhat reminiscent of AhR2a and AhR2b, and which is closely related to AhR paralogs in cormorant AhR1 gene, however, the result of (Lavine et al., 2005) show that it is closely related to AhR paralogs in rainbow trout (*Oncorhyncus mykiss*), the reason maybe is that they did not add the sequence of cormorant into the phylogenetic tree. However, marine and terrestrial mammals just have a single AhR gene which belongs to the AhR1 lineage (Karchner et al., 1999), interesting that fishes have more AhR genes than other vertebrates because they have retained AhR2 genes, this maybe caused by that a fish-specific whole-genome duplication event in their early evolutionary past (Hahn et al., 2006). The structural and functional diversity of AhR protein may confer species- and strain-specific difference in the sensitivity to toxic AhR ligands and it is possible that numerous, possibly diverse, physiological roles are partitioned among multiple AhRs and AHRRs. In addition, AhR2 genes from fish form a separate clade. Similarly, AhR1 genes from fish form another separate clade.

In addition, phylogenetic analysis of AhR from zebrafish, Fugu, and killifish along with mammalian AhR suggests that the difference in AhR diversity between mammal and fish is the result of gene and genome duplications coupled with lineage specific gene loss (Hahn, 2002; Karchner et al., 2005). Salmonids, which have undergone additional genome duplication, have even greater AhR diversity, including two AhR1 gene and four AhR2 genes in the Atlantic salmon (*Salmo salar*) (Hansson et al., 2003; Hansson et al., 2004).

The subtree of AhR genes form a monophyletic group that placed as the most basal lineage. The next diverging lineage consists of AHRR genes from an amphibian, bony fishes, and several mammalian species, that was strongly supported with a bootstrap value of 100% (Fig. 2B). Furthermore, zebrafish is notable for possessing two AHRR paralogs (zfAHRR1 and zfAHRR2), and grouped together, suggesting that they arose by gene duplication after the divergence of the zebrafish and killifish lineages. Moreover, appear to be co-orthologs of the mammalian AHRR; it is maybe the result of the fish-specific whole genome duplication (Evans et al., 2005). Not unexpectedly, the zebrafish AHRR (zfAHRR) genes clustered most closely with other fishes AHRR compared to amphibian and mammalian AHRRs, which received the support from Roy et al. (2006). Furthermore, zfAHRR1 more tightly clustered with the other fish AHRR genes than zfAHRR2 gene, with 97% bootstrap support using our methods. However, Evans et al. (2005) using maximum parsimony with the complete AHRR sequences, and got the reverse results that zfAHRR2 is more closed to other fish AHRR genes than zfAHRR1, the difference maybe caused by using the different sequence of AHRR genes, I think further study with more taxa and full-length sequences will be necessary to resolve this issue conclusively.

Until now, An AHRR gene has not been identified in any other earlier diverging vertebrates or invertebrates, including an invertebrate chordate or a deuterostome. So, maybe the AHRR is a vertebrate-specific member of the AhR subfamily within the PAS gene family, consistent with the close phylogenetic relationship of AHRR to AhR, the structures of the AHRR and AhR genes are highly similar. The AHRR maybe arose from a duplication of an ancestral AhR gene. For better understanding the divergence time which occurred in vertebrate or chordate evolution, it needs await the characterization of genomes from cartilaginous and jawless fishes (Hahn et al., 2009). Together, zebrafish AHRR1 and AHRR2 both belong to the AHRR clade within the larger AhR family.

All the AhRs contain HLH (helix-loop-helix), PAS (Per-ARNT-Sim) and PAS_3 three well-conserved domains (see Supplementary Table 3) involve in substrate binding. The first domain located in the N-terminal region of the molecule, consists of the bHLH (basic helix-loop-helix) domain found in many transcription factors (e.g. MyoD, c-myc, and Max) (Murre et al., 1989; Kadesch, 1992; Olson and Klein, 1994). The second domain is very similar to the Drosophila circadian rhythm gene per and the Drosophila single-minded protein sim and, therefore, is referred to as the PAS domain (Hoffman et al., 1991; Takahashi, 1992). The third domain, located at the C-terminal end of the molecule, is glutamine (Q)-rich. The ligand-binding function apparently resides in the PAS region of AhR (Dolwick et al., 1993). However, AHRR (see Supplementary Table 3) only has HLH and PAS, the two conserved domains.

Our results further supporting the hypothesis that AhR is an ancient protein, which is well conserved in vertebrates and invertebrates, indicating that its play a critical function throughout evolution (Karchner et al., 2002). In brief, the present study reveals that AhR1 and AhR2 genes in fish are less conserved (d_N values for clade B, C is 0.056 ± 0.008, 0.118 ± 0.012 respectively.) compared with other aquatic animals AhR genes (d_N values for clade A, D and E clade are 0.026 ± 0.008, 0.028 ± 0.008 and 0.026 ± 0.006, respectively). Additionally, from Fig. 2B and compare the d_N value between AhR1 and AhR2 of fish, we can conclude that AhR2 is less conserved than AhR1. Furthermore, AhR2 of zebrafish is required for TCDD toxicity during development (Carney et al., 2006), whether this is unique to zebrafish or is common in fish remains to be determined; and the results of Karchner et al. (1999) show that two Atlantic killifish AhR genes displayed different tissue-specific patterns of expression; AhR1 transcripts were pri-

marily expressed in brain, heart, ovary, and testis, while AhR2 transcripts were equally abundant in many tissues. Thus, we can suppose that AhR2 gene maybe play more important role in mediation process of toxicology in fishes. Obviously that the d_N value (0.250 ± 0.024) of AHRR is the biggest in these clades, because that this clade includes several phylogenetic groups. Together, All the values of $d_N/d_S < 1$, are calculated using the d_N and d_S values (Tables 2 and 3), which indicates a functional constraint on this gene product too.

