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Identification of cDNAs from Japanese pufferfish (*Fugu rubripes*) and Atlantic salmon (*Salmo salar*) coding for homologues to tetrapod prion proteins

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Abstract We identified cDNAs coding for homologues to tetrapod prion proteins (PrPs) in Atlantic salmon (*Salmo salar*) and Japanese pufferfish (*Fugu rubripes*), which were termed 'similar to PrPs' (stPrPs). Besides significant sequence homologies the fish stPrPs display characteristic structural features in common with tetrapod PrPs. In addition, two stPrPs were shown to be highly expressed in brain tissue. None of the so far identified PrP-homologues of fish resembles doppel. Hence, the duplication of the PrP gene, which generated doppel, may have occurred not in fish but later in the tetrapod lineage. The identification of fish PrPs provides a basis to address concerns about a possible susceptibility of fish to prion infections.
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Key words: Atlantic salmon; *Fugu rubripes*; Prion

1. Introduction

The prion protein (PrP) is a ubiquitous protein expressed most prominently in brain tissue [1]. PrPs are intensively studied because the presence of the normal cellular isoform of PrP is an absolute requirement for the replication of the causative agents of transmissible spongiform encephalopathies (TSEs) [2], which according to the prion hypothesis are essentially composed of a misfolded form of the PrP [1]. Its physiological role is however still largely unclear. Several lines of evidence implicate a neuroprotective role for PrP. PrP binds copper in vivo [3,4] and an associated superoxide dismutase activity may protect neurons against oxidative stress [5]. PrP was further shown to transduce neuroprotective signals by interacting with stress-inducible protein 1 [6]. So far PrPs were identified in mammals, birds, and amphibians. The characterisation of fish PrPs may promote the current understanding of the molecular evolution of PrPs and may help to elucidate structure/function relationships for PrPs.

Moreover, in the past, economically grown fish species were exposed to prions of mammalian origin through feeding with contaminated meat and bone meal. The identification of fish PrPs provides a scientific basis to address concerns about a possible susceptibility of fish to prion infections.

2. Materials and methods

2.1. Fish DNA and RNA

Fugu DNA and RNA from brain tissue was obtained from the Human Genome Mapping project Resource Centre. Total RNA was extracted from the brains and other organs of Atlantic salmon (8–12 cm total body length) using the Trizol reagent (Gibco BRL, Life Technologies, Grand Island, NY, USA).

2.2. cDNA synthesis and PCR

Total RNA (2.5 μg) was reverse transcribed using the First strand cDNA synthesis kit (Amersham Biosciences, Uppsala, Sweden). PCRs were carried out in a total volume of 50 μl containing 2.5 U of Taq polymerase Ampli Taq Gold (Applied Biosystems, Foster City, CA, USA), 1×buffer II (supplied with the Taq Polymerase), 2.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.2 μM of each primer.

2.3. RACE PCR

For amplification of 5′- and 3′-ends of cDNA, RACE PCR was performed using the Smart RACE cDNA Amplification Kit (Clontech, Palo Alto, CA, USA) according to the manufacturers instructions. The gene-specific primers designed for 5′-PCR of fugu stPrP-1 ('similar to PrPs') cDNA were: outer gene-specific primer 5′-GTGTAACCACCAACCGCACCACCAGG and inner gene-specific primer: 5′-GGCTGAGAACCGGATGGTTTTGATGG. For 3′-PCR the outer gene-specific primer was 5′-GCAGGTAGATACCCAGGCCAGGGAGG and the nested primer 5′-CGGACGGATCTCCTGAAAGACAAGGG.

The outer gene-specific primer designed for amplification of the 5′-end of Atlantic salmon cDNA was 5′-GCTGGGTACGGGTTGGGGTTCCTATT and the nested primer 5′-CGTCTGTGTGTTTGATGTTTTCGGCG.

For amplification of the 3′-end of salmon the outer gene-specific primer was 5′-CTGGGGGATATCCAAACCAGAACCCA and the nested primer 5′-GCCAATCCTGGGGGATTATCCAAACC.

