HOW RIBOSOMES SHAPE PROTEIN FOLDING

CASEY MESKOVICH
Department of Chemical Engineering, University of Illinois Urbana-Champaign

ABSTRACT
Protein folding is a fundamental biological process essential to cellular function, yet its mechanisms (particularly those occurring during synthesis) remain incompletely understood. Traditionally viewed as a post-translational event, mounting evidence now reveals that protein folding often begins co-translationally, with the ribosome playing a direct and active role. This review examines how ribosomes contribute to co-translational folding by providing a confined, highly regulated environment within the exit tunnel that shapes early folding events, guides hydrophobic collapse, and coordinates folding with translation speed. Structural and thermodynamic studies reveal that the ribosome minimizes entropic penalties and promotes stable intermediate states, thus enhancing folding efficiency and fidelity. Furthermore, ribosome-associated chaperones assist in preventing misfolding and aggregation. These insights not only reshape our understanding of the central dogma of protein science but also have profound implications for disease research, including cancer and neurodegenerative disorders, and offer new targets for therapeutic intervention aimed at regulating proteostasis during translation.

THE PROBLEM

Proper protein folding is crucial for cellular function, and errors in this process are linked to a variety of debilitating diseases, including neurodegenerative disorders like Alzheimer's and certain types of cancer. Despite this, the process by which proteins fold into their functional structures remains one of the most complex and poorly understood aspects of cellular biology.

Although significant advances have been made in predicting the final three-dimensional shapes of proteins from their amino acid sequences, the mechanism by which these shapes form during protein synthesis remains largely elusive. Once unfolded, many proteins fail to refold easily, tend to misfold and aggregate, and require the help of chaperones to attain their correct structure (Samatova, Komar, & Rodnina, 2023). This directly negates the central dogma of protein science, which is that the amino acid sequence of the protein defines its three-dimensional structure (Nature Publishing Group, 2024). This indicates that most proteins do not fold into their active forms freely in solution; instead, their folding begins while they are still being synthesized by the ribosome—the cellular machinery responsible for protein translation (Samatova et al., 2023).

AN INTRODUCTION TO RIBOSOMES AND PROTEIN SYNTHESIS

To understand how ribosomes influence protein folding, it's important to first grasp their role in protein synthesis. A ribosome is a cellular organelle found in the cytoplasm or bound to the endoplasmic reticulum and is primarily responsible for protein synthesis. Because of their essential role, cells contain a large number of ribosomes. The exact number varies depending on the cell type and its level of protein synthesis activity (Nature Publishing Group). For instance, a single mammalian cell can house up to ten million ribosomes (British Society for Cell Biology).

A ribosome is made up of two subunits, deemed "large" and "small." The large subunit is about twice the size of the small subunit and functions mainly as a catalyst, whereas the small subunit is typically a decoder (British Society for Cell Biology). The small subunit binds to messenger RNA (mRNA) and reads its nucleotide sequence, ensuring that the information is translated correctly. Once the small subunit attaches to the mRNA, the large subunit then locks onto the small subunit, forming a functional ribosome complex. This assembly creates a scaffold where the process of translation takes place (Genome.Gov).

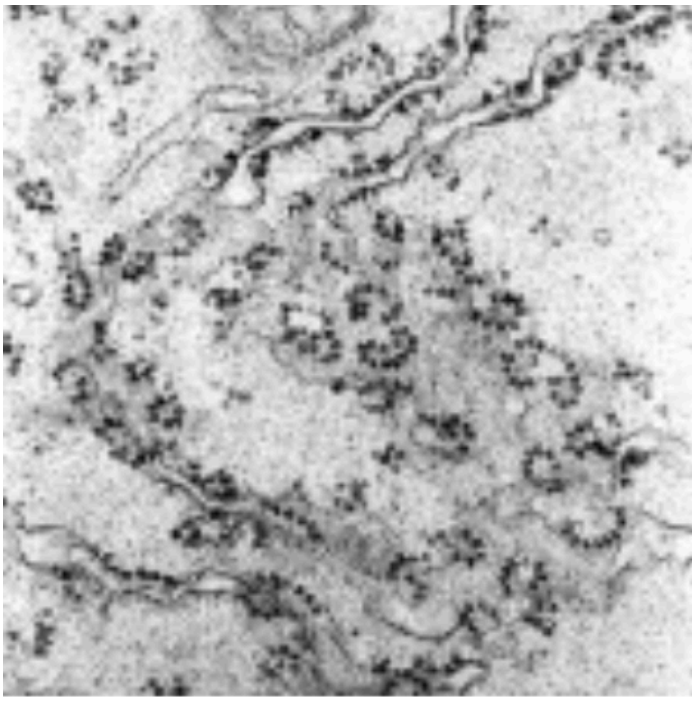

Figure 1: An electron microscope image showing part of the rough endoplasmic reticulum in a plant root cell from maize, in which the dark spots are ribosomes (British Society for Cell Biology)

Translation begins when the ribosome encounters the start codon of an mRNA strand. As the ribosome moves along the mRNA, transfer RNA (tRNA) molecules enter the ribosome, bringing along specific amino acids. The large subunit catalyzes the formation of peptide bonds between the amino acids, elongating the polypeptide chain. The ribosome continues reading the mRNA, adding one amino acid at a time until it reaches a stop codon, at which point translation halts, and the newly synthesized polypeptide is released (British Society for Cell Biology).

