

Illuminating the impact of N-terminal acetylation: from protein to physiology

Corresponding Author: Dr Nina McTiernan

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The article titled "Illuminating the impact of N-terminal acetylation: from protein to physiology" by Nina McTiernan, Ine Kjosås, and Thomas Arnesen provides a comprehensive review of N-terminal acetylation (NTA), a prevalent protein modification occurring predominantly co-translationally in eukaryotic cells.

NTA affects approximately 50-80% of eukaryotic proteomes and is catalyzed by N-terminal acetyltransferases (Nats). These enzymes transfer an acetyl group from acetyl-coenzyme A to the amino group at the protein's N-terminus, altering its characteristics and influencing various biological processes such as protein folding, localization, complex formation, and degradation. The review highlights that the competition between NATs and other modifying enzymes can significantly influence protein fate, a novel aspect neglected in previous reviews.

The manuscript is well-structured and concisely written. The figures are informative and understandable based on the figure captions. The article categorizes eight NATs (NatA to NatH) based on their substrate specificity, mechanism of action, and subcellular localization.

NatA: The major NAT, responsible for acetylating about 40% of the human proteome, primarily acting co-translationally.

NatB: Acetylates proteins with retained initiator methionine and polar or acidic residues, accounting for 21% of human proteome modifications.

NatC: Similar to NatB but targets hydrophobic or basic amino acids.

NatD: Selectively acetylates histones and requires specific sequences for activity.

NatE: Acts co-translationally on proteins with small residues in the second position.

NatF: Membrane-bound NAT involved in modifying transmembrane proteins.

NatG: Exclusive to plants, associated with plastids.

NatH: The most recently discovered NAT, involved in the maturation of actin in animal cells.

Next, the authors comprehensively describe the current knowledge on the function of NTA for the fate of specific substrate proteins. This knowledge sets the stage for summarizing the impact of the specific Nats on the cellular and organismal levels. Based on this information, the authors summarize and explain the relevance of NTA in diverse human diseases.

The review emphasizes how NTA is crucial for proper protein function and how this has been linked to various physiological processes and diseases, including cardiac dysfunction and neurodegenerative disorders. In summary, this review underscores the significance of N-terminal acetylation in cellular biology, detailing its mechanisms, enzyme interactions, and implications for health and disease.

I have only very minor comments, predominantly focusing on describing NAA50 function in eucaryotes (l 115f). I missed information about the potential internal Lys-acetylation activity of NAA50 described by several authors. Furthermore, the reader might benefit from dissecting the function of NAA50 and HYPK on NatA activity in different organisms e.g., Arbustner et al., Plant Physiology 2024 in this chapter or the chapter on NatA.

Reviewer #2

(Remarks to the Author)

Arnesen and coworkers provide a timely and clearly described and illustrated review on the function, biology and disease relevance of eukaryotic N-terminal acetyltransferases. I believe that this review will be of general interest to readers of Nature Communications.

Some suggested edits for clarity are listed below.

1. Line 48: when discussing how NatA is anchored to the ribosome, the yeast and human cryo-EM structures that were recently reported should be referenced.
2. Line 64: the sentence that reads "While substrates Nt-acetylated by multisubstrate NATs are often observed in both Nt acetylated and non-Nt-acetylated states, NatB substrates typically have an Nt acetylation stoichiometry of nearly 100%, despite representing a broad substrate pool." Seems contradictory. Can this sentence be rewritten. Also does this only apply to NatB. If not, it is not clear why this statement is in the NatB section.
3. Line 228: the authors should also discuss the recent findings of Kumar et al. (PMCID: PMC10857481) demonstrating that N-terminal acetylation of α -synuclein is protective against its degradation.
4. Line 266: the sentence that begins "By promoting interactions..." should begin a new paragraph.
5. Line 283: should consider reading "depletion of NatA activity resulted in a 4-fold increase..."
6. Line 712: suggest changing "induced" to "was correlated with"
7. Line 758: suggest changing "have been solved" to "have been determined"
8. Line 769: It's not clear what the authors mean by "protein shielding." Can the authors please elaborate.
9. Line 795: suggest changing to "suitable small molecule drug-like inhibitors"

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Point-by-point response to referees

Reviewer #1:

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I have only very minor comments, predominantly focusing on describing NAA50 function in eucaryotes (l 115f). I missed information about the potential internal Lys-acetylation activity of NAA50 described by several authors. Furthermore, the reader might benefit from dissecting the function of NAA50 and HYPK on NatA activity in different organisms e.g., Armbuster et al., Plant Physiology 2024 in this chapter or the chapter on NatA.

Response: We appreciate the reviewer's suggestions. Due to the limitations on word count and reference, we had to omit the KAT activities of NAA50 or NAA10, as our focus is on N-terminal acetylation and its impact. We have updated the NAA50

chapter to include its function on NatA activity across different organisms (including Armbruster et al., Plant Physiol, 2024, Weidenhausen et al., Structure, 2021, Armbruster et al., Plant Physiol, 2020 for plant NAA50 and Deng et al., Structure, 2019, Knorr et al., Nat Struct Mol Biol, 2019, Van Damme et al., Proteomics, 2015 for yeast NAA50), and this is also discussed in the chapter on the cellular and organismal impact of N-terminal acetylation.

Reviewer #2:

Arnesen and coworkers provide a timely and clearly described and illustrated review on the function, biology and disease relevance of eukaryotic N-terminal acetyltransferases. I believe that this review will be of general interest to readers of Nature Communications.

Some suggested edits for clarity are listed below.

1. Line 48: when discussing how NatA is anchored to the ribosome, the yeast and human cryo-EM structures that were recently reported should be referenced.

Response: We agree with the reviewer and have included references for the yeast and human NatA-ribosome structures: PMID: 30559462, PMID: 39227397, and PMID: 39169182.

2. Line 64: the sentence that reads “While substrates Nt-acetylated by multisubstrate NATs are often observed in both Nt acetylated and non-Nt-acetylated states, NatB substrates typically have an Nt acetylation stoichiometry of nearly 100%, despite representing a broad substrate pool.” Seems contradictory. Can this sentence be rewritten. Also does this only apply to NatB. If not, it is not clear why this statement is in the NatB section.

Response: We thank the reviewer for pointing this out. We included this information in the NatB section because NatB substrates are usually 100% or near 100% Nt-acetylated, which stands in contrast to the other multisubstrate NATs (NatA and NatC) whose substrates are often present in both Nt-acetylated and non-acetylated state. We have rewritten this statement to improve clarity.

3. Line 228: the authors should also discuss the recent findings of Kumar et al. (PMCID: PMC10857481) demonstrating that N-terminal acetylation of α -synuclein is protective against its degradation.

Response: In the section referred to by the reviewer, we focus on the effect Nt-acetylation has on α Syn aggregation, while the recent findings of Kumar et al. regarding degradation of α Syn is discussed in the stability section (line 306-308 in revised manuscript) and the disease section (line 615-619).

4. Line 266: the sentence that begins “By promoting interactions...” should begin a new paragraph.

Response: We have included a new paragraph.

5. Line 283: should consider reading “depletion of NatA activity resulted in a 4-fold increase...”

Response: We have amended the sentence according to the reviewer's suggestion.

6. Line 712: suggest changing "induced" to "was correlated with"

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8. Line 769: It's not clear what the authors mean by "protein shielding." Can the authors please elaborate.

Response: We thank the reviewer for pointing this out. We have changed this to "protein degradation shielding" to make the sentence more clear.

9. Line 795: suggest changing to "suitable small molecule drug-like inhibitors"

Response: We have amended the sentence according to the reviewer's suggestion.