The Topogenic Contribution of Uncharged Amino Acids on Signal Sequence Orientation in the Endoplasmic Reticulum*

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Signal sequences for insertion of proteins into the endoplasmic reticulum induce translocation of either the C- or the N-terminal sequence across the membrane. The end that is translocated is primarily determined by the flanking charges and the hydrophobic domain of the signal. To characterize the hydrophobic contribution to topogenesis, we have challenged the translocation machinery in vivo in transfected COS cells with model proteins differing exclusively in the apolar segment of the signal. Homo-oligomers of hydrophobic amino acids as different in size and shape as Val19, Trp19, and Tyr22 generated functional signal sequences with similar topologies in the membrane. The longer a homo-oligomeric sequence of a given residue, the more N-terminal translocation was obtained. To determine the topogenic contribution of all uncharged amino acids in the context of a hydrophobic signal sequence, two residues in a generic oligoleucine signal were exchanged for all uncharged amino acids. The resulting scale resembles a hydrophobicity scale with the more hydrophobic residues promoting N-terminal translocation. In addition, the helix breakers glycine and proline showed a position-dependent effect, which raises the possibility of a conformational contribution to topogenesis.

Proteins destined for the endoplasmic reticulum (ER) are synthesized with a hydrophobic signal sequence of typically 10–20 uncharged, mainly apolar amino acids. This sequence is recognized by the signal recognition particle, which targets the nascent chain-ribosome complex via the signal recognition particle receptor to the ER membrane (1). The ribosome binds to the translocon, a gated pore made of several copies of the translocon, specifically contacting Sec61, a particle receptor to the ER membrane (1). The ribosome binds to the translocon, a gated pore made of several copies of the translocon, specifically contacting Sec61, in a manner that leads to translocation of either the C terminus or the N terminus across the membrane (5). Cleaved signals of secretary and type I membrane proteins (e.g. glycoporin) and signal anchor sequences of type II membrane proteins (e.g. transferrin receptor) translocate the C-terminal sequence, whereas the reverse signal anchors of cytochrome P-450, microsomal epoxide hydrolase, and opsin, for example, translocated the N-terminal sequence. The end of the signal that is translocated is determined by several factors. Charged residues flanking the apolar segment of the signal influence the insertion process in a manner that induces the more positive end to stay on the cytoplasmic side (6, 7). However, the charge distribution is not generally sufficient to determine the orientation and to generate a unique topology (8, 9). Hydrophilic sequences N-terminal of the signal may inhibit their translocation if they fold in the cytosol before targeting is completed (10). Similarly, we have recently observed that glycosylation at sites near the signal sequence can influence topogenesis by glycan attachment to polypeptide segments that are transiently exposed to the ER lumen (11).

In addition, the apolar segment of the signal itself makes a significant contribution to orienting the signal within the translocon and the membrane. In diagnostic mutant constructs, an increased fraction of N-terminal translocation was obtained with increasing length and hydrophobicity of this segment (12–15). The influence of oligoleucine signals of different lengths on the topology was additive with the effects of flanking charges and of the N-terminal hydrophilic sequence (14). The topogenic contribution of the hydrophobic sequence was also shown to be important for natural proteins, since the correct and unique insertion of the signals of the vasopressin precursor (Nexo/Ccyc) and of microsomal epoxide hydrolase (Nexo/Ccyc) was compromised upon extending or shortening the apolar sequence, respectively (16).

In most constructs, both the length of the hydrophobic domain and its total hydrophobicity were altered simultaneously, hampering the distinction between these two factors with respect to their influence on topogenesis. Summing up the hydrophathy indices for different sequences did not yield a good correlation between total hydrophobicity and the resulting topologies (14, 15). The analysis suggested that neither the length of the apolar segment nor its hydrophobicity alone is responsible for the observed effects on insertion behavior. In fact, it was observed that orientation was also strongly affected by the distribution of hydrophobicity within the apolar segment, since the more hydrophobic terminus appeared to be preferentially translocated (15). Furthermore, it is obvious that two different sequences of the same length also differ in properties other than just hydrophobicity, such as the shape of the molecule and the propensity to assume α-helical conformation.

