Cloning and epitope mapping of a functional partial fusion receptor for human cytomegalovirus gH

Brenda R. Baldwin,1, 2 Chen-Ou Zhang2 and Susan Keay1, 2, 3

1, 2Molecular and Cell Biology Program1 and Division of Infectious Diseases2, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA
3Research Service, VA Maryland Health Care System, 10 N. Greene St, Rm 3B-184, Baltimore, MD 21201, USA

A cDNA clone encoding a partial putative human cytomegalovirus (HCMV) gH fusion receptor (CMVFR) was previously identified. In this report, the cDNA sequence of CMVFR was determined and the role of this CMVFR in HCMV/cell fusion was confirmed by rendering fusion-incompetent MOLT-4 cells susceptible to fusion following transfection with receptor cDNA. Blocking experiments using recombinant gH or either of two MAbs (against recombinant gH or purified viral gH:gL) provided additional evidence for the role of gH binding to this protein in virus fusion. An HCMV-binding domain of 12 aa in the middle hydrophilic region of CMVFR was identified by fusion blocking studies using synthetic receptor peptides. The 1368 bp cDNA of CMVFR contained a predicted ORF of 345 aa with two potential membrane-spanning domains and several possible nuclear localization signals. A search of sequence databases indicated that CMVFR is a novel protein. Further characterization of this cell membrane protein that confers susceptibility to fusion with the viral envelope should provide important information about the mechanism by which HCMV infects cells.

Introduction

More than 60% of the general adult population is infected with the betaherpesvirus human cytomegalovirus (HCMV) (Ho, 1991). HCMV rarely causes disease in immunocompetent individuals; however, in immunocompromised individuals (including allograft recipients and patients with AIDS), HCMV infection can cause sight-threatening and/or life-threatening illness (Ackermann et al., 1988; Betts et al., 1975; Ho, 1977; Ho et al., 1975; Pass et al., 1978; Rubin et al., 1977; Whelchel et al., 1979). HCMV may also be a cause of atherosclerosis (Melnick et al., 1995) or coronary restenosis following angioplasty (Speir et al., 1994; Zhou et al., 1996).

HCMV infection of cells occurs in several steps, including attachment, pH-independent fusion of the viral envelope with the cell membrane and penetration of the capsid, followed by transportation of the capsid to the nucleus (Compton et al., 1992; Michelson et al., 1977; Reynolds, 1978; Smith & de Harven, 1974). Three HCMV envelope glycoprotein complexes have been identified that may be involved in HCMV infection: gcl (55 and 93–116 kDa) consisting primarily of gB, the major envelope glycoprotein and essential for virus infectivity; gcll (47–52 and > 200 kDa), which is unique to CMV; and gcIII (86:32 and 145 kDa) consisting primarily of gH:gL, which is essential for virus infectivity (Britt & Auger, 1986; Forrester et al., 1992; Gretch et al., 1988; Kari & Gehrz, 1988; Lehner et al., 1989; Nowak et al., 1984; Pereira et al., 1982; Rasmussen et al., 1985). Cell proteins that are possibly involved in HCMV attachment and entry include heparan sulfate proteoglycan (Compton et al., 1993) and two cell membrane proteins. One of these proteins, a 30–36 kDa protein (Adlish et al., 1990; Taylor & Cooper, 1990), which may be annexin II (Wright et al., 1994, 1995), has been shown to bind to gB (Pietropaolo & Compton, 1997). The other is a 92–5 kDa protein that binds to gH:gL (Keay & Baldwin, 1991).

Our laboratory previously reported that antibodies that mimic gH:gL and bind to the 92-5 kDa cell membrane protein...
inhibit HCMV/cell fusion specifically (Keay & Baldwin, 1991). In addition, others have found that gH:gL is involved in cellular egress of infectious virions (Desai et al., 1988), as well as cell-to-cell spread of virus (Rasmussen et al., 1991; Milne et al., 1998), a function shared by other herpesvirus gH:gL homologues (Forrester et al., 1992).

