The membrane topology of the endoplasmic reticulum (ER) and sarcoplasmic reticulum (SR) Ca\textsuperscript{2+}-ATPases were investigated using in vitro transcription/translation of fusion vectors containing DNA sequences encoding putative membrane-spanning domains. The sequences of these Ca\textsuperscript{2+}-ATPases are identical except for the COOH-terminal end, which contains an additional predicted transmembrane segment in the ER ATPase. The MO and M1 fusion vectors (Bamberg, K., and Sachs, G. 1994 J. Biol. Chem. 269, 16909–16919) encode the NH\textsubscript{2}-terminal 101 (MO vector) or 139 (M1 vector) amino acids of the H,K-ATPase \( \alpha \) subunit followed by a linker region for insertion of putative transmembrane sequences and, finally, the COOH-terminal 177 amino acids of the H,K-ATPase \( \beta \) subunit containing five N-linked glycosylation consensus sequences. The linker region was replaced by the putative transmembrane domains of the Ca\textsuperscript{2+} ATPases, either individually or in pairs. Transcription and translation were performed using [\( ^{35} \text{S} \)]methionine in a reticulocyte lysate system in the absence or presence of canine pancreatic microsomes. The translated fusion protein was identified by autoradiography following separation using SDS-polyacrylamide gel electrophoresis. When testing single transmembrane segments, this method detects signal anchor activity with MO or stop transfer activity with M1. The first four predicted SERCA transmembrane domains acted as both signal anchor and stop transfer sequences. A construct containing the fifth predicted transmembrane segment was able to act only as a stop transfer sequence. The sixth transmembrane segment did not insert cotranslationally into the membrane. The seventh was able to act as both a signal anchor and stop transfer sequence, and the eighth showed stop transfer ability in the M1 vector. The ninth transmembrane segment had both signal anchor and stop transfer capacity, whereas the tenth transmembrane segment showed only stop transfer sequence properties. The eleventh transmembrane sequence, unique to the ER Ca\textsuperscript{2+} ATPase, had both signal anchor and stop transfer properties. These translation data provide direct experimental evidence for eight or nine of the 10 or 11 predicted transmembrane sequences in the current topological models for the SR or ER Ca\textsuperscript{2+} ATPases, respectively.

The euca ryotic P type ATPases consist of a single catalytic \( \alpha \) subunit and, on occasion, a second, \( \beta \), subunit (1). All of these P type ATPase \( \alpha \) subunits have a hydropathy profile indicative of a polypeptide integral membrane protein (Fig. 1). The mammalian Na,K-, H,K-, and Ca\textsuperscript{2+}-ATPases seem to share a characteristic hydropathy profile despite widely differing amino acid composition (1). This profile is also similar to that of the fungal H\textsuperscript{+}-ATPases, with the exception that the H5 and H6 regions are more hydrophobic and also distinct with an intervening hydrophilic sequence (2). Based on this hydropathy profile, the catalytic subunit of these enzymes has a large cytoplasmic domain, a membrane domain composed of several membrane-spanning segments, and a small extracytoplasmic domain (3–5). Hydropathy predictions are unequivocal in defining the first four putative transmembrane segments but are less certain in the COOH-terminal one-third of these pumps. Whereas the SR\textsuperscript{2+} Ca\textsuperscript{2+}-ATPase was thought to have 10 transmembrane segments (3), both the Na,K- and H,K-ATPases were predicted to have eight transmembrane segments when they were sequenced (4, 5). The Neurospora H\textsuperscript{+}-ATPase has been proposed to have 8, 10, or 12 such segments (2, 6, 7). No standard algorithm predicts 10 segments. In particular, only a single transmembrane segment is predicted for the H5/6 region of the mammalian pumps, in contrast to the two segments predicted for the Neurospora H\textsuperscript{+}-ATPase (2).

Numerous experimental methods have been used to determine the composition of the membrane domain of these pumps, such as protease cleavage, epitope mapping, mutagenesis, chemical labeling and in vitro translation scanning (8–18). It appears that no single method provides unequivocal data.

