Structure and functions of profilins

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Abstract Profilins are small actin-binding proteins found in eukaryotes and certain viruses that are involved in cell development, cytokinesis, membrane trafficking, and cell motility. Originally identified as an actin sequestering/binding protein, profilin has been involved in actin polymerization dynamics. It catalyzes the exchange of ADP/ATP in actin and increases the rate of polymerization. Profilins also interact with polyphosphoinositides (PPI) and proline-rich domains containing proteins. Through its interaction with PPIs, profilin has been linked to signaling pathways between the cell membrane and the cytoskeleton, while its role in membrane trafficking has been associated with its interaction with proline-rich domain-containing proteins. Depending on the organism, profilin is present in a various number of isoforms. Four isoforms of profilin have been reported in higher organisms, while only one or two isoforms are expressed in single-cell organisms. The affinity of these isoforms for their ligands varies between isoforms and should therefore modulate their functions. However, the significance and the functions of the different isoforms are not yet fully understood. The structures of many profilin isoforms have been solved both in the presence and the absence of actin and poly-L-proline. These structural studies will greatly improve our understanding of the differences and similarities between the different profilins. Structural stability studies of different profilins are also shedding some light on our understanding of the profilin/ligand interactions. Profilin is a multifaceted protein for which a dramatic increase in potential functions has

been found in recent years; as such, it has been implicated in a variety of physiological and pathological processes.

Keywords Actin polymerization · Phosphoinositides · Poly-L-proline · Profilin

Profilins are small, 14- to 17-kDa proteins expressed in eukaryotes (Tseng et al. 1984; Sonobe et al. 1986; Kwiatkowski and Bruns 1988; Magdolen et al. 1988; Widada et al. 1989; Valenta et al. 1991a, b) and certain viruses (Machesky et al. 1994). Its isoforms comprise 100–131 amino acids. The structure of profilin and some isoforms have been solved both with and without ligands. Although the amino acid sequence varies substantially between profilin isoforms or between profilin from different organisms, structural studies have shown that they assume similar three-dimensional (3D) structures (Fig. 1). Indeed, most profilins consist of seven beta sheets and four helices.

Profilin was originally identified as being involved in actin polymerization (Carlsson et al. 1977), and its role as a key regulator of F-actin dynamics has been well established. Other profilin ligands have also been identified, including proline-rich domain-containing proteins and phosphatidylinositol polyphosphates. The difference in sequences between profilin isoforms results in profilin having different affinities for its ligands.

The importance of profilins for normal cell proliferation and differentiation, growth, motility, and cytokinesis is well documented. Gene disruption and antisense studies in *Dictyostelium* revealed that profilin isoforms compensate for each other's function (Haugwitz et al. 1994). Mutants expressing only one isoform did not show any changes in phenotype. However, mutants lacking both isoforms showed various defects, such as affected motility, increased

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Fig. 1 Profilin-I isoforms from different organisms showing a similar helix (red) and strand (cyan) structure (PDB database: 1PFL, 1KOK, 2PRF, and 3NUL) with the loops highlighted in green

cell size ($\times 10$), increased accumulation of actin, impaired cytokinesis, and developmental arrest before fruiting body formation. These phenotypic defects were rescued by either of the profilin isoforms. The *chickadee* gene in *Drosophila*, which codes for profilin, is essential for female fertility and affects bristle development (Verheyen and Cooley 1994). Gene disruption studies have suggested that an allelic variant of *chickadee* (*Chk^{bin}*) in the same locus is responsible for both male and female fertility and that *chickadee* does not rescue this phenotype (Akiyama and Okada, 1993).

Profilin-I gene knockout studies have suggested that in the mouse, unlike *Dictyostelium*, profilin isoforms do not compensate for each other's function (Witke et al. 2001). The profilin-I null mutant was found to be embryonic lethal, while heterozygous (ko/wt) embryos showed reduced survival. These heterozygous (ko/wt) mouse for profilin-I had a 50% reduction in expression level but survived. These results suggest that profilin-I could be affecting mouse development in a dose-dependent manner. (Witke et al. 2001).