Preliminary characterization of the 92.5 kDa cell membrane protein indicates that it is a constitutively phosphorylated glycoprotein with primarily N-linked sugar residues (mannose and complex oligosaccharides) (Keay & Baldwin, 1992). Experiments using specific kinases or phosphatase inhibitors also indicated that phosphorylation of this receptor may be important in mediating HCMV infection (Keay & Baldwin, 1996). Furthermore, this 92.5 kDa receptor protein appears to mediate transmembrane signalling events, resulting in increased inositol triphosphate and intracellular calcium ion concentrations, as well as Sp1 and NF-kB activation following exposure to HCMV or cross-linked MAbs that bind to the receptor (Keay et al., 1995; Yurochko et al., 1997).

We previously isolated cDNA clones encoding part of the 92.5 kDa receptor and demonstrated that purified recombinant proteins expressed by HB101 Escherichia coli could block HCMV/cell fusion and plaque formation (Baldwin et al., 1996). We therefore sought to determine whether this cloned receptor protein could also mediate HCMV/cell fusion by transfecting fusion-incompetent cells (MOLT-4, unpublished data) with the receptor cDNA and by correlating expression of cell membrane receptor protein with the ability to fuse with octadecyl rhodamine B chloride (R18)-labelled HCMV. The sequence of the functional receptor cDNA was determined and synthetic peptides were generated to map the region involved in HCMV/cell fusion.

Methods

**Antibodies.** MAbs 4-3-5 and 6-5-1 (MAb) that mimic gH:gL of HCMV were generated and purified as previously described (Keay et al., 1988). BALB/c mouse immunoglobulin M (IgM)-kappa chain control antibody and IgG, control antibody (both from Southern Biotechnology) were purified by the same method. MAbs 1G6 (kindly provided by Lucy Rasmussen, Division of Infectious Diseases, Stanford Medical School, Stanford, CA, USA) and 11-1-1 (kindly provided by William Britt, Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL, USA) that are directed against HCMV gH:gL or recombinant gH, were generated as previously described (Rasmussen et al., 1984; Simpson et al., 1993) and purified using protein A Sepharose (Zymed) according to the manufacturer’s instructions.

**Recombinant gH.** Recombinant HCMV gH (rgH) was produced using a vaccinia virus vector (VV-gH; kindly provided by William Britt) and transfection of African green monkey kidney (AGMK) cells. Briefly, AGMK cells were inoculated with VV-gH at an m.o.i. of 30 in Eagle’s minimum essential medium (MEM) containing 2.5% foetal bovine serum (FBS), 1% antibiotic/antimycotic and 1% l-glutamine; the inoculated medium was incubated at 37 °C, 5% CO₂ for 24-48 h and the rgH was then harvested and purified as previously described for HCMV gB (Simpson et al., 1993; Britt et al., 1995). Mock gH was prepared by the above procedure from cells that had not been inoculated with VV-gH.

**Cell and viral cultures.** Human embryonic lung (HEL) fibroblasts (ATCC CCL-137) and AGMK cells (BioWhittaker (70-104B)) were grown in MEM containing 10% FBS, 1% antibiotic/antimycotic and 1% l-glutamine (Keay et al., 1988). MOLT-4 cells (ATCC CRL-1582) were grown in RPMI-1640 medium containing 10% FBS, 1% antibiotic/antimycotic and 1% l-glutamine. HCMV strain AD169 was passaged and harvested as previously described (Keay et al., 1995). Virus titre was determined by plaque formation in HEL cells (Keay et al., 1988).

**Isolation of HEL cDNA clones.** Clones were isolated from two HEL cDNA Jgt11 expression libraries (Clontech) using 4-3-5 and 6-5-1 as previously described (Baldwin et al., 1996).

