Cytochrome P450 1 Genes in Birds: Evolutionary Relationships and Transcription Profiles in Chicken and Japanese Quail Embryos

Maria E. Jönsson¹,², Bruce R. Woodin², John J. Stegeman², Björn Brunström¹

¹Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden, ²Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, United States of America

Abstract

Background: Cytochrome P450 1 (CYP1) genes are biomarkers for aryl hydrocarbon receptor (AHR) agonists and may be involved in some of their toxic effects. CYP1s other than the CYP1As are poorly studied in birds. Here we characterize avian CYP1B and CYP1C genes and the expression of the identified CYP1 genes and AHR1, comparing basal and induced levels in chicken and quail embryos.

Methodology/Principal Findings: We cloned cDNAs of chicken CYP1C1 and quail CYP1B1 and AHR1. CYP1Cs occur in several bird genomes, but we found no CYP1C gene in quail. The CYP1C genomic region is highly conserved among vertebrates. This region also shares some synteny with the CYP1B region, consistent with CYP1B and CYP1C genes deriving from duplication of a common ancestor gene. Real-time RT-PCR analyses revealed similar tissue distribution patterns for CYP1A4, CYP1A5, CYP1B1, and AHR1 mRNA in chicken and quail embryos, with the highest basal expression of the CYP1As in liver, and of CYP1B1 in eye, brain, and heart. Chicken CYP1C1 mRNA levels were appreciable in eye and heart but relatively low in other organs. Basal transcript levels of the CYP1As were higher in quail than in chicken, while CYP1B1 levels were similar in the two species. 3,3',4,5,5'-Pentachlorobiphenyl induced all CYP1s in chicken; in quail a 1000-fold higher dose induced the CYP1As, but not CYP1B1.

Conclusions/Significance: The apparent absence of CYP1C1 in quail, and weak expression and induction of CYP1C1 in chicken suggest that CYP1Cs have diminishing roles in tetrapods; similar tissue expression suggests that such roles may be met by CYP1B. Tissue distribution of CYP1B and CYP1C transcripts in birds resembles that previously found in zebrafish, suggesting that these genes serve similar functions in diverse vertebrates. Determining CYP1 catalytic functions in different species should indicate the evolving roles of these duplicated genes in physiological and toxicological processes.

Introduction

Members of the cytochrome P450 (CYP) superfamily of enzymes are present in most organisms, including bacteria, archaea, plants, fungi, and animals. They catalyze oxidative metabolism of various endogenous and exogenous compounds. Endogenous substrates include eicosanoids, cholesterol, bile acids, steroids, biogenic amines, vitamin D3, and retinoids [1,2]. Enzymes in the CYP1, CYP2, CYP3, and CYP4 families also metabolize exogenous compounds, such as plant or fungal secondary metabolites, environmental pollutants, and pharmaceuticals [3,4]. The CYP1 enzymes have been studied extensively because they can generate reactive and sometimes carcinogenic metabolites from environmental pollutants (e.g., polycyclic aromatic hydrocarbons, PAHs), but the interest in their endogenous functions is growing [e.g., [5]].

Genes in four CYP1 subfamilies - CYP1A, CYP1B, CYP1C, and CYP1D - are expressed in fish and amphibians, while mammalian species express CYP1A, CYP1B, and in some cases CYP1D genes [6,7,8,9,10,11,12]. In fish and the frog Xenopus tropicalis, expression of CYP1A, CYP1B and CYP1C genes is induced by exposure to agonists of the aryl hydrocarbon receptor (AHR), among the most potent being 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3,3',4,4',5-pentachlorobiphenyl (PCB126); CYP1D genes do not seem to be inducible by AHR agonists [6,7,10,13].

Avian species vary substantially in sensitivity to embryo toxicity of halogenated aromatic hydrocarbons that activate the AHR [14,15]. Chicken embryos are particularly sensitive to these compounds and the effects of exposure in ovo include reduced hatchability, developmental abnormalities, and induction of CYP1A-catalyzed enzyme activity [16,17,18]. Japanese quail embryos are considerably less sensitive than chicken embryos to TCDD and PCB126, both in terms of embryo toxicity and ethoxysresorufin O-deethylase (EROD) induction [19,20,21]. The difference in sensitivity has been attributed to variations in a few amino acid residues in the AHR [15,22].

Citation: Jönsson ME, Woodin BR, Stegeman JJ, Brunström B (2011) Cytochrome P450 1 Genes in Birds: Evolutionary Relationships and Transcription Profiles in Chicken and Japanese Quail Embryos. PLoS ONE 6(12): e28257. doi:10.1371/journal.pone.0028257

Editor: John A. Craft, Glasgow Caledonian University, United Kingdom

Received October 12, 2011; Accepted November 4, 2011; Published December 2, 2011

Copyright: © 2011 Jönsson et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Funding to MEJ and BB was from the Carl Tryggers Stiftelse and The Swedish Research Council Formas. Funding for BRW and JJS was from the United States National Institutes of Health (National Institute of Environmental Health Sciences), grants R01ES015912 and P42ES007381 to JJS. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: maria.jonsson@ebc.uu.se

Published October 12, 2011; Accepted November 4, 2011; Published December 2, 2011
Birds have two CYP1A genes, CYP1A4 and CYP1A5, which are orthologous to mammalian CYP1A1 and CYP1A2 [23] and which are inducible by AHR agonists [9,24]. At least some bird species also express CYP1B1; the constitutive localization of CYP1B1 mRNA has been determined in embryonic chicken (Gallus gallus) and quail (Coturnix coturnix japonica) [8], but the inducibility of CYP1B1 in birds has not been reported. A CYP1C gene was identified recently in the chicken genome [25], and CYP1C mRNA has been determined in embryonic chicken (Gallus gallus) and the mallard duck (Anas platyrhynchos) genomes. Expression of bird CYP1Cs at the transcript or protein level has not been studied at all.

The objectives of this work were to define some features of CYP1 genes in birds, particularly the CYP1B and CYP1C genes. We cloned cDNAs of quail CYP1B1, chicken CYP1C1, and quail AHR1, and determined basal mRNA expression profiles of the full complement of CYP1s and AHR1 in chicken and quail embryos. Induction of CYP1s was studied in early embryos and yolk sac membranes after in ovo exposure to PCB126. We also compared synteny around CYP1B and CYP1C genes in birds to those in other vertebrate species. The results indicate remarkable conservation of some features of CYP1 genes among vertebrates, although differences were also found among birds, and between birds and other vertebrates.