2.4. DNA sequencing analysis

PCR products were cloned using the TOPO TA Cloning kit (Invitrogen, Paisley, UK). Sequence analysis was performed on both strands, following the method by Sanger et al. [7], using the Big Dye RR Terminator Ampli Taq sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an automated sequencer (Applied Biosystems 373 A). Several sequencing primers were applied to obtain the complete sequence information. The sequences have been deposited at GenBank. The accession numbers are AY141106 (fugu stPrP-1), AY188583 (fugu stPrP-2), and AY141107 (salmon stPrP).

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2.5. Northern-blot analysis

Total fugu brain RNA and salmon RNA from various organs was fractionated by agarose gel electrophoresis, transferred to nylon membranes and hybridised with fugu- and salmon PrP-specific hybridisation probe, respectively. The final autoradiographies were performed by exposing XAR-5 films (Kodak, Chalon-sur-Saone, France) for 2 h.

2.6. Computer assisted analysis

The signal sequence was predicted by using the prediction tool SignalP V1.1 at http://www.cbs.dtu.dk/services/SignalP/ [8], and putative GPI-anchor-sites were analysed by using the 'Big-PI Predictor' at http://mendel.imp.univie.ac.at/sat/gpi/gpi_server.html [9].

Alignments were carried out with the Clustal W programme using the Gonnet algorithms and default settings followed by hand adjustments.

3. Results

The fugu genome database (http://fugu.jgi-psf.org/) was screened for potential homologues to tetrapod PrPs. Two closely related candidate sequences were found with significant homologies to tetrapod PrPs, which were subsequently termed stPrP-1 and -2. The first candidate stPrP-1 was present on scaffold 96 and would code for a putative protein with significant homology to tetrapod PrPs. By Northern-blot analysis of total fugu brain RNA using a fugu stPrP-1-specific probe a single band of high abundance with a size of approximately 2.7 kb (Fig. 1) was detected.

The predicted amino acid sequence of the fugu stPrP-1 is 450 amino acids (aa) in length and has all features previously described for members of the PrP protein family [10,11], namely a signal sequence, two lysine-clusters, a Gly-Pro-rich region, a hydrophobic region, two cysteine residues potentially involved in the formation of an intramolecular disulfide bond, a glycosylation site, and a putative GPI-anchor-site (Figs. 2 and 3). Furthermore, similar to tetrapod PrPs the fugu homologue stPrP-1 is a relatively basic protein with a p*I* of 9.57.

The stPrP-1 sequence contains only one potential glycosylation site. However, in the fresh water pufferfish *Tetraodon nigroviridis*, a closely related species, the Contig_38492_1 was found to represent a part of the Tetraodon stPrP-1 coding region, corresponding to aa 247–426 of the *Fugu rubripes* stPrP-1. In the *T. nigroviridis* stPrP-1, a second glycosylation site is located in a similar position to other already known PrPs, underlining even more strongly the homologies between stPrP-1 and tetrapod PrPs (Fig. 3).

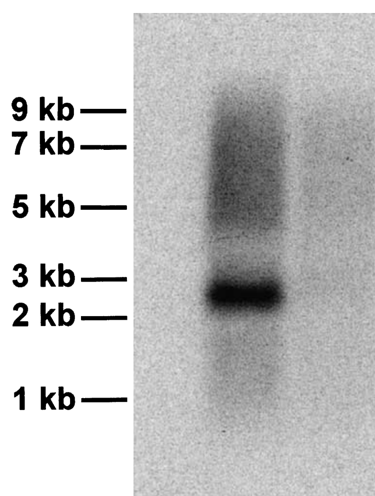

Fig. 1. Northern-blot analysis of *F. rubripes* stPrP-1 expression in brain tissue. Total RNA (10 μg), prepared from fugu brain tissue, was fractionated by agarose gel electrophoresis, transferred to nylon membranes and hybridised with a 586 bp fugu PrP-specific hybridisation probe.

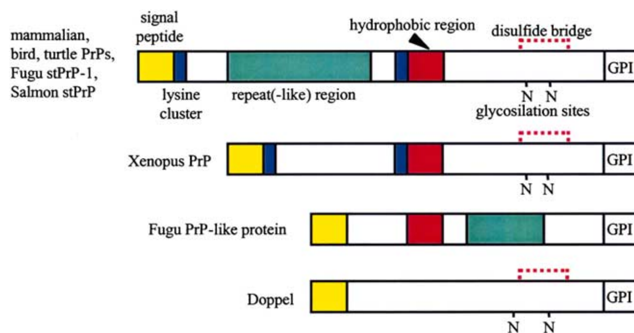

Fig. 2. Schematic presentation of characteristic structural PrP features. Note that the differences in size between fish stPrPs and tetrapod PrPs are not reflected.

