Review

Protein N-terminal acetyltransferases: when the start matters

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The majority of eukaryotic proteins are subjected to N-terminal acetylation (Nt-acetylation), catalysed by N-terminal acetyltransferases (NATs). Recently, the structure of an NAT–peptide complex was determined, and detailed proteome-wide Nt-acetylation patterns were revealed. Furthermore, Nt-acetylation just emerged as a multifunctional regulator, acting as a protein degradation signal, an inhibitor of endoplasmic reticulum (ER) translocation, and a mediator of protein complex formation. Nt-acetylation is regulated by acetyl-coenzyme A (Ac-CoA) levels, and thereby links metabolic cell states to cell death. The essentiality of NATs in humans is stressed by the recent discovery of a human hereditary lethal disease caused by a mutation in an NAT gene. Here, we discuss how these recent findings shed light on NATs as major protein regulators and key cellular players.

NATs: in the middle of everywhere

The majority of all cytoplasmic eukaryotic proteins are subjected to Nt-acetylation (see Glossary) [1,2]. Nt-acetylation is catalysed by a set of enzyme complexes, the NATs. NATs transfer an acetyl group from Ac-CoA to the α-amino group of the first amino acid residue of a protein (Figure 1). During the past decade, researchers have described how these enzymes function in a complex and specific system of Nt-acetylation. The combination of detailed *in vitro* knowledge and recently developed powerful proteomic tools for studying *in vivo* global Nt-acetylation states has yielded new insights into the evolution and molecular functions of the NATs. Furthermore, the first structure of a eukaryotic NAT was recently resolved, showing an NAT enzyme bound to its peptide substrate [3] and providing an understanding of the sequence-specific acetylation of protein N termini.

Recent studies have revealed several specific molecular functions for Nt-acetylation, including regulation of protein degradation through recruitment of ubiquitin ligases [4], prevention of protein translocation from the cytosol to the ER [5], mediating protein complex formation [6] and membrane attachment of small GTPases involved in organelle trafficking [7].

Through its substrate, Ac-CoA, acetylation is connected to cellular metabolism. Ac-CoA is a key metabolite that acts as a carbon-source rheostat regulating the acetylation of histones by lysine acetyltransferases (KATs), and thereby induces expression of growth genes [8]. Interestingly, Ac-CoA has also been shown to act as a signalling molecule by regulating the degree of Nt-acetylation of apoptosis-regulatory proteins [9], thus opening an exciting link between cellular metabolic states, Nt-acetylation and cell death.

Studying Nt-acetylation by positional proteomics has yielded the unexpected findings that Nt-acetylation occurs both co- and post-translationally [10,11]. Accumulating evidence supports a potential role of NATs in catalyzing lysine acetylation [12], making these enzymes combined NATs and KATs [1,13]. Furthermore, NATs can also act as cellular regulators independent of their catalytic activities [14,15], implying that NATs might thus act as moonlighting proteins.

The first human genetic disorder caused by a mutation in an NAT gene was reported in 2011. Individuals suffering from the associated Ogden syndrome have global developmental delays and die during infancy. This further highlights the general biological importance of NATs and Nt-acetylation [16].

Here, we overview the recent knowledge on the molecular functions of NATs and Nt-acetylation. These include the multiple biochemical activities of NATs, their structural characterisation and insights gained from proteome-wide

Glossary

Ac-CoA: acetyl-coenzyme A
KAT: lysine acetyl transferase. An enzyme that catalyses the addition of an acetyl group to the N-ε-amino group of lysines.
NAA10-60: protein N-α-acetyltransferase 10-60 gene. The genes encoding the NAT proteins.
Nt-acetylation: protein N-α-acetylation. Addition of an acetylgroup to the N-α-amino group of a polypeptide (Figure 1).
Naa10-60p: protein N-α-acetyltransferase 10-60 protein. The individual subunits of the NATs. NatA subunits are named from Naa10–15, NatB subunits are named from Naa20–25 and so forth (Figure 2).
NAT: N-terminal acetyltransferase. An enzyme or enzymatic complex that catalyses Nt-acetylation.
NatA–F: N-terminal acetyltranferase A–F. Different types of NATs, named from A to F (Figure 2).

Prefixes are used to indicate species, for example hNatC is human NatC, and yNaa10p is yeast Naa10p

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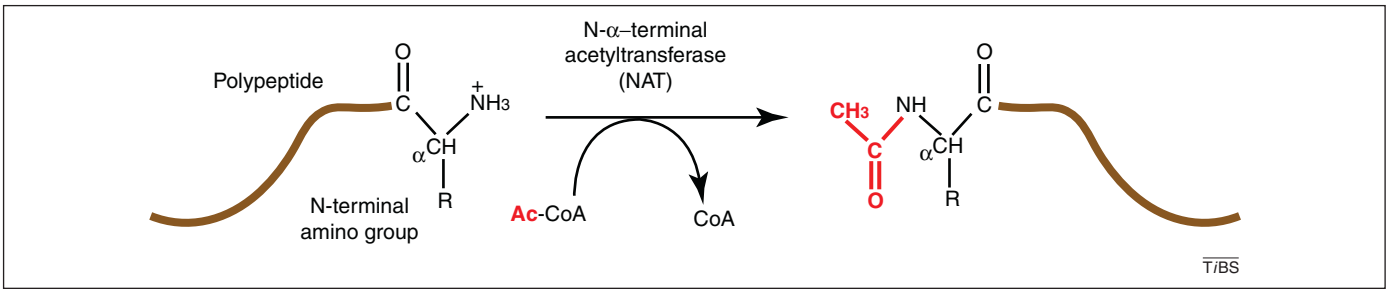

Figure 1. Nt-acetylation. An NAT acetylates the N-terminal α-amino group of a protein or polypeptide by transferring an acetyl moiety (Ac) from Ac-CoA. The acetylation removes the positive charge of the N-terminal amino group, thus changing the chemical properties of the protein N terminus.