Immunogold labeling in fibroblast cells showed that profilin was markedly enriched in lamellipodia or pseudopodial lobes, and a constituent of the microfilament structures was observed to be associated with cellular

membranes (Buss et al. 1992). Immunofluorescence staining showed that profilin is expressed in ruffling areas of the peripheral lamellae and nascent stress fibers of spreading cells and that profilin is absent in peripheral belts of stationary cells growing in epithelioid sheets (Mayboroda et al. 1997). Similarly, localization studies using profilin–green fluorescent protein (GFP) fusion molecules with mammalian cells showed profilin localization in areas of high actin dynamics, such as leading lamellae and ruffles induced by epidermal growth factor (Wittenmayer et al. 2000), and that it is a component of intracellular vesicles (Dong et al. 2000). Profilin has also been localized in intranuclear bodies, such as speckles, Cajal bodies, and gems (Giesemann et al. 1999; Skare et al. 2003). The toxicity produced by the overexpression of actin is also alleviated by profilin when it is co-expressed (Magdolen et al. 1988). Profilin is involved in transporting actin into the nucleus via exportin 6 and is associated with the snRNP complex and the regulation of transcription factor expression (Skare et al. 2003). This finding adds another dimension to this small actin-binding protein. Profilin was one of the proteins found to be expressed when living cells are subjected to hyperoxia (Vorum et al. 2004), but the function of the overexpression or regulation during stress is not yet understood (Gareus et al. 2006).

Profilin isoforms

Four profilin genes have been reported in the mouse and humans to date. The isoforms are generally expressed by different genes; however, differentially spliced isoforms are also known to exist. Human, bovine, mouse, and rat, profilin-II has been shown to be alternatively spliced into profilin-IIA and -IIB (Di Nardo et al. 2000; Lambrechts et al. 2000b). In humans, profilin-I is expressed in all cells, whereas other isoforms are tissue-specific. Profilin IIA and IIB are brain specific and shown to be required for neuronal development (Witke et al. 2001). Profilin-II forms complexes with proteins known to be involved in membrane trafficking, such as synapsin and dynamin-I. Profilin-III in humans and mouse are expressed in the testis and kidney and exclusively in developing spermatids (Braun et al. 2002). Profilin-III and -IV show only 30% amino acid identity among themselves and with other mammalian profilins (Obermann et al. 2005). Profilin-IV plays a major role in acrosome formation and sperm morphogenesis. Results of Northern blot and in situ transcript hybridization analyses suggest that profilin-III and -IV are transcribed in the germ cells. However, the timing of their expression during post-natal development of rat testis and in the rat spermatogenic cycle was distinct. In the human testis, profilin-IV mRNA expression correlates with the presence of germ cells. Profilin-III and -IV may regulate testicular actin cytoskeleton dynamics and play a role in acrosome generation and spermatid nuclear shaping. (Obermann et al. 2005)

The biochemical and structural characteristics of profilin-I and -II from different organisms have been compared. Gieselmann et al. (1995) reported that human profilin-I shows approximately a fivefold higher affinity for actin than profilin-II. In the mouse, profilin-II has been shown to have a higher affinity for dynamin-I than profilin-I (Witke et al. 1998). Structural analyses of human profilin isoforms by radiography suggest that the substitution of profilin-I S29 by Y29 in profilin-II contributes to the higher affinity of profilin-II for proline-rich sequences (Nodelman et al. 1999). Although human profilin-I and -II fold into similar 3D structures, the surface properties, such as exposure of hydrophobic patches (Fig. 2), and biochemical properties of each isoform are distinct. However, further detailed structural and functional studies are required to reveal the significance of the presence of profilin isoforms in eukaryotes.

