Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Membrane Integration and Intracellular Transport of the Coronavirus Glycoprotein E1, a Class III Membrane Glycoprotein*

(Received for publication, February 23, 1988)

Thomas Mayer, Teruko Tamura, Matthias Falk, and Heiner Niemann
From the Institut für Medizinische Virologie der Justus-Liebig-Universität, Frankfurter Str. 107, D-6300 Giessen, Federal Republic of Germany

The E1-glycoprotein \(M_r = 28,014; 228\) amino acids) of mouse hepatitis virus A59 is a class III membrane glycoprotein which has been used in this study as a model system in the study of membrane integration and protein transport. The protein lacks an NH\(_2\)-terminal cleavable signal sequence and spans the viral membrane three times. Hydrophobic domains I and III could serve as signal sequences for cotranslational membrane integration. Domain I alone was sufficient to translocate the hydrophilic NH\(_2\)-terminus of E1 across the membranes as evidenced by glycosylation of a newly introduced N-glycosylation site. The COOH-terminal part of E1 involving amino acids Leu\(^{124}\) to Thr\(^{326}\) was found to associate tightly with membranes at the post-translational level, although this part of the molecule lacks pronounced hydrophobic sequences. Membrane protection assays with proteinase K showed that a 2-kDa hydrophilic fragment was removed from the COOH terminus of E1 indicating that the protein is largely embedded into the membrane. Microinjection of \textit{in vitro} transcribed capped and polyadenylated mRNA into CV-1 cells or into secretory AtT20 pituitary tumor cells showed that the E1-protein accumulated in the Golgi but was not detectable at the plasma membrane or in secretory granules. The 28 NH\(_2\)-terminal hydrophilic amino acid residues play no role in membrane assembly or in intracellular targeting.

Various NH\(_2\)-terminal portions of E1 were fused to Ile\(^{140}\) of the cytoplasmic N-protein of mouse hepatitis virus. The resulting hybrid proteins were shown to assemble into membranes \textit{in vitro} and were detected either in the rough endoplasmic reticulum or transient vesicles of microinjected cells.

Membrane proteins have been divided into three groups based on their specific orientation in the membrane (Wickner and Lodish, 1985; Garoff, 1985). According to this classification of E1-glycoprotein of MHV* A59 belongs to the group III proteins which span a membrane several times (Armstrong et al., 1984; Rottier et al., 1986). The E1-protein has three functional domains. The ectodomain representing the 28 NH\(_2\)-terminal amino acids is hydrophilic and carries exclusively O-linked oligosaccharides which exhibit, in conjunction with the terminal amino acid sequence Ser-Ser-Thr-Thr, blood group M activity (Niemann et al., 1984b). A hydrophilicity analysis of E1 according to Kyte and Doolittle (1982) reveals four internal hydrophobic stretches (Fig. 1) that span the viral membrane three times and presumably contribute to the rigidity of the viral membrane. The carboxy-terminal part of E1 interacts with the viral nucleocapsid and thus plays an important role in the stages of virus formation (Sturman et al., 1986).

Cell fractionation studies of MHV A59-infected cells indicated that the E1 protein was synthesized on membrane-associated polysomes (Niemann et al., 1982). In contrast to most other viral glycoproteins the E1 protein could not be detected at the plasma membrane of infected cells other than in the form of virus particles. The intracellular distribution of E1 was restricted to perinuclear regions (Doller and Holmes, 1980) and thus paralleled the sites at which budding of coronavirus particles was observed at early stages of infection (Becker et al., 1967; Holmes et al., 1981; Tooze et al., 1984). Recent studies showed that this intracellular accumulation of the E1-protein in smooth vesicles is not due to an interaction of E1 with other coronavirus proteins but is an integral feature of the E1-protein itself (Machamer and Rose, 1987; Rottier and Rose, 1987; Niemann et al., 1987).

