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SPOTLIGHT

MICAL2 fine-tunes Arp2/3 for actin branching

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The ARP2/3 complex promotes branched actin networks, but the importance of specific subunit isoforms is unclear. In this issue, Galloni, Carra, et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202102043) show that MICAL2 mediates methionine oxidation of ARP3B, thus destabilizing ARP2/3 complexes and leading to disassembly of branched actin filaments.

Remodeling of branched actin networks enables cell protrusion and sensing of the environment and is essential for cell motility. Migrating cells such as fibroblasts, immune cells, and metastatic cancer cells rely on actin dynamics to generate pushing, pulling, and squeezing forces to propel themselves. Therefore, studying the processes regulating assembly and disassembly of actin filaments is key to understanding cell locomotion in health and disease. One of the most important catalyzers of actin assembly is the Arp2/3 complex, which drives lamellipodia formation and cell protrusion. Arp2/3-generated actin networks are also important for endocytic trafficking, membrane remodeling during vesicle internalization, cargo sorting, and membrane excision (1). The seven-protein ARP2/3 complex contains two unconventional actin-related proteins (ARP2 and ARP3) and five additional subunits (ARPC1–5). Mammals express two isoforms of three of the subunits (ARP3/ARP3B, ARPC1A/ARPC1B, and ARPC5/ARPC5L), resulting in functional diversity depending on the specific isoforms incorporated into the ARP2/3 complex; however, despite some intriguing roles described in muscle development (2) and platelet function (3), little is known about the biological significance of these isoforms.

The nucleation activity of ARP2/3 complex is regulated at multiple levels to ensure that new actin generation is spatially and temporally controlled. Activation is controlled by Wiskott Aldrich Syndrome Protein (WASP)–family proteins, which are themselves part of multi-protein complex machines (4). WASP-family protein complexes detect multiple inputs such as membrane phospholipids, protein–protein interactions, or post-translational modifications, and act as signaling hubs to regulate branched actin nucleation. Other proteins, such as cortactin or coronin, also modulate branch stability in an antagonistic manner (5). ARP2/3 can be post-translationally modified by phosphorylation and interaction with negative regulators, whereas actin itself is regulated by targeted oxidation of methionine residues (6). How these feedback loops that control ARP2/3 activity are coordinated with cell function is an intense area of research.

Molecule interacting with CasL (MICAL) proteins have emerged as important mediators of targeted protein oxidation (6). MICAL proteins (MICAL1–3) are flavin adenine dinucleotide–binding monooxygenases capable of oxidizing target proteins (including actin), either directly or through generation of diffusible H_2O_2, which in turn oxidizes proteins in close proximity. Actin oxidation occurs on two methionine residues (Met44 and Met47), resulting in F-actin disassembly and increased cofilin-mediated F-actin severing. Although actin is the best characterized MICAL substrate, there remains the intriguing possibility of the existence of additional targets that regulate cytoskeleton dynamics.

In this issue, Galloni, Carra, et al. evaluated the ability of ARP2/3 complexes, containing either ARP3 or the ARP3B isoform (i.e., isocomplexes), to promote actin assembly, and determined isoform-specific differences in their activity and molecular regulation (7). As a model system, the authors used HeLa cells infected with vaccinia virus to study actin branching, given that this virus induces actin tail nucleation in the host cells. They noticed that in cells lacking ARP3, the localization of GFP-ARP3 or GFP-ARP3B to actin tails was comparable, and both isoforms were similarly incorporated into ARP2/3 complexes (Fig. 1). However, the length of the actin tails in ARP3B-expressing cells was shorter than in ARP3-expressing counterparts. Given that ARP3 and ARP3B isocomplexes were equivalent in their ability to induce actin polymerization in vitro, these data pointed to a faster disassembly rate as the potential cause underlying shorter actin tails in ARP3B-expressing cells. Indeed, by tracking photoactivatable actin to study its dynamics, the researchers confirmed that the rate of filament disassembly was faster in ARP3B-expressing cells.

To identify the molecular basis for the differences between ARP3 and ARP3B, the authors tested a series of ARP3 and ARP3B chimeric proteins, which revealed the importance of ARP3B amino acids 281–418 in mediating the functional differences with ARP3. In particular, Met293 was essential for ARP3B to generate short actin tails. Given that MICAL enzymes promote actin filament disassembly through oxidation of actin Met44 and Met47, Galloni, Carra, et al.

