Stable expression of rabies virus glycoprotein in Chinese hamster ovary cells


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The rabies virus glycoprotein (G protein) has several important functions and is a major antigenic stimulus of the host immune system following rabies virus infection or vaccination. We developed a model system for studying the role of N-linked glycosylation in the intracellular transport and antigenicity of this molecule. The full-length cDNA of the G protein of the ERA strain of rabies virus was inserted into the eukaryotic shuttle vector pSG5 and then stably transfected into wild-type Chinese hamster ovary (CHO) cells and mutant CHO cell lines defective in glycosylation. Transfected wild-type CHO cells expressed the G protein (detected by immunofluorescence) on the cell surface in a manner similar to rabies virus-infected cells. The transfected wild-type CHO cells were shown by immunoprecipitation to produce a protein of 67K that comigrated with the fully glycosylated G protein isolated from virus-infected cells or purified virions. Treatment of the transfected cell lines with tunicamycin completely blocked surface expression and resulted in the intracellular accumulation of the G protein, suggesting that the presence of N-linked oligosaccharides is important for transport of this glycoprotein to the plasma membrane. The G protein cDNA was also expressed in the lectin-resistant CHO cell lines Lec 1, Lec 2 and Lec 8. In these cells initial N-linked glycosylation does occur, but later steps in processing of the oligosaccharides are blocked. In each case, the G protein was expressed on the surface of lectin-resistant CHO cells in a similar manner to expression on wild-type CHO cells. This suggests that various different N-linked oligosaccharide structures support intracellular transport of this glycoprotein. Thus, stably transfected CHO cell lines will provide a useful model system for further studies of the role of N-linked glycosylation in trafficking and antigenicity of the rabies virus G protein.

Introduction

The rabies virus glycoprotein (G protein) is the only protein on the surface of the mature virus and plays a critical role in virus infection (Dietzschold et al., 1978; Sokol et al., 1971). It is the viral attachment protein and is therefore important in determining the tissue tropism of the virus (Iwasaki et al., 1973; Perrin et al., 1982; Wunner et al., 1984). Following attachment to the host cell, the G protein facilitates fusion of the viral envelope with endocytic vesicle membranes (Mifune et al., 1982; Reagan & Wunner, 1984). The G protein is also involved in budding and the release of virus particles from host cells (Mifune et al., 1982; Reagan & Wunner, 1984). Finally, the G protein is a major antigenic stimulus of the host immune system during infection and vaccination, specifically inducing neutralizing antibodies (Cox et al., 1977; Wiktor et al., 1973) and cytotoxic T cells (Celis et al., 1988a, b).

G protein cDNAs from the ERA and CVS rabies virus strains have been cloned and expressed in bacteria (Anilionis et al., 1981; Yelverton et al., 1983) and also in eukaryotic cells (Kieny et al., 1984; Lecocq et al., 1985; Prehaud et al., 1989). Kieny et al. (1984) used a vaccinia virus recombinant containing the cDNA for the ERA strain G protein. This membrane protein has a single transmembrane domain and the mature protein contains 505 amino acids (Anilionis et al., 1981; Yelverton et al., 1983). The predicted G protein amino acid sequences from both strains have three potential N-linked oligosaccharide acceptor sites of the form Asn-X-Ser/Thr, only two of which are glycosylated (Dietzschold, 1977; Wunner et al., 1985). There are no known O-linked oligosaccharides (Dietzschold, 1977; Wunner et al., 1985). At least one N-linked oligosaccharide has been
predicted to be of the complex type by composition analysis (Dietzschold, 1977; Schlumberger et al., 1973).