In this study we have used \textit{in vitro} transcription/translation and microinjection techniques in combination with indirect immunofluorescence to study the membrane transport properties of the E1-polypeptide in more detail. We show that the E1-protein accumulates in perinuclear regions of fibroblasts and secretory cells. Based on the expression of various E1-mutants we show that deletions or additional N-glycosylation of the amino-terminal domain of E1 do not effect the Golgi-specific transport block. Internal hydrophobic domains I and III could mediate cotranslational integration of the polypeptide into microsomal membranes. An E1-mutant lacking all three hydrophobic domains associates with membranes also post-translationally. We show that fusion proteins between various parts of the E1 and a cytoplasmic protein integrate into membranes cotranslationally and accumulate in membranes of the RER and perinuclear vesicles.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

Experimental Strategy and Construction of Mutants of the E1-gene of MHV A59—To study the membrane assembly

* Portions of this paper (including "Experimental Procedures," Fig. 9, and one table) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

1 This work was supported by Grant Nr 175/5 from the Deutsche Forschungsgemeinschaft and by Fonds der Chemischen Industrie.

2 To whom reprint requests should be addressed.

3 The abbreviations used are: MHV, mouse hepatitis virus; RER, rough endoplasmic reticulum; ACTH, adrenocorticotropic hormone; WGA, wheat germ agglutinin; SDS, sodium dodecyl sulfate.
process of the E1-protein of MHV A59 we used in vitro synthesis of capped and polyadenylated E1-specific mRNA from pSP65 vectors (Krieg and Melton, 1984) and its subsequent translation in the presence of translocation-competent microsomal membranes. To obtain polyadenylated transcripts, an oligo(dA-dT) fragment derived from pSVa970 (Min Jou et al., 1980) was inserted downstream from the E1 coding sequences (Niemann et al., 1984a) as detailed in the Miniprint Section. Membrane translocation was assessed (i) by protection of the translocated domains from attack of exogenous proteinase K; (ii) by cosedimentation of the translated products with the microsomal fraction at neutral or alkaline pH; (iii) by glycosylation of a newly introduced N-glycosylation site at the NH₂ terminus of the E1-protein. Based on predictions of the secondary structure of the E1-protein (Fig. 1B; Rottier et al., 1986) and on the hydrophobicity (Fig. 1A; Kyte and Doolittle, 1982) we introduced additional restriction sites into the E1-gene by site-directed mutagenesis. These sites were used to construct a set of deletion mutants and fusion proteins as indicated in Fig. 1C. To analyze the intracellular distribution of the individual proteins, the corresponding mRNA was microinjected into various cell types and the proteins were visualized by indirect immunofluorescence.

The Hydrophilic NH₂-terminal Domain of the E1-protein Plays No Role in Membrane Integration nor in Establishing the Topology—Fig. 2 shows the results obtained by in vitro translations of E1-specific mRNA carrying mutations within the hydrophilic NH₂-terminal region. In agreement with published data (Niemann and Klenk, 1981) the wild type E1-protein (M, 26,014) is not glycosylated when microsomal membranes are added to the translation mixture. This observation supports the structural model of the E1-protein which suggests that the potential sequon (-Asn*-Phe-Ser-) is embedded in the membrane and thus not accessible for the glycosyl transferase. Proteinase K treatment of the transla-

![Fig. 1. Mutants of the E1-protein. Panel A, hydrophathy plot of the E1-polypeptide according to the program of Kyte and Doolittle (1982). The positions of restriction sites used for the construction of mutants are indicated. Restriction sites marked with a star were introduced by site-directed mutagenesis. Panel B, amino acid sequence of the E1-polypeptide shown in the single letter code. Amino acid changes resulting from the generation of restriction sites are indicated. Charged residues are indicated by + or – underneath the sequence. Open boxes show sequences with α-helical probability according to Eisenberg et al. (1984). Dots indicate the location of bends determined by the programs of Chou and Fasman (1978) and Cid et al. (1982). Panel C, construction of E1 deletion mutants and fusion genes. Black boxes indicate regions encoding hydrophobic domains. Dotted areas correspond to sequences encoding the MHV JHM nucleoprotein. Restriction sites used for the construction of fusions are depicted. Filled triangles symbolize mutations made to introduce N-glycosylation sites.](image-url)
tion products obtained in the presence of membranes yielded a truncated 24,000-dalton form. Rottier et al. (1985) have shown that this species represents the E1-protein lacking a 2,000-dalton fragment from the carboxyl-terminal end. To assess luminal exposure of the NH2-terminal domain, an N-glycosylation site (Asn-Thr-Thr) was introduced into this region by site-directed mutagenesis. The resulting polyepitope, designated E1Asn, was indeed glycosylated in the presence of membranes, as indicated by the formation of a 29,000-dalton species. The proteolytic cleavage product from this glycosylated species was larger (M, 26,500) than that of Elwt, again demonstrating that in the absence of detergent the proteolytic attack occurred exclusively within the carboxyl-terminal part of the E1-molecule. In the presence of detergent the E1Asn-species was degraded to a 15,600-dalton fragment as also obtained from Elwt, indicating that the N-glycosylation site was removed (data not shown).

