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Getting membrane proteins into shape

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In this issue, Ji et al.¹ show how a multipass membrane protein that initially inserts into the endoplasmic reticulum in a mostly inverted topology is post-translationally dislocated, re-inserted, and folded with the help of ATP13A1, a P-type ATPase.

About 3,000 membrane proteins in the human genome are woven back and forth multiple times across the lipid bilayer. These multipass membrane proteins have critical functions including signaling (e.g., G protein-coupled receptors), nutrient and metabolite transport, ion homeostasis, and membrane-related biochemistry. For decades, the prevailing model for eukaryotic multipass protein insertion was elegant in its simplicity: transmembrane domains (TMDs) are sequentially inserted one-by-one through a translocation channel as each TMD emerges from a channel-associated ribosome.^{2,3} The past few years have seen the emergence of new machinery and mechanisms that paint a more complex picture.⁴ The new study by Ji et al.¹ adds substantively to this shifting paradigm by revealing an active pathway for rectifying the topology of an initially mis-inserted multipass membrane protein.

The key initial observation by Ji et al. was that the 6-TMD protein ABCG2 is initially produced with only TMD1 inserted in the correct topology (Figure 1A). Most other parts of the protein are on the wrong side of the membrane, a conclusion deduced from an elegant series of topology assays relying on compartment-restricted modification reactions. Further detective work revealed that the mark-

edly hydrophilic TMD2 fails insertion completely and translocates into the ER lumen, causing TMD3 through TMD6 to adopt an inverted topology. Simply increasing the hydrophobicity of TMD2 completely avoids this problem, a solution not available to evolution because a hydrophilic TMD2 is critical to ABCG2 structure and function. Thus, the “first draft” of ABCG2 topology is almost entirely wrong. Remarkably, pulse-chase and compartment-restricted modification experiments provide compelling evidence that topologically incorrect ABCG2 is a precursor of mature cell-surface ABCG2.

How does the cell post-translationally rectify this topologically bungled ABCG2 precursor? Although a full answer will require considerably more investigation, Ji et al. have identified a critical first step in this process (Figure 1A). A key recognition element of an incorrect ABCG2 topology proved to be a conserved di-basic motif just downstream of TMD6. In mature ABCG2, this motif faces the cytosol (thereby fitting the “positive-inside” rule for membrane protein topology), whereas the motif faces the endoplasmic reticulum (ER) lumen when ABCG2 first completes co-translational insertion. Ji et al. find that the top interaction partner selective for topologically immature

ABCG2 is ATP13A1, implicating it in topological rectification.

This idea was attractive because of recent work showing that ATP13A1 is a widely conserved ER-resident ATPase that dislocates (to the cytosol) N- and C-terminal TMDs with short ER-luminal tails containing basic amino acids.⁵ Ji et al. now show that ATP13A1 acts on TMD6 of precursor ABCG2 in the same way—it uses an acidic groove to recognize the di-basic motif next to TMD6 and extracts it to the cytosol in an ATP-driven reaction. With TMD6 now out of the membrane, earlier TMDs can re-orient and re-insert, although the mechanisms involved are not known. If TMD6 cannot be extracted (e.g., in the absence of ATP13A1), subsequent re-orientation and maturation is inefficient. The requirement for ATP13A1 can be bypassed by preventing TMD6 insertion using a downstream small folded domain, thereby mimicking the dislocated state. This suggests that downstream re-orientation steps do not rely on ATP13A1, perhaps relying on yet unidentified factors.

A model emerges in which a first draft produced through co-translational and vectorial N-to-C insertion of ABCG2 is post-translationally rectified in a C-to-N direction. This begins by dislocating the last



