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Why a protein switches sides during translation

Sequences within growing transmembrane proteins determine when loops reverse direction to cross the ER membrane.

Focal Point

(Left to right) Arthur Johnson, Pen-Jen Lin, Candice Jongsma, Shuren Liao, and Martin Pool reveal how nascent transmembrane proteins snake back and forth across the ER membrane. As shown in the schematic diagram, their findings suggest that the ribosome (blue) initially fits snugly on top of the translocon (yellow) so that the newly made protein chain unreels into the interior of the ER (left). But the translation of a transmembrane segment (TMS1) opens up the ribosome and shuts the translocon pore, redirecting the nascent chain toward the cytoplasm (center). The system switches back when a second transmembrane segment (TMS2) is translated (right).

PHOTOS COURTESY OF JUDIT VAHIDMANI [JOHNSON], LISA EUBANKS [JONGSMA, LIAO, LIN], AND ARTHUR JOHNSON [POOL]

Multipass transmembrane proteins weave back and forth across the lipid bilayer. Two papers by Lin et al. (1, 2) reveal that information encoded within the proteins helps define this alternating pattern.

A protein destined for the plasma membrane usually starts out in the ER. As translation of the protein's mRNA begins, the newly made polypeptide slips into the ER membrane. As the protein elongates, it crisscrosses the membrane so that its hydrophilic loops are properly oriented either outside or inside the ER. A cell accomplishes this nifty stitching without allowing calcium ions to spill from the ER. During translation, a ribosome sits on a translocon (3), a pore that can open and close. However, researchers don't understand how the ribosome–translocon complex prevents calcium leaks. Another important question, says Arthur Johnson, senior author on both papers, "is how the system alternates delivery of these loops to opposite sides of the ER membrane at the proper time."

Previous studies on proteins with a single transmembrane segment (TMS) suggest that translation of that segment causes the nascent chain to reverse direction (4). But it was unclear whether this mechanism would enable a growing protein chain to feed through the membrane multiple times. To find out, Lin et al. allowed ribosomes to translate mRNA snippets that coded for two or three TMSs. The researchers tracked the resulting protein chains by tagging them with a fluorescent dye that goes dark when it encounters an iodide ion. By introducing iodide ions into the cytoplasm or the ER, the team could determine which side of the ER membrane the growing strands were exposed to. Each time a TMS was translated, the elongating chain reversed course, growing toward the cytosol instead of the ER interior or vice versa. Altering a TMS's amino acid sequence, length, or orientation didn't prevent it from causing a reversal, nor did changing how hydrophobic it was.

> "All of these structural changes are happening while the protein is still being made."

Translation of a TMS triggers a structural shift in the ribosome–translocon complex, the researchers conclude. When the ribosome sits flush on the translocon, which has its pore open, the nascent chain feeds into the ER. Translation of a TMS causes the pore to close and the ribosome to morph into an open state that directs the growing chain into the cytosol. The next TMS to come along prods the ribosome to slam down on the translocon and the pore to reopen, so that the nascent chain once again protrudes into the ER interior. This mechanism positions the growing protein's hydrophilic loops on one side of the ER membrane or the other without causing a calcium leak.

In their second study, Lin et al. investigated how a TMS triggers these changes. Shortly after being translated, each TMS slides into a tunnel in the ribosome that is lined by ribosomal RNA and a few proteins. The researchers discovered that, when the segment is opposite a protein in the tunnel called L17, the TMS coils into an α-helix. Whenever this happened, the growing protein chain at the membrane switched its destination, suggesting that TMS folding triggers the ribosome–translocon complex to reshape. Supporting that conclusion, Lin et al. showed that a soluble protein, the hormone prolactin, does not fold near L17 and does not warp the complex.

The findings indicate that a transmembrane protein controls when its loops reverse course. TMSs trigger the ribosome–translocon complex to contort and enable the lengthening protein to switch direction. "All of these structural changes are happening while the protein is still being made," says Johnson. A question the researchers would like to pursue is how TMS folding leads to alterations in the ribosome and translocon. They speculate that L17 and ribosomal RNA might help communicate the TMS shape change from the ribosome tunnel to the surface of the organelle.

1. Lin, P.-J., et al. 2011. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201103117.
2. Lin, P.-J., et al. 2011. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201103118.
3. Skach, W.R. 2009. *Nat. Struct. Mol. Biol.* 16:606–612.
4. Liao, S., et al. 1997. *Cell.* 90:31–41.