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Profilin, and Vascular Diseases

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1. Introduction

Actin is a highly dynamic network. It is essential for several important activities, such as muscle contraction and transmembrane signaling (Luna & Hitt, 1992; Salmon, 1989). Actin consists of actin filaments and a variety of associated proteins (Schmidt & Hall, 1998). Many proteins associated with the actin cytoskeleton control actin assembly and disassembly. These proteins regulate actin assembly at multiple levels, including the organization of actin monomers into actin polymers (Schmidt & Hall, 1998).

One key actin-regulatory protein is profilin, which associates with polymerization of actin. Profilin is a ubiquitous small (12–15 kDa) actin-binding protein expressed in eukaryotes (Kwiatkowski & Bruns, 1988; Magdolen et al., 1988; Sonobe et al., 1986; Tseng et al., 1984; Valenta et al., 1991a, b; Widada et al., 1989) and some viruses (Machesky et al., 1994). Profilin plays an important role in the regulation of actin polymerization in a number of motility functions (Haarer & Brown, 1990). The ability of profilin to bind to many ligands suggests that profilin is involved in signal transduction and may link transmembrane signaling to the control of the microfilament system (Korenbaum et al., 1998; Pantaloni & Carlier, 1993).

Early biochemical studies indicated that profilin interacts with actin in a 1:1 ratio and participates in the addition of monomers at the free barbed end of the filament then disassociates at the barbed end (Pantaloni & Carlier, 1993). Latest work has suggested several more functions of profilin aside from its monomer-sequestering ability. Profilin promotes the exchange of adenine nucleotide bound to actin monomer and also effectively lowers the critical concentration of monomer actin for polymerization of actin (Borisy & Svitkina, 2000; Theriot & Mitchison, 1993). It also promotes nucleotide exchange on an actin monomer by lowering the affinity of the actin monomer for its bound nucleotide by 1000-fold (Goldschmidt-Clermont et al., 1991).

It became progressively clear that profilins are vital constituents of the cytoskeleton. Additionally, the role of profilins in several cytoskeleton-based processes of clinical relevance has been proven. Several studies showed abnormal profilin levels in some pathological conditions. For example, high levels of profilin expression have been reported in human gastric cancer (Tanaka et al., 1992). On the contrary, profilin-I has been described as a tumor suppressor in some other types of cancer such as breast cancer (Das et al., 2009;

Zuo et al., 2007). Another clinical problem in which profilins may be involved is the lateral spreading of some infectious diseases (Pistor et al., 1995; Smith et al., 1996; Zeile et al., 1996). Moreover, profilins got a clinical consideration in other unexpected milieu. In this regard, profilins have been reported as major allergens implicated in pollen and food allergies in approximately 20% of type I allergy patients (Ebner et al., 1995; Valenta et al., 1991c, 1992). Furthermore, we (Hassona et al., 2010, 2011; Moustafa-Bayoumi et al., 2007) and others (Caglayan et al., 2010; Romeo & Kazlauskas, 2008; Romeo et al., 2004, 2007) have shown that profilin-I is an unexpectedly novel molecule that plays a highly significant role in vascular problems that predict a higher risk for developing arteriosclerosis, hypertension, stroke, heart failure, and finally death. Therefore, the aim of this chapter is to shed light on the significance of profilin-I via understanding the molecular and cellular aspects of this molecule, and its role in the vascular diseases.

2. Profilin family

2.1 Gene expression, products & intracellular localization of profilins

So far, there are four profilin genes that have been identified in the mouse and humans. Normally, the isoforms are expressed by diverse genes; nevertheless, differentially spliced isoforms are known to be present as well. It has been reported that in human, bovine, mouse, and rat, profilin-II is alternatively spliced into profilin-IIA and -IIB (Di Nardo et al., 2000; Lambrechts et al., 2000). In humans, profilin-I is expressed in every cell, while other isoforms are expressed in specific tissues. For example, profilin-IIA and -IIB are found to be brain specific and they are essential for neuronal development (Witke et al., 2001). Profilin-II complexes with other proteins such as synapsin and dynamin-I, well- known proteins that implicated in membrane trafficking. In addition, in humans and mouse profilin-III has been shown to be expressed in the testis and kidney and entirely in developing spermatids (Braun et al., 2002). At the amino acid level profilin-III and -IV exhibited only 30% identity among themselves and with other mammalian profilins (Obermann et al., 2005). Profilin-IV plays a key role in acrosome production and sperm morphogenesis. The same study by Obermann et al. proposes that profilin-III and -IV are transcribed in the germ cells. Yet, the expression timing was different during the rat testis post-natal development and in the rat spermatogenetic cycle. In the human testis, there is a correlation between profilin-IV mRNA expression and the presence of germ cells. Profilin-III and -IV may control testicular actin cytoskeleton dynamics and be a factor in acrosome production and spermatid nuclear shaping (Obermann et al. 2005).

Additionally, in *Caenorhabditis elegans* three profilin isoforms, profilin-I, profilin-II, and profilin-III, have been reported, among them profilin-I is crucial; however, profilin-II and profilin-III are not (Polet et al., 2006). As evident by immunostaining expression patterns for the profilin isoforms was different. At the early stages of embryogenesis, profilin-I confines to the cytoplasm and to the cellular contacts, while at the later stages of embryogenesis it confines to the nerve ring. At the late stages of embryogenesis, it has been shown that profilin-III expresses exclusively in the muscle cell walls. On the other hand, during adulthood, profilin-I is expressed in the neurons, the vulva, and the somatic gonad, profilin-II in the intestinal wall, the spermatheca, and the pharynx, and profilin-III, as dots, in the muscle cells of the body wall (Polet et al., 2006). Furthermore, two profilin isoforms (I and II) have been identified in *Dictyostelium amoebae*; profilin-I is fundamental

for growth and development, where profilin-II is not. Moreover, it has been reported that *Saccharomyces cerevisiae* and *S. pombe* have only a single profilin isoform (Ezezika et al., 2009; Magdolen et al., 1988).

Based on the small sizes of profilin (15 kDa) and the profilactin complex (57 kDa) one might expect that they can easily diffuse to the nucleus. Nonetheless, profilin ordinarily is excluded from the nucleus and can be found only in the cytoplasm. Either the most part of profilin is bound in the cytoplasm and only a small portion can diffuse freely or there is a particular export mechanism that can actively take the profilin out of the nucleus (Witke, 2004). Recently, Stuven et al., (2003) reported a profilin-specific exportin present in the mammalian cells. Exportin 6 identifies the actin-bound profilin only, as a cargo and moves it out of the nucleus. The reasons for the existence of this profilactin-specific exportin still unclear, but this finding proposes that the nuclear levels of profilin and actin should be strictly regulated (Witke, 2004). Conversely, there are numerous reports about a nuclear fraction of profilin. For example, it has been reported that profilin-I is linked with subnuclear structures such as ribonuclear particles and Cajal bodies, and anti-profilin antibodies interfere with splicing *in vitro*. This implies a role for profilin-I in pre-mRNA processing (Skare et al., 2003). Also, it has been proposed that in the nucleus profilin-I and profilin-II interact with the survival of motor neuron (SMN) protein, a nuclear factor that is mutated in spinal muscular atrophy (Giesemann et al., 1999). SMN is important for splicing regulation yet it is not known whether this requires profilin binding or not. Still, in cell culture, co-localization of profilin-I and profilin-II with SMN in nuclear gems has been established (Giesemann et al., 1999).

To date the nuclear localization of profilins is a mystifying finding. Only a role for profilin and actin in splicing, chromatin remodeling or transcriptional regulation can be speculated. A more detailed understanding of the dynamics and properties of nuclear profilin and actin is required. It is possible that in the nucleus these proteins are considered necessary momentarily during the cell cycle or, particularly, in cells experiencing transcriptional activity changes (Witke, 2004).

2.2 Structural aspects of profilin

All recognized profilins share common structural and biochemical properties, though the amino acid sequences of the analogous isoforms in distantly related species may demonstrate less than 25% homology (Schlüter et al., 1997). Numerous studies on profilins from different origins demonstrate that they have highly similar tertiary structures (Fedorov et al., 1994, 1997; Metzler et al., 1993; Schutt et al., 1993; Thorn et al., 1997; Vinson et al., 1993) (Figure. 1). The profilin polypeptide consists of 100-131amino acids (Krishnan & Moens, 2009) and it is folded into a central β-pleated sheet formed of 5–7 antiparallel β-strands (Schlüter et al., 1997). On one side, this core is flanked by N- and C-terminal α-helices, with both termini next to each other, and on the opposed side by an extra α -helix attached to either additional α -helix or a small β-strand (Schlüter et al., 1997) (Figure. 1).

It has been reported that there are three groups of ligands characterize profilins: (1) G-actin and actin-related proteins (Machesky et al., 1994; Schutt et al., 1989; Tobacman et al., 1983) (2) polyphosphoinositides (Lassing & Lindberg, 1985, 1988) (3) poly-L-proline (PLP) with the exception of *Vaccinia* profilin (Kaiser et al., 1989; Lindberg et al., 1988; Tanaka & Shibata 1985), existing either as a peptide or as a sequence motif in particular proteins.

In this context, Gieselmann et al., (1995) showed that human profilin-I exhibits about five folds higher affinity for actin than profilin-II. Radiography analyses of the structures of human profilin isoforms imply that the substitution of profilin-I S29 by Y29 in profilin-II participates in the higher affinity of profilin-II for proline-rich sequences (Nodelman et al., 1999). In spite of the similarity in the 3D structures of human profilin-I and -II, the surface characteristics, such as exposure of hydrophobic patches (Figure 2), and biochemical properties of each isoform are different (Krishnan & Moens, 2009).

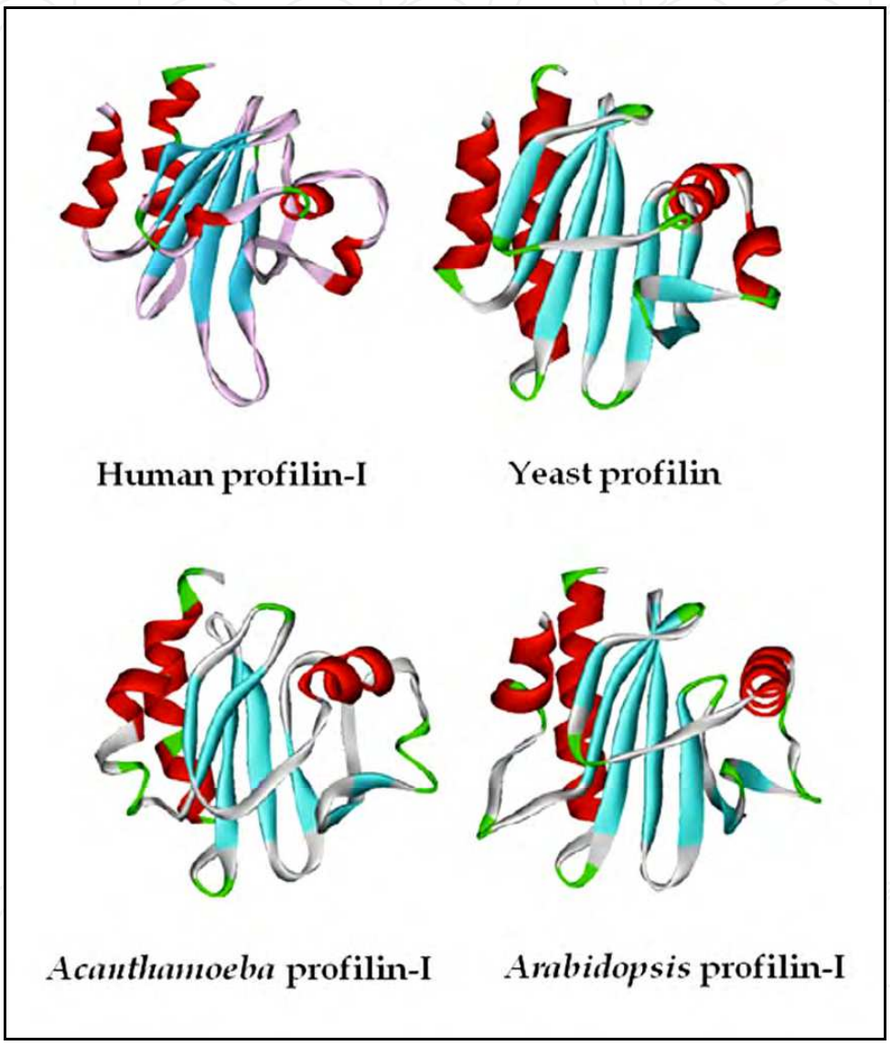


Fig. 1. Profilin-I isoforms from different organisms showing a similar helix (red) and strand (cyan) structure (PDB database: 1PFL, 1KOK, 2PRF, and 3NUL) with the loops highlighted in green, adapted from Krishnan & Moens, (2009) with permission.

