CellPress

# Global Resource Distribution: Allocation of Actin Building Blocks by Profilin

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http://dx.doi.org/10.1016/j.devcel.2014.12.022

**How cells regulate the distribution of a limited pool of actin between two competing structures has long been a mystery. Complementary studies from Suarez et al. (2015) and Rotty et al. (2015) now show that profilin controls the partitioning of actin monomers between competing actin networks assembled by Arp2/3 complex and formins or Ena/VASP.**

A central question in biological studies is how the sizes of different subcellular structures are controlled when they are assembled competitively from a finite pool of common building blocks (Hyman et al., 2014). For example, in eukaryotic cells, diverse architectures of filamentous actin must be assembled side by side from a limited pool of actin monomers within a shared cytoplasm (Michelot and Drubin, 2011). These actin networks include parallel arrangements of filaments such as those found in filopodia, antiparallel arrangements such as those in stress fibers and contractile rings, and branched filament arrays such as those at the leading edge and sites of endocytosis. The formation of these different networks is initiated and maintained by distinct sets of actin nucleation and elongation machineries. For instance, the Arp2/3 complex nucleates formation of branched actin filament networks, whereas formins, Ena/VASP, and other nucleators build unbranched, linear arrays. This dichotomy is on full display in budding and fission yeast, where there are only two main actin structures, patches and cables, which are assembled separately by the Arp2/3 complex and by formins, respectively. In mammalian cells, the situation is more complex, yet there is clear evidence that Arp2/3 complex and formins each play lead roles in the assembly of specific actin structures.

How yeast and mammalian cells regulate the distribution of the limited pool of actin monomers between different nucleation systems has been an open question for some time. Recent work in yeast from the Kovar lab has shown that acute pharmacological disruption of the Arp2/3 complex leads to actin cable overgrowth, and, reciprocally, pharmacological or genetic disruption of formins leads to overgrowth of patches (Burke et al., 2014). Now two new studies identify profilin as the molecular "gatekeeper" controlling this homeostatic balance between different nucleation systems and demonstrate that profilin "steers" actin monomers toward formins and Ena/VASP proteins, enabling them to compete against the more-abundant Arp2/3 complex for a limited pool of actin (Figure 1).

Profilin is an abundant and evolutionarily conserved actin monomer-binding protein found in all eukaryotic systems (Blanchoin et al., 2014). It suppresses spontaneous actin polymerization and blocks monomer addition onto pointed ends of filaments but freely allows addition to barbed ends. It has two well-characterized binding surfaces, one that interacts with actin monomers and one that interacts with specific proline-rich motifs present in proteins such as formins and Ena/VASP (Blanchoin et al., 2014). Estimated cellular concentrations suggest that the yeast Arp2/3 complex outnumbers active formins by ~15-fold (Suarez et al., 2015). Based on cell staining, it is likely that Ena/VASP proteins are also far less abundant than the Arp2/3 complex. This raises the question of how formins and Ena/VASP compete with the Arp2/3 complex to build actin structures. Given that profilin has been shown to play a critical role in vitro in enabling formins to accelerate actin filament polymerization (Kovar et al., 2006), and that it has been shown to be critical in vivo for formin-dependent actin assembly (Chang et al., 1997), the investigators in both studies asked whether profilin maintains the balance between formin- and Arp2/3 complex-assembled actin cellular structures and gives formins a fighting chance to get their share of the actin.

To put this question to the test, Rotty and colleagues (2015) raised and lowered profilin levels in both wild-type and Arp2/3 complex-deficient mammalian fibroblasts and then quantitatively assessed cell morphology and filamentous actin levels. Their analysis showed that cells genetically deficient in Arp2/3 complex had wild-type levels of total actin but lacked lamellipodia and showed defective protrusion dynamics. The Arp2/3 complex-deficient cells also exhibited an overgrowth of unbranched actin filament arrays, and this phenotype could be suppressed by further depletion of profilin. Overproduction of profilin had opposite effects, i.e., enhancing linear actin arrays in both the wild-type and Arp2/3 complex-deficient cells. In an in vitro reconstitution system, exogenous profilin inhibited actin nucleation by Arp2/3 complex and N-WASP VCA domain, demonstrating that profilin not only promotes formin- and Ena/VASP-dependent actin assembly, as was shown previously, but also inhibits Arp2/3 complex-mediated actin assembly. Interestingly, in cells lacking the Arp2/3 complex, treatment with the formin inhibitor SMIFH2 did not suppress overgrowth of the unbranched actin structures, suggesting either that profilin works through Ena/VASP or other proteins to produce these linear actin structures or that the anti-formin compound does not reach all classes of formins.

