NATure of actin amino-terminal acetylation

Peter A. Rubenstein^{a,1} and Kuo-kuang Wen^a

Actins constitute a highly structurally conserved family of proteins found in virtually all eukaryotic cells, in which they participate in processes such as production of contractile force, structural stabilization of the cell, cell motility, endocytosis, and exocytosis (1). The actin monomer, or G-actin, has a nucleotide-binding cleft separating two large domains. Each of these is separated into two subdomains, with the N terminus appearing as an arm which originates from subdomain 1 (Fig. 1). In the context of the actin filament (F-actin), a two-stranded helix, subdomain 1 is located on the filament exterior. This position allows the N-terminal region to be a site of interaction for myosin and a number of other actin-binding regulatory proteins (2, 3). Actins are characterized by an acidic N-terminal region consisting of two to four acidic amino acids in which the N-terminal amino acid is N-acetylated. However, in mature actins, the initiator methionine is missing. In the early 1980s, a series of papers reported the discovery of a unique processing pathway leading to the production of the pure actin (4–6). For class I actins, in which the initiator methionine directly precedes the eventual N-terminal acid residue, the methionine is acetylated. Then the acetyl-methionine is removed proteolytically, exposing the N-terminal acidic amino acid, which is then acetylated to produce the mature form of the protein. Examples of these actins are the beta and gamma cytoplasmic actins found in mammalian cells. For class II actins, which include the striated and smooth muscle actins in mammalian cells, the N-terminal processing is more complex. These proteins are produced from genes which encode a Met-Cys-acidic residue N terminus. For these actins, following removal of the initiator acetyl-methionine, the new N-terminal cysteine is N-acetylated. The acetyl-cysteine is then removed proteolytically to expose the eventual N-terminal acidic residue, which is then acetylated to produce the mature actin N terminus. The conservation of this unique processing pathway coupled with the demonstrated involvement of the N terminus in actin filament function suggests that proper N-terminal processing of actin may have significant functional interactions. Since the

discovery of this processing pathway, however, neither the identity of the enzyme that carries out the final acetylation step of the pathway nor the functional significance of this processing has been established. In PNAS, two papers from the same group [Drazic et al. (7) and Goris et al. (8)] make significant contributions toward solving these two questions.

A number of protein N-acetyltransferases had previously been characterized, but no enzyme had been found that would acetylate the N terminus of actin following removal of its initiator methionine residue. Here, Drazic et al. (7) show that a previously poorly described enzyme called NAA80/NATH or NAT6/Fus2 (9) had the requisite activity for such a task. Based on a series of in vitro studies, they first demonstrated that the enzyme had its highest activity with a peptide beginning ME but also showed high activity on peptides beginning DDDI and EEEL, the N termini of beta and gamma cytoplasmic actins. Subsequently, they demonstrated that in NAA80 knockout tissue culture cells only the acetylation of the two actins was essentially affected, and reintroduction of NAA80 into the cell restored actin acetylation.

Goris et al. (8) extend these studies to the kinetic and structural levels. Initially the enzyme was thought to work via a ping-pong mechanism (10). Goris et al. (8) demonstrate that the enzyme forms a ternary complex containing actin and the acetyl-CoA after which acetylation occurs. Recognition of this mechanism led to the isolation of an inhibitor which approximated the ternary complex when bound to the enzyme. The authors cocrystallized the inhibitor with the *Drosophila* form of NAT6, with the same specificity as the human enzyme, and determined the structure of the complex via X-ray crystallography. They demonstrated that the specificity of the enzyme resided in a more open active site cleft and a much more cationic surface in this cleft to accommodate the N-terminal negatively charged residues than in other N-acetyltransferases. The difference in specificity of the enzyme in vitro and in vivo was surprising. In vitro, the highest activity was in acetylating an N-terminal Met when the next

^aDepartment of Biochemistry, University of Iowa Carver College of Medicine, Iowa City, IA 52242

Author contributions: P.A.R. and K.-k.W. wrote the paper.

The authors declare no conflict of interest.

Published under the PNAS license.

See companion articles on pages 4399 and 4405.

¹To whom correspondence should be addressed. Email: peter-rubenstein@uiowa.edu.

Published online April 9, 2018.

