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Post-translational Modification and Regulation of Actin

Jonathan R. Terman[1] and **Anna Kashina**[2]

[1]Departments of Neuroscience and Pharmacology, The University of Texas Southwestern Medical Center, NA4.301/6000 Harry Hines Blvd., Dallas, TX 75390 USA

[2]Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 143 Rosenthal, 3800 Spruce Street, Philadelphia, PA 19104 USA

Abstract

Many of the best-studied actin regulatory proteins use non-covalent means to modulate the properties of actin. Yet, actin is also susceptible to covalent modifications of its amino acids. Recent work is increasingly revealing that actin processing and its covalent modifications regulate important cellular processes. In addition, numerous pathogens express enzymes that specifically use actin as a substrate to regulate their hosts cells. Actin post-translational alterations have been linked to different normal and disease processes and the effects associated with metabolic and environmental stressors. Herein, we highlight specific co- and post-translational modifications of actin and discuss the role that these modifications play in regulating actin dynamics.

Introduction

The actin cytoskeleton enables diverse cellular behaviors and so a critical goal is to understand the factors that specify its organization and dynamic properties. Small molecules such as Mg^{2+} and ATP have long been known to modulate actin and an increasing number of specific proteins are well-known for their actin regulatory abilities [1]. These effectors, along with environmental (e.g., reactive metals such as copper) and well-known biologically-produced (e.g., cytochalasins) toxins, physically associate and use non-covalent mechanisms to control actin. However, actin is also regulated through the covalent alteration of its amino acids. Although less-well appreciated, these co- and post-translational modifications (PTMs) of actin are widely employed, occur through enzymatic and non-enzymatic mechanisms, regulate the monomer-polymer equilibrium and organization of actin, and direct both physiological and pathological processes. Characterizing these modifications constitutes a rapidly expanding area within cell biology and actin studies. Below, we provide an overview of these actin PTMs and discuss recent progress on characterizing actin regulatory enzymes.

Acetylation

When actin was initially sequenced by amino acid hydrolysis, it was found to be N-terminally acetylated [2]. Subsequent studies unraveled actin's N-terminal acetylation machinery and revealed processing by specialized enzymes, including aminopeptidases that

Correspondence: Jon Terman (jonathan.terman@utsouthwestern.edu; Voice: 214-648-1464; Fax: 214-648-1801). Anna Kashina (akashina@vet.upenn.edu; Voice: 215-746-0895; Fax: 215-573-5189).

remove the N-terminal Met and on occasion the second amino acid, and acetyltransferases that sequentially modify the first, second, or third actin residue (Figures 1–3, Tables 1, S1–3). While such acetylation appears non-essential in lower eukaryotes [3], maturation and maintenance of actin's structural and functional properties in multiple species often requires N-terminal acetylation (Table S2). Acetylation also facilitates actomyosin interactions in muscles and may determine actin ubiquitylation and metabolic fate (Table S2).

Recent advances have also exposed other actin acetylation sites (Figure 3, Tables 1, S1–3) and diverse processes mediated by multiple acetyltransferases and deacetylases [4–6]. For example, the histone deacetylase HDAC6 specifically associates with actin and participates in actin rearrangements in vivo [7–9] and nuclear actin directly interacts with histone acetyltransferase during transcriptional regulation [10]. Thus, accumulating evidence suggests that acetylation may play important roles in modulating actin's role in cell movement, intracellular transport, and transcriptional regulation.

