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Mechanochemistry in Translation

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Abstract

As the influence of translation rates on protein folding and function has come to light, the mechanisms by which translation speed is modulated have become an important issue. One mechanism entails the generation of force by the nascent protein. Cotranslational processes, such as nascent protein folding, the emergence of unfolded nascent chain segments from the ribosome's exit tunnel, and insertion of the nascent chain into or translocation of the nascent chain through membranes, can generate forces that are transmitted back to the peptidyl transferase center and affect translation rates. In this Perspective, we examine the processes that generate these forces, the mechanisms of transmission along the ribosomal exit tunnel to the peptidyl transferase center, and the effects of force on the ribosome's catalytic cycle. We also discuss the physical models that have been developed to predict and explain force generation for individual processes and speculate about other processes that may generate forces that have yet to be tested.

Graphical Abstract

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Mechanical forces are propagated through continuous material bodies, and their effects are mediated by direct contact. When these mechanical forces initiate, catalyze, or sustain a chemical reaction, the phenomenon is termed mechanochemistry.¹ Mechanochemistry is prevalent in both biological and nonbiological materials. These forces are classified as either tensile forces (force vectors pulling on the ends of a molecule in equal magnitude and opposite directions), compressive forces (force vectors pushing at the ends of a molecule in equal magnitude and opposite directions), or shear forces (force vectors pushing on the sides of a molecule in an unaligned fashion), and each manifests different effects on diverse chemical processes.² For example, mechanical forces have been used to perform solvent-free reactions,^{3–6} reduce reaction times,⁷ increase yields,⁷ and improve or control the selectivity of reactions.⁸ Visually striking examples include polymers that can change color in response to tensile and shear forces^{9,10} and mechanochemical events that elicit light emission through soundwave excitation of liquid in the phenomenon of sonoluminescence.¹¹

These forces are no less important in biology.¹² They are ubiquitous, operating across all kingdoms of life and functioning over a wide range of spatial scales. Plant cells use turgor pressure, the force within the cell that pushes the plasma membrane against the cell wall, to maintain the cell's shape and stability and drive growth.¹³ Stem cells differentiate into specific phenotypes based in part on the elasticity (the ability of a material to return to its original state upon removal of a distorting force) of the substrate on which they are grown.¹⁴ Intracellular processes involving biomolecular binding events can also rely on mechanical forces that affect allostery, protein unfolding, and DNA loop formation.^{15–17} In addition, cells evolved to have their own internal mechanochemical machines, including the eukaryotic motor proteins kinesins, myosins, and the bacterial protein SecA. Kinesins, which convert chemical energy to mechanical force by hydrolyzing ATP to move along microtubules, exert piconewtons of force that moves vesicles or organelles inside cells.¹⁸ Myosins hydrolyze ATP to pull against actin filaments and cause muscle contraction,¹⁹ and SecA pulls on the nascent chain segments translationally arrested by the SecM sequence, allowing translation to restart.²⁰ Recently, mechanochemistry has been discovered to occur in translation, the process by which the genomic information encoded in a messenger RNA (mRNA) is converted into a protein molecule. This process involves the catalytic action of the ribosome to covalently link amino acids together. Both experimental and theoretical studies have confirmed that forces generated during translation can modulate the catalytic action of the ribosome at the peptidyl transferase center (PTC), where amino acids are covalently linked together.^{21,22}

In this Perspective, we discuss the sources of mechanical force acting on nascent proteins during their synthesis, how these forces are transmitted along the ribosome, and the consequences for translation-associated processes. We also emphasize the theoretical aspects of mechanochemistry in translation and what we believe to be fruitful future directions for this field.

SOURCES OF MECHANICAL FORCE GENERATION DURING TRANSLATION

To understand a mechanochemical system, three essential features must be delineated: the energetic source of the mechanical force, the means by which this force is transmitted, and

the consequence for chemical transformations (Figure 1a). Of these three features, the sources of force generation that occur during translation (“cotranslational”) have been the most thoroughly studied. Four cotranslational sources of tensile force have been identified (Figure 1b). These include entropic forces arising from unstructured nascent chain segments emerging from the exit tunnel,²² forces arising from the free energy of domain folding,²³ the cotranslational translocation of nascent chains across membranes,²⁴ and their insertion into membranes.²¹ Below, we explore recent discoveries concerning each of these mechanical force-generating processes.

Unstructured Nascent Chain Segments Generate a Tensile Force.