To identify the features within the hydrophobic segment of the signal that influence topogenesis and favor N- or C-terminal translocation, we have tested the effect of homo-oligomeric sequences of hydrophobic residues other than leucine. In addition, we tested the effect of individual residues of all uncharged amino acids within the context of a generic oligoleucine sequence. The resulting ranking of amino acids with respect to their effect on N-terminal translocation resembles a hydrophobicity scale yet differs from all existing major scales. This insertion scale may be useful to improve topology prediction.

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1 The abbreviation used is: ER, endoplasmic reticulum.
and to characterize the functional properties of the inside of the translocation pore.

**EXPERIMENTAL PROCEDURES**

cDNA Constructs—The plasmids encoding H1ΔQ16 and H1ΔQ23 have been described previously (14). H1ΔQ derivatives with hydrophobic sequences Ala16, Ile16, Val16, Phe16 (Leu-Val), Ala16, Tyr16, Met16, and Met22 were generated by annealing two complementary oligonucleotides encoding the sequence MPQF and the respective hydrophobic sequences with 5′ and 3′ sticky ends for ligation into a KpnI and a BamHI site, respectively. For example, to construct H1ΔQ23, oligonucleotides CATTGGGACCACGAGTGTGCTGTCGTGATGTTGATGGGACAACTACGACCACC with the templates H1ΔQ19 and H1ΔQ23 were generated by annealing two complementary oligonucleotides CATGGGACCACGAGTGTGCTGTCGTGATGTTGATGGGACAACTACCTACACGACAATCACCACC with the templates H1ΔQ19, H1ΔQ23, and H1ΔQ22, respectively. Polymerase chain reaction products were digested with KpnI and a BamHI and ligated into the expression vector pECE (17) with a modified NcoI site to test the topogenic properties of different hydrophobic sequences.

For analysis with endo-N-acetylglucosaminidase H-sensitive, N-terminal translocation (14). This construct was therefore a model to test the insertion behavior of a variety of derivatives with different hydrophobic sequences.

Constructs were transiently expressed in COS-7 cells, labeled with [35S]methionine for 30 min, immunoprecipitated, and analyzed by SDS-gel electrophoresis and fluorography. Their topologies with respect to the membrane (schematically shown in Fig. 1B) were derived from the glycosylation state of the proteins and from their extractability with 0.1% saponin. Translocation of the C terminus is apparent by glycosylation of the products. Translocation of the N terminus results in unglycosylated polypeptides, which can be distinguished from molecules that failed to insert by extraction with 0.1% saponin. This treatment allows soluble polypeptides to be released into the medium, whereas the membranes remain sufficiently intact to retain integrated proteins. As shown in Fig. 1C (lanes 1–4), both the endo-β-N-acetylglucosaminidase H-sensitive, glycosylated form and the unglycosylated form of H1ΔQ16 were completely resistant to extraction into the saponin supernatant (S) and were recovered with the remainder of the cells (C). This indicates that they were integral membrane proteins. In contrast, H1ΔQA16 was entirely extracted with saponin (Fig. 1C, lanes 5–8). Thus, a sequence of 16 alanines is not functional as a signal sequence for targeting and insertion into the ER membrane.

H1ΔQ with the wild-type transmembrane segment (lanes 9–12) produced a small fraction of unglycosylated, inextractable polypeptides corresponding to proteins spanning the membrane with an exoplastic N terminus. The majority of the polypeptides were glycosylated. However, a significant fraction was slightly smaller and saponin-extractable. This material is the product of signal peptidase cleavage, as has previously been observed for H1Δ translated in vitro (18). There, the cleavage site had been identified by sequencing to be between glycine and serine at the end of the apolar segment. Depending upon the presence or absence of the N-terminal tail, the signal sequence is most likely positioned differently within the translocan or the membrane, exposing a cryptic site to signal peptidase. Interestingly, H1Δ was not significantly cleaved in vitro (14, 19), possibly because the kinetics of leaving the range of signal peptidase is much faster in vivo. Mutation of the N-terminal flanking arginine to glutamine in H1ΔQ appeared to further enhance accessibility of the cleavage site, resulting in more than 50% cleavage in vivo.

