

Effects of Mutations in Three Domains of the Vesicular Stomatitis Viral Glycoprotein on Its Lateral Diffusion in the Plasma Membrane

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Abstract. The lateral mobility of the vesicular stomatitis virus spike glycoprotein (G protein) and various mutant G proteins produced by site-directed mutagenesis of the G cDNA has been measured. Fluorescence recovery after photobleaching results for the wild type G protein in transfected COS-1 cells yielded a mean diffusion coefficient (D) of $8.5 (\pm 1.3) \times 10^{-11}$ cm²/s and a mean mobile fraction of 75% ($\pm 3\%$). Eight mutant proteins were also examined: dTM14, lacking six amino acids from the transmembrane domain; TA2, lacking an oligosaccharide in the extracellular domain; QN2, possessing an extra N-linked oligosaccharide in the extracellular domain; CS2, possessing a serine instead of a cysteine at residue 489 in the cytoplasmic domain, preventing palmitate addition to the glycoprotein; TMR-stop, lacking the entire cytoplasmic domain except an arginine at residue 483; and three chimeric proteins, G μ , G23, and GHA, containing in

place of the 29 amino acid wild type cytoplasmic domain the cytoplasmic domains from the surface IgM from the spike protein of the infectious bronchitis virus or from the hemagglutinin protein of the influenza virus, respectively. The mean D for the mutant proteins varied over a relatively small range, with the slowest mutant, G23, exhibiting a value of $11.3 (\pm 1.4) \times 10^{-11}$ cm²/s and the fastest mutant, GHA, having a D of $28.6 (\pm 4.5) \times 10^{-11}$ cm²/s. The mean mobile fraction similarly varied over a small range, extending from 55 to 68%. None of the mutations resulted in the more rapid diffusion characteristic of membrane proteins embedded in artificial bilayers. Therefore, it appears that the cytoplasmic and transmembrane domains themselves contribute little to restraining the lateral mobility of this integral membrane protein when expressed in transfected cells.

SINCE the advent of fluorescence recovery after photobleaching (FRAP)¹ (14), considerable effort has been devoted to elucidating the mechanisms that restrict the lateral mobility of molecules in cell membranes. Lateral diffusion measurements of proteins reconstituted into artificial bilayers indicate diffusion coefficients (D) ~ 5 –500 times greater than those of the same proteins in cell membranes (14). Thus, the question arises as to what cellular components inhibit or completely restrict lateral diffusion and with which structural regions of integral membrane proteins do these components interact. Until recently the majority of lateral diffusion studies were restricted to naturally occur-

ring proteins. In this study, using cDNAs encoding the wild type and eight mutants of the vesicular stomatitis virus (VSV) spike glycoprotein (G protein), we transfected cells and measured the lateral diffusion of G proteins possessing specific alterations in the cytoplasmic, transmembrane, and extracellular domain. This system permits study of defined alterations in the structure of a single integral membrane protein while avoiding the cytopathic effects inherent in viral infection. Furthermore, other viral proteins (e.g., the matrix or M protein) with possible diffusion limiting properties are not present in transfected cells, permitting study of the G mutants alone with endogenous cellular proteins.

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1. *Abbreviations used in this paper:* D, diffusion coefficient; FRAP, fluorescence recovery after photobleaching; G protein, spike glycoprotein of the vesicular stomatitis virus; NBD-PC, 1-acyl-2-(N-4-nitro-benzo-2oxa-1,3 diazole aminocaptoyl) phosphatidylcholine; VSV, vesicular stomatitis virus.

Materials and Methods

Cells

Initial cultures of COS-1 cells were obtained from the line originally established by Gluzman (12). Subsequent cultures were purchased from the American Type Culture Collection (Rockville, MD). Cells were grown in Dulbecco-Vogt's modified Eagle medium supplemented with 5% heat-inactivated defined FCS (Hyclone Laboratories, Logan, UT) and penicillin (100 U/ml) streptomycin (100 μ g/ml); (GIBCO, Grand Island, NY).

Transfection

Cells plated on coverslips in 35-mm dishes were transfected by incubation at 37°C in 0.75 ml Tris-saline containing 10 µg/ml of the appropriate plasmid and DEAE-dextran (2×10^6 mol wt; Pharmacia Fine Chemicals, Div. Pharmacia Inc., Piscataway, NJ) at a concentration of 500 µg/ml. After 30 min the Tris-DNA solution was removed. 1 ml of complete media containing 100 µM chloroquine (Sigma Chemical Co., St. Louis, MO) was then added and the dishes returned to the 37°C incubator. After 2.5–3.5 h, the coverslips were rinsed twice with complete media and placed in a new dish containing 2 ml of fresh media (2). FRAP measurements were performed 36–47 h later. Plasmids encoding the wild type and the mutant proteins CS2, dTM14, TA2, QN2, Gµ, GHA, and G23 were prepared as previously described (1, 2, 23, 26, 28). The plasmid for TMR-stop was prepared using similar techniques.

