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

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N-terminal acetylation is a highly abundant protein modification in eukaryotic cells. This modification is catalysed by N-terminal acetyltransferases acting co- or post-translationally. Here, we review the eukaryotic N-terminal acetylation machinery: the enzymes involved and their substrate specificities. We also provide an overview of the impact of N-terminal acetylation, including its effects on protein folding, subcellular targeting, protein complex formation, and protein turnover. In particular, there may be competition between N-terminal acetyltransferases and other enzymes in defining protein fate. At the organismal level, N-terminal acetylation is highly influential, and its impairment was recently linked to cardiac dysfunction and neurodegenerative diseases.

N-terminal (Nt) acetylation is a prevalent protein modification that affects about 50–80% of eukaryotic proteins[1–6]. Nt-acetylation involves the transfer of an acetyl moiety ($COCH_3$) from acetyl-coenzyme A (Ac-CoA) to the amino group (NH_3^+) of the alpha carbon at the extreme N-terminus of a polypeptide or protein (Fig. 1). This reaction is catalysed by N-terminal acetyltransferases (NATs) and introduces a bulky and hydrophobic segment to the formerly positive NH_3^+ group. In eukaryotes, eight NATs have been identified (NatA–NatH) (Fig. 2)[6]. The different NATs are composed of one or several subunits, each termed N-alpha-acetyltransferases (NAAs), with a designated numbered suffix. Nt-acetylation can occur either co-translationally (NatA–NatE) or post-translationally (NatF–NatH). In brief, the co-translational NATs are conserved among all eukaryotes, whereas the post-translational NATs are mostly found in multicellular eukaryotes—animals and plants (Fig. 2)[6]. NatG, comprising a family of GNAT enzymes, is unique for plastids in plants[7,8], NatF has a Golgi localisation in animals in contrast to its plasma membrane localisation in plants[9,10], whilst the cytosolic NatH is unique for the animal kingdom[11]. Common for all the NATs is that their substrate specificities are predominantly determined by the first two to four amino acids of a substrate protein[6,8].

N-terminal acetyltransferases—masters of the Nt-acetylome

NatA was the first identified NAT, discovered in *S. cerevisiae*[12] and later in multicellular eukaryotes[4,13]. This is the major NAT, responsible for Nt-acetylating ~40% of the human proteome[1]. NatA works co-translationally and is composed of the catalytic subunit NAA10 and the auxiliary subunit NAA15. NAA15 anchors the complex to the ribosome[14–17] and modulates the substrate specificity[18]. NatA targets small Nt-amino acids (Ala, Ser, Thr, Val, Gly and Cys) exposed *after* the initiator methionine (iMet) has been cleaved off by a methionine aminopeptidase (MetAP)[1,19–21]. Recent cryo-EM structures uncovered that NatA and MetAPs concurrently bind close to the peptide tunnel exit of the ribosome, allowing for rapid and coordinated enzyme processing of the nascent polypeptide[16,17]. Huntingtin-interacting protein K (HYPK) is stably associated with the NatA complex in human and plant cells[22,23]. Paradoxically, HYPK inhibits NatA in vitro[24,25] while promoting its activity in vivo[22,23,26,27]. The inhibitory effect of HYPK is likely relieved in vivo through an allosteric change in NatA-HYPK binding upon NACα recruitment of NatA at the ribosome[17].

NatB acts co-translationally through its catalytic subunit NAA20, tethered to the ribosome by the auxiliary subunit NAA25, which wraps around NAA20[28–32]. As NatB Nt-acetylates 21% of the human proteome, it is also one of the major NATs. NatB operates specifically towards proteins with a retained iMet followed by a polar or acidic amino acid; Met-Asp, Met-Asn, Met-Glu, or Met-Gln-starting proteins[33]. NatB substrates typically have an Nt-acetylation stoichiometry of nearly 100%, unlike substrates of other multisubstrate NATs, which are often observed in both Nt-acetylated and non-Nt-acetylated states[33].

The ternary NatC complex is composed of the catalytic subunit NAA30 and two auxiliary subunits—the ribosome-anchoring subunit NAA35 and the smaller subunit NAA38 with a less understood

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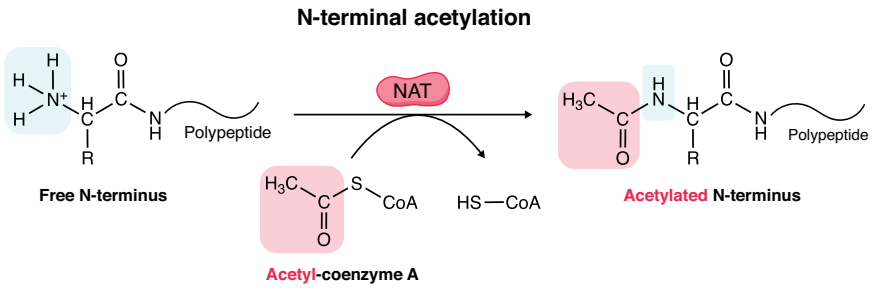

Fig. 1 | Protein N-terminal acetylation reaction. N-terminal acetyltransferases (NATs) catalyse the transfer of an acetyl moiety (red) from acetyl-coenzyme A (Ac-CoA) to the α-amino group (blue) at the extreme N-terminus of a polypeptide or protein.

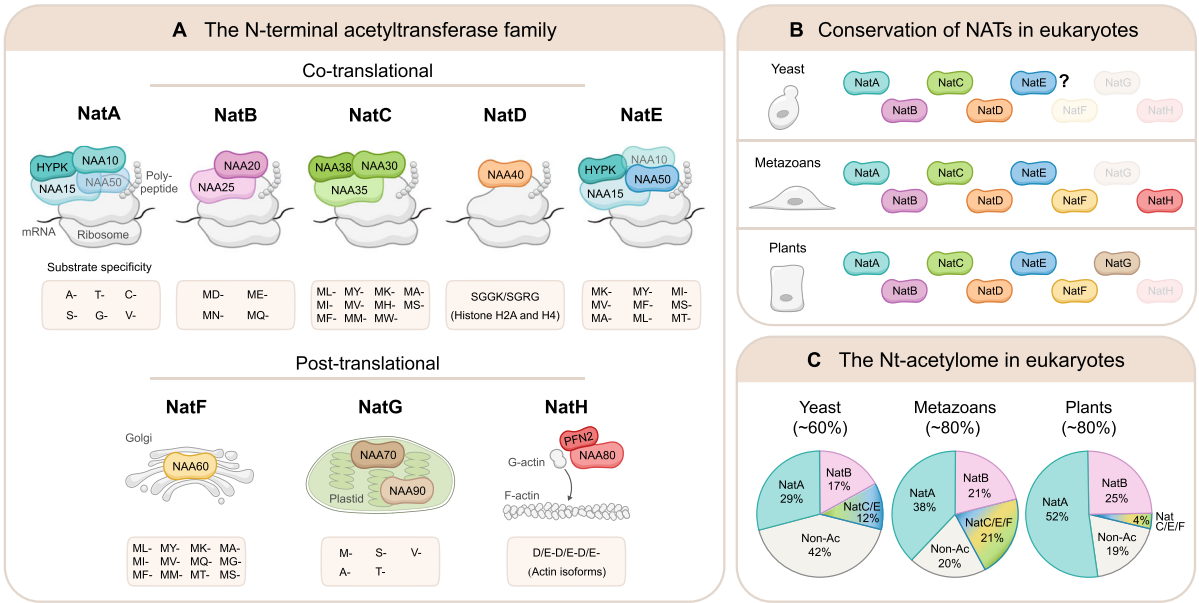

Fig. 2 | NAT machinery, conservation and Nt-acetylome in eukaryotes. A The eukaryotic family of N-terminal acetyltransferases (NATs) comprises eight enzyme types (NatA–NatH). NatA–NatE act co-translationally, modifying nascent polypeptides during their synthesis on the ribosome. In contrast, NatF–NatH operate post-translationally, targeting proteins after their synthesis[6]. NatF is localised on the cytosolic side of the Golgi apparatus (and of the plasma membrane in plants), modifying transmembrane proteins[9,10]. NatG is associated with plastids[7,8], and NatH specifically Nt-acetylates actins[11]. The catalytic subunits of each NAT are designated NAA10–NAA90, with some NATs requiring auxiliary subunits (NAA15, HYPK, NAA25, NAA35, NAA38 and PFN2) for ribosome anchoring and modulation of enzymatic activity. Each NAT complex exhibits distinct substrate specificity, primarily determined by the first two to four amino acids. The indicated substrate specificity is defined by human and/or yeast NATs (and appears to be similar in plants), except for NatG, which is based on plant enzymes[6,8]. **B** All co-translational NATs (NatA–NatE) are conserved from yeast to metazoans and plants, but yeast NatE is likely catalytically inactive. NatF is present in both plants and metazoans, whereas NatG is exclusive to plants and NatH is only found in metazoans[6]. **C** Approximately 50–80% of the eukaryotic proteome is N-terminally acetylated (Nt-acetylome). In yeast, metazoans and plants, NatA accounts for the largest share of the Nt-acetylome, followed by NatB. NatC, NatE and NatF have overlapping substrate specificities in vitro, with varying coverage of the Nt-acetylome in eukaryotes[4–6].

