You Got to Know When to Hold (or Unfold) 'Em…

Daniel N. Hebert,[1,3,*] Kshama D. Chandrasekhar,[1] and Lila M. Gierasch[1,2,3,*]
[1]Department of Biochemistry and Molecular Biology
[2]Department of Chemistry
[3]Program in Molecular and Cellular Biology
University of Massachusetts, Amherst, Amherst, MA 01003, USA
*Correspondence: dhebert@biochem.umass.edu (D.N.H.), gierasch@biochem.umass.edu (L.M.G.)
http://dx.doi.org/10.1016/j.molcel.2012.09.022

In this issue of *Molecular Cell*, Hoffmann et al. (2012) demonstrate that the ribosome-associated bacterial chaperone Trigger Factor assists in the maturation of ribosome-attached nascent chains by acting as both a holdase and an unfoldase.

As a protein emerges from the ribosome, it may begin exploring its folding landscape contemporaneously with translation. Thus, in-cell folding can be vectorial, with the N-terminal portion of a newly synthesized chain folding while the C-terminal portion is still being synthesized. Many factors, including chaperones and processing enzymes, associate with the ribosome to form a "greeting committee" with privileged access to the nascent chain, leading researchers to ask what their impact is on its folding. Do they simply delay folding of the N-terminal segments until later parts of the chain emerge? Or do they actively facilitate folding, by either promoting the formation of native structure or undoing improperly folded structures? Trigger Factor (TF) is an abundant folding factor of the bacterial cytoplasm that is known to bind ribosomes at a site adjacent to the polypeptide exit site, perfectly positioning it for early intervention, and indeed, earlier work showed that it shields the emerging polypeptide, measured by protection from proteolysis (Hoffmann et al., 2006). In this issue, Hoffmann et al. (2012) demonstrate that TF plays a direct role in the folding of nascent chains: TF delays conformational exploration of ribosome-attached nascent chains. And more strikingly, TF was found to facilitate unfolding of pre-established folded structures, suggesting that it plays a dual role both in confinement of the maturing protein and in reversing folding miscues to enable the nascent chain to more efficiently find the native fold.

TF was first identified in 1987 by Bill Wickner and coworkers based on its ability to maintain the *Escherichia coli* protein OmpA in a state that was competent for export and proper integration into the outer membrane; TF at that time was thought to be specific for secreted proteins (Crooke and Wickner, 1987). In the ensuing years, work from several laboratories has revealed that the role of TF is more general: it is now understood to be a key ribosome-associated chaperone and an integral member of the nascent chain greeting committee (Hoffmann et al., 2010). The structure of TF is compatible with either a "holdase" function, i.e., delaying folding to ensure that critical sequence determinants have emerged from the ribosome, or an "unfoldase" function, actively unfolding the polypeptide to ensure that it has the best chance of exploring conformational space and reaching its native state. Intriguingly, this chaperone has no ATP dependence and therefore cannot expend ATP-derived energy to perform its job. TF is comprised of three domains, an N-terminal domain that harbors a ribosome-binding motif, a peptidyl-prolyl isomerase (PPIase) domain, and a C-terminal domain (physically located between the other two domains) (Ferbitz et al., 2004). The fact that the N- and C-terminal domains present significant hydrophobic surface on the interior of a concave cavity, and the ability of the PPIase domain to catalyze peptide-bond isomerization, lends credence to a central role for TF in facilitating folding.

A variety of cleverly chosen model folding substrates including fast (seconds) as well as slow (minutes) folders were employed by Hoffmann et al. (2012) to monitor the influences of TF on protein folding. A SecM translation-arrest sequence was used to trap nascent chain-ribosome complexes and thus simulate cotranslational processes, enabling the authors to monitor the influence of TF on the maturation and conformational freedom of ribosome-arrested chains. One of the methods employed to monitor folding was the formation of disulfide bonds. This was possible in a bacterial in vitro translation system by creating an artificial oxidizing environment, which enabled the covalent trapping of conformations via intramolecular disulfides between Cys residues close in space. Modifying free thiol disappearance by PEGylation allowed direct observation of the rate of disulfide formation (Lu and Deutsch, 2005). The acquisition of protease resistance and enzymatic activity was also used to follow the folding of the model substrates and characterize the impact of TF on the folding process.

Hoffmann et al. (2012) observed that the conformational freedom of a nascent chain was limited when it was proximal to the ribosome. The ribosome directly affected conformational dynamics for regions up to 100 amino acids in length (Figure 1). These observations are generally consistent with recent single-molecule (Kaiser et al., 2011) and computational modeling (O'Brien et al., 2011) studies.

The impact of TF on the nascent chain appears to start where the ribosome's influence leaves off, i.e., when the nascent chain is greater than ~100 amino acids in length (Figure 1). Not only was TF binding able to retard the folding process by acting as a holdase, but it also displayed unfolding activity capable of reverting

aberrant conformations. The ability of TF to unfold a substrate required that TF be able to bind the ribosome, presumably to enhance its affinity for the nascent chain and to provide early substrate access, as a TF mutant in which the ribosome-binding motif was mutated was defective in unfoldase activity.