Antibodies

Douglas S. Lyles (Bowman Grey School of Medicine, Wake Forest University, Winston-Salem, NC) kindly provided a mouse anti-VSV protein monoclonal antibody (19). Lissamine rhodamine B sulfonyl chloride (Molecular Probes, Junction City, OR) was covalently attached to the antibody as follows: the pH of purified IgG (minimum concentration of 1 mg/ml) in PBS was adjusted to pH 9.0 with 1 M sodium bicarbonate buffer (pH 9) in a ratio of 4:1 (vol/vol). A rhodamine stock solution was prepared in acetone and added to the antibody solution in 5 aliquots at 10-min intervals. The final rhodamine/antibody molar ratio was 20:50, the higher ratios being used for the lower antibody concentrations. The conjugated antibody was separated from free dye by gel filtration chromatography using a Sephadex G-50 column (Pharmacia Fine Chemicals). The conjugated antibody was dialyzed exhaustively against PBS.

Fab fragments of rhodamine conjugated anti-G antibody were prepared by papain digestion (immobilized papain; Pierce Chemical Co., Rockford, IL) for 1 h at 37°C. The reaction products were passed over a protein A affinity column (Affi-Gel Protein A; Bio-Rad Laboratories, Richmond, CA) to remove any undigested and partially digested material containing the F_c moiety. SDS gel electrophoresis gave bands characteristic of Fab fragments and confirmed that no significant amount of intact IgG was present. The resulting solution was concentrated using a Centricon-10 (Amicon Corp., Scientific Sys. Div., Danvers, MA) and dialyzed against PBS.

Lipid Labeling

COS-1 cells were labeled with 1-acyl-2-(*N*-4-nitrobenzo-2-oxa-1,3 diazole amino caproyl) phosphatidylcholine (NBD-PC; Avanti Polar Lipids, Inc., Birmingham, AL) as follows. A 25 nM suspension of NBD-acyl-PC in PBS was sonicated for 1 h at 4°C. This suspension was incubated with cells for 1 h at 4°C; cells were then washed with PBS, and colabeled with rhodamine anti-G Fab fragments if required as described below.

FRAP Measurements

Coverslips to be labeled with antibody were washed twice in PBS, soaked 5 min in PBS containing 1% BSA (Sigma Chemical Co.) to reduce nonspecific staining, stained by inverting the coverslip on a 15-µl droplet of an anti-G-Fab fragment (in PBS) solution, and mounted on metal slides. Coverslips were discarded after no more than 70 min on the FRAP microscope stage. All procedures were carried out at room temperature. FRAP measurements and subsequent calculations of *D* were performed as previously described (15).

Results

Lateral Diffusion of Wild Type G

We initially measured the diffusion coefficient of the wild type G protein to establish a baseline for comparison with the mutant G proteins. The diffusion coefficients (*D*) varied considerably; however, ~75% of the sampled cells exhibited *D*s ranging from 1.0 to 10.0 × 10⁻¹¹ cm²/s and 50% of the sampled data fell in the narrow range extending from 4.0 to 8.0 × 10⁻¹¹ cm²/s (Fig. 1 A, top). Similarly the mobile fraction showed a strong central tendency with 50% of the cells

exhibiting recovery ranging from 80 to 100%. Overall the mean *D* was 8.5 (± 1.3) × 10⁻¹¹ cm²/s and the mean mobile fraction was 75% (± 3%).

We also measured the lateral diffusion of G protein in VSV-infected COS-1 cells. Previous experiments (16, 27) demonstrated that G protein in VSV-infected cells has a diffusion coefficient of (4–8.5) × 10⁻¹⁰ cm²/s, regardless of the time after infection. Our results indicate a strong time dependency with diffusion decreasing or disappearing altogether at 6 h after infection. Overall, for those cells that did exhibit diffusion, we calculated a mean *D* of 2.3 × 10⁻¹⁰ (± 1.3) cm²/s and a mean mobile fraction of 59% (± 10). We have at present no explanation to account for the discrepancy in results. A possible source of restraint to lateral mobility may lie in the SV-40 used to transform the CV-1 cells that give rise to COS-1 cells. Reidler et al. (27) found that G protein in VSV-infected SV3T3 cells (3T3 cells transformed by SV-40) exhibited a remarkably reduced mobile fraction (from 68 to 29%).

