Molecular phylogenies and evolutionary behavior of AhR (aryl hydrocarbon receptor) pathway genes in aquatic animals

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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 major phylogenetic groups of mollusc, amphibian, fish and aquatic mammal. 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 nonsynonymous nucleotide substitutions per nonsynonymous 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 by gene duplication events. The d_N values indicate that AhR pathway genes are well conserved in aquatic animals, except for CYP1A gene. Furthermore, the d_N value indicates that AhR pathway genes are less conserved in fish than in any other aquatic animals, and they likely go through an adaptive evolution within aquatic animals.

Key words

1 Introduction

Persistent organic pollutants (POPs) are organic compounds of natural or anthropogenic origin 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 groups：(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 enter aquatic ecosystems through effluent, atmospheric deposition, runoff, and groundwater and have become ubiquitous in the biosphere (Swain 1988). Now, they have seriously threatened the health of aquatic animals, even the health of human, so this problem has caught the worldwide attention.

A series of studies have revealed that AhR pathway play a pivotal role in the mediation process of 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 other POPs. In the regulation pathway of AhR, the main target molecular is CYP1 and CYP2. Cytochrome P450 1A1 (CYP1A1) is one of the xenobiotic metabolizing enzymes (XMEs), which is induced by TCDD and PAHs etc. A large body of literature has revealed the mechanisms 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 1997). Nevertheless, when there is a ligand, then the liganded AhR 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 genes including CYP1A1, cell cycle regulation gene (p27) and others to enhance their expression. AhR needs ligand for nuclear translocation and heterodimerization with ARNT (Mimura et al. 1999a), and Mimura and Fujii-Kuriyama (2003a) find 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 essential 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 differences in the spectrum of toxicity observed, for example, the LD_{50} for acute TCDD exposure varies from 1μg/kg in the guinea pig , 20

to 40μg/kg in the rat, 70μg/kg in the monkey, 114μg/kg in the mouse and rabbit, and 5000μg/kg in the hamster (Poland and Knutson 1982a). In addition, the diversity of the AhR pathway genes in different aquatic animals 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 genes, the gene's diversity and the patterns of selection, and infers the evolutionary behavior of AhR pathway genes and the protein constitute the pathway in the major aquatic animals. 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 animals, and understanding the functional evolution of these genes is essential to predict and interpret species differences 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 pathway (Detailed 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 the supplementary table 1). These major steps are formation of cytosolic complex, translocation of AhR, dimerization of AhR and induction of Cyp1A. The species and strains, taxonomy, abbreviations 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 (version 1.81, 2000; 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 sets using Gblocks 0.91b (Castresana 2000) with default parameters, and the filtered sequences were

concatenated. In addition, all trees were rooted with 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 parameters 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 were 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 nonsynonymous nucleotide substitutions within and between the major aquatic animal groups.

The frequencies of synonymous nucleotide substitutions per synonymous site (silent; d_S) and nonsynonymous nucleotide substitutions per nonsynonymous site (amino acid-changing; d_N) (Table 2 and Table 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://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 obtained from NJ, MP and ML algorithms were found to be highly congruent. Therefore, in this article, we just discussed the results based on the NJ tree analysis.

3.1 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), the 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 loss of HSP90 most likely results in an improperly folded or destabilized receptor protein HSP90. One subunit 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).

HSP90 protein is well conserved within aquatic animals. The phylogenetic tree (Fig. 2 A) demonstrates the close evolutionary relationship of HSP90 gene 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. Within the fish clade, there are two types of Hsp90 genes, namely Hsp90a and Hsp90b, which encode two similar cytosolic isoforms, since it has been proposed that HSP90α and HSP90β evolved by duplication of a common ancestral gene more than 500 million years ago, close to the time of emergence of vertebrates (Krone and Sass 1994; Moore et al. 1987). Despite the

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).

For example, the zebrafish HSP90α gene is strongly expressed following heat shock,

whereas the HSP90β is only weakly upregulated 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). 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

type of chinook salmon should belong to the type of HSP90a, which received support

from Palmisano, Winton et al (1999). However, there is an exception that the HSP90a

gene types of bastard halibut maybe belong to one pair of alleles of HSP90 gene.