ADP-Ribosylation

Both eukaryotes and bacterial pathogens express different ADP-ribosyltransferases that transfer ADP-ribose moieties from nicotinamide adenine dinucleotide (NAD) to specific actin residues (Figure 2, Tables 1, S2–3; reviewed in [11,12]). Perhaps the best-characterized ADP-ribosyltransferase is the *Clostridium botulinum* C2 toxin which ADP-ribosylates non-muscle G-actin on Arg-177, inducing cell rounding and actin network disruption (Table S2). This ADP-ribosylation inhibits actin polymerization. Moreover, ADP-ribosylated G-actin appears to cap the barbed end of actin, thereby inhibiting polymerization of non-modified actin. ADP-ribosylation of Arg-177 also reduces actin ATP hydrolysis and the nucleation activity of the Gelsolin-actin complex (Table S2). In contrast, another bacterial ADP-ribosyltransferase *Photorhabdus luminescens* TccC3, modifes a different residue on actin, Thr-148, and increases F-actin levels (Table S2). Eukaryotic ADP-ribosyltransferases affect actin in different ways – from the effects of Transferase A, which ribosylates Arg-95 and Arg-372 and delays filament formation, to arginine-specific ADP-ribosyltransferase, which modifies Arg-206 in G-actin to alter polymerization (Table S2). Enzymes that reverse actin ADP-ribosylation (ADP-ribosylactin Hydrolases) have also been described although they remain poorly understood [13].

Arginylation

Life at actin's N-terminus has proven to be even more complex after a recent discovery that actin is arginylated (Figures 1–3, Tables 1, S2–3; [14,15]). Arginylation is an enigmatic modification mediated by arginyltransferase Ate1 that has emerged as a global biological regulator. In-depth analysis reveals that actin and actin-binding proteins constitute a large subset of intracellular arginylation targets [16] and preventing arginylation leads to cell migration and myofibril contractility defects [14,17–19]. Ate1 knockout mice die with heart, vasculature [20] and neural crest morphogenesis [18] defects, reminiscent of those resulting from cell migration abnormalities in various mouse models (reviewed in [21]). In cells, lack of arginylation collapses the leading edge lamella and reduces F-actin without significantly affecting total actin levels [22].

While decreased F-actin levels and other cellular defects may be mediated by multiple arginylation-dependent events, the leading edge collapse is due to a lack of N-terminally arginylated β-actin [14]. Re-introduction of N-terminally arginylated β-actin into Ate1 knockout cells rescues their leading edge defect unlike a non-arginylated version. Remarkably, this effect appears to be highly specific to β and not γ-actin. A specialized mechanism selectively removes arginylated γ-actin by targeting differences in actin coding sequences and translation rates [23], thus ensuring that no arginylated γ-actin is found in

vivo. The reasons for this bias relate to the mechanisms maintaining the functional distinction between different actins, and are not fully understood.

Since N-terminal arginylation neighbors a residue typically modified by acetylation (Asp-2), these two N-terminal modifications are likely to be mutually exclusive. Given acetylation's prevalence and the strict regulation of actin arginylation, a specialized mechanism (undoubtedly regulated by particular intracellular events) likely co-translationally targets actin for either acetylation or arginylation.

Actin is also modified by internal arginylation (Figure 3, Tables 1, S2), which is predicted to affect polymerization and actin-binding protein interactions. It is unclear whether this arginylation occurs within intact proteins (which is chemically possible for some residues) or following proteolysis, which may generate functional or degradatory actin fragments. Further identifying actin arginylation sites and the mechanisms and physiological consequences of these modifications constitute exciting directions for future studies.

Crosslinking

Another means that bacteria use to modify actin is through crosslinking. The diarrheal pathogen *Vibrio cholerae* induces cell rounding and F-actin loss by crosslinking actin into oligomers with the MARTX and VgrG-1 enzymes (Tables 1, S2–3; Reviewed in [12]). Other types of crosslinking proteins including transglutaminases (TGases) also affect actin, altering Gln-41 and inducing crosslinking with specific amino acid residues (Tables 1, S1–3). TGases also crosslink actin to other proteins and small molecules, making them useful tools for studying actin (Table S1). In contrast to the actin crosslinking catalyzed by MARTX and VgrG-1, TGase-mediated crosslinking often stabilizes actin, making it more resistant to depolymerization, fragmentation, and proteolytic degradation, and has been linked to insulin secretion [24], pollen tube growth [25], growth factor signaling [26,27], apoptosis [28], and neuronal and endothelial cell form and function [29,30]. For example, the pollen TGase is thought to control the transition between short/unstable and stable bundles of F-actin at the apical and base domain boundary of growing pollen tubes [25]. Specific actin regulatory proteins including myosins are also indirectly affected by TGases and exhibit decreased binding and ATPase activity towards transglutaminated actin [25].

