

## Review

Laurenz Rabl and Elke Deuerling\*

# The nascent polypeptide-associated complex (NAC) as regulatory hub on ribosomes

<https://doi.org/10.1515/hsz-2025-0114>

Received February 7, 2025; accepted March 13, 2025;

published online April 2, 2025

**Abstract:** The correct synthesis of new proteins is essential for maintaining a functional proteome and cell viability. This process is tightly regulated, with ribosomes and associated protein biogenesis factors ensuring proper protein production, modification, and targeting. In eukaryotes, the conserved nascent polypeptide-associated complex (NAC) plays a central role in coordinating early protein processing by regulating the ribosome access of multiple protein biogenesis factors. NAC recruits modifying enzymes to the ribosomal exit site to process the N-terminus of nascent proteins and directs secretory proteins into the SRP-mediated targeting pathway. In this review we will focus on these pathways, which are critical for proper protein production, and summarize recent advances in understanding the cotranslational functions and mechanisms of NAC in higher eukaryotes.

**Keywords:** ribosome-associated factors; cotranslational protein modification; methionine aminopeptidases (MetAPs); N-terminal acetyltransferases (NATs); cotranslational protein transport; signal recognition particle (SRP)

## 1 Introduction

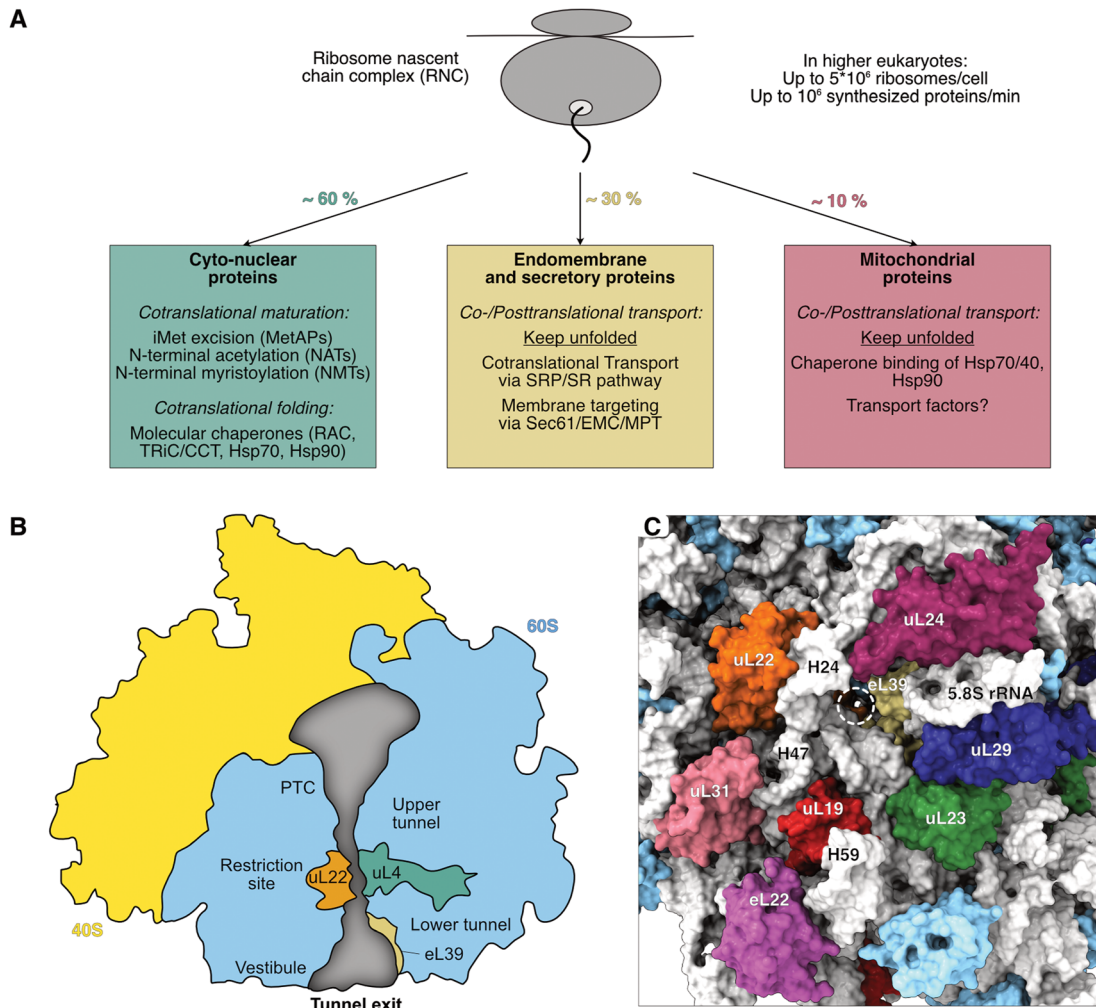
The synthesis of new proteins on ribosomes is crucial for refreshing and adapting the cell's proteome to changing conditions. Ribosomes, complex macromolecular devices composed of ribosomal RNA (rRNA) and ribosomal proteins (r-proteins), decode messenger RNA (mRNA) sequences to synthesize polypeptide chains based on the genetic code. About 60 % of newly made proteins encode cytonuclear

proteins while approx. 10 % are imported into mitochondria (Bykov et al. 2020) and approx. 30 % are endomembrane/secreted proteins that are cotranslationally targeted to the endoplasmic reticulum (ER) (Akopian et al. 2013) (Figure 1A). When newly synthesized polypeptides emerge from the ribosomal exit tunnel, they are immediately met by a variety of conserved protein biogenesis factors (Gamerding and Deuerling 2024). These factors, often ribosome-associated, guide the nascent chains through the early stages of modification and folding, and, if needed, direct the proteins to their appropriate cellular compartments. Correct protein processing is critical for maintaining a healthy proteome and cellular homeostasis, as improperly folded, modified, or mislocalised proteins can lead to protein aggregation and dysfunction, contributing to various diseases such as neurodegenerative disorders or cancer (McTiernan et al. 2025; Øye et al. 2025).

The vast majority of cytonuclear proteins are modified at their N-terminus as soon as the nascent chain exits the ribosomal tunnel (Figure 1A). Enzymes such as methionine aminopeptidases (MetAPs), N-terminal acetyltransferases (NATs), and N-myristoyltransferases (NMTs) play pivotal roles in modifying nascent polypeptide chains. MetAPs remove the initiating methionine from nascent proteins, a process that facilitates subsequent modification by NATs and NMTs, and determines protein stability, as the amino acid identity and modification status of a proteins' N-terminus has been recognized as a determinant of its half-life in the cell (Varshavsky 2024). NATs acetylate the N-terminus of nascent chains, influencing protein folding (Kang et al. 2012), stability (Hwang et al. 2010), localization (Behnia et al. 2004; Forte et al. 2011; Setty et al. 2004), and protein and membrane interactions (Dikiy and Eliezer 2014; Gao et al. 2016; Scott et al. 2011). NMTs catalyze the attachment of myristoyl groups to specific glycine residues, promoting membrane association and signal transduction (Tate et al. 2024; Traversono et al. 2013). This ensemble of enzymes acts as soon as the N-terminus of a protein emerges from the ribosomal tunnel and prior to other processing pathways such as folding or transport (Kramer et al. 2019). Thus, the activities of these modifying enzymes must be tightly coordinated at the ribosomal exit site in a spatial and timely manner,

\*Corresponding author: **Elke Deuerling**, Department of Biology, University of Konstanz, D-78457 Konstanz, Germany, E-mail: [elke.deuerling@uni-konstanz.de](mailto:elke.deuerling@uni-konstanz.de). <https://orcid.org/0000-0002-8905-0260>

**Laurenz Rabl**, Department of Biology, University of Konstanz, D-78457 Konstanz, Germany. <https://orcid.org/0000-0002-3736-9800>



**Figure 1:** Overview of protein biogenesis. (A) Overview of protein production pathways and conserved ribosome-associated protein biogenesis factors. (B) Schematic of the ribosomal tunnel, subdivided in the upper tunnel, restriction sites formed by uL22 and uL4, and the lower tunnel and vestibule. Ribosomal protein eL39 decorates the lip of the tunnel exit. PTC: Peptidyl transferase center. (C) Surface representation of molecular model of human 80S ribosome (PDB: 4UG0). Close-up of tunnel exit region with indicated ribosomal proteins and rRNA elements. Remaining 60S r-proteins in light blue, rRNA in grey. Location of ribosomal tunnel is depicted as a dashed white circle.

ensuring that cytonuclear proteins are correctly processed as they are synthesized. How this complex choreography of N-terminal modifications of nascent chains is organized, was enigmatic until recently (see below). Subsequently, molecular chaperones including the ribosome-associated complex (RAC), TRiC/CCT, Hsp70s and Hsp90s can bind cotranslationally to the growing polypeptides to support folding steps to the native state (Kramer et al. 2019).

About 30 % of newly made proteins encode secretory or endomembrane proteins that require appropriate targeting to the endoplasmic reticulum ER (Figure 1A). Transport of newly synthesized proteins carrying a hydrophobic ER-signal sequence (SS) or transmembrane domain (TMD) in the N-terminus is organized by the signal recognition particle (SRP) and the signal recognition particle receptor (SR) at the

ER membrane in a GTP-dependent manner. SRP binds to ribosomes and to signal sequences on nascent polypeptides as they emerge from the ribosome and initiates SR-mediated targeting of ribosome-nascent chain complexes (RNCs) to the Sec61 translocase at the ER-membrane (Akopian et al. 2013; Rapoport et al. 2017).

About 10 % of newly synthesized proteins are destined for mitochondria encoded by nuclear DNA and synthesized on cytosolic ribosomes (Bykov et al. 2020) (Figure 1A). Mitochondrial targeting signals can display variation and can be located N-terminally or internally (Bykov et al. 2020). Approximately 60–70 % of nuclear-encoded mitochondrial proteins carry a cleavable mitochondrial targeting sequence (MTS), a short peptide containing positively charged basic residues that facilitates the transport of a protein to the

mitochondria (Roise et al. 1986; Vögtle et al. 2009). That far little is known about specific transport factors for mitochondrial proteins. Albeit cotranslational mitochondrial import is considered to play a role for especially inner mitochondrial membrane proteins (Williams et al. 2014), it is assumed that many of the mitochondrial precursor proteins remain in an unfolded state and are posttranslationally transported to mitochondria with the help of cytosolic chaperones such as Hsp/Hsc70 and Hsp90, which prevent premature folding and aggregation. A very recent study suggests that co-chaperones of Hsc70 (St13 and Stip1) and Hsp90 (p23 and Cdc37) directly bind to the MTS of nascent mitochondrial proteins to facilitate chaperone retention at the mature domain (Juszkiewicz et al. 2025).