Nt-acetylation profiles. We further discuss how recent findings in the field of Nt-acetylation and NATs underpin the NATs as cardinal players in the cell.

Evolutionary development of Nt-acetylation

Nt-acetylation occurs in all kingdoms of life. In eubacteria, Nt-acetylation is rare and has been mainly linked to ribosomal proteins, stress response and to the expression of ectopic proteins in *Escherichia coli* [17,18]. The acetyltransferases RimI, RimJ and RimL in *Salmonella enterica* serovar Typhimurium and YhY in *E. coli* are believed to be homologues of eukaryotic NATs [19,20]. In the haloarchaea *Halobacterium salinarum* and *Natrosomonas pharaonis* both cytoplasmic and membrane proteins are N-terminally processed by aminopeptidases and NATs. Fourteen to nineteen percent of haloarchaeal N termini are Nt-acetylated. Nt-acetylation is mainly restricted to serine and alanine residues, thus resembling the specificity of eukaryotic Nt-acetyltransferase complex A (NatA) [21]. The *Sulfolobus solfataricus* ssArd1, a homologue of eukaryotic NatA component Naa10p, displays more promiscuous substrate specificity than eukaryote NatA complexes [22]. An evolutionarily conserved over-representation of serine or alanine in the second position of proteins in *E. coli*, Archaea, lower and higher eukaryotes [10] suggests that NATs may have developed from an Archaean NatA-like complex, and evolved to more restricted functions during evolution [22].

In the lower eukaryote *Saccharomyces cerevisiae*, 50–70% of all proteins are Nt-acetylated, whereas 70–90% of proteins in human cell lines are Nt-acetylated [1,20,23]. For the plant kingdom, Nt-acetylation is highly abundant, with 70–75% of proteins being Nt-acetylated in *Arabidopsis thaliana* [24,25]. Interestingly, in *Arabidopsis*, proteins can be post-translationally Nt-acetylated after import into the chloroplast, once their targeting sequence is cleaved off [25]. No NAT has been ascribed to catalyse the Nt-acetylation of proteins in the chloroplast.

The eukaryotic NAT-machinery

A group of NATs, NatA–E, perform most Nt-acetylations in eukaryotes. These NATs differ both in subunit composition as well as in substrate specificity, and combined, they act on the majority of eukaryotic proteins (Figures 2 and 3). The NatA–E enzymes and their substrate specificities seem to be at least partially conserved throughout eukaryotic evolution [1,25–29]. Higher eukaryotes also express NatF, which, due to its distinct substrate preference, is responsible for an overall increase in protein acetylation observed in higher eukaryotes when compared to lower eukaryotes [23].

NatA

NatA is composed of the catalytic subunit Naa10p (Ard1p) and the auxiliary subunit Naa15p (Nat1p) (Figure 2). NatA is conserved from lower to higher eukaryotes both with respect to subunit composition and substrate specificity [1]. NatA acetylates Ser-, Ala-, Thr-, Gly-, Val- and Cys- N termini after the initiator methionine is removed by methionine aminopeptidases (Figure 3) [1,30,31]. These N termini are over-represented in eukaryotes, therefore, NatA is the major NAT in view of the overall number of its potential substrates (Figure 3).

In yeast, deletion of the Naa10p or Naa15p subunits gives identical phenotypes: slow growth on nonfermentable carbon sources, failure to sporulate, failure to enter G_0 phase, decreased survival after heat shock, and sensitivity to various chemical stressors [32]. Interestingly, in human cell lines, knockdown of hNaa10p and hNaa15p gives both increased and decreased growth, and pro- and antiapoptotic effects [9,33] (this is further discussed below).

Naa10p and Naa15p are associated with ribosomes, suggesting a model where NatA performs cotranslational acetylation of nascent polypeptides [34,35]. Indeed, in yeast it was shown that yNaa15p anchors the NatA complex to the ribosome [35]. However, in humans, large fractions of hNaa10p and hNaa15p are also found to be nonribosomal, and thus might have functions other than cotranslational Nt-acetylation. Recently, it was shown that hNaa10p post-translationally acetylates β-actin and γ-actin independent of an hNaa10p–hNaa15p complex. Interestingly, these actin N termini are acidic, pointing to a different substrate specificity of hNaa10p when acting independently of hNaa15p [36]. Whether the change in substrate specificity of hNaa10p with or without hNaa15p is a general feature will be a question for future studies.

Higher eukaryotes display a more complex system of NatA subunits than found in lower eukaryotes. Gene duplications of both of h*NAA10* and h*NAA15* have resulted in the orthologues *NAA11* (*ARD2*) and *NAA16* (*NAT2*), respectively, both of which make functional gene products that might form different active hNatA complexes [37]. Thus, there are at least four possible variants of the hNatA complex, with the hNaa10p–hNaa15p dimer considered to be the most abundant [37]. Alternative splicing further produces several distinct mammalian Naa10p variants, adding to NatA complexity [38].