The importance of the N-linked oligosaccharides of the G protein is perhaps twofold. First, glycosylation may be necessary for immunogenicity. The G protein synthesized by bacteria, which fail to glycosylate proteins, is ineffective as a vaccine in animal models (Yelverton et al., 1983), whereas cloned G protein expressed and glycosylated in eukaryotic cells and glycosylated G protein purified from rabies virions are both effective as vaccines (Kieny et al., 1984; Prehaud et al., 1989; Wiktor et al., 1984; Wunner et al., 1983). However, the glycosylation requirement for immunogenicity and antigenicity is not resolved by this comparison. For example, mouse monoclonal antibodies which define antigenic sites on the G protein (Dietzschold et al., 1988; Lafon et al., 1983, 1984; Prehaud et al., 1988; Seif et al., 1985) may not require glycosylation of their targets for optimal binding (Wunner et al., 1985). Alternatively, as for other viral glycoproteins (Leavitt et al., 1977; Machamer et al., 1985; Norrild & Pedersen, 1982; Pizer et al., 1980; Vidal et al., 1989), glycosylation may be necessary for the biological function, intracellular transport and surface expression of the G protein in infected cells, although this has not yet been demonstrated.

To examine the effects of global changes in N-linked glycosylation on intracellular transport, surface expression and antigenicity of the G protein, we generated and characterized stably transfected Chinese hamster ovary (CHO) cell lines that express G protein from cloned cDNA. The effect of blocking glycosylation with tunicamycin or of altering the N-linked oligosaccharide structure using lectin-resistant CHO cells was studied. This approach, combined with site-directed mutagenesis of G protein cDNA, similar to that described for the G protein of vesicular stomatitis virus (VSV) (Machamer et al., 1985), will be used in future studies to examine these relationships in more detail.

Methods

Cell lines and tissue culture. Wild-type CHO cells (CHO-K1D) were obtained from Dr Stomato, Wistar Institute. Wild-type (Pro-5) and lectin-resistant CHO cell lines (Lee 1, Lee 2 and Lee 8) (Stanley et al., 1975) were obtained from the American Type Culture Collection. The CHO-K1D cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS), 2 mM-glutamine, 100 international units (IU)/ml of penicillin, 100 µg/ml of streptomycin and 0.25 µg/ml of amphotericin B. All other cell lines were grown in alpha-MEM supplemented as above. Transfected cell lines were maintained in complete medium containing 0.5 mg/ml of active G418 (Gibco). For some experiments, cells were cultured overnight in complete medium containing the glycosylation inhibitor tunicamycin (5 µg/ml).

Antibodies. Rabies virus G protein-specific monoclonal antibodies 101-1 (antigenic site II) and 509-6 (antigenic site I) from the Wistar Institute were described previously (Dietzschold et al., 1988; Flamand et al., 1980; Lafon et al., 1983) and monoclonal antibodies 62-36-7, 62-80-6, 62-15-2, 62-11-6 and 62-11-4 were generously provided by J. Smith, Centers for Disease Control, Atlanta, Ga., U.S.A. (Smith et al., 1984). Hybridoma cell lines producing IgG mouse monoclonal antibodies OKT11 and anti-Lewis* (151-6-a-7-9) were obtained from the American Type Tissue Culture Collection. Hybridoma culture supernatant was concentrated 20-fold by ammonium sulphate precipitation and subsequent dialysis. Polyclonal antibody to wild-type CHO cells was produced by three sequential weekly intraperitoneal immunizations of eight BALB/c mice with 5 x 10⁵ CHO-K1D cells. Immune serum was obtained 1 week after the last immunization, pooled and stored in aliquots at -20 °C.

Plasmid construction. The full-length cDNA of the G protein (ERA strain) was excised from the pG155pro plasmid (Kieny et al., 1984) with BglII and ligated into the unique BglII site of the pSG5 eukaryotic expression vector (Stratagene) (Green et al., 1988) to produce pSG5rabgp (Fig. 1). Plasmid pSG5rabgp was used to transform Escherichia coli HB101 and constructs containing the insert in the sense and antisense orientations were isolated, characterized and amplified using standard techniques (Sambrook et al., 1989).

Transfection. CHO cells were cotransfected with 25 µg of pSG5rabgp and 1 µg of pSV2neo (Southern & Berg, 1982) by the calcium phosphate precipitation method (Sambrook et al., 1989) with a 2 min glycerol shock at 4 h (Sambrook et al., 1989). After 48 h the medium was replaced with fresh complete medium containing 1 mg/ml of active G418 and thereafter the medium was replaced approximately every 2 days with fresh complete medium containing G418. After approximately 14 days G418-resistant colonies were isolated, amplified and screened for expression of rabies virus G protein by indirect immunofluorescence. Clonal cell lines were isolated by repetitive subcloning by limiting dilution.