## 2 The ribosomal exit tunnel

Newly synthesized polypeptides exit the ribosome into the cytosol via a tunnel in the large ribosomal subunit (Figure 1B). This tunnel, approximately 100 Å long and 10–20 Å wide, is predominantly lined by core rRNA (28S in higher eukaryotes), with its geometry shaped by extensions of ribosomal proteins uL4 and uL22 forming two narrow constrictions of about 10 Å in eukaryotes (Dao Duc et al. 2019). At the tunnel's rim, where it widens to about 20 Å, ribosomal protein eL39 contributes to its structural features (Nissen et al. 2000) (Figure 1B).

The tunnel can be divided into the upper chamber close to the PTC, the central part with the major constriction sites, and the lower tunnel and vestibule (Samatova et al. 2024). Overall, the ribosomal tunnel possesses an electronegative potential ranging from –8 mV to –22 mV, reflecting its rRNA-dominant composition (Lu et al. 2007). Although the tunnel's geometry prevents extensive protein folding, computational studies suggest that the confinement within the tunnel promotes formation of  $\alpha$ -helical structures (Ziv et al. 2005), which have been observed before and after the constriction sites (Agirrezabala et al. 2017; Bhushan et al. 2010; Lu and Deutsch 2005). More complex structures such as zinc-finger domains (Nilsson et al. 2015) and  $\beta$ -hairpin motifs (Kosolapov and Deutsch 2009) have been observed in the lower tunnel and vestibule. The vestibule even allows folding of simple tertiary-structures like partially folded three-helix bundles (Nilsson et al. 2017).

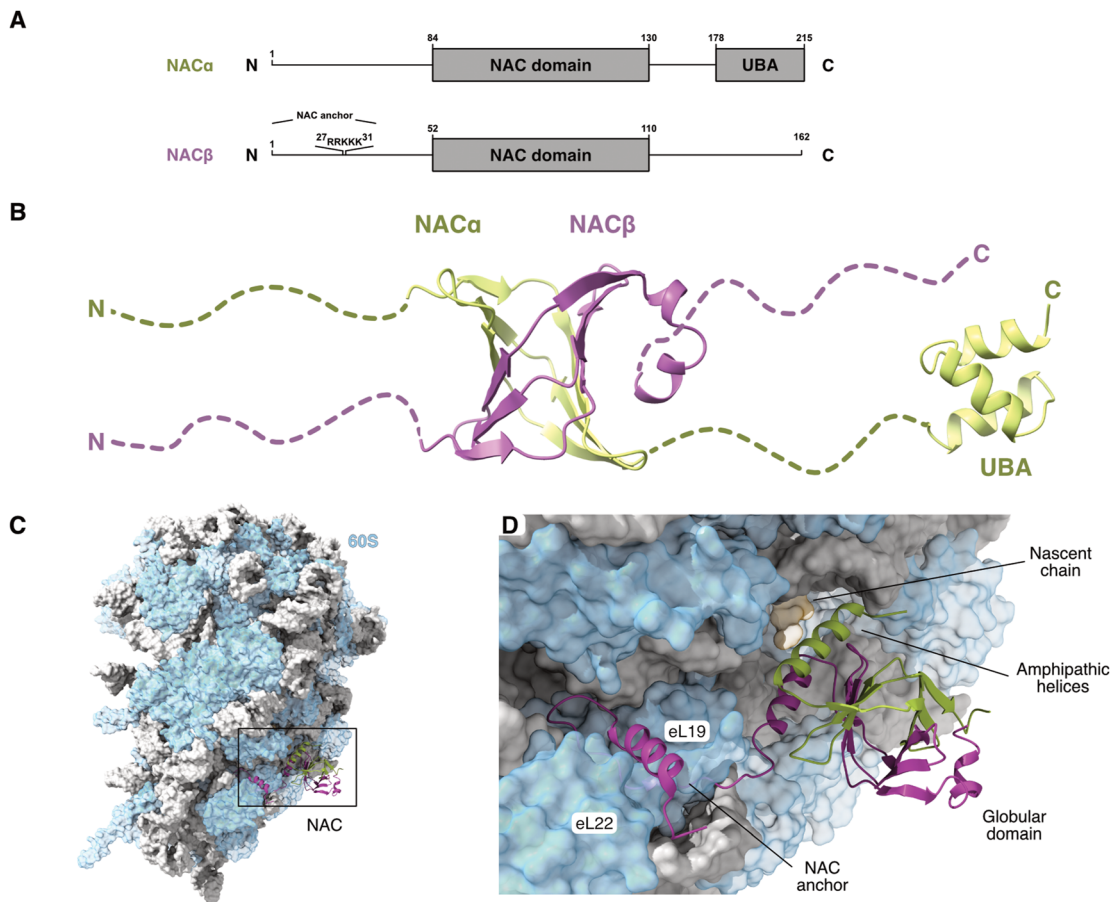
The ribosomal surface surrounding the tunnel exit (Figure 1C) functions as a biogenesis factor-binding platform, facilitating efficient handover of nascent chains to processing enzymes, targeting factors, and chaperones. These factors have at least partially overlapping binding sites and compete for timely and specific access to the nascent chain (Gamerding et al. 2024; Kramer et al. 2009). Unlike the interior tunnel, the tunnel exit is predominantly

composed of ribosomal proteins (universal: uL22, uL23, uL24, uL29; eukaryote-specific: eL19, eL22, eL31 and eL39), with minor contributions of rRNA elements (5.8S rRNA and helices H24, H47, and H59 of 28S rRNA) (Khatter et al. 2015). Ribosomal proteins uL23 and uL29, collectively dubbed the universal ribosome docking site, have been implicated in the binding of various proteins including Sec61 (Beckmann et al. 2001; Voorhees et al. 2014), SRP (Pool et al. 2002; Voorhees and Hegde 2015), NAC (Jomaa et al. 2022; Kramer et al. 2002), and MetAP1 (Gamerding et al. 2023; Nyathi and Pool 2015). Additionally, a second adaptor site comprising eL31 and uL22 has been identified as the binding site for RAC (Peisker et al. 2008), NAC (Pech et al. 2010), and the Signal recognition particle receptor (SR) (Halic et al. 2006). Well-regulated binding of factors to the tunnel exit is crucial, as emphasised by the fact, that premature factor association is actively prevented during ribosome biogenesis (Greber et al. 2012).

## 3 The nascent polypeptide associated complex (NAC)

The nascent polypeptide-associated complex (NAC) is a heterodimer of NAC $\alpha$  and NAC $\beta$ , conserved throughout eukaryotes (Wiedmann et al. 1994). Disruption of NAC gene expression is embryonically lethal in mice (Deng and Behringer 1995), flies (Markesich et al. 2000), and worms (Bloss et al. 2003). NAC is highly abundant and expressed approximately in a 1:1 stoichiometry to ribosomes (Kulak et al. 2014; Raue et al. 2007).

NAC $\alpha$  and NAC $\beta$  show substantial homology and dimerize via their NAC domains, forming a  $\beta$ -barrel-like structure with a hydrophobic core (Figure 2A and B) (Liu et al. 2010; Wang et al. 2010). The two N-termini and the two C-termini protrude as flexible arms from the globular domain. NAC's C-terminals arms act as recruiting devices for protein biogenesis factors (see below). The C-terminal arm of NAC $\alpha$  features a three-helix bundle characteristic of ubiquitin-binding (UBA) domains (Figure 2A and B). These domains typically contain a hydrophobic patch on the solvent-accessible surface formed by helices  $\alpha$ 1– $\alpha$ 3, mediating the ubiquitin interaction (Mueller and Feigon 2002). However, the NAC $\alpha$  UBA domain does not bind to ubiquitin (Andersen et al. 2007), but rather acts as a general protein-interaction module for recruitment of a subset of ribosome-associated proteins (Jomaa et al. 2022; Lentzsch et al. 2024; Minoia et al. 2024). The two N-terminal NAC arms that protrude from the NAC globular domain are involved in ribosome binding with the N-terminus of NAC $\beta$  as the major ribosome anchor of this complex (Gamerding et al. 2019; Jomaa et al. 2022; Wegrzyn et al. 2006).



**Figure 2:** NAC domain architecture, fold, and ribosome-binding mode. (A) Domain architecture of human NAC with annotated ribosome binding motif. In humans, NAC $\beta$  exists in a prominent short isoform (as depicted here) and a long isoform with an extended N-terminus (not shown). Start and stop of annotated domains according to AlphaFold3 structure prediction below. (B) AlphaFold3 structure prediction (Abramson et al. 2024) of human NAC heterodimer. NAC $\alpha$  in light green, NAC $\beta$  in purple. Prediction of unstructured N- and C-termini not shown. There are depicted as dashed lines. (C) Molecular model of cryo-EM structure of human NAC (NAC $\alpha$  in light green, NAC $\beta$  in purple) bound to an RNC translating an SS-containing nascent chain in an SRP-pre-handover state (PDB: 7QWR). Surface representation of 60S r-proteins in light blue, rRNA in grey, and nascent chain in light orange. (D) Close up of NAC bound to the tunnel exit.

NAC binds in a 1:1 stoichiometry to ribosomes with very high affinity ( $K_D = 1$  nM) (Jomaa et al. 2022). Given its approximately equimolar concentration, the majority of ribosomes are NAC-bound (Kulak et al. 2014; Raue et al. 2007). NAC can interact with empty ribosomes and RNCs displaying nascent chain still buried within the ribosomal tunnel by inserting its NAC $\beta$  N-terminus into the exit tunnel. Thus, it is assumed that it can contact nascent chains soon after translation initiation (Gamerding et al. 2019). In this conformation, NAC blocks the access to the exit tunnel for nascent chain modifying and targeting enzymes, preventing unproductive binding before the nascent chain is exposed (Gamerding et al. 2019). As elongation proceeds, NAC $\beta$  N-terminus is displaced from the tunnel interior by the growing polypeptide and occupies its canonical high-affinity binding site outside the tunnel. Recent cryo-EM data showed

that the NAC $\beta$  N-terminus (“NAC anchor”) wraps around eL22 and contacts eL19 and 28S rRNA (Figure 2D) (Jomaa et al. 2022; Lin et al. 2020). This interaction aligns with earlier biochemical data, showing that deletion of the N-terminus or mutations in the conserved RRK(X<sub>n</sub>)KK motif located in the N-terminus of NAC $\beta$  abolish stable ribosome association (Figure 2A) (Pech et al. 2010; Wegrzyn et al. 2006). Additionally, two amphipathic, antiparallel helices, which form only in presence of the ribosome, attach the NAC globular domain to the exit tunnel by forming a low-affinity interaction with the 28S rRNA (Jomaa et al. 2022; Lin et al. 2020) (Figure 2C).