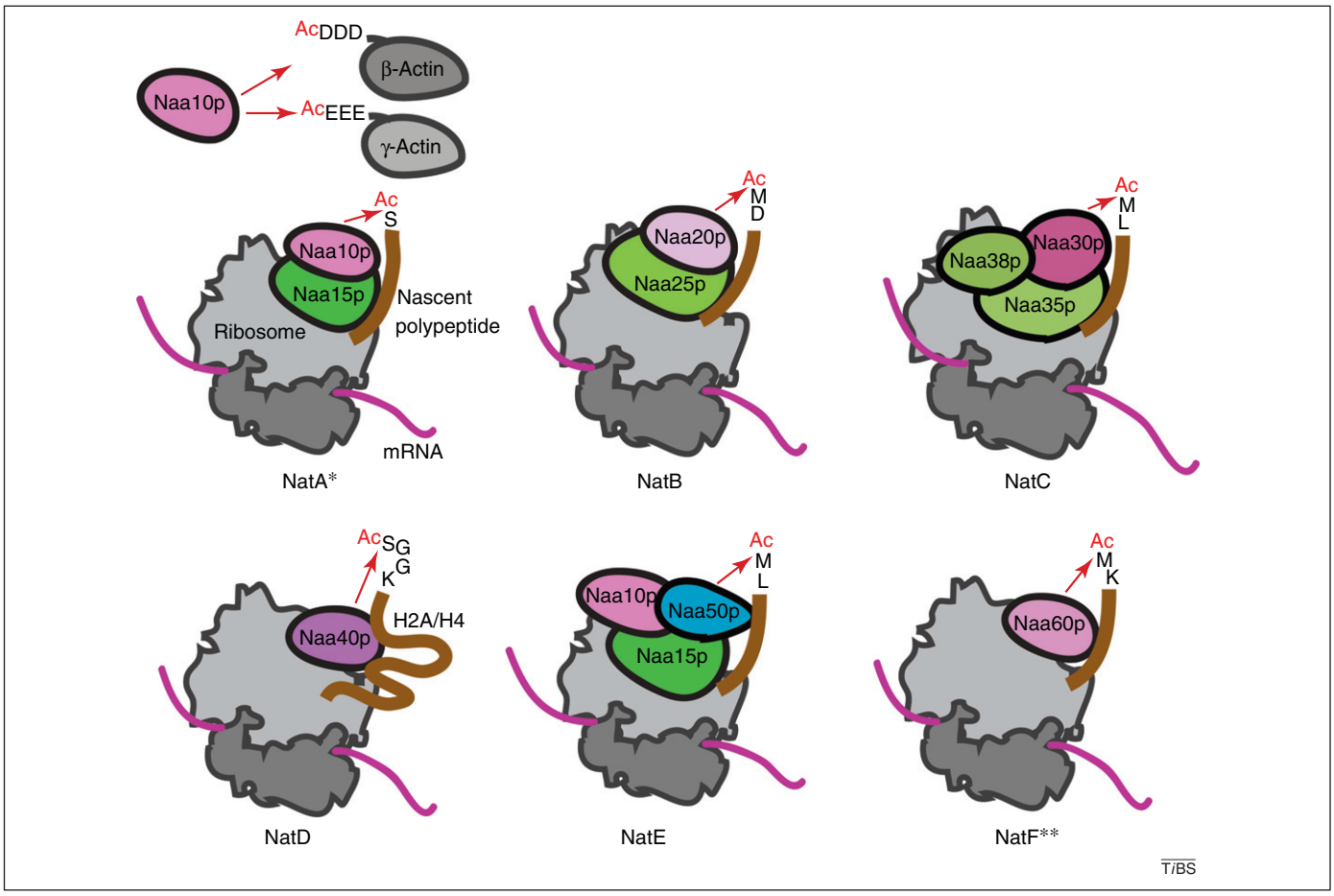

Figure 2. The eukaryotic NAT types. Subunit composition and substrate specificity of the eukaryotic NAT types are conserved from yeast to humans. The NATs are found on the ribosome in both humans and yeast. In humans, they are also found in nonribosomal forms. * In humans, Naa10p also acetylates β- and γ-actin post-translationally. **NatF is only present in higher eukaryotes.

NatA has several different interaction partners. The Huntingtin interacting protein K (HYPK) interacts with hNatA, potentially on the ribosome, where it affects Nt-acetylation of a subset of NatA substrates. Interestingly, depletion of HYPK, hNaa10p or hNaa15p increases the tendency for aggregation of Huntingtin, the cause of Huntington's disease [39]. hNaa10p has also been found to interact with hypoxia-inducible factor (HIF)-1α, and this interaction is proposed to inhibit hNaa10p-mediated activation of β-catenin transcriptional activity [40].

NatB

The NatB complex consists of the catalytic subunit Naa20p (Nat3p) and the auxiliary subunit Naa25p (Mdm20p) (Figure 2). This complex is conserved from yeast to humans, both with respect to subunit composition and substrate specificity. NatB subunits are ribosome-associated in humans and yeast, but in humans a large portion of the NatB subunits are also present in a nonribosomal form. NatB acetylates the N-terminal methionine of substrates with Met-Asp-, Met-Glu-, Met-Asn- or Met-Gln- N termini (Figure 3) [26,31,41,42].

Deletion mutants y*NAA20*-Δ (*nat3*-Δ) and *yNAA25*-Δ (*mdm20*-Δ) show slow growth, the inability to form functional actin cables, defects in vacuolar and mitochondrial inheritance and random polarity of budding [32]. Indeed, actin and tropomyosin require NatB-mediated Nt-acetylation for proper function in terms of actomyosin regulation and actin–tropomyosin binding [42]. In human cells, depletion of hNaa20p and hNaa25p leads to cell cycle arrest [26], and depletion of hNaa20p leads to p53 activation and apoptotic sensitisation [41].

NatC

The NatC complex is composed of the catalytic subunit Naa30p (Mak3p) and the auxiliary subunits Naa35p (Mak10p) and Naa38p (Mak31p) (Figure 2). In yeast, all three subunits are found on the ribosome, whereas in humans, they are also found in nonribosomal forms [27,43]. NatC acetylates the N-terminal methionine of substrates starting with Met-Leu-, Met-Phe-, Met-Ile- and Met-Trp- (Figure 3) [27,43,44]. Mutation of yNatC subunits gives identical analogous phenotypes as for yNatA and yNatB depletion, including diminished growth at 37 °C in media without fermentable carbon sources, lack of maintenance or assembly of the viral L-A dsRNA viral particle *gag*, and telomere elongation [43,44]. In human cell cultures, depletion of hNatC subunits leads to reduced viability and p53-dependent apoptosis [27].