3.3. Heterodimerization of AhR

When AhR binds to ligand, it translocates to the nucleus and dissociates from the Hsp90 complex to form a heterodimer with ARNT. The AhR/ARNT heterodimer binds to the XRE sequence in the promoter region of target gene encoding drug-metabolizing enzymes, including CYP1A1, quinone reductase, etc., and alters their expression (Kikuchi et al., 2003). ARNT belongs to the bHLH-PAS (basic helix-loop-helix–Per-ARNT-Sim) family. In addition to binding with AhR, ARNT also interacts with SIM1 (Single Minded 1), SIM2 (Single Minded 2), HIF1α (hypoxia-inducible factor 1α), CHF1 (Cardiovascular helix-loop-helix factor 1) and EPAS1 (Endothelial PAS domain protein 1) to regulate neurogenesis, the hypoxia response, cardiovascular development and pathological angiogenesis. Therefore, ARNT maybe serve as a central player in regulating these diverse signaling pathways (Taylor and Zhulin, 1999; Swanson, 2002; Mimura and Fujii-Kuriyama, 2003).

The daphnia ARNT1 and ARNT2 formed a monophyletic group, ARNT genes from other animals form another clade, composed of two subtrees, namely ARNT1 and ARNT2 subtree. Many mammals, including rodents and humans, express two closely related ARNT paralogs: ARNT1 is widely expressed (Hoffman et al., 1991), and ARNT2 is expressed primarily in the central nervous system and kidney of adult animal (Drutel et al., 1996). Except baikal seal, it just has only one ARNT, and it is grouped with ARNT1 clade rather than the ARNT2 clade, this consistent with the result of (Kim et al., 2005). Moreover, Common cormorant ARNT2 is orthologous to mammalian ARNT2 and paralogous to ARNT1; and frog ARNT1 and ARNT2 are more closed to mammal than fishes. Surprisingly, in contrast to AhR gene, ARNT have two isoforms (ARNT1 and ARNT2) in mammal, but fish apparently express only one single ARNT gene, although in different species, this may be either ARNT1 or ARNT2, for example, in killifish and zebrafish, ARNT2 is the only ARNT detected (Tanguay et al., 2000), and unlike mammalian ARNT2, which are restricted to specific tissue types, the teleost ARNT2 genes are ubiquitously expressed; In addition, ARNT2 has the ability to dimerize with the liganded AhR in vitro in Hepa-1 cell culture lines (Dougherty and Pollenz, 2008), and like AhR gene, ARNT gene duplication also occurred prior to the divergence of teleosts and tetrapods, at least 400 million years ago (Doolittle et al., 1996). On the contrary, The results of (Prasch et al., 2004) show that zebrafish ARNT2 is not essential for mediating TCDD developmental toxicity in zebrafish and suggest that alternate dimerization partners exist for zfAhR2 in vivo, and further proved by (Antkiewicz et al., 2006); furthermore, Prasch et al. (2006) confirmed that zebrafish ARNT1 play an essential role in mediating TCDD developmental toxicity, whether the expression pattern of ARNT genes is especial for zebrafish, further study of this interesting protein is eagerly awaited; while a different ARNT, likely an ARNT1 predominates in rainbow trout and scup (Powell and Hahn, 2000). Interestingly, however, the recently sequenced Fugu rubripes genome appears to encode two forms of ARNT (Rowatt et al., 2003) suggesting that, at least in some fish species, two ARNT genes do exist.

In addition, we can find that rainbow trout ARNTa and ARNTb cluster in one clade (from Fig. 2C), and results of (Necela and Pollenz, 1999) show that they are divergent mainly in their C-terminal domains, ARNTb contains a C-terminal domain rich in glutamine and asparagine (QN), whereas the C-terminal domain of rtARNTa is rich in proline, serine, and threonine (PST), rainbow trout ARNTb is involved in signaling events at many developmental stages, while the functionality of the dominant negative rainbow trout ARNTa is restricted (Sojka and Pollenz, 2001). So, we can conclude that they maybe result from the alternative splicing of the transcript from a single gene; it is consistent with some studies previously (Pollenz et al., 1996).