2.3 Profilin ligands

Despite its relatively small size, many profilin ligands have by now been recognized, such as actin and actin-related proteins, polyphosphoinositides, PLP, annexin-I, and the list still increasing (Schlüter et al., 1997). Recently, there are more than 50 described profilin-binding

ligands from diverse origins. However, this represents only a part of the real number of profilin-binding partners. Figure 3 shows the identified profilin ligands in mammalian cells. These do not include only molecules of focal contacts that could link profilin directly to actin polymerization such as VASP (vasodilator-stimulated Phosphoprotein) or Mena (mouse homolog of Drosophila enabled) (Gertler et al., 1996; Parast & Otey, 2000; Reinhard et al., 1995) but also include other molecules such as nuclear-export receptors (Boettner et al., 2000; Camera et al., 2003), regulators of endocytosis and membrane trafficking (Witke et al., 1998), Rac and Rho effectors molecules (Alvarez-Martinez et al., 1996; Miki et al., 1998; Ramesh et al., 1997; Suetsugu et al., 1998; Watanabe et al., 1997; Witke et al., 1998; Yayoshi-Yamamoto et al., 2000) and synaptic scaffold proteins (Mammoto et al., 1998; Miyagi et al., 2002; Wang et al., 1999). While a small number of these interactions demonstrated a physiological relevance, the recognition of profilin-interacting proteins could explain the unpredicted roles of profilin in mammalian cells. The profilin-ligands binding might help in linking different pathways to cytoskeletal dynamics via a mechanism that still unknown. Instead, the profilin–ligand interaction might work independently of actin to control the ligands directly (Witke, 2004).

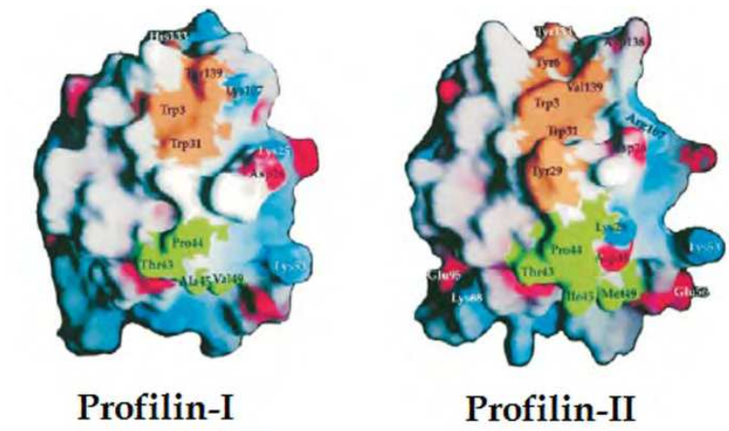


Fig. 2. Structure of human profilin-I and –II: differences in the surface-charge distribution might account for the ligand-binding specificity of profilin-I and -II. Colored regions highlight amino acid residues that are different in profilin-I and -II. Non-conserved residues are shown in blue; conserved residues are shown in brown, adapted from Witke, (2004) with permission.

Among this large number of profilin ligands we will focus on the binding of profilin to some of those ligands believed to be of relevant role in vascular problems such as actin and ligands in Rho/Rac pathway.

2.3.1 Profilin, actin & cytoskeleton

In vitro, Profilins can interact with and sequester actin monomers, in that way diminishing the concentration of free actin monomers that are accessible for filament elongation (Carlsson et al., 1977). They refill the pool of ATP-actin monomers via rising the nucleotide exchange rate by 1000-fold in comparison with that rate obtained from simple diffusion (Goldschmidt-Clermont et al., 1992). The profilin–ATP–actin complex can bind to the fast growing, barbed, or plus end of the actin filament and liberate the ATP–actin monomer, which is after that added to the filament (Figure 4). As a result, the elongating filament is made of ATP-actin. Down the filament, the ATP is slowly hydrolyzed via the actin intrinsic

ATPase activity. This produces ADP–actin in the older part of the filament. ADP–actin can be liberated gradually from the pointed or minus end of the filament by depolymerization or at faster rate by actin-depolymerizing proteins (Witke, 2004).

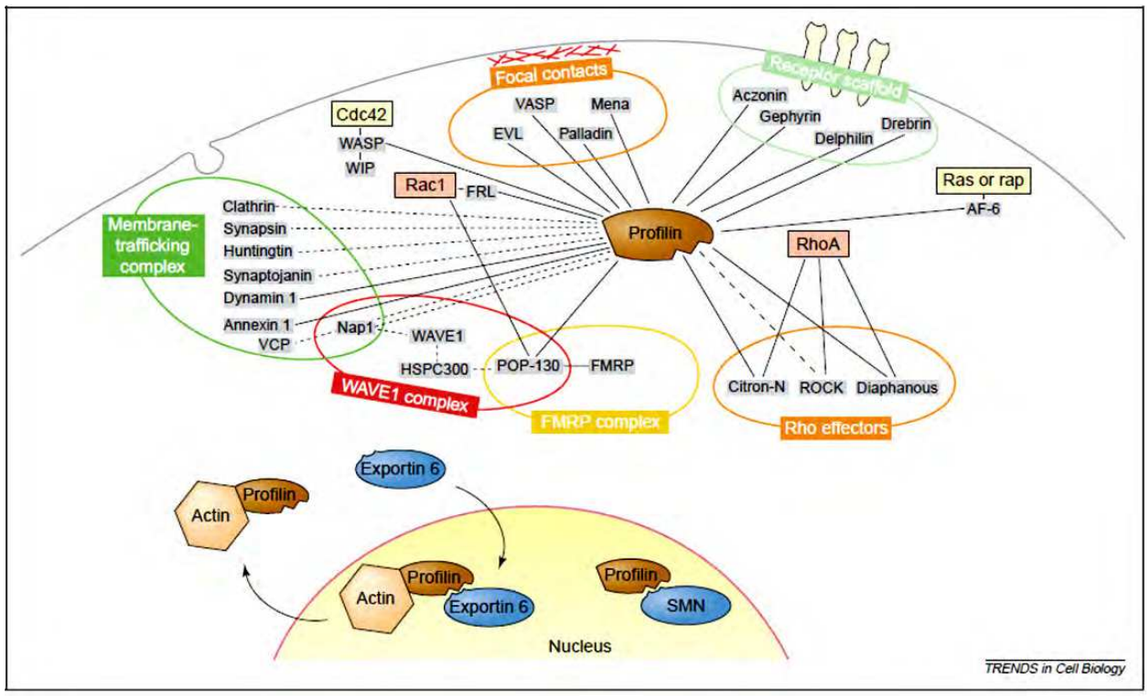


Fig. 3. Network of molecular interactions of profilin. Proteins that are known to interact with profilin are grouped according to their cellular location or the complexes in which they are found. Some of the profilin ligands are shared among different complexes (indicated by the intersecting fields), which suggests a crosstalk among signaling platforms, with profilin as the common denominator. Several links exist to small GTPases such as Rac1, RhoA, cdc42, Ras and Rap that are part of pathways that signal to the actin cytoskeleton. For simplicity, the term profilin is commonly used for profilin-I and profilin-II. Direct interactions between profilin and the ligands are indicated by unbroken lines, whereas potentially direct interactions are indicated by broken lines. Abbreviations: AF-6, All-1 fusion partner from chromosome 6; EVL, Ena VASP like; FMRP, fragile X mental retardation protein; FRL, formin-related gene in leukocytes; HSP, heat-shock protein; Mena, mouse homolog of Drosophila enabled; POP, partner of profilin; SMN, survival of motor neuron; VASP, vasodilator-stimulated phosphoprotein; VCP, valosine-containing protein; WASP, Wiskott–Aldrich syndrome protein; WAVE, WASP family verprolin-homologous protein; WIP, WASP-interacting protein, adapted from Witke, (2004) with permission.

It is worth noting that the presence of other G-actin binding proteins, such as thymosin β4 or any of the ADF family members can alter these processes (Pantaloni & Carlier, 1993). Additionally, capping the plus end of the filaments inhibits the addition of the profilin-actin complexes and consequently limits the activity of profilin to a simple sequestering effect (Pantaloni & Carlier, 1993; Perelroizen et al., 1996; Pring et al., 1992). Thus, the presence of other G-actin binding and/or capping proteins could regulate the profilin effect on cellular actin (Schlüter et al., 1997).

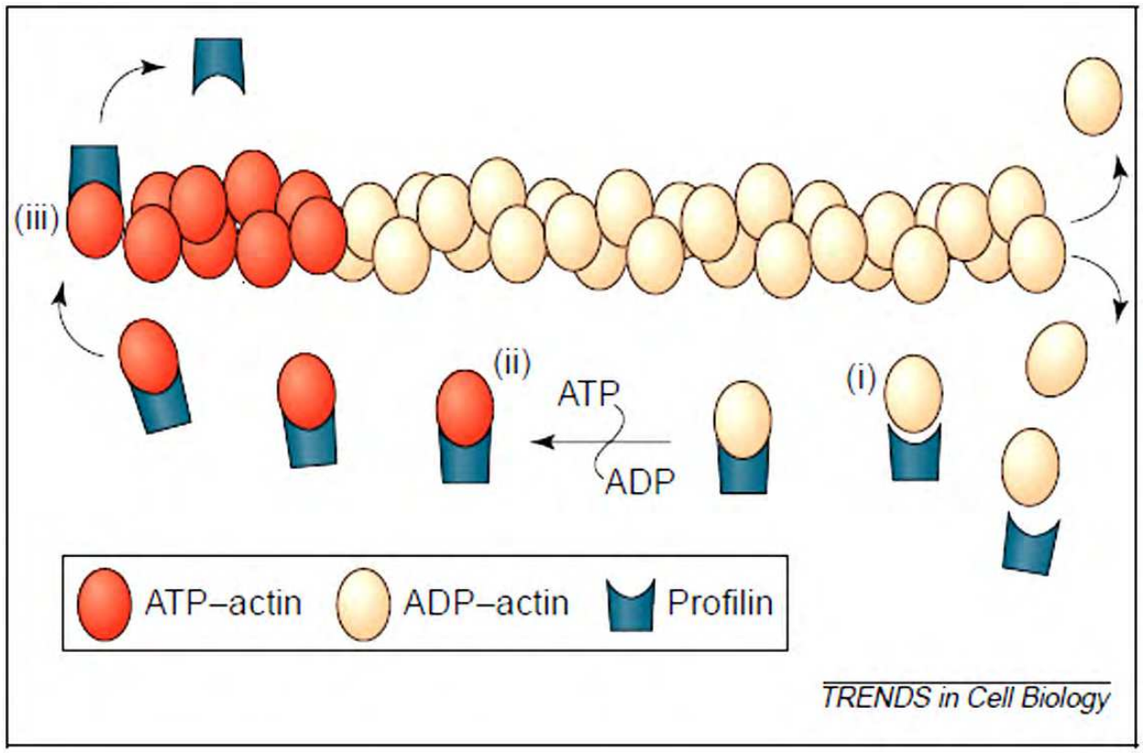


Fig. 4. Role of profilins in actin polymerization. An actin filament consists of two α-helical protofilaments. *In vitro*, three major functions have been identified for profilins in the regulation of actin polymerization. (i) Profilins can bind to and sequester actin monomers, thereby decreasing the concentration of free actin monomers that are available for filament elongation. (ii) Profilins replenish the pool of ATP–actin monomers (red) by increasing the rate of nucleotide exchange on the bound actin monomer 1000-fold compared with the rate of exchange based on simple diffusion. (iii) The profilin–ATP–actin complex can interact with the fast growing end of the actin filament and release the ATP–actin monomer, which is then added to the filament. 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 (orange) in the older part of the filament. ADP–actin can be released slowly from the end of the filament by depolymerization or at an accelerated rate by 'actin-depolymerizing proteins' (not shown), adapted from Witke, (2004) with permission.