NatD

NatD consists of the catalytic unit Naa40p (Nat4) (Figure 2) [29,45,46]. Naa40p differs conceptually from most other NATs in two respects. First, only two substrates

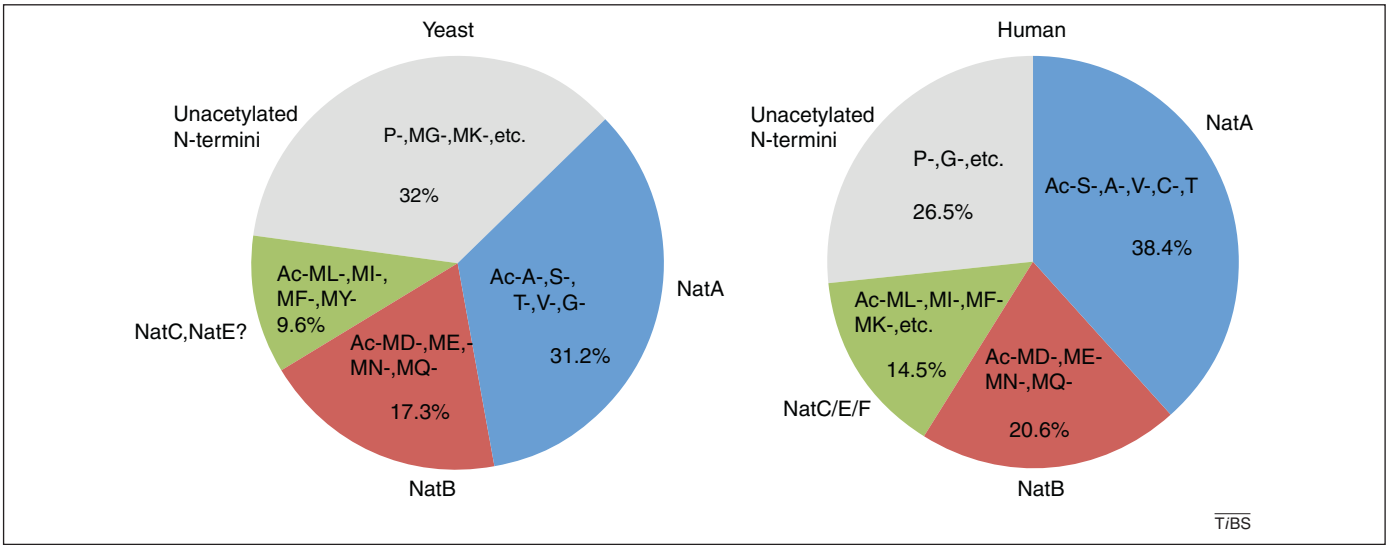

Figure 3. The Nt-acetylome of yeast and humans. Based on (i) the distribution of the first two amino acids of all yeast and human N-terminal protein sequences (SwissProt version 56.0); (ii) the experimentally determined percentage of Nt-acetylation (full or partial) of each type of N terminus [23]; and (iii) the substrate specificities of the NatA–NatF enzymes, the theoretical Nt-acetylomes are calculated. For Met-His-, Met-Arg-, and Met-Trp-, where experimental data of Nt-acetylation are lacking, the corresponding values of Met-Lys-, Met-Lys-, and Met-Phe- are used, respectively, due to structural similarities. NatD only acetylates two substrates, histones H2A and H4 (Ser-), and is not depicted.

have been identified both in yeast and humans: the histones H2A and H4 [29,45]. Second, whereas the substrate specificity for the other NATs lies within the first 2–5 residues of the substrate, the *in vivo* substrate specificity of Naa40p lies within the first 30–50 residues [46]. Although Naa40p acetylates histones, it differs from KATs because it is a NAT, and it is at least partially associated with ribosomes, where it might acetylate histones cotranslationally on the very N-terminal residue [29,46]. In yeast, *naa40*-Δ strains display minor phenotypes, including sensitivity to certain drugs inhibiting protein synthesis and mitosis. A synthetic growth defect was observed when deletion of *naa40* was combined with the addition of more net positive charges in the H4-histone tails. The authors therefore suggested that Nt-acetylation might play a role in regulating charge patches on the histone tails [46].

NatE

NatE is composed of Naa50p (Nat5p/San) and the NatA subunits Naa10p and Naa15p (Figure 2) [35,47]. Naa50p has been found to acetylate a specific set of N termini different from those acetylated by the NatA activity of Naa10p (Figure 3), namely, initiator methionines followed by a hydrophobic amino acid (similar to NatC) [28,37]. Also, Naa50p depletion does not give NatA-depletion-like phenotypes [35]. Therefore, the acetylation activity of Naa50p is considered distinct from the NatA activity, and named NatE. Naa50p acts in several processes, including sister chromatid cohesion [48,49], and chromosome resolution [50]. Furthermore, Chu and colleagues have observed that depletion of hNaa50p from HeLa cells increases regrowth of microtubules [51]. In yeast, no phenotype has been observed after Naa50p depletion [35].

Recently, the structure of a ternary hNaa50p–peptide–CoA complex was resolved, making it the first resolved protein structure of a substrate-bound NAT [3]. The structure reveals that the Met-Leu- substrate peptide sits in a hydrophobic pocket of hNaa50p, pointing it into the active site. The side-chains of Met^1 and Leu^2 form van der Waals interactions with the hNaa50p residues found in the hydrophobic pocket. This pocket is more constricted than similar binding sites found in KATs, explaining why hNaa50p strongly prefers N-terminal methionines to lysine side chains. A series of hydrogen bonds anchors the N-terminal peptide to the enzyme, and most of the enzyme residues responsible for these peptide backbone interactions are also conserved in hNaa10p, suggesting a conserved mode of binding of the N-terminal peptides to NATs [3].

