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Four members of the *Sox* gene family in channel catfish

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The homologous sequences of human or mouse *SOX1*, *SOX4* and *SOX11*, and one novel *Sox* gene (named *Cef-SoxN*) were identified in the genome of channel catfish *Ictalurus punctatus*. Identification of these genes is a potential step in understanding development regulations including sex determination in channel catfish.

Key words: *Sox* genes; channel catfish; sexual development; gene family.

During the past 20 years, the channel catfish, *Ictalurus punctatus* Rafinesque has become the most important food fish cultured in the U.S.A. with production levels reaching a high of 256·5 million kg in 1998. However, despite the economic importance of this species, little genetic information is available. There are few genetic markers and none are available to allow selection of broodstock or manipulation of sex. Although progress has been made recently in establishing a banded karyotype for channel catfish (Zhang *et al.*, 1998), attempts to identify sex chromosomes have not been successful (LeGrande, 1981; Zhang *et al.*, 1998). Male channel catfish grow faster than females and attempts to use genetic manipulations such as creating YY males to produce all-male populations are hampered by the lack of markers for sex genotype (Davis *et al.*, 1990; Tiersch *et al.*, 1992).

The search for the testis determinant on the mammalian Y chromosome has led to the discovery of a gene called *SRY* (' sex-determining region Y '), the sex-determining gene of mammals (Sinclair *et al.*, 1990; Koopman *et al.*, 1991). *SRY* encodes a DNA binding protein that contains a 79 amino acid motif, the high motility group (HMG) box, which has a role as a transcription factor in the developing gonad that begins a cascade leading to differentiation of the testis. *SRY* is the founding member of a gene family, called *Sox* (*SRY*-like HMG box) genes. At present, at least 30 different *Sox* genes have been cloned. Most of these have not been characterized. Although *Sox* genes are conserved, few have been identified in fish; these include *SoxP1* (Ito *et al.*, 1995) and *Sox9* from rainbow trout, *Oncorhynchus mykiss* Walbaum (Takamatsu *et al.*, 1997), and *Zf-Sox19* from zebrafish, *Danio rerio* Hamilton-Buchanan (Vriz & Lovell-Badge, 1995). *Sox* genes share a common HMG box DNA-binding domain, originally identified in the transcription factor UBF (upstream binding factor). The sex-determining gene *SRY* is conserved and functional only in mammals. However, the *Sox9* gene is widely accepted to be involved in sex determination among mammals, chickens and potentially fish (i.e. rainbow trout). The *Sox3* gene is suggested to be another candidate ancestor gene for *SRY* in humans as suggested by Graves (1998).

Previous studies using DNA hybridization have demonstrated the phylogenetic conservation of a portion of the *SRY* gene in channel catfish (Tiersch *et al.*, 1992). In the

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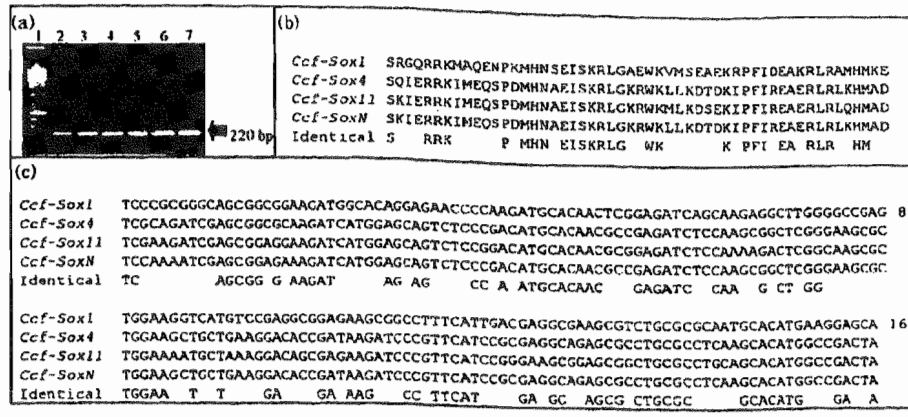

FIG. 1. The *Sox* genes of channel catfish amplified by degenerate polymerase chain reaction (PCR). (a) DNA fragments amplified from genomic DNA of male and female channel catfish with primers targeting *Sox* genes. Lane 1, 100 bp DNA ladder maker; lanes 2–4, male; lanes 5–7, female. (b) Comparison of deduced amino acid sequences of the conserved HMG box regions of four *Sox* genes in channel catfish. (c) The DNA sequence alignments of the four *Sox* genes. GenBank access numbers are AF000960, AF000961, AF000962 and AF000963. The degenerate primer sequences were: 5'CGATGGATCCATGAA(C/T)GC(A/T/C)TT(C/T)AT(G/A/T)GT(A/G/T/C)GG3', and 5'GCGCGAATTCGG(A/G/T/C)(C/T)(G/T)(A/G)TA(C/T)TT(A/G)TA-(A/G)T(C/T) (G/A/T)GG3', as described by Denny *et al.* (1992) with addition from the present study of sites for the restriction enzymes *Eco*R I or *Bam*H I placed into the 5' end of each primer. The thermal cycler was programmed as follows: 94° C for 4 min initial denaturation; 94° C for 30 s, 50° C for 40 s, and 72° C, for 1·5 min, for 35 cycles. The PCR products were cloned into pBluescript and sequenced using a Ready Reaction Cycle Sequencing Kit (Perkin Elmer) and autosequencer (ABI 310, Perkin Elmer). All nucleotide sequences were analyzed using the Blast programme to determine similarity with other *Sox* genes listed by the National Center for Biotechnology Information.

