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SPOTLIGHT

Profilin affects microtubule dynamics via actin

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Profilin binds microtubules in vitro. However, a new study by Vitriol and colleagues (https://doi.org/10.1083/jcb.202309097) now suggests that effects of profilin on microtubule dynamics in cells are indirect and result from its impact on actin dynamics rather than its direct binding to microtubules.

Actin and microtubule dynamics are essential for key cellular processes such as cell migration, cell division, and transport of organelles or vesicles. Although the activities of these two distinct cytoskeletal networks are often studied one at a time, the idea that their activities are intertwined and that they influence each other's dynamics has been around for over four decades (1). Nevertheless, the exact mechanisms through which changes in the actin cytoskeleton influence the microtubule network (and vice versa) still remain incompletely understood. Precise assembly and remodeling of these cytoskeletal networks are orchestrated by a plethora of actin- or microtubule-binding proteins whose activities are tailored to specific cell types and functions. More recently, proteins that are capable of binding both microtubules and actin filaments have been identified. Examples of such proteins include profilin and formin (2, 3). Understanding how these proteins mediate actin-microtubule crosstalk is crucial for elucidating the interlinked dynamics between the two cytoskeletal systems. Addressing this longstanding question in this issue of *Journal of Cell Biology*, Cisterna et al. shed light on the molecular mechanisms underlying profilin's regulation of microtubule dynamics (4).

Initially identified as an actin monomer binding protein, our knowledge about profilin's multifaceted effects in actin dynamics has expanded considerably over the past two decades (5). Profilin facilitates actin treadmilling by binding actin monomers, promoting nucleotide exchange, and enabling their addition specifically at filament barbed ends (but not pointed ends). Moreover, it also enhances the rate of barbed-end elongation of formin-bound filaments (6) and favors the assembly of formin-mediated linear actin structures over Arp2/3-mediated, branched actin structures (7, 8). Notably, profilin can also directly interact with free filament barbed ends, promoting their depolymerization. While its role in regulating actin dynamics is well established and widely acknowledged, its impact on microtubule dynamics has been debated. In this Spotlight, we highlight the recent study from the Vitriol lab aimed at addressing this question (4).

Purified profilin binds to the sides of microtubules and enhances their growth in vitro (2). These effects are conserved for profilins from yeast, fruit fly, and mammals. However, the effects of profilin on microtubule dynamics in vivo have sparked significant controversy, with conflicting reports suggesting both enhancement and inhibition of growth (9–13). These discrepancies have led to the suggestion that profilin's impact on microtubule dynamics may be context dependent or influenced by cell type and function. Despite these conflicting findings, there is a consensus that changes in cellular profilin concentration can lead to alterations in microtubule dynamics, specifically via microtubule acetylation and stabilization. While many studies have previously examined the effects of profilin on actin or microtubule cytoskeletons individually, very few studies have explored the simultaneous effects of perturbing profilin and actin on microtubule dynamics.

The Vitriol laboratory specializes in mechanisms underlying regulation of actin dynamics in cells, with a special emphasis on the role of profilin. In their recent manuscript, they seek to bridge the gap between profilin, actin, and microtubule dynamics. Specifically, they inquire whether the previously purported effects of profilin on microtubule dynamics may have been indirectly induced by alterations in the actin cytoskeleton rather than arising from direct binding of profilin to microtubules.

In contrast to previous studies, which typically assessed profilin's effects based on end-point snapshots of microtubule and actin dynamics, Vitriol and colleagues investigated the dynamic and time-sensitive implications of prolonged changes in profilin and the filamentous actin (F-actin) network. Contrary to prior in vitro findings that suggested a direct regulatory role of profilin on microtubules, their experiments, which involved prolonged disruption of actin filament assembly and actomyosin contractility in neuronal cells, suggest that profilin's impact on microtubules might

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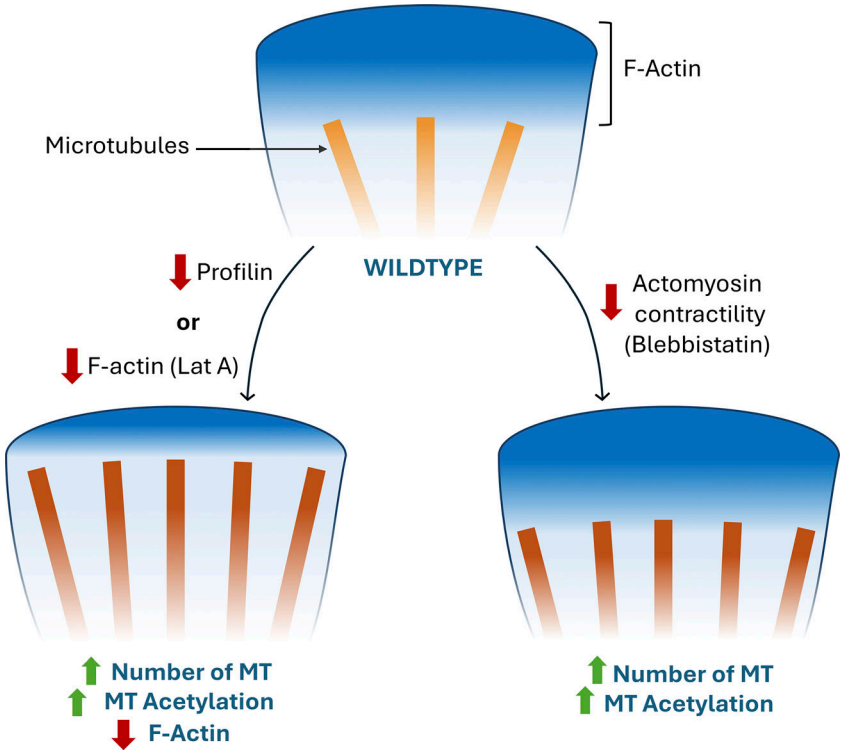