**Isolation of insert and subcloning into vectors.** The purified cDNA inserts (purification described by Baldwin et al., 1996) were cleaved from the phage by digestion with EcoRI (Gibco BRL), run on a 1.4% NuSieve agarose gel (FMC Bioproducts) and purified by Wizard PCR preps (Promega). These inserts were then subcloned into pSecTagA, B or C (encodes an Ig kappa chain leader sequence for transport to the cell membrane) (Invitrogen) and/or pGEX-4T-1 (Pharmacia) which was previously digested with EcoRI and treated with calf intestinal alkaline phosphatase (Pharmacia).Competent One Shot TOP10 Escherichia coli (Invitrogen) was transformed with 611-pSecTagA, B or C, and competent BL21 E. coli was transformed with 611-pGEX-4T-1, 131-pGEX-4T-1 or 31-pGEX-4T-1 as previously described (Baldwin et al., 1996). Subclones containing inserts were identified by digestion of the plasmid DNA with EcoRI and analysis on a 1% agarose gel. The protein encoded by 611-pSecTagA was designated CMVFR.

**DNA sequencing and sequence analysis.** Plasmid DNA was isolated by the alkaline lysis method (Birnboim & Doly, 1979). Nucleotide sequences of the plasmid inserts were determined using the dideoxy chain termination method (Sequenase Version 2.0; Amersham) or cycle sequencing (ThermoSequenase; Amersham). Oligonucleotides were synthesized at the Biopolymer Core Facility at the University of Maryland. Nucleotide and amino acid sequence analyses were performed using the University of Wisconsin Genetics Computer Group version 8.0 program.

**Epitope constructs and peptide expression.** The 611 cDNA was isolated from 611-pGEX-4T-1 by digestion with EcoRI followed by PsIL (Gibco BRL) or PlEL (New England Biolabs). The resulting four overlapping segments (A–D; Fig. 5 a) were then blunt-ended by T4 DNA polymerase (Gibco BRL), ligated to EcoRI linkers (New England Biolabs) and digested with an excess of EcoRI prior to insertion into pGEX-4T-1 (which had been previously digested with EcoRI and treated with calf intestinal alkaline phosphatase). Plasmid DNA was isolated from transformed BL21 E. coli and sequenced using 5’ and 3’ pGEX primers (Pharmacia) to verify the presence and frame of the inserts.

Segments A–D of CMVFR and control proteins [no insert or FR611 (Baldwin et al., 1996)] were expressed as fusion proteins linked to glutathione S-transferase (GST) using the pGEX expression system. Cultures were grown in 2 × YTAG medium (Pharmacia Biotech, 1997) for 5 h at 37 °C, induced with 0.1 mM IPTG for 2 h at 37 °C, pelleted and resuspended in PBS containing 1 mg/ml lysozyme, and put on ice for 30 min. DTT (5 mM) and 1% N-lauroyl-sarcosine were added to the lysate, which was then sonicated and 1% Triton X-100 was added for a 30 min incubation at room temperature. GST-linked proteins and GST protein alone were purified using glutathione Sepharose 4B (Pharmacia), rinsed several times with PBS and eluted with glutathione elution buffer [10 mM reduced glutathione in 50 mM Tris–HCl (pH 8.0)].

Peptides based on the amino acid sequence of a portion of the B and D segments [designated 1 (CRHGMSSCRGFLPRHFRT), 2 (STPL-...]}
SIQNCQLGKLVSQHL, 1–11A (CRHGMSSCRGL), 1–15A (CRHGMSSCRGLFRR), 1–18A (CRHGMSSCRGLFRRPRH), [1–3]–18A (CRHGMSSCRGLFRRPRH), [1–6]–18A (CRHGMSSCRGLFRRPRH) and 1–10B (FRLPRHRFRHT) were synthesized and purified by the Biopolymer Core Facility at the University of Maryland.

