Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 307 (2003) 165–171
BBRC
www.elsevier.com/locate/ybbrc

Alternative splicing and differential expression of *P450c17* (*CYP17*) in gonads during sex transformation in the rice field eel

Hongshi Yu, Hanhua Cheng, Yiqing Guo, Laixin Xia, and Rongjia Zhou*

Molecular and Developmental Genetics Laboratory, Department of Genetics and Center for Developmental Biology, College of Life Sciences, Wuhan University, Wuhan 430072, PR China

Received 25 April 2003

Abstract

Several mechanisms were used in determination of the development of the male or female of vertebrates. The genes for determination of sequential hermaphrodite sex are unknown. Here, we reported cloning, alternative splicing, and expression patterns of the *CYP17* gene of the rice field eel, a teleost fish with a characteristic of nature sex reversal. The *CYP17* gene of the rice field eel was clustered into the *CYP17* gene group of all the other vertebrates, especially into the fish subgroup. Four isoforms of the *CYP17* were generated in gonads by alternative splicing and polyadenylation. Alternative splicing events of all these isoforms occurred in 3′ regions, which encoded three different sizes (517, 512, and 159 aa) of proteins. RT-PCR results indicate specific expression in gonads of these isoforms. Northern blot analysis shows that expression patterns of the *CYP17* (dominantly expressed in testis, less in ovary, and the least in ovotestis) are consistent with the sex reversal process of the rice field eel. In situ hybridization further shows its specific expression in germinal lamellae, the gonadal epithelium of the gonads. These findings indicate that *CYP17* is differentially regulated in a sex- and developmentally specific manner, suggesting that the *CYP17* potentially has important roles in gonad differentiation during sex reversal of the rice field eel.

Keywords: Alternative splicing; Sex reversal; CYP17; The rice field eel

Morphologically distinct males and females are observed throughout the animal kingdom. A variety of mechanisms are used in determination/differentiation of two sexes, including X- and Y-chromosome heterogametes in male mammals, Z- and W-chromosome heterogametes in female birds, and a temperature dependent sex determination in reptiles. Some species are hermaphrodite, while others can belong sequentially first to one sex and then to the other. Molecular and evolutionary mechanisms for such a variety of strategies are still not completely understood, although several genes involved in sexual development are identified, including *SRY*, *SOX9*, *SF1*, *DAX1*, *WT1*, and *DMRT1* [1–11]. The *SRY* is a major testis-determining gene and only conserved in mammals [7,9]. The *SOX9* exists in vertebrates for both sex differentiation and chondrogenesis [4,6,11]. *DMRT1* is the only one characterized to date containing a DM domain that is conserved and functionally related among phyla, at least in *Drosophila* (*doublesex*), *Caenorhabditis elegans* (*mab-3*), and vertebrates (*DMRT1*/*DMY*) [8,12–14]. However, the cascade of sex determination/differentiation in vertebrates is still waiting for answer. Studies on alternative sex differentiation systems for comparison and compensation are helpful in understanding the evolution of sexual development in vertebrates.

The developing testis produces testosterone which is an important hormone in male sex differentiation, responsible for the stabilization and differentiation of the Wolffian ducts into seminal vesicles, epididymides, and vasa deferens. Testosterone is synthesized from cholesterol in a series of steps requiring several enzymes, including P450c17 (CYP17, or 17α-hydroxylase/c17,20-lyase). The gene *CYP17* has been identified in several species, including mammals, frogs, chicken, Songbird, rainbow trout, spiny dogfish shark, and Japanese eel [15–23]. The *CYP17* is mainly expressed in both gonads

* Corresponding author. Fax: +86-27-8788-2661.
E-mail address: rjzhou@public.wh.hb.cn (R. Zhou).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)01137-9

and adrenals, consistent with a role in gonadal steroidogensis.

The rice field eel, *Monopterus albus*, taxonomically belongs to teleosts, the family Synbranchidae of the order Synbranchiformes (Neoteleostei, Teleostei, and Vertebrata), and it is also the only representative species of the group of Synbranchidae in China. This freshwater fish is not only an economically important species of southeast Asia for food production, but also a good model for comparative genomic studies of distantly related vertebrate processes, such as sexual development, because of its special evolutionary status, relatively small genome size, and natural sex reversal from female via intersex into male during its life [24]. Several genes potentially involving in sexual development in the rice field eel have been identified in our laboratory, including two *Sox9* [25], *Sox17* [26], and *Dmrt1* genes (manuscript submitted). To get further insight into the evolutionary and developmental mechanisms of sexual differentiation in this special species, we report the cloning of *P450c17* (*CYP17*) and first finding of its alternative splicing in gonads, as well as its expression pattern during sex transformation.

