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Direct Redox Regulation of F-Actin Assembly and Disassembly by Mical

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Different types of cell behavior, including growth, motility, and navigation, require actin proteins to assemble into filaments. Here, we describe a biochemical process that was able to disassemble actin filaments and limit their reassembly. Actin was a specific substrate of the multidomain oxidation-reduction enzyme, Mical, a poorly understood actin disassembly factor that directly responds to Semaphorin/Plexin extracellular repulsive cues.

The actin cytoskeleton underlies a diverse array of cellular behaviors (1), but its regulatory mechanisms are incompletely understood. Recently, an actin regulator—the multidomain cytosolic protein Mical—was shown to directly bind and disassemble individual and bundled actin filaments (F-actin) (2). Although still poorly understood, Mical-mediated actin remodeling alters cell morphology and navigation in response to one of the largest families of extracellular guidance cues, the semaphorins and their plexin receptors (3–5). To determine whether F-actin is a Mical substrate, we directly modified Mical on their conserved pointed-end, which is critical for filament assembly. Mical posttranslationally oxidized the methionine 44 residue within the D-loop of actin, simultaneously severing filaments and decreasing polymerization. This mechanism underlying actin cytoskeletal collapse may have broad physiological and pathological ramifications.

In vitro actin biochemical assays, only very low, substoichiometric levels of Mical were required for F-actin disassembly (Fig. 1B), supporting the idea that a catalytic/posttranslational mechanism underlies Mical-mediated F-actin disassembly. This Mical-treated actin failed to repolymerize even after removal of Mical/NADPH (Fig. 1, C and D), indicating that Mical stably modifies actin to alter polymerization. We next wondered whether Mical, as an oxidoreductase enzyme, simply released diffusible oxidants to non-specifically alter polymerization. However, Mical does not alter polymerization of other proteins such as tubulin (2), and preventing Mical-actin interactions abolished Mical’s effects on actin (Fig. 1E and figs. S1 and S2). Indeed, unlike oxidases, which generate diffusible oxidants, most Mical-class monoxygenases/hydroxylases directly bind and are activated by their substrates (6). Likewise, Mical selectively binds F-actin (2) and increased its enzymatic activity by >100-fold in an F-actin-dependent manner (Fig. 1A) (7). In contrast to wild-type actin, which showed a 32-dalton difference after Mical treatment (Fig. 2A), M44LM47L actin was resistant to Mical modification and exhibited a whole mass similar to control actin (fig. S9). Thus, M44 and M47 are the only altered residues on Mical-treated actin and are required for Mical to posttranslationally modify actin. We thus used these purified mutant actins to determine whether modification of M44 and/or M47 induced Mical-mediated F-actin disassembly. All three mutant actins polymerized like wild-type actin and bound Mical (Fig. 3C). In contrast, F-actin generated by either the wild type or M47L depolymerized in the presence of Mical/NADPH, but filaments formed

represent the addition of two oxygens (16 daltons each) to actin. Further mass analysis revealed a substantial difference in the Mical/NADPH–treated actin peptide δFQQVMVGMGQK (figs. S3 and S4), and that actin’s methionine (M) 44 and M47 amino acid residues each had a mass increase of 16 daltons (Fig. 2B and fig. S5). Moreover, Mical selectively modified only these two methionines and not any of actin’s 14 other methionines (figs. S3, S4, and S6). Free methionine was also not a Mical substrate (Fig. 1F). M44 and M47 are poorly accessible to diffusible solvents, including oxidants, when actin is present in filaments (7–10), further indicating that these amino acid modifications are unlikely to be nonspecific. Thus, Mical selectively adds 16 daltons (the equivalent of one oxygen) to both actin M44 and M47.

The M44 and M47 residues of actin are phylogenetically conserved and invariant among cardiac, muscle, and cytoplasmic actins (Fig. 3A) and lie within the D-loop (residues 39 to 51) of the subdomain 2 portion of actin (Fig. 3B), a region that mediates actin-actin contacts and polymerization (11, 12). To confirm that M44 and M47 are the functionally relevant sites on Mical-modified actin, we mutated each site, substituting chemically related leucine for methionine residues (Fig. 3A). We then expressed and purified wild-type, M44L, M47L, and the double mutant M44LM47L actin proteins (figs. S7 and S8). In contrast to wild-type actin, which showed a 32-dalton difference after Mical treatment (fig. S9), M44LM47L actin was resistant to Mical modification and exhibited a whole mass similar to control actin (fig. S9). Thus, M44 and M47 are the only altered residues on Mical-treated actin and are required for Mical to posttranslationally modify actin. We thus used these purified mutant actins to determine whether modification of M44 and/or M47 induced Mical-mediated F-actin disassembly. All three mutant actins polymerized like wild-type actin and bound Mical (Fig. 3C). In contrast, F-actin generated by either the wild type or M47L depolymerized in the presence of Mical/NADPH, but filaments formed

