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# Cloning and expression of a novel human profilin variant, profilin II

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We have isolated a 1.7 kbp cDNA encoding a 140 amino acid protein (15.1 kDa, pI 5.91) with a high sequence similarity (62%) to human profilin (profilin I). We have termed this variant profilin II. Northern blot analysis showed that profilin II is highly expressed in brain, skeletal muscle and kidney and less strongly in heart, placenta, lung and liver. In addition, three different transcript lengths were detected. Only one transcript of profilin I was found. The expression level of this was low in brain and skeletal muscle, medium in heart and high in placenta, lung, liver and kidney.

Profilin variant; cDNA cloning; Tissue expression; *Homo sapiens*

## 1. INTRODUCTION

Profilin is a 12–15 kDa protein that initially was isolated from bovine spleen [1] and subsequently found to be expressed in several mammalian tissues such as thymus, brain, platelets [2–4], thyroid gland [5], macrophages [6], liver, spleen, uterus, heart, lung and kidney [7]. Human profilin shows 95% identity to bovine profilin and the amino acid exchanges are mainly conservative [7]. To date, all tissues examined express an 0.9 kb mRNA transcript with no indication of alternative splicing [7].

The function of profilin is controversial. Traditionally, profilin has been assigned to inhibit actin polymerization by binding of actin monomers [8], but it has recently been found that profilin has the potential to promote actin polymerization and filament stabilization [9,10]. Recent studies indicate that profilin may play an important role in cell signalling [11,12] and a model has been suggested where profilin binds tightly to phosphatidylinositol 4,5-bisphosphate ($PIP_2$) in the cell membrane. Binding of EGF to its receptor on the cell membrane activates the endogenous protein tyrosine kinase that phosphorylates itself and other proteins including phospholipase C-$\gamma_1$. The activated phosphorylated phospholipase C-$\gamma_1$ subsequently hydrolyses the profilin bound $PIP_2$ to DAG and $IP_3$ with the effect that profilin is released from the membrane to the cytosole where it may catalyse the exchange of ADP with ATP on G-actin thereby promoting the stabilization of actin filaments [10].

We have isolated a 1.7 kb cDNA predicting a 15 kDa protein with 62% identity to human profilin (profilin I). Profilin I and this variant (profilin II) may thus represent members of a larger protein family. We here report the expression of each profilin variant in various human tissues.

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*Abbreviations:* AMA, transformed amnion cells; bp, base pair(s); DAG, diacylglycerol; EGF, epidermal growth factor; $IP_3$, inositol trisphosphate; pI, isoelectric point; $PIP_2$, phosphatidylinositol 4,5-bisphosphate.

## 2. MATERIALS AND METHODS

### 2.1. *Preparation of cDNA Libraries*

RNA was prepared from cell monolayers by the guanidiniumthiocyanate/CsCl method [13]. mRNA was purified from transformed human amnion (AMA) cells using poly-dT 'push columns' (Stratagene) and double-stranded cDNA was prepared according to Gubler [14]. The cDNA was size-fractionated on low melting temperature agarose (FMC BioProducts) gels in fractions ranging from 300–1000, 1000–2400, 2400–4000 and >4000 bp. The *Xho*I-poly-$dT_{18}$ (GAGAGAGACTATCTCGAGC-TC-poly-$dT_{18}$) primed cDNA library was constructed in λ-ZAP II (Stratagene) and amplified as described [13].

### 2.2. *cDNA cloning*

A cDNA called R3 was isolated by excising a random area of about 1 $cm^2$ from a screening plate with subsequent self-excising of the resulting λ-ZAP II phages in pBluescript. The R3 cDNA was used as probe to screen $5 \times 10^5$ recombinant plaques, resulting in the detection of 8 positive clones. The largest insert was about 1.7 kbp. The cDNA fragment was subcloned into M13 BM20 or 21 (Boehringer Mannheim) and grown in large scale on *E. coli* strain JM105. Directional deletions were generated using the *Bal*31 exonuclease (Amersham) and the resulting fragments were recloned into M13 BM20 or 21 previous to sequencing by the dideoxy nucleotide chain termination technique [15] using [α-$^{35}$S]dATP and the T7 DNA polymerase (Amersham). The sequence was determined from both strands. DNA computer analyses were made on a VAX 2000 workstation using the Staden [16], the UWGCG program packages [17] and the ALMA alignment editor [18].

