

# Ribosome-tethered molecular chaperones: the first line of defense against protein misfolding?

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Folding of many cellular proteins is facilitated by molecular chaperones. Analysis of both prokaryotic and lower eukaryotic model systems has revealed the presence of ribosome-associated molecular chaperones, thought to be the first line of defense against protein aggregation as translating polypeptides emerge from the ribosome. However, structurally unrelated chaperones have evolved to carry out these functions in different microbes. In the yeast *Saccharomyces cerevisiae*, an unusual complex of Hsp70 and J-type chaperones associates with ribosome-bound nascent chains, whereas in *Escherichia coli* the ribosome-associated peptidyl-prolyl-*cis-trans* isomerase, trigger factor, plays a predominant role.

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## Abbreviations

<b>PFD</b>	prefoldin
<b>PPlase</b>	peptidyl-prolyl- <i>cis-trans</i> isomerase
<b>RAC</b>	ribosome-associated complex
<b>SRP</b>	signal recognition particle
<b>TF</b>	trigger factor
<b>TRiC</b>	tailless complex polypeptide ring complex
<b>Zuo</b>	zuotin

## Introduction

While a protein contains within its complete amino acid sequence all of the information necessary for attaining its functional three-dimensional structure, a newly synthesized protein faces additional challenges in reaching its native state within the crowded environment of the cell [1]. Being vectorial, the protein synthesis process itself is problematic. Although some domains of a nascent chain might be capable of folding spontaneously, the folded structure cannot be obtained until an entire domain is synthesized [2]. This time lag increases the chance that hydrophobic sequences normally buried in the interior of the protein will be exposed, leading to protein aggregation [3].

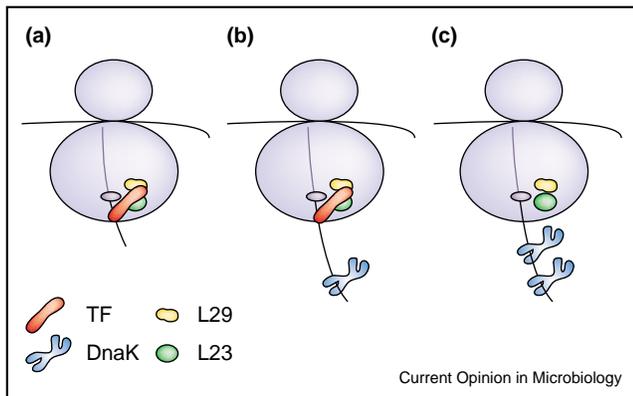
Around 40 amino acids of the nascent chain are protected from the cytosol by the ribosome exit tunnel [4]. Recent evidence indicates that after the chain leaves the tunnel, molecular chaperones bind, preventing aggregation. Both prokaryotic and eukaryotic chaperones have evolved to associate specifically with ribosomes and bind to polypeptide chains that have just emerged from the tunnel. In addition, non-ribosome-bound chaperones act on longer nascent chains, either during the process of translation, or after they have been released from the ribosome. Although chaperones carrying out these functions might be structurally unrelated, like all chaperones they share the ability to interact transiently with short stretches of amino acids that are predominantly hydrophobic in nature. However, their mode of action might differ. The interaction of, for example, Hsp70 and chaperonin-type chaperones, with polypeptide substrates, is regulated by binding and hydrolysis of ATP. Chaperonins act as oligomers, providing a cavity in which entire polypeptide chains are sequestered, whereas others, such as Hsp70s and trigger factor (TF), act as monomers [5].

In this review, we focus on recent advances in understanding the mechanisms of action of molecular chaperones that interact with nascent chains (molecular chaperones are comprehensively reviewed in [6,7]). We compare and contrast the chaperone systems in two diverse model systems, the prokaryote *Escherichia coli* and the unicellular eukaryote *Saccharomyces cerevisiae*. As in any comparison between systems that present different advantages and challenges, experimental approaches are not entirely parallel. However, striking similarities in the strategies used by the two organisms emerge, even though the individual players are quite different.