References and Notes

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Fig. 1. Mical directly modifies F-actin. (A) The Mical Redox domain alone with NADPH disassembles F-actin. (B) Low amounts of Mical, in comparison with actin, disassembles F-actin. S, soluble (G-actin); P, pellet (F-actin). \( n \geq 2 \) separate experiments per condition. [Actin] = 2 \( \mu M \); [Mical] = 1 \( \mu M \) to 1 nM; [NADPH] = 400 \( \mu M \). (C and D) Mical/NADPH—treated actin (green) does not repolymerize after removal of (C) NADPH or (D) Mical and NADPH. Modified actin (D, right) migrates normally and is not degraded. (E) Compartmentalized chambers with a membrane allowing small molecules, but not Mical, access to F-actin abolished disassembly (top), as compared with chambers with a punctured membrane (bottom). (F) Mical’s enzymatic activity (as determined by conversion of NADPH to NADP\(^+\)) is substantially increased by F-actin, but not G-actin or other proteins [bovine serum albumin (BSA)], or free methionine (Met).

Fig. 2. Mical oxidizes actin M44 and M47. (A) Mical/NADPH treatment induces a 32-dalton shift in the whole mass of both \( \alpha \)-actin and \( \beta \)-actin (Actin5C). (B) Spectra comparisons of the unmodified and modified (+32) peptide (figs. S3 and S4) reveals 16-dalton increases on both M44 and M47. Individual amino acid bonds were broken from both ends of the peptide chain (\( y_{0-11}, b_{0-11} \)) and the mass determined for generated fragments. For example, comparing \( y_7 \) (green arrowhead) and \( y_7 \) (red arrowhead) fragments between and within samples, shows a +16 mass increase on the \( y_7 \) ion (M44). Comparing \( b \) ions (breaking the peptide in the opposite direction) also reveals similar M44 and M47 mass increases. This Mical-modified spectrum (asterisk) is characteristic of methionine oxidation (fig. S5). (C) Methionine and its +16 form (methionine sulfoxide).
Fig. 3. Mical disassembles F-actin by oxidizing M44. (A) M44 and M47 (residue numbers from Rabbit) are conserved from yeast to humans. Rb, Rabbit; Dm, Drosophila; Hs, human. (B) The structure of monomeric actin including M44 and M47 in the D-loop of the pointed end of the actin monomer. Protein Data Bank (PDB) ID is 2ZWH (13). (C) Co-sedimentation reveals that WT, M47L, M44L, and M44LM47L actins polymerize (dots, P fraction) and bind Mical (rectangles, P fraction). In contrast, Mical/NADPH disassembles WT and M47L but not M44L and M44LM47L actins (arrows, P fraction).

Fig. 4. Mical-mediated M44 oxidation severs F-actin and triggers remodeling. (A) Actin filament formation involves amino acids within the D-loop at the pointed end of one actin monomer, associating with residues at the barbed end of another actin monomer. The M44 residue is situated in this D-loop at a critical interface between adjacent actin monomers. Mical (d, yellow arrowheads) activation by NADPH oxidizes M44 (MetO), disrupting actin-actin interactions to cut filaments. (B) Time-lapse TIRF microscopy images reveal that individual actin filaments are cut (arrows) by Mical/NADPH but not by either Mical or NADPH only (movies S1 to S4). (C to F) GFP Actin5C M44L-marked single-cell clone ([C], arrow) mutant for actin5C. (D) to (F) Increasing Mical in single bristle cells (D) generates F-actin reorganization and branching, which ([E] to [F]) is suppressed by actin M44L expression. Mean ± SEM, n > 10 bristle cells per genotype.
Mismatch Repair, But Not Heteroduplex Rejection, Is Temporally Coupled to DNA Replication

Hans Hombauer, Anjana Srivatsan, Christopher D. Putnam, Richard D. Kolodner*

In eukaryotes, it is unknown whether mismatch repair (MMR) is temporally coupled to DNA replication and how strand-specific MMR is directed. We fused Saccharomyces cerevisiae MS6H with cyclins to restrict the availability of the Msh2-Msh6 mismatch recognition complex to either S phase or G2/M phase of the cell cycle. The Msh6-S cyclin fusion was proficient for suppressing mutations at three loci that replicate at mid-S phase, whereas the Msh6-G2/M cyclin fusion was defective. However, the Msh6-G2/M cyclin fusion was functional for MMR at a very late-replicating region of the genome. In contrast, the heteroduplex rejection function of MMR during recombination was partially functional during both S phase and G2/M phase. These results indicate a temporal coupling of MMR, but not heteroduplex rejection, to DNA replication.