Results

Cloning and sequence comparisons

Using primers targeting the predicted chicken CYP1C1 [25] we cloned and determined the sequence of a cDNA covering the full coding region (1637 bp) of the transcript (GenBank: JN656933). The cloned chicken CYP1C1 nucleotide and deduced amino acid sequences showed 99.6% and 99.0% sequence identity to the predicted transcript and protein, respectively. Figure 1 shows the deduced amino acid sequence of chicken CYP1C1 aligned with X. tropicalis CYP1C1, and zebrafish (Danio rerio) CYP1C1 and CYP1C2 [7]. CYP1C-like sequences found in the genomes of the turkey, mallard duck, and anole lizard (Anolis carolinensis) are also included in Fig. 1. Chicken CYP1C1 showed 93% and 87% amino acid sequence identity with the corresponding regions of the turkey and mallard CYP1Cs, and the identity was higher in the substrate recognition site (SRS) regions (94% and 93% for turkey and mallard CYP1C). Compared with X. tropicalis CYP1C1, chicken CYP1C1 exhibited 57% and 68% sequence identity in the full length protein and SRS regions, respectively. The anole and chicken CYP1Cs showed 54% and 69% sequence identity in the full length protein and SRS regions, respectively. Zebrafish CYP1C1 and CYP1C2 showed only 51% and 47% identity with chicken CYP1C1 in the full protein whereas slightly higher identities were observed in the SRS regions (54% for CYP1C1 and 49% for CYP1C2).

Extensive cloning efforts did not uncover a CYP1C ortholog in quail. PCR was performed with combinations of 12 forward and 10 reverse primers targeting CYP1C1 regions that are conserved between the chicken, turkey, and mallard duck. In the reactions we used quail cDNA from whole embryos and from tissues which have a high CYP1C1 expression in zebrafish (eye, brain, and heart [7]), or genomic DNA from a 4-day-old whole quail embryo. Amplification of quail cDNA using the quantitative real-time RT-PCR primers designed for chicken CYP1C1 did yield a product, but that product was part of CYP1B1.

A cDNA resembling CYP1B1 was cloned from quail (GenBank: JN656934), and a sequence with close similarity to CYP1B1 was identified also in the zebrafish (Tainiostigma guttulata) genome. The cloned quail CYP1B1 sequence was 950 bp long, corresponding to approximately 60% of a complete coding CYP1B1 sequence, and the predicted protein included SRS 2–6 (Fig. 2). The deduced amino acid sequence of quail CYP1B1 showed 99% identity with the corresponding region of the known chicken CYP1B1, and the SRS regions available in both predicted proteins (SRS 3–6) were identical. The quail CYP1B1 and the predicted zebra finch CYP1B1 showed 96% amino acid identity for the cloned segment and for the SRS regions. Quail CYP1B1 also showed 69%, 60%, and 58% sequence identity with same region of CYP1B1s in human, X. tropicalis, and zebrafish, and higher degrees of identity in the SRS regions (75%, 65%, and 69%, respectively).

We also cloned a cDNA for quail AHR1 (corresponding to amino acid numbers 231–395 of chicken AHR1), which includes most of the AHR ligand-binding domain (GenBank: JN656935). Figure 3 shows the translated cloned quail AHR1 sequence aligned with AHR proteins in seven birds, X. tropicalis, human, mouse, and zebrafish. Two clades of AHR proteins have been identified in fish and birds, the AHR1s and AHR2s. The quail AHR1 showed 99% sequence identity with AHR1 in other birds, while lower identities (62–72%) were obtained when compared to the AHR2 in chicken, albatross, and cormorant. Quail AHR1 showed 82% sequence identity to a third predicted AHR (AHR1B-like) protein found in the chicken genome (located next to AHR2 on chromosome 7). The quail AHR1 sequence showed 70%, 84%, and 75% identity to the AHR1A, AHR1B, and AHR2 proteins in zebrafish, respectively. (Accession numbers of all CYP1C1, CYP1B1, and AHR genes mentioned here are shown in Table 1).

Dioxin response elements

Putative dioxin response elements (DREs) were sought in the promoter regions of the chicken, turkey, and mallard CYP1C genes, using the sequence 5′-TG/NCGGTG-3′ [26,27]. Within 10 kb upstream from the start codons of the CYP1C genes two putative DREs were found in chicken (at −458 and −1671 bp) and one was found in turkey (at −9193 bp). In the mallard duck genome database (version 1, Pre Ensembl) a fragment in the beginning of the CYP1C gene is unidentified (including approximately the first 140 nucleotides downstream from the start codon); putative DREs were found located at about 3600, 5500, and 5800 bp upstream from the 5′-edge of the unidentified region.

Synteny

In order to examine the degree of conservation of the genomic region around the CYP1C locus we identified the three genes closest on either side of CYP1C1 in chicken (RPUSD2 - CASC5 - RAD51 - [CYP1C1] - FAM82A2 - GCHFR - DNAJC17), and localized the genomic position of orthologs to these genes in various species (Fig. 4). Our results indicate that all seven genes have the same order in X. tropicalis, anole, chicken, and mallard, while CASC3 is absent in turkey (Fig. 4). Mouse and human have the same arrangement of these genes, except that CYP1C1 is missing (Fig. 4). In the zebrafish genome, a segment including RPUSD2, CASC5, and RAD51 was found located 900 kb downstream from FAM82A2, GCHFR, and DNAJC17 on chromosome 5 (and CYP1C1 was missing). In zebrafish, RAD51 and FAM82A2 were found next to each other on chromosome 20 whereas the two CYP1C paralogs are arranged in tandem on another chromosome [7] (the zebrafish CYP1Cs were mapped to chromosome 17 in previous zebrafish genome assemblies, but this mapping has not been confirmed in Zv9 as yet). No shared synteny with chicken is found near upstream from the two zebrafish CYP1Cs, but orthologs to GCHFR and DNAJC17 are located...
downstream from the CYP1Cs (Fig. 4). Zebrasfish RPUSD2 and CASC5 were found on chromosomes 17 and 1, respectively. Three-spined stickleback showed a syntenic arrangement similar to that of zebrafish for these genes (in the stickleback RPUSD2 and the CYP1Cs are located on the same chromosome 3.3 Mb apart).

In all species examined here, CYP1B1 was found adjacent to a FAM82A2 paralog, FAM82A1 (or FAM82A; Fig. 4).

In chicken and zebra finch, no CYP1D1 ortholog was found in the region of TMC1, ALDH1A1, and ANAX1, which is the location for CYP1D1 in rhesus monkey, zebrafish, anole lizard, X. tropicalis, and other species [12,28]. Neither was CYP1D1 found by blast searches in the chicken and zebra finch genomes.

