nature structural & molecular biology

The ribosome as a platform for co-translational processing, folding and targeting of newly synthesized proteins

Günter Kramer[1], Daniel Boehringer[2], Nenad Ban[2] & Bernd Bukau[1]

© 2009 Nature America, Inc. All rights reserved.

The early events in the life of newly synthesized proteins in the cellular environment are remarkably complex. Concurrently with their synthesis by the ribosome, nascent polypeptides are subjected to enzymatic processing, chaperone-assisted folding or targeting to translocation pores at membranes. The ribosome itself has a key role in these different tasks and governs the interplay between the various factors involved. Indeed, the ribosome serves as a platform for the spatially and temporally regulated association of enzymes, targeting factors and chaperones that act upon the nascent polypeptides emerging from the exit tunnel. Furthermore, the ribosome provides opportunities to coordinate the protein-synthesis activity of its peptidyl transferase center with the protein targeting and folding processes. Here we review the early co-translational events involving the ribosome that guide cytosolic proteins to their native state.

Ribosomes decode genetic information and convert it into the amino acid sequences of proteins. Ribosomes account for as much as 30% of total cell mass, with up to 10^5 and 10^6 ribosomes in bacteria and mammalian cells, respectively[1]. In growing cells, most ribosomes are active in translation, synthesizing polypeptide chains at rates of about 20 amino acids per second in bacteria and 5–9 amino acids per second in eukaryotes. Ribosomes are large ribonucleoprotein complexes composed of two subunits with a total molecular mass ranging between ~2.4 MDa (bacteria) and ~4 MDa (eukaryotes). The small subunit is involved in the decoding of mRNA. The large subunit harbors the peptidyltransferase center, composed of ribosomal RNA (rRNA), that forms the active site responsible for peptide bond formation. It also contains the ribosomal exit tunnel for nascent polypeptides (**Fig. 1a**). An mRNA transcript may be translated by several ribosomes simultaneously, and a recent cryo–electron tomography analysis of active polysomes revealed that the ribosomes are arranged in a staggered or pseudohelical organization, with the polypeptide exit sites facing outward[2]. This arrangement maximizes the distances between nascent chains emerging from neighboring ribosomes, thereby preventing unfavorable interactions between them.

The ribosomal environment for nascent polypeptides

The peptide exit tunnel has a length of 80–100 Å and a diameter of approximately 10 Å at its narrowest and 20 Å at its widest points[3–5]. The tunnel resembles a tube, able to accommodate a peptide stretch of approximately 30 amino acids in extended conformation or, if the formation of secondary structure is possible, up to 60 amino acids in an α-helical conformation[6–8]. In bacterial ribosomes, the tunnel wall is formed predominantly by 23S rRNA and looped-out segments of the ribosomal proteins L4, L22 and L23. There is a pronounced constriction in the tunnel about 30 Å from the peptidyltransferase center. At this point, a conserved β-hairpin loop of L22 comes into close proximity to an extended loop of L4 (**Fig. 1b**,**c**). At its distal end, the ribosomal exit tunnel widens up[3–6,9–11]. The rim of the exit point is composed of RNA, a ring of four ubiquitously conserved ribosomal proteins (L22, L23, L24 and L29) and additional kingdom-specific proteins (see below). Some of these proteins constitute major interaction sites for various factors involved in nascent chain processing, folding and targeting.

Translation speed can affect protein folding

Translation is physically and functionally coupled to the folding and targeting of newly synthesized proteins. Several mechanisms of communication allow the adjustment of the translation speed to folding and targeting processes. Variations of translation rates may result from local stable mRNA structure or the presence of rare or slowly translated codons in the translated mRNA, which may have drastic effects on the folding efficiency of newly synthesized proteins[12]. For instance, replacement of rare codons by more frequent ones in the genes from *Escherichia coli* or *Saccharomyces cerevisiae* results in faster translation but reduces the specific activity of the encoded proteins[13,14]. In fact, folding efficiency of a multidomain protein in *E. coli* is perturbed by synonymous substitutions of rare codons by others with highly abundant tRNAs[15]. A silent mutation in the human gene *ABCB1* (also known as *MDR1*) causes the encoded P-glycoprotein to fold differently, indicating that the altered conformation is caused by perturbation of the timing of folding and translation[16]. Thus, the speed of protein synthesis can affect protein-folding pathways. Accordingly, codons translated at a lower speed are

[1]Zentrum für Molekulare Biologie der Universität Heidelberg, DKFZ-ZMBH Allianz, Heidelberg, Germany. [2]Institut für Molekularbiologie und Biophysik, ETH Zürich, Zürich, Switzerland. Correspondence should be addressed to B.B. (bukau@zmbh.uni-heidelberg.de).

Published online 3 June 2009; doi:10.1038/nsmb.1614

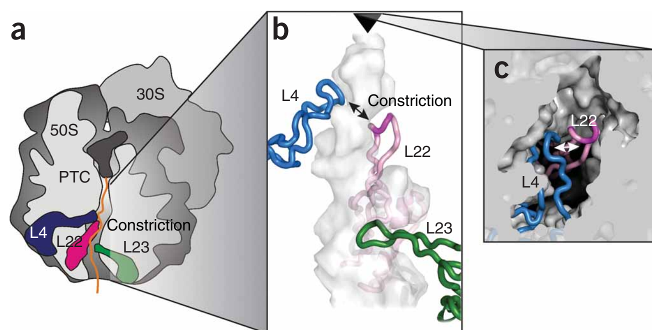

Figure 1 The path of the nascent chain through the ribosomal exit tunnel. (**a**) Schematic drawing of the ribosome (gray) sliced along the tunnel, showing the path of the nascent chain (orange) from the peptidyl transferase center (PTC) to the exit site. Proteins that interact with the nascent chain are color coded (L4, blue; L22, magenta; L23, green). (**b**) Outside view of the ribosomal tunnel (transparent gray surface). The loops of ribosomal proteins L4 (blue) and L22 (pink-magenta) form the narrowest constriction along the tunnel (arrow). Residues of protein L22 that are important for SecM stalling are highlighted in magenta. Ribosomal protein L23 at the exit of the tunnel is shown in green. (**c**) Blow up of the constriction point viewed from the PTC along the tunnel.

© 2009 Nature America, Inc. All rights reserved.

found predominantly in regions encoding domain boundaries and specific secondary-structure elements such as β-strands and random coil regions[17]. Such regions occur in numerous eukaryotic and prokaryotic genomes, indicating a universal selective pressure for their maintenance[18].

Nascent chains can promote translational pausing

The peptide exit tunnel is not a passive environment for the passage of nascent polypeptides but provides structural features to regulate protein synthesis and facilitate association of factors. Furthermore, interactions with the tunnel may promote the initiation of secondary structures in nascent chains.

The feedback regulation of translation via tunnel interactions uses sequence information from the nascent chains and leads to transient translational pausing. Such pausing can have beneficial effects for the coordination of ribosome-associated factors or the co-translational folding of nascent peptides[19]. Positively charged lysine or arginine residues in the nascent chain can slow down or even arrest translation, probably by charge-specific interactions between the tunnel and the nascent peptide[10,20]. Accordingly, translation of the poly(A) tail of mRNAs lacking a stop codon results in synthesis of polylysine, which causes translation arrest and stimulates degradation of the nascent polypeptide by the proteasome[21,22].

Other short peptide stretches can cause transient translational arrest by specific interactions with the exit tunnel. For example, in *E. coli*, where transcription and translation are coupled, the 24-amino-acid regulatory peptide TnaC can transiently stall translation in the presence of tryptophan, thus preventing transcriptional termination and promoting the expression of the downstream gene within the same operon[23]. Another example is the 170-amino-acid secreted SecM peptide that regulates the translation of the downstream *secA* gene in response to the secretion status of the cell[24]. During synthesis, the nascent SecM polypeptide interacts with the ribosomal exit tunnel to transiently arrest translation; SecA-mediated transport of SecM across the cytoplasmic membrane seems to overcome this arrest and circumvent the translation of the downstream *secA* gene[24,25]. The SecM nascent chain induces translation arrest through its C-terminal stalling sequence (150-FxxxxWIxxxxGIRAGP-166), which interacts with ribosomal 23S RNA (bases A2058 and A749–753) and projections of L22 and L4 (refs. 26,27; **Fig. 1b**,**c**). Interestingly, although Pro166 of SecM is essential for the arrest, the Pro166-tRNA remains at the ribosomal A-site without forming a polypeptide bond with the peptidyl-$tRNA^{Gly}$ located at the P-site[28].

Sequences that cause translational pausing are not limited to bacteria. For example, the nascent arginine attenuator peptide from yeast can cause co-translational stalling in both rabbit reticulocyte lysates and wheat-germ extracts, indicating that this mechanism might be conserved[29].

