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PERSPECTIVE

From Alpha to Beta – a co-translational way to fold?

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ABSTRACT

Protein folding in the cell is largely a co-translational process occurring during protein synthesis on the ribosome. It has become evident that co-translational folding is characteristic to almost every protein in the cell of pro- and eukaryotic origin that are single and multidomain, single and multisubunit, cytosolic, secretory and membrane. Co-translational protein folding begins very early during the process of polypeptide chain synthesis on the ribosome, with some secondary structure elements forming inside the ribosomal tunnel and some tertiary structures forming inside the vestibule (lower/wider) region of the ribosomal exit tunnel. However, many details of co-translational folding remains incompletely understood. New data show that folding of a β-barrel protein begins with formation of an α-helix inside the ribosome that rearranges into a β-hairpin structure as the growing peptide reaches the wider/vestibule region of the exit tunnel. While it was previously suggested that such scenario can take place on the ribosome, the new data provide the first experimental evidence in support of this notion.

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Folding *in vivo* vs folding *in vitro* in a test tube

A major area of progress in the field of protein folding in recent years resulted from a shift from considering and investigating protein folding *in vitro*/in solution to a comprehensive analysis of protein folding *in vivo*/on the ribosome. This shift came with the realization that *in vivo* protein folding is a co-translational process and that protein synthesis rates may be evolutionarily tuned to optimize protein folding, thus allowing proteins to circumvent deep kinetic traps during the folding on the ribosome, therefore helping to avoid misfolding and aggregation [1–4]. However, many details of the co-translational folding process remain only partially understood and both the mechanism and the pathway of co-translational folding remain subjects of intense studies and debates these days [1–4].

What new study tells us about the key differences between in vivo *and* in vitro *protein folding processes?*

New data published by Agirrezabala *et al* [5] provide important insights and into the mechanism of co-translational folding of the small, single-domain, β-barrel *Escherichia coli* protein, the cold shock protein A (CspA). CspA is comprised of 70 amino acids (aa) that fold into five β-strands connected by loops. In solution (*in vitro*), CspA, folds very rapidly (time constant, τ = 4 msec) by an apparent two-state mechanism, involving only native and denatured states [6, 7]. The study of such small proteins like CspA with simple folding trajectories has led to the considerable progress in understanding the early events of protein folding in solution as well as understanding of the general relationships between protein topology and folding kinetics. However, the folding trajectory of the CspA on the ribosome was not known. Obviously, CspA wouldn't be able to fold at the same rate on the ribosome, given the protein synthesis rates in *E. coli* of about 10–20 amino acids per second.

To monitor the timing of folding of CspA during ongoing translation Agirrezabala *et al* used the reconstituted rapid *in vitro* translation system, force profile analysis (FPA) and a combination of

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photoinduced electron transfer (PET) and PET with fluorescence correlation spectroscopy (FCS) (PET-FCS) approaches [5]. Furthermore, the authors utilized the cryo-electron microscopy (cryo-EM) analysis of the ribosome bound nascent chains. As such they were able to provide comprehensive readout of the *in vivo* folding process and detailed snapshots of CspA nascent chains conformations during the course of protein synthesis on the ribosome.

Importantly, the authors demonstrated that a β-barrel protein CspA starts to fold as α-helix inside the ribosomal exit tunnel. CspA nascent polypeptide chain was found then to undergo a conformational switch from an α-helical to a β-stranded conformation (Figure 1).

This example clearly highlights the differences between *in vitro* and *in vivo* folding pathways and illustrates how the co-translational nature of the protein folding on the ribosome alters the folding trajectory of a protein from a rapid two-state pathway to a complex translation-dependent landscape. This study further supports the view that translation is not merely a process of sequential addition of amino acids to the growing polypeptide chain, but a process that may also influence (and define) the mechanism of protein folding in the cell.

Ribosome has been proposed to generate an α-helical conformation at the C-terminal end of the growing nascent chains

Stereochemical analysis of the ribosomal transpeptidation reaction performed earlier by Lim and Spirin in the mid 1980s [8], allowed these authors to suggest that the decoding process can impact protein folding and that the ribosome may facilitate generation of an α-helical conformation at the C-terminal end of the growing nascent chain. It must be noted that the central event in peptide bond formation is the nucleophilic attack of the amino group of the second amino acid on the ester carbon of the initiating amino acid methionine and/or the amino acid of the peptidyl-tRNA in the P site, in case the ribosome already moved down the mRNA by one (or more codons) after the initial initiation event [9]. As such, transpeptidation reaction is considered as a nucleophilic Sn2 substitution reaction, passing through a tetrahedral intermediate [9]. Therefore, it was suggested that a stereochemically universal mechanism of the reaction should exist for all 20 amino acid residues, both in the attacked (donor) and in the attacking (acceptor) substrates [8]. Following this assumption, Lim and Spirin found that only one unique conformation of the tetrahedral intermediate would be sterically compatible with all