Recent studies in *Caenorhabditis elegans* and human cell lines uncovered NAC’s fundamental role and mechanism as a central regulatory hub in protein synthesis by regulating the processing pathways of nascent proteins (Gamerding



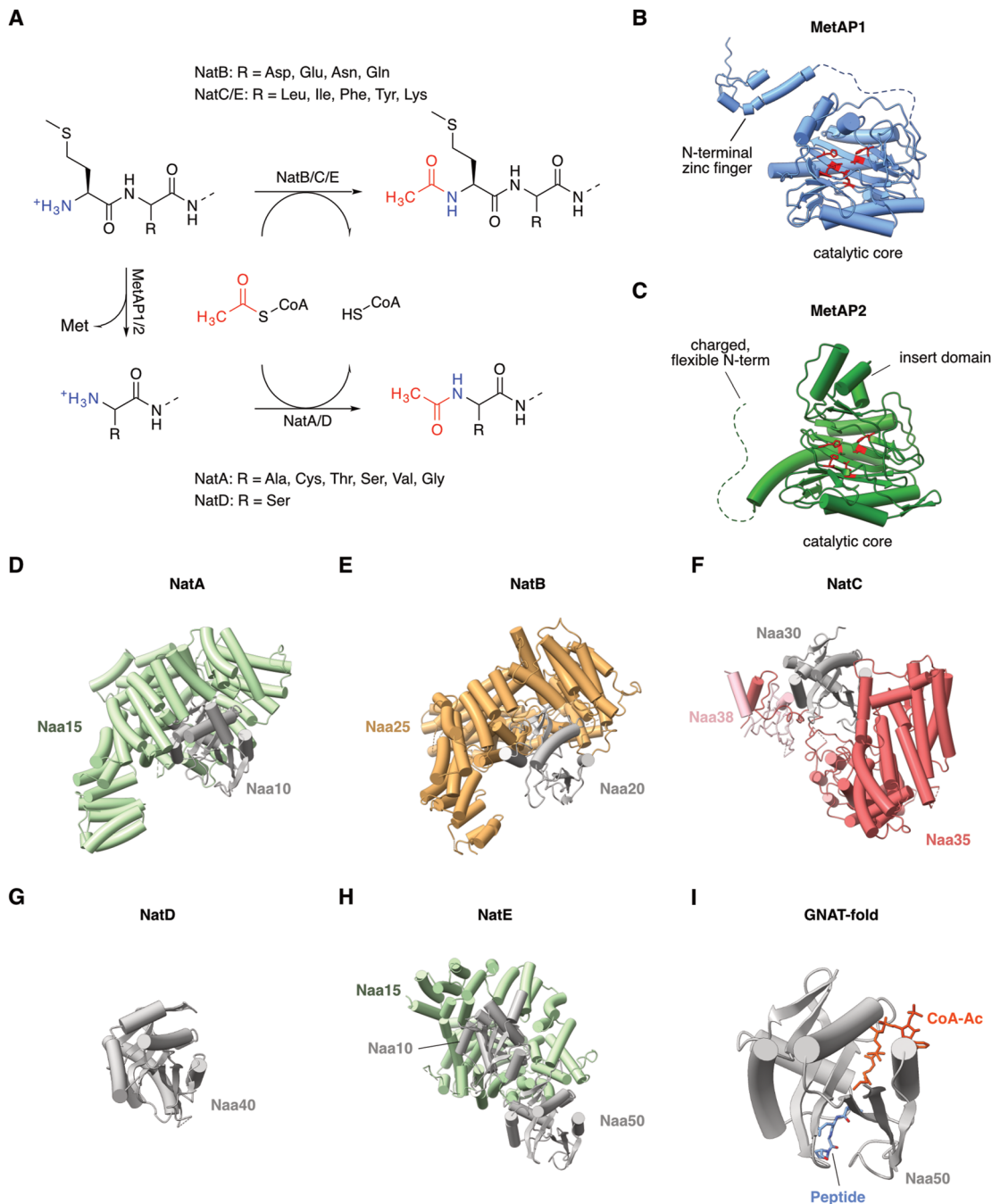
et al. 2023; Jomaa et al. 2022; Klein et al. 2024a; Lentzsch et al. 2024). NAC not only recognizes the type of nascent proteins produced by ribosomes but also orchestrates their precise modifications and cellular localization by recruiting appropriate cellular factors to ensure the correct fate and function of new proteins.

## 4 NAC regulates initiator methionine excision (NME) and N-terminal acetylation (NTA) by NatA/E

Newly synthesized proteins initially carry an N-terminal initiator methionine (iMet). This initiator methionine is cleaved cotranslationally, provided the second amino acid is small and uncharged (Ala, Cys, Thr, Ser, Val, Gly or Pro) (Figure 3A). Depending on organism, up to 70 % of proteins undergo NME, a conserved process from bacteria to higher eukaryotes (Giglione et al. 2004). In eukaryotes NME is carried out by two metalloproteases, methionine aminopeptidase 1 and 2 (MetAP1 (Addlagatta et al. 2005) and MetAP2 (Liu et al. 1998)), which have largely overlapping substrate specificities (Xiao et al. 2010) (Figure 3A–C). Both enzymes share a catalytic domain that adopts a characteristic pita-bread fold with conserved metal-binding sites and an active site of similar topology. In contrast to MetAP2, MetAP1 has an extended N-terminus containing a zinc finger domain, which has been implicated in its ribosome binding (Gamerding et al. 2023; Vetro and Chang 2002; Zuo et al. 1995). Conversely, MetAP2 features a charged N-terminal extension and an  $\alpha$ -helical insertion (insert domain) within its catalytic domain (Figure 3B and C). Cryo-EM studies of MetAP2 (Klein et al. 2024b) and MetAP2-like proteins Arx1 (Greber et al. 2012) and EBP1 (Bhaskar et al. 2021; Kraushar et al. 2021; Wild et al. 2020) have identified the insert domain as the main mediator of ribosome binding. Compared to ribosomes, MetAP1 and MetAP2 are relatively scarce in the cell, present at substoichiometric ratios of at least 1:10 in yeast (Raue et al. 2007) and human cell lines (Geiger et al. 2012; Kulak et al. 2014), precluding quantitative loading of ribosomes of these enzymes for NME. How MetAP1 associates with ribosomes *in vivo* and whether there is any preference for MetAP1 or 2 to bind the translation machinery was unknown until recently. A recent study showed that MetAP1 is the main enzyme associated with ribosomes in human cells and *C. elegans*, while MetAP2 seemingly serves as back up when cells are depleted for MetAP1 function (Gamerding et al. 2023).

Another very common cotranslational modification is N-terminal acetylation which neutralises the basic behaviour of the N-terminal primary amine (Figure 3A). The irreversible addition of an acetyl moiety from acetyl-CoA to a substrate occurs in up to 80 % of the proteome, including both cytosolic proteins, as well as transmembrane and organellar proteins (Aksnes et al. 2019). In eukaryotes, a set of five protein complexes called N-terminal acetyltransferases (NATs) - NatA, NatB, NatC, NatD, and NatE - act cotranslationally on cytosolic proteins, each with a distinct substrate spectrum (Polevoda et al. 2008, 2009) (Figure 3D–H). Like methionine processing enzymes, NATs are relatively low in abundance compared to ribosomes (Geiger et al. 2012; Kulak et al. 2014; Raue et al. 2007).

NatA, a heterodimer consisting of catalytic subunit Naa10 and ribosome-binding subunit Naa15 (Figure 3D), acts downstream of NME by MetAPs and acetylates neo-N-termini starting with Ala, Cys, Thr, Ser, Val or Gly (~40 % of human proteome) (Arnesen et al. 2005, 2009). Interestingly, in higher eukaryotes NatA activity is regulated by Huntingtin Interacting Protein K (HYPK) (Arnesen et al. 2010). HYPK contains an N-terminal ubiquitin associated (UBA) domain, that binds Naa15 with high affinity, and inhibits NatA through its C-terminal domain, which sterically blocks the catalytic centre of Naa10 (Gottlieb and Marmorstein 2018; Weyer et al. 2017). NatD is a highly specific NAT that acetylates SGRG-starting N-termini found in histones H2A and H4 (Hole et al. 2011). If the nascent protein maintains the iMet, the N-termini are acetylated by NatB, NatC, and NatE depending on the identity of the second amino acid. Glx-Asx-type N-termini (MD-, ME-, MN- and MQ-starting), which comprise approximately 20 % of the human proteome, are processed by NatB, a heterodimer of the catalytic subunit Naa20 and the ribosome-binding subunit Naa25 (Polevoda et al. 2003) (Figure 3E). Together, NatC and NatE account for the remaining methionine-starting N-termini (canonical substrates: ML-, MI-, MF-, MY-, MK-starting), adding up to about 20 % of the human proteome. Proteomics studies, however, suggest that these enzymes may acetylate a broader range of N-termini (MM-, MS-, MT-, MA-, MV-, MH-, MW-starting) (Van Damme et al. 2011, 2016, 2023). NatC is a heterotrimer composed of the catalytic subunit Naa30, the ribosome-binding subunit Naa35, and an additional auxiliary subunit Naa38 (Deng et al. 2023) (Figure 3F). In contrast, Naa50 serves as the catalytic subunit of NatE and binds the ribosome by attaching to the NatA complex (Deng et al. 2019) (Figure 3H). Naa50 can co-bind with HYPK to NatA but, unlike Naa10, is not fully inhibited by HYPK's C-terminus (Deng et al. 2020a). All Nat catalytic subunits share a conserved GCN5-related-N-acetyltransferase (GNAT) fold (Figure 3I). Substrate recognition is determined by the geometry and



