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Views and Reviews

Structure and Function of Profilin

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INTRODUCTION

Profilin is a ubiquitous actin monomer-binding protein, found in organisms from yeast to man. Biochemical studies indicate that profilin can regulate actin polymerization and have suggested some possible mechanisms. Early studies in *Acanthamoeba*, showing that profilin could reduce the rate and extent of actin polymerization, were consistent with a model in which free actin monomer is in equilibrium with both actin-profilin complexes and actin-actin complexes (i.e., filaments). Tobacman and Korn [1982] calculated that while profilin would not bind much actin monomer under physiological conditions, it could dramatically amplify any change in monomer concentration caused by other events in the cell.

Electron microscopic studies of actin assembly in vitro revealed that profilin does more than simply sequester monomers [Pollard and Cooper, 1984]. It also affects the two ends of the actin filament differently, retarding polymerization more at the "pointed" than at the "barbed" end (these names refer to the arrowhead appearance of actin filaments decorated with myosin heads). Profilin might accomplish this by binding to the surface of the actin monomer that makes contact with the pointed end of the filament, blocking interaction at that end. The other surface of the monomer would remain free to interact with the barbed end. A change in conformation in the monomer upon binding at the barbed end could then reduce its affinity for profilin. The functional consequence would be to increase the bias in actin polymerization toward the already favored barbed end of pre-existing filaments by more strongly inhibiting pointed-end polymerization and nucleation of new filaments.

RELATION TO THE PI CYCLE

The actions of profilin proposed above could take place passively, i.e., in response to other changes in the cell that do not involve a change in the affinity or availability of profilin for binding to actin. Recent studies indicate, however, that profilin activity may be regulated. These studies have also raised the possibility that profilin functions in intracellular signalling. Polyphosphoinositides (PIP_2 and to a lesser extent PIP) can bind to profilin and dissociate the actin-profilin complex [Lassing and Lindberg, 1988a]. Goldschmidt-Clermont et al. [1990] have shown that profilin can block the cleavage of PIP_2 by phospholipase C. Thus, the PI cycle may be linked via profilin to actin polymerization. (See minireviews of Stossel [1989], Forscher [1989], and a coming review by J.A. Cooper, *Annu. Rev. Physiol.*, in preparation; also, see discussion below.)

AFFINITY

Whereas the PI cycle might affect profilin availability, a number of other factors, including the nucleotide (see below) and divalent metal bound to actin, loss of terminal amino acids of actin, and a protein stabilizing factor [see Larsson and Lindberg, 1988], may alter the affinity of profilin for actin. It is not clear whether the affinity is regulated in vivo, however.

The effect of nucleotide on affinity is intriguing [see Lal and Korn, 1985]. Actin with bound ADP has higher affinity for profilin than actin with bound ATP, and the lack of bound nucleotide in the actin-profilin complexes isolated from mammals might be responsible for the high affinity of those complexes. Profilin can affect the state of the actin-bound nucleotide, decreasing its hydrolysis and increasing its rate of exchange. This

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might represent a physiological mechanism for ordinarily keeping the affinity of profilin for actin low, and transiently increasing the affinity when there's an increase in monomer with bound ADP (during depolymerization, for instance).

IN VIVO FUNCTION

The role of profilin in regulating actin polymerization within the cell has been examined during platelet activation and chemoattractant stimulation of neutrophils. Lassing and Lindberg [1988b] have shown that blocking PIP_2 cleavage with neomycin also interferes with the increase in actin polymerization upon platelet activation, and suggest that this is because of the interaction of PIP_2 with profilin (see above). There are transient increases in actin-profilin complexes [Lind et al., 1987] and in membrane-associated profilin [Hartwig et al., 1989] when platelets are activated with thrombin, suggesting that profilin may be a mediator of the induction of actin assembly. It is tempting to try and construct a series of events from such findings; for instance, thrombin might cause a G-protein-mediated activation of phospholipase C, which could result in a transient lowering of the concentration of PIP_2 and the release of profilin from the membrane. Profilin would then be free to form a complex with actin, facilitating the breakdown of the existing actin network in preparation for the subsequent polymerization that gives rise to the platelet shape change. Then profilin could return to the membrane as PIP_2 levels are increased by subsequent steps in the PI cycle.