## Ribosome-tethered chaperones: the players

Bacteria and yeast have specialized chaperones that interact directly with ribosomes [8,9]. As described below, both TF of *E. coli* and Ssb of *S. cerevisiae* are bound to ribosomes in a stoichiometry of approximately 1:1, regardless of their translational status. Both are crosslinked to short nascent chains (56 and 54 amino acids for TF and Ssb, respectively), indicating that they are poised to bind polypeptides as they exit the ribosome (Figures 1 and 2). The presence or absence of the nascent chain alters the interaction of both TF and Ssb with the ribosome, as indicated by differences in the salt-resistance of the ribosome-chaperone interaction. These comparisons suggest that both organisms have evolved a general chaperone system that functions on the ribosome to chaperone nascent chains. However, TF and Ssb share no sequence similarity. TF

Figure 1



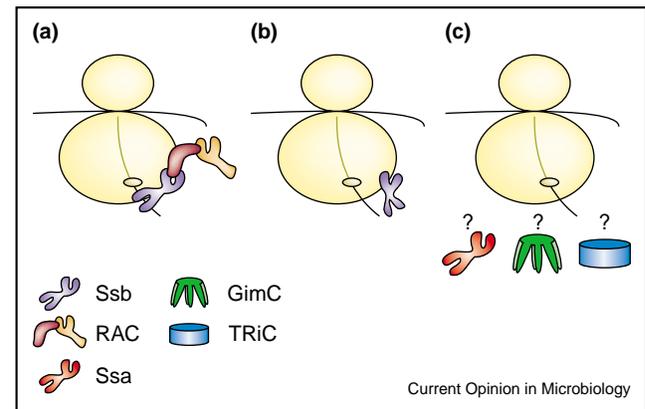
Model of early protein folding by chaperones in the *E. coli* cytosol. **(a)** In wild-type *E. coli* cells, TF binds to the ribosome, close to the ribosomal proteins L23 and L29, near the exit tunnel. The specific association of TF to the ribosome is dependent upon L23. [33\*\*]. Here, TF can interact with short nascent chains as they exit the ribosome tunnel. **(b)** DnaK, although it does not bind directly to the ribosome, interacts with longer nascent chains that are still bound to the ribosome [21,22]. The model places DnaK and TF on the same nascent chain; however, there are currently no results to support this. **(c)** The synthetic lethality observed for TF and DnaK suggests there is functional overlap between them. For example, when TF is absent, DnaK binds to more nascent chains, including chains of a shorter length [21,22].

belongs to the peptidyl-prolyl-isomerase family; Ssb belongs to the heat shock protein 70 (Hsp70) family. Below, we outline what is known about these two specialized members of these large, unrelated protein families.

Although it has been known for some time that TF possesses peptidyl-prolyl-*cis-trans* isomerase (PPIase) activity [8,10,11], the binding of TF to peptides is not dependent on the presence of proline residues. Although binding occurs in the PPIase domain [12\*\*], it is not yet known whether PPIase activity is required for the TF chaperoning of nascent chains. Such analysis will be challenging, as the domain containing the PPIase active site is the same domain as that required for general chaperone activity.

Current evidence implicates the Hsp70 Ssb as the main chaperone interacting with nascent chains on the ribosome in *S. cerevisiae*, as it can be crosslinked to nascent chains of between 54 and 152 amino acids [9,13\*\*]. However, just as TF is not a typical PPIase, Ssb is not a typical Hsp70. First, its fundamental biochemical properties are unusual. It has a high  $K_m$  for ATP, as well as an unusually high intrinsic ATPase activity [14]. In addition, direct *in vitro* demonstration of peptide-binding ability of Ssb has remained elusive, even though simple assays for peptide binding of many Hsp70s have been successful. However, experimental evidence, including analysis of chimeras with Ssb and other Hsp70s, and direct

Figure 2



Model of chaperone function on the yeast ribosome. **(a)** In wild-type yeast cells, Ssb and RAC (Zuo–Ssz complex) bind to the ribosome, where Ssb is positioned to interact with nascent chains emerging from the ribosome exit tunnel during protein synthesis [9,13\*\*]. **(b)** Without RAC, Ssb remains associated with ribosomes but is unable to interact with nascent chains [20\*\*]. **(c)** Other cytosolic chaperones, such as Ssa, GimC and TRiC, might interact with nascent chains in the absence of Ssb and RAC. Although these chaperones have been shown to function in the folding of newly synthesized proteins [28,31], there is no evidence that these chaperones interact with ribosome-bound nascent chains in yeast.

demonstration of a requirement of the peptide-binding domain *in vivo*, strongly indicate that Ssb is capable of binding unfolded protein, and that this binding is necessary for *in vivo* function [15,16\*]. Perhaps association with the ribosome is required for Ssb to be competent for binding polypeptides.