Materials and methods

Animals. The rice field eels were obtained from markets in the Wuhan area in China. The sexes were confirmed by microscopic analysis of their gonad sections.

RACE analysis and cloning of P450c17. SMART cDNAs were reverse transcribed from the RNAs of gonads of the rice field eel, using the SMART cDNA library construction kit (Clontech). 5′ RACE was performed using common SMARTIII primer, 5′ AAGCAGTGGTAT CAACGCAGAGTGGCCATTACGGCCGGG 3′, and P450 domain primer, 5′ TCTTTCCCGTTTCTGGTC 3′ designed based on the partial sequence we cloned. We performed 3′ RACE using common CDSIII primer 5′ ATTCTAGAGGCCGAGGCGGCCGACATG-$d(T)_{30}N_{-1}N$ 3′ (N = A, G, C, or T; N_{-1} = A, G, or C) and P450 domain primer, 5′ CAAAGTCATCATTGTCAACCAGCAC3′. After the PCR, nested PCRs were done using the same 5′ primer SMARTIII and nested primer 5′ ATATTTGCTGGAAGGCCAAGAACTG3′. PCR cycling conditions were: 35 cycles, with 30 s, 94 °C; 40 s, 64 °C or 56 °C, 120 s, 72 °C, in a 20 μl reaction mix containing 10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM dNTP, 0.2 μM each primer, and 1 U *Taq* DNA polymerase. Full lengths of cDNAs alternatively spliced were verified by following RT-PCR and sequencing analysis.

RT-PCR. Reverse transcription PCR was used to amplify individual isoforms of the *CYP17* gene from different tissues of the rice field eel. Reverse transcription was performed using M-MLV RT system (Promega, USA) with 0.5 μg of oligo$(dT)_{12–18}$ and 2 μg of total RNA in a 25 μl reaction. PCR were performed in a 20 μl reaction mix containing 10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM dNTP, 0.2 μM each primer, 1 U *Taq* DNA polymerase and 1 μl first-strand cDNA products. Amplification conditions were: 94 °C, 40 s; 62 °C (*CYP17a*), or 51 °C (*CYP17b*), or 48 °C (*CYP17c*), 40s; 72 °C, 100 s for 35 cycles, and 94 °C, 30 s; 58 °C, 40 s; 72 °C, 40 s for 20 cycles for *β-actin*. Primers are as the following:

CYP17a, 5′ CCGGAATTCATGGATATAACTTGGTTTCTA 3′ and 5′ CCTCTCGAGTTACGCCTGGCACTTGTTT 3′;
CYP17b, 5′ CCGGAATTCATGGATATAACTTGGTTTCTA 3′ and 5′ CCTCTCGAGTTATCCAGGATGACAAAG 3′;
CYP17c, 5′ CCGGAATTCATGGATATAACTTGGTTTCTA 3′ and 5′ CCTCTCGAGCTAGACAGGAGTGTACTTAC 3′; and
β-actin, 5′ TCCCTGTATGCCTCTGGT 3′ and 5′ ATGTCACGC ACGATCTCA 3′.

Sequence and phylogenetic analysis. All *CYP17* DNA and protein sequences from all species were aligned using Vector NT and analyzed by BLAST of GenBank online. A phylogenetic tree was constructed using Neighbor-joining method (Clustal X, 1000 runs) and viewed with TREE-view 1.6.6.

Southern and Northern blotting hybridization. Genomic DNAs were extracted from the blood of the rice field eel according to routine protocol, digested with *Eco*RI, electrophoresed in 0.8% agarose gel, and blotted onto a nitrocellulose filter. The filters were probed with the [α-^{32}P]dCTP-labeled *CYP17a2* cDNA (1.6 kb including the conserved P450 domain) and autographed. Northern blotting was performed according to routine protocol, except that hybridization at 42 °C was performed in ULTRAhyb solution (Ambion) with [α-^{32}P]dCTP-labeled *CYP17a2* (1.6 kb including the conserved P450 domain) cDNA as a probe.

