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Breakthroughs and Views

Autonomous and heteronomous positioning of transmembrane segments in multispanning membrane protein☆

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Abstract

Polypeptides synthesized by membrane-bound ribosomes are cotranslationally integrated into the endoplasmic reticulum membrane. Transmembrane segments are positioned in the membrane via two distinct modes. In the autonomous mode, hydrophobic segments are integrated into the membrane based on the characteristics of the segment. In the heteronomous mode, a segment that is not inserted into the membrane by itself is forced into a transmembrane disposition by other segments. This unexpected insertion is achieved by a signal–anchor sequence with N_{exo}/C_{cyto} topology that translocates the preceding segment. Structural and functional diversities of transmembrane segments in multispanning proteins are acquired via this mode. Such a heteronomous positioning of polypeptide segments might occur not only in the integration process of membrane proteins but also in the general folding processes of soluble proteins.

Polypeptide chains fold into specific higher-order structures. Through a long evolutionary process, the amino acid sequences of proteins have been established to be able to adopt their unique higher structures. Integral membrane proteins possess transmembrane (TM) segments that span the hydrophobic core of biomembranes. TM segments often form α-helical conformations and the majority of them are highly hydrophobic in order to be stable in the hydrophobic environment of the membrane. There is a significant population of TM segments, however, that are not very hydrophobic [1]. The clarification of the underlying principles for positioning TM segments in the membrane will be a significant step towards understanding protein architecture and the folding process. Furthermore, it will facilitate the prediction of membrane topologies of a large number of membrane proteins reported from various genome projects.

Major parts of the biomembrane in eukaryotic cells belong to the secretory pathway, in which membrane proteins originate from the endoplasmic reticulum (ER) and are then transported towards the Golgi apparatus and plasma membrane via vesicular transport. Those membrane proteins are synthesized by ribosomes bound to the ER membrane and are cotranslationally inserted into the membrane. The nascent polypeptide chain emerges from the ribosomes and enters into the protein translocation channel, the so-called "translocon," which provides an aqueous environment in the membrane [2]. The ER targeting of the nascent polypeptide chain is determined by the hydrophobic segment (H-segment) of the signal sequences. The H-segments are recognized by the signal recognition particle (SRP) at the exit of the ribosome, which arrests polypeptide chain elongation and targets the ribosomes to the ER [3]. On the ER membrane, the H-segment is released from the SRP by the SRP-receptor and then enters into the translocon [3]. The translocon is a sophisticated folding machinery for membrane proteins, through which polypeptide segments are correctly positioned on the membrane. The present paper is an overview of the framework of membrane topogenesis of integral proteins on the ER and provides a concept of heteronomous insertion of the TM segment in addition to autonomous membrane insertion.

☆ *Abbreviations:* ER, endoplasmic reticulum; H-segment, hydrophobic segment; N-domain, amino-terminal domain; TM, transmembrane.

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Autonomous and heteronomous positioning of TM segments in the membrane

During synthesis of the membrane proteins, the first H-segment is recognized by the SRP, which targets the ribosome to the ER membrane. The segment tends to be inserted into the translocon and triggers polypeptide translocation based on the hydrophobic characteristics of the segment. An H-segment can also stop ongoing translocation through the translocon, acting as a stop-transfer sequence, and thus become a TM segment. Such autonomous positioning of H-segments via sequential actions of insertion-start and -stop is considered to be the principle underlying membrane positioning [4]. As shown in Fig. 1A, the N-terminal H-segment starts the translocation of the following portion and the next H-segment stops the translocation (gray lines). Then, the third H-segment (black line) starts the insertion of the following portion. As a result, three hydrophobic segments get integrated into the membrane and the segment (blue line) that is not hydrophobic cannot be inserted into the membrane.