The other identified sequence related to tetrapod PrPs termed stPrP-2 was located on scaffold 155 and was found in close vicinity (2 kb distance) to the recently described fugu prion-like protein coding region [12]. The fugu stPrP-2 cDNA could code for a protein of 425 aa with a p*I* of 9.14. However, by Northern-blot analysis stPrP-2 expression in fugu brain tissue was below the limit of detection (data not shown). Thus, the expression of stPrP-2 in fugu tissues requires further analysis. Otherwise, fugu stPrP-2 is closely related to stPrP-1 (Table 1 and Fig. 3). Besides the sequence homologies structural features in common for stPrP-2 and tetrapod PrPs are a signal sequence, a Gly-Pro-rich region, two typically positioned cysteine residues, potential glycosylation sites, and a putative GPI-anchor. However, in contrast to stPrP-1 the part of stPrP-2, which aligns to the highly conserved hydrophobic region of tetrapod PrPs, is effectively disrupted by charged amino acids. Furthermore, stPrP-2 has untypically three potential N-linked glycosilation sites in its C-terminal part.

Having identified the fugu stPrP sequences we performed database searches for PrP homologues of other fish species. One *Salmo salar* EST (GenBank BG934458.1) of 695 nucleotides displayed a significant homology to both fugu stPrPs. The sequence information for the salmon stPrP was subsequently completed by RACE experiments. The salmon stPrP is with 605 aa residues even larger than the fugu stPrPs but displays similarly structural features characteristic for tetrapod PrPs including a basic p*I* of 9.46 (Fig. 3). As to be expected for a PrP homologue the 3.2 kb salmon stPrP mRNA was detectable by Northern-blot analysis in all organs examined but the expression was clearly most prominent in brain tissue (Fig. 4).