ARNT (see Supplementary Table 4) protein sequences show HLH, PAS and PAS_3 three conserved domains. PAS domains can also govern target gene specificity of different heterodimer (Zelzer et al., 1997). Dimers of individual PAS proteins bind specific DNA target sequence and interact with the basic region (Bacsi and Hankinson, 1996) or possibly other distinct regions of a protein (Pongratz et al., 1998), enabling transcriptional activation or repression. Recently, results of Partch et al. (2009) demonstrated that ARNT mainly uses a single PAS domain to interact with two coiled coil coactivators: TRIP230 and CoCoA, and illustrates how ARNT PAS-B is used to form critical interactions with both bHLH-PAS partners and coactivators that are required for transcriptional responses. The non-synonymous nucleotide substitution value suggests that these gene sequences are equally conserved in all the groups of aquatic animal (d_N values for clades A–G are 0.000 ± 0.000, 0.171 ± 0.013, 0.050 ± 0.009, 0.076 ± 0.009, 0.099 ± 0.009, 0.038 ± 0.008 and 0.090 ± 0.010, respectively). We can find that the d_N value of clade A is 0.000 ± 0.000, it might because daphnia ARNT1 and ARNT2 just belong to one pair of alleles or there has a alternative splicing of a single gene. However, the gene sequence from clade B shows a higher d_N value of 0.171 ± 0.013, indicating the unconserved nature. Besides, compare the d_N value between ARNT1 and ARNT2 in fishes, we can find that d_N value of ARNT1 (0.171 ± 0.013) is bigger than ARNT2 (0.099 ± 0.009), and zebrafish ARNT1 play an essential role in mediating TCDD developmental toxicity. Therefore, we can suppose that ARNT1 maybe takes place a positive selection when fishes encounter the environmental pollution. In conclusion, the value of $d_N/d_S < 1$, calculated using the d_N and d_S value (Tables 2 and 3), indicates a functional constraint on this protein and maybe play an important physiological function in aquatic animal. Indeed, Powell et al. (1999) have suggested that the high degree of sequence identity of the different isoform between species suggests substantial selective pressure for their strict conservation. 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), but ARNT2 is not essential in mediating POPs toxicity. Furthermore, low levels of ARNT could decrease the sensitivity of a particular tissue to agonist despite high AhR level (Schmidt and Bradfield, 1996). Collectively, different fish species utilize different ARNT isoforms, and the isoforms distribution is complicated and intriguing in teleost species. Also, characterization of ARNT in diverse species is a useful approach for evaluating the species differences in POPs toxicity, while the functional differences of ARNT could contribute to the interspecies differences in ligand-binding affinity of AhR. The possibility that the number, type, and expression patterns of different ARNT isoform may contribute to the variability, possibly via distinct interactions with other PAS-family proteins.

3.4. Induction of CYP1A

The ligand–AhR–ARNT heterodimer interacts with AhR response elements (XREs; also known as XREs or DREs) to activate or repress gene expression from target gene (Hahn et al., 2005, 2006). The best characterized target of the AhR pathway is Cyto-

chrome P4501A (CYP1A) gene, which is strongly induced by XREs (Whitlock, 1999). AhR–ARNT heterodimer has a broad affinity for polycyclic aromatic hydrocarbons, as well as aromatic amines, and some endogenous substrates (Gonzalez and Kimura, 2003; Teraoka et al., 2003). And they play a key role in biotransformation, detoxication 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) (Waxman, 1999), respectively.

The phylogeny tree (Fig. 2D) of CYP1A shows three distinct clades (clade A, CYP1A1 gene from fish; clade B, CYP1A1 gene from mammalian; and clade C, CYP1A2 gene from mammalian). The CYP1A subfamily appears to have originated early in the vertebrate lineage. Fish generally possesses a single CYP1A gene (Morrison et al., 1995, 1998); besides, we can find that gold fish clusters in a monophyletic group with other fish CYP1As, and more closely related to zebrafish CYP1A than to other fish CYP1As. However, Rainbow trout and salmonids are notable exceptions, this is consistent with the result of (Rabergh et al., 2000; Mahata et al., 2003). Mammalian, in contrast, generally possesses two paralogous CYP1A genes: CYP1A1 and CYP1A 2 (Kimura et al., 1984; Quattrochi et al. 1985). Fish CYP1As share significant sequence similarity with both CYP1A1s and CYP1A2s (Morrison et al., 1995) and display a combination of catalytic function characteristic of the mammalian isoforms (Gorman et al., 1998). However, fish CYP1As are considered more CYP1A1-like on the basis of slightly higher level of pairwise sequence identity and similarity in pattern of gene expression.

The induction of hepatic CYP1A is an important step in response to contaminants, such as PAHs. Researches show that four out of eight different XREs are functional in the control of CYP1A in the flounder. However, the CYP1A gene of killifish contains three consensus XREs (5′CACGC3′) within 1.6 kb of the putative transcriptional start site (Powell et al., 2004), in addition, two XREs at −613 bp and −1585 bp in common cormorant CYP1A5, and one XRE at −262 bp in chicken CYP1A5 conferred TCDD-responsiveness (Lee et al., 2009). Study of ZeRuth and Pollenz (2007) shows that XREs is not the sole determinant for regulation of aryl hydrocarbon receptor (AhR)-mediated gene and their function does not appear in an additive manner. In mouse, seven out of eight DREs are located 1.4 kb upstream of the CYP1A1 transcriptional start site and 12.6 kb upstream of the CYP1A2 start site. Furthermore, the DREs regulate both CYP1A1 and CYP1A2 expression in vivo and these two gene products do not cause many common dioxin-induced toxic endpoints (Nukaya et al., 2009). For these reasons, the activity of these response elements further enhances the evidence for considerable diversity in vertebrate CYP1A regulation (Lewis et al., 2004).