![Fig. 1. Construction of plasmid pSG5rabgp. Plasmid pSG5rabgp was produced by excision of the cDNA encoding the G protein from pG155pro using BglII and ligation of the purified fragment into the BglII site of pSG5. In pSG5, A represents the simian virus 40 (SV40) early promoter, B represents rabbit β-globin intron II, C represents the bacteriophage T7 promoter, D represents the SV40 polyadenylation signal, Amp* represents the ampicillin resistance gene and ori indicates the origin of replication. The hatched regions in pG155pro and pSG5rabgp represent the G protein cDNA.](attachment:image.png)
Southern blotting. Genomic DNA with a high Mr was extracted from cultured cells by standard methods (Sambrook et al., 1989), digested with EcoRI, separated by electrophoresis on a 0.8% agarose gel, and blotted onto Zeta-Probe nylon sheets (Bio-Rad). EcoRI was used because it is active in cutting genomic DNA and because both pSV2neo and pSG5rabgp have unique EcoRI restriction sites. Appropriate plasmid restriction fragments were purified twice by agarose gel electrophoresis and extracted from the gel with Gene-Clean (Bio 101). The purified fragments were then labelled with 32P[dCTP by nick translation following the manufacturer's instructions (BRL). The blot was treated with the labelled probe overnight at 65 °C in 0.5 M-NaHPO4, 1 mM-EDTA pH 7.2 containing 7% SDS, washed (two washes with 40 mM-NaHPO4, 1 mM-EDTA pH 7.2 containing 1% SDS and two washes with 40 mM-NaHPO4, 1 mM-EDTA pH 7.2 containing 5% SDS; 60 min per wash at 65 °C), and the probe that hybridized to the DNA was detected by exposure to Kodak XAR-5 X-ray film (Eastman Kodak).

Indirect immunofluorescence. Indirect immunofluorescence was performed on cells grown in eight-well tissue culture chamber slides (Nunc). For surface fluorescence, live unfixed cells were incubated for 45 min at 4 °C with primary antibody diluted in DMEM medium containing 30 mg/ml bovine serum albumin (DMEM-BSA). For cytoplasmic staining, cells were first permeabilized with acetone for 5 min at 4 °C and then air-dried before incubation with primary antibody. After three washes with DMEM-BSA, the cells were incubated at 4 °C for 45 min with the fluoresceinated F(ab')2 fragment of goat anti-mouse IgG (H + L) (Tago) diluted 1:50 in DMEM-BSA. The slides were washed as above and then covered with 50% glycerol in phosphate-buffered saline (pH 7.4) and a coverslip, and examined with a Leitz Orthoplan epifluorescence microscope (Wild Leitz USA).

Flow cytometry. Flow cytometry was performed on a Becton-Dickinson FACS Analyzer. Cells were removed from culture dishes with a dilute trypsin–EDTA solution (0.002% trypsin and 0.004% EDTA for 30 min at 37 °C), centrifuged and resuspended in medium for 2 h at 37 °C before staining. This method was used to minimize proteolysis of cell surface glycoproteins. Live cells were then treated in the same manner as described above for indirect immunofluorescence.

Immunoprecipitation. Logarithmically growing cells were metabolically labelled for 48 h with 0.25 μCi/ml of [14C]leucine in leucine-free DMEM (ICN) supplemented with 10% FCS, 2 mM-glutamine, 100 IU/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B. G protein was solubilized from transfected cells with lysis buffer (50 mM-Tris-HCl, 150 mM-NaCl, 5 mM-EDTA pH 7.4 with 0.5% NP40 and 200 μg/ml PMSF) as previously described (Wunner et al., 1980). Aliquots of solubilized protein containing approximately 2000000 c.p.m. in a total volume of 200 μl were incubated at 4 °C for 1 h with ascites fluid containing anti-G protein antibodies (1:100 final dilution) or with the indicated control antibodies. Fixed Staphylococcus aureus (Sigma) was then added (25 μl of a 10% suspension) and incubated at 4 °C for 1 h before centrifugation of the immune complexes. Immune complexes were washed five times in 15 mM-Tris–HCl, 0.5 M-NaCl, 5 mM-EDTA pH 7.4 with 5% sucrose and 1% NP40, electrophoresed on a 5 to 15% gradient SDS–polyacrylamide gel and analysed by fluorography with Amplify (Amersham).

