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# Genes encoding aromatases in teleosts: Evolution and expression regulation

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ABSTRACT

Cytochrome P450 aromatases, encoded by *cyp19a1* genes, catalyzes the conversion of androgens to estrogens and plays important roles in the reproduction of vertebrates. Vertebrate *cyp19a1* genes showed high synteny in chromosomal locations and conservation in sequences during evolution. However, amphioxus *cyp19a1* does not show synteny to vertebrate *cyp19a1*. Teleost fish possess two copies of the *cyp19a1* gene, which were postulated to result from a fish-specific genome duplication. The duplicated copies of fish *cyp19a1* genes evolved into the brain and ovarian forms of cytochrome P450 aromatase genes, *cyp19a1a* and *cyp19a1b*, respectively, with different regulatory mechanisms of expression, through subfunctionalization under long-term selective pressure. In addition to the estradiol (E2) auto-regulatory loop, there may be other mechanisms responsible for the high expression of aromatase in the teleost brain. The study of the two *cyp19a1* copies in teleost fish will shed light on the general evolution, function, and regulation of vertebrate *cyp19a1*.



## 1. Introduction

Aromatase is an enzyme complex composed of a cytochrome P450 aromatase, the product of the *cyp19a1* gene, and an NADPH-dependent cytochrome P450 reductase known as a ubiquitous flavoprotein (Simpson et al., 1994) that catalyzes the biosynthesis of estrogens from androgens. Thus, the expression or suppression of *cyp19a1* and aromatase activities could alter the ratio of gonadal sex steroids produced, which has been shown to control sexual differentiation and development in non-mammalian vertebrates (Kitano et al., 2000; Kwon et al., 2000; Chardard and Dournon, 1999; Rhen and Lang, 1994; Wibbels and Crews, 1994; Richard-Mercier et al., 1995; Elbrecht and Smith, 1992). In contrast to most other vertebrates, teleosts have two *cyp19a1* genes, *cyp19a1a*, encoding the ovarian form of aromatase, and *cyp19a1b*, encoding the brain form of aromatase (Tchoudakova and Callard, 1998; Tong et al., 2001). The duplicated *cyp19a1* genes are located on different chromosomes in the Nile tilapia, *Oreochromis niloticus* (Harvey et al., 2003), and each isoform has its own distinct regulatory mechanisms and physiological relevance (Kishida et al., 2001; Callard et al., 2001). There are several excellent reviews on aromatases in fish (Cheshenko et al., 2008; Diotel et al., 2010; Guiguen et al., 2010; Le Page et al., 2010). The present paper is intended to briefly summarize the current advancements in the study of *cyp19a1* in teleosts, including the origin, evolution, and regulation of *cyp19a1* genes.

## 2. Two copies of *cyp19a1* genes in teleosts

To date, studies have shown that there is only one copy of *cyp19a1* in most tetrapods, including mammals (Simpson et al., 1997) except pigs and peccaries (Corbin et al., 2007), chicken (McPhaul et al., 1988), Xenopus (Miyashita et al., 2000), and alligator (Gabriel et al., 2001). In addition, only one copy of *cyp19a1* was identified in Atlantic stingray (Ijiri et al., 2000), a cartilaginous fish.

In teleosts, duplicated copies of *cyp19a1* were first identified in goldfish (Tchoudakova and Callard, 1998) and subsequently confirmed in many other teleosts including zebrafish (Kishida and Callard, 2001; Tong et al., 2001), rainbow trout (Valle et al., 2002), orange-spotted grouper (Zhang et al., 2004b), channel catfish (Kazeto and Trant, 2005), medaka (Kuhl et al., 2005), and tilapia (Chang et al., 2005). One *cyp19a1* isoform is designated as *cyp19a1a* and the other as *cyp19a1b*. The former is mainly expressed in the ovary and encodes the ovarian aromatase P450aromA; the latter

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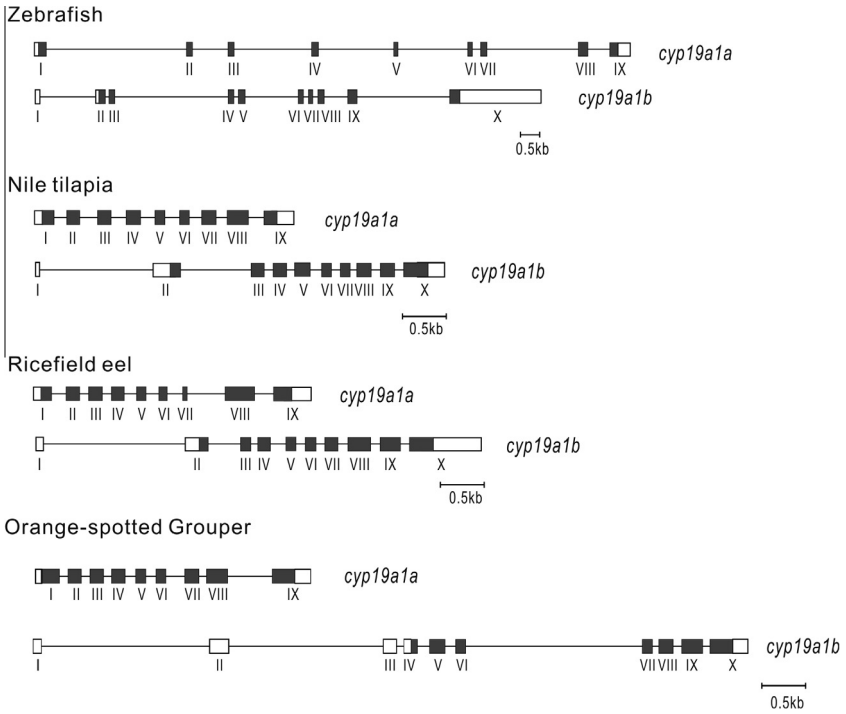


