

**Acknowledgments**

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# Where chaperones and nascent polypeptides meet

Véronique Albanèse and Judith Frydman

**Two recent studies provide evidence for a direct interaction between the ribosomal protein L23 at the exit tunnel of the ribosome and the bacterial chaperone trigger factor, and between the eukaryotic L23 homolog and the signal recognition particle. These findings indicate that the exit site of the ribosome may physically link translation to the cytosolic components that guide nascent polypeptides to their correct fate.**

Proteins are synthesized on the ribosome, an organelle-sized ribonucleoprotein complex that transforms the linear genetic code from nucleic acid into polypeptide chain. After synthesis, each polypeptide chain adopts a specific three-dimensional structure and performs a specific function in the cell. The crystal structure of the ribosome<sup>1</sup> has opened the door to understanding the mechanism of this complex machinery at high resolution and how it interacts with other cellular components.

As newly translated polypeptides emerge from the ribosome, they face a formidable task in the crowded environment of the cell — finding the right location in the cell and folding into their correct structure for proper function. While some newly synthesized polypeptide chains fold in the cytosol, others are targeted to intracellular compartments prior to folding. For instance, proteins destined for the secretory apparatus carry an N-terminal signal sequence, which is recognized cotranslationally by the signal recognition particle (SRP) and targets the protein to the endoplasmic reticulum (ER)<sup>2</sup>. Translation is temporarily halted upon engagement of SRP and resumes when the ribosome nascent chain complex docks onto the Sec61 translocon<sup>2</sup>.