Consistent with the size of the deletions, the two mutants El14-23 and El14-28 generated integral membrane proteins that were about 2,500 or 3,000 daltons smaller than the Elwt-peptide. Both peptides were efficiently integrated into the membranes. Proteolysis gave products that were again about 2,000 daltons smaller than the original peptides indicating that their overall structure in the lipid bilayer remained unaltered. In the presence of detergent all E1-mutants were degraded to the 15,600-dalton species indicating that the NH2-terminus was removed under such conditions. As indicated by the size of this fragment and further evidence below, additional cleavage in detergent also removed parts of the COOH-terminal tail.

The E1-protein Contains More than One Signal Sequence—

To analyze which of the internal hydrophobic domains was essential for membrane integration, we produced mutants in which one or more of these domains were deleted. The results are summarized in Fig. 3. A deletion of the first hydrophobic domain, as present in El14-50 (M, 20,100), neither prevented membrane integration nor did it alter the orientation of the protein in the membrane, as indicated by the proteolytic removal of the typical 2,000-dalton fragment. Analysis of El14-23 (M, 15,000), retaining solely the first hydrophobic sequence, did not yield any detectable protected fragment. The results obtained with preprolactin control mRNA (Fig. 3C) indicated that the membrane preparation was not leaky for the protease. The El14-50-Asn molecule, carrying the newly created N-glycosylation site, yielded a glycosylated 18,500-dalton species. Treatment with endo-β-N-acetylglicosaminidase H created a third molecular species which was somewhat larger than the nonglycosylated form. The cotranslational addition of increasing amounts of an acceptor peptide for N-glycosylation (benzoyl-Asn-Leu-Thr) was introduced into this gene (Bause, 1983) revealed that only one of the two sites was glycosylated (data not shown).

The El23-123-Asn (Fig. 3A) lacked all three hydrophobic domains and provided the NH2-terminal glycosylation site as a reporter group for luminal exposure. This peptide was not glycosylated and was completely degraded by the protease even in the absence of detergent. This finding excludes the possibility that smaller E1-peptides could diffuse through the membrane and provides further evidence that the COOH-terminal hydrophilic part of E1 was not intrinsically resistant to the protease.

Mutant El14-80 (Fig. 3C), retaining hydrophobic domain III, was inserted into the membranes. Treatment with the protease revealed that it was not secreted but remained anchored in the membranes. This domain seemed to be sufficient to stabilize the carboxyl-terminal part of the molecule within the membrane, since protease treatment removed only the COOH-terminal 2,000-dalton fragment from El14-80. In the absence of detergent, however, the E14-80 molecule was degraded to an 8,500-dalton species. The size of this product in comparison to that obtained from El14-194 under detergent conditions (15,500 daltons) indicates that in both instances the resistant fragments contained hydrophobic sequences and parts from the COOH-terminal part of the E1-molecule. The deletion of amino acids 154-194 made the COOH-terminal region susceptible to protease K even in the absence of detergent, as evidenced by the release of a 4,500-dalton fragment yielding a peptide of almost the same size as the product obtained in the presence of detergent.

When part of the hydrophobic domain II was deleted, as shown in Fig. 3C for El165-80-Asn, the overall topology of the mutant protein remained unaltered. Protease cleavage removed a 6,500-dalton fragment and thus did not occur at the original site around amino acid 205, but about 40 amino acids displaced toward the NH2-terminus yielding a protected fragment of about 21 kilodaltons. We interpret these findings to mean that part of the domain II helps to stabilize the COOH-terminal tail of E1 in the membranes.