Mapping of the Na,K-ATPase with epitope insertion has been interpreted as indicating eight transmembrane segments (19). A mobile COOH-terminal pair of transmembrane segments has been proposed that become exposed on the outside surface upon heating, based on tryptic access and phosphorylation at Ser-938 by protein A kinase (20). A similar heat-dependent translocation of the last extracytoplasmic loop was detected during trypsinization of the Na,K-ATPase (21). Insertion of epitopes into the Na,K-ATPase provided evidence for the first eight transmembrane segments but was not used to determine the location of the last two hydrophobic segments (22). Epitope mapping of the SR Ca\textsuperscript{2+}-ATPase showed that the postulated extracytoplasmic loop between H7 and H8 was indeed present in the interior of the SR vesicles (13, 14). Tryptic digestion of this ATPase provided experimental evidence for the first eight transmembrane segments that had been predicted, but an unexpected cleavage between H9 and H10 was observed (12). Mutagenesis to generate Ca\textsuperscript{2+} transport mutants provided evidence that H4, H5, H6, and H8 were in the membrane domain (15–18). The crystal structure of the SR
Ca\textsuperscript{2+}-ATPase at 14-Å resolution shows that the membrane domain can accommodate 10 membrane-spanning segments (23). Tryptic cleavage showed the presence of eight transmembrane segments in the gastric H,K-ATPase (24). Extraductoplasmonic labeling with Covalent reagents provided evidence for the same segments (24, 25). However, in vitro translation showed the presence of an additional pair of transmembrane segments, to give an experimental consensus for 10 membrane segments in this enzyme (9). In vitro translation of regions of the Neurepandra enzyme showed that the COOH-terminal domain contained six membrane insertion sequences (8) although it was concluded that pairwise insertion had to occur (26). A similar result was obtained using the five long sequence and all the other fragments.

The particular Ca\textsuperscript{2+}-ATPase DNA used in this study is from the ER (28). This SERCA2 pump differs from the SERCA1 pump from the SR only by having 42 more amino acids on the COOH terminus as a result of alternative RNA splicing (3, 28). The 11 possible transmembrane domains were predicted using a moving average of 11 amino acids. The hydropathy profile and Doolittle hydropathy profile is shown at the top of this figure. These sequences used for insertion are underlined and doted lines for the H5 long sequence and solid lines for all the other fragments.


caption: Membrane Topology of SERCA ATPases

**EXPERIMENTAL PROCEDURES**

Vector Design—The 5'-3' cDNA constructs used here encode the first 101 (M0 vector) or the first 139 (M1 vector) amino acids of the NH\textsubscript{2}-terminal portion of the \( \alpha \) subunit of the gastric H,K-ATPase, a linker sequence containing two restriction sites (BglII and HindIII) for insertion of the variable sequences, and finally, the last 177 amino acids of the H,K-ATPase \( \beta \) subunit. After translation in the absence or presence of microsomes, the mobility shift on SDS-PAGE due to glycosylation of the five N-glycosylation consensus sequences of the \( \beta \) region is used to determine the location of the COOH terminus of the fusion protein. Any inserted sequence that causes M0 to be glycosylated is characterized as a signal anchor sequence. Likewise, any sequence that prevents entry of the COOH terminus of M1 into the interior of the microsome, thereby preventing the usual glycosylation of this vector, is characterized as a stop transfer sequence. M0 is used to find signal anchor sequences, and M1 is used to find stop transfer sequences. These constructs have been used previously to characterize the membrane spanning segments of the H,K-ATPase \( \alpha \) subunit (9) and of a P type ATPase from Helicobacter pylori (31).

Plasmid Construction—The cloning plasmid used for both vectors was pGEM7zf+ (\( \Delta \)HindIII) in which the HindIII site in the polylinker had been removed. The construction of the M0 and M1 vectors in this plasmid has been described in detail (9).

Variable Segment—The sequences containing putative transmembrane segments, identified as sequential hydrophobic segments (Hn, where n is any number) of the Ca\textsuperscript{2+}-ATPase, were synthesized by polymerase chain reaction (PCR) and ligated into the BglII and HindIII sites of the M0 and M1 vectors. Sense primers contained the BglII site, and antisense primers contained the HindIII site. The appropriate sequence for the primers and conditions for the amplification were determined using the Oligo 4.0 primer analysis software (National Biosciences, Inc., Plymouth, MN). The different sequences used to analyze the topology of the Ca\textsuperscript{2+}-ATPase are presented in Table I. These sequences were based on a variety of predictive algorithms as shown in Table II.