4. Discussion

In summary, we describe the identification and characterisation of closely related cDNAs from *F. rubripes* and *S. salar* coding for proteins with significant homologies to tetrapod PrPs. In addition, the fugu *stPrP-1* and salmon *stPrP* genes are shown to be highly expressed in brain tissue. The sequence

```
Human          MANLG--CWMLVLFVATWSDLGLC----KKRPKPGG----------WNTGG----------------------------------------------------------------SR-YPGQGSP   44
Cow            MVKSHIGSW--ILVLFVAM-WSDVGLC-KKRPKPGG-----G----WNTGG----------------------------------------------------------------SR-YPGQGSP   47
Chicken        MARLLTTCCLLALLLAACTDVALS----KK-GK--GKPS--GGG--WGAGS----------------------------------------------------------------HR-QPSYPRQ   49
Turtle         MGRYRLTCWIVVLLVVMWSDVSFS----KK-GK--GK---GGGG--GNTGS----------------------------------------------------------------NR-NPNYPSN   48
Xenopus        MPQSLWTC-LVLISLICTLTVS-S----KKSG--GGK--SKTGG--WNTGS----------------------------------------------------------------NR-NPNYP--   46
Fugu stPrP-1   MGRL---CEVVLLSLLLLFLLNTKATWAKKSGSSGGK---KTGSS-TNWGS----------RTPSKPSGSQ-PNRNSNPYPSGGSYPHPGTGQ-SNP-GGYPRQNPASNPVGGSPNQ-YPGRTNP  104
Fugu stPrP-2   M-KL-SLLSLLSLSLL-LFHAHFS-LG-RKSGGTSGK-GSSSNKSSSSNNE-KT--KTSS------NAG---NRNNQGFVSESGSP-KQQGDNAPGGGGY--------GHGGY-----------P   87
Salmon stPrP   MGKL---CELALVSLALLALLHTDSTWAKRSGSS---SSSSKKTASSNRGSDKKPPKTSNTQTGSYPRQPQSPNRNPNPYPAGGSYPGRGTNQNQNPAGGYP-------AAGGYPNQQYPGRANP  112
Tetraodon      -----------------------------------------------------------------------------------------------------------------------------
Fugu-PrP-like  MSSQQKLPLTLTLCLLLLASLLPSSE-ARRGG--GGF-GRGGGGRSWG-RS--------------------------------------------------------------------------   46

Human          GGNR------------------------------------------------------------------------YPPQGGGGWGQPHGGGWG-QP----------------------------   68
Cow            GGNR------------------------------------------------------------------------YPPQGGGGWGQPHGGGWG-QP----------------------------   71
Chicken        PGYP------------------------------------------------------------------------HNPGYPHNPGYPHNPGYPHN-----------------------------   73
Turtle         PGYP------------------------------------------------------------------------QNPGYPRNPSYPHNPAYPPNPAYPPN-----------------------   78
Xenopus        -G-----------------------------------------------------------------------------------GYPGNTGG--S-----------------------------   56
Fugu stPrP-1   GGYPNQNPAGG--------------------------GYPNQNPARGNYPN--QYPAGGSNPNQ-YPGRAGTNQGGYPNQYPPAGGYPGQGGYPAAGRYPGQ-----------------------  177
Fugu stPrP-2   GGF-------GGR-----------------------------------------------------------------------GGH-GYGVYGH------------------------------  103
Salmon stPrP   GGYPNQNPAAGGYPAAGGNPAAGGYPNQQYPGRANPGGYPNQNPAAGGYPAAGGNPAAGGYPNQQYPGRA--NPGGYPNQNPAAGGYPAAGGNPVAGGYPNQQYPGRANPGGYPNQNPAAGGYPA  235
Tetraodon      -----------------------------------------------------------------------------------------------------------------------------
Fugu-PrP-like  -----------------------------------------------------------------------------------------------------------------------------   46

Human          ---------------------------HGGGWGQPH--------------------------------GGGWGQPHG-GG---------------------------------------------   88
Cow            ---------------------------HGGGWGQPH--------------------------------GGGWGQPHG-GG---------------------------------------------   91
Chicken        -------------------------PGYPHNPGYPQ--------------------------------NPGYPHNPGYPG---------------------------------------------   96
Turtle         -------------------------PGYPHNPSYPR--------------------------------NPSYPQNPGYPG---------------------------------------------  101
Xenopus        -----------------------------------------------------------------------------------------------------------------------------   56