Although TF is thought to function alone as a monomer on ribosomes [17], both genetic and biochemical experiments indicate that the function of the yeast ribosome-associated chaperone Ssb requires two additional proteins, Zuotin (Zuo) and Ssz. All Hsp70s require a J-type co-chaperone, and Ssb is no exception; Zuo was proposed to be the J-protein partner of Ssb [18]. Surprisingly, Zuo was recently found to form a very stable complex, termed 'ribosome-associated complex' (RAC), with Ssz, another Hsp70 family member [19\*]. The evidence for a critical role for RAC in Ssb function is compelling. Ssb could not be crosslinked to nascent chains on ribosomes lacking RAC, but the crosslink from Ssb to nascent chains was restored upon addition of RAC (Figure 2). In addition, Zuo containing an alteration in its J domain, even though bound to Ssz, could not restore the crosslink by Ssb, suggesting that Zuo is indeed the J-protein partner of Ssb [20\*\*].

These biochemical data are supported by genetic studies. The absence of Ssb, Ssz or Zuo causes identical defects: slow growth, particularly at low temperatures, and

hypersensitivity to both aminoglycoside translation inhibitors and high concentrations of salt. Furthermore, there is no additive effect on growth when strains lack any two, or all three chaperones, suggesting that all three function together in a common process [13<sup>••</sup>,20<sup>••</sup>].

But how this unusual triad functions together remains a mystery. Certainly, Ssz is not simply playing a role as a 'traditional' Hsp70; a Ssz truncation lacking its entire putative peptide-binding domain is functional *in vivo*, indicating that peptide binding is not a critical function of Ssz [13<sup>••</sup>]. This idea is consistent with the failure to observe crosslinking of Ssz with nascent chains. Ssz might function as a cofactor/regulator of Zuo, perhaps by modifying the ability of Zuo to interact with Ssb. Overexpression of Zuo or Ssb can partially compensate for the lack of Ssz, but Ssz overexpression cannot compensate for the absence of either Zuo or Ssb [20<sup>••</sup>], indicating a more central role for Ssb and Zuo than for Ssz.

### Non-ribosome-associated chaperones: taking over part of the workload

Both *E. coli* and *S. cerevisiae* have evolved ribosome-associated chaperones. But are they functionally unique *in vivo*? Analysis of the *E. coli* system revealed an extensive overlap in function with the abundant Hsp70, DnaK. Remarkably, the lack of TF has almost no effect on cell growth. Similarly, the lack of DnaK has surprisingly little deleterious effect on growth between 30°C and 37°C. However, the absence of both TF and DnaK is lethal [21,22], suggesting that DnaK can take over TF function when absent. Indeed, normally DnaK interacts with nascent chains on the ribosome, as was demonstrated by re-precipitation of DnaK-bound polypeptides with anti-puromycin antibodies [21], but cannot be crosslinked to very short nascent chains [8] (Figure 1). Deletion of either gene resulted in increases in substrates bound by the reciprocal protein [21,22]. However, DnaK is unlikely to 'take the place' of TF, as it is not recruited to ribosomes in the absence of TF [23].

Although there are similarities between TF and DnaK, there are also differences, as would be expected for such structurally different molecules. Both chaperones recognize hydrophobic residues and both prefer that these residues be flanked by positively charged ones. However, the binding of TF encompasses more amino acids and there is no preference for the position of the positively charged residues [12<sup>••</sup>,24]. TF, unlike DnaK, can retard the folding and export of some proteins [25,26<sup>•</sup>]. TF has PPIase activity; recently DnaK was shown to accelerate the *cis-trans* isomerization of non-prolyl peptide bonds [27<sup>•</sup>]. The biological significance of these activities remains unresolved.