**Transfection of MOLT-4 cells.** Vector pSecTagA and pSecTagA-, B- or C-containing 611 cDNA in E. coli were purified using a Qiagen plasmid maxi kit. MOLT-4 cells were then transected by electroporation. Briefly, 6 × 10^6 MOLT-4 cells in complete medium were mixed with 20 µg cDNA, incubated for 10 min at 37 °C and then put on ice for 5 min. The culture was then electroporated in 0.4 cm Gene Pulser cuvettes (Bio-Rad) at 260 V and 960 µF and put on ice for 2 min. Cells were transferred into T25 flasks with 10 ml complete medium and incubated for 48–72 h at 37 °C. Further incubation for 2–4 weeks at 37 °C with complete medium containing Zeocin (50 µg/ml; Invitrogen) was used to select transected cells.

**Immunofluorescence assay.** MOLT-4 cells transfected with 611-pSecTagA or controls (pSecTagA alone, 611-pSecTagB or 611-pSecTagC, or non-transfected MOLT-4 cells) were plated onto 8-well LabTek slides (Nunc) and incubated for 2 days at 37 °C. The cells were then spun at 220 × g for 20 min, carefully fixed with 50% acetone/50% ethanol for 10 min at room temperature, and incubated with 25 µg MAb 4-3-5 in PBS for 2 h at 37 °C, followed by 1:200 dilution of fluorescein isothiocyanate-labelled goat anti-mouse Ig (Caltag) in PBS for 2 h at 37 °C. The cells were then examined for fluorescence using a Nikon diaphot inverted phase microscope.

**Fusion assay.** HCMV (m.o.i. of 20) was labelled with a fluorescent amphiphile (R16) as previously described (Keay & Baldwin, 1991), then incubated with transfected MOLT-4 cells [611-pSecTagA or controls (pSecTagA alone, 611-pSecTagB or C, or non-transfected MOLT-4 cells)] for 30 min at 37 °C. Cells were rinsed with PBS and quantification of the fluorescent signal was obtained using a Zeiss axioplan microscope connected to a Percepsy image analyser.

For fusion blocking experiments using MAbS directed against purified HCMV gH:gL or rgH, R16-labelled HCMV was preincubated with IgG, 11-1-1 or control (IgGg) antibody at varying concentrations (10, 30 or 100 µg) for 30 min at 37 °C prior to inoculation of HEL cells; following an additional incubation for 30 min at 37 °C, the fluorescent signal was quantified by image analysis. For fusion blocking experiments using rgH, purified VV-gH protein (5 or 17 µg) was preincubated with HEL cells for 45 min at 37 °C prior to inoculation of HEL cells with R16-labelled HCMV, incubation for 30 min at 37 °C and quantification of the fluorescent signal.

For fusion blocking experiments using synthesized peptides, R16-labelled HCMV was preincubated first with equimolar amounts of receptor peptide 1 or 2 (30 µg = 12 µmol, 60 µg = 24 µmol, 100 µg = 40 µmol) for 1 h at 37 °C prior to incubation with HEL cells for 30 min at 37 °C and quantification of the fluorescent signal. Receptor peptides derived from 1 (1–11A, 1–15A, 1–18A, 1–3–18A, 1–6–18A, 1–10B) or PBS control were then used in fusion blocking experiments in varying amounts (30 µg = 19, 16, 14, 18 or 20 µmol, respectively; 60 µg = 38, 32, 28, 32, 40 or 40 µmol, respectively; 100 µg = 63, 53, 47, 49, 53 or 60 µmol, respectively) as above.

**Plaque assay.** HCMV (100 p.f.u. per well) was preincubated with varying amounts (10, 30 or 100 µg) of peptides 1, 2, 1–11A, 1–15A, 1–18A, 1–3–18A, 1–6–18A or 1–10B at 37 °C for 1 h prior to inoculation of HEL cells and a standard plaque assay was performed as previously described (Keay et al., 1988).