Glucose-mediated Modifications

Actin is covalently modified by sugars. The best known of these modifications, N- and O-linked glycosylation, are mainly confined to proteins within secretory/membrane-associated pathways/organelle lumens and thus would not typically modify actin. However, rats exposed to alcohol in utero exhibit glycosylated actin [31], suggesting that under some condition such glycosylation can occur.

Actin is readily susceptible to O-GlcNAcylation (O-GlcNAc) (Figure 2, Tables 1, S2–3), which occurs within the cytoplasm and nucleus and is controlled by two conserved enzymes that add or remove *O*-GlcNAc in response to stimuli. Although poorly understood, actin O-GlcNAcylation has been implicated in modulating cardiac [32] and skeletal [33] muscle contraction, in cardioprotection [34], and in tissue changes in diabetics [35], as has another glucose-mediated actin PTM, glycation (the non-enzymatic attachment of glucose to lysine residues). Notably, purified actin is susceptible to glycation, as is actin in diabetic patients and animal models [36–39], where glucose decreases polymerization and lowers F-actin levels [39–41]. Since F-actin normally deforms cells and facilitates their passage through vessels, it is interesting in light of the effects of actin glycation that diabetic retinopathy is characterized by decreased cell deformability and retinal microcapillary blockage [39].

Methylation

Actin from many species is normally methylated on His-73 (Figure 2, Tables 1, S2–3). Recent structural analyses indicate that His-73 methylation regulates actin's interdomain flexibility and stability (Table S2). These effects are thought to be due in part to methylated His-73 slowing the inorganic phosphate (Pi) release after ATP hydrolysis, leading to F-actin stabilization. Actin methylating enzymes have been identified [42,43], although it is unclear if they methylate His-73. Methylation also occurs in some species on a specific actin lysine residue (Lys-326; Tables 1, S2–3) and is post-translationally added to arginylated actin residues (Tables 1, S2–3; [44]). Interestingly, decreased actin methylation occurs following cellular transformation with the Src oncogene [45], further indicating that understanding the mechanisms that regulate actin methylation and its effects constitute important research directions.

Oxidation, Nitrosylation, and other Redox-related Modifications

Shortly after identifying actin, Straub and his colleagues noted that the "addition of an oxidizing agent prevents that polymerization of globular actin, [and] it even destroys polymerized actin" [46]. Since that time numerous reactive oxygen, nitrogen, and lipid reagents have revealed that actin is susceptible to Redox-mediated PTMs including oxidation, nitrosylation, nitration, carbonylation, and glutathionylation (Figures 1–3, Tables 1, S1–3). Yet, this susceptibility to Redox intermediates varies considerably depending on actin's state, including its nucleotide and salt binding condition and/or whether actin is present in filamentous versus globular form (Table S1). Thus, particular conditions might make actin more susceptible to Redox regulation in vivo and may even contribute to disease processes. For instance, it has long been noted that oxidative stress affects cellular actin organization and generates Redox-related actin PTMs (Table S1). Likewise, numerous pathologies/diseases are associated with Redox-related actin PTMs (Table S1). However, similar PTMs occur under normal conditions and following activation of specific signaling pathways (Table S1). Actin may even serve to protect against oxidative stressors including those that result from normal oxidative metabolism (Table S1). Thus, Redox-mediated actin PTMs are still poorly understood and do not simply induce a common effect – and so critical goals are to characterize the sites on actin that are modified by Redox intermediates and the specific effects they elicit.