The other hexadecamer sequences tested in the context of H1ΔQ, i.e. Ile16, Phe16, Met16, and Val16, produced functional
signal sequences giving rise to very few polypeptides that were both unglycosylated and extractable (Fig. 2). In comparison with Leu16, only Ile16 generated a larger fraction of polypeptides with a translocated N terminus (86%; Fig. 2, lanes 3 and 4). All others exclusively translocated their C terminus (lanes 7–12). An alternating sequence of leucines and valines, (LV)\(_{8}\), yielded an intermediate distribution with 40% N-terminal translocation. The signal-anchor of H1ΔQV\(_{16}\) and H1ΔQ(LV)\(_{16}\) was almost completely cleaved.

In order to determine the relative topogenic preference of homo-oligomers of valine, phenylalanine, and methionine, we generated constructs with extended sequences (Val\(_{14}\) to Val\(_{25}\), Phe\(_{16}\) to Phe\(_{25}\), Met\(_{22}\)). In addition, we also analyzed oligomers of tryptophan (Trp\(_{19}\) and Trp\(_{21}\)) and tyrosine (Tyr\(_{22}\)), i.e. of the amino acids with the most bulky side chains. Residues that are less hydrophobic than alanine were not tested, since they are unlikely to yield functional signal sequences. All of the sequences tested were capable of targeting and inserting the protein into the ER membrane, although not always completely (Fig. 2, lanes 19–42). As observed before for oligoleucine sequences (14) (Fig. 3), longer oligomers of valine, tryptophan, or phenylalanine produced increasing fractions of polypeptides with N-terminal translocation (Figs. 2 and 3). The length dependence of orientation for the different amino acids is approximately parallel but shifted relative to each other (Fig. 3). Fifty percent N-terminal translocation is obtained with approximate lengths of 14 (leucine), 19 (valine and tryptophan), and 23 residues (phenylalanine).

In the oligovaline series, an increasing population of products was found not to be targeted (i.e. was not glycosylated but was extractable), indicating that the functionality of the signal sequence was reduced with increasing length. Yet, long hydrophobic sequences per se did not cause poor functionality, since even 25-mers of leucine (14) and phenylalanine (Fig. 2) were completely integrated. Incomplete insertion was also obtained for constructs with Trp\(_{19}\), Trp\(_{21}\), and Tyr\(_{22}\). In the case of Tyr\(_{22}\), however, even glycosylated polypeptides without apparent signal cleavage were partially extracted, suggesting that an oligotyrosine sequence may not be capable of efficiently anchoring a protein in the membrane. From the quantitation of the results (Fig. 3), the following ranking of the amino acids with respect to their ability as a homo-oligomer to translocate the N terminus can be derived: Ile > Leu > Val > Trp > Tyr > Phe > Met.

Signal Cleavage Does Not Influence Topogenesis—We have recently observed that glycosylation can influence topogenesis of proteins with ambiguous topogenic determinants (11). This indicated that, within the translocon, the nascent polypeptides undergo dynamic reorientation, which can be influenced by protein modifications occurring simultaneously. Since signal cleavage, like glycosylation, occurs cotranslationally, it might also affect topogenesis. To test this possibility, we compared the insertion behavior of the signals Val\(_{16}\), (Leu-Val)\(_{16}\), Leu\(_{16}\), and Ile\(_{16}\) with sequences carrying individual mutations designed to prevent or to allow signal cleavage.

The most probable reason why Val\(_{16}\), (Leu-Val)\(_{16}\), and wild type, but not Leu\(_{16}\) and Ile\(_{16}\), allow signal cleavage is the presence of valines near the C terminus that fit into position –3 or −1 relative to the cleavage site where signal peptidase requires small, uncharged residues (20). Indeed, mutation of the last two valines of valine (14) and phenylalanine (Fig. 2) were ineffective. Most importantly, the mutations did not significantly affect the ratio of N- to C-terminal translocation, as is evident from quantitation of the products (values indicated in Fig. 4). Thus, signal cleavage did not detectably influence topogenesis. Therefore, cleavage of a construct does not interfere with the analysis of the topogenic properties of different sequences.

