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SPOTLIGHT

Capping protein regulators of actin assembly in budding yeast

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Cellular functions of actin capping protein (CP) regulators are poorly understood. Di Pietro and colleagues (https://doi.org/10.1083/jcb.202306154) shed unprecedented light on this topic using budding yeast. Two proteins with CPI (capping protein interacting) motifs recruit CP to sites of actin assembly, while a third contributes to CP turnover.

Actin assembly at the plasma membrane powers multiple cellular processes, including fluid-phase endocytosis, clathrin-mediated endocytosis (CME), virus budding, pseudopod formation, and ameboid and mesenchymal motility in many eukaryotic organisms (1). Budding yeast (*Saccharomyces cerevisiae*) has been an important model organism for understanding the molecular mechanisms involved in the multi-step process of CME, which down-regulates the numbers of membrane receptors and transporters (2). In CME and other types of actin-based motility, actin filaments polymerize at discrete locations on the plasma membrane, often nucleated by the Arp2/3 complex. Structural and regulatory components of the actin cytoskeleton play important roles in CME and other actin-based processes (2), and many of the components are conserved across eukaryotes. One of the conserved components is actin capping protein (CP), a heterodimer of subunits Cap1 (α) and Cap2 (β). CP binds to and "caps" barbed ends of actin filaments, blocking addition and loss of actin subunits at that end (3).

Regulators of CP include a diverse set of proteins with CP-interacting (CPI) motifs. These motifs bind directly to CP and modulate its binding to actin filament barbed ends. Among the first CPI motif proteins studied was the *Dictyostelium* protein CARMIL (Capping Arp2/3 Myosin Linker) (4). CPI motifs are found in several other protein families, whose amino-acid sequences are otherwise unrelated to CARMIL. CARMILs and other CPI-motif proteins are conserved across a wide range of organisms. In general, they bind directly to CP and regulate actin assembly at a cellular membrane via their interaction with CP (5).

Initial biochemical studies of CP led to speculation that CP in cells is constitutively active and moves about the cytoplasm by diffusion, binding to and capping barbed ends in a stochastic manner. However, many cells contain equimolar amounts of V-1, a small diffusible protein that binds directly to CP with high affinity and sterically blocks its ability to bind to barbed ends (6). CPI-motif proteins bind to a different surface of CP and allosterically weaken CP interactions with both V-1 and barbed ends. The inhibitory effects of CPI-motif proteins on CP are not complete; CPI–CP complexes are still able to cap barbed ends and bind V-1 under conditions that are probably in the physiological range (7). The combined actions of V-1 and CPI-motif proteins raise the possibility, proposed by Hammer and colleagues (6), that membrane-targeted CPI-motif proteins recruit CP to specific sites by binding to CP/V-1 complex and then activate CP by promoting dissociation of the V-1 inhibitor.

The Di Pietro group has led the field in elucidating the functions of CPI-motif proteins in budding yeast. This issue of *JCB* contains their latest contribution (8), which advances the field in several important ways. Budding yeast have three CPI-motif proteins—Aim21, Bsp1 (CPI motif discovered in this work), and twinfilin (Twf1). The powerful molecular and genetic tools of budding yeast allowed the Di Pietro group to design compelling experiments to study the three proteins individually and in different combinations. Importantly, specific CPI mutations in each of the three proteins lead to internalization defects of Mup1, a methionine transporter that localizes to the plasma membrane. Mup1 is a native endocytic cargo, so this observation demonstrates the physiological significance of CPI–CP interactions for CME in yeast.

The results lead to strong conclusions that answer important questions about molecular and cellular mechanisms. First, the Di Pietro group shows that the CPI-motif proteins Aim21 and Bsp1 recruit active CP to sites of actin assembly and CME at the plasma membrane; the two proteins synergize in this recruitment function. Thus, CP is not just diffusing about the cytoplasm, colliding with and capping barbed ends only in stochastic encounters. Second, the actin capping activity of CP bound to the CPI motif proteins is less than that of free CP, but this lower level of activity is still sufficient for a significant level of cellular function. Third, the group shows that Twf1, a third CPI-motif protein, functions downstream of Aim21 and Bsp1 to regulate CP

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turnover, which ultimately leads to actin filament disassembly. However, in contrast to Aim21 and Bsp1, Twf1-CPI appears not to work through allosteric inhibition of capping activity.

These results are particularly important because the set of CPI-motif proteins in other organisms contains even more members (5). CPI-motif protein families have distinct biochemical and structural properties, which are conserved through eukaryote evolution. Individual cells are likely to express two or more CPI-motif proteins. In part, their individual functions may be a property of the cellular location, which is specified by domains other than the CPI motif. An interesting open question for the future is whether and how different CPI-motif proteins at one location compete with one another for binding CP. The competition may lead to synergistic or antagonistic effects at different locations, leading to distinct effects on various biological processes, as indicated by the current study.

The role of twinfilin has been a particularly challenging question. Previous studies with animal cells led to views of twinfilin as promoting (9) or antagonizing (10) the barbed-end actin capping activity of CP. In the current study, a major conclusion is that budding yeast twinfilin Twf1 promotes turnover of CP, thus antagonizing capping of barbed ends.

In addition to recruiting CP to sites of endocytosis, the Di Pietro study also demonstrates that the Bsp1 CPI motif mediates CP localization to the budding yeast actomyosin ring at the neck between the mother and daughter cell. Thus, CP localization to specific subcellular locations via interaction with CPI motifs is an expanding theme for actin assembly and actin-based motility.

One issue of CP regulation not addressed in this study is the role of V-1, the steric inhibitor of actin capping by CP. V-1 appears not to be present in budding or fission yeast, based on genome sequence analyses and biochemical approaches. The apparent absence of V-1 raises the possibility that CPI-motif proteins evolved to be the major mechanism in yeast for modulating the actin capping activity of CP for physiological function.

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