Tissue distribution patterns of CYP1 and AHR1 mRNA
Basal levels of CYP1 and AHR1 expression were determined in liver, choioallantoic membrane (CAM), eye, brain, heart, and yolk sac membrane (YSM) in chicken and quail sampled on incubation day 13 and 11, respectively (equivalent developmental stages).
stages). Overall, the distribution patterns were very similar in the two species. In both chicken and quail, the liver showed the strongest expression of CYP1A4 followed by CAM and eye (Fig. 5). CYP1A5 was considerably more strongly expressed in liver than in other tissues and CYP1B1 was strongly expressed in eye, brain, and heart in both species (Fig. 5). However, our results suggest the levels of CYP1A4 and CYP1A5 mRNA were much higher in quail than in chicken, whereas CYP1B1 was expressed at roughly similar levels in the two species. The expression levels of AHR1 mRNA were fairly similar in liver, CAM, eye, brain, and heart in chicken while a somewhat larger variation was observed for AHR1 expression among these tissues in quail (Fig. 5). YSM showed the lowest AHR1 expression in both species (Fig. 5). We also analyzed CYP1C1 mRNA expression in chicken; the eye showed the highest level followed by heart while other organs showed relatively low levels (Fig. 5). The reference gene, elongation factor
1 alpha (EF1A), seemed to be expressed at a similar level in chicken and quail, and in both species the six different tissues showed only small variations in EF1A mRNA expression levels (Fig. 5).

The expression patterns of the various CYP1s within a tissue were compared in chicken and quail (Fig. 6). The results suggest that the CYP1As and CYP1B1 were expressed to a roughly similar level in chicken liver whereas the CYP1As were much more strongly expressed than CYP1B1 in quail liver (Fig. 6). In both species CYP1A4 appeared to be the most strongly expressed and CYP1A5 the most weakly expressed of the CYP1s in CAM, whereas CYP1B1 seemed to be the dominant CYP1 transcript in eye, brain, and heart (Fig. 6). The expression patterns in YSM varied between the two species, CYP1B1 looking more strongly expressed than the CYP1s in chicken YSM, whereas the opposite was observed in quail.

Expression patterns of CYP1 and AHR1 mRNA during early chicken embryo development

Expression of the four CYP1s and AHR1 was determined in whole-body samples of unexposed chicken embryos collected on incubation days 1, 2, 3, 5, and 7 (Table 2). We found that all transcripts were expressed at all sampling time points. During the whole 7-day period studied a similar pattern appeared: CYP1B1 was the most strongly expressed and CYP1A5 the second most strongly expressed of the CYP1s, while expression of CYP1A4 and CYP1C1 was considerably weaker than that of CYP1B1 (Table 2).

The time course of CYP1 mRNA expression in whole-body samples from incubation day 3 to 7 is shown in Figure 7. The day 1 and day 2 embryos gave small total RNA yields, and less RNA was used in the assay for these samples than for older embryos. The embryos from the first two sampling times were therefore not included in the time course analysis. Within the study period CYP1B1, CYP1C1, and AHR1 expression levels were relatively stable, while expression of CYP1A4 and CYP1A5 showed an increase from day 5 to day 7 (Fig. 7).

Table 3 shows the levels of CYP1 and AHR1 expression in whole-body, YSM, and CAM samples on day 7. While CYP1B1 was the most strongly expressed of the CYP1s in the whole-body samples and CAM, CYP1A5 showed the strongest expression in YSM (Table 3). In CAM the AHR1 was more strongly expressed than the CYP1s.

CYP1 mRNA induction by PCB126

The effect of PCB126 on CYP1 mRNA expression was determined in YSM and whole-body of chicken and quail embryos sampled 24 hours after injection. The control levels in whole-body and YSM were roughly similar in chicken and quail except for the level of CYP1A4 in YSM, which was much higher in quail than in chicken. Chicken and quail eggs were injected with 0.2 and 200 μg PCB126 kg⁻¹, respectively, doses that were shown by Brunstrom and Halldin [19] to induce hepatic EROD activity to a similar level in embryos of the two species. In chicken the PCB126 exposure induced expression of all four CYP1s in both
Table 1. GenBank or Ensembl accession numbers of the studied transcritps.

| Species             | Gene   | Number                      |
|---------------------|--------|-----------------------------|
| Chicken             | CYP1C1 | JN656933 (cloned)           |
| Turkey              | CYP1C1-like | ENSMGAG00000015774         |
| Mallard duck        | CYP1C1-like | ENSAPLGO0000001387         |
| Anole lizard        | CYP1C1-like | ENSACAG00000013750         |
| Xenopus tropicalis  | CYP1C1 | HQ018042                    |
| Zebrafish           | CYP1C1 | NM001020610                 |
| Zebrafish           | CYP1C2 | NM001114849                 |
| Japanese quail      | CYP1B1 | JN656934 (cloned)           |
| Chicken             | CYP1B1 | XP419515                    |
| Zebra finch         | CYP1B1-like | XP002191325                |
| Human               | CYP1B1 | NP000095                    |
| Anole lizard        | CYP1B1-like | XP003216002                |
| Xenopus tropicalis  | CYP1B1 | HQ018041                    |
| Japanese quail      | AHR1   | HM053555, JN656935 (cloned) |
| Chicken             | AHR1   | AAF70373                    |
| Turkey              | AHR1   | XP003207170                 |
| Albatross           | AHR1   | BAC87795                    |
| Zebra finch         | AHR1   | XP00218864                  |
| Common tern         | AHR1   | AF192503                    |
| Cormorant           | AHR1   | BAD01477                    |
| Mallard duck        | AHR1   | AF192501                    |
| Xenopus tropicalis  | AHR1   | CX900378                    |
| Mouse               | AHR    | NM013464                    |
| Human               | AHR    | AAH70080                    |
| Chicken             | AHR1-like | ENSGALG00000004322         |
| Zebrafish           | AHR1A  | NP571103                    |
| Zebrafish           | AHR1B  | AAY42958                    |
| Chicken             | AHR2   | XP421887                    |
| Albatross           | AHR2   | BAC87796                    |
| Cormorant           | AHR2   | BA64245                     |
| Zebrafish           | AHR2   | CAK11168                    |
| Japanese quail      | EF1A   | JN656936 (cloned)           |

doi:10.1371/journal.pone.0028257.t001

YSM and whole-embryos (Fig. 8). Chicken CYP1C1 was induced 2-fold compared with the control in both sample types, but the control level of this transcript was 10-fold higher in the whole-embryos than in the YSM. In quail, exposure to the 1000 times 2-fold compared with the control in both sample types, but the CYP1C1 showed induction in whole-embryos and a tendency for induction in YSM, whereas CYP1B1 expression was not significantly affected by the PCB126 exposure in quail (Fig. 8).

Discussion

This study deals with CYP1 genes and their expression in birds, focusing particularly on members of the CYP1B and CYP1C subfamilies. Phylogenetic analyses identify two major subclades in the vertebrate CYP1 family, one comprising the CYP1As and CYP1Ds and the other comprising the CYP1Bs and CYP1Gs [13,29]. Our results establish that the CYP1B/C subclade, as well as the CYP1A/I subclade, occurs in birds (although CYP1D genes seem to be missing). Genes related to the vertebrate CYP1 genes have been found in the sea urchin Strongylocentrotus purpuratus (CYP1-like genes) and in the sea squirts Ciona intestinalis and Ciona savignyi (CYP1E1 and CYP1F1/CYP1F); Fig. 9), suggesting that CYP1-like genes were present in animals even before the Cambrian explosion [25,30]. Goldstone et al. [25] found that the sea squirt genes can be assigned to either the CYP1A/ID subclade (CYP1E1) or the CYP1B/C subclade (CYP1F1) whereas the sea urchin genes do not fall into either of these subclades. This suggests that the two CYP1 subclades were established in early chordates (Fig. 9) [25].