Unstructured polymers experience a pulling force as they move from a confining volume to an open space because during this process they gain configurational entropy and lower their free energy.²⁵ This movement from a highly confining environment to a less confined space mirrors what happens to nascent protein segments as they emerge from the ribosome (Figure 2). As a nascent protein is elongated, it passes through the exit tunnel that is 80–100 Å in length and, on average, 15 Å in diameter.²⁶ The first 60 Å of the tunnel proximal to the PTC is too narrow to permit tertiary structure formation within the nascent chain. After nascent chain segments enter the last 30 Å of the tunnel, a “folding vestibule” with wider dimensions, they can begin to fold.^{27–29}

On the basis of this analogy of a polymer leaving a confined space, it was hypothesized²² that unfolded nascent chains exiting the ribosome tunnel would experience an entropic tensile force. An experimental approach and four levels of theory (as described below) were used to test this hypothesis. First, the extension of the nascent chain in the tunnel was determined experimentally with and without an additional unstructured N-terminal sequence attached to the nascent chain. A cysteine residue located in the nascent chain segment within the exit tunnel can be PEGylated (i.e., covalently modified by a polyethylene glycol molecule) in an amount linearly related to its distance from the PTC.³⁰ Thus, the fraction of PEGylated nascent chains was measured in these experiments, and the position of the reporter cysteine within the tunnel was estimated.²² The presence of the additional unstructured nascent chain segment relocated the reporter cysteine ~6 Å farther from the PTC, consistent with an entropic pulling force stretching the nascent chain segment inside the tunnel.²²

All-atom and coarse-grained simulations of these ribosome–nascent chain complexes were then performed to calculate the force that would give rise to the experimentally observed extension.²² All-atom simulations showed that the longer nascent chain generated a force that was experienced 90 Å away at the C-terminus of the nascent chain. However, these simulations did not reach equilibrium, preventing an accurate force measurement. The coarse-grained simulations did converge, however, and indicated that a force between 0.5 and 1.9 pN was generated for the protein containing the additional unstructured N-terminus. As an independent estimate of force generation, a polymer model³¹ was used to calculate the force required to stretch a polymer in an inert tube over the distances estimated from the PEGylation experiments. Depending on the persistence length of the nascent chain, this model estimates forces of 2.4–5.3 pN occur, while a second model³² treating the nascent

protein as an elastic rod in a high-force regime (which approximates confinement in the ribosome tunnel) estimates forces of ~1.5 pN. These results are consistent with the tensile forces calculated from the simulations,²² providing theoretical and computational evidence that as unstructured nascent chain segments emerge from the exit tunnel they can generate piconewtons of force that are transmitted back to the PTC in what can be viewed as a form of mechanical allostery.

Protein Folding Generates Forces.

While unstructured nascent chain segments can cause forces upon exiting the tunnel, other cotranslational processes involving changes in free energy should also generate force, primarily a tensile force. One such process is folding of the nascent chain. The cotranslational folding of the human-engineered Top7 domain was found to generate enough force to accelerate translation when translational “arrest” (i.e., very slow peptide bond formation) was induced by the SecM nascent protein sequence.²³ (SecM is a naturally occurring 17-residue protein sequence that dramatically slows translation when it is present in the exit tunnel via a mechanism involving extensive spatio-specific interactions with the exit tunnel wall^{33,34} and a proline residue at the PTC.³⁵) In these experiments, the translated mRNA sequence contained an N-terminal Top7 domain, followed by an unstructured linker of variable length, the SecM arrest sequence, and a C-terminal green fluorescent protein (GFP) to act as a reporter of expression of full-length protein (Figure 3a).²³ Goldman and co-workers²³ found that GFP was produced only when linkers of 15–22 residues were used, and not at shorter or longer linker lengths. They hypothesized that shorter linker lengths did not allow enough of the Top7 domain to emerge from the exit tunnel to allow it to fold and generate a force, and at longer linker lengths, the domain was folded but was too far from the ribosome surface to press against it and generate sufficient force to relieve arrest²³ (Figure 3b). This molecular hypothesis was later confirmed by simulations.³⁶

To estimate the magnitude of the pulling force generated by Top7 cotranslational folding, laser optical tweezers were applied to individual Top7 molecules in the absence of the ribosome.²³ Top7 generates approximately 12 pN of force at its midpoint of stability, when it is folded half the time, although the axis along which the force is applied can affect the force a domain can generate.^{37,38} If the free energy of folding is the primary determinant of force generation, this suggests 12 pN could be the force generated cotranslationally, as well.

Many groups have since used arrest sequence assays developed by the von Heijne lab²¹ to investigate features of cotranslational folding, including domain folding pathways,^{39–41} locations of folding in the exit tunnel,^{42–44} and how factors such as chaperones⁴⁵ affect folding and force generation. In these assays, different proteins are covalently attached to a SecM sequence, with a linker of controllable length between them. When sufficient force is generated by the cotranslational process under study, an increase in the fraction of full-length protein produced occurs, typically quantified by gel electrophoresis. A recent study combining this assay and coarse-grained simulations of ribosome–nascent chain complexes demonstrated that simulated force measurements can reproduce the fraction of full-length protein measured in the experiment⁴⁰ (Figure 4a). Thus, there is a growing body of evidence

that cotranslational protein folding can generate tensile forces that act on nascent chain segments inside the exit tunnel.

