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Ribosome-associated chaperones as key players in proteostasis

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***De novo* protein folding is delicate and error-prone and requires the guidance of molecular chaperones. Besides cytosolic and organelle-specific chaperones, cells have evolved ribosome-associated chaperones that support early folding events and prevent misfolding and aggregation. This class of chaperones includes the bacterial trigger factor (TF), the archaeal and eukaryotic nascent polypeptide-associated complex (NAC) and specialized eukaryotic heat shock protein (Hsp) 70/40 chaperones. This review focuses on the cellular activities of ribosome-associated chaperones and highlights new findings indicating additional functions beyond *de novo* folding. These activities include the assembly of oligomeric complexes, such as ribosomes, modulation of translation and targeting of proteins.**

Protein biogenesis is supported by a robust chaperone network

Ribosomes synthesize proteins based on the genetic information delivered by mRNAs. After synthesis of the first 35–40 amino acids, nascent chains exit the ~100-Å-long ribosomal tunnel. At that stage, multiple factors that directly bind to ribosomes interact with the emerging nascent polypeptides. Besides chaperones, processing enzymes cotranslationally modify the N termini of a subset of nascent peptides. In addition, targeting factors such as the signal recognition particle (SRP) initiate the delivery of secreted polypeptides to the membrane for translocation [1].

Folding of nascent proteins can start cotranslationally as the polypeptide exits the ribosomal tunnel, but it is only completed when the entire sequence is available on release from the ribosome. The ribosomal tunnel has a diameter between 10 and 20 Å and therefore it allows the passage of unfolded polypeptides or of peptides in an α-helical conformation but largely prevents the formation of structural elements involving long-distance interactions within the peptide chain [2]. Moreover, *de novo* protein folding is challenging because during synthesis, the sequence information of a nascent protein is incomplete and continuously changing; during elongation, approximately four and 20 new amino acids are added per second in eukaryotes and bacteria, respectively. In addition, the high concentration of molecules in the cytosol (300–400 mg/ml) causes excluded volume effects that enhance the non-native intra- and intermolecular contacts of the newly synthesized polypeptides and thereby increases the risk of misfolding and aggregation [3]. The cellular strategy to safeguard *de novo* protein folding involves a set of molecular chaperones to prevent such off-pathway reactions [4–8].

Chaperones involved in the *de novo* folding of cytosolic proteins can be divided into two groups based on their cellular localization (Figure 1). The first group comprises chaperones that dynamically bind to both the ribosome and the emerging polypeptide, thereby controlling early folding steps during translation. Strikingly, cells from different kingdoms of life have evolved diverse types of ribosome-associated chaperones that work through different structural and mechanistic principles. For instance, bacteria possess the chaperone trigger factor (TF) (Figure 1a), whereas eukaryotes have two different types of ribosome-associated systems that support *de novo* folding pathways (Figure 1b,c). In yeast, the first system consists of the ribosome-associated complex (RAC) and the Hsp70 chaperone Ssb. RAC is a stable complex formed by the Hsp40 chaperone zuotin (Zuo), and the Hsp70 chaperone Ssz. The second system is the heterodimeric nascent polypeptide-associated complex (NAC) (Figure 1b). Whereas NAC is highly conserved from yeast to humans, mammalian RAC (mRAC) consists of the Zuo homolog MPP11 and an Ssz-like protein Hsp70L1 (Figure 1c) [9] but lacks a ribosome-bound Ssb-like protein. Instead, mammalian cytosolic Hsp70 is recruited to nascent polypeptides via mRAC [10].

The second group of chaperones that acts on newly synthesized proteins mainly comprises the cytosolic chaperones of the Hsp70/40 and Hsp60/10 families, which are present in pro- and eukaryotes, as well as prefoldin, which is present in Archaea and eukaryotes (Figure 1). Both ribosome-associated and cytosolic chaperones cooperate to form a robust network for *de novo* protein folding. This network is best studied in *Escherichia coli*. Whereas the Hsp60/10 chaperone system GroEL/GroES is essential under all conditions, cells with individual deletions of the genes encoding TF and the Hsp70 protein DnaK are viable [11–13]. However, simultaneous deletion of genes encoding TF and DnaK causes cell death at 30°C and above [11,12]; this synthetic lethality can be suppressed by GroEL/GroES overexpression, which emphasizes the overlapping substrate pools and chaperone functions of these three systems [14,15].

Trigger factor

TF is by far the best-understood ribosome-associated chaperone and its role in cotranslational protein folding is well

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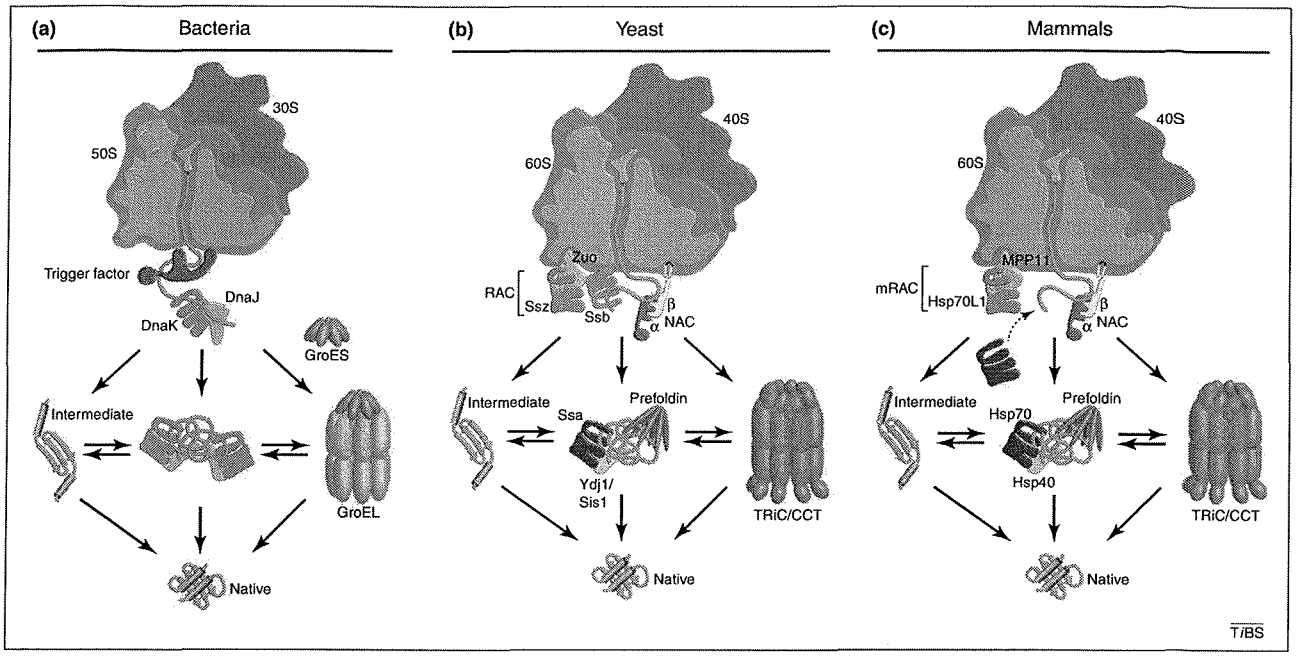