**Figure 3:** The human set of N-terminal modification enzymes acting cotranslationally on nascent polypeptides. (A) MetAPs cleave the iMet of cyto-nuclear nascent polypeptides, provided the second amino acid is small and uncharged (R = Ala, Cys, Thr, Ser, Val, Gly, Pro). Using Ac-CoA as a coenzyme, NatA acetylates these N-termini (R = Ala, Cys, Thr, Ser, Val, Gly). NatD specifically acetylates the N-termini of histones H2A and H4 (SGRG-starting). Methionine-retaining N-termini are acetylated cotranslationally by a combination of NatB, NatC, and NatE. (B and C) molecular models of (B) human MetAP1 (in blue, PDB: 2B3K) and (C) human MetAP2 (in green, PDB: 1BN5). Crystal structures are aligned to their common catalytic core. Key, conserved catalytic and metal-binding residues are depicted in red. MetAP1 N-terminal zinc-finger domain is visualized via AlphaFold3 modelling (Abramson et al. 2024). (D–H) Molecular models of human NATs acting cotranslationally aligned to their catalytic subunit, which is accessible for substrates from below. (D) Crystal structure of NatA (ribosome-binding subunit Naa15 in light green, catalytic subunit Naa10 in grey; PDB: 6C9M). (E) Cryo-EM structures of NatB (ribosome-binding subunit Naa25 in brown, catalytic subunit Naa20 in grey; PDB: 6VP9). (F) Cryo-EM structure of NatC (ribosome-binding subunit Naa35 in red, auxiliary subunit Naa38 in pink, and catalytic subunit Naa30 in grey; PDB: 7MX2). (G) Crystal structure of NatD (Naa40) in grey (PDB: 4U9W). (H) Cryo-EM structure of NatE (ribosome-binding subunit Naa15 in light green, and catalytic subunits Naa10 and Naa50 in grey; PDB: 6PPL). Structure is aligned to Naa50. (I) Close-up of human Naa50 (grey) to illustrate GNAT fold of NATs with substrate peptide (blue) and Ac-CoA (red) in catalytic site (PDB: 3TFY).

composition of the substrate binding pocket (Deng et al. 2020b, 2021, 2023; Grunwald et al. 2020; Liszczak et al. 2013; Magin et al. 2015).

Approximately 40 % of the mammalian proteome undergoes N-terminal methionine removal by MetAPs and subsequent acetylation by NatA in a strictly cotranslational and sequential manner. Recent studies revealed that NAC is essential to coordinate the function of both enzymes (Figure 4A). NAC employs its long and flexible C-terminal arms to recruit MetAP1 and NatA to the ribosome tunnel exit to form the quaternary complex, enabling their substrate-specific cotranslational N-terminal maturation activity (Figure 4A–D). Cryo-EM studies showed that NAC forms a multienzyme complex with MetAP1 and NatA early in translation, positioning their active sites to facilitate efficient sequential processing of the nascent protein (Gamerding et al. 2023; Klein et al. 2024a; Lentzsch et al. 2024). Additionally, NAC alleviates inhibitory interactions from the NatA regulatory protein HYPK, activating NatA on the ribosome and ensuring cotranslational Nt-acetylation (Lentzsch et al. 2024). The quaternary complex of NAC, MetAP1, and NatA forms a semicircular arrangement around the ribosome tunnel exit, with the catalytic sites of the processing enzymes directed to the tunnel exit with distances of 5 nm and 6 nm respectively (Figure 4A). This spatial organisation is well-suited for initiator methionine excision and subsequent acetylation.

The long, flexible C-terminal arm of NAC $\beta$  (Figure 2B) actively recruits MetAP1 to ribosomes to form a methionine excision complex for nascent proteins (Figure 4A–D). Structural studies have identified a conserved hydrophobic motif (<sup>146</sup>VPDLV<sup>150</sup> in human NAC $\beta$ ) at the end of the NAC $\beta$  arm, which specifically interacts with the N-terminal zinc-finger domain of MetAP1 (Gamerding et al. 2023). Experimental data from *in vivo* models confirm that this interaction is essential for the enzyme's ribosome binding and for facilitating N-terminal methionine excision by MetAP1 in cells. Additionally, the association of NAC's globular domain with the ribosomal tunnel exit is critical for proper enzyme docking (Figure 4D) (Gamerding et al. 2023). The NAC $\alpha$  C-terminal UBA domain and a helix (H2) following the NAC globular domain play distinct roles in NatA/E recruitment (Figure 4A–C). The UBA domain captures the enzyme complex. Notably, the binding to NAC $\alpha$  H2 releases the inhibitory block of HYPK from the Naa10 active site while both UBA domains of NAC and HYPK remain anchored to distinct locations on Naa15 (Figure 4A).

The high-affinity interactions between NAC $\beta$ 's C-terminal hydrophobic motif with MetAP1 and NAC $\alpha$ 's C-terminal UBA domain with NatA/E facilitate efficient ribosome association of these N-terminal maturation enzymes (Figure 4A–D). This

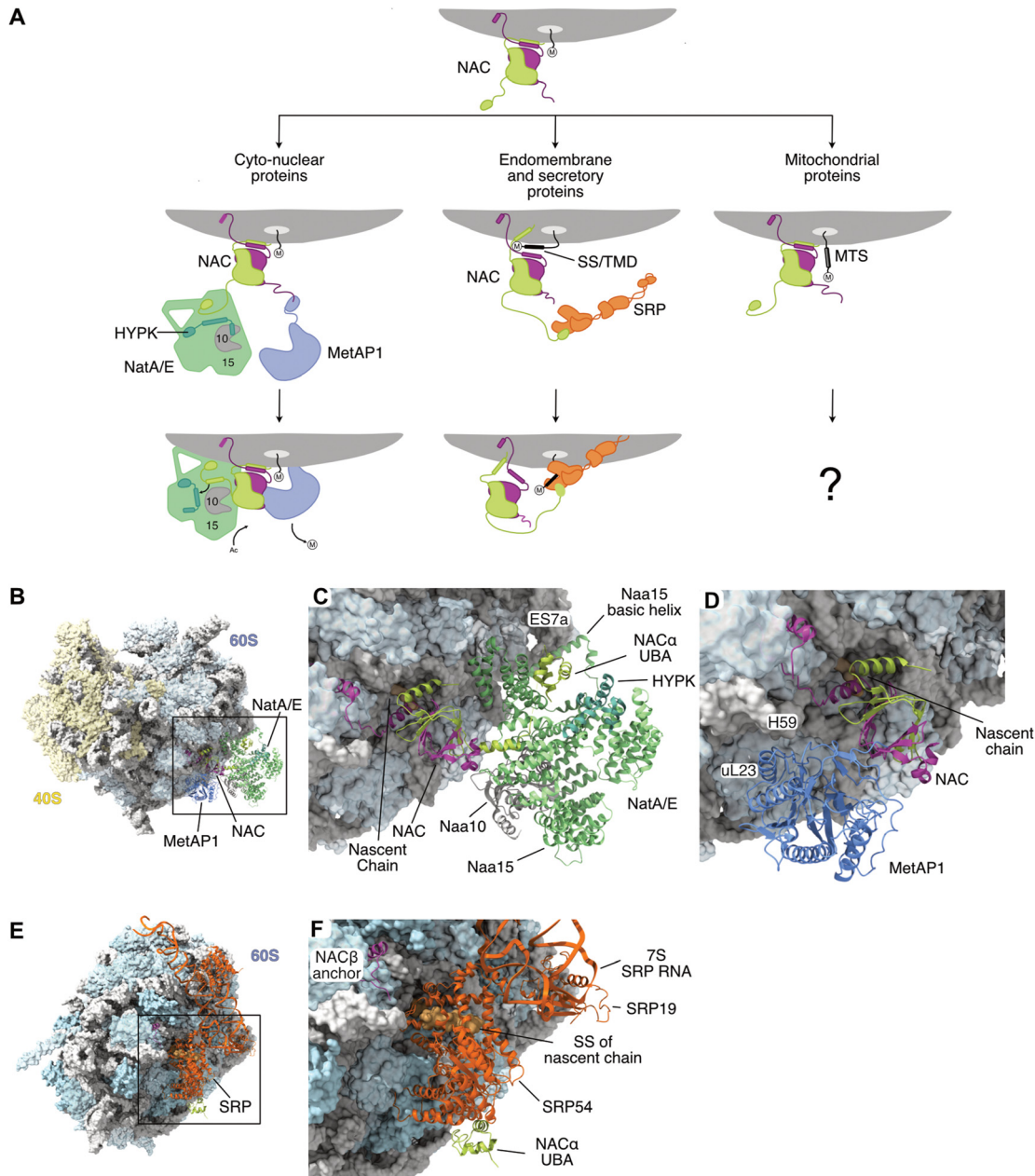
highlights the importance of precise spatial organisation of processing enzymes at the tunnel for efficient N-terminal maturation. Consistent with the distances of the catalytic sites, *in vitro* reconstitution of this process demonstrated that NME occurs at a nascent chain length of approximately 60 amino acids, while N-terminal acetylation by NatA takes place at around 95 amino acids (Lentzsch et al. 2024). Moreover, *in vitro* analyses suggest a significant co-dependency of MetAP1 and NatA for optimal catalytic activity showing that NatA activity was enhanced in the presence of MetAP1 in an *in vitro* reconstitution assay (Lentzsch et al. 2024). These findings support the notion that the quaternary complex organized by NAC offers a unique N-terminal processing environment, where the position and activity of the respective enzymes are finetuned by each other's presence.

Interestingly, N-terminal processing of cyto-nuclear proteins differs between yeast and higher eukaryotes. Yeast MetAP1 and NatA both rely on the extension segment ES27L<sub>a</sub> for correct positioning on the ribosome which is prerequisite for their cotranslational activity (Fujii et al. 2018; Knorr et al. 2019, 2023; Shankar et al. 2020). Moreover, NAC knock-out in yeast is viable and does not result in a growth phenotype (Reimann et al. 1999) suggesting plasticity of cotranslational protein biosynthesis pathways in lower eukaryotic organisms.

