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# Molecular cloning and gene expression of *Foxl2* in the Nile tilapia, *Oreochromis niloticus*

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**Abstract**

A *Foxl2* cDNA was cloned from the Nile tilapia ovary by RT-PCR and subsequent RACE. Alignment of known *Foxl2* sequences from vertebrates confirmed the conservation of the *Foxl2* open reading frame and protein sequences, especially the forkhead domain and C-terminal region, while some homopolymeric runs of amino acids are found only in mammals but not in non-mammalian vertebrates. RT-PCR revealed that *Foxl2* is expressed in the tilapia brain (B), pituitary (P), gill, and gonads (G), with the highest level of expression in the ovary, reflecting the involvement of Foxl2 in B–P–G axis. Northern blotting and in situ hybridization also revealed an evident sexual dimorphic expression pattern in the gonads. *Foxl2* mRNA was mainly detected in the granulosa cells surrounding the oocytes. The ovarian expression of *Foxl2* in tilapia begins early during the differentiation of the gonads and persists until adulthood, implying the involvement of *Foxl2* in fish gonad differentiation and the maintenance of ovarian function.


*Keywords:* *Foxl2*; cDNA cloning; Gene expression; Sexual dimorphic pattern; RT-PCR; Northern blot; In situ hybridization; Nile tilapia

The forkhead family of transcription factors is conserved in evolution and known to play critical roles in the regulation of cellular differentiation and proliferation [1]. Members of this group exhibit tissue-specific expression patterns and are in general involved in cell-type determination and differentiation. In the differentiation process, forkhead proteins are often involved in sustaining the proliferation of determined precursor cells, as well as in the expression of differentiated traits. In many cases, genes responsible for differentiation processes during embryonic development are later recycled to control metabolism in the adult [2].

*Foxl2* is a putative transcription factor involved in ovarian development and function. Its mutation in humans is responsible for the blepharophimosis syndrome, characterized by eyelid malformations and premature ovarian failure (POF) [3,4]. A recent study also found that it can interact with the gonadotropin releasing hormone (GnRH) receptor activating sequence to regulate GnRH receptor gene expression [5]. A comparative analysis of the *Foxl2* sequences of 10 vertebrate species, including 7 from mammals and 3 from fish, revealed that the entire open reading frame (ORF) is under purifying selection leading to strong protein conservation. Recent data on *Foxl2* transcript and protein expression indicate *Foxl2* (1) to be the earliest known sex dimorphic marker of ovarian determination/differentiation in vertebrates, and (2) to have, at least in mammals, an ovarian expression persisting until adulthood. The conservation of its sequence and pattern of expression suggests that *Foxl2* might be a key factor in the early development of the vertebrate female gonad and involved later in adult ovarian function [4–6].

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*Foxl2* has been well characterized in several mammals, such as human, mouse, and goat. Partial genomic sequences of *Foxl2* which lie in the forkhead domain were also obtained from chicken and red-eared slider turtle. In situ hybridization and RT-PCR analysis revealed a sexually dimorphic expression pattern in chicken and turtle, as well as in mouse: *Foxl2* was detected in early ovaries of all three species around the time of sex determination [6]. Complete genomic sequences of *Foxl2* are also available for several fish species, such as pufferfish, tetraodon, and zebrafish. However, to date, no full-length *Foxl2* cDNA has been cloned from any non-mammalian vertebrate. Here we report the isolation of *Foxl2* cDNA and its gene expression determined by RT-PCR, Northern blotting, and in situ hybridization in a teleost fish, the Nile tilapia.

## Materials and methods

*Animals.* Nile tilapia, *Oreochromis niloticus*, were kept in recirculating freshwater tanks at 26 °C prior to use. To obtain fry, artificially fertilized eggs were cultured in recirculating water at 26 °C. All genetic females (XX) and males (XY) were obtained by artificial fertilization of eggs from normal females (XX) with sperm from either sex reversed males (XX) or super males (YY), respectively. Genetic XX males were generated by treating fry with 17α-methyltestosterone (10 μg/g diet) from 7 to 20 days after hatching (dah) according to the method of Kobayashi et al. [7].

