A stable start

Gibbs, Daniel J.; Bailey, Mark; Etherington, Ross D.

DOI:
10.1016/j.tcb.2022.02.004

Document Version
Peer reviewed version

Citation for published version (Harvard):
Gibbs, DJ, Bailey, M & Etherington, RD 2022, 'A stable start: cotranslational Nt-acetylation promotes proteome stability across kingdoms', *Trends in Cell Biology*, vol. 32, no. 5, pp. 374-376.
https://doi.org/10.1016/j.tcb.2022.02.004

A stable start: co-translational Nt-acetylation promotes proteome stability across kingdoms

Daniel J. Gibbs[1,*], Mark Bailey[2] and Ross D. Etherington[1]

[1] School of Biosciences, University of Birmingham, Edgbaston, B15 2TT, UK
[2] Plant Sciences Department, Rothamsted Research, Harpenden, AL5 2JQ, UK

[*]Correspondence: d.gibbs@bham.ac.uk (Gibbs, D.J.)

Twitter: @DJ_Gibbs

ORCID IDs:

D.J.G: 0000-0002-4100-6682
M.B: 0000-0003-1843-9348
R.D.E: 0000-0002-2909-4771

Keywords: Acetylation, N-terminus, co-translational modification, proteolysis, N-degron pathway, proteostasis

Abstract

Two recent studies show that co-translational N-terminal protein acetylation (NTA) promotes proteome stability in humans[1] and plants[2] by masking non-acetylated N-degrons that would otherwise destabilise proteins. This is in contrast to previous findings linking NTA to degradation, suggesting this widespread mark has complex and context-specific functions in regulating protein half-lives.

Main text

N-terminal (Nt-) protein acetylation (NTA) is a highly abundant modification found on up to 90% of soluble proteins in plants and animals, typically occurring co-translationally via the catalytic action of ribosome-tethered Nt-acetyltransferases (NATs)[3]. NATA is the predominant eukaryotic NAT, accounting for approximately 40% of total Nt-acetylated proteins by modifying non-bulky Nt-amino acid residues following iMet cleavage. Nascent proteins that retain iMet are instead Nt-acetylated by different conserved NATs, mainly NATB and C, and several other enzymes with dual Nt- and Lysine-(Lys-)acetyltransferase and post-translational activities have recently been characterised [3, 4]. In contrast to Lys-acetylation, NTA is irreversible and persists throughout the protein's life. This widespread prevalence and permanency suggests a general role for this modification in proteome homeostasis, rather than substrate-specific signalling.

NTA has been linked to diverse protein functions, including subcellular targeting, protein interactions, folding, and turnover [3]. NTA of certain proteins was previously shown to conditionally target them for proteolysis via the Ac/N-degron pathway in yeast and humans, as part of a quality control mechanism important for destroying misfolded proteins and regulating multicomplex subunit stoichiometries [5-7]. However, the universality of this pathway remains unknown, and several reports have shown that NTA can in fact enhance protein stability. Moreover, a proteome-wide analysis of yeast N-degrons [8] indicated that Nt-hydrophobicity, rather than NTA, is a bigger determinant of protein destabilisation, and that NTA can promote protein stability by preventing degradation through the separate Arg/N-degron pathway [7-9]. Two recent studies in human cells [1] and the model plant Arabidopsis [2] have now addressed on a larger scale a role for NTA in cellular proteostasis, by focussing on

downstream consequences of NATA activity. Both studies reveal a widespread role for protein NTA in promoting proteome stability, rather than degradation, uncovering a conserved protective function for this modification (Figure 1).

To identify E3 ubiquitin ligases that recognise Ac/N-degrons, Mueller *et al.* (2021)[1] developed a mass-spec-based pipeline for enriching protein N-termini from HeLa cells, and then used NTA and non-NTA variants of N-termini from proteins that are normally highly Nt-acetylated to capture binding partners from cell lysates. Here, they observed robust enrichment of several E3s of the IAP family that was specific to the non-NTA versions. They named these proteins “cryptic IAP binders”, since they are usually acetylated, which masks them from interaction. IAP E3 ligases contain BIR domains that recognise IBM motifs in target proteins, which are non-acetylated neo-N-termini that are exposed post-translationally by endo-proteolysis or signal-peptide processing. Analysis of known IBMs revealed that they invariably initiate with the same residues that are targeted by NATA during translation, indicating that cryptic IAP binders might be NATA substrates. Supporting this, RNAi depletion of NATA, followed by affinity capture using IAPs as bait, identified several normally Nt-acetylated proteins that were specifically ubiquitinated by IAPs only in their non-acetylated form. Since these cryptic IAP binders mimic the *de novo* N-terminal protein fragments that displace caspases from BIR domains to promote apoptosis, authors monitored apoptotic prevalence in NATA RNAi cells, which revealed a significantly increased number of cells undergoing this process compared to WT or NATB-depleted lines. This study reveals a role for NATA-mediated acetylation in proteome stabilisation by protecting proteins from IAP-mediated proteolysis, and suggests a previously unknown function for IAPs as regulators of protein quality control. Moreover, it implicates NATA in the prevention of ectopic apoptosis by masking N-termini that would otherwise trigger caspase activation through binding to IAPs.

