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# On the origin and evolution of vertebrate and viral profilins

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**Abstract The three dimensional structures of profilins from invertebrates and vertebrates are remarkably similar despite low sequence similarity. Their evolutionary relationship remains thus enigmatic. A phylogenetic analysis of profilins from Deuterostoma indicates that profilin III and IV isoforms each form distinct groups. Profilin IV is most related to invertebrate profilins and originated prior to vertebrate evolution whereas separation of profilin I, II and III isoforms occurred early in vertebrate evolution. Viral profilins are most similar to profilin III. In silico analysis of representative profilin gene structures corroborates the phylogenetic result and we discuss this in terms of biochemical differences.**


*Keywords:* Profilin isoforms; Phylogenetic analysis; Profilin gene structure; Viral profilin

## 1. Introduction

Profilins are multifunctional single domain proteins. With few exceptions, all profilins bind to actin, long proline-rich sequences and polyphosphoinositides [1]. The latter interaction keeps profilin in an inactive state whereas the two former may contribute to actin dynamics. Profilins sequester actin when barbed ends are capped [2]. When these ends are free, low profilin concentrations increase elongation rates by adding actin (in complex with profilin) to the fast growing filament ends [2,3], high profilin concentrations, however, increase depolymerization at these ends [4]. Their interaction with proline-rich ligands may further modulate actin polymerization. The combination of these functional properties makes profilins important regulators of actin cytoskeletal dynamics required for various cell motility processes [1]. Next to the vertebrate founding member of this protein family: profilin I [5], various profilins were identified in several species from the eukaryotic kingdom and in viruses. More recently, paralogues have been characterized in bovine, human, mouse and rat: the two splice variants profilins IIa and IIb, profilin III and profilin IV [6–11]. Previous phylogenetic analysis separated the profilins largely into two groups: profilins from vertebrates and from invertebrates. The latter includes homologues from plants and fungi [12]. Such studies have pointed to the fact that sequence similarity between profilins from invertebrate and vertebrate species is low, yet there is a remarkable conservation of 3D-structure and function [13,14]. For instance, the biochemically and structurally well-characterized profilins I and II from *Acanthamoeba* and Mammalia adopt the same fold [13] and bind actin, polyphosphoinositides and poly-L-proline sequences [15], albeit that, in given species, for some isoforms differences in affinities for these ligands exist [8,16,17]. Aligning the primary structures of *Acanthamoeba* and Mammalian profilins, however, yields a similarity score lower than 25% and is thus in the twilight zone of evolutionary relationship based on sequence comparison.

This raises the question on how the vertebrate profilins originated. Below we argue that the more recently identified profilins III and IV isoforms may yield clues to this answer. Profilin IV arose prior to vertebrate evolution and this isoform seems to be more related to invertebrate profilins, whereas phylogenetic analysis and in silico analysis of gene structures suggests profilins I, II and III evolved from an ancestral Deuterostoma profilin. The viral profilins are related to the vertebrate profilins and profilin III seems to be the closest relative. In addition, we pinpoint distinct features in the profilin sequences typical for a particular isoform class. This can be useful for classifying profilins during annotation, but may also have significance for understanding biochemical properties of these isoforms.

## 2. Materials and methods

Public databases were mined using protein sequences of human profilins I, IIa, IIb, III and IV. In most cases the non-redundant protein database (http://www.ncbi.nlm.nih.gov), the genome sequence (http://www.ncbi.nlm.nih.gov/Genomes), Ensembl (http://www.ensembl.org) as well as EST-sequences (http://www.ncbi.nlm.nih.gov) were searched with BLAST. In some cases, secondary BLAST searches were done with other profilin sequences including those from the echinodermate *Strongylocentrotus purpuratus*. Profilin homologues from *Homo sapiens*, *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis*, *Danio rerio* (zebrafish) and *S. purpuratus* were retrieved (see Supplementary Table 1). In case the protein was not annotated we assigned the roman number of the closest human homologue. Protein sequences were aligned with T-Coffee [18] (see Supplementary material) and manually edited using BioEdit [19]. Distances in Table 1 were derived using ClustalW. Neighbor-joining phylogenetic trees were constructed using TreeCon [20]