The domain properties that determine how much force is generated have been identified in two recent studies. Farias-Rico and co-workers⁴⁶ experimentally determined the dependence of force generation on protein size, thermodynamic stability, and net charge of the cotranslationally folded protein. A second study by Leininger and co-workers³⁶ used coarse-grained simulations to assess the influence of a protein domain's thermodynamic stability, size, and topology, as well as translation speed, on force generation. Both studies found that increasing the thermodynamic stability of a specific, cotranslationally folded domain correlates with an increase in the generated force (Figure 5). Applying the arrest sequence assay to a library of mutated S6 protein domains arrested on the ribosome resulted in an approximately linear relationship between the fraction of full-length protein produced from each construct and the mutated domain's stability in the absence of the ribosome, indicating that greater stability correlated with greater forces.⁴⁶ Leininger and co-workers³⁶ studied five different proteins at 10 different stabilities in a set of coarse-grained simulations where the force on the C-terminal nascent chain residue was calculated. For each protein, they found the same trend at a fixed linker length: force increases with increasing domain stability but plateaus after the domain's folded state is ~3 kcal/mol more stable than the unfolded state. To explain this trend, they created a statistical mechanical model that assumes that the folded (N) and unfolded (U) states each generate a characteristic force ($F_{N,L}$ and $F_{U,L}$) at a given linker length (L). The force due to folding at any linker length can then be written as the product of the difference between these characteristic forces and the probability that the domain is in the folded state at linker length L [denoted by the conditional probability $P(N|L)$], which, when converted to a free energy, yields the relation

$$\Delta\langle\text{force}\rangle = (F_{N,L} - F_{U,L})P(N|L) = \frac{F_{N,L} - F_{U,L}}{1 + e^{\beta\Delta G_{UN,L}}} \quad (1)$$

where $G_{UN,L}$ is the free energy of folding at a given length and $\beta = 1/(k_B T)$, where k_B is the Boltzmann constant and T is the temperature. Across the five simulated proteins, eq 1 quantitatively describes the observed trends.³⁶ Equation 1 says that at a fixed linker length, as the stability of a domain increases (whether due to mutations or changes in the environment), the most rapid increase in force occurs around the midpoint of folding, and further increases in force eventually decay exponentially outside this region because further increases in stability do not appreciably change the percentage of folded protein molecules.³⁶

The results of Farias-Rico and co-workers and Leininger and co-workers at first seem contradictory. One was fit to a linear equation, and the other exhibits sigmoidal behavior; however, these differences can be reconciled. First, the free energy used on the x -axis of the Farias-Rico plot was not measured on the ribosome; instead, it is the free energy of the folded domain in the absence of the ribosome (i.e., the protein isolated in a test tube). The folded state is less stable when close to the ribosome surface, especially at linker lengths when folding is first possible.⁴⁷ Thus, using free energies in the absence of the ribosome, rather than the true free energies on the ribosome, could skew whether the experimental

fraction full-length data appear linear or sigmoidal. Second, all of the point mutants used in the experimental study of S6 are less stable than the wild type, which could make it difficult to sample the plateau region that Leininger and co-workers found when native-state stability increased.³⁶ As part of this Perspective, we have taken the Farias-Rico data set⁴⁶ and fit it to eq 1 and have found excellent agreement, suggesting the data are better described by eq 1 (Figure 5, solid lines) than by a linear equation. By fitting the data to the functional form of eq 1, we are modeling the common assumption that the fraction of full-length protein produced is a function of the probability that the domain is folded. This new analysis of the Farias-Rico data suggests that there is an onset of a plateau region, as expected both computationally and theoretically, and if S6 mutants more stable than the wild-type sequence had been used, then the plateau would have been observed experimentally.