Figure 1. **Schematic representation illustrating changes in microtubule and actin networks resulting from genetic and pharmacological perturbations.** Top: Wild-type cells with normal microtubule (light orange) and actin (blue) networks. Bottom left: Knocking out profilin 1 or exposure to latrunculin A (LatA) leads to increase in both the number, acetylation (dark orange), and stabilization of microtubules, as well as a reduction in F-actin. Bottom right: Treatment with the myosin inhibitor blebbistatin leads to more microtubules and increased microtubule acetylation without affecting the total amount of filamentous actin. MT: microtubule.

largely be indirect (Fig. 1). This is likely attributable to an adaptive homeostatic response. Cisterna et al. (4) first quantified the effects of knocking out profilin 1 on both actin and microtubule networks. Surprisingly, they observed similar phenotypes upon treatment of their wild-type cells with prolonged (but not acute) treatment of Latrunculin A (LatA), which binds actin monomers and sequesters them from polymerization, but does not affect microtubule dynamics. Importantly, the authors note that a 3-h LatA treatment had no effects on microtubule assembly or organization, but a longer, overnight treatment reproduced the profilin 1 knockout phenotype. This indicates that the long-term loss of the F-actin network influences microtubule dynamics, regardless of the underlying cause, whether due to suppression of profilin 1 expression or direct disruption of actin dynamics. These observations are consistent with previous studies, which reported that in cellular regions with high actin turnover, such as axonal neurite outgrowths, profilin 1 depletion significantly reduces retrograde flow and microtubule growth speed (9). Conversely, in cellular compartments with low rates of actin dynamics/turnover, no significant differences in microtubule growth speed were observed.

To further elucidate the specific mechanism through which changes in actin dynamics influence microtubule dynamics, Cisterna et al. (4) disrupted actomyosin contraction by treating cells with the myosin II-inhibitor, blebbistatin, for 24 h. Once again, they observed similar changes in the microtubule network as seen with the profilin 1 knockout. Importantly, this treatment did not alter the total F-actin content of cells, leading them to conclude that changes in the microtubule cytoskeleton are governed by actomyosin contractility rather than due to direct changes in actin dynamics themselves. Taken together, this study establishes that the primary effects of profilin 1 depletion do not occur via profilin's direct modulation of microtubules, but rather via prolonged changes in the actin network, which in turn lead to compensatory adjustments in the microtubule cytoskeleton. Therefore, in cells, the effects of profilin on microtubules cannot be completely uncoupled from its influence on actin dynamics.

The authors further explored profilin's potential effects on a third class of cytoskeletal network, namely the intermediate filaments. They discovered that knocking out profilin led to increased expression of neurofilament heavy chain, suggesting that, akin to the microtubule cytoskeleton, the intermediate filament network might also be able to dynamically adapt to compensate for changes in the actin cytoskeleton.

The compelling findings presented in the highlighted study emphasize the pressing need for deeper exploration into the underlying molecular mechanisms and signaling pathways governing the interplay among the three cytoskeletal networks. In summary, Vitriol and colleagues conclude that changes in contractile actin networks, which can be triggered by profilin 1 depletion, LatA-mediated inhibition of actin assembly, or myosin II inhibition, can cause adaptive responses in microtubule dynamics. In the future, it will be important to further validate their proposal by employing other independent methods of altering cell mechanics, such as applying external force or modifying the activity of proteins that control actin filament length (e.g., cofilin) or filament crosslinking (e.g., alpha-actinin and fascin). Cisterna et al. (4) also provide a cautionary note, emphasizing that a regulatory protein with major effects on one of the cytoskeletal networks could indirectly impact the dynamics of other networks. They further demonstrate that experimental interpretations can vary depending on cell type (neuronal vs. non-neuronal) and subtle differences between experimental conditions. While bottom-up reconstitution studies with purified proteins provide a robust platform for investigating specific biochemical mechanisms, the discrepancies among the discussed studies highlight the potential challenges in connecting in vivo results with in vitro observations (and vice versa), especially in cases which might elicit adaptive responses in cells that are challenging to replicate in vitro.

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