**Fig. 1.** **Analysis of protein topology.** A, N-terminal sequences of the constructs with homo-oligomer hydrophobic signals (H1ΔQX\(_{16}\)) and of their precursors. B, possible topologies with respect to the ER membrane. Upon insertion, either the C terminus (a and b) or the N terminus (c) will be translocated. Signal cleavage (arrow) may release the nascent protein to the ER membrane results in a soluble cytosolic product. C, N-terminal sequences of A\(_{16}\), Leu\(_{16}\), and H1ΔQ, ER insertion of H1ΔQL\(_{16}\), H1ΔQA\(_{16}\), and H1ΔQ. Transfected COS-7 cells were labeled with [\(^{35}\)S]methionine for 30 min and then extracted with saponin. The saponin extracts (S) and the residual cells (C) were subjected to immunoprecipitation, with (+) or without (−) endo-β-N-acetylglucosaminidase H de-glycosylation, and analyzed by SDS-gel electrophoresis and fluorography after treatment. The C-terminal domain contains two glycosylation sites, one of which is within 20 residues of the apolar signal domain and not always completely modified. The positions of molecular mass markers of 38, 26, and 20 kDa are indicated. L\(_{16}\), Leu\(_{16}\); A\(_{16}\), Ala\(_{16}\); wt, wild type.
Topogenic Contribution of Uncharged Amino Acids as Part of a Hydrophobic Signal—The hydrophobic segment of natural signal sequences is not exclusively composed of hydrophobic amino acids; it may also contain polar, uncharged residues. To determine the contribution of all uncharged amino acids in the context of a hydrophobic signal sequence on its orientation, two residues in the generic oligoleucine signal of H1DQL16 or H1DQL19 were exchanged for all uncharged residues (H1DQX2/16 or H1DQX2/19; Fig. 5). The nonleucine residues were placed at positions n and n + 5, which places them on opposite sides of an α-helix. The expression products are shown in Fig. 5 (A and B), and the resulting topologies are quantified in Fig. 6. The residues that were tested in both H1DQX2/16 and H1DQX2/19 showed the same relative behavior: Leu > Trp > Tyr > Cys > Ala > Thr. Only isoleucine and valine increased the fraction of polyoleptides with a translocated N terminus in comparison with an oligoleucine sequence. Tryptophan had no effect, whereas all the other amino acids reduced N-terminal translocation to various degrees. The amino acids that most effectively reduced N-terminal translocation are the most polar uncharged residues histidine, glutamine, and asparagine and the helix breakers proline and glycine.

In the initial series H1DQX2/16 and H1DQX2/19, the nonleucine residues were placed in positions 4 and 9 from the C-terminal end of the hydrophobic sequence. To test whether the position in the second half of the hydrophobic sequence is important for topology, constructs were made in which Thr, Ser, Gly, Asn, Gln, His, and Pro, i.e. the residues most strongly affecting the topogenic behavior of the apolar sequence, were placed at positions 4 and 9 from the N-terminal end (H1DQX2/19N; Fig. 5C and Fig. 6, circles). Moving these residues from the C-terminal to the N-terminal half of the signal inverts the hydrophobicity gradient along the hydrophobic sequence, which was expected to result in a slight reduction of N-terminal
translocation (15). This effect was observed for asparagine, whereas for threonine, serine, glutamine, and histidine, no statistically significant difference could be observed. In the case of proline and glycine, however, N-terminal translocation was significantly increased in H1DQ2/19N versus H1DQ2/19. Since proline and glycine are the two strongest helix breakers, this result suggests that the conformation of the hydrophobic sequence also influences topogenesis.

**DISCUSSION**

Signal sequences have two distinct functions: to target the nascent chain-ribosome complex to the ER membrane and to initiate translocation of the sequence on either one of its ends. The first function is to recruit the signal recognition particle in an interaction that requires a sufficiently hydrophobic core of the signal (1, 21). After targeting to the ER membrane, the signal is recognized by the Sec61 translocation complex, leading to a tight junction between the ribosome-nascent chain complex and the translocon (22). Photocross-linking analysis revealed that upon complete insertion into the channel, signal sequences are precisely positioned with respect to the protein components of the channel and lipids, suggesting a specific binding site of protein-protein interactions at the interface between the channel and the surrounding lipids (5, 23). The hydrophobic segment of the signal was found to specifically contact the transmembrane helices 2 and 7 of Sec61p in yeast (24). An additional component, TRAM (translocating chain-associating membrane protein), was shown by *in vitro* cross-linking to be in contact with signal sequences (2, 25, 26) and in particular with the N-terminal hydrophilic segments (27). In reconstitution assays, TRAM was found to be particularly important for signals with relatively short N-terminal hydrophilic segments (25). The TRAP (translocon-associated protein) complex has also been shown to be in proximity to translocating signal sequences (28, 29), and although it is not essential for translocation, its true role is yet to be elucidated. Recognition of the signal sequence by a binding site in or closely associated with the translocon is likely to be a key event in defining the orientation of the signal and thus whether the N terminus or the C terminus of the protein is translocated across the membrane.