Domain size, electrostatics, and topology also have consequences for force generation during cotranslational folding. The size of the domain influences the linker length at which force is generated, with larger domains generating force only at longer lengths, presumably because smaller domains are able to fold closer to the ribosome surface or deeper inside the exit tunnel than larger ones.^{46,48} However, the size of the domain is not predictive of the maximum force it can produce, based on results from computer simulations.³⁶ Electrostatics also plays a role in determining linker lengths at which force is generated. Domains with more positive net charges generate forces earlier than those that have large net negative charges.⁴⁶ Even intrinsically disordered proteins (IDP) exhibit such effects, in which a negatively charged IDP was found to generate a pulling force while a positively charged IDP was found to generate no force.⁴⁹ These results are consistent with the experimental finding by the Deutsch group that electrostatic potentials along the tunnel are negative,⁵⁰ due to the large RNA content of the ribosome. Thus, the ribosome tends to repel negatively charged domains and protein segments while attracting positively charged ones. An insightful approach to evaluating the effect of topology on force generation is circular permutation. In this approach, the domain size and the relative positioning of ordered secondary structural elements in Cartesian space are automatically maintained, and iso-stability is maintained through specific simulation parameters; however, the ordering of secondary structural elements and tertiary contacts along the primary structure are shuffled (Figure 6a,b). An *in silico* study³⁶ found that different topologies created in this way can dramatically modulate the maximum force generated by the folded domain (Figure 6c). Simulations of two nascent proteins attached to the ribosome reveal that the cotranslational folding of β -hairpins located at the C-terminal end of a domain pushes the more N-terminal portions of the domain into the ribosomal surface, generating more force than circular permutants of the domain that have other structural elements at the C-terminus.³⁶ Thus, experiments and simulations on arrested ribosomes reveal domain stability, topology, size, and electro-statics can determine the linker length at which folding and force are generated and the magnitude of that force.

Continuous Translation Can Decrease the Magnitude of the Tensile Force.

The experimental arrest sequence assay utilizes arrested ribosomes, which are at equilibrium, while continuous translation is out of equilibrium. Therefore, the tensile forces may differ between these two cases. To study this possibility, Leininger and co-workers³⁶ simulated the continuous translation of two proteins and found that arrested ribosome

measurements are a good approximation of continuous behavior when folding occurs faster than amino acid addition. When folding is slower than amino acid addition, however, folding of the domain occurs at longer linker lengths (Figure 6d), which decreases the force (Figure 6e). Surprisingly, even a delay in folding by a few residues can cause the force to disappear (Figure 6d,e). Using a database of estimated folding times for *Escherichia coli* cytosolic proteins, some 34% of the *E. coli* proteome falls into the latter situation.^{51,52} Thus, translation speed can modulate tensile forces on the ribosome, and we anticipate that an appreciable fraction of proteins that exhibit a force on arrested ribosomes will exhibit a decreased force during continuous synthesis.

Three Features Are Required for Cotranslational Folding Force Generation.

Through their set of simulations, Leininger and co-workers³⁶ were able to discern the necessary conditions for force generation and create a predictive statistical mechanical model. For a folding domain to generate forces that are transmitted to the PTC at a given linker length, the domain must (i) be folded, (ii) be in contact with the ribosomal surface, and (iii) have no slack in the linker protein segment. The probability of all of these prerequisites occurring can be represented by the conditional probability

$P\left(\frac{L_{ee}}{L_C} > 0.73 | L, N, T\right)$, where L , N , and T specify the linker length, the fact that the domain is folded, and the fact that the domain is in contact with the ribosome, respectively. The term $\frac{L_{ee}}{L_C} > 0.73$ states that the ratio of the linker sequence's end-to-end distance (L_{ee}) to its

contour length (L_C) is greater than the threshold of 0.73 used to define the absence of slack (0.73 is not arbitrary; it is the boundary between the intermediate- and high-force regimes of the extension curve of a wormlike polymer model chain stretched in an inert cylinder³⁶). The effects of translation rates on force are accounted for by expressing the probability of folding at a given length [$P(N|L)$] in terms of the folding (k_N), unfolding (k_U), and subsequent amino acid addition [$\omega_A(L+1)$] rates, resulting in the expression

$$P(N|L) = \frac{\omega_A(L+1)P(N|L-1) + k_N(L)}{k_N(L) + k_U(L) + \omega_A(L+1)}.^{53}$$

As in eq 1, assuming that the folded and unfolded states each produce a characteristic force (F_N and F_U , respectively), the force due to folding is then

$$\Delta\langle\text{force}\rangle = (F_N - F_U)P\left(\frac{L_{ee}}{L_C} > 0.73 | L, N, T\right) \frac{\omega_A(L+1)P(N|L-1) + k_N(L)}{k_N(L) + k_U(L) + \omega_A(L+1)} \quad (2)$$

Equation 2 provides the ability either to predict how changes in translation–elongation or domain folding kinetics influence force generation or to fit experimental data to extract underlying parameters in this equation. Tellingly, if any one of the three aforementioned conditions is removed from eq 2, the model predicts the wrong force trends, indicating each is a necessary condition to give rise to force at the molecular level.³⁶ Additionally, eq 2 emphasizes that elongation kinetics can modulate force generation during translation.³⁶

Membrane Insertion and Translocation Can Generate Forces.