Functional overlap of the Ssb system with other yeast chaperones is less clear. Cells lacking this system are

viable, but have severe growth defects, indicating that other chaperones are normally unable to completely compensate for their absence. But, of course, the cytosol of *S. cerevisiae* contains additional general chaperones that might be able to partially function in place of Ssb (Figure 2). Considering the role of DnaK in *E. coli*, other Hsp70s, such as Ssa are obvious candidates. No cross-linking of Ssa to ribosome-bound nascent chains has been observed [9], but evidence does exist for the importance of Ssa in folding of newly synthesized polypeptides. A temperature-sensitive *ssa*-deficient strain has reduced specific activity of a cytosolic enzyme ornithine transcarbamylase [28]. In addition, the cytosol of both *E. coli* and *S. cerevisiae* contains chaperones that are oligomeric rings with central cavities, GroEL and TRiC (tailless complex polypeptide ring complex), respectively, that normally encapsulate substrate proteins. Although, at this point there is no evidence that these chaperonins interact with nascent chains on ribosomes in either yeast or bacteria, the mammalian homolog of TRiC has been found associated with ribosome-bound nascent chains [29,30]. Of particular interest in eukaryotic cells is the chaperone complex GimC (known as prefoldin [PFD] in mammalian cells). It is believed to bind newly synthesized substrate proteins such as actin, transferring them to TRiC [31]. Recently, the crystal structure of PFD from *Methanobacterium thermoautotrophicum* has been solved, revealing a novel structure: PFD is a multisubunit protein with a structure resembling a jellyfish, with subunits held together at one end by  $\beta$  strands. Six long coiled coils extend from this end like tentacles, their ends containing hydrophobic patches, predicted to interact with unfolded protein substrates [32].

### The ribosome-chaperone interaction

Although the existence of ribosome-associated chaperones has been known for some time, only very recently has progress been made in defining the chaperone-ribosome interaction. Crosslinks were obtained between L23 and L29 (ribosomal proteins located at the exit tunnel) and TF [33<sup>••</sup>]. The interaction with L23 proved to be the critical one. The absence of L29 had no obvious effect on the TF-ribosome interaction, whereas substitution of specific residues of L23, exposed on the ribosome surface, disrupted TF binding to the ribosome, as well as binding of purified L23 to TF. A positive correlation was observed between the association of TF with the ribosome in L23 mutants and growth, suggesting that ribosome association of TF is required for its effective action.

Analysis of the yeast ribosome-associated chaperone machinery of Ssb/Zuo/Ssz is less clearly defined. But it is known that Ssb and Zuo bind independently to the ribosome, whereas Ssz is tethered through its association with Zuo [18,19<sup>•</sup>]. Zuo function is correlated with its ability to associate with ribosomes, as its central highly charged region is necessary for both ribosome association

and function [18]. In addition, the expression of Ssb is tightly coupled to the translational status of the cell, as its expression is co-regulated with ribosomal protein genes [34,35]. Precisely defining the docking sites of the yeast chaperones will be of interest to determine the extent of the parallels between the yeast and bacterial systems.

The ribosome association of the structurally distinct TF and Ssb suggests a conserved role for chaperones in nascent chain folding. The connection between chaperones and the ribosome might extend beyond folding of the newly synthesized protein, for example, to translation. The sequence of a nascent chain can influence translation through its interaction with the exit tunnel [36\*,37\*]. That the translating ribosome can sense the progress of a nascent chain and relay a message ahead to the chaperone machinery is an intriguing possibility. Conceivably, such signaling could generate a conformational change, activating the chaperone. A precedent for such a change in mammalian ribosomes exists in the case of signal recognition particles (SRPs), which mediates targeting of ribosome–nascent-chain complexes to the endoplasmic reticulum (ER) [38\*\*]. When SRP contacts its receptor, it is repositioned on the ribosome. The recent advances in determining the structures of both prokaryotic and eukaryotic ribosomes should help in identifying any such regulatory interactions involving chaperones [39–41].

