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Incorporation of a Charged Amino Acid into the Membrane-Spanning Domain Blocks Cell Surface Transport But Not Membrane Anchoring of a Viral Glycoprotein

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The membrane-spanning domain of the vesicular stomatitis virus glycoprotein (G protein) consists of a continuous stretch of 20 uncharged and mostly hydrophobic amino acids. We examined the effects of two mutations which change the amino acid sequence in this domain. These mutations were generated by oligonucleotide-directed mutagenesis of a cDNA clone encoding the G protein, and the altered G proteins were then expressed in animal cells. Replacement of an isoleucine residue in the center of this domain with a strongly polar but uncharged amino acid (glutamine) had no effect on membrane anchoring or transport of the protein to the cell surface. Replacement of this same isoleucine residue with a charged amino acid (arginine) generated a G protein that still spanned intracellular membranes but was not transported efficiently to the cell surface. The protein accumulated in the Golgi region in about 50% of the cells, and about 20% of the cells had detectable protein levels in a punctate pattern on the cell surface. In the remaining cells the protein accumulated in a vesicular pattern throughout the cytoplasm. Models which might explain the abnormal behavior of this protein are discussed.

The techniques of in vitro mutagenesis combined with the expression of mutated genes allow the systematic analysis of protein structure and function. We have begun to use this approach to study the structural requirements of a membrane-spanning domain using the vesicular stomatitis virus (VSV) glycoprotein (G protein) as a model membrane-spanning protein. The G protein precursor consists of a single polypeptide chain of 511 amino acids including an N-terminal signal sequence of 16 amino acids (reviewed in reference 16). Like other proteins which are transported to the plasma membrane, G protein is synthesized in ribosomes bound to the rough endoplasmic reticulum (ER). As the nascent protein chain is inserted across the lipid bilayer, two asparagine-linked oligosaccharide chains are attached to amino acid residues 179 and 336. Before the protein is inserted completely across the bilayer, a membrane-spanning domain (residues 463 to 482) becomes anchored in the bilayer. A highly charged, cytoplasmic domain of 29 amino acids extends from the membrane-spanning domain to the COOH terminus of the protein (Fig. 1).

Membrane-spanning domains have been identified in many viral and cellular proteins by using a computer-assisted analysis which determines the average hydrophobicity of the amino acids within a "window" which is moved along the protein sequence (for example, see reference 9). The membrane-spanning domain in the VSV glycoprotein is readily identified by such analyses, and it is typical of such domains in other viral and cellular proteins (9, 18). It consists of a continuous stretch of 20 uncharged amino acids and has a strongly hydrophobic character overall. The function of this domain has been demonstrated directly by showing that its addition to a secretory protein is sufficient to anchor the hybrid protein in the membrane (5).

Analysis of the amino acids present in a variety of membrane-spanning domains has shown that charged and strongly polar amino acids are generally excluded. Indeed, in the computer program devised by Kyte and Doolittle to find membrane-spanning regions, the free-energy increases associated with transferring a polar residue (glutamine) or a charged residue (arginine) into the hydrophobic environment of the membrane are assumed to be nearly equivalent (9). However, other estimates indicate that the free-energy differences are much higher for charged amino acids than for polar amino acids (for example, see reference 24). Because we were interested in defining the minimal alterations which would disrupt the function of a membrane-spanning domain, we examined the expression of a mutated cDNA clone that encodes a G protein with a strongly polar amino acid (glutamine) or a charged amino acid (arginine) substituted for an isoleucine residue in the middle of this membrane-spanning domain. Surprisingly, neither amino acid substitution blocked the membrane-spanning function of this domain, and the glutamine substitution had no effect on transport of the protein to the cell surface. In contrast, substitution of arginine blocked almost all transport of the protein to the cell surface.

MATERIALS AND METHODS

Oligonucleotide-directed mutagenesis. The method used for in vitro mutagenesis was modified from Zoller and Smith (25) and carried out as described previously (13). A phage designated mp8-G4 carries the negative strand of the VSV cDNA clone encoding the G protein incorporated into the phage genome at the unique *Bam*HI site. DNA prepared from this phage was used as a template for in vitro mutagenesis. The synthetic oligonucleotide primer (16-mer, Fig. 1B) was synthesized manually by the phosphotriester method, and the 19-mer (Fig. 1B) was synthesized by the phosphite-triester procedure on a Systec Microsyn model 1450A. After deblocking, both oligonucleotides were purified by electrophoresis on a 12% polyacrylamide gel. All further procedures were as described previously (13) except that 5 U of the Klenow fragment of DNA polymerase I was included in each reaction. Phage DNAs with the desired mutation were