PCR—A typical 100-\( \mu \)l reaction contained 1 ng of the template, 0.3 \( \mu \)M of each primer, 200 \( \mu \)M deoxynucleotides (Pharmacia Biotech Inc.) in the buffer supplied by the manufacturer and 1 unit of Vent polymerase (New England Biolabs, Inc.). The PCR products were digested with BglII and HindIII and gel-purified for ligation into the expression vectors.

Ligation—Ligation and transformation of plasmids were performed according to standard protocols (32).

Vector Amplification and Purification—The expression plasmids con-
tain the different protein sequences were replicated in Escherichia coli XL-1 blue (Stratagene) and isolated using anion exchange columns (Qiagen, Chatsworth, CA). Inserts were screened by PCR with the T7 sense primer and the downstream PCR2 primer (33). The inserts in all plasmids were verified by dideoxy sequencing. Sequence analysis was done with the help of GENEWORKS software (version 2.0, Intelligentech, Inc.).

**In Vitro Translation**—The protein was synthesized from isolated plasmid cDNA using the TNT™ kit in vitro transcription/translation system (Promega) in the presence of [35S]methionine according to the manufacturer’s suggestions. This method allows transcription and translation to be performed in a single step. Translation reactions that contained canine pancreatic microsomes (Promega) were centrifuged through 250 mM sucrose, 50 mM Tris, pH 7.5, before electrophoresis. The translation products were separated on 10% Laemmli gels that were subsequently fixed in 45% methanol, 10% acetic acid, washed for 1 h in the same solution, and then dried and subjected to autoradiography (34). The products formed in the presence of microsomes were run in lanes next to lanes containing the translation products formed in their absence.

**Autoradiography**—Gels were placed into a cassette containing Kodak X-Omat AR x-ray film and were exposed for 12–96 h at room temperature.

**Materials**—All chemicals were analytical grade or better. Molecular biology reagents were obtained from Promega, Pharmacia, and New England Biolabs.

**RESULTS**

Behavior of H,K-ATPase NH₂-terminal and Ca²⁺-ATPase NH₂-terminal Sequences—As previously demonstrated (9), the M0 vector translation product shows no shift in apparent molecular weight in the absence or presence of microsomal membranes, indicating that the β region of the fusion protein does not membrane-insert. The M1 vector product has a shift of approximately 12.5 kDa in apparent molecular mass because the glycosylation consensus sequences in the β fragment access the interior of the microsomes. The M1 region thus acts as a signal anchor sequence (Fig. 2). Constructs in which the NH₂-terminal sequences of MO and M1 were replaced with the corresponding sequences of the Ca²⁺-ATPase performed identically to the HK vectors (data not shown). Subsequent experiments used the standard HK M0 and M1 vectors. The SERCA sequences that were inserted into the variable region of the MO and M1 vectors are listed in Table I.

**Membrane Insertion during Translation**—The finding of signal anchor activity for a specific sequence indicates that this sequence inserts into the ER membrane during translation and is therefore likely to be membrane-inserted in the mature protein. The presence of sequence only able to behave as a stop transfer signal does not necessarily imply membrane insertion of this sequence in the mature protein unless it is preceded by a signal anchor sequence.

We investigated a hydrophobic sequence of the soluble part of the sheep cyclo-oxygenase (35). This sequence (positions 290–317) has been shown by crystal analysis (36) to be buried inside the structure of the enzyme and is not membrane-spanning even though it has a hydrophobicity level similar to Ca H7. This sequence had no effect in the M0 vector but almost completely prevented the glycosylation of M1 (Fig. 2). Because in the native protein there is no sequence preceding this stretch of amino acid that can act as a signal anchor sequence, the stop transfer activity of this region of the protein as demonstrated in our constructs is not diagnostic of a membrane-spanning segment in the cyclo-oxygenase.

