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Channel catfish, *Ictalurus punctatus* (Rafinesque), tetraspanin membrane protein family: identification, characterization and phylogenetic analysis of tetraspanin 3 and tetraspanin 7 (CD231) transcripts

Hung-Yueh Yeh · Phillip H. Klesius

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Abstract Tetraspanins, a large cell surface protein superfamily characterized by having four transmembrane domains, play many critical roles in physiological and pathological processes. In this study, we report the identification, characterization and phylogenetic analysis of the channel catfish tetraspanin 3 and tetraspanin 7 (CD231) transcripts. The full-length nucleotide sequences of tetraspanin 3 and tetraspanin 7 cDNA have 1,453 and 1,842 base pairs, respectively. Analysis of the nucleotide sequences reveals that each has one open reading frame (ORF). The ORF of tetraspanin 3 appears to encode 241 amino acids with calculated molecular mass of 26.8 kDa, while the ORF of tetraspanin 7 potentially encodes 251 amino acids with calculated molecular mass of 27.9 kDa. By comparison with the human counterparts, the channel catfish tetraspanin 3 and tetraspanin 7 peptides have four transmembrane domains, three intracellular domains and two (small and large) extracellular domains. In addition, several characteristic features critical for structure and functions in mammalian tetraspanins are also conserved in channel catfish tetraspanin 3 and tetraspanin 7. The transcripts were detected by RT-PCR in restrictive organs. These results with those from our previous studies on other channel catfish tetraspanins provide important information for further investigating the roles of various tetraspanins in channel catfish infection with microorganisms.

Keywords Channel catfish · *Ictalurus punctatus* · Tetraspanin 3 · TSPAN3 · Tetraspanin 7 · TSPAN7 · CD231

H.-Y. Yeh · P. H. Klesius
Aquatic Animal Health Research Unit, Agricultural Research Service, United States Department of Agriculture, 990 Wire Road, Auburn, AL 36832-4352, USA

Present Address:
H.-Y. Yeh (✉)
Poultry Microbiological Safety Research Unit, Richard B. Russell Research Center, Agricultural Research Service, United States Department of Agriculture, 950 College Station Road, Athens, GA 30605-2720, USA
e-mail: hungyueh.yeh@ars.usda.gov

Tetraspanins (TSPAN), also called four transmembrane domain proteins, are one of cell surface protein superfamilies. The TSPAN family has more than 30 members in human (Hemler 2005, 2008; Berditchevski and Odintsova 2007; Levy and Shoham 2005; Pols and Klumperman 2009; Charrin et al. 2009; Huang et al. 2005). The common features of these proteins include the following: (1) TSPAN are relatively evolutionarily conserved and are expressed ubiquitously, (2) the large extracellular loop of TSPAN contains Cys–Cys–Gly and Pro–X–X–Cys–Cys motifs, and two or four cysteine residues, and (3) TSPAN with their partners form so-called tetraspanin-enriched microdomains (TEM) (Berditchevski 2001; Hemler 2005, 2008; Berditchevski and Odintsova 2007; Levy and Shoham

2005). Although a limited number of the TSPAN members have been studied, these proteins have many impacts on various physiological and pathological processes. In physiological condition, these proteins involve in modulation of immune signaling (Hemler 2005; Levy and Shoham 2005), regulation of intracellular protein trafficking (Berditchevski and Odintsova 2007), regulation of integrin-dependent cell migration (Berditchevski 2001), tissue differentiation (Boismenu et al. 1996) and egg-sperm interactions (Le Naour et al. 2000; Miyado et al. 2000). On the other hand, many pathogens take advantage of TEM for them to establish infection, such as CD81 acts as a receptor on the hepatocyte surface for the hepatitis C virus envelope protein E2 (Cormier et al. 2004; Pileri et al. 1998) and for *Plasmodium* sporozoite infectivity (Silvie et al. 2003). Shrimp tetraspanin 3 mediates the entry of white spot syndrome virus (Gui et al. 2012).

In teleost fish, only a few of TSPAN have been identified and characterized—Atlantic salmon and rainbow trout CD9 (Fujiki et al. 2002), *Dasyatis akajei* CD9 (Zhu et al. 2006), zebrafish CD81 (Yoder and Litman 2000) and channel catfish CD81 (Yeh and Klesius 2009) and CD63 (Yeh and Klesius 2010a). Channel catfish production is the most important aquaculture industry in the southeastern United States (USDA 2007). However, little information on other channel catfish TSPAN gene members is available. In this report, we describe the isolation, characterization, phylogenetic analysis and tissue expression distribution of the channel catfish TSPAN3 and TSPAN7 transcripts.

Materials and methods

Fish

Channel catfish (NWAC103 strain) used in this study were maintained as previously described (Lim et al. 2010). As previously described, spleen, anterior kidney, liver, intestine, skin and gill were collected after catfish were euthanized by immersion in tricaine methanesulfonate (Yeh and Klesius 2010b). The use of fish in the experiment was approved by the Institutional Animal Care and Use Committee of the USDA ARS Aquatic Animal Health Research Unit in Auburn, AL.

