Review Article

Translation Rates and Protein Folding

Anton A. Komar [1,2*], **Ekaterina Samatova** [3] and **Marina V. Rodnina** [3*]

*1 - **Center for Gene Regulation in Health and Disease,** Department of Biological, Geological and Environmental Sciences, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115, USA*
*2 - **Department of Biochemistry and Center for RNA Science and Therapeutics,** School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA*
*3 - **Max Planck Department of Physical Biochemistry,** Max Planck Institute for Multidisciplinary Sciences, 37077 Goettingen, Germany*

Correspondence to Anton A. Komar and Marina V. Rodnina: *a.komar@csuohio.edu (A.A. Komar), rodnina@mpinat.mpg.de (M.V. Rodnina)*
https://doi.org/10.1016/j.jmb.2023.168384
Edited by J. Buchner

Abstract

The mRNA coding sequence defines not only the amino acid sequence of the protein, but also the speed at which the ribosomes move along the mRNA while making the protein. The non-uniform local kinetics – denoted as translational rhythm – is similar among mRNAs coding for related protein folds. Deviations from this conserved rhythm can result in protein misfolding. In this review we summarize the experimental evidence demonstrating how local translation rates affect cotranslational protein folding, with the focus on the synonymous codons and patches of charged residues in the nascent peptide as best-studied examples. Alterations in nascent protein conformations due to disturbed translational rhythm can persist off the ribosome, as demonstrated by the effects of synonymous codon variants of several disease-related proteins. Charged amino acid patches in nascent chains also modulate translation and cotranslational protein folding, and can abrogate translation when placed at the N-terminus of the nascent peptide. During cotranslational folding, incomplete nascent chains navigate through a unique conformational landscape in which earlier intermediate states become inaccessible as the nascent peptide grows. Precisely tuned local translation rates, as well as interactions with the ribosome, guide the folding pathway towards the native structure, whereas deviations from the natural translation rhythm may favor pathways leading to trapped misfolded states. Deciphering the 'folding code' of the mRNA will contribute to understanding the diseases caused by protein misfolding and to rational protein design.

© 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Introduction

Protein synthesis and folding are fundamental processes that ensure cell viability, fitness, and the ability of the organism to respond to environmental cues. At each round of translation elongation, the ribosome adds an amino acid to the C-terminus of the nascent peptide. The growing peptide moves down the exit tunnel of the ribosome and starts to fold inside the narrow space of the tunnel. The environment of the tunnel and the vectorial appearance of the nascent peptide from the N- to the C-terminus define the pioneering round of the nascent protein folding on the ribosome. Interactions with the ribosome inside and outside of the tunnel favor formation of

0022-2836/© 2023 The Author(s). Published by Elsevier Ltd.This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Please cite tts article as: A.A. Komar, E. Samatova and M.V. Rodnina, Translation Rates and Protein FoldingJournal of Molecular Biology, https://doi.org/10.1016/j.jmb.2023.168384

local compact structures such as α-helices, destabilize and delay formation of native protein folds, and induce formation of folding intermediates that are not observed in solution (for reviews, see[1–4]). The timescales of translation and protein folding govern the partitioning between natively folded, functionally active conformations and misfolded off-pathway intermediates that have to be removed by the quality control machinery of the cell. This interplay is made even more complex by the fact that kinetics of translation is not uniform, with some codons and codon contexts leading to delays in protein synthesis. Generally, local translation rates can be modulated by many factors, including synonymous codon usage, tRNA pools (including tRNA isoacceptors and isodecoders, as well as tRNA modifications), mRNA secondary structures and amino acid sequences in nascent peptides (for review, see[5]). Early observations suggested that rare codon clusters lead to translational pauses and those, in turn, modulate protein production and folding, leading to the hypothesis that translational pauses might serve as "interpunctuations" during nascent chain folding on the ribosome (reviewed in[6–8]). Since then, enormous progress in bioinformatics, ribosome profiling, as well as biophysical and structural techniques provided strong support in favor of this concept. In a broader sense, mRNA context appears to encode the conserved pace of translation which is important to maintain correct protein folding. This concept is referred to as a "folding code of mRNA" or "translational tuning"[8–10]; however, the exact mechanisms of how variation of the translation rates results in correctly folded or misfolded protein structures remains unclear. In this review, we will discuss how different factors that define local translation speed modulate protein folding with the focus on synonymous codons and amino acid charges in nascent peptides.

Modulation of Translation Rates by Synonymous Codons

Among the factors affecting local translation rates, synonymous codons, i.e. codons that encode the same amino acid, but are decoded by different tRNAs, are studied best. Frequencies of synonymous codons differ between genomes, as well as among individual mRNAs in the genome, which is known as codon usage bias. While the view that synonymous codons affect both translation and cotranslational folding are widely accepted, in most cases interpretation of the results in terms of translational tuning relies on assumptions. The first assumption is that a non-uniform distribution of synonymous codons along the mRNA indeed changes the local translation rates, thereby generating a unique pattern of discontinuous synthesis, referred to as a translation rhythm. Second, altered translation rhythm is assumed to affect cotranslational protein folding on the ribosome. Third, alterations in the protein conformational ensemble arising during the pioneering round of protein folding on the ribosome must endure after the protein is released into the cytosol. Deviations in translation kinetics, e.g. due to replacements of rare for frequent codons, should lead to changes in protein's function or enhanced protein aggregation/degradation. In principle, the same set of assumptions holds true for any type of translational pauses; in the following, we will summarize the experimental evidence underlying the links between translation rhythm and protein folding/misfolding for synonymous codon clusters.

Non-uniform distribution of synonymous codons and translation kinetics

According to the neutral theory of molecular evolution,[11] synonymous codon substitutions should be largely unaffected by the selective pressure, because they encode the same amino acid; hence, synonymous codons distribution along the mRNA should be random. However, studies of single nucleotide variants in human genome suggest that in many cases rare codon clusters are substantially more abundant than expected based on random selection and their positions are highly conserved.[12,13] This implies that synonymous codons have an important evolutionary conserved role in mRNA homeostasis. In fact, substantial evidence indicates that codon usage modulates mRNA stability, translation efficiency and fidelity as well as protein folding, and these effects are sometimes linked, e.g., low translational efficiency may lead to mRNA degradation and translation errors can result in protein misfolding.[14,15] However, if the positions of synonymous codons in the mRNA are changed, the resulting protein can misfold even if translation remains efficient and error-free, and the challenge is to understand why this happens.

The next question is whether rare codon clusters indeed lead to translational pauses. Early experiments revealed local variations in translation rates[16,17] and suggested that the presence of rare codons in domain linkers served to slow down translation at these regions.[17] The development of ribosome profiling[18] enabled genome-wide analysis of ribosome pausing events at a codon resolution. While locating the ribosome pause sites turned out to be sensitive to details of experimental protocols,[19,20] an improved ribosome profiling method identified pause sites at rare codon clusters[20] and suggested that these pauses are conserved.[21] The patterns of ribosome pausing during synthesis of paralogous domains tend to be significantly more similar than those of non-paralogous domains, suggesting that translation rhythm is not only conserved but also crucial to retain folding pathways and facilitate efficient folding.[21] Thus, the assumption that

synonymous codons can lead to local translation delays appears to be valid for many mRNAs.