Fig. 1. The actin monomer (**A**, Protein Data Bank ID code 2BTF) and the filament (**B**, ref. 15). The N-terminal peptide (red) and the ADP bound in the nucleotide cleft (cyan) are shown. The four subdomains are denoted on the monomer, and M denotes the individual monomers in the filament.

amino acid was acidic. However, elimination of NAA80 showed the accumulation of actin in which the Met had been removed and the N-terminal amino group was exposed. Subsequent work showed that in the cell NATB was responsible for the initial Met acetylation, and NAA80 then carried out the final acetylation following removal of the acetyl-Met. This result underscores the importance of establishing the activity of a protein *in vivo*, where the biochemical context may be very different from what occurs *in vitro*, to gain insight into its true function. The studies carried out by this group did not specifically demonstrate that this enzyme carried out the ultimate acetylation of the class II actin amino-terminal residue. However, the demonstrated specificity of the enzyme would make it the likely candidate for class II processing. Furthermore, *Drosophila melanogaster* actins are class II actins, and the NAA80 used for the crystallization studies was the *Drosophila* homolog. This enzyme was also shown to have the same substrate specificity as the human form, further supporting this hypothesis.

Discovery of a posttranslational protein modification and the manner in which it is carried out is often much easier to establish than the functional significance of that modification. Drazic et al. (7) made a series of observations that shed light on the functional significance of N-terminal actin acetylation. They showed NAA80 distributed diffusely throughout the cytoplasm instead of associating with ribosomes as do most NATs. They demonstrated that NAA80 knockout cells displayed faster motility and faster gap closure in a wound-healing assay. They also showed the acetylation was important for controlling cell morphology. Elimination of the enzyme resulted in a decreased G/F actin ratio and an increase in filopodia- and lamellipodia-containing cells, all consistent with the increased cell motility they observed. To gain insight into these differences in cell behavior, the authors purified actin from control and knockout cells and compared their polymerization properties. Absence of acetylation did not affect overall actin polymerization rates. It also did not affect spontaneous filament

nucleation or Arp2/3 and formin-dependent nucleation. However, it caused a twofold slower filament elongation rate from preformed actin seeds compared with acetylated actin; and with mDia1, but not mDia2, formin the unacetylated actin polymerized only about 40% as fast as did the acetylated actin. Of the two formins, only the mDia 1 shows a strong elongation activity as well as nucleation. The effect of altered acetylation on filament elongation is unexpected, because the N terminus is far removed from the monomer–monomer interaction sites that result in filament formation. Thus, the acetylation effects can only occur through propagated conformational changes from the amino terminus to the interfaces that are important in longitudinal strand and in cross-strand contacts within the actin filament. In the latter case, the possibility of the N terminus' affecting strand–strand interactions in the middle of the actin filament has been demonstrated (11).

The identification of actin N-terminal acetylating enzyme, its structure determination, the ability to eliminate it from cells, and the demonstration that its absence affects actin function at the cell and molecular level opens the door to many avenues of investigation that should provide new insight into the regulation of actin function. Elimination of the N-acetyl group from actin would introduce a positive charge, thereby decreasing net negative charge density of the amino-terminal fragment. Earlier studies based on genetically altered yeast actin demonstrated that the degree of negative charge density affects actin-activated myosin ATPase activity (2). Decreased negative charge density also increases the propensity of the actin to form spontaneous bundles. Changes in myosin activation could easily be tied to alterations in cell motility associated with NAA80 elimination, while increased bundling could very well lead to the decreased G/F actin ratio and increased filament stability that would facilitate lamellipodia and filopodia formation as observed in the knockout cells. The ability to isolate active nonacetylated actin allows testing of these hypotheses specifically in the context of actin posttranslational processing, which was not possible earlier. Further investigation of

these effects could provide significant new insight into allosteric regulation of actin function.

Knockout of NAA80 knockouts was studied in relatively undifferentiated cell-culture models where constraints on actin cytoskeletal function are often not as constrictive as in well-differentiated tissue. It would be very instructive to examine the effects of NAA80 elimination in a live mouse where effects of elimination of actin acetylation might be more drastic, especially in tissues like inner ear hair cells where the actin machinery is very fragile. No such mouse studies apparently have yet been done. However, such a mouse is evidently commercially available. NAA80, also Fus2, was located to a portion of the chromosome that contained a cluster of tumor-suppressor genes that have been hypothesized to work together to suppress tumor growth (12). Having now identified the activity of Fus2, and using a live mouse model, one could examine tumor behavior in a knockout animal to determine the extent to which the enzyme helps facilitate tumor-suppressing activity. The enhanced motility and increase in lamellipodia and filopodia observed in the knockout cells would be consistent with a cancer cell with increased metastatic potential, in concordance with the idea that NAA80 has a tumor-suppressor function.