RNA isolation and rapid amplification of cDNA end (RACE) library construction

Total RNA from channel catfish tissues was isolated by using a Tri reagent (Molecular Research Center,

Table 1 Oligonucleotide primers used for PCR amplification in this study

Primer	Sequence	Direction	*Tm* (°C)	Use
TSPAN3-219F	5′-AGCATTTGTGTGATCGCCTGCCGAAG-3′	Forward	74	3′-RACE
TSPAN3-222F	5′-AGCCATCATTATTGCCGTGGCTGTGG-3′	Forward	74	3′-RACE and RT-PCR
TSPAN3-81R	5′-TGCAGGTGGTGTTTGCTTTGCAGCAT-3′	Reverse	74	5′-RACE
TSPAN3-755R	5′-CTTCGGCAGGCGATCACACAAATGCT-3′	Reverse	74	5′-RACE and RT-PCR
TSPAN7-702F	5′-GCTGAACTCGTGGCAGGCATTTCTGG-3′	Forward	74	3′-RACE
TSPAN7-829F	5′-GGCATGCTGCTTGTCACGCTACATCA-3′	Forward	73	3′-RACE and RT-PCR
TSPAN7-729R	5′-AGCCAGAAATGCCTGCCACGAGTTCA-3′	Reverse	74	5′-RACE
TSPAN7 1158R	5′-GCCAAAAGCCTGCAAACGCTCCACTT-3′	Reverse	74	5′-RACE and RT-PCR
GeneRacer 5′Primer (Invitrogen)	5′-CGACTGGAGCACGAGGACACTGA-3′	Forward	74	5′-RACE
GeneRacer 3′Primer (Invitrogen)	5′-GCTGTCAACGATACGCTACGTAACG-3′	Reverse	78	3′-RACE
β-Actin-F	5′-GACTTCGAGCAGGAGATGGG-3′	Forward	72	RT-PCR
β-Actin-R	5′-AACCTCTCATTGCCAATGGTG-3′	Reverse	69	RT-PCR
GeneRacer RNA Oligo (Invitrogen)	5′-CGACUGGAGCACGAGGACACUG ACAUGGACUGAAGGAGUAGAAA-3′			
GeneRacer Oligo dT (Invitrogen)	5′-GCTGTCAACGATACGCTACGTAAC GGCATGACAGTGT$_{24}$-3′			

Inc., Cincinnati, OH) according to the manufacturer's instructions. Tissues used in this study included spleen, anterior kidney, intestine, liver, skin and gill. The quality and quantity of total RNA were determined by using RNA 1200 chips on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). Both 5′- and 3′-RACE full-length ends were generated by using a GeneRacer kit (Invitrogen, Carlsbad, CA) per manufacturer's instructions. Briefly, for 5′-RACE, 5 μg of total RNA was treated with calf intestine phosphatase (to remove phosphate at the 5′-end), then with tobacco acid pyrophosphatase (to get rid of cap structure at the 5′-end) and ligated to a GeneRacer RNA Oligo provided in the kit. The treated RNA was reverse transcribed into cDNA with random hexamers using a SuperScript III RT kit (Invitrogen). For 3′-RACE, the same amount of total RNA was directly reverse transcribed into cDNA with GeneRacer Oligo dT primer using the same SuperScript III RT kit (Invitrogen). The sequences of the GeneRacer RNA Oligo and the GeneRacer Oligo dT are listed in Table 1.

TSPAN gene amplification

Both 5′- and 3′-RACE of TSPAN3 and TSPAN7 transcripts were PCR amplified. The PCR mixtures (50 μl per reaction) contained the following reagents (in final concentrations): 1× PrimeSTAR HS PCR premix (TaKaRa, Madison, WI), 300 μM each of gene-specific primer and GeneRacer primer, and cDNA template. The amplification was performed on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA) with the following parameters: 98 °C for 2 min, followed by 35 cycles of 98 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The primers for PCR amplification are listed in the Table 1. Expressed sequence tag (EST) accession no. CK418505 was used for TSPAN3 primer design, while the contiguous sequence assembled from EST accession nos. FD340406, FD351218 and FD325369 was used for TSPAN7 primer design. The amplified PCR products were purified by agarose gel electrophoresis and ligated into the pSC vector, followed by transformation of the ligated vector into the Solo Pack® competent *Escherichia coli* cells (Agilent Technologies) according to the manufacturer's instructions. At least ten colonies per PCR product were randomly selected for DNA sequencing.

DNA sequencing and bioinformatics

The DNA sequencing reactions were performed at the USDA ARS Genomics and Bioinformatics Research Unit in Stoneville, MS with an ABI3730×1 Genetic Analyzer (Applied Biosystems, Foster City, CA). Chromatograms were edited, trimmed and analyzed also at the USDA ARS Genomics and Bioinformatics Research Unit in Stoneville, MS. The amino acid sequence was deduced from nucleotide sequences using Transeq (Rice et al. 2000) and aligned with other TSPAN amino acid sequences deposited in GenBank using ClustalW2 (Larkin et al. 2007). ExPASy server (Gasteiger et al. 2005) was used to calculate the TSPAN3 and TSPAN7 peptide molecular masses and

Table 2 Identity of tetraspanin 3 and tetraspanin 7 amino acid sequences among species (%)

	Channel catfish	Atlantic salmon	Zebrafish	Western clawed frog	Red jungle fowl	Human	Mouse
Channel catfish		68	73	46	45	41	41
Atlantic salmon	78		76	45	47	44	43
Zebrafish	77	75		41	45	41	41
Western clawed frog	65	63	59		86	84	82
Red jungle fowl	65	64	61	90		90	88
Human	67	64	61	89	92		96
Mouse	68	64	61	89	92	97	

Identity in percentage between two species was calculated by the ClustalW2 program (Larkin et al. 2007) via http://www.ebi.ac.uk. The right-hand and left-hand corners show the identity of tetraspanin 3 and tetraspanin 7 amino acid sequences among species, respectively

pI and to analyze the *N*-glycosylation sites. The signal peptide site in the amino acid sequence was detected using the SignalP 3.0 (Bendtsen et al. 2004). Transmembrane topology of the TSPAN peptides was predicted via TMHMM Web Server (version 2.0) (Krogh et al. 2001). Phylogenetic relationships of TSPAN amino acid sequences from various species were analyzed by the MEGA 4.0 software (Tamura et al. 2007) based on the ClustalW2 alignment results.