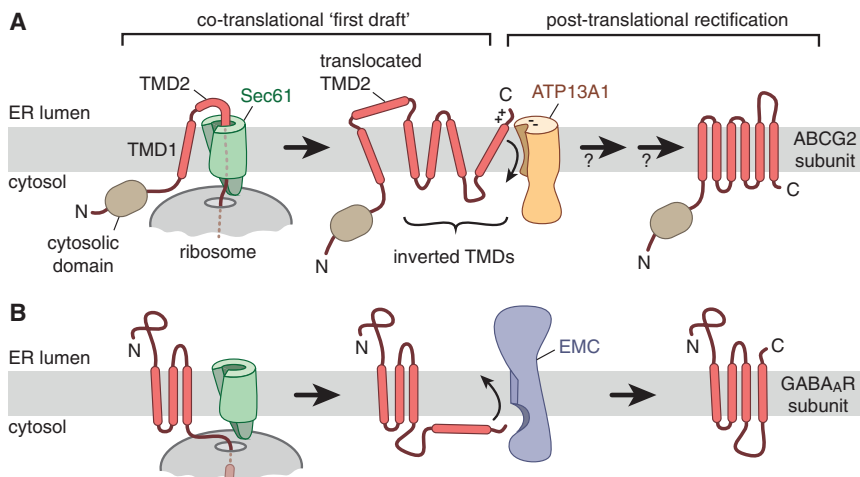


Figure 1. Topology of some membrane proteins involves two stages

(A) Co-translational translocation of ABCG2 at the ER membrane results in its semi-hydrophilic second transmembrane domain (TMD2) being transported into the lumen rather than inserted into the lipid bilayer. This causes downstream TMDs to be inverted. The inverted topology is recognized by ATP13A1, a P5-type ATPase that detects lumen-exposed positive charges flanking the final TMD and dislocates this TMD to the cytosol. This dislocation reaction initiates subsequent TMD reorientation and insertion steps, which are not well understood, to achieve the final correct topology.

(B) Co-translational translocation of the GABA_A receptor subunit ends before the final TMD is inserted. This topologically incomplete product requires a rectification step that is mediated by a TMD insertase called EMC.

TMD and culminates with insertion (from the ER lumen) of the initially problematic TMD2. Reserving the insertion of TMD2 until everything else is in place makes conceptual sense because its stability in the membrane critically depends on interactions with several other TMDs. This final insertion reaction occurs from the ER lumen, a process that is not only unprecedented in current paradigms of membrane protein biogenesis but for which no insertion machinery is yet known. Determining how this insertion reaction occurs will be an interesting future goal, especially as it seems to be linked in some unclear way to formation of a functional ABCG2 dimer.

One might have hypothesized that a semi-hydrophilic TMD2 would be temporarily “held” in the membrane via an intramembrane chaperone, a strategy that seems to be used for other multipass proteins.^{6,7} Perhaps TMD2 of ABCG2 cannot access this mechanism due to its particularly high hydrophilicity relative to the amphiphilic binding site of the PAT complex, a translocon-associated intramembrane chaperone. TMDs of varying hydrophilicity, including many whose residence in the membrane is strongly disfavored, are critical for the folding and function

of most multipass proteins. It therefore seems that temporarily accommodating such semi-hydrophilic nascent TMDs is a widespread and ancient problem that may have evolved multiple solutions, including intramembrane chaperones and post-translational insertion. Finding the factors and mechanisms for the handling of high-hydrophilicity TMDs is an important goal.

The work of Ji et al. underscores the idea that membrane proteins are often not made correctly during the first attempt. Although this has been appreciated for a long time, especially with problematic proteins such as CFTR, it now seems that erroneous first drafts are not always degraded. Instead, they can be post-translationally rectified, and in some cases such as ABCG2, the rectification process is a critical part of their biogenesis. An analogous but simpler situation where the topologically incorrect co-translational product is rectified post-translationally was recently described for a subset of multipass proteins with a TMD close to the C terminus⁸ (Figure 1B). Here, the final TMD is too close to the termination codon to be inserted co-translationally, resulting in a partially inserted translation product.

This final insertion reaction is mediated by a TMD insertase called EMC, an ER-resident member of the universally conserved Oxa1 insertase family.⁹ Whether EMC participates in later stages of ABCG2 re-orientation warrants investigation.

Thus, depending on the substrate, multipass membrane protein rectification can involve TMD dislocation by ATP13A1 or TMD (re)insertion mediated by EMC. The combined effect of these two factors of opposite activity seems to be crucial for membrane protein homeostasis. For example, EMC mediates insertion of N- and C-terminal TMDs, generally disfavoring translocation of TMD-flanking positive charges to the ER lumen.¹⁰ However, EMC can erroneously insert TMDs intended for the opposite topology or intended for the mitochondria. These incorrectly inserted TMDs would be flanked by lumen-facing positive charges, resulting in ATP13A1-mediated dislocation⁵ to allow a second attempt at correct insertion. These opposing activities combine to improve the fidelity of membrane protein topology and localization. Given this intimate relationship, a (dynamic) EMC-ATP13A1 complex might operate to both generate and rectify a protein’s topology at the ER.

It is now clear that producing the extraordinarily diverse multipass membrane proteome cannot be accomplished by a one-size-fits-all mechanism. Some might well follow the simple rules of sequential TMD insertion, but others need help from intramembrane chaperones, multiple insertases acting on different parts of the protein, and as it now emerges, a rectification process to finalize a protein’s topology. When considered with the added problem of assembling multi-subunit complexes, one can expect the discovery of yet additional new factors and principles in this re-emergent area of cell biology.

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DECLARATION OF INTERESTS

The author is a member of the *Molecular Cell* advisory board.

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