Previously, it has been considered that the profilins effect on nucleotide exchange on actin directly regulates their ability to promote filament assembly at the plus end. Polymerization of filament is coupled with the actin-bound ATP hydrolysis and thus far, profilins are unique microfilament associated proteins that can work as nucleotide exchange factors. Polymerization of ATP-actin occurs more rapidly and at a lower critical concentration than ADP-actin (Pollard, 1986). Nonetheless, profilin isoforms I and III in *Arabidopsis* are unable to speed up the rate of nucleotide exchange on G-actin yet still reduce the critical concentration at the plus ends of filaments, similar to vertebrate profilin (Perelroizen et al., 1996). These data demonstrate that the major effect of profilins on actin polymerization cannot be linked with their capacity to work as nucleotide exchange factors.

In vivo, the global view that the main biological function of profilin was observed in its actin sequestering effect became debatable, principally due to the finding that the concentration of profilin in cells and its actin-binding affinity are inadequate to stabilize the G-actin pool (Babcock & Rubenstein 1993; Goldschmidt-Clermont et al., 1991; Machesky & Pollard, 1993; Sohn & Goldschmidt-Clermont, 1994). Generally, the data obtained from cells with different profilin levels are in harmony with the notion that in lower eukaryotes the central role of profilin is to sequester G-actin, whereas in higher eukaryotes this is mainly done via other G-actin binding proteins such as thymosin β4 (Safer et al., 1991), and profilins are mostly implicated in the actin filament dynamics control (Sohn & Goldschmidt-Clermont, 1994).

Based on this notion, lower eukaryotes deficient in profilins should exhibit an increase in F-actin, however in higher eukaryotes this would not be the principal outcome. Compatible with this model, *S. pombe* cells with profilin overproduction showed undetectable amount of actin filaments, and are incapable of forming a contractile ring (Balasubramanian et al., 1994). In *S. cerevisiae* cells harmful effects due to actin overexpression, could be compensated by profilin overexpression (Magdolen et al., 1993). Conversely, several studies reported about filament-stabilizing or -regulating functions of profilin in higher eukaryotes. For example, the overall F-actin content and stability were elevated whereas; a considerable amount of F-actin was shifted from stress fibers to the cortical actin network in Chinese hamster ovary cells overexpressing profilin (Finkel et al., 1994). Likewise, actin filaments were stabilized against cytochalasin D and latrunculin in baby hamster kidney cells overexpressing birch profilin (Rothkegel et al., 1996). In addition, a shift in F-actin from stress fibers to thick peripheral actin filament bundles with a corresponding increase in cellular adhesion to fibronectin has been reported in cultured human endothelial cells overexpressing profilin (Moldovan et al., 1997).

Although these findings indicated a differential role of profilins between lower and higher eukaryotes, a few studies showed contradictory data to these reports (Cao et al., 1992; Edwards et al., 1994; Staiger et al., 1994). Consequently, a final conclusion on the validity of the assumption regarding differential functions of profilins in higher and lower eukaryotes needs to be confirmed with further experimentations (Schlüter et al., 1997).

2.3.2 Profilin & Rho/Rac pathway

Rho/Rac signaling pathway represents one of the well-known pathways in the regulation of actin cytoskeleton, as indicated by the Rac1-dependent membrane ruffling and RhoA-stimulated stress-fiber formation (Nobes & Hall, 1995). Although there is no any report about the direct interaction between profilins and Rho and/or Rac or any other small GTPases, many of the profilin ligands are well-recognized Rho/Rac effector molecules (Witke, 2004). In this regard, our recent data showed that profilin overexpression in vascular smooth muscle cells (VSMC) of transgenic mice results in vascular remodeling and hypertension. These were associated with increased Rho-GTPase activity and Rho-dependent coiled-coil kinase (ROCK) expression (Hassona et al., 2010; Moustafa-Bayoumi et al., 2007). As well, it has been reported that ROCK is a part of the profilin-II complex in the brain (Witke et al., 1998) and this binding is significant in the regulation of neurite outgrowth by ROCK (Da Silva et al., 2003). Furthermore, two other proteins that connect profilin to the Rac pathway were recognized in the profilin-II complex in the brain, Nck-associated protein (Nap 1) and partner of profilin (POP)-130 (Witke et al., 1998). GTP–Rac1

interacts with POP-130 and can detach the tetrameric WAVE 1 [Wiskott–Aldrich syndrome protein (WASP) family verprolin-homologous protein]1 complex, resulting in the activation of actin polymerization by WAVE1. Yet, the role of profilin binding to POP-130 is not apparent however it is possible that profilin might manage the complex formation between WAVE1 and POP-130 and between FMRP (fragile X mental retardation protein) and POP-130, in the same way as Rac1 (Witke, 2004).

Additional small-GTPase-binding molecules that can interact with profilin are the Rho-binding molecules, mouse homologs of the *Drosophila* gene diaphanous (mDia1, mDia2 and mDia3) which are known as potent nucleators of actin polymerization (Wallar & Alberts, 2003). Generally, the diaphanous protein exists in an inactive conformation due to folding back of its N terminal GTPase-binding domain onto its C-terminal Dia-autoregulatory domain resulting in association and autoinhibition. RhoA binding to the N terminus releases the autoinhibition and activates actin nucleation (Alberts, 2002). Profilin binding occurs through the proline-rich formin homology domain that present in the core of that diaphanous molecule (Watanabe et al., 1997). Yet, the significance of that binding is not clear. One interesting possibility is that diaphanous can move actin after it has been sequestered by profilin and activate actin polymerization (Li & Higgs, 2003). However, this is limited by the argument that the studies of profilin-diaphanous binding used truncated versions of diaphanous, rather than the full-length protein. *In vivo*, it has been suggested that large complex of diaphanous oligomers is present as well (Li & Higgs, 2003), which via diaphanous monomers can interact with profilin and/or profilactin molecules. Nevertheless, the structure and regulation of this enormous signaling platform for actin nucleation need to be understood (Witke, 2004).

2.3.3 Ligands binding sites

In this section we will discuss the binding sites of the main profilin ligands, actin, phatidylinositol 4,5-bisphosphate (PtdIns 4,5-P), and PLP. Initially, profilin binds with high affinity (micromolar range) to G-actin in a 1:1 stoichiometric complex (Schlüter et al., 1997). The amino acid motif LADYL in the C-terminal α-helix was first proposed to be implicated in actin binding depending on (1) the presence of this motif in most of profilins, (2) the presence of homologous sequences in a range of actin-binding proteins such as DNase I, fragmin, gelsolin, severin, villin and the vitamin D-binding protein (Binette et al., 1990; Tellam et al., 1989; Vandekerckhove, 1989; Vinson et al., 1993). Nevertheless, this hypothesis was neglected due to (1) the absence of this sequence in mammalian profilins, (2) the ability of Saccharomyces profilin to interact with actin even after deletion of this motif (Haarer et al., 1993). Now, the LADYL-motif is believed to be a central element in the dense structure of these proteins (Ampe & Vandekerckhove, 1994; Fedorov et al., 1994; Haarer et al., 1993; McLaughlin & Weeds, 1995). Studies on bovine profilin-I and β-actin showed that the actin binding sites on profilin are localized in the α-helix 3, the proximal part of α-helix 4, and in the β-strands 4, 5 and 6 (Schutt et al., 1993) (Figure 5). These residues bind to subdomains 1 and 3 on the actin molecule; however, they do not exhibit a conserved sequence motif (Thorn et al., 1997). In the bovine complex, Phe375 appears to be a key residue that interacts with Ile73, His119, Gly121 and Asn124 on the profilin side (Schutt et al., 1993). Similarly, other studies on *Acanthamoeba* reported that actin-related proteins such as Arp2 interact with profilin using the same binding site (Kelleher et al., 1995; Machesky, 1997).

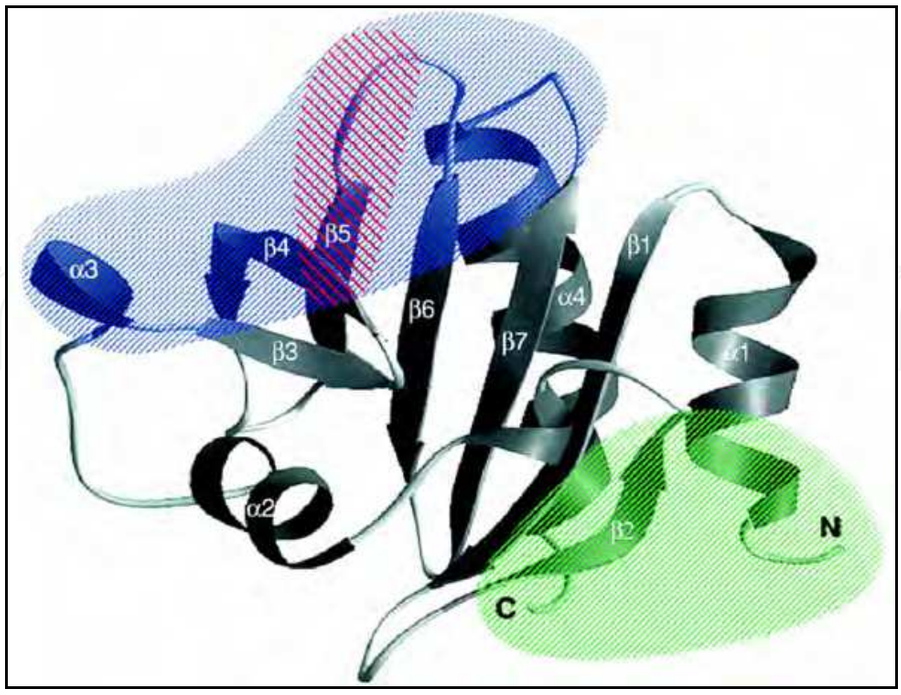


Fig. 5. Topographical relation of the main ligand binding domains as seen on the X-ray structure of bovine profilin (Schutt et al., 1993). The binding domains of actin and actin related proteins (blue; Schutt et al., 1993) and PtdIns 4,5-P_2 (red; Sohn et al., 1995) overlap, while that for proline-cluster sequences (green; Metzler et al., 1994) is located at the opposite side of the profilin molecule, adapted from Schlüter et al., (1997) with permission.

On the other hand, studies on *Acanthamoeba* described a positively charged area opposing both termini, placed in the G-actin binding site as the binding motif of the second key ligand of profilin, PtdIns 4,5-P_2 (Fedorov et al., 1994) (Figure 5). This was supported by mutation studies on *Saccharomyces* profilin and human profilin-I. Point mutations in this region diminished the binding affinity of profilin to PtdIns 4,5-P_2 (Haarer et al., 1993; Sohn et al., 1995). In line with the observation that the binding sites of G-actin and PtdIns 4,5-P_2 on profilin overlap (Figure 5), it has been reported that these ligands compete with each other for binding to profilin (Lassing & Lindberg, 1985; 1988; Machesky et al., 1990). In addition, other reports showed that binding of PtdIns 4,5-P2 results in a conformational change in profilin and disrupts the profilin-actin complex (Raghunathan et al., 1992). Also, it has been revealed that profilin can bind a variety of phosphatidylinositol and the binding affinity of human profilin-I to phosphatidylinositol 3,4-bisphosphate (PtdIns 3,4-P_2), and phosphatidylinositol 3,4,5-trisphosphate (PtdIns 3,4,5-P_3) is higher than its affinity to PtdIns 4,5-P_2 (Lu et al., 1996). Furthermore, phosphoinositide (PI) 3-kinase activity may be regulated by profilin through direct binding to the p85 subunit of this enzyme (Singh et al., 1996). PI 3-kinase has no effect on the binding of actin to profilin (Singh et al., 1996), signifying that the binding sites of actin and p85 on profilin are different.