NatF

In 2011, a new NAT was identified, NatF, which is defined by the Naa60p enzyme (Figure 2) [23]. NatF is found in higher, but not in lower eukaryotes, and contributes to the higher abundance of Nt-acetylation in humans as compared to yeast (Figure 3). Naa60p displays a substrate specificity that is partly overlapping with Naa30p (NatC) and Naa50p (NatE) (Figure 3), namely Met-Leu-, Met-Phe-, Met-Ile- and Met-Trp-. In addition Naa60p acetylates initiator methionines followed by lysine, whereas such N termini are rarely acetylated in yeast [23].

To summarise, the NATs differ in subunit composition, substrate preferences and phenotypes. Although there is some redundancy between the different NATs with respect to substrate specificity, they seem to have evolved into specific biological roles. Future studies will aim at investigating the specific biological roles of the different NATs.

The various functions of Nt-acetylation

For decades after its discovery [52], the functional role of Nt-acetylation has remained unknown. Still we do not fully understand why a majority of eukaryotic proteins carry this modification (Box 1). However, several key findings made during the past 2 years point to several molecular mechanisms in which Nt-acetylation participates (Figure 4).

Box 1. Outstanding questions

- What are the biological functions of Nt-acetylation? The number of Nt-acetylated proteins is immense, but the biological role of Nt-acetylation is still largely elusive. On a molecular level, Nt-acetylation steers diverse protein functions, including protein degradation, interactions and localisation (Figure 4), but besides this, little is known about the potential general cellular as well as physiological functions of Nt-acetylation.
- What underlies the evolutionary development of the complex and specific NAT system? NATs seem to have increased in complexity throughout evolution. Why have different NATs evolved, and why are some parts of the machinery highly conserved, whereas other NATs first show up later in evolution, remain open questions. Also, are there more NATs, possibly modifying specific substrates, besides those already described?
- What is the exact interplay between Ac-CoA, Nt-acetylation and metabolism? The recent finding that NatA is regulated by Ac-CoA levels points to an exciting link between cellular metabolic states, Nt-acetylation and cell death. This raises the questions whether Ac-CoA levels regulate Nt-acetylation in general, and whether Nt-acetylation through this is a player in the regulation of cellular metabolism?
- Is Nt-acetylation reversible? Nt-acetylation has hitherto been considered irreversible. If Nt-acetylation can be reversed through (yet undiscovered) Nt-deacetylases (as is the case for lysine acetylation of proteins where lysine acetylases and lysine deacetylases work together), it could modify proteins more dynamically than the static model of Nt-acetylation that is assumed today.
- Is there interplay between the NATs and other ribosome-associated protein biogenesis factors? A fraction of the NATs are attached to the ribosome, where they are assumed to perform cotranslational acetylation. Do the NATs interact with other ribosome biogenesis factors to regulate processing and folding of nascent polypeptides, and can the NATs themselves be regulated on the ribosome, for example so that the correct NAT is situated on the correct ribosome where translation of its substrate occurs?
- Can NATs be utilised in diagnostics and treatment of disease? Several studies have linked hNaa10p (NatA) to cancer, although the proposed roles of hNaa10p vary from oncoprotein to tumor suppressor. More work is needed to understand if and when NatA might be targeted in cancer chemotherapy.

Protein stability

Varshavsky *et al.* have provided evidence that Nt-acetylation in some yeast proteins creates degradation signals [4]. The ubiquitin ligase Doa10 recognises these Nt-acetylated proteins and marks them with ubiquitin for subsequent proteasomal degradation, thus shedding new light on the N-end rule pathway of protein stability and degradation (Figure 4a) [53]. The authors have suggested that Nt-acetylation might act as a quality control mechanism to degrade unfolded or misfolded proteins, and to regulate protein stoichiometries. These findings break with the previous conception that Nt-acetylation protects proteins from degradation [54], and thus they shed new light on what has been called the N-end rule pathway of protein stability and degradation [53]. Nonacetylated N-terminal amino groups can be Nt-ubiquitylated and subsequently degraded. Nt-acetylation is thus suggested to block Nt-ubiquitylation, thereby stabilising proteins [55]. Both hypotheses link Nt-acetylation to protein stability or protein degradation, and both might represent real events in the cell; each however applying to specific proteins under defined conditions. Two recent studies, one on NatB in yeast [56] and one on NatA in human cells [9], have suggested that Nt-acetylation in general neither positively nor negatively affects protein stability. Thus, future in-depth studies are required to assess whether the effect of Nt-acetylation on protein stability is general, protein-specific or depends on factors such as cellular localisation and biological context.

Interaction mediator

Nt-acetylation has also been shown to be a direct mediator for protein-membrane interactions (Figure 4b) and protein–protein interactions (Figure 4c). Earlier studies have suggested that NatC-mediated Nt-acetylation of the GTPases Arl3p and Grh1p creates an N-terminal membrane anchor, attaching the GTPases to the Golgi membrane where they function in organelle traffic [57,58]. Likewise, Nt-acetylation mediates association of the tRNA methyltransferase Trm1p-II with the inner nuclear membrane [59].

Recently, Schulman *et al.* have shown how Nt-acetylation of the potential NatC substrate and E2-ligase Ubc12 mediates interaction with the E3-ligase Dcn1 by burying the Ubc12 N terminus in a hydrophobic binding pocket in Dcn1 (Figure 4c) and subsequently neddylating the Dcn1 substrate Cul1 [6]. Another protein–protein interaction requiring Nt-acetylation is Tfs1p inhibition of carboxypeptidase Y (CPY) [60]. Thus, Nt-acetylation can regulate specific cellular processes through determining protein localisation and protein interactions.