The arrest sequence assay has been used to obtain trends in force arising from a variety of processes; however, it was first applied to monitor cotranslational insertion of transmembrane helices into a membrane.²¹ When transmembrane helices insert into a membrane via the Sec translocon, they produce a biphasic pulling force profile, with fraction full-length maxima at linker lengths of 30 and 40 residues.²¹ The first peak was hypothesized to occur when the transmembrane helix enters the Sec translocon, and the second peak was suggested to arise from the helix partitioning into the surrounding bilayer. This hypothesis was supported by a study that combined the arrest sequence assay with coarse-grained molecular dynamics simulations that reproduced the biphasic profile and gave a detailed description of the molecular mechanisms of force generation⁵⁴ (Figure 4b,c). Several factors were found to contribute to the strength of this tensile force. The force generated by C-terminal transmembrane helices is sensitive to its interactions with more N-terminal transmembrane helices. Upon mutation of interacting residues, the fraction of full-length protein varied, indicating the force is a function of these interactions. The fraction of full-length protein produced was also modulated by varying the length of the unstructured loops between the helices⁵⁵ and the topological orientation of the helices across the membrane.⁵⁶ In summary, the intra- and intermolecular interactions of the nascent chain with itself and the surrounding environment during membrane insertion all contribute to the tensile forces generated.

Transmembrane proteins are not the only proteins that interact with the Sec translocon; secretory proteins also pass through it while crossing the membrane to enter the lumen of the endoplasmic reticulum. Secretory proteins that contain charged residues may be subject to electrostatic forces across the endoplasmic reticulum membrane. This hypothesis was investigated using the arrest sequence assay. For a series of secretory proteins, with varying numbers and types of charges, biphasic fractions of full-length profiles were observed.²⁴ The first phase contained a large peak presumably reflecting the electrostatic force caused by the transmembrane electrical potential. The second peak may reflect interactions between charged residues and the surface potential at the membrane. Additionally, the fraction of full-length protein produced increased with the number of charged residues or charge density. Finally, a physics-based model that was a function of the transmembrane electrical potential and the membrane surface potential was able to quantitatively reproduce the experimental data derived from sequences containing a single stretch of negatively charged residues.²⁴ However, the model was inaccurate when predicting the trends in the presence of mixed positive and negative charges in the sequence, indicating that additional features beyond transmembrane electrical potential and membrane surface potential need to be incorporated into the model. Such features might include hydrophobic and electrostatic interactions between the nascent chain and translocon, electrostatic interactions between the nascent chain and ribosome, or electrostatics within the nascent chain.²⁴

EXPERIMENTAL BIAS TOWARD DOMAINS WITH C-TERMINAL HAIRPIN STRUCTURES

Simulations show that C-terminal β -hairpins generate disproportionately large forces during cotranslational folding because they push the nascent protein into the ribosome surface.³⁶ Motivated by these observations, we surveyed the literature of experimental studies to determine how frequently structures containing C-terminal hairpin structures have been used in arrest sequence assays. A C-terminal hairpin structure is defined as two antiparallel secondary structural elements, each containing at least four residues and forming at least three van der Waals contacts, connected by a loop, that are also the last two secondary structure elements in the domain (Figure 7). Of the 24 unique domains that have been studied using the arrest sequence assays,^{23,39–46,57,58} 21 of them contain a C-terminal hairpin. We then examined what percent of cytosolic *E. coli* domains (using crystal structures from the Protein Data Bank⁵²) have C-terminal hairpins. We find that $21.8 \pm 2.4\%$ (error bar = 95% confidence interval computed from bootstrapping; $n = 1210$ domains) of the domains in these proteins contain a C-terminal hairpin. Thus, there is a 4-fold experimental bias ($88\% = 21/24$ vs 22%) toward reporting results on domains with C-terminal hairpins. This over-representation might come from underlying correlations, especially as most experimentally studied domains are small. Therefore, we examined whether small domains are naturally enriched in C-terminal hairpin structures. When we defined small domains as those with fewer than 100 residues, intermediate domains as those with 100–200 residues, and large domains as those with more than 200 residues, we find that $19 \pm 4\%$ of small domains, $25 \pm 4\%$ of intermediate domains, and $19 \pm 5\%$ of large domains contain C-terminal hairpin structures. As the domains studied experimentally have a median size of 81 residues, we can rule out the possibility that there is a bias of C-terminal hairpin structures in small domains that would explain their over-representation in experimental studies. Alternatively, domains without C-terminal hairpins might give poor signal-to-noise ratios because of the smaller forces they tend to generate and for this reason might be left unreported in the literature. Regardless of the reason, these results indicate the proteins studied thus far are not representative of the *E. coli* cytosolic proteome on the whole.