*Cloning of Foxl2 cDNA.* A 669-bp *Foxl2* cDNA fragment was amplified from pooled ovaries of fish 15 dah by RT-PCR using two degenerate primers (Table 1: Foxl-F1 and Foxl-R2). These degenerate primers were designed from the conserved forkhead domain and the C-terminal region of known cDNA sequences from mammals and genomic sequences of zebrafish and fugu, respectively.

Then 5′- and 3′-RACE were performed to obtain the 5′- and 3′-cDNA ends of *Foxl2* using the SMART RACE Kit (Clontech) according to the manufacturer's instructions. Six gene-specific primers (Table 1: from Foxl-F3 to Foxl-R8) were designed and used for RACE.

After the sequencing of the RACE products, a pair of gene-specific primers (Table 1: Foxl-F9 and Foxl-R10) was designed based on the end sequences of the cDNA and used to amplify the full-length cDNA.

Table 1
Sequence of primers used in RT-PCR and RACE

| Primer | Sequence |
|---|---|
| Foxl-F1 | 5′-GAGAAG(AC)G(TGC)CT(CT)ACGCTGTCCGG-3′ |
| Foxl-R2 | 5′-CCCA(AG)TA(AT)GAGCA(AG)TGCATCAT-3′ |
| Foxl-F3 | 5′-GCCGCGGGAGGGCGGCGGCGAGAGAA-3′ |
| Foxl-F4 | 5′-TTCCAGCCGGGGAAGGCCTTGTTC-3′ |
| Foxl-F5 | 5′-TCTCCTCCAGTAACGGAGCCGGCCTT-3′ |
| Foxl-R6 | 5′-TCGCCGCCGCCCTCCCGCGGCACCTT-3′ |
| Foxl-R7 | 5′-GAACAAGGCCTTCCCCGGCTGGAA-3′ |
| Foxl-R8 | 5′-AAGGCCGGCTCCGTTACTGGAGGAGA-3′ |
| Foxl-F9 | 5′-GGACAGTTTTCGACCCACTGGTGT-3′ |
| Foxl-R10 | 5′-AGGAGGTGAAGTATCCCCGTGCAG-3′ |
| β-Actin-F | 5′-GGCATCACACCTTCTACAACGA-3′ |
| β-Actin-R | 5′-ACGCTCTGTCAGGATCTTCA-3′ |

Then the product was sequenced again to confirm the nucleotide sequence.

Reverse transcription was carried out using SuperScript II (Gibco-BRL) and mRNA from adult tilapia ovaries according to the manufacturer's instructions. PCRs were run at 94 °C (1 min), 55–68 °C (1 min), and 72 °C (1.5 min) for 36 cycles using a Perkin–Elmer 480 thermal cycler.

*Nucleotide sequencing.* Bi-directional sequencing was performed by the dideoxy chain termination method using an ABI Prism 370 genetic analyzer with denatured, double-stranded DNA as template in combination with a Big Dye 3.1 DNA polymerase-based sequencing kit (Pharmacia).

*Phylogenetic analysis.* Alignments of nucleotide sequences and deduced protein sequences were performed with the multiple alignment software DNAstar and ClustalX. ClustalX was also employed to calculate trees using the N–J method [8]. TREEVIEW was used to display the phylogenetic tree [9]. The values represent bootstrap scores for 1000 trials, indicating the credibility of each branch. Except the tilapia *Foxl2* sequences (AY554172), all the *Foxl2* protein sequences were obtained from GenBank and public genome resources. Their GenBank accession numbers are: human (AF301906), goat (AY112725), mouse (AF522275), rat (AC105826), pig (AY340971), cow (AY340970), rabbit (AY340972), ciona (AJ534400), fugu (CAAB01001061), tammar wallaby (AY340969), red-eared slider turtle (AY155353), and rainbow trout (EST sequences: CA354643 and CA341688). The chicken Foxl2 sequences were obtained from the TIGR *Gallus gallus* Gene Index GgGI TC Report [http://tigrblast.tigr.org/tgi/ TC94008 (N-terminal), TC71544 (C-terminal)] and UCSC Genome Bioinformatics Site (http://genome.ucsc.edu/, February, 2004 assembly, the 35 amino acid gap between N- and C-terminal). The zebrafish and tetraodon sequences were obtained from public genome resources by interrogating them with the fugu *Foxl2* sequences.