Codon usage also has an effect on the global translation rates. Approaches involving substitutions of rare with frequent synonymous codons, known as codon optimization, are widely used to enhance protein production, in particular in heterologous hosts with different codon usage bias.[22] Currently, an array of codon optimization tools is available, which however rarely consider the effects of synonymous codon usage on protein folding and primarily focus on enhancing protein yield. However, maximizing the yield often results in incorrect protein folding,[23–25] thereby reducing protein activity and increasing risks of manufacturing malfunctioning protein, including protein therapeutics. Notably, even minor changes in protein structure can result in altered immunogenicity,[26] underscoring the significance of the problem.[27] Carefully designed engineering of synonymous codons along mRNA allowing to restore natural pace of translation not only enhances protein yield, but also improves protein folding and stability.[28,29]

While many rare codons slow down translation, not every synonymous mutation leads to a marked change in ribosome profiling pattern or displays fitness effects and, vice versa, not every pause in ribosome profiling is due to synonymous codons. Moreover, interpretation of synonymous codon effects is often complicated by confounding factors, such as changes in mRNA structure and stability or increased misreading,[14,15] which have profound effects on the translation rate and yield. Prolonged translation pauses may result in stalled complexes that are recognized by the quality control machinery of the cell, which disassembles such complexes and degrades the incomplete protein. On the other hand, mild pausing effects of synonymous codons can be enhanced by the context, including mRNA structures, the vicinity of bicodon motifs that further delay translation, or the charges in nascent peptides. The interplay between these various factors ensures the optimum yield of functional protein in the cell.

Synonymous codons and protein folding

The first evidence for the effect of synonymous codon substitutions on protein folding came from *in vitro* experiments using *Escherichia coli* chloramphenicol acetyltransferase (CAT).[30] In that study, synonymous replacements of sixteen consecutive rare codons in the CAT gene led to faster translation of the mutated mRNA region, but decreased the specific activity of the enzyme by about 20%, indicating that a fraction of CAT molecules was misfolded.[30] Notably, recent computer simulation studies also suggested that rare codon substitutions should reduce CAT activity by about 20%.[31] A number of subsequent studies over the last decade provided ample evidence that synonymous codon substitutions can affect proteins' conformation and substrate specificity, aggregation propensity, phosphorylation profiles, as well as intracellular processing and targeting.[32–40]

One notable study connects synonymous codon usage with cellular phenotypes in *Neurospora crassa.* Altered codon usage in the circadian clock gene *frequency* (FRQ) mRNA impaired FRQ activity and completely abolished circadian rhythms.[34] Replacing rare with frequent codons in this region altered protein stability, phosphorylation profiles, and protease sensitivity of FRQ. Similarly, codon 'optimization' of the open reading frame of a component of the Drosophila circadian clock encoded by the *period* (Per) gene abolished the circadian locomotor rhythm due to impairment of PER activity and reduction of PER phosphorylation.[37]

Another elegant study showed how rational design of synonymous codons within a given sequence can be used to modulate protein conformations.[36] The authors engineered a fluorescent protein consisting of the N- (yellow) and C-terminal (cyan) half-domains competing to form a folded domain with the central half-domain, which allowed quantifying the folding outcome using a fluorescence readout. The properties of the resulting protein depended on the synthesis rates of the inter-half-domain linker, which was modulated by differential synonymous codon composition of the mRNA.[36] These studies provide a first step towards using synonymous codons for design of proteins with the same sequence but different structures.

One study may be of particular interest, because it provides links between synonymous codon usage, local and global translational kinetics, co- and post-translational folding, and protein conformation and stability.[28] In this study, eye lens protein γB-crystalline was used as model protein, because translation of this two-domain protein is a non-uniform process with specific pauses optimized to tune its synthesis and folding in the cell.[17,28] Synonymous codon substitutions altered both local and global translation rates and protein conformation, resulting is different sensitivity to spontaneous proteolysis in cells (Figure 1). Alterations in mRNA stability and misreading levels were ruled out by control experiments, suggesting that the observed effects were likely due to the codon-dependent changes in translation and protein folding.[28] Real-time fluorescence and FRET measurements showed that when the natural translation rhythm was changed, folding of the N-terminal domain of γB-crystallin was delayed. In part, this could be explained by slower translation and delayed emergence of the nascent peptide from the exit tunnel. However, even when the full N-terminal domain emerged from the ribosome, its folding was still significantly slower when translation rhythm was distorted. This suggests that the ensemble of peptide conformations exiting the tunnel depends on the speed of synthesis.[28] Moreover, 2D NMR revealed that proteins produced from mRNAs with a different

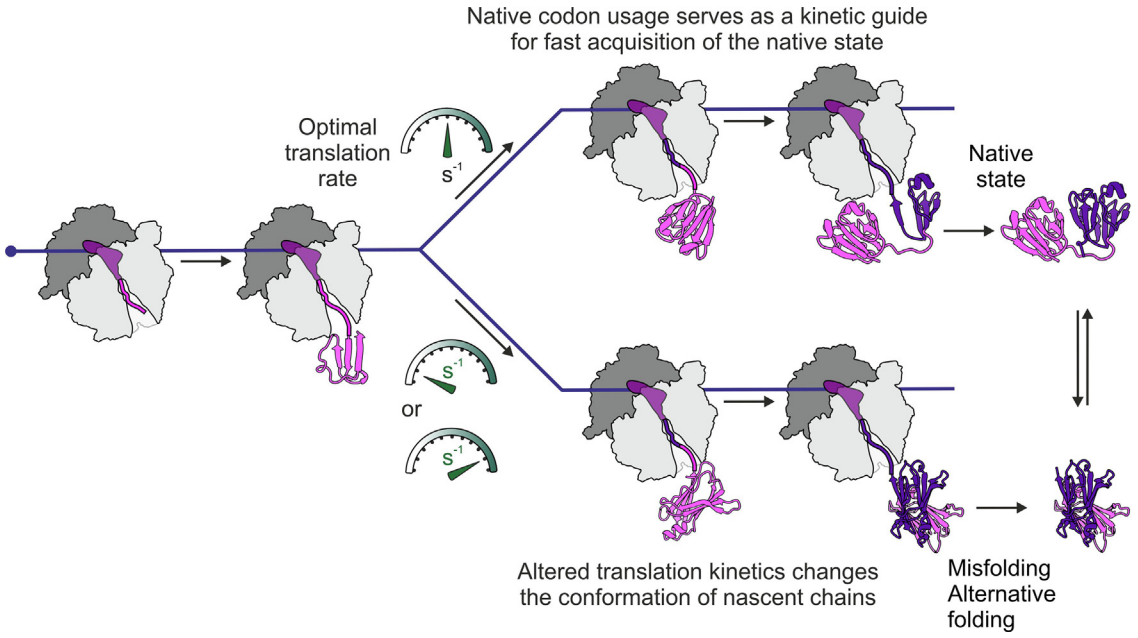

Figure 1. Translational tuning of protein folding. Optimal, native rhythm of translation ensures correct folding (upper branch). Shown is the structure of γB-crystallin[38] (for visual clarity, the sizes of the ribosome and the nascent protein are not drawn to scale). Altered translation kinetics can lead to misfolding (lower branch; the misfolded structure shown is an artistic imagination). Misfolded proteins can refold co- or post-translationally, with or without the help of molecular chaperones, but is some cases become trapped in stable non-native, aggregation-prone states.