Conversely, the profilin-PtdIns 4,5-P2 complex can be hydrolyzed only via phospholipase Cγ1 (PLCγ1). Phosphorylation and activation of this lipase as a result of transmembrane signaling (Goldschmidt-Clermont et al., 1990; 1991) leads to the conclusions that (1) profilins

are implicated in the metabolism of phosphoinositide and (2) hydrolysis of PtdIns 4,5-P2 causes profilin to move out from the membrane to the cytosol where it can bind to actin or other ligands. These conclusions propose that profilin–phosphoinositide binding plays a vital role *in vivo* (Janmey et al., 1995; Ostrander et al., 1995).

Mutation (Björkegren et al., 1993; Haarer et al., 1993) and NMR (Archer et al., 1994; Metzler et al., 1994) analyses described the binding site of profilin to the third main ligand, PLP as a hydrophobic patch including the NH- and COOH-terminal α-helices and the upper face of the antiparallel β-sheet, opposing to the actin/PtdIns 4,5-P2 binding region (Figure 5). The binding of PLP to profilins has no effect on the interaction with G-actin or PtdIns 4,5-P_2 (Archer et al., 1994; Kaiser et al., 1989), indicating that PLP has a distinct binding site (Figure 4). Expediently, this specific PLP-profilin binding is used for profilins purification (Kaiser et al., 1989; Lindberg et al., 1988). For effective profilin binding, it has been proposed that 6 continuous prolines would be sufficient (Metzler et al., 1994). Nevertheless, other reports demonstrated that at least 8–10 prolines are required for efficient binding (Domke et al., 1997; Machesky & Pollard, 1993; Perelroizen et al., 1994; Petrella et al., 1996).These proline stretches may be interrupted by single glycine residues (Domke et al., 1997; Lambrechts et al., 1997) and may be capable of simultaneous binding of two profilins (Lambrechts et al., 1997), depending on the ability of profilin to oligomerize (Babich et al., 1996).

The first recognized ligand for PLP was VASP, a focal adhesion molecule that was reported to interact directly with F-actin (Jockusch et al., 1995; Reinhard et al., 1995), and it also described as a substrate of both cGMP- and cAMP-dependent protein kinases in platelets (Halbrügge et al., 1990). VASP has a central proline-rich domain with a single copy and a 3-fold tandem repeat of a remarkable (G)P_5 motif (Haffner et al., 1995). This motif is both required and sufficient for profilin binding (Domke et al., 1997; Lambrechts et al., 1997; Reinhard et al., 1995). Another PLP-binding ligand similar to VASP is a VASP-related mouse protein, Mena (Gertler et al., 1996). Additional PLP-biding ligands are the formin-related proteins, *S. cerevisiae* Bni1p and Bnr1p, *S. pombe* Cdc12p, *Drosophila* cappuccino and p140mDia, the mammalian homologue of the *Drosophila* protein diaphanous (Chang et al., 1997; Evangelista et al., 1997; Imamura et al., 1997; Manseau et al., 1996; Watanabe et al., 1997). These proteins have a proline-rich domain with numerous proline stretches consisting of 5–13 residues and a C-terminal consensus sequence of approximately 100 amino acids (Castrillon & Wasserman, 1994). Due to the high specific binding of Bni1p, Bnr1p and p140mDia to the GTP-bound form of Rho family members (Kohno et al., 1996; Imamura et al., 1997; Watanabe et al., 1997); they perhaps represent significant connectors between signal transduction, profilin and the cytoskeleton. Furthermore, adenylyl cyclase-associated protein (CAP) has been described as PLP-binding ligand. CAP has a $G(P)_6$ $G(P)_5$ motif and it can bind to profilin (Domke et al., 1997; Lambrechts et al., 1997). Nevertheless, other studies demonstrated that CAP exists in a folded configuration (Lambrechts et al., 1997) and hence its binding to profilin may be firmly regulated.

2.3.4 Regulation of profilin-ligands binding

The important factors that could help in understanding the process of profilin-ligand binding regulation include the structural requirements for the binding of profilin to the ligand, the binding specificity of ligands to different profilins and the mechanisms of ligand

release. Initially, the structural requirements for the profilin-ligand binding are not completely understood. In spite of binding of profilin to an extremely diverse group of ligands either directly or as part of a larger complex, the binding sites on both profilin and ligands appear to be well conserved. The majority of ligands are believed to interact with the PLP domain of profilin that contains the N- and C-terminal helices. The only exception, so far, to this model is gephyrin, which appears to bind to a special profilin domain (Giesemann et al., 2003). All profilin ligands are characterized by the presence of stretched or nearly stretched proline-rich domains that are required for profilin binding. Still, a contiguous prolines stretch is insufficient. Depending on the data obtained from *in vitro* studies using synthetic PLP peptides of different length high-affinity binding requires a decamer as a minimum, (Perelroizen et al., 1994) however this cannot be extended to cover proteins or to be used for recognizing or evaluating the ability of profilin to bind to a ligand. A lot of profilin ligands contain in their proline-rich domains proline repeats of no more than three or four successive prolines. Further amino acids, mostly glycines, appear to be capable of replacing proline, and an efficient profilin-binding domain appears to include numerous repeats that have the consensus sequence ZPPX (where Z=P, G or A; and X= any hydrophobic amino acid) (Witke et al., 1998).

The second important factor in regulating the binding of profilins to their ligands is the binding specificity of ligands to the different profilins. Previous reports showed that the interaction of ligands with profilin-I and profilin-II occurs in a highly specific manner (Witke et al., 1998) and it looks likely that it is not only the PLP-binding domains but also other complex binding parameters have to be considered. Comparative studies on the structures of mammalian profilin-I and profilin-II indicated that they are approximately superimposable (Nodelman et al., 1999). Nevertheless, the distribution of surface charges in profilin-I and profilin-II is significantly different and this perhaps participates in the ligand-binding specificity (Figure 2). Eventually, identifying the structural features of different profilin complexes will be helpful to understand the basis of specificity.

Finally, the profilin- ligands binding should be a dynamic process and the mechanisms of ligand release under physiological conditions have to be determined. For example, actin can be released from profilactin complex via PtdIns(4,5)P_2, and an analogous mechanism might be used for ligand binding regulation. For instance, PtdIns(4,5)P_2 can regulate the interaction between dynamin 1 and profilin-II, but not the Mena–profilin or VASP–profilin complexes. Regulation of Mena, VASP, and other ligands binding might be achieved in different ways such as profilin or ligand phosphorylation (Witke, 2004).

2.4 Role of profilin in signal transduction

Profilins bind to several ligands, and a lot of these ligands are part of various complexes or interact with each other as well (Figure 3). This results in an intimate crosstalk among these complexes that can substitute and distribute components and, thus, could assimilate signals from other signaling pathways such as small-GTPase and phosphoinositide pathways. In these signaling platforms profilins appear to be a common denominator (Witke, 2004). Figure 6 is a schematic representation demonstrating various interactions between profilin, the microfilament system and different signaling pathways.

Profilins are linked to the phosphatidylinositol cycle and in turns to the receptor tyrosine kinase pathway through their binding to PtdIns 4,5-P_2. Profilin-bound PtdIns 4,5-P_2 is

resistant to hydrolysis by phospholipase Cγ1 (Goldschmidt-Clermont et al., 1990). However, this resistance can be overcome after activating phospholipase via receptor tyrosine kinases-dependant phosphorylation (Goldschmidt-Clermont et al., 1991). This activation process results in PtdIns 4,5-P_2 hydrolysis with subsequent formation of other two second messengers, diacylglycerol and inositol 1,4,5-trisphosphate. Additionally, profilin releases from the membrane, which might initiate fast, local actin polymerization. Conversely, activated phospholipase Cγ1 cannot hydrolyze other PI 3-kinase activity products such as PtdIns 3,4-P_2 and PtdIns 3,4,5-P_3 ,that bind to profilin with a higher affinity than PtdIns 4,5-P_2. Consequently, it has been revealed that PtdIns 3,4-P_2 and PtdIns 3,4,5-P_3 may regulate phospholipase Cγ1-controlled turnover of PtdIns 4,5-P_2 (Lu et al., 1996).

In addition, the profilin ligands of the formin-related proteins such as p140mDia connect the GTPase-related signaling cascade, which is also coupled with the PtdIns 4,5-P_2 signaling pathway to the microfilament system. The small GTPases of the Rho family are active members that are involved in regulating the cytoskeleton-based processes such as cell morphology, adhesion and cytokinesis (Tapon & Hall, 1997). Most likely, these formin-related proteins are down-stream effectors of Rho in this cascade (Evangelista et al., 1997; Watanabe et al., 1997).

Also, the microfilament system is linked to the adenylyl cyclase-related pathway via substrates of the cAMP/cGMP-dependent protein kinases such as VASP/Mena family (Butt et al., 1994; Gertler et al., 1996) and the putative profilin ligand CAP, which is an adenylyl cyclase activator (Fedor-Chaiken et al., 1990; Field et al., 1990; Toda et al., 1985). This linking can be executed through either direct binding of CAP and VASP proteins to actin (Freeman et al., 1995; Gieselmann & Mann, 1992; Gottwald et al., 1996; Hubberstey et al., 1996; Reinhard et al., 1992) or recruiting profilin and profilin-actin complexes to areas of dynamic actin remodelling via the interaction of VASP proteins with cell contact proteins such as zyxin and vinculin (Brindle et al., 1996; Gertler et al., 1996; Reinhard et al., 1995, 1996).

Furthermore, annexin I could be involved in this crosstalk depending on previous reports that described the sensitivity of annexin I-profilin binding to PtdIns 4,5-P_2 and actin (Alvarez-Martinez et al., 1996). On top of that the annexins activity is controlled by the free Ca^{2+} level, which is adjusted via PtdIns 4,5-P_2 hydrolysis upon the action of the activated phospholipase Cγ 1 (Figure 6). In addition to annexin I, Ca^{2+} level will affect various Ca^{2+}-actin-binding and –severing proteins which slice the actin filaments and create new plus ends to which profilin-actin complexes can be added (Schlüter et al., 1997) (Figure 6).

Interestingly, in mesangial cells extracellular profilin was shown to bind specifically to a putative receptor and stimulates AP-1, a key element in signal transduction that is involved in the regulation of the transcription of several genes and cell growth (Tamura et al, 2000).

With the current large number of profilins ligands the future challenge is to determine their role in this complicated signaling crosstalk. One possibility is that profilins may act as regulators for the composition of the complexes and facilitate entrance or exit of certain ligands. Additionally, they might act as direct regulators for the ligands activities. Identification of all profilins molecular interactions, their ligands, and recognizing the structure of these complexes will be helpful to understand the mechanisms by which profilins can control this diverse signaling complexes (Witke, 2004).

