NAA80 is actin’s N-terminal acetyltransferase and regulates cytoskeleton assembly and cell motility

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Actin, one of the most abundant proteins in nature, participates in countless cellular functions ranging from organelle trafficking and pathogen motility to cell migration and regulation of gene transcription. Actin’s cellular activities depend on the dynamic transition between its monomeric and filamentous forms, a process exquisitely regulated in cells by a large number of actin-binding and signaling proteins. Additionally, several posttranslational modifications control the cellular functions of actin, including most notably N-terminal (Nt)-acetylation, a prevalent modification throughout the animal kingdom. However, the biological role and mechanism of actin Nt-acetylation are poorly understood, and the identity of actin’s N-terminal acetyltransferase (NAT) has remained a mystery. Here, we reveal that NAA80, a suggested NAT enzyme whose substrate specificity had not been characterized, is Nt-acetylating actin. We further show that actin Nt-acetylation plays crucial roles in cytoskeletal assembly in vitro and in cells. The absence of Nt-acetylation leads to significant differences in the rates of actin filament depolymerization and elongation, including elongation driven by formins, whereas filament nucleation by the Arp2/3 complex is mostly unaffected. NAA80-knockout cells display severely altered cytoskeletal organization, including an increase in the ratio of filamentous to globular actin, increased filopodia and lamellipodia formation, and accelerated cell motility. Together, the results demonstrate NAA80’s role as actin’s NAT and reveal a crucial role for actin Nt-acetylation in the control of cytoskeletal structure and dynamics.

Humans express six highly conserved actin isoforms, which account for ~5% of the total protein in most cells and ~20% in muscle cells (1). Actin’s abundance is paralleled by its crucial role in countless cellular functions (2), which are regulated in cells by a large number of actin-binding proteins (ABPs) (3). An essential group of actin regulators is those that control the initiation of actin polymerization, known as “actin nucleators” (4). By overcoming the rate-limiting step in actin polymerization, i.e., nucleation, these proteins control the spatiotemporal polymerization of actin as well as the specific morphology of actin networks (2, 5). Two types of nucleators, actin-related proteins (the Arp2/3 complex) and formins, mediate most nucleation activities in cells. The Arp2/3 complex is responsible for the formation of branched actin networks (6), whereas formins mediate the assembly of unbranched filament structures such as filopodia and stress fibers (7). Other than ABPs, several forms of posttranslational modifications (PTMs) regulate actin’s function, among which N-terminal (Nt)-acetylation is the most widespread. Protein Nt-acetylation, affecting 80–90% of all proteins in humans, is emerging as a key regulator of cell function during translation. In contrast, actin, the most abundant protein in the cytoplasm of animal cells, is Nt-acetylated posttranslationally and following a unique multistep mechanism that has remained poorly characterized. Here, we describe the discovery of actin’s N-terminal acetyltransferase (NAT), NAA80. We further demonstrate that actin Nt-acetylation plays essential roles in filament assembly, cytoskeleton organization, and cell motility, resulting in a net increase in the ratio of monomeric to filamentous actin and fewer lamellipodia and filopodia. These effects converge to reduce cell hypermotility. This work establishes the role of Nt-acetylation for the most abundant cytoskeletal protein in animals and reveals a NAT acting posttranslationally and on a single dedicated substrate.

Significance

More than 80% of human proteins are N-terminal (Nt)-acetylated during translation. In contrast, actin, the most abundant protein in the cytoplasm of animal cells, is Nt-acetylated posttranslationally and following a unique multistep mechanism that has remained poorly characterized. Here, we describe the discovery of actin’s N-terminal acetyltransferase (NAT), NAA80. We further demonstrate that actin Nt-acetylation plays essential roles in filament assembly, cytoskeleton organization, and cell motility, resulting in a net increase in the ratio of monomeric to filamentous actin and fewer lamellipodia and filopodia. These effects converge to reduce cell hypermotility. This work establishes the role of Nt-acetylation for the most abundant cytoskeletal protein in animals and reveals a NAT acting posttranslationally and on a single dedicated substrate.