combination of synonymous codons were structurally different despite the unchanged amino acid sequence and, furthermore, a more natural translation rhythm led to a considerable conformational diversity in purified γB-crystallin with respect to its oxidation state. This may indicate that folding of γB-crystallin may require dynamic transitions to establish a correct pattern of disulfide bonds, whereas the reduced conformation formed as a result of a mistuned translation is entangled in an alternative, unnatural conformation. This work suggested that synonymous substitutions alter the landscape of accessible protein conformations both on and off the ribosome[28] (Figure 1). Together, these results support the hypothesis that synonymous codon usage may serve as a secondary code for protein folding in the cell.

Synonymous codons in disease

There are several examples that illustrate the link between synonymous codon usage and disease. For example, a single synonymous mutation of a frequent isoleucine ATC codon to the rare ATT codon encoded by the multidrug resistance 1 (MDR1) gene alters P-glycoprotein substrate specificity.[32] This study was the first to demonstrate that naturally occurring synonymous variants encoding the same amino acid can nevertheless result in proteins with different structural and functional properties and explained previously noted differences in P-glycoprotein pharmacokinetics in individuals carrying this mutation.

Subsequent studies provided further examples of naturally occurring single synonymous mutations linked to disease states and explained hitherto puzzling cellular phenotypes.[41–43] For example, the pathogenic effect of a single valine GTG to GTA synonymous mutation in the *F9* gene (encoding blood coagulation factor FIX) associated with hemophilia B remained unclear,[44] because it could not be explained by changes in mRNA levels/stability or alternative splicing, which is a common cause of many diseases associated with synonymous mutations. It turned out that the mutation changes the rate of FIX synthesis, which alters its structure and processing and decreases extracellular protein levels.[43] Further examples of synonymous codon substitutions in a plethora of disease-relevant proteins include cystic fibrosis transmembrane conductance regulator (CFTR),[45] Kirsten Ras oncogene homolog from the mammalian Ras gene family (KRAS),[46,47] or the estrogen receptor-alpha protein in breast cancer cells.[48] These data shed light on the potential roles of single nucleotide variants in human genome that can be used for risk assessment and diagnostics of diseases.

Charged amino acids in the nascent chain

In addition to synonymous codons, other factors can modulate local translation rates, such as patches of charged amino acids and specialized

stalling sequences in nascent peptides, as well as the secondary structure elements in the mRNA (reviewed in[5,49,50]). These effects are often hard to identify due to the lack of simple signatures in ribosome profiling. While ribosome pausing at some mRNA contexts, e.g. bicodons and mRNA secondary structures, are well characterized (reviewed in[5]), their effects on protein folding are unclear. In contrast, charged amino acid regions in nascent peptides are known to play an important role in modulating protein folding.[51] Positively and negatively charged amino acid patches may have a different effect depending on their location in the exit tunnel. Analysis of 551,705 protein sequences from different organisms suggested that positively charged sequences are generally less abundant than negatively charged ones[52] and there is a strong evolutionary pressure against supercharged proteins with charges greater than 14. Patches of positively charged amino acids are more frequent at the N-terminus of proteins.[52,53] Positively charged amino acids upstream of the A-site codon slow down translation independent of the codon identity or distribution,[51] and there is a linear correlation between the length of the positively charged segment and the length of translation pause.[54] One model for the translational slowdown is that positive charges exert force on the peptidyl transferase center that increases the distance between amino acids attached to the CCA ends of P- and A-site tRNAs and hence increases the barrier for peptide bond formation.[55] We note that cryo-EM studies of ribosome complexes with Lys-tRNA in the A site did not observed any deviations from the canonical tRNA positions.[56] This suggests that the effect of positive charges on the peptidyl transferase center is more likely indirect, due to signaling through the tunnel, consistent with the observation that the maximum effect is observed when the peptide linker connecting the charged patch with the tRNA is about 10 amino acids long.[51] Such linker would bring the charges into the vicinity of the constriction site of the ribosome formed by ribosomal proteins uL4 and uL22. Therefore an alternative explanation for the experimental data[51] is that the repulsion between the positive charges in the nascent peptide and in uL4 and uL22 prevents formation of the active conformation of the peptidyl transferase center, akin to rearrangements induced by different conformations of CspA nascent chain.[57] We also note that a delay in translation when a nascent peptide reaches the constriction site may be a more general effect, as genome-wide ribosome profiling in mammalian cells revealed elevated ribosome density at the 5th mRNA codon.[58,59] The effect was attributed to the geometry of the constriction site, as deletion of the tunnel loop of the ribosomal protein L4 diminishes translational pausing at the 5th codon.[58] Poly(Lys) stretches can also inhibit translation, but in this case translation stalling requires a string of consecutive AAA codons and is not observed on the alternative Lys codon AAG, indicating that the peptide effects are unlikely to play a key role.[60–62]

Stretches of negatively charged residues in the nascent peptide may cause even more dramatic effects, leading to translational arrest and destabilization of ribosome complexes, a process referred to as intrinsic ribosome destabilization (IRD).[63,64] The effect depends on the location of the charged sequence in the nascent peptide and on the upstream peptide context, with the upstream positive charges enhancing the inhibitory effect, whereas hydrophobic residues counteracting it.[63] Translation arrest is greatly reduced if the IRD sequence is located > 30 amino acids away from the N-terminus of the nascent protein. It is likely that as the nascent protein starts to fold, the developing tension is sufficient to alleviate the translation arrest by the IRD, akin to the rescue effect of folding on the SecM-like stalling sequences.[49] Natural strong IRD-like sequences contain alternating negatively charged residues and prolines, but they usually do not stall translation, because they are mostly found in the middle of open reading frames, where the ribosome is stabilized by the presence of the nascent peptide in the tunnel.[64] A similar sequence motif comprised of negatively charged residues, Gly and Pro was identified as universally conserved stalling site using ribosome profiling datasets from phylogenetically diverse eukaryotes (yeast, fruit fly, zebra fish, mouse and human).[65] The relevance of such translation stop sites for protein folding is unclear, but given that a pause in translation may result in attenuation of protein folding, such pauses may play the same role as rare codon clusters.

How can local translation rates affect folding of a mature protein?