### 2.3. *Northern blot analysis*

Poly(A)$^+$ RNA from cultured AMA cells and SV40 transformed

MRC-5 fibroblasts (MRC-5 V2) was purified from total RNA and subjected to gel electrophoresis on a horizontal 1% formaldehyde/agarose gel [13] and transferred to nylon membranes (Hybond N, Amersham). A human multiple tissue blot containing 2 $\mu$g mRNA per lane was purchased from Clontech. Hybridizations were made with [$\alpha$-$^{32}$P]dATP labelled random primed probes covering the whole or parts of the 1.7 kbp cDNA insert. The blots were also hybridized with a 60 mer 5'-end labelled oligodeoxyribonucleotide specific for the human profilin I transcript and a 50-mer oligodeoxyribonucleotide recognizing the $\beta$-actin transcript. Probes were removed from the filter by the addition of boiling water with 0.1% SDS. Conditions of hybridization and washing were in general as previously described [19], but the final high stringency wash (15 mM NaCl, 1.5 mM $Na_3$citrate, 65°C) was performed for 1 h.

## 3. RESULTS

In the execution of a random cDNA cloning project we have identified a cDNA (R3) which encodes a protein with a high similarity to human profilin [7]. The R3 clone did not contain the complete coding region but a subsequent screening using the R3 clone as probe resulted in the purification of a 1.7 kbp cDNA containing the complete coding region (Fig. 1). An open reading frame from position 14 to position 433 encodes a predicted protein of 140 amino acids with a molecular mass of 15.1 kDa and a pI of 5.91. The high similarity to human profilin [7] was found only at the amino acid level, whereas little or no similarity was found between the DNA sequences. We will refer to the hitherto known profilin [7] as profilin I and our cloned variant is then termed profilin II.

We analysed the expression of both human profilin transcripts in different tissues and cell lines (Figs. 2 and 3), and found that two or more bands hybridized with the profilin II clone in each tissue examined. The shortest transcript (arrow 1 in Fig. 2A) was seen in all tissues with the strongest expression in kidney and the weakest in pancreas. A longer transcript (arrow 2 in Fig. 2A) was also observed in all the tissues, with a high expression in brain, skeletal muscle and kidney and low expression in placenta, lung, liver and pancreas. In brain and skeletal muscle an additional even longer transcript was expressed (arrow 3 in Fig. 2A). Identical hybridization patterns were observed with probes covering either the coding region (nucleotides 1–600) or the 3'-untranslated region (nucleotides 600–1693). The same blot was hybridized with an oligonucleotide recognizing human profilin I, Fig. 2B. This oligonucleotide hybridized to a transcript of about 0.9 kb which was barely detectable

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   1  GAAGGGCTCGAAGATGGCCGGTTGGCAGAGCTACGTGGATAACCTGATGTGCGATGGCTG   60
   1              M  A  G  W  Q  S  Y  V  D  N  L  M  C  D  G  C    16

  61  CTGCCAGGAGGCCGCCATTGTCGGCTACTGCGACGCCAAATACGTCTGGGCAGCCACGGC   120
  17   C  Q  E  A  A  I  V  G  Y  C  D  A  K  Y  V  W  A  A  T  A    36

 121  CGGGGGCGTCTTTCAGAGCATTACGCCAATAGAAATAGATATGATTGTAGGAAAAGACCG   180
  37   G  G  V  F  Q  S  I  T  P  I  E  I  D  M  I  V  G  K  D  R    56

 181  GGAAGGTTTCTTTACCAACGGTTTGACTCTTGGCGCGAAGAAATGCTCAGTGATCAGAGA   240
  57   E  G  F  F  T  N  G  L  T  L  G  A  K  K  C  S  V  I  R  D    76

 241  TAGTCTATACGTCGATGGTGACTGCACAATGGACATCCGGACAAAGAGTCAAGGTGGGGA   300
  77   S  L  Y  V  D  G  D  C  T  M  D  I  R  T  K  S  Q  G  G  E    96

