The lateral mobility of some membrane proteins is determined by their ectodomains

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INTRODUCTION

Many membrane spanning proteins exhibit much lower lateral mobility in plasma membranes than in reconstituted artificial membranes (1). The cytoplasmic domain plays an important role in restricting the lateral mobility of some membrane proteins. For example, the interaction of the cytoplasmic domain of the major erythrocyte membrane protein, Band 3, with the red cell cytoskeleton significantly reduces its lateral mobility, and cytoplasmic domain truncations of either or both subunits of the heterodimeric class II major histocompatibility complex (MHC) molecules significantly increase their lateral diffusion coefficients (2). In the case of Band 3, interactions of the large cytoplasmic domain with membrane associated cytoskeleton prevent long range lateral diffusion while permitting rotation of a fraction of these anion transporters. However, direct cytoplasmic domain interactions with the cytoskeletal cortex apparently do not restrict the mobility of all membrane proteins. For example, truncation of the cytoplasmic domain of the epidermal growth factor receptor (3) and the MHC Type I antigens (4) had little effect on their lateral mobility. To further elucidate the rules governing the lateral mobility of membrane proteins, we studied the lateral mobility of wild type and mutant vesicular stomatitis virus (VSV G) spike glycoproteins after transient expression in transfected COS cells. Full details of this work have been reported in two publications (5, 6).

MATERIALS AND METHODS

All the wild type and chimeric protein constructs were incorporated into SV40 based vectors (7–9) for transient expression in COS-1 cells after transfection as previously described (5, 6). Fluorescence recovery after photobleaching (FRAP) measurements were done 40–50 h after transfection and after labeling of the cells by rhodamine conjugated Fab fragments of appropriate antibodies.

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RESULTS AND DISCUSSION

Major mutations of VSV G do not produce large lateral mobility changes

We investigated the lateral mobility of the vesicular stomatitis virus (VSV G) spike glycoprotein and a number of mutant proteins (5, 6). The mutations included dTM14, lacking six amino acids from the transmembrane domain and TMR-stop, lacking the entire cytoplasmic domain except an arginine at residue 483 (see Fig. 1). Neither of these mutations resulted in the large lateral coefficients ($D \cong 10^{-8}$ cm^2/s) characteristic of membrane proteins embedded in artificial bilayers (1), indicating that the cytoplasmic and transmembrane domains themselves contribute little to restraining the lateral mobility of this integral membrane protein when expressed in transfected cells. Mobile fractions in all these experiments were similar.

Changing the mode of membrane anchorage does not produce large changes in lateral mobility

In contrast to many membrane spanning proteins, a number of glycosyl-phosphatidylinositol (GPI) linked proteins exhibit large lateral diffusion coefficients ($\geq 10^{-9}$ cm^2/s). For example, the lateral mobility of VSV G is low, whereas Thy-1, a small GPI linked protein, exhibits high lateral mobility (Fig. 1). To determine if the GPI linkage was a major determinant of the high lateral mobility of these lipid-linked proteins, we measured the lateral diffusion of chimeric membrane proteins composed of normally transmembrane proteins that were converted to GPI linked proteins, or GPI linked proteins that were converted to membrane spanning proteins. This strategy is exemplified by the constructs G-Thy and Thy-G (Fig. 1, top panel). G-Thy, a GPI linked glycopro-