The flanking charges of the signal are most likely to exert their effect on orienting the peptide by electrostatic interactions with the translocon. It is less clear how the apolar segment affects topogenesis. The effect of a hydrophobicity gradient along the apolar sequence, as observed by Harley *et al.* (15), could be explained by a similar gradient in the signal binding site of the translocon. A more hydrophobic surface near the luminal side would result in preferential orientation of the signal with its more hydrophobic end pointing toward the ER lumen. How longer and/or more hydrophobic sequences without hydrophathy gradients induce increased N-terminal translocation is not obvious.

**FIG. 5.** Topogenic contribution of uncharged amino acids in the context of an oligoleucine sequence. Constructs H1ΔQX2/16, H1ΔQX2/19, and H1ΔQX2/19N, as illustrated, were expressed in COS-7 cells and analyzed as described in the legend to Fig. 2.
FIG. 6. Quantitation of the topologies of H1ΔQX<sub>2/19</sub>, H1ΔQX<sub>2/16</sub>, and H1ΔQX<sub>2/19N</sub>. Multiple experiments similar to those shown in Fig. 5 were quantified using a PhosphorImager. The fraction of poly peptides with a translocated N terminus (i.e. unglycosylated and inextractable) as a percentage of the total inextractable products is plotted for each amino acid X in H1ΔQX<sub>2/16</sub> (A, bars), H1ΔQX<sub>2/19</sub> (B, bars), and H1ΔQX<sub>2/19N</sub> (B, open circles). The mean and S.D. of three or four determinations are shown.

### TABLE I

Topogenic preference of amino acids for N-terminal translocation versus hydrophobicity and α-helix propensity scales

| Topogenesis   | Hydrophobicity scales | α-Helix propensity |
|---------------|-----------------------|--------------------|
| Homo Mixed   | KD       | GES     | ARH | PGH | C | WW | LD | CF |
| I            | I        | F       | F   | M   | W | I   | W  | E  |
| L            | V        | M       | M   | L   | F | V   | F  | L  |
| VW           | L        | I       | F   | M   | L | I   | W  | L  |
| Y            | F        | LV      | LVW | I   | L | M   | L  | F  |
| M            | C        | C       | M   | A   | Y | A   | C  | M  |
| M            | A        | A       | G   | T   | W | G   | G  | Q  |
| A            | G        | T       | T   | G   | W | G   | G  | Y  |
| T            | T        | G       | Y   | T   | C | G   | G  | C  |
| S            | S        | S       | S   | AHP | S | S   | T  | D  |
| G            | W        | P       | Q   | P   | R | EP  | T  | H  |
| N            | Y        | Y       | N   | Y   | K | AH  | S  | R  |
| Q            | P        | H       | P   | G   | Q | DY  | H  | T  |
| H            | H        | Q       | H   | S   | Q | N   | P  | Q  |
| P            | DENQ     | N       | K   | R   | N | Q   | R  | C  |
|             | K        | E       | E   | H   | K | R   | N  | Y  |
|             | R        | K       | D   | N   | D | R   | K  | N  |
|             | D        | R       | E   | D   | K | K   | P  |
|             | R        | K       | D   | E   | E | P   |    |
|             | D        |        |     |     |    |      |    |

To analyze the topogenic properties of the apolar signal sequence, we challenged the insertion machinery in vivo, using transfected COS-7 cells, with a variety of diagnostic constructs differing exclusively in the hydrophobic segment of the signal. This segment was either a homo-oligomer of a hydrophobic amino acid or oligoleucine sequences with two interspersed uncharged amino acids. The behavior of homo-oligomers showed that the machinery can cope with sequences that differ dramatically in size and shape, e.g. oligovaline versus oligotryptophan. Oligotryptophan and oligotirosine sequences were sufficiently hydrophobic to be recognized by the signal recognition particle and by the translocon. This is not true, since various hydrophobicity scales differ considerably in the classification of these two residues (Table I). According to some criteria, tryptophan is considered the most hydrophobic amino acid, whereas according to others it is ranked below serine and threonine. Similarly, tyrosine is regarded as a very hydrophilic residue by some scales. Although the oligotirosine sequence was functional for targeting and insertion, it appeared not to be an efficient membrane anchor, since glycospayed (and therefore initially integrated) poly peptides were partially extractable by saponin. Furthermore, our results confirm as a general rule what was previously observed for oligoleucine sequences of 7–25 residues (14); the longer a homo-oligomeric sequence of a residue, the higher the proportion of proteins with a translocated N terminus.