present report, polymerase chain reaction (PCR) and degenerate primers are used to identify the partial nucleotide sequence of four members of the *Sox* gene family present in channel catfish.

Degenerate primers designed from a consensus sequence of other known *Sox* genes yielded a major band of ~220 bp in each sex when amplified with DNA of male and female channel catfish [Fig. 1(a)]. A faint band of ~600 bp was observed also. The PCR products of 220 bp from male channel catfish were cloned and sequenced. Of the five clones that were sequenced, two sequences were identical [Fig. 1(b), (c)]. These clones shared 98% agreement with the amino acid sequence of the HMG box of the mouse *Sox1* (Wright *et al.*, 1993) and were designated as *Ccf-Sox1* (*Sox1* for channel catfish) (Table I). The third clone was 96% identical to mouse *Sox4* (Farr *et al.*, 1993) and 98% identical to human *SOX4* (Denny *et al.*, 1992; Wright *et al.*, 1993), and was designated as *Ccf-Sox4*. The fourth clone shared 98% agreement with mouse *Sox11* (Jay *et al.*, 1995) and human SOX11 (Wright *et al.*, 1993). This gene was designated *Ccf-Sox11*. The fifth clone was 96% identical to human *SOX4*, and also 94% identical to mouse *Sox11*. This could represent a novel *Sox* gene in channel catfish, and was tentatively designated as *Ccf-SoxN*. On the basis of deduced amino acid sequences shared within HMG boxes, *Ccf-Sox4*, *Ccf-Sox11* and *Ccf-SoxN* [Figure 1(b)] formed a group, consistent with the grouping of sub-family C (Wright *et al.*, 1993) described for mice, while *Ccf-Sox1* was consistent with the sub-family B grouping. Gene expression studies in mice have demonstrated that *Sox1*, *Sox2* and *Sox3* were expressed in the developing nervous system and urogenital ridge (Collingnon *et al.*, 1996). Expression of *Sox4* was observed in murine lymphocyte lines and in the murine thymus, which is consistent with a role in the regulation of lymphoid differentiation (van de Wetering *et al.*, 1993). Patterns of expression for *SOX11* are consistent with the hypothesis that this gene is important in the

TABLE I. Sequence agreement among *Sox* genes identified from channel catfish and known *Sox* genes from mice and humans

Catfish gene	Catfish gene sequence agreement			Human gene	Sequence agreement	Mouse gene	Sequence agreement
	Ccf-Sox4	*Ccf-Sox11*	*Ccf-SoxN*				
Ccf-Sox1	53%	58%	55%			*Sox1*	98%
Ccf-Sox4		91%	98%	*SOX4*	98%	*Sox4*	96%
Ccf-Sox11			92%	*SOX11*	98%	*Sox11*	98%
Ccf-SoxN				*SOX4*	96%	*Sox11*	94%

developing nervous system (Jay *et al.*, 1995). Identification of *Sox* genes in channel catfish provides data for studying evolution and function of these genes and the developmental biology of channel catfish.

Mechanisms of sex determination in fishes are diverse and poorly understood. Sex phenotype can be manipulated by use of hormones to improve yields and profits, yet the genes determining sex, and even the chromosomes bearing the sex determining genes, have not been identified in the majority of fishes. The study of sex determination has been addressed in channel catfish using hormonal manipulation and breeding studies, and monosex populations and novel combinations of genotype and phenotype, including XY females and YY males (which are fertile in channel catfish) have been produced. Due to the faster growth rates of male catfish compared with female catfish, production in commercial aquaculture could be increased by the use of YY male broodstock to produce all-male progeny. At present, the identification of individual YY males requires 3 or 4 years, during which time the fish reach sexual maturity and the sex ratio of the progeny is determined. Using molecular techniques to identify the sex of immature catfish would eliminate the delay and could shed light on sex-determining mechanisms in vertebrates. *Sox* genes in other organisms have various roles in development, which may allow identification of sex-determining mechanisms in channel catfish without prior identification of sex chromosomes. Also it might be informative to determine if particular *Sox* transcripts are produced in each sex of fishes during sex development. With increased application of techniques for physical mapping of genes, association of the *Sox* gene family with pathways of sexual development might provide a useful starting point for localization of sex-determining genes in vertebrate species lacking heteromorphic sex chromosomes.

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