Ota et al. [5] demonstrated a novel topogenic mode, the heteronomous membrane insertion of a segment that is not very hydrophobic. As shown in Fig. 1B, the blue segment is not inserted into the membrane by itself, because it is not hydrophobic. The following b-segment, which possesses the characteristics to be inserted in the N_{exo}/C_{cyto} orientation, mediates insertion of the blue segment into the membrane. Eventually, three hydrophobic and one hydrophilic TM segments are positioned in the membrane. Although positioning of the polar and charged amino acid residues is unfavorable in the hydrophobic core of the membrane, it can be facilitated via this mode.

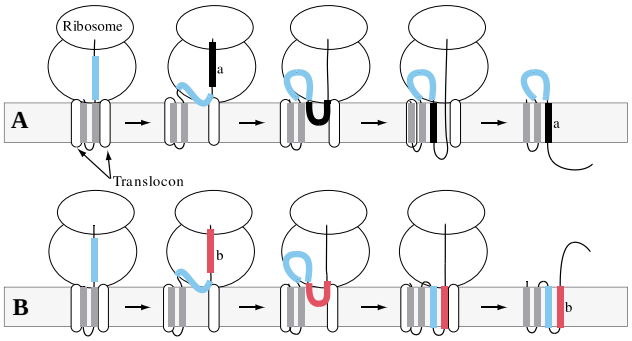

Fig. 1. Autonomous and heteronomous membrane insertion of TM segments. The two hydrophobic N-terminal segments are inserted by the autonomous function. (A) The a-segment is also autonomously inserted into the translocon and mediates translocation of the following portion. The blue segment remains on the cytoplasmic side of the membrane. (B) The b-segment, which possesses a strong N_{exo}/C_{cyto} orientation of SA-I, mediates the heteronomous insertion of the preceding blue segment.

Signal–anchor sequence with N_{exo} /C_{cyto} orientation

The unexpected topology is conferred by the signal-anchor sequence that mediates the translocation of the preceding portion [6]. Generally, H-segments of signal sequences can form both orientations (Fig. 2). When the following segments are translocated through the translocon, it results in an N_{cyto}/C_{exo} orientation. If the sequence is cleaved off, it becomes a signal peptide (SP); if not, it becomes a signal-anchor sequence (termed here type II signal–anchor, SA-II). In contrast, when the amino-terminal domain (N-domain) is translocated, it becomes a signal-anchor sequence with an N_{exo}/C_{cyto} orientation (termed here type I signal–anchor, SA-I). SA-I has been found with various TM segments [7]; diverse members of microsomal cytochrome P450 superfamilies [8], NADPH cytochrome P450 reductase [9], synaptotagmin family [6], neuregulin [7], etc. Almost all N-terminal TM segments of the G-protein coupled receptor family are also SA-I sequences. Via the SA-I sequence, even a large dihydrofolate reductase domain of more than 200 residues, which was fused at the N-terminus of synaptotagmin II, can be translocated through the membrane in a cell-free system (Kida, unpublished observation).

The positively charged residues and stable folding of the N-domain prevent translocation of the N-domain [7]. Insertion of positive charges in the N-terminal flanking region of the H-segment changes an SA-I sequence into an SA-II sequence [10–12]. The longer H-segment translocates the N-domain with the more positive charges [13]. In the case of P450, only shortening of the H-segment results in inversion of the topology [10]. Generally, the hydrophobicity of the H-segment as well as the length influences the orientation of the segment [7,13]. In mouse synaptotagmin II (Syt II), the positively charged residues just after the H-segment are also crucial for translocation of the N-domain (Fig. 3, +) [6]. The positive charges fix the C-terminus at the entrance of the translocon. When the positively charged residues are replaced with asparagine residues, the SA-I orientation is inverted to the SA-II orientation.

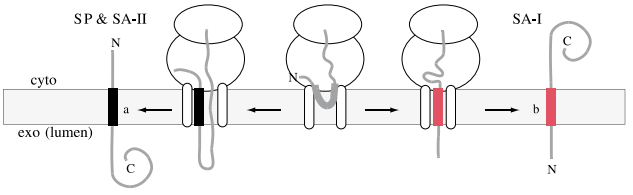

Fig. 2. Signal sequence orientation. The orientation is determined by the side of the H-segment that is translocated through the translocon. The a-segment mediates translocation of the following portion to be a signal peptide (SP) or type II signal–anchor sequence (SA-II). The b-segment translocates the preceding portion to be a type I signal–anchor (SA-I).

