Transmembrane Orientation and Topogenesis of the Third and Fourth Membrane-spanning Regions of Human P-Glycoprotein (MDR1)

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ABSTRACT

Understanding how the multidrug resistance phenotype is manifest in human cancer cells will require insight into the mechanism of assembly, transmembrane topology, and intracellular trafficking of human P-glycoprotein (MDR1). Previously, we showed that MDR1 amino terminus biogenesis occurred through an unexpected interaction between novel topogenic sequence subtypes and that transmembrane topology of corresponding amino and carboxy halves was not equivalent. We now investigate topology and topogenic activities of the third and fourth transmembrane regions (TM3 and TM4) of human MDR1 using protease protection of defined reporter epitopes expressed in Xenopus laevis oocytes. As was previously observed for TM1 and TM2, determinants in TM3 and TM4 exhibited cooperativity in directing proper assembly and transmembrane orientation. The signal sequence encompassing TM3 required residues from TM4 to realign translation of the MDR1 chain into the endoplasmic reticulum (ER) lumen. Remaining residues from TM4 terminated translocation and established a polytopic transmembrane topology in which TM3 and TM4 both spanned the membrane in the orientation predicted by hydropathy-based models. Remarkably, when translocating sequentially into the ER lumen, neither TM4 alone nor TM4 together with TM3 efficiently terminated translocation. Thus, MDR1 biogenesis required both the presence of these sequences and their proper orientation with respect to the ER translocation apparatus. This conclusion was supported by experiments in which TM3 and TM4 topology was reproduced in a defined chimeric protein which mimicked native MDR1 presentation. These additional variations on simple themes of protein biogenesis utilized by MDR1 demonstrate that events of complex protein biogenesis may be dissected and studied using protein chimeras with defined translocation properties.

INTRODUCTION

Transmembrane topology of most eukaryotic polytopic IMPs is established at the ER membrane coincident with protein synthesis (1–4). One model for generating polytopic topology proposes that independent topogenic sequences with properties similar to S sequences, ST, or SA sequences direct sequential translocation and membrane integration events through interactions with receptor proteins at the ER membrane (5–7). Support for this model comes from two types of studies: those in which IMPs of predicted polytopic topology were generated by expression of chimeras encoding S, ST, and/or SA sequences (e.g., S-ST-S, SA-ST-SA, and SA-SA-SA) (4, 8, 9) and other studies in which initial topogenic sequences of naturally occurring polytopic proteins such as bovine rhodopsin, uracil permease, and the acetylcholine receptor were found to be functionally indistinguishable from conventional S, SA, or ST sequences (10–12).

Human P-glycoprotein, MDR1, is a polytopic integral membrane protein which exports a diverse group of cytotoxic drugs from human cells (13–15). It is also a member of the ABC protein superfamily which performs a wide variety of cellular transport functions (16, 17). It was previously thought, based on hydropathy analysis and homology with bacterial hemolysin B, that MDR1 and eukaryotic relatives [i.e., CFTR (cystic fibrosis transmembrane conductance regulator) and STE 6 (sterile 6)] exhibited a tandem repeat structure with each half of the molecule containing six hydrophobic membrane-spanning helices and a large cytosolic ATP-binding domain (18–21). Recent studies have called into question not only this tandem repeat topological model (22–24) but also current hypotheses concerning how the ABC transport superfamily is assembled into membranes (25).

In vivo studies of reporter-protein chimeras and in vitro studies of both full-length and truncated human MDR1 have demonstrated that the carboxy terminus spans the membrane four rather than six times as predicted by hydropathy (22). Topology of the first and second versus the seventh and eighth putative transmembrane helices (TM1-TM2 versus TM7-TM8, respectively) demonstrated an asymmetric rather than symmetric topology for homologous halves of the protein (25). In addition, initial events in MDR1 topogenesis exhibited an unexpected complexity in that they were not directed by independent SA or ST sequences (25). Instead, two independent topogenic sequences (TM1 and TM2, respectively) functioned in concert to establish integration of the nascent chain into the lipid bilayer. Studies by Zhang and Ling (23) using cell-free analysis of truncated murine P-glycoprotein also demonstrated a carboxy terminus topology which spanned the membrane only four times. Similar in vitro studies of hamster P-glycoprotein demonstrated two alternate topologies for the amino terminus, one which spanned the membrane six times and a second which spanned only four times (24).

