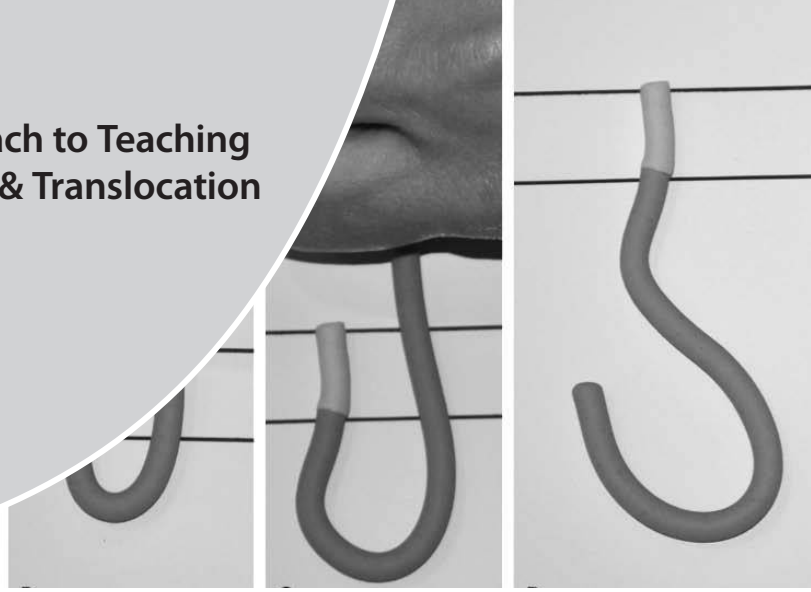
HOW TO DO IT

A Hands-on Approach to Teaching Protein Translation & Translocation into the ER

MICHELLE L. LABONTE



To view the color version of this article, including color photographs, go to dx.doi.org/10.1525/abt.2013.75.3.10.

Abstract

The process of protein translation and translocation into the endoplasmic reticulum (ER) can often be challenging for introductory college biology students to visualize. To help them understand how proteins become oriented in the ER membrane, I developed a hands-on activity in which students use Play-Doh to simulate the process of protein insertion into the ER membrane. After completing the hands-on activity, students are better able to solve problems in which they have to predict the membrane orientation of a protein.

Key Words: *Translation; endoplasmic reticulum; transmembrane protein; hands-on activity; introductory college biology; intermediate college biology.*

The location of a mature protein within or outside a cell is determined by signal sequences composed of either continuous or discontinuous stretches of amino acids within the protein. Proteins destined for the ER initially include an ER signal sequence, a continuous stretch of amino acids located within the newly synthesized polypeptide chain. Depending on the location of the signal sequence, it will either be removed from or retained within the mature form of the protein. Proteins destined for the ER are initially translated by ribosomes located in the cytosol. As the newly synthesized polypeptide chain emerges from the ribosome, an ER signal sequence is detected by a signal recognition particle (SRP) in the cytosol. The interaction of the SRP and the signal sequence causes translation to halt as the entire complex consisting of ribosome, mRNA, polypeptide chain, and SRP relocates to the ER membrane. The SRP then interacts with an SRP receptor on the ER membrane and translation resumes, during which time the polypeptide is translocated through a channel in the ER membrane (Alberts et al., 2009). The number and orientation of signal sequences within a polypeptide chain determine the final location of the mature protein. Because N-terminal ER signal sequences are cleaved by a signal peptidase located in the ER membrane, a polypeptide chain with a single N-terminal signal sequence will end up as a soluble protein in the ER lumen. A single-pass type I membrane protein is oriented in the ER membrane such that the N-terminal region of the protein is located in the ER lumen and the C-terminal region of the protein is located in the cytosol. A single-pass type II membrane protein has the N-terminal region of the protein in the cytosol and the C-terminal region of the protein in the ER lumen (Lodish et al., 2012). The presence or absence of additional signal sequences will determine whether a protein will remain in the ER or continue through the secretory pathway to the Golgi apparatus or plasma membrane.