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Data deposition: The mass spectrometry proteomics data in Table S1 have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository (dataset ID PXD006856; user name: reviewer9589@ebi.ac.uk; password: LMHvnmII).

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Actin Nt-acetylation affects cell motility. (A) NAA80-NT was immunoprecipitated from HeLa cells and used in Nt-acetylation assays with [14C]-Ac-CoA and peptides representing a variety of N termini. (B) NAA80-eGFP expressed in HeLa and HAP1 cells shows a cytosolic distribution. (C) Phosphoimaging of NAA80 KO1 cell lysates incubated with [14C]-Ac-CoA and purified recombinant MBP-NAA80, suggesting the incorporation of [14C]-Ac at the N terminus of actin. (D) The isoform and Nt-acetylation specificity of Acβ-actin and Acγ-actin antibodies is confirmed in dot blot assays with peptides representing the unacetylated and acetylated N termini of β- and γ-actin. (E) NAA80 expression is required for actin Nt-acetylation. Immunoblot analysis of HAP1 control and NAA80 KO1 cells transfected with empty V5 plasmid, wild-type NAA80-V5, or the catalytically inactive mutant NAA80mut-V5. The samples were probed with Acβ-actin, Acγ-actin, pan-actin, V5, and GAPDH antibodies. (F) Actin acetylation is eliminated in NAA80-KO cells and restored upon reexpression of active NAA80. HAP1 control cells were transfected with an empty eGFP vector, and NAA80 KO1 cells were additionally transfected with NAA80-eGFP or NAA80mut-eGFP and stained with Acβ-actin or Acγ-actin antibodies and analyzed by IF. The general presence of F-actin in the analyzed cells was visualized by Rhodamine phalloidin staining (Bottom Row). Of note, the cells shown here are from separate samples not subjected to antibody labeling. (Scale bars, 10 μm.)

Acidic N termini (DDDI and EEEI) (Fig. 1A and Fig. S1B). This preference for the DDDI substrate peptide and EEEI as the second best was observed both in NAA80 immunoprecipitated from HeLa cells (Fig. 1A) and in recombinantly expressed and purified NAA80 (Fig. S1B). Among all the proteins expressed in animals, these N termini uniquely correspond to those of cytoplasmic β- and γ-actin, respectively, after the intermediate step of posttranslational cleavage of the N-terminal acetylmethionine (Fig. S1C) (17).

NAA80-eGFP expressed in human HAP1 cells displayed a diffuse cytosolic distribution (Fig. 1B) and, contrary to most NATs, did not associate with ribosomes (Fig. S1D) (8, 18), suggesting that it targets its substrate(s) posttranslationally. To identify NAA80-specific substrates in cells, we generated two HAP1 NAA80-KO cell lines (KO1 and KO2) using the CRISPR/Cas9 system (Fig. S24). Phosphoimaging of NAA80 KO1 cell lysates treated with purified NAA80 and isotope-labeled [14C]-acetyl CoA revealed a single band at ~43 kDa, corresponding to the expected molecular mass of actin (Fig. 1C). Actin’s identity was confirmed using N-terminal proteomics (9, 19) by quantitatively assessing the Nt-acetylation status of 402 unique N termini from control and NAA80 KO1 cell lysates. Only peptides corresponding to the fully processed N termini of β- and γ-actin showed altered Nt-acetylation levels in KO cells (Fig. S2B and Table S1). No other proteins, including Arps (ARPC2, CAZA2, TPM1, and TPM4), displayed any difference between control and KO1 cells (Table S1). This can be explained by the fact that the N termini of these proteins do not match NAA80’s substrate specificity. The lack of matching substrate sequence also applies to other Arps, including all isoforms of the Arp2/3 complex, capping protein, VASP, myosin, tropomyosin, and formin. Furthermore, both actin isoforms were 100% and 0% Nt-acetylated in control and NAA80 KO1 cells, respectively. Isoform- and Nt-acetylation–specific actin antibodies recognized an ~43-kDa band in control but not NAA80 KO1 cells (Fig. 1D and E). The Acβ-actin and Acγ-actin antibody signals reappeared in KO cell lysates transfected with NAA80 (Fig. 1E) but not in cells transfected with a catalytically inactive mutant, NAA80mut (W105F, R170Q, G173D, Y205F) (Fig. 1E and Fig. S3), confirming that catalytically active NAA80
is absolutely required for actin Nt-acetylation in cells. Of note, the Ac-β-actin is a weaker antibody in our hands than Ac-γ-actin. Both signals are almost equally strong in rescue experiments (Fig. 1 D and E), suggesting a more effective rescue for β-actin and thus confirming the substrate specificity of NAA80 we observed in our in vitro experiments (Fig. 1F and Fig. S3), showing that only control cells and NAA80-eGFP–transfected N4A480-KO cells were positive for Ac-β-actin and Ac-γ-actin antibody staining.