Co- and Post-translational Interaction of the E1-mutants with Microsomal Membranes—To analyze whether membrane integration of the individual mutants was coupled to translation, we examined peptides, to which membranes had been

![Fig. 3. Membrane translocation of E1-mutants lacking individual hydrophobic domains.](image-url)
added before or after their synthesis, for cosedimentation with the membranes at neutral or alkaline pH. The results of Fig. 4 show that all the molecular species retaining one of the hydrophobic domains integrated exclusively at the cotranslational level and were present in the pellet fraction. The finding that the peptides E1A45–132 and E1A4–80 were not released at alkaline pH further supports our conclusion that the hydrophobic domains I and III function simultaneously as signal and stop transfer sequences.

In contrast, peptide E1A23–123, although lacking all three internal hydrophobic domains, clearly associated with the membranes at the co- and post-translational level at either pH.

**Membrane Assembly of E1-N Fusion Proteins**—We have constructed four E1-N fusion proteins containing NH2-terminal E1-specific sequences fused via the amino acid indicated to Ile465 of the nucleoprotein of MHV JHM (Fig. 1C). The results summarized in Fig. 5 revealed that all peptides with the exception of E1-N(3–145) were integrated and anchored in the membranes.

As demonstrated by the analyses of E1-N(64–145) and E1-N(80–145), the second hydrophobic domain or the remainder of it was also embedded into the membranes and thus protected against proteolytic attack yielding products of 10,400 and 11,200 daltons, respectively. Fragments of this size could not be derived from the nucleoprotein, since no proteolytic degradation products could be identified from E1-N(3–145). E1-N(207–145) yielded fragments in the protease protection assay that were indistinguishable from the corresponding fragment derived from E1wt, indicating the identical membrane topology of the fusion protein. The topology of the NH2 termini was verified by analyzing the corresponding variants carrying the newly created N-glycosylation site (data not shown).

**Intracellular Transport Properties of the E1-protein and Its Mutants**—The in vitro synthesized mRNA was capped and polyadenylated in order to increase its half-life after microinjection into eucaryotic cells (Huez et al., 1981; Drummond et al., 1985). The intracellular targeting of the E1-proteins was studied by indirect immunofluorescence as detailed under “Experimental Procedures.”

In agreement with published data (Machamer and Rose, 1987; Niemann et al., 1987; Rottier and Rose, 1987), the E1-protein accumulated in perinuclear regions of the injected cells (Fig. 6B). In double-labeling experiments these regions could not be distinguished from those recognized by the Golgi-specific rhodamine-labeled wheat germ agglutinin (Fig. 6A). The specific distribution of E1 was observed in about 50% of the injected cells while the remaining cells did not respond with any synthesis of E1-protein.

No E1 could be detected on the surface of injected cells as judged by the failure of staining with polyclonal E1-specific antibodies against virus particles and purified by elution from Western blots. In addition, no staining was obtained with antibodies directed against a synthetic peptide consisting of the eight NH2-terminal amino acids (Ser-Ser-Thr-Thr-Gln-Ala-Pro-Glu) of E1 (data not shown). Even at late stages after injection or when 3-fold larger amounts of RNA (3 μg/ml) were injected, E1 was absent from the plasma membrane. In such instances also the nuclear membrane and the RER of
Golgi-specific Transport of the El-glycoprotein

The amino-terminal domain of El does not alter its intracellular transport properties.

In addition, a deletion of most of the hydrophilic NH_{2}-terminal domain had no influences on the intracellular targeting as shown for the ElA4-28-protein in Fig. 6, G and H.