Three profilin isoforms, PFN-1, PFN-2, and PFN-3, have been identified in *Caenorhabditis elegans*, of which PFN-1 is essential and PFN-2 and PFN-3 are not (Polet et al. 2006). Immunostaining has revealed different expression patterns for the profilin isoforms. In embryos, PFN-1 localizes to the cytoplasm and to the cell–cell contacts

during the early stages of embryogenesis, and to the nerve ring during later stages of embryogenesis. During late embryogenesis, PFN-3 expression has been specifically detected in the walls of muscle cells. In adult worms, PFN-1 is expressed in the neurons, the vulva, and the somatic gonad, PFN-2 in the intestinal wall, the spermatheca, and the pharynx, and PFN-3, in a dot-like fashion, in the body wall muscle cells (Polet et al. 2006). *Dictyostelium* amoebae has two profilin isoforms (I and II); profilin-I is essential for growth and development, whereas profilin-II is not. *Saccharomyces cerevisiae* and *S. pombe* are known to each have only a single profilin isoform (Magdolen et al. 1988; Ezezika et al. 2009).

Profilin and actin polymerization dynamics

Profilins can bind to and sequester actin monomers, thereby decreasing the concentration of free actin monomers that are available for filament elongation (Carlsson et al. 1977). They replenish the pool of ATP-actin monomers by increasing the rate of nucleotide exchange by 1000-fold compared with the rate of nucleotide exchange based on simple diffusion (Goldschmidt-Clermont et al. 1992). The profilin–ATP–actin complex can interact with the fast-growing, barbed, or plus end of the actin filament and release the ATP–actin monomer, which is then added to the filament (Fig. 3). Consequently, the elongating filament consists of ATP-actin. Along the filament, the ATP is slowly hydrolyzed by the intrinsic ATPase activity of actin, which generates ADP–actin in the older part of the filament. ADP–actin can be released slowly from the pointed or minus end of the filament by depolymerization or at an accelerated rate by actin-depolymerizing proteins (Witke 2004).

The crystal structure of the bovine beta-actin–profilin complex has been solved (Fig. 4a; Chik et al. 1996). Mutation studies have confirmed that the C-terminal residue of actin, F375, plays a major role in profilin binding (Lambrechts et al. 2002). In addition, five other residues in profilin, namely Y59, I73, H119, G121, and N124, are also important for binding actin, and any mutation of any of these (Fig. 4b) impairs the actin binding ability of profilin (Eads et al. 1998; Nodelman et al. 1999; Ezezika et al. 2009).

Intracellular pathogens, such as *Listeria monocytogenes* and *Shigella flexneri*, use actin polymerization as a mechanism to propel themselves through the cytoplasm and to spread to neighboring cells without entering the extracellular space. These pathogens can induce polarized polymerization of actin on their surface, which pushes them forward, leaving an actin comet tail behind. In *L. monocytogenes*, the bacterially encoded membrane protein ActA

Fig. 2 Two 180° views of human profilin-I and II isoforms showing hydrophobic surfaces (green) and hydrophilic surfaces (yellow). PDB database: 1PFL and 1D1J-chain A

is essential for this type of motility (Kocks 1994). Based on profilin-depletion experiments, the recruitment of profilin to ActA seems to be an important step in bacterial motility (Theriot et al. 1994). However, the results of an in vitro motility assay using *L. monocytogenes* and purified components, such as actin, capping protein, cofilin, and the actin-related protein (Arp)2/3, suggested that profilin was not essential for bacterial movement but that it could increase its efficiency (Loisel et al. 1999).

Profilin and phosphatidylinositol 4, 5-bisphosphate [PI(4, 5)P₂]

The interaction of profilin with PI(4,5)P₂ is based on the binding of the negatively charged headgroup of the phosphoinositide to basic amino acids (Fig. 5). The more positively charged *Acanthamoeba* profilin-II isoform has

the highest affinity for PI(4, 5)P₂. Similarly, the more basic human profilin-I isoform interacts better with PI(4, 5)P₂ than profilin-IIA (Lambrechts et al. 1997). Yu et al. (1992) compared the PI(4, 5)P₂ binding sites of profilin and gelsolin and postulated that residues 126–136 (KCYEMSHLRR) of human profilin-I are a modified version of the PI(4, 5)P₂-binding motif in gelsolin (KSGLYKK). Using photoactivatable homologs of PI (4, 5)P₂, Chaudhary et al. (1998) subsequently hypothesized that the carboxy terminal basic residues in human profilin-I is involved in contacting the negative head groups of PI(4, 5)P₂. A competition between poly-L-proline and PI (4, 5)P₂ for binding to profilin (Lambrechts et al. 1997) has been observed, which is consistent with the proposal that the carboxy terminus of profilin is involved in PI(4, 5)P₂ binding (Cedergren-Zeppezauer et al. 1994). Based on a comparison of the crystal structure of the two *Acanthamoeba* profilin isoforms, it has been proposed that a surface