**Actin Nt-Acetylation Controls Cell Motility.** Next, we explored the impact of actin Nt-acetylation in cells. Since the actin cytoskeleton is a major factor in cell motility (20), we tested HAP1 control and NAA480-KO cells in several independent migration assays. We found that the lack of actin Nt-acetylation resulted in increased motility of HAP1 NAA480-KO cells. In wound-healing assays, NAA480 KO1 cells closed the gap ~12 h faster than control cells (Fig. 2 A and B), with cell-front velocity increasing from 6.4 ± 0.7 to 10 ± 0.6 μm/h (Fig. 2C). We observed similar results for both KO cell lines in an Incucyte ZOOM system for live-cell imaging, showing an increase of cell-front velocity from 7 to 9 μm/h and 11 μm/h for NAA480 KO1 and KO2 cells, respectively (Fig. S4 and Movie S1). To investigate the migratory properties of single cells, we performed a chemotaxis assay by seeding cells on a transparent porous membrane in medium containing 1% FBS. Cells were attracted to the bottom side by a medium containing 10% FBS, and the number of migrating cells was normalized to the growth rates of each cell line (Fig. S4). NAA480 KO1 cells showed an ~50% increase in chemotactic motility compared with control cells (Fig. 2D). In addition to the chemotactic assay for directed migration, we also measured random cell migration in parallel wells in which both chambers contained 10% FBS. In this assay, we also observed increased random motility of NAA480 KO1 cells (Fig. 2E), consistent with an increased capacity of these cells to generate force (31). Similar results were obtained with NAA480 KO2 cells (Fig. S4 D and E). Taken together these data demonstrate that cells deficient in actin Nt-acetylation have increased motility.

**Actin Nt-Acetylation Affects Cytoskeletal Morphology.** The actin cytoskeleton regulates not only cell motility but also cell shape and morphology (2). Thus, we compared the morphology of the actin cytoskeleton in HAP1 control and NAA480-KO cells by phalloidin staining. Based on the hypermotility phenotype of NAA480-KO cells, we were specifically interested in the formation of cell protrusions, such as filopodia and lamellipodia, that are usually linked to cell motility. As anticipated, NAA480-KO cells displayed an increase in both the number and length of filopodia-like structures (Fig. 3 A–D). These cells showed an approximately fourfold increase in the number of filopodia-like structures, defined as phalloidin-stained protrusions with a length ≥0.5 μm (Fig. 3C). The average filopodia length increased from 1 μm in control cells to 5 μm in NAA480-KO cells (Fig. 3D). Confirming the specificity of this phenotype, the reexpression of NAA80 in NAA480-KO cells reduced the number of filopodia-like structures nearly to control levels, whereas the expression of the catalytically inactive mutant NAA80mut did not (Fig. S5A). As in the other experiments described above, the NAA480 KO2 clone showed similar behavior (Fig. S5B). Consistent with the apparent elevated number of filamentous actin structures in NAA480-KO cells, we observed a significant reduction in the ratio of globular-actin to filamentous-actin (G/F) (21), from 0.73 in HAP1 control cells to 0.55 in NAA480 KO1 cells (Fig. 3E). This effect was reversed by the expression of wild-type NAA80 but not NAA80mut in both NAA480-KO cell lines (Fig. S5C), demonstrating that NAA80-dependent actin Nt-acetylation directly impacts the transition between G- and F-actin.