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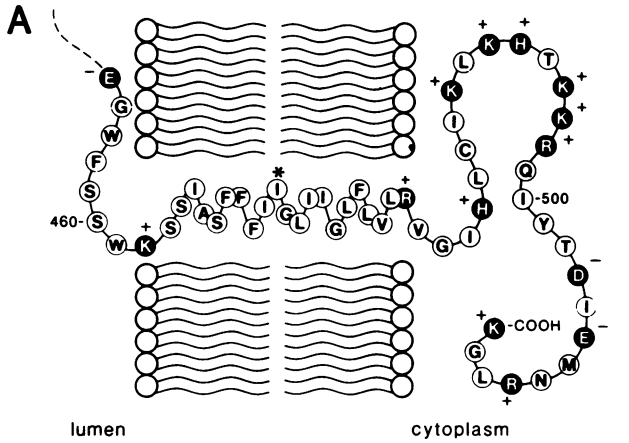

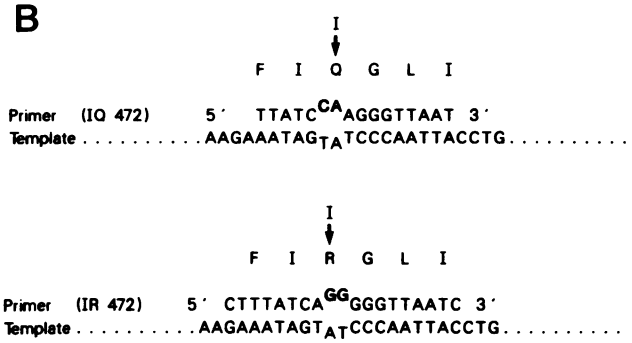

FIG. 1. (A) Diagram illustrating the transmembrane domain and flanking amino acid sequences in the VSV glycoprotein. Amino acids are indicated in the single-letter code and charged residues are indicated by reversed lettering; amino acid numbers are indicated starting from the NH_2 terminus of the protein. The isoleucine residue indicated by an asterisk was changed to either glutamine or arginine as described in the text. (B) Oligonucleotide-directed mutagenesis. The synthetic oligonucleotides (16-mer and 19-mer) used as primers on the mp8-G4 DNA are shown hybridized to the template DNA. Incorporation of the mismatched bases into the DNA sequences will change the codon for isoleucine (ATA) to that for glutamine (CAA) or arginine (AGG) as indicated.

identified by hybridization to ^{32}P-labeled primer. The melting point of each primer hybridized to the corresponding mutant DNA was at least 10°C higher than that of the primer hybridized to the parental template DNA. After mutant phages were identified, replicative-form phage DNA was prepared. A *Bam*HI fragment containing the mutated G gene was excised from the replicative-form DNA and cloned into the simian virus 40-based expression vector JC119 (20). Colonies of *Escherichia coli* containing the plasmid DNAs with the appropriate mutations were identified first by hybridization to the 5' ^{32}P-labeled primers. These were then screened for the proper orientation for expression. The nucleotide sequence at the site of the mutation was then verified (12). Recombinant DNA procedures have been described previously (13, 14).

Transfection of COS cells. Subconfluent monolayers of COS cells (about 2×10^6 cells) on 5-cm dishes were transfected as follows. Plasmid DNA (5 μg) was added to 1 ml of Tris-saline (0.14 M NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.9 mM Na_2HPO_4, 25 mM Tris hydrochloride [final pH, 7.4]), and then DEAE-dextran (molecular weight, 2×10^6; Pharmacia Fine Chemicals) was added to a final concentration of 500 μg/ml. Cells were then washed twice with Tris-saline and incubated with the DNA. After 30 min at 37°C, the DEAE-dextran-DNA was removed, and the cells were incubated in 2 ml of Dulbecco-Vogt modified Eagle medium containing 5% fetal calf serum and 100 μM chloroquine (Sigma Chemical Co.) for 3 h at 37°C. The medium was removed, and the cells were washed twice with Tris-saline. Cells were incubated an additional 40 h in 5 ml of Dulbecco-Vogt modified Eagle medium with 5% fetal calf serum before labeling or fixation.

Biosynthetic labeling, immunoprecipitation, and indirect immunofluorescence. Biosynthetic labeling with [^{35}S]methionine and [^{3}H]palmitic acid were as described previously (15). Immunoprecipitations and indirect immunofluorescence were carried out essentially as described previously (15), except that a 1:75 dilution of rhodamine-conjugated wheat germ agglutinin (WGA) was used to stain the Golgi apparatus (Virtanen et al. [23]).