Results

Isolation and characterization of cell lines expressing G protein

Recombinant plasmids containing the G protein in either the sense or antisense orientation were isolated, characterized and purified. These were then each individually transfected into wild-type CHO cells along with pSV2neo. Following selection with G418, individual colonies and polyclonal populations of stably transfected resistant cells were obtained.

To confirm the integration of G protein cDNA into cellular genomic DNA, a Southern blot was performed with DNA from polyclonal G418-resistant populations of CHO-K1D cells transfected with either the sense or antisense versions of pSG5rabgp (Fig. 2). Probing the Southern blot of EcoRI-cleaved cellular genomic DNA with a 32P-labelled pSG5rabgp BgIII fragment containing the G protein cDNA revealed the presence of a broad band centred at 8 kb for the antisense-transfected CHO-K1D cells (lane 7) and two relatively discrete bands of 9 kb and 5 kb for the sense-transfected CHO-K1D cells (lane 8). Plasmid pSG5rabgp has a single EcoRI restriction site which is outside the region of the rabiolabelled BgIII fragment probe, therefore if no rearrangement of the plasmid DNA occurred following integration each copy of integrated DNA should yield a single band on the Southern blot. Our results showing a few bands of various sizes (lanes 7 and 8) therefore indicate random integration of relatively few copies of G protein cDNA into the CHO cell genomic DNA (Southern & Berg, 1982). In contrast, no bands were detected using DNA from untransfected CHO-K1D cells (lane 5) or from cells transfected with only pSV2neo, the plasmid containing the selectable marker (lane 6). When the first probe was stripped from the blot and the blot re-probed with a 32P-labelled BglII–BamHI restriction fragment of pSV2neo containing neomycin resistance (neo8) coding sequences, hybridization bands were specifically detected for both the sense- and antisense-transfected cells, as well as for the cells transfected with pSV2neo alone (lanes 14 to 16). This demonstrates the specificity of the probes used in this experiment and that plasmid DNA containing the selectable marker was also integrated into the cellular genomic DNA of the appropriate cell lines.

The polyclonal population of CHO-K1D cells transfected with the sense version of pSG5rabgp was examined for expression of the G protein by immunofluorescence (Fig. 3). When live, intact cells were stained with monoclonal antibody 101-1 (b), approximately 20% of the cells were positive, and 10% were highly positive. The fluorescence pattern of the positively stained cells was uniform and diffuse and was qualitatively similar to that seen when the cells were stained with anti-CHO cell polyclonal antisemur (c) and (d). In contrast, 101-1 did not bind to untransfected CHO-K1D cells (a) or to cells transfected with G protein cDNA in the antisense orientation (data not shown). Neither of the isotype-matched negative control anti-
Fig. 2. Analysis of wild-type and transfected CHO-K1D cell lines by Southern blotting. Genomic DNA was isolated, digested with EcoRI, separated by agarose gel electrophoresis and blotted onto Zeta-Probe, as described in Methods. The blot was probed with either 32P-labelled G protein cDNA (a) or with a 32P-labelled segment of the neo8 gene (b). The sizes of the labelled lambda DNA standards (kb; lanes 1 and 9) are indicated on the left. Lanes 2 to 4 and 10 to 12 contain 50, 20 and 10 pg of EcoRI-digested pSG5rabgp (sense orientation) plasmid DNA, respectively. The remaining lanes contain cellular genomic DNA. Lanes 5 and 13, untransfected CHO-K1D cells; lanes 6 and 14, CHO-K1D cells transfected with pSV2neo alone; lanes 7 and 15, CHO-K1D cells cotransfected with pSG5rabgp (antisense orientation) and pSV2neo; lanes 8 and 16, CHO-K1D cells cotransfected with pSG5rabgp (sense orientation) and pSV2neo.