Fig. 3 Schematic representation of actin polymerization and ADP/ATP exchange by profilin. Red, solid ribbon Profilin, ribbon with helices in red and sheets in cyan actin, grey spheres ADP, red spheres ATP

with a positive electrostatic potential, formed by residues 71, 80, 81, and 115, corresponding to human profilin residues 74, 88, 90, and 125, were the main PI(4, 5) P_2 binding sites in *Acanthamoeba* profilin (Fedorov et al. 1994). This surface largely overlaps with the actin binding surface. Hence, this model explains the observed competition between actin and PI(4, 5) P_2 for binding to profilin (Fig. 5; Lassing and Lindberg 1985). Mutagenesis of the yeast homolog partially confirmed this model with residue 71, but not residue 80, being implicated in phosphoinositide binding (Haarer et al. 1993).

Based on the *in silico* model of profilin-II derived from profilin-I, Lambrechts et al. (1997) suggested that Glu56 in

mammalian profilin-IIA was responsible for the weaker interaction of this isoform because the negative charge of the Glu56 residue reduces the large, positively charged surface around the hypothetical PI(4, 5) P_2 binding site. In human profilin, however, only Arg88—and not Arg74—are argued to be involved in PI(4, 5) P_2 -binding since only the mutant in Arg88 showed decreased inhibition of PI(4, 5) P_2 hydrolysis by PLC γ (Sohn et al. 1995).

In addition to Arg88, Arg136 in the carboxy terminal helix makes a major contribution to PI(4, 5) P_2 -binding. Given that the R136D mutant, but not R88A, displays wild-type actin binding activity, it has been proposed that the PI(4, 5) P_2 and actin binding sites only partly overlap (Fig. 6).

Fig. 4 **a** A ribbon diagram of the atomic structure of profilin (red) co-crystallized with monomeric actin (yellow). For clarity, the ATP in the actin cleft is not represented. **b** Ribbon and stick model showing the actin and profilin contacts, with the essential amino acids highlighted as green sticks (profilin) and red sticks (actin residues). PDB database: 1HLU

Fig. 5 Profilin structure highlighting the binding sites for phosphatidylinositol 4, 5-bisphosphate [PI(4,5)P₂]. *Green sticks* Residues involved in PI(4,5)P₂ binding, *solid ribbon* backbone of profilin. PDB database: 1PFL

There is also an association between PI(4, 5)P₂-binding and the binding of proline-rich ligands. Indeed, the profilin-IIA mutant W3A, which is defective in poly-L-proline binding, shows increased PI(4, 5)P₂-binding. Based on observed conformational changes following poly-L-proline and PI(4, 5)P₂-binding, Lambrechts et al. (2002) proposed that the correct orientation of the terminal α -helices is important for ligand binding. This is strengthened by the fact that the addition of a myc-tag to the carboxy terminal helix of profilin-IIA abolishes poly-L-proline binding completely (Lambrechts et al. 2002).

Fig. 6 Human profilin-I showing actin and poly-L-proline binding sites overlapping with PI(4,5)P₂ binding sites

In vitro studies have revealed that PI(4, 5)P₂ dissociates actin:profilin complexes (Lassing and Lindberg 1985) and demonstrated the interaction between profilin-I and PI(4, 5)P₂ in both micelles and vesicles (Lassing and Lindberg 1988; Goldschmidt-Clermont et al. 1990). PI(4,5)P₂ was shown to disrupt the ‘profilactin’ complex, suggesting a mechanism by which ATP–actin monomers are released; this release, in turn, could promote local actin polymerization. More recently, Lu et al. (1996) showed that phosphatidylinositol (3,4)-bisphosphate and phosphatidylinositol (3,4,5)-triphosphate bind to profilin with even higher affinity than PI(4, 5)P₂ and that phosphatidylinositol (3,4,5)-triphosphate inhibits profilin sequestering activity much better than PI(4, 5)P₂.