A striking interaction between nascent chains and the ribosomal tunnel regulates the association of the signal recognition particle (SRP). In *E. coli*, nascent chains too short to reach the end of the ribosomal exit tunnel can nevertheless increase the affinity of SRP for ribosomes by approximately 100-fold[30]. Signal transfer from the inside of the tunnel to the ribosomal surface occurs via a loop in L23 that reaches into the exit tunnel. This active recruitment of SRP to the ribosome is independent of a signal sequence in the nascent chain. In yeast, SRP also shows higher affinity for actively translating ribosomes harboring nascent chains buried in the exit tunnel than for vacant ribosomes, but only if the ribosomes are translating a membrane anchor sequence[31]. Together, these findings show that ribosomes can transmit information about the presence of a nascent chain from their interior to the surface, to control the interaction with the SRP.

The ribosome initiates folding

Several studies suggest that ribosomes promote the formation of secondary structure in nascent chains. In a published study, Förster resonance energy transfer (FRET) pairs placed at opposite ends of a nascent transmembrane segment were used to measure the degree of folding in the tunnel of eukaryotic ribosomes[32]. Strikingly, the distance between the labels was as short inside the tunnel as when the transmembrane domain was inserted into the membrane as an α-helix, suggesting that the ribosome can promote helix formation. In another study, PEGylation of cysteines placed at defined positions was used to measure the relative compaction of arrested nascent chains[11]. Distinct zones of secondary-structure induction or stabilization within the tunnel were detected, predominantly in regions close to the peptidyl transferase center (PTC) and near the protein exit channel. Similar analyses suggest that co-translational acquisition of secondary structure, and even some tertiary structure, might occur within the last 20 Å of the tunnel, where it widens up[9,33].

Enzymatic processing of nascent chains

Many cellular proteins are subjected to chemical modifications, some of which occur during protein biosynthesis. Among the various factors that interact with nascent chains are enzymes involved in N-terminal deformylation or methionine excision, two proteolytic pathways needed to produce diverse N termini in proteins, or enzymatic modification by acetylation[34,35] (**Fig. 2**). Other modifications such as N-myristoylation have also been suggested to occur co-translationally; however, mechanistic details on how this might be coupled to translation are lacking[36].

Methionine aminopeptidase and peptide deformylase. Methionine aminopeptidases (MAPs) are essential in all kingdoms of life[35]. This protein family catalyzes the removal of N-terminal methionine from nascent chains, a reaction controlled by the sequence of the nascent chain itself.

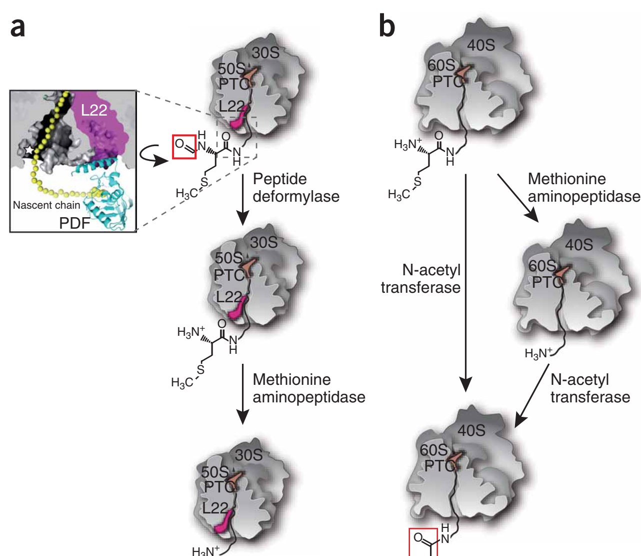

Figure 2 N-terminal co-translational enzymatic processing and modification of nascent polypeptides in the vicinity of the ribosome. (**a**) In bacteria, the ribosome-associated peptide deformylase (PDF) removes the formyl group of the N-terminal formyl methionine of nascent proteins. This process is a prerequisite for the proteolytic removal of the unmasked methionine by methionine aminopeptidase (MAP). The enlargement shows PDF (cyan ribbon) bound to ribosomal protein L22 (magenta) next to the ribosomal tunnel exit (white star). The second interaction site of PDF on the ribosome, protein L32, is not visible in this view of the ribosome, which is sliced along the tunnel. The path of the nascent chain is indicated by yellow spheres. (**b**) In eukaryotes, the N-terminal methionine of nascent polypeptides is often removed by methionine aminopeptidase. Irrespective of whether this enzymatic processing occurs, ribosome-associated N-acetyltransferases can transfer an acetyl moiety to the N-terminal amine group of the nascent polypeptide.

Several lines of evidence suggest that some MAP family members interact with ribosomes. N-terminal methionines can be removed co-translationally when the nascent chains reach a minimal length of just 40 amino acids[37]. Furthermore, yeast MAPs have been found to be associated with ribosomes[38,39]. The MAP-ribosome interaction sites are not known to date, but a 50–100-amino-acid N-terminal extension was suggested to facilitate the proper interaction of yeast MAP with the large ribosomal subunit[39,40].

Bacteria, mitochondria and plastids initiate translation by the binding of a specialized initiator tRNA charged with formylmethionine[35]. N-formylation is assumed to block the reactive amino group to prevent unfavorable side reactions and to enhance the efficiency of translation initiation. The bacterial enzyme peptide deformylase (PDF) is an essential protein that co-translationally removes the formyl group of nascent proteins (**Fig. 2a**), a step required for the subsequent action of the MAP to excise the N-terminal methionine[41–43]. *Escherichia coli* PDF associates with the large subunit of the ribosome through a C-terminal helical extension that binds to a groove between ribosomal proteins L22 and L32, located next to the ribosomal exit tunnel[44] (**Figs. 2a** and **3**). Binding of PDF to the ribosome positions its active site for interaction with the emerging nascent polypeptides, and this is functionally important *in vivo*—expression of a C-terminally truncated and ribosome binding–deficient PDF in cells lacking full-length PDF reduces the viability and growth rate of cells under PDF-limiting conditions[44].

Acetyltransferases. N-terminal acetylation is another protein modification believed to occur co-translationally in eukaryotes[45]. Although found only rarely on prokaryotic or archaeal proteins, it is one of the most frequent protein modifications in eukaryotes, occurring on approximately 80–90% of the cytosolic mammalian proteins and about 50% of yeast proteins. Despite being so widespread, the significance of acetylation is not well understood. The reaction is catalyzed by several non-essential hetero-oligomeric acetyltransferases (NATs), which differ in their substrate specificities (**Fig. 2b**). Several lines of evidence indicate co-translational acetylation of nascent chains and suggest that NATs might be ribosome associated[46,47]. N-terminal acetylation of 40–70-amino-acid nascent polypeptides by a mainly polysome-bound acetyltransferase was observed in rat liver extracts[48]. In reticulocyte lysates, the N-terminal methionine excision by MAP and the acetylation of the N-terminal glycine of ovalbumin occurs when the nascent chain has a length of only 41–47 amino acids[49]. NatA from yeast is bound to vacant as well as active ribosomes[50] and can be cross-linked to nascent polypeptides[51]. Recent evidence shows that NatB and NatC are also ribosome associated[50]. Furthermore, copurification experiments suggest that ribosomal proteins Rpl25p (or

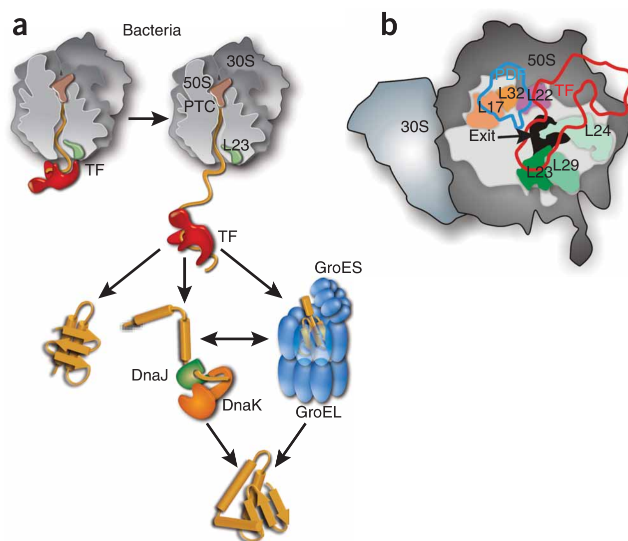

Figure 3 Model of bacterial chaperones involved in *de novo* protein folding. (**a**) The ribosome-bound chaperone Trigger factor interacts with nascent chains at the ribosome. Trigger factor (TF) can stay bound and move with the nascent chain for a limited time to promote the folding of cytosolic proteins. Besides the interaction with Trigger factor, some proteins need further folding assistance by either the Hsp70 chaperone system, consisting of DnaK together with its cochaperone DnaJ and the nucleotide-exchange factor GrpE, or by the Hsp60 chaperonin GroEL together with its cochaperone GroES. For some proteins, productive folding requires consecutive binding steps and shuttling between both chaperone systems. (**b**) The tunnel exit (black) of the large ribosomal 50S subunit, showing the ribosomal proteins surrounding it and associated enzymes. The surface of the ubiquitously conserved proteins L23, L29, L24 (green) and L22 (magenta) is indicated. Bacterial specific proteins L17 and L32 are colored yellow. The projections of PDF (blue) and TF (red) on the bacterial ribosomal surface are shown as outlines.