Since we observed a hypermotility phenotype for N4A480-KO cells (Fig. 3), we quantified the number of cells showing at least one distinguishable lamellipodium supported by microspikes or filopodia-like structures. To efficiently study these cell protrusions in HAP1 cells, which typically grow in clusters, we included only cells at the borders of the clusters in quantifications. These were scored as either positive or negative for the presence of lamellipodia. In agreement with the increased cell-front velocity in the motility assays, we observed that twice as many NAA480-KO cells as control cells formed lamellipodia (32 vs. 16%) (Fig. 3 F–H). These results further emphasize the importance of actin Nt-acetylation in the control of cytoskeletal dynamics.

**Nt-Acetylation–Dependent Actin Polymerization and Stability.** To address the mechanism behind the altered cytoskeleton organization phenotypes of N4A480-KO cells, we analyzed the recovery rates of cytoskeletal structures in cells treated with the actin-depolymerizing drug latrunculin A (LatA). Within 60 min of LatA treatment the actin appeared to be fully depolymerized in control and NAA480-KO cells, and washout of the drug resulted in the recovery of actin filament structures, but the time of recovery was significantly delayed for NAA480-KO cells compared with control cells (Fig. S6), consistent with a direct role of actin Nt-acetylation in actin polymerization.

We next explored the in vitro effect of actin Nt-acetylation on the polymerization/depolymerization properties of actin alone or...
in the presence of some of the most common actin-assembly factors in cells. Cytoplasmic actin (a mixture of β and γ isoforms) was purified from control and NAA80-KO cells, and the presence or absence of Nt-acetylation was verified by Western blotting (Fig. 4A) (22). The time course of actin polymerization measured using the pyrene-actin polymerization assay revealed no significant
difference in the polymerization rate of acetylated β-γ-actin (Ac-actin) vs. nonacetylated β-γ-actin (non-Ac-actin) (Fig. 4B). However, the elongation of 1.5-μM β-γ-actin filament seeds (±Nt-acetylation) in the presence of 0.5-μM β-γ-actin monomers (i.e., above the critical concentration for monomer addition at the barbed end but below that of the pointed end) was more than twofold faster for Ac-actin than for non-Ac-actin (Fig. 4C). In contrast, the depolymerization rate of 0.1-μM filament seeds (i.e., below the critical concentration of both the pointed and barbed ends) was approximately twofold faster for Ac-actin than for non-Ac-actin, suggesting that, at least in the absence of other factors, actin filament structures formed by non-Ac-actin might be more stable (Fig. 4D). Because both elongation and depolymerization are approximately twofold faster for Ac-actin than for non-Ac-actin, whereas total polymerization is unchanged, nucleation seems to be mostly unaffected by Nt-acetylation. In cells, nucleation is greatly enhanced by the Arp2/3 complex (3, 5). However, we also found no difference in the relative polymerization rate of Ac-actin and non-Ac-actin induced by Arp2/3 complex (Fig. 4E and Fig. S7 A and B). We next analyzed two formins, mDia1 and mDia2, that, while closely related in sequence, have very different actin-assembly properties. We observed no significant difference in the relative polymerization rates of Ac-actin and non-Ac-actin induced by mDia2 (Fig. 4D and Fig. S7 A and C), a formin that has strong nucleation but no elongation activity (23, 24). In contrast, mDia1, which has both strong nucleation and strong elongation activities (23, 24), the polymerization rate was approximately twofold higher for Ac-actin than for non-Ac-actin (Fig. 4G and Fig. S7 A and D). Because Nt-acetylation-dependent differences appear to be emphasized during formin-mediated elongation, we analyzed the role of profilin (isomers 1 and 2), known to virtually stop pointed-end elongation of actin alone while dramatically accelerating barbed-end elongation by formins (24). As expected, mDia1 accelerated the elongation rate of β-γ-actin filament seeds from profilin-1-actin compared with the elongation of seeds from actin alone, but this effect was greater for Ac-actin than for non-Ac-actin (Fig. 4H). Curiously, no significant difference was observed with profilin2-actin (Fig. 4I), suggesting that mDia1 processes profilin-1-actin more efficiently than profilin-2-actin. We note, however, that these assays detect the incorporation into filaments of the small fraction of pyrene-labeled actin, which binds profilin with weaker affinity than unlabeled actin (24), so the effects of profilin may be far more pronounced than indicated by the bulk assays. Together, these results suggest that the differences in filament assembly between Ac-actin and non-Ac-actin remain mainly from an Nt-acetylation-dependent increase in the rates of filament depolymerization and elongation, whereas nucleation appears to be mostly unaffected (spontaneous or Arp2/3 complex-driven).