### Ribosome-associated chaperones in mammalian cells?

In mammalian cells, evidence for the interaction of ribosome-bound nascent chains has existed for many years. These include Hsc70 (an Hsp70), PFD and TRiC [29–31, 42,43]. However, no specialized ribosome-associated member of these families has been identified. A protein related to Zuo, Zrf1/MIDA1, has been found in mammalian cells and is thought to play a role in regulating cell growth [44,45]. Whether it also associates with ribosomes, or plays a role in protein synthesis is unknown. Another mammalian J protein, Mtj1, shares some features with Zuo. Mtj1 traverses the ER membrane, associating with ribosomes docked to the translocation complex on the cytosolic side and the Hsp70 BiP on the luminal side. Like Zuo, the interaction of Mtj1 with ribosomes involves a highly charged region of the protein [46\*\*].

### Conclusions

In summary, although structurally distinct, ribosome-tethered molecular chaperones that interact with nascent polypeptide chains exist in both yeast and bacteria. Future work should resolve whether these different chaperones mechanistically function the same and whether in either or both systems their function is more intimately entwined with the process of translation than we currently envision. In addition, a better understanding of early protein folding events in mammalian cells

will reveal the extent of conservation of molecular chaperone function.

### Update

Using a fluorescently labeled TF, Maier *et al.* [47] recently determined that the association and dissociation of TF with the ribosome were slow processes. The dynamics of the ribosome interaction are significantly different from the previously characterized fast binding and release of TF with substrates [12\*\*]. The authors suggest that these differences in dynamics would allow TF to remain bound to the ribosome while interacting with many sites on the nascent chain during its complete synthesis.

### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Minton A: **Implications of macromolecular crowding for protein assembly.** *Curr Opin Struct Biol* 2000, **10**:34-39.
  2. Jaenicke R: **Protein folding: local structures, domains, subunits, and assemblies.** *Biochemistry* 1991, **30**:147-161.
  3. Dobson C, Karplus M: **The fundamentals of protein folding: bringing together theory and experiment.** *Curr Opin Struct Biol* 1999, **9**:92-101.
  4. Malkin LI, Rich AJ: **Partial resistance of nascent polypeptide chains to proteolytic digestion due to ribosomal shielding.** *J Mol Biol* 1967, **26**:329-346.
  5. Bukau B, Horwich AL: **The Hsp70 and Hsp60 chaperone machines.** *Cell* 1998, **92**:351-366.
  6. Frydman J: **Folding of newly translated proteins *in vivo*: the role of molecular chaperones.** *Annu Rev Biochem* 2001, **70**:603-649.
  7. Hartl F, Hayer-Hartl M: **Molecular chaperones in the cytosol: from nascent chain to folded protein.** *Science* 2002, **295**:1852-1858.
  8. Hesterkamp T, Hauser S, Lutcke H, Bukau B: ***Escherichia coli* trigger factor is a prolyl isomerase that associates with nascent polypeptide chains.** *Proc Natl Acad Sci USA* 1996, **93**:4437-4441.
  9. Pfund C, Lopez-Hoyo N, Ziegelhoffer T, Schilke BA, Lopez-Buesa P, Walter WA, Wiedmann M, Craig EA: **The molecular chaperone Ssb from *S. cerevisiae* is a component of the ribosome-nascent chain complex.** *EMBO J* 1998, **17**:3981-3989.
  10. Scholz C, Stoller G, Zarnt T, Fischer G, Schmid FX: **Cooperation of enzymatic and chaperone functions of trigger factor in the catalysis of protein folding.** *EMBO J* 1997, **16**:54-58.
  11. Stoller G, Rucknagel KP, Nierhaus KH, Schmid FX, Fischer G, Rahfeld JU: **A ribosome-associated peptidyl-prolyl *cis/trans* isomerase identified as the trigger factor.** *EMBO J* 1995, **14**:4939-4948.
  12. Patzelt H, Rudiger S, Brehmer D, Kramer G, Vorderwulbecke S, Schaffitzel E, Waitz A, Hesterkamp T, Dong L, Schneider-Mergener J *et al.*: **Binding specificity of *Escherichia coli* trigger factor.** *Proc Natl Acad Sci USA* 2001, **98**:14244-14249.