　　All the HSP90 proteins show two well conserved domains, namely HATPase_c

and HSP90. 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). Further more, HSP90 and

the proteasome are playing a pivotal role in modulating AhR signaling and Cyp1A

responses(?) in trout hepatocytes (Wiseman and Vijayan 2007). The HSP90 genes of

invertebrates (clade A) were observed to be less conserved than those of vertebrates

(clades A, B, C, D, E, F, 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 (Table 2 and 3), which indicates a functional constraint on this gene product. In addition, Rutherford et al. (2007) found that Hsp90 as a homodimer, which associates with co-chaperones 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 other POPs), the AhR complex translocates into the nucleus and the AhR dissociates from HSP90 complex to form a heterodimer with its partner molecule, ARNT (Poland and Knutson 1982b). 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, and PAHs etc (Ma 2001).

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. 1999b). These results indicate that AhR and AHRR form a regulatory feedback loop (Mimura and Fujii-Kuriyama 2003a). Recently, Evans et al. (2008) propose a mechanism of AHRR action involving "transrepression" of AhR signalling through protein-protein interactions rather than through 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.

The phylogenetic tree (as seen in Fig. 2B) of AhR gene 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 result of Hahn et

al. (1997). However, marine and terrestrial mammals just have a single AhR gene

which belongs to the AhR1 lineage (Karchner et al. 1999), X. laevis AhR1a and

AhR1b are somewhat reminiscent of AhR2a and AhR2b, and closely related to AhR

paralogs in rainbow trout (*Oncorhyncus mykiss*) (Abnet et al. 1999). 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. In addition, AhR2 genes from fish

form a separate clade. Similarly, AhR1 genes from fish form another separate clade.

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 zebrafish and other

animal AHRRs, distinct from the AhR. Zebrafish possess AHRR1 and AHRR2 that

are likely the result of the fish-specific whole genome duplication (Evans et al. 2005).

Phylogenetic analysis of AhRs from zebrafish, Fugu, and killifish along with

mammalian AhRs suggests that the differences in AhR diversity between mammals and fish are the result of gene and genome duplications coupled with lineage specific gene loss (Hahn 2002; Karchner et al. 2005). Salmonids, which have undergone an 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).

All the AhRs contain HLH (helix-loop-helix), PAS (Per-ARNT-Sim) and PAS_3 three well-conserved domains (see supplementary table 3) involved 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) (Kadesch 1992; Murre et al. 1989; 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) just has HLH and PAS two conserved domains.

Consistent with the hypothesis that AhR is an ancient protein, which is well conserved in vertebrates and invertebrates, indicating its pivotal function throughout evolution (Karchner et al. 2002), the present study also reveals that AhR2 sequences are less conserved in fish (d_N values for clade C is 0.118 ± 0.012) compared to others AhR genes (d_N values for clade A, B, D and E clade are 0.026 ± 0.008, 0.056 ± 0.008,

0.028 ±0.008 and 0.026± 0.006, respectively). All the values(?) of $d_N/d_S < 1$, are calculated using the d_N and d_S values (Table 2 and 3), which indicates a functional constraint on this gene products too. Although in mammals the single AhR (AhR1 ortholog) is required for TCDD 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.

3.3 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 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). 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 may serve as a central player in regulating these diverse signaling pathways (Mimura and Fujii-Kuriyama 2003b; Swanson 2002; Taylor and Zhulin 1999).

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. Surprisingly, we can find that killifish ARNT2 resembles both

mammalian ARNT forms more closely than rainbow trout ARNTb. Thus, there may

be regulatory and/or functional diversity of ARNT among vertebrate taxa. Differences

that affect aryl hydrocarbon signal transduction, which could include the spatial and

temporal expression of multiple ARNT isoforms, may contribute to interspecific

variations in sensitivity to dioxin-like compounds (Powell et al. 1999). The two

rainbow trout ARNT proteins can result from alternative splicing of the transcript

from a single gene (Powell and Hahn 2000).