Fugu stPrP-1   ---------------------GGYP-QYPAAGGYPNQRSP-----YQYPA--------AGGYPVRTGNTGPGWGQPGGHPGGYPGGYPGGYPGGAVGGYTNWNPNNKILSP-------------R  254
Fugu stPrP-2   -------------------------PGYGG-HGYG------VYGHPGY--GGHGF-HGRGGY-------KPGDG-P--YRGSYGGGYY-DY----GGRYINQNPNNQILSPHYVNSFGDGGRGGR  178
Salmon stPrP   AGGNPVAGGYPNQQYPGRANPGGYPNQNPAAGGYPNQNPGRGGVNPGYPAGGH----PAGGYPVRGGNT--GWGQAGGYPGGGTG---GGYQ-------PNWNPNNKILSP-------------R  331
Tetraodon      ----------------------------------------------------------------------------------------------AVGGYTNWNPNNQILSP-------------R
Fugu-PrP-like  ----------------------------------------------------------------------------------------------------------QGQSR-------------G   52

Human          WG-Q--------GG----GTHSQWN--KP--SKPKTNMKH-MAGAAAAGAVVGGLGGYVLGSAMSRPIIHFGSDYEDRYYRENM-HRYPNQVYYRPMD---EYSNQ--------NNFVHDCV--  180
Cow            WG-QPHGGG--GWG--QGGTHGQWN--KP--SKPKTNMKH-VAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYYRENM-HRYPNQVYYRPVD---QYSNQ--------NNFVHDCV--  191
Chicken        WG-QGYNPS--SGG--SYHNQKPW---KP----PKTNFKH-VAGAAAAGAVVGGLGGYAMGRVMSGMNYHFDSPDEYRWWSENS-ARYPNRVYYR-DYSS--PVPQ--------DVFVADCF--  193
Turtle         GGGQHYNPA--GGG-TNFKNQKPW---KP--DKPKTNMKA-MAGAAAAGAVVGGLGGYALGSAMSGMRMNFDRPEERQWWNENS-NRYPNQVYYKE-YNDR-SVPE--------GRFVRDCL--  203
Xenopus        WGQQPYNPS----G---YN--KQW---KP--PKSKTNMKS-VAIGAAAGAI----GGYMLGNAVGRMSYQFNNPMESRYYNDY-YNQMPNRVYRPM-YRGEEYV--------SEDRFVRDCY--  149
Fugu stPrP-1   FGGGGYMPG--MGG-SPFSQSVQSMGYKP--KSPSFAKKA-MV-AAGVGAVAGMAVGYGLG-RFPRPHFGFRSPSEEYYYNNYMYHRYGTQSTDEKDY-GRDYVYKPPPRAESYENFMDRCM--  367
Fugu stPrP-2   RGSAFYDKVKVMGR-SPSHE-----------SK-GFGHTA--ARAASGGAVAKMAVDYGLG-RFPRPPFQFQSPEEEYYYNHYMYRTYGVKSTDANDY-SRDYFYSKP--LENYERHMNTCMK-  282
Salmon stPrP   YGG-GYGYGG-MGGGSPFSRSVQGMGQYPSTQSKGFAKKAFM--AAGVGALAGMAVGYGLG-RFPRPHFNFRNPQEEQSYNNYMYSKYGERSTDENDY-GRDYTYKVPPRAESYDSFMDTCMKR  449
Tetraodon      FGGGGYMPG--MGG-SPFSQSVQAMGYKP--KSPSFAKKA-MV-AAGVGAVAGMAVGYGLG-RFPRPHFGFRSPSEEYYYNNYMYRQYGLKSTDERDY-GRDYVYKLPPRAESYEQFMDRCM--
Fugu-PrP-like  WGGGGAGQA--YRPVQ--------------SSNPS-AGK--VAGAAAAGAI----GGAMIGSALSRPGYGYGGYGGYGMGYGGYGGGYG--GGYGGGY-GRGYGYPRGGY--SNE---------  139

Human          NIT-IKQRTV----------------------------------------------------------TTTTKGENFT---ETDVKMME--RVVEQMCITQYERESQAYY--QRGS---------  230
Cow            NIT-VKEHTV----------------------------------------------------------TTTTKGENFT---ETDIKMME--RVVEQMCITQYQRESQAYY--QRGA---------  241
Chicken        NIT-VTEYSI--------------------------------------------------GPAAKKNTSEAVAAANQTEV-EMENKVVT--KVIREMCVQQYREY-------RLAS---------  248
Turtle         NNT-VTEYKI-----------------------------------------------------------DPNENQNVTQV-EVR--VMK--QVIQEMCMQQYQQY-------QLAS---------  247
Xenopus        NMS-VTEYII-----------------------------------------------------------KPTEGKNNSELNQLDTTV-KS-QIIREMCITEYRR----------GS---------  193
Fugu stPrP-1   NRTDLLKDKG-----------------------------------------------------------GKTDGDDDTVGIEEIGYPALVDQMKSRRCVEEYMAYSEQFLEKQA-----------  422
Fugu stPrP-2   NI-ELLPEENQQKSNKPALYTPSSPVLAPNNDTDNNTAADNSSTSTTPNHPNQPEAKPPSP-TAQIPSESTADRDSDTVSIVEIGYPALIEQIKLRKCLERYMVYSEKYLEM-RGGAQ-------  397
Salmon stPrP   ND--LLQDQGRDSQATPNNQKNN-PQGANSQNMPITPEMGNSSPASNQEGTDKV--KPLTPAPDEIAGEGKEEVDGPTVSIEEIGYPALVEQLKNRKCVELYINHSERFLEKQ-------TAERS  562
Tetraodon      NRTNLLKDQG-----------------------------------------------------------SSKAEDNDTVSIEEIGYPALIEQMKSRRCLEEYMAKSEK----------------
Fugu-PrP-like  --------------------------------------------------------------------------------------PEGSGDM-------EY-YYS-------GTSSSPA-----  158

Human          ------------------SMVLFSSPPVI--LLIS-FLIFLIVG                                                                                   253
Cow            ------------------SVILFSSPPVI--LLIS-FLIFLIVG                                                                                   264