References and Notes

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F-actin is thus a direct and specific substrate for Mical. This biochemical reaction alters actin at a specific amino acid residue, disrupting actin-actin associations and fragmenting filaments. Also, this posttranslationally modified actin no longer polymerizes normally, differentiating Mical’s effects from other F-actin disassembly factors such as cofilin, which physically disassembles F-actin, recycles actin monomers, and promotes actin assembly (20). Furthermore, Mical modifies the pointed end of actin proteins, and not the fast-growing, membrane-proximal barbed end (Fig. 4A), providing a logic by which actin reassembly and branching (3) follows Semaphorin/Plexin–Mical–mediated F-actin collapse (fig. S14). These results together present a specific oxidation-dependent mechanism (Fig. 4A and fig. S15) that selectively regulates actin dynamics and cellular behavior.

Supporting Online Material
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by M44L actin were resistant to Mical/NADPH and did not depolymerize (Fig. 3C). F-actin generated by M44LM47L was also resistant to Mical (Fig. 3C), revealing that Mical modifies actin M44 to induce F-actin disassembly.

Methionine (2-amino-4-thiomethylbutanoic acid) is susceptible to the addition of an oxygen group on its sulfur atom, which generates methionine sulfoxide (MetO) (Fig. 2C). Our results, including the identification of MetO containing peptide fragments (fig. S5), suggest that Mical directly converts M44 to MetO44, which disassembles F-actin and alters actin polymerization. The D-loop region containing M44 on the pointed end of one actin monomer mediates the contact with the barbed end of the adjacent actin monomer (Fig. 4A) (11, 13–16). Thus, M44 oxidation would be expected to affect the interaction between the pointed and barbed ends of individual actin subunits (12) and perhaps lead to F-actin disassembly. To examine whether such a mechanism underlies Mical’s effects, we performed additional actin biochemical assays and visualized individual actin filaments directly using both real-time total internal reflection fluorescence (TIRF) and electron microscopy (EM). Indeed, Mical cut actin filaments into multiple smaller pieces (Fig. 4B, figs. S10 and S11, and movies S1 to 4), indicating that Mical-mediated M44 oxidation disrupts the association between individual actin monomers and thereby disassembles F-actin and alters repolymerization.

MICALs control the organization of actin in neurons, muscles, and bristles in vivo and mammalian cells in vitro (fig. S12) (3). A dominant mutation in the M44 residue (M44T) of skeletal muscle fibers is associated with actin accumulation and aggregation [neuraminic myopathy (19)], and we sought to determine whether M44 was necessary for Mical-mediated F-actin remodeling in vivo. Mical-mediated F-actin alterations, including that Mical is both activated and required for Semaphorin/Plexin–induced F-actin disassembly and remodeling, have been well characterized by using model Drosophila bristle processes (2), so we generated mutant bristle cells in which we replaced wild-type actin with M44L actin (Fig. 4C and fig. S13). As in vitro (Fig. 3C), actin M44L incorporated into filaments in vivo (fig. S13). In contrast, replacing wild-type actin with actin M44L suppressed the branching and shortening of bristles characteristic of elevated Mical activity (Fig. 4, D to F) and generated Mical loss-of-function–like straight and tip-altered bristles (fig. S13) (2). Thus, Mical-mediated F-actin alterations in vivo, as in vitro, require the M44 residue of actin.

F-actin is thus a direct and specific substrate for Mical. This biochemical reaction alters actin at a specific amino acid residue, disrupting actin-actin associations and fragmenting filaments. Also, this posttranslationally modified actin no longer polymerizes normally, differentiating Mical’s effects from other F-actin disassembly factors such as cofilin, which physically disassembles F-actin, recycles actin monomers, and promotes actin assembly (20). Furthermore, Mical modifies the pointed end of actin proteins, and not the fast-growing, membrane-proximal barbed end (Fig. 4A), providing a logic by which actin reassembly and branching (3) follows Semaphorin/Plexin–Mical–mediated F-actin collapse (fig. S14). These results together present a specific oxidation-dependent mechanism (Fig. 4A and fig. S15) that selectively regulates actin dynamics and cellular behavior.