**Membrane Domain of the ER Ca²⁺-ATPase**—The hydrophobicity plot using the Kyte and Doolittle algorithm is presented in Fig. 1, with the putative membrane-spanning segments shaded. The first eight membrane spanning segments have been shown to be present by trypsinolysis of SR Ca²⁺-ATPase vesicles, and H9 was also found to be membrane-associated. The last hydrophobic sector of the ER Ca²⁺-ATPase is absent from the SR Ca²⁺-ATPase. Previous biochemical data and hydropathy guided our choice of vectors in the investigation of the signal anchor and stop transfer properties of different regions of the Ca²⁺-ATPase. Since the sequences of the ER and SR Ca²⁺-ATPases are identical except for the COOH-terminal end, our data, with the exception of the last transmembrane sequence, apply to both pumps.

H1 and H2—The putative H1 segment (positions 61–83) inserted into the MO vector promotes the glycosylation of the β sequence, acting as a strong signal anchor. If this segment is inserted into the M1 vector, it prevents the glycosylation of the β region, suggesting that it can also act as a stop transfer signal. It is therefore able to membrane-insert in either orientation (Fig. 3).

The H2 transmembrane domain of the ATPase (positions 82–109) inserted into M1, prevents the glycosylation of the β region, acting as a strong stop transfer sequence. When inserted into MO, it promotes the glycosylation of the β region. This sequence appears less effective in promoting glycosylation than the H1 sequence, which is the natural signal anchor sequence. Hence, it is a better stop transfer than signal anchor sequence.

Translation of the H1/H2 construct resulted in the absence of glycosylation, predicted from a sequence containing a signal anchor and a stop transfer segment pair. The in vitro translation properties of these segments correspond to what is expected both from the hydropathy plot and from biochemical analysis of the SR Ca²⁺-ATPase.

H3 and H4—When inserted into the MO vector, both the H3 (positions 261–282) and H4 (positions 285–324) regions of the Ca²⁺-ATPase promoted glycosylation of the β sequence, showing that both of these hydrophobic stretches can act as signal anchor sequences. The glycosylation obtained with H3 is greater than with H4. H3 is the natural signal anchor sequence. When inserted into the M1 vector, H4 acts as a stop transfer sequence corresponding to its predicted role in assembly of the native enzyme. H3 can also act as a stop transfer sequence. The translation product of the H3/H4 sequence was mostly nonglycosylated in MO, as expected by the combined activity of the signal anchor and stop transfer sequences. These
data are illustrated in Fig. 4. H5 and H6—Various sequences coding in the region of the putative fifth and sixth transmembrane domains, H5 (positions 761–785 and 742–792: long) and H6 (positions 777–824) were inserted into the M0 and M1 vectors. Various hydropathy algorithms predict that this region contains only a single membrane spanning sequence (see Table II). The putative transmembrane domain H5 (positions 761–785) showed no membrane insertion properties. A longer H5 fragment (H5 long, positions 742–792) showed some stop transfer activity. Hence only the sequence coding for the amino acids between positions 742 and 792 had any membrane insertion properties, acting as a stop transfer in the M1 vector (Fig. 5). The diagnostic significance of this finding is questionable, since there is no pairing signal anchor sequence preceding H5. No membrane insertion was found with the sequence containing H6 (positions 777–824).

The isolation of a fragment containing H5-H6, beginning at residue Leu-776 and probably ending at residue Lys-835, following trypsinolysis of intact, cytoplasmic side out vesicles (12) showed that these sequences contain a pair of membrane embedded segments in the intact SR Ca\textsubscript{2+}-ATPase.

H7 and H8—The H7 (positions 824–880) transmembrane domain inserted into M0 and M1 vectors acted both as a signal anchor and as a stop transfer sequence. Its ability to induce glycosylation of the \textit{b} sequence was less than that of H1 or H3 (Fig. 6). As also shown in Fig. 6, the putative transmembrane domain H8 (positions 881–932 or 889–921) did not show signal