Using a cellulose-bound peptide library, the authors found that peptide binding to trigger factor (TF) was independent of proline residues, even though it occurred through the peptidyl-prolyl-*cis-trans* isomerase (PPIase) domain and an amino acid alteration that enhanced PPIase activity also enhanced peptide binding. In addition, using fluorescence spectroscopy it was determined that the affinity of TF for peptides in solution was very low, suggesting that binding of TF to the ribosome is important for creating a high local concentration of substrates for TF.

13. Hundley H, Eisenman H, Walter W, Evans T, Hotokezaka Y, Wiedmann M, Craig E: **The *in vivo* function of the ribosome-associated Hsp70, Ssz1, does not require its putative peptide-binding domain.** *Proc Natl Acad Sci USA* 2002, **99**:4203-4208.
- Using crosslinking experiments, the authors demonstrate that Ssb can interact with nascent chains just long enough to extend from the exit tunnel, whereas no crosslink of Ssz was observed. In addition, the authors show that the putative peptide-binding domain of Ssz is not required for *in vivo* function. Less than 2% of the normal amount of Ssz and Zuo are required for normal function *in vivo*. Together, these results suggest that Ssz functions in an unprecedented way, together with Zuo, to promote Ssb function in the folding of nascent chains.
14. Lopez-Buesa P, Pfund C, Craig EA: **The biochemical properties of the ATPase activity of a 70-kDa heat shock protein (Hsp70) are governed by the C-terminal domains.** *Proc Natl Acad Sci USA* 1998, **95**:15253-15258.
15. James P, Pfund C, Craig E: **Functional specificity among Hsp70 molecular chaperones.** *Science* 1997, **275**:387-389.
16. Pfund C, Huang P, Lopez-Hoyo N, Craig E: **Divergent functional properties of the ribosome-associated molecular chaperone Ssb compared to other Hsp70s.** *Mol Biol Cell* 2001, **12**:3773-3782.
- Although *in vitro* binding of peptide was not detectable for Ssb, using a chimeric Ssb protein containing the peptide-binding domain of another Hsp70, Ssa, and single amino acid alterations in the putative Ssb peptide-binding cleft, the authors make a case that peptide binding is an essential activity of Ssb *in vivo*.
17. Patzelt H, Kramer G, Rauch T, Schonfeld H-J, Bukau B, Deuerling E: **Three-state equilibrium of *Escherichia coli* trigger factor.** *Biol Chem* 2002, **383**:1611-1619.
18. Yan W, Schilke B, Pfund C, Walter W, Kim S, Craig EA: **Zuotin, a ribosome-associated DnaJ molecular chaperone.** *EMBO J* 1998, **17**:4809-4817.
19. Gautschi M, Lilie H, Funschilling U, Mun A, Ross S, Lithgow T, Rucknagel P, Rospert S: **RAC, a stable ribosome-associated complex in yeast formed by the DnaK-DnaJ homologs Ssz1p and zuotin.** *Proc Natl Acad Sci USA* 2001, **98**:3762-3767.
- This study provided the first evidence of Ssz working with Zuo. Ssz was found to heterodimerize with Zuo, forming a stable complex associated with ribosomes. Analysis of an *ssz1* deletion strain showed it was phenotypically identical to a strain lacking *zuo1*.
20. Gautschi M, Mun A, Ross S, Rospert S: **A functional chaperone triad on the yeast ribosome.** *Proc Natl Acad Sci USA* 2002, **99**:4209-4214.
- Together with Hundley *et al* 2002 [13\*], this paper establishes Ssb, Zuo and Ssz as a trio of chaperones working together on the ribosome. Using a crosslinking approach, the authors show that both Zuo and Ssz are required for Ssb to interact with nascent chains and that Zuo having a single amino acid alteration in its J domain is not functional in this assay. This conclusion was supported by genetic analysis showing an identical phenotype for strains lacking any one, two or all three chaperones.
21. Teter SA, Houry WA, Ang D, Tradler T, Rockabrand D, Fischer G, Blum P, Georgopoulos C, Hartl FU: **Polypeptide flux through bacterial Hsp70: DnaK cooperates with trigger factor in chaperoning nascent chains.** *Cell* 1999, **97**:755-765.
22. Deuerling E, Schulze-Specking A, Tomoyasu T, Mogk A, Bukau B: **Trigger factor and DnaK cooperate in folding of newly synthesized proteins.** *Nature* 1999, **400**:693-696.
23. Kramer G, Ramachandiran V, Horowitz PM, Hardesty B: **The molecular chaperone DnaK is not recruited to translating ribosomes that lack trigger factor.** *Arch Biochem Biophys* 2002, **403**:63-70.
24. Rudiger S, Germeroth L, Schneider-Mergener J, Bukau B: **Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries.** *EMBO J* 1997, **16**:1501-1507.
25. Lee HC, Bernstein HD: **Trigger factor retards protein export in *Escherichia coli*.** *J Biol Chem* 2002, **277**:43527-43535.
