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SPOTLIGHT

A step-by-step guide to fragmenting bundled actin filaments

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There has long been conflicting evidence as to how bundled actin filaments, found in cellular structures such as filopodia, are disassembled. In this issue, Chikireddy et al. (https://doi.org/10.1083/jcb.202312106) provide a detailed in vitro analysis of the steps involved in fragmentation of fascin-bundled actin filaments and propose a novel mechanism for severing two-filament bundles.

Since cellular environments constantly change, the ability to rapidly assemble and disassemble filamentous (F-actin) cytoskeletal networks is necessary for a range of behaviors including endocytosis, division, growth, and migration (1). The cell utilizes an array of biochemically distinct actin binding proteins that are differentially associated with diverse F-actin networks that dictate network architectures and dynamic properties. Understanding the molecular mechanisms that underlie the dynamics of F-actin structures is necessary for modifying cellular behavior, but disentangling these effects within the cell is difficult. As such, the use of complex in vitro reconstitution assays paired with measurements of binding, unbinding, disassembly, and network remodeling can provide important mechanistic insights.

Significant work has focused on F-actin network assembly, but equally important are the mechanisms by which networks are disassembled and/or remodeled. Turnover of actin filaments help dictate the mechanical properties of networks, network lifetimes, and the ability of the cell to rapidly adapt to its environment. ADF/cofilin is generally recognized as a major player in mediating the disassembly of F-actin networks in eukaryotic cells. Decades of work from many labs (2) has elaborated the molecular mechanisms underlying cofilin-mediated F-actin disassembly on single actin filaments (Fig. 1 A). The initial association of ADF/cofilin to an actin filament constrains the helical twist of the filament, which facilitates cooperative binding of additional cofilin that ultimately generates regions of cofilin-coated and cofilin-free filament. Differences in filament twist at boundaries of bare and ADF/cofilin-decorated segments drive fragmentation due to local stress accumulation at mechanical discontinuities (3). Because fragmentation occurs preferentially at boundaries, poorly decorated filaments sever more readily. As such, factors that affect the ability of cofilin to bind and spread along the filament can affect cofilin severing efficiency. As different actin binding proteins can influence various aspects of the binding and severing efficiency of cofilin, evaluating cofilin function in the presence of actin binding proteins is vital to understanding F-actin network disassembly in the cell.

Complex F-actin architectures perform specific cellular tasks vital to survival. For example, bundling or crosslinking of multiple actin filaments by actin binding proteins generates diverse F-actin network architectures for various functions, including filopodia, stress fibers, and the cytokinetic ring. Diverse F-actin crosslinking proteins have specific properties that affect the architecture and dynamics of different networks. These properties include the affinity of the crosslinker to filaments, the crosslinking length of the bundler, the twist the bundling factor confers upon binding, and the orientation of the filaments in the network, which can be parallel, antiparallel, or mixed polarity. Parallel F-actin bundles are a common architecture found in highly dynamic protrusive cellular structures such as filopodia (Fig. 1 B), microspikes, and microvilli. Fascin is an F-actin bundling protein found in filopodia with a small crosslinking distance that tightly bundles parallel filaments (4). Therefore, understanding the mechanisms underlying disassembly of dynamically bundled F-actin networks is critical. However, understanding how ADF/Cofilin acts on bundles has proved difficult, with some studies reporting that bundling factors synergize with cofilin to promote disassembly (5–7), potentially due to sparse decoration with cofilin or torsional strain from crosslinkers. Conversely, others have reported that bundled filaments are more resistant to disassembly by cofilin (8, 9).

In Chikireddy et al., the authors have taken a methodical, carefully controlled approach to dissecting the molecular mechanism of cofilin-mediated disassembly of bundled actin filaments (Fig. 1 C). They

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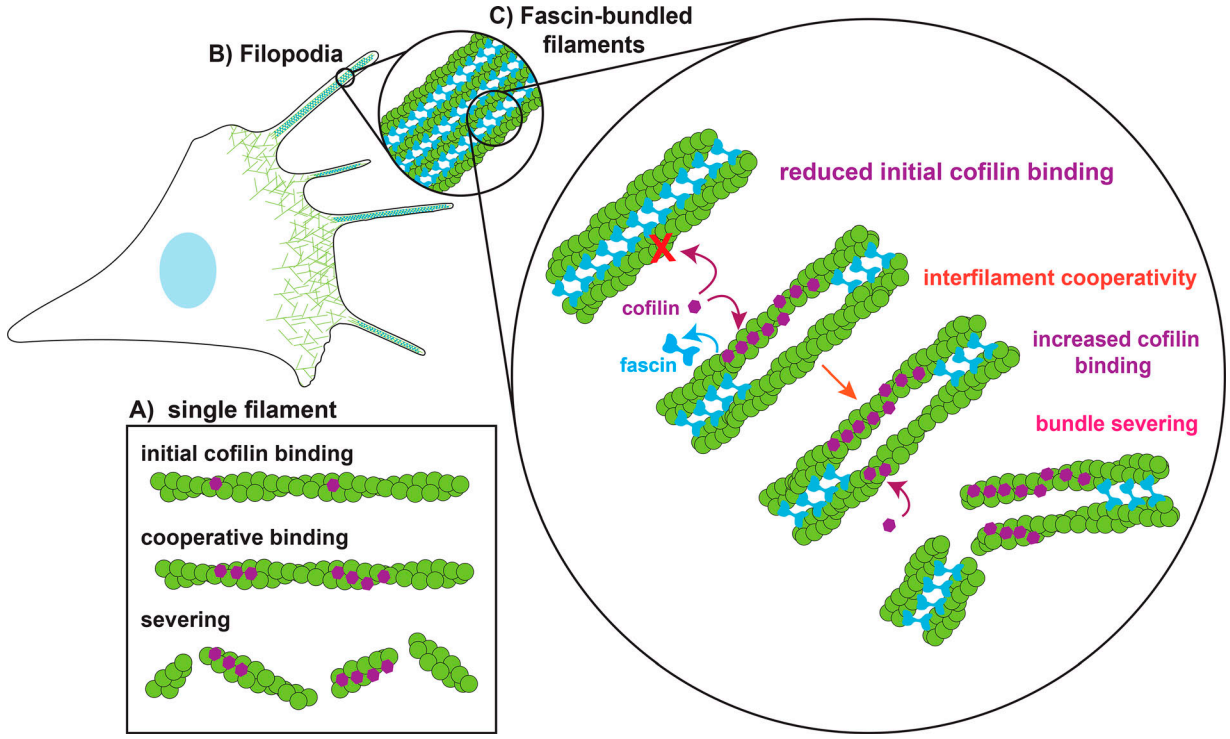