## 5 NAC's role in SRP-dependent ER targeting

Approximately one third of the proteome is targeted to the ER, with the majority relying on the cotranslational action of the signal recognition particle (SRP) (Akopian et al. 2013). SRP is a universally conserved ribonucleoprotein composed of six protein subunits (SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72) and a 7S SRP RNA in eukaryotes (Walter and Blobel 1980, 1982). Notably, SRP is much less abundant in the cytosol compared to ribosomes, with a ratio of at least 1:10 (Kulak et al. 2014). When a hydrophobic targeting signal of a protein destined to the ER, either an N-terminal SS or TMD, emerges from the ribosomal tunnel exit, it is recognized and bound by SRP54 (Voorhees and Hegde 2015). Once bound by SRP, the RNC is delivered to the Sec61 translocon in ER-membrane via the ER-membrane associated SRP receptor (SR) in a GTP-dependent manner. SRP then dissociates from SR, completing the targeting cycle (Jomaa et al. 2021; Kobayashi et al. 2018; Lee et al. 2018).

The SS/TMD recognition is a critical step in ER targeting. However, *in vitro* SRP also binds to signal-less ribosome nascent chain complexes (RNCs) suggesting a regulatory mechanism at the ribosome *in vivo* (Chartron et al. 2016; Flanagan et al. 2003; Kalies et al. 1994). Two studies provided



**Figure 4:** NAC regulates cotranslational protein processing and targeting. (A) Model of NAC (NACα in light green, NACβ in purple) as a regulatory hub on the ribosome, orchestrating cotranslational protein processing. MetAP1 in blue, Naa15 in green, Naa10 in grey, HYPK in cyan, SRP in orange. (B–D) Molecular model of cryo-EM structure of quaternary complex of NAC, MetAP1, NatA/E-HYPK, and RNC displaying a model protein (PDB: 9F1D). Surface representations of 40S r-proteins in light yellow, 60S r-proteins in light blue, rRNA in grey, and nascent chain in light brown. Naa50 is not depicted for clarity. (C) Close-up of NAC and NatA/E-HYPK. (D) Close-up of NAC and MetAP1. (E) Molecular model of cryo-EM structure of NAC (NACα in light green, NACβ in purple), SRP (orange), and RNC displaying a signal sequence in post-handover state of the nascent chain (PDB: 7QWQ). Surface representations of 60S r-proteins in light blue, rRNA in grey, nascent chain in light brown. NAC globular domain is not resolved in this structure. (F) Close-up of NAC and SRP at the ribosomal tunnel exit.

evidence *in vivo* that the binding of SRP to secretory nascent chains is tightly regulated by NAC (Gamerding et al. 2015; Jomaa et al. 2022) (Figure 4A). Via globular domain docking, NAC prevents incorrect association of SRP to RNCs without SS by sterically blocking SRP access to the ribosomal exit site.

For ribosomes engaged in secretory protein translation, the release of NAC's globular domain from the tunnel exit is necessary to allow access of SRP (Figure 4A). This detachment is triggered by hydrophobic ER-targeting signals in the growing polypeptide which interact with a hydrophobic



pocket of NACs globular domain beneath the two  $\alpha$ -helices that tether the globular domain to the ribosome exit site (Figure 2C). This interaction destabilizes the ribosome-binding platform, causing the NAC globular domain to detach, thereby eliminating its antagonistic effect on SRP (Figure 4A). However, NAC remains associated with the ribosome via its N-terminal NAC $\beta$  anchor, which binds outside the SRP docking region (Figure 4E and F). Importantly, there is a second activity of NAC on SRP binding. Structural and biochemical analyses revealed that the UBA domain of NAC $\alpha$  specifically interacts with the NG-domain of SRP54 for SRP recruitment (Jomaa et al. 2022) (Figure 4A, E and F). It is suggested that this enhances the local concentration of SRP, which is about 10-fold less abundant than ribosomes (Kulak et al. 2014), and allows SRP to scan the ribosomal exit site for binding access and the nascent chains for the presence of a signal sequence.

Notably, proteins destined for the endoplasmic reticulum (ER) appear to be exempt from NME and NTA, as N-terminal processing disfavours translocation (Forte et al. 2011). This phenomenon can be explained by the conformational change of NAC's globular domain induced by hydrophobic ER targeting signals. Signal sequence exposure and interactions with the hydrophobic pocket in NACs globular domain detaches this domain from the ribosomal tunnel exit (Figure 4A) and thereby disrupts efficient MetAP1 binding and subsequent acetylation of nascent proteins.

## 6 Other roles of NAC

Yeast NAC has also been linked to cotranslational targeting to mitochondria, acting as a stimulatory factor for import. Deletion of NAC genes is possible in yeast and cells display mild mitochondrial targeting defects, consistent with decreased levels of mitochondria-associated ribosomes (Alamo et al. 2011; Fünfschilling and Rospert 1999; George et al. 1998, 2002; Yogev et al. 2007). OM14, a yeast-specific outer mitochondrial membrane protein, serves as an NAC receptor, enhancing cotranslational import and interacting with the TOM complex to facilitate nascent polypeptide handover to the translocation pore (Lesnik et al. 2014; Schulte et al. 2023). Similar mechanisms remain to be found in other eukaryotic species (Figure 4A). In *C. elegans*, NAC depletion triggers the mitochondrial stress response, at least partially due to mistargeting of ER-bound substrates to mitochondria (Gamerding et al. 2015). A CRISPRi screen in human cell lines implicated NAC in the biogenesis of polytopic outer mitochondrial membrane proteins (Muthukumar et al. 2024).

Moreover, NAC has long been considered a ribosome-associated chaperone (Deuerling et al. 2019; Shen et al. 2019). Knockout of NAC and RAC in yeast results in a synthetic cell viability defect associated with wide-spread aggregation, particularly of ribosomal proteins (Koplin et al. 2010; Ott et al. 2015). Cotranslational ubiquitylation is also elevated in NAC-depleted cells, primarily for aggregation-prone proteins, membrane-targeted, and mitochondrial species, indicative of a protective role during translation (Duttler et al. 2013; Wang et al. 2013). NAC also exhibits chaperone activity off the ribosome, colocalizing with aggregates during aging and after heat shock. Additionally, overexpression of NAC delays polyQ aggregation and facilitates luciferase refolding in worms (Kirstein-Miles et al. 2013; Shen et al. 2019). *In vitro*, NAC prevents aggregation of substrates such as polyQ, firefly luciferase, A $\beta$ -40, and  $\alpha$ -synuclein in an ATP-independent manner, characteristic of holdase-type chaperone. For polyQ- and luciferase-type aggregates, this activity was partly attributed to charged interactions with the NAC $\beta$  N-terminus, suggesting that NAC can shuttle between ribosome-associated and posttranslational chaperone roles (Kirstein-Miles et al. 2013; Martin et al. 2018; Shen et al. 2019). However, the precise mechanism of NAC's chaperone activity and substrate recognition remains unexplored.

## 7 Concluding remarks

Positioned at the forefront of protein production, NAC plays an essential role in maintaining proteome integrity in eukaryotes by regulating the access of different protein biogenesis factors including MetAP1 for N-terminal methionine excision, NatA/E for N-terminal acetylation, and SRP for transport of nascent proteins to the ER. It is unclear if NAC is also involved in the binding of other NATs. Moreover, NAC's role in regulating further cotranslational modifications including N-terminal myristoylation by N-terminal myristoyl transferases (NMT1 and NMT2) is unknown (Duronio et al. 1989; Wilcox et al. 1987; Yang et al. 2005). N-terminal myristoylation occurs downstream of iMet cleavage by MetAP1/2 and directly competes with NatA for its substrates (Castrec et al. 2018). It is tempting to speculate that NAC coordinates also NMT1/2 binding to ensure efficient and specific processing of nascent chains. Further open questions are (i) regarding the purpose and functionality of the NAC's tunnel insertion, and how this relates to NAC's function as a molecular control hub at the ribosome tunnel exit, (ii) NAC's role in the handling of mitochondrial proteins, and (iii) if NAC's chaperone activity aids folding of nascent proteins given its ability to efficiently suppress protein aggregation *in vivo* and *in vitro*.

**Acknowledgments:** We apologize to those whose work could not be cited owing to space constraints. We thank Dr. Christina Schlatterer for proof reading the manuscript.

**Research ethics:** Not applicable.

**Informed consent:** Not applicable.

**Author contributions:** All authors have accepted responsibility for the entire content of this manuscript and approved its submission. E.D. and L.R. wrote the manuscript and designed the figures.