Lateral Diffusion of Mutant Forms of G

We examined eight mutant forms of G protein: QN2, possessing an asparagine instead of a glutamine at residue 280, creating a new glycosylation signal yielding a protein with three N-linked oligosaccharides; TA2, containing an alanine in place of a threonine at residue 338, disrupting the asparagine-glycine-threonine glycosylation signal, and thus preventing oligosaccharide addition at asparagine 336; dTM14, a mutant lacking six amino acids from the middle of the transmembrane domain; TMR-stop, lacking the entire cytoplasmic domain except an arginine at residue 483; CS2, possessing a serine instead of a cysteine at residue 489 in the cytoplasmic domain, preventing palmitate addition to the glycoprotein; G23, a chimera possessing the extracellular and transmembrane domains of G protein and the first three cytoplasmic domain amino acids from G protein followed by 128 amino acids from the cytoplasmic domain of the E1 glycoprotein of the infectious bronchitis virus; Gµ, containing the surface IgM cytoplasmic domain (Lys-Val-Lys) in place of the 29 amino acid wild type sequence; and GHA, a chimera containing extracellular and transmembrane domains of G protein followed by the cytoplasmic domain from the hemagglutinin protein of the influenza virus. Previously, correct construction of the mutant and wild type plasmids was determined by digestion with restriction endonucleases and by DNA sequence analysis. Furthermore, immunoprecipitation of the mutant proteins, followed by SDS-PAGE analysis, verified that the cDNAs encoded a protein of the predicted molecular weight (1, 23, 24, 26, 28, 29). The results in terms of distributions of *D* and mobile fraction values over the cell population examined are given in Fig. 1 (A–C) and summarized in Table I. FRAP measurements on these proteins yielded mean diffusion coefficients ranging from 11.3 × 10⁻¹¹ cm²/s to 28.6 × 10⁻¹¹ cm²/s and mean mobile fractions from 55 to 68% (see Table I).

On the whole each mutant exhibited a wider range of diffusional behavior than the wild type G protein, with standard errors of the mean equaling two and three times that of the wild-type. Two exceptions to this large variation in diffusion coefficients were QN2 [*D* = 13.1 (± 2.0) × 10⁻¹¹ cm²/s] and G23 [*D* = 11.3 (± 1.4) × 10⁻¹¹ cm²/s], both of