The extensive work on the mechanism of cotranslational protein folding (reviewed in[2,66–67]) suggests how altered translation rates can lead to misfolding. The folding energy landscape on the ribosome differs dramatically from that of protein re-folding in solution (Figure 2). Cotranslational compaction of the N-terminal amino acids of the nascent protein is vectorial and begins in the narrow exit tunnel, which limits the conformational space to mostly local intrachain contacts. Inside the tunnel, nascent chains remain dynamic and the different conformations can rapidly interconvert.[68] However, as the nascent chain grows, some of the earlier states become inaccessible, because interactions within a longer nascent chain offer a more stable fold. The irreversibility due to the increase of the chain length may trap non-native intermediates on the path to the misfolded state (Figure 2). Further increase in the chain length may preclude refolding of incorrectly formed states, because the metastable states that exchange between native and non-native folds are no longer available.[69] Misfolding may be global or local, such as changes in loop

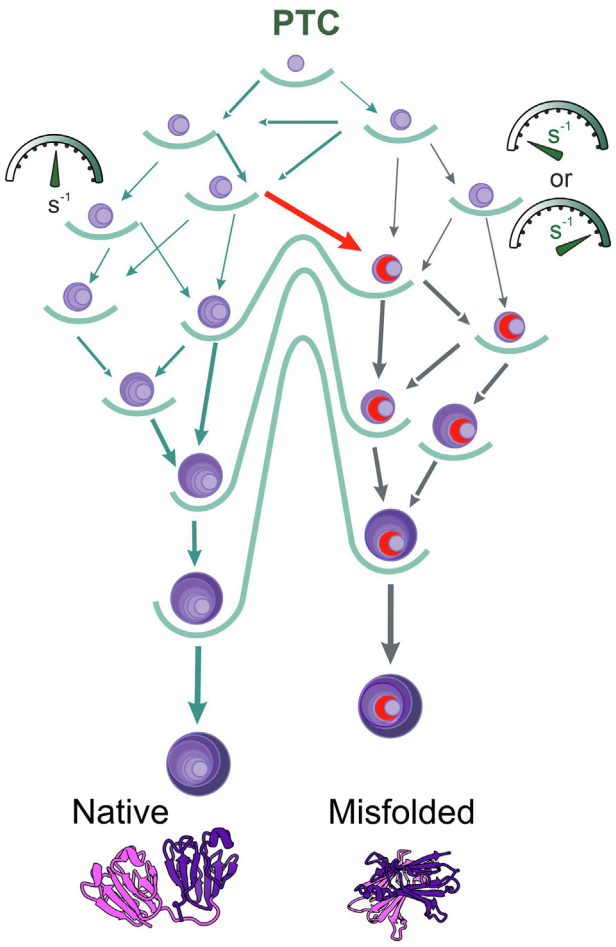

Figure 2. The energy landscape of protein folding on the ribosome. Nascent chain compaction begins with a few rapidly interconverting folding intermediates (small purple balls). As the nascent chain grows (represented by the increasing ball size), some of the previously allowed conformations become inaccessible, thereby providing a barrier separating the pathways leading to correct folding (green arrows) from misfolding (gray arrows). Altered (higher or lower) translation rate opens the time window that favors partitioning to the misfolded state (red arrow). Misfolding may be global or local, such as formation of entangled states (illustrated by the red color in misfolded protein conformations).

conformations or formation of entangled states.[31] Depending on the protein, these intermediates can be converted – co- or post-translationally, with or without the help of molecular chaperones – into the native protein state through reshuffling reactions or become trapped in stable non-native, aggregation-prone states.

For the above kinetic partitioning model to explain misfolding, the rates of chain elongation and fluctuations in nascent protein conformations should be comparable. Translation rates vary, on average, between 1 and 20 aa/s depending on the organism, codon identity and context, whereas secondary and tertiary structure formation typically occurs on a μs to ms time scale.[70,71] The large difference in the time scales would suggest that cotranslational folding takes place under quasi-equilibrium conditions.[72,73] Furthermore, because ribosome-bound nascent chains are highly dynamic,[57,68,69,74–77] any folding/misfolding events should be transient and reversible. This implies that emerging protein domains should be able to assume a post-translational refolding pathway as soon as they have moved far enough from the ribosome surface, and there is some evidence in favor of this notion.[68,78–80] Furthermore, many misfolding defects can be removed by chaperones that interact with nascent chains on the ribosome and with the released proteins in solution. However, as described above, there are numerous examples demonstrating that misfolded conformations formed cotranslationally persist after the release of the protein from the ribosome and in some cases the proteins do not attain their native conformations even their refolding in solution.[81] This raises a question of how any alterations in translation speed that do

not change the amino acid sequence of the protein can affect translation given that the time domains of the two reactions hardly overlap.

One approach to understand the link between the translation and folding time scales is through computer simulations.[31,73,82–84] An early study combining chemical kinetic equations for co-translational folding with genomic and proteomic data suggested that folding of about one third of *E. coli* cytosolic proteins is under kinetic control and that the majority of the co-translational folding domains within these proteins are influenced by translation kinetics.[82] One important observation from computer simulations is that non-native interactions involving C-terminal residues can drastically slow down cotranslational folding into native structures.[84] This notion resonates with the conclusions of biochemical/structural studies suggesting that formation of the native domain structures is delayed by the ribosome.[69,85,74–77] Furthermore, slower folding may be a consequence of a vectorial folding pathway on the ribosome that proceeds through other intermediates than refolding in solution.[57,72,78,86–89] Such non-native intermediates may be destabilized and more prone to misfolding. Indeed, force measurements indicated that stalling of ribosomes with incomplete nascent chain induces rapid formation of off-pathway intermediates that refold very slowly, whereas on-going translation prevents the peptide from entering these local energy minima by rapidly increasing the length of the peptide.[69] Thus, the rate of translation could be evolutionary adjusted to avoid conformationally trapped intermediates by modulating the transition towards longer peptides that can form native contacts.[83,84] Moreover, nascent chain contacts with the proteins lining up the tunnel walls, uL4, uL22, uL23, uL24 and uL39 can have an effect. While uL4 and uL22 may affect folding indirectly, due to coordinating the activity of the peptidyl transferase center,[57] interactions of nascent peptide with uL23, uL24 and uL39 delay and modulate folding.[74,90] Forming and resolving these transient interactions, which change as the peptide moves down the tunnel, may provide a link between the translation and folding timescales. Thus, non-equilibrium processes due to vectorial nature of translation, delayed folding into native structures, and interactions with the tunnel walls can explain the formation and retention of alternative conformations depending on the translation speed.

