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Research paper

Distribution analysis of profilin isoforms at transcript resolution with mRNA-seq and secondary structure in various organs of *Rattus norvegicus*

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ABSTRACT

Profilin (*Pfn*) is an actin binding protein, ubiquitously found in mammals and is essential for the actin polymerization in cells. In brain, it plays a pivotal role in neurogenesis and synapse formation by interacting with various proteins. Four *Pfn* isoforms have been identified in mammals. This study presents the identification and transcriptional expression of various *Pfn* isoforms (*Pfn*1, *Pfn*2, *Pfn*3 and *Pfn*4) in brain, heart, kidney, liver, and muscle and testis of *Rattus norvegicus.* Organs have been classified into groups based on some similarities. Group I includes brain and testis, Group II includes skeletal muscle and heart, while Group III includes kidney and liver. *Pfn*1 has been identified in all groups, *Pfn*2 and *Pfn*3 have been identified in group I, group III and in one organ (skeletal muscle) of group II. To the best of the authors knowledge, no report of *Pfn*1 and *Pfn*2 presence in testis, *Pfn*3 in brain, liver and skeletal muscle, *Pfn*4 in kidney and skeletal muscle exists to date. Transcriptional expression showed variations among expression level of different *Pfn* isoforms in various organs with respect to the control gene GADPH. We hypothesize that this could be attributed to profilin isoform specific mRNA structure and corresponding motifs, which generally contribute to similar or varied decay rates, cellular localization, post transcriptional regulation pattern and ligand binding.

1. Introduction

Pfn ubiquitously found in mammals and are small proteins of molecular weights of 14–16 kDa, which play a pivotal role in the modulation of actin microfilaments dynamics (Carlsson et al., 1977; Tilney et al., 1983; Pollard and Cooper, 1986; Rao et al., 2013). *Pfn* family is critical for temporal and spatial regulation of the actin filament growth, a central process in cell shape alteration and cellular mobility. In addition to this, *Pfn* is also critical in metastasis of tumor cells, maintenance of cell structure integrity, as well as signal transduction of growth factors (Ding and Roy, 2013). *Pfn* is present in muscular cells, as well as in non-muscular cells, with very high assembly of actin molecules. *Pfn* binds in a 1:1 complex with the monomeric actin which can cause the shift of balance between actin filaments and their associated subunits. In animal cells, this process is carried out under the regulation of many large, multidomain proteins of the formin families and Ena/VASP that can bind to the actin, profilin as well as profilin-actin complexes that are thought to be members of various signalling pathways (Krause et al., 2003; Krebs et al., 2001; Watanabe et al., 1997). Loss of *Pfn*1 in mice is lethal during early development (Witke et al., 2001).

In addition to binding to actin and the polyproline stretches of formins and Ena/VASP and other proteins, *Pfn* shows binding affinity to the acidic phospholipids, some of which are also involved in signal transduction. Other than actin monomer, *Pfn* has high binding affinity with interactive partners like Phosphatidylionositol 4,5 bisphosphate (PIP2) (Sohn et al., 1995), Poly-L-proline (PLP) rich domains (Björkegren et al., 1993; Gibbon et al., 1998), vasodilator stimulated phosphoprotein (VASP) (Haffner et al., 1995; Reinhard et al., 1995), formin like protein (Frazier and Field, 1997; Kamei et al., 1998), Arp2/3 complex (Mullins et al., 1998; Loisel et al., 1999), rho-associated, coiled-coil-containing protein kinase 1 (ROCK1), Dynamin, WAVE regulatory complex and neuroligin. Pfn regulates the ADP-actin monomer in Srv2/CAP pathways (Mattila et al., 2004), Pfn2 specific isoform is involved in Rho/Rac pathway (Da Silva et al., 2003), Pfn1 showed its association in PI3K/AKT pathway (Das et al., 2009).

*Pfn*s are thought to be involved in linking the transduction of signals with the actin filament formation and were found to localize with actin, VASP, WAVE and formins in membrane, opposed to the dynamic regions of cells (Jockusch et al., 2007). However, the presence of various actin based motility processes in cells, such as locomotion, morphogenesis of cellular protrusions, cytokinesis, membrane trafficking and adhesion complexes, probably require regional modulation and specific fine tuning of *Pfn* ligand interactions (Polet et al., 2007).

Four *Pfn* isoforms have been identified and expressed in tissue specific manner. The present study aimed to identify the presence of *Pfn*1, *Pfn*2, *Pfn*3 and *Pfn*4 in brain, heart, kidney, liver, skeletal muscle and testis of *Rattus norvegicus.* Since, mRNA structure broadly influences gene regulation and the nature of profilin mRNA structures or effects of sequence variation (leading to isoform formation) in them is not known. For this purpose, online web servers were also utilized for secondary

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structure prediction as well as structural motif analysis of the profilin isoforms.