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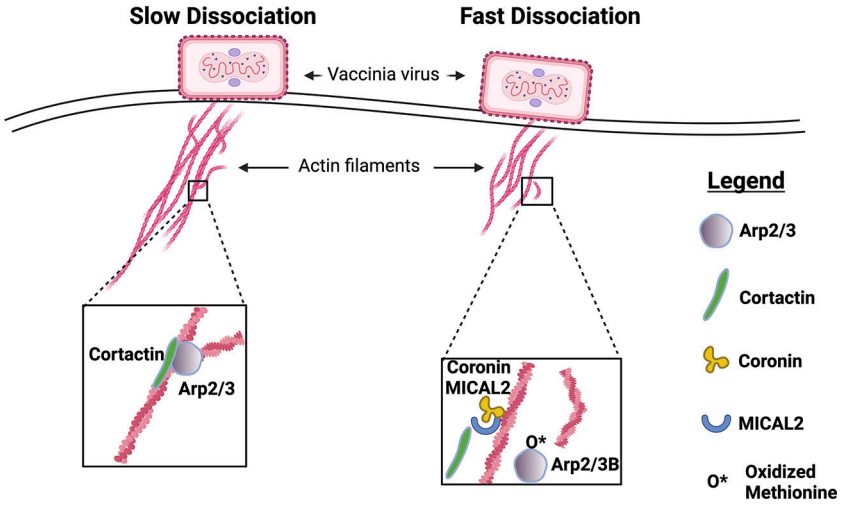

Figure 1. **Vaccinia virus surfs on the outside of the cell, forming an actin tail in the cytoplasm that aids its propulsion.** Arp2/3 complex is involved in initiating the branched actin structures and shows slow dissociation from the branches when it is stabilized by the linker protein cortactin. When an Arp2/3 complex containing the ARP3B isoform of ARP3 forms, the dissociation is enhanced, as ARP3B is subject to oxidation by MICAL2, which is recruited to branches by coronin, causing cortactin displacement and rapid branch dissociating leading to shorter actin tails.

decided to investigate the possibility that MICAL-induced oxidation of Met293 in ARP3B inhibits ARP3B activity. Fluorescently tagged MICAL2, but not MICAL1, was recruited to vaccinia-induced actin tails at a position relatively distant from the virus itself, similar to the actin-binding protein coronin (8). Down-regulation of MICAL2, but not MICAL1, increased actin tail stability and suppressed the short actin tail phenotype induced by ARP3B overexpression. Using an antibody raised against oxidized Met293, the researchers confirmed that ARP3B oxidation was reduced following MICAL2 knockdown. Recruitment of MICAL2 to actin tails was dependent on coronin 1C expression, and silencing of coronin 1C resulted in actin filament stabilization and reversal of ARP3B-induced actin tail shortening comparable to MICAL2 knockdown. Thus, coronin 1C recruitment of MICAL2 results in ARP3B oxidation on Met293, leading to dissociation of ARP2/3B isocomplexes and consequent actin networks destabilization.

Interestingly, the authors noted that the actin nucleation promoting factor cortactin, which stabilizes ARP2/3-mediated branch points along actin filaments, was required for actin tail destabilization in ARP3B overexpressing cells but was not necessary for localization of coronin 1C or MICAL2 to actin tails. One possibility is that cortactin supports local MICAL2-mediated oxidation of ARP3B at branch points to induce filament de-branching, rather than bulk actin filament depolymerization that would result from direct actin oxidation. Since MICAL proteins are directed to specific cytoskeleton locations by interacting with Myosin 5A (9) and Myosin 15 (10), the consequences of MICAL activity on actin cytoskeleton organization and function may be fine-tuned by specific MICAL subcellular localization and interacting partners.

Given that actin binds directly to the catalytic monooxygenase and calponin homology domains of MICAL proteins to increase enzyme activity and promote methionine oxidation, it is not entirely surprising that the actin-related ARP3B protein can be oxidized by MICAL2. However, the location of Met293 in ARP3B is not analogous to the Met44 or Met47 residues of actin, which raises questions regarding the mechanism of ARP3B oxidation by MICAL2. Structural modeling of the MICAL3–actin complex positions the actin loop containing Met44 and Met47 near the enzyme active site (11). ARP3B may interact with MICAL2 differently to bring Met293 close to the active site for direct oxidation, or H_2O_2 produced by MICAL2 might diffuse and oxidize highly concentrated nearby proteins. If this second possibility were true, then it is also possible that additional protein targets (e.g., coronin 1C, cortactin, additional ARP2/3 subunits) might also be oxidized on Met or Cys residues. Since the effects of MICAL1 on actin are counteracted via reduction of the oxidized Met residues by the sulfoxide reductase enzyme SelR (12), it remains to be determined if ARP3B can be similarly reactivated.

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