**Western blot and dot blot analyses.** Segments A–D and control proteins were run on 10% Tricine–SDS–polyacrylamide gels (Schagger & von Jagow, 1987) and transferred in Towlbin buffer (25 mM Tris, 192 mM glycine, pH 8.3) containing 10% methanol to either nitrocellulose or PVDF membranes (both from Bio-Rad). Nitrocellulose blots were blocked in 2% polyvinylpyrrolidone-40 in PBS for 1 h at room temperature, incubated with 50 µg 4-3-5 or control BALB/c IgM in PBS for 2 h at 37 °C, followed by 1:2000 alkaline phosphatase-labelled goat anti-mouse Ig in PBS for 1 h at room temperature, and developed with BCIP/NBT (Kirkegaard and Perry). PVDF blots were stained (50% methanol, 0·1% Coomassie R250) and destained (30% ethanol, 5% glacial acetic acid) and the degree of staining was used to determine protein concentration.

For dot blots, varying amounts (1, 2 or 4 µg) of peptides 1, 2, 1–11A or 1–10B were blotted onto nitrocellulose membranes. Membranes were then blocked in 15% Blotto (4-3-5 and IgM blocker; Keay et al., 1988) or 20% Blotto/1% Tween-20 (6-5-1 blocker) for 1 h at room temperature and incubated with 25 µg 4-3-5, 6-5-1 or BALB/c IgM control in PBS for 2 h at 37 °C followed by 1:250 horseradish peroxidase-labelled goat anti-mouse IgM (Zymed) in PBS for 1 h at room temperature prior to development with a-chloronaphthol.

**Statistical analysis.** Significant differences between independent means were determined at the 95% confidence level (P < 0·05) using a statistical program from Decision Analyst.

**Accession number.** The accession number for the nucleotide sequence of CMVFR is AF169251.

**Results**

**Sequencing of clones that express partial HCMV fusion receptor**

Three clones were previously identified from two different HEL 4g11 expression libraries and shown to bind specifically to both MAB3 (Baldwin et al., 1996). The nucleotide sequences of these cDNA were determined to be identical; the 1368 bp cDNA and derived amino acid sequences are shown in Fig. 1.

As determined by a ‘21-residue window’ with a mean hydrophobicity value greater than 0·42 (Eisenberg et al., 1984), two possible transmembrane regions exist within the predicted 345 aa ORF of the receptor peptide: aa 73–93 (mean hydrophobicity value = 0·53) and aa 161–181 (mean hydrophobicity value = 0·48) (Fig. 1). Three putative nuclear localization signals were also found throughout the cDNA. N-linked glycosylation sites were not found, however, indicating that the N-linked glycosylation sites implied by previous receptor characterization studies (Keay & Baldwin, 1992) are located in other regions of the receptor protein. Furthermore, neither an AUG translation initiation codon nor a polyadenylation signal was present, indicating that the 5′ and 3′ ends may not be present in the cDNA of the partial receptor. Throughout the sequence, numerous cysteines were evident, suggesting many possible disulfide bonds.

Database searches performed by use of the Pearson and Lipman Blastn, Blastp and Blastx programs (Altschul et al., 1990) indicated that the receptor gene may be interrupted by an intronic sequence and also localized on human chromosome.
Transfection of MOLT-4 cells with cDNA encoding partial receptor protein

Previously, we had shown that expressed proteins FR131 and FR611 could block both HCMV/cell fusion and HCMV infectivity (Baldwin et al., 1996). To confirm that the protein encoded by the cDNA could mediate virus fusion, MOLT-4 cells were transfected with 611-pSecTagA (in-frame), 611-pSecTagB or 611-pSecTagC vector control. An immunofluorescence assay using MAb 4-3-5 binds was first determined by Western blot. MAb 4-3-5 did not bound specifically to polypeptide B–GST (aa 204–345, 48 kDa), while BALB/c IgM and D–GST (aa 146–345, 48 kDa), while BALB/c IgM and secondary control antibodies did not bind to these two polypeptides (blots not shown). In comparison, 4-3-5 did not bind to show detail of cell membrane binding.