All the CYP1As (see Supplementary Table 5) show the p450 well conserved domain. CYP1A gene from fish is observed to be less conserved (d_N for clade A = 0.246 ± 0.017) than the CYP1A gene from mammal (d_N for clade B = 0.125 ± 0.015, C = 0.158 ± 0.015, respectively), and fish species vary widely in their sensitivity to POPs; thus, we may conclude that the CYP1A of fishes maybe go through adaptive evolution under the environmental contaminant pressure. In addition, compare the d_N value between CYP1A1 and CYP1A2 in mammal, we can find that d_N value of CYP1A2 (0.158 ± 0.015) is bigger than CYP1A1 (0.125 ± 0.015), and mammal CYP1A2 play an important role in mediating POPs toxicity, therefore, we can suppose that CYP1A2 maybe takes place a greater adaptive evolution than CYP1A1. Surprisingly, this gene is not like other genes in the AhR pathway, the value of $d_N/d_S > 1$, calculated using the d_N and d_S value (Tables 2 and 3), consistent with the results of Goldstone and Stegeman (2006), suggesting that gene conversion and positive selection may have been the dominant processes of sequence evolution, and there may be an adaptive evolution on this gene. This may be because the evolutionary history of the CYP1A superfamily appears to be extremely complex; the reason is that gene and genome duplication, gene amplification and conversion, gene structure rearrangements, gene loss, horizontal gene transfer, and convergent evolution all contribute to the evolution of CYP1A (Werck-Reichhart and Feyereisen, 2000). So CYP1A gene might play a more important role for predicting and interpreting species differences in sensitivity to toxicity caused by POPs. In addition, many studies have demonstrated that there are marked interindividual differences in humans in both levels of hepatic CYP forms and the inducibility of these enzymes. Interindividual differences can be due to environmental factors (e.g. diet, cigarette smoking, and exposure to enzyme inducing pollutants), physiological factors (e.g. age, disease, and endocrine homeostasis) and to polymorphisms in CYP genes, nuclear receptors, regulatory proteins, and transporters (Tang et al., 2005).

4. Conclusions

The phylogenetic analysis suggests that the gene duplication has substantially contributed to the diversity of AhR pathway genes across aquatic animals. This study also indicates that the AhR pathway genes value of $d_N/d_S < 1$, indicates a functional constraint on these gene products. The AhR pathway genes productions are ancient protein that is conserved in vertebrates and invertebrates, indicating its important function throughout evolution. But CYP1A gene is an exceptant, so maybe it plays a more important role in the species difference in sensitivity to toxicity caused by POPs. In addition, the non-synonymous nucleotide substitution (d_N) value indicates that AhR pathway genes are less conserved in fish than in other animals, and fish possesses more gene isoforms than other aquatic animals (see Table 4, summarized from four steps of AhR pathway). Furthermore, according to the expression patterns of zebrafish (see Table 4) and the value of d_N (Table 3), we find that the gene isoforms with the higher value of d_N play more important role in the process of development toxicology in zebrafish. This indicates that AhR pathway genes likely go

Table 4
Isoform numbers of AhR pathway genes and the expression patterns of zebrafish. This table mainly summarized the expression patterns of the five genes (Hsp90, AhR + AHRR, 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 comprise of mollusc, amphibian, fish, and aquatic mammal). "–" indicates that there have no data at present.

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

through an adaptive evolution within aquatic animals under the pressure of environmental pollution.

In addition, fish species vary widely in their sensitivity to POPs. The number, type, and expression pattern of AhR pathway genes may contribute to interspecies difference in aryl hydrocarbon toxicity, possibly through distinct interaction with additional PAS-family proteins. Veldhoen et al. (2008) results show that AhR gene involves the autoimmune, so it may help fish to adapt to the various stimuli of environmental pollutants. These discoveries give us a novel insight into the role of AhR pathway genes in the process of toxicology regulation. We hope that this research may provide an access to the better understanding of the toxicology mechanism of POPs in aquatic animals, and bring fresh idea for further study of the mechanism of POPs toxicology.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of the manuscript entitled.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2009.09.012.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and Psi-Blast: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Antkiewicz, D., Peterson, R., Heideman, W., 2006. Blocking expression of AhR2 and ARNT1 in zebrafish larvae protects against cardiac toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Sci. 94, 175–182.

Bacsi, S.G., Hankinson, O., 1996. Functional characterization of DNA-binding domains of the subunits of the heterodimeric aryl hydrocarbon receptor complex imputing novel and canonical basic helix-loop-helix protein–DNA interactions. J. Biol. Chem. 271, 8843–8850.

Carney, S.A., Chen, J., Burns, C.G., Xiong, K.M., Peterson, R.E., Heideman, W., 2006. Aryl hydrocarbon receptor activation produces heart-specific transcriptional and toxic responses in developing zebrafish. Mol. Pharmacol. 70, 549–561.

Carver, L.A., Bradfield, C.A., 1997. Ligand-dependent interaction of the aryl hydrocarbon receptor with a novel immunophilin homolog in vivo. J. Biol. Chem. 272, 11452–11456.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552.

Csermely, P., Schnaider, T., So ti, C., Prohászka, Z., Nardai, G., 1998. The 90-kDa molecular chaperone family structure, function, and clinical applications. Pharmacol. Therapeut. 79, 129–168.

Cumanova, A., Gilca, G., Orlova, N., 2007. The Fate of Persistent Organic Pollutants in the Environment. Springer, Dordrecht.

Denison, M.S., Pandini, A., Nagy, S.R., Baldwin, E.P., Bonati, L., 2002. Ligand binding and activation of the Ah receptor. Chem.-Biol. Interact. 141, 3–24.