function[34–36]. A recent study revealed that human NAA38 broadens NatC's substrate specificity and increases its thermostability by repositioning NAA30's peptide binding loop to enhance catalysis and ordering an N-terminal fragment of NAA35, respectively[37]. NatC, similar to NatB, co-translationally acetylates iMet-retained proteins; however, with a specificity towards hydrophobic or basic amino acids in the second position. This includes Met-Leu, Met-Ile, Met-Phe, Met-Tyr, Met-Val, Met-Met, Met-Lys, or Met-His-starting proteins in human cells and, additionally, Met-Trp, Met-Ala and Met-Ser-starting proteins in yeast[5,34,38–40].

Some NATs are highly selective, meaning that their active site only accommodates Nt-acetylation of substrates with very specific sequences. NatD is a selective NAT that co-translationally acetylates histones H2A and H4, requiring a four-residue Nt-sequence Ser-Gly-Arg-Gly (Ser-Gly-Gly-Lys in yeast H2A) after MetAP processing[41–43]. A few other substrates with a corresponding Nt-sequence may also be Nt-acetylated by NatD[44]. NatD activity was first observed in *S. cerevisiae*, where a NatA knockout (KO) strain contained Nt-acetylated Ser of histone H2A and H4, despite Nt-Ser being a canonical NatA substrate, revealing the presence of a selective NAT for histones[12]. Another distinct feature is that NatD consists solely of the catalytic unit NAA40, which is believed to be ribosome-binding as no auxiliary partners are identified[41–43].

NAA50, the catalytic subunit of NatE, operates either bound to NatA on the ribosome or individually in the cytosol[14,45–47]. In humans, NAA50 binds to NatA via NAA15, inducing a conformational change in NAA10 that reduces NatA activity in vitro. Moreover, HYPK binding to NatE inhibits NAA50 in vitro by altering its substrate-binding site[48]. NatE acts co-translationally on proteins with a small amino acid in the second position, but unlike NatA, only if the iMet is retained (Met-Ala, Met-Ser, Met-Thr and Met-Val)[47]. However, such N-termini are commonly processed by MetAP, suggesting that Nt-acetylation by NatE shields some substrates from iMet removal if they are Nt-acetylated first. This portrays an interplay between NatA, NatE and MetAP in regulating Nt-acetylation of specific substrates, although the molecular mechanism is

unclear. Interestingly, NatE has overlapping substrate specificity with NatC (and NatF) towards some hydrophobic or basic residues following the retained iMet (Met-Lys, Met-Tyr, Met-Phe, Met-Leu, Met-Ile)[47,49,50], but the potential substrate redundancy is not fully understood. In *A. thaliana*, NAA50 exhibits canonical NatE substrate specificity and plays a vital role in plant immunity independent of NatA regulation[51–53]. In contrast, yeast NAA50 is catalytically inactive, but several NatA substrates have reduced Nt-acetylation in *naa50*-lacking yeast, indicating NAA50 has a scaffolding role aiding NatA catalysis[15,47,54].

The first membrane-bound NAT identified was NatF, comprising the catalytic NAA60 enzyme as a monomer[9,55]. In human cells, NatF is associated with the Golgi apparatus through a unique C-terminal segment that inserts the protein at the cytosolic surface of the Golgi membrane[56]. In plants, NAA60 is associated with the plasma membrane, also through a C-terminal anchor[10]. Given its subcellular localisation, it is reasonable to assume NatF is a post-translational NAT acting specifically on transmembrane proteins[9,10]. NatF also has a preference for iMet-starting proteins when a Leu, Ile, Phe, Tyr, Val, Met, Lys, Gln, Thr, Ala, Gly, or Ser residue is in the second position[9,55]. Thus, NatF has overlapping substrate specificity with NatC and NatE, and together they Nt-acetylate 21% of the human proteome. However, the redundancy in vivo may be lower than it appears in vitro, considering NatF localises to the Golgi and Nt-acetylates transmembrane proteins. Additionally, the catalytic subunits of NatC, NatE and NatF differ in shape, hydrophobicity and electrostatic surface, suggesting these seemingly overlapping NATs likely have substrate determinants beyond the identity of the first two amino acids[36].

Another organellar NAT is NatG, which is exclusive to the plant kingdom and localised in plastids[7]. The catalytic enzyme of NatG was initially named NAA70, but later an additional nine plant-specific enzymes with putative dual NAT and lysine acetyltransferase (KAT) activities were identified, called GNAT1-10 [8]. Today, NAA70 refers to GNAT4-7 and GNAT10, whilst GNAT1-3 is called NAA90—all functioning as plastid NATs, collectively called NatG[8,57]. NAA70 is present on the luminal (GNAT4, 5, 7 and 10) and cytosolic side (GNAT6) of plastids, whereas NAA90 (GNAT1-3) is exclusively in the plastid lumen. NAA70 post-translationally Nt-acetylates proteins starting with Met, Ala, Ser, Thr, or Val[7,8,57]. NAA90 in vivo substrates are mostly Ala, Ser, or Thr-starting proteins and there seems to be redundancy between GNAT1 and GNAT2[58].

The most recently discovered NAT is the animal kingdom NatH, consisting of the catalytic NAA80 enzyme. NatH acts post-translationally in the cytosol and is highly selective towards β- and γ-actin in non-muscle cells[11,59,60]. During translation, cytoplasmic β- and γ-actin are first co-translationally Nt-acetylated by NatB. After translation, a two-step maturation process is initiated. First, the actin maturation protease (ACTMAP) cleaves the Nt-acetylated iMet, producing a novel acidic N-terminus: Asp-Asp-Asp- for β-actin and Glu-Glu-Glu- for γ-actin[61]. NAA80 then completes maturation by Nt-acetylating the acidic N-terminus[11]. This post-translational maturation of actins, including cleavage and NAA80 activity, is exclusively found in animal cells[6,11]. ACTMAP and NAA80 also mature actins in muscle cells in a similar manner[60–63]. NAA80 stably associates with profilin (PFN), which binds globular (G-) actin and regulates actin filament formation[62]. Although PFN1 is more abundant in most cells, NAA80 (and ACTMAP) preferentially bind to the less abundant PFN2[64]. The NAA80/PFN2 complex increases NAA80's activity compared to NAA80 alone or with PFN1, suggesting that PFN2 chaperones actin Nt-acetylation by NAA80. Also, NAA80 merely associates with G-actin, not filamentous (F-) actin, indicating that the NAA80/PFN2 complex Nt-acetylates G-actin prior to its incorporation into F-actin[64].

Destinies of N-terminally acetylated proteins

Through the introduction of a hydrophobic and bulky acetyl group, Nt-acetylation alters the N-terminal characteristics of proteins, thereby potentially influencing their function and fate. The functional implications for proteins receiving Nt-acetylation include protein folding and aggregation, protein-protein interaction, subcellular targeting and stability and turnover (Fig. 3)[6]. The diverse functional implications reflect the broad substrate pool undergoing Nt-acetylation, and the functional consequences are dependent on the specific target protein rather than a unique role of Nt-acetylation[6]. Different effects of Nt-acetylation could also be causally linked; for instance, protein degradation observed due to the presence or absence of an Nt-acetyl group could be secondary to aberrant folding, membrane binding, or protein complex formation.

