JBC EDITORS' PICK HIGHLIGHT

Forcing the ribosome to change its message

DOI 10.1074/jbc.H120.013747

Sarah E. Leininger[‡], **Carol Deutsch**[§1], **and Edward P. O'Brien**[‡¶2]

From the [‡]*Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania 16802, the* [§]*Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and the* [¶]*Bioinformatics and Genomics Graduate Program, Institute for Computational and Data Sciences, Pennsylvania State University, University Park, Pennsylvania 16802*

Edited by Karen G. Fleming

Mechanical forces can be generated when nascent protein segments are integrated into a membrane. These forces are then transmitted through the nascent protein to the ribosome's catalytic core, but only a few biological consequences of this process have been identified to date. In this issue, Harrington *et al.* present evidence that these forces form a conserved mechanism to influence the efficiency of ribosomal frameshifting during translation of viral RNA, indicating that mechanical forces may play a broader regulatory role in translation than previously appreciated.

Mechanical forces are ubiquitous in biology, occurring on a wide range of spatial and temporal scales and functional roles. The mechanisms underlying mechanical force generation on the ribosome, and their consequences for translation and co-translational processes, are at an early and exciting stage of discovery. To date, four co-translational processes that generate force have been identified, including entropic pulling generated by unstructured nascent chain segments emerging from the ribosome exit tunnel (1), co-translational domain folding (2, 3), insertion of protein segments into a membrane (4), and protein passage through the translocon into the endoplasmic reticulum lumen (5). Many more sources of force have been hypothesized (6). These forces can relieve ribosomal stalling and increase translation rates (1, 2, 4), thereby allowing proteins to fold or interact with the translocon, a protein-lined membrane pore, at the proper time and chain length before continuing synthesis. Harrington *et al.* (7), in this issue, demonstrate a new effect of mechanochemical allostery. They show, for the first time, that mechanical forces generated by translocon-mediated membrane integration facilitate programmed ribosomal frameshifting in viruses, which results in production of a different protein (7) (Fig. 1). Moreover, they find that the novel mechanism responsible appears to be topological isomerization of the emerging nascent protein (7).

Ribosomes usually need to maintain a fixed reading frame while translating the information in an mRNA into a protein molecule. Some mRNA sequences, however, have evolved to let the ribosome shift its reading frame under certain circumstances, thus allowing multiple, unique proteins to be stoichiometrically produced from the same mRNA (8). This is especially important for viruses, whose genome size is constrained by the limited space within the viral capsid, but also occurs in all three kingdoms of life (8). The enveloped Sindbis virus, which is part of the larger evolutionarily related alphavirus class, uses a single transcript to encode its five structural proteins. In ~16% of nascent chains, the ribosome frameshifts and instead produces a sixth protein, a virulence factor (7). Alphaviruses contain conventional frameshifting regulators, including a "slippery" poly-U section of RNA and a downstream RNA secondary structure that provides a pause site for translation. Now a third key, and distinctly different regulator, has been uncovered: Mechanical force modulates the efficiency of programmed frameshifting.

In an elegant series of biochemical and computational studies, Harrington *et al.* first demonstrate that different topologies of Sindbis virus's capsid protein, E2, exist in membranes. They observed two different glycosylation patterns, which they interpret as two different topological populations based on a combination of experiments. The dominant E2 topology has a single transmembrane segment; a second population, formed only 20% of the time, has two transmembrane segments. Because the frequency of populating this alternative topology is similar to the frequency of frameshifting (about 16%), the authors hypothesize that integration of the second transmembrane segment (denoted TM2) is linked to frameshifting. To test this hypothesis, they designed two mutants— one that increased the propensity of TM2 to insert into the membrane and one that decreased it. They indeed found that the former led to more frameshifting, and the latter had less frameshifting. This suggests that events that happen to nascent chain segments at or near the translocon are communicated more than 10 nm to the A- and P-sites of the ribosome.

Mechanical force, whose magnitude is proportional to the probability of membrane insertion (4), provides a natural mechanism for such long-range communication. Thus, changing the length of the sequence between TM2 and the poly-U slip site should alter the force experienced on the tRNA when this slip site is being translated (4) and consequently alter the efficiency of frameshifting. To test this prediction, the authors inserted or deleted various numbers of amino acids along the

The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

[1] Supported by National Institutes of Health Grant R01 GM052302. To whom correspondence may be addressed. E-mail: cjd@pennmedicine.upenn.edu.

[2] Supported by National Science Foundation Grant MCB-1553291 and National Institutes of Health Grant R35 GM124818. To whom correspondence may be addressed. E-mail: epo2@psu.edu.

