Cofilin-induced structural changes in actin filaments stay local

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Actin is a major cytoskeletal protein that plays crucial roles in a number of biological events involving force generation and shape changes. Actin monomers are polymerized into actin filaments, which serve as a core of the actin cytoskeleton together with many associated proteins. Although purified actin can be spontaneously polymerized under physiological conditions in test tubes, assembly and disassembly of actin are spatially and temporally controlled within cells. For example, concerted directional assembly of actin filaments can push membranes and organelles, whereas disassembly of actin filaments contributes to cytoskeletal remodeling and recycling of disassembled actin monomers for a new round of actin polymerization. Therefore, coordinated regulation of actin assembly and disassembly is often required to achieve normal cellular behaviors. Particularly, actin filament disassembly is a challenging task in the cytoplasm. Once actin is polymerized, slow spontaneous dissociation of actin subunits from filaments limits the rate of overall actin turnover. In addition, the cytoplasm generally contains high concentrations of actin monomers that can increase net actin assembly. One of the factors that promote actin filament disassembly is the actin depolymerizing factor (ADF)/cofilin family of proteins, which is expressed in various cell types across eukaryotes and involved in cellular processes that require dynamic rearrangement of the actin cytoskeleton, such as cell migration, cytokinesis, and morphogenesis (1, 2). ADF/cofilin (hereafter referred to as cofilin) promotes actin depolymerization and enhances actin turnover (3–5). Cofilin binds to the side of actin filaments at a 1:1 (cofilin:actin subunit) molar ratio in a cooperative manner such that clusters of cofilin-decorated regions are generated. Then, filament severing occurs frequently at or near boundaries between cofilin-decorated and bare regions on the filament (6, 7). Therefore, cofilin severs actin filaments most efficiently when cofilin binds to filaments at low densities (8). However, the mechanism of filament severing at the edges of cofilin clusters remains unclear. Cofilin-bound actin filaments are structurally different from bare actin filaments (9–11), which led to a hypothesis that structural discontinuities at the boundaries between cofilin-bound and bare regions generate mechanically fragile points (12). However, other studies demonstrate that cofilin-induced structural changes are propagated into bare regions (13, 14). To clarify this problem, a collaborative group led by De La Cruz and Sindelar (15) has recently analyzed structural variations of actin filaments with cofilin clusters using cryo-electron microscopy and demonstrated that cofilin-induced structural changes are limited within two actin subunits at the boundaries. In PNAS, Huehn et al. (16) further determine near-atomic structures of cofilin and actin at the boundaries and show that cofilin only induces...
structural changes on actin subunits locally by direct contacts without affecting neighboring actin subunits in a bare region. The high-resolution structure of actin subunits at the margins of cofilin clusters provides clues to understanding the mechanism of cofilin-induced actin filament severing.

Actin filaments are polarized two-stranded helical polymers. Polymerization of actin in a stacking manner generates two filament ends with distinct biochemical properties: pointed (or minus) and barbed (or plus) ends (18) (Fig. 1A). Cofilin contacts two longitudinally positioned actin subunits on the same protofilament and changes the twist of the filament (9). Cofilin also alters the conformation of actin subunits such that longitudinal contacts between the two actin subunits are disrupted (10, 11). Even with the cofilin-induced disturbance in actin–actin bonds, cofilin-saturated actin filaments are not readily fragmented (8) because cofilin acts as a cross-bridge that stabilizes the two longitudinal actin subunits. When only a single cofilin molecule is bound to a filament, Huehn et al. (16) find that only the upper (the pointed-end side) actin subunit adopts the cofilin-induced conformation, such that the longitudinal actin–actin bond is disrupted only between the upper cofilin-bound subunit (i in Fig. 1B) and the adjacent longitudinally positioned subunit (+2 in Fig. 1B). Thus, a single cofilin makes a fragile point on only one protofilament without affecting the opposite protofilament, which is not sufficient to cause efficient severing.