*Analysis of Foxl2 expression by RT-PCR.* Total RNA (2.0–5.0 μg) was isolated from various tissues of adult fish containing either post-vitellogenic ovary or spermiating (sperm released when gentle pressure was applied to the abdomen) testis and treated with DNase I (Invitrogen, Carlsbad, CA, USA). Then, cDNA was synthesized and RT-PCR was employed for the analysis of *Foxl2* expression. Reverse transcription was carried out using SuperScript II (Gibco-BRL) and mRNA from adult tilapia tissues according to the manufacturer's instructions. The total RNA isolated from tissues was used as the initial template for RT-PCR. The PCR consisted of 2 min at 94 °C, followed by 33 cycles of 94 °C (30 s), 58 °C (30 s), and 72 °C (1 min), ending with 10 min of extension at 72 °C. A pair of *Foxl2* gene-specific primers (Foxl-F3 and Foxl-R6) was used to amplify 484-bp cDNA fragments by PCR.

A 342-bp tilapia β-actin fragment was amplified (27 cycles) to test the quality of the cDNA used in the PCR with a pair of β-actin primers (Table 1: β-actin-F and β-actin-R).

All the PCR products were electrophoresed on 1.5% agarose gels and the gels were stained with ethidium bromide to visualize the bands.

*Northern blot analysis.* Total RNA was extracted from the brain and gonads of adult fish using Isogen (Nippon Gene, Toyohama, Japan). Poly(A)$^+$ RNAs were purified using Oligotex-dT30 (Takara, Otsu, Shiga, Japan). Five micrograms of each mRNA was electrophoresed on a 1.5% formaldehyde agarose gel and transferred onto a nylon membrane (Hybond-N+, Amersham). Hybridization and signal analysis were performed as described previously [10,11].

*In situ hybridization.* Gonads were dissected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4, 4% PFA) at 4 °C overnight. After fixation, the tissues were embedded in paraffin and cross-sections were cut at 5 μm. Probes of sense and antisense digoxigenin (DIG)-labeled RNA strands were transcribed in vitro from linearized pGEM-T easy-*Foxl2* cDNA (AY554172) using a RNA labeling kit (Roche). In situ hybridization was performed as described previously [7].

## Results

### *Nile tilapia Foxl2 sequences*

The isolated *Foxl2* cDNA is 1745-bp long, with an ORF encoding a protein of 303 amino acids (aa). The nucleotide and deduced aa sequences of the isolated cDNA clone are shown in Fig. 1. Tilapia *Foxl2* contains the characteristic 110-aa DNA-binding domain, the forkhead which is also known as a "winged helix." However, it does not contain a polyalanine tract (A), nor does it contain any glycine, and proline repeats. It shows greatest similarity to the fugu (96.1%), tetraodon (95.1%), and zebrafish (79.6%) *Foxl2s*. It also shows around 60% similarity to mammalian *Foxl2s*.

### *Phylogenetic analysis*

Alignment of the fish, chicken, and mammalian Foxl2 sequences revealed that Foxl2 is rather conserved in terms of protein sequence, especially in the forkhead domain and the C-terminal region, while homopolymeric runs of aa, such as the polyalanine (A) tract and proline (P) and glycine (G) repeats, are less conserved. These homopolymeric runs are found in mammals but are absent in fish as well as in other non-mammalian vertebrates, including chicken (Fig. 2). Based on a complete alignment of 11 complete Foxl2 sequences and 4 partial sequences, a phylogenetic tree of Foxl2 was constructed using human FOXL1 as an outgroup. The high conservation of Foxl2s in vertebrates, especially