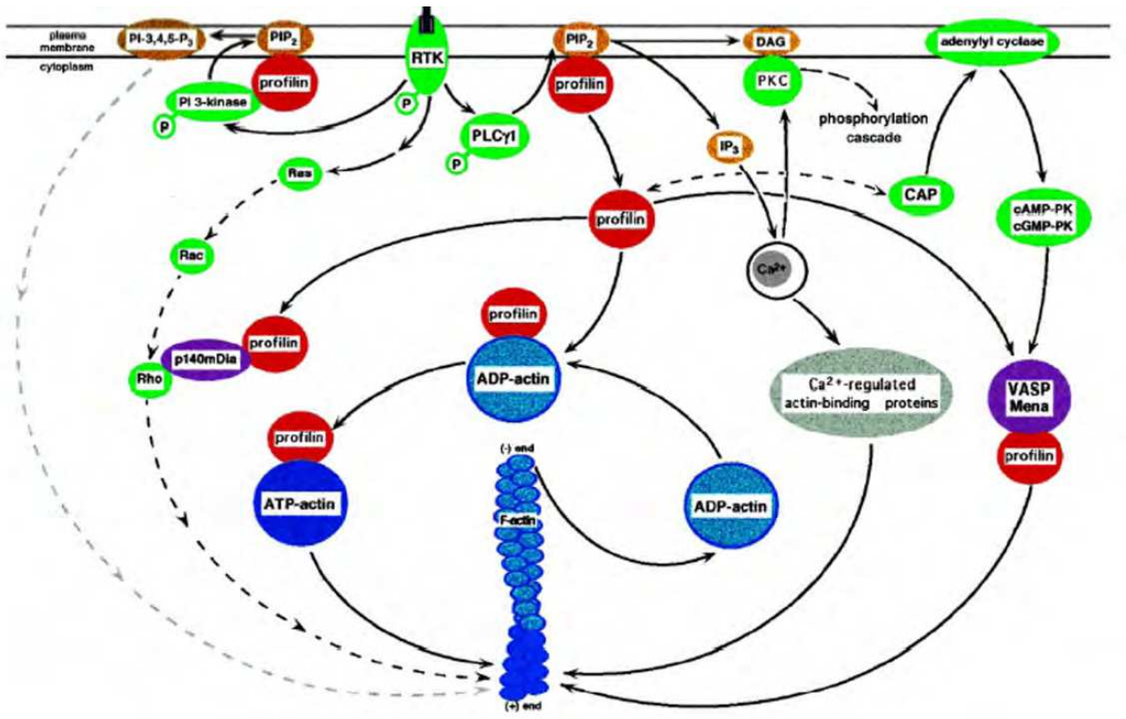


Fig. 6. The involvement of profilin (red) in different signaling routes. This schematic drawing depicts only the main connections established so far. Molecules and second messengers of the polyphosphoinositide signaling pathway are indicated in yellow, protein members of signaling routes are marked green, proline-cluster proteins identified as profilin ligands are marked purple, the actin cycle is seen in blue, Ca^{2+} in intracellular stores and Ca^{2+} regulated microfilament proteins are marked grey. For simplicity, the solid arrows indicate either direct interactions between components, as shown by biochemical assays, or point to pathways. Broken arrows indicate suspected or indirect interactions. Abbreviations: PI-3,4,5-P_3: phosphatidylinositol 3,4,5-trisphosphate; PIP_2: phosphatidylinositol 4,5-bisphosphate; RTK: receptor tyrosine kinase; DAG: diacylglycerol; PLCγ 1: phospholipase Cγ 1; cAMP/cGMP- PK: cAMP/cGMP dependent protein kinase; IP_3: inositol 1,4,5-trisphosphate, adapted from Schlüter et al., (1997) with permission.

3. Profilin & vascular diseases

3.1 Role of profilin in vascular smooth muscle & endothelial cells

3.1.1 Profilin & vascular smooth muscle cells migration & proliferation

Migration of smooth muscle cell takes place throughout vascular development, as a result of vascular injury, and throughout atherogenesis. Throughout vascular development, platelet-derived growth factor promotes migration of pericyte or other precursors of smooth muscle that is required for the formation of correct vessel wall (Hellstrom et al., 1999). Clinically, vascular injury takes place after angioplasty, vascular stent implantation, or organ transplantation. In vascular injury in animals, thickening of intima and media has been attributed to VSM proliferation and migration from media to intima (Clowes et al., 1989; Majesky & Schwartz, 1990; Reidy, 1992). Throughout atherogenesis, VSMCs migrate to

occupy the intima, either from the media (Murry et al., 1997) or from the circulation via $CD34^+$ hematopoietic progenitor cells migration, resulting in smooth muscle progenitor cells (Yeh et al., 2003). Figure 7 shows the inner lining of a normal artery.

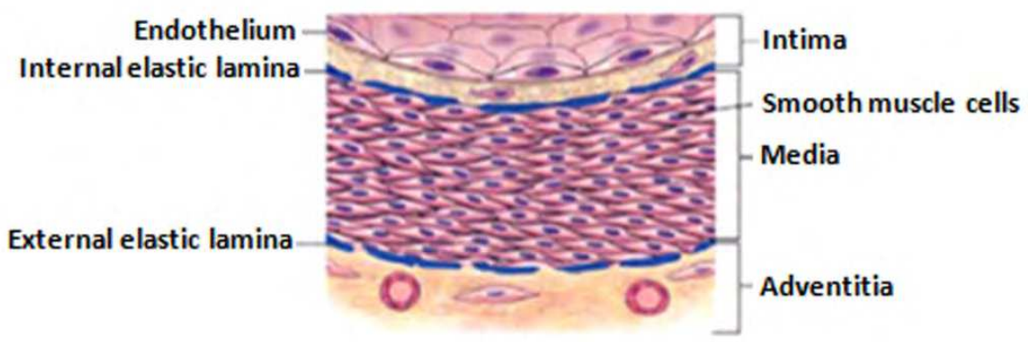


Fig. 7. Inner lining of normal artery

VSMC migration requires the extension of lamellipodia toward the stimulus via actin polymerization, trailing edge detachment via focal contacts degradation, and force generation via myosin II in the cellular body to drive the cell forward (Gerthoffer, 2007). Initiation of new filaments nucleation is achieved by actin-related proteins such as ARP2/3 complex interacting with the minus end and elimination at the plus end of capping proteins that are PIP_2-sensitive. Extension of new actin filaments is improved by formin-related proteins such as mDia1 and mDia2 that operate along with profilin on the plus end. Activation of The formins mDia1 and mDia2 is achieved by RhoA and Cdc42, respectively. Profilin released from the binding sites of membrane phospholipid enhances nucleotide exchange on G-actin monomers and promotes actin polymerization. Stimulation of filament branching is accomplished via activating WAVE complex and WASP by Rac and Cdc42, respectively. WAVE and WASP increase nucleation and branching through activating actin-related proteins such as ARP2/3 complex. Severing of Actin-filament by gelsolin is stimulated by Ca^{2+}, and nucleation is favored via liberating gelsolin from plus ends of F-actin by PtdIns 4,5-P2. Stimulation of actin depolymerization is executed by cofilin at the minus end. Cofilin acts to limit the filaments length and to induce the existing filaments turnover. These operations have been reported to be sufficient for force generation to expand the leading edge of the cell toward the stimulus (Mogilner & Oster, 2003; Prass et al., 2006). Consistent with these findings our recent data confirmed the significant role of profilin-I in VSMC migration. Migration assays performed on VSMC isolated from the aorta of transgenic mice that overexpress the cDNA of profilin-I or profilin-I-dominant negative mutant (88R/L) and nontransgenic controls showed that the rate of cell migration of profilin-I VSMCs is significantly higher than that of the control and 88R/L. Conversely, 88R/L mice exhibited a significantly lower rates compared to nontransgenic controls (Figure 8) (Hassanain HH, unpublished).

On the other hand, it has been shown that profilin plays a vital role in the proliferation and differentiation of normal cell. Disruption in the profilin results in embryonic lethality due to gross impairment in growth, motility, and cytokinesis in single cells (Haugwitz et al., 1994; Witke, 2004; Witke et al., 2001). Also, profilin-1 was demonstrated to exert cellular responses such as DNA synthesis and increasing the binding activity of AP-1 DNA in mesangial cells via activating putative cell surface receptors (Tamura et al., 2000).

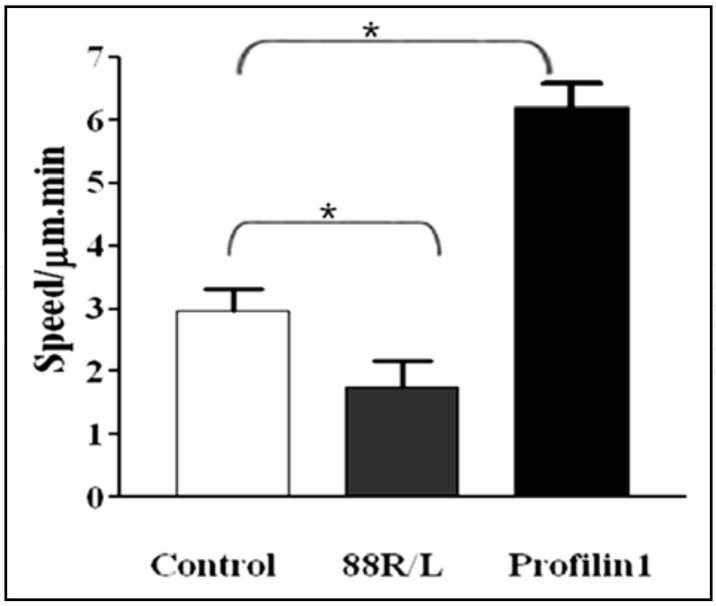


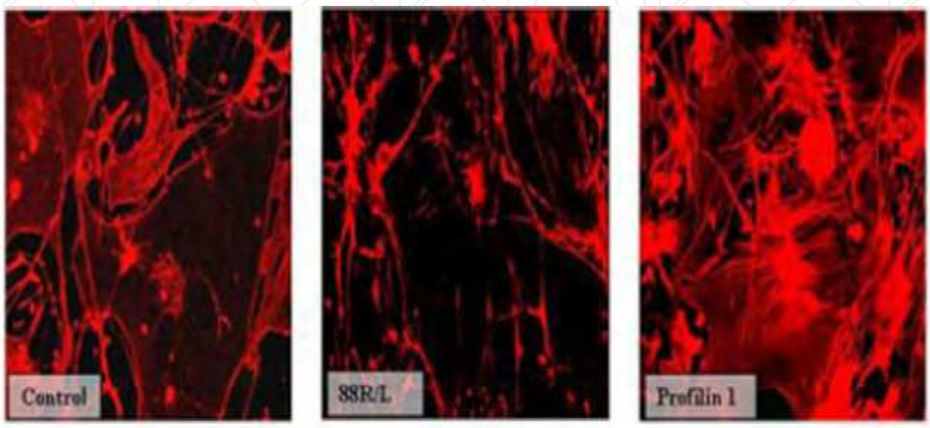
Fig. 8. The MetaMorph image analysis of the mean speed of the individual cells (µm/min) of profilin-I, 88 R/L and control VSMCs. The differences in mean were determined by ANOVA. *P < 0.05, compared with corresponding control, is considered to be significant (Hassanain HH, unpublished).