Intracellular Transport of El-N Fusion Proteins—Fig. 7 shows the intracellular distribution of newly synthesized El-N-proteins. A monoclonal antibody directed against the nucleoprotein was used to detect the fusion proteins. In agreement with the observation that El-N(3-145) did not integrate into the membranes in vitro (Fig. 5), the polypeptide was found dispersed throughout the cytoplasm of the injected cell (Fig. 7A). In contrast, El-N(64-145) containing the first and part of the second hydrophobic domain accumulated in membranes of the RER (Fig. 7C) as indicated by double-labeling with a polyclonal antibody binding to the carboxyl-terminal domain of canine ribophorin I (Fig. 7D). Therefore, both antibodies bound to epitopes that were located at the cytoplasmic face of the RER. The El-N(80-145)-protein containing the first two membrane-spanning domains accumulated

the injected cells contained El-protein (data not shown). This observation indicates that the El-protein is accumulating rapidly in membranes of the Golgi and piles up in the RER only after the former membranes are saturated (Tooze et al., 1984).

To determine whether the perinuclear accumulation of El-protein was a phenomenon restricted to fibroblasts, we injected mRNA into AtT20-cells, a transformed mouse pituitary gland cell line secreting ACTH. Again, the El-protein was present in the Golgi region of the injected cells (Fig. 6D). No El was detectable at the cell surface (not shown), and no El was present in peripheral secretory granules that were labeled with antibodies against ACTH (Fig. 6C).

Fig. 6, E and F, show that the ElAsn mutant protein also accumulated in Golgi-like compartments. Since the ElAsn species was efficiently glycosylated in the in vitro assay, it is highly likely that this glycosylation also occurs in vivo. This would then allow the conclusion that cotranslation N-glycosylation of the amino-terminal domain of El does not alter its intracellular transport properties.

In addition, a deletion of most of the hydrophilic NH_{2}-terminal domain had no influences on the intracellular targeting as shown for the ElA4-28-protein in Fig. 6, G and H.

Intracellular Transport of El-N Fusion Proteins—Fig. 7 shows the intracellular distribution of newly synthesized El-N-proteins. A monoclonal antibody directed against the nucleoprotein was used to detect the fusion proteins. In agreement with the observation that El-N(3-145) did not integrate into the membranes in vitro (Fig. 5), the polypeptide was found dispersed throughout the cytoplasm of the injected cell (Fig. 7A). In contrast, El-N(64-145) containing the first and part of the second hydrophobic domain accumulated in membranes of the RER (Fig. 7C) as indicated by double-labeling with a polyclonal antibody binding to the carboxyl-terminal domain of canine ribophorin I (Fig. 7D). Therefore, both antibodies bound to epitopes that were located at the cytoplasmic face of the RER. The El-N(80-145)-protein containing the first two membrane-spanning domains accumulated
in perinuclear membranes (Fig. 7E) which were not labeled with the ribophorin-specific antibody (not shown). Some of the El-N containing compartments were stained by the Golgi-specific lectin (Fig. 7F). The intracellular distribution El-N(207-145) followed basically the pattern specific for the RER. The labeled structures, however, seemed to have a more vesicular character. By using WGA in similar double-labeling experiments it became obvious that these vesicles were not closely associated with Golgi compartments. It is feasible to assume that these vesicular structures represent transient vesicles which are derived from the RER and constitute the primary sites of virus maturation in the infected cell (Becker et al., 1987; Tooze et al., 1984).

**DISCUSSION**

We have analyzed the topogenic signals and the intracellular transport properties of the glycoprotein El of MHV A59, a class III membrane glycoprotein.

One of the models for the biosynthesis of polytopic membrane proteins suggests that these multispanning proteins are translocated into the endoplasmic reticulum membrane by alternating signal and stop transfer sequences (Friedlander and Blobel, 1985; Kopito and Lodish, 1985). Recently Zerial et al. (1987) have demonstrated that foreign peptides could replace the internal signal and anchor sequence of the human transferrin receptor. These studies suggested that the hydrophobic character and the position in the molecule rather than the actual amino acid composition determine the character of a transmembrane sequence. In light of these findings we did not attempt to take the internal hydrophobic domains of the El-protein of MHV A59 completely out of their context by transferring them into different proteins. Instead, we have constructed deletion mutants and fusion proteins which retained authentic El-sequences either from the NH2 terminus or from the COOH terminus. We show here that the domains I and III could function as signal and stop transfer sequences determining the topology of the El-molecule (Fig. 8).

