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Profilin: Multi-functional Roles of an Actin Binding Protein

The profilin (Pfn) family of proteins were originally characterized and studied as regulators of actin polymerization. First identified in 1976[1,2], these small (14-17 kDa) proteins exist as four isoforms in humans (Pfn1-4)[3]. Pfn1 is expressed in most cell types, Pfn2 is primarily localized to the brain, and Pfn3 and Pfn4 are localized to the testes[4]. Pfn expression is essential for the embryonic development of mice[5,6]. However, forty years later, the role of Pfn proteins in regulating actin polymerization, activating intracellular signal transduction pathways via binding to polyphosphoinositides[3,6], regulating microtubule end turnover[7], binding ligands via poly-L-proline domains[3,6], and potentially suppressing tumorigenicity is still being investigated[8-10]. This newsletter describes the different biological interactions that Pfns are involved in and how these interactions affect actin polymerization.

Profilins and Actin Polymerization

Cellular processes such as trafficking, motility, division, and growth require remodeling of the actin cytoskeleton. Pfns regulate actin polymerization and can both inhibit and promote actin polymerization[11]. Pfns bind to monomeric G-actin in a 1:1 ratio with a binding affinity of 0.1 µM, effectively sequestering the G-actin from incorporation into growing filaments (Fig. 1). Notably, the intracellular concentration of Pfns has been estimated to be 10-80 µM, which is not sufficient to maintain the high concentrations of G-actin found in the cell[12-14]. Pfns can also bind the barbed end of F-actin, albeit with a reduced affinity (25 µM)[15-17]. In addition, Pfn1 catalyzes the nucleotide exchange of ADP for ATP on G-actin by 1000-fold, which resupplies the pool of ATP-G-actin for barbed end growth[6,18,19]. Indeed, when the barbed ends of an actin filament are exposed, the ATP-actin:Pfn complex promotes filament elongation by loading ATP-actin onto the barbed end of the actin filament, followed by disassociation of the bound Pfn protein[6,17]. However, in the presence of end-capping proteins bound to the barbed ends of actin polymers, it has been observed in vitro that Pfn acts as an actin sequestering protein that prevents the formation of actin filaments and promotes F-actin depolymerization[11,20]. Depolymerization of F-actin was also observed in vivo upon microinjection of Pfn into either Swiss 3T3 fibroblasts or rat kidney cells[21].

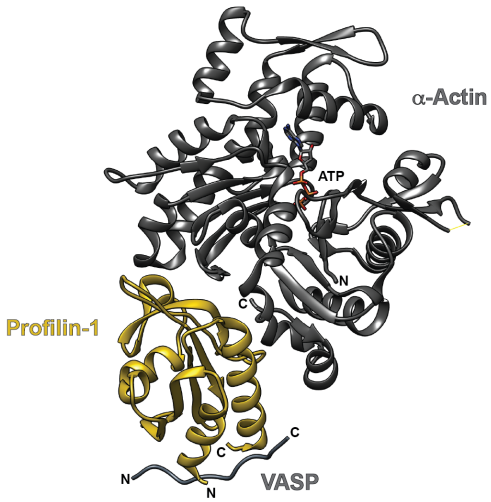

Figure 1. The crystal structure of human profilin-1 (yellow) bound on opposite sides to α-actin (grey) and the peptide from the poly-proline site of human VASP. Image was created with UCSF Chimera[30] (PDB 2PAV).

While the exact molecular mechanisms by which Pfns promote and inhibit actin polymerization are complex, Pfns appear to act as a gatekeeper in regulating the activities of the actin nucleation-promoting factors, formins and Arp2/3 complex[22]. Formins direct the formation of linear, unbranched filaments, while the Arp2/3 complex and the VCA domain of WASP stimulate the nucleation of branched actin filaments. Recent studies show that the Pfns favor formin-mediated actin polymerization and inhibit Arp2/3-mediated actin polymerization[22,23]. While the exact molecular mechanisms underlying Pfn-mediated inhibition of Arp2/3 activities are unknown, it is speculated that Pfns provide actin monomers for formin-mediated activity, while directly or indirectly competing with the VCA domain of WASP for actin monomer binding[23].