In situ hydridization analysis. For in situ hybridization to gonadal sections, antisense and sense RNA probes were prepared separately from a region including P450 domain of *CYP17a2* (1.6 kb) of the rice

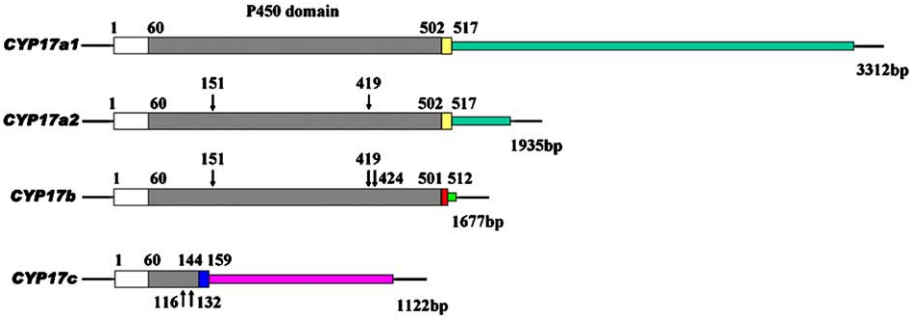

Fig. 1. Diagram illustrating the isoforms of the *CYP17* gene of the rice field eel generated by alternative splicing and polyadenylation. The *CYP17* is transcribed to form different isoforms of mRNAs: *CYP17a1*, *CYP17a2*, *CYP17b*, and *CYP17c*, which may code P450c17 proteins with different amino acids (numbers above each line), respectively. P450 domains are indicated by shaded boxes. Sequences from aa 1–501 or 502 are common among the transcripts except of the isoform *CYP17c*. Alternatively spliced regions in 3′ region are showed by different colors. The numbers in the end under the lines indicate nucleotide numbers of these cDNAs. The numbers with arrowheads indicate the different amino acids compared with *CYP17a1*. GenBank Accession Nos. are: AY224681–AY224684 for *CYP17a1–CYP17c*.