2. Material and methods

2.1. Animal care and usage

Animal care and experimental procedure were carried out in accordance with ethical review board (ERB) of Forman Christen College Lahore (FCCU), Pakistan and all the efforts were made to minimize the animal suffering. Rats were dissected under general anesthetic conditions and organs were surgically separated. Fresh tissues were immediately processed for RNA extraction.

2.2. Total RNA extraction

Total RNA was extracted from brain, heart, kidney, liver, muscle, pancreas and testis of *R. norvegicus* using the TRIzol® Reagent (Life technologies, Thermo Fisher Scientific USA) by following manufacturer's protocol. RNA qualitative and quantitative analyses were done on Nanodrop 2000 (Life technologies, Thermo Fisher Scientific USA) of all RNA samples. RNA was stored at −80 °C for further use.

2.3. cDNA synthesis and gene amplification

Quantified RNA was used for first strand cDNA synthesis. 1 μg of total RNA was used to prepare the cDNA library by using oligo (dT) and M-MLV reverse transcriptase (Life technologies, Thermo Fisher Scientific USA #K1631) according to manufacturer's protocol. Primers used for the amplification of target cDNA were designed from conserved region of related sequences available in databank (www.ncbi.nlm.nih.gov). Genbank information was used for designing of *Rattus norvegicus* primers. All sequences were aligned using clustalW (www.ebi.ac.uk/Tools/msa/clustalw2/) and primers were designed with the help of primer designing tool of the National Centre for Biotechnology Information databank (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Polymerase chain reaction mixture (25 μl) was prepared by adding 1 μl of first strand cDNA, 2.5 μl 10× Taq buffer (Life technologies, Thermo Fisher Scientific USA), 2 μl $MgCl_2$ (2.5 mM), 2 μl dNTP mix (2.5 mM), 2 μl forward primer (10 μM), 2 μl reverse primer (10 μM) (Table 1), 1.5 μl Taq Polymerase (1 U). PCR analysis was carried out using thermocycler (Nyx Technik Amplitronyx Series 4 A4, GMI USA). The amplification conditions are as follows: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 58 °C for 30 s and extension at 72 °C for 1 min. Final extension was carried out at 72 °C for 7 min (Fig. 1).

2.4. Gene sequencing analysis

PCR products were resolved on 1.2% agarose gel electrophoresis. PCR products of accurate size were purified from gels using gel purification kit (FavorPrep™, BiOSETTIA USA). Cleaned products were sequenced by Sanger sequencing (Eurofins USA) with gene specific primers.

2.5. Structural analysis of the profilin mRNA transcripts

RNA secondary structures were predicted using RNA fold webserver (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) by feeding FASTA formatted profilin mRNA sequences and minimum free energy and partition functional parameters. Secondary structure in the usual dot-bracket notation and 2D form was obtained. Thermodynamic description according to the loop-based energy model was also obtained, using RNAeval web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAeval.cgi) at the backend. Ensemble free energy or partition function folding along with positional entropy was also acquired (Gruber et al., 2008; Lorenz et al., 2011).

RNA promo (http://genie.weizmann.ac.il/pubs/rnamotifs08/rnamotifs08_predict.html) was used to predict structural motifs in the sequences using Vienna algorithm. Specific and relatively short candidate structures were identified and used as seeds for inferring motifs, by means of a probability oriented algorithm. These were then overlaid on the already predicted whole RNA structures, for inferring locality and significance with respect to certain region of the mRNA. Intra and inter-isoform comparison of the motifs was also carried out to pinpoint possible similarities and differences which may be responsible for the varied and similar localities of the mRNA in different rat organs.

2.6. Quantitative real time PCR analysis of Pfn (1, 2, 3 and 4)

Quantitative real time PCR primers for *Pfn* isoforms were designed using GenScript Real-time PCR (TaqMan) Primer Design (https://www.genscript.com/ssl-bin/app/primer) and are given in Table1. The reactions were carried out in CFX real time PCR detection system (Bio-rad USA). Each 20 μl reaction contained SYBR green mix (Life technologies, Thermo Fisher Scientific USA), 0.75 μl each forward primer (10 μM) and reverse primer (10 μM) (Table 2), 1 μl cDNA and molecular grade water. The housekeeping gene GAPDH was used as internal control for all real time experiments (Table 2). The cycling parameters are as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 57 °C for 6 s and extension at 72 °C for 1 min. This was followed by plate read for the detection of fluorescence at 72 °C. Lastly, final extension was carried out at 72 °C for 3 min. The specificity of PCR amplification was determined by melt curve analysis followed by 65 °C for 30 s, 95 °C for 5 s and 25 °C for 30 s. All the samples were preceded in triplicate and all experiments were repeated at least three times.

2.7. Statistical analysis

All the results of QRT-PCR were expressed as mean ± (S.D). One-way analysis of variance (ANOVA) was followed by Tukey's HSD post hoc test was used to analyze that the data, with the statistical

Table 1
Gene specific and Real time gene specific primers sequence for the Pfn1, Pfn2, Pfn3 and Pfn4.