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AGA GAG AGG AAG GKC TGG TGC GTG TCG CGT ACT GAT CCG CAA AAT TAC AAT TAA GAT TTC      60
TCC GTG CGC AAT TTG GAG GAC AGT TTT CGA CCC ACT GGT GTA TCC TGA GAT TCT GCA GCG     120
CAG CAT TTC ACC GAT CGA GAC AGA AGC GTC TGA AAG GTG TTC TTT TTC AAA GTT TAG ACC     180
GGA CTC GTT TGT TTT GGT GTG TGC GCA ATG ATG GCC ACT TAC CAA AAC CCG GAG GAT GAC     240
                                    M   M   A   T   Y   Q   N   P   E   D   D    (11)
GCA ATG GCC CTA ATG ATC CAC GAC ACC AAC ACG ACC AAG GAG AAA GAG CGC CCA AAA GAG     300
 A   M   A   L   M   I   H   D   T   N   T   T   K   E   K   E   R   P   K   E    (31)
GAG CCG GTT CAG GAC AAA GTC TCT GAG AAG CCG GAT CCG TCC CAG AAA CCG CCG TAC TCC     360
 E   P   V   Q   D   K   V   S   E   K   P   D   P   S   Q   K   P   P   Y   S    (51)
TAT GTC GCT CTC ATT GCC ATG GCT ATC CGG GAG AGC TCC GAG AAG CGC CTC ACG CTG TCC     420
 Y   V   A   L   I   A   M   A   I   R   E   S   S   E   K   R   L   T   L   S    (71)
GGC ATA TAC CAG TAT ATA ATC ACC AAA TTC CCC TTC TAC GAG AAG AAC AAG AAA GGT TGG     480
 G   I   Y   Q   Y   I   I   T   K   F   P   F   Y   E   K   N   K   K   G   W    (91)
CAG AAC AGC ATC AGA CAC AAC CTC AGT CTT AAC GAA TGC TTC ATA AAG GTG CCG CGG GAG     540
 Q   N   S   I   R   H   N   L   S   L   N   E   C   F   I   K   V   P   R   E   (111)
GGC GGC GGC GAG AGA AAG GGG AAT TAC TGG ACC CTC GAC CCA GCC TGT GAG GAC ATG TTC     600
 G   G   G   E   R   K   G   N   Y   W   T   L   D   P   A   C   E   D   M   F   (131)
GAG AAG GGG AAC TAC AGG CGA CGC CGC AGG ATG AAG CGG CCT TTC AGA CCC CCA CCA ACG     660
 E   K   G   N   Y   R   R   R   R   R   M   K   R   P   F   R   P   P   P   T   (151)
CAC TTC CAG CCG GGG AAG GCC TTG TTC GGA GGG GAC AGC TAT GGC TAC CTT TCT CCA CCC     720
 H   F   Q   P   G   K   A   L   F   G   G   D   S   Y   G   Y   L   S   P   P   (171)
AAG TAC CTG CAG TCT AGC TTT ATG AAC AAC TCC TGG TCG TTG GGC CAG CCA CCC ACT CCG     780
 K   Y   L   Q   S   S   F   M   N   N   S   W   S   L   G   Q   P   P   T   P   (191)
ATG TCC TAC ACA TCC TGT CAG ATG GCC AGC GGC AAC GTG AGT CCG GTG AAC GTC AAG GGG     840
 M   S   Y   T   S   C   Q   M   A   S   G   N   V   S   P   V   N   V   K   G   (211)
CTG TCA GCC CCC TCA TCA TAT AAC CCT TAC TCC CGG GTG CAG AGC ATG GCG CTC CCC AGC     900
 L   S   A   P   S   S   Y   N   P   Y   S   R   V   Q   S   M   A   L   P   S   (231)
ATG GTG AAC TCT TAC AAT GGC ATG AGT CAC CAT CAC CAT CCC CAT CAT ACG CAG CAG CTG     960
 M   V   N   S   Y   N   G   M   S   H   H   H   P   H   H   T   Q   Q   L   (251)
AGC CCT GCC ACC GCG GCA CCA CCT CCG GTC TCC TCC AGT AAC GGA GCC GGC CTT CAG TTC    1020
 S   P   A   T   A   A   P   P   P   V   S   S   S   N   G   A   G   L   Q   F   (271)
GCG TGC TCC CGC CAG CCC GCG GAG CTC TCC ATG ATG CAC TGC TCA TAC TGG GAA CAC GAG    1080
 A   C   S   R   Q   P   A   E   L   S   M   M   H   C   S   Y   W   E   H   E   (291)
ACC AAA CAC TCG GCG TTA CAC ACG AGG ATT GAT ATT TGA AGT TTA CCA ACT GGT CCG TGC    1140
 T   K   H   S   A   L   H   T   R   I   D   I   *                               (303)
AAA AAT GAA GAC TCT GGG AGA GAG AGA CAG AGA GGT AAT TCC TGT GAT TTC AAA ACA GAG    1200
CCG ACA GTA TTT TGA AGA GAC CAC TCT GAC TCA GAT CTC TTC GGT TCC AAG CTG ACA TCT    1260
AAA TGC CGA CGG GGA GCT ATA TGC ACG AGG TGA TGC TGA GAA AGT TTT ATG AAA TGG AAA    1320
CAG GAG GTA AAG GAG TCA TCG GCT GCT CAC TCA TCA GGT AGC ACA ACC TCT GGG AGC CAT    1380
TTC GTG CGC CTG TAT TAC ACT TGG ACT GAT GGC CAT AAC TCG GTA TTG TAA AAT CAA AAT    1440
TGT GGA ATG AAA AAC TGA TTA TTT TTA ATG GAT AGA CAT TGC TGA CAT CGG TCC AGC TGT    1500
ATT TTT GGT GTG CGT AAT GAT TTA TCT CAG CTT TCC TTC GGC TTA CTG CCG AAA GAC GTC    1560
CTA ATG AAG TCA GAG TTT TAA TGT GTG ATT AAA TCA GAG CTA ATT TTG GCT TTC TTA GTA    1620
AGC TGC ACC GAA AAA GGC CTG CAC GGG GAT ACT TCA CCT CCT GCT ATA GTT GGA CAC TTT    1680
GTT AGG TTT AAA AGG GTG GGT AGA GAT CCA GCA ACT ACC AGA AAA AAA AAA AAA AAA AAA    1740
AAA AA                                                                            1745
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Fig. 1. Nucleotide and amino acid sequences of Nile tilapia *Foxl2* cDNA (GenBank Accession No. AY554172). The translated amino acid sequence is shown in standard one-letter code below the nucleotide sequence. Amino acid residues are numbered in parentheses.