Figure 1. Chaperone networks involved in *de novo* protein folding. The concept of chaperone systems that assist *de novo* protein folding is conserved across all kingdoms of life. In **(a)** *Escherichia coli*, **(b)** *Saccharomyces cerevisiae* and **(c)** mammals, chaperones bind directly to ribosomes (grey) to act on nascent polypeptides (orange). These ribosome-associated chaperones comprise trigger factor in bacteria and the eukaryotic α–β heterodimer nascent polypeptide-associated complex (NAC). In addition, the heat shock protein (Hsp)70/40-based systems Zuo/Ssz/Ssb and MPP11/Hsp70L1 localize to ribosomes in yeast and mammals, respectively. The ribosome-associated complex (RAC) consists of Ssz and zuotin (Zuo) in yeast [48]. MPP11 and Hsp70L1 form mammalian RAC (mRAC). Ssb homologs are restricted to fungi, whereas mammalian RAC recruits cytosolic Hsp70 to nascent polypeptides [10]. Members of the Hsp70/40 and Hsp60/10 chaperone families act downstream of ribosome-associated chaperones on a subset of newly synthesized proteins that require further folding assistance. These include the DnaK/DnaJ and GroEL/GroES chaperone systems in bacteria, and Ssa-Ydj1/Sis1 and the chaperonin TRiC/CCT in yeast [4,11,13,30,64]. Hsp70–Hsp40 chaperones and TRiC/CCT also interact with newly synthesized proteins in mammalian cells. Specialized chaperones, such as prefoldin, participate in *de novo* folding of certain substrates [65,66].

characterized. This chaperone is only found in bacteria and chloroplasts. It consists of three domains: an N domain, a peptidyl-prolyl *cis–trans* isomerase (PPIase) domain and a C domain. TF adopts an extended three-dimensional conformation with the C domain positioned in the center of the molecule (Figure 2a,b) [16,17]. The N domain uses a conserved signature motif to mediate binding to the ribosome via ribosomal protein (r-protein) L23 at the rim of the ribosomal tunnel exit (Figure 2b,d). The TF N domain, together with the C domain, forms an open cavity with two protruding arms [16–18]. The PPIase domain is located at the distal end of TF. It has been suggested that this domain prolongs the residence time of TF on nascent chains and thereby may contribute to delay cotranslational folding (discussed later). However, the PPIase activity of TF is not essential *in vivo* and TF variants lacking the isomerase function show a chaperone activity that is comparable to wild type TF [15,19,20].

TF exists in a monomer–dimer equilibrium. In its monomeric state, TF associates transiently with ribosomes and hunches over the ribosomal tunnel, facing its central cavity towards the tunnel exit. Therefore, TF is ideally positioned to capture emerging chains (Figure 2d). TF has multiple binding sites for substrates throughout its interior, which is lined by several hydrophobic side chains that can provide multiple hydrophobic contact sites for an unfolded polypeptide chain (Figure 2c) [16,21,22]. Indeed, the residence time of TF on translating ribosomes (between 10 and 50 s) correlates with the hydrophobicity of the nascent polypeptide [23]. In addition to the hydrophobic contacts, the cavity also exposes hydrophilic side chains; these might be involved in electrostatic contacts to substrates. Accordingly, it has been shown that TF binds to the folded and positively charged r-protein S7 [24,25]. Thus, the substrate interface of TF is versatile, using different types of interactions, which could explain its broad substrate spectrum (discussed later).

TF accommodates the substrate in its interior, which provides a protective environment to prevent proteins from aggregating or being degraded (Figure 2d) [26,27]. In addition, ribosome-bound TF can prevent premature and incorrect folding of proteins during synthesis. For example, it retards cotranslational folding of recombinantly expressed firefly luciferase in *E. coli* cells, thereby enhancing the total yield of active luciferase [23,28]. By contrast, it is unclear whether TF can also promote cotranslational folding processes. The local accumulation of folding-competent polypeptide segments within the TF cavity may favor the formation of key contacts in the nascent chain and thus drive initial folding steps. On the basis of its crystal structure, TF can accommodate entire protein domains or even small proteins (with a size up to 130 aa) in its central cavity [16]. Indeed, it has been shown that purified TF stimulates the refolding of denatured glyceraldehyde 3-phosphate dehydrogenase to its native state in the absence of ribosomes [29]. However, whether this also holds true for the folding of nascent polypeptide chains awaits experimental proof.