	Primer ID	Primer sequence	Annealing temperature	Amplicon size
Complete gene primers	Pfn1: NM_022511.2	F = 5′CCCCGAGCTCTCTGCCTT′3 R = 5′TATCTGTCCATCCAGCCCCA′3	57	665 bp
	Pfn2: NM_030873.1	F = 5′AGTGCGAAGGGCTCGAAG′3 R = 5′CCCCTAATACTTAACAGTCTGCCTA′3	57	479 bp
	Pfn3:NM_001109487.1	F = 5′TTACTGGGACTG ACTGA GCGG′3 R = 5′TGGTTGGTAGGGAGACCAGAAT′3	57	470 bp
Real time gene specific primers	Pfn1	F = 5′ACTGCCAAGACGCTAGTCCT′3 R = 5′GAGGTCAGTACTGGGAACGC′3	57	112 bp
	Pfn2	F = 5′CGGGAAGGTTTCTTTACCA′3 R = 5′GACCAAGACTCTCCCAGCTC′3	57	168 bp
	Pfn3	F = 5′GGTGATGGCGTACTGGATG′3 R = 5′ATCCGCCAATCAGATCGT′3	57	151 bp

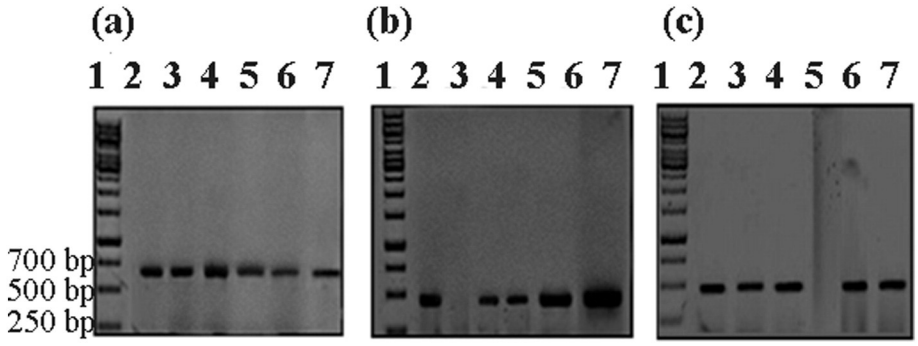

Fig. 1. Full length gene amplification of Pfn1, Pfn2 and Pfn3 in different organs. (a) Full length gene amplification of Pfn1 in *Rattus norvegicus*, M represents the 1 Kb ladder, 1 = Pfn1 identified in brain, 2 = Pfn1 identified in heart, 3 = Pfn1 identified in kidney, 4 = Pfn1 identified in skeletal muscle, 5 = Pfn1 identified in liver and 6 = Pfn1 identified in testis. (b) Full length gene amplification of Pfn2 in *Rattus norvegicus*, M represents the 1 Kb ladder, 1 = Pfn2 identified in brain, 2 = Pfn2 not identified in heart, 3 = Pfn2 identified in skeletal muscle, 4 = Pfn2 identified in liver, 5 = Pfn2 identified in testis and 6 = Pfn2 identified in kidney. (c) Full length gene amplification of Pfn3 in *Rattus norvegicus*, M represents the 1 Kb ladder, 1 = Pfn3 identified in brain, 2 = Pfn3 identified in testis, 3 = Pfn3 identified in liver, 4 = Pfn3 not identified in heart, 5 = Pfn3 identified in skeletal muscle and 6 = Pfn3 identified in kidney.

significance at (<0.05). *P means value <0.05 and **P indicates value <0.005. Data were analyzed using SPSS v20.0 (IBM).

3. Results

3.1. Quantitative real time PCR

In group I, transcriptional expression level of GAPDH (control) was similar to the *Pfn*1 in brain. Expression of *Pfn*2 was higher than the GAPDH (control) and *Pfn*1 but lower when compared with the *Pfn*3. So, *Pfn*3 is highly expressed in brain tissue of *Rattus norvegicus*, as compared with the *Pfn*2 and *Pfn*1 (Fig. 2a). In testis expression level of GAPDH (control) was similar with *Pfn*2 while the transcriptional expression of both GAPDH (control) and *Pfn*2 was lower than *Pfn*1, *Pfn*3 and *Pfn*4. *Pfn*1 and *Pfn*3 both have similar expression level and both have lower level of expression as compared to *Pfn*4. *Pfn*4 level was observed highest among control, *Pfn*1, *Pfn*2 and *Pfn*3 (Fig. 2b).

In the skeletal muscle of group II, expression level of GAPDH, *Pfn*2 and *Pfn*3 was similar and higher then *Pfn*1, however transcriptional level of *Pfn*4 was highest when compared with Control, *Pfn*1, *Pfn*2 and *Pfn*3 (Fig. 2c). In heart, transcription expression of only *Pfn*1 was observed that was relatively more in comparison of GAPDH (Fig. 2d).