RT-PCR

RT-PCR assay was performed to determine the expression profile of TSPAN3 and TSPAN7 in various channel catfish tissues as described previously (Yeh and Klesius 2009, 2010a). β-Actin was used as an internal control. The PCR products were analyzed in 2 % agarose gel electrophoresis and stained with ethidium bromide. Images were recorded by a

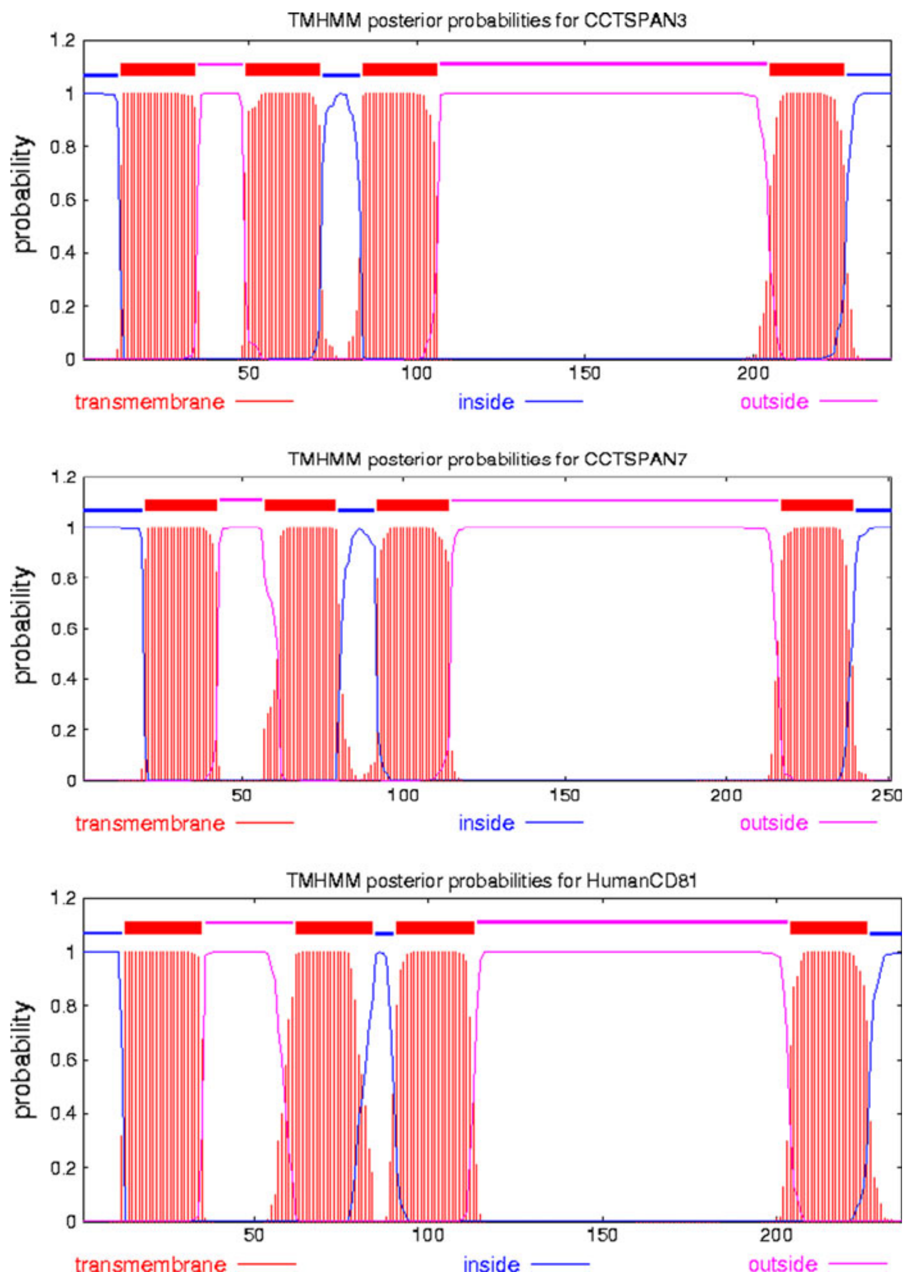

Fig. 1 Prediction of transmembrane topology of channel catfish TSPAN3 and TSPAN7. The deduced amino acid sequences were analyzed via TMHMM Server (version 2.0) (Krogh et al. 2001). Human tetraspanin CD81 is included for comparison. (Color figure online)

(a)