Actin has five cysteines that are highly susceptible to Redox intermediates – including reactive oxygen, nitrogen, and lipid species (Tables 1, S1–2). Cys-374 is the most susceptible, undergoing oxidation, glutathionylation, carbonylation, and nitrosylation. For instance, oxidation of Cys-374 occurs upon air-exposure, aging, and freeze-thawing of purified actin, inducing disulfide bond formation between actin monomers (Table S2). Reducing agents such as DTT are typically added to purified actin to reverse these effects. Redox modification of Cys-374 has also been linked to intramolecular disulfide bond formation, decreased polymerization rates, increased critical concentrations, filament weakening, and abnormal actin function within irreversible sickled cells (Table S2). However, Cys-374 is used to label actin (e.g., pyrene-actin) and its modification in some cases allows for relatively normal actin properties (Table S1). Thus, this Redox-modified Cys-374 needs further characterization, as do other Redox-susceptible cysteines including Cys-272 and Cys-285, whose modification has been linked to decreased polymerization and altered interactions with actin regulatory proteins (Tables 1, S2–3).

Some of the 16 methionines within actin are also susceptible to oxidation (Tables 1, S2–3) and this oxidation has long been postulated to functionally impair actin [47,48]. Indeed, the specific oxidation of the Met-44 residue of actin has recently been found to disassemble F-

actin and inhibit its polymerization [49]. Met-44 is within the D-loop of actin that is critical for actin subunit contacts. Oxidizing Met-44 introduces a negative charge into the monomer-monomer contact region and severs F-actin [49]. As with other Redox sensitive residues within actin that exhibit different Redox susceptibility depending on actin conformation and ionic conditions (as discussed above, Table S1), Met-44 is buried within F-actin and poorly accessible to diffusible oxidants [48–52]. This raises the possibility that particular in vivo conditions might make Met-44 more susceptible to Redox-regulation and it is interesting that Met-44 is oxidized in vivo in response to oxidative stress (Table S2). Specific tyrosine, histidine, and tryptophan residues of actin are also susceptible to Redox-induced modifications, although the functional significance of these modifications are unknown (Tables 1, S2–3).

While a range of non-specific chemicals and Redox intermediates are known to affect actin, it has been unclear whether Redox processes that specifically target actin also exist. For example, enzymes that generate reactive oxygen, nitrogen, and lipid species have been linked to actin modification (Table S1), but because of the non-specific nature of these enzymes that diffusibly release reactive species, actin constitutes only one of a number of proteins that are modified. Likewise, because of its abundance, actin is more likely to be non-specifically modified and may even “soak-up” reactive species and/or serve to protect the cell from Redox intermediates. Yet, some of these enzymes including nitric oxide synthase (NOS), NADPH oxidase, and 5-lipooxygeanse associate/co-localize [53–65] and have their activity regulated by actin [66–69]. Still, the specific roles of these diffusible Redox generators in regulating actin remain poorly understood.

Recent work has identified a Redox enzyme, the multi-domain flavoprotein monooxygenase Mical, that specifically and directly modifies actin [49,70]. Mical, which is a cytosolic enzyme that mediates semaphorin-dependent cellullar guidance, uses its C-terminus to associate with the semaphorin receptor plexin and its N-terminus monooxygenase (Redox) enzymatic domain to bind to F-actin [71,72]. Mical is activated by F-actin binding and specifically oxidizes Met-44 and Met-47 to disassemble F-actin and decrease polymerization [49]. Interestingly, Mical’s active site likely “fits” between monomers to access and modify these poorly-accessible residues [48–52]. Mutating Met-44 disrupts Mical-mediated actin regulation in vitro and in vivo [49] and leads to actin accumulation defects in both model organisms and human patients that mimic the effects of mutating Mical [49,72,73].

Phosphorylation

Phosphorylation is a major post-translational regulator, so it is perhaps no surprise that actin is heavily phosphorylated. Yet, despite the identification of 35 different phosphorylation sites (Figures 1–3, Tables 1, S1–3), actin phosphorylation in higher eukaryotes is still poorly understood.

Actin phosphorylation has been best characterized in Dictyostelium and in the slide mold Physarum (Table S2), which may have their own machinery for phosphorylation-dependent actin regulation. In Dictyostelium, phosphorylation occurs on Tyr-53, a conserved residue adjacent to the DNase I binding loop (D-loop) (residues 40–50) that has been implicated in actin subunit-subunit contacts (Table S2; [74]). Such phosphorylation interferes with polymerization, likely due to stabilization of actin’s D-loop by hydrogen bonding with the phosphate group. This actin phosphorylation appears to be critical for Dictyostelium to respond to specific signals and transition into a dormancy state.