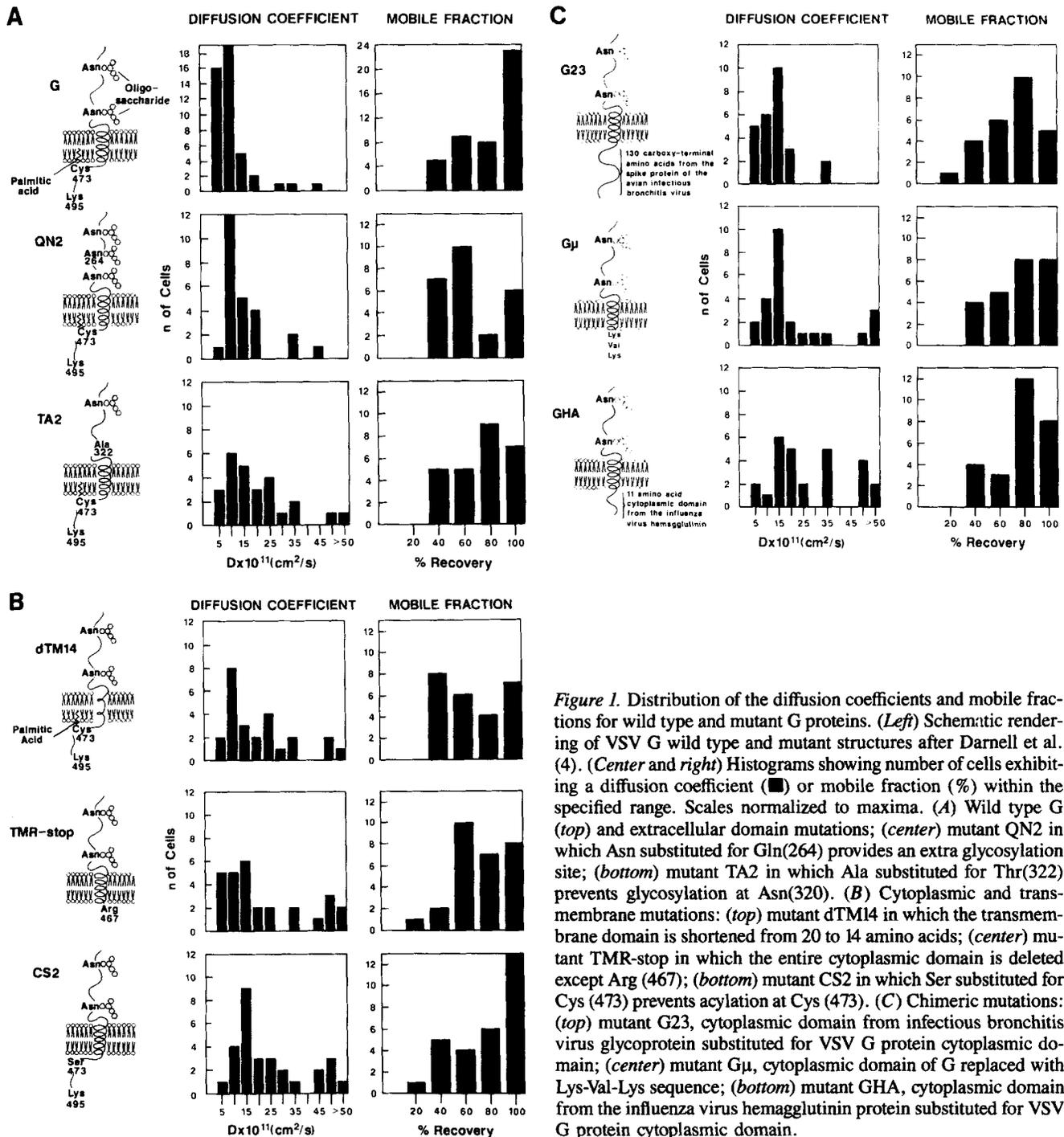


Figure 1. Distribution of the diffusion coefficients and mobile fractions for wild type and mutant G proteins. (*Left*) Schematic rendering of VSV G wild type and mutant structures after Darnell et al. (4). (*Center and right*) Histograms showing number of cells exhibiting a diffusion coefficient (■) or mobile fraction (%) within the specified range. Scales normalized to maxima. (*A*) Wild type G (*top*) and extracellular domain mutations; (*center*) mutant QN2 in which Asn substituted for Gln(264) provides an extra glycosylation site; (*bottom*) mutant TA2 in which Ala substituted for Thr(320) prevents glycosylation at Asn(320). (*B*) Cytoplasmic and transmembrane mutations: (*top*) mutant dTM14 in which the transmembrane domain is shortened from 20 to 14 amino acids; (*center*) mutant TMR-stop in which the entire cytoplasmic domain is deleted except Arg (467); (*bottom*) mutant CS2 in which Ser substituted for Cys (473) prevents acylation at Cys (473). (*C*) Chimeric mutations: (*top*) mutant G23, cytoplasmic domain from infectious bronchitis virus glycoprotein substituted for VSV G protein cytoplasmic domain; (*center*) mutant Gμ, cytoplasmic domain of G replaced with Lys-Val-Lys sequence; (*bottom*) mutant GHA, cytoplasmic domain from the influenza virus hemagglutinin protein substituted for VSV G protein cytoplasmic domain.

which showed fairly consistent diffusional behavior (see Fig. 1, A middle and C top).

In our experiments we noticed large temporal variations in the fluorescence intensity within the region interrogated by the measuring laser beam of the FRAP apparatus. Subsequent monitoring of the cell surface with the FRAP instrument without bleaching indicated that the variations in fluorescence intensity were due to fluctuations at the cell surface unrelated to FRAP bleaching. These fluctuations made it impossible to analyze some of the FRAP curves. In an attempt to investigate the source of fluorescence variation, we observed live cells with digitized fluorescence microscopy (5).

G-transfected cells stained with rhodaminated IgG gave bright staining, particularly of structures presumed to be microvilli. Digital analysis revealed that fluorescence intensity changes within selected areas were directly related to the movement of these putative microvilli. However, we were unable to attribute fluorescence intensity changes to microvillar movement when using the dimmer image provided by the rhodaminated Fab fragment.

Control Studies

To rule out the possibility of a systematic effect on the diffusion coefficient or mobile fraction due to the length of

Table I. Mean Diffusion Coefficients (D) and Mobile Fractions (MF) for Wild Type G and Mutant G Proteins*

Protein	Mutation	D ($\times 10^{11}$ cm ² /s)	MF	n
Wild type G	None	8.5 \pm 1.3	75 \pm 3	45
QN2	Asn substituted for Gln(264) provides extra glycosylation site	13.1 \pm 2.0	55 \pm 5	25
TA2	Ala substituted for Thr(322) prevents glycosylation at Asn(320)	17.5 \pm 2.6	65 \pm 4	26
dTM14	Transmembrane domain shortened from 20 to 14 amino acids	19.0 \pm 3.3	62 \pm 5	25
TMR-stop	Entire cytoplasmic domain deleted except Arg(467)	20.7 \pm 3.4	65 \pm 4	28
CS2	Ser substituted for Cys(473) prevents acylation at Cys(473)	22.6 \pm 2.8	66 \pm 5	29
G23	Cytoplasmic domain from infectious bronchitis virus glycoprotein substituted for VSV G protein cytoplasmic domain	11.3 \pm 1.4	64 \pm 5	26
G μ	Cytoplasmic domain replaced with Lys-Val-Lys	21.1 \pm 4.3	66 \pm 4	25
GHA	Cytoplasmic domain from the influenza virus hemmagglutinin protein substituted for VSV G protein cytoplasmic domain	28.6 \pm 4.5	68 \pm 4	27

*COS-1 cells previously plated on coverslips and transfected with the appropriate vector were labeled with an Fab fragment of a rhodaminated anti-G protein monoclonal antibody and subjected to FRAP measurement as previously described (15). Values are \pm SEM.

time after transfection, we plotted D and the mobile fraction versus the time after transfection with wild type cDNA (data not shown). No correlation was found. Similarly, we plotted the mobile fraction versus D; again, no correlation was apparent (data not shown). These experiments demonstrated that neither the level of expression of the vector nor the state of replication of the vector, both of which vary with time, affect the lateral diffusion of G protein.