The location of a mature protein within or outside a cell is determined by signal sequences composed of either continuous or discontinuous stretches of amino acids within the protein.

In teaching introductory cell and molecular biology college courses, I found that students had trouble understanding the process of protein translocation through the ER membrane. Given the literature on the effectiveness of hands-on learning (Satterthwait, 2010), I developed an activity in which students use Play-Doh to simulate and visualize protein insertion into the ER membrane. By spending time manipulating Play-Doh proteins, students are better able to determine the correct membrane orientation of proteins even after the hands-on activity has been completed.

Procedure

Before beginning the hands-on activity, I give a short lecture on protein translation and translocation into the ER in which I cover ER signal sequences, the translocation channel, the signal peptidase, and some examples of secreted proteins as well as single- and multi-pass membrane proteins (Alberts et al., 2009). To begin the hands-on activity, I draw the ER membrane on the white board and label the cytosol and the ER lumen. I make my own Play-Doh version of a secreted protein using light blue for the N-terminal

The American Biology Teacher, Vol. 75, No. 3, pages 211–213. ISSN 0002-7685, electronic ISSN 1938-4211.
DOI: 10.1525/abt.2013.75.3.10

Figure 1. A Play-Doh version of a polypeptide chain that will ultimately result in a mature secreted protein. The N terminal and C terminal regions of the protein and the signal sequence are indicated. See *ABT* online for color figure.

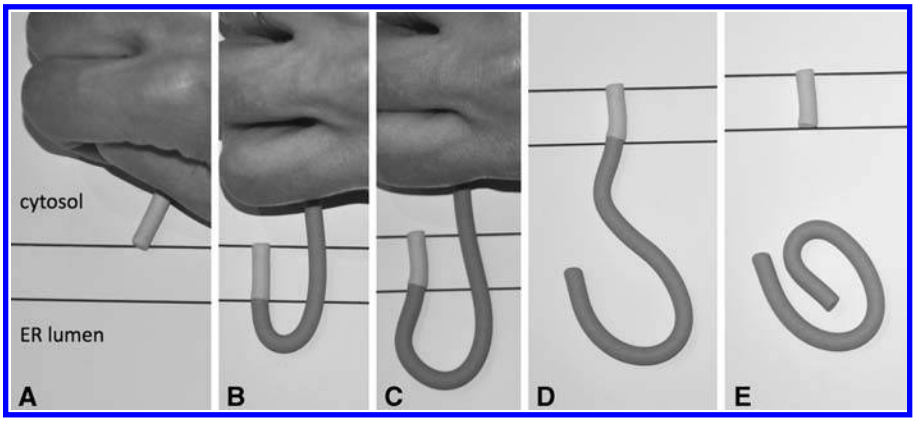


Figure 2. Simulation of the translation and translocation of a secreted protein. (A) Signal sequence appears from the ribosome (hand). (B) Translated polypeptide chain is translocated through ER membrane. (C) Translation and translocation continue. (D) Translation and translocation have finished. (E) N terminal signal sequence is cleaved. See *ABT* online for color figure.

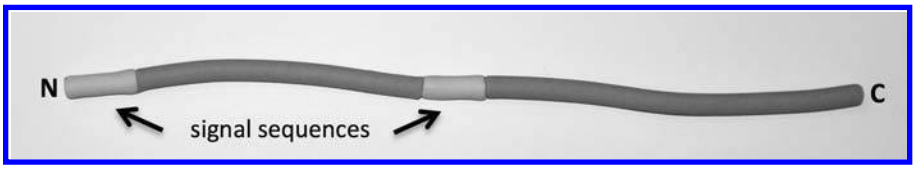


Figure 3. A Play-Doh version of a polypeptide chain that will ultimately result in a type I membrane protein. The N terminal and C terminal regions of the polypeptide chain and the signal sequences are indicated. See *ABT* online for color figure.