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                      ←--CPD---→←--------TM-------→←------SEL------→←--------
Mouse                 MGQCGITSSKTVLVFLNLIFWGAAGILCYVGAYVFITYDDYDHFFEDVYTLFPAVVIIAV 60
Human                 MGQCGITSSKTVLVFLNLIFWGAAGILCYVGAYVFITYDDYDHFFEDVYTLIPAVVIIAV 60
Red jungle fowl       MGQCGITSSKTVLVFLNLIFWAAAGILCYVGAYVFITYDDYDHFFEDVYTLIPAVIIIAV 60
Western clawed frog   MGQCGLISSKTVLVFLNLIFWAAAGILCYVGAYVFITYDDYDHFFEDVYTLIPGVIIIAA 60
Zebrafish             M-DCGIITSKTILLLLSLIFWAAGAALAYVGSYVIKSYNNFEDFMSDRHTLIPAAIIIGV 59
Atlantic salmon       M-DCGIITSKTVLLLLNIIFWAAGGALAYVGSYVIKSYNNFDNFLEDKYTLIPAAIIITV 59
Channel catfish       M-DCGIIASKTVLLLLSLIFWAAGSALAYVGAYVISSYKNFDDFRVDKYSVIPAAIIIAV 59
                      *  **   *** *  *  *** *   * *** **   *     *  *     *   *

                      TM-------→←----CPD----→←--------TM--------→←------------
Mouse                 GALLFIIGLIGCCATIRESRCGLATFVFILLLVFVTEVVVVVLGYVYRAKVENEVDRSIQ 120
Human                 GALLFIIGLIGCCATIRESRCGLATFVIILLLVFVTEVVVVVLGYVYRAKVENEVDRSIQ 120
Red jungle fowl       GTLLFIIGLIGCCATIRESRCGLATFVIILLLVFITEVVVVVLGYIYRAKVEDEVDHSIQ 120
Western clawed frog   GTLLFIIGLIGCCATIRESRCGLATFVVILLLVFVTEVVVVVLGYIYRAKVEDEVDNTIA 120
Zebrafish             AVVMFIIGFVGCCATLRESKVGLGLFLIIIMLIFAAEVTAFVFGIIYRGRIRGDLEKSMN 119
Atlantic salmon       GVVMFIIGTVGCCATLRESKVGLSFFLMILLAMFAAEVAALVFAFIYQGRIKGDLEQSMS 119
Channel catfish       AVVMFIIGIIGCCATLRESKVGLGFFLLLILLMFAAEVTAFVFGIIYKGRINGDLEKTMN 119
                          ****  ***** ***  **  *       *  **   *    *

                      -----------------------------------------------LEL-----
Mouse                 KVYKTYNGTDSDAASRAIDYVQRQLHCCGIHNYSDWENTDWFKETKNQSVPLSCCRETAK 180
Human                 KVYKTYNGTNPDAASRAIDYVQRQLHCCGIHNYSDWENTDWFKETKNQSVPLSCCRETAS 180
Red jungle fowl       KVYNGYNGTNPDAASRAIDYVQRQLRCCGIHNYTDWENTVWFKQTKNNSVPLSCCKATLS 180
Western clawed frog   NVFNQYNGISPDSASRAIDYVQRQLHCCGIDNYLDWENTPWFSEAKNNSVPLSCCRNYVF 180
Zebrafish             DVFLKYDGLNSE--THAVDYLQSQLECCGVKNQTDWTLTSWFAQH-NNTVPQSCCKANMT 176
Atlantic salmon       DVFQKYDGENSE--TRAVDYLQSQLQCCGVQTYMNWTTTPWFSSS-NNTVPISCCKRNNT 176
Channel catfish       EVFDKY-GDESE--TRAVDYLQTQLECCGVLNYTDWSSRPWFSSH-NNSVPHSCCKAN-T 174
                       *   * *        * ** * ** ***      *    **    *  ** ***

                      ------------------------------→←--------TM--------→←-----
Mouse                 SCNGSLANPSDLYAEGCEALVVKKLQEILMHVIWAALAFAAIQLLGMLCACIVLCRRSRD 240
Human                 NCNGSLAHPSDLYAEGCEALVVKKLQEIMMHVIWAALAFAAIQLLGMLCACIVLCRRSRD 240
Red jungle fowl       NCTGSLTRPMDLYSEGCEALVVKKLQEIMMYVIWAALAFAAIQLLGMLCACIVLCRRSRD 240
Western clawed frog   NCTGSMNKPGDLYSEGCKALVVEKLQEIMMYVIWAALAFAAIQLLGMLCACIVLCRRTRD 240
Zebrafish             QCTGQLSQPDLLNTQGCEAKLEQVLQDVLSYAMLVILGFAIIKFFGMLSVCVITCKS-KK 235
Atlantic salmon       ECTGKLDQPDLLNTQGCEVKLEQLLQDVMSYAMLVILGFAIIKFFGMLSVCVITCKSGRR 236
Channel catfish       TCTGQFNHPEVLNTHGCKTKLEELLQNVLTYAMFVIMGFAIIKFFGMISICVIACRS-SR 233
                       * *    *  *   **       **            ** *   **   *   *