Mapping of the functional region of the receptor for fusion with HCMV

The polypeptide containing the epitope to which MAb 4-3-5 binds was first determined by Western blot. MAb 4-3-5 bound specifically to polypeptide B–GST (aa 204–345, 42 kDa) and D–GST (aa 146–345, 48 kDa), while BALB/c IgM and secondary control antibodies did not bind to these two polypeptides (blots not shown). In comparison, 4-3-5 did not bind to show detail of cell membrane binding.

4 since 100% homology over 261 bp was found to be a sequence-tagged genomic clone (STS4-411, 374 bp). Even though CMVFR appears to be novel, the sequence of the ORF. Potential membrane-spanning domains are boxed, cysteines are indicated by +, putative nuclear localization signals are underlined and the stop codon is indicated by an asterisk.

**Fig. 1.** Nucleotide sequence of the CMVFR cDNA and derived amino acid sequence of the ORF. Potential membrane-spanning domains are boxed, cysteines are indicated by +, putative nuclear localization signals are underlined and the stop codon is indicated by an asterisk.
bind to polypeptide A–GST (aa 1–203, 48 kDa) or C–GST (aa 1–145, 42 kDa), indicating that the epitope to which 4-3-5 binds is located within aa 204–345. Two peptides from the 5’ end of this region were then synthesized [peptides 1 (aa 204–224) and 2 (aa 225–244) (Fig. 5a)] to further define the epitopes for the MAbs. Dot blot analysis indicated strong binding of the MAbs (both 4-3-5 and 6-5-1) specifically to peptide 1 in a dose-dependent manner (Fig. 5b). Two smaller peptides from this region (1–11A, 1–10B) were also synthesized; one of which (1–11A, aa 204–214) was specifically recognized by both 4-3-5 and 6-5-1 antibodies in a dose-dependent manner (data shown for 4-3-5, Fig. 5b). These findings indicate that the epitopes to which both 4-3-5 and 6-5-1 bind are located within aa 204–214.

To determine if the same peptides that contained the MAbs 4-3-5-binding epitope were also able to block HCMV fusion, R18-labelled virus was preincubated with equimolar concentrations of peptide 1 or 2 for 1 h at 37 °C prior to inoculation of HEL cells. As shown in Fig. 6, peptide 1 blocked fusion in a dose-dependent manner [relative fluorescence: 100 µg (0.22 ± 0.09, P = 0.01); 60 µg (0.54 ± 0.15, P = 0.05); or 30 µg (0.88 ± 0.08, P > 0.20) as compared to 0 µg control (0.95 ± 0.11)] whereas the same concentrations of peptide 2 did not inhibit fusion [0.03 ± 0.07, P > 0.20; 0.90 ± 0.07, P > 0.20; 0.92 ± 0.12, P > 0.20; as compared to control (0.95 ± 0.11), respectively]. Like peptide 2, equimolar concentrations of the smallest segments of 1 (11A and 10B, data not shown) were not able to inhibit HCMV/cell fusion; however, when R18-labelled HCMV was preincubated with larger segments of 1 [1–15A (aa 204–218), 1–18A (aa 204–221), 1–31–18A (aa 207–221) or 1–63–18A (aa 210–221)] at 30, 60 or 100 µg (0 µg for controls), there was a significant decrease in fusion [values for each peptide, respectively: 0.69 ± 0.01 (P = 0.01), 0.89 ± 0.02 (P > 0.20), 0.90 ± 0.02 (P > 0.20) as compared to control of 0.90 ± 0.02; 0.31 ± 0.02 (P = 0.01), 0.64 ± 0.03 (P = 0.01), 0.75 ± 0.01 (P = 0.01), as compared to 0.90 ± 0.02; 0.24 ± 0.02 (P = 0.01), 0.45 ± 0.02 (P = 0.01), 0.57 ± 0.03 (P = 0.01), as compared to 0.92 ± 0.01; and 0.22 ± 0.01 (P = 0.01), 0.38 ± 0.01 (P = 0.01), 0.51 ± 0.02 (P = 0.01), as compared to 0.92 ± 0.01 (Fig. 6)].