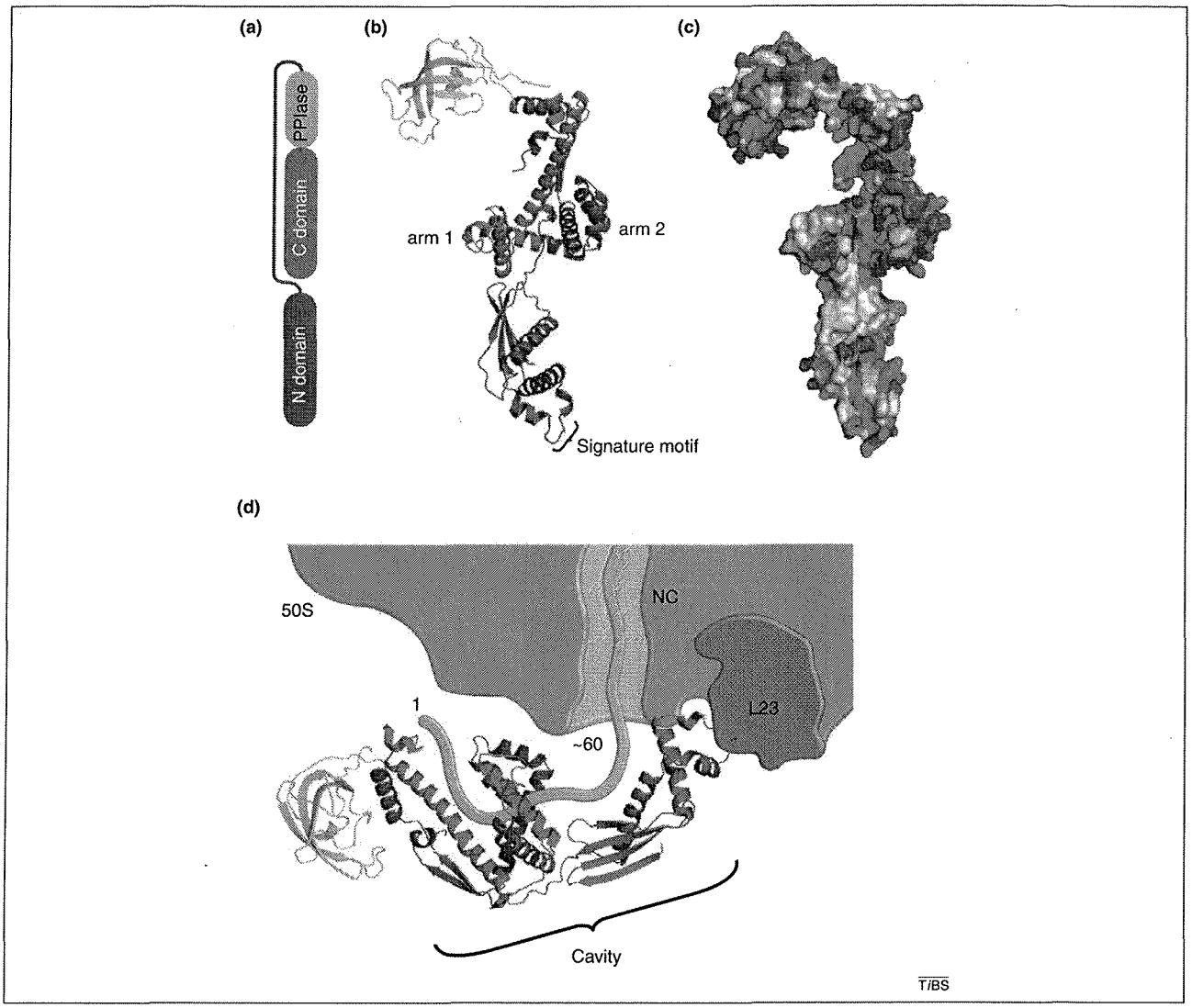

Figure 2. Trigger factor (TF) from *Escherichia coli*. **(a)** Domain organization of TF. **(b)** Ribbon diagram of the crystal structure of TF (PDB 1W26). The N domain (red) contains the TF signature motif (GFRxGxxP) in a loop region between two α-helices and is connected to the peptidyl-prolyl *cis–trans* isomerase (PPIase) domain (green) via an extended linker. The C domain (blue) is located in the center of the molecule and forms two arm-like protrusions. The N domain and both arms of the C domain together form a cavity for nascent polypeptide chains. **(c)** Surface charge distribution of TF for the same orientation as in **(b)**. Positively and negatively charged residues are shown in blue and red, respectively. **(d)** Structural model of TF bound to the large ribosomal subunit (grey) based on [16]. The main contact between TF [colors as in **(a)** and **(b)**] and the large ribosomal subunit (grey) involves the signature motif in the N domain of TF and the ribosomal protein L23 (dark grey). *In vivo*, TF is preferentially recruited to translating ribosomes carrying nascent chains (orange) longer than 100 amino acids (aa), which have ~60 aa exposed outside the ribosomal tunnel. TF structures were prepared using PyMOL (DeLano Scientific LLC) based on [16].

What are the substrates of ribosome-bound TF?

The first attempts to uncover TF substrates identified proteins that misfold and aggregate in the absence of TF and the cooperating DnaK (Hsp70) system [11,13]. Although deletion of TF alone does not cause protein aggregation, an additional reduction in DnaK levels results in substantial protein misfolding and aggregation. More than 300 different aggregation-prone protein species were found in TF- and DnaK-deficient cells. The proteins identified are involved in many different cellular processes, range in size from 16 to 140 kDa, and are specifically enriched in large (>40 kDa) multidomain proteins. Moreover, a proteome-wide analysis of the GroEL interactome revealed that ~250 cytosolic proteins interact with GroEL during folding, but that this number increases substantially in cells lacking TF and DnaK [30]. The data suggest a strong overlap of substrate pools for TF, DnaK and GroEL, which explains the robustness of the chaperone network for newly synthesized proteins in *E. coli*.