The effect of Nt-acetylation on protein folding and aggregation

Initial studies demonstrated that Nt-acetylation stabilises the α-helix formation of peptides by providing hydrogen bond formation to the main-chain NH-group[65]. This was later supported by structural studies of certain polypeptides[66,67]. Nt-acetylation was also suggested to have a global role in protein folding due to the accumulation of misfolded proteins in NatA-depleted yeast cells[68].

Nt-acetylation of alpha-Synuclein (αSyn) is associated with enhanced folding and decreased aggregation. Aggregation of αSyn is involved in the development of Parkinson's disease, and as a Met-Asp-starting protein, it is likely Nt-acetylated by NatB[33,69]. Several in vitro studies have shown that non-acetylated αSyn is more prone to aggregation compared to Nt-acetylated αSyn[70–72]. Nt-acetylation of αSyn preserves its native conformation by stabilising an α-helix structure formed by the N-terminus[72,73]. Additionally, it prevents aggregation and fibril assembly by disrupting the hydrogen bonds present in αSyn oligomers[70,72,73]. The fibrils formed by Nt-acetylated or non-acetylated αSyn are also morphologically different; the non-acetylated variant has more β-sheet formation compared to the acetylated variant, which is known to increase fibrillation[71]. Hence, Nt-acetylation aids in correct conformation and prevents aggregation.

Huntingtin (Htt) is another protein prone to aggregation, which is believed to cause neuronal death, leading to Huntington's disease[74]. Htt is found Nt-acetylated in mammalian cells[74] and demonstrated to be a NatA substrate in vitro[75]. HYPK, which is a part of the NatA complex, acts as a chaperone for Htt and HYPK knockdown (KD) increases Htt aggregation[22]. HYPK is also essential for normal NatA-mediated Nt-acetylation in plants and human cells[22,23,26]. Both wild type and a pathogenic variant of Htt are detected as Nt-acetylated in human cells[74,76] and initial findings indicated that intact NatA activity prevents cellular Htt aggregation[22]. However, a recent in vitro study of purified Htt variants suggests that Nt-acetylation of Htt increases its aggregation propensity compared to non-acetylated Htt[75], emphasising that the molecular mechanisms underlying the effects of HYPK and NatA on Htt folding and aggregation remain to be clarified.

Nt-acetylation mediates interactions with proteins and subcellular structures

Whether dependent of folding effects or not, Nt-acetylation can promote protein-protein interactions beyond self-aggregation. A classic example is the impact of Nt-acetylation on tropomyosin function. Early studies suggested that a native and Nt-acetylated tropomyosin is essential for obtaining an α-helical structure[67,77,78] and thereby functionality, including filament formation and binding to F-actin[79–81]. Different tropomyosin isoforms are Nt-acetylated by NatA or NatB depending on their sequence[33,82]. A recent intein-based expression method using human cells demonstrated by direct comparison that Nt-acetylated tropomyosin binds F-actin more strongly than the non-acetylated tropomyosin[82]. In agreement with these direct protein-level effects, impaired tropomyosin Nt-acetylation has been linked to several cytoskeletal phenotypes in yeast and mammalian cells[28,29,33].

In yeast and humans, the E2 neddylation enzymes UBE2F and UBE2M (Yeast: Ubc12) are Nt-acetylated by NatC. This increases their

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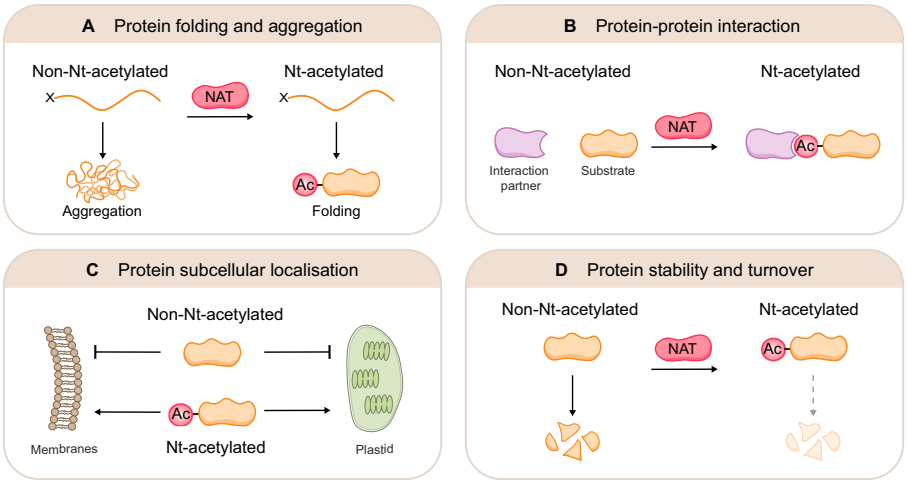

Fig. 3 | N-terminal acetylation and its impact on protein fate. N-terminal acetylation has varying effects depending on the function of the substrate. Here, four main functions of Nt-acetylation are summarised. **A** Nt-acetylation may facilitate correct protein folding, maintaining protein solubility and function, while the non-Nt-acetylated variant is prone to aggregation[22,68,70,71]. **B** Protein-protein interactions may depend on Nt-acetylation, increasing the ability to bind hydrophobic binding grooves and enhancing the formation of functional protein complexes[28,82,84]. **C** Some substrates depend on Nt-acetylation to achieve correct subcellular localisation, ensuring they reach their functional destinations within the cell[35,88–91]. **D** Nt-acetylation can protect proteins from degradation, contributing to their stability and longevity[39,94,95]. However, some Nt-acetylated proteins may be targeted for degradation through conditional mechanisms, such as correct stoichiometry, folding and protein-protein interactions[111,112].

binding affinity towards the hydrophobic pocket of DCNL E3 ligases, forming a cognate E2/E3 complex that promotes cullin neddylation[83,84]. Another example is how Nt-acetylation of the yeast transcriptional regulator Sir3 stabilises its structure, enhancing its interaction with nucleosomes[85,86]. A global proteomics analysis uncovered that yeast lacking NatA displayed reduced thermostability of ribosomes and proteasomes, suggesting that Nt-acetylation of ribosomal and proteasomal proteins is important for their folding and interactions in these large complexes[87].

By promoting interactions with specific proteins or structures, Nt-acetylation may also facilitate correct subcellular localisation. In *A. thaliana*, Nt-acetylation of various chloroplast precursor-proteins is suggested to play an important role in directing them to the chloroplasts[88]. Similarly, in yeast and human cells, NatC-mediated Nt-acetylation of Arl8b is required for lysosomal membrane association[35,89]. Furthermore, ARFRP1 (Yeast: Arl3p) requires Nt-acetylation to be Golgi-localised through protein-protein interaction with SYS1 (Yeast: Sys1p), a Golgi multi-pass membrane protein[90,91].

Nt-acetylation—imprinting protein stability and conditional protein degradation

A function of Nt-acetylation that has gained prominence lately is to prevent protein degradation by shielding the N-terminus, which otherwise (in its non-acetylated state) may act as an N-degron. An N-degron is an N-terminal sequence that is recognised as a degradation signal by ubiquitin (Ub) E3 ligases, marking the protein for degradation by ubiquitination of a nearby lysine residue[92,93]. Several recent efforts have elucidated the major protective role of Nt-acetylation[39,94,95]. In *A. thaliana*, depletion of NatA activity resulted in a 4-fold increase in protein turnover via the ubiquitin-proteasome system, leading to the identification of the non-AcX^2 (X = Ala, Ser, or Gly) N-degron[23,95]. However, not all non-acetylated Ala-, Ser-, or Gly-starting proteins tested were targeted for degradation, emphasising that there are several determinant parameters, such as an accessible lysine for polyubiquitination near the exposed N-terminus[93]. In human cells, a handful of NatA substrates were shown to interact with inhibitor of apoptosis (IAP) E3 ligases, but only the non-acetylated form[94]. In addition, non-modified NatA substrates (Gly-, Ala-, Ser-, Thr- and Cys-) may act as N-degrons for the E3 ligases CRL2-ZER1/ZYG11B[96–98]. Hence, classic NatA substrates that fail to undergo Nt-acetylation can be targeted for degradation by E3 ligases (IAPs or CRL2-ZER1/ZYG11B) in the recently named GASTC/N-degron pathway[93,99]—ensuring protein quality control and protein homoeostasis.