To further define the mechanism of biogenesis of human MDR1, we have used a Xenopus oocyte expression system (26) to examine topology of polypeptides generated from cDNA clones containing a reporter of translocation engineered into sequential sites of the MDR1-coding region. With this technique we confirmed the previously reported transmembrane topology of TM1 and TM2 (25) and demonstrated that TM3 and TM4 (in contrast to TM9 and TM10) spanned the membrane in the orientation predicted by hydropathy-based models. An internal signal sequence encompassing TM3 and a significant portion of TM4 (residues 139–226) reinitiated translocation of the MDR1 nascent chain. Although TM4 along with its flanking sequences terminated translocation when it was preceded by TM3 in its native context, TM4 lacked efficient de novo stop transfer activity. Finally, native topology of TM4 and/or TM3 was reconstituted in a complex protein chimera, demonstrating the requirement for both the presence as well as proper orientation of these sequences with respect to the ER translocation apparatus.

MATERIALS AND METHODS

Materials. DNA-modifying enzymes were purchased from New England Biolabs. Oligonucleotides were synthesized at the University of California, San Francisco, Biomedical Resource Center. Tran35S-label was purchased from ICN Radiochemicals, and proteinase K was purchased from Boehringer.
Mannheim. Additional reagents were purchased from Sigma or Fisher Scientific unless otherwise specified.

Construction of cDNA Clones. All cDNA constructs were placed under control of the SP6 in plasmid SP64T (22).

Clones TM1-2.P, TM1-3*17.P, TM1-3*17.P, and TM1-4.P (Fig. 1A) were generated by using the PCR to amplify MDR1 fragments using a "sense" oligonucleotide (ATTTAGTGCTACTAAG) homologous to the SP6 promoter [pSPMDRA2 template (22)] and antisense oligonucleotides: 1, CCCACCATCCGTCACCTAAACCAGCTTCCCTCCTGCG; 2, ACTGATGCTGACACATCATGACCCCGCCACG; 3, GACAGCAGCGGTACCCCAAGAACAGGACTGATGGCCAA; and 4, ACTGATGCTGACACATCATGACCCCGCCACG encoding a BstEII restriction site at base pairs 500, 660, 680, and 830, respectively, in the MDR1-coding sequence. PCR fragments were digested with HindIII and BsrEII, gel purified, and ligated into the HindIII-BsrEII-digested S.LST.gG.P (22) vector, thus fusing the P reporter (derived from bovine prolactin) at amino acid residues 165, 217, 224, and 276 of MDR1, respectively.

Plasmid TM1.P has been previously described (25).

Plasmid TM3*8.P was constructed by digesting a PCR-generated fragment (sense oligonucleotide, GTGCGCTGACCGGAAGACAAATACACAA; antisense oligonucleotide 4 above, MDR2 template) with BstEII and ligating into the BsrEII site between globin-derived and P reporters of plasmid S.gG.P (8, 27) to generate S.gG.TM3*8.P. S.gG.TM3*8.P was digested with BsaI (at codon 143 in the MDR1-coding sequence), Neol (translation start codon), and Klenow fragment, and the plasmid was closed.

To construct plasmids S.LST.gG.TM3.P, S.LST.gG.TM3*19.P, S.LST. gG.TM3-4.P, and S.LST.gG.TM4.P (Fig. 1D), pSPMDRA2 was digested with PvuII and BsaAI (codons 139–209), PvuII (codons 139–239), ApaLI and RsaI (codons 165–276), or BsaAI and RsaI (codons 209–276), respectively, blunted with Klenow as necessary, and ligated to the oligonucleotide linker.
Fig. 2. Xenopus oocyte expression and topology of MDR1 fusion proteins. Plasmids identified above the autoradiograms were transcribed in vitro and expressed in XO containing [35S]methionine. XO homogenates were digested with PK in the presence or absence of 1% Triton X-100 (det) as indicated and immunoprecipitated with anti-prolactin antisera. Prolactin-reactive fragments protected from PK in the absence but not presence of detergent are indicated (lanes 2 and 1, downward arrowheads). Topology of the fusion proteins at the ER membrane is diagramed beneath the autoradiograms. N, MDR1 amino terminus; hatched ovals, putative TM regions; P, prolactin-derived reporter. Cytosol and ER faces of the membrane are indicated. Small circle, three N-linked glycosylation sites between TM1 and TM2. The percentage of 35S-labeled P-reactive chains protected from PK digestion (corrected for methionine content) as compared to total P-reactive chains is indicated (% P translocated).

encoding a BstEII restriction site (CTGCCGTGACCGCAG). Fragments were digested with BstEII, gel purified, and ligated into the BstEII site of plasmid S.L. ST.gG.P previously described (8). L, ST, and G represent a β-lactamase-derived passenger domain, the IgM stop transfer sequence, and a modified chimpanzee α-globin domain, respectively (8). The S sequence is derived from preprolactin (28).