| Inserts | Amino acids | Positions |
|---------|-------------|-----------|
| M0/M1   | -VYETG-     | 61–83     |
| Ca H1   | -LLVRILLACISFVLAWFEEGEE- | 61–109 |
| Ca H2   | -EETITAFEVPFVILLIVANAIAVGWQE- | 61–109 |
| Ca H1–2 | -LLVRIL----- | 261–282 |
| Ca H3   | -SKVSLICIAVNIINGHFNPD- | 285–324 |
| Ca H4   | -GGSWIRGAIYFRIAVALVAAIPEGLPAVITTCALGR- | 261–324 |
| Ca H3-4 | -SKVSL------ | 761–785 |
| Ca H5   | -RYLISNNVBEVVCIFLTAALGFPGALIPVQELVNLVLWNLVTGLPAVITTCALGR- | 742–792 |
| Ca H6   | -TAALGFPGALIPQELVNLVLWNLVTGLPAVITTCALGR- | 777–824 |
| Ca H5-6 | -IVAAVE------ | 742–824 |
| Ca H7   | -KEPLISGWLFFRYLAIGCYVGAATGGVAAWVF1ADGGFPRSVFQSLHFLQCKEDNP- | 824–880 |
| Ca H8   | -IFESPYNMTALSVLVTVIGCNALNSLENQSL- | 889–921 |
| Ca H7-8 | -KEPLIS------ | 924–921 |
| Ca H9   | -RMPWENIWLVLGICLSLMLHFLILYVE- | 923–950 |
| Ca H10  | -PLPLFIQPTFLNTQIVMLKISPLFVIMDTEIKFVPVANLEPGKECAQ- | 951–999 |
| Ca H9-10 | -RMPWFE------ | 923–999 |
| Ca H11  | -ATRPSCLLSACTDGSIPFVLLMPVPVWYSTDINPSDFWQ- | 1001–1043 |

**COOH-terminal \( \beta \)**

\[-SFLAGYSPAAQVDNINCNTSKYTYPFQCEFSPAHPWNHTK5CFATADMLNCSGLTDP-SF6GKEKGKFCFIHMKMIVRFLPSNTPPPRVDCTFLDMPHQALT-\]

**FIG. 3.** In vitro translation of the vectors MO and M1 with insertion of the Ca H1, Ca H2, and the putative membrane pair Ca H1–2. The figure shows the autoradiograms of the proteins resulting from translation of the different MO and M1 constructs (Ca H1, Ca H2, and Ca H1–2) in the absence (−) and presence (+) of microsomes followed by SDS-PAGE.

**FIG. 4.** In vitro translation of the vectors MO and M1 with insertion of the Ca H3, Ca H4, and the membrane pair Ca H3–4. The figure shows the autoradiograms of the translated proteins resulting from translation of the different MO and M1 constructs (Ca H3, Ca H4, and Ca H3–4) in the absence (−) and presence (+) of microsomes followed by SDS-PAGE.
The predictions were performed using unmodified algorithms (38–42). The Kyte-Doolittle was based on an average of 11 amino acids.

| Predicted membrane segments | Membrane segments found by |
|-----------------------------|---------------------------|
| Raai/Agrons | Eisenberg | Klein | Kyte/Doolittle |
| H1 | Leu^{96}–Phe^{98} | Leu^{96}–Glu^{90} | Leu^{97}–Ala^{91} | Leu^{97}–Ala^{96} |
| H2 | Thr^{99}–Trp^{107} | Ala^{92}–Trp^{107} | Phe^{92}–Val^{109} | Phe^{92}–Val^{109} |
| H3 | Met^{102}–Val^{128} | Leu^{92}–Val^{128} | NP^{a} | NP^{a} |
| H4 | Val^{126}–Gly^{127} | Val^{126}–Val^{128} | Val^{126}–Phe^{128} | Val^{126}–Asn^{125} |
| H5 | Trp^{129}–Thr^{133} | Ile^{129}–Cys^{131} | Ile^{129}–Val^{131} | Ile^{129}–Val^{131} |
| H6 | NP | Ile^{129}–Ala^{135} | NP^{a} | NP^{a} |
| H7 | Asn^{137}–Phe^{138} | Ile^{137}–Val^{134} | NP^{a} | NP^{a} |
| H8 | Val^{136}–Ser^{137} | Val^{136}–Phe^{138} | Val^{136}–Phe^{138} | Val^{136}–Phe^{138} |
| H9 | Met^{138}–Leu^{146} | Met^{139}–Leu^{146} | Met^{139}–Leu^{146} | Met^{139}–Leu^{146} |
| H10 | NP | NP | NP | NP |
| H11 | Leu^{1008}–Ile^{1013} | Ala^{1010}–Val^{1013} | Gly^{1012}–Met^{1013} | Gly^{1012}–Met^{1013} |

* H^{a}, only predicted.
# NP, not predicted.
\( ^{b} \) ND, not determined.
\( ^{c} \) NF, not found.