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Table 1
Calculated distances in percent identity of deuterostome profilins (for nomenclature see legend Fig. 1)

| | Homo 1 | Mus 1 | Gallus 1 | Xenopus 1 | Danio 1 | Strongyloc. 1 | Homo 2 | Mus 2a | Gallus 2a | Xenopus 2 | Danio 2a | Homo 2b | Mus 2b | Gallus 2b | Danio 2b | Homo 3 | Mus 3 | Gallus 3 | Danio 3 | Homo 4 | Mus 4 | Gallus 4 | Xenopus 4 | Danio 4 | Strongyloc. 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Homo 1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mus 1 | 95 | | | | | | | | | | | | | | | | | | | | | | | | |
| Gallus 1 | 76 | 77 | | | | | | | | | | | | | | | | | | | | | | | |
| Xenopus 1 | 54 | 54 | 57 | | | | | | | | | | | | | | | | | | | | | | |
| Danio 1 | 60 | 59 | 56 | 51 | | | | | | | | | | | | | | | | | | | | | |
| Strongyloc. 1 | 24 | 15 | 20 | 16 | 23 | | | | | | | | | | | | | | | | | | | | |
| Homo 2a | 61 | 62 | 58 | 52 | 69 | 18 | | | | | | | | | | | | | | | | | | | |
| Mus 2a | 61 | 62 | 58 | 52 | 69 | 18 | 99 | | | | | | | | | | | | | | | | | | |
| Gallus 2a | 61 | 62 | 58 | 53 | 69 | 18 | 98 | 99 | | | | | | | | | | | | | | | | | |
| Xenopus 2a | 60 | 60 | 57 | 52 | 66 | 22 | 80 | 80 | 80 | | | | | | | | | | | | | | | | |
| Danio 2a | 60 | 61 | 58 | 57 | 73 | 26 | 82 | 82 | 82 | 73 | | | | | | | | | | | | | | | |
| Homo 2b | 62 | 63 | 59 | 51 | 68 | 20 | 93 | 92 | 92 | 74 | 76 | | | | | | | | | | | | | | |
| Mus 2b | 61 | 62 | 57 | 49 | 67 | 19 | 89 | 90 | 89 | 72 | 73 | 94 | | | | | | | | | | | | | |
| Gallus 2b | 61 | 62 | 59 | 52 | 67 | 20 | 87 | 87 | 88 | 72 | 72 | 93 | 89 | | | | | | | | | | | | |
| Danio 2b | 58 | 59 | 56 | 56 | 72 | 26 | 76 | 76 | 76 | 69 | 94 | 74 | 71 | 70 | | | | | | | | | | | |
| Homo 3 | 43 | 43 | 38 | 34 | 40 | 13 | 41 | 41 | 41 | 43 | 41 | 43 | 40 | 39 | 40 | | | | | | | | | | |
| Mus 3 | 42 | 42 | 39 | 35 | 37 | 8 | 37 | 37 | 37 | 42 | 38 | 39 | 36 | 37 | 37 | 88 | | | | | | | | | |
| Gallus 3 | 43 | 42 | 40 | 40 | 43 | 16 | 46 | 46 | 46 | 45 | 48 | 44 | 42 | 43 | 44 | 54 | 57 | | | | | | | | |
| Danio 3 | 35 | 36 | 35 | 40 | 36 | 18 | 34 | 34 | 34 | 35 | 38 | 35 | 34 | 35 | 39 | 29 | 35 | 35 | | | | | | | |
| Homo 4 | 20 | 18 | 8 | 17 | 15 | 17 | 11 | 11 | 11 | 13 | 14 | 10 | 10 | 5 | 16 | 8 | 20 | 10 | 6 | | | | | | |
| Mus 4 | 9 | 3 | 4 | 18 | 7 | 17 | 6 | 6 | 6 | 8 | 11 | 6 | 6 | 5 | 12 | 2 | 5 | 5 | 3 | 83 | | | | | |
| Gallus 4 | 5 | 4 | 13 | 5 | 6 | 17 | 5 | 5 | 5 | 6 | 10 | 6 | 5 | 6 | 11 | 11 | 12 | 2 | 10 | 55 | 52 | | | | |
| Xenopus 4 | 5 | 4 | 4 | 10 | 3 | 13 | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 4 | 4 | 7 | 6 | 11 | 3 | 50 | 49 | 56 | | | |
| Danio 4 | 3 | 3 | 3 | 7 | 4 | 9 | 5 | 5 | 5 | 7 | 6 | 9 | 3 | 3 | 7 | 7 | 8 | 3 | 4 | 47 | 46 | 44 | 48 | | |
| Strongyloc. 4 | 7 | 9 | 9 | 14 | 3 | 12 | 3 | 3 | 3 | 6 | 6 | 3 | 3 | 3 | 6 | 7 | 3 | 4 | 4 | 41 | 45 | 48 | 50 | 48 | |