Proteolysis of microsomes containing G protein. Subconfluent COS cell monolayers (10^7 cells on 10-cm dishes) were transfected with 30 μg of plasmid DNA. After 40 h the cells were scraped off the dishes, suspended in 3 ml of Tris-saline that had been diluted to 0.1× normal concentration (see above), and broken by 30 to 40 strokes of Dounce homogenization. Samples (1 ml) were incubated with trypsin (100 μg/ml; Worthington Diagnostics), with trypsin plus Nonidet P-40 (100 μg/ml and 1%, respectively), or without addition at 37°C for 30 min. Trypsin inhibitor (100 μg/ml; Worthington Diagnostics) was then added to block further proteolysis. The samples were then sedimented through 10% sucrose (10 ml) in Tris-saline in a Beckman SW41 rotor at 38,000 rpm for 4 h at 4°C. The pellets were dissolved in a solution containing 1% Nonidet P-40, 0.4% deoxycholate, 66 mM EDTA, and 10 mM Tris hydrochloride (pH 7.4) and centrifuged for 1 min in a Microfuge to remove nuclei and debris. The supernatants were then immunoprecipitated with rabbit anti-VSV as described previously (15).

RESULTS

Mutagenesis and expression. The cloned cDNA encoding the VSV G protein is conveniently expressed in a monkey fibroblast cell line (COS cells [4]) by using a vector in which the G protein-coding sequence is placed downstream from the simian virus 40 late promoter (14). Transfection of COS cells (which support extensive replication of the transfected DNA) results in a high level of G protein synthesis after 2 days. The transport and modification of the G protein expressed in this system appear normal (14). Although only about 5% of the transfected cells express the G protein, the level of expression in the positive cells is high, facilitating localization of G protein by indirect immunofluorescence. This level of expression is also suitable for analysis of G protein processing and modification by biosynthetic labeling and immunoprecipitation.

To create VSV G proteins containing charged or polar amino acids in the center of the 20-amino-acid membrane-spanning domain, we used oligonucleotide-directed mutagenesis (13, 25) to change the nucleotide sequence of the region of a cDNA clone encoding this domain. The isoleucine codon (ATA) encoding the indicated amino acid in the transmembrane domain (amino acid 472 in G protein, Fig. 1A and B) was changed to a codon specifying arginine (AGG) or glutamine (CAA). The mutagenesis was carried out as described previously (13) with mutagenic oligonucleotide primers 16 and 19 nucleotides long (Fig. 1B). The

template DNA was the mp8-G4 clone carrying the negative strand of the cDNA specifying G protein in the phage genome. After identification of the phage carrying the desired mutation, the mutated cDNA was subcloned from the double-stranded phage replicative-form DNA into the simian virus 40-based expression vector pJC119 (20). The two plasmid DNAs expected to encode the altered G proteins were designated pIQ472 and pIR472 to indicate the amino acid number and the changes introduced in the single-letter code (I, isoleucine; R, arginine; Q, glutamine). These plasmids will be referred to subsequently as pIQ and pIR.

Localization of the altered proteins by indirect immunofluorescence. Initial experiments showed that expression of pIQ or pIR DNA in COS-1 cells resulted in the synthesis of a single species of immunoprecipitable G protein which comigrated with authentic G protein on sodium dodecyl sulfate-polyacrylamide gels. To determine if these proteins were being transported to the cell surface and to examine their intracellular distribution, we carried out double-label indirect immunofluorescence on transfected COS cells. Figure 2 shows examples of the results obtained when cells transfected with DNA of pIR or pIQ were examined by double-label indirect immunofluorescence. All cells synthesizing the G protein containing the glutamine substitution in the transmembrane domain showed bright surface immunofluorescence as well as a typical internal pattern of Golgi region fluorescence. This pattern was indistinguishable from that observed for wild-type G protein expressed in the same cells (14, 15).

In contrast, the majority of the cells expressing the G protein with the arginine substitution in the transmembrane domain showed strong internal fluorescence but did not have any detectable G protein on the cell surface. Also, the internal localization of this protein showed considerable cell-to-cell variability. Generally, the mutant protein appeared to be concentrated in a Golgi-like region to one side of the nucleus or in a more widespread vesicular pattern (Fig. 2 and 3). However, in about 20% of the cells we detected low levels of surface fluorescence (Fig. 2) which appeared to be concentrated in patches on the plasma membrane on the same side of the cell as the Golgi region or over the Golgi region. The pattern of surface fluorescence was always different from the pattern of internal Golgi fluorescence (Fig. 2).