Clones S.gG.TM3.P, S.gG.TM3-4.P, and S.gG.TM4.P (Fig. 1c) and S.gG.TM3-18.P were generated by digesting plasmids S.L.ST.gG.TM3.P, S.L.ST.gG.TM3-4.P, S.L.ST.gG.TM3-18.P, and S.L.ST.gG.TM4.P, respectively with BstXI (globin coding region) and PstI. Gel-purified fragments were ligated into a BstXI-PstI-digested -phosphatase S.gG plasmid vector (28).

Clone TM3-18.P was generated by sequentially digesting S.gG.TM3-18.P with BbsI, Klenow fragment, and PstI and then ligating the gel-purified fragment into an Ncol, Klenow-blunted, PstI-digested Sp64 vector derived from plasmid BPI (22). Clone TM3.P was generated by digesting S.gG.TM3.P with BbsI and Klenow fragment, and cloning into SF10, calf intestinal phosphatase.

Oocyte Expression and PK Digestion. mRNA was transcribed in vitro with SP6 RNA polymerase (New England Biolabs) as previously described (22). Transcription mixture (2 μl) was added to 0.5 μl of a 10-fold concentration of Tran35S-label and injected into freshly harvested stage VI XO (50 nl/oocyte) as described previously (8). Following incubation at 18°C for 3–5 h, oocytes were homogenized on ice in ~3 volumes of homogenization buffer (0.25 μl sucrose-50 mM Tris, pH 7.5-50 mM potassium acetate-5 mM Mg1-5 mM DTT). CaCl2 was added to a final concentration of 10 mM and rapid transfer into 10 volumes of 0.1 M Tris, pH 8.0-1% SDS preheated to 100°C.

Immunoprecipitation. Oocyte homogenates were diluted into 1 ml of 0.1 M NaCl-0.1 M Tris, pH 8.0-10 mM EDTA-1% Triton X-100. Antisera (1 μl, antiprolactin antisera; U.S. Biochemicals), anti-globin antisera, or anti-β-lactamase antisera (gift of C. N. Chang) and protein A Affigel (5 μl; Bio-Rad) was added, and samples were rotated at 4°C for 6–10 h. Beads were washed three times with 0.1 M NaCl-0.1 M Tris, pH 8.0-10 mM EDTA-1% Triton X-100 and twice with 0.1 M NaCl-0.1 M Tris, pH 8.0, prior to SDS-polyacrylamide gel electrophoresis.

Image Analysis and Quantitation. SDS-polyacrylamide gels were fluorographed on En3Hance (Dupont) prior to autoradiography. Autoradiograms were digitized using an AGFA Focus Color Plus Scanner (Adobe Photoshop software). Band intensities were individually determined by measuring mean pixel density minus background multiplied by total area.

RESULTS

A Prolactin-derived Reporter Maps MDR1 Transmembrane Topology through the First Four Transmembrane Helices. To study the transmembrane topology of MDR1 we used a reporter of translocation (P), derived from the secretory protein prolactin, which lacks intrinsic translocation activity (27) and faithfully reflects transmembrane topology as directed by upstream conventional topogenic sequences signal and ST sequences in both bitopic and polytopic proteins (8, 12, 22). This reporter was engineered at sequential sites in the MDR1-coding sequence (plasmids TM1.P, TM1-2.P, TM1-3-8.P, TM1-3-18.P, and TM1-4.P, Fig. 1A). Topology of the reporter was determined by Xenopus oocyte expression, digestion with PK, and immunoprecipitation with anti-prolactin antisera.