Lateral diffusion of the lipid probe, NBD-PC, in the plasma membrane of COS-1 cells in both mock-transfected cells and in cells transfected with the gene for wild type G was also measured to assess the possible effect of the amount of G protein expressed at the plasma membrane. This experiment required double labeling. First, all cells in the culture were labeled with NBD-PC; then the culture was counter stained with rhodamine anti-G Fab. Rhodamine optics allowed identification of those cells in the culture that were expressing wild type G at the plasma membrane. Subsequent FRAP measurements with fluorescein optics allowed measurement of the NBD-PC diffusion coefficient in the transfected cells. Control values were obtained from nonexpressing cells in the same culture or in separate mock transfected cultures that had been labeled with NBD-PC. No significant difference was found in the lipid analogue lateral diffusion coefficients between mock-transfected or nonexpressing cells and transfected cells (data not shown).

The effect of possible multivalent aggregates of anti-G Fab fragments was tested by labeling the cells with Fab preparations that had been previously centrifuged at 100,000 g. No appreciable change in the measured diffusion coefficients was effected by this procedure.

Discussion

Transfection of vectors, containing wild type and mutated genes of various proteins, into selected recipient cells offer model systems in which structure-function relationships can be examined. Two previous studies have examined the effect of site-directed truncations of the cytoplasmic domain on the

lateral diffusion coefficient of the H-2 murine histocompatibility antigen (9) and the epidermal growth factor receptor (21). Both studies detected little effect of such truncations on the lateral diffusion coefficient of these proteins. This study extends this line of experimentation to another integral membrane protein, the VSV G protein, examining the effects of alterations in the extracellular, transmembrane, and cytoplasmic domains on the lateral mobility of G protein. We studied G proteins with a mutation in the extracellular domain that either lacked or contained an additional N-linked oligosaccharide. We also examined a G protein that lacked six of the 20 amino acid residues from the membrane-anchoring domain yet has the same extracellular and cytoplasmic domains of the wild type G protein (1). In addition, two types of mutations in the cytoplasmic domain of the G protein were examined, one that eliminates the site for palmitate acylation and another type that examines the effect of substituting the cytoplasmic domains from different integral membrane proteins for the cytoplasmic domain of the G protein.

In considering the behavior of the wild type and mutant G proteins in the plasma membrane, it is helpful to recall the possible sources of restraint to protein lateral mobility. Membrane proteins will exhibit decreased diffusion through: (a) increased protein concentration leading to crowding within the membrane as well as decreased membrane fluidity; (b) self-association, leading to dimer, trimer, and other larger multimers; (c) specific or nonspecific association with other membrane proteins, leading to larger, more slowly diffusing protein complexes; and (d) association with extracellular or cytoplasmic structures that could prevent or decrease diffusion.

Several million copies of G can be expressed in transfected cells, prompting the consideration that this amount of protein added to the plasma membrane may result in reduced diffusion coefficients of membrane components. However, similar NBD-PC diffusion coefficients were measured in mock-transfected and wild type G-transfected cells indicating that this amount of additional plasma membrane protein did not

appreciably obstruct the diffusion of the diI probe or generally decrease plasma membrane fluidity. This is a plausible result considering the following comparison. In a COS cell having 2×10^6 plasma membrane copies of G in a $2,000 \mu\text{m}^2$ surface area, G is expressed at a surface density of $1,000/\mu\text{m}^2$. Acetylcholine receptor in plaques can be found in surface densities of $10,000/\mu\text{m}^2$ (31). This receptor most likely occupies a far larger cross-sectional area in the membrane plane allowing us to estimate that in transfected cells G occupies a maximum of 2–4% of the available membrane area. Theoretical treatments show that such an increment in fractional area coverage is too small to appreciably affect probe lateral diffusion (see, for example, reference 32).

The self-aggregation state of G is not well established. In reconstituted bilayer systems, energy transfer studies suggest that G exists as a monomer and any unusual treatment converts it to a multimer (Parce, W., personal communication). In infected cells detergent solubilization of a naturally occurring secreted form of G protein fails to reveal anything but monomer G. On the other hand, monoclonal antibody quickly patches G at the cell surface, indicating either a repeated epitope on the molecule or a multimeric complex (Parce, W., personal communication). Furthermore, recent studies suggest that G forms oligomers at least during transport to the surface of infected cells (18). The hemagglutinin of influenza virus exists in mature form as a trimer (35) which is assembled before transport out of the endoplasmic reticulum (11).

The generalized increase in D values seen with mutants could be attributed to reduced oligomerization of the G protein due to conformational changes induced by the various mutations. Reduced patching upon addition of monoclonal anti-G IgG might be expected when more monomeric antigen is present. Qualitatively no gross alteration in either the patch pattern or its relationship with subjacent spectrin was noticed when transfected cells were expressing G or G μ .

With respect to association with other membrane proteins, no such interactions have been demonstrated, but as discussed below, relatively weak interactions with other membrane proteins cannot be excluded.