In addition, PI(4, 5)P₂, bound to profilin, can only be hydrolyzed by phospholipase C γ 1 (PLC γ 1) when this lipase is phosphorylated and activated, which occurs in response to transmembrane signaling (Goldschmidt-Clermont et al. 1990; 1991). This leads to two, not mutually exclusive scenarios: (1) profilins are involved in phosphoinositide metabolism and (2) PI(4, 5)P₂ hydrolysis causes translocation of profilin from the membrane to the cytosol where it can interact with actin or other ligands. These scenarios suggest an important role for profilin–phosphoinositide interaction in vivo (Janmey et al. 1995; Ostrander et al. 1995). The structural basis for this interaction has, however, only been partly resolved.

Profilin and poly-L-proline

Profilins bind poly-L-proline stretches, and many proteins containing proline-rich domains have been identified as ligands. However, the *Vaccinia* virus (Machesky et al. 1994) and mouse profilin-IIB spliced form do not show any binding affinity to poly-L-proline (Di Nardo et al. 2000). The enabled/vasodilator stimulated phosphoprotein (Ena/VASP) family is a well-established ligand for profilin (Reinhard et al. 1995; Gertler et al. 1996; Lambrechts et al. 2000a). In neuronal cells, profilin-II has been shown to interact with ligands involved in signal transduction, membrane trafficking, and the recycling of vesicles, such as ROCK2, synapsins, POP-130/CyFIP1, and dynamin-1 (Gareus et al. 2006).

The poly-L-proline binding site has been investigated in crystallographic, mutagenesis, and spectroscopic studies, revealing that a hydrophobic pocket between the amino and carboxy terminal α -helices forms the binding site for poly-L-proline (Fig. 7; Bjorkegren et al. 1993; Haarer et al. 1993; Ostrander et al. 1999).

H133 and W3 are two of the amino acids that have been shown to be involved in binding. Deletion of the last three residues in yeast profilin produces a 100-fold decrease in

Fig. 7 Profilin structure showing the binding of poly-L-proline peptide in the groove between the two alpha helices of the N and C terminus. PDB database: 1AWI-chain A

poly-L-proline affinity. Kaiser and Pollard (1996) reported that the deletion and substitution of as few as three residues at the C-terminus of amoeba profilin reduced poly-L-proline binding.

Profilin mutants unable to bind poly-L-proline but still capable of binding actin and $\text{PI}(4,5)\text{P}_2$ are lethal, suggesting that poly-L-proline binding is an essential function of profilin. However, the functional mechanisms and cellular pathways regulated by profilin–poly-L-proline interaction remain unclear.

Phosphorylation of profilin

Calf profilin is phosphorylated in the presence of $\text{PI}(4, 5)\text{P}_2$ (Hansson et al. 1988; Singh et al. 1996) at serine 137 by protein kinase C (PKC); in contrast, plant profilin has been reported to be phosphorylated on multiple tyrosine residues. However, the precise role of such phosphorylations is not yet understood (Guillen et al. 1999).

So far in this article, we have highlighted the importance of poly-L-proline binding and of the carboxy terminal residues involved in this binding. Phosphorylation of the carboxy terminal serine of profilin may therefore be an important regulatory switch controlling profilin interactions with ligands containing proline-rich domains.

Spectrofluorimetric, immunoprecipitation, and Western blot studies have revealed that the phosphorylation of profilin increases its affinity towards actin and poly-L-proline (Sathish et al. 2004). However, the opposite result was obtained by Shao et al. (2008) with S137D mutant: the S137D mutant failed to interact with the proline-rich domain-containing huntingtin protein, and there was no profilin-induced huntingtin aggregate formation observed.

Further, Sathish et al. (2004) observed increased levels of the actin critical monomer concentration in the presence of phosphoprofilin, indicating that phosphoprofilin may be a better actin sequester. Binding isotherm data and Scatchard plot analysis of the changes in profilin tryptophan fluorescence in the presence of increasing amounts of poly-L-proline revealed that phosphoprofilin possesses a high affinity towards poly-L-proline sequences (Sathish et al. 2004).

