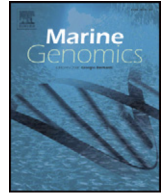
Marine Genomics 3 (2010) 157–163

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen



# Cloning of cDNA and prediction of peptide structure of Plzf expressed in the spermatogonial cells of *Labeo rohita*

Chinmayee Mohapatra [a], Hirak Kumar Barman [a,*], Rudra Prasanna Panda [a], Sunil Kumar [b], Varsha Das [a], Ramya Mohanta [a], Shibani Dutta Mohapatra [a], Pallipuram Jayasankar [a]

[a] *Fish Genetics & Biotechnology Division, Central Institute of Freshwater Aquaculture (Indian Council of Agricultural Research), Kausalyaganga, Bhubaneswar 751002, Odisha, India*
[b] *Bioinformatics Centre, Institute of Life Sciences, Nalco Square, Bhubaneswar-751023, Odisha, India*

ARTICLE INFO

*Article history:*
Received 29 June 2010
Received in revised form 31 August 2010
Accepted 3 September 2010

*Keywords:*
Plzf
BTB domain
3D structure
BTB homo-dimer
Spermatogonial stem cell
*Labeo rohita*

ABSTRACT

The promyelocytic leukemia zinc finger (Plzf) gene containing an evolutionary conserved BTB (bric-a-brac/tramtrack/broad complex) domain plays a key role in self-renewal of mammalian spermatogonial stem cells (SSCs) via recruiting transcriptional co-repressors. Little is known about the function of Plzf in vertebrate, especially in fish species. To gain better understanding of its role in fishes, we have cloned Plzf from the testis of *Labeo rohita* (rohu), a commercially important freshwater carp. The full-length cDNA contains an open reading frame (ORF) of 2004 bp translatable to 667 amino acids (aa) containing a conserved N-terminal BTB domain and C-terminal $C_2H_2$-zinc finger motifs. *L. rohita* Plzf, which is phylogenetically related to *Danio rerio* counterpart, abundantly expressed in spermatogonial stem cells (SSCs). A three-dimensional (3D) model of BTB domain of Plzf protein was constructed by homology modeling approach. Molecular docking on this 3D structure established a homo-dimer between two BTB domains creating a charged pocket containing conserved aa residues: L33, C34, D35 and R49. Thus, Plzf of SSC is structurally and possibly functionally conserved. The conserved aa residues in the cleft resulting from Plzf BTB self-association are likely to be the binding platform for interaction with recruited co-repressor peptides. The identified Plzf could be the first step towards exploring its role in rohu SSC behavior.

© 2010 Elsevier B.V. All rights reserved.

## 1. Introduction

The promyelocytic leukemia zinc finger (Plzf, also known as zfp145) was first discovered by virtue of its involvement in a t(11;17) chromosomal translocation as a fusion protein with the retinoic acid receptor α (RARα) in acute promyelocytic leukemia (APL) (Chen et al., 1993). Patients harboring such translocation are resistant to retinoic acids therapy. Plzf is a nuclear phosphoprotein (Reid et al., 1995) containing C-terminal nine *kruppel*-$C_2H_2$-type zinc fingers capable of recognizing specific DNA sequences (Barna et al., 2002) and an N-terminal BTB (bric-a-brac/tramtrack/broad complex) domain, also known as POZ (poxvirus and zinc finger) domain, named for its presence in the *Drosophila* proteins BTB (Zollman et al., 1994) as well as its homology with several POZ (Bardwell and Treisman, 1994). BTB motif of 120 amino acid (aa) residues is associated with RARα. Both BTB and zinc finger domains are highly conserved throughout eukaryotes. BTB domain forms homo-dimerization, hetero-dimerization between different BTB-harboring proteins and is involved in transcriptional repression (Bardwell and Treisman, 1994; Chen et al., 1993; Li et al., 1997). Several nuclear co-repressor proteins: SMRT, N-CoR, Sin-3, LYRIC/AEG-1 and in turn histone deacetylases (HDACs) are recruited in the transcriptional complex via its BTB domain (David et al., 1998; Grignani et al., 1998; He et al., 1998; Hong et al., 1997; Lin et al., 1999; Thirkettle et al., 2009). Plzf has been described as a repressor for Hox-containing genes during embryogenesis (Barna et al., 2002; Felicetti et al., 2004). These evidences indicate that the transcriptional repressor function of Plzf is mediated through altering local and long-range chromatin topology.