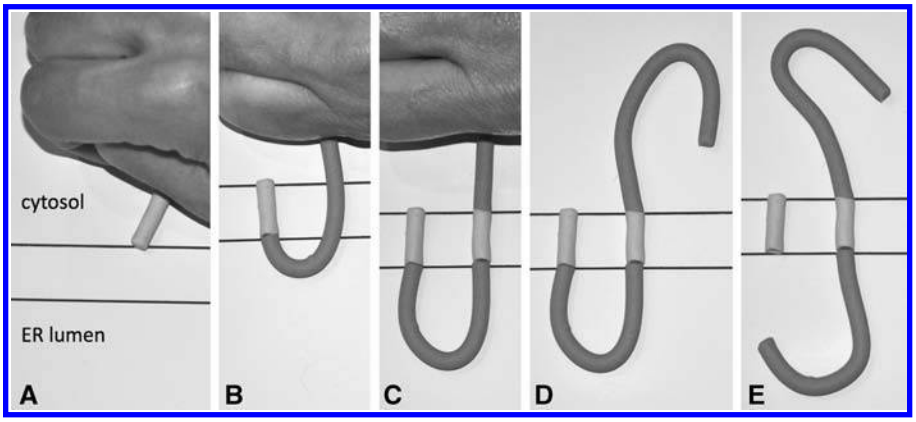


Figure 4. Simulation of the translation and translocation of a type I membrane protein. (A) Signal sequence appears from the ribosome (hand). (B) Translated polypeptide chain is translocated through ER membrane. (C) Translocation halts as second signal sequence enters ER membrane. (D) Translation continues. (E) N terminal signal sequence is cleaved. See *ABT* online for color figure.

signal sequence and dark blue for the rest of the protein (Figure 1). Next, I ask students to draw the ER membrane on paper and I instruct everyone to make a Play-Doh version of the polypeptide chain in Figure 1. I ask students to put their Play-Doh polypeptide aside while they watch me demonstrate how it would be translated and inserted through the ER membrane.

Using my hand as a ribosome, I conceal the polypeptide chain. I then allow the N terminal region of the polypeptide chain to leave my hand, simulating translation. I talk to the students about how the light blue signal peptide would be recognized by the SRP, translation would be temporarily stopped, and the whole complex of ribosome, SRP, mRNA, and partially translated polypeptide chain would be brought over to the SRP receptor at the ER membrane. Once at the ER membrane on the white board (Figure 2A), I simulate how translation would resume by letting out more of the polypeptide (now the dark blue section) from my hand.

As translation continues, I show the newly translated polypeptide chain entering the translocation channel in the ER membrane (Figure 2B). For simplicity of demonstration, I do not draw the translocation channel but instead have an image of it projected while I demonstrate the process on the white board. Translation continues (Figure 2C) and the rest of the polypeptide chain enters the ER lumen (Figure 2D). The signal peptide is cleaved by the signal peptidase (Figure 2E). I then ask the students to simulate the process individually at their desks.

Next, I move on to a single-pass type I membrane protein. I ask students to make their own version of the polypeptide chain (Figure 3) so that we can simulate the translation and translocation process together.

Using the same technique described above, I use my hand as a ribosome and demonstrate how a type I membrane protein becomes inserted in the ER membrane. The N-terminal signal sequence is revealed as the polypeptide chain emerges from the ribosome, and translation halts as the complex moves to the ER membrane (Figure 4A). Translation then resumes and the newly synthesized polypeptide chain is translocated through the ER membrane (Figure 4B). Translation and translocation continue until a second signal sequence emerges from the ribosome and subsequently gets stuck in the ER membrane (Figure 4C). The remaining C-terminal region of the polypeptide chain

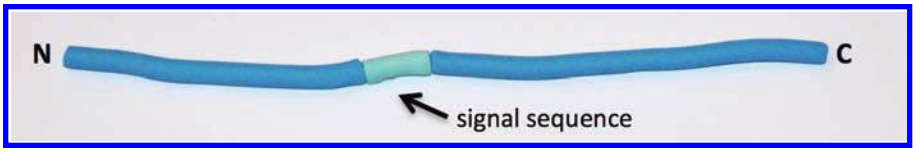


Figure 5. A Play-Doh version of a polypeptide chain that will ultimately result in a type II membrane protein. The N terminal and C terminal regions of the polypeptide chain and the signal sequence are indicated.