One unresolved question concerns the structure of the putative misfolded states. A recent multiscale modeling of three *E. coli enzymes* (type III chloramphenicol acetyltransferase, D-alanine–D-alanine ligase B and dihydrofolate reductase) suggests that synonymous mutations alter kinetic partitioning between co- and post-translational folding/misfolding pathways.[31] Near-native intermediates formed along such altered pathway represent long-lived kinetic traps that may display topological entanglements that render a fraction of the protein inactive. The distortions are local, for example in the proximity of the enzyme active site, but otherwise display an overall native fold, which would explain why they do not aggregate and avoid recognition by the chaperones and the cell degradation machinery. Whether such entangled states would represent a typical outcome of the altered translation kinetics for any given protein remains to be established, but the simulations provide a good starting point to test their predictions by e.g. mutational analysis and enzymatic assays. On the other hand, translation pauses, particularly caused by synonymous codons, can delay rearrangements at quite common boundaries between different secondary structures, such as, e.g., β-strand → coil and coil → β-strand.[91] Also formation of native and non-native disulfide bonds can be affected by the translation delays and timing of compaction events.[92,93] Analysis of the vast amount of sequence and structural data (using 1343 protein chains) available for *E. coli* suggested that the backbone dihedral angle of an amino acid is statistically dependent on the identity of the codon encoding it.[94] Remarkably, this correlation allowed the authors to predict protein backbone dihedral angles with a lower error than when using amino acid sequence information alone.[95] The Coding Sequence and Structure (CSandS) database containing information on 4406 proteins including 786 *E. coli* proteins, 890 human and 301 yeast proteins, similarly allowed the authors to conclude that synonymous codons have distinct propensity for different protein secondary structure types and this propensity is significantly different from those favored by the amino acids they encode.[96] Overall, these results support the premise that synonymous codons encode more information than merely an amino acid identity and can predict the conformation of nascent peptides and their propensity to fold and misfold.

Conclusions

The work of many groups over the last decade demonstrated that mRNA harbors additional layers of structural information beyond the amino acid sequence of proteins. The signals provided by the codon context and mRNA structure translate into a precisely tuned periods of rapid protein synthesis separated by pauses. This rhythm of translation plays an important role in determining the protein yield and activity. However, a number of questions remains open. While there are numerous examples of synonymous codons modulating protein folding, it remains unclear why some synonymous variants alter translation pace and protein conformations, whereas others are neutral. Here, the codon context including neighboring codons/amino acids, potential mRNA secondary structures, the

respective tRNA concentrations, and the translation rates upstream and downstream of the pause site may be important[9,97,98]; further systematic work will be required to understand the prevalence and importance of these signals. Moreover, the impact of translation pauses on folding of any given protein depends on the exact nature of cotranslational folding intermediates for this protein. Predicting such intermediates and understanding how alteration of the translation rhythm changes the structure and the population distribution of intermediates is one of the major challenges of future research.

One obvious bottleneck for future studies is the scarcity of reliable structural information on cotranslational folding intermediates inside the exit tunnel, which limits our understanding of both the nature of folds as well as their interactions with the ribosome. Given the transient and dynamic nature of these putative intermediates, their structural elucidation presents a formidable challenge. Even more difficult would be to visualize the misfolded intermediates and trace their folding landscape on and off the ribosome. However, with the accumulating evidence on potential medical implications of synonymous variants, such structural work would help to predict the potential impact of synonymous variations on protein conformations and to understand the potential disease causes. In this respect, it appears important to implement codon-specific structural information[94] into structural databases and protein structure prediction algorithms and to test these predictions experimentally. Understanding of how synonymous codons – in their given context – affect protein folding may open a path for alternative design strategies based on the translation rhythm without changing an amino acid sequence.

Notably, factors others than synonymous variants can affect local translation speed. Indeed, tRNA concentrations seem to be even better predictors of translation speed than relative codon usage.[96] In principle, any pause irrespective of its source could have the same effect on protein folding. This implies that also mRNA secondary structures (which slow down translation while the ribosome unwinds them) should affect protein folding, but there are no studies of such effects so far. Another major challenge is to understand the position-specific effects of charges in nascent chains on cotranslational folding, ribosome stalling and destabilization[63] and the cellular response to such stalling events. In contrast to transient ribosome pausing events, which are quickly resolved yet still affect protein folding, prolonged ribosome stalling caused by negatively charged patches in nascent peptides can lead to ribosome collisions with the next ribosome, thereby eliciting quality control responses coupling translation to ribosome recycling and protein degradation pathways. Interactions of nascent chains with the ribosome can also allosterically alter the conformation of the peptidyl transferase center.[57] The crosstalk between the nascent peptide conformation and the structure of the active sites of the ribosome remains another open question that has to be clarified. Whatever the results, it is likely that future experiments will provide exciting insights into folding code of mRNAs and into the cross-talk between the functional centers of the ribosome, nascent peptide conformation and dynamics, and the activity of the protein in the cell.

CRediT authorship contribution statement

Anton A. Komar: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. **Ekaterina Samatova:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Marina V. Rodnina:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

DATA AVAILABILITY

No data was used for the research described in the article.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The work was funded by the European Research Council (ERC) Advanced Investigator Grant RIBOFOLD (proposal number 787926) the DFG Leibniz Prize, and the Max Planck Society to MVR. AAK is supported by the National Institutes of Health (NIH) grants HL151392 and GM128981.

Received 20 October 2023;
Accepted 2 December 2023;
Available online xxxx

Keywords:
cotranslational protein folding;
protein synthesis;
synonymous codons;
protein folding energy landscape;
protein misfolding