This scheme, however, is almost certainly inadequate to explain all of the phenomena. It looks from an examination of quantitation done by Lind et al. [1987] and Hartwig et al. [1989] (both dependent on a number of assumptions) that there isn't enough profilin to account for the unpolymerized actin in resting platelets, nor are changes in membrane-bound profilin or actin-profilin complex upon activation large enough to have much impact in the scheme proposed above (unless multiple oscillations in the PI cycle are invoked) [Berridge and Galione, 1988]. Similar conclusions are reached by Southwick and Young [1990] studying stimulated neutrophils.

Another problem is that Markey et al. [1981] obtained the opposite result, a decrease rather than a transient increase in levels of the actin-profilin complex upon platelet activation (as do Southwick and Young [1990] in neutrophils). Since considerable time elapses between cell lysis and the measurement of complexes, this assay may be particularly susceptible to in vivo artifact.

This scheme is also incomplete because it doesn't include the contribution of gelsolin. Gelsolin also interacts with PIP_2 and forms transient complexes with actin during platelet activation [Stossel, 1989]. Since there is little formation of actin-profilin complex when platelets are activated with ADP instead of thrombin, but gelsolin-actin complexes are formed in both cases [Lind et al., 1987], it may be that gelsolin is the main player. Profilin's role might then be to modulate gelsolin effects by competing with gelsolin for PIP_2 binding [Janmey and Stossel, 1989]. Alternately, profilin and gelsolin might have additive effects.

It's not even clear how important PIP_2 is as a messenger, as Bengtsson et al. [1988] found ways of independently inducing actin polymerization and breakdown of PIP_2 in neutrophils. However, additive effects in that system, as well as the finding of Hansson et al. [1988] that protein kinase C can phosphorylate profilin in PIP_2-stimulated manner, suggest that PIP_2 may play at least a modulatory role.

It's also possible that regulation in the other direction is going on; the amount of profilin bound to membranes in resting platelets is enough to bind a substantial fraction of the PIP and PIP_2. Thus it is reasonable to propose that profilin might regulate the PI cycle by interfering with PIP_2 breakdown.

PROFILIN NULL MUTANTS

The above platelet studies are a good first step in moving from in vitro studies of profilin to an investigation of its in vivo function. Genetic studies are another approach to this question. Magdolen et al. [1988] reported that the yeast profilin gene was essential. However, we found we could obtain viable yeast profilin null mutants by altering the sporulation conditions, allowing us to study a more informative null phenotype [Haarer et al., 1990]. These cells grow about a third as fast as wild-type yeast and have defects reminiscent of those seen in temperature-sensitive actin mutants [Novick and Botstein, 1985]; they are large, round, have budding defects, and show delocalized actin distribution and deposition of chitin (a normally highly localized cell-wall component). These and other aspects of the actin mutant phenotype, taken together with earlier immunofluorescence studies of wild-type yeast (in which actin localization correlated with the changing sites of cell-wall secretion during the cell cycle), led to the conclusion that actin is required for polarized secretion, e.g., during budding. Therefore, our results with profilin null mutants may reflect profilin's role in reorganizing actin during the cell cycle. We are looking for an event in yeast analogous to platelet activation so that synchronous changes in response to a specific stimulus can be compared in wild-type and mutant yeast.

SEQUENCE COMPARISONS

The sequences of *Acanthamoeba,* yeast, *Dictyostelium,* cow, human, and mouse profilin have been determined. Whereas some organisms appear to have a single profilin gene (yeast, human), others have more than one (*Dictyostelium,* M. Schleicher and A. Noegel, personal communication; *Acanthamoeba*). The *Acanthamoeba* isoforms are highly conserved. The mammalian profilins are extremely highly conserved (greater than 90% identity), and are slightly larger (~15 kD) than the yeast and *Acanthamoeba* profilins (~13 kD). N-terminal sequence is reasonably conserved among species, the central stretch is less conserved, and the C-terminal half is again conserved in the sense that yeast shows similarity to both *Acanthamoeba* and mammals. (These latter two show little similarity to each other in the C-terminal half but have similar hydropathy plots.)