Folding of cytosolic proteins is complicated by the vectorial nature and the rela-

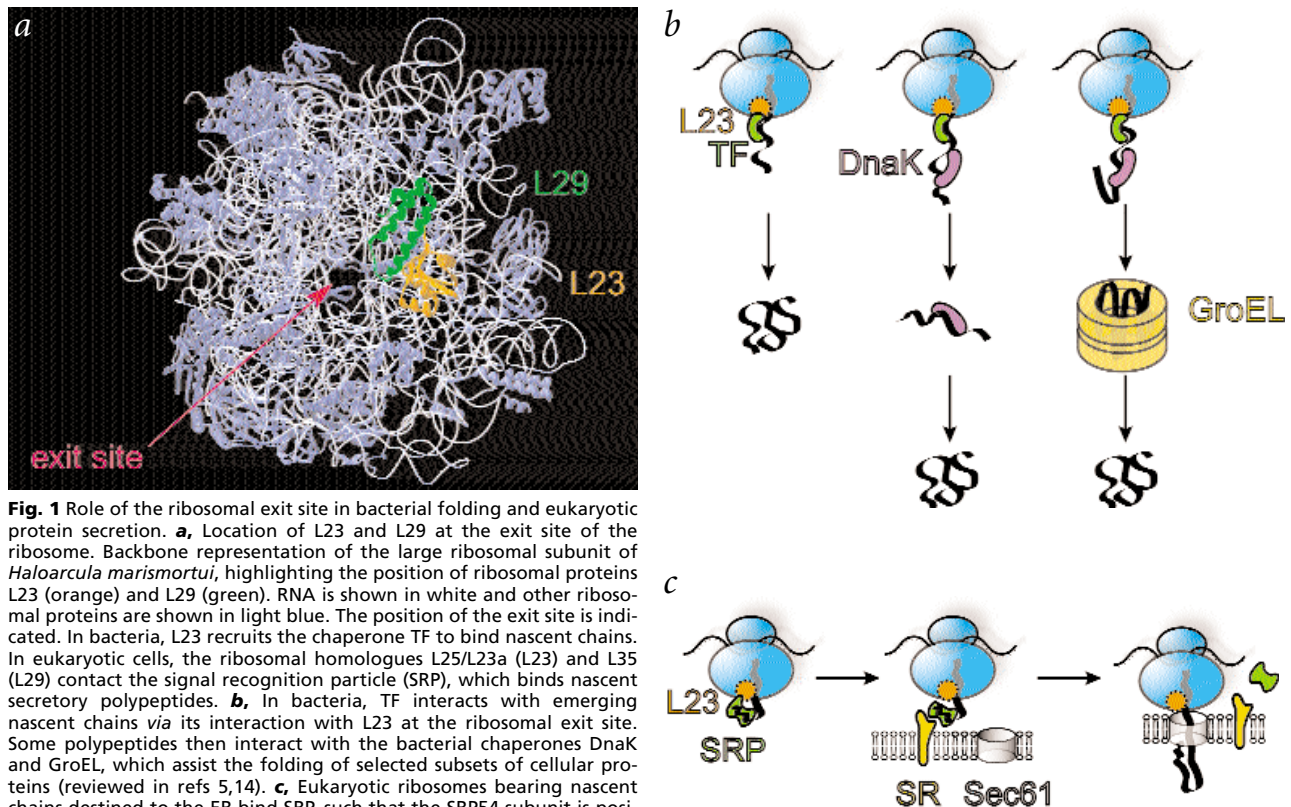
tively slow elongation rate of the translation process. The nascent chain is initially constrained in the 100 Å long ribosomal exit tunnel, which is too narrow (~15 Å) to allow either folding or aggregation to occur<sup>1,3</sup>. However, after emerging from the ribosome, the partially synthesized, aggregation-prone polypeptide is exposed to the crowded cellular milieu<sup>4–6</sup>. These nascent polypeptides interact cotranslationally with several cellular proteins called molecular chaperones, which recognize and bind exposed hydrophobic sequences, thereby preventing aggregation and facilitating folding to the native state<sup>4–6</sup>. Molecular chaperones also bind and stabilize stress-denatured proteins, thus raising the question of how these chaperones are recruited to the ribosome-bound polypeptide. In the simplest model, chaperones could recognize the unfolded polypeptide in a stochastic manner, just as they would recognize any other unfolded protein in the cytosol. Alternatively, folding and translation could be coordinated by the specific recruitment of chaperones to the site of protein synthesis. Indeed, studies in eukaryotic cells provide evidence for the existence of mechanisms that couple translation and chaperone-mediated folding<sup>7,8</sup>. In a recent issue of *Nature*, Kramer *et al.*<sup>9</sup> provide evidence that, in a bacterial system, molecular