The two subclades, multiple subfamilies, and several pairs of paralogs indicate that the CYP1 gene family has undergone several duplication events. Duplication of whole genomes, genomic segments, or single genes are believed to be important for evolution of new functions [31]. Genes are also lost over time of evolution, supposedly as they become superfluous. CYP1D1 is expressed in fish, X. tropicalis, and the cynomolgus monkey (Macaca fascicularis), while it is a pseudogene in human and some other mammals [6,12,13,28]. Surprisingly, CYP1D has yet to be found in any bird species; whether it was lost early in the avian line is an important question. The CYP1Gs appear to have been lost in mammals generally [29].

Our findings hint that the role of CYP1Is is weakening also in birds. Chicken, quail, turkey, and mallard duck belong to the superorder Galloanserae (orders Galliformes and Anseriformes), while zebra finch belongs to the superorder Neoaves (order Passeriformes), which appears to have undergone a rapidly radiating evolution, comprising 95% of extant species [32]. Among the species studied, there was no clear evolutionary trend in the presence/absence of CYP1C1, i.e., it was found in chicken, turkey, and mallard, but not in their close relative quail. Furthermore, no hit for CYP1C was obtained in blast searches of the zebra finch genome database. Turkey CYP1C was predicted to be a pseudogene in Ensembl, having one small intron (5'-CCCC-3'). This could be an inaccurate prediction due to sequencing error since removal of one cytosine and use of the intron as a codon results in a translated protein sequence highly similar to the chicken and mallard CYP1Cs (Fig. 1). However, only one putative DRE was found within 10 kb upstream from turkey CYP1C (at about 9 kb upstream from the start codon), which raises a question about the inducibility of this gene via the AHR. The mallard CYP1C gene had three putative DREs in the upstream promoter region, but gene prediction is uncertain since part of the promoter sequence (and the start codon) was unidentified. In the zebra finch two CYP1s that are not CYP1C orthologs were found at other places on chromosome 5. These genes were CYP2R1 (vitamin D 25-hydroxylase) and CYP46A1 (cholesterol 24-hydroxylase) and the regions around these CYP1s exhibited a high degree of shared synteny in zebra finch, chicken, and turkey. In the zebra finch, genomic rearrangement appears to have occurred precisely at the location equivalent to that of CYP1C1 in the other birds (Fig. 4). Whether this represents true genomic rearrangement rather than misassembly is not clear at present. That some CYP1C1 roles may be diminishing in birds is suggested also by the relatively low basal level of CYP1C1 mRNA expression in chicken embryos (except in the eye and heart) and that CYP1C1 is only slightly inducible by PCB126 in birds, while CYP1C1 is relatively strongly inducible in fish [7,10,33,34,35]. To resolve the evolutionary fate of CYP1C1 in birds, this gene needs to be studied in a larger number of species.

The region around the CYP1C locus shows a high degree of shared synteny in many vertebrate species, including the anole lizard. The genes next to CYP1C1, RADS1 and FAM82A2, code for...
highly conserved proteins with developmental functions, i.e., DNA repair (RAD51) and differentiation and apoptosis (FAM82A2). These two genes contain several putative DREs in their promoter regions (within about 2 kb upstream from the start site) in zebrafish, chicken, X. tropicalis and human. Interestingly, an ortholog of FAM82A2, FAM82A1 (FAM82A) was found located next to CYP1B1 on the chromosome in all species examined here (Fig. 4). This could mean that the regions of CYP1B1 and CYP1C1 derive from two copies generated by genome duplication in early vertebrates.

Constitutive expression

The tissue distribution profiles for basal levels of CYP1A, CYP1B, and CYP1C mRNA in chicken and quail embryos (Fig. 5) were astonishingly similar to those in adult zebrafish. In both fish and birds there are distinct differences in mRNA expression patterns between the subclades, with CYP1A and CYP1D in fish being more highly expressed in the liver and CYP1B/CYP1C being more highly expressed in eye, heart, and brain [7,13]. Expression of CYP1A4, CYP1A5, and AHR1 has been determined also in the cormorant (Phalacrocorax carbo), and the mRNA expression profiles of these genes in liver, heart, and brain were almost identical to those in chicken and quail embryos [36]. The tissue distribution of the two bird CYP1As also showed some similarity to the distribution of the two CYP1As in mammals, in which CYP1A1 (ortholog of CYP1A4) is widely expressed whereas CYP1A2 (ortholog of CYP1A5) is expressed strongly only in the liver [37].

Regarding distribution of CYP1B1 in mammals, heart and brain show high expression levels in mice, while in human CYP1B1 is highly expressed in heart and weakly expressed in brain [38]. Unlike the quite strong CYP1B1 expression in the bird embryo liver, CYP1B1 is weakly expressed in the mammalian liver [38]. It is a curious finding that the eye is the tissue where CYP1C1 was most highly expressed in the 13-day chicken embryo. Notably the two CYP1Cs show a high expression also in the adult zebrafish eye [7]. The roles of CYP1C in the eye are not known. In mammals, CYP1B1 is critical for normal eye development [39,40]. CYP1Cs might share this role in other vertebrates.

The relatively high level of CYP1B1 expression over the course of development in most tissues (excluding liver) in both chicken and quail embryos suggests that it plays a role in developing birds. CYP1B1 mRNA has been localized (by in situ hybridization) to the developing eye, neural system, somites, and other structures in chicken embryos [8]. In embryonic zebrafish the basal level of CYP1B1 mRNA expression peaks at approximately 32 hours after fertilization, in a period of organogenesis and differentiation, while CYP1A expression peaks about three weeks after fertilization, and expression of the CYP1Cs peaks just after hatching [33]. Choudhary et al. [41] found that in developing mice CYP1B1 is expressed during neural patterning and somitogenesis, organogenesis, and later fetal stages, whereas CYP1A1 is expressed during gastrulation only, while CYP1A2 expression was not detected at all. Thus CYP1B1 appears to be important in early development in zebrafish, chickens, and mice.