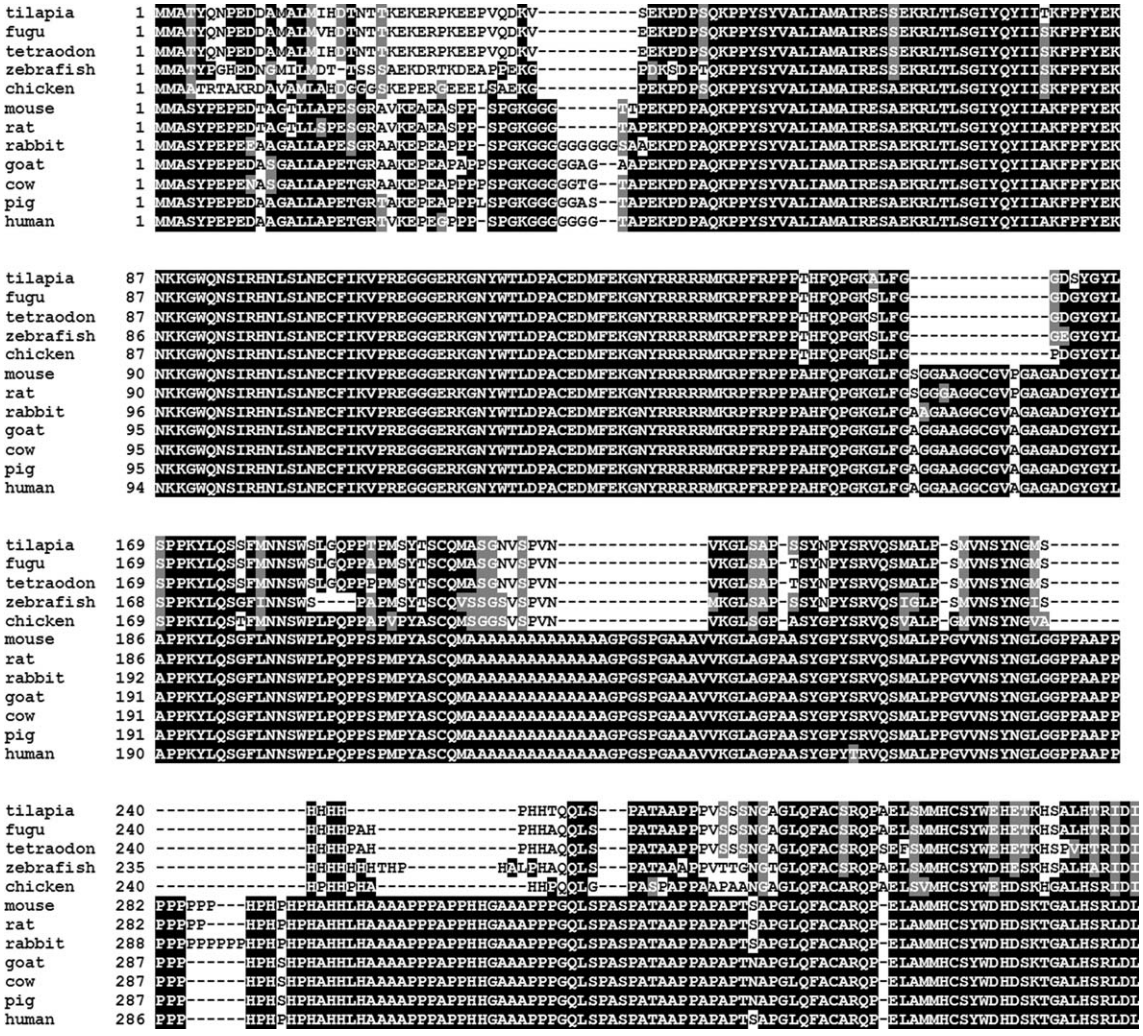


Fig. 2. Alignment of the amino acid sequences of tilapia Foxl2 with other Foxl2s from vertebrates. For GenBank accession numbers, refer to Materials and methods. BOXSHADE (http://www.ch.embnet.org/software/BOX_form.html) was used to make this figure.

among mammals and among fish, was clearly reflected by the short distance among different species (Fig. 3).

*Tissue distribution of Foxl2 mRNA*

Analysis of the tissue distribution pattern of *Foxl2* in tilapia by RT-PCR revealed that the gene is expressed in the brain, pituitary, gill, and gonads, with the highest level in the ovary (Fig. 4).

*Northern blot*

In adult tilapia, *Foxl2* showed a sexually dimorphic expression pattern in the gonads. A single transcript of about 2.0 kb was detected in the ovary, whereas no band was detected in the testis. On the other hand, the same expression levels were found in both male and female brain (Fig. 5). This is consistent with the tissue distribution revealed by RT-PCR.

*In situ hybridization*