It is likely that the major reduction in the G lateral diffusion coefficients relative to the values expected in simple bilayers is due to the interactions of this protein either directly or indirectly (via other membrane proteins) with structures peripheral to the plasma membrane (10, 14, 15, 17). For example, in VSV-infected cells, Reider et al. (27) have demonstrated that the M protein in infected cells does lower the mobile fraction of G protein, but no effect on the mobility of the diffusing population was observed.

In this context, it is of interest to inquire which parts of the G molecule are involved in these proposed mobility retarding interactions. In general, any change in the cytoplasmic domain generated at most an approximate threefold increase in the diffusion coefficient, as occurred with GHA [D = $28.6 (\pm 4.5) \times 10^{-11} \text{ cm}^2/\text{s}$]. G μ , CS2, GHA, and TMR-stop all possessed mean diffusion coefficients in the range of $20.7\text{--}28.6 \times 10^{-11} \text{ cm}^2/\text{s}$. Shortening the transmembrane domain, as with dTM14, also exacted a small increase in the diffusion coefficient, raising the mean value to $19.0 (\pm 3.3) \times 10^{-11} \text{ cm}^2/\text{s}$. Changes in extracellular glycosylation (each oligosaccharide having a mass of $\sim 3,000$ D and comprising ~ 15 residues) elicited a still smaller effect, barely affecting

the diffusion coefficient in the case of QN2 [D = $13.1 (\pm 2.0) \times 10^{-11}/\text{s}$]. Similarly, G23, with its 130 amino acid cytoplasmic domain showed a marginal increase in diffusion. These effects on lateral diffusion rates are relatively minor when compared with the fact that membrane proteins diffuse at least 100-fold faster in simple lipid bilayers with no significant immobile fraction (34).

It should be noted that the variation in lateral diffusion coefficients between the mutants does not correlate at all with variation in transport rates from endoplasmic reticulum to Golgi seen for some of the mutants (measured as half-time for acquisition of endo H resistance; see Table II). This lack of correlation can be explained by two possibilities: (a) although lateral mobility may be necessary for intracellular transport in order to allow clustering into specialized regions of the endoplasmic reticulum and the Golgi complex, lateral diffusion is not a rate-limiting determinant of intracellular transport; (b) lateral diffusion is the rate-limiting factor affecting intracellular transport, but the mechanisms in the endoplasmic reticulum and the Golgi complex governing lateral diffusion differ from those at the plasma membrane; hence, mutations altering lateral mobility at the plasma membrane may not significantly affect lateral mobility in intracellular membranes. The disparate environments found in the lumen of the endoplasmic reticulum and the extracellular matrix render the latter hypothesis plausible.

The fact that none of the mutations tested produced a large (order of magnitude) change in the protein diffusion coefficients restricts the search for the factors restraining the lateral mobility of certain membrane proteins. It is likely that the cytoplasmic domain is not a crucial region in determining the mobility of this protein. These results are consistent with the previous work on the mouse H-2 antigen (9) and the epidermal growth factor receptors (21), in which cytoplasmic deletions elicited only small changes in protein diffusion. It should be noted that in cases where the membrane protein is known to be anchored by cytoskeletal components, such as band 3 in the erythrocyte membrane (for review see reference 3) or the band 3 analogue in kidney epithelium (7), mutations in the cytoplasmic domain could be expected to increase the lateral diffusion markedly if they interfered with the interaction of band 3 with ankyrin or with ankyrin ana-

Table II. Lack of Correlation of the Lateral Diffusion Coefficient for Wild Type and Mutant G Proteins with Their Rates of Transport from Endoplasmic Reticulum to the Golgi*

Protein \ddagger	D ($\times 10^{11} \text{ cm}^2/\text{s}$) \S	(Transport rate) $^{-1}$	Reference
		<i>min</i>	
Wild type G	8.5	15–20	8, 29
QN2	13.1	15–20	36
TA2	17.5	15–20	36
dTM14	19.0	15–20	6
CS2	22.6	15–20	5
G23	11.3	25	8
G μ	21.1	110–120	8
GHA	28.6	55–60	8

* Measured as the half-time for acquisition of endo H resistance.

\ddagger As defined in Table I.

\S As stated in Table I; standard errors given in Table I.

logues. It is also clear that portions of the cytoplasmic domain are crucial for the clustering in coated pits that precedes internalization of the low density lipoprotein receptor (20) and epidermal growth factor receptor (22) and for efficient transport of some proteins to the cell surface after biosynthesis (6, 26, 30, 37).

Second, the small change in diffusion coefficient seen with dTM14 raises questions regarding the importance of the transmembrane domain to diffusion. As previous experiments have suggested that the transmembrane deletion leaves the cytoplasmic and extracellular domains unaltered (1), the reduction from 20 to 14 amino acids in the membrane-spanning domain must alter the alpha-helical structure in this domain. The failure of the conformational change to exact a significant alteration in diffusive behavior suggests that the transmembrane domain plays little or no direct role in limiting lateral mobility of G in the plasma membrane.