based on Poisson corrected evolutionary distances (using 500 bootstrap samples). Maximum likelihood trees were created using TREE-PUZZLE [21] using 25 000 puzzling steps and Gamma distributed rates (eight categories) to model rate heterogeneity.

Information on gene structures was retrieved in Ensembl using profilin as a keyword. The chicken profilin II gene structure is only partially given in Ensembl. It was further derived from the gene sequence on NCBI contig **NW_060426.1** after a BLAST search. Additionally, contigs of *S. purpuratus* (**NW_811840.1**, **NW_814035.1** contigs of profilin 1) (**NW_861595.1** contig of profilin IV) were used to find possible exons of the profilins of this species. Given the scarcity of genome and EST information about reptilians, profilins from reptiles were not further considered.

We also performed a phylogenetic analysis with four human profilins and with profilin sequences from an invertebrate animal (*Caenorhabditis elegans*), a protist (*Acanthamoeba castellanii*), the slime mold *Dictyostelium discoidum* and the plant *Arabidopsis thaliana*. We choose these organisms because they express more than one isoform (unlike for instance *Saccharomyces cerevisiae* and *Drosophila melanogaster*). Additionally, sequences of some viral profilins were included (see Supplementary Table 1).

## 3. Results and discussion

### 3.1. Profilins I, II, III and IV are present in different vertebrate classes

Mammals have four profilin genes, one of which gives rise to two variants by alternative splicing. We investigated whether other vertebrates also have these profilin paralogues and splice variants. Therefore, we searched profilin sequences in the NCBI databases either as protein entries or using the protein sequences of the five human profilin isoforms with TBLASTN in the EST-database. In addition, the Ensembl database was systematically screened and in some cases searched with a sequence via the TBLASTN option. Profilins I, II and IV homologues are readily identified in mammals, birds, *Xenopus* and fish (see Supplementary Table 1). We could not find a protein or gene sequence nor an EST-sequence for profilin III in *Xenopus*. One potential, unannotated homologue was identified in Zebrafish (on chromosome 2 in Ensembl) and there are more than 50 *D. rerio* EST's for profilin III present in the NCBI database. Additionally, we identified potential profilin III homologous in two other fish species: *Squalus acanthias* (EST **gi:91047822**) and *Leucoraja erinacea* (EST **gi:48692220**). These findings suggest that profilin III originated prior to the evolution to amphibia and the lack of profilin III in frog reflects the fact that this gene has not yet been uncovered in the present version of the genome project or that it got lost in frogs. Finally, we also searched for profilins from echinodermates and urochordates where we only found evidence for two variants in *S. purpuratus* (see below for annotation) one being very similar to profilins from other sea urchins (a.o. *Anthocidaris crassispina*) and the other to a profilin in the urochordate *Ciona savignyi*.

In mammals, the profilin II gene gives rise to two isoforms due to alternative splicing [6,8]. To reveal conserved profilin II gene structures, we searched the Ensembl entries for chicken, *Xenopus* and zebrafish. The profilin IIb isoform from zebrafish is annotated in Ensembl. For chicken and frog this is not the case. A possible exon for chicken profilin IIb is however present 200 bp downstream of the stop codon of the profilin IIa specific exon and this is at an equivalent position as in the human, mouse and zebrafish profilin II gene. In the frog genome, there is no evidence for a IIb exon located at a similar position, but we identified a region potentially coding for the C-terminus of frog profilin IIb, at approximately 484 kb from the stop codon in exon IIa. Since we did not find matching profilin IIb EST-sequences for chicken nor for *Xenopus*, the identification of this splice variant in birds and *Xenopus* awaits further research.