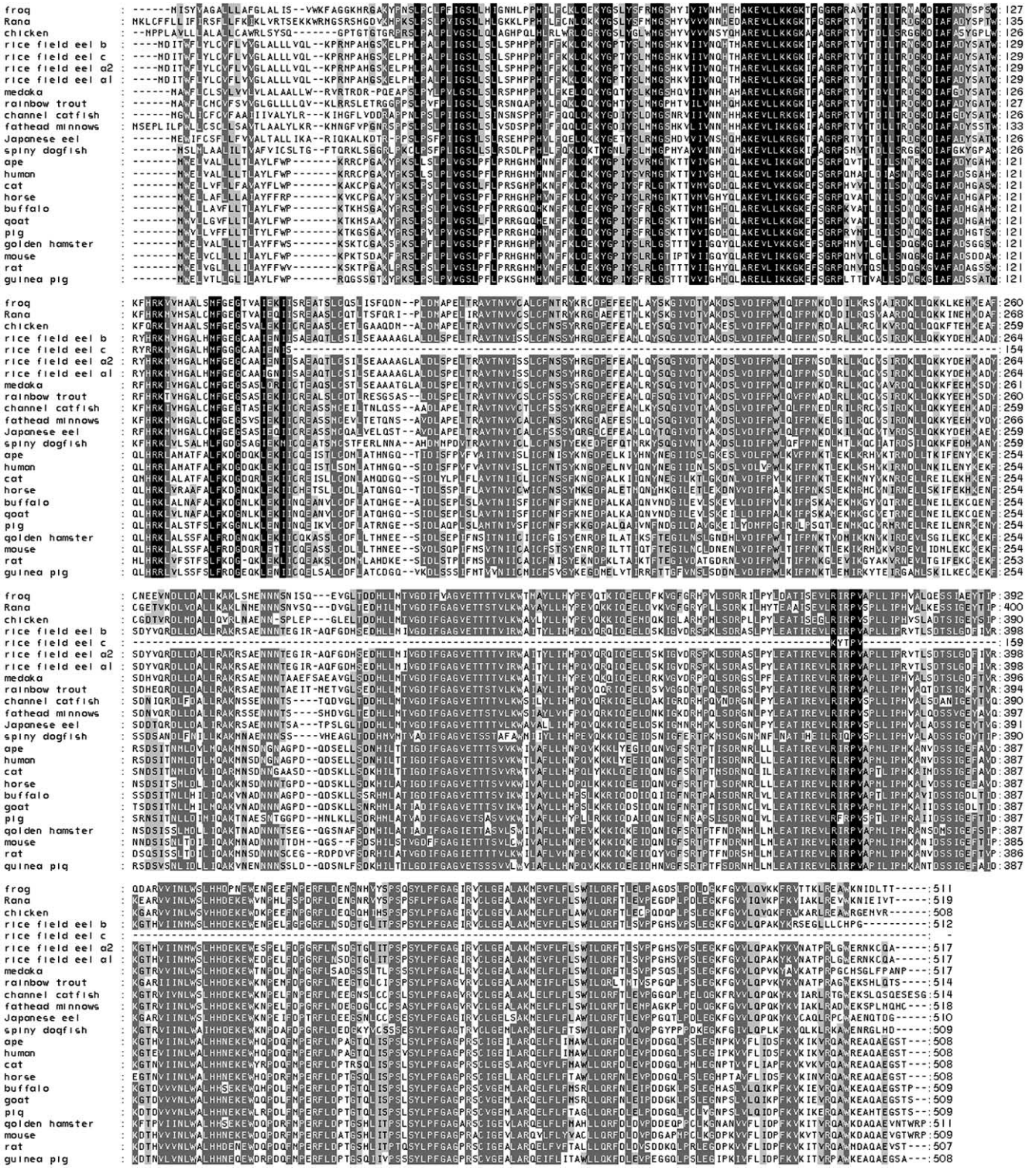

Fig. 2. Alignment of amino acid sequences of the *CYP17* genes from the rice field eel, ape (monkey) (AAG10599), buffalo (AAG02227), cat (AAG02226), channel catfish (O73853), chicken (P12394), pig (S22339), fathead minnows (CAC38768), frog (AAG42003), goat (AAF65823), golden hamster (P70687), horse (BAA06350), human (AAA52140), medaka (P70085), mouse (AAL12229), guinea pig (Q64410), rainbow trout (P30437), Rana (O57525), rat (NP036885), spiny dogfish (Q92113), and Japanese eel sequence from [18].

field eel and labeled with digoxigenin-UTP, using SP6 or T7 RNA polymerase separately (SP6 for production of sense probe, T7 for antisense probe). Gonads tissues were cryosectioned and the sections were immediately hybridized (42 °C) and hybridization signals were detected by NBT/BCIP system according to the manufacturer's instructions (Boehringer).

Results

The CYP17 is alternatively spliced in gonads of the rice field eel

In an attempt to isolate the *CYP17* gene from gonads of the rice field eel to further understand molecular mechanisms involved in sex reversal, we first used a 5′ and 3′ RACE analysis approach. While the 5′ RACE showed one band after PCR and gel running, multiple bands were observed in 3′ RACE and nested PCR. All these bands were cloned into the vector for sequencing. After we obtained both 5′ and 3′ half regions of *CYP17* sequence, which overlap in the P450 domain region, full length of the *CYP17* sequence was amplified by PCR based on the sequence information of the 5′ and 3′ ends of this gene. Interestingly, four 3′ regions of different *CYP17* transcripts were obtained. After sequencing analysis confirmation, three of them (*CPY17a1*, *CPY17b*, and *CPY17c*) were alternatively spliced forms and one transcript (*CPY17a2*) was generated by alternative polyadenylation. These alternative transcripts were further confirmed by PCR amplification and sequencing of full length of their cDNAs, respectively. These isoforms may encode different lengths (517, 512, and 159 aa) of proteins.

The 5′ region and P450 domain are common and alternative splicing sites occurred only in the 3′ region (Fig. 1). *CYP17c* was truncated in the P450 domain as alternative splicing. The highest level of conservation was within the P450 domain of the proteins, especially in the 5′ region of the domain, when compared with those

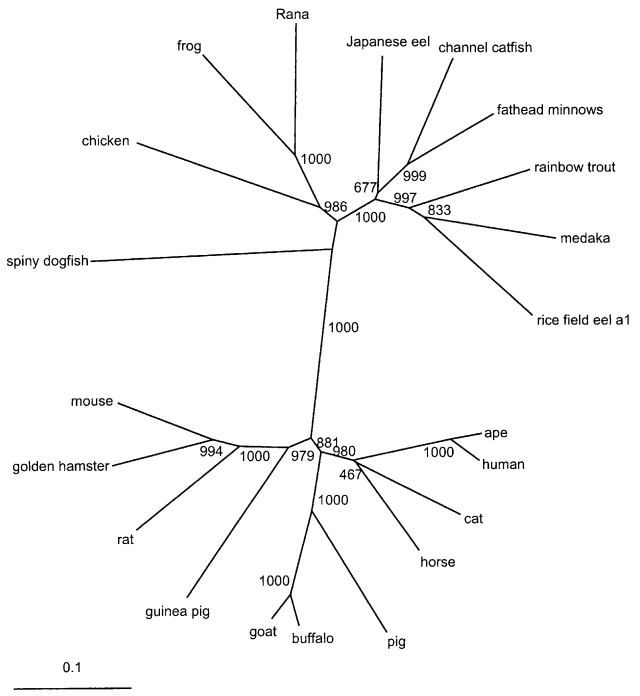

Fig. 3. Phylogenetic tree connecting all other CYP17 proteins of vertebrates. Neighbor-joining method was used to construct this tree (1000 runs). The CYP17 tree consists their taxonomy. GenBank accession numbers are the same as in Figs. 1 and 2.