References

1. Liutkute, M., Samatova, E., Rodnina, M.V., (2020). Cotranslational folding of proteins on the ribosome. *Biomolecules* **10**, 10.
2. Bustamante, C., Alexander, L., Maciuba, K., Kaiser, C.M., (2020). Single-molecule studies of protein folding with optical tweezers. *Annu. Rev. Biochem.* **89**, 443–470.
3. Waudby, C.A., Burridge, C., Cabrita, L.D., Christodoulou, J., (2022). Thermodynamics of co-translational folding and ribosome-nascent chain interactions. *Curr. Opin. Struct. Biol.* **74**, 102357
4. Trovato, F., O'Brien, E.P., (2016). Insights into cotranslational nascent protein behavior from computer simulations. *Annu. Rev. Biophys.* **45**, 345–369.
5. Samatova, E., Daberger, J., Liutkute, M., Rodnina, M.V., (2020). Translational control by ribosome pausing in bacteria: how a non-uniform pace of translation affects protein production and folding. *Front. Microbiol.* **11**, 619430
6. Komar, A.A., (2009). A pause for thought along the co-translational folding pathway. *Trends Biochem. Sci.* **34**, 16–24.
7. Komar, A.A., (2016). The Yin and Yang of codon usage. *Hum. Mol. Genet.* **25**, R77–R85.
8. Komar, A.A., (2021). A code within a code: how codons fine-tune protein folding in the cell. *Biochemistry (Mosc.)* **86**, 976–991.
9. Liu, Y., Yang, Q., Zhao, F., (2021). Synonymous but not silent: the codon usage code for gene expression and protein folding. *Annu. Rev. Biochem.* **90**, 375–401.
10. Chaney, J.L., Clark, P.L., (2015). Roles for synonymous codon usage in protein biogenesis. *Annu. Rev. Biophys.* **44**, 143–166.
11. Kimura, M., (1983). The Neutral Theory of Molecular Evolution. UK Cambridge University Press, Cambridge.
12. Zhao, Z., Fu, Y.X., Hewett-Emmett, D., Boerwinkle, E., (2003). Investigating single nucleotide polymorphism (SNP) density in the human genome and its implications for molecular evolution. *Gene* **312**, 207–213.
13. Chamary, J.V., Parmley, J.L., Hurst, L.D., (2006). Hearing silence: non-neutral evolution at synonymous sites in mammals. *Nature Rev. Genet.* **7**, 98–108.
14. Hanson, G., Coller, J., (2018). Codon optimality, bias and usage in translation and mRNA decay. *Nature Rev. Mol. Cell Biol.* **19**, 20–30.
15. Drummond, D.A., Wilke, C.O., (2008). Mistranslation-induced protein misfolding as a dominant constraint on coding-sequence evolution. *Cell* **134**, 341–352.
16. Krasheninnikov, I.A., Komar, A.A., Adzhubei, I.A., (1991). Nonuniform size distribution of nascent globin peptides, evidence for pause localization sites, and a contranslational protein-folding model. *J. Protein Chem.* **10**, 445–453.
17. Komar, A.A., Jaenicke, R., (1995). Kinetics of translation of gamma B crystallin and its circularly permutated variant in an in vitro cell-free system: possible relations to codon distribution and protein folding. *FEBS Lett.* **376**, 195–198.
18. Ingolia, N.T., Ghaemmaghami, S., Newman, J.R., Weissman, J.S., (2009). Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* **324**, 218–223.
19. Hussmann, J.A., Patchett, S., Johnson, A., Sawyer, S., Press, W.H., (2015). Understanding biases in ribosome profiling experiments reveals signatures of translation dynamics in yeast. *PLoS Genet.* **11**, e1005732.
20. Mohammad, F., Green, R., Buskirk, A.R., (2019). A systematically-revised ribosome profiling method for bacteria reveals pauses at single-codon resolution. *Elife* **8**, e42591
21. Nissley, D.A., Carbery, A., Chonofsky, M., Deane, C.M., (2021). Ribosome occupancy profiles are conserved between structurally and evolutionarily related yeast domains. *Bioinformatics* **37**, 1853–1859.
22. Gustafsson, C., Govindarajan, S., Minshull, J., (2004). Codon bias and heterologous protein expression. *Trends Biotechnol.* **22**, 346–353.
23. Cortazzo, P., Cervenansky, C., Marin, M., Reiss, C., Ehrlich, R., Deana, A., (2002). Silent mutations affect in vivo protein folding in Escherichia coli. *Biochem. Biophys. Res. Commun.* **293**, 537–541.
24. Perach, M., Zafrir, Z., Tuller, T., Lewinson, O., (2021). Identification of conserved slow codons that are important for protein expression and function. *RNA Biol.* **18**, 2296–2307.
25. Liu, K., Ouyang, Y., Lin, R., Ge, C., Zhou, M., (2022). Strong negative correlation between codon usage bias and protein structural disorder impedes protein expression after codon optimization. *J. Biotechnol.* **343**, 15–24.
26. Katneni, U.K., Alexaki, A., Hunt, R.C., Hamasaki-Katagiri, N., Hettiarachchi, G.K., Kames, J.M., et al., (2022). Structural, functional, and immunogenicity implications of F9 gene recoding. *Blood Adv.* **6**, 3932–3944.
27. Sauna, Z.E., Richards, S.M., Maillere, B., Jury, E.C., Rosenberg, A.S., (2020). Editorial: immunogenicity of proteins used as therapeutics. *Front. Immunol.* **11**, 614856
28. Buhr, F., Jha, S., Thommen, M., Mittelstaet, J., Kutz, F., Schwalbe, H., et al., (2016). Synonymous codons direct cotranslational folding toward different protein conformations. *Mol. Cell* **61**, 341–351.
29. Punde, N., Kooken, J., Leary, D., Legler, P.M., Angov, E., (2019). Codon harmonization reduces amino acid misincorporation in bacterially expressed proteins and improves their immunogenicity. *AMB Express* **9**, 167.
30. Komar, A.A., Lesnik, T., Reiss, C., (1999). Synonymous codon substitutions affect ribosome traffic and protein folding during in vitro translation. *FEBS Lett.* **462**, 387–391.
31. Jiang, Y., Neti, S.S., Sitarik, I., Pradhan, P., To, P., Xia, Y., et al., (2023). How synonymous mutations alter enzyme structure and function over long timescales. *Nature Chem.* **15**, 308–318.
32. Kimchi-Sarfaty, C., Oh, J.M., Kim, I.W., Sauna, Z.E., Calcagno, A.M., Ambudkar, S.V., et al., (2007). A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* **315**, 525–528.
33. Zhang, G., Hubalewska, M., Ignatova, Z., (2009). Transient ribosomal attenuation coordinates protein synthesis and co-translational folding. *Nature Struct. Mol. Biol.* **16**, 274–280.
34. Zhou, M., Guo, J., Cha, J., Chae, M., Chen, S., Barral, J. M., et al., (2013). Non-optimal codon usage affects expression, structure and function of clock protein FRQ. *Nature* **495**, 111–115.
35. Hu, S., Wang, M., Cai, G., He, M., (2013). Genetic code-guided protein synthesis and folding in Escherichia coli. *J. Biol. Chem.* **288**, 30855–30861.
36. Sander, I.M., Chaney, J.L., Clark, P.L., (2014). Expanding Anfinsen's principle: contributions of synonymous codon