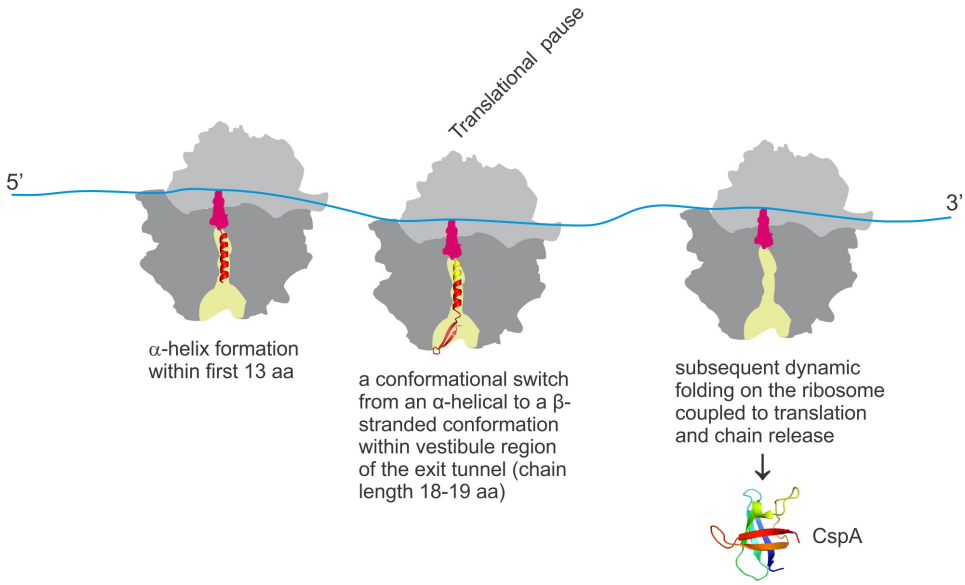

Figure 1. A stepwise vectorial co-translational folding of a β-barrel protein CspA begins with formation of an α-helix inside the ribosomal tunnel. An α-helix inside the ribosome rearranges into a β-hairpin structure as the growning peptide reaches the wider, so-called vestibule region of the exit tunnel. A translational pause may facilitate this transition. The nascent chain remains highly dynamic until its release from the ribosom

400 possible pairs of the 20 reacting amino acid residues and at the same time capable of cleaving into a planar trans-peptide group. This intermediate appeared to have torsion angles phi and psi similar to that found in α-helix [8]. Thus, these authors suggested that the α-helical conformation for the C-proximal section of the nascent polypeptide inside the ribosome tunnel would be more probable than other conformations. Several questions immediately arose with this assumption, and the most important one, – if the ribosome facilitates generation of an α-helical conformation in the growing nascent chain inside the tunnel, how then and at what point in time other secondary structures (and specifically β-strands) will be formed? It was suggested that the α-helical conformation will be retained inside the tunnel and rearranged into other structures as the nascent peptide will be emerging from the ribosome into aqueous solution [8].

Remarkably, the possibility of α-helical structure formation inside the tunnel for the natural α-helical regions (found in different proteins) was experimentally demonstrated 30 years after the original Lim and Spirin's theoretical suggestion [10–12].

Why the new study is important?

However, until the study by Agirrezabala *et al* it has not been shown that the ribosome can generate an α-helix inside the tunnel for a mainly β-protein and that this α-helix can rearrange during the course of translation into a β-hairpin structure. Therefore, this manuscript provides the first experimental evidence in support of this notion. PET kinetics study performed by Agirrezabala *et al* also showed that the compaction of the nascent CspA chain into an α-helix occurs, when the polypeptide reaches the length of about 13 aa [5]. Addition of the next 5 aa of the CspA nascent chain leads to a rapid conformational rearrangement of the nascent chain (Figure 1). The nascent chain then gradually moves into the lower vestibule region of the tunnel, which is wide enough to accommodate formation of the β-hairpin structure.

Thus, a β-barrel protein starts to fold inside the ribosomal tunnel into initially α-helical conformation and then undergoes several conformational rearrangements in the course of ongoing translation before emerging from the ribosome. Interestingly, rearrangement of the high-PET intermediate into a less compact state coincides with the translation pausing at about aa 19, which may facilitate this transition [5]. It was previously suggested that translation pausing at the boundaries between different secondary structures may facilitate the conversion of the α-helical structure generated by the ribosome into other structures [1]. However, direct experimental evidence in support of this consideration was lacking. Therefore, this report not only demonstrates that conversation of an α-helix into a β-structure may occur during ongoing translation, but also provides additional data suggesting that this transition may be facilitated by translational pausing. However, whether the later is indeed the case remains to be established.

This study represents a substantial step forward in our understanding of the process of co-translational protein folding in the cell and opens up several new avenues of research in the field. Future experiments should also answer a question, how widespread is this phenomenon and whether the conversation of an α-helix into a β-structure is taking place for other mainly β proteins.

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Disclosure statement

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Author contributions

A. A. K. prepared and wrote the manuscript.

Data Availability Statement

This perspective article does not have any data. The data availability policy is not applicable to this manuscript.

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