THE ROLE OF RIBOSOMES IN CO-TRANSLATIONAL FOLDING

While ribosomes are most commonly known for their primary function in protein synthesis, recent studies have shown that they also play a crucial and active role in the folding of nascent

proteins (proteins that have been synthesized, but have not yet folded into their final shape). This process, known as co-translational folding, refers to the folding of a protein as it is being synthesized by the ribosome. Unlike post-translational folding, where proteins fold after they are completely synthesized, co-translational folding happens simultaneously with translation. This allows the nascent polypeptide chain to begin adopting its functional three-dimensional structure while it is still attached to the ribosome (Samatova et al., 2023).

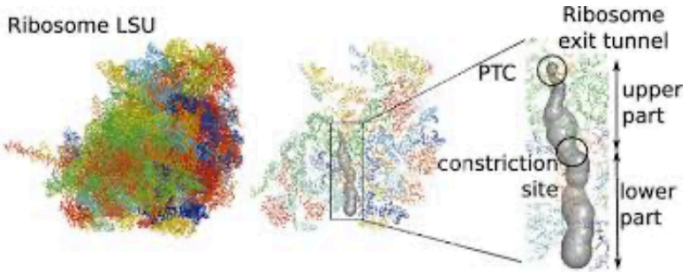

Figure 2: A ribosome, showing the ribosome exit tunnel (Duc, Batra, Bhattacharya, Cate, & Song, 2019)

In co-translational folding, the ribosome provides a highly structured environment that promotes proper folding as the polypeptide chain emerges. Proteins begin to form secondary structures inside the exit tunnel of the ribosome. The tunnel is about 100 Å long and 10–30 Å wide (Samatova et al., 2023). The size of the tunnel restricts the protein's ability to fold prematurely or adopt incorrect structures. This tunnel, which is only large enough to accommodate a few amino acids at a time, ensures that the folding process is gradual and controlled. Furthermore, this confinement helps facilitate the hydrophobic collapse—a step in the protein folding process where hydrophobic (water-repelling) amino acid residues come together in the protein's interior to form a compact core (Rich, 2007). The ribosome's tunnel, by limiting the available space, may guide this process, helping to expose hydrophobic regions at the right moment while preventing their exposure to the aqueous cellular environment.

The tight confines of the ribosomal exit tunnel also serve to coordinate the folding of the nascent polypeptide with the rate of translation. The speed at which the ribosome moves along the mRNA dictates the time scale over which the protein emerges and begins to fold (Samatova et al., 2023). As the polypeptide extends, it interacts with the tunnel walls, which helps control the rate of compaction. This coordination ensures that folding begins in a spatially constrained manner, preventing the protein from adopting non-native structures before sufficient sequence information has been synthesized.

Moreover, the ribosome-associated chaperones (proteins that assist in protein folding) often interact with the nascent protein as it exits the tunnel (Samatova et al., 2023). These chaperones bind to specific regions of the protein, stabilizing it as it folds into its correct configuration. They can help to prevent improper folding or aggregation by stabilizing the partially folded intermediates that form within the exit tunnel. This combination of spatial restriction and chaperone assistance provides a powerful mechanism for ensuring that proteins fold correctly and efficiently while still attached to the ribosome.

Another critical aspect of the exit tunnel is its ability to adapt dynamically to the needs of the protein being synthesized. Recent studies from Samatova et al. have suggested that the shape and size of the exit tunnel may vary depending on the specific protein being synthesized. For example, larger, more complex proteins might encounter different structural features of the tunnel compared to smaller proteins, which could influence how they fold.

This adaptability suggests that the ribosome provides a responsive folding environment. It not only offers a spatially constrained space but also adjusts to accommodate the specific folding requirements of different nascent proteins (Samatova et al., 2023). In some cases, the ribosome itself might actively contribute to the folding process by making specific structural adjustments or by modulating the translation rate in response to the protein's folding needs. For instance, if the emerging protein requires more time to adopt a critical fold, the ribosome may slow its translation rate, allowing the protein to fold more effectively within the confines of the exit tunnel.

THE THERMODYNAMICS OF CO-TRANSLATIONAL FOLDING

Recent experiments using nuclear magnetic resonance (NMR) spectroscopy and simulations of ribosome-nascent chain complexes (RNCs) have provided new insights into how the ribosome influences protein folding on a molecular level. In multiple studies within the Nature Publishing Group, researchers purified ribosome-nascent chain complexes and investigated the structural dynamics of the emerging polypeptide using high-resolution techniques. By measuring hundreds of interatomic distances and combining this data with simulations of millions of atoms, they were able to construct experimentally derived models of the nascent chain at different stages of synthesis.