**Fig. 1.** Schematic diagram showing the relative positions of introns and exons of teleost *cyp19a1* genes, as modified from our previous report (Zhang et al., 2008). The exons are boxed and indicated by Roman numerals at the bottom, and the introns are depicted by lines. The translated exons are shaded. The genomic structure was determined by comparing the sequences of the corresponding cDNA and genomic DNA, and the following sequences were downloaded from *Entrez* (NCBI): Nile tilapia *cyp19a1a* gene (AF472620) and cDNA(U72071); Nile tilapia *cyp19a1b* gene (AF472621) and cDNA(AF295761); zebrafish *cyp19a1a* gene (NM131154) and cDNA(NC007129); zebrafish *cyp19a1b* gene (NM131642) and cDNA (NC007136); ricefield eel *cyp19a1a* gene (EU841366) and cDNA (EU252487); ricefield eel *cyp19a1b* gene (EU840259) and cDNA (EU252488); orange-spotted grouper *cyp19a1a* gene (Li, 2006) and cDNA (AY510711); orange-spotted grouper *cyp19a1b* gene (Li, 2006) and cDNA (AY510712).

is mainly expressed in the brain and encodes the brain aromatase P450aromB. In Japanese eel and European eel, teleosts belonging to an ancient group of elopomorphs (Ijiri et al., 2003; Jeng et al., 2005), only one copy of *cyp19a1* has been identified (Tzchori et al., 2004), most likely due to the loss of the other copy of *cyp19a1* during the evolution of this lineage (Cheshenko et al., 2008). Although it was once postulated that only one copy of *cyp19a1* is present in ricefield eel (Yu et al., 2008; Guiguen et al., 2010), we recently identified duplicated copies of *cyp19a1* in ricefield eel: *cyp19a1a*, predominantly expressed in the ovary, and *cyp19a1b*, predominantly expressed in the brain (Zhang et al., 2008). The sizes of *cyp19a1b* and *cyp19a1a* genes vary highly among teleosts, mostly due to the length of introns; however, the gene structures are highly conserved, with 8 introns and 9 exons in *cyp19a1a* and 9 introns and 10 exons in *cyp19a1b* (Fig. 1).

## 3. Syntenic analysis of *cyp19a1* loci in vertebrates

The evolutionary relationship of *cyp19a1* genes was examined with syntenic analysis in gnathostome vertebrates, with the exception of the platypus due to the incompleteness of its sequences. As information on *cyp19a1* in agnathostome vertebrates is still lacking, it was not included in this analysis. Among the representative vertebrates examined, the *cyp19a1* loci showed a highly conserved synteny, with the same neighboring genes and arrangement and without the insertion of other genes in most vertebrates (Fig. 2). The conserved syntenic region around the *cyp19a1* loci could be categorized into two conserved gene blocks: AP4E1/Tnfaip813/CYP19/GLDN/DMXL2 and SCG3/LysMD2/TMOD2/TMOD3/LED1/MAPK6/GNB5. In teleosts, duplicated copies of *cyp19a1* are present on different chromosomes and are associated with the conserved gene blocks. The syntenic region of *cyp19a1a* in teleosts contains gene blocks Tnfaip813/cyp19a1a/GLDN/DMXL2 and CG3/LysMD2/TMOD2/TMOD3/LED1/MAPK6, whereas the syntenic region of *cyp19a1b* contains gene block cyp19a1b/AP4E1/GNB5. Compared to tetrapods, the genes in the syntenic regions of *cyp19a1a* and *cyp19a1b* in teleosts appear to be complementary, and the sum of genes in both syntenic regions of *cyp19a1a* and *cyp19a1b* in teleosts (except the stickleback) is equal to the genes in the two conserved gene blocks around the *cyp19a1* loci of tetrapods.

The syntenic analysis of *Cyp19a1* loci also revealed some particular chromosomal rearrangements in mammals. In the horse genome, the two conserved gene blocks in the syntenic region around the *Cyp19a1* locus are inversed. Tandem duplication of *Cyp19a1* occurred in the cow genome, another mammalian species, in addition to pigs and peccaries (Corbin et al., 2007), which contains multiple copies of *Cyp19a1*. In the mouse genome, the syntenic region around the *Cyp19a1* locus only contains the conserved gene block Tnfaip813/CYP19/GLDN/DMXL2 but not the other one, implying that the Tnfaip813/CYP19/GLDN/DMXL2 gene block has undergone transposition (Chiang et al., 2001). Similarly in the genome of stickleback, the syntenic region around the *cyp19a1a* locus only contains the same gene block, Tnfaip813/CYP19/GLDN/DMXL2, as in mouse but not the other conserved gene block as in other teleosts. This finding suggests that the gene block containing *cyp19a1a* has also undergone transposition and the underlying mechanisms for the transposition of *cyp19a1* may be conserved in stickleback and mouse.

## 4. The origin and evolution of *cyp19a1* in teleosts

The well-conserved synteny of both *cyp19a1a* and *cyp19a1b* in teleosts with *cyp19a1* in tetrapods supports the idea that the teleost-duplicated *cyp19a1* genes arose from a whole-genome duplication event (Chiang et al., 2001). Furthermore, *cyp19a1* in gnathostome vertebrates appears to have evolved from the same ancestral *cyp19* gene, around which there are presumably two conserved gene blocks, AP4E1/Tnfaip813/CYP19/GLDN/DMXL2 and