HOW FORCES ARE TRANSMITTED FROM THE EXIT PORT TO THE PTC OF THE RIBOSOME

All four known sources of force generation take place 60–110 Å from the PTC. How can a pulling force generated from so far away be transmitted to the PTC, and is any of that force attenuated along the way, for example, due to thermal motion or nonspecific interactions of the nascent chain with the ribosome? Two mechanisms of force transmission from the cytoplasmic end of the exit tunnel to the PTC have been proposed: through the protein backbone or through the exit tunnel walls³⁵ (Figure 8).

A series of simulations performed by Fritch and co-workers²² addressed both of these questions showing that force transmission occurs primarily through the backbone of the nascent protein and the tensile force is not attenuated during transmission to the PTC. Fritch and co-workers first ran all-atom and coarse-grained simulations of an unstructured nascent

chain on a ribosome with all of the ribosomal interaction sites fixed, thus preventing any force from being transmitted allosterically via the exit tunnel walls, and measured the forces on the C-terminal residue attached to the P-site tRNA at the PTC and on a reporter cysteine residue 31 residues away at the exit port of the tunnel. The tensile forces at these two opposite ends of the tunnel were statistically indistinguishable, demonstrating that all of the force generated by the portion of the nascent chain outside of the tunnel was transmitted along the protein backbone to the PTC. Next, Fritch and co-workers allowed ribosomal interaction sites composing the walls of the exit tunnel to fluctuate but harmonically restrained the fourth nascent chain residue from the PTC, thereby preventing the transmission of force to the PTC via the protein backbone. In this case, no tensile force was experienced at the PTC, indicating there was no force transmission through the tunnel wall. While these simulations confirm that force is transmitted through the protein backbone with little attenuation, they do not definitively rule out transmission through the exit tunnel walls due to the long equilibration times required for large all-atom simulations. However, these results do suggest that any force transmission through the exit tunnel walls is likely to be minor compared to that transmitted through the protein backbone.²²

EFFECTS OF MECHANICAL FORCE ON RIBOSOME CATALYSIS

Forces are generated and transmitted through the nascent chain segments in the exit tunnel to the C-terminus of the protein, but how can such tensile forces modulate the chemistry at the PTC of the ribosome?

The PTC has three distinct functional locations, the A, P, and E sites that can accommodate tRNA molecules. The key steps of the ribosome catalytic cycle include accommodation at the A site that allows entry of an aminoacylated tRNA into the ribosome, peptide bond formation between the peptidyl-tRNA located at the P site and the amino acid at the A site, the simultaneous translocation of the tRNAs at the P and A sites to the E and P sites, and release of the tRNA from the E site, which completes the catalytic cycle. The rate of any one of these steps could potentially be altered by mechanical forces, thereby affecting the overall rate of protein synthesis. To date, only the effect of tensile force on the peptide bond formation step has been examined.

Circumstantial evidence from cryo-electron microscopy structures of ribosomes translationally slowed by SecM strongly implies that tensile forces can alter the structural configuration of the reactants at the P site, thereby slowing the rate of peptide bond formation. Specifically, the SecM sequence has been observed to shift the P-site amino acid 2 Å away from the A-site amino acid.³⁴ When the SecA motor protein pulls on the SecM sequence in vivo, the resulting force presumably shifts the P site back into place and thereby accelerates peptide bond formation.²⁰ Similarly, when optical tweezers have been used to apply force, they too relieve SecM-arrested ribosome–nascent chain complexes, with larger forces able to relieve arrest more quickly.²³ These arrest peptides are an extreme case of variation in translation rates and are extremely rare in nature, as only 12 have been identified of 100000 unique proteins.⁵⁹ Thus, it is important to understand the effects of force on translation in non-arrested cases.

The influence of piconewtons of tensile force on peptide bond formation by the ribosome was studied using quantum mechanical/molecular mechanical calculations in which peptide bond formation was simulated in the absence or presence of 1.9 pN of tensile force applied to the nascent chain. The free energy profile of the reaction revealed that the transition-state barrier decreases in the presence of a tensile force (Figure 9).²² This suggests the intriguing and potentially fundamental concept that forces generated by the elongating nascent peptide can accelerate catalysis by the ribosome for non-arresting nascent chain sequences. Thus, tensile forces can modulate the rate of peptide bond formation by lowering the energy barrier to peptide bond formation.