                      CPD---------→
Mouse                 PAYELLITGGTYA 253
Human                 PAYELLITGGTYA 253
Red jungle fowl       PAYELLITGGTYA 253
Western clawed frog   PAYELLITGGTYA 253
Zebrafish             NEYQPLYA----- 243
Atlantic salmon       TGYQALYV----- 244
Channel catfish       NDYQPLYS----- 241
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Fig. 2 Multiple alignment of the deduced channel catfish TSPAN3 (**a**) and TSPAN7 (**b**) peptide sequences against those of other species deposited in the GenBank database. The potential structural domains of both peptides are indicated above the sequences—CPD (cytoplasmic domain), TM (transmembrane domain), SEL (small extracellular loop) and LEL (large extracellular loop). The characteristic conservative Cys–Cys–Gly and Pro–X–X–Cys–Cys motifs in LEL are *highlighted*. Gaps were introduced in the sequences denoted by *hyphens*. Identical amino acids among species are indicated as *asterisks* below the sequences. GenBank accession numbers of each species are as follows: **a** TSPAN3: Atlantic salmon, ACI33716; channel catfish, GU120083; human, NP_005715; mouse, BAE40182; red jungle fowl, XP_001232465; western clawed frog, NP_001017023; and zebrafish, NP_001002748. **b** TSPAN7: Atlantic salmon, NP_001135033; channel catfish, GU120084; human, CAB65594; mouse, NP_062608; Northern pike, ACO14473; red jungle fowl, XP_416779; western clawed frog, NP_989350; and zebrafish, NP_999939. (Color figure online)

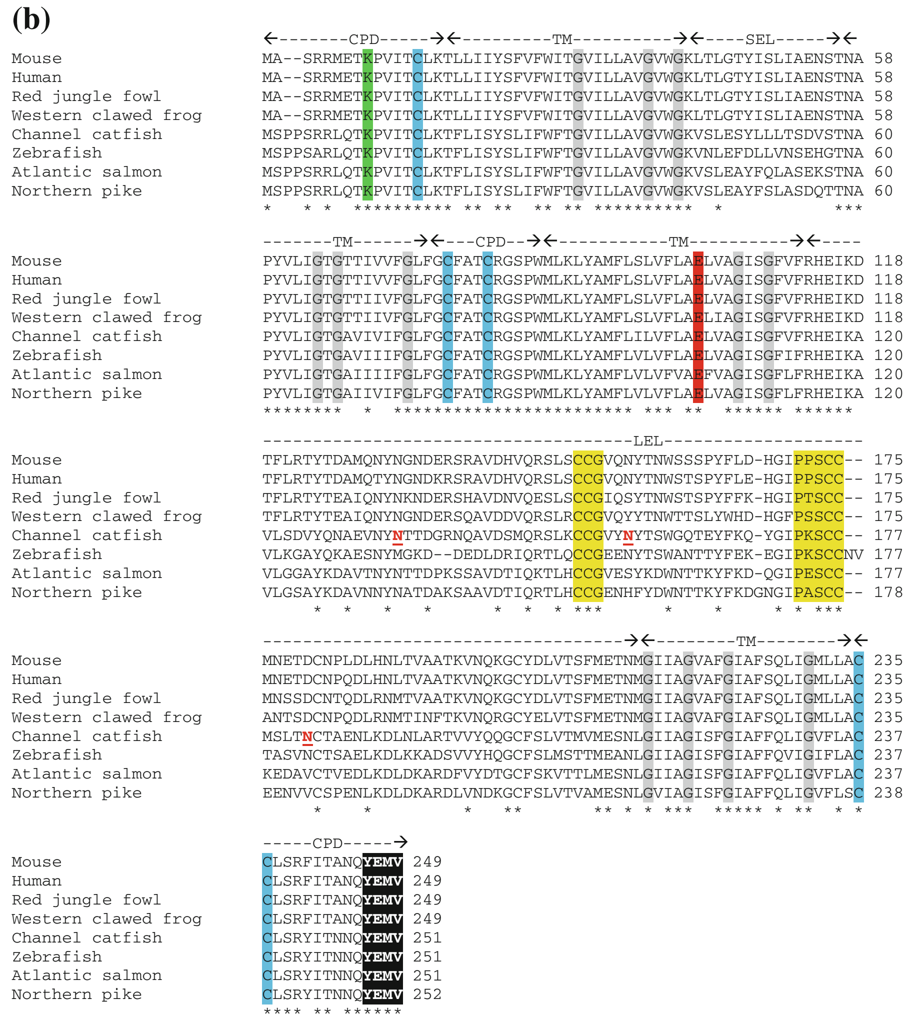
KODAK Gel Logic 440 Imaging System (v. 4.0.3, Eastman Kodak, Rochester, NY) and processed with AlphaView SA software (v. 3.4.0).

Results and discussion

In our previous studies, we have identified and characterized two channel catfish TSPAN transmembrane proteins: CD81 and CD63 (Yeh and Klesius 2009, 2010a). We further explored the channel catfish TSPAN gene family by searching the expressed sequence tags (EST) deposited in the GenBank database (www.ncbi.nlm.nih.gov/EST) and found that channel catfish has at least 14 TSPAN genes/proteins. In this communication, we, in conjunction with the RACE method (Frohman et al. 1988), cloned and sequenced the channel catfish TSPAN3 and TSPAN7 transcripts.