In a separate study, Linster *et al.* (2022)[2] investigated global functions for NATA in Arabidopsis, which they previously showed regulates hormone-responsive stress resilience [10]. RNAi-mediated knockdown of NATA triggered a four-fold increase in global protein degradation rates, which correlated with enhanced total protein ubiquitination and proteasome activity. A vast majority of the proteins displaying faster degradation were NATA substrates. Remarkably however, steady-

state protein levels were unaltered due to a concomitant four-fold increase in the translation of mRNAs encoding NATA-targeted polypeptides, via upregulated TOR kinase activity. This indicates a feedback mechanism whereby cells replenish destabilised non-acetylated NATA targets though enhancing their synthesis rates, which may be critical for proteome homeostasis. Next, a plant-optimised tandem fluorescent timer system was used to monitor half-lives of specific proteins, which demonstrated that: (1) non-NATA targets were unaffected, and (2) diverse NATA substrates were unstable as a *direct* consequence of their non-acetylated N-termini. Authors proposed that non-acetylated N-termini function as nonAc-X^2/N-degrons (where X^2 represents NATA-targeted residues), and that co-translational NTA is required for masking these degrons to prevent proteolysis. Since NATA activity was previously shown to be reduced by the stress phytohormone ABA [10], it is tempting to speculate that stress-induced decreases in NTA may trigger physiological responses by increasing turnover of a specific subset of proteins.

These two studies identify NATA-mediated NTA as a critical modification for promoting proteome stability, rather than instability, across eukaryotes. This indicates that the Ac/N-degron pathway [5] may be constrained to a more specific set of protein substrates than previously appreciated, similar to the Arg/N-degron pathway, which is important for signal-responsive control of regulatory proteins [9]. Several key questions now remain. Does NTA of proteins that retain iMet during translation also act in a protective manner, or is this function specific to NATA-substrates? Does post-translational NTA impact protein stability, or is it speciailly linked to co-translational modification? Furthermore, what E3 ligases are responsible for degradation of non-acetylated NATA-substrates in plants? Plants do not contain canonical IAP-like E3 ligases [2], which suggests different systems are in operation. It will also be of interest to determine if human or yeast NATA enzymes are stress-responsive, like Arabidopsis NATA [10], and to consider whether use of this enzyme for signal-responsive proteome remodelling has convergently evolved in metazoans, fungi and plants.

NTA has received increased attention in recent years, and its role in controlling protein half-lives has been controversial. What was once considered an inert and non-regulatory chemical modification is now shown to intrinsically determine the half-life of a large proportion of proteins during their synthesis, which has important implications

for co-translational protein quality control and stress-responsive proteostasis across eukaryotic kingdoms.

Acknowledgments:

This work was supported by a European Research Council Starting Grant (ERC-StG 715441-GasPlaNt) and Biotechnology and Biological Sciences Research Council grant (BB/V008587/1) to D.J.G.

References

1. Mueller, F., et al., *Overlap of NatA and IAP substrates implicates N-terminal acetylation in protein stabilization.* Sci Adv, 2021. **7**(3).
2. Linster, E., et al., *Cotranslational N-degron masking by acetylation promotes proteome stability in plants.* Nature Communications, 2022. **13**(1): p. 810.
3. Aksnes, H., R. Ree, and T. Arnesen, *Co-translational, Post-translational, and Non-catalytic Roles of N-Terminal Acetyltransferases.* Mol Cell, 2019. **73**(6): p. 1097-1114.
4. Bienvenut, W.V., et al., *Dual lysine and N-terminal acetyltransferases reveal the complexity underpinning protein acetylation.* Mol Syst Biol, 2020. **16**(7): p. e9464.
5. Hwang, C.S., A. Shemorry, and A. Varshavsky, *N-terminal acetylation of cellular proteins creates specific degradation signals.* Science, 2010. **327**(5968): p. 973-7.
6. Shemorry, A., C.S. Hwang, and A. Varshavsky, *Control of protein quality and stoichiometries by N-terminal acetylation and the N-end rule pathway.* Mol Cell, 2013. **50**(4): p. 540-51.
7. Park, S.E., et al., *Control of mammalian G protein signaling by N-terminal acetylation and the N-end rule pathway.* Science, 2015. **347**(6227): p. 1249-1252.
8. Kats, I., et al., *Mapping Degradation Signals and Pathways in a Eukaryotic N-terminome.* Molecular Cell, 2018. **70**(3): p. 488-501.e5.
9. Gibbs, D.J., et al., *The eukaryotic N-end rule pathway: conserved mechanisms and diverse functions.* Trends Cell Biol, 2014. **24**(10): p. 603-11.
10. Linster, E., et al., *Downregulation of N-terminal acetylation triggers ABA-mediated drought responses in Arabidopsis.* Nat Commun, 2015. **6**: p. 7640.

Figure Legend

Figure 1. NATA-mediated Nt-acetylation (NTA) and protein stability. (**A**) Nascent polypeptides with X^2 residues in position two (corresponding to A,V,S,T,C,G) have their iMet cleaved by Methionine Amino-Peptidases (MetAPs). (**B**) Exposed X^2 residues are co-translationally acetylated (Ac) by NATA. For many proteins this has a stabilizing effect on their half-life [1,2], whilst for some it triggers degradation via the Ac/N-degron pathway [5-7]. (**C**) Reduced NATA activity leads to unacetylated X^2 N-terminal residues, which can stabilise certain Ac/N-degron pathway substrates. In human cells, many non-acetylated NATA substrates are bound and ubiquitinated by IAP E3-ligases, and degraded via the 26S proteasome. This triggers ectopic apoptosis via caspase activation, but also contributes to protein quality control (PQC). Absence of NTA in humans and yeast can also redirect Ac/N-degron pathway substrates to the Arg/N-degron pathway via Arg/N-recognin E3 ligases [7,8]. In Arabidopsis, NATA depletion leads to proteasomal degradation of non-acetylated proteins, via currently unknown E3 ligases, by exposing non-Ac$X^{2/}$N-degrons. This turnover is counterbalanced by an increase in NATA-substrate translation through enhanced TOR kinase activity. Thus, in both humans and plants, NTA by NATA plays a broad role in promoting proteome stability, which suggests the Ac/N-degron pathway may be constrained to a smaller set of proteins.