In *S. cerevisiae* and mammals, a basic or hydrophobic residue in the first position of a protein is known to be a destabilising N-degron, recognised by UBR Ub E3 ligases[92,100]—known as the Arg/N-degron pathway. Arg/N-degrons are divided into type 1: Arg, Lys and His (basic residues), and type 2: Phe, Trp, Tyr, Leu and Ile (hydrophobic residues). Notably, Asp, Asn, Gln, Glu and Cys can be post-translationally modified into Nt-Arg (Type 1) following deamidation (Asn to Asp, Gln to Glu) or oxidation (Cys), and by direct arginylation (Asp, Glu)[93]. In *S. cerevisiae*, Ubr1 is the responsible Ub E3 ligase (N-recognin), while in mammals there are at least four UBR enzymes: UBR1, UBR2, UBR4 and UBR5[93,100]. Furthermore, UBR4 works in a complex with KCMF1[101]. NatC Nt-acetylates iMet followed by a hydrophobic or basic residue and such N-termini are also recognised by UBR Ub E3 ligases when not Nt-acetylated. Consequently, NatC-mediated Nt-acetylation protects these N-termini from UBR-recognition and proteasomal degradation of the protein[39]. In NatC KO cell lines, several NatC substrates are degraded by UBR1, UBR2, or UBR4-KCMF1 due to the lack of Nt-acetylation. Genome-wide unbiased CRISPR KO screening uncovered UBR components as strong positive genetic interactors of NatC subunits, and removal of UBR Ub E3 ligases reversed NatC KO phenotypes, revealing a major role of NatC in shielding proteins from degradation[39].

Normal co-translational protein processing rarely produces proteins recognised by the Arg/N-degron pathway. N-termini like Phe, Leu, Asp, Glu, and Arg are usually not exposed because MetAP only cleaves the iMet if the second amino acid is small. An exception is some Met-hydrophobic N-termini that leave the ribosome non-Nt-acetylated and can be targets for the Arg/N-degron pathway[6]. Instead, many Arg/N-degron pathway targets are generated through post-translational processing by proteases like caspases or signal peptidases[93]. Another notable exception is Met-Cys-starting proteins. After canonical co-translational Met-cleavage, the resulting Cys N-terminus may be

oxidised by ADO (in mammalian cells) or PCOs (in plants), followed by Nt-arginylation and proteasomal degradation by UBRs[93,102–106]. These represent key oxygen-sensing pathways in multicellular eukaryotes, where regulatory proteins such as RGS4/5 (G-protein signalling regulators) in humans and ERF-VII transcription factors in plants are stabilised during hypoxia. Cys N-termini can also be Nt-acetylated by NatA, and NatA-mediated Nt-acetylation and ADO-mediated Nt-oxidation are mutually exclusive modifications[21]. For most Cys-starting proteins, there seems to be no competition between Nt-acetylation and Nt-oxidation due to the substrate specificities of NatA and ADO; Cys-acidic/polar N-termini are preferentially Nt-acetylated, while Cys-aromatic/basic N-termini are oxidised. For a few Cys-starting proteins that are substrates of both NatA and ADO, Nt-acetylation could prevent ADO-mediated oxidation and subsequent arginylation and degradation[21]. In addition, unmodified Cys-starting N-termini could be targeted by the previously mentioned CRL2-ZER1/ZYG11B E3 ligases[97].

NatB-mediated acetylation is suggested to protect certain substrates from proteasomal degradation[107–109]. However, NatB is not considered a main factor in proteostasis, as no global effect on protein stability was observed in NatB-deficient yeast cells[110]. In mice, NatB-mediated acetylation is believed to shield procaspase-8 and -9 from UBR4- and UBR1-mediated degradation, respectively, facilitating apoptosis activation[109]. In *D. melanogaster*, citrate metabolism has been linked to increased protein stability due to increased NatB-mediated Nt-acetylation in male flies[107]. In fruit flies with reduced NatB activity, phenotypes like decreased fertility and spermatid differentiation and increased proteasomal turnover were observed; however, these were rescued by dUBR1 KD[107]. This suggests that NatB protects key proteins involved in fertility and spermatid differentiation from proteasomal degradation, although the involved NatB substrate(s) remain unidentified. In addition, NatB was recently revealed as a regulator of αSyn, and the absence of NatB was shown to increase the proteasomal turnover of αSyn[108]. Ube2w, an E2 Ub-conjugating enzyme, is suggested to be involved in the degradation of non-acetylated αSyn[108]. However, further evidence is needed, as the causality remains unclear, particularly since no physical interaction has been identified between non-acetylated NatB-type N-termini and Ub ligases. In summary, these recent efforts indicate that Nt-acetylation leaves an imprint on the proteome, promoting protein stability. While NatA and NatC are proposed to have a more general role in protein stability, NatB appears to shield only a subset of proteins from degradation.

Conditionally, Nt-acetylated proteins can be recognised by E3 ligases through another N-degron pathway, called the Ac/N-degron pathway[111,112]. Under normal conditions, the Nt-acetylated protein terminus may not be exposed due to folding, interactions with membranes, or other proteins (see above). Aberrant folding, protein complex formation, etc. may expose such Nt-acetylated termini to E3 ligases, which can subsequently target these proteins for degradation. The first Ac/N-degrons were discovered in yeast, where Nt-acetylated Met-, Ala-, Ser-, Thr-, or Val-starting proteins were shown to be recognised by the E3 ligases Doa10 or Not4[111,112]. Ac/N-degron conditionality and shielding by protein complex formation were demonstrated for the *S. cerevisiae* Cog1 subunit of the oligomeric Golgi (COG) complex and the *S. pombe* Hcn1 subunit of the APC/C (anaphase-promoting complex) Ub ligase[111]. A well-known human protein degraded through the Ac/N-degron pathway is the RGS2 protein[113]. RGS2 is a Met-Gln-starting protein that is Nt-acetylated by NatB and only recognised by MARCHF6 (Yeast orthologue: Doa10) in its Nt-acetylated form[113]. MARCHF6 KD stabilised RGS2 expression, while overexpression of MARCHF6 destabilised RGS2. Furthermore, MARCHF6 pulls down RGS2 only in its acetylated form[113]. Overall, this supports that the acetyl group is essential for MARCHF6 recognition. In vivo, RGS2 is fully Nt-acetylated by NatB and forms a complex with $G\alpha_q$-protein, which shields its N-terminus in the complex. Thus, the N-terminus is only exposed and susceptible for degradation when the protein-protein complex is not in stoichiometric balance[113]. Recently, the recognition domain (Ac/N-domain) of MARCHF6, which specifically binds to and mediates the degradation of Nt-acetylated substrates like RGS2 and PLIN2, but not the non-acetylated counterparts, was identified[114].

Combined, the Ac/N-degron, GASTC/N-degron and Arg/N-degron pathways may control a large number of proteins in an Nt-acetylation-dependent manner. While co-translational Nt-acetylation is likely to shield a major part of the proteome from being targeted by the GASTC/N-degron- and Arg/N-degron pathways, the Ac/N-degron pathway may ensure protein quality control and correct protein-protein stoichiometry (Fig. 4)[99].

Cellular and organismal impact of N-terminal acetylation

Nt-acetylation is evolutionarily conserved among eukaryotes[1]. Although a complete understanding of its physiological roles is still lacking, studies in various species have shown that NATs significantly impact cellular function as well as organismal development and physiology. In most cases, we may assume that NAT impairment reduces Nt-acetylation which mediates cellular and physiological effects. However, we cannot exclude the possibility that some effects may arise from non-canonical functions of the NATs, not involving protein Nt-acetylation[6].