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 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. The nonsynonymous

nucleotide substitution values suggest that these gene sequences are equally conserved

in all the groups of aquatic animals (d_N values for clades A, B, C , D, E, F, and 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, maybe because daphnia ARNT1 and ARNT2 just belong to one pair of

alleles. However, the gene sequences from clade B show a higher d_N value of 0.171 ±

0.013, indicating the unconserved nature. The value of $d_N/d_S<1$, calculated using the

d_N and d_S values (Tables 2 and 3), indicates a functional constraint on this protein.

Indeed, Powell et al. (2000) have suggested that the high degree of sequence identity of the different isoforms 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). Additionally, low levels of ARNT could decrease the sensitivity of a particular tissue to agonist despite high AhR levels (Schmidt and Bradfield 1996). So, we can find that there is specificity interaction between ARNT and AhR isoforms.

3.4 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. 2006; Hahn et al. 2005). The best characterized targets of the AhR pathway are Cytochrome P4501A (CYP1A) genes, which are strongly induced (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). And they play a central 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), respectively (Waxman 1999).

The phylogeny tree (Fig.2D) for the CYP1A shows three distinct clades (clade A, CYP1A1 genes from fish; clade B, CYP1A1 genes from mammalian; and clade C, CYP1A2 genes from mammalian). The CYP1A subfamily appears to have originated early in the vertebrate lineage. Fish generally possess a single CYP1A gene (Morrison et al. 1995; Morrison et al. 1998); Rainbow trout and salmonids are notable exceptions (Mahata et al. 2003; Rabergh et al. 2000). 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 functions characteristic of the mammalian isoforms (Gorman et al. 1998). However, fish CYP1As are considered more CYP1A1-like on the basis of slightly higher levels of pairwise sequence identity and similarities in patterns 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. The activity of these response elements enhances the evidence for considerable diversity in vertebrate CYP1A regulation (Lewis et al. 2004). All the CYP1A (see supplementary table 5) show the p450 well conserved domain. CYP1A genes from fish are observed to be less conserved (d_N for clade A= 0.246 ± 0.017) than the CYP1A genes from mammalian

(d_N for clade B= 0.125 ± 0.015, C= 0.158 ± 0.015, respectively). Surprisingly this

gene is not like other genes in the AhR pathway, the value of d_N / d_S **> 1**, calculated

using the dN and dS values (Tables 2 and 3), consistent with the results of Goldstone

and Stegeman (2006), suggesting that gene conversion and positive selection maybe

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; gene and genome duplication,

gene amplification and conversion, gene structure rearrangements, gene loss,

horizontal gene transfer, and convergent evolution have all been implicated in CYP1A

evolution (Werck-Reichhart and Feyereisen 2000). So CYP1A gene maybe play an

more important role for predicting and interpreting species differences in sensitivity to

toxicity caused by POPs.

4 Conclusions

The phylogenetic analysis suggests that the gene duplication has contributed substantially to the distribution of genes for AhR pathway across aquatic animals genomes. This study also indicates that the AhR pathway genes value of $d_N/d_S<1$, indicates a functional constraint on these genes product. 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 differences in sensitivity to toxicity caused by POPs. In addition, the non-synonymous nucleotide substitution (d_N) values indicate that AhR pathway genes are less conserved in fish than in any other animals relatively, and fish posses 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 through an adaptive evolution within aquatic animals.

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 differences in aryl hydrocarbon toxicity, possibly through distinct interactions with additional PAS-family proteins. Veldhoen et al. (2008) results show that AhR gene involves the autoimmune, so it may help fishes 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 offer some fresh ideas for further study of

the mechanism of POPs toxicology.

Supplementary material

Supplementary materials are available online.