Two clones, TM1.P and TM1-3-18.P, generated protease-protected, P-reactive fragments in the absence of detergent (Fig. 2, lanes 2 and 11, downward arrowheads). Clone TM1.P (lane 1, M, 32,000) generated multiple fragments as described previously (25). Full-length unglycosylated chains (lane 1, M, 32,000) were variably glycosylated and cleaved upon translocation into the ER lumen (lane 2, downward arrowheads). Full-length glycosylated transmembrane chains (lane 1, M, 35,000 and 38,000) were more easily visualized upon darker exposure. Glycosylation was confirmed by digestion with endoglycosidase H (data not shown). Nascent chains generated from clone TM1-3-17.P were also translocated into the ER lumen, generating a protease-protected and cleaved, M, 15,000 P-reactive fragment (lane 10, upward arrowhead).
Fig. 3. Characterization of signal sequence activity of TM3 in Xenopus oocytes. Clones, labeled above the autoradiogram, were transcribed in vitro, expressed in Xenopus oocytes, digested with PK in the presence or absence of detergent (det), and immunoprecipitated with anti-prolactin antisera as indicated. Only clone TM3*P exhibited signal sequence activity as demonstrated by a P-reactive fragment protected from protease (lane 8, downward arrowhead). Percentage of prolactin reactivity recovered following PK digestion is shown.

Transmembrane Topology of TM3 and TM4 Is Not Directed by Sequential Actions of Independent Signal and Stop Transfer Sequences. The internal signal sequence identified within TM3 and 4 is consistent with a model of polytopic protein biogenesis in which independent signal and ST sequences act sequentially to direct complex topology. Therefore, we attempted to identify ST sequence activity which could terminate translocation and direct the polytopic topology observed in Fig. 2.

ST sequences terminate translocation and integrate the nascent chain into the ER membrane (5, 35, 36) and have been defined using mutagenesis and chimeric cassettes (37–39). To test for ST activity in TM3 and 4 of MDR1 we generated a series of chimeric clones (diagramed in Fig. 1C) containing coding regions of TM4, TM3 and TM4, or TM3 downstream of a conventional amino-terminal signal sequence (S) in the previously described chimeric cassette S.gG.X.P (8). Stop transfer activity was determined by XO expression, PK digestion, and quantitation of the efficiency with which MDR-1 sequences terminated translocation and established a transmembrane topology. The IgM-derived ST was tested as a control for ST activity in this assay (clone S.gG.ST.P).

As shown in Fig. 4A, protease digestion degraded essentially all full-length chains (>95%) from clone S.gG.ST.P (lanes 1 versus 2 and 4 versus 5) and generated an M, 25,000 globin-reactive fragment (lane 2, upward arrowhead). Thus, these chains exhibited a uniform transmembrane orientation with the globin reporter in the ER lumen and the P reporter in the cytosol (N-trans or Type 1 topology). In contrast, chains generated from clone S.gG.TM4.P failed to terminate translocation. Fifty percent of full-length chains were protected from PK digestion and remained immunoreactive to both anti-globin and anti-prolactin antisera (Fig. 4B, lanes 1, 2, 4, and 5, downward arrowheads). TM4 by itself, therefore, exhibited only weak ST activity. TM3 also failed to terminate translocation, since 76% of full-length chains (clone S.gG.TM3.P) were protected from PK digestion (Fig. 4C, lanes 1, 2, 4, and 5). When TM3 and TM4 were tested together (clone S.gG.TM3–4.P) >50% of chains were fully translocated (Fig. 4D). The sequence TM3*19 (clone S.gG.TM3*19.P) also lacked ST activity (data not shown). Thus, despite their ability to terminate translocation during native MDR1 biogenesis, TM3 and/or TM4 do not exhibit ST activity when translocating sequentially into the ER lumen.

Polytopic Topology of TM3 and TM4 of MDR1 Is Reconstituted in a Defined Transmembrane Chimeric Protein. In native MDR1 the internal signal sequence encoded by TM3*17 emerges from the ribosome as part of a nascent transmembrane chain anchored in the membrane through the cooperative actions of TM1 and TM2 (25). To test whether topology observed for TM3–4 could be reproduced in a defined chimera, the coding sequences of TM3, TM3*19, or TM3–4 were engineered between globin- and prolactin-derived passagengers in the previously characterized chimeric protein, S.L.ST.gG.P (8), generating clones S.L.ST.gG.TM3.P, S.L.ST.gG.TM3*17.P, and S.L.ST.gG.TM3–4.P, respectively (Fig. 1D). L represents a previously described β-lactamase-derived passenger domain (8). The S, ST, gG, and P domains are described above. Translocation, initiated by the S sequence, is terminated by the ensuing ST sequence. TM3 and TM4 thus emerge from the ribosome on the cytosolic surface of the ER membrane as part of a transmembrane nascent chain in an orientation mimicking their native state in MDR1. Clone S.L.ST.gG.S.P, containing a second prolactin-derived signal sequence, was used to confirm the fidelity of reinitiation of translocation by a bona fide signal sequence.