Dolwick, K.M., Swanson, H.I., Bradfield, C.A., 1993. In vitro analysis of Ah receptor domains involved in ligand-activated DNA recognition. Proc. Natl. Acad. Sci. USA 90, 8566–8570.

Doolittle, R., Feng, D., Tsang, S., Cho, G., Little, E., 1996. Determining divergence times of the major kingdoms of living organisms with a protein clock. Science 271, 470–477.

Dougherty, E., Pollenz, R., 2008. Analysis of AH receptor–ARNT and AH receptor–ARNT2 complexes in vitro and in cell culture. Toxicol. Sci. 103, 191–206.

Drutel, G., Kathmann, M., Heron, A., Schwartz, J., Arrang, J., 1996. Cloning and selective expression in brain and kidney of ARNT2 homologous to the Ah receptor nuclear translocator (ARNT). Biochem. Biophys. Res. Commun. 225, 333–339.

Evans, B.R., Karchner, S.I., Franks, D.G., Hahn, M.E., 2005. Duplicate aryl hydrocarbon receptor repressor genes (AHRR1 and AHRR2) in the zebrafish *Danio rerio*: structure, function, evolution, and AhR-dependent regulation in vivo. Arch. Biochem. Biophys. 441, 151–167.

Evans, B.R., Karchner, S.I., Allan, L.L., Pollenz, R.S., Tanguay, R.L., Jenny, M.J., Sherr, D.H., Hahn, M.E., 2008. Repression of aryl hydrocarbon receptor (AhR) signaling by AhR repressor: role of DNA binding and competition for AhR nuclear translocator. Mol. Pharmacol. 73, 387–398.

Finn, R.D., Tate, J., Mistry, J., Coggill, P.C., Sammut, S.J., Hotz, H.-R., Ceric, G., Forslund, K., Eddy, S.R., Sonnhammer, E.L.L., Bateman, A., 2008. The Pfam protein families database. Nucleic Acids Res. 36, D281–D288.

Fitch, W.M., 1971. Toward defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. 20, 406–416.

Flaveny, Colin, Perdew, Gary H., Miller III, Charles A., 2009. The aryl hydrocarbon receptor does not require the p23 co-chaperone for ligand binding and target gene expression in vivo. Toxicol. Lett. 189, 57–62.

Fujii-Kuriyama, Y., Mimura, J., 2005. Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes. Biochem. Biophys. Res. Commun. 338, 311–317.

Goldstone, H., Stegeman, J., 2006. A revised evolutionary history of the CYP1A subfamily: gene duplication, gene conversion, and positive selection. J. Mol. Evol. 62, 708–717.

Gonzalez, F.J., Kimura, S., 2003. Study of P450 function using gene knockout and transgenic mice. Arch. Biochem. Biophys. 409, 153–158.

Gorman, N., Walton, H.S., Sinclair, J.F., Sinclair, P.R., 1998. CYP1A-catalyzed uroporphyrinogen oxidation in hepatic microsomes from non-mammalian vertebrates (chick and duck embryos, scup and alligator). Comp. Biochem. Physiol. C-Toxicol. Pharmacol. 121, 405–412.

Goryo, K., Suzuki, A., Carpio, C., Siizaki, K., Kuriyama, E., Mikami, Y., Kinoshita, K., Yasumoto, K., Rannug, A., Miyamoto, A., 2007. Identification of amino acid residues in the Ah receptor involved in ligand binding. Biochem. Biophys. Res. Commun. 354, 396–402.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Gupta, R., 1995. Phylogenetic analysis of the 90 kD heat shock family of protein sequences and an examination of the relationship among animals, plants, and fungi species. Mol. Biol. Evol. 12, 1063–1073.

Hahn, M.E., 2002. Aryl hydrocarbon receptors: diversity and evolution. Chem.-Biol. Interact. 141, 131–160.

Hahn, M.E., Karchner, S.I., Shapiro, M.A., Perera, S.A., 1997. Molecular evolution of two vertebrate aryl hydrocarbon (dioxin) receptors (AhR1 and AhR2) and the PAS family. Proc. Natl. Acad. Sci. USA 94, 13743–13748.

Hahn, M., Karchner, S., Franks, D., Merson, R., 2004. Aryl hydrocarbon receptor polymorphisms and dioxin resistance in atlantic killifish (*Fundulus heteroclitus*). Pharmacogenetics 14, 131–143.

Hahn, M.E., Merson, R.R., Karchner, S.I., 2005. Xenobiotic receptors in fish: structural and functional diversity and evolutionary insights. Biochem. Mol. Bio. Fishes 6, 191–228.

Hahn, M.E., Karchner, S.I., Evans, B.R., Franks, D.G., Merson, R.R., Lapseritis, J.M., 2006. Unexpected diversity of aryl hydrocarbon receptors in non-mammalian vertebrates: insights from comparative genomics. J. Exp. Zool. A. Comp. Exp. Biol. 305, 693–706.

Hahn, M., Allan, L., Sherr, D., 2009. Regulation of constitutive and inducible AhR signaling: complex interactions involving the AhR repressor. Biochem. Pharmacol. 77, 485–497.

Hansson, M.C., Wittzell, H., Persson, K., von Schantz, T., 2003. Characterization of two distinct aryl hydrocarbon receptor (AhR2) genes in atlantic salmon (*Salmo salar*) and evidence for multiple AhR2 gene lineages in salmonid fish. Gene 303, 197–206.