FUTURE DIRECTIONS

This Perspective has focused entirely on tensile forces acting on nascent proteins because this is the only class of force reported in the literature. Therefore, a fundamental question for future research will be to ask whether compressive and shear forces act on nascent proteins to modulate translation. Any time the exit tunnel is obstructed there is the potential to generate compressive forces. The magnitude and consequences of such forces on nascent chain elongation rates and folding are ripe for investigation. One intriguing possibility is that steric and/or electrostatic obstruction, especially at the narrow constriction of the exit tunnel, might occur with the binding of antibiotics⁶⁰ in the tunnel or the interaction of nascent chain sequence motifs with particular regions of the tunnel. Shear forces exist when there is mass flow in a system and instantaneous shear forces can arise as thermal motion stochastically moves two portions of a molecule in opposite directions. During translation, the binding and unbinding of cotranslationally acting factors, such as chaperones and enzymes that interact with the nascent chain, could induce hydrodynamic flows that create shear stress on the nascent protein. Translation occurs in the cytoplasm; therefore, cytoplasmic streaming is another source of mass flow inside cells that might generate shear forces as the flow passes across the ribosome.⁶¹ These situations therefore represent potential scenarios in which compressive or shear forces are created. The influence of such forces on cotranslational processes has not yet been investigated and is a promising area of future research.

The strong experimental bias toward studying force generation by proteins that have C-terminal hairpin structures is concerning as it suggests the possibility that the insights gained from the studies to date might not be generalizable to the broader proteome of organisms. It is important for the field to minimize such bias by studying a broader, more representative, set of proteins. Ideally, a random selection of globular protein domains from *E. coli* and yeast should be examined experimentally.

One of the largest gaps between almost all current experimental studies of force generation on the ribosome and what happens *in vivo* is that translationally arrested ribosomes are used. While some proteins will exhibit rapid folding relative to typical codon translation times, others can exhibit comparable time scales. In the latter case, as was found in computer simulations, the tensile forces generated can be attenuated or disappear altogether. Thus, delineating the boundary between domains that exhibit force during continuous translation and those that do not would benefit the field. This will identify proteins more likely to

undergo mechanochemical modulation on the ribosome under physiological translation conditions.

While four different processes (the emergence of an unstructured nascent chain, the folding of domains, trans-location across membranes, and the insertion into membranes) have been shown to generate tensile force, any one of 11 different cotranslational processes⁶² known to act on nascent proteins has the potential to generate a force. Several untested sources that might generate a force are listed in Figure 1b (red). A guiding principle concerning the potential for each of these processes to generate a pulling force comes from statistical mechanics, where the equilibrium tensile force generated is proportional to the free energy gradient in the system. Thus, the greater the change in free energy per unit distance, the larger the force will be. For example, this rule predicts that domains that fold cooperatively (i.e., without intermediates) will tend to generate forces larger than those generated by domains that fold less cooperatively. In the latter case, the change in the free energy of folding is spread out over more linker lengths than in the former case. This statistical mechanical rule also predicts that larger forces will be generated by cotranslationally acting factors that bind to the nascent chain with stronger binding affinities, as they have greater free energies of binding.

Force generation, transmission, and chemical consequences drive many molecular processes. Recent discoveries now reveal that mechanochemistry is also relevant to translation, a key step in the biogenesis of proteins across all of the kingdoms of life. The next several years of investigation in this area should be fruitful as many of the aforementioned fundamental questions can be addressed with existing experimental technology and theoretical and computational tools. Promoting further interactions and communication between experimental and theoretical researchers will accelerate the pace of discovery in this field.

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Figure 1.

Cotranslational processes generate force that acts on the catalytic core of the ribosome. (a) Cotranslational processes involve the nascent chain (green) and occur outside or at the cytoplasmic end of the ribosomal exit tunnel (red region), and the forces they generate can be transmitted through the nascent chain inside the exit tunnel (purple region) to the PTC (located in the blue region), where they can act on peptide bond formation and potentially other substeps of the ribosome's catalytic cycle. (b) Four cotranslational processes have been shown to generate forces (blue). Many other cotranslational processes and interactions have the potential to generate forces because they cause changes in free energy but have yet to be tested (red).

Figure 2.

Unstructured nascent chain segments can generate a pulling force. The nascent chains in panels a and b are the same length; however, the N-terminus in panel a remains within the exit tunnel where it is more confined, while the N-terminus in panel b has left the exit tunnel and is free to adopt a wide variety of conformations. This increases the entropy of the system and decreases the free energy of the system, generating an entropic pulling force transmitted back to the PTC.

Figure 3.