Acknowledgements

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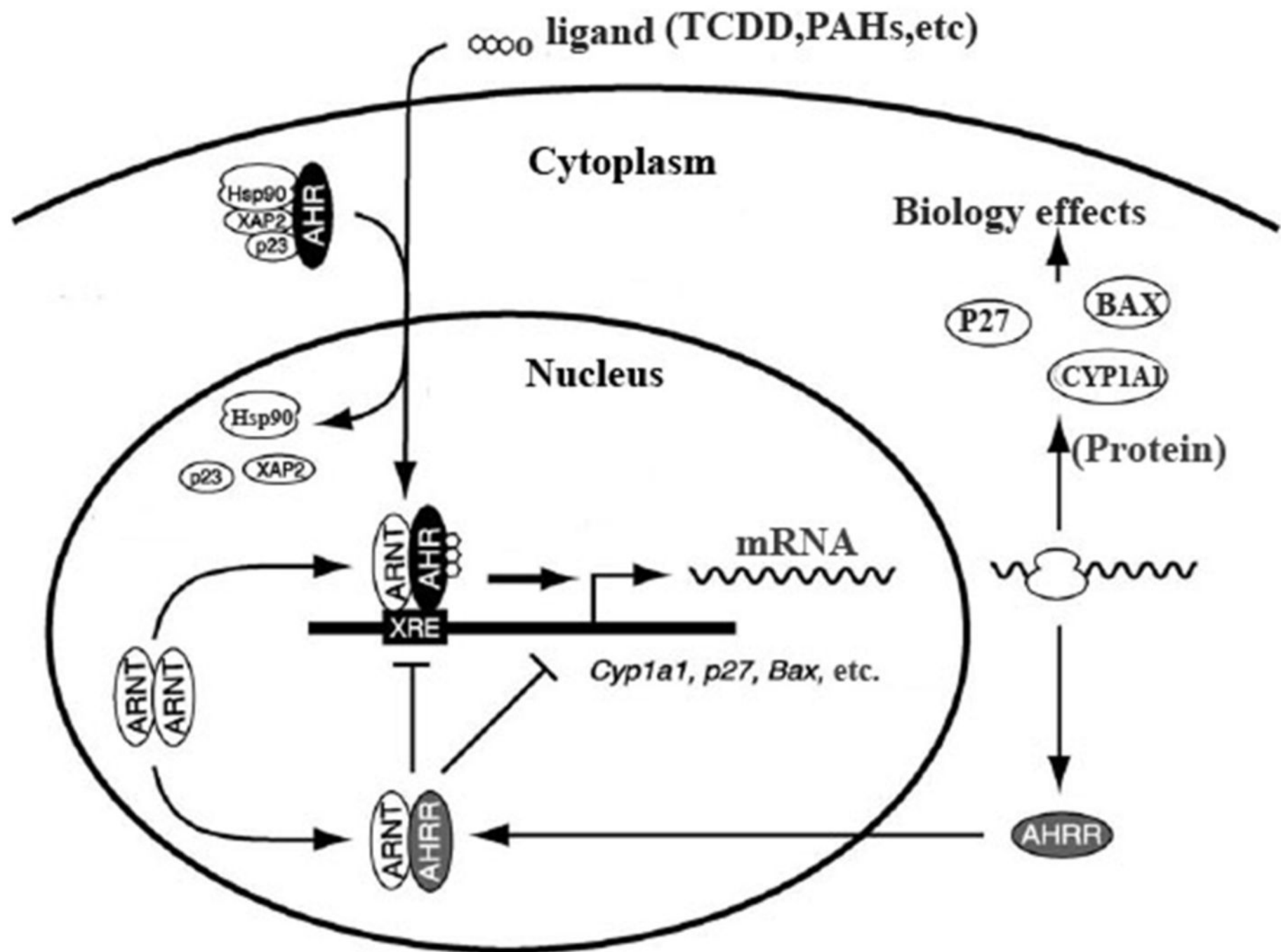
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