Figure 1. **Mechanisms for single filament and two-filament bundle fragmentation by cofilin. (A)** On a single actin filament (A), cofilin binds, spreads, and severs filaments. **(B and C)** In fascin-bundled actin filaments (C), as commonly found in filopodia (B), the initial binding and spreading of cofilin is inhibited. Once fascin dissociates, cofilin can bind along one filament and through "interfilament cooperativity" promotes cofilin binding to the adjacent filament at a faster rate than the initial binding event or on individual filaments. Severing of the first filament, then the second filament in the bundle results in bundle fragmentation.

found that actin filaments bundled by fascin are severed more slowly by cofilin than unbundled single filaments. Inhibition of severing correlates with bundle size, whereby larger bundles exhibit slower severing. The authors systematically evaluated the steps involved in bundle disassembly, namely: the initial binding of cofilin, the cooperative binding of cofilin clusters along a filament, and ultimately the severing of the bundle. The authors found that filaments bundled by fascin reduce the initial binding of cofilin to a filament as well as the rate of cofilin cluster growth. For a bundle to be severed, individual filaments within the bundle must be bound and severed by cofilin. However, inhibition of cofilin binding presents a conundrum. If initial cofilin binding is strongly inhibited, how does cofilin binding to adjacent filaments occur with sufficient frequency to result in bundle severing? To evaluate this, the authors characterized cofilin binding and severing of two-filament bundles. They found that while the initial binding event of cofilin on the first bundled filament is much slower than on isolated single filaments, the initiation rate of cofilin binding on the second filament in the bundle is higher than on unbundled filaments. The authors postulate that the increase in initiation rate on the second bundled filament is due to "interfilament cooperativity," where cofilin binding to one filament in a bundle confers favorable conditions for cofilin binding to the second filament in the bundle. The authors propose that the local twist conferred by cofilin binding is transmitted to the adjacent filament in the bundle, which would both relax the torsional stress induced by the cofilin cluster on the first filament and make the second filament more favorable for cofilin binding. Under these conditions, adjacent filaments containing cofilin clusters are severed, resulting in severing of the entire bundle.

The "interfilament cooperativity" model for cofilin-mediated bundle severing is compelling but raises questions. How is the change in filament twist conferred on the adjacent filament? Cryo-EM studies suggest that the effects of cofilin binding on filament twist do not extend more than one actin subunit from the cofilin boundary in the same filament (10), so it is surprising that cofilin binding could affect another filament in the bundle. Similar cryo-EM studies will likely be required to confirm the change in twist of the adjacent filament. Additionally, it is unclear how this mechanism occurs in larger bundles with more than two filaments and if "interfilament cooperativity" extends to multiple filaments in a bundle, or only in the filaments most proximal to the cofilin-bound filament. It will be interesting to evaluate how other actin filament bundling proteins, such as α-actinin, filamen, or fimbrin, modify the dynamics of filament turnover in different F-actin network architectures. More complex F-actin network architectures with mixed bundling factors, branched versus linear networks, and parallel, anti-parallel, or mixed orientation filaments will also be of interest to investigate at the level of cofilin binding, spreading and severing events.

It is important to consider the mechanisms that can be used to modify the turnover rate of individual filaments and networks in cells (2). There are many cofactors that affect the rate of cofilin binding, the time from cofilin binding to severing, as well as the rate of cofilin-mediated depolymerization. Evaluating how factors affect cofilin-mediated turnover in F-actin networks such as

bundles is an important future direction to pursue. Consistent with this, recently published work (7) evaluated the effect of Mical, a protein that oxidizes F-actin, on fascin-bundled actin filaments and found a synergy between Mical and cofilin in facilitating bundle disassembly by shortening, thinning, and severing of fascin bundles. While cofilin binding to Mical-treated fascin-bundled actin filaments was not specifically analyzed, previous work indicated an increased rate of binding of cofilin to Mical-treated actin filaments (11), and a similar mechanism may be acting here. Taking the approach used here (12) of sophisticated imaging of reconstituted systems with increasingly complex mixtures of components with careful observation of effects on binding, spreading, and severing by cofilin will provide critical insights into F-actin network disassembly mechanisms.

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