Two general mechanisms remain to explain reduced diffusion coefficients of many plasma membrane components. In the case of the G glycoproteins, the large extracellular domain of 462 residues may be the principal determinant of its lateral diffusion in COS-1 cells. The determinant apparently does not reside solely in the two $\sim 3,000$ -D oligosaccharides as neither addition of one chain nor deletion of one chain appreciably affects lateral diffusion. This, in spite of the fact that the charge and hydration of such polysaccharide "trees" suggests that they extend from the protein body ~ 35 Å into the extracellular space. To understand the nature of these restraints, it will be important to examine other alterations in this domain that retain the capacity to be transported to the cell surface. While the Fab ligand could conceivably also contribute to the reduced mobility of G and the various mutants, several recent experiments argue that the mobility restraints observed are due to interactions involving the membrane protein itself with the extracellular matrix. McCloskey and Poo (25) have shown that the lateral mobility of the F_c receptor in rat basophilic leukemia cells is about the same whether measured in the presence or absence of the IgE ligand. Furthermore, cellular FRAP measurements of the Thy 1 antigen using monoclonal IgG antibodies (13) and the H-2 murine histocompatibility antigen using Fab fragments of anti-H-2 antibody have (8) produced values in excess of 1×10^{-9} cm²/s for a fraction of these proteins; such results suggest that rapid diffusion can be measured by FRAP in the presence of a bound antibody label.

The alternative to the ectodomain restraints to lateral diffusion would be a second system of membrane proteins (2a) that interact both with peripheral structures and with certain membrane proteins, such as G, to reduce the lateral diffusion coefficients of such proteins. Such a system has been invoked by Singer et al. (33) to recognize those membrane proteins cross-linked into "patches".

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References

- Adams, G. A., and J. K. Rose. 1985. Structural requirements of a membrane-spanning domain for protein anchoring and cell surface transport. *Cell*. 41:1007-1015.
- Adams, G. A., and J. K. Rose. 1985. Incorporation of a charged amino acid into the membrane-spanning domain blocks cell surface transport but not membrane anchoring of a viral glycoprotein. *Mol. Cell. Biol.* 5:1442-1448.
- Appar, J. R., M. F. Mescher. 1986. Agorins: major structural proteins of the plasma membrane skeleton of P815 tumor cells. *J. Cell Biol.* 103: 351-360.
- Branton, D., C. Cohen, and J. Tyler. 1981. Interaction of cytoskeletal proteins on the human erythrocyte membrane. *Cell*. 24:24-32.
- Darnell, J., H. Lodish, and D. Baltimore. 1986. *Molecular Cell Biology*. Scientific American Book, New York. 585 pp.
- DiGiuseppi, J., R. Inman, A. Ishihara, K. Jacobson, and B. Herman. 1985. Applications of digitized fluorescence microscopy to problems in cell biology. *Biotechniques*. 3:394-403.
- Doyle, C., M. G. Roth, J. Sambrook, and M.-J. Gething. 1985. Mutations in the cytoplasmic domain of the influenza virus hemagglutinin affect different stages of intracellular transport. *J. Cell Biol.* 100:704-714.
- Drenckhahn, D., K. Schluter, D. P. Allen, and V. Bennett. 1985. Colocalization of band 3 with ankyrin and spectrin at the basal membrane of intercalated cells in the rat kidney. *Science (Wash. DC)*. 230:1287-1289.
- Eddidin, M., and T. Wei. 1982. Lateral diffusion of H-2 antigens on mouse fibroblasts. *J. Cell Biol.* 95:458-462.
- Eddidin, M., and M. Zuniga. 1984. Lateral diffusion of wild-type and mutant L^d antigens in L cells. *J. Cell Biol.* 99:2333-2335.
- Elson, E. L., and J. A. Reidler. 1979. Analysis of cell surface interactions by measurements of lateral mobility. *J. Supramol. Struct.* 12:481-489.
- Gething, M. J., K. McCammon, and J. Sambrook. 1986. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of fold in intracellular transport. *Cell*. 46:939-956.
- Gluzman, Y. 1981. SV40-transformed simian cells support the replication of early SV40 mutants. *Cell*. 23:175-182.
- Ishihara, A., Y. Hou, and K. Jacobson. 1987. The Thy-1 antigen exhibits rapid lateral diffusion in the plasma membrane of rodent lymphoid cells and fibroblasts. *Proc. Natl. Acad. Sci. USA*. 84:1290-1293.
- Jacobson, K. 1983. Lateral diffusion in membranes. *Cell Motil.* 3:367-373.
- Jacobson, K., D. O'Dell, and J. T. August. 1984. Lateral diffusion of an 80,000-dalton glycoprotein in the plasma membrane of murine fibroblasts: relationships to cell structure and function. *J. Cell Biol.* 99:1624-1633.
- Johnson, D. C., M. J. Schlessinger, and E. L. Elson. 1981. Fluorescence photobleaching recovery measurements reveal differences in envelopment of sindbis and vesicular stomatitis viruses. *Cell*. 23:423-431.
- Koppel, D. E. 1981. Association dynamics and lateral transport in biological membranes. *J. Supramol. Struct. and Cell Biochem.* 17:61-67.
- Kreis, T., and H. Lodish. 1986. Oligomerization is essential for transport of vesicular stomatitis viral glycoprotein to the cell surface. *Cell*. 46:929-937.
- Lefrancois, L., and D. S. Lyles. 1982. The interaction of antibody with the major surface glycoprotein of vesicular stomatitis virus. I. Analysis of neutralizing epitopes with monoclonal antibodies. *Virology*. 121:157-167.
- Lehrman, M. A., J. L. Goldstein, M. S. Brown, D. W. Russell, and W. J. Schneider. 1985. Internalization-defective LDL receptors produced by genes with nonsense and frameshift mutations that truncate the cytoplasmic domain. *Cell*. 41:735-743.
- Livneh, E., M. Benveniste, R. Prywes, S. Felder, Z. Kam, and J. Schlessinger. 1986. Large deletions in the cytoplasmic kinase domain of the epidermal growth factor receptor do not affect its lateral mobility. *J. Cell Biol.* 103:327-331.
- Livneh, E., R. Prywes, O. Kasheles, N. Reiss, I. Sasson, Y. Mory, A. Ullrich, and J. Schlessinger. 1986. Reconstitution of human EFG-receptors and its deletion mutants in cultured hamster cells. *J. Biol. Chem.* 261: 12490-12497.
- Machamer, C. E., R. Z. Florkiewicz, and J. K. Rose. 1985. A single N-linked oligosaccharide at either of the two normal sites is sufficient for transport of vesicular stomatitis virus G protein to the cell surface. *Mol. Cell. Biol.* 5:3074-3083.
- Machamer, C. E., J.-L. Guan, R. Florkiewicz, and J. K. Rose. 1986. Role of N-linked glycosylation in the intracellular transport of transmembrane proteins. *Microbiology (Wash. DC)*. 1986:292-296.
- McCloskey, M., Z.-Y. Liu, and M. M. Poo. 1984. Lateral electromigration and diffusion of F_cε receptors on rat basophilic leukemia cells: effect of IgE binding. *J. Cell Biol.* 99:778-787.
- Puddington, L., C. E. Machamer, and J. K. Rose. 1986. Cytoplasmic domains of cellular and viral integral membrane proteins substitute for the cytoplasmic domain of the vesicular stomatitis virus glycoprotein in transport to the plasma membrane. *J. Cell Biol.* 102:2147-2157.