Hansson, M.C., Wittzell, H., Persson, K., von Schantz, T., 2004. Unprecedented genomic diversity of AhR1 and AhR2 genes in Atlantic salmon (*Salmo salar*). Aquat. Toxicol. 68, 219–232.

Hoffman, E.C., Reyes, H., Chu, F.F., Sander, F., Conley, L.H., Brooks, B.A., Hankinson, O., 1991. Cloning of a factor required for activity of the Ah (dioxin) receptor. Science 252, 954–958.

Kadesch, T., 1992. Helix-loop-helix proteins in the regulation of immunoglobulin gene transcription. Immunol. Today 13, 31–36.

Karchner, S.I., Powell, W.H., Hahn, M.E., 1999. Identification and functional characterization of two highly divergent aryl hydrocarbon receptors (AhR1 and AhR2) in the teleost *Fundulus heteroclitus* evidence for a novel subfamily of ligand-binding basic helix loop Helix-Per-Arnt-Sim (bHLH-PAS) factors. J. Biol. Chem. 274, 33814–33824.

Karchner, S.I., Franks, D.G., Powell, W.H., Hahn, M.E., 2002. Regulatory interactions among three members of the vertebrate aryl hydrocarbon receptor family: AhR repressor, AhR1, and AhR2. J. Biol. Chem. 277, 6949–6959.

Karchner, S.I., Franks, D.G., Hahn, M.E., 2005. AhR1B, a new functional aryl hydrocarbon receptor in zebrafish: tandem arrangement of AhR1b and AhR2 genes. Biochem. J. 392, 153–161.

Karchner, S.I., Franks, D.G., Kennedy, S.W., Hahn, M.E., 2006. The molecular basis for differential dioxin sensitivity in birds: role of the aryl hydrocarbon receptor. Proc. Natl. Acad. Sci. USA 103, 6252–6257.

Karchner, S.I., Jenny, M.J., Tarrant, A.M., Evans, B.R., Kang, H.J., Bae, I., Sherr, D.H., Hahn, M.E., 2009. The active form of human aryl hydrocarbon receptor (AhR) repressor lacks exon 8, and its Pro(185) and Ala(185) variants repress both AhR and hypoxia-inducible factor. Mol. Cell. Biol. 29, 3465–3477.

Kazlauskas, A., Poellinger, L., Pongratz, I., 1999. Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (aryl hydrocarbon) receptor. J. Biol. Chem. 274, 13519–13524.

Kazlauskas, A., Sundstrom, S., Poellinger, L., Pongratz, I., 2001. The hsp90 chaperone complex regulates intracellular localization of the dioxin receptor. Mol. Cell. Biol. 21, 2594–2607.

Kikuchi, Y., Ohsawa, S., Mimura, J., Ema, M., Takasaki, C., Sogawa, K., Fujii-Kuriyama, Y., 2003. Heterodimers of bHLH-PAS protein fragments derived from AhR, AhRR, and ARNT prepared by co-expression in *Escherichia coli*: characterization of their DNA binding activity and preparation of a DNA complex. J. Biochem. 134, 83–90.

Kim, E., Iwata, H., Suda, T., Tanabe, S., Amano, M., Miyazaki, N., Petrov, E., 2005. Aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT) expression in Baikal seal (*Pusa sibirica*) and association with 2,3,7,8-TCDD toxic equivalents and CYP1 expression levels. Comp. Biochem. Physiol. C-Toxicol. Pharmacol. 141, 281–291.

Kimura, S., Gonzalez, F.J., Nebert, D.W., 1984. The murine Ah locus. comparison of the complete cytochrome P1-450 and P3-450 cDNA nucleotide and amino acid sequences. J. Biol. Chem. 259, 10705–10713.

Krone, P.H., Sass, J.B., 1994. HSP 90 alpha and HSP 90 beta genes are present in the zebrafish and are differentially regulated in developing embryos. Biochem. Biophys. Res. Commun. 204, 746–752.

Lavine, J., Rowatt, A., Klimova, T., Whitington, A., Dengler, E., Beck, C., Powell, W., 2005. Aryl hydrocarbon receptors in the frog *Xenopus laevis*: two AhR1 paralogs exhibit low affinity for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicol. Sci. 88, 60–72.

Lee, J., Kim, E., Iwata, H., 2009. Dioxin activation of CYP1A5 promoter/enhancer regions from two avian species, common cormorant (*Phalacrocorax carbo*) and chicken (*Gallus gallus*): association with aryl hydrocarbon receptor 1 and 2 isoforms. Toxicol. Appl. Pharmacol. 234, 1–13.

Lewis, N., Williams, T.D., Chipman, K., 2004. Functional analysis of xenobiotic response elements (XREs) in CYP 1A of the european flounder (*Platichthys flesus*). Mar. Environ. Res. 58, 101–105.

Ma, Q., 2001. Induction of CYP1A1. The AhR/DRE paradigm: transcription, receptor regulation, and expanding biological roles. Curr. Drug Metab. 2, 149–164.

Mahata, S.C., Mitsuo, R., Aoki, J.Y., Kato, H., Itakura, T., 2003. Two forms of cytochrome P450 cDNA from 3-methylcholanthrene-treated European eel *anguilla anguilla*. Fish Sci. 69, 615–624.

Mimura, J., Fujii-Kuriyama, Y., 2003. Functional role of AhR in the expression of toxic effects by TCDD. BBA 1619, 263–268.