In Group III, transcriptional level of GAPDH was lower than *Pfn*1, *Pfn*2, *Pfn*3 and *Pfn*4 in kidney. *Pfn*1 was expressed at low concentration as compared to *Pfn*2, *Pfn*3 and *Pfn*4; while the expression level of *Pfn*2 was higher as compared to control, *Pfn*1 and *Pfn*3 and lower than the *Pfn*4. Expression level of *Pfn*3 was higher than *Pfn*1 and control while lower then *Pfn*2 and *Pfn*4 transcriptional level was highest among all other in kidney tissue (Fig. 2e). In liver sample expression at the transcriptional level of control is lower among *Pfn*1, *Pfn*2 and *Pfn*3. While the expression level of *Pfn*2 was highest as compared to control, *Pfn*1 and *Pfn*3; *Pfn*1 was expressed at low concentration as compared to *Pfn*2 and *Pfn*3. Expression level of *Pfn*3 was higher than *Pfn*1 and control while lower then *Pfn*2 (Fig. 2f). While no transcriptional expression was observed for *Pfn*4.

Table 2
Pfn isoforms (Pfn1, Pfn2, Pfn3 and Pfn4) identified in mentioned organs of *Rattus norvegicus*.

	Organ name	Pfn1 (665 bp)	Pfn2 (470 bp)	Pfn3 (479 bp)	Pfn4 (141 bp)
Group1	Brain	+	+	+	−
	Testis	+	+	+	+
Group2	Skeletal muscle	+	+	+	+
	Heart	+	−	−	−
Group3	Liver	+	+	+	−
	Kidney	+	+	+	+

3.2. Relative fold expression

3.2.1. Group I

Relative fold expression in brain for GAPDH (control) and *Pfn*2 was up-regulated (1-fold) which was highest among *Pfn*2 and *Pfn*3; however expression of *Pfn*2 (2.25-fold) and for *Pfn*3 (2.22-fold) gradually decreased while no expression for *Pfn*4 have been observed (Fig. 3a). Testis fold expression of control and *Pfn*3 was up regulated (1-fold), *Pfn*1 was slightly down regulated as compared to control and *Pfn*3 (0.8-fold), while less regulated observed in *Pfn*2 (0.26-fold) and for *Pfn*4 (0.15-fold) (Fig. 3b).

3.2.2. Group II

Relative fold expression at transcript level was highest for GAPDH (control) (1-fold), while for *Pfn*1 was down regulated (0.69-fold) so far expression of *Pfn*2 was 0.43-fold increased and transcript level of *Pfn*3 was 0.35 fold increased; 0.0007 fold increased for *Pfn*4 (Fig. 3c). In heart, transcript relative fold expression was observed similar for GAPDH (control) and *Pfn*1 (1-fold). While no relative expression was observed for *Pfn*2, *Pfn*3 and *Pfn*4 (Fig. 3d).

3.2.3. Group III

Relative fold expression at transcription level in the kidney sample showed that level of *Pfn*1 and *Pfn*4 was higher in comparison of *Pfn*2 and *Pfn*3. Least comparative fold expression was observed for *Pfn*2 (0.068-fold) and *Pfn*3 (0.184-fold) (Fig. 3e). In liver, control and *Pfn*1 showed up-regulated transcript level (1-fold), while the transcript level of *Pfn*3 and *Pfn*4 was observed to be amplified 0.33 and 0.085 fold respectively (Fig. 3f).

3.3. mRNA structure shows varied profile with conserved structure motifs

RNA structure is vital for gene function and regulation. Transcript analysis has been limited to primary sequence and expression level. However, with advancement in computational biology techniques and availability of sophisticated tools and webservers, it is feasible to annotate and compare gene transcripts based on RNA secondary structure (Fig. 4). Structural analysis of the mRNAs will definitely assist in identifying and validating profilin mRNA motifs, which might play crucial role in diverse cellular processes.

4. Discussion

Here, we present the detailed study on isolation of profilin isoforms (*Pfn*1, *Pfn*2, *Pfn*3 and *Pfn*4) in rat organs (brain, kidney, liver, muscle, testis and heart). Because the objectives of our study were identification,