© 2009 Nature America, Inc. All rights reserved.

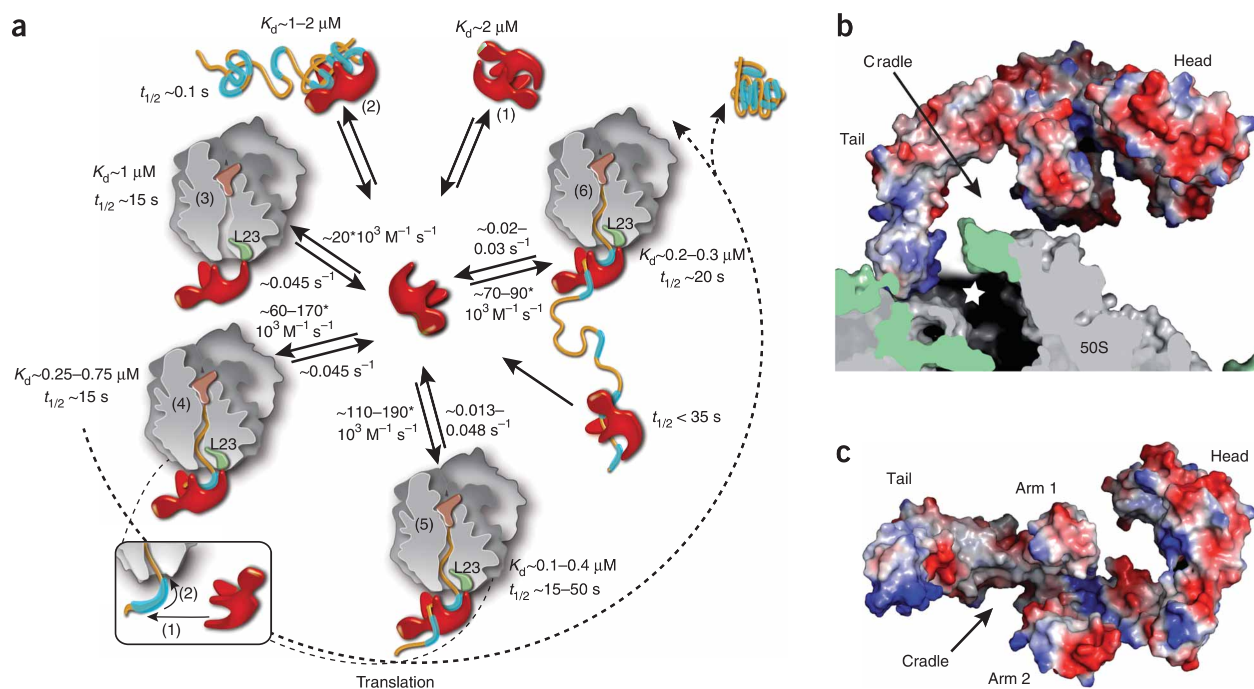

Figure 4 Interaction of Trigger factor with nascent chains. (**a**) Model of the functional cycle of Trigger factor (red). Unbound Trigger factor exists in a monomer-dimer equilibrium (1). Trigger factor may interact with folding proteins in the cytosol (2) or bind to vacant ribosomes (3). Trigger factor association is accelerated by the presence of a peptide chain emerging from the ribosome (4). The affinity of Trigger factor can be substantially increased by the presence of longer nascent chains exposing hydrophobic sequences (cyan). Such increase in affinity is caused by a several-fold accelerated association rate and decreased dissociation rate and can cause a prolonged $t_{1/2}$ of Trigger factor at the ribosome of up to 50 s (5). The association rate of Trigger factor binding to ribosomes eventually decreases when a large nascent polypeptide is exposed on the ribosomal surface, presumably as a result of steric hindrance. Trigger factor may remain associated with some nascent chains even after its dissociation from the ribosomal binding site (6). Rate constants determined for Trigger factor interactions with ribosomes or ribosome nascent chain complexes are from ref. 56. Inset, binding sites in the nascent chain increase Trigger factor's association rate to ribosomes, presumably by a fast interaction with the nascent chain (1) followed by ribosome binding (2). (**b**) Model of *E. coli* Trigger factor bound to an *E. coli* ribosome nascent chain complex derived from a combination of EM and crystallographic data. The crystal structure of Trigger factor is shown in surface representation (colored by electrostatic potential) on the *E. coli* ribosome (rRNA, grey; proteins, green) and sliced along the tunnel (white star). The Trigger factor arms and the ribosome-bound tail form a cradle that might allow folding of the nascent chain. (**c**) The crystal structure of Trigger factor (colored as in **b**) viewed from the tunnel.

© 2009 Nature America, Inc. All rights reserved.

L23) and Rpl35p (or L29), both positioned in close proximity to the ribosomal polypeptide exit tunnel, might have a role in NatA binding to the ribosome.

Ribosome associated chaperones in co-translational folding

In principal, newly synthesized polypeptide chains of cytosolic proteins have the potential to start the folding process co-translationally[52]. Within the cellular environment, however, they interact with a large arsenal of molecular chaperones that guide the folding process. At the forefront are chaperones that transiently associate with ribosomes and are thereby optimally positioned to assist folding during ongoing synthesis. They cooperate with cytosolic chaperones of the Hsp60, Hsp70 and Hsp90 families that act downstream in the folding process[52]. Strikingly, prokaryotes and eukaryotes have evolved structurally unrelated ribosome-associated chaperone systems (**Figs. 3** and **5**). Whereas prokaryotes use the chaperone Trigger factor, eukaryotes have evolved Hsp70 and J-protein–based systems and the heterodimeric nascent polypeptide-associated complex (NAC).

The bacterial Trigger factor. Trigger factor is the only ribosome-associated chaperone for which we have significant mechanistic insights. The chaperone activity of Trigger factor crucially depends on its transient association with the ribosome[53] (**Fig. 4a**). In the absence of nascent chains, Trigger factor cycles on and off the ribosome with a mean residence time of 11–15 s[54–56]. Depending on their length, sequence and folding status, nascent chains can accelerate Trigger factor association with ribosomes and delay its dissociation, leading to an overall increase in affinity of 9-fold to 30-fold[55–57]. In this manner, the nascent chains themselves control the timing of Trigger factor binding to the ribosome. This mechanism presumably relies on Trigger factor's ability to rapidly bind and release peptides enriched in aromatic and basic residues, allowing it to sample for binding sites on the growing nascent chains[58,59]. Such a mechanism enables Trigger factor to discriminate between vacant and translating ribosomes and to preferentially associate with ribosome–nascent chain complexes (RNCs).

An elegant FRET study showed that Trigger factor may stay associated with newly synthesized polypeptide chains for up to 35 s, even when dissociated from the ribosome, provided that the polypeptide has high overall hydrophobicity[55]. The kinetic features of Trigger factor allow its rapid reloading to RNCs[55,56]. Such cycling may be particularly relevant for the assisted folding of multidomain proteins, which are the preferred clients for Trigger factor *in vivo*[60]. For such proteins, one could envision that, through consecutive loading, several molecules of Trigger factor could simultaneously associate with a nascent chain[61].