Spermatogenesis is a complex process comprising mitotic and meiotic cell-cycle events of testis. The mitotic germ cells in the adult testis are the spermatogonial stem cells (SSCs), which originate from the primordial germ cells (PGCs) during embryogenesis. SSCs are capable of undergoing self-renewal and differentiation throughout the life-time to produce spermatozoa. Very little is known about spermatogonial renewal and differentiation in the fish testis. Establishment of *in vitro* culture system for SSCs could be an effective approach to explore molecular mechanisms controlling the proliferation and differentiation of SSCs because it is difficult to investigate SSC behavior *in vivo*. Even though mice and hamster spermatogonia and SSCs could be isolated efficiently for long-term culture (Kanatsu-Shinohara et al., 2005, 2008), such an efficient protocol in other species including fish is lacking. Recently, the primary culture system for fish SSC has been established (Nobrega et al., 2009; Shikina et al., 2008). One of the major obstacles in the progress of SSC biology

* Corresponding author. Fax: +91 6742465407
*E-mail address:* hkbarman68@hotmail.com (H.K. Barman).

1874-7787/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.margen.2010.09.002

research has been the lack of sufficient information about phenotypic and genotypic markers specific to different developmental stages of spermatogonial cells. SSCs share some, but not all, phenotypic characteristics of other stem cells (Kubota et al., 2003).

*Labeo rohita*, popularly known as rohu, is the most commercially important carp species in India as well as in neighboring countries (Barman et al., 2003). Recently, we have identified some novel ESTs preferentially expressed in rohu testis and spermatogonial cell by subtractive and suppressive hybridization (unpublished, GenBank Accession nos: GR911356–GR911432). These novel transcripts could be used as genetic-markers to identify fish spermatogonial cells. With the best of our knowledge, the full-length sequence information for any transcripts with known functions linked to SSC proliferation and/or differentiation in *L. rohita* is completely lacking.

Plzf expression was identified in male germ cells (Buaas et al., 2004; Costoya et al., 2004). The rapid exhaustion of proliferative spermatogonial cells was evidenced in Plzf-null mice (Costoya et al., 2004). The critical role of Plzf was demonstrated by repressing the transcription of Kit, a hallmark of spermatogonial differentiation (Filipponi et al., 2007). The epigenetic regulation of the methylated histone in Plzf-expressing mouse spermatogonial cells was envisaged (Payne and Braun, 2006). These evidences suggested that Plzf controls the tight balance between spermatogonial self-renewal and differentiation. Work on expression of markers on fish spermatogonia that may help identify the stem cell pools has been extremely limited (Nobrega et al., 2009), and this is a major obstacle for isolation and characterization of highly pure population of fish SSCs. On the other hand, some rodent markers for SSCs were not applicable to humans (He et al., 2010). Hence, isolation, characterization and functional analyses of fish-specific phenotypic markers are essentially required. From the evidences described above, Plzf may be a marker for rohu SSCs, as it is for mouse SSCs.

In this study, we have cloned and sequenced the Plzf cDNA from the testis of *L. rohita*. The N-terminal BTB domain and C-terminal zinc fingers were identified. Tissue distribution analysis revealed its abundance in spermatogonial cells. We have also predicted the three-dimensional (3D) structure of BTB domain by homology modeling. Deduced aa sequence of *L. rohita* Plzf cDNA was compared with homologous sequences of mammals and chicken from GenBank. Further, we have performed molecular docking on this 3D model so as to understand its homo-dimerization properties. These results are discussed in the context of previously identified Plzf of other species.

## 2. Materials and methods

### 2.1. RNA Extraction and cDNA preparation

Total cellular RNA was extracted from *L. rohita* testis using TRIzol solution according to the manufacturer's instructions. RNA samples were treated with DNaseI (Bangalore Genei, India) to eliminate DNA contamination. The treated RNA was extracted with phenol and chloroform, precipitated and resuspended in RNase-free water. RNA quality and quantity was verified by denaturing gel electrophoresis and spectrophotometric readings. The cDNA was synthesized using Prime Script™ reverse transcriptase (Takara) and oligodT primers as per manufacturer's protocol.