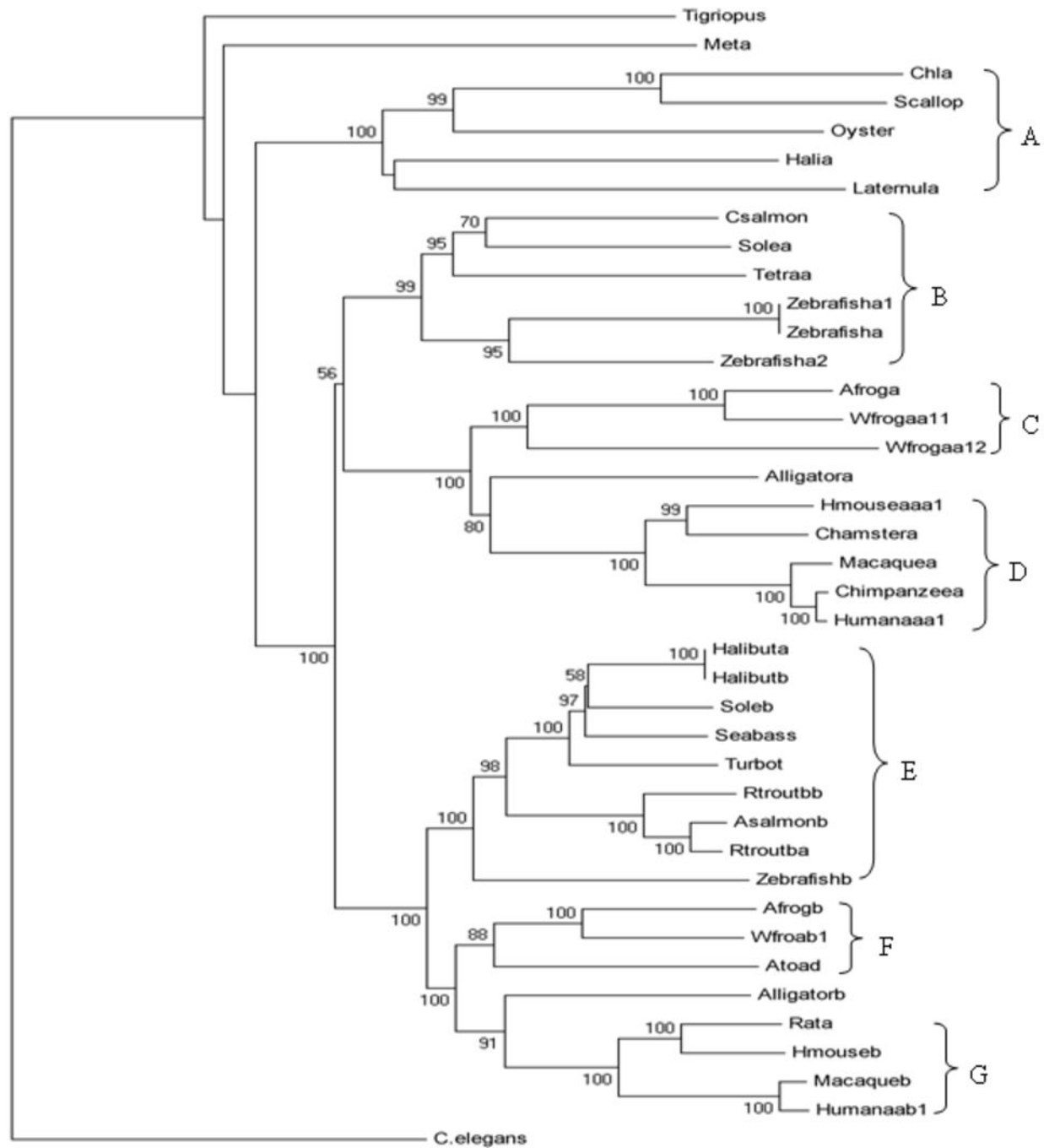TH17-cell-mediated autoimmunity to environmental toxins. Nature 453, 106-109.