Cotranslational protein folding generates forces. (a) Arrest sequence assays use protein constructs similar to that shown. They start with a domain of interest at the N-terminus, attach a linker of variable length, attach an arrest sequence, and occasionally have a reporter domain such as GFP at the C-terminus. (b) Experiments and simulations have shown that there is a narrow range of linker lengths where folding forces occur. Simulations have shown in molecular detail that short linkers (top) do not allow the domain to cotranslationally fold, so no force is generated³⁶ (and arrest sequence assays exhibit a correspondingly small fraction of full-length protein produced). Long linkers (bottom) allow the domain to fold; however, the domain has fewer contacts with the ribosome that are needed to generate force, and there is more slack in the linker, which weakens its ability to transmit force if any is generated. Linkers of an ideal length (middle) allow the domain to fold and push against the ribosome, with little slack in the linker, making force generation and transmission as

efficient as possible. It is at these lengths where higher fractions of full-length protein are observed in arrest sequence assays.

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Figure 4.

Experimental and simulation results are in good agreement for cotranslational folding and insertion. (a) Simulation and experimental results agree well for the cotranslational folding of a Titin I27 domain. Panel a modified from ref 40. (b) Simulation and (c) experimental results show similarly aligned force peaks for the cotranslational insertion of polyleucine hydrophobic segments into a membrane. Blue and green dotted lines added for emphasis. Panels b and c modified from ref 54.

Figure 5.

Statistical mechanical model (eq 1) that can accurately describe the results from varied data sets. Equation 1 was developed to describe simulation data (blue) of protein 1F0Z and utilizes the free energies of the folded protein on the ribosome at a given linker length. The experimental arrest sequence data (red) report the fraction of full-length protein produced vs free energies of mutated S6 protein in the absence of the ribosome. However, the ribosome is known to destabilize folded domains. To account for this, we added a constant value of 5.75 kcal/mol to all of the experimentally reported stabilities, which shifts the free energy of the domains such that reported half-maximum fraction full length is at $G_{UN,L} = 0$. We find the

$$\text{model } f_{FL} = \frac{f_{FL,N} - f_{FL,U}}{1 + e^{\beta(\Delta G_{UN,L} + 5.75)}} \text{ [where } f_{FL} \text{ is the fraction of full-length protein produced}$$

and $f_{FL,N}$ and $f_{FL,U}$ are the characteristic fractions of full-length protein produced if the domain was in the folded (N) and unfolded (U) states, respectively] describes the trend in the experimental data well. Note that the left y-axis corresponds to the force for the data presented as blue circles while the right y-axis corresponds to the fraction full-length data presented as red triangles. Data for S6 taken from ref 46. Data for protein 1F0Z and the model taken from ref 36.

Figure 6.

Topology and translation speed modulate the forces generated during cotranslational folding.

(a) Linear representations of wild-type and rewired protein 1P9K, with helices shown as cylinders and strands represented by arrows. (b) Crystal structures of these domains, colored from red (N-terminus) to blue (C-terminus). (c) The presence of C-terminal β -hairpins leads to the generation of large forces. (d) In the case of continuous synthesis, folding of the 2IST domain is delayed by six residues. (e) When the translation rate is faster than the folding rate (purple triangles and red squares), folding domains (data for 2IST with a C-terminal linker of variable length) generate less force because they fold at longer linker lengths. Figure modified from ref 36.

Figure 7.

Examples of domains (a) with and (b) without C-terminal hairpins. For this analysis, we defined C-terminal hairpin structures as the last two secondary structural elements in a domain, each with at least four residues, which are antiparallel and connected by a loop and form at least three van der Waals contacts. Hairpin-containing structures are PDB entries 1QYS, 3GB1, 1A6J, and 1LOU (from left to right, respectively). Structures lacking hairpins are 1BIA, 1NY8, 2JSO, and 1DXE (from left to right, respectively).

Figure 8.

Nascent protein backbone that is the primary route of force transmission through the exit tunnel. Fritch and co-workers used a series of simulations to show that force is transmitted through the protein backbone (black arrow) and not the tunnel walls (yellow arrow).

Figure 9.

Tensile forces reduce the free energy barrier to peptide bond formation. (a) Structural representation of the reaction corresponding to peptide bond formation, with the movement of electrons colored red. (b) Force vector acting on the P-site residue. The ribosome (gray) is cropped to show only the portions near the PTC. The P-site residue is colored blue (the other nascent chain residues have been omitted for the sake of clarity), and the P-site tRNA is colored cyan. When in an extended conformation, the nascent chain pulls at an angle 15° below the long axis of the exit tunnel (the yellow arrow shows the direction of the force vector reported in ref 22), which reduces the free energy barrier to peptide bond formation between the P-site residue and the A-site residue (red), which is attached to its tRNA (magenta). (c) Quantum mechanical/molecular mechanical calculations show that when tensile forces are present (in this case due to the entropic pulling force arising from a longer

unstructured nascent chain as compared to a shorter one), a lower free energy barrier to peptide bond formation occurs. Panels a and c modified from ref 22.

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