 301  GCCAACATACAATGTGGCTGTCGGCAGAGCTGGTAGAGCATTGGTTATAGTCATGGGAAA   360
  97   P  T  Y  N  V  A  V  G  R  A  G  R  A  L  V  I  V  M  G  K   116

 361  GGAAGGTGTCCACGGAGGCACACTTAACAAGAAAGCATATGAACTCGCTTTATACCTGAG   420
 117   E  G  V  H  G  G  T  L  N  K  K  A  Y  E  L  A  L  Y  L  R   136

 421  GAGGTCTGATGTGTAAGCAGCCTCTCCCCATCTACCTAGCAACTGTCTTCATCAACAACC   480
 137   R  S  D  V  ***                                              140

 481  CTAATTATGGTCACAATGCTACCAAACTGTAGATGGTAGCTAATTTTTCTTTACCTATTT   540
 541  TCTAATGTCATGATTCCTGTTTGCCCAATGGATCATTTGTATGTTAACCACTGTATGTAA   600
 601  CCAACCCTTATCTGGCAACATAATTGCAGCACAATAATGATTTGCATGATACCTTGAAAT   660
 661  TGGGGGGAGGGGGCATGCCAAGTTGGGCATCACTTTGTCTTAGCAATTAATGGGATATTG   720
 721  ATTACTAAAATAAGTTAATATTAAGCAAGGTGCCGGTTGTACAATCTCTGATCAGTGTCT   780
 781  TTTCAGCACTTTGAGCATTTACTTGGCTCATTTAGTCTTCCTTTTGTAGCGCATGGTTGG   840
 841  GAGGAAAAAGTGCATGCATCATTCCTTCACTCTTCTCTTTTTCCCGCCCCCCCCTCCCTT   900
 901  CGCACATAGGCATTTGGTTTGCTTCCATCTTTTTTTATGCAGTGCCTGTTTTTTTTTTAAC   960
 961  CAATTAAAATCCCTTTTGTTGATGAGCTATTGAGAGCTGCAGTAGTTTGCTTTTAGTATT  1020
1021  GTTGTTGCACTTGAGCAGAGACAAACCTTTATTCATAGTGTCTACAGGACATATGAAGAG  1080
1081  TGCAATGGCAAAACAAGAGCAAAAAGCACTTCCTCCCATGACCTTACAGTAACCATACTG  1140
1141  ATTGAATCCCCAGGGACATTCCATCATTGCAATAGCTCAGATTTTTCTTCCTTTTTCTTT  1200
1201  GCACACCAGCTCTACTCTTTAGTAAAATTGTAAAAGGCTGCCATTATGGACATTAGGTAT  1260
1261  CCCAACATAACCATCTGGAGTGTGTCCAGTTTGTTCTTCATAGGACCAATTTTTATTTGC  1320
1321  AGCTTGAGTTTTTATATGAAGTTGCATTATTGTGGACTTGGCTGTCCTTGAATTTTTTTC  1380
1381  ATATGTATTCTGTGCCATACTATTGTTAAAATGAACTGTTGCTATTGTGAGATGGATTTT  1440
1441  AACTGACCTATTAAGGGTTTCTTTCGAATGGCACTACTTTAGGGACATTCTAGTATTTGC  1500
1501  TTCTATTGTTTGGGCCTTGTGGATAATGTACAGATTTAAAAACAAATCTTGTTGCTGATT  1560
1561  TGTCCATTTCTTTCCCTGCACTTTGTTACATCTGGGATACAGTCTAACTCATCTGATTTA  1620
1621  ATATGCATTTAAAAAAATGCCATAACTATTAAACACCTTGTTTACAGACAGATGAAATAA  1680
1681  ATTTATTCCAACC-Poly A                                           1693
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Fig. 1. Nucleotide sequence and predicted amino acid sequence of profilin II. The cDNA is 1693 bp and codes for a 140 amino acid protein between the start codon at position 14 (underlined) and the stop codon at position 434 (indicated with asterisks). The putative polyadenylation signal, AATAAA (underlined), is located 12 bp upstream from the poly A tail. The molecular mass of the predicted protein is 15.1 kDa and the calculated pI is 5.91. The nucleotide sequence has been submitted to the GenBank/EMBL Data Bank with accession number L10678.