To determine whether the heterogeneous internal staining pattern of the G protein specified by pIR corresponded to the Golgi region, we performed double-label indirect immunofluorescence on transfected cells to detect the G protein and the Golgi region in the same cells. Rhodamine-conjugated wheat germ agglutinin was used to detect the Golgi region. The Golgi region as well as lysosomes is labeled strongly with this lectin, apparently because it has a high affinity for terminal *N*-acetylglucosamine residues present on partially processed or degraded oligosaccharides (23). Figure 3 shows examples of three typical fluorescence patterns seen for the mutant G protein (panels A, B, and C) and the WGA-stained regions of the same cells (panels D, E, and F). About half of the cells showed the G protein in a typical Golgi pattern or an apparently enlarged Golgi region (panels A and B, respectively), corresponding well with the wheat germ agglutinin pattern (panel D). In panel C (typical of the remainder of the cells), the stain for G protein appeared to be localized in numerous vesicles throughout the cytoplasm. The WGA staining of the same cells appeared also to be vesicular and displayed a similar pattern (panel F).

Oligosaccharide processing on the mutant G proteins. Because the transport of the G protein containing arginine in the transmembrane domain appeared to be largely blocked, we decided to examine the processing of the oligosaccharides on this protein to obtain more information on the site at which it was blocked. The enzyme endoglycosidase H (endo H) will cleave the high-mannose oligosaccharides added in the rough ER, but it will not cleave the oligosaccharides once they have been processed beyond a certain point in the Golgi apparatus (7). Thus, the rate of acquisition of endo H resistance can be used to measure the kinetics of protein

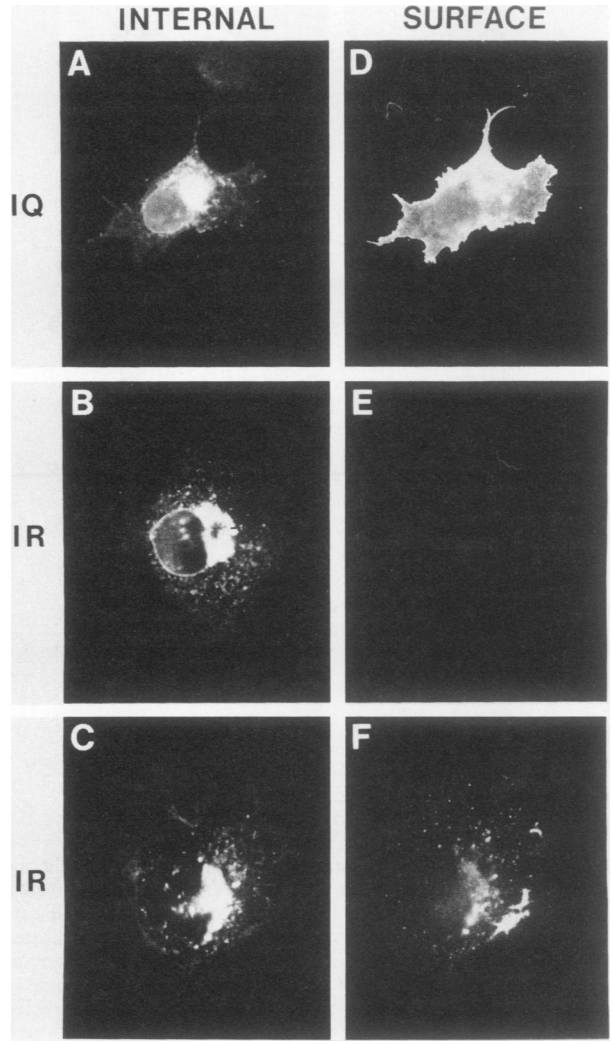

FIG. 2. Double-label indirect immunofluorescence showing cell surface and internal staining for mutant G proteins. COS cells were transfected with pIQ472 DNA or pIR472 DNA, and the mutant proteins were detected by indirect immunofluorescence after 48 h. The cell surface G protein was labeled with rabbit anti-VSV and rhodamine-conjugated goat anti-rabbit immunoglobulin G before permeabilization with detergent. The internal G protein was labeled after permeabilization with guinea pig anti-VSV serum followed by fluorescein isothiocyanate-conjugated goat anti-guinea pig immunoglobulin G. The same field is shown for each pair of internal and surface labelings. The top panels show typical internal (A) and surface (D) immunofluorescence patterns for a cell expressing the G protein specified by pIQ472. The remaining panels show two typical internal (B and C) and surface (E and F) labeling patterns observed for the mutant protein specified by pIR472.