Fig. 3. Indirect immunofluorescence of wild-type- and pSG5rabgp (sense orientation)-transfected CHO-K1D cells. Wild-type- and sense-transfected cells were grown on eight-well slides, stained unfixed with the indicated primary antibody (G protein-specific mouse monoclonal antibody 101-1 or mouse anti-CHO cell polyclonal antibody) followed by fluorescein-labelled anti-mouse IgG secondary antibody, and examined by fluorescence microscopy as described in Methods. (a) Untransfected cells and monoclonal antibody 101-1; (b) transfected cells and monoclonal antibody 101-1; (c) untransfected cells and anti-CHO polyclonal antibody; (d) transfected cells and anti-CHO polyclonal antibody. Bar marker represents 25 μm.

bodies, OKT11 and anti-Lewisα, bound to any of the cells tested (data not shown). The polyclonal population of pSG5rabgp (sense orientation)-transfected cells was further examined with six additional G protein-specific monoclonal antibodies. Similar to the results found with monoclonal antibody 101-1, approximately 20% of the polyclonal population of transfected cells was positive with each of the G protein-specific monoclonal antibodies tested (data not shown). Thus, the G protein epitopes recognized by these antibodies were appropriately expressed at the cell surface. Similar results were obtained with a clonal derivative of wild-type CHO cells, Pro-5 (Stanley et al., 1975), transfected with pSG5rabgp (data not shown).

The polyclonal population of cells transfected with pSG5rabgp (sense orientation) was subcloned by repetitive limiting dilution and screened for expression of G protein by indirect immunofluorescence. Clone T3.30.11, which expressed a high level of G protein when examined by indirect immunofluorescence with all
of the anti-G protein antibodies, was further analysed by flow cytometry. A uniform population of brightly fluorescent cells stained positively with both the 101-1 and 509-6 monoclonal antibodies (Fig. 4). No staining was seen when clone T3.30.11 was incubated with OKT11, an isotype-matched control antibody of irrelevant specificity. CHO cells transfected with G protein cDNA in the antisense orientation were negative with all antibodies tested except for the anti-CHO polyclonal serum (Fig. 4). The T3.30.11 clone and three other carefully studied clones were found to express the G protein stably; no diminution in expression of the protein was noted either by indirect immunofluorescence or by flow cytometry after more than 6 months of continuous culture.

Transfected CHO cells were metabolically labelled with [3H]leucine and detergent-soluble cellular proteins were immunoprecipitated with G protein-specific monoclonal antibodies (Fig. 5). A band at 67K was observed for three independently isolated sense-transfected clonal cell lines (lanes 6 to 8). This band comigrates with authentic 67K G protein specifically immunoprecipitated from rabies virus-infected baby hamster kidney (BHK) cells (lanes 1 and 2) and also with the G protein from purified rabies virions (lane 4). No corresponding band was seen following immunoprecipitation of the non-transfected cells (lane 5) or when proteins from infected cells were immunoprecipitated with an isotype-matched negative control antibody (lane 3). Similarly, no proteins were specifically immunoprecipitated from antisense-transfected cells (data not shown). These results suggest that the 67K protein immunoprecipitated from the transfected cells is identical to fully glycosylated authentic G protein.

**Role of glycosylation in intracellular transport of G protein**

To assess the role of the two N-linked oligosaccharides in intracellular transport of the G protein, the polyclonal population of stably transfected CHO cells was treated with tunicamycin; this compound specifically and completely blocks N-linked glycosylation of proteins (see Fig. 6). Abundant surface staining of G protein was observed by indirect immunofluorescence in both non-permeabilized and permeabilized cells that were not treated with tunicamycin (Fig. 7a and b). However, when the transfected cells were grown in the presence of 5 μg/ml of tunicamycin for 16 h, immunofluorescence was observed only if the cells were first permeabilized (c and d). Similar results were obtained with each of the four transfected clonal cell lines expressing G protein which were examined (data not shown). These findings indicate that G protein lacking both N-linked oligosaccharides is not transported or is inefficiently transported to the plasma membrane and instead accumulates intracellularly.