Figure 4. Synteny of CYP1C and CYP1B1 regions in various species. Data were collected from the current assembly versions of the genome databases in Ensembl (http://www.ensembl.org/info/about/species.html): Zebrafish (version Zv9), X. tropicalis (version JGI 4.2, scaffolds: GL173137 and GL173263), anole lizard (version AnoCar2.0, scaffold GLG32641), chicken (version 2.1, Chr. 5), mallard duck (version 1.0, scaffold 2370), turkey (version UMD2, Chr. 5), zebra finch (version taeGut3.2.4, Chr. 5), mouse (version NCBI/M37, Chr. 2), and human (version GRCh37, Chr. 15) Zebrafish CYP1C1 and CYP1C2 have been mapped to chromosome 17 in previous zebrafish genome assemblies [7], but this mapping has not been confirmed in Zv9 as yet. The synteny of CYP1B1 is shared by all species shown here. Chr = chromosome. doi:10.1371/journal.pone.0028257.g004
Endogenous substrates for CYP1 enzymes include various eicosanoids, estradiol, retinoids, and uroporphyrinogen and melatonin (reviewed by [1,4,5]). Chambers et al. [8] found that CYP1B1 can catalyze a step in the formation of retinoic acid, and suggested it is involved in retinoid-mediated patterning. The CYP1s also have been suggested to metabolize endogenous AHR ligands that could play roles in development and differentiation [42]. One molecule which could have this function is the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole (FICZ) [43]. FICZ activates the AHR at hormonal levels and is metabolized by human CYP1A1, CYP1A2, and CYP1B1 with an extraordinarily high efficiency [44]. In conclusion, the CYP1 enzyme functions may include synthesis and degradation of endogenous AHR agonists and other signaling molecules.

The AHR and CYP1 mRNA induction

Induction of CYP1A, CYP1B, and CYP1C genes and most toxic effects of TCDD are mediated via the AHR. The AHR genes are divided into two clades, AHR1 and AHR2. Mammals have a single AHR1 gene and no AHR2 gene, while fish and birds have both AHR1 and AHR2 genes [36,45]. In zebrafish, AHR-dependent toxicity and CYP1 induction are mediated principally via AHR2, whereas in birds AHR1 seems to be prominent in these roles [27,36,45]. We found that AHR1 mRNA was expressed at relatively high levels in a variety of tissues in both quail and chicken (Fig. 5). In the cormorant, AHR1 mRNA expression shows a wider distribution and higher level than AHR2 mRNA expression [36]. Features of the AHR may explain differences in sensitivity to dioxin-like compounds in certain mouse strains [46], and in birds, where for instance turkey and quail are much less sensitive than chicken [19,47]. Frogs show a low sensitivity to dioxin toxicity and have an AHR with low dioxin affinity [48,49]. The differing sensitivities to dioxin of mouse strains were shown to be due to differences in specific amino acid residues in the AHR ligand binding domain [46]. Similarly, differences in two amino acid positions in the ligand binding domain were shown to distinguish common tern (resistant) and chicken (susceptible) AHR1s [22]. The identity of the amino acids at these two positions predicted the sensitivity in a wide range of bird species [15]. Our results confirm those of Head and co-workers [15], showing that quail AHR1 has valine in position 324 and alanine in position 380 (the same as seen in the tern AHR1), while these positions have isoleucine and serine in chicken (Fig. 3). The turkey AHR1 has isoleucine and alanine in these positions, but also differs from the chicken and common tern AHR1 by having an isoleucine instead of threonine in position 297; the mallard AHR1 has two threonines instead of two alanines in the positions 256–257, but otherwise is identical to the quail AHR1 in the ligand binding domain (Fig. 3 and [15]). In addition to features associated with a resistant AHR, i.e., a low sensitivity to developmental toxicity of dioxins [50] and weak EROD response to PCB126 observed in...
embryo liver [19], quail exhibited a weaker CYP1 mRNA induction by PCB126 than chicken despite the 1000 times higher dose given to quail. The basal levels of hepatic CYP1A4 and CYP1A5 expression were much higher in quail embryos than in chicken embryos at similar stages, whereas basal CYP1B1 expression looked largely similar in the two species. The significance of this difference between the genes is not understood. However, assuming there is a maximal capacity to synthesize the transcript for a given CYP1, the relative induction level would be low when compared to a high basal level. A higher basal level in the quail could explain the “weaker” induction of CYP1A4 in YSM of quail than of chicken. Hence, a lower “fold-induction” level of a CYP1 gene could reflect a high constitutive level of expression rather than a low responsiveness. However this would not explain the weaker induction of CYP1A5 in quail vs. chicken, and the induction of CYP1B1 in chicken but not in quail (Fig. 8). Rather these differences could be related to differences in AHR affinity for PCB126 not compensated for by the higher dose given to quail. In addition to AHR affinity of the inducer, the level of CYP1 induction depends on the number of functional DREs in the gene promoters, epigenetic factors, interaction of the AHR with nuclear receptors (e.g., estrogen receptor), cofactors, the AHR repressor, etc. [26,51,52,53,54,55]. Because the CYP1s are likely to be expressed in different cell types, it would be informative to study

### Figure 6. Expression patterns for CYP1 mRNA in various tissues in unexposed embryonic chicken and Japanese quail.

Samples were collected at a similar developmental stage in chicken and quail (on incubation day 13 and 11, respectively). Levels of mRNA expression for CYP1A4, CYP1A5, and CYP1B1 in chicken and Japanese quail, and for CYP1C1 mRNA in chicken were determined by real-time RT-PCR. Data were normalized (calculated by E\(^{-D_{Ct}}\)) and results are shown as percentage of the gene with the highest level of expression within a tissue (mean±SD). Statistical differences among transcript levels within a tissue were determined by one-way ANOVA followed by Tukey’s multiple comparisons test and are shown by different letters (p<0.05); n = 3–4 for chicken and n = 3 for quail.

doi:10.1371/journal.pone.0028257.g006

### Table 2. Relative levels of basal expression for CYP1 and AHR1 mRNA in early chicken embryos.

| Transcript | Day 1  | Day 2  | Day 3  | Day 5  | Day 7  |
|------------|--------|--------|--------|--------|--------|
| CYP1A4     | 0.4±0.5| 0.2±0.0| 0.1±0.1| 0.1±0.1| 0.9±0.1|
| CYP1A5     | 45±32  | 39±22  | 11±15  | 4±1    | 28±2   |
| CYP1B1     | 100±137| 100±9  | 100±31 | 100±45 | 100±13 |
| CYP1C1     | 1.6±1.4| 2.3±0.9| 0.4±0.2| 0.5±0.1| 0.7±0.4|
| AHR1       | 51±56  | 158±86 | 81±30  | 24±7   | 57±4   |

Whole-body samples of unexposed chicken embryos were collected on developmental days 1, 2, 3, 5, and 7 and analyzed by real-time RT-PCR. Data were normalized (calculated by E\(^{-D_{Ct}}\)) and results are shown as percentage of the CYP1B1 mRNA level (n=3). The embryos analyzed were staged according to Hamburger and Hamilton [63] and http://msucares.com/poultry/reproductions/poultry_chicks_embryo.html: Day-1 samples were taken after 31 h of incubation (stage 9, seven somites). At this stage the nervous system, eye, and heart have begun to develop. On day two (sampled at 50 h: stage 16, 19–22 somites) heart beats can be observed. On day three (sampled at 74 h: stages 20–21, 40–43 somites) nose, legs, and wings begin to appear. On day five (stage 27) the beak and reproductive organs start to form and sex differentiation occurs. On day seven (stage 31) feather papillae begin to appear.

doi:10.1371/journal.pone.0028257.t002
cell-specific induction of the CIPIs, and CYP1C1 in particular, in chicken.