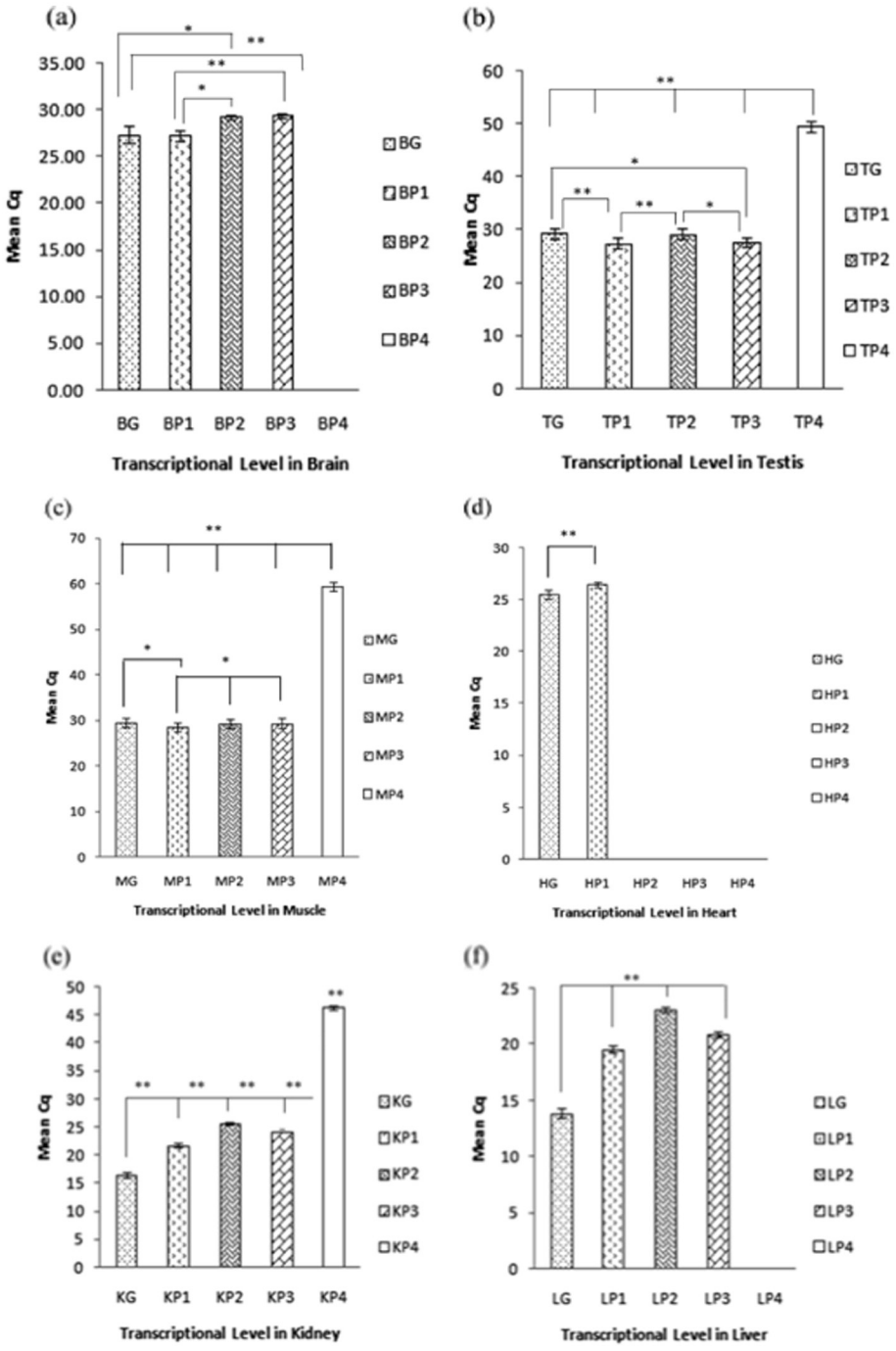

Fig. 2. Gapdh and Pfngenes expression have been observed in mentioned organs of *Rattus norvegicus* by using Real-time PCR Group I: (a) Expression level in brain, BG, BP1, BP2, BP3 and BP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in brain. (b) Expression level in testis, TG, TP1, TP2, TP3 and TP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in testis. Group II: (c) Expression level in muscle, MG, MP1, MP2, MP3 and MP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in skeletal muscle. (d) Expression level in heart, HG, HP1, HP2, HP3 and HP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in heart. Group III: (e) Expression level in kidney, KG, KP1, KP2, KP3 and KP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in kidney. (f) Expression level in liver, LG, LP1, LP2, LP3 and LP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in liver.

secondary structure and expression analysis of above mentioned *Pfn* isoforms. Previously, it was reported that *Pfn*1 expressed in all organs except heart, skeletal muscle and testis (Behnen et al., 2009; Lambrechts et al., 2000), whereas our findings revealed that *Pfn*1 present in all designated groups (I, II, III). Similarly, *Pfn*2 was identified in all organs of group I and III and skeletal muscle (group II), and that was reported in testis before this present study. *Pfn*3 and *Pfn*4 known as "somatic profilins" and so far were accounted in testis only (Obermann et al., 2005), however, presently *Pfn*4 was identified also in skeletal muscle and kidney in addition to testis.