Mimura, J., Ema, M., Sogawa, K., Fujii-Kuriyama, Y., 1999. Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. Genes Dev. 13, 20–25.

Monostory, K., Pascussi, J.M., 2008. Regulation of drug-metabolizing human cytochrome P450s. Acta Chim. Slov. 55, 20–37.

Morrison, H.G., Oleksiak, M.F., Cornell, N.W., Sogin, M.L., Stegeman, J.J., 1995. Identification of cytochrome P-450 1A (CYP1A) genes from two teleost fish, toadfish (*Opsanus tau*) and scup (*Stenotomus chrysops*), and phylogenetic analysis of CYP1A genes. Biochem. J. 308, 97–104.

Morrison, H.G., Weil, E.J., Karchner, S.I., Sogin, M.L., Stegeman, J.J., 1998. Molecular cloning of CYP1A from the estuarine fish *Fundulus heteroclitus* and phylogenetic analysis of CYP1 genes: update with new sequences. Comp. Biochem. Physiol. C-Toxicol. Pharmacol. 121, 231–240.

Murre, C., McCaw, P.S., Baltimore, D., 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. Cell 56, 777–783.

Necela, B., Pollenz, R., 1999. Functional analysis of activation and repression domains of the rainbow trout aryl hydrocarbon receptor nuclear translocator (rtARNT) protein isoforms. Biochem. Pharmacol. 57, 1177–1190.

Nei, M., Gojobori, T., 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. 3, 418–426.

Norstrom, R.J., Simon, M., Muir, D.C.G., Schweinsburg, R.E., 1988. Organochlorine contaminants in arctic marine food chains: identification, geographical distribution and temporal trends in polar bears. Environ. Sci. Technol. 22, 1063–1071.

Nukaya, M., Moran, S., Bradfield, C., 2009. The role of the dioxin-responsive element cluster between the CYP1A1 and CYP1A2 loci in aryl hydrocarbon receptor biology. Proc. Natl. Acad. Sci. USA 106, 4923–4928.

Okey, A.B., 2005. Toxicological implications of polymorphisms in receptors for xenobiotic chemicals: the case of the aryl hydrocarbon receptor. Toxicol. Appl. Pharmacol. 207, 43–51.

Olson, E.N., Klein, W.H., 1994. bHLH factors in muscle development: dead lines and commitments, what to leave in and what to leave out. Genes Dev. 8, 1–8.

Padmini, E., Usha Rani, M., 2009. Seasonal influence on heat shock protein 90[alpha] and heat shock factor 1 expression during oxidative stress in fish hepatocytes from polluted estuary. J. Exp. Mar. Biol. Ecol. 372, 1–8.

Palmisano, A.N., Winton, J.R., Dickhoff, W.W., 1999. Sequence features and phylogenetic analysis of the stress protein Hsp90[alpha] in chinook salmon (*Oncorhynchus tshawytscha*), a poikilothermic vertebrate. Biochem. Biophys. Res. Commun. 258, 784–791.

Partch, C., Card, P., Amezcua, C., Gardner, K., 2009. Molecular basis of coiled coil coactivator recruitment by the aryl hydrocarbon receptor nuclear translocator (ARNT). J. Biol. Chem. 284, 15184–15192.

Perdew, G.H., 1988. Association of the Ah receptor with the 90-kDa heat shock protein. J. Biol. Chem. 263, 13802–13805.

Poland, A., Knutson, J.C., 1982. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. 22, 517–554.

Pollenz, R., Sullivan, H., Holmes, J., Necela, B., Peterson, R., 1996. Isolation and expression of cDNAs from rainbow trout (*Oncorhynchus mykiss*) that encode two novel basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) proteins with distinct functions in the presence of the aryl hydrocarbon receptor. Evidence for alternative mRNA splicing and dominant negative activity in the bHLH/PAS family. J. Biol. Chem. 271, 30886–30896.

Pongratz, I., Antonsson, C., Whitelaw, M.L., Poellinger, L., 1998. Role of the PAS domain in regulation of dimerization and DNA binding specificity of the dioxin receptor. Mol. Cell. Biol. 18, 4079–4088.

Posada, D., Buckley, T., 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.

Posada, D., Crandall, K., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Powell, W.H., Hahn, M.E., 2000. The evolution of aryl hydrocarbon signaling proteins: diversity of ARNT isoforms among fish species. Mar. Environ. Res. 50, 39–44.

Powell, W.H., Karchner, S.I., Bright, R., Hahn, M.E., 1999. Functional diversity of vertebrate ARNT proteins: identification of ARNT2 as the predominant form of ARNT in the marine teleost, *Fundulus heteroclitus*. Arch. Biochem. Biophys. 361, 156–163.

Powell, W., Morrison, H., Weil, E., Karchner, S., Sogin, M., Stegeman, J., Hahn, M., 2004. Cloning and analysis of the CYP1A promoter from the atlantic killifish (*Fundulus heteroclitus*). Mar. Environ. Res. 58, 119–124.

Prasch, A.L., Heideman, W., Peterson, R.E., 2004. ARNT2 is not required for TCDD developmental toxicity in zebrafish. Toxicol. Sci. 82, 250–258.

Prasch, A., Tanguay, R., Mehta, V., Heideman, W., Peterson, R., 2006. Identification of zebrafish ARNT1 homologs: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity in the developing zebrafish requires ARNT1. Mol. Pharmacol. 69, 776–787.