ER translocation

A recent study has presented a novel link between Nt-acetylation and protein targeting (Figure 4d). Nt-acetylation has been found to be necessary for cytosolic retention, because selected Nt-acetylated proteins are no longer post-translationally translocated to the ER [5]. In support of this, *in silico* analyses correlating the N-terminal processing status (i.e. N-terminal methionine removal and Nt-acetylation) and the presence of signal peptides (that target proteins to the ER) have revealed a strong correlation between protein translocation and lack of Nt-acetylation.

In conclusion, these data imply that Nt-acetylation is a very diverse protein modifier, with a potentially wide range of functional outcomes. Important tasks for follow-up studies will be to elaborate to what extent Nt-acetylation works through general mechanisms, or whether the molecular roles of Nt-acetylation are as diverse as the proteins being acetylated.

Other biochemical activities of NATs

The biochemical activity of the NATs in performing Nt-acetylation has been thoroughly documented *in vitro* and *in vivo* as described above. However, there is also evidence supporting a potential role for NATs in catalysing lysine acetylation. Lim *et al.* and Shin *et al.* have demonstrated that hNaa10p is capable of acetylating lysine residues in β-catenin and myosin light chain kinase (MLCK), respectively [61,62]. hNaa10p is also able to autoacetylate itself at K136, and this is essential for its activity and cellular function [12]. Furthermore, hNaa50p is capable of

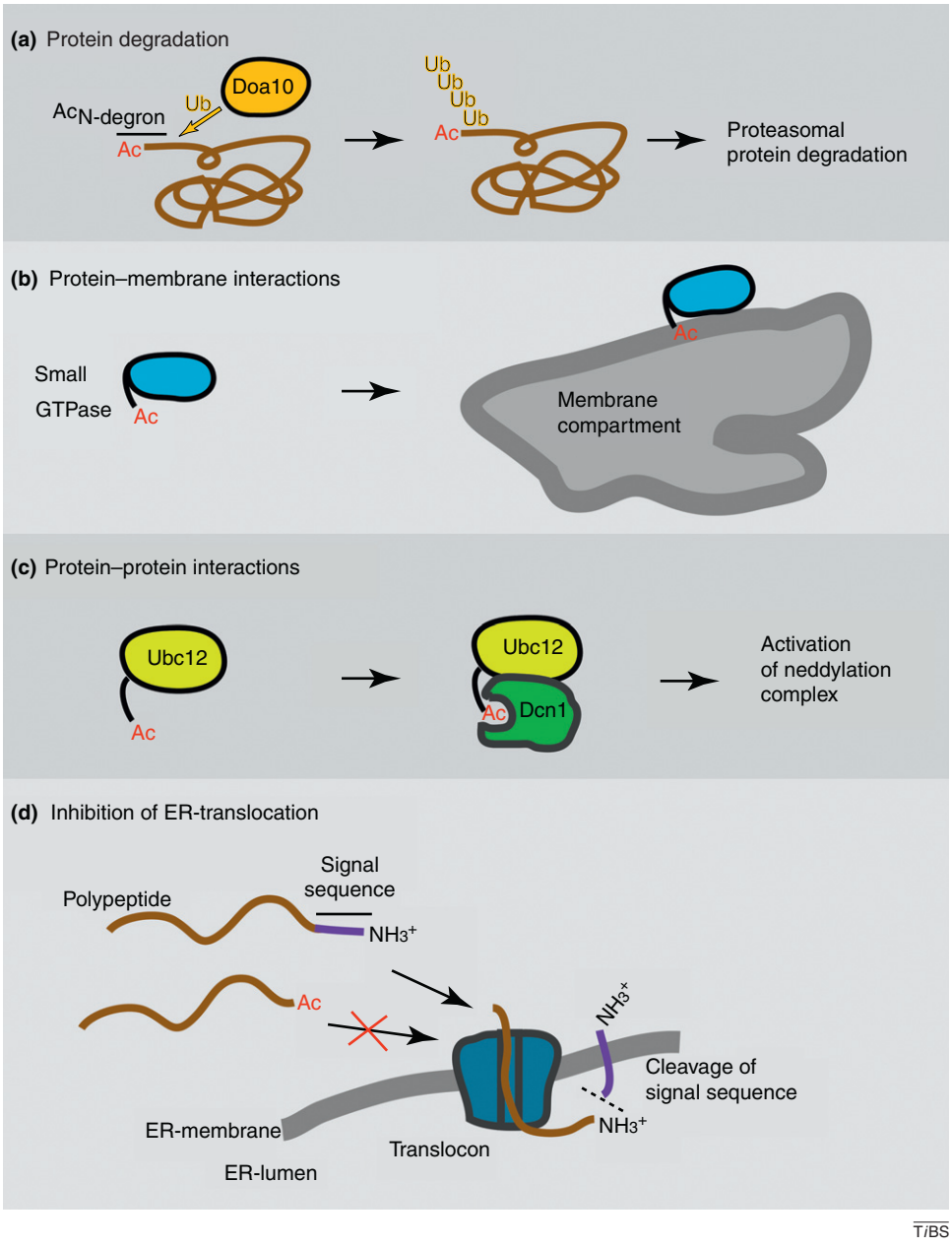

Figure 4. Molecular functions of Nt-acetylation. Nt-acetylation has been shown to provide its target with different molecular functions. **(a)** Protein degradation. In yeast, Nt-acetylation of specific proteins creates an ^{Ac}N-degron that is recognised by the ubiquitin ligase Doa10p, and following ubiquitylation the protein is degraded. **(b)** Protein–membrane interactions. Nt-acetylation functions as a part of an N-terminal membrane anchor, for example, for the small GTPase Arl3p in yeast. **(c)** Protein–protein interactions. Nt-acetylation facilitates the interaction between the E3 ligase Ubc12 and the E2 ligase Dcn1. This interaction is needed for activation of the enzymatic complex, and subsequent neddylation of downstream substrates. **(d)** Protein translocation into the ER. Nt-acetylation of an ER signalling sequence has been shown to inhibit protein translocation into the ER. In addition, the N termini of ER-translocation signalling sequences tends towards being unsuitable for Nt-acetylation by the known NAT-types.

performing both Nt-acetylation and lysine acetylation, including autoacetylation *in vitro*, although the latter at a much lower rate as compared to its Nt-acetylation activity [28,51]. Although their main function is likely to be Nt-acetylation, these enzymes might indeed be combined NATs and KATs depending on their context.