A recent study using selective ribosome profiling provided a global analysis of the nascent interactome of TF for the first time [31]. The technique combines crosslinking of endogenously synthesized tagged TF to nascent polypeptides and affinity purification of ribosome–TF complexes and subsequent identification of the mRNA that is being read by TF-bound ribosomes. This elegant approach revealed new fundamental features of the cotranslational activity of TF. The first surprise was that recruitment of TF

to translating ribosomes is delayed *in vivo* until nascent peptides reach a length of ~100 aa. This means that, assuming that 30–40 aa of the nascent chains are buried in the ribosomal tunnel, TF must bind to emerging peptides only once they have exposed at least 60–70 aa outside the ribosome (Figure 2d). The finding that TF does not interact with short nascent polypeptides *in vivo* contradicts *in vitro* crosslinking studies that suggest that TF contacts peptides immediately on their exit from the ribosomal tunnel [21]. This apparent contradiction is resolved by taking into account that additional factors interact with nascent proteins *in vivo*. The initial exclusion of TF, which only correlates with the length of the polypeptides, provides a time window to allow processing enzymes to access nascent proteins [31]. These enzymes are required, for example, to remove the formyl moiety and the initiator methionine from the N termini of nascent polypeptide chains. The second important finding from the ribosome profiling study is that TF interacts with all newly synthesized proteins, except for those inserted into the cytoplasmic membrane via SRP targeting. By contrast, nascent outer membrane β-barrel proteins (Omps) were among the strongest TF interactors during their synthesis on ribosomes. This suggests that the chaperone activity of *E. coli* TF is particularly important for keeping Omps in a translocation-competent conformation, so that they can be efficiently exported by the Sec machinery (Figure 5a) [31]. It should be mentioned that this finding is in agreement with pioneering studies by Wickner and coworkers, who initially identified TF in a reconstituted *in vitro* translocation experiment as a cytosolic component that maintains proOmpA in a transport-competent conformation for delivery into inside-out membrane vesicles [32]. However, because TF is also evolutionarily conserved in Gram-positive bacteria that lack an outer membrane, it is obvious that TF is required to assist in the folding of many cytosolic proteins as well.

More than one function for TF?

Without doubt, TF is a chaperone for nascent polypeptides, but there might be another function for the nonribosomal dimeric form of TF in the cytosol (Figure 5a). Martinez-Hackert and Hendrickson discovered that TF also associates with proteins that are normally assembled into large complexes [25]. More than 60 different full-length proteins, including many r-proteins such as the 30S protein S7, were copurified with recombinant TF from *E. coli* lysates. Subsequent structural analysis of TF from *Thermotoga maritima* (TF-*Tm*) in a 2:2 stoichiometric complex with the r-protein S7-*Tm* revealed that TF-*Tm* is able to bind and stabilize almost natively folded S7 proteins. The structural data indicate that TF-*Tm* masks large areas of the surface of S7, which are substantially buried on assembly into the 30S ribosomal subunit. Therefore, it was suggested that TF might act as an assembly factor for large protein complexes and ribosomes (Figure 5a). However, the physiological importance of an assembly function of TF remains unclear because cells lacking TF show only a very mild defect in ribosome biogenesis at elevated temperatures. Nevertheless, it is an attractive hypothesis that TF has more than one function. Considering that TF is present in a 1–2 molar excess over ribosomes [33] and preferentially associates with translating ribosomes after synthesis of the first 100 aa, enough TF is available to serve both activities: cotranslational chaperoning of newly made proteins and posttranslational assembly support for large complexes.

Eukaryotic ribosome-associated chaperones

In eukaryotes, a Hsp70/40 system and NAC are assumed to function in cotranslational protein folding (Figure 1b,c). However, their mechanisms of action and structures are barely characterized. As with bacterial TF, these eukaryotic factors are abundant cellular proteins that can cycle on and off the ribosome and may have additional functions in the cytosol or nucleus. Moreover, the presence of nascent polypeptides enhances ribosome association of these chaperones, although no kinetic data exist so far [34].

NAC

NAC has been described as the initial factor that interacts with nascent polypeptides as they emerge from ribosomes and prevents them from forming incorrect interactions [35]. The complex is widely conserved from Archaea to man. However, Archaea only have a homodimeric NAC formed by two α-subunits, whereas yeast and higher eukaryotes form an α–β heterodimer (Figures 1b,c and 3). Crystal structures of truncated NAC variants from archaeal and human complexes suggest that homo- and heterodimers of NAC are formed via their homologous six-stranded β-barrel-like NAC domains, one of which is present in α- and β-NAC subunits, respectively (Figure 3b). In contrast to β-NAC, the α-NAC subunit contains a C-terminal ubiquitin-associated (UBA) domain [36–38], suggesting that there could be different functions for the two subunits [4,36]; however, the role of the UBA domain is unknown.

No structural information for the NAC ribosome-binding element exists. However, it is known that the N terminus of the β-NAC subunit of eukaryotic NAC harbors a conserved ribosome-binding motif (Figure 3a) and ribosome binding might involve the predicted adjacent α-helical element as well. Crosslinking data suggest that there may be multiple anchor sites for NAC on ribosomes, including the r-protein Rpl25, the yeast homolog of bacterial L23 [39–41]. The conserved r-protein L23/Rpl25 serves as a multifunctional docking site and coordinates other ribosome-associated factors such as TF (in bacteria), SRP and the Sec translocation pore [7]. Importantly, ribosome binding seems to be a prerequisite for NAC interaction with nascent polypeptides, because amino acid substitutions in the ribosome-binding motif of β-NAC that diminish ribosome association also prevent crosslinking to nascent chains *in vitro* [35,39]. Although β-NAC mediates association of the heterodimeric complex with ribosomes, both NAC subunits contact nascent polypeptides. However, the substrate binding sites in the individual NAC subunits are not yet identified.