Chicken        ------------------GIQLHPADTWLAVLLLLL--TTLFAMH                                                                                  273
Turtle         ------------------GVKLLSDPSLM--LIIMLVIFF--VMH                                                                                  270
Xenopus        ------------------GFKVLSNPWLI--LTITLFVYF--VIE                                                                                  216
Fugu stPrP-1   ---------------RNRCGPLSFGAIQLFSSLL-LL-SSTLLLQ                                                                                  450
Fugu stPrP-2   -------------RLETGSQMVLVG---VTSTLLMLMNSSMLH                                                                                    424
Salmon stPrP   SSSSSNNNNNNKNVNSRSNPLGQGLVQLITSLLMVL-SSILLLH                                                                                   605
Tetraodon      --------------------------------------------
Fugu-PrP-like  ---------------Y--------SSIIIITSLTALLMGHW--VASI                                                                                180
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Fig. 3. Alignment of PrP amino acid sequences from different species. Amino acid positions identical in five species or more are shaded in grey. Conserved cysteine residues involved in the formation of disulfide bridges and N-linked glycosilation sites are shaded in red. The N-linked glycosilation sites of fugu stPrP-2 and salmon stPrP are marked in green. No numbering is given for the deduced *T. nigroviridis* PrP sequence because it is still incomplete.

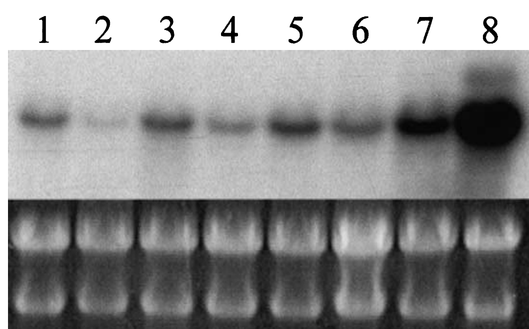

Fig. 4. Northern-blot analysis of tissue-specific expression of salmon stPrP. Lane 1, muscle; 2, liver; 3, skin; 4, gills; 5, kidney; 6, spleen; 7, heart; 8, brain. Total RNA (10 μg), prepared from the indicated tissues, was fractionated by agarose gel electrophoresis, transferred to nylon membranes and hybridised with a 703 bp salmon PrP-specific hybridisation probe. The lower part of the figure shows the ethidium bromide-stained gel before the transfer to demonstrate that equal amounts of qualitatively comparable RNAs were loaded.

similarities of fugu and salmon stPrPs to tetrapod PrPs and the conservation of structural features indicates that fish PrPs may fold in part like tetrapod PrPs.

A striking feature of the fugu and salmon stPrPs is the length and heterogeneity of the Gly-Pro-rich region, which resembles the repeat region of mammalian PrPs. It consists of 201, 141, and 70 aa for the salmon stPrP, the fugu stPrP-1, and stPrP-2, respectively. In contrast, among tetrapod PrPs the longest repeat region reported to date is that of turtle with 60 aa, whereas in mammals or birds it extends only over a region of 32–54 residues and is entirely missing in the African clawed frog. Histidine residues present in tetrapod PrP repeat regions, which may be involved in copper binding, are among the fish stPrPs only found in the Gly-Pro-rich region of *F. rubripes* stPrP-2. However, native chicken PrP does not bind copper despite the histidine residues in its repeat region [13], which underlines the potential functional diversity of this PrP domain in different species.

Suzuki et al. [12] reported recently a cDNA coding for a putative prion-like protein in *F. rubripes*. However, in contrast to fugu stPrP-1 and salmon stPrP reported here the expression of fugu prion-like mRNA was essentially confined to the eye and was hardly detectable in the central nervous system or in any other organ. In addition, the suggested prion-like protein lacks characteristic key features of PrPs; the lysine clusters, the glycosylation site(s) and the conserved cysteine-residues are missing. Moreover, the only repeat (-like) region of this PrP-like protein is atypically positioned C-terminally of the hydrophobic region (Fig. 3).