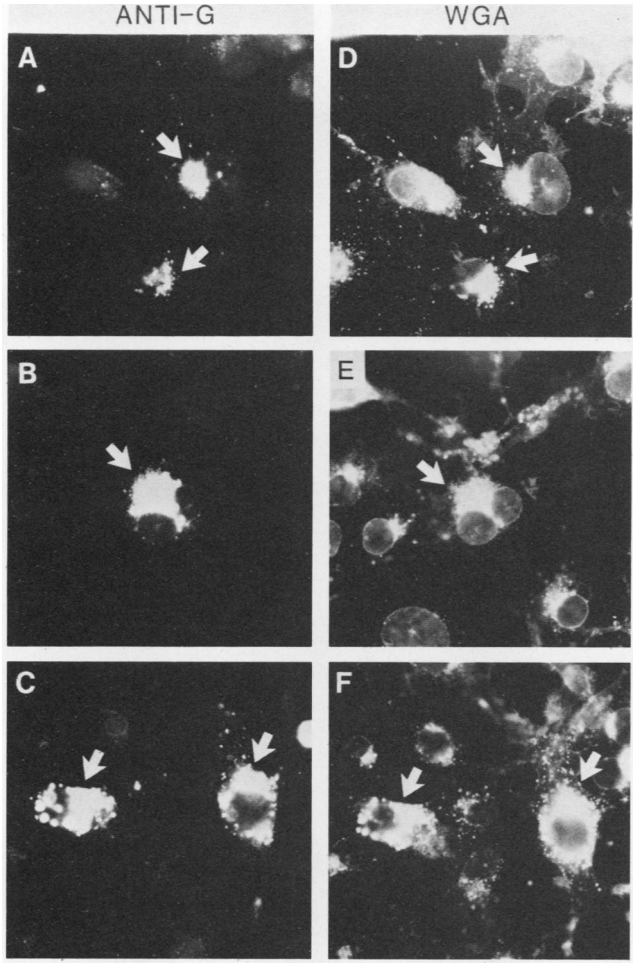

FIG. 3. Double-label immunofluorescence staining for mutant G proteins and for the Golgi apparatus. The G protein was labeled with rabbit antiserum and fluorescein-conjugated goat anti-rabbit immunoglobulin G after permeabilization of the cells. The Golgi apparatus was labeled with rhodamine-conjugated wheat germ agglutinin (WGA). Arrows indicate the regions containing G protein (A, B, and C) and the corresponding regions stained by wheat germ agglutinin (D, E, and F).

transfer from the ER to the site in the Golgi apparatus where endo H resistance is acquired (7).

To analyze the acquisition of endo H-resistant oligosaccharides by the mutant G protein containing arginine in the transmembrane domain, we carried out a pulse-chase experiment (Fig. 4). Cells transfected with DNA of pIR, pIQ, or pSVGL (encoding wild-type G protein) were pulse labeled with [^{35}S]methionine for 15 min followed by the indicated chase periods with unlabeled methionine. The wild-type G protein and the G protein specified by pIQ were completely resistant to endo H by 1 h. Another experiment (data not shown) indicated that both of these proteins acquired endo H-resistant sugars with a half time of about 15 min (14). In contrast, little or no endo H resistance was acquired by the G protein specified by pIR even after a 3-h chase. This result suggests that the transport of the mutant protein is blocked in the ER or at an early point in the Golgi apparatus. To determine if the mutant G proteins encoded by pIR or pIQ were secreted, we analyzed immunoprecipitated material from the medium. No secreted material was found under conditions (14) in which the majority of a truncated G protein lacking the transmembrane and cytoplasmic domains was found in the medium (data not shown).

G protein containing arginine in the transmembrane domain spans cellular membranes. One possible explanation for the failure of the G protein specified by pIR to be transported to the cell surface in most cells is that the protein was transferred completely into the lumen of the ER in a form that was not secreted. Earlier studies have shown that the carboxy terminus (cytoplasmic domain) of wild-type G protein is susceptible to protease digestion when microsomes from VSV-infected cells are subjected to proteolysis (2, 8).

To determine whether the G protein with arginine in the transmembrane domain was still a membrane-spanning protein, we carried out a proteolysis experiment similar to that used previously to detect the cytoplasmic tail of G protein in transfected COS cells (5). A crude microsome preparation was obtained from a Dounce homogenate of [^{35}S]methionine-labeled COS cells that had been transfected with DNA encoding wild-type G protein (pSVGL) or pIR DNA. This homogenate was incubated in the presence or absence of trypsin or in the presence of trypsin plus detergent (to permeabilize the microsomes). Microsomes were then centrifuged to remove proteolytic fragments and trypsin, solubilized with detergent, and subjected to immunoprecipitation and gel electrophoresis. The results (Fig. 5) show that the wild-type G protein and the mutant G protein with arginine in the transmembrane domain have a short segment of the polypeptide chain (presumably the COOH terminus) that is susceptible to proteolysis. The decrease in apparent molecular size (about 2,500 daltons) is similar for both proteins.