Multiple functions have been described for NAC, including roles as a transcription activator, a mediator of ER stress and a triage factor for transport to mitochondria and the ER [42]. However, most of these potential NAC functions still lack compelling *in vivo* evidence. The observation

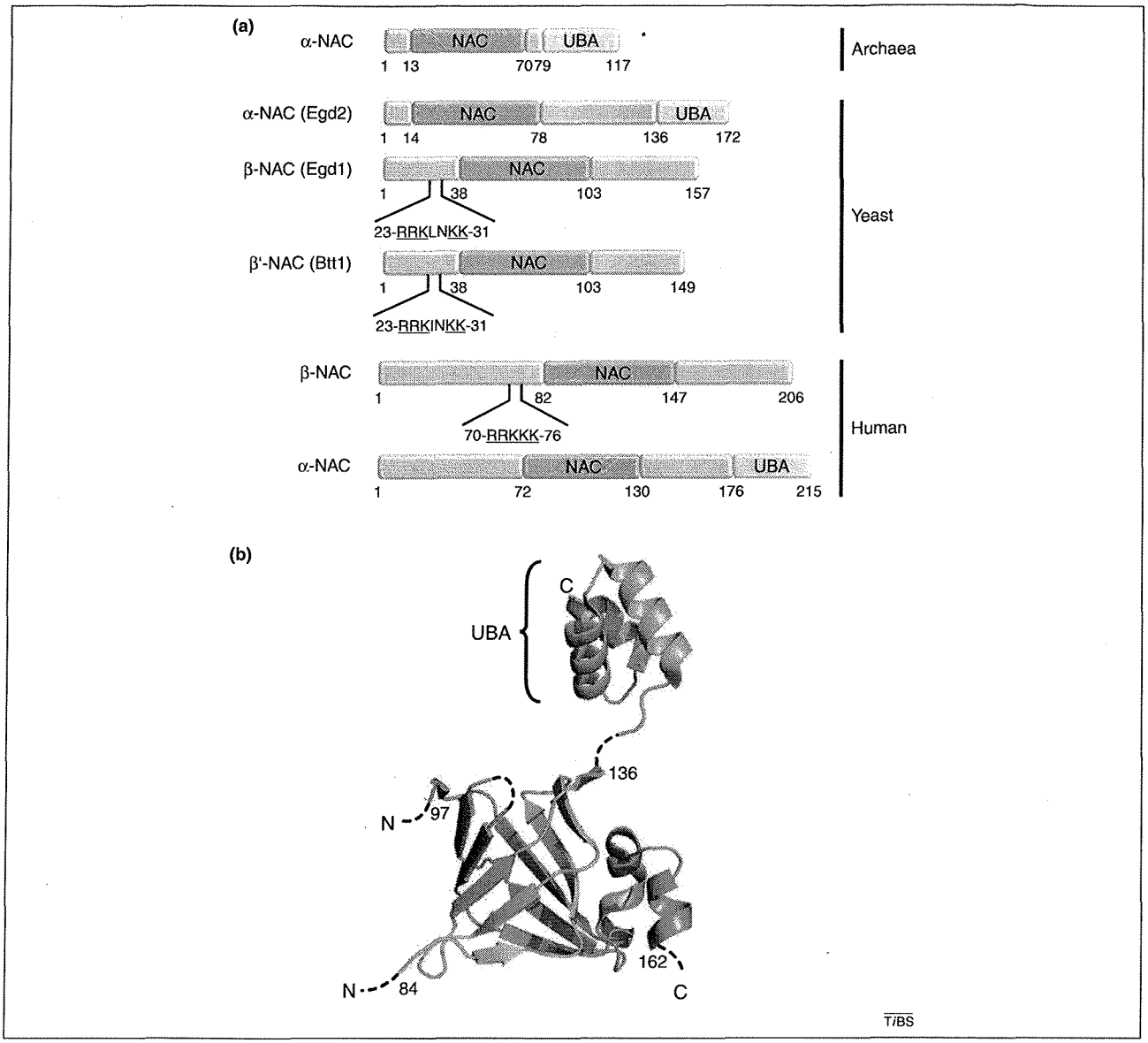

Figure 3. The nascent polypeptide-associated complex (NAC). **(a)** Schematic representation of the conserved domains of archaeal, yeast (*Saccharomyces cerevisiae*) and human NAC. The conserved NAC domains (green) and the ubiquitin-associated (UBA) domain (yellow) of α-NAC are indicated. The conserved NAC signature motif, which mediates ribosome binding [39], is indicated for the yeast and human β-NAC subunits. **(b)** Structural model of NAC. α-NAC (blue) and β-NAC (orange) form a stable heterodimer via the NAC domains. The UBA domain is derived from the crystal structure of archaeal NAC (PDB 1TR8) [36] and was modeled on the human NAC domain heterodimer [PDB 3LKX, aa 84–136 of α-NAC (NACA) and aa 97–162 of β-NAC (BTF3) isoform A] [37]. Broken lines indicate unresolved parts of the molecule. The figure was generated using PyMOL (DeLano Scientific LLC).

that NAC quantitatively associates with ribosomes in 1:1 stoichiometry, crosslinks to nascent proteins and protects them from proteolysis suggests that this complex might be a component of the chaperone network in eukaryotes that is dedicated to support *de novo* folding processes [7,34]. Moreover, loss of NAC function in yeast results in no detectable phenotype; however, the absence of NAC significantly exacerbates the growth defects of cells lacking the ribosome-associated Hsp70 homolog Ssb at 30°C and strongly enhances aggregation of newly synthesized proteins [43]. This suggests that NAC is genetically and functionally connected to the second ribosome-attached chaperone system. Because of the robustness of the chaperone network, loss of NAC alone might be compensated for in yeast. By contrast, NAC mutations in mice, fruit flies and *Caenorhabditis elegans* cause developmental defects and early embryonic lethality, suggesting additional irreplaceable NAC functions in higher eukaryotes [44–46].

A study by Frydman and colleagues further supports the hypothesis that NAC acts as an integral component of the chaperone network that promotes folding of newly synthesized proteins. They systematically identified cotranslational substrates of NAC via the mRNAs associated with ribosome–NAC complexes [47]. The data revealed that NAC binds to virtually all translating ribosomes and contacts every nascent polypeptide made in

yeast. In contrast to higher eukaryotes, yeast has three NAC subunits (Figure 3a): two β-NAC (Egd1 and Btt1) and one α-NAC (Egd2), which give rise to different NAC homo- and heterodimers with different substrate specificities [45,47]. Btt1-subunit homo- and heterodimers predominantly bind to ribosomes that translate mitochondrial or ribosomal proteins. By contrast, Egd1 and Egd2, which form the most abundant NAC heterodimer in yeast, show a preference for ribosomes that translate metabolic enzymes and secretory and membrane proteins [47]. By comparing the interactome of NAC (Egd1/Egd2) with that of SRP, Frydman and coworkers found that a subset of nascent secretory proteins interacts with both factors. Although yeast cells lacking NAC do not have pronounced impairments in targeting of proteins to their appropriate compartments, some secreted polypeptides require NAC to efficiently interact with SRP, whereas others are prevented from interacting with SRP when NAC is present [47]. Therefore, yeast NAC appears to act as a triage factor for certain secretory nascent polypeptides that are targeted by SRP to the Sec translocation pore (Figure 5b).