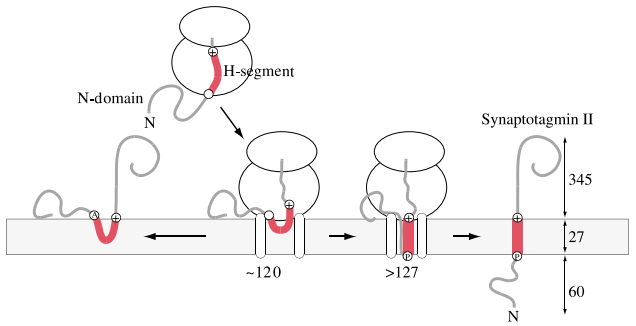

Fig. 3. Topogenesis of the SA-I sequence. Nascent polypeptide of the 120-residue N-terminal of Syt II is targeted to the ER membrane but its N-domain remains on the cytoplasmic side. The nascent chain of more than 127 residues is integrated into the membrane. The positive charges just behind the H-segment are essential for N-domain translocation (+). The N-domain translocation occurs just after the H-segment and the positive charges emerge from the ribosome. The amino acids with a high turn propensity for the TM segment (such as Pro, Lys Asp, etc.) are also essentially located just in front of the H-segment (P). Without these residues, the H-segment is inserted into the membrane in a loop configuration (A). Numbers indicate the number of residues in the polypeptide chain.

Certain amino acid residues are required for N-domain translocation at the specific position between the N-domain and the H-segment of the Syt II molecule (Fig. 3, P) [14]. When all the three proline residues within this region were changed to alanine, neither the N-domain nor the following portions were translocated through the translocon and anchored into the membrane with a loop conformation (Fig. 3, A). At least one proline residue within this region is essential for translocation of the N-domain. The proline can be replaced with polar residues, irrespective of the charges, but hydrophobic and less polar residues are not favorable for N-domain translocation. Trp and Ser had an intermediate effect. This character correlates strongly with the turn-propensity scale for TM segments, which is experimentally determined [14]. H-segments with the characteristics described above can exert the topogenic function of SA-I sequence.

Kida et al. [6] studied the timing of membrane integration of mouse Syt II. Syt II possesses a 60-residue hydrophilic N-domain, which is translocated through the membrane from the C-terminus to the N-terminus (Fig. 3). When truncated mRNA coding for only the 120-residue N-terminal sequence is translated in a cell-free system, the nascent polypeptide chain can be targeted to the membrane and be in close vicinity of the translocon subunit, while the N-domain remains on the cytoplasmic side of the membrane. This is the productive intermediate, as the N-domain is readily translocated when the polypeptide is released from the ribosome by puromycin. At this length, the C-terminus of the H-segment is just emerging from the ribosome, given the fact that there are 40 residues in the ribosome. When the nascent chain is more than 127 residues, the N-domain is fully translocated to the lumenal space, thus, indicating that full translocation of the N-domain requires a longer segment than does the ER targeting. Because the positive charges following the H-segment contribute to N-domain translocation, they must be recognized by a factor, which is likely to be translocon.

The membrane integration process of the SA-I protein is a cotranslational process. The H-segment and the following charged region must be recognized by the folding machinery at the appropriate time during polypeptide chain elongation. If the polypeptide is synthesized in the absence of the membrane in a cell-free system and incubated with the full-active ER membrane after translation, it can no longer be inserted into the membrane. Heinrich et al. [15] demonstrated that the translocon is essential not only for polypeptide chain translocation across the membrane but also for insertion of the H-segment into the lipid environment, by partitioning the segment into the lipid environment. Even the membrane anchoring of the hydrophobic loop in Fig. 3 requires the translocon.