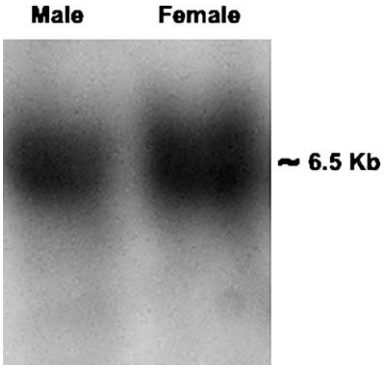

Fig. 4. Southern blot of genomic DNA from blood cells of both male and female rice field eels after hybridization with [α-^{32}P]dCTP-labeled *CYP17a* cDNA as a probe.

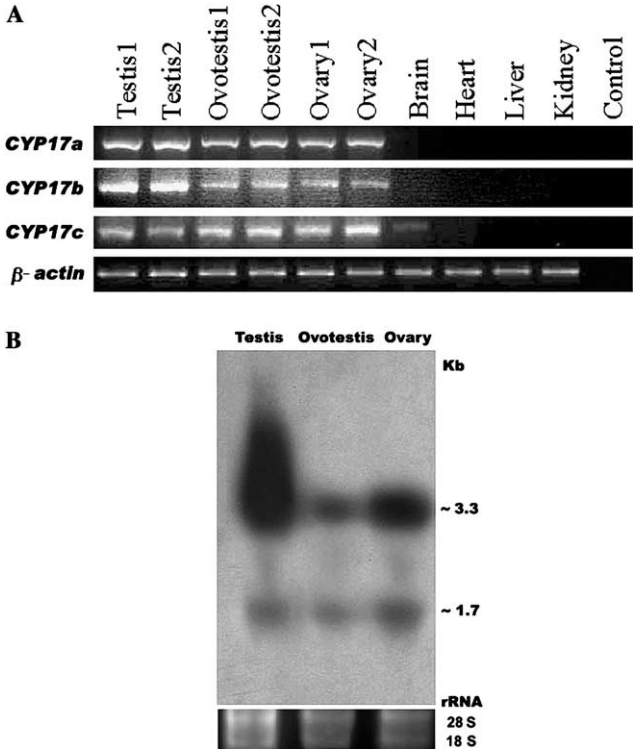

Fig. 5. RT-PCR and Northern blot analysis of the expression of the rice field eel *CYP17*. (A) RT-PCR of the *CYP17* of the rice field eel shows their expression in three kinds of gonads. There is no expression in other tissues besides a faint band observed in brain for the *CYP17c*. RT-PCR with *β-actin* primers (bottom panel) was used as a control. A negative control with water was also included in each experiment. RT-PCRs were done repeatedly twice in each tissue for confirmation. (B) Northern blot analysis of the *CYP17* expression in testis, ovotestis, and ovary of the rice field eel. The *CYP17* expression is dominant in testis, less dominant in ovary, but of low expression in ovotestis of intersex. 18S and 28S rRNA bands are shown at the bottom as RNA loading control.