Previous studies of the carbohydrate composition of purified G protein suggested that at least one of the two N-linked oligosaccharides is of the complex variety and contains sialic acid (Dietzschold, 1977; Schlumberger et al., 1973). To examine the importance of the type of oligosaccharide present on the G protein for proper intracellular transport, the cDNA was transfected into three lectin-resistant mutants of Pro-5 CHO cells (Lec 1, Lec 2 and Lec 8; see Fig. 6). These cells allow initial N-linked glycosylation to occur, but later steps in processing of the oligosaccharides are blocked. Lec 2 cells are markedly defective in the sialylation of oligosaccharides; complex type oligosaccharides of G protein expressed in these cells are therefore expected to lack terminal sialic acid. In contrast, the oligosaccharides synthesized on G protein produced in Lec 1 cells are expected to be limited to the high mannose type (Stanley et al., 1975), whereas those synthesized in Lec 8 cells could be of either the high mannose or hybrid variety (Stanley et al., 1975). Following transfection of pSG5rabgp into each of the lectin-resistant cell lines, the G418-resistant cells were
isolated and examined by indirect immunofluorescence with G protein-specific antibodies. In each case, the lectin-resistant cell lines were found to express the transfected G protein on their plasma membrane (data not shown). The degree of surface expression of the G protein for each of the three lectin-resistant cell lines was comparable to that observed above for the transfected wild-type CHO cells. For each lectin-resistant transfected cell line, tunicamycin blocked surface expression of G protein (data not shown). These results indicate that although some type of N-linked glycosylation is critical for intracellular transport and surface expression of G protein, the specific structures of the N-linked oligosaccharides on this glycoprotein are less important in this process.

Discussion

Both wild-type and lectin-resistant CHO cell lines transfected with G protein cDNA express this protein in a stable manner without any apparent cytotoxic effect. The transfected wild-type CHO cells specifically produce a protein of 67K (Fig. 5), which agrees with the Mr of the G protein isolated from infected cells or purified virions (Wiktor et al., 1973; Wunner et al., 1985). Since previous studies demonstrated that the unglycosylated protein migrates faster than the 67K fully glycosylated viral protein (Wunner et al., 1985), the present result suggests that the protein produced by the transfected cells is appropriately glycosylated. Using indirect immunofluorescence and flow cytometry, the expressed protein was detected on the plasma membrane of the transfected cells (Fig. 3 and 4) in a manner similar to that found with virus-infected cells (Lodmell & Ewalt, 1987; Wiktor & Koprowski, 1978; Wiktor et al., 1984). The configuration of the transfected protein appears to be similar to that of the virus-produced protein, since all of the G protein-specific monoclonal antibodies tested were able to recognize the protein on the surface of transfected cells.

Glycosylation of several viral G proteins is important for their proper folding, expression, transport and function (Basak & Compans, 1983; Gibson et al., 1978; Leavitt et al., 1977; Norrild & Pedersen, 1982; Pizer et al., 1980; Sodora et al., 1989; Vidal et al., 1989). We examined the role of N-linked glycosylation in the intracellular transport of G protein by using both the glycosylation inhibitor tunicamycin and three lectin-resistant CHO cell lines with well characterized glyco-
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Fig. 6. The biosynthetic pathway for N-linked glycosylation. The positions in the normal pathway which are blocked by tunicamycin and by the lectin-resistant mutants Lec 1, Lec 2 and Lec 8 are indicated. The anomeric linkages are deleted for simplicity. Examples of high mannose, hybrid, asialo-complex and sialylated complex oligosaccharides are also noted.