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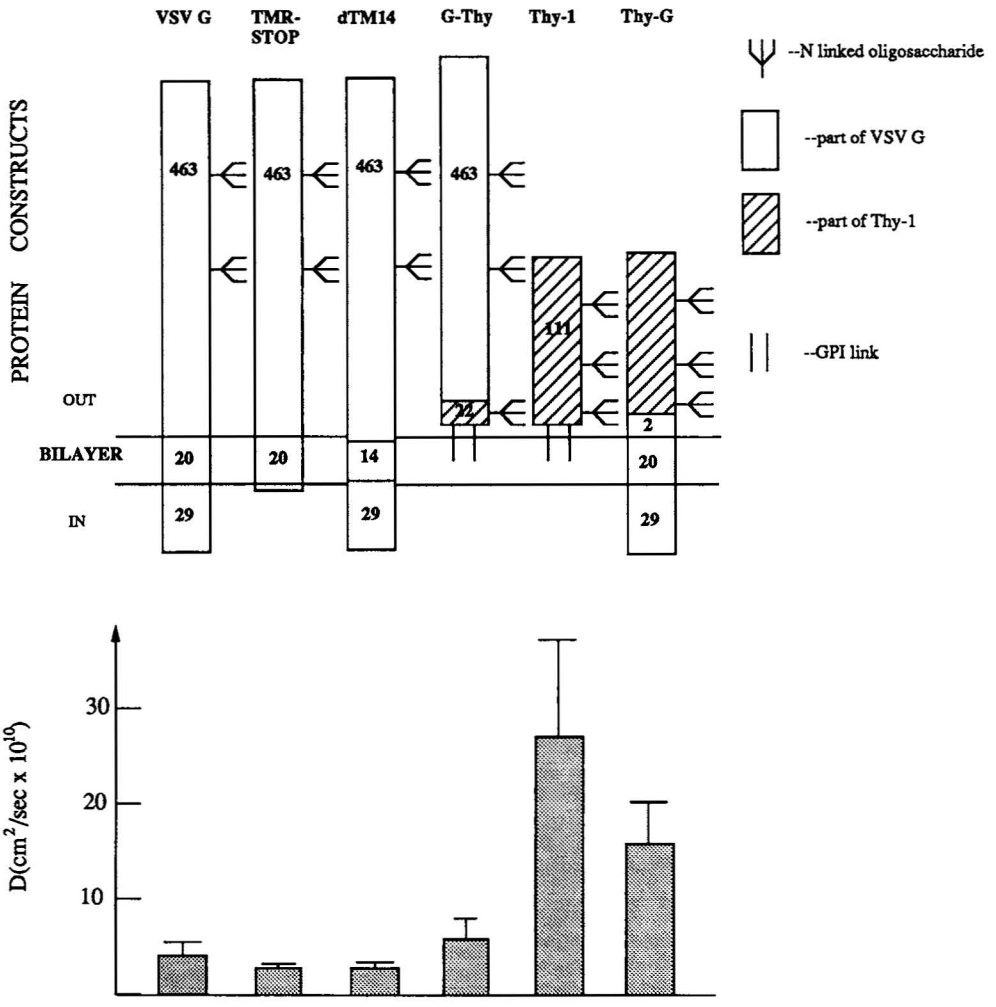

FIGURE 1 Lateral mobility of chimeric constructs of VSV G and Thy-1. (*Top panel*) Schematic of protein constructs with the number of amino acids in the various domains and the number of glycosylation sites indicated. (*Bottom panel*) Diffusion coefficient of each protein construct.

tein, has the entire VSV G protein ectodomain and a 22 amino acid CH_2-terminus of the mature Thy-1 protein including the GPI linkage, whereas Thy-G is a chimeric construct which has the coding sequence of Thy-1 ectodomain fused with that of the VSV G transmembrane and cytoplasmic domains.

The results of this experiment were surprising (Fig. 1, bottom panel). Providing a lipid linkage to the G ectodomain (G-Thy) increased its lateral mobility only slightly compared to VSV G giving it a D value well below that characteristic of many GPI-linked proteins. Providing transmembrane anchorage to a normally GPI linked protein (Thy-G) resulted in only a less than twofold reduction in D, compared to that for Thy-1 itself. Because changing the mode of membrane anchorage mode produced only marginal (less than twofold) changes in the lateral diffusion coefficient, we conclude that the nature of the ectodomain determines the lateral mobility of proteins of this type.

To account for these data, we propose that the lack of interaction of the ectodomain of normally GPI linked proteins with other cell surface components allows diffusion that is constrained only by the movement of the membrane anchor in the plane of the bilayer. In contrast, cell surface interactions of the ectodomain of membrane spanning proteins, exemplified by the VSV G glycoprotein, serve to reduce their lateral diffusion coefficients by 10-fold with respect to many GPI-linked proteins. This reduction in D can be accounted for a model in which "sticky," rapid exchange interactions occur between the ectodomains of the diffusing proteins and a system of largely immobile "post" proteins whose cytoplasmic domains are anchored or entrapped by the membrane associated cytoskeleton. The identity of the

putative posts, which would have mobility properties similar to erythrocyte Band 3, and the nature of the "sticky" interactions remain to be elucidated.

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