chaperones are not only functionally, but also structurally coupled to translation.

The role of chaperones in *de novo* folding is best characterized in *Escherichia coli*. Three bacterial chaperones are thought to participate in the folding of newly translated polypeptides in the cytosol: trigger factor (TF), the Hsp70 DnaK and the chaperonin GroEL (Fig. 1b). TF appears to be the first player in the folding of nascent chains, recognizing relatively short hydrophobic stretches and protecting them from aggregation<sup>10</sup>. DnaK can then bind longer chains and allow larger polypeptides to fold<sup>11,12</sup>. TF and DnaK seem to have partially overlapping functions, and mutants that are defective in the chaperone functions of both proteins exhibit a synthetic lethal phenotype<sup>11,12</sup>. Finally, GroEL functions post-translationally to assist folding of a subset of cytosolic proteins<sup>13</sup>.

What is the mechanism that brings chaperones to bind nascent chains? Because of its early association with translating polypeptides and the presence of a ribosome-targeting domain, TF appeared to be a good candidate to gain insight into the interaction of chaperones with ribosomes. Kramer *et al.*<sup>9</sup> demonstrate that binding of TF to nascent chains is mediated by a direct and specific interaction of this chaperone with the ribosomal protein L23.





**Fig. 1** Role of the ribosomal exit site in bacterial folding and eukaryotic protein secretion. **a**, Location of L23 and L29 at the exit site of the ribosome. Backbone representation of the large ribosomal subunit of *Haloarcula marismortui*, highlighting the position of ribosomal proteins L23 (orange) and L29 (green). RNA is shown in white and other ribosomal proteins are shown in light blue. The position of the exit site is indicated. In bacteria, L23 recruits the chaperone TF to bind nascent chains. In eukaryotic cells, the ribosomal homologues L25/L23a (L23) and L35 (L29) contact the signal recognition particle (SRP), which binds nascent secretory polypeptides. **b**, In bacteria, TF interacts with emerging nascent chains via its interaction with L23 at the ribosomal exit site. Some polypeptides then interact with the bacterial chaperones DnaK and GroEL, which assist the folding of selected subsets of cellular proteins (reviewed in refs 5,14). **c**, Eukaryotic ribosomes bearing nascent chains destined to the ER bind SRP, such that the SRP54 subunit is positioned in the vicinity of the eukaryotic homologue of L23 (L25/L23a). Upon binding to the SRP (SR) receptor in the ER membrane, the complex is rearranged so that the contact with L23a is lost. The ribosome–nascent chain complex is subsequently transferred to the Sec61 translocation channel, which also contacts L23 at the ribosomal exit site<sup>16</sup>.

The authors engineered a TF with a UV-activated crosslinker located in its ribosomal interaction domain. Crosslinking experiments identified two interacting ribosomal proteins, L23 and L29. Using strains of *E. coli* that lack functional forms of these proteins, they show that L23 is necessary for the association of TF with ribosomes. Specifically, this interaction is mediated by two short motifs: Val-Ser-Glu (residues 16–18) in L23 and Phe-Arg-Lys (residues 44–46) in TF. Importantly, they demonstrate that binding of TF to the ribosome is required for its subsequent binding to the nascent chain. Furthermore, using a genetic approach, they show that the TF–L23 interaction is essential for the chaperone function of TF *in vivo*. Notably, the crystal structure of the ribosome places L23 near the exit site where the nascent chain emerges from the ribosome into the cytosol (Fig. 1a). The association of TF with the ribosomal exit site raises interesting questions regarding the dynamic nature of the interaction between TF, the ribosome and the nascent chains. For example, does TF remain associated with the ribosome while scanning the nascent polypeptides or does it escort the nascent chain as it moves

away from the exit site? Future studies should also clarify how newly made polypeptides interact with chaperones downstream of TF, such as DnaK or GroEL.

The finding that the ribosome may play a key role in recruiting the folding machinery has important implications for our understanding of chaperone-mediated *de novo* folding. It remains to be investigated whether a similar mechanism also applies to the cotranslational association of chaperones with nascent chains in eukaryotic cells. The cytosolic folding machinery in eukaryotes appears to be somewhat different and more complex than in prokaryotes<sup>5,6,14</sup>. The eukaryotic cytosol lacks TF homologs, but several other cytosolic chaperones have been shown to associate with translating ribosomes and/or nascent chains, including members of the Hsp70 family and the eukaryotic chaperonin TRiC/CCT<sup>5,6,14</sup>. Although it is unclear how many of these chaperones are directly interacting with ribosomes, another recent study also implicates the exit site of the eukaryotic ribosome as the physical contact point for nascent chain-binding components. In a recent issue of *Science*, Pool *et al.*<sup>15</sup> show that the SRP54 subunit in SRP,

which associates with the signal sequence of the nascent chain destined for the ER, is positioned in close proximity to the eukaryotic homologs of L23 and its neighboring proteins L29, L25/L23a and L35 in the ribosome (Fig. 1c). The engagement of the ribosome–nascent chain–SRP complex with the SRP receptor in the ER membrane alters the interaction between the ribosomal proteins and SRP54, presumably to facilitate transfer of the ribosome–nascent chain complex to the Sec61 translocation channel. Strikingly, the Sec61 translocon also contacts the L23 and L29 homologs at the ribosomal exit site<sup>16</sup>.

The studies of Kramer *et al.*<sup>9</sup> and Pool *et al.*<sup>15</sup> raise the exciting possibility that ribosomal proteins—and perhaps even ribosomal RNA—could be key players in linking translation and folding. Instead of simply delivering polypeptides into the cytosol, the ribosome appears to actively participate in determining the subsequent fate of the translated polypeptide. Although it is tempting to speculate that the ribosomal exit site provides a scaffold that recruits all the proteins needed to regulate the early interactions involving the nascent chains, it is not clear how these different proteins in

such a scaffold could bind simultaneously without steric conflict. Even more intriguing is the possibility that the ribosome itself may 'sense' the nascent chain before it exits the ribosomal tunnel and thus recruit the appropriate proteins at the right time to take care of the nascent chain<sup>17,18</sup>.