27. Reidler, J. A., P. M. Keller, E. L. Elson, and J. Lenard. 1981. A fluorescence photobleaching study of vesicular stomatitis virus infected BHK cells. Modulation of G protein mobility by M protein. *Biochemistry*. 20:1345-1349.
28. Rose, J. K., G. A. Adams, and C. J. Gallione. 1984. The presence of cysteine in the cytoplasmic domain of the vesicular stomatitis virus glycoprotein is required for palmitate addition. *Proc. Natl. Acad. Sci. USA*. 81:2050-2054.
29. Rose, J. K., and J. E. Bergmann. 1982. Expression from the cloned cDNA of cell-surface and secreted forms of the glycoprotein of vesicular stomatitis virus in eucaryotic cells. *Cell*. 30:753-762.
30. Rose, J. K., and J. E. Bergmann. 1983. Altered cytoplasmic domains affect intracellular transport of the vesicular stomatitis virus glycoprotein. *Cell*. 34:513-524.
31. Saltpeper, M. M., and R. H. Loning. 1985. Nicotinic acetylcholine receptor in vertebrate muscle: properties, distribution and neural control. *Prog. Neurobiol. (NY)*. 25:297-325.
32. Saxton, M. 1982. Lateral diffusion in an ardupelago: effect of impermeable patches on diffusion in a cell membrane. *Biophys. J.* 39:165-173.
33. Singer, S. J., J. F. Ash, L. Bourguignon, M. Heggnes, and D. Louvard. 1978. Transmembrane interactions and the mechanisms of transport of proteins across membranes. *J. Supramol. Structure*. 9:357-373.
34. Vaz, W. L. C., F. Goodsaid-Zalduondo, and K. Jacobson. 1984. Lateral diffusion of lipids and proteins in bilayer membranes. *FEBS (Fed. Eur. Biochem. Soc.) Letters*. 174:199-207.
35. Wiley, D. C., J. J. Skehel, and M. D. Waterfield. 1977. Evidence from studies with a cross-linking reagent that the hemagglutinin of influenza virus is a trimer. *Virology*. 79:446-448.
36. Wills, J. W., R. V. Srinivas, and E. Hunter. 1984. Mutations of the Rous sarcoma virus *env* gene that affect the transport and subcellular location of the glycoprotein products. *J. Cell Biol.* 99:2011-2023.
37. Zuniga, M., B. Malissen, M. McMillan, P. R. Brayton, S. S. Clark, J. Forman, and L. Hood. 1983. Expression and function of transplantation antigens with altered or deleted cytoplasmic domains. *Cell*. 34:535-544.