We previously found that the patterns of induction of CYP1A, CYP1B, and CYP1C were similar in zebrafish and X. tropicalis after exposure to PCB126 [6,33]. In zebrafish embryos CYP1A, 1B1, 1C1, and 1C2 were induced 280-, 23-, 23-, and 40-fold versus the control and in tadpoles CYP1A, 1B1, and 1C1 were induced up to 90-, 3-, and 8-fold versus the control [6,33]. Together with the present results (Fig. 3) these findings indicate the CYP1A genes are more responsive to PCB126 than the CYP1B/CYP1C genes in developing animals. Thus, a strong inducibility appears to be an evolutionarily conserved feature of the CYP1As, which could have to do with their functions.

Conclusions

In this study we establish that CYP1C1 is present in some birds. We show that CYP1C1 mRNA is rather highly expressed in the chicken embryo eye. CYP1B1 appears to have a high developmental expression in both chicken and quail. The similar distribution patterns of CYP1C and CYP1B transcripts in chicken and zebrafish imply that these CIPIs may serve similar functions in diverse vertebrates. Together with the absence of CIPIs in mammals, the apparent absence of CYP1C1 in quail, and weak expression and induction of CYP1C1 mRNA in chicken suggests that CIPIs have diminishing roles in tetrapods, which may be met by CYP1B1. Determining catalytic functions of CYP1 proteins in different species should indicate the evolving roles of these duplicated genes in physiological and toxicological processes. The studies reported here expand our view of the likely history and role of CIPIs.

Methods

Eggs

Fertilized eggs from chicken (White Leghorn) and Japanese quail were obtained from local Swedish breeders (OVA Production AB, Vittinge, and Olstorps Konserverfabrik, Fargelanda, respectively). Eggs were incubated at 37.5°C and 60% relative humidity with automatic turning every 6 hours until sampled. The experiments of this study were approved by Uppsala Ethical Committee for Research on Animals (Uppsala district court; permit number C 282/9).

Cloning and synteny

Complementary DNA of chicken CYP1C1 was cloned using primers targeting the predicted full coding transcript. Total RNA was extracted from whole-body homogenate of chicken embryos (incubated for 5 days) using RNA STAT 60 (Tel. Test Inc., Friendswood, TX, USA). Subsequently mRNA was isolated from the total RNA using MicroPoly(A)Purist™ Kit (Ambion Inc., Austin, TX, USA), and cDNA was synthesized using the Omniscript reverse transcriptase kit with random hexamer primers (Eurofins MWG Operon, Huntsville, AL, USA). Amplification of cDNA was performed using the Advantage® 2 polymerase PCR kit according to instructions provided by the manufacturer (Clontech Laboratories Inc., Mountain View, CA, USA). The PCR products were resolved on a 1% agarose gel. A product of approximately 1600 bp was isolated and ligated into the pGEM-T Easy Vector (Promega, Madison, WI, USA), and the
construct was transformed into *Escherichia coli* (TOP 10 Kit, Invitrogen, Carlsbad, CA, USA). Plasmids were purified from cultures of positive clones and sequenced (Eurofins MWG Operon). The sequences obtained were assembled using Sequencher® (Gene Codes Corporation, Ann Arbor, MI, USA), resulting in a consensus sequence corresponding to the full coding part of the predicted chicken CYP1C1.

We also cloned partial sequences of quail CYP1B1, AHR1, and EF1A using primers designed to target conserved regions of the chicken orthologs. Total RNA was prepared from whole-body homogenate of 4-day-old quail embryos using the Aurum™ total RNA fatty and fibrous tissue kit (Bio-Rad Laboratories Inc., Hercules CA, USA) and the RNA was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad). Quail cDNAs were amplified using the gene-specific primers with Advantage® 2 polymerase PCR kit (Clontech Laboratories Inc.). The PCR products were sequenced by Uppsala Genome Center (Rudbeck Laboratory, Uppsala) and sequences obtained were aligned using ClustalW in BioEdit [56]. The cloned sequences of chicken CYP1C1 and quail CYP1B1, AHR1, and EF1A were assigned the following GenBank accession numbers: JN656933, JN656934, JN656935 and JN656936, respectively.

Seeking an ortholog for CYP1C1 in quail we designed primers (12 forward and 10 reverse) targeting regions conserved between chicken CYP1C1 and the CYP1C1 predictions in turkey and mallard duck. In addition to cDNA from embryonic day 4 we used pooled cDNA from eye, brain, and heart (collected on embryonic day 11). A CYP1C1 gene was also sought using genomic DNA isolated from quail whole-body homogenate (embryonic day 4) with DNeasy Blood & Tissue Kit (Qiagen). We also amplified quail cDNA using the quantitative real-time RT-PCR primers designed for chicken CYP1C1 (Table 4), but the product obtained was of CYP1B1. Consequently, we did not find any ortholog for CYP1C1 in quail neither in genomic DNA nor in cDNA made from total RNA.
The deduced amino acid sequences of the cloned cDNA were aligned with homologous sequences in other species and sequence identities were examined after pair-wise alignments using BioEdit. The SRS regions were localized out from Lewis et al. [57]. The synteny of \textit{CYP1C} and \textit{CYP1B} genes was determined in zebrafish, \textit{X. tropicalis}, chicken, turkey, mallard duck, zebra finch, mouse, and human using the genome databases in Ensembl.

**Figure 9. Evolutionary history of the CYP1 family genes.** The panel shows the presence of CYP1-like genes and CYP1A, CYP1B, CYP1C, CYP1D, CYP1E and CYP1F subfamily genes in various echinozoan, tunicate, and vertebrate classes. Suggested events of gene duplication and gene loss are shown by bullets. Ten CYP1-like genes have been identified in the genome of the sea urchin \textit{S. purpuratus} and three CYP1A-like genes in the genome of the anole lizard. These genes have not been further studied. Genes within brackets indicate known or suspected absence in at least one species within the taxon (birds, mammals). Data were collected from Godard et al. [29], Goldstone et al. [25], Jönsson et al. [7], Goldstone et al. [13], Gao et al. [35], Jönsson et al. [6], and the anole lizard genome database (http://www.ensembl.org/Anolis_carolinensis/Info/Index). Footnote: \(^1\) CYP1D1 is a pseudogene in human, is expressed in macaques [12,13], and appears to be absent in the mouse.

\[\text{doi}:10.1371/journal.pone.0028257.g009\]

