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A Flip Turn for Membrane Protein Insertion

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The transmembrane domains in a membrane protein must be recognized and correctly oriented before their insertion into the lipid bilayer. Devaraneni et al. (2011) generate snapshots at different stages of membrane protein biogenesis, revealing a dynamic set of steps that imply an unexpectedly flexible membrane insertion machinery.

Integral membrane proteins typically acquire their topology and insert into the membrane cotranslationally as the polypeptide is emerging from a ribosome. These critical biosynthetic events occur at a membrane-embedded translocon through which the nascent membrane protein is translocated (Osborne et al., 2005). The ribosome translocon complex (RTC) is responsible for recognizing the transmembrane domains (TMDs) of membrane proteins, orienting them in the correct topology and inserting them into the lipid bilayer. Whereas the sequence features of a TMD and its flanking domains that influence topology have been extensively characterized (von Heijne, 2006), far less is known about how this information is decoded by the RTC to ensure accurate membrane protein topogenesis. In this issue of *Cell*, Devaraneni et al. (2011) demonstrate that both a TMD and the RTC can experience dynamic conformational changes during topogenesis, providing new insights into the complex process of membrane protein biogenesis.

A mechanistic understanding of TMD insertion requires high-resolution information about RTC structure, the nascent polypeptide within the RTC, and how their relative configurations change over time. Because these events occur cotranslationally, the process is necessarily rapid and dynamic, severely complicating most methods of analysis. The traditional way to circumvent this temporal problem is by assembling ‘‘stalled’’ translocation intermediates of defined polypeptide lengths in vitro. Examination of these presumptive intermediates by structural, biochemical, and biophysical methods has provided much of our current insights into protein translocation and membrane insertion (Osborne et al., 2005).

However, cohesive models for membrane protein insertion have been challenging to derive because different studies have employed different assays, probes, and substrates, yielding sometimes contradictory findings. To tackle this problem, Devaraneni et al. (2011) simultaneously employ multiple types of probes (Figure 1A) on successively longer RTCs of a model protein with a TMD that achieves the type II orientation (with the N terminus facing the cytosol and C terminus translocated across the membrane). Each probe is designed to assess the location of specific residues in the nascent chain relative to the RTC, the membrane, or both. Though not all assays are informative at every length, this extensive and systematic strategy nonetheless provides ‘‘snapshots’’ of each nascent chain-RTC intermediate. When these snapshots are stitched together, the resulting ‘‘stop motion animation’’ allows the authors to propose a model for how a single-spanning membrane protein inserts into the lipid bilayer (Figure 1B).

The model for insertion of a type II TMD consists of four coordinated and dynamic steps. First, at short nascent chain lengths, the TMD initially inserts in a

"head-first," or a type I, orientation (with the N terminus translocated across the membrane). This orientation is maintained through successively longer lengths, with additional polypeptide accumulating at the cytosolic side of the RTC. Next, an uncharacterized change in the RTC structure, including an altered ribosome-translocon junction, appears to occur. Third and most impressively, the TMD undergoes a dramatic 180° flip to reorient to a type II topology. This orientation is then maintained as the polypeptide elongates, with the TMD eventually inserting into the lipid bilayer (Figure 1B).

The most remarkable implication of this study is the dramatic cotranslational reorientation of a TMD within the RTC. This suggests that a TMD can sample two distinct topologies and that the first orientation a TMD acquires is not necessarily its final one. This is consistent with earlier studies that suggested a similar inversion during insertion of an artificial poly-leucine TMD (Goder and Spiess, 2003). In fact, the final topology of even complex polytopic proteins may not be firmly decided until downstream sequences have been decoded (Seppälä et al., 2010). Such reorientation requires that the RTC be sufficiently dynamic and flexible to accommodate different states of the nascent chain. At the very least, the RTC must provide enough space for the TMD to convert from a type I to a type II orientation without compromising translocon integrity. This implies a more complex interplay between the components of the RTC and the polypeptide than previously appreciated, an idea substantiated by other examples suggesting that the ribosome (Berndt et al., 2009; Mariappan et al., 2010) and translocon (Liao et al., 1997; Pool, 2009) readjust to accommodate different polypeptides.