In Physarum, a Ca^{2+}-dependent actin kinase (actin-fragmin kinase, AFK) modifies a cluster of actin threonine residues (Thr 201–203), facilitating filament elongation by weakening interactions with fragmin, a gelsolin-related protein that controls filament length (Table S2).

Protein phosphatases PP1 and PP2A specifically reverse this phosphorylation and its effects [75]. These threonine residues are phylogenetically conserved on the surface facing the pointed end of filaments (Figures 1, 3), suggesting that their phosphorylation may facilitate subunit addition to the pointed end. As in Dictyostelium, this phosphorylation is regulated during stimuli-induced transitions to dormancy and likely represents an adaptation mechanism to modulate sporulation and germination through rapid actin rearrangements (Table S1). An analogous form of regulation may mediate actin rearrangements in mammals through casein kinase I, which phosphorylates actin and exhibits AFK-like characteristics, including Ser/Thr specificity and Ca^{2+} dependence (Table S1).

In mammals, a vast collection of actin phosphorylation sites is emerging from proteomics of numerous cell types, disease conditions, and following various stimuli (Tables S1–2). For example, insulin stimulates actin phosphorylation on Tyr and Ser residues by unknown kinases, leading to impaired DNase I binding. Phosphorylation by PAK1, a p21-activated kinase, is linked to stress fiber dissolution and F-actin redistribution. Phosphorylation triggered by membrane-associated and Src kinases also exert negative effects on actin polymerization. Yet, few reports examine direct kinase-mediated actin phosphorylation. The most extensive work in this regard involves phosphorylation by cAMP-dependent protein kinase (PKA) and Ca^{2+}/phosphoinositide-dependent protein kinase (PKC), which produce opposing effects on actin: phosphorylation by PKA interferes with polymerization, while PKC-mediated phosphorylation induces it [76,77]. These results suggest a dynamic interplay between signaling cascades mediated by these two kinases in actin-dependent morphological changes.

Ubiquitylation and SUMOylation

Protein ubiquitylation is characterized by the covalent attachment of ubiquitin/ubiquitin-like proteins to specific target proteins and is best known for tagging proteins for degradation (polyubiquitylation). Actin, too, is ubiquitylated (Figures 1–3, Tables 1, S2–3) by specific ubiquitin ligases including MuRF-1, UbcH5, and Trim32 [78–81], which decrease actin levels [81]. Such polyubiquitin-mediated actin degradation is linked to muscle remodeling and atrophy [79–81], although myofibrillar proteins like myosin appear to protect actin from this degradation [78,80]. Ubiqiuitin-dependent actin degradation also occurs co-translationally on slowly synthesized arginylated actin, representing a means to degrade incorrectly arginylated actin [23] and perhaps other PTM actins. In contrast to polyubiquitylation, monoubiquitylation is thought to confer stability and differential subcellular localization to actin (Tables 1, S2–3; [82,83]) and occurs in many organisms including Drosophila, where muscle actin is monoubiquitylated on every seventh filament subunit, which may regulate muscle contraction [84]. Actin is also modified by the small ubiquitin-like modifier (SUMO) (Tables 1, S2–3), which retains actin in the nucleus [85–87]. Other small ubiquitin-like actin modifications including ISGylation [88] have also been identified but their effects are unknown.

Other Actin Post-translational Modifications

Actin, like other proteins, is susceptible to isomerization of aspartyl residues (isoaspartylation) and deamidation of asparaginyl residues, modifications linked to protein aging and inactivation that occur through non-enzymatic processes (Tables 1, S2–3;[89]). Actin is also a substrate for enzymes such as protein L-isoaspartyl methyltransferase (PIMT) that convert isoaspartyl residues back to aspartyl residues, and PIMT-KO mice exhibit a higher prevalence of isoapartylated actin [89,90]. Fatty acylation/alkanoylation, which includes such modifications as myristoylation and palmitoylation, also occur on actin, although it is unknown if these are enzyme-driven modifications [91,92]. Likewise, actin