**Table 4. Sequences of all real-time RT-PCR primers used in the experiments.**

| Species/Transcript | GenBank Acc. No. | Forward primer (5' to 3') | Reverse primer (5' to 3') | Product size |
|--------------------|------------------|---------------------------|---------------------------|-------------|
| Chicken            |                  |                           |                           |             |
| \textit{CYP1A4}    | NM205147.1       | ACTGCCAGGAGAAAGGACAG      | TCAAACCTGCCCCCAACAG       | 97          |
| \textit{CYP1A5}    | NM205146.1       | TTCCCATCAGGCAAGCAAGA      | GTCGCCTGATCTGTTGACAGC     | 109         |
| \textit{CYP1B1}\(^1\) | XM419513.2       | CATCTCTCTCATCTAACAAGA     | GTACAGGAAAGCAGTGTGAG     | 130         |
| \textit{CYP1C1}    | JN656933         | TGGCCCATACCCCTTGCAATCAT  | ACTGCCACTGTTGCAAGAAGC     | 99          |
| \textit{AHR1}      | NM204118.1       | GCTGCTAGAAAAAAAGTGGTGT   | ATTTCTCATCTACCCCTT       | 148         |
| \textit{EF1A}      | NM204157.2       | GATGCTATACAAAAATGTTGTTGC | GCTGCTAGAAAAAAAGTGGTGT   | 140         |
| Japanese quail     |                  |                           |                           |             |
| \textit{CYP1A4}    | GQ906939.1       | GCAAGTGAACACGAGAAGGAGA   | ACCACCTCTGACCCCTGTAAC    | 111         |
| \textit{CYP1A5}    | GQ906938.1       | GCAAGTGAACACGAGAAGGAGA   | ACCACCTCTGACCCCTGTAAC    | 111         |
| \textit{CYP1B1}\(^1\) | JN656934         | CATCTCTCTCATCTAACAAGA     | GTACAGGAAAGCAGTGTGAG     | 130         |
| \textit{AHR1}      | JN656935         | GCTGCTAGAAAAAAAGTGGTGT   | ATTTCTCATCTACCCCTT       | 142         |
| \textit{EF1A}      | JN656936         | GATGCTATACAAAAATGTTGTTGC | GCTGCTAGAAAAAAAGTGGTGT   | 140         |

\(^1\)The same primer pair was used for chicken and quail \textit{CYP1B1}. doi:10.1371/journal.pone.0028257.t004
CYP1 mRNA expression in chicken and quail embryos

Basal levels of CYP1 mRNA were determined in unexposed chicken embryos sampled after various times of incubation (1, 2, 3, 5, and 7 days). Whole embryos and YSM were sampled separately (day 3, 5, and 7). In addition, CAM was collected from chicken at embryonic day 7. Finally, liver, CAM, eye, brain, heart, and YSM were collected from chicken and quail at embryonic day 13 and 11, respectively. All samples were frozen in liquid nitrogen and stored at −80°C.

Inducibility of the CYP1s was examined after exposure to PCB126 by egg injection. Injection solutions were prepared by dissolving PCB126 in a peanut oil:lecithin mixture (10:1, w:v) which was emulsified in water (1:1.5, v:v) by ultra-sonication. Equivalent peanut oil:lecithin:water emulsions without PCB126 were prepared for controls. The emulsions were injected into the yolks of embryonated eggs after 3 (quail) and 4 (chicken) days of incubation. The volumes injected were 20 μl (quail) and 100 μl (chicken) corresponding to 290 μg PCB126/kg for quail and 0.2 μg PCB126/kg for chicken, doses that are high enough to induce hepatic EROD activity [19]. After injection, the holes in the shells were sealed with melted paraffin wax and the eggs were returned to the incubator. The embryos were sampled 24 hours later. Whole embryos and YSM were sampled separately, frozen in liquid nitrogen, and stored at −80°C.

Quantitative real-time RT-PCR

Total RNA was isolated and DNase-treated using the Aurum™ total RNA fatty and fibrous tissue kit (Bio-Rad) according to Bio-Rad’s instructions. The purity and quantity of RNA were determined spectrophotometrically (260/280 and 260/230 nm ratios were generally 2 or above) using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). Total RNA was reverse transcribed using the iScript cDNA Synthesis kit (Bio-Rad).

Gene-specific quantitative real-time RT-PCR primers for chicken and quail CYP1A4, CYP1A5, CYP1B1, AHR1, and EF1A, and for chicken CYP1C1, were synthesized by Sigma-Aldrich (St. Louis, MO, USA) (Table 4). The predicted amplicon length was 75–150 bp. PCR was conducted using a Rotor Gene 6000 real-time PCR machine (Qiagen, Hilden, Germany). The 20-μl PCR reaction mixtures consisted of iQ SYBR Green Supermix (Bio-Rad), forward and reverse primers (5 pmoles of each; Table 4) and cDNA derived from 90 ng of total RNA. All samples were analyzed in duplicate with the following protocol: 95°C for 10 min followed by 30–40 cycles (cycle numbers varying with transcript levels) of 95°C for 15 s and 62°C for 43 s. At the end of each PCR run a melt curve analysis was performed in the range from 55°C to 95°C.

Calculations and statistics

Finding a reference gene which is stable during development or which does not vary among tissues is difficult. Therefore, in some cases basal levels of CYP1 and AHR1 mRNA expression were calculated without normalization to an internal control (indicated in figure and table legends). In these calculations we used the equation E−CT where E = PCR efficiency and CT = threshold cycle [58,59]. The effect of PCB126 on mRNA expression was determined after calculation of E−ΔACT [59]. EF1A was used as a reference gene for both quail and chicken; in neither of the two species EF1A was significantly affected by the PCB126 exposure. Mean values of E for within-experiment amplion groups were determined by the LinRegPCR program using data within 10% of the group median [60,61]. The E values obtained ranged from 1.83 to 1.92. Outliers were excluded based on the Grubbs test [62]. Statistical analyses were performed using Prism 5 by GraphPad Software Inc. (San Diego, CA, USA) with log-transformed data. The statistical methods used were Student’s t test and one-way ANOVA followed by Tukey’s or Dunnett’s post hoc tests. Data were log-transformed before statistical analysis when the variances differed between groups. In the figures data are shown as mean±SD. Numbers of biological replicates used (n) are given in the figure legends.

Author Contributions

Conceived and designed the experiments: MEJ JJS BB. Performed the experiments: MEJ BRW BB. Analyzed the data: MEJ. Contributed reagents/materials/analysis tools: MEJ JJS BB. Wrote the paper: MEJ JJS BB.