The ribosome-associated Hsp70/40 system

The ribosome-associated Hsp70/40 chaperone system is only found in eukaryotes. The conserved core element is the stable heterodimeric RAC. In *Saccharomyces cerevisiae*, this system is complemented by another ribosome-associated Hsp70, Ssb, whereas in higher eukaryotes cytosolic Hsp70 acts together with the ribosome-bound mRAC system (Figures 1b,c and 4) [9,10,48,49].

There are no structural data for RAC, but hydrogen–deuterium exchange experiments, combined with mutational analysis, gave insights into the overall architecture of the complex [50]. The N terminus of Zuo stably binds to the Hsp70 protein Ssz, mainly via contacts with the C-terminal substrate-binding domain (SBD) and some contacts with the N-terminal nucleotide-binding domain (NBD) of Ssz (Figure 4a). Furthermore, Zuo has a J domain that stimulates the ATPase activity of Ssb and a charged region that allows RAC to bind the ribosome near the ribosomal protein Rpl31 at the ribosomal tunnel exit [51]. In contrast to canonical J-domain proteins, Zuo requires stable interaction with Ssz to act as a co-chaperone for Ssb [48,52]. Thus far, RAC association with nascent

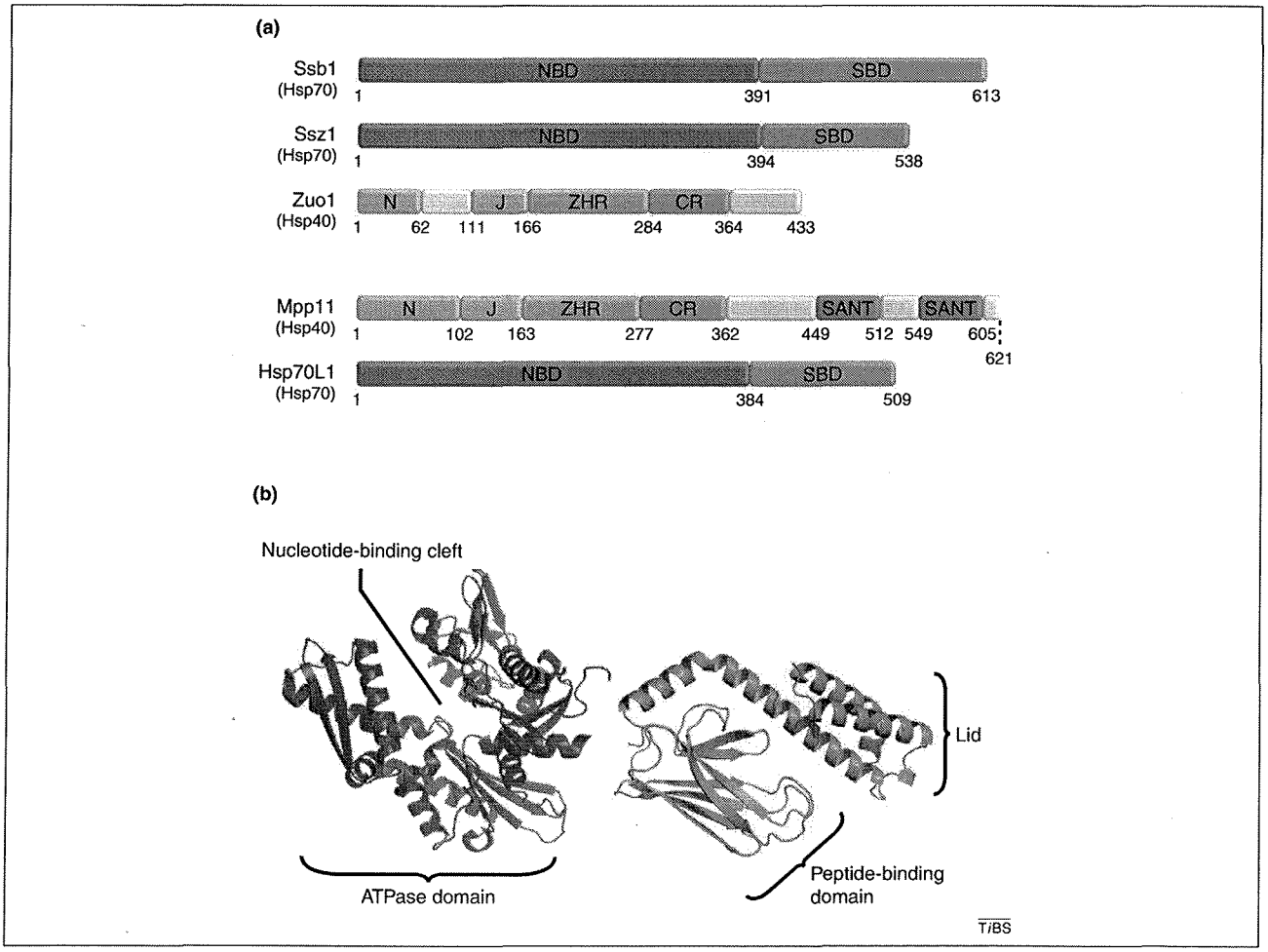

Figure 4. RAC–Ssb from yeast and its mammalian homolog mRAC. (a) Schematic representation of the conserved domains of yeast Ssb and RAC (Ssz and Zuo) and mammalian RAC (MPP11 and Hsp70L1). NBD, nucleotide-binding domain; SBD, substrate-binding domain; N, N-terminal domain; J, J domain; ZHR, Zuo homology region; CR, charged region; SANT, SANT domain. (b) Structural model of Ssb1 from *S. cerevisiae*. The ATPase domain (PDB 3GL1) is shown in blue. A model of the C-terminal substrate-binding domain (green) was generated using I-TASSER protein structure and function prediction [67]. The figure was generated using PyMOL (DeLano Scientific LLC).

polypeptides has never been shown, but it is essential for stimulating the binding of Ssb to nascent substrates.