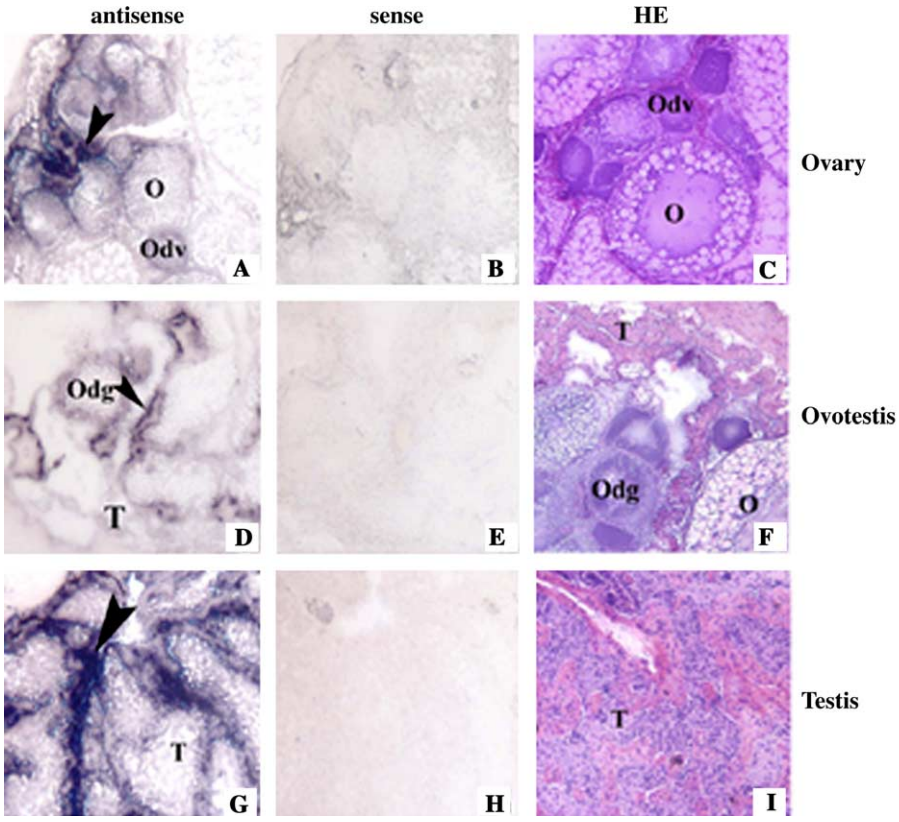

Fig. 6. Expression analysis of *CYP17* of the rice field eel by in situ hybridization to gonad sections of female, intersex, and male rice field eel. (A), (D), and (G), antisense probed for *CYP17* shows expression of these transcripts in gonadal lamellae or epithelium (arrowheads) of female, intersex, and male, respectively. Sense probing as control (B), (E), and (H) and H.E. staining (C), (F), and (I) in the gonad samples of the three sexes are shown on the right panel. O, ova; Odv, developing ova; T, seminiferous tubules; and Odg, degenerating ova.

of mammals, amphibians, fishes, and birds (Fig. 2). These transcripts of *CYP17* of the rice field eel are clustered into the *CYP17* gene group of all the other vertebrates, especially into the fish subgroup (Fig. 3). In the genome of the rice field eel, an identical single band (about 6.5 kb) was observed in both male and female DNAs, when the *CYP17* as a probe to hybridize the genomic DNAs digested with enzyme *Eco*RI (Fig. 4).

CYP17 is specifically expressed in gonads and dominantly in the testis

RT-PCR was carried out on gonads of female, intersex, and male and other adult tissues to analyze individual transcript expression of the *CYP17* during gonadal transformation from female via intersex to male of the rice field eel. All these isoforms were specifically expressed in the three kinds of gonads (Fig. 5A), but a very faint band was observed in brain. Northern blot analysis was used to investigate differences in expression among the three forms of gonads (Fig. 5B). Dominant expression of the 3.3 kb form (*CYP17a1*) was observed in testis, less in ovary, and at low levels in ovotestis, and another band of 1.7 kb (*CYP17b*) was also observed. A very faint band was detected between the bands of 1.7 and 3.3 kb (*CYP17a2*), while *CYP17c* was not detected by Northern blot analysis, but RT-PCR shows its expression, suggesting that it is the lowest expressed isoform. These data show that the four isoforms have different amounts of expression in the transcriptional level.

The CYP17 was expressed in the gonadal lamellae

In order to gain insight into the role of the *CYP17* gene in sex differentiation in this species, we analyzed the gene expression patterns in the three forms of gonads by in situ hybridization to gonad sections (Fig. 6). In all the three sexes, *CYP17* expressions were restricted to the gonadal lamellae with bipotential capacity to form testis, ovotestis, and ovary, and there was no expression in the developing germ cells.

Discussion

Although alternative splicing is known to increase diversity of expression mRNA transcripts, functional significance for the vast majority of alternative splicing events is unknown. We report here for the first time that

the *CYP17* gene of the rice field eel is alternatively spliced in gonads. Alternative splicing events of genes involved in sexual development have been observed in a few occasions. The *Drosophila Dsx* gene controls somatic sexual differentiation by producing alternatively spliced mRNAs with different 3′ regions encoding related sex-specific protein DSX^m in males and DSX^f in females [3]. Some other genes are also alternatively spliced and play a crucial role in sexual development in mammals, for example *WT1* [2,5]. Recent studies have shown that the SRY and SOX factors play a role in pre-mRNA splicing in mammalian cells [27]. Moreover, our recent studies have also shown that the *DMRT1* is alternatively spliced in gonads of a number of vertebrate species (manuscript submitted). Thus, it seems that regulation at the transcriptional level, especially by alternative splicing, is an important mechanism governing the sex determination/differentiation cascade. Although the *CYP17* genes of several vertebrates (including mammals, frogs, chicken, Songbird, rainbow trout, spiny dogfish shark, and Japanese eel) have been identified, alternative splicing events of these genes have not been reported. The identification of alternative splicing of *CYP17* gene in the rice field eel and their specific expression patterns in sexual development will help in understanding sexual differentiation of this species.