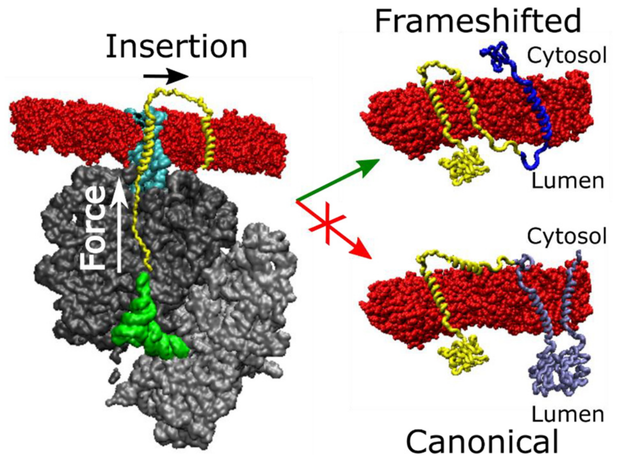

Figure 1. When TM2 (*yellow helix*) inserts (*black arrow*) into the membrane (*red*), it generates a force (*white arrow*) that is transmitted through the nascent chain to the tRNA (*green*) and promotes frameshifting (*green arrow*), producing the TransFrame protein (*dark blue*) instead of the canonical form of the polyprotein (*lavender*) that is synthesized when insertion of TM2 does not occur (*red arrow*). The structure on the left was created from Protein Data Bank entry 4V6M. The hypothetical protein membrane structures on the right were created from Protein Data Bank entries 4V6M and 1WYK.

nascent chain to modify the distance between TM2 and the slip site. All such mutants drastically decreased the rate of frameshifting, suggesting that the Sindbis structural polyprotein has evolved an optimal chain length to ensure force generation and promote efficient frameshifting. As a further test, the authors ran coarse-grained molecular dynamics simulations of the WT and mutant proteins and found that the simulated pulling force was highest for the mutant that experimentally exhibited the most integration of TM2 into the membrane, and the pulling force was lowest for the mutant that exhibited the least integration of TM2. Finally, Harrington *et al.* examined the sequences of six related alphavirus polyproteins and found that all of them had marginally hydrophobic transmembrane segments 44–52 residues upstream of a slip site, suggesting that the use of force to aid in frameshifting may be an evolutionarily conserved mechanism across alphaviruses.

These results identify a new biological role for co-translationally generated mechanical forces and add to a growing appreciation of a role for mechanochemistry during translation. At the molecular level, forces acting on the nascent chain have been shown to change the relative orientations of the A- and P-site amino acids, changing the free energy barrier to peptide bond formation (1), or disrupt stalling sequence interactions between the nascent chain and the exit tunnel wall (9). In the case of the E2 protein, however, the frameshifting observed suggests that the force most likely modifies the interactions between the tRNA and the mRNA. This opens up the possibility that other translational phenomena dependent on these interactions, such as the read-through of stop codons, could exhibit a force dependence as well. Thus, by providing evidence that mechanical force is a conserved mechanism by which viruses can influence frameshifting, Harrington *et al.* have shown that mechanochemistry on the ribosome has more wide-reaching regulatory effects than previously assumed. There are many other co-translational processes that have the potential to generate forces or be influenced by them. Exploring and understanding these possibilities is an exciting area of future research and, as done in this study, requires the combined efforts of experimentalists and theorists.

References

1. Fritch, B., Kosolapov, A., Hudson, P., Nissley, D. A., Woodcock, H. L., Deutsch, C., and O'Brien, E. P. (2018) Origins of the mechanochemical coupling of peptide bond formation to protein synthesis. *J. Am. Chem. Soc.* **140,** 5077–5087 CrossRef Medline
2. Goldman, D. H., Kaiser, C. M., Milin, A., Righini, M., Tinoco, I., Jr., and Bustamante, C. (2015) Mechanical force releases nascent chain–mediated ribosome arrest *in vitro* and *in vivo*. *Science* **348,** 457–460 CrossRef Medline
3. Leininger, S. E., Trovato, F., Nissley, D. A., and O'Brien, E. P. (2019) Domain topology, stability, and translation speed determine mechanical force generation on the ribosome. *Proc. Natl. Acad. Sci. U.S.A.* **116,** 5523–5532 CrossRef Medline
4. Ismail, N., Hedman, R., Schiller, N., and von Heijne, G. (2012) A biphasic pulling force acts on transmembrane helices during translocon-mediated membrane integration. *Nat. Struct. Mol. Biol.* **19,** 1018–1022 CrossRef Medline
5. Ismail, N., Hedman, R., Lindén, M., and von Heijne, G. (2015) Charge-driven dynamics of nascent-chain movement through the SecYEG translocon. *Nat. Struct. Mol. Biol.* **22,** 145–149 CrossRef Medline
6. Leininger, S. E., Narayan, K., Deutsch, C., and O'Brien, E. P. (2019) Mechanochemistry in translation. *Biochemistry* **58,** 4657–4666 CrossRef Medline
7. Harrington, H. R., Zimmer, M. H., Chamness, L. M., Nash, V., Penn, W. D., Miller, T. F., 3rd, Mukhopadhyay, S., and Schlebach, J. P. (2020) Cotranslational folding stimulates programmed ribosomal frameshifting in the alphavirus structural polyprotein. *J. Biol. Chem.* 295, 6798–6808 CrossRef Medline
8. Dinman, J. D. (2006) Programmed ribosomal frameshifting goes beyond viruses. *Microbe Wash. DC* **1,** 521–527 CrossRef Medline
9. Gumbart, J., Schreiner, E., Wilson, D. N., Beckmann, R., and Schulten, K. (2012) Mechanisms of SecM-mediated stalling in the ribosome. *Biophys. J.* **103,** 331–341 CrossRef Medline