undergoes non-enzymatic cysteine acylation [93] and interacts directly with membrane lipids (Table S1). Actin also undergoes proteolytic cleavage (Table S1) and in some cases proteolysis and fatty acylation may occur together. For example, actin is a caspase substrate and such digestion triggers actin N-myristoylation, mitochondrial targeting, and participation in apoptosis [94]. Sulfation of actin also occurs [95] and while it is unclear how sulfation affects actin, sulfotransferases bind and may use F-actin to mobilize and allow the sulfation of hydroxysteroids, such as cholesterol, at specific intracellular locations [96]. Recent work has also revealed that actin is susceptible to S-sulfhydration of its cysteines, a modification that enhances polymerization and results from cystathionine gamma-lyase (CSE)-mediated hydrogen sulfide (H_2S) generation [97]. Finally, numerous other covalent protein modifications [98], including those chemically-induced (e.g., succinylation; Table S1), and newly-discovered naturally occurring ones (e.g, malonylation; Tables 1, S2–3), may also play in vivo roles in regulating actin through enzymatic and non-enzymatic means.

Conclusions

As more and more studies identify new post-translational regulatory mechanisms and protein modifications, actin – one of the most essential and abundant intracellular proteins – has emerged as a major target of such PTMs. These observations further increase actin's functional and regulatory complexity, although much remains to be understood of these modifications and their physiological/pathological roles. Additional complexity may result from PTMs of the same or neighboring sites on actin. For example, modifications such as phosphorylation, nitration, and O-GlcNAcylation can alternatively affect the same residues, while others work sequentially, such as methylation of arginylated actin residues (Tables 1, S2–3; [44]). Different PTMs may also facilitate or oppose one another by regulating access to modifying enzymes and/or chemical groups. Notably, numerous neighboring residues within actin's subdomain 2 are post-translationally modified, including Met-44 (oxidation) and Tyr-53 (phosphorylation) (Tables 1, S2–3), indicating that this important region is extensively regulated, perhaps by inter-related or mutually exclusive means. Understanding the individual effects and relationships of different PTMs constitutes an exciting future direction. Likewise, developing new methods for detecting and characterizing specific PTMs will also further our understanding of the roles and hierarchical relationship between the multiple PTMs of actin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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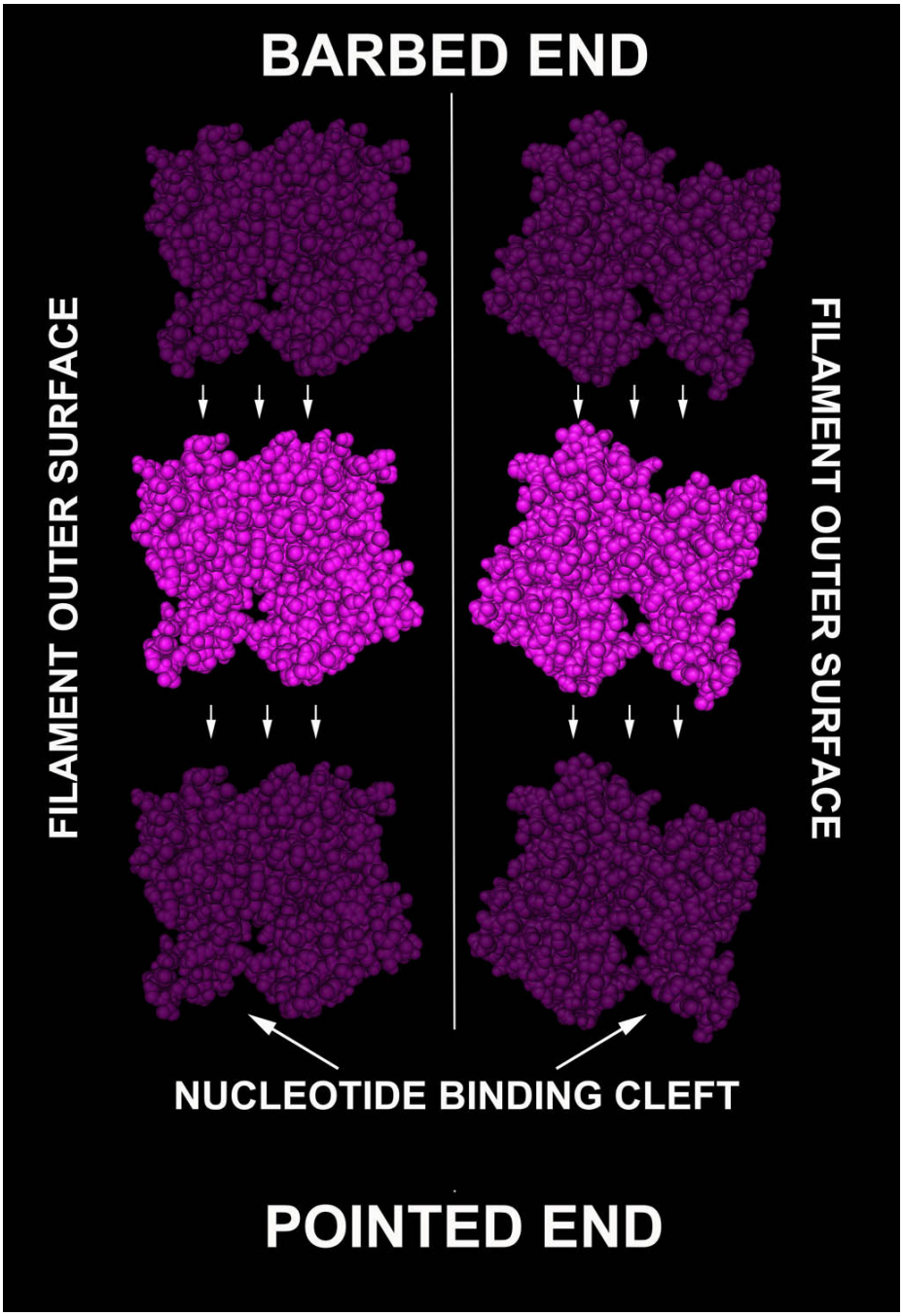