To ascertain which population of cells in the developing gonads expresses *Foxl2*, in situ hybridization was performed using ovaries and testes from 50 to 100 dah tilapia. Specific signals were observed in the granulosa cells surrounding the previtellogenic oocytes but not in the oocytes (Figs. 6A and B). In contrast, no specific signals were detectable in testis (Fig. 6C). These results are consistent with the results of Northern blot analysis for gonads.

## Discussion

The conservation of *Foxl2* sequences has been well demonstrated in mammals as well as in fish. Meanwhile, some differences were found between fish and mammalian *Foxl2*, such as the lack of homopolymeric runs of aa

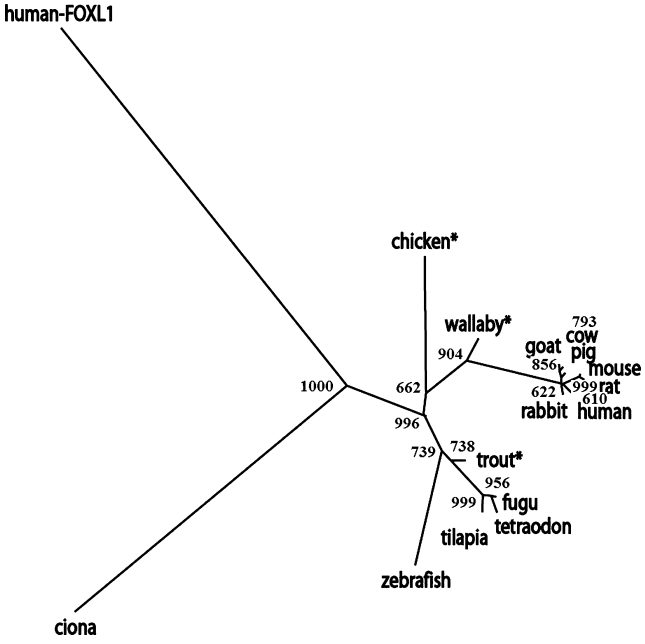


Fig. 3. Phylogenetic tree of *Foxl2* in vertebrates. The tree was rooted using human FOXL1 as the outgroup. Branch lengths are proportional to the number of amino acid changes on the branch. Partial sequences (*) may have artificially short branches. For GenBank accession numbers, refer to Materials and methods.