The actin amino-terminal fragment has been a source of surprise in terms of structure and function. One is its unique processing, largely maintained across evolution, the manner in which this is carried out, and its effect on actin function. Another puzzle, with respect to the two cytoplasmic actins, is their conservation across mammalian species, considering there are only four amino acid differences between the two isoforms and three of these are aspartic acid for glutamic acid substitutions. These changes would at most be expected to produce small differences in charge density and size. Nevertheless, experiments with the purified isoforms show these can cause differences in polymerization rates (13), and cells have clearly been able to use these differentially to carry out different functions. Recently, Kashina and coworkers (14) demonstrated that the nucleotide sequence of the two genes may play a significant factor in differential actin utilization. Whereas elimination of beta actin is lethal, these authors found that editing the beta actin gene to produce gamma actin did not cause lethality. The ability to manipulate genes for actin and the ability to control processing should allow new avenues of research concerning mechanisms of actin function and the importance of factors such as the above-mentioned three in various aspects of that function.

- 1 Pollard TD, Cooper JA (2009) Actin, a central player in cell shape and movement. *Science* 326:1208–1212.
- 2 Cook RK, Root D, Miller C, Reisler E, Rubenstein PA (1993) Enhanced stimulation of myosin subfragment 1 ATPase activity by addition of negatively charged residues to the yeast actin NH2 terminus. *J Biol Chem* 268:2410–2415.
- 3 Sutoh K, Mabuchi I (1986) Improved method for mapping the binding site of an actin-binding protein in the actin sequence. Use of a site-directed antibody against the N-terminal region of actin as a probe of its N-terminus. *Biochemistry* 25:6186–6192.
- 4 Redman K, Rubenstein PA (1981) NH2-terminal processing of Dictyostelium discoideum actin in vitro. *J Biol Chem* 256:13226–13229.
- 5 Rubenstein PA, Martin DJ (1983) NH2-terminal processing of Drosophila melanogaster actin. Sequential removal of two amino acids. *J Biol Chem* 258:11354–11360.
- 6 Rubenstein PA, Martin DJ (1983) NH2-terminal processing of actin in mouse L-cells in vivo. *J Biol Chem* 258:3961–3966.
- 7 Drazic A, et al. (2018) NAA80 is actin's N-terminal acetyltransferase and regulates cytoskeleton assembly and cell motility. *Proc Natl Acad Sci USA* 115:4399–4404.
- 8 Goris M, et al. (2018) Structural determinants and cellular environment define processed actin as the sole substrate of the N-terminal acetyltransferase NAA80. *Proc Natl Acad Sci USA* 115:4405–4410.
- 9 Zegerman P, Bannister AJ, Kouzarides T (2000) The putative tumour suppressor Fus-2 is an N-acetyltransferase. *Oncogene* 19:161–163.
- 10 Wang H, Liu L, Hanna PE, Wagner CR (2005) Catalytic mechanism of hamster arylamine N-acetyltransferase 2. *Biochemistry* 44:11295–11306.
- 11 Feng L, et al. (1997) Fluorescence probing of yeast actin subdomain 3/4 hydrophobic loop 262–274. Actin-actin and actin-myosin interactions in actin filaments. *J Biol Chem* 272:16829–16837.
- 12 Gatphayak K, Knorr C, Chen K, Brenig B (2004) Structural and expression analysis of the porcine FUS2 gene. *Gene* 337:105–111.
- 13 Bergeron SE, Zhu M, Thiem SM, Friderici KH, Rubenstein PA (2010) Ion-dependent polymerization differences between mammalian β - and γ -nonmuscle actin isoforms. *J Biol Chem* 285:16087–16095.
- 14 Vedula P, et al. (2017) Diverse functions of homologous actin isoforms are defined by their nucleotide, rather than their amino acid sequence. *eLife* 6:e31661.
- 15 Oda T, Iwasa M, Aihara T, Maéda Y, Narita A (2009) The nature of the globular- to fibrous-actin transition. *Nature* 457:441–445.