Heteronomous insertion by SA-I in multispanning proteins

In multispanning membrane proteins, the internal SA-I sequence can confer a TM topology onto a hydrophilic segment located on its N-terminal side [5]. Ota et al. [16] systematically evaluated the topogenic functions of 14 TM segments of an anion exchanger (band 3 of erythrocyte) and reported that the TM4, TM6, and TM8 segments possess a topogenic preference of SA-I. The TM3 segment, which is not as hydrophobic and cannot be inserted into the membrane by itself, is inserted into the membrane only in the presence of the TM4 segment [5]. Ukaji et al. [17] recently demonstrated that the N-terminal TM segment of glucose-6-phosphatase is an SA-I sequence and that TM2 is inserted into the membrane by the SA-I function of TM3. The intrinsic topogenic preference of the SA-I sequence allows the weakly hydrophobic segment to be inserted into the membrane. In model constructs that include two SA-I sequences with a hydrophilic segment between them (Fig. 4), a TM topology is conferred onto the hydrophilic segment (blue segment) [5]. The unexpected topology is demonstrated using various constructions, glycosylation site scanning, and site-specific proteolysis. It has been further demonstrated that the hydrophilic segments are not fixed in the membrane and can move in relation to the membrane plane. Although these findings clearly indicate that TM segments do not need to be hydrophobic, such a configuration would be thermodynamically highly unstable and has never been predicted according to traditional theory.

Several TM segments within multispanning membrane proteins possess a strong intrinsic topogenic

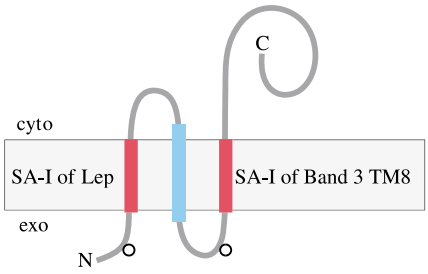

Fig. 4. Engineered TM topology of the hydrophilic segment. The hydrophilic segment of prolactin is inserted into the membrane by the following SA-I sequences of TM8 segment of band 3. Both of the created glycosylation sites in the lumen are glycosylated (circles) [5]. The N-terminal SA-I is from the leader peptidase (Lep) of *Escherichia coli*.

character and can exert these functions, irrespective of the context and force unexpected topologies onto the preceding segments. The heteronomous positioning of weakly hydrophobic segments in the membrane was suggested by studies of the internal SA-I sequence. Similarly, internal SA-II sequences should also result in heteronomous positioning of weakly hydrophobic segments; a segment that is insufficient to stop an ongoing translocation might be fixed in the translocon if the following H-segment possesses a strong preference for the SA-II sequence. Several examples of the contribution of internal SA-II sequences on heteronomous positioning have been suggested by statistical analysis of structurally well-defined multispanning membrane proteins (Mr. H. Araki, Dr. K. Nakai, Tokyo University, personal communication).

The heteronomous insertion mode enables diverse sequence multiplicity of TM segments. The polar and/or charged residues within the TM segments exhibit(s) key functions of integral membrane proteins; for example, ion transport, signal transduction, and receptor functions, etc. If membrane integration of the polypeptide segment is defined only by the hydrophobic interaction between polypeptide segments and membrane lipids, such sequence multiplicity cannot be achieved. Furthermore, it is reasonable to hypothesize that the amino acid sequences of the membrane proteins evolved on the basis of the bio-machinery.

Implications for protein folding

Due to cotranslational topogenesis through the translocon, the polypeptide chain need not search a large number of possible structures to reach the final folded structure, but it is sequentially committed to the final topology by cotranslational recognition of the TM segments. Some TM segments are positioned, depending on the distal effect of other TM segments. This consideration is important not only for membrane protein topogenesis but also for soluble protein folding. Some segments acquire the three-dimensional conformation based on their own character, but others are positioned by the distal effect in a heteronomous manner. The cotranslational recognition and the heteronomous positioning of certain segment would be a key for describing the polypeptide chain folding.

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