**Use of Large Language Models, AI and Machine Learning Tools:** None declared.

**Conflict of interest:** The authors state no conflict of interest.

**Research funding:** German Science Foundation (DFG) grants SPP 2453/P07 and 537004599 to E.D. and SFB969/A01.

**Data availability:** Not applicable.

## References

- Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., Ronneberger, O., Willmore, L., Ballard, A.J., Bambrick, J., et al. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* 630: 493–500.
- Addlagatta, A., Hu, X., Liu, J.O., and Matthews, B.W. (2005). Structural basis for the functional differences between type I and type II human methionine aminopeptidases. *Biochemistry* 44: 14741–14749.
- Agirrezabala, X., Samatova, E., Klimova, M., Zamora, M., Gil-Carton, D., Rodnina, M.V., and Valle, M. (2017). Ribosome rearrangements at the onset of translational bypassing. *Sci. Adv.* 3: e1700147.
- Akopian, D., Shen, K., Zhang, X., and Shan, S.-O. (2013). Signal recognition particle: an essential protein-targeting machine. *Annu. Rev. Biochem.* 82: 693–721.
- Aksnes, H., Ree, R., and Arnesen, T. (2019). Co-translational, post-translational, and non-catalytic roles of N-terminal acetyltransferases. *Mol. Cell* 73: 1097–1114.
- Alamo, M.d., Hogan, D.J., Pechmann, S., Albanese, V., Brown, P.O., and Frydman, J. (2011). Defining the specificity of cotranslationally acting chaperones by systematic analysis of mRNAs associated with ribosome-nascent chain complexes. *PLoS Biol.* 9: e1001100.
- Andersen, K.M., Semple, C.A., and Hartmann-Petersen, R. (2007). Characterisation of the nascent polypeptide-associated complex in fission yeast. *Mol. Biol. Rep.* 34: 275–281.
- Arnesen, T., Anderson, D., Baldersheim, C., Lanotte, M., Varhaug, J.E., and Lillehaug, J.R. (2005). Identification and characterization of the human ARD1–NATH protein acetyltransferase complex. *Biochem. J.* 386: 433–443.
- Arnesen, T., Starheim, K.K., Van Damme, P., Evjenth, R., Dinh, H., Betts, M.J., Ryningen, A., Vandekerckhove, J., Gevaert, K., and Anderson, D. (2010). The chaperone-like protein HYPK acts together with NatA in cotranslational N-terminal acetylation and prevention of Huntingtin aggregation. *Mol. Cell. Biol.* 30: 1898–1909.
- Arnesen, T., Van Damme, P., Polevoda, B., Helsens, K., Evjenth, R., Colaert, N., Varhaug, J.E., Vandekerckhove, J., Lillehaug, J.R., Sherman, F., et al. (2009). Proteomics analyses reveal the evolutionary conservation and divergence of N-terminal acetyltransferases from yeast and humans. *Proc. Natl. Acad. Sci. U S A* 106: 8157–8162.
- Beckmann, R., Spahn, C.M.T., Eswar, N., Helmers, J., Penczek, P.A., Sali, A., Frank, J., and Blobel, G. (2001). Architecture of the protein-conducting channel associated with the translating 80S ribosome. *Cell* 107: 361–372.
- Behnia, R., Panic, B., Whyte, J.R.C., and Munro, S. (2004). Targeting of the Arf-like GTPase Arl3p to the golgi requires N-terminal acetylation and the membrane protein Sys1p. *Nat. Cell Biol.* 6: 405–413.
- Bhaskar, V., Desogus, J., Graff-Meyer, A., Schenk, A.D., Cavadini, S., and Chao, J.A. (2021). Dynamic association of human Ebp1 with the ribosome. *RNA* 27: 411–419.
- Bhushan, S., Gartmann, M., Halic, M., Armache, J.-P., Jarasch, A., Mielke, T., Berninghausen, O., Wilson, D.N., and Beckmann, R. (2010).  $\alpha$ -Helical nascent polypeptide chains visualized within distinct regions of the ribosomal exit tunnel. *Nat. Struct. Mol. Biol.* 17: 313–317.
- Bloss, T.A., Witze, E.S., and Rothman, J.H. (2003). Suppression of CED-3-independent apoptosis by mitochondrial  $\beta$ NAC in *Caenorhabditis elegans*. *Nature* 424: 1066–1071.
- Bykov, Y.S., Rapaport, D., Herrmann, J.M., and Schuldiner, M. (2020). Cytosolic events in the biogenesis of mitochondrial proteins. *Trends Biochem. Sci.* 45: 650–667.
- Castrec, B., Dian, C., Ciccone, S., Ebert, C.L., Bienvenut, W.V., Le Caer, J.-P., Steyaert, J.-M., Giglione, C., and Meinel, T. (2018). Structural and genomic decoding of human and plant myristoylomes reveals a definitive recognition pattern. *Nat. Chem. Biol.* 14: 671–679.
- Chartron, J.W., Hunt, K.C.L., and Frydman, J. (2016). Cotranslational signal-independent SRP preloading during membrane targeting. *Nature* 536: 224–228.
- Dao Duc, K., Batra, S.S., Bhattacharya, N., Cate, J.H.D., and Song, Y.S. (2019). Differences in the path to exit the ribosome across the three domains of life. *Nucleic Acids Res.* 47: 4198–4210.
- Deng, J.M. and Behringer, R.R. (1995). An insertional mutation in the BTF3 transcription factor gene leads to an early postimplantation lethality in mice. *Transgenic Res.* 4: 264–269.
- Deng, S., Gardner, S.M., Gottlieb, L., Pan, B., Petersson, E.J., and Marmorstein, R. (2023). Molecular role of NAA38 in thermostability and catalytic activity of the human NatC N-terminal acetyltransferase. *Structure* 31: 166–173.e4.
- Deng, S., Gottlieb, L., Pan, B., Supplee, J., Wei, X., Petersson, E.J., and Marmorstein, R. (2021). Molecular mechanism of N-terminal acetylation by the ternary NatC complex. *Structure* 29: 1094–1104.e1094.
- Deng, S., Magin, R.S., Wei, X., Pan, B., Petersson, E.J., and Marmorstein, R. (2019). Structure and mechanism of acetylation by the N-terminal dual enzyme NatA/Naa50 complex. *Structure* 27: 1057–1070.e1054.
- Deng, S., McTiernan, N., Wei, X., Arnesen, T., and Marmorstein, R. (2020a). Molecular basis for N-terminal acetylation by human NatE and its modulation by HYPK. *Nat. Commun.* 11: 818.
- Deng, S., Pan, B., Gottlieb, L., Petersson, E.J., and Marmorstein, R. (2020b). Molecular basis for N-terminal alpha-synuclein acetylation by human NatB. *eLife* 9: e57491.
- Deuerling, E., Gamberdinger, M., and Kreft, S.G. (2019). Chaperone interactions at the ribosome. *Cold Spring Harb. Perspect. Biol.* 11, <https://doi.org/10.1101/cshperspect.a033977>.
- Dikiy, I. and Eliezer, D. (2014). N-terminal acetylation stabilizes n-terminal helicity in lipid- and micelle-bound  $\alpha$ -synuclein and increases its affinity for physiological membranes. *J. Biol. Chem.* 289: 3652–3665.
- Duronio, R.J., Towler, D.A., Heuckeroth, R.O., and Gordon, J.I. (1989). Disruption of the yeast N-myristoyl transferase gene causes recessive lethality. *Science* 243: 796–800.

- Duttler, S., Pechmann, S., and Frydman, J. (2013). Principles of cotranslational ubiquitination and quality control at the ribosome. *Mol. Cell* 50: 379–393.
- Flanagan, J.J., Chen, J.-C., Miao, Y., Shao, Y., Lin, J., Bock, P.E., and Johnson, A.E. (2003). Signal recognition particle binds to ribosome-bound signal sequences with fluorescence-detected subnanomolar affinity that does not diminish as the nascent chain lengthens. *J. Biol. Chem.* 278: 18628–18637.
- Forte, G.M.A., Pool, M.R., and Stirling, C.J. (2011). N-terminal acetylation inhibits protein targeting to the endoplasmic reticulum. *PLoS Biol.* 9: e1001073.
- Fujii, K., Susanto, T.T., Saurabh, S., and Barna, M. (2018). Decoding the function of expansion segments in ribosomes. *Mol. Cell* 72: 1013–1020.e1016.
- Fünfschilling, U. and Rospert, R. (1999). Nascent polypeptide-associated complex stimulates protein import into yeast mitochondria. *Mol. Biol. Cell* 10: 3289–3299.
- Gamerding, M. and Deuerling, E. (2024). Cotranslational sorting and processing of newly synthesized proteins in eukaryotes. *Trends Biochem. Sci.* 49: 105–118.
- Gamerding, M., Hanebuth, M.A., Frickey, T., and Deuerling, E. (2015). The principle of antagonism ensures protein targeting specificity at the endoplasmic reticulum. *Science* 348: 201–207.
- Gamerding, M., Jia, M., Schloemer, R., Rabl, L., Jaskolowski, M., Khakzar, K.M., Ulusoy, Z., Wallisch, A., Jomaa, A., Hunaeus, G., et al. (2023). NAC controls cotranslational N-terminal methionine excision in eukaryotes. *Science* 380: 1238–1243.
- Gamerding, M., Kobayashi, K., Wallisch, A., Kreft, S.G., Sailer, C., Schlömer, R., Sachs, N., Jomaa, A., Stengel, F., Ban, N., et al. (2019). Early scanning of nascent polypeptides inside the ribosomal tunnel by NAC. *Mol. Cell* 75: 996–1006.e1008.
- Gao, J., Barroso, C., Zhang, P., Kim, H.-M., Li, S., Labrador, L., Lightfoot, J., Gerashchenko, M.V., Labunsky, V.M., Dong, M.-Q., et al. (2016). N-terminal acetylation promotes synaptosomal complex assembly in *C. elegans*. *Genes Dev.* 30: 2404–2416.
- Geiger, T., Wehner, A., Schaab, C., Cox, J., and Mann, M. (2012). Comparative proteomic analysis of eleven common cell lines reveals ubiquitous but varying expression of most proteins. *Mol. Cell. Proteomics* 11, M111.014050.
- George, R., Beddoe, T., Landl, K., and Lithgow, T. (1998). The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria in vivo. *Proc. Natl. Acad. Sci. U S A* 95: 2296–2301.
- George, R., Walsh, P., Beddoe, T., and Lithgow, T. (2002). The nascent polypeptide-associated complex (NAC) promotes interaction of ribosomes with the mitochondrial surface in vivo. *FEBS Lett.* 516: 213–216.
- Giglione, C., Boularot, A., and Meinel, T. (2004). Protein N-terminal methionine excision. *Cell. Mol. Life Sci.* 61: 1455–1474.
- Gottlieb, L. and Marmorstein, R. (2018). Structure of human NatA and its regulation by the Huntingtin interacting protein HYPK. *Structure* 26: 925–935.e928.
- Greber, B.J., Boehringer, D., Montellese, C., and Ban, N. (2012). Cryo-EM structures of Arx1 and maturation factors Rei1 and Jjj1 bound to the 60S ribosomal subunit. *Nat. Struct. Mol. Biol.* 19: 1228–1233.
- Grunwald, S., Hopf, L.V.M., Bock-Bierbaum, T., Lally, C.C.M., Spahn, C.M.T., and Daumke, O. (2020). Divergent architecture of the heterotrimeric NatC complex explains N-terminal acetylation of cognate substrates. *Nat. Commun.* 11: 5506.
- Halic, M., Gartmann, M., Schlenker, O., Mielke, T., Pool, M.R., Sinning, I., and Beckmann, R. (2006). Signal recognition particle receptor exposes the ribosomal translocon binding site. *Science* 312: 745–747.
- Hole, K., Van Damme, P., Dalva, M., Aksnes, H., Glomnes, N., Varhaug, J.E., Lillehaug, J.R., Gevaert, K., and Arnesen, T. (2011). The human N-alpha-acetyltransferase 40 (hNaa40p/hNatD) is conserved from yeast and N-terminally acetylates histones H2A and H4. *PLoS One* 6: e24713.
- Hwang, C.-S., Shemorry, A., and Varshavsky, A. (2010). N-terminal acetylation of cellular proteins creates specific degradation signals. *Science* 327: 973–977.
- Jomaa, A., Eitzinger, S., Zhu, Z., Chandrasekar, S., Kobayashi, K., Shan, S.-o., and Ban, N. (2021). Molecular mechanism of cargo recognition and handover by the mammalian signal recognition particle. *Cell Rep.* 36: 109350.
- Jomaa, A., Gamerding, M., Hsieh, H.-H., Wallisch, A., Chandrasekaran, V., Ulusoy, Z., Scaiola, A., Hegde, R.S., Shan, S.-O., Ban, N., et al. (2022). Mechanism of signal sequence handover from NAC to SRP on ribosomes during ER-protein targeting. *Science* 375: 839–844.
- Juszkiewicz, S., Peak-Chew, S.-Y., and Hegde, R.S. (2025). Mechanism of chaperone recruitment and retention on mitochondrial precursors. *Mol. Biol. Cell* 36: ar39.
- Kalies, K.-U., Görlich, D., and Rapoport, T.A. (1994). Binding of ribosomes to the rough endoplasmic reticulum mediated by the Sec61p-complex. *J. Cell Biol.* 126: 925–934.
- Kang, L., Moriarty, G.M., Woods, L.A., Ashcroft, A.E., Radford, S.E., and Baum, J. (2012). N-terminal acetylation of  $\alpha$ -synuclein induces increased transient helical propensity and decreased aggregation rates in the intrinsically disordered monomer. *Protein Sci.* 21: 911–917.
- Khatner, H., Myasnikov, A.G., Natchiar, S.K., and Klaholz, B.P. (2015). Structure of the human 80S ribosome. *Nature* 520: 640–645.
- Kirstein-Miles, J., Scior, A., Deuerling, E., and Morimoto, R.I. (2013). The nascent polypeptide-associated complex is a key regulator of proteostasis. *The EMBO J.* 32: 1451–1468.
- Klein, M., Wild, K., and Sinning, I. (2024a). Multi-protein assemblies orchestrate co-translational enzymatic processing on the human ribosome. *Nat. Commun.* 15: 7681.
- Klein, M.A., Wild, K., Kišonaitė, M., and Sinning, I. (2024b). Methionine aminopeptidase 2 and its autoprolysis product have different binding sites on the ribosome. *Nat. Commun.* 15: 716.
- Knorr, A.G., Mackens-Kiani, T., Musial, J., Berninghausen, O., Becker, T., Beatrix, B., and Beckmann, R. (2023). The dynamic architecture of Map1- and NatB-ribosome complexes coordinates the sequential modifications of nascent polypeptide chains. *PLoS Biol.* 21: e3001995.
- Knorr, A.G., Schmidt, C., Tesina, P., Berninghausen, O., Becker, T., Beatrix, B., and Beckmann, R. (2019). Ribosome–NatA architecture reveals that rRNA expansion segments coordinate N-terminal acetylation. *Nat. Struct. Mol. Biol.* 26: 35–39.
- Kobayashi, K., Jomaa, A., Lee, J.H., Chandrasekar, S., Boehringer, D., Shan, S.-O., and Ban, N. (2018). Structure of a prehandover mammalian ribosomal SRP-SRP receptor targeting complex. *Science* 360: 323–327.
- Koplin, A., Preissler, S., Ilina, Y., Koch, M., Scior, A., Erhardt, M., and Deuerling, E. (2010). A dual function for chaperones SSB–RAC and the NAC nascent polypeptide-associated complex on ribosomes. *J. Cell Biol.* 189: 57–68.
- Kosolapov, A. and Deutsch, C. (2009). Tertiary interactions within the ribosomal exit tunnel. *Nat. Struct. Mol. Biol.* 16: 405–411.