Topology of these clones was determined by XO expression, PK digestion, and immunoprecipitation with anti-lactamase, anti-globin, and anti-prolactin antisera. Clone S.L.ST.gG.S.P generated three pep-
MDR1 BIOGENESIS

A

S.gG.ST.P

PK - + + + + +
Det - - - - - -
Ab G G G P P P

45 kD

30 kD

14 kD

% Transmembrane 95%

B

S.gG.TM4.P

PK - + + + + +
Det - - - - - -
Ab G G G P P P

45 kD

30 kD

14 kD

% Transmembrane 50%

C

S.gG.TM3+2P

- + + - - +
G G + P P P

45 kD

30 kD

14 kD

% Transmembrane 24%

D

S.gG.TM3-4-P

PK - + + + + +
Det - - - - - -
Ab G G G P P P

45 kD

30 kD

14 kD

% Transmembrane 36%

DISCUSSION

Polypeptide reporters of translocation have been widely used to study transmembrane topology of prokaryotic polytopic IMPs (40–43) based on periplasmic and cytoplasmic differences in reporter enzymatic activity. Here we report an analogous system utilizing a passive reporter of translocation which allows in vivo analysis of eukaryotic protein topology. Xenopus oocytes provide an in vivo expression system capable of generating mature functional MDR1 protein (26) which is amenable to rapid expression of cDNA constructs and analysis of ER-derived vesicles containing newly synthesized protein (8).

Using this technique we have studied topology of the corresponding amino and carboxy hydrophobic domains of MDR1. The current data confirm the previously reported topology of TM1 and TM2 and demonstrate that TM3 and TM4 also span the ER membrane in the orientation predicted by hydropathy-based structural models (18). However, using the same expression system, reporter domain and assay conditions, the corresponding region of the MDR1 carboxy terminus exhibited a different topology, which was confirmed using epitope-specific antisera (22). The current study thus further demon-

Fig. 4. Stop transfer activity of TM3- and TM4-derived sequences. Clones labeled above the autoradiograms were expressed in XO, digested with PK with or without detergent (det) present, and immunoprecipitated with anti-globin (G) or antiprolactin (P) antisera. Full-length chains recovered in the absence of PK (lanes 1 and 4, arrowheads) were compared with those recovered following PK digestion (lanes 2 and 5) to determine the fraction of chains in a transmembrane orientation. The percentage of transmembrane chains determined by averaging results with both antisera is indicated.

Consistent with its activity in MDR1, TM3 alone failed to reinitiate chain translocation (Fig. 5B, lane 8). In contrast, TM3' + 19 translocated the P passenger (Fig. 5C, lanes 7 and 8, downward arrowheads) with equal efficiency to that observed in its native MDR1 context. Finally, TM3 and TM4 together (Fig. 5D) completely terminated chain translocation and reestablished the P reporter in a cytosolic orientation. Each of the MDR1-derived sequences in these defined heterologous chimeras thus exhibited the translocation activity and topological phenotype that was observed in the intact MDR1 amino terminus.
Fig. 5. Reconstitution of MDR1 TM3 and TM4 topology in a heterologous chimeric protein. Clones were expressed in X0, digested with PK with and without detergent (det), and immunoprecipitated with anti-lactamase (L), anti-globin (G), and anti-prolactin (P) antisera as indicated. Cleavage of the P reporter from the nascent chain (A, clone S.L.ST.gG.S.P; B, clone S.L.ST.gG.TM3−19.P) generated a fragment reactive to globin and lactamase antisera (A and C, lanes 1 and 4, upward arrowheads) and a second fragment reactive to prolactin antisera (A and C, lane 7, downward arrowhead). Translocation of the prolactin fragment was confirmed by protection from PK digestion in the absence of detergent (A and C, lane 8, downward arrowheads). Translocation of the lactamase passenger by the N-terminal signal sequence was confirmed in all clones by recovery of an M, 23,000, lactamase-reactive fragment following PK digestion (A−D, lane 2). After methionine content was corrected for, translocation efficiencies of the P passenger were 67 and 73% for clones S.L.ST.gG.S.P and S.L.ST.gG.TM3−19.P, respectively. Topology of the chimeric proteins generated by these clones is schematically diagramed beneath each autoradiogram.
strates a topological model of MDR-1 in which corresponding TM regions in the amino (TM3 and TM4) and carboxy (TM9 and TM10) terminal halves are asymmetric.