Profilins and Phosphoinositide Binding

Polyphosphoinositides modulate actin polymerization, at least partially through an interaction with Pfns. Pfns interact with both phosphatidylinositol 4,5-bisphosphate (PI(4,5) P2) and phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5) P3)[24-26]. Binding of PI(4,5)P2 to Pfn is believed to prevent Pfn from binding G-actin, thereby increasing the pool of G-actin available for Arp2/3-mediated branched filament formation.

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Alternatively, an increase in Pfn following PI(4,5)P2 hydrolysis by phosphorylated phospholipase C could decrease the G-actin pool and promote formin-mediated actin polymerization[27]. To date, phosphoinositide-mediated control of Pfn-regulated actin assembly has only been observed in vitro.

Profilins and Poly-L-Proline

Pfns also recognize poly-L-proline stretches via their N- and C-terminal helices, domains distinct from the actin binding domain (Fig. 1). The capacity of Pfns to bind poly-L-proline enables binding to over 50 different ligands in multiple organisms[6]. While several Pfn binding partners are involved in actin regulation, other newly discovered partners are involved in endocytosis, nuclear export, and Rac/Rho effector protein signaling[6]. These vastly different binding partners suggest that Pfns may be regulated or involved in other non-actin-related pathways. The physiological relevance of these interactions is not well understood and awaits elucidation.

Profilins and Cancer

Dynamic changes in the actin cytoskeleton occur during metastasis and cancer cell invasion. Several breast cancer cell lines with higher levels of expression for Pfn1 have reduced tumorigenicity, motility, and invasiveness[8,28]. Reduced binding of Pfn to actin in one breast cancer cell line exhibited reduced suppression of tumorgencity[29]. Pfn overexpression reduces the invasiveness of some breast cancer cells lines and orthotopic cancer systems[8,28]. These results suggest that the control of cytoskeleton dynamics by Pfns can play an important role in inhibiting cancer cell proliferation and migration and warrant further investigation.

Summary

Despite decades of research, the functionality and binding partners of the actin binding protein Pfn are not completely understood. Indeed, Pfns exert a complex regulation on actin, both able to inhibit and promote actin filament formation. Perhaps even more intriguing are recent reports that Pfn's functions go far beyond regulating actin cytoskeletal dynamics to include roles in controlling microtubule dynamics, binding proline domain-containing ligands, and activating a multitude of intracellular signaling pathways via binding to polyphosphoinositides. Pfns also offer potential as a drug target in anti-cancer therapies. To help researchers unravel Pfn's various functions and binding partners, Cytoskeleton, Inc. offers purified actin and actin binding proteins, including profilin, Acti-stain phalloidins, functional assay kits, and F-actin live cell imaging probes.

Actin Products

Actin Products	Amount	Cat. #
Profilin 1 (recombinant human no tag)	1 X 100 µg 1 x 500 µg 1 x 1 mg	PR02-A PR02-B PR02-XL2
Actin Protein (>95% pure): rabbit skeletal muscle	1 x 1 mg 5 x 1 mg	AKL95-B AKL95-C
Actin Protein (>99% pure): rabbit skeletal muscle	4 x 250 µg 2 x 1 mg 5 x 1 mg 10 x 1 mg 20 x1 mg	AKL99-A AKL99-B AKL99-C AKL99-D AKL99-E
Spirochrome SiR-Actin Kit	50 nmol	CY-SC001
Spirochrome SiR700-Actin Kit	35 nmol	CY-SC013
Acti-stain™ 488 Phalloidin	300 slides	PHDG1
Acti-stain™ 555 Phalloidin	300 slides	PHDH1
Phalloidin (rhodamine)	500 ul	PHDR1

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Actin Biochem Kits

Actin Biochem Kit	Reactions	Cat. #
Actin Binding Protein Spin-Down Assay Biochem Kit: rabbit skeletal muscle actin	30-100 assays	BK001
Actin Binding Protein Spin-Down Assay Biochem Kit: human platelet actin	30-100 assays	BK013
Actin Polymerization Biochem Kit (fluorescence format): rabbit skeletal muscle actin	30-100 assays	BK003
G-Actin/F-actin In Vivo Assay Biochem Kit	30-100 assays	BK037