One key observation from these studies is that the unfolded nascent polypeptide on the ribosome is significantly more solvated (i.e., surrounded by water molecules) and structurally expanded than an isolated polypeptide in solution. The ribosome provides a confined space that affects the hydration state of the nascent chain: water molecules bound to the protein have lower entropy (a lower degree of disorder) compared to free water molecules. This reduction in entropy makes the solvated nascent polypeptide more ordered, which in turn reduces the entropy of the entire ribosome-nascent chain complex.

This greater order in the solvated nascent chain on the ribosome contrasts with the higher entropy of freely solvated polypeptides, which are typically more disordered in solution (Nature Publishing Group, 2024). The entropic penalty of folding—a process that reduces the degree of disorder in the protein chain—is typically discouraging when folding occurs in free solution.

However, on the ribosome, the entropic cost of folding is significantly reduced. Since the unfolded nascent chain is already more ordered due to solvation effects in the confined ribosomal environment, the transition to folded intermediates is less entropically costly. This makes co-translational folding (coTF) energetically more favorable, pushing the nascent polypeptide toward stable, soluble, and partially structured conformations even before translation is completed.

As the nascent chain emerges from the ribosome, the protein enters a series of partially folded intermediate states, which are stabilized by the ribosomal environment and its associated factors. These intermediates are a hallmark of co-translational folding, a process in which the protein adopts its functional conformation through a series of sequential steps during translation. The ribosome plays an essential role in shaping the folding process by stabilizing these intermediates and guiding the nascent protein along a defined folding pathway.

In contrast to folding in isolation, where intermediates tend to form transiently and are less stable, co-translational folding promotes the formation of stable intermediates that are thermodynamically more favorable. These co-translational intermediates are typically less prone to misfolding and aggregation, as they are formed in the more controlled, confined space provided by the ribosome (Nature Publishing Group, 2024). As the nascent chain lengthens, the intermediates progressively convert into the fully folded, active structure of the protein.

The ability of the ribosome to stabilize these partially folded states is crucial for efficient protein synthesis. The ribosome's exit tunnel, by constraining the nascent chain, ensures that the polypeptide is not able to fold indiscriminately or prematurely into an energetically unfavorable conformation. Instead, folding occurs sequentially and in a more controlled manner, with each elongation step allowing the protein to move along a defined folding pathway toward its final, active form (Nature Publishing Group, 2024).

The unique structural and solvation environment within the ribosomal exit tunnel also influences the thermodynamics of protein folding. The entropic penalty of folding is lower in the ribosome-bound state compared to isolated polypeptides in free solution, which is a critical factor in promoting the formation of co-translational folding intermediates. This results in a more favorable folding process overall, as proteins can fold in a more efficient and controlled manner while they are still being

synthesized (Samatova et al., 2023).

Interestingly, this finding challenges previous assumptions about how folding on the ribosome works. Contrary to the idea that the ribosome might simply provide a "scaffolding" for protein synthesis, recent evidence suggests that the loss of entropy associated with the solvated unfolded polypeptide on the ribosome is a key mechanism by which the ribosome actively stabilizes partially folded intermediates. These intermediates, which form earlier in the synthesis process, eventually lead to the protein adopting its final functional conformation. This thermodynamic shift pushes the protein towards folding at an earlier stage, aiding in the overall efficiency of protein synthesis.

IMPLICATIONS

The understanding of ribosome-mediated protein folding has broad implications for both basic and applied biological research. First, it underscores the efficiency of protein synthesis within cells. By guiding proteins through partially folded intermediates as they are synthesized, the ribosome reduces the chances of misfolding and aggregates forming, ensuring proteins achieve their correct structure more efficiently. This is particularly important for cells with high protein production demands, such as neurons and muscle cells, where rapid and accurate protein folding is crucial for cellular function.

In addition, aberrant protein folding and translation processes are increasingly recognized as factors in cancer development. Cancer cells often exhibit elevated levels of protein synthesis and rely heavily on chaperone systems to maintain proteostasis under stressful conditions. Disruptions in ribosome-mediated folding can contribute to oncogenesis by allowing the accumulation of dysfunctional proteins that promote uncontrolled growth. Targeting the ribosome's role in protein folding could therefore offer novel strategies for cancer therapy, either by selectively impairing the folding of oncogenic proteins or by exploiting vulnerabilities in the cancer cell's heightened dependence on proteostasis mechanisms.

Finally, the ribosome's influence on protein folding also opens new avenues for drug development. By targeting the ribosome's exit tunnel or associated chaperones, researchers could develop therapies that optimize protein folding during translation, potentially preventing misfolding and improving the efficiency of drug discovery efforts related to protein-based diseases. This could lead to the creation of small molecules or biologics that specifically assist in the proper folding of disease-related proteins.

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