Figure 1. Structural model of the actin subunits and their fit within the filament structure and intersubunit interactions

Front and back view of the same actin subunit is shown in each left and right panel, respectively. The key functional areas are indicated. Structure shown is based on alpha cardiac actin, PDB identifier 2A42.

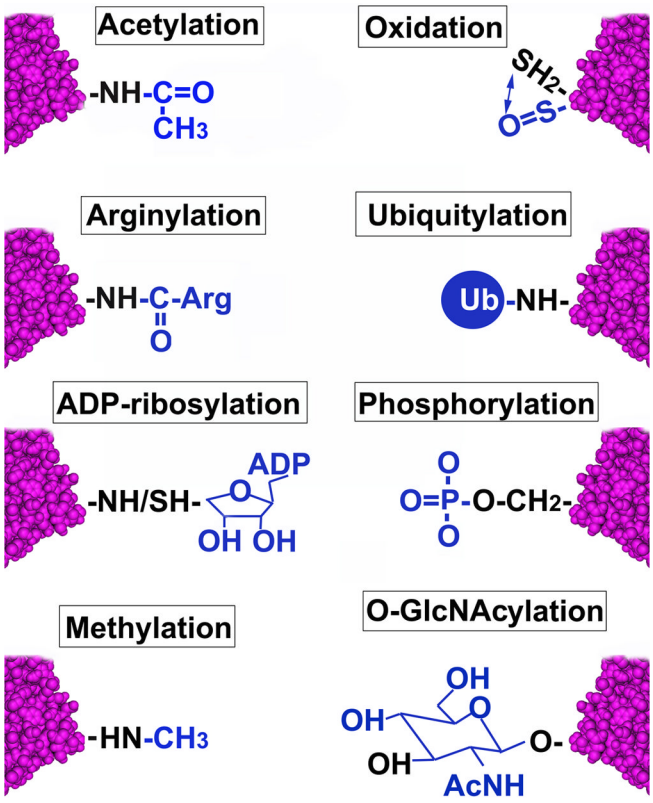

Figure 2.
Chemistries of the major actin modifications.