Real-time PCR analysis for the transcriptional expression of *Pfn* isoforms in Group I, Group II, and in Group III showed that RNA levels vary among these groups. However, possibility cannot be ruled out that response to extracellular signals and ligand binding affinity of the isoforms may also modulate the different pathways, leading to differential expression of *Pfn* isoforms among brain, liver, heart, kidney, muscle and testis. *Pfn*1 shows association with various signalling cascades that regulates its expression differently among different organs (Murk et al., 2012). Currently transcriptional level was observed more elevated in muscle and slightly less in brain and testis, whereas *Pfn*1 was

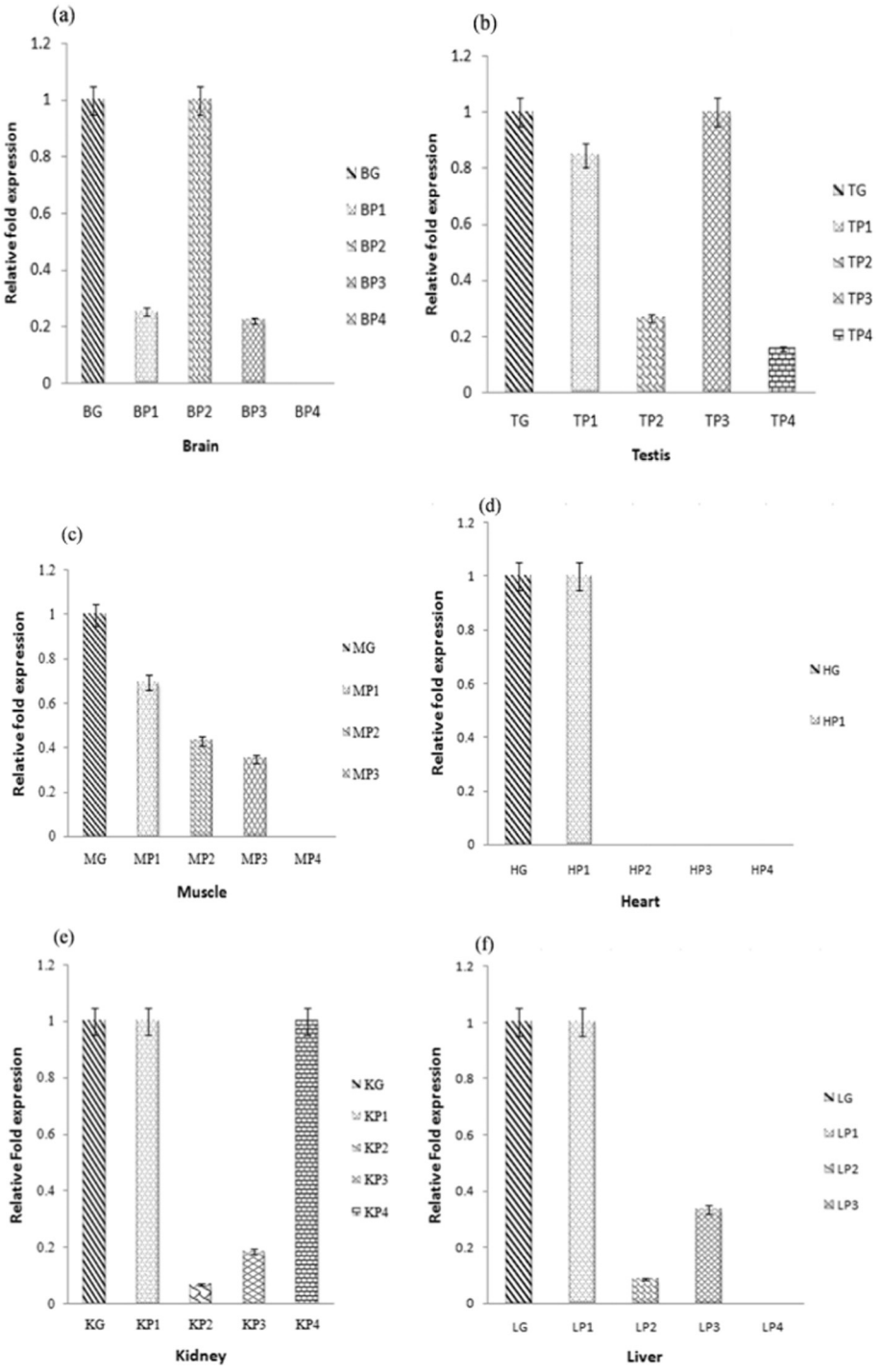

Fig. 3. Gapdh and Pfnrelative fold expression have been observed in mentioned organs of *Rattus norvegicus* by using Real-time PCR. Group I: (a) relative fold expression in brain, BG, BP1, BP2, BP3 and BP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in brain. (b) relative fold expression in testis, TG, TP1, TP2, TP3 and TP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in testis. Group II: (c) relative fold expression in muscle, MG, MP1, MP2, MP3 and MP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in skeletal muscle. (d) relative fold expression in heart, HG, HP1, HP2, HP3 and HP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in heart. Group III: (e) relative fold expression in kidney, KG, KP1, KP2, KP3 and KP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in kidney. (f) relative fold expression in liver, LG, LP1, LP2, LP3 and LP4 represents the level of Gapdh, profilin1, profilin2, profilin3 and profilin4 in liver.

moderately expressed in heart and least expression was observed in kidney and liver. Transcriptional level of *Pfn*2 was high in brain, muscle and in testis while relatively low expression was observed in kidney and liver. Expression of *Pfn*3 was observed to be higher in brain, testis and muscle while less in liver and kidney. *Pfn*4 transcriptional expression was also observed in kidney, skeletal muscle as well as in testis.