The structure of the actin filament with a single cofilin molecule suggests a mechanism of cooperativity in cofilin binding to actin filaments. Cofilin binds to actin filaments with a positive cooperativity and forms clusters on actin filaments. A single-molecule study using fluorescence microscopy showed that binding of a cofilin to an actin filament facilitates binding of a second cofilin molecule within ~24 actin subunits from the initial binding site (19). The near-atomic structure of the single-cofilin–bound actin filament by Huehn et al. (16) suggests that a second cofilin molecule actually binds right next to the first cofilin molecule to the pointed-end side on the same protofilament (Fig. 1B) because this is the only unoccupied cofilin-binding site where the actin conformation is altered by the first cofilin molecule. If such adjacent binding reactions continue toward the pointed ends just like closing a zipper, it is consistent with the atomic force microscopy observation of an asymmetric growth of cofilin clusters toward the pointed ends (13). However, the fluorescence microscopy observation demonstrated symmetric growth of cofilin clusters toward both ends (7). This discrepancy needs to be solved by further investigation. Another remaining question is how cofilin-induced structural changes are propagated into another protofilament. Within cofilin clusters, both actin protofilaments are decorated by cofilin (16). Although a single cofilin molecule does not induce a structural change on the other protofilament, a short cofilin cluster may affect laterally to facilitate cofilin binding to the other protofilament.

Huehn et al. also analyzed structures of actin filaments with cofilin clusters (more than five cofilin molecules) and successfully determined near-atomic structures of actin and cofilin at the barbed-end side of cofilin clusters. Cofilin alters conformation of all actin subunits that contact cofilin within the cluster without affecting conformation of actin subunits outside of the cluster, demonstrating a sharp transition of actin conformation at the boundaries. Although they were not able to determine near-atomic structure of the boundary at the pointed-end side, the conformational transition is also abrupt at the pointed-end side (15), suggesting that conformation of actin subunits is similar to equivalent subunits in a single-cofilin–bound filament. Importantly, at the barbed-end boundary, a lower actin subunit (~2 in Fig. 1C) also adopts a cofilin-bound conformation, whereas an equivalent subunit in a single-cofilin–bound filament remains in a conformation of a bare actin filament (Fig. 1B), suggesting that cofilin clustering induces a conformational change of the terminal actin subunit at the boundaries. Unlike at the pointed-end boundary, the sharp transition at the barbed-end boundary does not disrupt a longitudinal actin–actin bond. Nevertheless, the longitudinal actin–actin bond at the barbed-end boundary appears strained and may be less stable than the bond in a bare actin filament (Fig. 1C, gray small squares). These structures clearly indicate that cofilin-induced structural changes on actin are limited by direct contacts causing abrupt structural changes at the boundaries of bare and cofilin-decorated segments.

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The data reported by Huehn et al. (16) identify that the pointed-end side of the boundaries between bare and cofilin-decorated segments is the most fragile point in the actin filament with cofilin clusters (Fig. 1C, large arrows), suggesting a mechanism of cofilin-induced filament severing. Cofilin disrupts the longitudinal actin–actin bond but, at the same time, binds to two actin subunits to form a longitudinal cross-bridge (10, 11). When cofilin clusters on a filament, the actin subunits (+3 and +4 in Fig. 1C) at the pointed-end side of the boundary lose the longitudinal actin–actin bonds with the adjacent actin subunits (+5 and +6 in Fig. 1C) in the bare region without a cofilin cross-bridge. Cofilin clusters on both protofilaments will generate a fragile point that is likely to break apart. The longitudinal actin–actin bond of the actin subunits (~2 and ~3 in Fig. 1C) at the barbed-end side of the boundary is not completely disrupted, but this is another fragile point where severing can occur (Fig. 1C, small arrows). This model is consistent with the fluorescence microscopy observations that severing preferentially occurs at or near the pointed-end side of cofilin clusters (7, 20). However, the real-time atomic force microscopy observations demonstrated that ~80% of severing occur either within the bare or cofilin-decorated region near the boundaries (13). Therefore, additional efforts are required to determine the relationship between cofilin-induced structural changes on actin and precise location of filament severing.

Further investigation on the transient states from cofilin–actin binding to filament severing should advance our understanding on the molecular mechanism of the cofilin-dependent actin dynamics and help to resolve some discrepancies among researchers. Structural information on cofilin-bound actin filaments should also help to understand how cofilin modulates functions of auxiliary factors for actin disassembly (for example, coronin, actin-interacting protein 1, and cyclase-associated protein) and how cofilin competes with actin filament stabilizing factors such as tropomyosin.

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