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SPOTLIGHT

Cappin' or formin': Formin and capping protein competition for filament ends shapes actin networks

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How cells assemble distinct actin networks from shared cytoplasmic components remains an important unresolved question. In this issue, Wirshing et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202209105) demonstrate how capping protein and formin competition for actin filament barbed ends controls the assembly of branched and linear actin networks.

Cells assemble a variety of actin filament networks for distinct fundamental processes such as cell motility, cytokinesis, endocytosis, vesicular transport, and cell shape maintenance. Each of these actin networks is assembled from a shared pool of actin monomers and actin-binding proteins into structures with distinct geometry, dynamics, and composition. How cells are able to sort actin-binding proteins and assemble the proper amount of actin into each network for its optimal function has been an active area of research and a burning question that remains unresolved (1). In this issue, Wirshing et al. (2) use a powerful combination of in vivo and in vitro approaches to demonstrate how a competition between formins and actin capping protein (CP) for the fast-growing actin filament barbed end directs optimal Arp2/3 complex- and formin-mediated actin assembly into two distinct structures that exist right next to each other in the cytoplasm of the budding yeast cell (Fig. 1).

Work over the past 20 yr has established several mechanisms responsible for assembly of distinct actin networks with different structures and functions from a common cytoplasmic pool of components. First, network architecture is determined by the type of actin nucleation factor, where Arp2/3 complex builds branched actin networks, while formins and Ena/VASP proteins assemble linear actin arrays. Second, competition and cooperation between several key actin-binding proteins directs their sorting between actin networks, further defining each network's composition (1). Third, profilin-mediated competition for a limited supply of actin monomers controls the distribution of actin monomers between different actin networks (3–5). Fourth, competition among actin filament barbed end binding proteins, such as CP and Ena/VASP, has been shown to be important for controlling linear versus branched actin assembly (6, 7). Despite these advances, actin network sorting mechanisms remain poorly understood because of incomplete knowledge of kinetic parameters driving the competition among different network components and the challenges of resolving multiple actin networks in complex animal cells.

In the present study, Wirshing et al. (2) follow up on the recent advances in understanding the mechanisms of CP and formin competition for the actin filament barbed ends (8, 9) and investigate physiological consequences of this competition for actin assembly in a favorable model organism, budding yeast. Unlike complex animal cells, during interphase, budding yeast contain only two distinct actin networks: actin patches and actin cables. Actin patches contain branched actin networks assembled by the Arp2/3 complex at the sites of clathrin-mediated endocytosis. Actin cables are linear actin bundles assembled by two formins, Bnr1 and Bni1, for polarized vesicular transport. Significantly, formins and CP compete with each other for binding actin filament ends and in cells display mutually exclusive localization, with formins absent from the patches and CP absent from the cables. However, until the present study, the exact kinetic mechanism of this competition and its consequences for composition, organization, and function of actin patches and cables remained unclear.

While competition of formins and CP for the actin filament barbed end had been demonstrated 20 yr ago (1), two more recent studies (8, 9) elaborated on the biochemical mechanism of this competition. It turns out that vertebrate formins and CP can simultaneously bind to the filament end in the assembly termed the decision complex and stimulate each other's dissociation in a process called associative competition mechanism. In the present study, Wirshing et al. (2) set out to determine whether this mechanism applies to yeast CP and formins and whether this mechanism plays a role in the proper assembly of actin networks in live yeast cells.

Through elegant biochemical reconstitution studies with all yeast components, Wirshing et al. (2) demonstrate that indeed yeast CP protein Cap1/2 stimulates dissociation of formins from actin filament ends, presumably via associative competition

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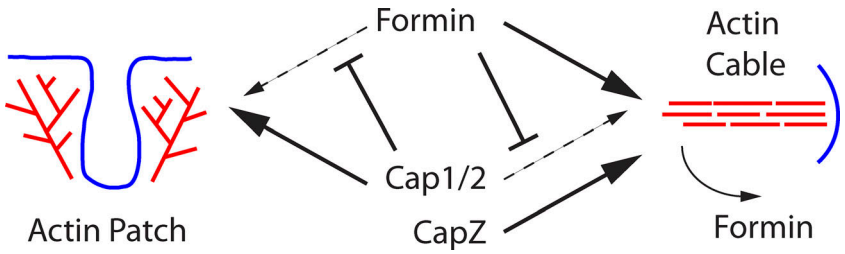

Figure 1. **Competition between formins and capping protein for actin filament barbed ends controls the assembly of branched and linear actin networks.** Competition for actin filament barbed ends prevents localization of yeast capping protein Cap1/2 to linear actin cables assembled by formins and localization of formins to branched actin networks in endocytic actin patches assembled by the Arp2/3 complex. Vertebrate capping protein CapZ displaces formins and inhibits cable assembly.

mechanism, but does so much less efficiently (almost two orders of magnitude slower) than its vertebrate counterpart CapZ. Conversely, the authors found that yeast formins are unable to displace CP from actin filament ends.

To determine the physiological consequences of this CP-formin competition, Wirshing et al. (2) examined the assembly of actin cables and patches either in cells overexpressing yeast or vertebrate CP or in yeast cells lacking CP (cap2Δ). They found that over 30-fold overexpression of yeast CP had no effect on actin cables, while similar overexpression of vertebrate CapZ inhibited cable formation. This fits nicely with the parameters measured in vitro, as formins assemble cables faster than yeast CP can displace them from barbed ends. In contrast, vertebrate CP can displace formins at rates comparable to those of cable assembly, although, for technical reasons, incorporation of CapZ into cables expected from this model could not be directly visualized.

But what happens to the assembly of actin cables and patches in the absence of CP? Wirshing et al. (2) found that in cap2Δ cells, more actin was assembled into actin patches at the expense of actin cable assembly. While increased actin patch assembly has been previously observed (1), diminished cables is the novel observation missed by earlier studies, which may potentially explain polarity defects observed in fission yeast lacking CP (10). Chemical inhibition of Arp2/3 complex restored actin cables in cells lacking CP, suggesting that diminished cables in the absence of CP are the result of depletion of cytoplasmic actin monomer pool due to over-assembly of actin patches, similar to a mechanism originally proposed by Burke et al. (3).

Remarkably, in the absence of CP, actin patches also took on cable-like characteristics, recruiting formins and tropomyosin, which are normally absent from patches, similar to the findings recently reported in fission yeast (11). Thus, CP shields patches from formins, consistent with the inability of yeast formins to displace CP from barbed ends observed in vitro. In contrast, recruitment of tropomyosin into patches without CP is surprising given a well-established role of actin-bundling protein fimbrin in excluding tropomyosin from patches (1). Wirshing et al. (2) resolved this contradiction by demonstrating that tropomyosin in cap2Δ cells is recruited to patches only after fimbrin starts leaving patches and filaments presumably grow longer. Thus, all four mechanisms controlling the assembly of distinct actin networks outlined above contribute to actin assembly defects in live yeast lacking CP.

While the findings in the current study fit nicely with the proposed competition mechanisms of actin assembly in the yeast model, the future challenge is to examine the physiological consequences of these mechanisms in more complex animal cells. Specifically, does associative competition mechanism, with faster rates of displacement of formins by vertebrate CP, play a role in shaping actin networks in animal cells, or is the activity of CP in cells sufficiently tamed by factors inhibiting CP? Resolving these questions requires supplementing classic studies on the role of CP in actin network assembly with measuring kinetic parameters in in vitro reconstitution systems as well as knowing intracellular protein concentrations.

Acknowledgments

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