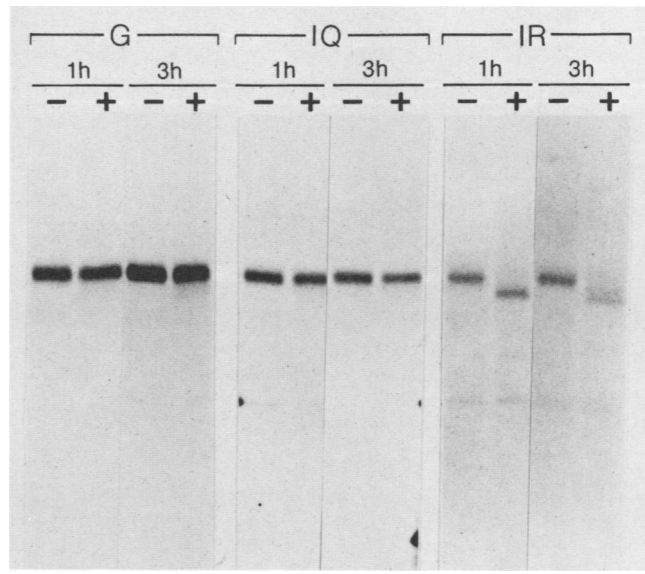

FIG. 4. Acquisition of endo H-resistant oligosaccharides by mutant and wild-type proteins. COS-1 cells (three 5-cm dishes for each DNA) were transfected with 5 μg of pSVGL DNA (encoding the wild-type G protein) or the DNAs designated pIQ472 and pIR472. After 40 h the cells were pulse labeled with 100 μCi of [^{35}S]methionine. The cells were then washed and transferred to medium containing 10 mM methionine. Cells were harvested at the indicated times. Immunoprecipitates of the G proteins were divided in half and either digested with endo H (+) or mock digested (−) before electrophoresis on a 15% polyacrylamide gel containing sodium dodecyl sulfate.

Similar results were obtained for the mutant G protein encoded by pIQ (data not shown). Trypsin probably cleaves at a site(s) within the cluster of four lysines and one arginine (residues 491 to 498) in the cytoplasmic domain. In control experiments it was found that rat growth hormone (a secreted protein [5]) and a secreted form of G protein containing a precise deletion of the transmembrance domain (G. A. Adams and J. K. Rose, manuscript in preparation) were not cleaved by trypsin after digestion of microsomes.

Fatty acid is esterified to the mutant proteins. Palmitic acid is normally esterified to the G protein at the single cysteine residue (i.e., residue 489) in the cytoplasmic domain (10, 13). This relatively late modification probably occurs as G protein enters the Golgi complex (19). If the protein specified by pIR were transported to the Golgi apparatus, we might expect it to obtain this modification of its cytoplasmic domain. To determine whether either mutation in the transmembrane domain affected palmitate addition, we transfected COS cells with DNA of pIR, pIQ, and pSVGL (encoding wild-type G protein). Parallel dishes were labeled with either [^{3}H]palmitic acid or [^{35}S]methionine, and the labeled proteins were then analyzed following immunoprecipitation and gel electrophoresis. The results of this experiment (Fig. 6) show that the fatty acid esterification of both mutant proteins is apparently normal, since the ratio of [^{3}H]palmitic acid to [^{35}S]methionine labeling is similar in all three cases.

Recombination of the mutation in pIR into the wild-type background. One potential artifact of oligonucleotide-directed mutagenesis is the introduction of mutations at sites in the mutagenized DNA distant from the intended site. Because the template for mutagenesis included the entire coding region of the G protein, we were concerned that the transport defect in the protein specified by pIR might have been due to such a secondary mutation. Such a model was ruled out as follows.