In most cases it is unclear to what degree the sequence differences in these profilins will be reflected in functional differences. Profilins are grossly similar in function as they all can make one-to-one complexes with actin monomer. The *Acanthamoeba* isoforms appear to have the same affinity for actin, but a different affinity for PIP_2 [Machesky et al., 1989]. We have been able to complement the profilin null mutation in yeast by introducing an *Acanthamoeba* profilin gene on a high-copy-number plasmid, indicating functional similarity (unpublished results). In contrast to mammals, it has not been possible to isolate a high-affinity actin-profilin complex from *Acanthamoeba,* but this isn't necessarily due to differences in the primary sequence (see above).

Sequence comparisons are useful in trying to decide which amino acids may be important for the interaction of profilin with actin and PIP_2. A sequence near the C terminus (LADYLIG) that is absolutely conserved among yeast and *Acanthamoeba* (but not mammalian) profilins also shows up in other actin-binding proteins such as gelsolin [Tellam et al., 1989]. It is therefore a good candidate for an actin-binding site. Crosslinking and other data also place an actin-binding site near the C-terminus of profilin [Vandekerckhove et al., 1989]. The homology to gelsolin is interesting, as it suggests that profilin, like gelsolin, binds to the face of the actin monomer that is exposed at the barbed filament end. This fits with the explanation offered above for differential effects of profilin on the two actin filament ends.

Basic amino acids are thought to be important in an electrostatic interaction of profilin with PIP_2, since the phosphates are required for binding (i.e., profilin doesn't interact with PI). Profilin binds to mixed lipid micelles with a stoichiometry of about one profilin to five PIP_2's [Machesky et al., 1989] indicating that more than one basic amino acid may be involved. Looking for conservation in the position of positively charged amino acids may therefore be a way to find residues that are important in PIP_2 binding. One region of profilin does show conservation of positive charge; basic residues in yeast align with those at *Acanthamoeba* positions 66, 71, 75, 80, 81, and 90. (An alignment gap in this region complicates the question for mammalian profilins.) Two *Acanthamoeba* profilin isoforms are 80% similar in sequence but have an order of magnitude difference in their affinity for PIP_2 [Machesky et al., 1989]. These isoforms have differences in basic amino acids in only three positions (residues 24, 50, and 66). Taken together, these data suggest that position 66 may be one of the residues involved in PIP_2 binding. The profilin isoform with the lower affinity has a histidine instead of an arginine in that position, which would be less likely to carry a positive charge at physiological pH. A lysine is found in this position in yeast and mammalian profilins.

STRUCTURE

Magnus et al. [1988] are determining the three-dimensional structure of *Acanthamoeba* profilin, and Schutt et al. [1989] are determining the structure of a mammalian actin-profilin complex. Mammalian profilin at low resolution appears similar in shape to *Acanthamoeba* profilin. The arrangement in the actin-profilin crystal looks like an "untwisted" actin filament with profilin in the interstices, leading Schutt et al. [1989] to propose that profilin regulates filament structure or assembly in a previously unsuspected way. Since profilin does not cosediment with filaments, this structure might only represent a transient state, and might indicate that profilin does not block all of the sites of actin-actin interaction.

SUMMARY AND FUTURE DIRECTIONS

In vitro studies suggest ways that profilin may regulate actin polymerization. Future studies will expand our understanding of how this activity is involved in cell function. At present, there is evidence for a link with the PI cycle, but it is far from clear what the role of this interaction is; this question is being actively pursued. Genetic studies, using site-directed mutagenesis for instance, should provide insight into in vivo function, and may help in determining whether all of profilin's actions result from its interaction with actin, or whether it has other, independent effects (on PIP_2, for example). The three-dimensional structures now being determined will be invaluable in interpreting results of these site-directed mutagenesis studies.

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REFERENCES

Bengtsson, T., Rundquist, I., Stendahl, O., Wymann, M.P., and Andersson, T. (1988): Increased breakdown of phosphatidylinositol 4,5-bisphosphate is not an initiating factor for actin assembly in human neutrophils. J. Biol. Chem. 263:17385–17389.

Berridge, M.I., and Galione, A. (1988): Cytosolic calcium oscillators. FASEB J. 2:3074–3082.

Forscher, P. (1989): Calcium and polyphosphoinositide control of cytoskeletal dynamics. Trends Neurosci. 12:468–474.

Goldschmidt-Clermont, P.J., Machesky, L.M., Baldassare, J.J., and Pollard, T.D. (1990): The actin-binding protein profilin binds to PIP_2 and inhibits its hydrolysis by phospholipase C. Science 247:1575–1578.