NatA is essential in higher eukaryotes, and NatA removal causes lethality in several model organisms such as *T. brucei*, *C. elegans*, *D. melanogaster*, *A. thaliana*, *D. rerio* and human cells[6]. Contrastingly, NatA is dispensable in yeast, but NatA deletion strains have defects in growth, sporulation, mating, transition to G_0 phase and increased stress sensitivity[1,12]. More recently, a multi-level study of *S. cerevisiae* implicated NatA in systemic adaptation control and maintenance of genome integrity[110]. Specifically, NatA activity regulates Sir3- and Orc1-mediated gene silencing and expression of transposons, mitochondrial genes, pheromone response genes and sub-telomeric genes[110]. Yeast cells lacking NatA also showed upregulation of the ubiquitin proteasome system[115]. In *M. musculus* and *D. rerio*, NAA10 is crucial for normal development. Morpholino-mediated KD of zebrafish *naa10* resulted in increased lethality, while surviving morphants had impaired growth and developmental defects[116]. *Naa10* KO mice also displayed developmental abnormalities, including partial embryonic lethality, growth failure, brain defects and cardiac anomalies[117,118]. Surprisingly, *Naa10* KO mice did not show global Nt-acetylome impairment, leading to the discovery of a compensatory paralogue, denoted *Naa12*, which exerts NatA activity when bound to NAA15[118]. Furthermore, mice lacking both *Naa10* and *Naa12* resulted in embryonic death. NatA deficiency in *A. thaliana* causes embryonic lethality[4,119], incomplete endosperm cellularisation[120] and increased drought resistance[4] (Fig. 5A). HYPK is a positive regulator of NatA activity in *A. thaliana* and the rice plant *O. sativa*, with HYPK depletion causing developmental defects and increased stress tolerance, consistent with the phenotypes in NatA-depleted plants[23,27].

NatB is non-essential in yeast, like any other yeast NAT, but NatB deletion strains display the most severe phenotypes, including growth, cytoskeleton and mating defects[28,29]. These phenotypes may result from impaired actin cable formation, as NatB-dependent Nt-acetylation of tropomyosin is a prerequisite for its ability to bind and stabilise actin filaments[28,29]. Similarly, NatB KD in human and mouse cells causes proliferation arrest, impaired cytoskeleton organisation and decreased motility[30,33,109]. Mouse *Naa20* KO cells have reduced extrinsic apoptosis activation, likely due to NatB-mediated Nt-acetylation of procaspase-8, shielding it from UBR-mediated degradation[109]. Multi-omics analyses showed that NatB deletion in *S. cerevisiae* leads to protein aggregate accumulation and stress response induction, indicating a role of NatB in cellular proteostasis[110]. This is supported by findings in *D. melanogaster*, where NatB-mediated Nt-acetylation was found to be essential for spermatid differentiation, possibly by shielding key proteins from

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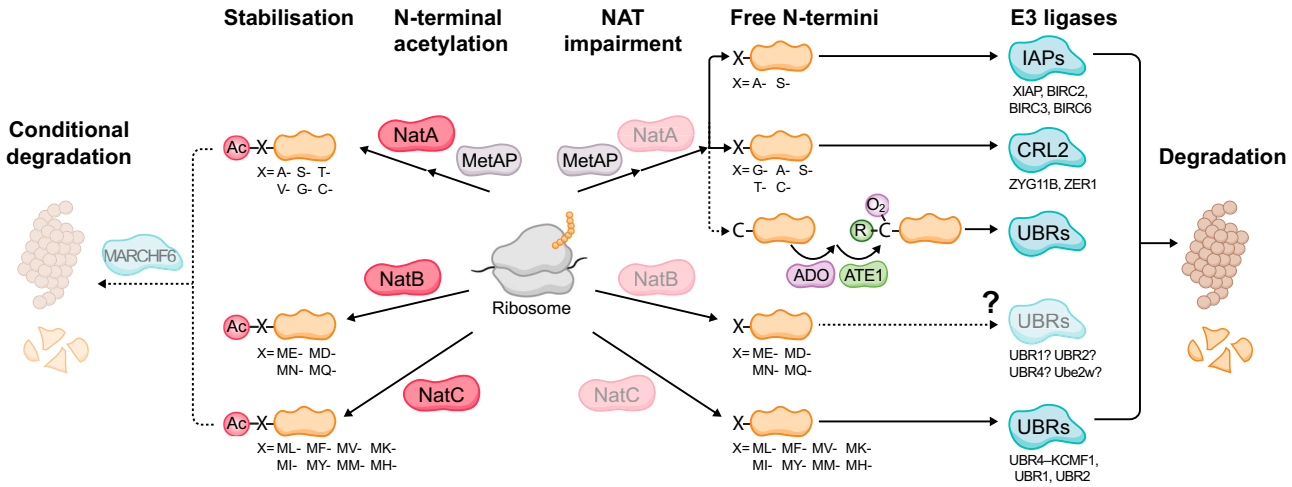

Fig. 4 | Nt-acetylation-dependent regulation of protein stability and degradation. The major co-translational NATs (NatA, NatB and NatC) Nt-acetylate nascent polypeptides at the ribosome. NatA Nt-acetylates small amino acids (Ala, Ser, Thr, Val, Gly, Cys) after the initiator methionine (iMet) is excised by MetAPs[1,20,21]. NatB Nt-acetylates N-termini with a retained iMet followed by Asp, Glu, Asn, or Gln[33]. Similarly, NatC acts on iMet-retained N-termini, but with a hydrophobic or basic amino acid in the second position (Leu, Ile, Phe, Val, Tyr, Met, His, or Lys)[38]. Nt-acetylation imprints protein stability by protecting proteins from proteasomal degradation mediated by ubiquitin (Ub) E3 ligases. However, the Ub E3 ligase MARCHF6 can recognise certain Nt-acetylated N-termini and confer conditional protein degradation through the Ac/N-degron pathway[113], potentially as a protein quality control mechanism. In cells with impaired NAT function, non-acetylated N-termini can be recognised by specific Ub E3 ligases and targeted for proteasomal degradation through the N-degron pathways[93]. In human cells, several Ub E3 ligases recognise non-Nt-acetylated NatA substrates. In the GASTC/N-degron pathway, inhibitor of apoptosis proteins (IAPs), including XIAP, BIRC2, BIRC3 and BIRC6, act on N-termini starting with Ala or Ser[94], while the Cullin 2-RING E3 Ub ligase (CRL2) substrate receptors, ZYG11B and ZER1, recognise Gly, Ala, Ser, Thr, or Cys-starting N-termini[96,97]. Additionally, UBRs can mediate the degradation of Cys-starting proteins through the Arg/N-degron pathway following oxidation by ADO and subsequent arginylation by ATE1[93]. This degradation route is likely less common for non-Nt-acetylated Cys-starting substrates of NatA, as NatA and ADO have distinct substrate preferences[21]. In *A. thaliana*, global proteome destabilisation was observed following NatA depletion, but the responsible Ub E3 ligases are not identified[95]. The Ub E3 ligases UBR4–KCMF1, UBR1 and UBR2 mediate degradation of non-Nt-acetylated NatC substrates[39]. NatB may shield a certain subset of proteins from degradation. Non-Nt-acetylated NatB substrates are potentially recognised by UBR1 in *D. melanogaster*[107] and by UBR1 and UBR4 in mice[109]. Human Ub ligases that recognise non-Nt-acetylated NatB substrates are not established, but Ube2w may be involved in degradation of non-acetylated αSyn in human cells[108].

dUBR1-mediated degradation[107]. Loss of NatB in *A. thaliana* is associated with pleiotropic developmental defects and growth retardation (Fig. 5A)[31,121]. Unlike the drought resistant NatA-deficient plants, NatB-deficient plants show increased sensitivity to drought, high osmolarity, high salinity and reductive stress, indicating NatB is essential for abiotic stress tolerance[31,122]. NatB is also implicated in pathogen resistance in plants[31,123]. A study found that NatA and NatB antagonistically regulate two proteoforms of the plant immune receptor SNC1, with NatA destabilising and NatB stabilising SNC1, affecting the immune response[123]. Recently, NatB was proposed to play a role in viral infection in yeast and human cells, where NatB activity facilitates Influenza A virus's suppression of host gene expression and viral polymerase activity[124].