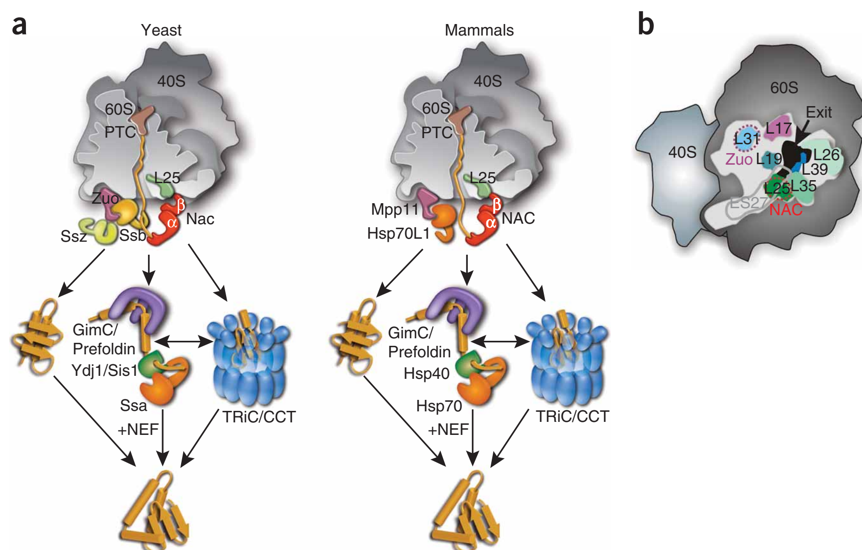

Figure 5 Model of eukaryotic chaperones presumably involved in *de novo* protein folding. (**a**) In yeast, two ribosome-associated systems interact with nascent polypeptides, the nascent chain–associated complex (NAC) and the Hsp70- and Hsp40-based triad system Ssb/Ssz/Zuo. In analogy to the bacterial system, some proteins need further assistance by cytosolic chaperones of the Hsp60 family of chaperonins (TriC/CCT) or the Hsp70 system consisting of Ssa1–4, together with the J-proteins Ydj1 or Sis1 and a nucleotide-exchange factor (NEF). The prefoldin–GimC complex is implicated in the folding of actin and tubulin. In higher eukaryotes, Mpp11, a member of the J-protein class of cochaperones recruits Hsp70L1 to ribosomes. (**b**) Illustration showing the tunnel exit (black) of the eukaryotic 60S subunit with the ribosomal proteins surrounding the tunnel exit and associated enzymes. The surface of the ubiquitously conserved proteins L25, L35, L26 (green) and L22 (magenta) is indicated. Eukaryote-specific proteins L31, L19 and L39 are colored blue. The suggested binding sites for Zuo and NAC on the eukaryotic ribosome are indicated by magenta and red ellipses, respectively. The projection of expansion segment 27 of ribosomal 28S rRNA is shown as gray outline.

© 2009 Nature America, Inc. All rights reserved.

The crystal structure of Trigger factor[62] greatly improved our understanding of how this chaperone works (**Fig. 4b**,**c**). Trigger factor folds into an elongated molecule with a dragon shape. The N-terminal domain (the 'tail') harbors a conserved loop sequence involved in ribosome binding, mainly through contacts with L23 close to the tunnel exit[62,63]. It is connected via a long linker to the peptidyl-prolyl isomerase (PPIase) domain (the 'head') at the other end of the protein. The C-terminal domain (the 'back') lies between the head and the tail, and forms a cradle with two protruding helical arms. In addition to the full-length crystal structure of *E. coli* Trigger factor, a fragment comprising its N-terminal domain was cocrystallized in complex with the archaeon *Haloarcula marismortui* 50S ribosomal subunit[62]. By superimposing full-length Trigger factor onto the structure of the 50S subunit, it was proposed that Trigger factor forms a hydrophobic cradle directly at the exit of the nascent polypeptide tunnel. This cradle is open on both sides and seems to be large enough to accommodate small globular domains, even when they are folded.

Recent cryo-EM work has revealed the structure of *E. coli* Trigger factor in complex with a translating ribosome[64]. The EM reconstruction shows that Trigger factor forms an arch over the tunnel exit, similar to the model derived by crystallography[62]. Nevertheless, it is important to note that Trigger factor has substantial rotational freedom in its ribosome-bound state[62,64], which may explain why Trigger factor was observed bound to the large ribosomal subunit in several other conformations, trapped by crystal contacts[65,66]. This flexibility allows Trigger factor to open up or close in over the exit tunnel, perhaps in response to the presence of a particular nascent chain, as suggested by nascent chain protease-protection assays[67,68].

How exactly Trigger factor supports the folding of nascent chains has not been conclusively answered. Studies using ribosome complexes with stalled nascent chains revealed that, over a stretch of at least 85 residues, the largely unfolded nascent polypeptide initially follows a rather defined path in the interior of Trigger factor, contacting all three domains[64,69]. This interior has, overall, a hydrophobic character, but it also exposes some polar and charged residues (**Fig. 4c**), thereby permitting interaction with stretches of nascent chains with differing chemical characteristics. The nascent chains can remain accommodated inside the protective cradle of ribosome-bound Trigger factor, even after folding[64]. Through this interaction Trigger factor can shield nascent chains from degradation by proteases[67,68]. Furthermore, Trigger factor was shown to improve the yields of correctly folded model substrates by reducing the speed of folding *in vivo* and *in vitro*[61]. It remains to be seen whether this reflects the general mode of action of this chaperone.

There are a number of open questions regarding the action of Trigger factor, including the enigmatic role of its PPIase domain. Its deletion or an active site mutation reduce the chaperone activity of Trigger factor only slightly[70–72]. How the substrates are transferred from Trigger factor to other chaperones that act in subsequent folding steps is another interesting future question in the field.

Ribosome-associated chaperones in eukaryotes. The ribosome-associated factors acting in eukaryotes are best characterized for *S. cerevisiae*, although their functional roles in protein folding remain enigmatic. Yeast contains two different systems, the Ssb/Ssz/Zuotin chaperone triad and NAC[73–76] (**Fig. 5a**).

Deletion of any single component of the triad causes similar cellular defects, including cold and salt sensitivity, slow growth and hypersensitivity toward aminoglycosides, indicating a functional interplay[77–79]. Ssb directly associates with ribosomes and has been reported to bind to a large fraction of nascent chains and to protect them from off pathways that eventually lead to misfolding and ubiquitination[74,75,77,80,81]. Additional ribosome-independent roles for Ssb are currently being discussed.

Ssz and Zuotin assemble into a stable heterodimeric complex termed the ribosome-associated complex (RAC). Ribosome association is mediated by Zuotin[77], which was shown to contact the ribosomal protein Rpl31 (ref. 82; **Fig. 5b**). As deletion of Rpl31 does not affect ribosome association of Zuotin, additional interaction sites are likely to exist. RAC acts as a cochaperone of Ssb, stimulating its ATPase activity, although a direct contact to the nascent chain has not been demonstrated so far. Although Zuotin can stimulate the ATPase activity of Ssb on its own, it requires Ssz for full activity[83,84]. Ssz is unusual because it lacks any detectable ATPase activity *in vitro*, and its function *in vivo* does not depend on its ability to bind ATP or peptide substrates[84].

An ortholog of yeast Zuotin has been recently described, the human J-protein MPP11 (refs. 85,86; **Fig. 5a**). MPP11 associates with ribosomes and forms a stable complex with Hsp70L1, a remote relative of Ssz in humans. Complementation analysis in yeast revealed that MPP11 cannot functionally interact with Ssb but, instead, teams up with the cytosolic Hsp70 Ssa and thereby partially rescues cells lacking Ssb and Zuotin. Thus, ribosome-associated chaperones belonging to the Hsp70 and J-protein class seem to be a general feature, found from yeast to humans.

Ssb is subject to further regulation in yeast by three nucleotide-exchange factors (NEFs) that accelerate the exchange of ADP for ATP: Fes1, the Hsp110 family member Sse and Snl1 (refs. 87–90). The roles of these NEFs in regulation of Ssb are still debated. They obviously accelerate the functional cycle of Ssb; however, whether this occurs in a regulated fashion, leading to substrate dissociation when needed, is unclear. Furthermore, in the case of Sse, it is tempting to speculate that its putative substrate binding site[91] is used to directly transfer Ssb-bound substrate onto Sse, thereby establishing a chaperone cascade.

The second ribosome-associated system is NAC, which binds nascent chains and ribosomes in a 1:1 stoichiometry[73,92,93]. In eukaryotes NAC is composed of α and β subunits, whereas in archaea NAC is formed by two α components[94]. The binding of eukaryotic NAC to ribosomes is mediated exclusively by the β subunit, whereas both subunits contact the nascent polypeptides[95]. So far, a putative role of NAC in protein folding is indicated only by its ribosome association and interaction with nascent chains[96,97]. Deletion of the NAC-encoding genes in *S. cerevisiae* causes only minor growth impairment at higher temperature in some strain backgrounds, and fission yeast devoid of NAC do not show any phenotype[98,99]. In contrast, a lack of NAC in nematodes, fruitflies and mice causes embryonic lethality[100–102].