(i). The NH2-terminal hydrophilic domain of El does not play a role in the membrane integration process or in determining the topology of the El-protein. No cleavable signal sequence is uncovered by the removal of this part of the El-molecule which notably shows the largest degree of heterogeneity among different strains of coronaviruses (Lappe et al., 1987; Rascheier et al., 1987; Bourne et al., 1984).

(ii). Hydrophobic domain I alone was sufficient to translocate the amino-terminal part of the El-molecule to the luminal side as demonstrated by the glycosylation of the newly created N-glycosylation site in ElA45-132-Asn. No glycosylation was observed when membranes were added post-translationally. The orientation of the ElA45-132 molecules is identical to that of the M2-protein of influenza virus (Lamb et al., 1986) but differs from that of other glycoproteins with internal uncleavable signal sequences such as the asialoglycoprotein receptor (Spiess and Lodish, 1986), the human transferrin receptor (Zerial et al., 1986), or the human glucose transporter (Mueckler and Lodish, 1986). At present we do not know whether domain I can translocate only NH2-terminal sequences of a limited size. While the El-proteins from the bovine and the avian coronaviruses have hydrophilic ectodomains containing 28 and 22 amino acids, respectively, the corresponding ectodomain of the El-preprotein from transmissible gastroenteritis virus is 46 amino acids in length. Interestingly, this polypeptide is synthesized with an additional NH2-terminal cleavable signal sequence of 17 amino acid residues (Lau et al., 1987).

(iii). The transmembrane domain I functioned as a stop transfer sequence, even though basic amino acid residues present in the cytoplasmic loop between domains I and II were removed together with domains II and III. Clearly, ElA45-312 was not secreted into the lumen since the native glycosylation site (AsnZ7-Phe-Ser) was not glycosylated in this deletion mutant or in a corresponding El-N fusion protein.

(iv). The presence of a second signal sequence within the third hydrophobic domain was demonstrated by the analysis of ElA4-80. This protein was inserted into the membrane exclusively at the cotranslational level, and the peptide exhibited the authentic orientation (Fig. 8). It has been shown previously that signal recognition particles exert a translational block as late as up to a point in the translation when two-thirds of the El-molecule (159 amino acids) have been synthesized (Rottier et al., 1985). These data are in agreement with our observation that the third domain indeed functions as a signal sequence.

Our conclusion that the hydrophobic domain II of the El-protein is not actively involved in the membrane insertion process is based on indirect evidence. First, the two polypeptides containing either a combination of domains I and II (present in mutant El-N(80-145)) or II and III (present in ElA4-50) assembled in the membrane in the original orientation. Second, ElA45-80 which lacked the first half of domain II was integrated efficiently into membranes with the authentic topology, as indicated by N-glycosylation of the NH2 terminus. We interpret these findings to mean that membrane integration and orientation of domain II are predetermined by the presence of domains I and III. However, our data do not exclude the possibility that domain II could function independently as a signal sequence.

The capability of the COOH-terminal tail of El to associate with membranes post-translationally was unexpected. This behavior may reflect the natural role of El as a matrix protein guiding the viral nucleocapsid to the place of virus budding (Sturman et al., 1980).

Our microinjection experiments indicated that the El-protein has an intrinsic signal for a retention in Golgi-like compartments in fibroblasts and secretary AT20 cells. This retention signal of the El-protein is functional in the absence of other viral proteins. Similar results have been obtained previously for the El-protein of avian infectious bronchitis virus (IBV) (Mschamer and Rose, 1987) and for the El-protein of MHV A59 using DNA expression vectors (Niemann et al., 1987; Rottier and Rose, 1987). In MHV A59-infected AT20 cells virus particles were shown to bud into pre-Golgi compartments and then share the secretory pathway with the secretory protein ACTH through the same Golgi stacks into
the trans-Golgi network. At this site the constitutive secretory pathway for the virus and the regulated secretory pathway for the hormone diverged (Tooze et al., 1987). We show here that this transport property was also shared by the isolated E1-protein, since it was not detected in secretory post-Golgi vesicles filled with ATCH.