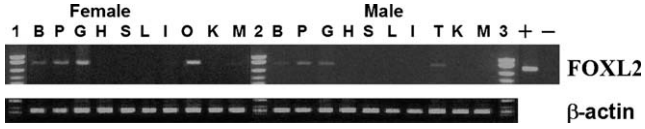


Fig. 4. RT-PCR analysis of *Foxl2* expression in various tissues of adult tilapia. B, brain; P, pituitary; G, gill; H, heart; S, spleen; L, liver; I, intestine; O, ovary; K, kidney; M, muscle; T, testis; + and –, positive and negative controls; and 1, 2, and 3, markers. Lower panel, β-actin as internal control.

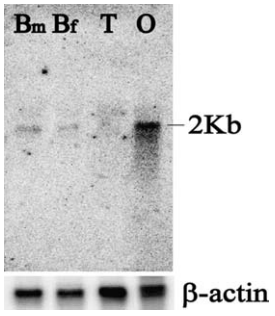


Fig. 5. Northern blot analysis of the expression of *Foxl2* in tilapia brain and gonads. The $^{32}$P-labeled tilapia *Foxl2* cDNA fragment was hybridized with 5 μg mRNA from testis (T) and ovary (O) and brain of male and female (Bf and Bm). The lower panel shows the same membrane after stripping and hybridization with the tilapia β-actin probe.

in fish [4]. The conservation of *Foxl2* sequences among vertebrates and the differences found between mammals and fish were further confirmed by this study. Formerly,

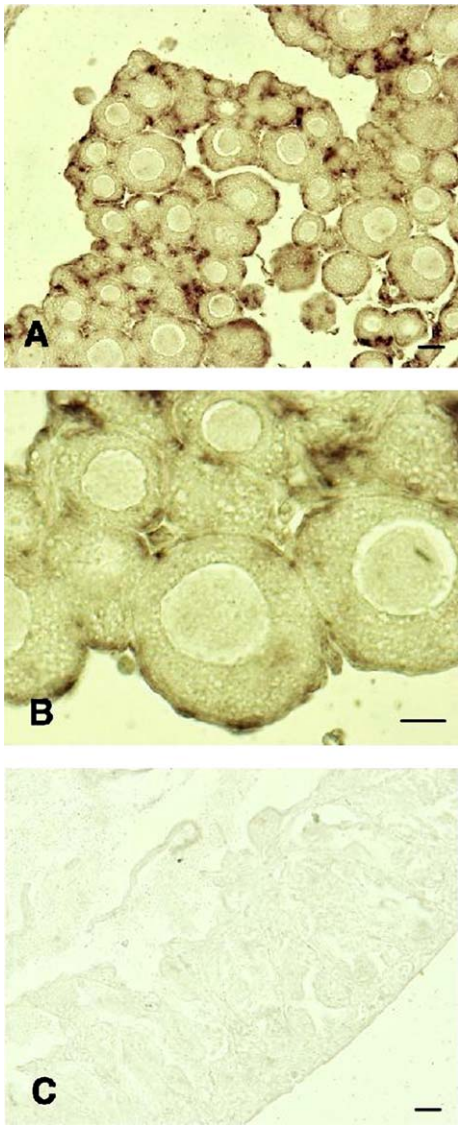


Fig. 6. Localization of *Foxl2* mRNA in gonads determined by in situ hybridization. (A) Ovary at 50 dah. Specific signals for *Foxl2* RNA were observed in follicle cells surrounding the previtellogenic oocytes and some interstitial cells. No signals were detected in oocytes. (B) Higher magnification of section A. Specific signals in follicle cells surrounding the oocyte were mainly found in pregranulosa cells. (C) Testis at 100 dah. No specific signals for *Foxl2* RNA were detectable. Scale bar, (A,C) 50 μm; (B) 20 μm.