### 3.2. Phylogenetic analysis clusters profilin IV isoforms in a very distinct group

We retrieved profilin sequences from species with ongoing or finished genome sequencing projects and that we considered as representatives of evolution of Deuterostoma: human (*H. sapiens*), mouse (*M. musculus*), chicken (*G. gallus*), *Xenopus*, zebrafish (*D. rerio*) and the two isoforms from the sea urchin *S. purpuratus* (see Supplementary Table 1). The protein sequences were aligned using T-Coffee (see Supplementary Fig. 1).

The distance matrix (Table 1) already suggests different groups of profilins exist. Additionally, we constructed a phylogenetic tree. Profilins I, IIa and IIb are highly similar (Table 1) and therefore, not surprisingly, cluster together in the tree (Fig. 1). However, in some cases, the observed topology may not present the true tree. For instance *D. rerio* profilin I derives from an internal node closer to profilin II isoforms than to profilin I sequences, and *D. rerio* profilins IIa and IIb are more similar to each other than to their respective orthologues (see also Supplementary material). Profilins III (with the exception of zebrafish profilin III) and IV each cluster in a distinct group, setting these groups clearly apart from profilins I and II and from each other. Especially for the profilin IV group this is very evident given the very low similarity with the other isoforms from vertebrates (cyano in Table 1). The Prof III group is positioned between the monophyletic group of vertebrate profilin I or II and one of the profilin isoforms from *S. purpuratus* (here assigned as profilin I because of its database entry: PROF1_STRPU, **NP_999760**). The position of sea urchin *S. purpuratus* profilin I in the phylogenetic tree suggests it may be an ancestor-like molecule for profilins I, II and III from vertebrates. Interestingly, we found that the other profilin homologue in sea urchin (**XP_791926**) is most similar to vertebrate profilins IV (Table 1, pink background). The urochordate *C. savignyi* also has a profilin belonging to this group. Taken together these findings suggest that separation of profilin IV from an ancestor profilin molecule occurred prior to evolution to vertebrates and that origination of vertebrate profilins I, II and III occurred early in vertebrate evolution.

A similar T-Coffee alignment with human and *S. purpuratus* profilins and profilin sequences from an invertebrate animal, a protist, a plant and a slime mold (see Section 2) and the resulting tree show an intriguing result (Fig. 2). As observed before [12], profilin paralogues from a single species tend to cluster together on the same branch. There are two exceptions to this. Both the human and the *S. purpratus* profilin IV orthologues cluster together and do not branch-off from the vertebrate profilin subtree. This suggests that the human profilin IV isoform is more similar to the other profilin IV genes than to the other human profilin isoforms (see also Table 1). Instead, the profilin IV isoforms are closer positioned to the invertebrate, protist plant and slime mold profilins. Thus the tree suggests that an ancestral profilin, similar to sea urchin profilin IV, duplicated and yielded a common ancestor for sea urchin profilin I and vertebrate profilins I, II and III.