**TABLE II**

| Predicted membrane segments | Membrane segments found by |
|-----------------------------|---------------------------|
| In vitro translation | Trypsin sites |
| Ca M5 short | Ca M6 | Ca M5–6 | Ca M5 long |
| Ca H5 | Ca H6 | Ca H5–6 | Ca H5 extended sequence |
| HM0 | HM1 | HM0 | HM1 | HM0 | HM1 | HM0 | HM1 |
| kDa | 49.5 | 32.5 | 49.5 | 32.5 |

**FIG. 5.** In vitro translation of the vectors MO and M1 with insertion of the Ca H5, Ca H6, the membrane pair Ca H5–6, and the Ca H5 longer construct. The figure shows the autoradiograms of the proteins resulting from translation of the different MO and M1 constructs (Ca H5, Ca H6, Ca H5–6, and the CA H5 extended sequence) in the absence (−) and presence (+) of microsomes followed by SDS-PAGE.

**DISCUSSION**

The different approaches that have been taken to define the membrane domain of the Ca^{2+}-ATPases have not provided full information on the number of transmembrane sequences. Most of the SERCA pump putative transmembrane domains appear to be amenable to in vitro translation analysis, as were those of the H,K-ATPase and the H. pylori P type ATPase (9, 31).

The translation results for the first four transmembrane segments show that they are able to act as both signal anchor and stop transfer sequences. H2 and H4 act as signal anchor sequences as demonstrated by glycosylation in the MO fusion protein despite the fact that their membrane orientation in the fusion protein is opposite to their orientation in the assembled enzyme. The same effect can be seen with H1 and H3 inserted into the M1 vector. Thus, the orientation of the segment appears not to be important in determining membrane insertion in these particular constructs. The same result has been found with the H,K-ATPase (9). These sequences were able to insert individually in contrast to the pairing apparently required by the membrane integration of H1 and H2 of Neurospora ATPase (26).

No region in the predicted H5, H6, or H5 with H6 acted as a signal anchor sequence. However, H5 with part of H6 was able to act as a stop transfer sequence. In order to interpret the appearance of only a stop transfer sequence, where a signal anchor and stop transfer pair was expected, we tested a sequence from cyclo-oxygenase containing a hydrophobic stretch of amino acids that is known not to be membrane-inserted (35, 36). It acted as a stop transfer sequence in M1 but not as a signal anchor sequence in M0. Therefore, the finding of a stop transfer sequence in the H5 region of the Ca^{2+}-ATPase, in the absence of a preceding signal anchor sequence, should not be interpreted as evidence for co-translational membrane inser-
Membrane Topology of SERCA ATPases