References

1. Choudhary D, Janson I, Stolov I, Sarfarazi M, Schenkman JB (2004) Metabolism of retinoic acid and arachidonic acid by human and mouse cytochrome P450 1B1. Drug Metab Dispos 32: 840–847.
2. Nebert DW, Dalton TP (2006) The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat Rev Cancer 6: 947–960.
3. Zhou SF, Zhou ZW, Yang LP, Gai JP (2009) Substrates, inducers, inhibitors and structure-activity relationships of human Cytochrome P450 2C9 and implications in drug development. Curr Med Chem 16: 3480–3675.
4. Nebert DW, Russell DW (2002) Clinical importance of the cytochromes P450. Lancet 360: 1155–1162.
5. Nebert DW, Karp CL (2008) Endogenous functions of the aryl hydrocarbon receptor (AHR): transcription of cytochrome P450 1 (CYP1)-metabolized xenobiotics and AHR biology. J Biol Chem 283: 36061–36065.
6. Jonsson ME, Berg C, Goldstone JV, Stegeman JJ (2011) New CYP1 genes in the frog Xenopus (Silurana) tropicalis: Induction patterns and effects of AHR agonists during development. Toxicol Appl Pharmacol 257: 11–17.
7. Shimada T, Sugie A, Shindo M, Nakajima T, Azuma E, et al. (2003) Tissue-specific induction of cytochromes P450 1A1 and 1B1 by poly cyclic aromatic hydrocarbons and polychlorinated biphenyls in engineered C57BL/6J mice of arylhydrocarbon receptor gene. Toxicol Appl Pharmacol 187: 1–10.
8. Uno Y, Uehara S, Murayama N, Yamazaki H (2011) CYP1D1, pseudogenized in human, is expressed and encodes a functional drug-metabolizing enzyme in cynomolgous monkey. Biochem Pharmacol 81: 442–450.
9. Goldstone JV, Jonsson ME, Behrendt L, Woodin BR, Jenny MJ, et al. (2009) Cytochrome P450 1B1, 1C2 and 1D1 genes in the killifish Fundulus heteroclitus: Basal expression and response of five killifish CYP1s to the AHR agonist PCB126. Aquat Toxicol 93: 234–243.
10. Shindoh Y, Takeuchi Y, Shimizu J, Azuma E, et al. (2003) Tissue-specific induction of cytochromes P450 1A1 and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in engineered C57BL/6J mice of arylhydrocarbon receptor gene. Toxicol Appl Pharmacol 187: 1–10.
11. Shimada T, Sugie A, Shindo M, Nakajima T, Azuma E, et al. (2003) Tissue-specific induction of cytochromes P450 1A1 and 1B1 by poly cyclic aromatic hydrocarbons and polychlorinated biphenyls in engineered C57BL/6J mice of arylhydrocarbon receptor gene. Toxicol Appl Pharmacol 187: 1–10.
12. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
13. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
14. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
15. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
16. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
17. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
18. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
19. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
20. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, et al. (1996) Effects of exposure of wild ducks to polychlorinated biphenyls (PCBs) and chlordane (CB) on growth and development of Mallard embryos. Arch Environ Contam Toxicol 42: 753–754.
19. Brunstrom B, Halldm K (1998) EROD induction by environmental contamin-
anants in avian embryo livers. Comp Biochem Physiol C 121: 213–219.
20. Cohen-Barnhouse AM, Zwiernik MJ, Link JE, Fitzgerald SD, Kennedy SW, et al. (2011) Sensitivity of Japanese quail (Coturnix japonica), Common pheasant (Phasianus colchicus), and White Leghorn chicken (Gallus gallus domesticus) embryos to in ovo exposure to TCDD, PCDF, and TCDF. Toxicol Sci 119: 93–103.
21. Yang Y, Wiseman S, Cohen-Barnhouse AM, Wan Y, Jones PD, et al. (2010) Effects of in ovo exposure of white leghorn chicken, common pheasant, and Japanese quail to 2,3,7,8-tetrachlorodibenzo-p-dioxin and two chlorinated dibenzo-p-dioxins (CYP1A) induction. Environmental toxicology and chemis-
try/SETAC 29: 1490–1502.
22. Karchner SI, Franks DG, Kennedy SW, Hahn ME (2006) The molecular basis for differential dioxin sensitivity in birds: role of the aryl hydrocarbon receptor. Proc Natl Acad Sci U S A 103: 6252–6257.
23. Goldstone HM, Stegerman JJ (2006) A Revised Evolutionary History of the CYP1A Subfamily: Gene Duplication, Gene Conversion, and Positive Selection. J Mol Evol 62: 708–717.
24. Gläsl D, Gannom M, Yutze Y, Bader D, Klinkenb (1996) Molecular cloning and expression of two novel avian cytochrome P450 1A1 enzymes induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Biol Chem 271: 33054–33059.
25. Goldstone JV, Goldstone HM, Morrison AM, Tarrant A, Kern SE, et al. (2007) Cytochrome P450 1 genes in early deuterostome (tunicates and sea urchin) and vertebrates (chicken and frog): origin and diversification of the CYP1 gene family. Mol Biol Evol 24: 2619–2631.
26. Zeruth G, Pollenz RS (2005) Isolation and characterization of a dioxin-Inducible CYP1A promoter/enhancer region from zebrafish (Danio rerio). Zebrafish 2: 100–113.
27. Lee JS, Kim EY, Iwata H, Franks DG, Karchner SI, et al. (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.
28. Yasui T, Kim EY, Iwata H, Franks DG, Karchner SI, et al. (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.
29. Godard CA, Goldstone JV, Said MR, Dickerson RL, Woodin BR, et al. (2005) Sensitivity of Japanese quail (Coturnix japonica), common pheasant (Phasianus colchicus), and White Leghorn chicken (Gallus gallus domesticus) embryos to in ovo exposure to TCDD, PCDF, and TCDF. Toxicol Sci 119: 93–103.
30. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using the 2(-Delta Delta C(T)) Method. Methods 25: 402–408.
31. Choudhary D, Jansson S, Schenkman JB, Sarfarazi M, Steivo (2003) Comparative expression profiling of 40 mouse cytochrome P450 genes in embryonic and adult tissues. Arch Biochem Biophys 430: 50–61.
32. Wei YD, Helleberg H, Rannug U, Rannug A (1998) Rapid and transient induction of CYP1A1 gene expression in human cells by the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole. Chem Biol Interact 110: 39–55.
33. Zeruth G, Pollenz RS (2007) Functional analysis of cis-regulatory regions within the dioxin-inducible CYP1A promoter/enhancer region from zebrafish (Danio rerio). Zebrafish 2: 100–113.
34. Lavine JA, Rowatt AJ, Klimova T, Whittington AJ, Dengler E, et al. (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: 69–72.
35. Jurek RE, Walker MK (1997) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on development of anuran amphibians. Environ Toxicol Chem 16: 239–240.
36. Ramakers C, Allan LL, Sherr DH (2009) Regulation of constitutive and inducible AHR signaling: complex interactions involving the AHR repressor. Biochem Pharmacol 77: 485–497.
37. Hahn ME, Allan LL, Sherr DH (2009) Regulation of constitutive and inducible AHR signaling: complex interactions involving the AHR repressor. Biochem Pharmacol 77: 485–497.
38. Chiaro CR, Patel RD, Marcus CB, Perdew GH (2007) Evidence for an aryl hydrocarbon receptor-mediated cytochrome P450 autoregulatory pathway. Mol Pharmacol 72: 1369–1379.
39. Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. Journal of Morphology 88: 49–92.