In line with the established role of profilin in cellular migration and proliferation, it has been shown that recombinant profilin-I stimulates DNA-synthesis and migration of both rat and human VSMCs in a concentration-dependent manner (Caglayan et al., 2010). The same study indicated that profilin-induced VSMCs migration is dependent on PI3K activity (Caglayan et al., 2010). Moreover, Cheng et al., (2011) found that profilin-I plays a key role in Angiotensin (Ang) II-induced VSMCs proliferation. They also suggested that Ang-II increases profilin-I expression and promotes VSMCs proliferation via activating AT1 receptor/JAK2/STAT3 pathway (Cheng et al., 2011). On the contrary, other studies described the involvement of phospho-extracellular signal-regulated kinase1/2 (P-ERK1/2) and phospho-c-Jun NH2-terminal kinase (P-JNK) in Ang-II-induced profilin-I expression (Zhong et al., 2011), and that PI3-kinase, Src, and, to a lesser extent, P-ERK1/2 are required for profilin-I-dependent VSMCs proliferation (Caglayan et al., 2010). Consequently, Cheng et al., (2011) proposed that the interaction of these signaling pathways mediating the role of profilin-I in VSMCs proliferation requires further investigation. Consistent with these data, we observed that the treatment of mouse aortic VSMCs with Ang-II (100 nM/10 min) resulted in increased profilin-I expression (Hassanain HH, unpublished)

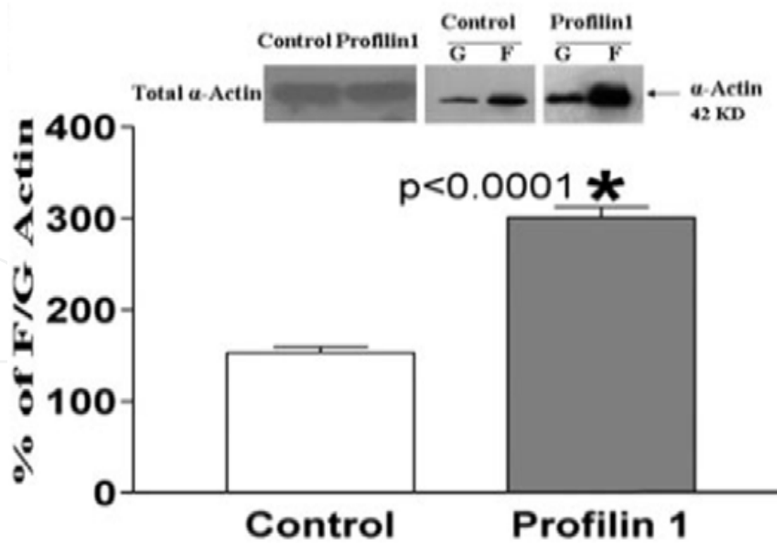
3.1.2 Profilin & vascular smooth muscle contraction

Regulation of smooth muscle contraction has been thought to be only dependent on the 20-kDa regulatory light chain of myosin (MLC20) that in turn modulates cross-bridge cycling of actomyosin. Numerous studies showed that contractile stimulation promotes actin polymerization in vascular and airway smooth muscle tissues (Cipolla & Osol, 1998; Jones et

al., 1999; D. Mehta & Gunst, 1999) and in cultured smooth muscle cells (An et al., 2002; Barany, et al., 2001; Hirshman & Emala, 1999). In addition, inhibition of actin polymerization by specific inhibitors such as latrunculin decreases the contractile stimuli- activated force development in smooth muscle (Cipolla & Osol, 1998; D. Mehta & Gunst, 1999; Youn et al., 1998). However, this does not affect contractile stimulation-induced MLC20 phosphorylation (34), suggesting that actin polymerization plays a central role during smooth muscle contraction. Tang & Tan, (2003) investigated the effect of profilin, the main actin-regulatory protein on the regulation of smooth muscle contraction. They demonstrated that profilin downregulation with antisense repressed force generation, without affecting MLC20 phosphorylation, signifying that profilin is crucial for smooth muscle contraction and that it does not regulate the activation of contractile protein. Yet, profilin downregulation repressed increases in the F-actin/G-actin ratio in return to agonist stimulation, showing that profilin is essential for actin dynamics during contractile stimulation of smooth muscle (Tang & Tan, 2003). In harmony with these finding our results showed higher expression of stress fibers and membrane ruffling in vascular smooth muscle cells from profilin-I transgenic mice compared with nontransgenic control and 88R/L. The 88R/L cells, however, showed lower expression of stress fiber formation and ruffling than the nontransgenic controls (Figure 9*A*) (Moustafa-Bayoumi et al., 2007). In addition, we confirmed these findings by assessing the ratio of F-actin/G-actin in the aortic smooth muscle cells from profilin-I. Our results showed a significant increase in F/G actin ratio in the aortic smooth muscle cells from profilin-I mice compared with the nontransgenic controls (Figure 9B) (Moustafa-Bayoumi et al., 2007). Furthermore, we showed that profilin-I plays a significant role in increased contractility and force development in the mesenteric arteries of profilin-I mice via activating Rho/ROCK pathway and MLC20 (Hassona et al., 2010). Activated Rho elevates MLC20 phosphorylation by *1*) directly phosphorylating MLC20 and *2*) phosphorylation and inhibition of the MBS of MLC20 phosphatase (Higgs & Pollard, 2001; Pollard & Borisy, 2003). This increases myosin contractility and tension contributing to stress fibers. In conclusion, our results indicate that overexpression of profilin-I in smooth muscle cells leads to increased contractility and force development via increasing actin polymerization (Moustafa-Bayoumi et al., 2007) and MLC20 activation(Hassona et al., 2010), which in turn induce mechanical stress that is considered as the main initiator for arterial stiffness and hypertension observed in these mice.



(a)



(b)

Fig. 9. Overexpression of profilin-I induced actin polymerization in vascular smooth muscle cells. Rhodamine-phalloidin staining of smooth muscle cell confluent monolayers shows increased stress fibers in vascular smooth muscle cells from profilin-I transgenic mouse as compared with nontransgenic control (*a*). The 88R/L cells, however, show lower expression of stress fiber formation than the control (*a*). Analysis of the F-actin/G-actin ratio shows significant increase in F-actin/G-actin (*F*/*G*) ratio in the aortic smooth muscle cells from profilin-I mice compared with the nontransgenic controls (*b*) (Moustafa-Bayoumi et al., 2007).

3.1.3 Profilin & vascular endothelial cells adhesion

Endothelial cells survival neatly depends on their ability to anchor to extracellular matrix proteins. Suppression of endothelial cell adhesion has been shown to induce apoptosis in these cells (Meredith et al., 1993; Re et al., 1994; Zang et al., 1995). It has been found that transient overexpression of profilin in cultured human aortic endothelial cells using replication-incompetent adenovirus enhances endothelial cells adhesion to the extracellular matrix via promoting the binding of extracellular fibronectin to its receptor on the surface of these cells. Additionally, it was revealed that profilin-mediated enhancement of endothelial cell adhesion has a protective role in situations of focal contacts disruption due to shear, stretch or other focal injuries (Moldovan et al., 1997).

Moreover, the authors, Moldovan et al., (1997) proposed that the profilin-mediated effect seems to be stimulated via recruiting integrins $\alpha_5\beta_1$ to the endothelial cell surface. Numerous mechanisms may explain this later effect. One possibility is that profilin might cause improvement in the access of receptor molecules to the cell surface. Instead, profilin might cause impairment in the internalization of membrane receptors. These effects may be achieved in 1) actin-dependent manner, where profilin might decrease receptor internalization via disrupting actin stress fibers or it might offer a stronger anchor for fibronectin receptor molecules in focal contacts via stabilizing actin filaments that are not stress fibers (Finkel et al., 1994), or 2) actin-independent manner, where profilin interacts

with PtdIns 4,5-P2 and inhibits its hydrolysis by phospholipase C (Goldschmidt-Clermont et al., 1990, 1991; Lassing & Lindberg, 1985). Increased concentrations of PtdIns 4,5-P2 could stimulate the stabilization of newly formed focal contacts including the fibronectin receptor via an unknown mechanism or profilin overexpression could overcome other actin-binding proteins for interacting with PtdIns 4,5-P2 and thus enhance their binding to actin filaments (Hartwig et al., 1995).

3.1.4 Role of profilin in vascular endothelial cells migration, proliferation & capillary morphogenesis

Vascular endothelial cell (VEC) migration is vital for capillary outgrowth from pre-existing blood vessels during angiogenesis (Bauer, et al., 2005). During cell migration, actin cytoskeleton reorganization is a dynamic process that includes both actin polymerization and depolymerization in an accurate spatiotemporal manner. Regulation of this actin remodeling process is achieved by a large number of actin binding proteins such as those involved in monomer sequestering, nucleating, elongating, severing, depolymerizing, and capping of actin filaments (Pollard & Borisy, 2003). Expression profiles in VEC experiencing capillary morphogenesis identified some of the key actin-binding proteins that have been previously involved in angiogenesis such as thymosin β4, profilin, gelsolin and VASP. Among these proteins, as a minimum thymosin β4 has been established as a proangiogenic molecule *in vivo* (Philp et al., 2004; Salazar et al., 1999). In addition, it has been reported that silencing profilin-I expression in human umbilical vein endothelial cells significantly decreases their capability of forming planar cord-like structures on matrigel (a commonly adopted *in vitro* representation for angiogenesis). These findings proposed for the first time that profilin-I might play a key role in VEC capillary morphogenesis (Ding et al., 2006).

In a more recent report for the same group they adopted a knockdown–knockin experimental system to stably express either fully functional form or mutants of profilin-I that are deficient in binding to actin and proteins containing polyproline domains, in a human dermal microvascular cell line. They showed that silencing endogenous profilin-I expression in this cell line results in slow rate of random migration, decreased membrane protrusion velocity and a significant reduction in matrigel-induced cord formation. These defects were rescued only via re-expression of fully functional but not any of the two ligand-binding deficient mutants of profilin-I. They also showed that loss of profilin-I expression in VEC inhibits three dimensional capillary morphogenesis, MMP2 secretion and ECM invasion. Disruption of actin and polyproline interactions of profilin-I inhibited VEC invasion through ECM, as well. They concluded that profilin-I regulates VEC migration, invasion and capillary morphogenesis through its binding to both actin and proline-rich ligands (Ding et al., 2009). Furthermore, they indicated that these *in vitro* findings pave the way for future *in vivo* studies to investigate the role of profilin-I in angiogenesis.

Interestingly, cutaneous wound healing experiments in our profilin-I and 88R/L transgenic mice showed a significant increase in blood vessel density in profilin-I transgenic mice compared to 88R/L transgenic mice and nontransgenic control at post wound day 7 (Figure 10) (Hassanain HH, unpublished). These data could indicate the importance of profilin-I in angiogenic reponse in VEC.

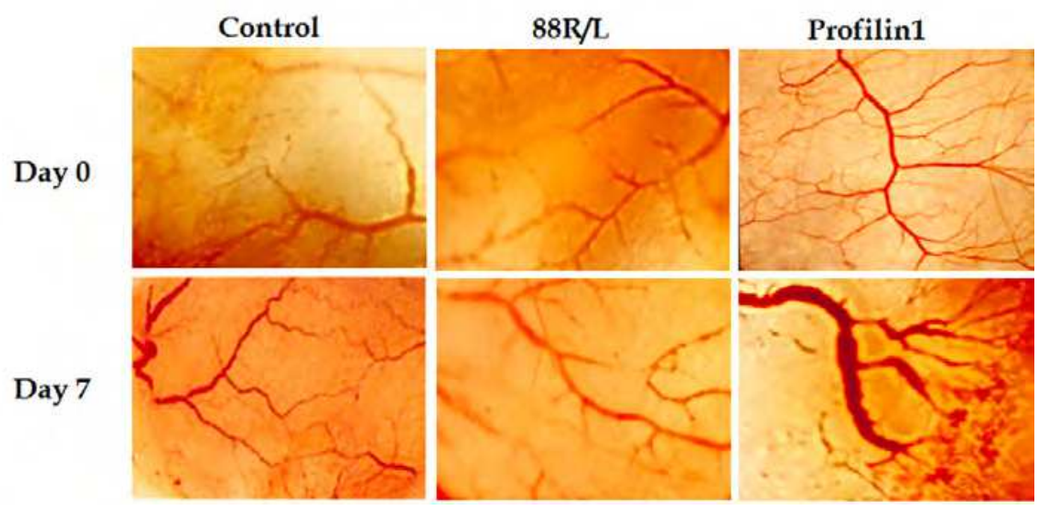


Fig. 10. Stimulation of angiogenesis in the wound area of profilin-I mice. Distribution of capillaries along the margin of the excision wound in Profilin-I, 88R/L and nontransgenic control mice at post wound days 0 and 7. High magnification of capillaries in the skin was obtained with a 2X objective lens light microscope (Hassanain HH, unpublished).