26. Maier R, Scholz C, Schmid FX: **Dynamic association of trigger factor with protein substrates.** *J Mol Biol* 2001, **314**:1181-1190.
- The authors present an extensive analysis of the interaction of trigger factor (TF) with substrate protein. The affinity of a permanently unfolded protein used as a test substrate was found to be lower for TF than for other chaperones, with both the binding and release occurring very rapidly.
27. Schiene-Fischer C, Habazettl J, Schmid FX, Fischer G: **The hsp70 chaperone DnaK is a secondary amide peptide bond cis-trans isomerase.** *Nat Struct Biol* 2002, **9**:419-424.
- The authors present evidence that DnaK can catalyse *cis-trans* isomerization of secondary amide bonds, and suggests a novel role for DnaK in protein folding utilizing this activity.
28. Kim S, Schilke B, Craig E, Horwich A: **Folding *in vivo* of a newly translated yeast cytosolic enzyme is mediated by the Ssa class of cytosolic yeast Hsp70 proteins.** *Proc Natl Acad Sci USA* 1998, **95**:12860-12865.
29. McCallum CD, Do H, Johnson AE, Frydman J: **The interaction of the chaperonin tailless complex polypeptide 1 (TCP1) ring complex (TRiC) with ribosome-bound nascent chains examined using photo-cross-linking.** *J Cell Biol* 2000, **149**:591-602.
30. Hansen WJ, Cowan NJ, Welch WJ: **Prefoldin-nascent chain complexes in the folding of cytoskeletal proteins.** *J Cell Biol* 1999, **145**:265-277.
31. Siegers K, Waldmann T, Leroux MR, Grein K, Shevchenko A, Schiebel E, Hartl FU: **Compartmentation of protein folding *in vivo*: sequestration of non-native polypeptide by the chaperonin-GimC system.** *EMBO J* 1999, **18**:75-84.
32. Siegert R, Leroux MR, Scheuffer C, Hartl FU, Moarefi I: **Structure of the molecular chaperone prefoldin: unique interaction of multiple coiled coil tentacles with unfolded proteins.** *Cell* 2000, **103**:621-632.
33. Kramer G, Rauch T, Rist W, Vorderwulbecke S, Patzelt H, Schulze-Specking A, Ban N, Deuerling E, Bukau B: **L23 protein functions as a chaperone docking site on the ribosome.** *Nature* 2002, **419**:171-174.
- This important paper provides the first evidence of a direct interaction between the peptide exit tunnel of the ribosome and the chaperone machinery. Using a crosslinking approach, the authors identify L23, a protein very close to the exit site, as critical for the interaction of trigger factor (TF) with the ribosome. Furthermore, the authors demonstrate that amino acid alterations in L23 that disrupt interaction with TF leads to increased aggregation of newly synthesized proteins in the absence of DnaK, suggesting a requirement for the TF-ribosome interaction.
34. Lopez N, Halladay J, Walter W, Craig EA: **SSB, encoding a ribosome-associated chaperone, is coordinately regulated with ribosomal protein genes.** *J Bacteriol* 1999, **181**:3136-3143.
35. Nelson RJ, Ziegelhoffer T, Nicolet C, Werner-Washburne M, Craig EA: **The translation machinery and seventy kilodalton heat shock protein cooperate in protein synthesis.** *Cell* 1992, **71**:97-105.
36. Gong F, Yanofsky C: **Instruction of translating ribosome by nascent peptide.** *Science* 2002, **297**:1864-1867.
- See annotation Nakatogawa and Ito (2002) [37\*].
37. Nakatogawa H, Ito K: **The ribosomal exit tunnel functions as a discriminating gate.** *Cell* 2002, **108**:629-636.
- This paper and Gong and Yanofsky (2002) [36\*] demonstrate that the sequence and location of the nascent polypeptide in the *Escherichia coli* ribosome can influence the translation of the polypeptide. Furthermore, the authors find suppressor mutations in 23S rRNA and the gene encoding L22 r-protein that can bypass elongation arrest caused by the sequence of the SecM nascent chain. Together, these results suggest that the ribosome, specifically the peptidyl transferase center and the peptide exit tunnel, can sense features of the nascent chain peptide and that the 'message' might be relayed to the rest of the ribosome.
38. Pool M, Stumm J, Fulga T, Sinning I, Dobberstein B: **Distinct modes of signal recognition particle interaction with the ribosome.** *Science* 2002, **297**:1345-1348.
- Crosslinking experiments were used to examine the interaction of ribosomes with SRP54, which interacts with ribosome-bound nascent chains. SRP54 contacts ribosomal proteins L23 and L35 situated near the exit site. The binding of SRP54 to its receptor, which is normally located in the ER membrane, in the presence of GTP, caused a rearrangement disrupting interaction with L23, predicted to lead to movement of the nascent chain away from the ribosome. These regulated interactions