Haarer, B.K., Lillie, S.H., Adams, A.E.M., Magdolen, V., Bandlow, W., and Brown, S.S. (1990): Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. J. Cell Biol. 110:105–114.

Hansson, A., Skoglund, G., Lassing, I., Lindberg, U., and Ingelman-Sundberg, M. (1988): Protein kinase C-dependent phosphorylation of profilin is specifically stimulated by phosphatidylinositol bisphosphate (PIP_2). Biochem. Biophys. Res. Commun. 150:526–531.

Hartwig, J.H., Chambers, K.A., Hopcia, K.L., and Kwiatkowski, D.J. (1989): Association of profilin with filament-free regions of human leukocyte and platelet membranes and reversible membrane binding during platelet activation. J. Cell Biol. 109: 1571–1579.

Janmey, P.A., and Stossel, T.P. (1989): Gelsolin-polyphosphoinositide interaction. J. Biol. Chem. 264:4825–4831.

Lal, A.A., and Korn, E.D. (1985): Reinvestigation of the inhibition of actin polymerization by profilin. J. Biol. Chem. 260:10132–10138.

Larsson, H., and Lindberg, U. (1988): The effect of divalent cations on the interaction between calf spleen profilin and different actins. Biochim. Biophys. Acta 953:95–105.

Lassing, I., and Lindberg, U. (1988a): Specificity of the interaction between phosphatidylinositol 4,5-bisphosphate and the profilin:actin complex. J. Cell. Biochem. 37:255–267.

Lassing, I., and Lindberg, U. (1988b): Evidence that the phosphatidylinositol cycle is linked to cell motility. Exp. Cell Res. 174: 1–15.

Lind, S.E., Janmey, P.A., Chaponnier, C., Herbert, T.-J., and Stossel, T.P. (1987): Reversible binding of actin to gelsolin and profilin in human platelet extracts. J. Cell. Biol. 105:833–842.

Machesky, L.M., Goldschmidt-Clermont, P.J., and Pollard, T.D. (1989): Heterogeneity among profilins with respect to their interactions with polyphosphoinositides. J. Cell Biol. 109: 268a.

Magdolen, V., Oechsner, U., Muller, G., and Bandlow, W. (1988): The intron-containing gene for yeast profilin (PFY) encodes a vital function. Mol. Cell. Biol. 8:5108–5115.

Magnus, K.A., Lattman, E.E., Kaiser, D.A., and Pollard, T.D. (1988): The crystal structure of *Acanthamoeba* profilin-I at 2.4 Å resolution. Biophys. J. 53:28a.

Markey, F., Persson, T., and Lindberg, U. (1981): Characterization of platelet extracts before and after stimulation with respect to the possible role of profilactin as microfilament precursor. Cell 23:145–153.

Novick, P., and Botstein, D. (1985): Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell 40:405–416.

Pollard, T.D., and Cooper, J.A. (1984): Quantitative analysis of the effect of *Acanthamoeba* profilin on actin filament nucleation and elongation. Biochemistry 23:6631–6641.

Schutt, C.E., Lindberg, U., Myslik, J., and Strauss, N. (1989): Molecular packing in profilin:actin crystals and its implications. J. Mol. Biol. 209:735–746.

Southwick, F.S., and Young, C.L. (1990): The actin released from profilin-actin complexes is insufficient to account for the increase in F-actin in chemoattractant stimulated polymorphonuclear leukocytes. J. Cell Biol. 110:1965–1973.

Stossel, T.P. (1989): From signal to pseudopod. How cells control cytoplasmic actin assembly. J. Biol. Chem. 264:18261–18264.

Tellam, R.I., Morton, D.J., and Clarke, F.M. (1989): A common theme in the amino acid sequences of actin and many actin-binding proteins? Trends Biochem. Sci. 14:130–133.

Tobacman, L.S., and Korn, E.D. (1982): The regulation of actin polymerization and the inhibition of monomeric actin ATPase activity by *Acanthamoeba* profilin. J. Biol. Chem. 257:4166–4170.

Vandekerckhove, J.S., Kaiser, D.A., and Pollard, T.D. (1989): *Acanthamoeba* actin and profilin can be cross-linked between glutamic acid 364 of actin and lysine 115 of profilin. J. Cell Biol. 109:619–626.