© 2009 Nature America, Inc. All rights reserved.
npg

In *S. cerevisiae* the ribosome-associated chaperones mentioned above (Ssb1, Ssb2, Ssz and Zuotin) are members of the cellular network of chaperones linked to protein synthesis (CLIPS)[80]. CLIPS-encoding genes are repressed under all stress conditions and transcriptionally co-regulated with the translational apparatus, suggesting roles in the folding of newly synthesized proteins[80,103]. Among the CLIPS are the chaperonin TRiC/CCT, the GimC–prefoldin complex, the prolyl isomerases Cpr6/7p and the Hsp70s Ssa1 and Sse1. Strikingly, all CLIPS are physically linked to the protein-synthesis machinery, that is, they comigrate with translating polysomes. Consistent with their specialized function, the deletion of any CLIPS-encoding gene increases cellular sensitivity to antibiotics that inhibit translation and to acetidine-2-carboxylic acid, a proline analog that is incorporated into newly made proteins and prevents their correct folding. On the other hand, CLIPS mutant cells are not sensitive to protein misfolding caused by heat shock, because yeast cells use a different set of stress-regulated chaperones for quality control of misfolded proteins[80,104].

Why have eukaryotes developed two distinct protein-folding systems, whereas bacterial chaperones do not show this specialization? Eukaryotes have a higher proportion of larger multidomain proteins, as well as more complex protein folds, which is likely to result in an increased need for a dedicated and efficient chaperone network[61]. Conversely, the evolution of a highly potent chaperone system for co-translational folding in eukaryotes may have allowed the coevolution of multidomain proteins that have highly complex folding pathways. Furthermore, the range of cellular functions for the chaperones has increased in eukaryotes (for example, protein transport into organelles and regulation of signaling pathways). The specialization of chaperone machines may ensure faithful assistance of the folding of newly synthesized proteins in the cytosol at all growth conditions.

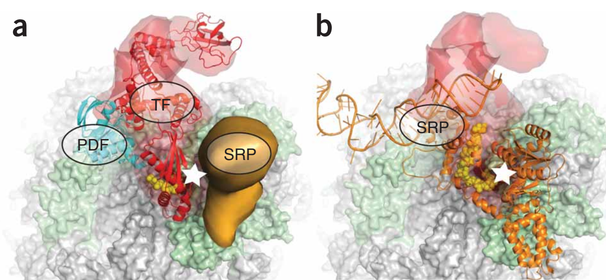

Figure 6 Spatial arrangement of nascent chain–processing factors around the tunnel exit (star). (**a**) For short nascent chains (yellow spheres) Trigger factor (TF, red ribbon) might function as a router, coordinating the nascent chain processing by peptidyl deformylase (PDF, blue ribbon) and sampling for signal sequences by the signal recognition particle (SRP). The flexibly bound SRP is represented by the EM density observed for SRP (orange surface) bound to the nontranslating 70S ribosome in a partially disordered state[110]. (**b**) Longer nascent chains displaying the signal sequence for protein translocation are bound by SRP (orange ribbon), which stably associates with the ribosome. In this conformation, SRP would overlap with the Trigger factor bound to a translating ribosome, as observed by EM (EM density shown in transparent red).

Interplay between processing, folding and targeting factors

Ribosomal L23 (Rpl25) as a general docking site for nascent chain–associated factors. Available crystal structures of large ribosomal subunits from bacteria and archaea show several proteins surrounding the exit of the nascent polypeptide tunnel (**Figs. 3b** and **5b**). These are the universally conserved proteins L22 (Rpl17), L23 (Rpl25), L24 (Rpl26) and L29 (Rpl35) together with kingdom-specific proteins. L17 and L32 are found only in bacteria, whereas Rpl19, Rpl31 and Rpl39 exist only in archaea and eukaryotes[3,5,105]. Interestingly, one of them, the ribosomal protein L23 (Rpl25), is involved in almost all interactions of the ribosome with ribosome-associated factors investigated so far, including the translocon, SRP, Trigger factor, NAC, ERj1, Oxa1 and NatA[50,62,63,96,106–112]. Thus, L23 (Rpl25) seems to constitute a general docking platform for various factors that transiently associate with ribosomes to act on nascent chains.

What might be the reason for the concentration of different factors on just one binding site? The binding to L23 could be used for the coordination of ribosome-associated factors in time and space. Accordingly, several factors may sequentially associate with the ribosomal exit site in a kinetically controlled fashion, with additional contacts to the nascent chains acting as codeterminants. In contrast, simultaneous binding of factors could be facilitated by adjacent binding sites located in close proximity to each other at L23. Consistent with this idea, structural modeling based on crystallographic data indicates that binding of PDF and Trigger factor may not be sterically exclusive[44,62] (**Figs. 3b** and **6a**). Therefore, PDF, the first enzyme that processes nascent chains, could bind to the ribosome next to Trigger factor and process nascent chains emerging from one of the lateral openings of the Trigger factor cradle. In this scenario, nascent polypeptides do not have to leave the Trigger factor cradle to be processed by PDF[44]. It will be interesting to determine whether MAP also joins in with Trigger factor and PDF at the ribosomal exit site.

The interplay of SRP with Trigger factor and NAC. A crucial process that takes place at the ribosome is the decision concerning the co-translational protein translocation into and across membranes versus folding in the cytosol. Co-translational protein translocation

involves the interaction of SRP with ribosomes[113], which allows SRP to recognize the hydrophobic signal sequence at the N termini of nascent chains as they emerge from the exit tunnel. The SRP–RNC complex interacts in a GTP-controlled fashion with the membrane-bound SRP receptor and is targeted to the translocon in the ER membrane[114]. In eukaryotes, this process is coupled to a transient arrest of translation.

An interplay of bacterial SRP and Trigger factor is indicated by cross-linking studies demonstrating competition between both factors for association with nascent chains harboring hydrophobic signal sequences[115–118]. Likewise, the appearance of a hydrophobic signal-anchor sequence in the nascent chain stabilizes SRP association with the ribosome while diminishing the association of Trigger factor with nascent chains; Trigger factor, on the other hand, inhibits binding of SRP to less hydrophobic signal sequences[115,117,119–122]. This relationship is further supported by *in vivo* observations showing that the absence of Trigger factor accelerates protein export, suppresses the need for the targeting factor SecB and increases the number of ribosomes at the membrane[123,124]. These findings indicate that Trigger factor directly or indirectly interferes with protein targeting to the Sec translocon.

The competitive association of SRP and Trigger factor with nascent chains does not seem to be due to a principally mutually exclusive binding of both factors to their ribosomal docking sites, as their concurrent association to ribosomes has been reported[119,125]. However, both factors share L23 as docking site, and structural modeling predicts significant clashes upon simultaneous binding to ribosomes displaying a signal sequence[64,110] (**Fig. 6**). Interaction of SRP with signal sequences within nascent chains generates additional contacts with the ribosomal surface including rRNA as well as ribosomal proteins L24, L22 and L32 (refs. 110,126). Massive structural rearrangements of at least one of the two factors would be required for simultaneous binding. Consistent with this idea is the observation that SRP undergoes substantial conformational changes during its activity at the ribosome[106,127], but how these relate to the interplay of SRP with other factors acting at the ribosome remains to be determined.

Another kind of interplay of SRP in eukaryotes is that with NAC. This protein complex has not only been suggested to be involved in co-translational protein folding, but also in negatively regulating the targeting function of SRP[97]. *In vitro*, the absence of NAC causes the irregular interaction of SRP with RNCs lacking signal sequences and, presumably as a consequence, the mistargeting of signal-less RNCs to and the translocation of these nascent chains across the endoplasmic reticulum membrane[128–130]. These results led to the proposal that NAC controls the fidelity of protein translocation to the ER by increasing the specificity of SRP. This interpretation is also supported by recent data suggesting that NAC and SRP compete for a common binding site at the ribosome[38]. It is intriguing that many features of NAC are shared with those of Trigger factor, although both factors are structurally unrelated and may use different mechanisms for their action at the ribosome.

Perspectives

A myriad of recently accumulated experimental evidence demonstrates that nascent chains are co-translationally welcomed by various ribosome-associated factors. These factors exert their function or increase their efficiency by specifically binding to the ribosome in the vicinity of the ribosomal exit tunnel. They are involved in the processing and modification of nascent chain N termini, and they assist co-translational protein folding and the targeting of the nascent membrane or exported proteins to the translocation machinery. However, we are only beginning to understand the mechanistic aspects of their function and their interaction with the ribosome. To better understand these processes and their dynamic interplay, it will be necessary to use interdisciplinary experimental approaches combining *in vivo* studies with detailed biophysical studies of binding affinities, single-molecule experiments and structural studies ranging from crystallography and EM to NMR. Undoubtedly, owing to the complexity of these processes and the large size of the ribosome, these experiments will present formidable challenges, but we can be certain that the future in this field will bring many exciting discoveries.

