Proteins must acquire and maintain a specific fold to execute their biochemical function(s). In solution, unfolded proteins typically find this native structure through a biased sampling of preferred intermediate conformations. However, the initial search for these structures begins during protein synthesis, and it is unclear how much interactions between the ribosome and nascent polypeptide skew folding pathways. In this issue, Jensen and colleagues use a ribosomal force-profiling assay to show that RNase H forms a similar folding intermediate on and off the ribosome. In conjunction with measurements of the rate of RNase H unfolding on and off the ribosome, their results show that ribosomal interactions have little impact on the folding pathway of RNase H. These findings suggest that the ribosome itself does not necessarily rewire protein folding reactions.

Although proteins sample a diverse array of conformations throughout their lifetime, most are energetically wired to seek their native conformational ensemble. The question of how proteins acquire these select few favorable conformations in such short order has remained a fundamental biochemical curiosity for many decades (1). Investigations of the folding and unfolding of purified proteins in solution have generally revealed that, to hasten their conformational search, unfolded proteins are funneled through a select set of preferred intermediate states on their way to the native conformation. However, far less is known about how protein folding plays out in the cell. Proteins begin their conformational search as the nascent polypeptide emerges from the ribosomal exit tunnel and begins to explore the cellular milieu, which is full of other biomolecules. Nevertheless, the extent to which interactions between the nascent chain, ribosome, and various other components of the proteostasis network distort energy landscapes and bias protein folding and misfolding trajectories is unclear. Emerging insights into these matters are sure to shed new light on the constraints of protein evolution and the molecular basis of disease.

Our current lack of insight into the mechanisms of cotranslational folding can be largely attributed to a dearth of suitable methodology. Most of the advanced spectroscopic techniques used to track the formation of protein structure in solution are incompatible with efforts to probe folding on the ribosome. In this issue, Jensen et al. (2) circumvent this limitation by using two emerging nonspectroscopic techniques to probe the cotranslational folding and unfolding of Escherichia coli RNase H. In solution, RNase H is known to form a series of folding intermediates that lead to the progressive stabilization of a mixed α/β core intermediate known as Icore (Fig. 1)(3, 4). On the ribosome, RNase H exhibits a slightly diminished thermodynamic preference for its native fold (5), although it was unclear how interactions between the nascent chain and ribosome impact kinetic folding pathways. Given the sizable body of prior knowledge on its folding mechanism, RNase H represents an excellent model system for comparative studies of protein folding pathways on and off the ribosome.

To investigate the impact of the ribosome on RNase H folding, the authors fused a G/S linker and a Sec M arrest peptide to the C terminus of RNase H, which stalls the ribosome at the point in which the protein emerges from the ribosomal exit tunnel. Using this stalled ribosome-nascent chain complex, the authors were able to compare the rate of RNase H unfolding on and off the ribosome using pulse proteolysis, which is a proteolytic technique to measure the fraction of folded protein (6, 7). Their results demonstrate that the rate of RNase H unfolding increases 10-fold on the ribosome. Interestingly, the magnitude of this increase completely accounts for the effect of the ribosome on the folding equilibrium of RNase H, which implies that the rate of folding is unchanged on the ribosome (Fig. 1). Moreover, the ribosome appears to have little impact on the dependence of the unfolding rate on the concentration of urea (kinetic m-value), which suggests that the ribosome does not alter the relative position of the rate-limiting conformational transition on the reaction coordinate (Fig. 1).

Although these findings alone cannot rule out the formation of unique cotranslational folding intermediates, they suggest that RNase H may take the same path(s) to the native state both on and off the ribosome. To explore this possibility further, the authors turned to an arrest peptide–based force-profiling assay. This assay reports the efficiency of ribosomal readthrough at the stall sequence, which is known to be stimulated by the mechanical tension generated by cotranslational transitions in the nascent chain (8, 9). Using a panel of mutants known to alter the stability of Icore (10), the authors show that readthrough and the corresponding tension on the ribosome are correlated with the propensity of RNase H to form this folding intermediate in solution. This observation provides experimental evidence suggesting that RNase H forms similar folding intermediates on and off the ribosome, an additional indication that the ribosome has limited impact on its folding pathway.

There are many lines of evidence suggesting that the tunability of translation kinetics enhances the fidelity protein folding in the cell, which implies that the translation machinery may manipulate folding pathways. Nevertheless, this work represents one of the relatively few experimental investigations of the direct impact of the ribosome on the conformational energy
landscape of the nascent polypeptide chain. It is perhaps surprising that the ribosome has so little impact on this folding reaction, considering that the weak interactions between the ribosome and nascent chain likely reshape the ensemble of unfolded structures that is accessible during translation. Nevertheless, these observations show that the ribosome need not alter the energetic barriers to folding—at least not at the late stages of synthesis, where most of the nascent chain has emerged from the exit tunnel. These results also show that the earliest paths to the native structure are likely to feature familiar intermediate structures. The ribosome therefore appears to avoid preferential interactions with nascent chain intermediates that may skew its energetic landscape, which is perhaps fortuitous given the diverse range of products it synthesizes.

The ribosome’s ability to avoid tangling with disordered nascent chains likely minimizes the potential for arbitrary polypeptide sequences to antagonize protein synthesis. It should also be noted that the retention of a single, efficient folding pathway could potentially promote protein evolvability. If energy landscapes were radically different on and off the ribosome, it may limit the number of sequences that are capable of both folding efficiently during translation and maintaining the native conformation after synthesis is complete. Nevertheless, establishing the generality of these findings will require further exploration of how cotranslational folding mechanisms vary across the proteome.

Conflict of interest—The author declares that he has no conflicts of interest with the contents of this article.

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