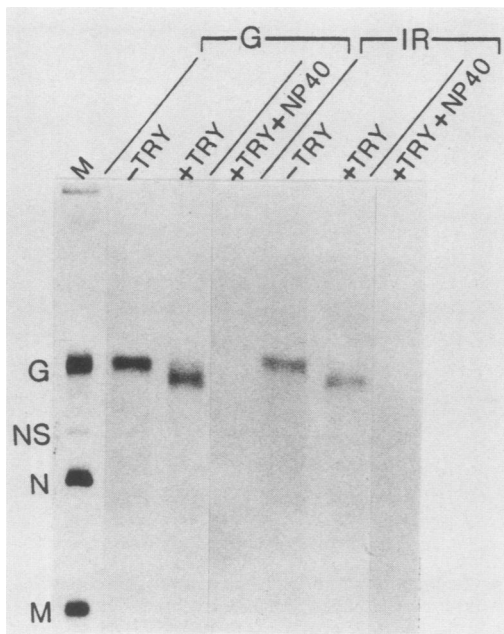

FIG. 5. Proteolysis of G proteins in a crude microsome preparation. Microsomes isolated from COS cells transfected with DNA of pSVGL and pIR472 were subjected to proteolysis with trypsin (TRY) in the presence or absence of Nonidet P-40 (NP40) and subjected to electrophoresis on a 15% polyacrylamide gel after immunoprecipitation. Additions were as indicated. Markers of VSV proteins are shown in lane M.

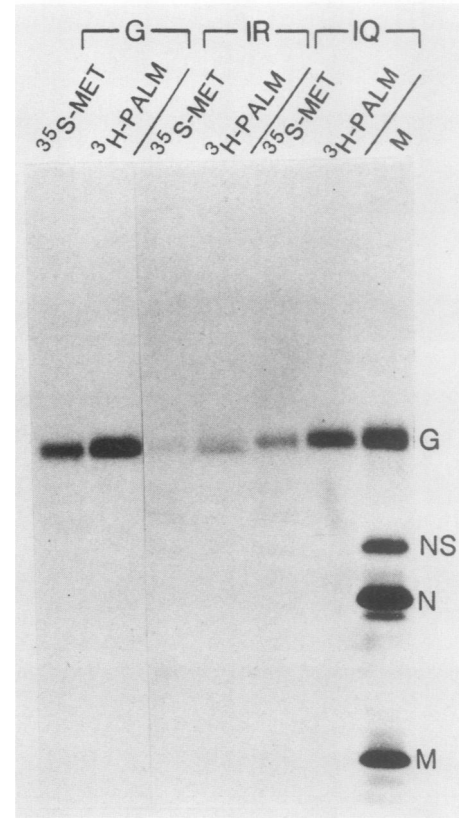

FIG. 6. [^{3}H]palmitic acid and [^{35}S]methionine labeling of wild-type G protein and the mutant G proteins. COS cells were transfected with the DNA of pSVGL, pIQ472, or pIR472 as indicated. Transfections were carried out in parallel on either 10-cm dishes (30 μg of DNA per 10^7 cells) for [^{3}H]palmitic acid labeling (1 mCi per dish, 6-h labeling period) or 5-cm dishes (5 μg of DNA per 2.5×10^6 cells) for [^{35}S]methionine labeling (50 μCi per dish, 1-h labeling period). Immunoprecipitates of cell-associated proteins were subjected to electrophoresis on a 15% polyacrylamide gel and subjected to fluorography for 5 days.

A 330-base-pair *Hin*fI fragment of the pIR DNA (containing nucleotides 1254 to 1584 of the G cDNA sequence [16]) was purified and sequenced (12). Only the expected two base changes (those changing the isoleucine codon to an arginine codon) were found in this fragment. Because the coding sequence for the G protein terminates at nucleotide 1565, we were able to conclude that there were no extra mutations in the coding region after nucleotide 1254. To recombine this fully sequenced portion of the coding region with the wild-type gene, we prepared a DNA fragment spanning from the unique *Hga*I site (nucleotide 1311) to the *Xho*I site at the 3′ end of the gene. This fragment was ligated to the 5′ *Bam*HI-*Hga*I fragment from the wild-type gene, and the recombinant gene was then cloned into the pSVGL2 expression vector (15) after removal of the insert with *Bam*HI and *Xho*I. The mutant G protein specified by this reconstructed plasmid showed intracellular distribution and endo H sensitivity identical to that of the G protein specified by pIR. This demonstrates that the substitution of arginine for isoleucine was the only change responsible for the transport defect.

Another feature of the expression of the G protein from pIR which should be noted is that the level of expression of

the mutant protein was about fourfold less than that of the wild-type protein expressed from the same vector. This lower level of expression was also observed with the recombinant described above, indicating that it was a consequence of the mutation. The reason for the lower level of protein expression is not known, but the pulse-chase experiment (Fig. 4) indicated that it was not due to rapid degradation of the mutant protein. Also, the lower level of expression is unlikely to be an artifact due to a change in the affinity of the polyclonal antibody for the mutant protein, because the mutant protein was recognized by at least two monoclonal antibodies which bind to different epitopes (data not shown).