TSPAN3 has been detected in a wide variety of cell types (Puls and Wright 2000; Tokoro et al. 2001) and plays roles in regulating proliferation and migration of

Fig. 2 continued



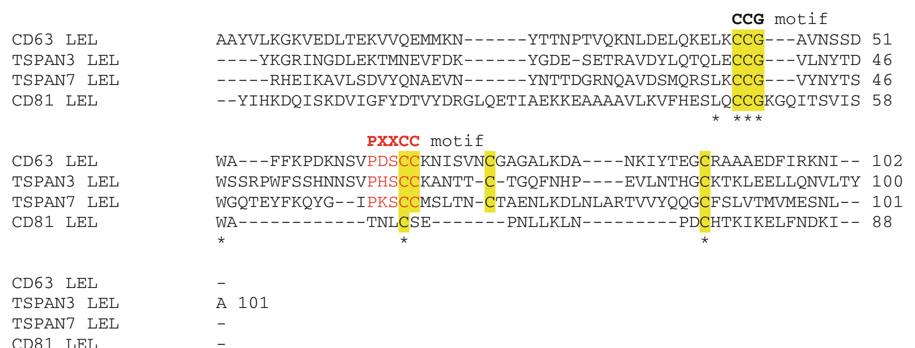


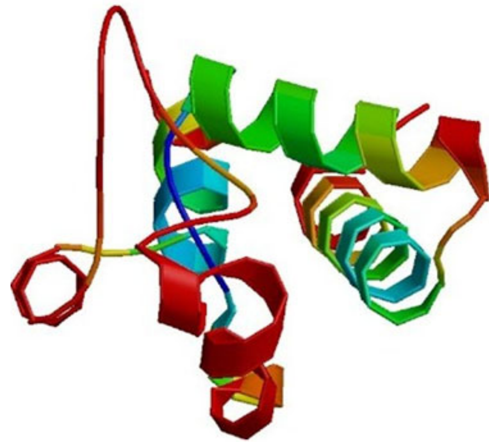
Fig. 3 Comparison of the large extracellular loops of channel catfish tetraspanin members: CD63 (FJ899742), CD81 (FJ205473), TSPAN3 (GU120083) and TSPAN7 (GU120084). Conserved cysteine residues and canonical Cys–Cys–Gly and Pro–X–X–Cys–Cys motifs are *highlighted*. (Color figure online)

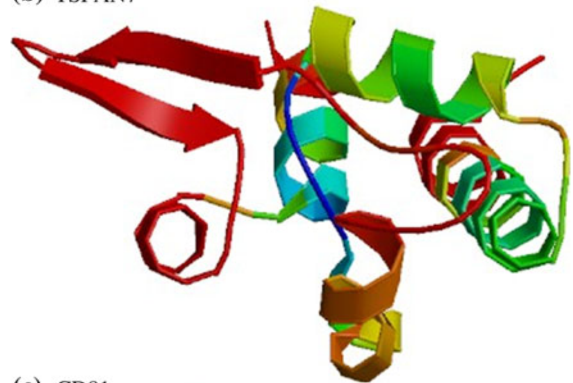
oligodendrocytes (Tiwari-Woodruff et al. 2001) as well as downregulating mouse dendritic cell function in association with T cell stimulation (Tokoro et al. 2001). The full-length of the channel catfish TSPAN3 cDNA consisted of 1,453 nucleotides, which included 5′- and 3′-untranslated regions (UTR) and an open reading frame (ORF) (GenBank accession no. GU120083). In the 5′-UTR, the sequence had a Kozak motif ($^{A}/_{G}$ NNATGG) (Kozak 1987). The 3′-UTR contained both polyadenylation signal sequences and

a 26-nucleotide polyadenylation tail, indicating that the sequence was likely to be full-length. The ORF appears to encode a 241-amino-acid-residue peptide with a calculated molecular mass and pI of 26,775.22 daltons and 6.48, respectively. Like mammalian TSPAN3, the channel catfish counterpart had two potential *N*-glycosylation sites at Asn^{148} and Asn^{162}.

TSPAN7 was first cloned from a human immature T cell line (Emi et al. 1993), followed from mouse brain neurons (Hosokawa et al. 1999). The function of this protein is not known, but has been implicated in the control of neurite outgrowth and association with X-linked mental retardation in human (Zemni et al. 2000). A recent study shows that the cellular prion protein interacts with bovine TSPAN7 (Guo et al. 2008). The full-length of the channel catfish TSPAN7 sequence was comprised of 1,842 nucleotides, which included 5′- and 3′-UTR and an ORF (GenBank accession no. GU120084). The 3′-UTR contained an mRNA instability motif, polyadenylation signal sequences and a 25-nucleotide polyadenylation tail, indicating that the channel catfish sequence was likely to be full-length. The ORF appears to encode a 251-amino-acid-residue peptide with a calculated molecular mass and pI of 27,900.70 daltons and 8.18, respectively. The peptide had three potential *N*-glycosylation sites at Asn^{134}, Asn^{157} and Asn^{182}.

When the deduced channel catfish TSPAN3 and TSPAN7 amino acid sequences were compared with their respective counterparts from other species, we noticed that the degree of conservation of TSPAN3 among species ranged from 73 % (vs. zebrafish) to 41 % (vs. mammals) and that of TSPAN7 was from 78 % (vs. Atlantic salmon) to 67–68 % (vs. mammals) (Table 2). When TSPAN3 and TSPAN7 amino acid sequences were further analyzed by the TMHMM program (Krogh et al. 2001), like mammalian counterparts (Puls and Wright 2000; Emi et al. 1993; Hosokawa et al. 1999; Todd et al. 1998; Tokoro et al. 2001), these channel catfish tetraspanins are transmembrane proteins and can be structurally divided into four hydrophobic transmembrane domains, three intracellular domains and two (small and large) extracellular loops (Fig. 1), consistent with the characteristics of the tetraspanin superfamily. As shown in Fig. 2, we observed several features that are critical for structure and functions in mammalian TSPAN are also conserved in the channel catfish counterparts: (1) tyrosine-based lysosomal sorting motifs (**Tyr**-Glu-Met-**Val** of TSPAN7 and **Tyr**-Gln-Pro-**Leu** for TSPAN3) as highlighted in black (Berditchevski 2001; Berditchevski and Odintsova 2007), (2) several