**Discussion**

The identity of the actin NAT has been a longstanding question since the unique processing of *Dictyostelium* and mammalian actins was discovered in 1981 and 1983, respectively (15, 25). Based on their N-terminal sequences after processing (26), the other actin isoforms, e.g., skeletal and cardiac muscle α-actin and smooth muscle α- and γ-actin, are also likely to be Nt-acetylated by NAA80 (Fig. S1C). Furthermore, the effects of actin Nt-acetylation likely vary among cell types. For instance, in muscle cells, where actin accounts for ~20% of the total cellular protein, Nt-acetylation could predictably have rather dramatic effects in muscle contraction and actomyosin force generation (27, 28).

We found that Nt-acetylation is responsible for pronounced and opposite effects on actin filament depolymerization and elongation dynamics, including formin-induced elongation. Thus, while Ac-actin elongates faster, it also depolymerizes faster, suggesting that cytoskeletal structures consisting of non-Ac-actin may last longer. In contrast, actin filament nucleation was mostly unaffected by Nt-acetylation. We note that filament nucleation is a one-time reaction, whereas both elongation and depolymerization are repetitive reactions that thus could be more sensitive to the cumulative effects of Nt-acetylation. The in vitro effects observed here with several actin assembly factors (4, 5, 24) likely converge to produce the global effects on cell migration and monomeric-to-filamentous actin ratios described above (2).

Due to actin’s broad spectrum of cellular activities, its specific N-terminal processing, and its evolution from bacterial precursors such as FtsA and MreB (29-31), we asked whether a correlation existed between actin’s unique N-terminal sequence and the expression of NAA80. Generally, eukaryotic proteins with N-terminal sequences beginning with Met-Asp or Met-Glu are Nt-acetylated cotranslationally by NatB, which is shared with other eukaryotic proteins beginning with Met-Asp/Met-Glu. (B) Taxonomic tree showing the coevolution of NAA80 and an acidic actin N terminal. For each species, filled red circles indicate the presence of an acidic N terminal in mature actin, and filled blue circles indicate the expression of Naa80. The full names of the organisms are given in Table S2.
studied how actin Nt-acetylation affects cell protrusions and migration, but cell migration depends not only on cell protrusions but also on adhesion and contraction. It is possible that adhesion and contraction are directly or indirectly influenced by the presence of actin’s Nt-acetyl group and thus Nt-acetylation may explain the overall impact on cell migration. Further studies will also shed light on the molecular mechanisms for the observed differences described here in actin polymerization by actin alone or driven by formin. Actin Nt-acetylation could directly decrease the affinity of actin toward formin, as was shown for other protein–protein interactions dependent on Nt-acetylation (13). The enhanced actin filament stability and the slower depolymerization rate of purified non–Ac-actin suggest that the actin filament structure itself could be affected. The lack of the Nt-acetyl group and a subsequently altered filament structure could potentially be sterically unfavorable for actin polymerization and depolymerization. Previous studies on yeast actin have shown that the stability of actin filament structures is affected by the negative charge density at the N terminus. Removing the two negatively charged acidic residues in yeast actin (MDSE) caused an increase in the number of actin strands forming filament bundles (36). Comparison of actin filament structures is affected by the negative charge and a subsequently altered filament structure could potentially be affected. The lack of the Nt-acetyl group and a subsequently altered filament structure could potentially be sterically unfavorable for actin polymerization and depolymerization. Previous studies on yeast actin have shown that the stability of actin filament structures is affected by the negative charge density at the N terminus. Removing the two negatively charged acidic residues in yeast actin (MDSE) caused an increase in the number of actin strands forming filament bundles (36). Comparison of actin filament structures is affected by the negative charge and a subsequently altered filament structure could potentially be affected.