3.2 Role of profilin in vascular remodeling & hypertension

Hypertension represents a major risk factor for cardiovascular events such as stroke and myocardial infarction. It is well established that hypertension leads to remodeling of large and small arteries (Folkow, 1982; Simon, 2004). Remodeling of the vasculature is an active process of structural changes that involves alterations in cellular processes, including growth and changes in the extracellular matrix integrin-cytoskeleton axis, resulting in an increase in the media-to-lumen ratio (Gimbrone et al., 1997; Intengan & Schiffrin, 2001). Physiological remodeling is an adaptive process occurring in response to hemodynamic changes and aging. However, when this process becomes maladaptive, it plays a role in hypertension's complications (Ming et al, 2002; Touyz, 2007). Increased mechanical strain/hypertension in the vessel wall triggers the hypertrophic signaling pathway resulting in structural remodeling of vasculature. Increased actin polymerization and stress fiber formation generate mechanical force that represents an important modulator of cellular morphology and function in a variety of tissues and is an important contributor to hypertrophy in the cardiovascular system (Ruwhof &van der Laarse, 2000). Also, it has been shown that actin polymerization within VSMCs in response to increased intravascular pressure is a novel mechanism underlying arterial myogenic behavior. The cytosolic concentration of G-actin is significantly reduced by an elevation in intravascular pressure, demonstrating the dynamic nature of actin within VSMCs and implying a shift in the F:G equilibrium in favor of F-actin. Profilin-I which is a key actin-regulatory protein that plays an essential role in regulating de novo actin polymerization, particularly actin treadmilling (Carlier & Pantaloni, 2007; Suetsugu et al., 1999) could be vital in regulating all of these vascular events. Indeed, our report in the Journal of Biological Chemistry (Moustafa-Bayoumi et al., 2007) established the feasibility of our proposal. We showed that elevated expression of profilin-I gene in VSMCs of profilin-I mice favoring F-actin induces stress fiber formation (Figure 11) and plays an important role in vascular hypertrophy by inducing

internal mechanical stress and triggering the hypertrophic signaling pathways, integrins-$\alpha_1\beta_1$/Rho-ROCK/MAPKs e.g. P-ERK and P-JNK, leading to vascular remodeling in both large (e.g. aorta) and small (e.g. mesenteric) arteries (Figure 12A, B) of profilin transgenic mice (Hassona et al., 2010; Moustafa-Bayoumi et al., 2007).

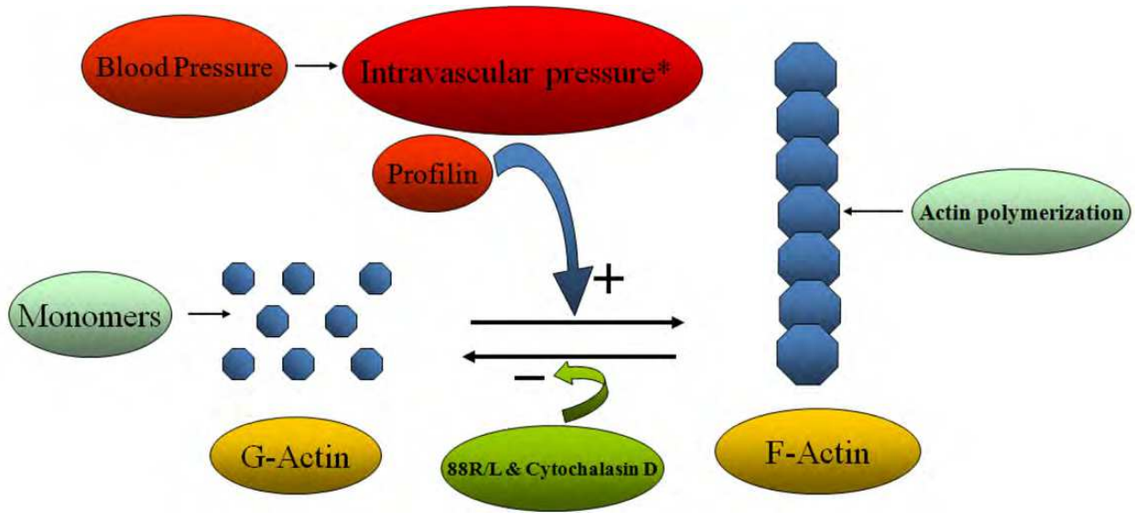


Fig. 11. Hypertension or increased profilin-I expression in VSMCs leads to a shift in the F:G equilibrium in favor of F-actin and an elevation in intravascular pressure. This pathway can be reversed by F-actin inhibitor, cytochalasin D or profilin-I mutant, 88R/L.

Consistent with our finding, very recent studies showed increased profilin-I expression in hypertrophic thoracic aorta and mesenteric arteries of spontaneously hypertensive rats with subsequent elevation in both P-ERK and P-JNK, suggesting that profilin-I may contribute to the vascular remodeling in these rats (Cheng et al., 2011; Zhong et al., 2011). In this context, previous studies suggested that mechanical stretch is closely related to JNK and ERK1/2 activation (Hu et al., 1997; Pyles et al., 1997). These cascades play an important role in remodeling of blood vessels, as well. In addition, this pathway is activated by Ang-II and has been implicated in the pathogenesis of cardiovascular diseases (P.K. Mehta & Griendling, 2007). Interestingly, it has been recently reported that profilin-I is a key component in the Ang-II-induced vascular remodeling (Cheng et al., 2011; Zhong et al., 2011).

As it was mentioned above that hypertension is a major cause of vascular remodeling. The primary aim of anti-hypertensive drugs, particularly Ang-converting enzyme inhibitors and Ang receptor subtype 1 antagonists, is to lower the blood pressure with the hope of reversing this remodeling (Schiffrin, 2001). Importantly, In our profilin-I model we demonstrate that the reverse can be true as well, i.e. alteration in cytoskeleton dynamics favoring increased actin polymerization can contribute to vascular adaptations with aging resulting in increased systolic blood pressure by the time the profilin-I mice were six months old (Figure 12C) (Moustafa-Bayoumi et al., 2007). The blood pressure in the profilin-I mice was elevated 25–30 mm Hg higher than nontransgenic controls. In agreement with our findings, it has been demonstrated that profilin speeds up the actin remodeling and accordingly improves the growth and invasion force of VSMCs resulting in increased vascular resistance and accelerated formation of pulmonary hypertension (Dai et al., 2006).

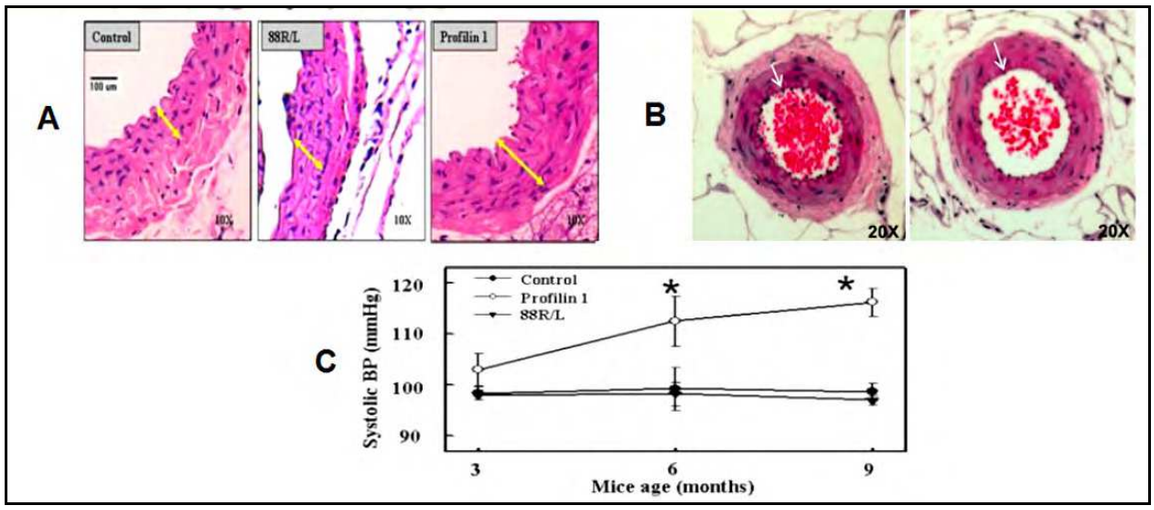


Fig. 12. Profilin overexpression induced vascular hypertrophy and hypertension. Hematoxylin and eosin staining shows clear signs of remodeling and vascular hypertrophy in the aorta of profilin-I transgenic mice (yellow arrows; **A**) and mesenteric arteries (white arrows; **B**). There are no differences, however, between 88R/L and nontransgenic control aortic sections (**A**). Tail cuff measurements of blood pressure show significant increase in the systolic blood pressure (BP) in profilin-I transgenic mice at 6 months and older compared with nontransgenic control mice (C) (Hassona et al., 2010; Moustafa-Bayoumi et al., 2007).

On the other hand, the blood pressure in 88R/L mice was below the control littermates; however, it did not reach statistical significance. The absence of a hypotensive phenotype in the 88R/L mice could be due to the lack of significant vascular remodeling as a result of decreased actin polymerization. Our results showed a decrease in stress fibers formation in 88R/L mice (Figure 9); however, these changes did not translate into significant alterations in the vasculature. This might be due to an activation of a compensatory mechanism to maintain the integrity of vessel structure and thus keep the blood pressure at a survival level. Additionally, our preliminary data showed that inhibition of profilin-I-induced stress fibers by cytochalasin D lowered blood pressure in profilin-I mice. As a pilot study the profilin-I mice were injected with a single dose of cytochalasin D (0.5 µg/gram body weight) which led to lowered blood pressure within 10 minutes in these mice from 140 mmHg to 70 mmHg and the effect was sustained for more than 1.5 hours. Then the mice were recovered without any sign of sickness. To make sure that cytochalasin D had no damaging effect on the endothelium, we assessed the functionality of the endothelium using Ach and wire-myography. Our results showed no damage in the endothelium after cytochalasin D treatment (Hassanain HH, unpublished). We should note that cytochalasin D was used before by other investigators in different studies with much higher doses and no toxicity was observed (Speirs & Kaufman, 1989).

Furthermore, stress fiber formation could affect the relaxation/contraction process of the smooth muscles, making it more constrictive and/or less responsive to vasodilators such as nitric oxide. That could be an important factor contributing to hypertension besides the vascular hypertrophy in the profilin-I transgenic mice. Our recent report in the American Journal of Physiology confirmed this proposal. We showed that vascular hypertrophy-

associated hypertension of profilin-I transgenic mice led to functional remodeling of peripheral arteries. Our results showed a significant increase in the contraction response of profilin-I mesenteric arteries toward phenylephrine and significant decreases in the relaxation response toward ACh and sodium nitrite compared with nontransgenic controls (Hassona et al, 2010). Additionally, inhibiting stress fibers formation with cytochalasin D significantly relaxes the phenylephrine-contracted mesenteric arteries, suggesting that the increased constriction of mesenteric arteries to phenylephrine could be because of the increased F- to G actin ratio; however, cytochalasin D treatment reduced this ratio (Hassona et al., 2010).

Moreover, it has been reported that in addition to the role of hypertension in vascular remodeling, there are pressure-independent genes that play a key role in vascular remodeling. This concept is supported by the observation that despite blood pressure control in hypertensive patients, the rate of restenosis (attributable to remodeling) remains high (Gurlek et al., 1995). In harmony with this concept we recently showed that normalization of blood pressure by selected anti-hypertensive agents is not enough to correct the structural and functional remodeling of profilin-I transgenic mice (Hassona et al., 2011). Our results demonstrated that there is only correction in the functional remodeling and signaling cascades of the mesenteric arteries of losartan- and amlodipine-treated, but not those of atenolol-treated profilin-I transgenic mice, where losartan and amlodipine decrease the F-actin and stress fibers formation, proposing that the stress fibers seem to play a major role in the development and progression of the vascular remodeling-associated hypertension. We finally concluded that profilin-I gene, which is the key player controlling stress fiber formation may be a good target to treat not only hypertension but also the vascular remodeling in hypertensive patients (Hassona et al., 2011).