- Kramer, G., Boehringer, D., Ban, N., and Bukau, B. (2009). The ribosome as a platform for co-translational processing, folding and targeting of newly synthesized proteins. *Nat. Struct. Mol. Biol.* 16: 589–597.
- Kramer, G., Rauch, T., Rist, W., Vorderwülbecke, S., Patzelt, H., Schulze-Specking, A., Ban, N., Deuerling, E., and Bukau, B. (2002). L23 protein functions as a chaperone docking site on the ribosome. *Nature* 419: 171–174.
- Kramer, G., Shiber, A., and Bukau, B. (2019). Mechanisms of cotranslational maturation of newly synthesized proteins. *Annu. Rev. Biochem.* 88: 337–364.
- Kraushar, M.L., Krupp, F., Harnett, D., Turko, P., Ambrozkiwicz, M.C., Sprink, T., Imami, K., Günigmann, M., Zinnall, U., Vieira-Vieira, C.H., et al. (2021). Protein synthesis in the developing neocortex at near-atomic resolution reveals Ebp1-mediated neuronal proteostasis at the 60S tunnel exit. *Mol. Cell* 81: 304–322.e316.
- Kulak, N.A., Pichler, G., Paron, I., Nagaraj, N., and Mann, M. (2014). Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nat. Methods* 11: 319–324.
- Lee, J.H., Chandrasekar, S., Chung, S., Hwang Fu, Y.-H., Liu, D., Weiss, S., and Shan, S.-O. (2018). Sequential activation of human signal recognition particle by the ribosome and signal sequence drives efficient protein targeting. *Proc. Natl. Acad. Sci. U S A* 115: E5487–E5496.
- Lentsch, A.M., Yudin, D., Gämderinger, M., Chandrasekar, S., Rabl, L., Scaiola, A., Deuerling, E., Ban, N., and Shan, S.-O. (2024). NAC guides a ribosomal multienzyme complex for nascent protein processing. *Nature* 633: 718–724.
- Lesnik, C., Cohen, Y., Atir-Lande, A., Schuldiner, M., and Arava, Y. (2014). OM14 is a mitochondrial receptor for cytosolic ribosomes that supports co-translational import into mitochondria. *Nat. Commun.* 5: 5711.
- Lin, Z., Gasic, I., Chandrasekaran, V., Peters, N., Shao, S., Mitchison, T.J., and Hegde, R.S. (2020). TTC5 mediates autoregulation of tubulin via mRNA degradation. *Science* 367: 100–104.
- Liszcak, G., Goldberg, J.M., Foyn, H., Petersson, E.J., Arnesen, T., and Marmorstein, R. (2013). Molecular basis for N-terminal acetylation by the heterodimeric NatA complex. *Nat. Struct. Mol. Biol.* 20: 1098–1105.
- Liu, S., Widom, J., Kemp, C.W., Crews, C.M., and Clardy, J. (1998). Structure of human methionine aminopeptidase-2 complexed with fumagillin. *Science* 282: 1324–1329.
- Liu, Y., Hu, Y., Li, X., Niu, L., and Teng, M. (2010). The crystal structure of the human nascent polypeptide-associated complex domain reveals a nucleic acid-binding region on the NACA subunit. *Biochemistry* 49: 2890–2896.
- Lu, J. and Deutsch, C. (2005). Folding zones inside the ribosomal exit tunnel. *Nat. Struct. Mol. Biol.* 12: 1123–1129.
- Lu, J., Kobertz, W.R., and Deutsch, C. (2007). Mapping the electrostatic potential within the ribosomal exit tunnel. *J. Mol. Biol.* 371: 1378–1391.
- Magin, R.S., Liszcak, G.P., and Marmorstein, R. (2015). The molecular basis for histone H4- and H2A-specific amino-terminal acetylation by NatD. *Structure* 23: 332–341.
- Markesich, D.C., Gajewski, K.M., Nazimiec, M.E., and Beckingham, K. (2000). Bicaudal encodes the Drosophila beta NAC homolog, a component of the ribosomal translational machinery. *Development* 127: 559–572.
- Martin, E.M., Jackson, M.P., Gämderinger, M., Gense, K., Karamonos, T.K., Humes, J.R., Deuerling, E., Ashcroft, A.E., and Radford, S.E. (2018). Conformational flexibility within the nascent polypeptide-associated complex enables its interactions with structurally diverse client proteins. *J. Biol. Chem.* 293: 8554–8568.
- McTiernan, N., Kjosås, I., and Arnesen, T. (2025). Illuminating the impact of N-terminal acetylation: from protein to physiology. *Nat. Commun.* 16: 703.
- Minoia, M., Quintana-Cordero, J., Jetzinger, K., Kotan, I.E., Turnbull, K.J., Ciccarelli, M., Masser, A.E., Liebers, D., Gouarin, E., Czech, M., et al. (2024). Chp1 is a dedicated chaperone at the ribosome that safeguards eEF1A biogenesis. *Nat. Commun.* 15: 1382.
- Mueller, T.D. and Feigon, J. (2002). Solution structures of UBA domains reveal a conserved hydrophobic surface for protein–protein interactions. *J. Mol. Biol.* 319: 1243–1255.
- Muthukumar, G., Stevens, T.A., Inglis, A.J., Esantsi, T.K., Saunders, R.A., Schulte, F., Voorhees, R.M., Guna, A., and Weissman, J.S. (2024). Triaging of  $\alpha$ -helical proteins to the mitochondrial outer membrane by distinct chaperone machinery based on substrate topology. *Mol. Cell* 84: 1101–1119.e1109.
- Nilsson, O.B., Hedman, R., Marino, J., Wickles, S., Bischoff, L., Johansson, M., Müller-Lucks, A., Trovato, F., Puglisi, J.D., O'Brien, E.P., et al. (2015). Cotranslational protein folding inside the ribosome exit tunnel. *Cell Rep.* 12: 1533–1540.
- Nilsson, O.B., Nickson, A.A., Hollins, J.J., Wickles, S., Steward, A., Beckmann, R., von Heijne, G., and Clarke, J. (2017). Cotranslational folding of spectrin domains via partially structured states. *Nat. Struct. Mol. Biol.* 24: 221–225.
- Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. (2000). The Structural basis of ribosome activity in peptide bond synthesis. *Science* 289: 920–930.
- Nyathi, Y. and Pool, M.R. (2015). Analysis of the interplay of protein biogenesis factors at the ribosome exit site reveals new role for NAC. *J. Cell Biol.* 210: 287–301.
- Ott, A.-K., Locher, L., Koch, M., and Deuerling, E. (2015). Functional dissection of the nascent polypeptide-associated complex in *Saccharomyces cerevisiae*. *PLoS One* 10: e0143457.
- Øye, H., Lundekvam, M., Caiella, A., Hellesvik, M., and Arnesen, T. (2025). Protein N-terminal modifications: molecular machineries and biological implications. *Trends in Biochemical Sciences*, <https://doi.org/10.1016/j.tibs.2024.12.012> (Epub ahead of print).
- Pech, M., Spreter, T., Beckmann, R., and Beatrix, B. (2010). Dual binding mode of the nascent polypeptide-associated complex reveals a novel universal adapter site on the ribosome. *J. Biol. Chem.* 285: 19679–19687.
- Peisker, K., Braun, D., Wölfe, T., Hentschel, J., Fünfschilling, U., Fischer, G., Sickmann, A., and Rospert, S. (2008). Ribosome-associated complex binds to ribosomes in close proximity of Rpl31 at the exit of the polypeptide tunnel in yeast. *Mol. Biol. Cell* 19: 5279–5288.
- Polevoda, B., Brown, S., Cardillo, T.S., Rigby, S., and Sherman, F. (2008). Yeast Na-terminal acetyltransferases are associated with ribosomes. *J. Cell. Biochem.* 103: 492–508.
- Polevoda, B., Cardillo, T.S., Doyle, T.C., Bedi, G.S., and Sherman, F. (2003). Nat3p and Mdm20p are required for function of yeast NatB Na-terminal acetyltransferase and of actin and tropomyosin. *J. Biol. Chem.* 278: 30686–30697.
- Polevoda, B., Hoskins, J., and Sherman, F. (2009). Properties of Nat4, an Na-acetyltransferase of *Saccharomyces cerevisiae* that modifies N termini of histones H2A and H4. *Mol. Cell. Biol.* 29: 2913–2924.
- Pool, M.R., Stumm, J., Fulga, T.A., Sinning, I., and Dobberstein, B. (2002). Distinct modes of signal recognition particle interaction with the ribosome. *Science* 297: 1345–1348.
- Rapoport, T.A., Li, L., and Park, E. (2017). Structural and mechanistic insights into protein translocation. *Annu. Rev. Cell Dev. Biol.* 33: 369–390.