### 2.2. Isolation of full-length Plzf cDNA and data analysis

The testis cDNA was amplified by polymerase chain reaction (PCR) with various combinations of forward and reverse primers (Supplementary Table 1) designed from the consensus sequence of *Danio rerio, Homo sapiens* and *Mus musculus* Plzf cDNA. The PCR reaction of 25 µl final volume was a mixture of 20 µl of PCR grade water, 0.5 µl of cDNA, 1 µl primer mixture (10 pM forward and 10 pM reverse), 2.5 µl of 10X PCR buffer with 15 mM $MgCl_2$ (Bangalore Genei, India), 0.5 µl of 10 mM dNTPs and 1.5 U of *Taq* DNA polymerase. The PCR program was as follows: 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 58 °C, and 1 min at 72 °C. The final elongation was at 72 °C for 7 min. PCR products were separated on a 1.5% agarose gel and desired bands were excised for purification using gel extraction kit (USB). The purified fragments were cloned into pGEMT easy vector (Promega) and then transformed into chemically competent DH5α cells. The positive inserts were sequenced using an automated ABI 310 genetic analyzer (Perkin-Elmer Applied Bios system). The sequences were verified as parts of Plzf using the BLASTN program (http://www.ncbi.nlm.nih.gov/blast). The sequence towards 5′-end was obtained following genome walking strategy using Genome Walker™ kit (Clontech) as per manufacturer's instructions. Briefly, the genomic DNA was extracted from *L. rohita* liver (Barman et al., 2003). Four restriction enzyme (*Eco*RV, *Dra*I, *Pvu*II and *Stu*I) digested libraries were constructed. RAPID amplification of cDNA ends (RACE) PCR was performed to obtain the 3′-end of the Plzf transcript using Smarter RCAE cDNA amplification Kit (Clontech) as per manufacturers' protocol. Gene-specific primers were designed from the obtained partial sequence of *L. rohita* Plzf (Supplementary Table 1). The amplified DNA fragments were cloned, sequenced and analyzed as described above. The amino acid sequence was deduced by Expasy translate tool (http://expasy.org/tools/dna.html) and verified using BLASTP at the same site. Phylogenetic analysis of the predicted Plzf aa sequences of *L. rohita* and homologous sequences from GenBank was performed using MEGA version 4.0 Neighbor Joining consensus trees were generated using Maximum Composite-Likelihood model with 500 replicates for bootstrap analysis (Tamura et al., 2007).

### 2.3. Cell culture

Rohu (*L. rohita*) spermatogonial cells were cultured as described with minor modifications (Kurita et al., 2004). Briefly, collagenase dissociated adult testis were cultured in L-15 medium containing MEM, 5% FBS, 1% rohu serum, GDNF (glial cell-derived neurotrophic factor), bFGF (basic fibroblastic growth factor), glucose, and insulin at 28 °C in the absence of feeder cells. RNA was extracted from spermatogonial cells (as described above) being propagated for 90 days with 6 passages.

### 2.4. Quantitative RT-PCR

Quantitative RT-PCR (qPCR) for Plzf and the house-keeping genes were performed in triplicate for each cDNA sample using Light Cycler-480 SYBR Green I kit (Roche Diagnostics) in a Light Cycler 480 RT-PCR instrument (Roche Diagnostics) as per manufacturers' instructions. In order to identify the most stable house-keeping gene across the tissue panel, gene expression stability measures (M values) were calculated by geNorm software as described (McCurley and Callard, 2008; Vandesompele et al., 2002). Relative mRNA levels of target gene (Plzf) were normalized to elongation factor 1-alpha (Elfa) expression for each sample, and normalized standard deviations were calculated. Primer annealing temperature for both Plzf and Elfa was 60 °C. Negative control PCR containing RNA template and Elfa primers were included for each sample to rule out the possibility of genomic DNA contamination.

### 2.5. Homology modeling

The Plzf protein of *L. rohita* (GenBank Accession no: GI 284468449) was used as target sequence. The three-dimensional (3D) structure of the Plzf protein of *L. rohita* was not yet available in data bank; hence the present exercise of developing the 3D model of the Plzf protein was undertaken. BLASTP search was performed against Brookhaven Protein Data Bank (PDB) with the default parameters to find out suitable templates for homology modeling (Altschul et al., 1990;

Download English Version:

https://daneshyari.com/en/article/2058267

Download Persian Version:

https://daneshyari.com/article/2058267

Daneshyari.com