Studies by Machamer and Rose (1987) demonstrated that the first transmembrane domain of the protein from the infectious bronchitis virus was responsible for its retention in the Golgi while a protein retaining only the third transmembrane domain was transported to the plasma membrane. Unfortunately, we were unable to detect E1-peptides after microinjection of mRNA encoding E1Δ45-132 and E1Δ4-80. At present we do not know whether this is due to an instability of the corresponding mRNA, whether the protein synthesized in vivo was degraded, or whether it was too dispersed throughout the cells to be detected with the antibodies.

The described modifications of the ectodomain of the E1-molecule had no influence on the E1-specific transport properties. To assess the applicability of parts of the E1-molecule to direct fusion proteins into the Golgi, we have microinjected mRNA encoding various parts of the E1-protein fused in frame to a carboxy-terminal part of the cytoplasmic N-protein of MHV JHM. Each of the fusion proteins containing one or more of the hydrophilic domains of E1 was detected in perinuclear membranes. The fusion proteins E1-N(64-145) and E1-N(80-145) were not transported into the Golgi indicating that particular nucleoprotein-specific sequences added to the cytoplasmic COOH terminus prevented release from the RER. Only in very few cells the intracellular distribution of E1-N(207-145) overlapped with the Golgi pattern as stained by WGA, and it was also different than the pattern obtained with RER-specific antibodies. We suggest that the compartments harboring the El-N(207-145) are transient vesicles which in the virus-infected cells are the sites of particle formation. Experiments involving immunoelectron microscopy on cells infected with recombinant vaccinia virus are currently in progress.

Acknowledgments—We thank David Meyer (UCLA, Los Angeles) and John Tooze (EMBL, Heidelberg) for antibodies directed against ribophorin and ACTH, respectively. We are indebted to Bernhard Dobberstein (EMBL, Heidelberg) for providing dog pancreatic membranes and for perinuclear staining. We thank Juan Ortín (Universidad Autónomas, Madrid) and Carl Blobel (University of California, San Francisco) for plasmids pSVa970 and pB4.