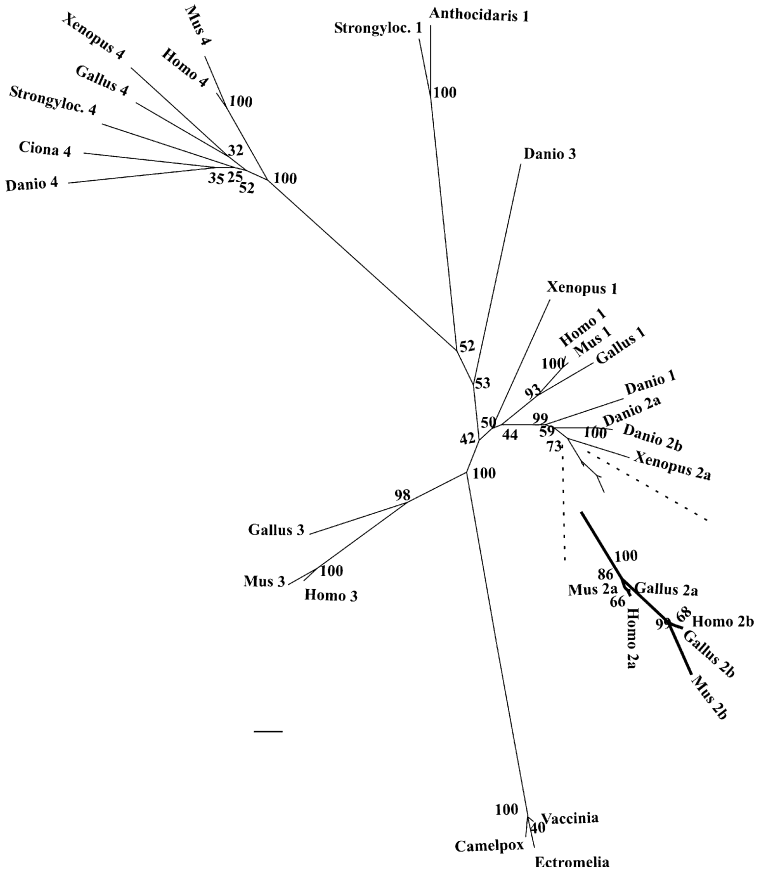


Fig. 1. Unrooted Neighbor-joining phylogenetic tree of profilins from selected Deuterostoma and from viruses. The name code is the genus name of the species concerned followed by an Arabic number indicating the profilin isoform. Strongyloc. indicates a profilin from *Strongylocentrotus purpuratus*. Profilins III (with the exception of *Danio rerio* profilin III) and IV cluster in separate groups whereas profilin I and II isoforms cluster together. Part of the profilin II branch is shown enlarged (lower right). Note that viral profilins branch off from the profilin III subtree. The percentage bootstrap support (500 samples) is indicated on the nodes. Maximum likelihood trees using more complex evolutionary models correcting for rate heterogeneity yielded similar results (data not shown).

### 3.3. The gene structures corroborate profilin IV grouping separately from profilins I and II

The gene structures of mouse profilin I and II were described previously [6,8,22]. Basically, the splice sites are conserved between mouse profilin I and II and thus these forms have homologous introns, with the exception that profilin II contains an additional exon encoding the C-terminus of splice variant IIb. The number of exons and the exon boundaries are conserved in human, chicken, *Xenopus* and zebrafish (Fig. 3). Two Contigs from the *S. purpuratus* genome (**NW_811840.1**, **NW_814035.1**) suggest that the exon structure of *S. purpuratus* profilin I is similar to the one of profilin I from mammals. In case of the profilin III gene structures the situation is more variable. Human and mouse profilin III have a single coding exon. In chicken the eight last amino acids of this isoform are encoded by a separate exon, whereas the *D. rerio* profilin III gene seems to have a similar structure as vertebrate profilin I and II genes. These findings suggest that, upon evolution to birds and mammals, the profilin III gene gradually lost introns. More importantly, it indicates that the more primitive profilin III genes have similar gene structures as their profilin I and II counterparts. This further supports the evolutionary relationship, suggested above, that a profilin I-like molecule from Deuterostoma gave rise to vertebrate profilins I, II and III.

The gene structures of profilin IV from human, mouse, chicken, *Xenopus* and zebrafish are also conserved (Fig. 3). They consist of four exons that have different lengths and, consequently, different boundaries compared to profilins I and II. This further demonstrates a more ancient relation of these genes. Interestingly, translation of contig **NW_861595.1** (region 4162–5488) from *S. purpuratus* suggests a similar structure for profilin IV from this species (three coding exons are readily identified, evidence for the fourth exon comes from the EST sequence). This again sets the profilin IV members apart from the other chordate profilins.