Fig. 7. Indirect immunofluorescence of G protein cDNA-transfected CHO-K1D cells treated with tunicamycin. Cells cotransfected with pSG5rabgp (sense orientation) and pSV2neo were grown on eight-well slides and incubated for 16 h in the presence or absence of 5 μg/ml of tunicamycin. Live or acetone-fixed (permeabilized) cells were then stained with G protein-specific mouse monoclonal antibody 101-1 and fluorescein-labelled secondary antibody and examined by fluorescence microscopy as described in Methods. (a) Live and (b) fixed cells incubated without tunicamycin; (c) live and (d) fixed cells incubated with tunicamycin. Bar marker represents 25 μm.

Tunicamycin treatment of transfected CHO cells blocked surface expression of G protein, indicating that glycosylation is essential for its proper transport to the plasma membrane (Fig. 7). Treatment of rabies virus-infected cells with tunicamycin prevents glycosylation of the G protein (Wunner et al., 1985), but the effect of tunicamycin on transport of this glycoprotein to the plasma membrane of infected cells or on production of infectious virions has not been studied. Our results suggest that unglycosylated G protein is not efficiently transported to the cell surface and therefore tunicamycin would greatly inhibit production of infectious virions by virus-infected cells. Similar results have been reported for the G protein of VSV-infected cells (Gibson et al., 1978; Kotwal et al., 1986; Leavitt et al., 1977). Deletion of both N-linked acceptor sites on VSV G protein by site-directed mutagenesis also blocked surface expression of the protein, whereas deletion of either site alone had little effect on translocation (Machamer et al., 1985; Machamer & Rose, 1988a; Rose & Bergmann, 1982). The N-linked oligosaccharides on VSV G protein have been proposed to promote translocation by allowing proper protein folding and by preventing formation of aberrant intermolecular disulfide bonds (Machamer & Rose, 1988a, b). The N-linked oligosaccharides on the rabies virus G protein may play a similar role; analogous studies with the G protein are currently under way using site-directed mutagenesis and stable transfection of the mutated G protein cDNAs.

Lectin-resistant CHO cell lines were used to examine whether certain types of N-linked oligosaccharides present on the G protein would affect surface expression of this glycoprotein. Whether the oligosaccharides were restricted to be of the high mannose type (Lec 1 cells),
hybrid type (Lec 8 cells) or complex type lacking the terminal sialic acid (Lec 2 cells), G protein was expressed on the surface of transfected cells in comparable amounts. These studies, however, do not exclude the possibility of different rates of intracellular transport of the variably glycosylated protein. Previous studies have shown that several different lectin-resistant cell lines infected with VSV all produce infectious virions, indicating that, as for the rabies virus G protein, the specific type of oligosaccharide present on VSV G protein is not critical for surface expression (Robertson et al., 1978).

Eukaryotic cell lines which stably express G protein, such as those described in this report and by Lecocq et al. (1985), offer several advantages over other available means to study this protein. Although intact rabies virus infects a broad range of tissue culture cells in vitro (Wunner, 1987), it is inconvenient to work with infected cells owing to the risk of human infection from live virus and because of the cytopathic effect of the virus on the host cells. Cells infected with recombinant vaccinia virus containing G protein cDNA can be used to study the protein without risk of human infection (Kieny et al., 1984), but the vaccinia virus vector also has a cytopathic effect on host cells. Finally, G protein has been expressed in E. coli, but the expressed protein does not undergo normal post-translational modification, including glycosylation; the unmodified protein produced by these cells has been found to be ineffective as a vaccine in animal models (Yelverton et al., 1983). In contrast, the approach described in this report offers some unique advantages. The ability to alter the glycosylation of G protein by expressing it in lectin-resistant cell lines will allow studies exploring the functional and antigenic significance of the oligosaccharides on this protein. In addition, site-directed mutagenesis of G protein can be used to delete and create N-linked oligosaccharide acceptor sites, permitting further study of its structure–function relationships. Since antigenic epitopes on the G protein are preserved in transfected cells, this suggests that comparison of wild-type and mutant G proteins in transfected mammalian cell lines will be useful for studying both humoral and cellular host immune responses to rabies virus (Bunschoten et al., 1989; Celis et al., 1988a; Eager et al., 1989; Morgeaux et al., 1989).

In summary, a model system for studying the rabies virus G protein is described which should be useful in a wide range of studies.

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