In addition to their catalytic activity, NATs can also act as cellular regulators independent of any catalytic activity. At least two such cases have been reported for hNaa10p: binding to cell-adhesion-regulating p21-activated kinase (PAK)-interacting exchange factor (PIX) proteins inhibits cell migration [14] and binding to DNA methyltransferase 1 (DNMT1) silences E-cadherin transcription and stimulates cell proliferation [15].

NATs and cellular metabolism

A central question is whether or not Nt-acetylation is a regulated process. Expression profiles and post-translational modifications of NATs indicate that NAT activities vary between biological contexts [63,64]. In 2011, Ac-CoA was presented as a regulator of hNaa10p activity, providing evidence that NAT activity can indeed be regulated for specific biological purposes. Yi *et al.* have shown that the antiapoptotic factor Bcl-xL partly mediates its effect

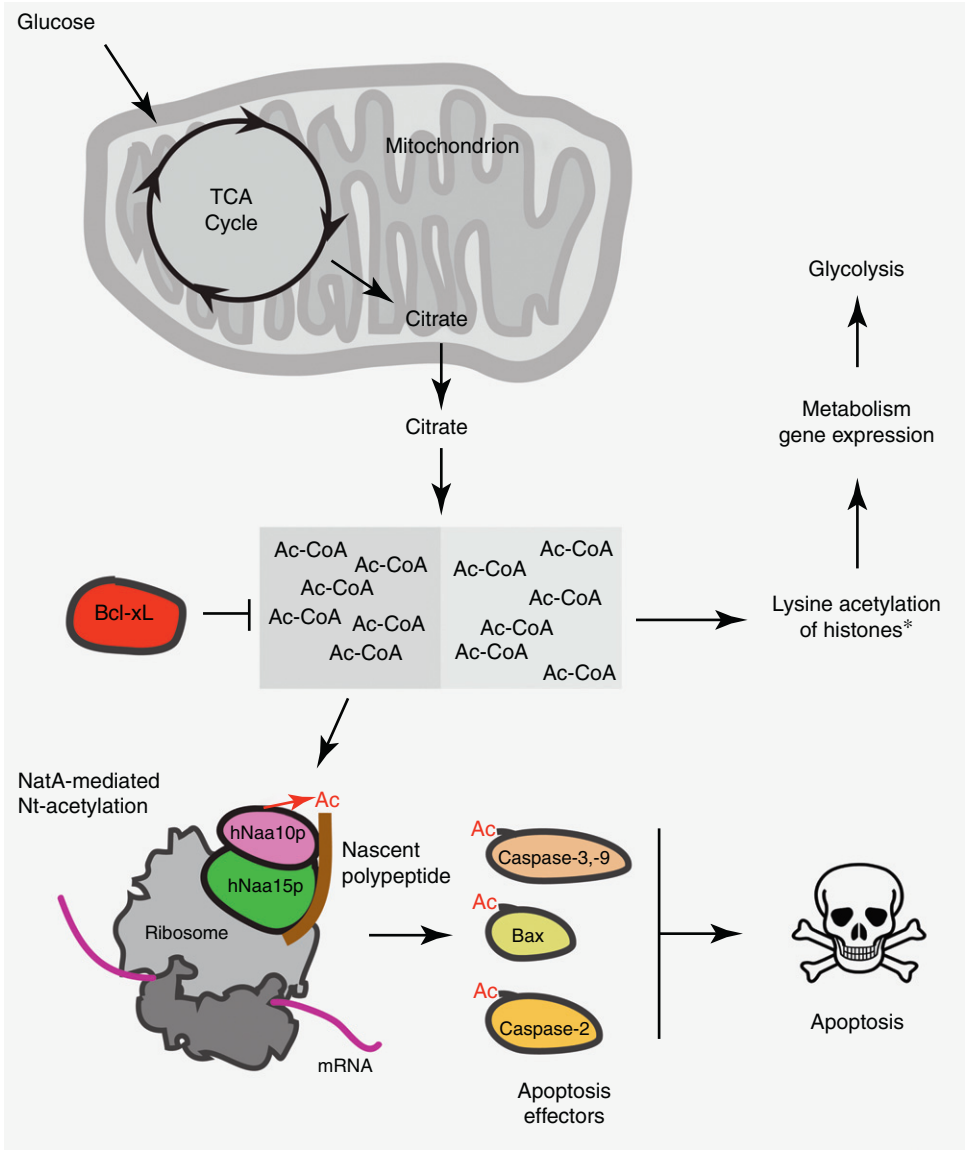

Figure 5. To be or not to be – Ac-CoA can determine cell fate through lysine – and Nt-acetylation. The oncogene Bcl-xL inhibits apoptosis through lowering Ac-CoA levels, and thus inhibiting hNaa10p-mediated acetylation of apoptosis effectors caspase-2,-3 and -9 and Bax. It has been suggested that Bcl-xL inhibits export of citrate from the mitochondria to the cytoplasm, and thus stops the conversion of citrate into Ac-CoA. Ac-CoA levels are earlier shown to regulate the KAT-mediated acetylation of histones (*this acetylation seems not to be affected by Bcl-xL), and subsequently activation of glycolysis through the transcription of metabolism genes. Interestingly, Ac-CoA levels depend on cellular glucose levels, and thus hNaa10p might connect metabolic cellular state and apoptotic response.