Sex transformation in the rice field eel occurs naturally during its life from female, via intersex, to male. During this process, which is genetically determined, the ovary will gradually transform into ovotestis, and then become a testis. The expression patterns of the *CYP17* (dominantly expressed in testis, less in ovary, and the least in ovotestis) are consistent with the sex reversal process of the rice field eel. Moreover, the expression of the gene is restricted to a key region of sex differentiation, the germinal lamellae (the gonadal epithelium), from where different germ cells will differentiate. Mouse *CYP17* expression begins to appear in the genital ridge at E11.5, just after *Sry* and *Dmrt1*, is abundant at E18.5, and is also expressed in Leydig cells of testis and theca cell of ovary [28]. While the Sry is a key testis-determining factor in mammals [7], and *Dmrt1*/*DMY* is a prime candidate for sex-determining gene in some fish species, such as the medaka [12,13], we reason that the *CYP17* may potentially have an important role in gonad differentiation of some vertebrate species with sex transformation characteristic, such as the rice field eel.

Acknowledgments

The authors thank Dr. Ken McElreavey of Institut Pasteur of France for his comments on the manuscript. The work was supported by the National Natural Science Foundation of China, Hubei Provincial Science and Technology Department, and Wuhan Municipal Science and Technology Department.

References

[1] J.C. Achermann, M. Ito, P.C. Hindmarsh, J.L. Jameson, A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans, Nat. Genet. 22 (1999) 125–126.

[2] S. Barbaux, P. Niaudet, M.C. Gubler, J.P. Grunfeld, F. Jaubert, F. Kuttenn, C.N. Fekete, N. Souleyreau-Therville, E. Thibaud, M. Fellous, K. McElreavey, Donor splice-site mutations in WT1 are responsible for Frasier syndrome, Nat. Genet. 17 (1997) 467–470.

[3] K.C. Burtis, B.S. Baker, *Drosophila* doublesex gene controls somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides, Cell 56 (1989) 997–1010.

[4] P. de Santa Barbara, N. Bonneaud, Boizet, B.M. Desclozeaux, B. Moniot, P. Südbeck, G. Scherer, F. Poulat, P. Berta, Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Mullerian hormone gene, Mol. Cell. Biol. 18 (1998) 6653–6665.

[5] A. Hammes, J.K. Guo, G. Lutsch, J.R. Leheste, D. Landrock, U. Ziegler, M.C. Gubler, A. Schedl, Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation, Cell 106 (2001) 319–329.

[6] J. Kent, S.C. Wheatley, J.E. Andrews, A.H. Sinclair, P. Koopman, A male-specific role for *SOX*9 in vertebrate sex determination, Development 122 (1996) 2813–2822.

[7] P. Koopman, J. Gubbay, N. Vivian, P. Goodfellow, R. Lovell-Badge, Male development of chromosomally female mice transgenic for SRY, Nature 351 (1991) 117–121.

[8] C.S. Raymond, C.E. Shamu, M.M. Shen, K.J. Seifert, B. Hirsch, J. Hodgkin, D. Zarkower, Evidence for evolutionary conservation of sex-determining genes, Nature 391 (1998) 691–695.

[9] A.H. Sinclair, P. Berta, M.S. Palmer, J.R. Hawkins, B.L. Griffiths, M.J. Smith, J.W. Foster, A.M. Frischauf, R. Lovell-Badge, P.N. Goodfellow, A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif, Nature 346 (1990) 240–244.

[10] A. Swain, V. Narvaez, P. Burgoyne, G. Camerino, R. Lovell-Badge, Dax1 antigonizes Sry action in mammalian sex determination, Nature 391 (1998) 761–767.

[11] V.P. Vidal, M.C. Chaboissier, D.G. De Rooij, A. Schedl, Sox9 induces testis development in XX transgenic mice, Nat. Genet. 28 (2001) 216–217.

[12] M. Matsuda, Y. Nagahama, A. Shinomiya, T. Sato, C. Matsuda, T. Kobayashi, C.E. Morrey, N. Shibata, S. Asakawa, N. Shimizu, H. Hori, S. Hamaguchi, M. Sakaizumi, DMY is a Y-specific DM-domain gene required for male development in the medaka fish, Nature 417 (2002) 559–563.

[13] I. Nanda, M. Kondo, U. Hornung, S. Asakawa, C. Winkler, A. Shimizu, Z. Shan, T. Haaf, N. Shimizu, A. Shima, M. Schmid, M. Schartl, A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*, Proc. Natl. Acad. Sci. USA 99 (2002) 11778–11783.