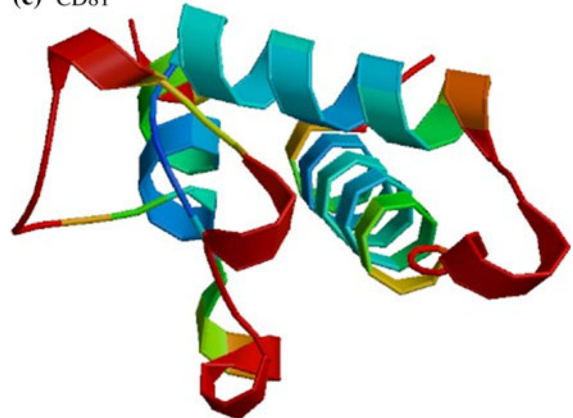


Fig. 4 Proposed three-dimensional structural models of channel catfish TSPAN3 and TSPAN7 large extracellular loops. Human CD81 large extracellular loop was used as a template (1g8qA) to model the three-dimensional structures of the counterparts of channel catfish TSPAN3 (**a**), TSPAN7 (**b**) and human CD81 (**c**). (Color figure online)

Cys residues in intracellular domains as highlighted in blue (Levy and Shoham 2005), (3) the positive-charged Lys residue in the first intracellular domain as highlighted in green (Todd et al. 1998), (4) the Gly residues in the transmembrane domains as highlighted in gray (Todd et al. 1998), and (5) the

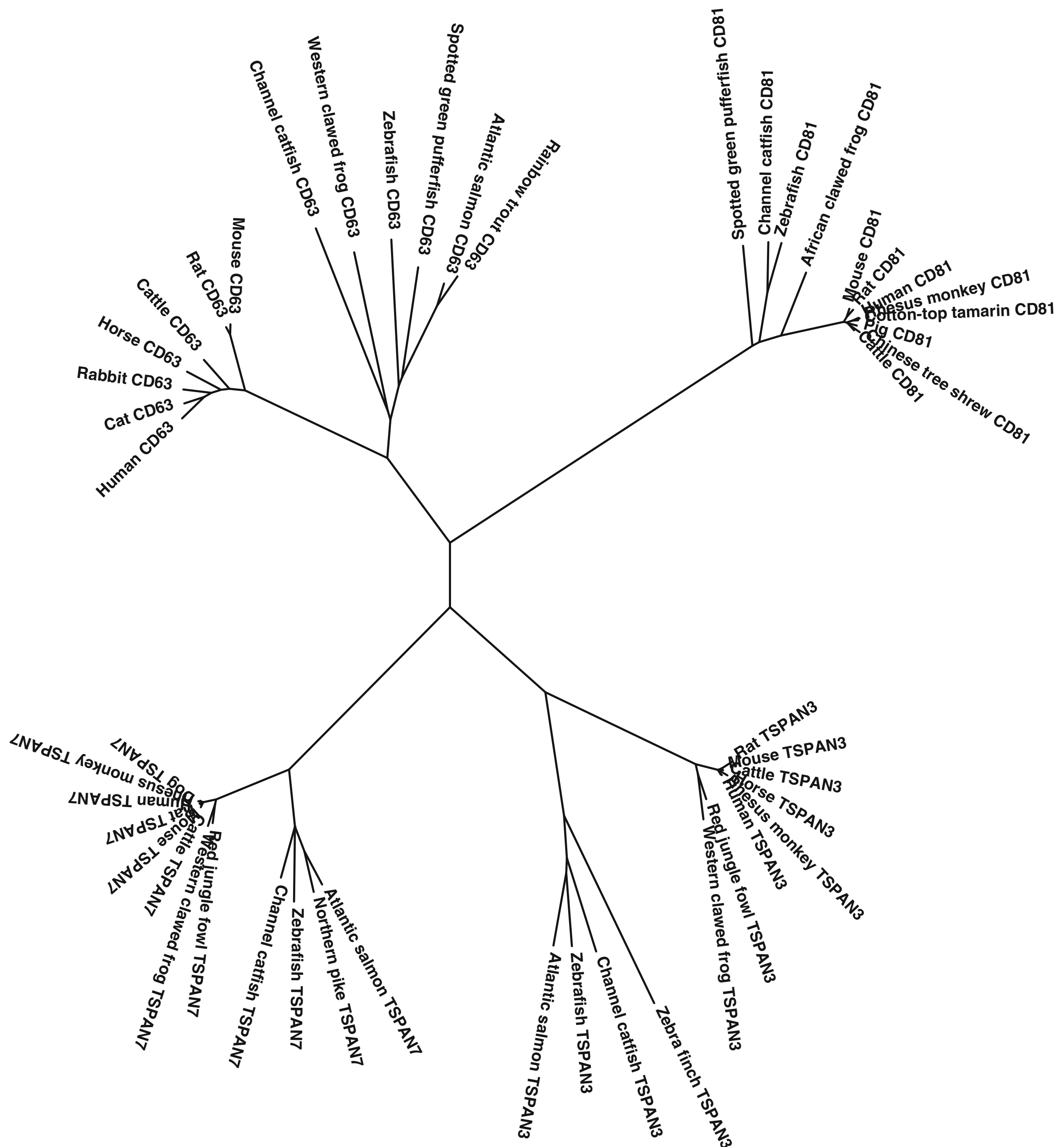


Fig. 5 Molecular phylogenetic relationships of the partial tetraspanin protein members among vertebrates. The tree was constructed with the neighbor-joining method (Poisson correction model) in the MEGA 4.0 software (Tamura et al. 2007) based on the alignment results from ClustalW2. The tree was built in 1000 bootstrap replicates with the value >50 % and complete gap deletion. The tetraspanin amino acid sequences used for this analysis are CD63 (Yeh and Klesius 2010a), CD81 (Yeh and Klesius 2009), tetraspanin 3 and tetraspanin 7 (see Fig. 2 legend)

negative-charged Glu residue in the transmembrane 3 domain as highlighted in red (Todd et al. 1998).