REFERENCES

Amsorae, W. (1982) Exp. Cell Res. 140, 31-37
Armstrong, J., Niemann, H., Simkens, S., Rottier, P., and Warren, G. (1984) Nature 309, 751-752
Bause, E. (1985) Biochim. J. 204, 321-336
Becker, W. B., McIntosh, I., Dees, J. H., and Chanock, R. (1967) J. Virol. 1, 1019-1027
Bonnassel, M. E. G., Brown, T. D. K., and Blinn, M. M. (1984) Virus Res. 1, 393-413
Burke, B., Griffiths, G., Reggio, H., Louvard, D., and Warren, G. (1982) EMBO J. 1, 1621-1628
Chou, P. Y., and Pasman, G. D. (1978) Adv. Enzymol. Relat. Areas Mol. Biol. 47, 247-254
Clé, H., Bunster, M., Arriagada, E., and Campea, M. (1982) FEBS Lett. 160, 247-254
Doller, E. W., and Holmes, K. V. (1980) Abstr. Annu. Meet. Am. Soc. Microbiol. Abstr. T150, p. 897
Drummond, D. R., Armstrong, J., and Colman, A. (1985) Nucieic Acids Res. 13, 7375-7394
Eisenberg, D., Schwartz, E., Konaromy, M., and Wall, R. (1984) J. Mol. Biol. 179, 125-152
Friedlander, M., and Blobel, G. (1985) Nature 318, 338-343
Garoff, H. (1985) Annu. Rev. Cell Biol. 1, 405-445
Gilmore, R., and Blobel, G. (1985) Cell 42, 497-505
Holmes, K. V., Doller, E. W., and Behnke, J. N. (1981) Adv. Exp. Med. Biol. 142, 133-149
Hutz, C., Bruck, C., and Cleuter, Y. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 908-911
Jackson, R. J., and Hunt, T. (1983) Methods Enzymol. 96, 50-74
Kopito, R. R., and Lodish, H. F. (1985) Nature 316, 234-238
Kriem, P. A., and Melton, D. A. (1984) Nucleic Acids Res. 12, 7657-7670
Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 488-492
Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105-132
Lisowski, M. K. (1970) Nature 227, 680-685
Lamb, R. A., Zobeidee, S. L., and Richardson, C. D. (1986) Cell 40, 621-636
Lappas, W., Hogue, B. G., and Brian, D. A. (1987) Virology 157, 47-57
Laude, H., Rasschaert, D., and Huet, J.-C. (1987) J. Gen. Virol. 68, 1687-1696
Machamer, C. E., and Rose, J. K. (1987) J. Cell Biol. 105, 1209-1214
Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Min Jou, W., Verhous, M., Devos, R., Saman, E., Fang, R., Huygens, M., Fierse, W., Theefall, G., Barber, C., Carey, N., and Enzagie, S. (1986) Cell 19, 685-696
Mueckler, M., and Lodish, H. F. (1966) Cell 44, 629-637
Niemann, H., and Klenk, H.-D. (1981) J. Mol. Biol. 153, 963-1010
Niemann, H., Bossekh, B., Evans, D., Rosing, M., Tamura, T., and Klenk, H.-D. (1984) Adv. Exp. Med. Biol. 173, 201-213
Niemann, H., Gayer, R., Klenk, H.-D., and Wirth, M. (1984a) Adv. Exp. Med. Biol. 173, 201-213
Niemann, H., Gayer, R., Klenk, H.-D., Linder, D., Sturm, S., and Wirth, M. (1984b) EMBO J. 3, 665-670
Niemann, H., Mayer, T., Wirth, M., and Tamura, T. (1987) Adv. Exp. Med. Biol. 218, 83-97
Olmsdet, J. B. (1981) J. Biol. Chem. 256, 11955-11957
Rasschaert, D., Celfi, G., and Laude, H. (1987) Biochimie 69, 391-400
Rottier, P., Tamura, T., Bossekh, C. B., Wege, H., Schwartz, R. T., and Niemann, H. (1985) J. Biol. Chem. 260, 15673-15679
Rottier, P. J. M., and Rose, J. K. (1977) J. Virol. 11, 2042-2045
Rottier, P., Armstrong, J., and Meyers, D. I. (1985) J. Biol. Chem. 260, 4646-4652
Rottier, P. J. M., Welling, W. G., Welling-Wester, S., Niester, H. G. M., Lenstra, J. A., and van der Zeijst, A. M. (1986) Biochemistry 25, 1335-1339
Skinner, M. A., and Siddell, S. G. (1983) Nucleic Acids Res. 11, 5045-5054
Spies, M., and Lodish, H. F. (1986) Cell 44, 177-185
Sturman, S. I., Holmes, K. V., and Behnke, J. (1990) J. Virol. 53, 449-462
Tamura, T., Bauer, H., Bizz, C., and Pipkorn, R. (1983) Cell 34, 687-696
Tooze, J., Tooze, S., and Warren, G. (1984) Eur. J. Cell Biol. 33, 281-283
Tooze, J., Tooze, S. A., and Fuller, S. (1977) J. Cell Biol. 105, 1215-1216
Wege, H., Bories, R., and Wege, H. (1984) J. Virol. 59, 1247-1248
Wickner, W. R., and Lodish, H. F. (1985) Science 220, 400-407
Zerial, M., Melanocon, F., Schneider, C., and Garoff, H. (1986) EMBO J. 5, 1543-1559
Zerial, M., Haytlebrooke, D., and Garoff, H. (1987) Cell 48, 147-155
Golgi-specific Transport of the El-glycoprotein

3 Experimental Procedures

3.1 Preparation of In-frame Translation Vectors

The in-frame translation vectors used in this study, pEF1059 and pEF1061, were constructed by inserting 5' EcoRI and 3' XbaI restriction sites into the containing vectors pEF1053 and pEF1054, respectively. The sequences of the 5' EcoRI and 3' XbaI sites were deduced from the published sequences of the corresponding restriction enzymes.

Fig. 3: Preparation of In-frame Translation Vectors

The in-frame translation vectors were used in this study, pEF1059 and pEF1061, to express the El-glycoprotein in mammalian cells. These vectors were constructed by inserting 5' EcoRI and 3' XbaI restriction sites into the containing vectors pEF1053 and pEF1054, respectively. The sequences of the 5' EcoRI and 3' XbaI sites were deduced from the published sequences of the corresponding restriction enzymes.