### 3.4. Biochemical implications of sequence divergence

Based on the gene structures it is evident that the phylogenetic analysis did not entirely correctly separate profilin I, IIa and IIb sequences. This is likely due to the fact that these proteins have the same length and are very similar. Yet the biochemical properties, with respect to polyphosphoinositide binding and poly-L-proline binding, of these profilin isoforms are very different (see e.g. [8,17] and Table 2). Obviously these differences are due to different sequence features, but apparently these are not sufficiently discriminative in the calculation of evolutionary distances. That is perhaps with the exception of the C-termini of profilins I, IIa and IIb that may be more characteristic for each particular subclass. Indeed, it turns

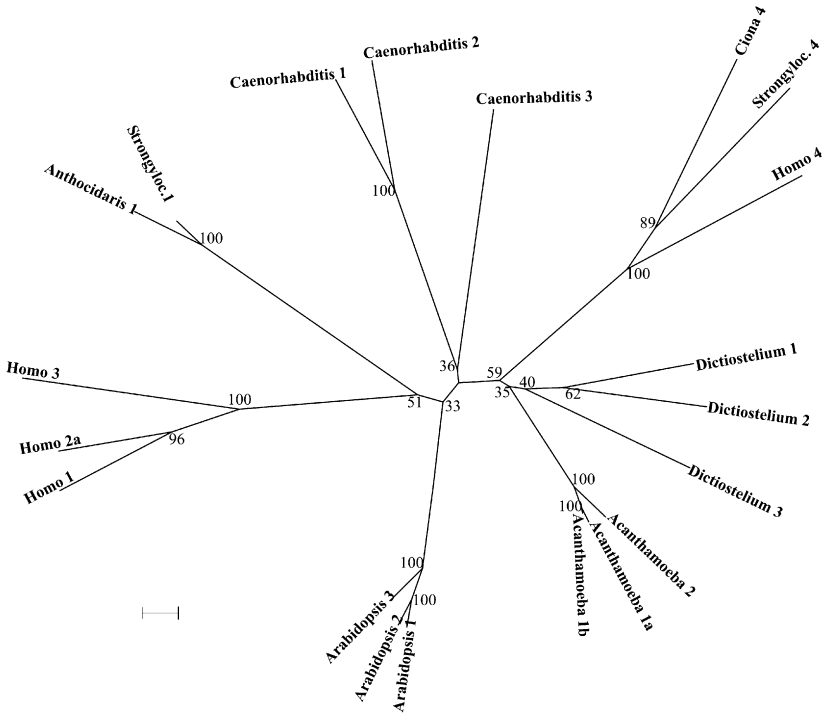


Fig. 2. Unrooted Neighbor-joining phylogenetic tree of profilin paralogues from selected species and from human. Note that the profilin IV sequences (represented by Homo sapiens, *Strongylocentrotus purpuratus* and *Ciona savignyi*) branch from the invertebrate subtree. The percentage bootstrap support (500 samples) is indicated on the nodes. Maximum likelihood trees yielded similar results (data not shown).