In the other study, Suarez and colleagues asked whether profilin regulates the competition between formins and the Arp2/3 complex in fission yeast. The authors used genetic approaches to

overexpress or reduce actin and profilin levels, employed the small-molecule-inhibitor CK666 to inactivate the Arp2/3 complex, and developed in vitro competition assays for actin assembly (Suarez et al., 2015). They found that the ratio of profilin to monomeric actin was critical for maintaining the normal balance between formation of actin cables and patches. Further, they reconstituted in vitro this competition between Arp2/3 complex and formins and showed that modulating the level of profilin could tune the balance between these two nucleation systems. Increasing levels of profilin shifted the distribution of actin assembly toward formins. Further, even in the absence of formins, profilin inhibited actin nucleation by the Arp2/3 complex. In addition, both Suarez et al. (2015) and Rotty et al. (2015) used mutants of profilin defective at its two binding surfaces to probe the mechanism. In both systems they found that the actin monomer-binding surface of profilin is required for its ability to suppress Arp2/3 complex-mediated actin assembly, while the polyproline-binding surface was required for enhancement of formin-mediated actin assembly.

Together, the two studies define profilin as a key factor in regulating the balance between different types of cellular actin networks produced by different nucleation systems. This in turn suggests that homeostasis in different cell types and organisms may be controlled by altering expression levels of profilin or by expressing specific isoforms of profilin that interact preferentially with specific formins and Ena/VASP proteins. In future studies, it will be important to determine mechanistically how profilin is inhibiting the Arp2/3 complex; perhaps this involves the previously described profilin-binding site on Arp2 (Mullins et al., 1998) and/or competition with specific nucleation-promoting factors (NPFs) of Arp2/3 complex. It will also be important to determine whether profilin inhibits nucleation by Arp2/3 complex using full-length NPFs, because in the present studies only small VCA fragments of N-WASP and *S. pombe* WASP/Wsp1p were used. This may be relevant because a previous study found that profilin inhibits *S. cerevisiae* Arp2/3 complex-mediated actin assembly stimulated by VCA domain but not by full-length Las17/WASP (Rodal et al., 2003). In addition, it will be important to consider how lipid inactivation of profilin might favor Arp2/3 complex-mediated actin assembly in specific locations of cells while allowing more global inhibition of Arp2/3 complex-mediated nucleation elsewhere in the cell. Finally, it will be important to quantify levels of free profilin, profilin-bound actin monomers, and free actin monomers in cells. With these numbers, a more complete understanding of the resource-distribution mechanism can be gained.

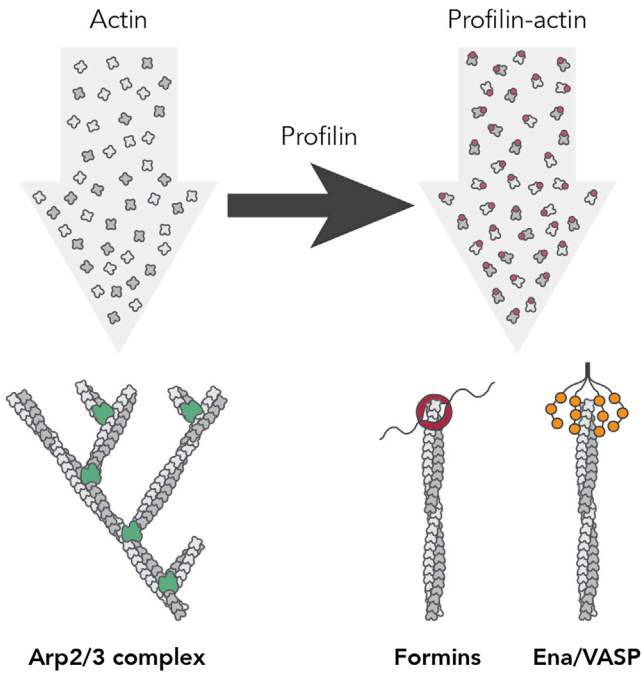


**Figure 1. Model Depicting Profilin as the Molecular Gatekeeper of Actin Building Block Distribution**

In cells, only a fraction of the actin monomer pool is bound by profilin. Profilin-actin subunits are used preferentially by formins and Ena/VASP to build unbranched (linear) actin structures, while free actin subunits are used more favorably by the Arp2/3 complex and its nucleation-promoting factors to construct branched filament networks. Thus, higher levels of profilin tip the balance toward formin- and Ena/VASP-mediated actin assembly.

## ACKNOWLEDGMENTS

This research was supported by a fellowship from the Leukemia and Lymphoma Society to J.L.H-R and a grant from the National Institutes of Health (GM083137) to B.L.G.

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