NatC is believed to exert important roles in organellar biology and energy regulation. In yeast, all three NatC subunits, NAA30, NAA35 and NAA38, are necessary for NatC-mediated Nt-acetylation[34]. Deletion of any yeast NatC subunits produces slow growth phenotypes, but unlike yeast NatA and NatB deletions, mating efficiency remains unaffected[34]. In contrast, the auxiliary subunits of NatC in *A. thaliana* are dispensable, as only the removal of NAA30, but not NAA35, impairs photosynthesis and growth (Fig. 5A)[88]. In *C. elegans*, NatC regulates the balance between reproductive growth and stress tolerance in response to nutrients and stressors[125,126]. NatC deficiency in human cells disrupts mitochondrial morphology and function and increases lysosomal content and cell granularity[38,39]. These phenotypes are likely linked to NatC's role in shielding the proteome from degradation, as they were reversed by UBR KD[39]. Furthermore, these findings were supported by studies in *D. melanogaster*, where NatC was shown to be essential for longevity, fertility and prevention of age-dependent motility loss[39]. The decreased longevity and motility in NatC deletion flies were rescued by muscle-specific overexpression of UbcE2M, the fruit fly homologue of the human NEDD8-conjugating enzymes UBE2M and UBE2F, which are degraded when lacking NatC-mediated Nt-acetylation. This supports a role of NatC in protecting against protein degradation and in normal muscle development in *D. melanogaster* (Fig. 5B)[39].

NatD-mediated Nt-acetylation of histone H2A and H4 is implicated in the regulation of cellular metabolism. Initial yeast studies showed only minor growth defects in *naa40*Δ strains under certain conditions[127]. A later study found that calorie restriction down-regulated yeast *naa40* and Nt-acetylation of histone H4, resulting in increased levels of H4 Arg3 methylation and induction of stress response genes such as PNC1, promoting yeast longevity. This implicates a role for naa40 in regulating cellular lifespan in yeast (Fig. 5C)[128,129]. In mouse hepatocytes, *Naa40* depletion increased cellular Ac-CoA levels, resulting in lipid synthesis induction and impaired insulin signalling[130]. In agreement with these findings, *NAA40* KD in *D. melanogaster* larval fat body, which is analogous to the mammalian liver, also coincided with increased lipid synthesis[130]. In the model insect *T. castaneum*, *NAA40* KD upregulated genes involved in lipid biosynthesis and arrested larval development, possibly due to epigenetic modulation of the steroid hormone ecdysone during metamorphosis[131]. Together, these studies highlight a role of NAA40 in lipid metabolism, although further studies are needed to fully understand the molecular mechanisms involved.

Although NAA50 (NatE) interacts with NatA, it has a different substrate specificity and loss of NAA50 produces distinct cellular phenotypes. In human and *D. melanogaster* cells, NAA50 removal causes aberrant sister chromatid cohesion, mitotic arrest and chromosome segregation defects[46,132,133]. This phenotype might be conserved among metazoans, as no phenotypes are observed in *naa50*Δ yeast strains and yeast NAA50 is likely catalytically inactive[14,54]. In *A. thaliana*, loss of NAA50 causes severe growth impairments and infertility (Fig. 5A)[51,134]. Moreover, NAA50 depletion renders plants hypersensitive to abscisic acid and osmotic stress, but increases pathogen

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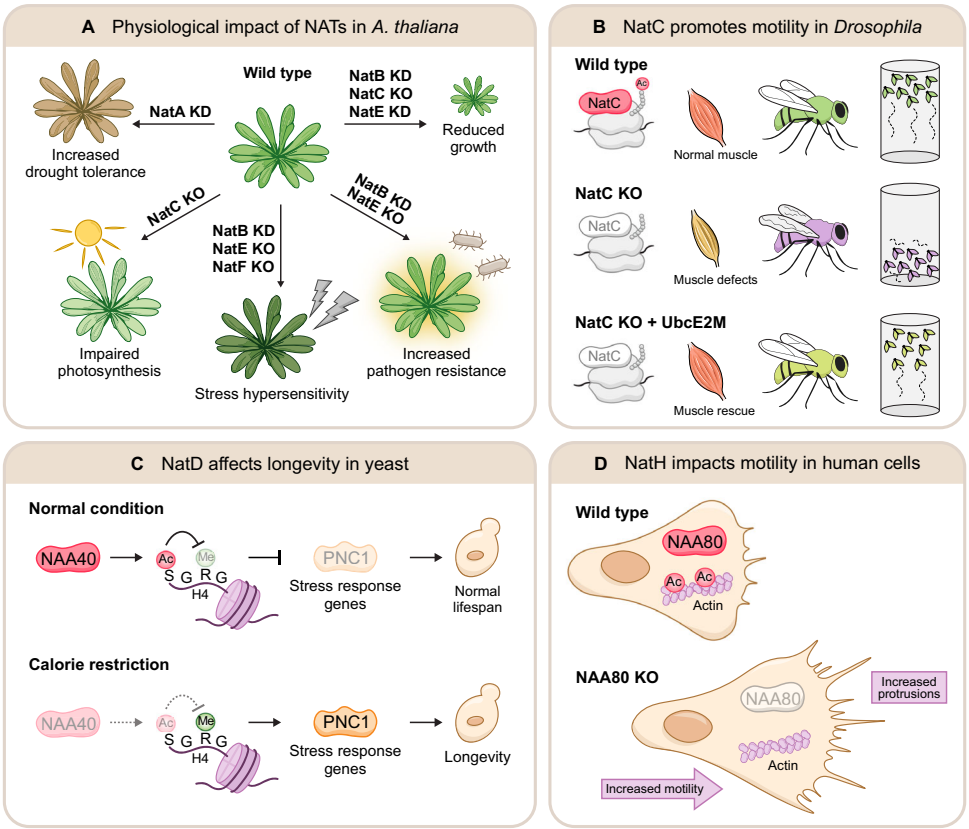

Fig. 5 | The cellular and organismal impact of Nt-acetylation. A Summary of the physiological impact of NATs in *A. thaliana*[57]. **B** NatC prevents age-dependent motility loss in *D. melanogaster*. NatC KO fruit flies exhibit muscle developmental defects and motility loss, and these phenotypes are rescued upon UbcE2M overexpression[39]. **C** In yeast, NatD-mediated Nt-acetylation (Ac) of histone H4 normally antagonises the methylation (Me) of H4 Arg3, which regulates yeast lifespan. Under calorie restriction, yeast NatD and Nt-acetylation of histone H4 are downregulated, resulting in increased levels of H4 Arg3 methylation and induction of stress response genes such as PNC1, which promotes yeast longevity[128]. **D** Post-translational Nt-acetylation of actin mediated by NAA80 in human cells affects cytoskeletal dynamics. Human NAA80 KO cells lacking Nt-acetylation of actin display more cell protrusions and increased cell motility, implicating NAA80 as a cell migration regulator[11]. KD knockdown, KO knockout.

resistance, suggesting NAA50 plays an important role in plant development and stress responses[51,53,134].

Similarly to NAA50, *NAA60* (NatF) KD in *D. melanogaster* induces chromosomal segregation defects during anaphase but displays normal metaphase, unlike the mitotic arrest observed in NAA50-depleted cells[55]. In HeLa cells, *NAA60* KD caused Golgi ribbon fragmentation[9], possibly linked to the impaired segregation phenotype, as Golgi fragmentation occurs in a controlled fashion during mitosis. Since NAA60 is Golgi-localised in human cells and Nt-acetylates transmembrane proteins, the fragmented Golgi phenotype may indicate that NAA60 itself or some of its transmembrane substrates are important for Golgi integrity[9]. NAA60 also promotes influenza A virus infection, likely by suppressing IFNα and IFN-stimulated genes[135]. Interestingly, the *A. thaliana* NAA60 orthologue is anchored exclusively to the plasma membrane and mediates high salt stress adaptation (Fig. 5A)[10].

While Nt-acetylation occurs on 20-30% of all plastid proteins[57], the physiological roles of the plastid-NatG family are not fully understood and their dual KAT and NAT function adds an extra layer of complexity. Studies of GNAT2 (NAA90) in *A. thaliana* showed that GNAT2 is required for state transitions and pathogen resistance, but likely through its lysine and serotonin acetyltransferase activity[57].

The most recently identified NAT, NAA80 (NatH), is exclusively found in animals where it post-translationally modifies cytosolic actins[11]. Since actins are NAA80's only known substrates, phenotypes resulting from NAA80 impairment likely result from lack of actin Nt-acetylation. Human *NAA80* KO cells exhibit several cytoskeleton-related phenotypes, including increased cell protrusions and accelerated motility, pointing to NAA80 as an essential cell migration regulator (Fig. 5D)[11]. Additionally, *NAA80* KO cells display increased F-actin levels and Golgi fragmentation, linked to NAA80's ability to modify actin, as reintroducing active NAA80 rescued the phenotype[136]. Interestingly, a *D. rerio naa80* KO model lacking Nt-acetylated actin showed no obvious effects on zebrafish viability, development, or behaviour[63]. However, *naa80*-depleted zebrafish had aberrant inner ear development and impaired hearing[63], consistent with hearing loss in humans with *NAA80* variants[137], suggesting *naa80* is important for normal hearing. A recent study suggested NAA80 plays a role in viral infection, where NAA80 acts as a host factor by promoting viral replication for several viruses such as Enterovirus 71[138].