Quattrochi, L.C., Okino, S.T., Pendurthi, U.R., Tukey, R.H., 1985. Cloning and isolation of human cytochrome P-450 cDNAs homologous to dioxin-inducible rabbit mRNAs encoding P-450-4 and P-450-6. DNA 4, 395–400.

Rabergh, C.M.I., Vrolijk, N.H., Lipsky, M.M., Chen, T.T., 2000. Differential expression of two CYP1A genes in rainbow trout (*Oncorhynchys mykiss*). Toxicol. Appl. Pharmacol. 165, 195–205.

Rowatt, Ashley J., Depowell, John J., Powell, Wade H., 2003. ARNT gene multiplicity in amphibians: characterization of ARNT2 from the frog *xenopus laevis*. J. Exp. Zool. Part B. 300B, 48–57.

Roy, N., Courtenay, S., Chambers, R., Wirgin, I., 2006. Characterization of the aryl hydrocarbon receptor repressor and a comparison of its expression in Atlantic tomcod from resistant and sensitive populations. Environ. Toxicol. Chem. 25, 560–571.

Rutherford, S., Knapp, J., Csermely, P., 2007. Hsp90 and developmental networks. In: Csermely, Peter, Vígh, László (Eds.), Molecular Aspects of the Stress Response: Chaperones, Membranes and Networks. Springer, New York, USA, pp. 190–197.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Schmidt, J.V., Bradfield, C.A., 1996. Ah receptor signaling pathways. Annu. Rev. Cell. Dev. Biol. 12, 55–89.

Sojka, K., Pollenz, R., 2001. Expression of aryl hydrocarbon receptor nuclear translocator (ARNT) isoforms in juvenile and adult rainbow trout tissues. Mar. Biotechnol. 3, 416–427.

Soshilov, A., Pandini, A., Bonati, L., Denison, M.S., 2006. Characterization of hsp90-binding to the PASB domain of the Ah receptor. FASEB J. 20, A963.

Swain, W.R., 1988. Human health consequences of consumption of fish contaminated with organochlorine compounds. Aquat. Toxicol. 11, 357–377.

Swanson, H.I., 2002. DNA binding and protein interactions of the AhR/ARNT heterodimer that facilitate gene activation. Chem.-Biol. Interact. 141, 63–76.

Takahashi, J.S., 1992. Circadian clock genes are ticking. Science 258, 238–240.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. Mega4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Tang, C., Lin, J.H., Lu, A.Y.H., 2005. Metabolism-based drug-drug interactions: What determines individual variability in cytochrome p450 induction? Drug Metab. Dispos. 33, 603–613.

Tanguay, R., Andreasen, E., Heideman, W., Peterson, R., 2000. Identification and expression of alternatively spliced aryl hydrocarbon nuclear translocator 2 (ARNT2) cDNAs from zebrafish with distinct functions. BBA 94, 117–128.

Taylor, B.L., Zhulin, I.B., 1999. PAS domains: internal sensors of oxygen, redox potential, and light. Microbiol. Mol. Biol. Rev. 63, 479–506.

Teraoka, H., Dong, W., Tsujimoto, Y., Iwasa, H., Endoh, D., Ueno, N., Stegeman, J.J., Peterson, R.E., Hiraga, T., 2003. Induction of cytochrome P450 1A is required for circulation failure and edema by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in zebrafish. Biochem. Biophys. Res. Commun. 304, 223–228.

Thompson, J., Gibson, T., Plewniak, F., Jeanmougin, F., Higgins, D., 1997. The Clustal-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Veldhoen, M., Hirota, K., Westendorf, A.M., Buer, J., Dumoutier, L., Renauld, J.-C., Stockinger, B., 2008. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453, 106–109.

Walisser, J.A., Bunger, M.K., Glover, E., Harstad, E.B., Bradfield, C.A., 2004. Patent ductus venosus and dioxin resistance in mice harboring a hypomorphic ARNT allele. J. Biol. Chem. 279, 16326–16331.

Waxman, D.J., 1999. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. Arch. Biochem. Biophys. 369, 11–23.

Werck-Reichhart, D., Feyereisen, R., 2000. Cytochromes P450: a success story. Genome Biol. 1, 1–9.

Whitlock, J.P., 1999. Induction of cytochrome P4501A1. Annu. Rev. Pharmacol. Toxicol. 39, 103–125.

Wiseman, S.B., Vijayan, M.M., 2007. Aryl hydrocarbon receptor signaling in rainbow trout hepatocytes: role of hsp90 and the proteasome. Comp. Biochem. Physiol. C-Toxicol. Pharmacol. 146, 484–491.

Yasui, T., Kim, E., Iwata, H., Franks, D., Karchner, S., Hahn, M., Tanabe, S., 2007. Functional characterization and evolutionary history of two aryl hydrocarbon receptor isoforms (AhR1 and AhR2) from avian species. Toxicol. Sci. 99, 101–117.

Zelzer, E., Wappner, P., Shilo, B.Z., 1997. The PAS domain confers target gene specificity of drosophila bHLH/PAS proteins. Genes Dev. 11, 2079–2089.

ZeRuth, G., Pollenz, R., 2007. Functional analysis of cis-regulatory regions within the dioxin-inducible CYP1A promoter/enhancer region from zebrafish (*Danio rerio*). Chem.-Biol. Interact. 170, 100–113.