The large extracellular loop of tetraspanins is associated with various cell surface proteins, and the complexes subsequently initiate signal transduction pathways. We furthermore compared the large extracellular loops of channel catfish CD81, CD63, TSPAN3 and TSAPN7. They overall show a relatively low degree of homology over the amino acid sequences, but four Cys residues and the Cys–Cys–Gly motif are conserved in all TSPAN examined (Fig. 3), indicating that the disulfide linkages form within the large extracellular loop, so that tertiary structure is conserved throughout evolution (Seigneuret et al. 2001). We also noticed that the Pro–X–X–Cys–Cys motif is conserved in TSPAN3, TSPAN7 and CD63 (Fig. 3). Because the three-dimensional structure of the large extracellular loop of human CD81 has been elucidated at 1.60 Å (template 1G8QA), we used this as a template to model the structure of the large extracellular loop of channel catfish TSPAN3 and TSPAN7 via SWISS-MODEL website. As seen in Fig. 4, the predictive structures of the large extracellular loops of channel catfish TSPAN3 and TSPAN7 show significant similarities to the counterpart of human CD81.

To determine the phylogenetic relationships of channel catfish TSPAN to other species, a total of 48 TSPAN amino acid sequences retrieved from the GenBank database were analyzed with the neighbor-joining method in the MEGA 4.0 software (Tamura et al. 2007) based on the ClustalW2 alignment result to infer a phylogenetic tree. As seen in Fig. 5, four well-segregated clads are observed, that is, TSPAN3, TSPAN7, CD63 and CD81. Further, each clad shows that the teleost and mammalian homologs form distinguishable subclusters supported by the bootstrap analysis. This result suggested that like mammals, the teleost fish have TSPAN genes.

The expression profile of channel catfish TSPAN3 and TSPAN7 transcripts in various tissues was determined. The sizes of the specific amplified fragments of TSPAN3, TSPAN7 and β-actin were 534, 330 and 203 base pairs, respectively. A recent study by Small et al. (2008) demonstrated that seven reference genes for real-time PCR vary across all channel catfish tissues examined. Based on the pooled standard error of the mean, β-actin (SEM = 0.11) is the second least variable after α-tubulin (SEM = 0.08) across tissues.

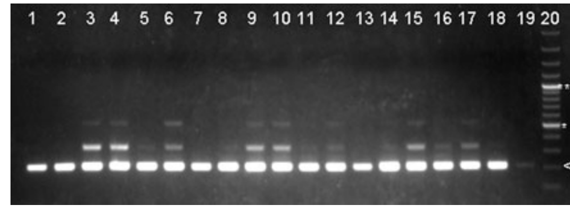


Fig. 6 Expression profile of TSPAN3 and TSPAN7 transcripts in channel catfish tissues ($n = 3$). Total RNA from various tissues of three individual channel catfish was used for RT-PCR assays. The sizes of amplified products were 534, 330 and 203 base pairs for TSPAN3, TSPAN7 and β-actin, respectively. Spleen, lanes 1, 7 and 13; anterior kidney, lanes 2, 8 and 14; liver, lanes 3, 9 and 15; intestine, lanes 4, 10 and 16; skin, lanes 5, 11 and 17; and gill, lanes 6, 12 and 18. Lane 19, no template control; and lane 20, 100-bp GeneRuler MW ladders (**, 1,000 bp; *, 500 bp and <, 200 bp) (Fermentas Life Sciences, Glen Bernie, MD)

Thus, use of β-actin in RT-PCR assays is suitable. As seen in Fig. 6, unlike in mouse that the TSPAN3 transcript is expressed ubiquitously (Puls and Wright 2000; Tokoro et al. 2001), the expression of the TSPAN3 transcript was detected in liver of three fish examined, while in intestine and gill of two fish, and skin in one fish. The reason is not known, but some tetraspanin expression is highly restricted in specific cell types (Angelisova et al. 1994; Maecker et al. 1997). The TSPAN7 transcript was detected in liver and intestine of three fish, but in gill of two fish and skin in one fish. These results suggest that both TSAPN3 and TSPAN7 transcripts are constitutively expressed in the restrictive tissues. It is also interesting to note that both TSPAN3 and TSPAN7 transcripts were detected in liver of three fish examined. The reason is yet to be determined, but it is possible that these tetraspanin proteins play critical roles in protein trafficking and cell adhesion in catfish liver.

In conclusion, the full-lengths of channel catfish TSPAN3 and TSPAN7 transcripts were identified, sequenced and characterized based on sequence alignment and phylogenetic analysis. The transcripts were detected by RT-PCR in restrictive tissues. These results with those our previous studies on CD81 and CD63 (Yeh and Klesius 2009, 2010a) provide important information for further studying the roles of various TSPAN in channel catfish infection with microorganisms.

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