FIGURE LEGENDS

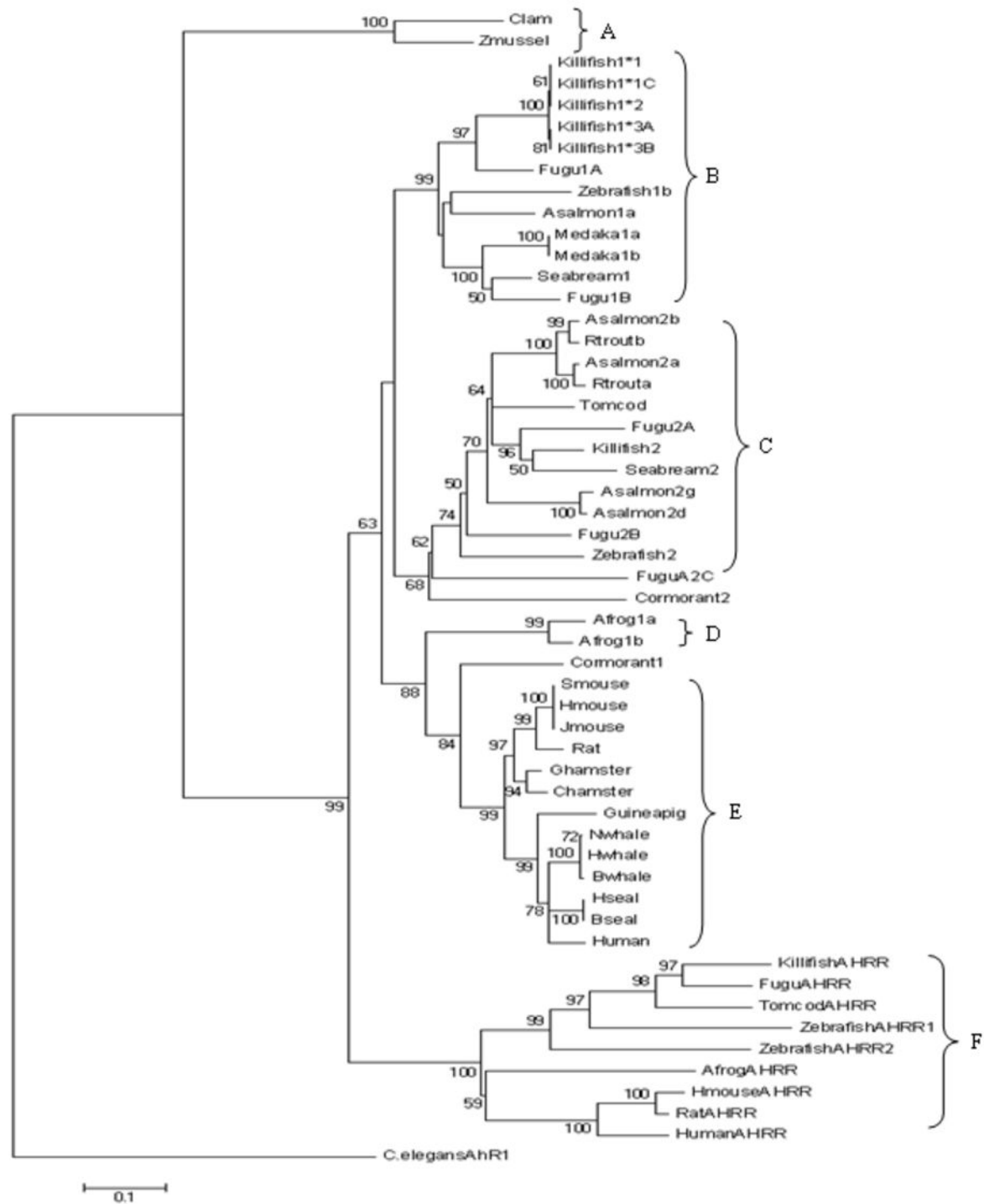Fig. 1. Regulation Mechanism of AhR pathway

