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CYTOSKELETON NEWS

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this issue

Actin Modifications and the Cytoskeleton

Actin, a highly expressed and ubiquitous cytoskeletal protein, is a major substrate for at least 17 post-translational modifications (PTMs)[1]. PTMs are highly dynamic and often reversible processes where a protein's functional properties are altered by addition of a chemical group or another protein to its amino acid residues. With roles in cell growth, motility, trafficking, and division, it is imperative to understand how actin's function is altered by PTMs. The aim of this newsletter is to summarize what is known about 3 important actin PTMs: arginylation, glutathionylation, and phosphorylation (Fig. 1).

Arginylation

Arginylation is mediated by arginyltransferase (Ate1) and involves the addition of arginine on the N-terminus of beta-actin by a peptide bond[2,3]. Arginylation can impact actin's function in several ways. For example, arginylation increases actin polymerization[3,4] and strengthens the actin filament network[3], the main structural support for maintaining dendritic spine morphology and size[5]. Blockade/loss of arginylation is associated with defects in cell migration and myofibril contraction[4,6-8] as well as collapse of leading edge lamella and reduced F-actin levels[3]. The leading edge collapse is specifically due to decreased N-terminally arginylated beta actin[4]. Besides the N-terminus, arginylation also occurs internally on the actin molecule on at least two residues[1]. Internal arginylation is predicted to affect polymerization and interactions between actin and actin binding proteins[1]. *Ex vivo* studies using fibroblasts cultured from Ate1 knockout mice also support a role for arginylation in actin function. Cells have slower rates of polymerization (faster nucleation/slower elongation), decreased F-actin staining, shorter actin filaments, and an increased number of intracellular actin aggregates[1,3] (Fig. 1). *In vivo*, Ate1 knockout mice have defects in cardiovascular development[9] and neural crest morphogenesis[7].

Glutathionylation

Glutathionylation is one of many reduction-oxidation (redox) PTMs that target two of actin's cysteine amino acid residues (Cys217, Cys374)[1]. Glutathionylation is a reversible PTM whereby glutathione is attached to an actin's cysteine residue via a disulfide bond, creating glutathione disulfide. Actin glutathionylation serves to protect actin, and thereby cells, from oxidative stress[10-12]. For example, actin glutathionylation is believed to participate in stabilization of axons and dendrites as well as neuron survival during periods of oxidative stress[11]. Furthermore, actin glutathionylation influences how cells' actin networks respond to growth factors, mediating actin polymerization and subsequent trafficking and re-arrangement of F-actin[13]. During oxidative stress, glutathionylation increases which decreases actin polymerization, resulting in reduced F-actin levels[1,13] (Fig. 1). Besides inhibiting F-actin formation, increased glutathionylation has also been linked to abnormal rearrangement of actin filaments[12,14]. Upon reversal of glutathionylation, actin polymerization increases[13].

Phosphorylation

Actin has at least 35 amino acid residues that can be modified by phosphorylation and this PTM can exert both negative and positive effects on polymerization[1]. For example, when actin's Tyr53 residue in the slime mold Dictyostelium is phosphorylated, polymerization decreases, likely through a disruption of actin subunit-subunit contact[15,16]. Conversely,

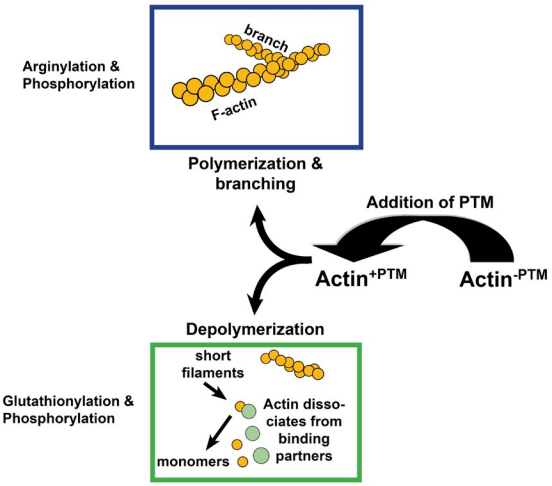

Figure 1: Post-translational modifications (PTMs) affect actin activity differentially. Arginylation promotes polymerization while glutathionylation decreases it. Phosphorylation can either increase or decrease actin polymerization, depending on the residue modified. Phosphorylation also affects actin binding.

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in the slime mold Physarum, actin fragmin kinase (AFK), a calcium-dependent enzyme, phosphorylates actin's Thr201-203 residues, leading to elongation of the actin filaments[17-20]. This elongation is believed to be a result of reduced interactions between fragmin and actin. Fragmin is related to the severing protein gelsolin and as such, controls filament length[17-20]. The effect of Thr phosphorylation is reversed by protein phosphatases PP1 and PP2A[21]. In both organisms, the changes in actin phosphorylation states are associated with cytoskeletal responses to extracellular events (e.g., locomotion, phagocytosis, signal transduction) and transition into a state of dormancy[1,15-18].

In mammals, proteomic analyses have revealed that multiple kinases phosphorylate actin and vary by cell type, disease conditions, and external stimuli. Unfortunately, many of the studies are correlational and do not report a direct relationship between a given kinase and actin phosphorylation[1]. For example, Ser and Tyr residues on actin are phosphorylated in response to insulin via unknown kinases, leading to reduced DNAse I binding[1] (Fig. 1). Likewise, activation of the p21-activated kinase PAK1 leads to actin phosphorylation which is correlated with loss of stress fibers and altered F-actin localization[22]. Similarly, Src kinase-driven phosphorylation of actin impairs actin polymerization[1,23]. Several known actin kinases are casein kinase I[1,24], cAMP-dependent protein kinase (PKA), and calcium/phosphoinositide-dependent protein kinase (PKC)[25,26]. Casein kinase I phosphorylates actin similar to AFK (targets Thr and Ser residues and is calcium-dependent). PKA and PKC act in an opposing manner with the former impairing polymerization and the latter stimulating it[27,28] (Fig. 1).

In summary, actin is a major cytoskeletal protein whose function is modulated by a variety of PTMs. Despite actin's relevance in all aspects of cell biology, our current understanding of how at least 17 different PTMs affect actin polymerization, stability, and binding is not complete. As new PTM tools are developed, we can look forward to greatly advancing our understanding of PTMs not only for actin, but for many other cytoskeletal proteins.

Actin Related Research Tools

Protein	Source	Purity	Cat. #	Amount
Actin Protein	Rabbit skeletal muscle	>99%	AKL99-A AKL99-B	4 x 250 ug 2 x 1 mg
Actin Protein	Human platelet, non-muscle	>99%	APHL99-A APHL99-B	2 x 250 ug 1 x 1 mg
Pre-formed Actin Filaments	Rabbit skeletal muscle	>99%	AKF99-A AKF99-B	1 x 1 mg 5 x 1 mg
Pyrene Actin Protein	Rabbit skeletal muscle	>99%	AP05-A AP05-B	1 x 1 mg 5 x 1 mg
Biotinylated Actin Protein	Rabbit skeletal muscle	>99%	AB07-A AB07-C	5 x 20 ug 20 x 20 ug

Kit	Cat. #	Amount
G-actin/F-actin In Vivo Biochem Kit™	BK037	30-100 assays
Actin Binding Protein Spin-Down Assay Biochem Kit™	BK013	30-100 assays
Actin Polymerization Biochem Kit™	BK003	30-100 assays

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