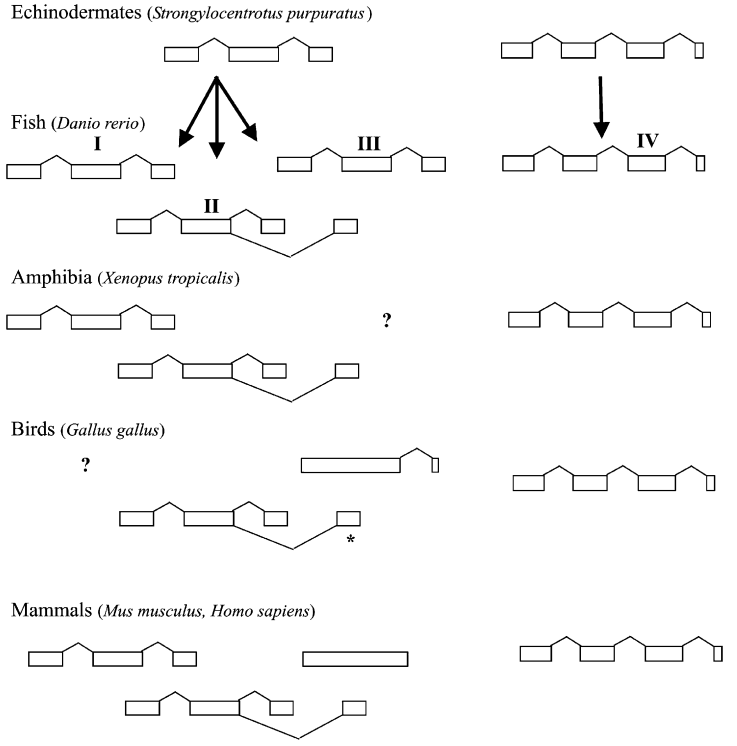


Fig. 3. Exon intron structure of profilin genes of the indicated classes of chordates and of Echinoidea. The species from which the information on gene structure was taken are indicated between brackets. The coding exons are shown as boxes and are drawn to scale. The introns are indicated by a broken line (not to scale). The question marks indicate that we have at present no information for the genes of chicken profilin I (several EST's of this form do exist) and *Xenopus* profilin III isoform. The asterisk indicates a potential exon for the C-terminus of profilin IIb isoform is present but as yet there is no evidence that this isoform is expressed. The profilin III gene structures evolved to an intron-less gene in mammals. For the other profilins the gene structures remained conserved but the gene structures of profilin IV isoforms are different from those of vertebrate profilin I, II and from the more primitive zebrafish profilin III and *S. purpuratus* profilin I. The gene structures of *X. tropicalis* and *D. rerio* profilin IV are not annotated in Ensembl. This finding is based on BLAST searches in the respective genome sequences.

Table 2
Known biochemical properties of vertebrate profilins

| | $K_d$ for Actin | $K_d$ for poly-L-proline | $K_d$ for PI-(4,5)-$P_2$ | Expression pattern |
|---|---|---|---|---|
| Human profilin I | 0.35 [8] | 200–300 μM [30] | 11 μM [31] | Ubiquitous |
| Mouse profilin I | ND | ND | ND | Ubiquitous [6] |
| Bovine profilin I | 0.34 μM [9] | >120 μM [32] | +[9] | ND |
| Rat profilin IIa | 0.38 μM [8] | 0.3 μM [8] | Low affinity [8,4] | ND |
| Mouse profilin IIa | + [6] | + [6] | ND | Brain, skeletal muscle [8,6] |
| Bovine profilin IIa | 0.36 μM [9] | 0.5 μM [32] | Low [9] | ND |
| Human profilin IIb | 0.6 μM [8] | Very low affinity [8] | Very low affinity [8] | Brain [7] |
| Mouse profilin IIb | No [6] | No [6] | ND | Liver, kidney [8] |
| Mouse profilin III | ND | ND | ND | Testis [8] |
| Rat profilin III | + [29] | + [29] | ND | Kidney, testis [29] |
| Mouse and human profilin IV | ND [11] | ND [11] | ND [11] | Testis [11] |

+: binds, but no quantitative data available.
No: no interaction found.
ND: not determined.
PI-(4,5)-P2 binding is relative to the $K_d$ for human profilin I.
Poly-L-proline binding for human profilin IIb is relative to rat profilin IIa.

out that especially the profilin IIa extreme C-terminal sequence is very well conserved. A systematic screening of sequences revealed that all vertebrate profilin IIa sequences in the non-redundant protein database and translated high quality EST sequences have the $^{129}$(S/A)MAKYLRD(S/M)GF$^{139}$ sequence at their extreme C-terminus. The same parts of vertebrate profilin I or IIb: (E/T/A)M(A/G)X(H/Y)LR(R/C)S(G/Q)Y (X indicates four or more substitutions) and X(L/M)(A/S/T)X(Y/H)LR(R/K/C)XXX, respectively, are less conserved. Clearly some of these residues may have structural roles, but this may also couple back to the functionality of profilins. Indeed it was shown that the profilin IIa and IIb isoforms, which only differ in their C-terminal part, have distinct biochemical properties (see Table 2 and [8]).