Oligomerization of profilins

Several studies (Babich et al. 1996, Mitterman et al. 1998, Wopfner et al. 2002) have reported the spontaneous oligomerization of profilins. In 1996, Babich et al. reported the presence of dimers and tetramers in profilin preparations isolated from human platelets. These authors suggested that the oligomers are formed via sulphhydryl and/or ionic bonds and proposed that profilin tetramers are the oligomeric state that binds actin. Oligomerization has also been reported for birch pollen and human and yeast profilins expressed and purified from *Escherichia coli* (Mitterman et al. 1998). These authors showed that the disulphide bond formation between profilin can be disrupted under reducing conditions but that both reduced and oxidized profilins bind to actin. Wopfner et al. (2002) found that profilin from mugwort pollen formed dimers and tetramers, and they suggested that the oligomeric forms may have a higher allergenic potential than monomers. Although there is evidence that profilin does form oligomers, the presence of these oligomers in cells and their functions remain to be elucidated.

Stabilities of profilin and profilin mutants

The stability of profilins has been investigated by urea denaturation, acid-induced denaturation, and/or thermal denaturation. The mid-point urea denaturation concentration for most of the profilin is approximately 3.5 M. However, some mutants and isoforms show large variations in stability. The denaturation of *S. cerevisiae* profilin in urea was monitored by fluorescence, and the mid-point urea concentration was found to be 3.4 M (Eads et al. 1998). These authors also reported a free energy change for unfolding of 5.9 and 1.8 kcal/mol. Urea-induced unfolding studies were performed on various mutations of *S. pombe* profilin (Lu and Pollard 2001). The mid-point urea denaturation concentrations for most of the profilin mutants varied from 3.3 to 4.5 M. However, the L121R mutant showed a mid-point of denaturation at much lower urea concentration (1.6 M). The stabilities of some profilin mutants have also been indirectly tested by the different

concentrations of urea used to elute them from the poly-L-proline column. Human profilin-I mutants Y6F and K25Q were eluted with 3.5 M urea, whereas wt profilin was eluted with 7.5 M urea (Sohn et al. 1995).

The mid-point of urea denaturation concentration reported for human profilin-I was 3.5 M (McLachlan et al. 2007), while that for the mutants R136D, R88A/R136D, and R88E/R136D were 3.2, 2.8 and 3.5 M, respectively (Lambrechts et al. 2002). Using acid-induced denaturation, an intermediate state was found in profilin-I at pH 4, with a free energy change of 2 kcal/mol (McLachlan et al. 2007). *Acanthamoeba* profilin-I and -II have a stability similar to that of human profilin-I despite their large difference in sequences (Kaiser and Pollar 1996).

Studies have also been carried out on plant profilin. The thermal unfolding of apple profilin gave a T_m of 65°C (Ma et al. 2006) while oxidized (polymeric) and reduced (monomeric) birch profilin gave a T_m of 42.6°C and 50.5°C, respectively (Mitterman et al. 1998). The mid-point urea denaturation concentration in *Z. maize* profilin wt was found to be slightly higher than that of human profilin-I with a concentration of 4 M (Karakesisoglou et al. 1996; Kovar et al. 2001), while the concentration for *Z. maize* profilin mutants Y6Q, K86A (Valenta et al. 1991a) Y6F (Ayscough 1998) and D8A (Ho et al. 1989) were 2.8, 3.7, 3.8, and 3.5 M urea, respectively. These studies clearly demonstrate that mutations may not change the overall folding of the proteins but could severely affect stability and function.