The various sequences in M0 and/or M1 vectors and their effect on translation

| Hydrophobic segment | Amino acid | Glycosylation in | M0 | M1 |
|---------------------|------------|------------------|----|----|
| Ca H1               | Leu<sup>41</sup> | Glu<sup>83</sup> | -<sup>a</sup> | -<sup>b</sup> |
| Ca H2               | Glu<sup>82</sup> | Glu<sup>109</sup> | + | - |
| Ca H1–1             | Leu<sup>41</sup> | Glu<sup>109</sup> | - | ND<sup>c</sup> |
| Ca H3               | Ser<sup>91</sup> | Pro<sup>292</sup> | + | - |
| Ca H5               | Gly<sup>825</sup> | Arg<sup>324</sup> | + | - |
| Ca H3–4             | Ser<sup>261</sup> | Arg<sup>224</sup> | - | ND |
| Ca H5               | Arg<sup>91</sup> | Ala<sup>785</sup> | + | - |
| Ca H5 lo            | Ile<sup>277</sup> | Leu<sup>926</sup> | - | - |
| Ca H6               | Thr<sup>277</sup> | Lys<sup>324</sup> | + | - |
| Ca H5–6             | Ile<sup>277</sup> | Lys<sup>324</sup> | - | ND |
| Ca H7               | Lys<sup>277</sup> | Pro<sup>306</sup> | + | - |
| Ca H8               | Ile<sup>290</sup> | Lys<sup>291</sup> | - | +/– |
| Ca H7–8             | Lys<sup>277</sup> | Lys<sup>324</sup> | + | ND |
| Ca H9               | Arg<sup>293</sup> | Gin<sup>299</sup> | + | - |
| Ca H10              | Pro<sup>291</sup> | Gin<sup>299</sup> | - | - |
| Ca H9–10            | Arg<sup>293</sup> | Gin<sup>299</sup> | - | ND |
| Ca H11              | Ala<sup>201</sup> | Ser<sup>1043</sup> | + | - |

<sup>a</sup> +, glycosylation.
<sup>b</sup> –, no glycosylation.
<sup>c</sup> ND, not determined.
<sup>lo</sup> extended sequence.

In vitro translation of the vectors MO and M1 with insertion of the Ca H9, Ca H10, the Ca H11, and the membrane pair Ca H9–10. The figure shows the autoradiograms of the lanes resulting from translation of the different MO and M1 constructs (Ca H9, Ca H10, Ca H11, and the Ca H9–10 pair) in the absence (−) and presence (+) of microsomes followed by SDS-PAGE.

![Image](image_url)

**FIG. 7.** In vitro translation of the vectors MO and M1 with insertion of the Ca H9, Ca H10, the Ca H11, and the membrane pair Ca H9–10. The figure shows the autoradiograms of the lanes resulting from translation of the different MO and M1 constructs (Ca H9, Ca H10, Ca H11, and the Ca H9–10 pair) in the absence (−) and presence (+) of microsomes followed by SDS-PAGE.

Table III

The various sequences in M0 and/or M1 vectors and their effect on translation

| Hydrophobic segment | Amino acid | Glycosylation in | M0 | M1 |
|---------------------|------------|------------------|----|----|
| Ca H1               | Leu<sup>41</sup> | Glu<sup>83</sup> | -<sup>a</sup> | -<sup>b</sup> |
| Ca H2               | Glu<sup>82</sup> | Glu<sup>109</sup> | + | - |
| Ca H1–1             | Leu<sup>41</sup> | Glu<sup>109</sup> | - | ND<sup>c</sup> |
| Ca H3               | Ser<sup>91</sup> | Pro<sup>292</sup> | + | - |
| Ca H5               | Gly<sup>825</sup> | Arg<sup>324</sup> | + | - |
| Ca H3–4             | Ser<sup>261</sup> | Arg<sup>224</sup> | - | ND |
| Ca H5               | Arg<sup>91</sup> | Ala<sup>785</sup> | + | - |
| Ca H5 lo            | Ile<sup>277</sup> | Leu<sup>926</sup> | - | - |
| Ca H6               | Thr<sup>277</sup> | Lys<sup>324</sup> | + | - |
| Ca H5–6             | Ile<sup>277</sup> | Lys<sup>324</sup> | - | ND |
| Ca H7               | Lys<sup>277</sup> | Pro<sup>306</sup> | + | - |
| Ca H8               | Ile<sup>290</sup> | Lys<sup>291</sup> | - | +/– |
| Ca H7–8             | Lys<sup>277</sup> | Lys<sup>324</sup> | + | ND |
| Ca H9               | Arg<sup>293</sup> | Gin<sup>299</sup> | + | - |
| Ca H10              | Pro<sup>291</sup> | Gin<sup>299</sup> | - | - |
| Ca H9–10            | Arg<sup>293</sup> | Gin<sup>299</sup> | - | ND |
| Ca H11              | Ala<sup>201</sup> | Ser<sup>1043</sup> | + | - |

<sup>a</sup> +, glycosylation.
<sup>b</sup> –, no glycosylation.
<sup>c</sup> ND, not determined.
<sup>lo</sup> extended sequence.

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