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picture story

The origin of silence

At eukaryotic telomeres and centromeres, DNA is condensed into a transcriptionally silent structure known as heterochromatin. Here, the lack of gene expression can 'spread' randomly into nearby active DNA and, as it can be inherited from generation to generation, may give rise to epigenetic effects whereby individuals with the same genes display different phenotypes. The yeast *Saccharomyces cerevisiae* also packages its chromosomal mating loci into heterochromatin to maintain mating type, but here silencing is maintained unambiguously. In a recent paper, Zhang et al. (*EMBO J.* **21**, 4600-4611; 2002) studied the structure and function of the yeast Orc1 protein, a subunit of the origin recognition complex (ORC), revealing a mechanism by which the silencing machinery can be recruited to mating loci.

In *S. cerevisiae*, each of the two chromosomal loci, dubbed HML and HMR, contain silencing elements bound by the replication complex ORC. Four silent information regulator (ySir) proteins are also needed to silence these chromosomal regions; ySir2p is an NAD-dependent histone deacetylase, while ySir3p and ySir4p bind to histone tails lacking acetyl groups. Efficient silencing may thus involve recruitment of ySir2p and histone deacetylation, which lowers gene expression and nucleates a ySir3p/ySir4p repressive structure. Similar processes underlie silencing at telomeres.

But how is it that silencing contained at mating loci, while it spreads at regions bordering telomeres? ORC may provide a clue, as the yeast Orc1 (yOrc1) protein subunit can bind directly to ySir1p via its N-terminal domain (NTD). Recruitment

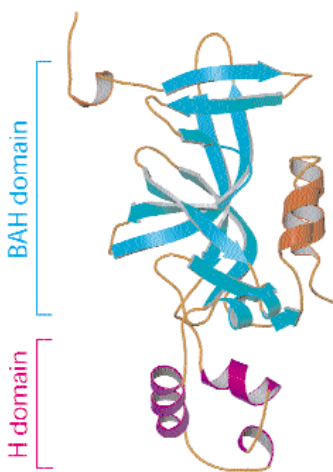


Figure courtesy of R.-M. Xu. Modified with permission from Zhang et al.

of ySir1p to the HML and HMR silencer elements via the ORC complex could thus bring about binding of the remaining Sir proteins and strengthen their association with the mating loci.

Zhang et al. found two domains in the structure of the conserved yOrc1p NTD: a bromodomain-adjacent homology (BAH) domain comprised mostly of β-strands, and a smaller helical H domain (left). The core BAH domain is relatively common based on sequence alignment, while the H domain is found in yOrc1p and ySir3p as well as in metazoan counterparts of Orc1. The authors found the yOrc1 H domain to be necessary for silencing (right, wild type, pink). Replacing the yeast Orc1 H domain with that from the human protein, which does not bind ySir1p well, abolishes silencing (right, ΔH domain, white). Chromatin immuno-precipitation experiments were used to probe ySir proteins distribution on the mating loci, and removing the yOrc1 H domain was found

to reduce recruitment of ySir2p, ySir3p and ySir4p, as well as ySir1p. At telomeres, however, deletion of the H domain in yOrc1p did not affect Sir protein recruitment, consistent with exclusive function at mating loci.

The yOrc1p H domain is thus necessary to recruit all Sir proteins to mating loci. Similar interactions between ORC1 and silencing components may occur in other metazoans: in *Drosophila melanogaster*, for example, Orc1 interacts with the chromatin modifier HP1. Zhang et al. were also able to pinpoint mutations in the BAH domain that suppress the silencing phenotype of histone mutants, offering insight into additional functions of this motif. The roles of BAH domains in DNA methyltransferases, histone deacetylases and ATP-dependent chromatin remodeling factors remain to be established, but are likely to reveal interesting links between nuclear processes.

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