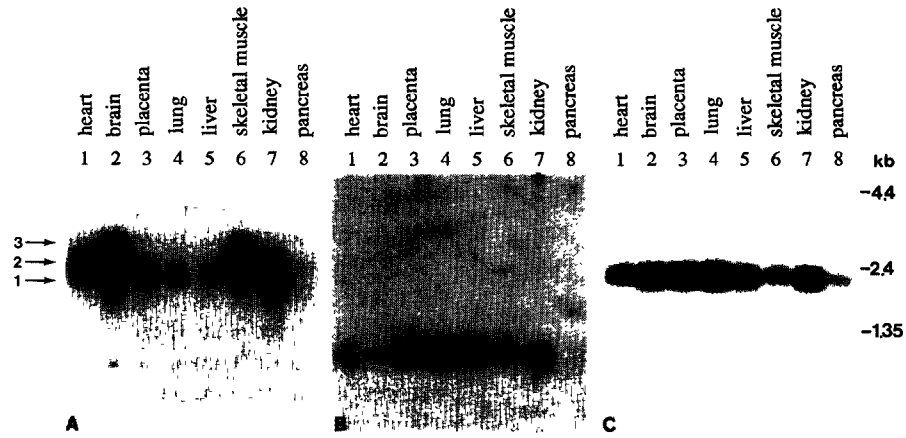


Fig. 2. Northern blot of mRNA from different tissues. Northern blot of 2 mg human poly(A)$^+$ RNA per lane from heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7) and pancreas (lane 8). (A) Hybridization with a random labelled probe covering the complete profilin II cDNA. (B) Hybridization with a 5′-labelled oligodeoxyribonucleotide recognizing the profilin I transcript. (C) Hybridization with a 5′-labelled oligodeoxyribonucleotide recognizing the $\beta$-actin transcript.

in pancreas but otherwise expressed in all tissues examined. The expression levels of the profilin I transcript in various tissues largely confirmed the expression pattern at the protein level in bovine tissues [20]. Hybridization of the Northern blot with an oligonucleotide complementary to the fifty 5′ bases of the coding region of human $\beta$-actin is shown in Fig. 2C as reference. Similar hybridizations were made to Northern blots of mRNA purified from cultured transformed amnion cells (AMA) and SV40 transformed MRC-5 fibroblasts (MRC-5 V2) (Fig. 3). Both cell types expressed profilin I and two of the profilin II transcripts.

## 4. DISCUSSION

Searches in the SwissProt and Mipsx protein sequence databases revealed, as shown in Table I, that profilin II has high similarity (62–64%) to human [7], mouse [21] and bovine [22] profilin. The alignment to human profilin I, pictured in Fig. 4, shows that 89 amino acids are identical and 51 are exchanged. However, both the mouse and bovine profilins have much higher similarities to human profilin I (95–96%) than they have to profilin II (63–64%) showing that both are homologous to human profilin I. In contrast, the similarity of both human profilins to invertebrate profilins, e.g. *Acanthamoeba* [23,24] is low (25–30%).

The profilin II transcripts are expressed in all the tissues and cell lines that we have tested, and the levels of expression are generally complementary to the levels of the profilin I transcripts. Thus, in tissues with a low expression of profilin I we find a high expression of profilin II and *vice versa*. This is true for brain, skeletal muscle, placenta, lung and liver and to some extent in heart, but less pronounced in kidney and pancreas. The different profilin II transcripts are not equally represented in the different tissues. The shortest transcript is highly expressed in kidney, whereas the medium and longest transcripts are more dominating in brain and skeletal muscle. The mechanism creating the size difference of the transcripts is unknown. All transcripts hybridize with both the coding and the noncoding regions of the profilin II cDNA, which may indicate, that the longer transcripts originate from extensions at the 5′- or the 3′-end, maybe resulting from the use of different polyadenylation sites. The expression pattern of the

Table I

Percent identity among profilins from various species

| | Human II | Human I | Mouse | Bovine | *Acanthamoeba* I | *Acanthamoeba* II |
|---|---|---|---|---|---|---|
| Human II | - | 62.1 | 63.6 | 62.9 | 25.6 | 29.6 |
| Human I | | - | 95.7 | 95.0 | 24.8 | 28.0 |
| Mouse | | | - | 96.4 | 23.2 | 24.8 |
| Bovine | | | | - | 26.4 | 28.0 |
| *Acanthamoeba* I | | | | | - | 83.2 |
| *Acanthamoeba* II | | | | | | - |

Sequence comparisons were performed among the two human profilins, I (human I, [7]) and II (human II, this study), the two *Acanthamoeba* profilins, *Acanthamoeba* I [23] and *Acanthamoeba* II [24] as well as mouse [21] and bovine [22] profilin.