through the inhibition of Ac-CoA production. Lowered levels of Ac-CoA lead to a reduction in Naa10p-mediated Nt-acetylation of the apoptotic mediators caspase 2, 3 and 9 and Bax, resulting in reduced apoptosis (Figure 5). The Bcl-xL mediated regulation of Ac-CoA does not alter ε-acetylation of histones H3 and H4, thus Ac-CoA levels can regulate acetylation in an acetyltransferase-specific manner [9]. These data not only show that a NAT can be regulated by Ac-CoA levels, but also suggest a potential mechanism where NatA-mediated Nt-acetylation links the metabolic cell state to cell death. Future work is needed to verify this hypothesis (Box 1).

Recently, a connection between energy levels and lysine acetylation was revealed. In yeast, lysine acetylation of histones increased as a response to glycolytic burst, mainly through the KATs picNuA4 and SAGA [65]. In human cells, it was further shown that the link between glucose and acetylation went through the conversion of glucose-derived citrate into Ac-CoA [66]. Ac-CoA was found to act as a carbon-source rheostat, regulating the expression of growth genes by modulating KAT-mediated acetylation of histones [8]. Thus, Ac-CoA levels might, to various extents, centrally regulate both lysine and Nt-acetylation and their associated downstream effects.

NATs in human pathology

The physiological impact of the NATs has only recently become evident. The first human genetic disorder caused by a mutation in a NAT gene was discovered in 2011 [16]. The resulting Ogden syndrome is caused by a mutation in

the gene encoding the catalytic subunit hNaa10p of the NatA complex. X-chromosome exon sequencing and a probabilistic disease–gene discovery algorithm, VAAST, have been used to identify a c.109T>C (p.Ser37Pro) variant in *NAA10*. Functional analysis has demonstrated a significantly impaired biochemical activity of hNaa10p-S37P compared with wild-type hNaa10p, suggesting this is the causative mutation for the syndrome. Affected boys suffer from global developmental delays and lethality during infancy. Although the exact mechanism linking defective hNaa10p to disease has not been elucidated, the Ogden syndrome highlights the physiological importance of NATs.

Several studies have linked NATs to cancer. hNaa10p in particular has been proposed as both an oncoprotein and a tumour suppressor. *NAA10* is overexpressed in different types of cancers such as colorectal cancer [67,68], lung cancer [15] and breast cancer [68]. Several studies have proposed various molecular explanations for the role of Naa10p as an oncoprotein. Knockdown studies have demonstrated that p53-dependent apoptosis is induced when cancer cells are depleted of hNaa10p [33]. hNaa10p also induces cyclin D1 transcription through c-Jun and c-Fos via the extracellular signal-regulated kinase (ERK)1/2 pathway because these two interact with β-catenin to activate cyclin D1 transcription [40]. Lee *et al.* have suggested that hNaa10p directly interacts with DNMT1 in an acetyltransferase-independent manner. This facilitates interaction between DNMT1 and its substrate DNA, thereby enabling DNMT1 to silence transcription of the tumour suppressor E-cadherin through promoter CpG methylation [15]. In contrast to these studies supporting the role of hNaa10p as an oncoprotein, there are several studies that indicate that hNaa10p has tumour suppressor properties. Yi *et al.* have reported that hNaa10p might promote cell death because hNaa10p-depleted cells do not undergo apoptosis induced by DNA damage [9]. Kuo *et al.* have reported that breast cancer cell lines that overexpress hNaa10p exhibit reduced cell growth and tumourigenicity [13]. This latter property of hNaa10p has been attributed to stabilization of tuberous sclerosis complex (TSC)2 through direct interaction and Nt-acetylation. Stabilisation and increased abundance of TSC2 suppress the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) signalling pathway and thereby promote autophagy, thus possibly reducing cell proliferation. In two independent studies, low expression of hNaa10p correlates to poor prognosis and aggressiveness of tumours in lung and breast cancer with more lymph node metastasis. Hua *et al*. have found that hNaa10p levels inversely correlate with the metastatic ability of lung cancer. They have suggested that hNaa10p inhibits cell migration through binding to PIX, and thus blocking PIX activation of the cell migration machinery [14]. Shin *et al*. have reported that reduced levels of hNaa10p also promote cell migration, but through inactivation of MLCK via direct binding of hNaa10p and N-ε-acetylation of MLCK [62]. Thus, the role of hNaa10p in cancer development seems to be dependent on the specific biological and pathological context. Further mechanistic and phenotypic studies are needed to clarify the role of hNaa10p in different cancer contexts, and whether hNaa10p can be utilised in the diagnosis or treatment of cancer (Box 1).

Concluding remarks

During the past 15 years, an elaborate system of NATs and Nt-acetylation has been revealed. The number of important contributions within the past 2–3 years shows that our understanding of Nt-acetylation is still only in its infancy (Box 1). NatA–F have been identified, but whether there are more NATs acetylating specific (minor) subsets of proteins remains an open question. Not much is known about whether NATs have developed into specific cellular tasks, but the role of NATs in particular pathways is emerging. The molecular role of Nt-acetylation is diverse, ranging from protein degradation, mediation of protein interactions to inhibition of ER translocation. Future studies will need to address whether the role of Nt-acetylation is as diverse as the immense number of different Nt-acetylated proteins, or whether more general roles exist. Furthermore, the role of Nt-acetylation in physiology and pathology is still elusive. The characterisation of the Ogden syndrome caused by a mutation in the h*NAA10* gene emphasises the physiological importance of Nt-acetylation. However, the challenge that remains is to single out specific mechanisms that mediate pathological effects of NAT-alterations.

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