- Raue, U., Oellerer, S., and Rospert, S. (2007). Association of protein biogenesis factors at the yeast ribosomal tunnel exit is affected by the translational status and nascent polypeptide sequence. *J. Biol. Chem.* 282: 7809–7816.
- Reimann, B., Bradsher, J., Franke, J., Hartmann, E., Wiedmann, M., Pohn, S., and Wiedmann, B. (1999). Initial characterization of the nascent polypeptide-associated complex in yeast. *Yeast* 15: 397–407.
- Roise, D., Horvath, S.J., Tomich, J.M., Richards, J.H., and Schatz, G. (1986). A chemically synthesized pre-sequence of an imported mitochondrial protein can form an amphiphilic helix and perturb natural and artificial phospholipid bilayers. *EMBO J.* 5: 1327–1334.
- Samatova, E., Komar, A.A., and Rodnina, M.V. (2024). How the ribosome shapes cotranslational protein folding. *Curr. Opin. Struct. Biol.* 84: 102740.
- Schulte, U., den Brave, F., Haupt, A., Gupta, A., Song, J., Müller, C.S., Engelke, J., Mishra, S., Mårtensson, C., Ellenrieder, L., et al. (2023). Mitochondrial complexome reveals quality-control pathways of protein import. *Nature* 614: 153–159.
- Scott, D.C., Monda, J.K., Bennett, E.J., Harper, J.W., and Schulman, B.A. (2011). N-terminal acetylation acts as an avidity enhancer within an interconnected multiprotein complex. *Science* 334: 674–678.
- Setty, S.R.G., Strohlic, T.I., Tong, A.H.Y., Boone, C., and Burd, C.G. (2004). Golgi targeting of ARF-like GTPase Arl3p requires its N-acetylation and the integral membrane protein Sys1p. *Nat. Cell Biol.* 6: 414–419.
- Shankar, V., Rauscher, R., Reuther, J., Gharib, W.H., Koch, M., and Polacek, N. (2020). rRNA expansion segment 27Lb modulates the factor recruitment capacity of the yeast ribosome and shapes the proteome. *Nucleic Acids Res.* 48: 3244–3256.
- Shen, K., Gamberdinger, M., Chan, R., Gense, K., Martin, E.M., Sachs, N., Knight, P.D., Schlömer, R., Calabrese, A.N., Stewart, K.L., et al. (2019). Dual role of ribosome-binding domain of NAC as a potent suppressor of protein aggregation and aging-related proteinopathies. *Mol. Cell* 74: 729–741.e727.
- Tate, E.W., Soddy, L., de la Lastra, A.L., Wang, M., and Lin, H. (2024). Protein lipidation in cancer: mechanisms, dysregulation and emerging drug targets. *Nat. Rev. Cancer* 24: 240–260.
- Traverso, J.A., Micallela, C., Martinez, A., Brown, S.C., Satiat-Jeunemaitre, B., Meinel, T., and Giglione, C. (2013). Roles of N-Terminal fatty acid acylations in membrane compartment partitioning: *Arabidopsis* h-Type thioredoxins as a case study. *Plant Cell* 25: 1056–1077.
- Van Damme, P., Evjenth, R., Foyn, H., Demeyer, K., De Bock, P.-J., Lillehaug, J.R., Vandekerckhove, J., Arnesen, T., and Gevaert, K. (2011). Proteome-derived peptide libraries allow detailed analysis of the substrate specificities of N-acetyltransferases and point to hNaa10p as the post-translational actin N-acetyltransferase. *Mol. Cell. Proteomics* 10: M110.004580.
- Van Damme, P., Kalvik, T.V., Starheim, K.K., Jonckheere, V., Myklebust, L.M., Menschaert, G., Varhaug, J.E., Gevaert, K., and Arnesen, T. (2016). A role for human N-alpha acetyltransferase 30 (Naa30) in maintaining mitochondrial integrity. *Mol. Cell. Proteomics* 15: 3361–3372.
- Van Damme, P., Osberg, C., Jonckheere, V., Glomnes, N., Gevaert, K., Arnesen, T., and Aksnes, H. (2023). Expanded *in vivo* substrate profile of the yeast N-terminal acetyltransferase NatC. *J. Biol. Chem.* 299: 102824.
- Varshavsky, A. (2024). N-degron pathways. *Proc. Natl. Acad. Sci. U S A* 121: e2408697121.
- Vetro, J.A. and Chang, Y.-H. (2002). Yeast methionine aminopeptidase type 1 is ribosome-associated and requires its N-terminal zinc finger domain for normal function *in vivo*. *J. Cell. Biochem.* 85: 678–688.
- Vögtle, F.N., Wortelkamp, S., Zahedi, R.P., Becker, D., Leidhold, C., Gevaert, K., Kellermann, J., Voos, W., Sickmann, A., Pfanner, N., et al. (2009). Global analysis of the mitochondrial N-proteome identifies a processing peptidase critical for protein stability. *Cell* 139: 428–439.
- Voorhees, R.M., Fernández, I.S., Scheres, S.H.W., and Hegde, R.S. (2014). Structure of the mammalian ribosome-Sec61 complex to 3.4 Å resolution. *Cell* 157: 1632–1643.
- Voorhees, R.M. and Hegde, R.S. (2015). Structures of the scanning and engaged states of the mammalian SRP-ribosome complex. *eLife* 4: e07975.
- Walter, P. and Blobel, G. (1980). Purification of a membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U S A* 77: 7112–7116.
- Walter, P. and Blobel, G. (1982). Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature* 299: 691–698.
- Wang, F., Durfee, L.A., and Huibregtse, J.M. (2013). A cotranslational ubiquitination pathway for quality control of misfolded proteins. *Mol. Cell* 50: 368–378.
- Wang, L., Zhang, W., Wang, L., Zhang, X.C., Li, X., and Rao, Z. (2010). Crystal structures of NAC domains of human nascent polypeptide-associated complex (NAC) and its αNAC subunit. *Protein Cell* 1: 406–416.
- Wegrzyn, R.D., Hofmann, D., Merz, F., Nikolay, R., Rauch, T., Graf, C., and Deuerling, E. (2006). A conserved motif is prerequisite for the interaction of NAC with ribosomal protein L23 and nascent chains. *J. Biol. Chem.* 281: 2847–2857.
- Weyer, F.A., Gumiero, A., Lapouge, K., Bange, G., Kopp, J., and Sinning, I. (2017). Structural basis of HypK regulating N-terminal acetylation by the NatA complex. *Nat. Commun.* 8: 15726.
- Wiedmann, B., Sakai, H., Davis, T.A., and Wiedmann, M. (1994). A protein complex required for signal-sequence-specific sorting and translocation. *Nature* 370: 434–440.
- Wilcox, C., Hu, J.-S., and Olson, E.N. (1987). Acylation of proteins with myristic acid occurs cotranslationally. *Science* 238: 1275–1278.
- Wild, K., Aleksic, M., Lapouge, K., Juare, K.D., Flemming, D., Pfeffer, S., and Sinning, I. (2020). MetAP-like Ebp1 occupies the human ribosomal tunnel exit and recruits flexible rRNA expansion segments. *Nat. Commun.* 11: 776.
- Williams, C.C., Jan, C.H., and Weissman, J.S. (2014). Targeting and plasticity of mitochondrial proteins revealed by proximity-specific ribosome profiling. *Science* 346: 748–751.
- Xiao, Q., Zhang, F., Nacev, B.A., Liu, J.O., and Pei, D. (2010). Protein N-terminal processing: substrate specificity of *Escherichia coli* and human methionine aminopeptidases. *Biochemistry* 49: 5588–5599.
- Yang, S.H., Shrivastav, A., Kosinski, C., Sharma, R.K., Chen, M.-H., Berthiaume, L.G., Peters, L.L., Chuang, P.-T., Young, S.G., and Bergo, M.O. (2005). N-myristoyltransferase 1 is essential in early mouse development. *J. Biol. Chem.* 280: 18990–18995.
- Yogev, O., Karniely, S., and Pines, O. (2007). Translation-coupled translocation of yeast fumarate into mitochondria *in vivo*. *J. Biol. Chem.* 282: 29222–29229.
- Ziv, G., Haran, G., and Thirumalai, D. (2005). Ribosome exit tunnel can entropically stabilize α-helices. *Proc. Natl. Acad. Sci. U S A* 102, pp. 18956–18961.
- Zuo, S., Guo, Q., Ling, C., and Chang, Y.-H. (1995). Evidence that two zinc fingers in the methionine aminopeptidase from *Saccharomyces cerevisiae* are important for normal growth. *Mol. Gen. Genet.* 246: 247–253.