Topogenic sequences which direct protein topology at the ER membrane can be defined if they function efficiently when engineered into appropriate heterologous contexts. This approach has been used to analyze functional determinants of topogenic sequences from secretory and bitopic proteins (e.g., S, ST, and SA sequences) (reviewed in Refs. 5 and 7). Similar studies of polytopic proteins have been more limited (10, 12, 22). Such an analysis is an important prerequisite to understanding the molecular events at the ER membrane which facilitate assembly of complex proteins such as the ABC transporters. In the case of MDR-1, novel variations on the simple themes of protein biogenesis (5) appear to occur at each location studied to date: TM1–2, TM3–4, and TM7–8 (22, 25). Here we show that interactions between sequence determinants of TM3 and TM4 significantly influenced both reinitiation and termination of translocation. Furthermore, topogenic activities of TM3 and TM4 depended on their orientation relative to the ER membrane and translocation apparatus.

Using a cell-free translation system, Zhang et al. (24) recently reported that hamster P-glycoprotein exhibited two different forms of amino terminus topology in which TM3 and TM4 spanned the membrane either once or twice (24). Our results indicate that the homologous region of human MDR1 exhibited a single topology and spanned the membrane twice. It should be noted that the current study examines cotranslational assembly of TM3 and TM4 into the ER membrane. Conceivably, subsequent synthesis of downstream topogenic sequences (e.g., encoded within TM5 and TM6) might alter interactions between TM3 and 4 and translocation components, posttranslationally modifying the topology demonstrated here. Such a topological change (e.g., posttranslational retrograde translocation) has been observed in one instance of a chimeric polytopic protein (9) and has also been reported in a secretory protein precursor (44). However, preliminary observations using MDR1-P reporter chimeras in XOs suggest that transmembrane topology of TM3 or TM4 is not altered by subsequent synthesis of TM5 and/or TM6, at least for the vast majority of chains. Differences in observed topology between human and hamster P-glycoproteins might also have resulted from the different expression systems used, the use of different reporters and fusion sites, or underlying topological differences between rodent versus human P-glycoproteins. Resolution of these questions will require direct comparison of these proteins.

Recent studies have shown that signal sequences open a large aqueous channel in the membrane (45) through which the nascent chain translocates to reach the ER lumen (46). Subsequent termination of translocation and integration of the chain into the membrane has also been proposed to occur through specific receptor-mediated events (47–50) in which channel closure and/or disassembly allows the nascent chain access to the lipid bilayer. The internal MDR1-derived sequence, TM3∗17, exhibited bona fide signal sequence activity and, thus, could be viewed as opening such a channel. When TM3 and TM4 engage the channel simultaneously (e.g., in their native context or in the clone S.L.S.T.gG.TM3–4.P), translocation was terminated in essentially 100% of chains. In contrast, when TM3 and TM4 engage the channel sequentially (e.g., through the channel opened by a preceding signal sequence in clone S.gG.TM3–4.P) they were unable to efficiently terminate translocation. These results suggest that during MDR1 biogenesis, TM3∗17 initiates translocation (possibly via opening a channel for translocation) in such a way that translocation can be terminated (via closing such a channel) by the remainder of TM4 only when TM3 and TM4 are correctly oriented with respect to channel proteins.

Several protein components required for nascent chain translocation across the ER membrane have recently been identified and cloned (51). Evidence suggests that translocation proteins, sec61P and TRAM (translocation-associated membrane protein), comprise critical components of the channel through which translocation occurs (52–54). Like other polytopic proteins studied, MDR1 utilizes the conventional signal recognition particle receptor-mediated mechanism for ER targeting and translocation (25). In addition, MDR1 associates with sec61P during assembly of multiple transmembrane segments. How MDR1 utilizes protein translocation machinery to effect proper assembly within the translocation channel and whether different TM regions require different subsets of channel components remain critical questions in understanding molecular events of MDR1 assembly at the ER membrane.

Finally, these results demonstrate the potential utility of using defined protein chimeras for reconstituting specific events in polytopic protein biogenesis and identifying resident ER proteins which direct these events. Future studies are being directed at identifying the molecular mechanisms underlying these unique variations observed for MDR1 biogenesis. Whether these variations result from cooperative interactions between sequences of the nascent chain (i.e., between TM3 and TM4), between the nascent chain and individual channel components and receptors, or both is currently under investigation.

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