RNA secondary structure of *Pfn* isoforms is another important aspect that possibly influences every step in the gene expression, yet the role of structural elements in rat profilin mRNAs remains unexplored. For this purpose, bioinformatics based analysis of the mRNA secondary structure was carried out. This helped shed light on the additional layer of information present in the protein fabricating gene transcripts, in the form of RNA structure. RNA secondary structure has critical roles in

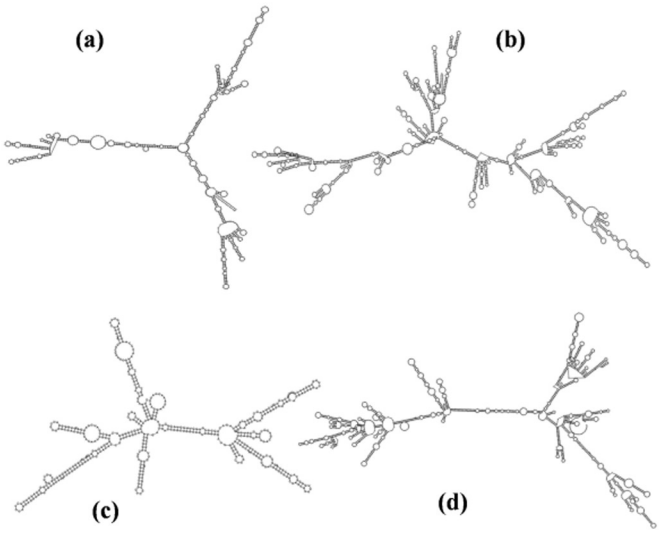

Fig. 4. mRNA secondary structures of the profiling isoforms 4 (a), 4 (b), 4(c) and 4(d).Secondary structures illustrate regions of similarity i.e. structure motifs as well as hairpin loops. Structure conservation of mRNA motifs could be linked to numerous comparable traits like cellular localization and expression.

processes ranging from ligand sensing (Tang et al., 2015) to the regulation of translation (Katz and Burge, 2003; Fernández-Miragall et al., 2006; Wan et al., 2011), poly-adenylation (Kwok et al., 2015) and splicing (Buratti and Baralle, 2004; Wan et al., 2011). The structure of messenger RNA is also important for post-transcriptional regulation, mainly because it affects binding of trans-acting factors (Rabani et al., 2008). However, due to lack of information about the in vivo structure of full-length mRNAs of rat profilins, little is known about this interrelation. We attempted to predict the structure of whole mRNA as well as predicted motifs that might influence expression of profilins. Secondary folds and motif structures (Supplementary Table 1) of the profilin mRNA isoforms seem to share some conserved regions as well as divergent parts. Structural folds, however, appear visually more conserved as compared to sequence, which shows little conservation (Supplementary Fig. 1). This is an initial attempt at deciphering the secondary structure of rat mRNA and link it to expression. Structural bioinformatics of RNA is still in its infancy and this information needs to be validated experimentally to capture the real essence of mRNA structure relation to gene expression. Overall loop and motif conservation in mRNA has a more important role in determining the expression, cellular localization and copy number of the gene transcripts, as it is universally accepted that structure conservation is more important than sequence (Chursov et al., 2012). It has been previously demonstrated that human Catechol-*O*-methyltransferase haplotypes control protein expression due to varied mRNA secondary structure (Nackley et al., 2006). Higher stability of the local stem loop structure of mRNAs is linked to low protein levels and overall expression. This analysis provided baseline information to further explore the relation of structure to expression of profilin isoforms.