Relevance for human disease

The NAT machinery plays an important role in human development and physiology, and aberrant Nt-acetylation is associated with human pathologies such as cancer, developmental syndromes and neurodegenerative diseases.

NATs in genetic disease

Pathogenic variants in different NAT genes can cause rare genetic diseases in humans. The lethal X-linked disorder Ogden syndrome, caused by a *NAA10* p.(Ser37Pro) variant, was first described in 2011[139]. The eight affected boys from two families exhibited developmental delay, aged appearance, hypotonia, craniofacial anomalies and cardiac arrhythmias, and died within 2 years of life. Biochemical studies revealed that the p.(Ser37Pro) variant had

reduced catalytic activity and impaired complex formation with both NAA15 and NAA50[139,140].

In the succeeding years, several pathogenic variants in *NAA10* and *NAA15*, encoding the NatA subunits, were identified as causes of congenital diseases in both males and females. These conditions are collectively known as *NAA10*- and *NAA15*-related syndromes[141–145]. Affected individuals exhibit phenotypic heterogeneity with regard to clinical features and severity, often including developmental delay, intellectual disability and cardiac anomalies[145]. Recent studies have used induced pluripotent stem cells (iPSCs) to investigate heart defects caused by *NAA10* and *NAA15* variants. iPSCs with NAA15 haploinsufficiency showed moderately impaired Nt-acetylation levels and reduced protein levels for many proteins, including ribosomal proteins. Four of these downregulated proteins are previously associated with autosomal dominant congenital heart disease, potentially contributing to the heart disease caused by defective NAA15[143]. In iPSC-derived cardiomyocytes expressing *NAA10* variants, long QT syndrome was linked to abnormal Cav1.2 channel gating properties[146]. Nevertheless, the molecular mechanisms underlying *NAA10*- and *NAA15*-related syndromes remain poorly understood. The phenotype variability may be influenced by several factors, such as genetic background differences, tissue-specific effects caused by skewed X-chromosome inactivation in females and the impact of different variants on the function and stability of numerous NatA substrates.

In recent years, pathogenic variants in other NAT genes have emerged. Biallelic *NAA20* variants were found in seven individuals from three families with a neurodevelopmental disorder, named *NAA20*-related syndrome[147,148]. These individuals exhibited overlapping phenotypes, including intellectual disability, developmental delay and microcephaly, with variable occurrence of congenital heart defects, ataxia, and epilepsy. Biochemical assays indicated that the *NAA20* variants impaired NatB complex formation and NatB-mediated Nt-acetylation.

A heterozygous nonsense variant in the *NAA30* gene was identified in an individual presenting with global developmental delay, autism spectrum disorder and a tracheal cleft[149]. These clinical manifestations were linked to aberrant NatC-mediated Nt-acetylation due to truncation of the catalytic subunit NAA30.

NAA60 was recently established as a causal gene for autosomal recessive primary familial brain calcifications (PFBC), a neurodegenerative disorder characterised by abnormal calcium deposition in the brain[150]. Ten individuals from seven families with autosomal recessive PFBC harboured biallelic missense variants or deletion variants in the *NAA60* gene. These variants attenuated NAA60-mediated Nt-acetylation, and the disease mechanism involved impaired cellular phosphate homeostasis associated with the loss-of-function *NAA60* variants[150].

A homozygous *NAA80* p.(Leu130Pro) variant affecting the actin NAT was identified in two brothers with high-frequency sensorineural hearing loss, developmental delay, muscle weakness and craniofacial dysmorphisms[137]. The *NAA80* variant showed reduced Nt-acetylation of actin subtypes and impaired actin dynamics in patient-derived cells. Interestingly, individuals with pathogenic β-actin and γ-actin variants display similar phenotypes, suggesting that the clinical features are linked to disrupted actin function[137].

Deficient Nt-acetylation by the major NATs—NatA, NatB and NatC—leads to overlapping global phenotypes, despite having different classes of substrates[1,33,38]. This may be attributed to their large substrate pools, potentially affecting thousands of proteins, resulting in pleiotropic effects and broad phenotypes like intellectual disability and developmental delay. In contrast, pathogenic variants in *NAA60* and *NAA80* appear to cause more specific clinical features[137,150], reflecting their specialised roles with limited substrate pools comprising transmembrane proteins and actins, respectively[9,11,59,60]. An overview of pathogenic NAT variants and associated phenotypes is shown in Fig. 6. So far, no disease-causing variants have been described in the catalytic subunits NAA40 (NatD) or NAA50 (NatE).

NATs in cancer

Several of the NATs are suggested to play critical roles in cancer development and progression. NATs are prevalently found upregulated in various types of cancers and are recognised as potential prognostic markers and therapeutic targets in cancer treatment.

Overexpression of NAA10 has been observed in multiple types of cancer and is associated with overall survival rates and disease recurrence in patients[151]. NAA10 is believed to play a key role in cancer cell proliferation and survival by modulating cellular processes such as cell cycle progression, migration, apoptosis and autophagy[152–158]. Increased NAA10 expression correlates with tumour aggressiveness and metastasis in lung[159], prostate[160,161], liver[162], renal[163] and colorectal cancer[164,165], indicating an oncogenic role. Conversely, NAA10 may act as a tumour suppressor in breast cancer and oral squamous cell carcinoma, where increased NAA10 expression correlates negatively with lymph node metastasis and positively with patient survival[166–168]. While the functions of NAA10 in malignancies are not well understood, several mechanisms have been proposed, some of which involve non-canonical roles of NAA10[151]. Depletion of NatA-mediated Nt-acetylation induces growth inhibition and apoptosis in cervical, colon and thyroid cancer cells. Furthermore, NatA KD sensitises cancer cells to drug treatment[169,170].

Early studies showed that NatB is essential for cell proliferation and survival in cancer cell lines, implicating its role in tumorigenesis[30,171]. Moreover, NAA20 was found upregulated in both a hepatocellular carcinoma (HCC) mouse model and HCC patients[171]. These findings were supported by recent studies, which proposed that the oncogenic role of NAA20 in HCC involves the regulation of autophagic and proliferative signalling pathways[172,173]. In triple-negative breast cancer, increased NAA20 expression correlates with poor patient survival and NAA20 depletion decreased cancer cell growth, migration and invasion[174]. Analogous to NAA20, NAA25 was found overexpressed in breast cancer and *NAA25* KD was correlated with apoptosis and decreased cell growth[175].

NatC KD has been shown to cause growth arrest and p53-dependent apoptosis in cancer cells, suggesting NatC is crucial for cancer cell proliferation and survival[35]. Notably, NAA30 is upregulated at the protein level in glioblastoma and glioblastoma-initiating cells (GICs)[176]. In vitro and in vivo analyses demonstrated that NAA30 depletion reduced cell growth and viability of GICs, possibly via the p53 pathway, indicating an important role of NAA30 in glioblastoma tumorigenesis.

Overexpression of the histone NAT NAA40 is associated with tumour growth and metastasis in various cancers, including breast[177], lung[178], liver[179] and colorectal cancer[180] and negatively impacts patient survival. NAA40 is considered a critical epigenetic modulator in cancer progression and may influence chemoresistance[177,178,181].

A pan-cancer analysis indicated NAA50 as an oncogene overexpressed in many cancers, including lung adenocarcinoma, with potential implications for cell proliferation and immune cell infiltration[182].