The fact that the removal of the Nt-acetyl group in N/4/480 KO cells in this study decreases the negative charge density may explain the phenotypes observed in vitro as well as in vivo.

In this study, the functional and cellular roles of the most conserved actin modification in animals have been revealed. We have also gained a deeper understanding of the cellular roles of Nt-acetylation. In contrast to the established NAT enzymes often acting on the ribosomes and targeting large groups of substrates (8), NAA80 is a specific postranslational eukaryotic NAT. An accompanying paper defines structural and cellular determinants essential for this specificity towards actin (38). Such a mode of action resembles the enzymes of other major protein modifications such as protein phosphorylation and lysine acetylation, and future studies will reveal whether actin Nt-acetylation also represents a key entry point for cellular regulation.

Materials and Methods

Detailed procedures for cell culturing, microscopy analysis, recombinant protein purification, actin purification, pyrene-based polymerization and depolymerization assays, Nt-acetylation assays, and Western blot analysis are described in SI Materials and Methods and Table S3.

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1. Sheterline P, Clayton J, Sparrow J (1995) Actin. Protein Profile 2:1–103. 2. Pollard TD, Cooper JA (2009) Actin, a central player in cell shape and movement. Science 326:1208–1212. 3. Pollard TD (2016) Actin and actin-binding proteins. Cold Spring Harb Perspect Biol 8: a018226. 4. Mullins RD, Heuser JA, Pollard TD (1998) The interaction of Arp2/3 complex with actin. Nucleation, high affinity pointed end capping, and formation of branching networks of filaments. Proc Natl Acad Sci USA 95:581–588. 5. Dominguez R (2016) The WH2 domain and actin nucleation: Necessary but insufficient. Trends Biochem Sci 41:478–490. 6. Swaney KF, Li R (2016) Function and regulation of the Arp2/3 complex during cell migration in diverse environments. Curr Opin Cell Biol 42:63–72. 7. Breitsprecher D, Goode BL (2013) Formins at a glance. J Cell Sci 126:1–7. 8. Aksnes H, Drazic A, Marie M, Arnesen T (2016) First things first: Vital protein marks by N-terminal acetylation and the N-end rule pathway. Science 352:973–977. 9. Park SE, et al. (2015) Control of mammalian G protein signaling by N-terminal acetylation and the N-end rule pathway. Science 347:1249–1252. 10. Scott DC, Monda JK, Bennett EJ, Harper JW, Schuman BA (2011) N-terminal acetylation acts as an avidity enhancer within an interconnected multiprotein complex. Science 334:674–678. 11. Hawang CS, Shemorry A, Varshavsky A (2010) N-terminal acetylation of cellular proteins creates specific degradation signals. Science 327:973–977. 12. Park SE, et al. (2015) Control of mammalian G protein signaling by N-terminal acetylation and the N-end rule pathway. Science 347:1249–1252. 13. Scott DC, Monda JK, Bennett EJ, Harper JW, Schuman BA (2011) N-terminal acetylation acts as an avidity enhancer within an interconnected multiprotein complex. Science 334:674–678. 14. Holmes WM, Mannecka BK, Gutkenst RN, Serio TR (2014) Loss of amino-terminal acetylation suppresses a prion phenotype by modulating global protein folding. Nat Commun 5:3833. 15. Rubenstein PA, Martin DJ (1983) NH2-terminal processing of Dictyostelium discoideum actin in vitro. J Biol Chem 258:11354–11360. 16. Gauthi M, et al. (2003) The yeast N(alpha)-acetyltransferase NatA is quantitatively essential for this specificity towards actin (38). Such a mode of action resembles the enzymes of other major protein modifications such as protein phosphorylation and lysine acetylation. Aksnes H, Drazic A, Marie M, Arnesen T (2016) First things first: Vital protein marks by N-terminal acetylation and the N-end rule pathway. Science 352:973–977.