Leucine is the most abundant amino acid in transmembrane and signal sequences (6, 30). It can thus serve as a generic hydrophobic matrix to assess the relative effect of interspersed noneleucine residues, even if they are polar and cannot possibly function as a targeting signal by themselves. The ranking order of residues with respect to promoting or allowing N-terminal translocation in an oligoleucine context can be seen to resemble a hydrophobicity scale. However, existing hydrophobicity scales, which are based on different theoretical calculations and/or various experimental measurements, differ considerably from each other (Table I). Our topogenic scale resembles most closely the hydropathy scale by Kyte and Doolittle (31). The main disagreements are the rankings of tryptophan, tyrosine, glycine, and proline.

One observation suggests, however, that the hydrophobicity of the side chains is not the only criterion influencing topogenesis. The helix breakers glycine and proline showed a position-dependent effect. Proline is a helix breaker because it cannot form a hydrogen bond between its amide nitrogen and the backbone carbonyl of a residue at position −4 in an α-helix. For
this reason, a proline is tolerated in positions 1–4 of a helix. In H1ΔQP_{219} and H1ΔQP_{218N}, there is one proline near the center of the hydrophobic sequence and a second one at position 4 either from the C terminus, where it destabilizes or kinks the α-helix, or from the N terminus, where it does not. In H1ΔQP_{218N}, the effect of the prolines on the topologies is smaller than in H1ΔQP_{219}, correlating with the conformational effect of the second proline. Similarly, the effect of glycine, which destabilizes an α-helix by increasing the conformational freedom, is reduced near the N terminus of the helix.

The conformation of the hydrophobic core of the signal may therefore play a role in topogenesis. There is clearly no correlation between the topogenic effects and the α-helix propensities of the amino acids as determined from the structures of soluble proteins (32) (Table I, column CF) or from circular dichroism analysis of peptides in aqueous solution (33). However, it has been shown that the helix propensities are dramatically different in a hydrophobic environment (33, 34). Testing the effect of different amino acids X on the helix content of peptides with the sequence KKAAXAAAAAXAAWAAAAKKKK-amide in n-butanol yielded a helicity scale that is quite similar to hydrophobicity scales (33) (Table I, column LD). This scale is also quite similar to the topogenic scale determined here. The major differences concern glycine, whose properties are position-dependent in our system, and tryptophan.

A recent study on the insertion of polypeptides with closely spaced conflicting signal sequences demonstrated that dynamic reorientation of polypeptides can occur within the translocon (11). This is also likely to happen for the signals analyzed here, since the topogenic determinants are somewhat ambiguous, resulting in a mixture of orientations. Signals that form a short, unstable, or kinked hydrophobic helix may reorientate more easily within the translocon than those with a long and stable helix. Since this correlates with preferential translocation of the C and the N terminus, respectively, it might indicate that the signal initially inserts into the translocon with its free N terminus and subsequently, depending on its flanking charges, tends to invert its orientation as the nascent polypeptide grows at the C-terminal end. A sizable N-terminal hydrophilic extension would be expected to inhibit initial insertion with the N terminus and reduce N-terminal translocation. This is indeed the case, since N-terminal translocation, which for the tailless protein H1ΔLeu_{25} amounted to ~80%, was entirely blocked for H1Leu_{25}, which carries the 40-residue N-terminal extension of the wild-type receptor (14). Only in combination with inverted flanking charges was the effect of the long hydrophobic sequence restored (H1–4ΔLeu_{19–21}).

In this study, we have presented a new scale ranking amino acids with respect to their tendency to induce N-terminal translocation of a signal sequence. The precise mechanism by which components of the ER membrane determine the final orientation of a protein remains unclear. However, this scale, determined in vitro, will be useful in giving a more accurate method to predict the behavior of amino acids in protein topogenesis.

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