Profilin and cancer

Janke et al. (2000) reported a reduced expression level in tumorigenic breast cancer cells compared to control cells. The identification of the differential expression of profilin genes in fetal rat stomach and normal adult rat stomach has led to the finding that profilin could be involved in the tumorigenic transformation of mucous cells (Tanaka et al. 1992). Experiments with the temperature-sensitive cell line tsFT101 derived from a mouse mammary carcinoma cell line revealed a wobble mutation in profilin at cysteine 39 complemented by a normal profilin gene, thereby providing evidence that profilin is involved in cancer suppression (Cao et al. 1997). Janke et al. (2000) reported that the level of profilin expressed was low in tumorigenic breast cancer cell lines and that the transfection of profilin-I cDNA into CAL51 cells, a tumorigenic breast cancer cell line, had a prominent effect on cell growth, cytoskeletal organization and spreading, and the suppression of tumorigenicity. Feldner et al. (2002) suggested that the suppressor effect of profilin on tumor cell migration might be through profilin's function in controlling actin dynamics. Gene

silencing studies have revealed that profilin-I is involved in endothelial cell migration and proliferation (Ding et al. 2006) while Zou et al. (2007) and Bae et al. (2009) have shown that profilin-I is also involved in breast cancer cell migration.

Metastasis is one of the major issues in cancer progression. The implied involvement of profilin in cell motility, proliferation, and development has been established unambiguously in lower and higher eukaryotes. When a pancreatic adenocarcinoma cell line (PaCa44) was treated with a chemotherapeutic agent, 5-aza-2'-deoxycytidine (DAC), with the aim of evaluating the effect of this drug on cell growth and protein expression, cell proliferation was strongly inhibited and profilin-I was one of the genes shown to be silenced (Cecconi et al. 2003). Using stable isotope labeling with amino acids in cell culture in pancreatic cancer cells, it has been confirmed that profilin-I is downregulated (Gronborg et al. 2006). The effect of profilin overexpression on the migration of breast cancer cells were studied by Roy and Jacobson (2004), who found that overexpressing profilin in BT474 cells prevented cell migration. They also observed that even a moderate level of profilin overexpression significantly impaired the ability of BT474 cells to spread on a fibronectin-coated substrate and migrate in response to epidermal growth factor (EGF). Furthermore, Wittenmayer et al. (2004) found that the profilin functional actin binding site is required to reduce the tumorigenicity of breast cancer cells.

The results from experiments using membrane-permeable profilin-I (PTD4-PfnI) suggest that profilin-I induces lamellipodia formation independently of the presence of EGF in primary bovine trabecular meshwork cells (Syriani et al. 2008). The effects are time- and dosage-dependent and specific to the profilin-I isoform: the H133S mutation in the poly-L-proline binding domain showed a reduced ability to induce lamellipodia, and the H199E mutation in the actin binding domain failed to induce membrane spreading (Syriani et al. 2008). Profilin-I levels were found to be lower in hepatocarcinoma (SMMC-7721) cells and the all-*trans* retinoic acid-induced inhibition of proliferation and migration was found to be mediated through profilin-I expression. The overexpression of profilin-I led to the inhibition of cell proliferation and migration, and RNAi-based PFN1 silencing was shown to rescue the inhibitory effect (Wu et al. 2006). The overexpression of wild-type and mutant profilin-I in breast cancer cell lines suggests that intact actin binding site of profilin-I is required to suppress cell motility and that both actin and poly-L-proline binding sites are required for profilin-induced enhancement in cell spreading (Zou et al. 2007).

Das et al. (2009) have recently demonstrated that the overexpression of profilin-I in breast cancer cells dramat-

ically suppresses growth-factor-induced PI(3,4,5)P₃ generation. Since membrane phosphoinositides are critical regulators of the actin cytoskeleton, profilin-I could be an important nexus between phosphoinositide-derived signaling and cell motility. Such evidence suggests that profilin-I is a potential cancer suppressor which controls cell migration and proliferation through modulation of actin dynamics.

Conclusions

Profilin was first identified as an actin-binding protein 32 years ago. Since then, its actin binding properties have been extensively studied. New functions have been identified during the last decade, and profilin now appears to occupy a major position at the crossroad of multiple cellular pathways involving cytoskeletal reorganization as well as membrane and nuclear trafficking, vesicle recycling, and signal transduction. However, very little is still known about the molecular mechanisms involving profilin in those many different roles. Deciphering the molecular signals that direct profilin and its isoforms toward specific functions is the task that lies ahead of us.

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