selection to rational protein design. *J. Am. Chem. Soc.* **136**, 858–861.
37. Fu, J., Murphy, K.A., Zhou, M., Li, Y.H., Lam, V.H., Tabuloc, C.A., et al., (2016). Codon usage affects the structure and function of the Drosophila circadian clock protein PERIOD. *Genes Dev.* **30**, 1761–1775.
38. Walsh, I.M., Bowman, M.A., Soto Santarriaga, I.F., Rodriguez, A., Clark, P.L., (2020). Synonymous codon substitutions perturb cotranslational protein folding in vivo and impair cell fitness. *Proc. Natl. Acad. Sci. USA* **117**, 3528–3534.
39. Yu, C.H., Dang, Y., Zhou, Z., Wu, C., Zhao, F., Sachs, M. S., et al., (2015). Codon usage influences the local rate of translation elongation to regulate co-translational protein folding. *Mol. Cell* **59**, 744–754.
40. Pechmann, S., Chartron, J.W., Frydman, J., (2014). Local slowdown of translation by nonoptimal codons promotes nascent-chain recognition by SRP in vivo. *Nature Struct. Mol. Biol.* **21**, 1100–1105.
41. Gartner, J.J., Parker, S.C., Prickett, T.D., Dutton-Regester, K., Stitzel, M.L., Lin, J.C., et al., (2013). Whole-genome sequencing identifies a recurrent functional synonymous mutation in melanoma. *Proc. Natl. Acad. Sci. USA* **110**, 13481–13486.
42. Edwards, N.C., Hing, Z.A., Perry, A., Blaisdell, A., Kopelman, D.B., Fathke, R., et al., (2012). Characterization of coding synonymous and non-synonymous variants in ADAMTS13 using ex vivo and in silico approaches. *PLoS One* **7**, e38864.
43. Simhadri, V.L., Hamasaki-Katagiri, N., Lin, B.C., Hunt, R., Jha, S., Tseng, S.C., et al., (2017). Single synonymous mutation in factor IX alters protein properties and underlies haemophilia B. *J. Med. Genet.* **54**, 338–345.
44. Knobe, K.E., Sjorin, E., Ljung, R.C., (2008). Why does the mutation G17736A/Val107Val (silent) in the F9 gene cause mild haemophilia B in five Swedish families? *Haemophilia* **14**, 723–728.
45. Kim, S.J., Yoon, J.S., Shishido, H., Yang, Z., Rooney, L.A., Barral, J.M., et al., (2015). Protein folding. Translational tuning optimizes nascent protein folding in cells. *Science* **348**, 444–448.
46. Lampson, B.L., Pershing, N.L., Prinz, J.A., Lacsina, J.R., Marzluff, W.F., Nicchitta, C.V., et al., (2013). Rare codons regulate KRas oncogenesis. *Curr. Biol.* **23**, 70–75.
47. Fu, J., Dang, Y., Counter, C., Liu, Y., (2018). Codon usage regulates human KRAS expression at both transcriptional and translational levels. *J. Biol. Chem.* **293**, 17929–17940.
48. Clusan, L., Percevault, F., Jullion, E., Le Goff, P., Tiffoche, C., Fernandez-Calero, T., et al., (2023). Codon adaptation by synonymous mutations impacts the functional properties of the estrogen receptor-alpha protein in breast cancer cells. *Mol. Oncol.* **17**, 1302–1323.
49. Ito, K., Chiba, S., (2013). Arrest peptides: cis-acting modulators of translation. *Annu. Rev. Biochem.* **82**, 171–202.
50. Wilson, D.N., Beckmann, R., (2011). The ribosomal tunnel as a functional environment for nascent polypeptide folding and translational stalling. *Curr. Opin. Struct. Biol.* **21**, 274–282.
51. Lu, J.L., Deutsch, C., (2008). Electrostatics in the ribosomal tunnel modulate chain elongation rates. *J. Mol. Biol.* **384**, 73–86.
52. Requiao, R.D., Fernandes, L., de Souza, H.J.A., Rossetto, S., Domitrovic, T., Palhano, F.L., (2017). Protein charge distribution in proteomes and its impact on translation. *PLoS Comput. Biol.* **13**, e1005549.
53. Duc, K.D., Song, Y.S., (2018). The impact of ribosomal interference, codon usage, and exit tunnel interactions on translation elongation rate variation. *Plos, Genetics* **14**, e1007166
54. Charneski, C.A., Hurst, L.D., (2013). Positively charged residues are the major determinants of ribosomal velocity. *PLoS Biol* **11**, e1001508
55. Leininger, S.E., Rodriguez, J., Vu, Q.V., Jiang, Y., Li, M.S., Deutsch, C., et al., (2021). Ribosome elongation kinetics of consecutively charged residues are coupled to electrostatic force. *Biochemistry* **60**, 3223–3235.
56. Jain, S., Koziej, L., Poulis, P., Kaczmarczyk, I., Gaik, M., Rawski, M., et al., (2023). Modulation of translational decoding by m(6)A modification of mRNA. *Nature Commun.* **14**, 4784.
57. Agirrezabala, X., Samatova, E., Macher, M., Liutkute, M., Maiti, M., Gil-Carton, D., et al., (2022). A switch from alpha-helical to beta-strand conformation during co-translational protein folding. *EMBO J* **41**, e109175
58. Han, Y., Gao, X., Liu, B., Wan, J., Zhang, X., Qian, S.B., (2014). Ribosome profiling reveals sequence-independent post-initiation pausing as a signature of translation. *Cell Res.* **24**, 842–851.
59. Haase, N., Holtkamp, W., Lipowsky, R., Rodnina, M., Rudorf, S., (2018). Decomposition of time-dependent fluorescence signals reveals codon-specific kinetics of protein synthesis. *Nucleic Acids Res.* **46**, e130.
60. Chandrasekaran, V., Juszkiewicz, S., Choi, J., Puglisi, J. D., Brown, A., Shao, S., et al., (2019). Mechanism of ribosome stalling during translation of a poly(A) tail. *Nature Struct. Mol. Biol.* **26**, 1132–1140.
61. Tesina, P., Lessen, L.N., Buschauer, R., Cheng, J., Wu, C. C., Berninghausen, O., et al., (2020). Molecular mechanism of translational stalling by inhibitory codon combinations and poly(A) tracts. *EMBO J.* **39**, e103365.
62. Koutmou, K.S., Schuller, A.P., Brunelle, J.L., Radhakrishnan, A., Djuranovic, S., Green, R., (2015). Ribosomes slide on lysine-encoding homopolymeric A stretches. *Elife* **4**, e05534
63. Chadani, Y., Niwa, T., Izumi, T., Sugata, N., Nagao, A., Suzuki, T., et al., (2017). Intrinsic ribosome destabilization underlies translation and provides an organism with a strategy of environmental sensing. *Mol. Cell* **68**, 528–539 e5.
64. Chadani, Y., Sugata, N., Niwa, T., Ito, Y., Iwasaki, S., Taguchi, H., (2021). Nascent polypeptide within the exit tunnel stabilizes the ribosome to counteract risky translation. *EMBO J.* **40**, e108299.
65. Chyzynska, K., Labun, K., Jones, C., Grellscheid, S.N., Valen, E., (2021). Deep conservation of ribosome stall sites across RNA processing genes. *NAR Genom Bioinform.* **3**, lqab038
66. Samatova, E, Komar, AA, Rodnina, MV, (2024). How the ribosome shapes cotranslational protein folding. *Curr. Opin. Struct. Biol.* **84**, 102740.
67. Cassaignau, A.M.E., Cabrita, L.D., Christodoulou, J., (2020). How does the ribosome fold the proteome? *Annu. Rev. Biochem* **89**, 389–415.
68. Liutkute, M., Maiti, M., Samatova, E., Enderlein, J., Rodnina, M.V., (2020). Gradual compaction of the nascent peptide during cotranslational folding on the ribosome. *Elife* **9**, e60895