The crystal structure of profilin I with poly-L-proline and mutagenesis of profilin I and IIa, allowed to define the binding pocket for proline-rich sequences. It engages the aromatic side chains of $W_3Y_6W_{31}H_{133}Y_{139}$ (profilin I numbering) [23–26]. These residues are mostly conserved between profilin I and IIa, which both bind poly-L-proline. In contrast, the aromatic or hydrophobic character of these residues and especially of those in the C-terminus is less or not conserved in profilins IIb, III and IV. Given that profilin IIb has low affinity poly-L-proline [8] it is tempting to speculate that profilins III and IV have lower affinity for such sequences but this remains to be biochemically proven (Table 2). In agreement with this, however, is the fact that biochemical analysis of *Vaccinia* virus profilin shows this isoform has lower affinity for poly-L-proline sequences when compared to mammalian profilin I [27]. It is of interest to note that several viruses express such a profilin isoform. Phylogenetic analysis clusters their sequences close to each other and positions them on the vertebrate profilin III branch (Fig. 1), suggesting these viral isoforms originated from a profilin III isoform.

Profilin IV members are readily recognizable because they are shorter. Compared to profilins I and II, the profilin IV isoforms have two deletions in the middle of the protein sequence. We note that *S. purpuratus* profilin I also has these two deletions. We mapped the position of these deletions on the 3D-structure of profilin I [13] and they correspond to loops between β-strands 4 and 5 and β-strands 5 and 6 (respectively yellow and magenta in Fig. 4 and Supplementary Fig. 1). With the exception of the most primitive *D. rerio* isoform, profilin

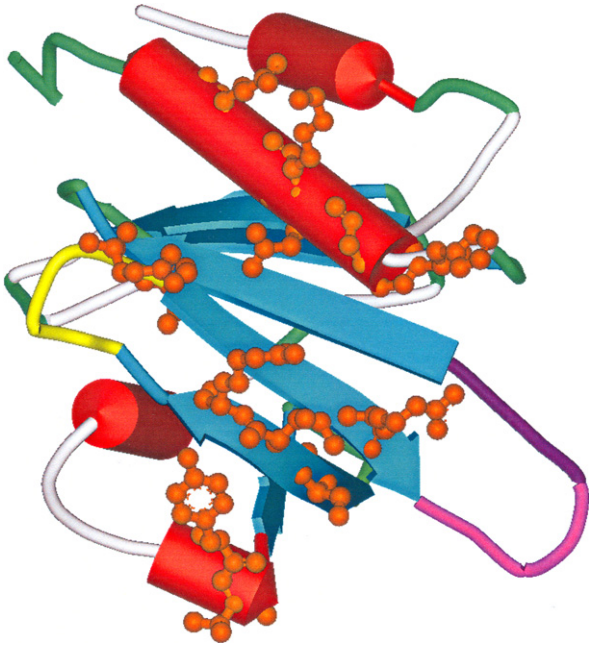

Fig. 4. Location of the profilin III and IV specific deletions on the 3D structure of profilin I. The loop deleted in profilin III is indicated in purple. The loops deleted in profilin IV are indicated with yellow and with magenta. The residues of profilin 1 implicated in actin binding are shown in ball and stick (orange).

III isoforms only lacks part of this second loop (purple in Supplementary Fig. 1). Obviously, this may have functional implications. Interestingly, the first loop missing only in profilin IV (yellow in Fig. 4), is located at the interface with actin in the profilin I-actin crystal structure (residues in orange ball and stick in Fig. 4) [28]. Unfortunately, the actin binding capacities of this isoform have not yet been reported, but given the deletion close to residues involved in actin binding this isoform may have altered actin binding capacities. The smaller deletion in profilin III does not abolish binding to actin (Table 2), however, its affinity has not yet been reported [29].

In conclusion, both phylogeny and gene structures suggest that the profilin IV isoforms are a distinct evolutionary group and that profilin IV arose prior to evolution to vertebrates. On the other hand, profilins I, II and III evolved during early

vertebrate evolution probably from an ancestor similar to sea urchin profilin I isoforms. The highly similar viral proteins share most recent common ancestry with profilin III isoforms. The fact that the separate profilin groups arose prior to or early in vertebrate evolution and that conservation is maintained, suggests they have a specific function. This is especially the case for profilins IIa, IIb, III and IV with a rather restricted expression pattern (Table 2) [6,8,10,11,29]. What these tissue specific functions are, remains to be discovered in future experiments.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2006.12.013.

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