[14] C.S. Raymond, M.W. Murphy, M.G. O'Sullivan, V.J. Bardwell, D. Zarkower, Dmrt1, a gene related to worm and fly sexual regulators, is required for mammalian testis differentiation, Genes Dev. 14 (2000) 2587–2595.

[15] H.R. Fevold, M.C. Lorence, J.L. McCarthy, J.M. Trant, M. Kagimoto, M.R. Waterman, J.I. Mason, Rat P450(17 alpha) from testis: characterization of a full-length cDNA encoding a unique steroid hydroxylase capable of catalyzing both delta 4- and delta 5-steroid-17,20-lyase reactions, Mol. Endocrinol. 3 (1989) 968–975.

[16] F. Freking, T. Nazairians, B.A. Schlinger, The expression of the sex steroid-synthesizing enzymes CYP11A1, 3beta-HSD, CYP17, and CYP19 in gonads and adrenals of adult and developing zebra finches, Gen. Comp. Endocrinol. 119 (2000) 140–151.
[17] M. Kagimoto, J.S. Winter, K. Kagimoto, E.R. Simpson, M.R. Waterman, Structural characterization of normal and mutant human steroid 17 alpha-hydroxylase genes: molecular basis of one example of combined 17 alpha-hydroxylase/17,20 lyase deficiency, Mol. Endocrinol. 2 (1988) 564–570.
[18] Y. Kazeto, S. Ijiri, T. Todo, S. Adachi, K. Yamauchi, Molecular cloning and characterization of Japanese eel ovarian P450c17 (CYP17) cDNA, Gen. Comp. Endocrinol 118 (2000) 123–133.
[19] L.B. Lutz, L.M. Cole, M.K. Gupta, K.W. Kwist, R.J. Auchus, S.R. Hammes, Evidence that androgens are the primary steroids produced by *Xenopus laevis* ovaries and may signal through the classical androgen receptor to promote oocyte maturation, Proc. Natl. Acad. Sci. USA 98 (2001) 13728–13733.
[20] H. Ono, M. Iwasaki, N. Sakamoto, S. Mizuno, cDNA cloning and sequence analysis of a chicken gene expressed during the gonadal development and homologous to mammalian cytochrome P-450c17, Gene 66 (1988) 77–85.
[21] N. Sakai, M. Tanaka, S. Adachi, W.L. Miller, Y. Nagahama, Rainbow trout cytochrome P-450c17 (17 alpha-hydroxylase/17,20-lyase), cDNA cloning, enzymatic properties and temporal pattern of ovarian P-450c17 mRNA expression during oogenesis, FEBS Lett. 301 (1992) 60–64.
[22] J.M. Trant, Isolation and characterization of the cDNA encoding the spiny dogfish shark (*Squalus acanthias*) form of cytochrome P450c17, J. Exp. Zool. 272 (1995) 25–33.
[23] P. Zhang, T.F. Nason, X.G. Han, P.F. Hall, Gene for 17 alpha-hydroxylase/C (17–20) lyase P-450: complete nucleotide sequence of the porcine gene and 5′ upstream sequence of the rat gene, Biochim. Biophys. Acta 1131 (1992) 345–348.
[24] R. Zhou, H. Cheng, R.T. Tiersch, Differential genome duplication and fish diversity, Rev. Fish Biol. Fisher. 11 (2002) 331–337.
[25] R. Zhou, L. Liu, Y. Guo, H. Yu, H. Cheng, X. Huang, T.R. Tiersch, P. Berta, Similar gene structure of two Sox9a genes and their expression patterns during gonadal differentiation in a teleost fish, rice field eel (*Monopterus albus*), Mol. Reprod. Dev. (2003) (in press).
[26] R. Wang, H. Cheng, L. Xia, Y. Guo, X. Huang, R. Zhou, Molecular cloning and expression of SOX17 in gonads during sex reversal in the rice field eel, a teleost fish with a characteristic of nature sex transformation, Biochem. Biophys. Res. Commun. 303 (2003) 452–457.
[27] K. Ohe, E. Lalli, P. Sassone-Corsi, A direct role of SRY and SOX proteins in pre-mRNA splicing, Proc. Natl. Acad. Sci. USA 99 (2002) 1146–1151.
[28] P. Zhang, N.A. Compagnone, C. Fiore, J.L. Vigne, P. Culp, T.J. Musci, S.H. Mellon, Developmental gonadal expression of the transcription factor SET and its target gene, P450c17 (17alpha-hydroxylase/c17,20 lyase), DNA Cell Biol. 20 (2001) 613–624.