What is the impact of TF action on the folding landscape of a nascent chain? By presenting a strongly interacting large binding surface, TF binding competes with intramolecular interactions. When the stability of the intramolecular glue is lower than the binding energy for interaction with TF, the polypeptide will unfold. Hoffmann et al. (2012) show that TF binding is not so strong as to destabilize a highly stable native state, such as that formed by dihydrofolate reductase bound to methotrexate or stabilized by lowering temperature, or a barnase mutant harboring stabilizing disulfides. The picture that emerges is that TF acts to unfold protein domains while a nascent chain is tethered to the ribosome, and that the impact of this function is to allow chains to continue sampling all possible conformations until the polypeptide has been fully synthesized.

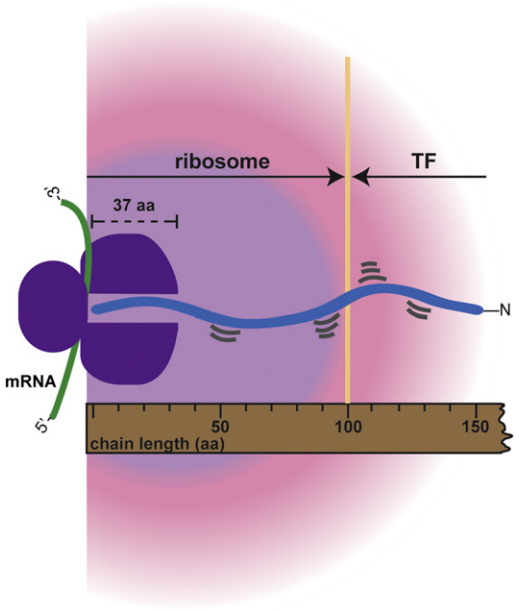

Figure 1. The Sphere of Influence of the Ribosome and TF on the Nascent Chain

The translating ribosome (dark purple structure) influences the conformational freedom of the nascent polypeptide (blue sinuous line) up to 100 amino acids in length from the ribosomal P site (light purple shading). Once a nascent chain reaches a length of greater than 100 amino acids, TF can hold or unfold it (pink shading). The ruler denotes amino acid chain length.

The elegant work of Hoffmann et al. (2012) nonetheless leaves many open questions about TF. Using this array of model substrates, the holdase and foldase functions of TF were preserved when the PPIase domain was removed. Previous work had clearly placed the TF PPIase domain in the FKBP (FK506-binding protein) family (Stoller et al., 1995). What is the true role of the TF PPIase domain in the biosynthesis of endogenous substrates? The fact that Hoffmann et al. (2012) relied on stalled, ribosome-attached nascent chains and an in vitro transcription/translation system alters the sequence of the client protein and the dynamics of its appearance from the ribosome. It will be important to validate their findings in systems that lack these unnatural conditions and use endogenous *E. coli* proteins. A very striking observation from this and the complementary study by Oh et al. (2011), which used ribosome profiling, was that TF action required a chain length of ~100 amino acids (~35 within the ribosome tunnel, and 65 outside). What is happening to the nascent polypeptide chain before this length? What is the hierarchy of greeting committee roles in shaping the folding landscape of the nascent chain?

Lastly, it will be of great interest to understand what factors play comparable roles in the folding of nascent eukaryotic protein chains. The prevalence of multidomain proteins in the proteomes of eukaryotes makes this question all the more pressing. There are suggestions throughout the literature that the nascent chain binding proteins (i.e., nascent chain-associated complex or NAC) are functionally analogous to TF (del Alamo et al., 2011). Yet no direct data have yet established this. We are eager to learn how general the roles of greeting committees for newly synthesized proteins are, and how common the features of the energy landscapes for cotranslational folding are.

REFERENCES

Crooke, E., and Wickner, W. (1987). Proc. Natl. Acad. Sci. USA *84*, 5216–5220.

del Alamo, M., Hogan, D.J., Pechmann, S., Albanese, V., Brown, P.O., and Frydman, J. (2011). PLoS Biol. *9*, e1001100. http://dx.doi.org/10.1371/journal.pbio.1001100.

Ferbitz, L., Maier, T., Patzelt, H., Bukau, B., Deuerling, E., and Ban, N. (2004). Nature *431*, 590–596.

Hoffmann, A., Merz, F., Rutkowska, A., Zachmann-Brand, B., Deuerling, E., and Bukau, B. (2006). J. Biol. Chem. *281*, 6539–6545.

Hoffmann, A., Bukau, B., and Kramer, G. (2010). Biochim. Biophys. Acta *1803*, 650–661.

Hoffmann, A., Becker, A.H., Zachmann-Brand, B., Deuerling, E., Bukau, B., and Kramer, G. (2012). Mol. Cell *48*, this issue, 63–74.

Kaiser, C.M., Goldman, D.H., Chodera, J.D., Tinoco, I., Jr., and Bustamante, C. (2011). Science *334*, 1723–1727.

Lu, J., and Deutsch, C. (2005). Nat. Struct. Mol. Biol. *12*, 1123–1129.

O'Brien, E.P., Christodoulou, J., Vendruscolo, M., and Dobson, C.M. (2011). J. Am. Chem. Soc. *133*, 513–526.

Oh, E., Becker, A.H., Sandikci, A., Huber, D., Chaba, R., Gloge, F., Nichols, R.J., Typas, A., Gross, C.A., Kramer, G., et al. (2011). Cell *147*, 1295–1308.

Stoller, G., Rücknagel, K.P., Nierhaus, K.H., Schmid, F.X., Fischer, G., and Rahfeld, J.U. (1995). EMBO J. *14*, 4939–4948.