ACKNOWLEDGMENTS

We thank members of the Bukau and Ban laboratories for critical reading of the manuscript. We acknowledge the help of Y. Cully, F. Gloge and A. Rutkowska in preparing parts of the figures. Work in the authors' laboratories is supported by grants from the Deutsche Forschungsgemeinschaft (DFG) to B.B. and G.K. (SFB 638, FOR967), the Swiss National Science Foundation (SNSF), the National Center of Excellence in Research (NCCR) Structural Biology programme of the SNSF and the ETH Research Grant TH-3/04-1 to N.B., and the Federation of European Biochemical Societies long-term fellowship to D.B.

Published online at http://www.nature.com/nsmb/

Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

© 2009 Nature America, Inc. All rights reserved.

1. Bashan, A. & Yonath, A. Correlating ribosome function with high-resolution structures. *Trends Microbiol.* **16**, 326–335 (2008).
2. Brandt, F. *et al.* The native 3D organization of bacterial polysomes. *Cell* **136**, 261–271 (2009).
3. Ban, N., Nissen, P., Hansen, J., Moore, P.B. & Steitz, T.A. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289**, 905–920 (2000).
4. Harms, J. *et al.* High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. *Cell* **107**, 679–688 (2001).
5. Nissen, P., Hansen, J., Ban, N., Moore, P.B. & Steitz, T.A. The structural basis of ribosome activity in peptide bond synthesis. *Science* **289**, 920–930 (2000).
6. Voss, N.R., Gerstein, M., Steitz, T.A. & Moore, P.B. The geometry of the ribosomal polypeptide exit tunnel. *J. Mol. Biol.* **360**, 893–906 (2006).
7. Picking, W.D., Picking, W.L., Odom, O.W. & Hardesty, B. Fluorescence characterization of the environment encountered by nascent polyalanine and polyserine as they exit *Escherichia coli* ribosomes during translation. *Biochemistry* **31**, 2368–2375 (1992).
8. Malkin, L.I. & Rich, A. Partial resistance of nascent polypeptide chains to proteolytic digestion due to ribosomal shielding. *J. Mol. Biol.* **26**, 329–346 (1967).
9. Kosolapov, A. & Deutsch, C. Tertiary interactions within the ribosomal exit tunnel. *Nat. Struct. Mol. Biol.* **16**, 405–411 (2009).
10. Lu, J., Kobertz, W.R. & Deutsch, C. Mapping the electrostatic potential within the ribosomal exit tunnel. *J. Mol. Biol.* **371**, 1378–1391 (2007).
11. Lu, J. & Deutsch, C. Folding zones inside the ribosomal exit tunnel. *Nat. Struct. Mol. Biol.* **12**, 1123–1129 (2005).
12. Tsai, C.J. *et al.* Synonymous mutations and ribosome stalling can lead to altered folding pathways and distinct minima. *J. Mol. Biol.* **383**, 281–291 (2008).
13. Crombie, T., Swaffield, J.C. & Brown, A.J.P. Protein folding within the cell is influenced by controlled rates of polypeptide elongation. *J. Mol. Biol.* **228**, 7–12 (1992).
14. Komar, A.A., Lesnik, T. & Reiss, C. Synonymous codon substitutions affect ribosome traffic and protein folding during *in vitro* translation. *FEBS Lett.* **462**, 387–391 (1999).
15. Zhang, G., Hubalewska, M. & Ignatova, Z. Transient ribosomal attenuation coordinates protein synthesis and co-translational folding. *Nat. Struct. Mol. Biol.* **16**, 274–280 (2009).
16. Kimchi-Sarfaty, C. *et al.* A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* **315**, 525–528 (2007).
17. Thanaraj, T.A. & Argos, P. Protein secondary structural types are differentially coded on messenger RNA. *Protein Sci.* **5**, 1973–1983 (1996).
18. Clarke, T.F., IV & Clark, P.L. Rare codons cluster. *PLoS One* **3**, e3412 (2008).
19. Marin, M. Folding at the rhythm of the rare codon beat. *Biotechnol. J.* **3**, 1047–1057 (2008).
20. Lu, J. & Deutsch, C. Electrostatics in the ribosomal tunnel modulate chain elongation rates. *J. Mol. Biol.* **384**, 73–86 (2008).
21. Ito-Harashima, S., Kuroha, K., Tatematsu, T. & Inada, T. Translation of the poly(A) tail plays crucial roles in nonstop mRNA surveillance via translation repression and protein destabilization by proteasome in yeast. *Genes Dev.* **21**, 519–524 (2007).
22. Dimitrova, L.N., Kuroha, K., Tatematsu, T. & Inada, T. Nascent peptide-dependent translation arrest leads to Not4p-mediated protein degradation by the proteasome. *J. Biol. Chem.* **284**, 10343–10352 (2009).

