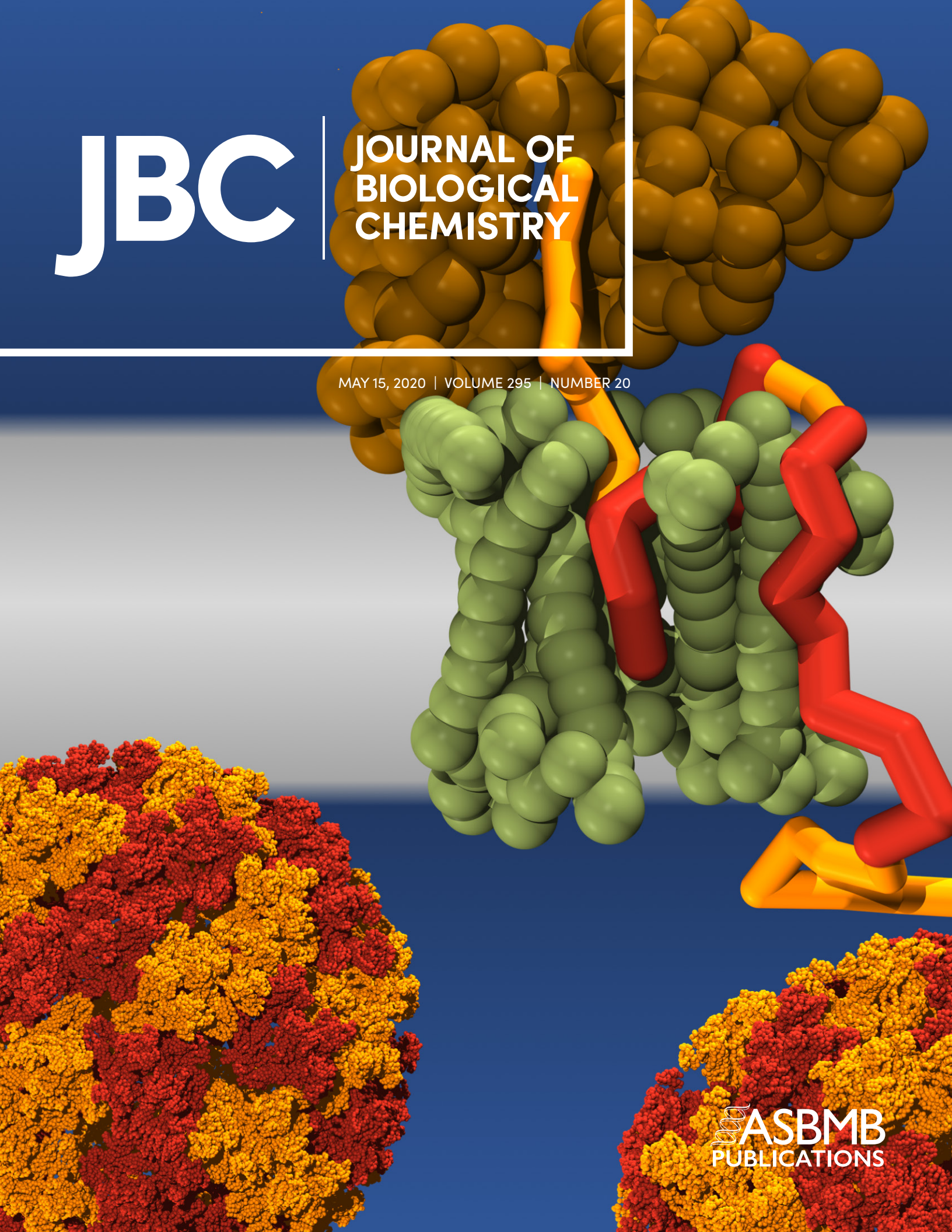
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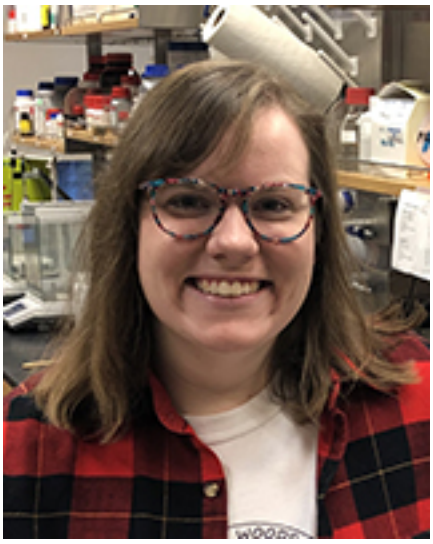
Haley R. Harrington



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How did you become interested in this topic?

I have always loved working with membrane proteins, but when I rotated in a virology lab during my first year of graduate school, I learned that I also loved working on viruses. My university has a very collaborative nature. I knew that my top two lab choices were starting a collaboration project, and I knew that was the project for me because it combined both of my interests so well.

Can you describe an exciting moment you experienced while doing this research?

I was finishing up the experiments for this paper at the same time I was preparing for my qualifying exams. I was tasked with practicing my presentation, and it's like everything came together to be the awesome story, and I was so proud of myself and all of the wonderful lab members and collaborators who made this work possible. It was truly exciting to see the story come together so clearly.

If you could go back in time and re-do this project, what advice would you give your past self?

Honestly, the biggest hurdle to this project was getting the cloning to work. As I am sure some people have experienced, cloning sometimes doesn't cooperate, and if I could go back I would tell myself the strategies that worked so that I didn't have to spend so much time troubleshooting, but hindsight is 20/20.

What do you hope to do next? Where do you seek scientific inspiration?

Right now I am keeping my options open and seeking opportunities to find what scientific career I am truly passionate about. I have had a number of scientific mentors throughout my career, and they all have different scientific careers now, so it's encouraging to know that we all have options and people we can talk to for advice.

Read Harrington's article on page 6798.

Forcing the ribosome to change its message

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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

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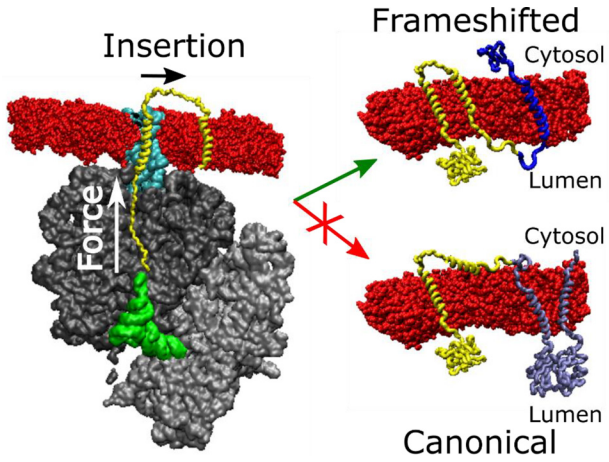

Figure 1. When TM2 (*yellow helix*) inserts (*black arrow*) into the membrane (*red*), it generates a force (*white arrow*) that is transmitted through the nascent chain to the tRNA (*green*) and promotes frameshifting (*green arrow*), producing the TransFrame protein (*dark blue*) instead of the canonical 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.

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