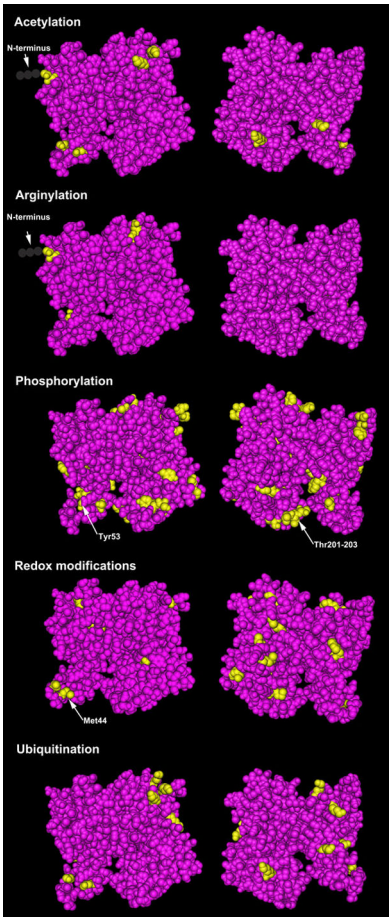

Figure 3. Structural model of the actin molecule (front and back view) with individually mapped sites for several major post-translational modifications

Residues found to undergo modifications are highlighted in yellow in each set of panels. Selected modification sites with defined effects are indicated by arrows in the corresponding panels. Structure shown is based on alpha cardiac actin, PDB identifier 2A42.

Table 1

Specific Post-translational modifications of actin

Modification	Known Sites on Actin (*=known functionally important modification)
Acetylation	Met-1*, Asp/Glu-2*, Asp-3*, Lys-50, Lys-61, Lys-68, Lys-191, Lys-326, Lys-328
ADP-Ribosylation	Arg-28, Arg-95*, Thr-148*, Arg-177*, Arg-206*, Arg-372*
Arginylation	Asp-3*, Ser-52, Ile-87, Phe-90, Gly-152, Leu-295, Asn-299
Carbonylation	His-40, His-87, His-173, Cys-374*
Crosslinking	Lys-50 (with Glu-270)*
Glutathionylation	Cys-217, Cys-374*
Isoaspartylation	Asp-25, Asp-179
Malonylation	Lys-61
Methylation	His-73*, Ile-87, Asn-299, Lys-326
Nitration	Tyr-53, Tyr-69*, Tyr-91, Tyr-198, Tyr-218, Tyr-240, Tyr-294, Tyr-362
Nitrosylation	Cys-217, Cys-257, Cys-285, Cys-374*
O-GlcNAcylation	Ser-52, Ser-155, Ser-199, Ser-232, Ser-323, Ser-368
Oxidation	Met-44*, Met-47, Trp-79, Met-82, Trp-86, Met-178, Met-190, Cys-217, Met-227, Cys-257, Met-269, Cys-272*, Cys-285*, Met-325, Trp-340, Met- 355, Trp-356, Cys-374*
Phosphorylation	Ser-14, Ser-33, Ser-52, Tyr-53*, Ser-60, Thr-66, Tyr-69, Thr-89, Tyr-91, Thr-148, Thr-160, Thr-162, Tyr-166, Tyr-169, Thr-186, Tyr-188, Tyr-198, Ser-199, Thr/Ser-201*, Thr-202*, Thr-203*, Tyr-218*, Thr-229, Ser- 233, Ser-239, Tyr-240, Thr-262, Tyr-294, Thr-297, Tyr-306, Thr-318, Ser- 323, Ser-324, Tyr-362, Ser-365
SUMOylation	Lys-68*, Lys-284*
Transglutamination	Gln-41*
Ubiquitylation	Lys-50, Lys-61, Lys-68, Lys-84, Lys-113, Lys-118, Lys-191, Lys-291, Lys- 315, Lys-326, Lys-328