69. Alexander, L.M., Goldman, D.H., Wee, L.M., Bustamante, C., (2019). Non-equilibrium dynamics of a nascent polypeptide during translation suppress its misfolding. *Nature Communs.* **10**, 2709.
70. Garbuzynskiy, S.O., Ivankov, D.N., Bogatyreva, N.S., Finkelstein, A.V., (2013). Golden triangle for folding rates of globular proteins. *Proc. Natl. Acad. Sci. USA* **110**, 147–150.
71. B. Manavalan, K. Kuwajima, J. Lee, PFDB: A standardized protein folding database with temperature correction, Sci. Rep. 9 (2019) 1588.
72. Holtkamp, W., Kokic, G., Jager, M., Mittelstaet, J., Komar, A.A., Rodnina, M.V., (2015). Cotranslational protein folding on the ribosome monitored in real time. *Science* **350**, 1104–1107.
73. O'Brien, E.P., Vendruscolo, M., Dobson, C.M., (2012). Prediction of variable translation rate effects on cotranslational protein folding. *Nature Communs.* **3**, 868.
74. Ahn, M., Wlodarski, T., Mitropoulou, A., Chan, S.H.S., Sidhu, H., Plessa, E., et al., (2022). Modulating co-translational protein folding by rational design and ribosome engineering. *Nature Communs.* **13**, 5441.
75. Liu, K., Rehfus, J.E., Mattson, E., Kaiser, C.M., (2017). The ribosome destabilizes native and non-native structures in a nascent multidomain protein. *Protein Sci.* **26**, 1439–1451.
76. Samelson, A.J., Jensen, M.K., Soto, R.A., Cate, J.H., Marqusee, S., (2016). Quantitative determination of ribosome nascent chain stability. *Proc. Natl. Acad. Sci. USA* **113**, 13402–13407.
77. Tan, R., Hoare, M., Welle, K.A., Swovick, K., Hryhorenko, J.R., Ghaemmaghami, S., (2023). Folding stabilities of ribosome-bound nascent polypeptides probed by mass spectrometry. *Proc. Natl. Acad. Sci. USA* **120**, e2303167120
78. Cabrita, L.D., Cassaignau, A.M.E., Launay, H.M.M., Waudby, C.A., Wlodarski, T., Camilloni, C., et al., (2016). A structural ensemble of a ribosome-nascent chain complex during cotranslational protein folding. *Nature Struct. Mol. Biol.* **23**, 278–285.
79. Guinn, E.J., Tian, P.F., Shin, M., Best, R.B., Marqusee, S., (2018). A small single-domain protein folds through the same pathway on and off the ribosome. *Proc. Natl. Acad. Sci USA* **115**, 12206–12211.
80. Jensen, M.K., Samelson, A.J., Steward, A., Clarke, J., Marqusee, S., (2020). The folding and unfolding behavior of ribonuclease H on the ribosome. *J. Biol. Chem.* **295**, 11410–11417.
81. Evans, M.S., Sander, I.M., Clark, P.L., (2008). Cotranslational folding promotes beta-helix formation and avoids aggregation in vivo. *J. Mol. Biol.* **383**, 683–692.
82. Ciryam, P., Morimoto, R.I., Vendruscolo, M., Dobson, C. M., O'Brien, E.P., (2013). In vivo translation rates can substantially delay the cotranslational folding of the Escherichia coli cytosolic proteome. *Proc. Natl. Acad. Sci. USA* **110**, E132–E140.
83. Tanaka, T., Hori, N., Takada, S., (2015). How Co-translational Folding of Multi-domain Protein Is Affected by Elongation Schedule: Molecular Simulations. *PLoS Comput. Biol.* **11**, e1004356.
84. Bitran, A., Jacobs, W.M., Zhai, X., Shakhnovich, E., (2020). Cotranslational folding allows misfolding-prone proteins to circumvent deep kinetic traps. *Proc. Natl. Acad. Sci. USA* **117**, 1485–1495.
85. Kaiser, C.M., Goldman, D.H., Chodera, J.D., Tinoco, I., Bustamante, C., (2011). The Ribosome Modulates Nascent Protein Folding. *Science* **334**, 1723–1727.
86. Khushoo, A., Yang, Z., Johnson, A.E., Skach, W.R., (2011). Ligand-Driven Vectorial Folding of Ribosome-Bound Human CFTR NBD1. *Mol. Cell* **41**, 682–692.
87. Liu, K.X., Maciuba, K., Kaiser, C.M., (2019). The ribosome cooperates with a chaperone to guide multi-domain protein folding. *Mol. Cell* **74**, 310.
88. Nilsson, O.B., Nickson, A.A., Hollins, J.J., Wickles, S., Steward, A., Beckmann, R., et al., (2017). Cotranslational folding of spectrin domains via partially structured states. *Nature Struct. Mol. Biol.* **24**, 221.
89. Kelkar, D.A., Khushoo, A., Yang, Z.Y., Skach, W.R., (2012). Kinetic analysis of ribosome-bound fluorescent proteins reveals an early, stable, cotranslational folding intermediate. *J. Biol. Chem.* **287**, 2568–2578.
90. Kudva, R., Tian, P., Pardo-Avila, F., Carroni, M., Best, R. B., Bernstein, H.D., et al., (2018). The shape of the bacterial ribosome exit tunnel affects cotranslational protein folding. *Elife* **7**, e36326
91. Ma, X.X., Wang, Y.N., Cao, X.A., Li, X.R., Liu, Y.S., Zhou, J.H., et al., (2018). The effects of codon usage on the formation of secondary structures of nucleocapsid protein of peste des petits ruminants virus. *Genes Genomics.* **40**, 905–912.
92. Robinson, P.J., Kanemura, S., Cao, X., Bulleid, N.J., (2020). Protein secondary structure determines the temporal relationship between folding and disulfide formation. *J. Biol. Chem.* **295**, 2438–2448.
93. Schulte, L., Mao, J., Reitz, J., Sreeramulu, S., Kudlinzki, D., Hodirnau, V.V., et al., (2020). Cysteine oxidation and disulfide formation in the ribosomal exit tunnel. *Nature Communs.* **11**, 5569.
94. Rosenberg, A.A., Marx, A., Bronstein, A.M., (2022). Codon-specific Ramachandran plots show amino acid backbone conformation depends on identity of the translated codon. *Nat. Communs.* **13**, 2815.
95. Ackerman-Schraier, L., Rosenberg, A.A., Marx, A., Bronstein, A.M., (2022). Machine learning approaches demonstrate that protein structures carry information about their genetic coding. *Sci. Rep.* **12**, 21968.
96. Saunders, R., Deane, C.M., (2010). Synonymous codon usage influences the local protein structure observed. *Nucleic Acids Res.* **38**, 6719–6728.
97. Stein, K.C., Frydman, J., (2019). The stop-and-go traffic regulating protein biogenesis: How translation kinetics controls proteostasis. *J. Biol. Chem.* **294**, 2076–2084.
98. Gamble, C.E., Brule, C.E., Dean, K.M., Fields, S., Grayhack, E.J., (2016). Adjacent codons act in concert to modulate translation efficiency in yeast. *Cell* **166**, 679–690.