DISCUSSION

The VSV G protein is synthesized on membrane-bound ribosomes and translocated across the ER membrane as a nascent chain (17). Transfer of the polypeptide chain stops when a hydrophobic segment of amino acids (designated a membrane-spanning domain or "stop transfer sequence" [1]) becomes anchored in the lipid bilayer. This domain occurs near the COOH terminus of the G protein (Fig. 1A) and consists of 20 amino acids flanked by lysine and arginine. We report here on the expression of a mutated cDNA clone that encodes a G protein with a strongly polar amino acid (glutamine) or a charged amino acid (arginine) substituted for an isoleucine residue in the middle of this membrane-spanning domain.

We anticipated that the presence of these amino acids might be sufficient to prevent anchoring of the polypeptide in the bilayer and result in its secretion. Secretion of G protein lacking the entire membrane-spanning and cytoplasmic domains was observed (14). However, neither mutant G protein is secreted, and proteolysis experiments on microsomes showed that both proteins span microsomal membranes (i.e., they have approximately the same size cytoplasmic domain available for protease cleavage as does the wild-type protein). Indeed, the mutant G protein containing glutamine in the transmembrane domain is transported to and apparently anchored at the cell surface just like the wild-type G protein.

Although the G protein containing the arginine substitution spans cellular membranes, it is not transported efficiently to the cell surface. Most of the protein accumulates intracellularly, and only a faint, patchy, surface pattern of this mutant G protein is observed on the cell surface in a small fraction of the cells. Because the oligosaccharides added to this mutant G protein remain endo H sensitive for up to 3 h, it seems likely that the protein is not reaching the Golgi compartment, where processing to endo H resistance occurs (7). However, at the resolution of a light microscope the mutant protein appeared to accumulate the Golgi region, and it was also modified with fatty acid, a modification that may occur in the Golgi apparatus (11, 19). Thus, the mutant protein may accumulate in an early Golgi compartment or perhaps at some point just before entering the Golgi apparatus. Analysis of the protein localization by electron microscopy will be required to determine the site of blockage more precisely.

Before we discuss models which might explain the behavior of this mutant G protein, it is worthwhile to consider a few features of known membrane-spanning domains. These domains are generally assumed to be α-helices by analogy with the multiple membrane-spanning segments of bacteriorhodopsin (6). Given that the width of the hydrophobic core of the membrane is about 3 nm (21), 20 amino acids are the minimum required to span the membrane as an α-helix, with each residue advancing the helix 0.15 nm. The transmembrane domains of proteins which span the membrane only once generally contain an uninterrupted sequence of at least 20 uncharged, nonpolar amino acids with a strongly hydrophobic character overall. Polar, uncharged amino acids such as glutamine are excluded, especially from the middle of these domains (18). In proteins which have multiple membrane-spanning domains, charged and polar residues apparently do occur in the membrane-spanning segments but are located on faces of the α-helices which are directed toward the protein interior and not on those faces which interact with lipid (3).

If we assume that the dimensions of the bilayer are fixed and that the membrane-spanning domain is α-helical, then it would seem likely that the arginine in the transmembrane domain of the mutant G protein (or a flanking charged residue) is buried in the interior of the membrane. Alternatively, the polypeptide chain might assume a much more extended conformation when the arginine is present. For example, the sequence between lysine 462 and the arginine substituted at amino acid 472 might be stretched across the membrane. Such a change might be expected to leave a larger cytoplasmic domain available for proteolysis. We have not observed this, but it is not clear that the proteolysis experiments are sensitive enough to detect it.

There are numerous possible explanations for the inefficient transport of the mutant G protein containing arginine in the transmembrane domain. The protein might disrupt membrane structure and even create an ion channel. Such membrane disruption could explain the expanded Golgi-like structures seen in many cells producing this protein. In fact, the appearance of the Golgi region is similar to that seen in cells treated with the ionophore monensin, which is known to cause conspicuous dilation of vacuoles derived from the Golgi apparatus (22). The transport of other proteins in these cells may also be blocked, and this can be examined. Other models might involve effects on G protein conformation or interaction with other membrane proteins. The presence of the mutant protein on the surface of some cells in a punctate or highly localized pattern suggests that it may not diffuse rapidly in the plane of the membrane. If rapid lateral diffusion in internal membranes is important for incorporation of the protein into transport vesicles, slow diffusion might be responsible for the inefficient cell surface transport. We plan to examine the rate of lateral diffusion of the mutant protein to test this model.

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