except sequences from mammals and fish, only partial genomic sequences of *Foxl2*, which lie in the forkhead domain, were available for chicken and turtle. However, as no complete *Foxl2* sequences from these vertebrate groups were available, it was impossible to judge whether such sequence differences between fish and mammals can be applied to all non-mammalian species. In this study, we successfully obtained two chicken *Foxl2* EST sequences encoding the N- and C-terminal regions, with only a 35 aa gap in the middle (from 166 to 200 of the chicken 305 aa *Foxl2*). Later on, we explored the UCSC Genome Bioinformatics Site

(http://genome.ucsc.edu/, February, 2004 assembly) by sequence homology search and successfully identified the 35 aa gap sequence. Alignment of vertebrate Foxl2s including the complete chicken Foxl2 sequences clearly answered the aforementioned question: the 14 aa polyalanine tract, as well as other homopolymeric runs of aa, such as proline and glycine repeats, is only conserved in mammals. These features are absent in non-mammalian vertebrates (Fig. 2). A similar situation was observed for the transcription factor *DAX1*. The three and half amino-terminal repeats with probable DNA-binding activity have been conserved among mammalian *DAX1s*, however, in non-mammalian vertebrates, including the Nile tilapia, the cloned *DAX1s* lack the 3.5 amino-terminal repeats [10]. Differences between mammalian and fish *Foxl2* have also been reported in the 5′-flanking regulatory region. After comparing a 1000-bp genomic sequence immediately upstream of the putative transcription start site from human and mouse, Udar et al. [12] identified 243 bp of the sequence that is highly conserved. However, this sequence is not conserved in *Fugu rubripes*. The disorder known as blepharophimosis/ptosis/epicanthus inversus syndrome (BPES), which is characterized by a distinctive eyelid abnormality, occurs in two forms. In type I BPES, eyelid abnormalities are associated with ovarian failure. Type II BPES shows only the eyelid defects. *Foxl2* was mutated to produce truncated proteins in type I BPES and larger proteins in type II. Individuals with type II BPES have an expansion of polyalanine repeats 3′ to the forkhead domain [13]. A major difference between mammals and fish is that fish lack a functional eyelid, which appeared later from amphibians. Furthermore, type II BPES has never been reported in non-mammalian vertebrates with a functional eyelid. Taking all these into consideration, we propose that the sequence differences between mammalian and non-mammalian *Foxl2* may be responsible for the differences in eyelid structure and function between these vertebrates.

RT-PCR revealed that the tilapia *Foxl2* is expressed in the brain (B), pituitary (P), gill, and gonads (G), with the highest levels found in the ovary. This expression pattern indicates that the B–P–G axis is the main target tissue of Foxl2 in fish. Recently, it was reported that Foxl2, together with Smads and AP1, interacts with a receptor activating sequence to regulate GnRH receptor gene expression in mammals [5]. It seems likely that fish Foxl2 executes its function through the transcriptional regulation of the GnRH–GtH–sex steroids pathway. It is interesting that *Foxl2* was also expressed in the gill, an organ unique to aquatic fish, as this may indicate a novel function of Foxl2 in vertebrate evolution.

By Northern blotting, a single transcript of about 2.0 kb was detected in the ovary and very weak bands were detected in both male and female brain, whereas no band was detected in the testis because of the relatively low sensitivity of the technique compared to RT-PCR. Consistently, in situ hybridization also gave strong signals in the differentiating (50 dah fish) and adult ovary whereas no signal was detected in the testis. In fact, the expression of *Foxl2* in the ovary might have started even earlier as the first 669-bp tilapia *Foxl2* cDNA fragment was amplified from pooled ovaries of 15 dah fish. The expression in the ovary is restricted to the somatic compartment: the granulosa (follicular) cells and some interstitial cells. No signal has been observed in the oocyte. This is very similar to the situation in mammals [3,14]. However, by RT-PCR, low levels of *Foxl2* transcript were detected in the adult tilapia testis. Whether these transcripts are translated into protein remains a question. Low levels of *Foxl2* transcripts have also been observed in the goat and mouse testis, at fetal and adult stages, but no protein could be found in any of those tissues [3,14,15].

Our data showed for the first time that the *Foxl2* ovarian expression in fish begins early in development and persists until adulthood, which is a conserved feature of vertebrates as well demonstrated in mammals, birds, and reptiles. On the other hand, the expression of Foxl2 in the brain, pituitary and gill of tilapia suggests other novel functions of this gene, particularly in the case of the gill which is unique to the aquatic teleost. Further studies are highly warranted.

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