Although we did not investigate the genomic organisation of the fugu and salmon *stPrP* genes in detail, our comparison of cDNA and genomic DNA sequences indicates that the fugu stPrP-1 coding region is located on a single exon, which is a feature in common with the fugu prion-like coding region and mammalian PrP genes. However, some stPrP-1 cDNAs sequenced showed the presence of a small in-frame intron of 30 nucleotides in length in the Gly-Pro-rich region, which could further contribute to the heterogeneity of this domain.

In mammalian genomes the *PrP* gene tandemly lines with its duplicate *doppel*. In contrast the two fugu *stPrPs* were found on different scaffolds. Because the human and fugu genome syntenies are highly conserved in limited lengths of chromosomal segments [14], it seems unlikely that the ancestors of stPrP-1 and stPrP-2 evolved directly into the present forms of PrP and doppel. Interestingly, the *stPrP-2* coding region is located in close vicinity to the *fugu prion-like* gene on scaffold 155. Furthermore, fugu homologues of KIAA0168 (RAS association protein family domain 2) and SLC231A (solute carrier family 23 member) are present on the same scaffold in direct neighbourhood to fugu prion-like and stPrP-2 but were not found on scaffold 96, which contains fugu stPrP-1. Given that in the human genome *KIAA0168* and *SLC231A* are located together with *PrP* and *doppel* on chromosome 20p13 it is tempting to speculate that genetic rearrangements in this region contributed to the evolution of tetrapod PrPs and doppel.

Taken together, the *F. rubripes* genome harbours at least three elements, *stPrP-1*, *stPrP-2*, and *fugu prion-like*, whose ancestors have potentially participated in the evolution of PrPs from fish to tetrapods. In terms of structural relatedness stPrP-1 comes closest to tetrapod PrPs. In terms of genomic localisation *stPrP-2* and *fugu prion-like* appear to be more closely linked to tetrapod PrPs, although both genes are hardly expressed in brain tissue. None of these so far identified elements resembles *doppel* (Fig. 2) [15]. Hence, the duplication of the *PrP* gene, which generated *doppel*, may have occurred not in fish but later in the tetrapod lineage.

An important factor controlling interspecies transmission of prion infections is the PrP homology between source and recipient species [16,17]. Although the C-terminal part of the fugu stPrP-1 is surprisingly most closely related to the bovine and human PrP with 25.0% and 24.8% aa identities (Table 1), respectively, based on the sequence homology alone, it seems unlikely that fish could contract a TSE by the uptake of

Table 1
Amino acid identities (%) for the C-terminal part of tetrapod PrPs[a] and PrP-related fish proteins

	Human	Cattle	Chicken	Turtle	*Xenopus*	F. PrP-1	F. PrP-2	S. PrP	F.-like
Human	–	90.8	40.4	41.8	35.5	24.8	21.3	17.7	16.3
Cattle	90.8	–	44.4	42.4	36.1	25.0	22.2	18.1	21.4
Chicken	40.4	44.4	–	59.3	39.0	17.8	16.0	25.0	21.4
Turtle	41.8	42.4	59.3	–	40.7	22.1	20.7	24.1	14.3
Xenopus	35.5	36.1	39.0	40.7	–	23.3	17.1	17.3	18.4
F. PrP-1	24.8	25.0	17.8	22.1	23.3	–	51.8	68.3	23.5
F. PrP-2	21.3	22.2	16.0	20.7	17.1	51.8	–	41.8	16.3
S. PrP	17.7	18.1	25.0	24.1	17.3	68.3	41.8	–	19.4
F.-like	16.3	21.4	21.4	14.3	18.4	23.5	16.3	19.4	–

[a]Corresponding to aa 90–230 of the human PrP, which form the proteinase K-resistant core of the disease-associated PrP isoform. F = fugu and S = salmon.

prions of mammalian origin. However, the multiple factors responsible for the so-called species barrier are neither fully investigated nor completely understood. Moreover, the situation appears to be more complex than previously anticipated because as shown for *F. rubripes* fish genomes may code for more than one protein with homologies to mammalian PrPs. The fugu and salmon PrP homologues presented in this study facilitate further analysis of the potential conversion of a PrP from fish into a disease-associated isoform by mammalian prions and is likely to accelerate the identification of PrPs from further fish species farmed for human consumption.

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