23. Gong, F. & Yanofsky, C. Instruction of translating ribosome by nascent peptide. *Science* **297**, 1864–1867 (2002).
24. Nakatogawa, H. & Ito, K. Secretion monitor, SecM, undergoes self-translation arrest in the cytosol. *Mol. Cell* **7**, 185–192 (2001).
25. Butkus, M.E., Prundeanu, L.B. & Oliver, D.B. Translocon "pulling" of nascent SecM controls the duration of its translational pause and secretion-responsive secA regulation. *J. Bacteriol.* **185**, 6719–6722 (2003).
26. Woolhead, C.A., Johnson, A.E. & Bernstein, H.D. Translation arrest requires two-way communication between a nascent polypeptide and the ribosome. *Mol. Cell* **22**, 587–598 (2006).
27. Nakatogawa, H. & Ito, K. The ribosomal exit tunnel functions as a discriminating gate. *Cell* **108**, 629–636 (2002).
28. Muto, H., Nakatogawa, H. & Ito, K. Genetically encoded but nonpolypeptide prolyl-tRNA functions in the A site for SecM-mediated ribosomal stall. *Mol. Cell* **22**, 545–552 (2006).
29. Fang, P., Spevak, C.C., Wu, C. & Sachs, M.S. A nascent polypeptide domain that can regulate translation elongation. *Proc. Natl. Acad. Sci. USA* **101**, 4059–4064 (2004).
30. Bornemann, T., Jockel, J., Rodnina, M.V. & Wintermeyer, W. Signal sequence-independent membrane targeting of ribosomes containing short nascent peptides within the exit tunnel. *Nat. Struct. Mol. Biol.* **15**, 494–499 (2008).
31. Berndt, U., Oellerer, S., Zhang, Y., Johnson, A.E. & Rospert, S. A signal-anchor sequence stimulates signal recognition particle binding to ribosomes from inside the exit tunnel. *Proc. Natl. Acad. Sci. USA* **106**, 1398–1403 (2009).
32. Woolhead, C.A., McCormick, P.J. & Johnson, A.E. Nascent membrane and secretory proteins differ in FRET-detected folding far inside the ribosome and in their exposure to ribosomal proteins. *Cell* **116**, 725–736 (2004).
33. Evans, M.S., Sander, I.M. & Clark, P.L. Cotranslational folding promotes β-helix formation and avoids aggregation *in vivo*. *J. Mol. Biol.* **383**, 683–692 (2008).
34. Martinez, A. *et al.* Extent of N-terminal modifications in cytosolic proteins from eukaryotes. *Proteomics* **8**, 2809–2831 (2008).
35. Giglione, C., Boularot, A. & Meinnel, T. Protein N-terminal methionine excision. *Cell. Mol. Life Sci.* **61**, 1455–1474 (2004).
36. Meinnel, T. & Giglione, C. Tools for analyzing and predicting N-terminal protein modifications. *Proteomics* **8**, 626–649 (2008).
37. Ball, L.A. & Kaesberg, P. Cleavage of the N-terminal formylmethionine residue from a bacteriophage coat protein *in vitro*. *J. Mol. Biol.* **79**, 531–537 (1973).
38. Raue, U., Oellerer, S. & Rospert, S. 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 (2007).
39. Vetro, J.A. & Chang, Y.H. 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 (2002).
40. Zuo, S., Guo, Q., Ling, C. & Chang, Y.H. Evidence that two zinc fingers in the methionine aminopeptidase from *Saccharomyces cerevisiae* are important for normal growth. *Mol. Gen. Genet.* **246**, 247–253 (1995).
41. Fry, K.T. & Lamborg, M.R. Amidohydrolase activity of *Escherichia coli* extracts with formylated amino acids and dipeptides as substrates. *J. Mol. Biol.* **28**, 423–433 (1967).
42. Pine, M.J. Kinetics of maturation of the amino termini of the cell proteins of *Escherichia coli*. *Biochim. Biophys. Acta* **174**, 359–372 (1969).
43. Adams, J.M. On the release of the formyl group from nascent protein. *J. Mol. Biol.* **33**, 571–574 (1968).
44. Bingel-Erlenmeyer, R. *et al.* A peptide deformylase-ribosome complex reveals mechanism of nascent chain processing. *Nature* **452**, 108–111 (2008).
45. Polevoda, B. & Sherman, F. Composition and function of the eukaryotic N-terminal acetyltransferase subunits. *Biochem. Biophys. Res. Commun.* **308**, 1–11 (2003).
46. Yamada, R. & Bradshaw, R.A. Rat liver polysome N α-acetyltransferase: isolation and characterization. *Biochemistry* **30**, 1010–1016 (1991).
47. Green, R.M., Elce, J.S. & Kisilevsky, R. Acetylation of peptidyl-tRNA on rat liver polyribosomes. *Can. J. Biochem.* **56**, 1075–1081 (1978).
48. Pestana, A. & Pitot, H.C. Acetylation of nascent polypeptide chains on rat liver polyribosomes *in vivo* and *in vitro*. *Biochemistry* **14**, 1404–1412 (1975).
49. Palmiter, R.D., Gagnon, J. & Walsh, K.A. Ovalbumin: a secreted protein without a transient hydrophobic leader sequence. *Proc. Natl. Acad. Sci. USA* **75**, 94–98 (1978).
50. Polevoda, B., Brown, S., Cardillo, T.S., Rigby, S. & Sherman, F. Yeast N^{α}-terminal acetyltransferases are associated with ribosomes. *J. Cell. Biochem.* **103**, 492–508 (2008).
51. Gautschi, M. *et al.* The yeast N^{α}-acetyltransferase NatA is quantitatively anchored to the ribosome and interacts with nascent polypeptides. *Mol. Cell. Biol.* **23**, 7403–7414 (2003).
52. Hartl, F.U. & Hayer-Hartl, M. Converging concepts of protein folding *in vitro* and *in vivo*. *Nat. Struct. Mol. Biol.* **16**, 574–581 (2009).
53. Kramer, G. *et al.* L23 protein functions as a chaperone docking site on the ribosome. *Nature* **419**, 171–174 (2002).
54. Maier, R., Eckert, B., Scholz, C., Lilie, H. & Schmid, F.X. Interaction of Trigger factor with the ribosome. *J. Mol. Biol.* **326**, 585–592 (2003).
55. Kaiser, C.M. *et al.* Real-time observation of Trigger factor function on translating ribosomes. *Nature* **444**, 455–460 (2006).
56. Rutkowska, A. *et al.* Dynamics of Trigger factor interaction with translating ribosomes. *J. Biol. Chem.* **283**, 4124–4132 (2008).
57. Raine, A., Lovmar, M., Wikberg, J. & Ehrenberg, M. Trigger factor binding to ribosomes with nascent peptide chains of varying lengths and sequences. *J. Biol. Chem.* **281**, 28033–28038 (2006).
58. Maier, R., Scholz, C. & Schmid, F.X. Dynamic association of Trigger factor with protein substrates. *J. Mol. Biol.* **314**, 1181–1190 (2001).
59. Patzelt, H. *et al.* Binding specificity of *Escherichia coli* Trigger factor. *Proc. Natl. Acad. Sci. USA* **98**, 14244–14249 (2001).
60. Deuerling, E. *et al.* Trigger Factor and DnaK possess overlapping substrate pools and binding specificities. *Mol. Microbiol.* **47**, 1317–1328 (2003).
61. Agashe, V.R. *et al.* Function of Trigger factor and DnaK in multidomain protein folding: increase in yield at the expense of folding speed. *Cell* **117**, 199–209 (2004).
62. Ferbitz, L. *et al.* Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins. *Nature* **431**, 590–596 (2004).
63. Kramer, G. *et al.* L23 protein functions as a chaperone docking site on the ribosome. *Nature* **419**, 171–174 (2002).
64. Merz, F. *et al.* Molecular mechanism and structure of Trigger factor bound to the translating ribosome. *EMBO J.* **27**, 1622–1632 (2008).
65. Schlünzen, F. *et al.* The binding mode of the Trigger factor on the ribosome: implications for protein folding and SRP interaction. *Structure* **13**, 1685–1694 (2005).
66. Baram, D. *et al.* Structure of Trigger factor binding domain in biologically homologous complex with eubacterial ribosome reveals its chaperone action. *Proc. Natl. Acad. Sci. USA* **102**, 12017–12022 (2005).
67. Hoffmann, A. *et al.* Trigger factor forms a protective shield for nascent polypeptides at the ribosome. *J. Biol. Chem.* **281**, 6539–6545 (2006).
68. Tomic, S., Johnson, A.E., Hartl, F.U. & Etchells, S.A. Exploring the capacity of Trigger factor to function as a shield for ribosome bound polypeptide chains. *FEBS Lett.* **580**, 72–76 (2006).
69. Lakshmipathy, S.K. *et al.* Identification of nascent chain interaction sites on Trigger factor. *J. Biol. Chem.* **282**, 12186–12193 (2007).
70. Scholz, C., Stoller, G., Zarnt, T., Fischer, G. & Schmid, F.X. Cooperation of enzymatic and chaperone functions of Trigger factor in the catalysis of protein folding. *EMBO J.* **16**, 54–58 (1997).
71. Kramer, G. *et al.* Functional dissection of *Escherichia coli* Trigger factor: unraveling the function of individual domains. *J. Bacteriol.* **186**, 3777–3784 (2004).
72. Merz, F. *et al.* The C-terminal domain of *E. coli* Trigger factor represents the central module of its chaperone activity. *J. Biol. Chem.* **42**, 31963–31971 (2006).
73. Wiedmann, B., Sakai, H., Davis, T.A. & Wiedmann, M. A protein complex required for signal-sequence-specific sorting and translocation. *Nature* **370**, 434–440 (1994).
74. Pfund, C. *et al.* The molecular chaperone Ssb from *Saccharomyces cerevisiae* is a component of the ribosome-nascent chain complex. *EMBO J.* **17**, 3981–3989 (1998).
75. Gautschi, M., Mun, A., Ross, S. & Rospert, S. A functional chaperone triad on the yeast ribosome. *Proc. Natl. Acad. Sci. USA* **99**, 4209–4214 (2002).
76. Wegrzyn, R.D. & Deuerling, E. Molecular guardians for newborn proteins: ribosome-associated chaperones and their role in protein folding. *Cell. Mol. Life Sci.* **62**, 2727–2738 (2005).
77. Gautschi, M. *et al.* RAC, a stable ribosome-associated complex in yeast formed by the DnaK-DnaJ homologs Ssz1p and zuotin. *Proc. Natl. Acad. Sci. USA* **98**, 3762–3767 (2001).
78. Hundley, H. *et al.* The *in vivo* function of the ribosome-associated Hsp70, Ssz1, does not require its putative peptide-binding domain. *Proc. Natl. Acad. Sci. USA* **99**, 4203–4208 (2002).
79. Yan, W. *et al.* Zuotin, a ribosome-associated DnaJ molecular chaperone. *EMBO J.* **17**, 4809–4817 (1998).
80. Albanèse, V., Yam, A.Y., Baughman, J., Parnot, C. & Frydman, J. Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. *Cell* **124**, 75–88 (2006).
81. Nelson, R.J., Ziegelhoffer, T., Nicolet, C., Werner-Washburne, M. & Craig, E.A. The translation machinery and 70 kDa heat shock protein cooperate in protein synthesis. *Cell* **71**, 97–105 (1992).
82. Peisker, K. *et al.* 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 (2008).
83. Huang, P., Gautschi, M., Walter, W., Rospert, S. & Craig, E.A. The Hsp70 Ssz1 modulates the function of the ribosome-associated J-protein Zuo1. *Nat. Struct. Mol. Biol.* **12**, 497–504 (2005).
84. Conz, C. *et al.* Functional characterization of the atypical Hsp70 subunit of yeast ribosome-associated complex. *J. Biol. Chem.* **282**, 33977–33984 (2007).
85. Hundley, H.A., Walter, W., Bairstow, S. & Craig, E.A. Human Mpp11 J protein: ribosome-tethered molecular chaperones are ubiquitous. *Science* **308**, 1032–1034 (2005).
86. Otto, H. *et al.* The chaperones MPP11 and Hsp70L1 form the mammalian ribosome-associated complex. *Proc. Natl. Acad. Sci. USA* **102**, 10064–10069 (2005).
87. Dragovic, Z., Shomura, Y., Tzvetkov, N., Hartl, F.U. & Bracher, A. Fes1p acts as a nucleotide exchange factor for the ribosome-associated molecular chaperone Ssb1p. *Biol. Chem.* **387**, 1593–1600 (2006).
88. Yam, A.Y., Albanese, V., Lin, H.T. & Frydman, J. Hsp110 cooperates with different cytosolic HSP70 systems in a pathway for *de novo* folding. *J. Biol. Chem.* **280**, 41252–41261 (2005).
89. Raviol, H., Sadlish, H., Rodriguez, F., Mayer, M.P. & Bukau, B. Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. *EMBO J.* **25**, 2510–2518 (2006).