Zuo homologs also exist in mammals (MPP11), but have some variations in their domain composition, including two additional SANT-like domains at the C-terminal end of the chaperone (Figure 4a) [9,49]. SANT domains are normally involved in DNA binding and chromatin remodeling [53]; however, their role in MPP11 is unknown. The Zuo homologs share the J domain and the charged region for ribosome binding. Thus, they are likely to function in a similar manner on ribosomes. This assumption is supported by the finding that human MPP11 can complement the phenotype caused by deletion of the gene encoding Zuo in yeast. Hsp70L1 was identified as the RAC partner (analogous to Ssz) for human MPP11 (Figures 1c and, 4a) [9,49].

Ssb is only found in fungal species, such as yeast. Its structure is typical of Hsp70 proteins: it contains an N-terminal ATPase domain connected via a linker to the C-terminal SBD (Figure 4b). Similar to classical cytosolic Hsp70 s, the Ssb reaction cycle is driven by co-chaperones. These include RAC, which stimulates ATP hydrolysis for tight substrate binding, and nucleotide exchange factors such as Sse1, Sln1 and Fes1, which exchange ADP for ATP, thus triggering substrate release [54]. Ssb associates with ribosomes at the ribosomal exit site in a 1:1 stoichiometry that is independent of RAC. Yeast cells lacking Ssb, RAC or both have a very similar phenotype, which includes sensitivity to high salt concentrations, low temperature and translation inhibitory drugs [54,55]. This suggests that these components form a functional unit on ribosomes.

As demonstrated recently, the loss of Ssb causes misfolding of newly synthesized proteins and the accumulation of protein aggregates [43]. Interestingly, protein aggregation was significantly enhanced in cells lacking NAC and Ssb simultaneously, although the pattern of aggregated proteins remained similar, suggesting overlapping substrate pools for the two ribosome-associated systems. Mass spectrometry analysis identified mainly r-proteins in the insoluble fraction of yeast cells lacking Ssb and NAC [43]. Strikingly, 52 out of 78 r-proteins were identified in the aggregates. This suggests that r-proteins are among the major substrates for RAC–Ssb and NAC.

Do RAC–Ssb and NAC play a role in ribosome biogenesis and the regulation of protein synthesis?

Cells lacking Ssb or both Ssb and NAC not only accumulate protein aggregates but also have strongly decreased levels of the 40S and 60S ribosomal subunits and reduced translational activity [43,56,57]. Moreover, combined deletion of genes encoding either Ssb or Zuo and Jjj1, a chaperone specific for maturation of the 60S ribosomal subunit, caused synthetic lethality in yeast. Several defects in ribosome biogenesis were discovered in cells lacking components of the RAC–Ssb and NAC systems. For instance, accumulation of a GFP-labeled r-protein in the nucleus revealed defects in the assembly and export of pre-60S subunits into the cytoplasm [43,56,57]. In addition, microarray analysis demonstrated that Ssb and Zuo influence pre-18S and pre-25S rRNA processing in the nucleus [43,56,57]. These data strongly support a role for RAC–Ssb and NAC in ribosome assembly. This function is consistent with the suggestion that Zuo, Ssb and NAC cycle between the cytoplasm and the nucleus, and with the observed transcriptional coregulation of Ssb and r-proteins [42,56,58,59].

How these chaperones function in ribosome biogenesis is an open question. Two models are plausible that are not mutually exclusive (Figure 5b). The first possibility is that the chaperones could bind to r-proteins during translation, remain bound to their substrates after synthesis, accompany r-proteins into the nucleus, and then release them directly onto rRNA for assembly. This is feasible, because many r-proteins are highly abundant, have complex conformations and only attain their correct structure on assembly into ribosomal particles. The second possibility is that these chaperones actively promote ribosomal maturation steps in the nucleus. This assumption is supported by the finding that Zuo interacts with nuclear ribosome precursors [56]. Given the complexity of the ribosome assembly process, much effort needs to be put into understanding the exact mechanisms and functions of these chaperones.

Are NAC and RAC–Ssb systems involved in managing protein aggregation?

An elegant screen for proteins that interact with different artificial aggregation-prone β-sheet proteins revealed that NAC associates with insoluble amyloid-like fibers in mammalian cells [60]. In addition, yeast Ssb associates with insoluble polyglutamine repeat proteins *in vivo* [61] and modulates the formation and toxicity of the Sup35 prion [62]. Although the physiological importance of these findings is not clear, an attractive hypothesis is that the NAC and RAC–Ssb systems not only cycle between different cellular compartments but also associate with different macromolecular assemblies in the cytosol (ribosomes and protein aggregates) to exert versatile chaperoning functions (Figure 5b). Finally, it should be mentioned that a recent study reported yet another ribosome-independent function of Ssb. This study showed that Ssb regulates the phosphorylation level of Snf1 protein kinase, thereby allowing an appropriate response to changing glucose concentrations [63].