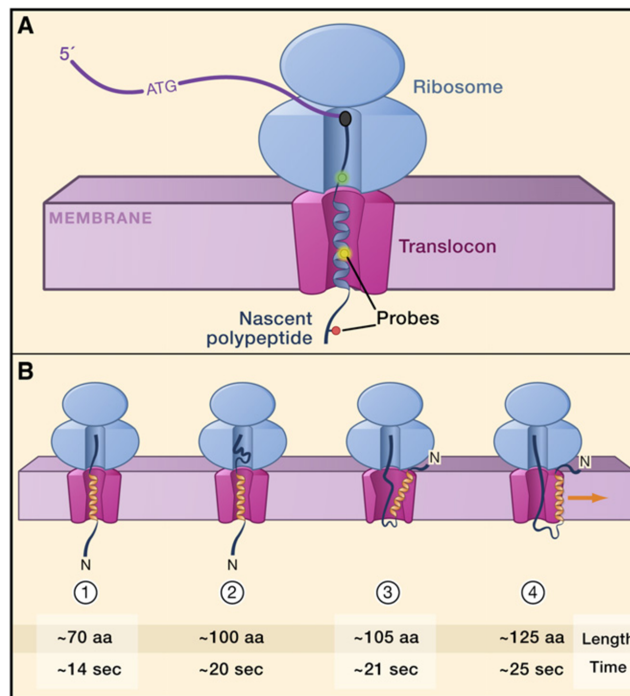

Figure 1. Probing Membrane Protein Insertion to Reveal Its Dynamic Steps

(A) Anatomy of a stalled ribosome translocon complex (RTC) that simulates a membrane protein insertion intermediate. A truncated transcript (purple) lacking an in-frame stop codon is translated to generate a nascent polypeptide (black) of defined length tethered to the ribosome via a tRNA (black circle) in the peptidyl transferase center. Natural and unnatural amino acid residues incorporated at specific sites in the nascent polypeptide are used to probe its location relative to the RTC and membrane. Examples include: cysteine (green), a probe for accessibility to cytosolic modification reagents; photocrosslinker (yellow), a probe for proximity to specific RTC proteins or membrane lipids; and fluorophore (red), an environmental probe for accessibility to collisional quenchers. In addition, accessibility to general or site-specific proteases can be used to evaluate the nascent chain position relative to the ribosome-translocon junction.

(B) Architecture of RTCs at different lengths of a nascent type II membrane protein. Approximate polypeptide length and the time it would take to synthesize it (at approximately five residues per second) are indicated. The ribosome and translocon are not to scale. The TMD (orange) inserts head-first into the translocon (1); downstream nascent chain accumulates on the cytosolic side of the RTC in a protected environment (2); and the TMD rotates 180° while staying in proximity to the translocon (3) and eventually inserts into the bilayer with further elongation (4). The critical inversion reaction (step 3) only occurs during type II membrane protein topogenesis.

The conserved central channel within the translocon is formed by the Sec61 complex. However, available structures of the bacterial homologs of the Sec61 complex do not provide an obvious explanation for how a dynamic reorientation could take place within its limited sized channel (Osborne et al., 2005). This implies that the minimal translocation channel might not suffice for the biogenesis of at least some types of substrates. It is therefore noteworthy that, in all organisms, the translocon is composed of more proteins than just the Sec61 channel, and the functions of such accessory factors remain largely obscure. Thus, a critical future goal will be to biochemically or genetically manipulate translocon composition and evaluate the consequences on membrane protein insertion using the high-resolution methods exemplified by Devaraneni et al. Only by having complete experimental control over both the substrate and the machinery can the next stage of insights be obtained.

The other key challenge will be to develop new methods capable of testing mechanistic models of insertion while maintaining the temporal features of cotranslational translocation. An intrinsic assumption by the present and previous studies is that successively longer stalled RTCs represent a temporal series. However, the time dimension is only approximated with this strategy; the actual measurements are made at equilibrium. Whether a shorter nascent chain necessarily reflects the kinetic history of a longer nascent chain remains to be experimentally validated on a case-by-case basis. Addressing this will require methods to either detect or trap otherwise highly transient intermediates postulated by studies using stalled RTCs.

For example, the model from Devaraneni and colleagues predicts that a type I orientation is briefly sampled before the final type II topology is achieved. How could this be tested in real time during cotranslational insertion of a full-length (nontruncated) type II membrane protein? One might imagine a means to "mark" the N terminus if it accesses the *trans* side of the membrane, thereby inferring that it had sampled that compartment. Indeed, glycosylation has been used as one

such mark (c.f., Goder and Spiess, 2003), but its dependence on a complex enzymatic reaction limits its broader application. Furthermore, the glycan's size is likely to alter the polypeptide's properties and influence its topogenesis. Analogous methods based on ubiquitin cleavage have also been used on the cytosolic side of the membrane (Cheng and Gilmore, 2006), but these can have similar limitations. Development of rapid and compact artificial reactions that can be topologically confined to one or the other side of the membrane would be ideal.

The currently available methods to study protein translocation and membrane protein insertion, used to great effect in the extensive efforts by Devaraneni et al. and others, are beginning to reach their limits. The insights provided by these static studies have led to various provocative but incompletely tested models of how key biosynthetic events occur as part of an intrinsically dynamic process. The field now waits for new methods that will unlock both the translocation machinery and the temporal constraints of cotranslational processes, thereby opening them to robust experimental manipulation.

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