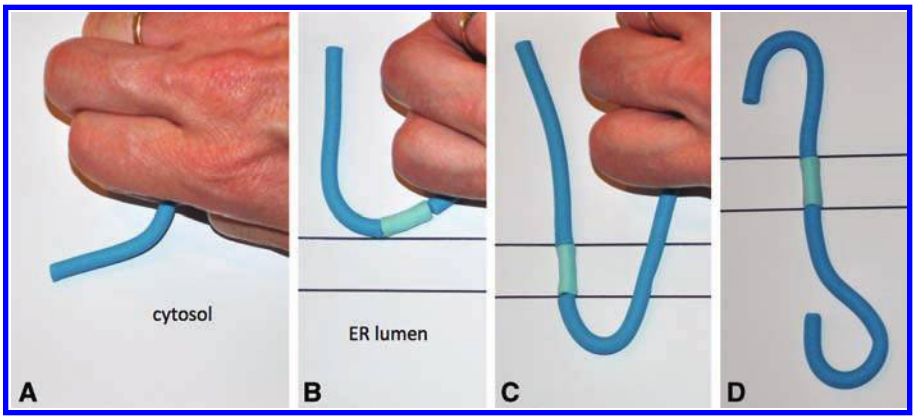


Figure 6. Simulation of the translation and translocation of a type II membrane protein. (A) N-terminal region of the polypeptide chain remains in the cytosol. (B) Signal sequence appears from the ribosome (hand). (C) Translated polypeptide chain is translocated through ER membrane. (D) Translation and translocation are complete and the signal sequence is retained in mature protein.

continues to be translated and remains in the cytosol (Figure 4D), and the N-terminal signal sequence is cleaved by the signal peptidase (Figure 4E). I have the students label the N and C terminal ends of the protein once they have completed the translation and translocation simulation.

Students are then asked to make a type II membrane protein. They begin by creating a polypeptide chain with a single internal signal sequence (Figure 5). After they have had a chance to simulate the translation and translocation of this polypeptide chain on their own, I demonstrate the process.

The N-terminal end of this polypeptide chain initially remains in the cytosol because it does not contain a signal sequence (Figure 6A). However, a signal sequence soon emerges and is recognized by the SRP. The entire complex then moves to the ER membrane (Figure 6B), where translation resumes and translocation of the protein begins (Figure 6C). The internal signal sequence remains part of the mature protein (Figure 6D). Students then do more complicated simulations such as multi-pass membrane proteins.

Conclusion

This hands-on activity is well received by students because it allows them to have fun while learning a difficult concept. Because students do not have access to Play-Doh during exams, I also spend time teaching them how to approach these problems without Play-Doh. I find that other, more traditional approaches to teaching this concept are better received after the students have had a chance to do the Play-Doh simulation on many different types of proteins. The Protein Translocation animation produced by Garland Science for *Essential Cell Biology, 3rd Ed.* (Alberts et al., 2009; video available at http://www.youtube.com/watch?v=PUy_Em5dXmc) is also a great resource to complement the Play-Doh simulation approach.

Acknowledgments

I thank the students in my BISC 110 Introductory Cellular and Molecular Biology courses at Wellesley College for enthusiastically taking part in this activity. In addition, I am grateful for the thoughtful suggestions and critiques provided by the anonymous reviewers.

References

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M. & others. (2009). *Essential Cell Biology, 3rd Ed.* New York, NY: Garland Science, Taylor & Francis.

Lodish, H., Berk, A., Kaiser, C.A., Krieger, M., Bretscher, A., Ploegh, H., Amon, A. & Scott, M.P. (2012). *Molecular Cell Biology, 7th Ed.* New York, NY: W.H. Freeman.

Satterthwait, D. (2010). Why are "hands-on" science activities so effective for student learning? *Teaching Science*, *56*(2), 7–10.

MICHELLE L. LABONTE is an Instructor in Biological Sciences Laboratory at Wellesley College, 106 Central Street, Wellesley, MA 02481; e-mail: mlabonte@wellesley.edu.