© 2009 Nature America, Inc. All rights reserved.

npg

90. Sondermann, H. *et al.* Prediction of novel Bag-1 homologs based on structure/function analysis identifies Snl1p as an Hsp70 co-chaperone in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **277**, 33220–33227 (2002).
91. Liu, Q. & Hendrickson, W.A. Insights into Hsp70 chaperone activity from a crystal structure of the yeast Hsp110 Sse1. *Cell* **131**, 106–120 (2007).
92. Fünfschilling, U. & Rospert, S. Nascent polypeptide-associated complex stimulates protein import into yeast mitochondria. *Mol. Biol. Cell* **10**, 3289–3299 (1999).
93. Rospert, S., Dubaquie, Y. & Gautschi, M. Nascent-polypeptide-associated complex. *Cell. Mol. Life Sci.* **59**, 1632–1639 (2002).
94. Spreter, T., Pech, M. & Beatrix, B. The crystal structure of archaeal nascent polypeptide-associated complex (NAC) reveals a unique fold and the presence of a ubiquitin-associated domain. *J. Biol. Chem.* **280**, 15849–15854 (2005).
95. Beatrix, B., Sakai, H. & Wiedmann, M. The α and β subunit of the nascent polypeptide-associated complex have distinct functions. *J. Biol. Chem.* **275**, 37838–37845 (2000).
96. Wegrzyn, R.D. *et al.* A conserved motif is prerequisite for the interaction of NAC with ribosomal protein L23 and nascent chains. *J. Biol. Chem.* **281**, 2847–2857 (2006).
97. Wiedmann, B., Sakai, H., Davis, T.A. & Wiedmann, M. A protein complex required for signal-sequence-specific sorting and translocation. *Nature* **370**, 434–440 (1994).
98. Andersen, K.M., Semple, C.A. & Hartmann-Petersen, R. Characterisation of the nascent polypeptide-associated complex in fission yeast. *Mol. Biol. Rep.* **34**, 275–281 (2007).
99. Reimann, B. *et al.* Initial characterization of the nascent polypeptide-associated complex in yeast. *Yeast* **15**, 397–407 (1999).
100. Bloss, T.A., Witze, E.S. & Rothman, J.H. Suppression of CED-3-independent apoptosis by mitochondrial βNAC in *Caenorhabditis elegans*. *Nature* **424**, 1066–1071 (2003).
101. Markesich, D.C., Gajewski, K.M., Nazimiec, M.E. & Beckingham, K. *bicaudal* encodes the *Drosophila* beta NAC homolog, a component of the ribosomal translational machinery*. *Development* **127**, 559–572 (2000).
102. Deng, J.M. & Behringer, R.R. An insertional mutation in the BTF3 transcription factor gene leads to an early postimplantation lethality in mice. *Transgenic Res.* **4**, 264–269 (1995).
103. Gasch, A.P. *et al.* Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* **11**, 4241–4257 (2000).
104. Craig, E.A., Eisenman, H.C. & Hundley, H.A. Ribosome-tethered molecular chaperones: the first line of defense against protein misfolding? *Curr. Opin. Microbiol.* **6**, 157–162 (2003).
105. Schuwirth, B.S. *et al.* Structures of the bacterial ribosome at 3.5 Å resolution. *Science* **310**, 827–834 (2005).
106. Pool, M.R., Stumm, J., Fulga, T.A., Sinning, I. & Dobberstein, B. Distinct modes of signal recognition particle interaction with the ribosome. *Science* **297**, 1345–1348 (2002).
107. Beckmann, R. *et al.* Architecture of the protein-conducting channel associated with the translating 80S ribosome. *Cell* **107**, 361–372 (2001).
108. Blau, M. *et al.* ERj1p uses a universal ribosomal adaptor site to coordinate the 80S ribosome at the membrane. *Nat. Struct. Mol. Biol.* **12**, 1015–1016 (2005).
109. Halic, M. *et al.* Structure of the signal recognition particle interacting with the elongation-arrested ribosome. *Nature* **427**, 808–814 (2004).
110. Schaffitzel, C. *et al.* Structure of the *E. coli* signal recognition particle bound to a translating ribosome. *Nature* **444**, 503–506 (2006).
111. Jia, L. *et al.* Yeast Oxa1 interacts with mitochondrial ribosomes: the importance of the C-terminal region of Oxa1. *EMBO J.* **22**, 6438–6447 (2003).
112. Mitra, K. *et al.* Structure of the *E. coli* protein-conducting channel bound to a translating ribosome. *Nature* **438**, 318–324 (2005).
113. Keenan, R.J., Freymann, D.M., Stroud, R.M. & Walter, P. The signal recognition particle. *Annu. Rev. Biochem.* **70**, 755–775 (2001).
114. Skach, W.R. Cellular mechanisms of membrane protein folding. *Nat. Struct. Mol. Biol.* **16**, 606–612 (2009).
115. Eisner, G., Koch, H.G., Beck, K., Brunner, J. & Muller, M. Ligand crowding at a nascent signal sequence. *J. Cell Biol.* **163**, 35–44 (2003).
116. Eisner, G., Moser, M., Schafer, U., Beck, K. & Muller, M. Alternate recruitment of signal recognition particle and Trigger factor to the signal sequence of a growing nascent polypeptide. *J. Biol. Chem.* **281**, 7172–7179 (2006).
117. Ullers, R.S. *et al.* Sequence-specific interactions of nascent *Escherichia coli* polypeptides with Trigger factor and signal recognition particle. *J. Biol. Chem.* **281**, 13999–14005 (2006).
118. Ullers, R.S. *et al.* Interplay of signal recognition particle and Trigger factor at L23 near the nascent chain exit site on the *Escherichia coli* ribosome. *J. Cell Biol.* **161**, 679–684 (2003).
119. Raine, A., Ivanova, N., Wikberg, J.E. & Ehrenberg, M. Simultaneous binding of Trigger factor and signal recognition particle to the *E. coli* ribosome. *Biochimie* **86**, 495–500 (2004).
120. Valent, Q.A. *et al.* Nascent membrane and presecretory proteins synthesized in *Escherichia coli* associate with signal recognition particle and Trigger factor. *Mol. Microbiol.* **25**, 53–64 (1997).
121. Lee, H.C. & Bernstein, H.D. The targeting pathway of *Escherichia coli* presecretory and integral membrane proteins is specified by the hydrophobicity of the targeting signal. *Proc. Natl. Acad. Sci. USA* **98**, 3471–3476 (2001).
122. Beck, K., Wu, L.F., Brunner, J. & Muller, M. Discrimination between SRP- and SecA/SecB-dependent substrates involves selective recognition of nascent chains by SRP and Trigger factor. *EMBO J.* **19**, 134–143 (2000).
123. Lee, H.C. & Bernstein, H.D. Trigger factor retards protein export in *Escherichia coli*. *J. Biol. Chem.* **277**, 43527–43535 (2002).
124. Ullers, R.S., Ang, D., Schwager, F., Georgopoulos, C. & Genevaux, P. Trigger factor can antagonize both SecB and DnaK/DnaJ chaperone functions in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **104**, 3101–3106 (2007).
125. Buskiewicz, I. *et al.* Trigger factor binds to ribosome-signal-recognition particle (SRP) complexes and is excluded by binding of the SRP receptor. *Proc. Natl. Acad. Sci. USA* **101**, 7902–7906 (2004).
126. Halic, M. *et al.* Following the signal sequence from ribosomal tunnel exit to signal recognition particle. *Nature* **444**, 507–511 (2006).
127. Buskiewicz, I.A., Jockel, J., Rodnina, M.V. & Wintermeyer, W. Conformation of the signal recognition particle in ribosomal targeting complexes. *RNA* **15**, 44–54 (2009).
128. Lauring, B., Sakai, H., Kreibich, G. & Wiedmann, M. Nascent polypeptide-associated complex protein prevents mistargeting of nascent chains to the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA* **92**, 5411–5415 (1995).
129. Lauring, B., Kreibich, G. & Wiedmann, M. The intrinsic ability of ribosomes to bind to endoplasmic reticulum membranes is regulated by signal recognition particle and nascent-polypeptide-associated complex. *Proc. Natl. Acad. Sci. USA* **92**, 9435–9439 (1995).
130. Möller, I. *et al.* A general mechanism for regulation of access to the translocon: competition for a membrane attachment site on ribosomes. *Proc. Natl. Acad. Sci. USA* **95**, 13425–13430 (1998).

© 2009 Nature America, Inc. All rights reserved.
npg