might serve as a model for understanding the action of ribosome associated chaperones.

39. Spahn CM, Beckmann R, Eswar N, Penczek PA, Sali A, Blobel G, Frank J: **Structure of the 80S ribosome from *Saccharomyces cerevisiae*-tRNA-ribosome and subunit-subunit interactions.** *Cell* 2001, **107**:373-386.
40. Nissen P, Hansen J, Ban N, Moore PB, Steitz TA: **The structural basis of ribosome activity in peptide bond synthesis.** *Science* 2000, **289**:920-930.
41. Ban N, Nissen P, Hansen J, Moore PB, Steitz TA: **The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution.** *Science* 2000, **289**:905-920.
42. Beckmann RP, Mizzen L, Welch W: **Interaction of Hsp70 with newly synthesized proteins: implications for protein folding and assembly.** *Science* 1990, **248**:850-856.
43. Thulasiraman V, Yang C-F, Frydman J: ***In vivo* newly translated polypeptides are sequestered in a protected folding environment.** *EMBO J* 1999, **18**:85-95.
44. Hughes R, Chan FY, White RA, Zon LI: **Cloning and chromosomal localization of a mouse cDNA with homology to the *Saccharomyces cerevisiae* gene *zuotin*.** *Genomics* 1995, **29**:546-550.
45. Shoji W, Inoue T, Yamamoto T, Obinata M: **MIDA1, a protein associated with Id, regulates cell growth.** *J Biol Chem* 1996, **270**:24818-24825.
46. Dudek J, Volkmer J, Bies C, Guth S, Muller A, Lerner M, Feick P, Schafer KH, Morgenstern E, Hennessy F *et al.*: **A novel type of co-chaperone mediates transmembrane recruitment of DnaK-like chaperones to ribosomes.** *EMBO J* 2002, **21**:2958-2967.  
The authors present the initial characterization of a mammalian J protein, Mtj1p, found in the ER membrane. Mtj1, containing a luminal J domain that interacts with the Hsp70 BiP and a cytosolic charged region that interacts with ribosomes, might serve as a bridge to recruit the Hsp70 to the Sec complex during translocation.
47. Maier R, Eckert B, Scholz C, Lilie H, Schmid FX: **Interaction of trigger factor with the ribosome.** *J Mol Biol* 2003, **326**:1181-1190.