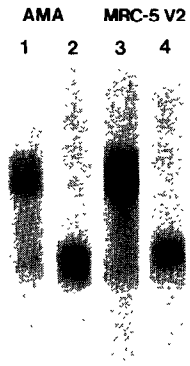


Fig. 3. Northern blot of mRNA from cultured cells. Northern blot of poly(A)$^+$ RNA purified from AMA cells (lanes 1 and 2) and SV40 transformed MRC-5 fibroblasts (MRC-5 V2) (lanes 3 and 4) hybridized with the profilin II cDNA (lanes 1 and 3) or an oligodeoxyribonucleotide recognizing the human profilin I transcript (lanes 2 and 4).

profilin I mRNA is similar to what was reported for bovine profilin by Buss and Jockusch [20] who used antibodies against calf thymus profilin (profilin I) to determine its tissue distribution. They found very little profilin I in skeletal muscle and brain, tissues that exhibit a low expression of profilin I mRNA but a high expression of profilin II mRNA (Fig. 2). Thus it is likely that profilin II serves a role in tissues where profilin I levels are low.

Profilin I has been suggested to inhibit actin polymerization by binding to actin monomers [8]. Until recently, there were six known isoforms of actin: cardiac muscle actin [25], skeletal muscle $\alpha$-actin [26], aortic smooth muscle $\alpha$-actin [27], $\beta$-actin [28], $\gamma$-actin [29] and enteric smooth muscle $\gamma$-actin [30]. The similarities among these actin variants are more than 94%. Additional unknown actin variants may exist as exemplified by the recently discovered actin-RPV [31] or centractin [32], which possesses only about 50% identity to the hitherto known actins. This new actin variant is located at centrosomes [32], and is a component of the dynactin complex [31]. Thus, the novel profilin variant may have a function in relation to some of these actin variants or to other yet undiscovered actin variants. Alternatively, since it recently has been suggested that profilin I may link cell signalling at the membrane level to reorganization of the cytoskeleton involving $PIP_2$ [9,11,12], it is possible that the new profilin variant possesses different binding affinity to $PIP_2$. This has been found in *Acanthamoeba*, where two profilin variants exist with similar affinities to actin [33] but differing with respect to their affinity to $PIP_2$ [34]. *Acanthamoeba* isoform II has a 10-to 50-fold higher affinity compared with isoform I [34]. However, neither of the two human isoforms possesses significantly higher similarity score to any of the *Acanthamoeba* profilins than the other human isoform (Table I). Both human profilins have slightly higher similarity scores to *Acanthamoeba* profilin II (28.0% and 29.6%, respectively) than to *Acanthamoeba* profilin I (24.8% and 25.6%, respectively). Thus, although it cannot be ruled out, there is no obvious indication for suggesting a difference in binding affinity to $PIP_2$ with respect to the two human profilins. Further biochemical studies are necessary to reveal the exact physiological role of profilin II.

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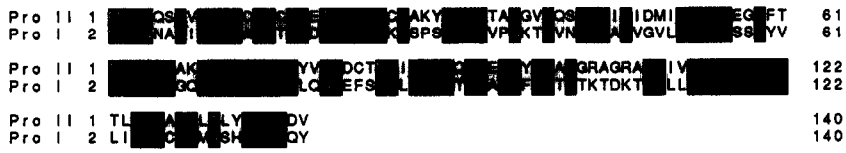


Fig. 4. Alignment of the amino acid sequences of human profilin II and I. The predicted human profilin II (ProII) is aligned to human profilin I (ProI) [7]. The identity is 62% and there are no gaps in the alignment.

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