3.3 Role of profilin in atherosclerosis & vascular complication in diabetes

Vascular endothelium dysfunction goes before, and may participate in atheroma formation in return to various cardiovascular risk factors such as diabetes (Johnstone et al., 1993; Tesfamariam et al., 1990;), hyperlipidemia (Chikani et al., 2004; Steinberg et al., 1997), and both local and systemic inflammatory mediators (Libby, 2002). Interestingly, Romeo et al., (2004) revealed that profilin-I levels are improved in the endothelium of diabetic aorta of both human and experimental animals. They also demonstrated that profilin overexpression in primary aorta EC was capable of triggering indicators of endothelial dysfunction such as apoptosis, ICAM-1 up-regulation, and decreased VASP phosphorylation. In addition, profilin was found to be required for LDL-mediated ICAM-1 up-regulation and it can be regulated by LDL/cholesterol signaling, but not high glucose (Romeo et al., 2004). Although, Clarkson et al., (2002) reported that exposure to high glucose was able to increase profilin-I mRNA in mesangial cells and in the diabetic rat kidney. Romeo et al., (2004) suggested that the inability of high glucose to enhance profilin-I protein levels in EC is in line with a multifactorial etiology of endothelial dysfunction coupled with the metabolic syndrome and may reveal the inadequate effect of glucose-lowering monotherapy to prevent macrovascular complications in type 2 diabetic patients (U.K. Prospective Diabetes Study (UKPDS) Group, 1998). On the other hand, our preliminary data showed that mouse aortic VSMCs treated with glucose (25 mM/24 hours) increased profilin-I expression (Hassanain HH, unpublished).

Furthermore, Romeo et al., (2004) showed that profilin was clearly increased in EC and macrophages within atherosclerotic lesions of apoE null mice. In a more recent report, the

same group specified the significance of profilin-I for atherogenesis *in vivo* as profilin-I heterozygosity resulted in protection from atherosclerosis in LDL receptor-null mice (Romeo et al., 2007). In this report, a variety of atheroprotective indicators were recognized in mice with heterozygous deficiency of profilin-I, as compared to profilin-I wild-type mice. Aortas from these heterozygous mice exhibited preserved activation of endothelial nitric oxide synthase (eNOS) and nitric oxide-dependent signaling, decreased expression of vascular cell adhesion molecule (VCAM)-1 and decreased accumulation of macrophage at the sites of injury. Correspondingly, profilin-I knockdown in cultured aortic ECs was able to protect against endothelial dysfunction induced by oxidized low-density lipoproteins (oxLDL). Additionally, macrophages from bone marrow of profilin-I-deficient heterozygous mice exhibited diminished internalization of oxLDL and oxLDL-induced inflammation. These studies concluded that profilin-I plays a vital role in early atheroma formation and that decreasing profilin-I levels is atheroprotective. Finally, profilin-I atheroprotective effect is mediated via combined mechanisms that depend on both endothelium and macrophages (Romeo et al., 2007).

Moreover, the same group addressed the pathways responsible for profilin-I gene expression in 7-ketocholesterol (oxysterol)-stimulated endothelial cells and in the diabetic aorta. They showed that oxysterol-binding protein-1 (OSBP1) is required for oxysterol-dependent nucleation and activation of the JAK2/STAT3 pathway, which in turn regulates profilin-I gene expression in endothelial cells. Similarly, diabetes increases the activation of STAT3 and its recruitment to the profilin-I promoter in large vessels *in vivo* (Romeo et al., 2008)

Very recently, it has been reported that profilin-I expression is markedly increased in human atherosclerotic plaques compared to the normal vessel wall (Caglayan et al., 2010). A correlation was found between profilin-I serum levels and the degree of atherosclerosis, as well. The atherogenic effects of profilin-I on VSMCs imply an auto-/paracrine role within the plaque. In addition, it was found that profilin-I acts as an extracellular ligand and triggers atherogenic effects in VSMCs including DNA synthesis and migration. Besides, profilin-1 stimulates typical signaling pathways such as the PI3K/AKT and RAS-RAf-MEK-ERK pathways. These findings revealed that profilin-I might play a critical role in atherogenesis and may represent a novel therapeutic target in human patients (Caglayan et al., 2010).

3.4 Role of profilin in age-associated vascular problems

Aging is a major risk factor for the development of vascular diseases, such as hypertension and arteriosclerosis, which lead to stroke and heart failure (Spagnoli et al., 1991). Aging is also linked with decreased stress tolerance. Susceptibility to a variety of physiological stresses such as infection, inflammation, and oxidative damage enhances with age and is causally coupled with clinical problems in the elderly (Starr et al., 2011). So far, the mechanism of age-related changes in vasculature has not been completely understood. On the top of that, the role of profilin in these age related changes remains largely unstudied.

Recently, it has been reported that protein nitration levels increased in aged mice compared to young mice. Also, particularly strong nitration was found in the pulmonary vascular endothelium during systemic inflammatory response syndrome (SIRS). Age- and SIRS-dependent increased protein nitration was evident in proteins related to the actin cytoskeleton that are responsible for maintaining pulmonary vascular permeability such as transgelin-2, LASP 1, tropomyosin, myosin and profilin-I. Recognizing the nitrated proteins

indicated important modifications to the vascular endothelial cytoskeleton, which potentially participates in the barrier dysfunction, enhanced vascular permeability, and pulmonary edema (Starr et al., 2011).

It has been established that deficiency in plasma fibronectin increases lung vascular permeability (Wheatley et al., 1993); consequently, as adhesion of endothelial cell to fibronectin depends on profilin expression (Moldovan et al., 1997), lack of functional profilin may be to some extent responsible for vascular permeability as a result of inefficient barrier integrity. These data can fairly elucidate the age-associated enhancement in susceptibility to systemic inflammation, acute lung injury, and respiratory failure (Starr et al., 2011).

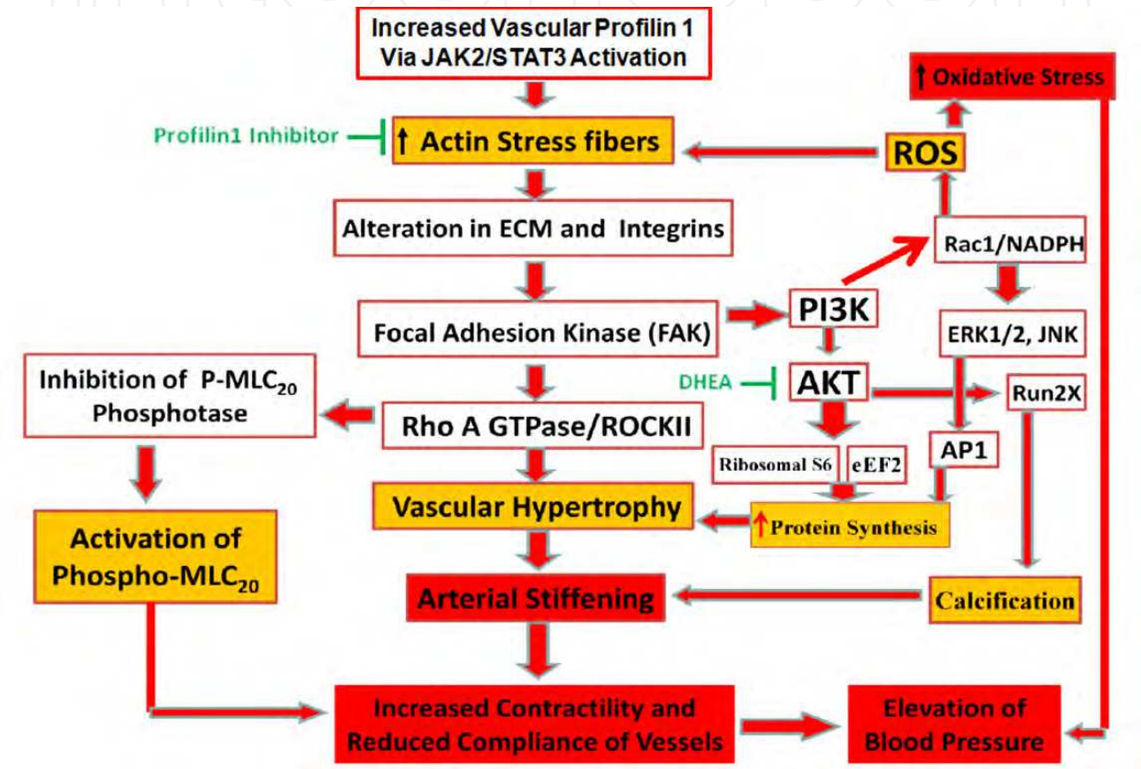


Fig. 13. JAK2/STAT3 pathway activation increases profilin-I (Romeo et al., 2008; Cheng et al., 2011) in the vessel media induced stress fiber formation and increased internal mechanical stress in the vessel walls (Moustafa-Bayoumi et al., 2007) which modulates changes in ECM and integrins (Abouelnaga et al., 2009; Hassona et al., 2010). These changes led to activation of FAK (Abouelnaga et al., 2009) that in turn activate Rho/ROCKII (Hassona et al, 2010; Moustafa-Bayoumi et al., 2007), PI3 kinase and AKT (Caglayan et al., 2010). Activation of Rac1/NADPH pathway (Abouelnaga et al., 2009) results in increased superoxide production and increases oxidative stress (Hassanain HH, unpublished) in vessel walls which could contribute to hypertension. The activation of Rho/ROCKII and AKT result in activation of MLC20 (Hassona et al., 2010), and increases in protein synthesis (Gingras et al., 1998; Kitamura et al., 1998; Ushio-Fukai et al., 1999) and calcification (Byon et al., 2008), respectively. These changes in the media of the vessel walls result in arterial stiffening and hypertension (Moustafa-Bayoumi et al., 2007). Profilin-I inhibitor can block the stress fiber formation in this pathway (Moustafa-Bayoumi et al., 2007) and dehydroepiandrosterone (DHEA) can inhibit AKT kinase pathway (Bonnet et al., 2009).

Conversely, other indirect evidence showed that profilin-I increased with age; a recent study using proteomic and genomic analyses of hippocampus from young and old rats showed a significant increase in profilin-I expression in aged rat hippocampus (Weinreb et al., 2007). Another study investigating differential protein expression profiles in chronically stimulated T cell clones found that profilin-I was widely and highly expressed in cytoplasm (Mazzatti et al., 2007). The study concluded that differential expression of profilin-I in aging may contribute directly to immunosenescence via disrupting the intracellular signaling and intercellular communication (Egerton et al., 1992; Witke et al., 1998). Consistent with these findings our preliminary data showed an increase in profilin-I expression in the aortic medial layers of older wild-type mice compared with young mice (Hassanain HH, unpublished).

Taken together, this review shed some light on the important role of profilin-I in vascular diseases. However, more studies need to be done in order to fully understand the profilin-I signaling pathway and its mechanism(s) of regulation. Figure 13 summarize some of the proposed signaling molecules involved in profilin-induced vascular complications.

4. Acknowlegements

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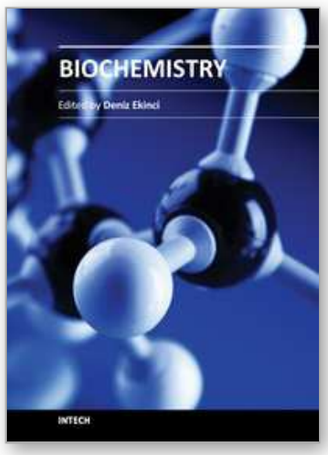

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Over the recent years, biochemistry has become responsible for explaining living processes such that many scientists in the life sciences from agronomy to medicine are engaged in biochemical research. This book contains an overview focusing on the research area of proteins, enzymes, cellular mechanisms and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in biochemistry. Particular emphasis is devoted to both theoretical and experimental aspect of modern biochemistry. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in biochemistry, molecular biology and associated areas. The book is written by international scientists with expertise in protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the book will enhance the knowledge of scientists in the complexities of some biochemical approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of biochemistry.

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