Fig. 2. NJ phylogenetic trees from AhR pathway genes (protein coding DNA

sequences) from diverse aquatic animals. Genes involved in (A) formation of

cytosolic complex, (B) translocation of AhR, (C) dimerization of AhR, (D) induction

of CYP1A. Bootstrap values <50% are not shown. Tree A is based on HSP90, B on

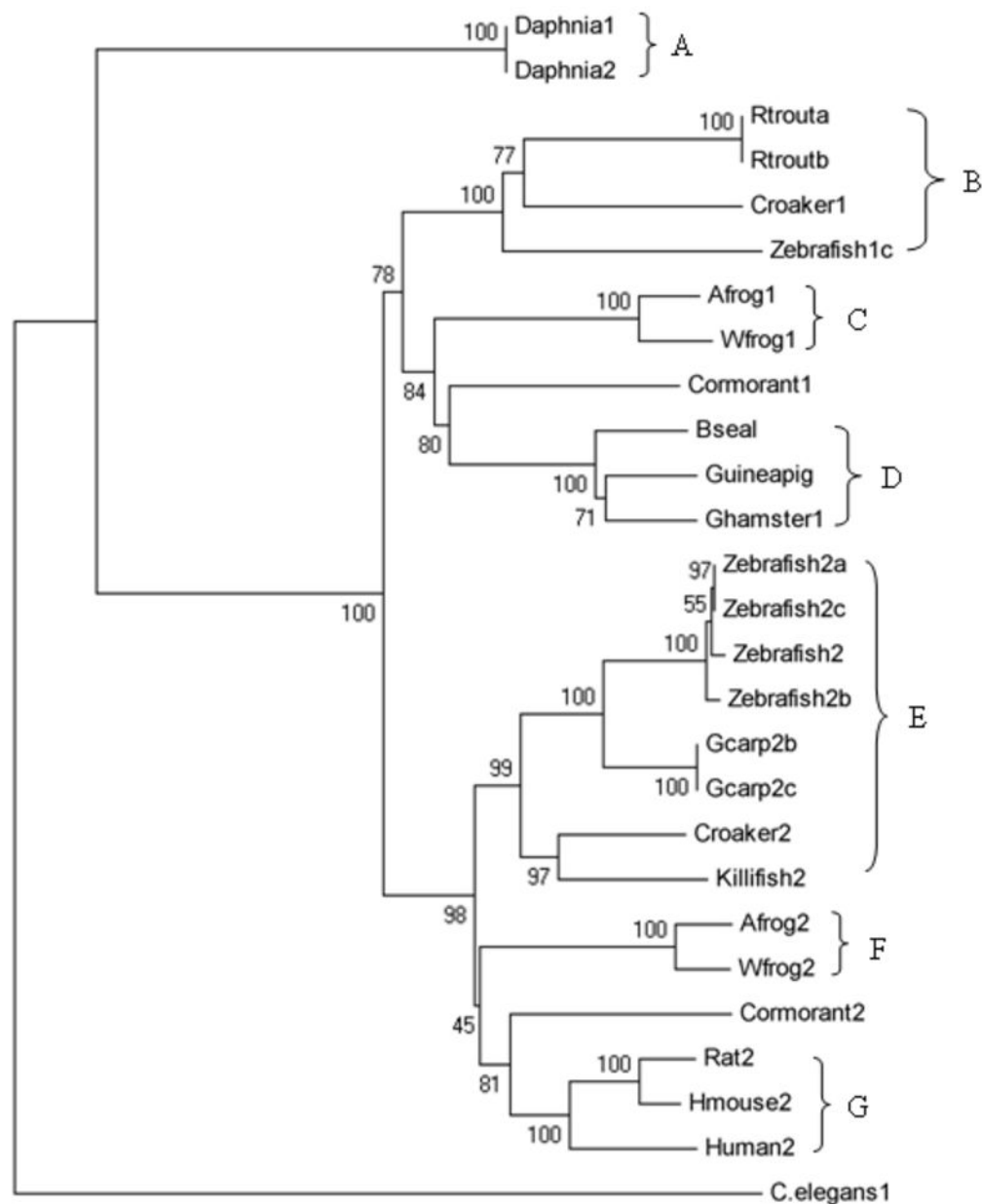AhR and AHRR, C on ARNT and D on CYP1A.

Hsp90

AHR+
AHRR

ARNT

CYP1A