Based on the information available and the data reported here, we support the view that *Pfn*1 and *Pfn*2 are important for actin-based neuronal plasticity and have a critical role in regulating actin dynamics, signalling and involved in discrete as well as cooperative activities. *Pfn*1 preferentially binds to PIP2 (Lambrechts et al., 1997), whereas *Pfn*2 interacts preferentially with the ROCK as well as PIP2 pathways, as we showed in this study (Da Silva et al., 2003), we propose that both interactions may involve in actin assembly. Network of profilins interaction with other ligands/proteins in the cell has been modeled using available information from the literature (Fig. 5). Furthermore, there is evidence for differential roles of *Pfn* isoforms (*Pfn*3 and *Pfn*4) in the performance of formins, a family of powerful actin regulators (Paul and Pollard, 2009). Formins drive the assembly of actin bound to diverse *Pfn* isoforms by significantly different rates (Neidt et al., 2008), indicating that there are indeed isoform specific differences in the interaction of *Pfn* 3 and *Pfn*4 with formins (Ezezika et al., 2009). Here, we show that only *Pfn*4 is bound to the formin mDia1 while the *Pfn*3 bound to formin mDia2 that regulates the same pathway of formin in different organs. It has already been shown that *Pfn*1 has a higher affinity for the membrane lipid PIP2 than *Pfn*2 (Lambrechts et al., 1997). Such differences may well be the basis for differential actin filament assembly, as required for either dendritic complexity or spine density. Both *Pfn* isoforms (*Pfn*1 and *Pfn*2) considered here are also involved in transmitting signals from the neuronal membrane downstream to the

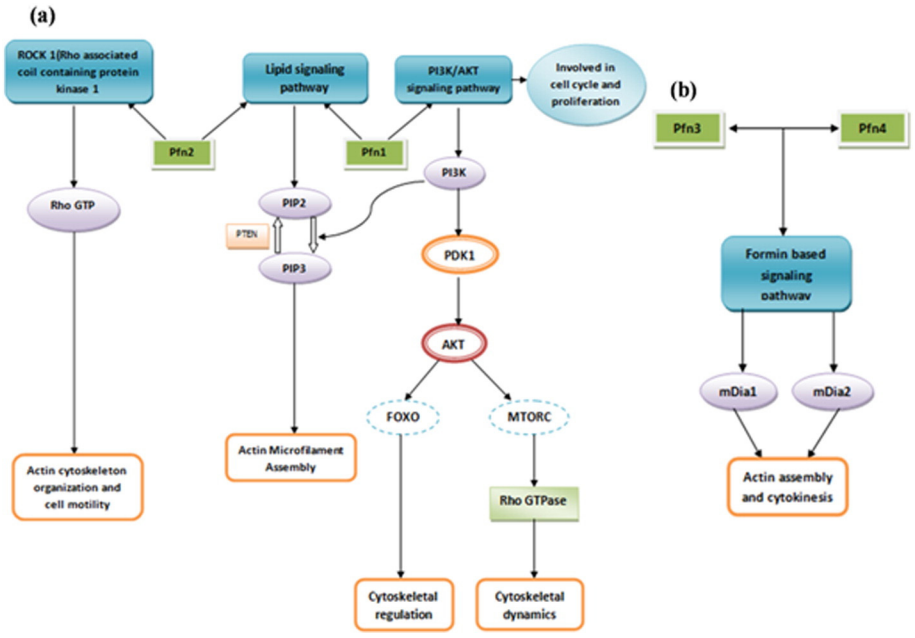

Fig. 5. Proposed model for profilin isoforms in various mentioned organs. These four protein reacts with different signalling pathways to regulate the actin dimerization. 5(a) Pfn1 is involved in PI3K/AKT and lipid signalling pathways. Pfn2 shows its involvement in ROCK1 signalling pathway as well as in lipid signalling pathway aslo that are responsible for actin cytoskeletal regulation and cell motality. 5(b) Pfn3 and Pfn4 are involved in formin based signalling pathways via mDia1 and mDia2 that regulates the actin assembly.

actin cytoskeleton. ROCK signalling by Rho GTP has been demonstrated to control actin cytoskeletal organization and cell mobility (Da Silva et al., 2003). Future research may help provide details about the signalling mechanism and signalling partners involved.

5. Conclusion

In order to analysis of *Pfn* isoforms at the transcriptional expression level, we evaluated that *Pfn* isoforms expressed at the various transcriptional level rates in different mentioned organs of *Rattus* by using real-time PCR. *Pfn* isoforms (*Pfn*1, *Pfn*2, *Pfn*3 and *Pfn*4) have been identified in various organs (brain, testis, liver, heart, kidney and skeletal muscle) of *Rattus* and their transcriptional level was varied in tissue sample. They were significantly varies by using ANOVA. *Pfn* is actin binding monomer, it also binds and interacts to poly-L-proline stretches of formins and Ena/VASP, phospholipids, PIP2, Arp2/3 complex (Mullins et al., 1998; Loisel et al., 1999), rho-associated, coiled-coil-containing protein kinase 1 (ROCK1), Dynamin, WAVE regulatory complex, neuroligin some of which are also involved in signal transduction and also associated with some pathways Srv2/CAP , Rho/Rac pathway, PI3K/AKT pathway. *Pfn*1 and *Pfn*2 involved in neuronal plasticity, while *Pfn*2 known as specifically "Neuronal *Pfn* isoform" however *Pfn*3 and *Pfn*4 known as "somatic isoforms".

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2016.05.023.

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