Emerging concepts of functional versatility

The myriad of recently accumulated data on the *in vivo* roles of ribosome-associated chaperones from pro- and eukaryotic kingdoms suggests that they are versatile and vital elements of the chaperone network and perform more than one function. There is a striking functional analogy among these diverse chaperones. They cotranslationally support *de novo* folding and also act through unknown mechanisms to regulate ribosome biogenesis and thereby control protein synthesis. The latter is intriguing, because it allows these chaperones to regulate the influx of all new proteins into the cellular proteome beyond just those that require their assistance for folding. In addition to their ribosome-associated cotranslational roles, TF and NAC are important for modulating transport processes across membranes, and Ssb and NAC associate with protein aggregates. Thus, ribosome-associated chaperones

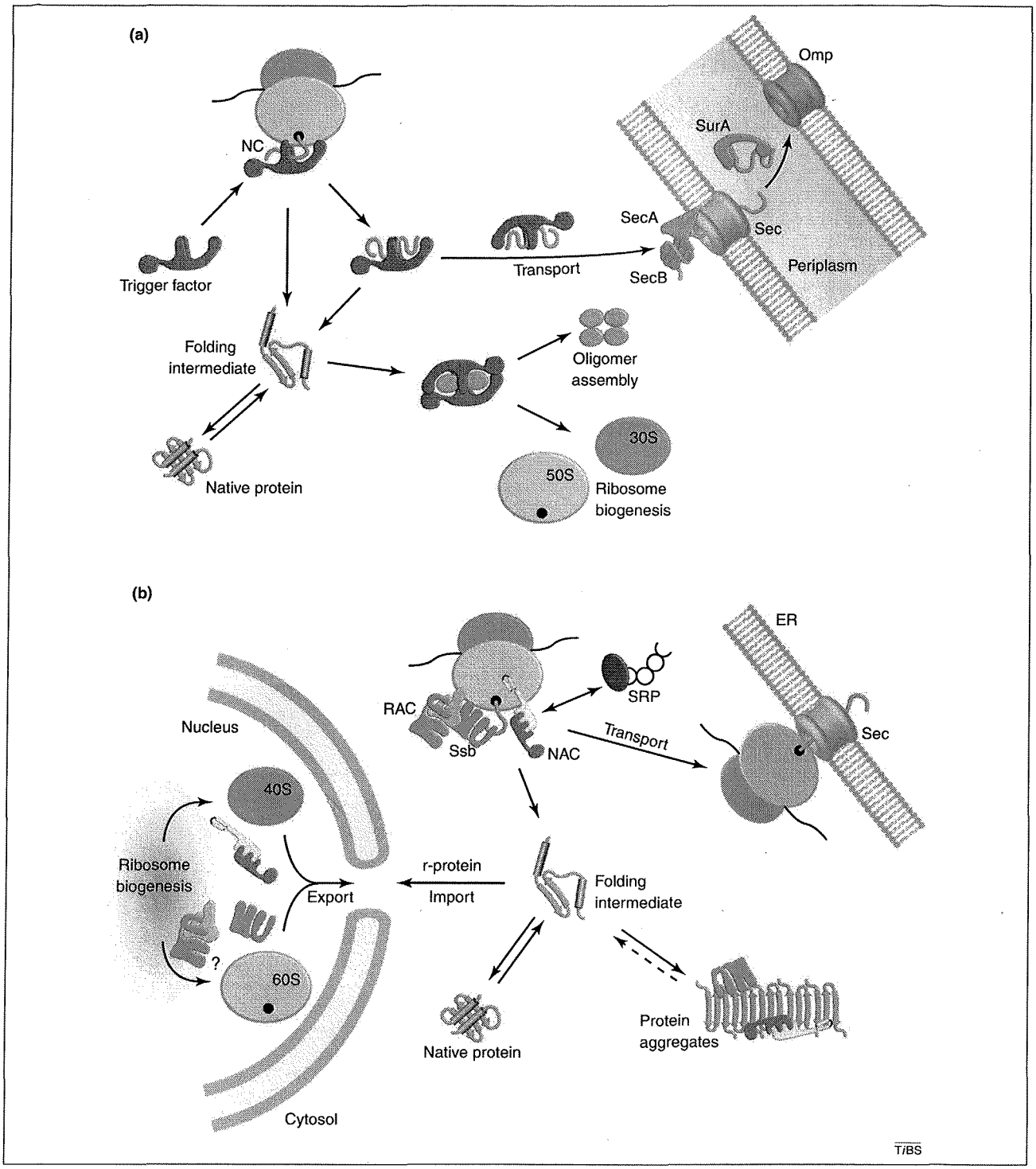

Figure 5. Ribosome-associated chaperones are functionally versatile and act in diverse cellular processes. **(a)** On the ribosome, TF protects cytosolic nascent polypeptides (orange) from aggregation, proteolysis and premature *de novo* folding [68]. In *E. coli*, TF strongly binds to outer membrane proteins (Omps) and supports targeting of translocation-competent Omp precursors to the cytoplasmic membrane for post-translational translocation through the secretion (Sec) translocation pore into the periplasm. This pathway probably involves the motor ATPase SecA and the translocation-dedicated chaperone SecB. In the periplasm, SurA, a chaperone with structural features similar to TF, assists Omp transport and membrane insertion [31]. In addition, nonribosomal TF facilitates the assembly of protein oligomers and the incorporation of ribosomal proteins into ribosome subunit precursors [25]. **(b)** In yeast, NAC and RAC–Ssb bind to ribosomes and interact with nascent polypeptide chains to assist *de novo* folding [4,11,13,30,64]. NAC may also modulate the interaction of SRP with nascent polypeptides and thus contribute to cotranslational targeting of secretory proteins to the ER membrane [47]. NAC (human) and Ssb were both found in association with amyloid-like aggregates [60,61], indicating a possible function in protein aggregation, disaggregation or aggregate clearance. In addition, RAC–Ssb and NAC have functions in the nucleus, including ribosome biogenesis [43,56].

are potent modulators of protein synthesis, folding, assembly and transport. Therefore, they represent key regulators of cellular proteostasis. A future challenge will be to dissect their individual roles in this complex network of interconnected biological processes that maintains proteostasis.

Acknowledgments

We apologize that we could not discuss all aspects of *de novo* folding and chaperone functions in depth and apologize to all our colleagues whose recent research was not or only very briefly discussed or not cited because of space constraints. We thank the members of the Deuerling laboratory for critically reading and discussing the manuscript. Research in our laboratory is supported by the SFB 969 of the German Science Foundation (DFG) and the Konstanz Research School Chemical Biology.

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