NATs in neurodegenerative diseases

As mentioned, pathogenic *NAA60* variants and thus impaired Nt-acetylation of transmembrane proteins, may cause the neurodegenerative disease PFBC with Parkinson's like symptoms[150]. Furthermore, aggregation of the neuronal protein αSyn plays a crucial role in the pathogenesis of Parkinson's disease and other synucleinopathies[71,183]. αSyn is found Nt-acetylated in brain tissue, and its N-terminus is likely a substrate of NatB. Nt-acetylation of αSyn affects its stability, aggregation process and neurotoxicity[71,184]. A recent study demonstrated that NatB is a strong regulator of endogenous αSyn in human cell lines[108]. Loss of NatB-mediated Nt-acetylation resulted in decreased stability of non-acetylated αSyn, while the degradation of αSyn might be rescued by depletion of the E2 Ub-conjugating enzyme Ube2w. Thus, these findings implicate that NatB has an indirect role in Parkinson's disease pathogenesis through its regulation of αSyn, and

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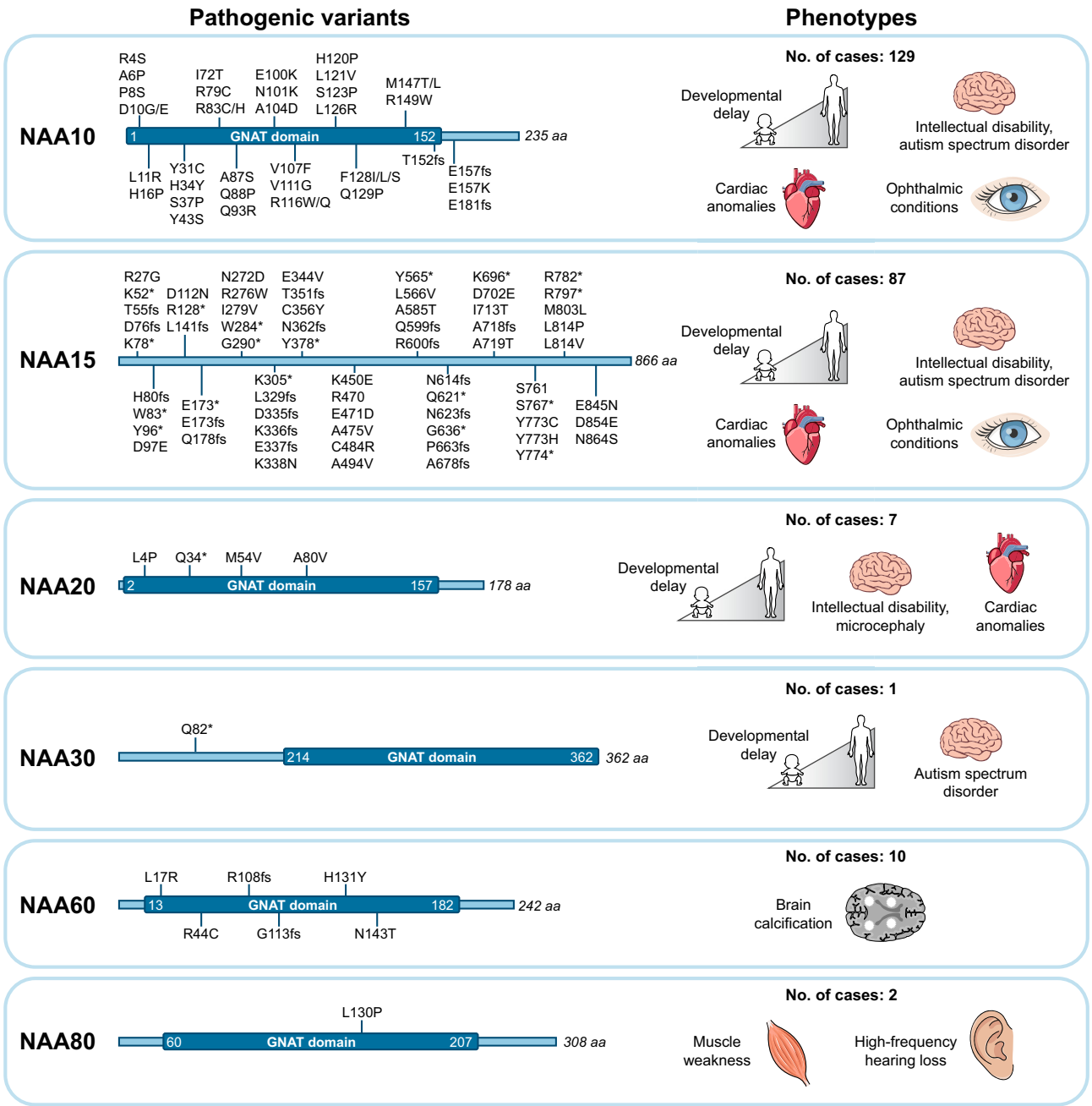

Fig. 6 | Overview of pathogenic NAT variants. Schematic representation of the pathogenic variants identified in various NAT subunits, their associated phenotypes and the number of affected individuals. Impairment of the major co-translational NATs (NatA-NatC) results in severe and general phenotypes[141–145,147–149]. In contrast, impairment of the specific post-translational NatF (NAA60) and NatH (NAA80) is linked to brain calcification[150] or abnormal hearing and muscles[137], respectively. The Gcn5-related N-acetyltransferase (GNAT) domains of the catalytic NAT subunits are shown in dark blue.

targeting NatB-mediated Nt-acetylation of αSyn could be a potential therapeutic strategy[108]. Huntington's disease is a neurodegenerative disorder linked to the aggregation of the Htt protein. In vitro work demonstrated that Htt is a substrate of NatA[75], but as described earlier, there are conflicting results regarding the impact of Nt-acetylation on Htt and its aggregation propensity[22,75]. Thus, NatA-mediated Nt-acetylation may impact Huntington's disease by affecting Htt aggregation, but the mechanism remains unclear.

Conclusions and outstanding questions

The machinery responsible for Nt-acetylation in eukaryotes is likely fully or nearly fully identified (Fig. 2). However, additional NATs may exist, potentially targeting distinct substrates not covered by the general multisubstrate NATs or specifically operating in organelles such as mitochondria. While the structures and *modus operandi* for most of these enzymes have been determined, there is limited knowledge on how this modification is regulated at the gene or post-translational level. Although Nt-acetylation is considered irreversible, it is possible that a hitherto unidentified N-terminal deacetylase (NDAC) could exist to regulate specific proteins.

The high abundance and patterns of Nt-acetylation in representative eukaryotic species are defined, but we only have direct mass spectrometry evidence for the Nt-acetylation of a minor fraction of the proteome[1,5,9,33,47]. Thus, increased coverage could uncover sub-patterns and substrates of special interest. The molecular roles of Nt-acetylation are diverse, with many different functions identified for

single proteins (Fig. 3). Recent investigations uncovered that protein degradation shielding may be a major constitutive function of Nt-acetylation in the plant and animal kingdoms[39,94,95]. Additionally, Nt-acetylation may create conditional degradation signals[93,111,112]. The different functions of Nt-acetylation can likely be causally linked for several Nt-acetylated proteins. For instance, the lack of Nt-acetylation may cause aberrant folding, hinder proper complex formation or proper membrane binding. This, in turn, may expose the non-acetylated N-terminus of the uncomplexed protein, which may be recognised by Ub E3 ligases and marked for degradation. Since the full range of molecular effects of Nt-acetylation is rarely known for each specific protein, it is often difficult to separate direct effects from indirect effects. A more comprehensive understanding of these events would be beneficial. Furthermore, identifying which Ub E3 ligases recognise which N-terminal sequences, with or without Nt-acetylation, is essential to fully comprehend the dynamics between NATs and Ub E3 ligases.

Nt-acetylation is essential for the function of many proteins, as reflected in the vital roles of NATs in physiology. NAT impairment in humans causes severe and general phenotypes for the major co-translational enzymes NatA-NatC[141–145,147–149], while brain calcification or abnormal hearing and muscles are observed for the specific post-translational enzymes NatF and NatH, respectively[137,150]. For cases where we observe specific (rather than general) phenotypes after NAT impairment, investigating the underlying molecular mechanisms could uncover important substrates and cellular pathways, enhancing our understanding of Nt-acetylation. Increased NAT expression is associated with poor prognosis in many cancers, and developing NAT inhibitors represents a promising avenue for potential anti-cancer treatment. While selective NAT inhibitors, such as bisubstrate analogues, have been developed for some enzymes[59,185,186], suitable small molecule drug-like inhibitors have yet to be developed. Overall, continued research into the regulatory mechanisms and dynamics of Nt-acetylation will be essential for enhancing our understanding of this pivotal protein modification as well as uncovering potential therapeutic targets.

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Competing interests

The authors declare no competing interests.

Additional information

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