Preincubation of HCMV with 10, 30 or 100 µg peptide 1 also significantly decreased plaque formation in a dose-dependent manner [33 ± 4%, P = 0.10; 47 ± 4%, P = 0.02; 64 ± 4%, P = 0.01, respectively] whereas preincubation with equimolar concentrations of peptide 2 [12 ± 1%, P > 0.20; 4 ± 7%, P > 0.20; 9 ± 6%, P > 0.20, respectively] had no
appreciable effect on plaque formation (Fig. 7). Peptides 1–18A, 1–3–18A, 1–6–18A and 1–15A also caused a significant decrease in plaque formation at 100 µg (47–60 µmol) (51 ± 4%, 58 ± 1%, 48 ± 6% and 31 ± 2%, respectively; \( P = 0.01 \) for all four peptides) (Fig. 7), although the smaller peptides 1–11A or 1–10B had no appreciable effect.

Discussion

In this paper, we report the sequencing and functional mapping of a partial cell membrane fusion receptor for HCMV gH. Although the cDNA clones did not contain the entire receptor, they were capable of mediating virus fusion in fusion-incompetent MOLT-4 cells. In addition, fusion was specifically blocked when these same transfected MOLT-4 cells were preincubated with recombinant HCMV gH, or when the \( R_{18} \)-labelled HCMV was preincubated with anti-rgH or anti-gH:gL antibodies, providing strong evidence that these clones encode a functional gH fusion receptor protein. The functional region for HCMV fusion was found to be within aa 210–221, a hydrophilic surface domain of CMVFR. In addition, HCMV infectivity was inhibited in a dose-dependent manner by a peptide from within this region. Mutagenic analysis may help to identify additional functional determinants in the partial receptor protein not identified by the peptide mapping studies.

The predicted amino acid sequence for the receptor protein indicates three putative nuclear localization signals. An emerging body of data indicates that ligand-induced cell membrane protein translocation to the nucleus can occur for certain polypeptide hormones and their receptors, including epidermal growth factor and growth hormone and their receptors (Holt et al., 1994; Lobie et al., 1994). It is conceivable that enveloped viruses similarly couple the nuclear translocation of viral proteins and their cell membrane receptors. For example, the binding of Epstein–Barr virus to its receptor, CR2, may result in the translocation of CR2 which has been shown to be present in the nuclei of Raji cells (Gauffre et al., 1992). In addition, matrix proteins of several enveloped viruses,
including CMV, influenza virus, Newcastle disease virus and human immunodeficiency virus (HIV) (Coleman & Peeples, 1993; Gallay et al., 1995; Mocarski, 1993; Ye et al., 1995), have been shown to undergo nuclear translocation within a short period of time following exposure to virus, as have the gB envelope glycoproteins from both herpes simplex virus-1 and HCMV (Radsak et al., 1990; Raviprakash et al., 1990). Although it is not known whether the translocation of viral proteins (Liu & Stinski, 1992; Roizman & Sears, 1993), some of which appear to be important for transactivation of viral genes, is cell membrane receptor-mediated, it is likely that cellular proteins are involved in the translocation process. The similarity between a region of CMV F and protamine is therefore of particular interest since protamine, which is a nuclear translocating DNA-binding protein that is concentrated in the acrosomal region of the head of mature sperm (Lyman et al., 1993), also appears to be involved in a membrane fusion event during fertilization (Liu et al., 1996).

Two possible transmembrane domains were also identified in the partial fusion receptor protein. It is notable that several cell membrane proteins that mediate fusion with enveloped viruses, including HIV-1 coreceptors (Deng et al., 1996; Feng et al., 1996), measles virus substance P receptor (Schroeder, 1986) and canine distemper virus CD9 receptor (Loffler et al., 1997), also have been shown to be multispan transmembrane proteins, suggesting that this configuration may be common to virus fusion receptor proteins.

The cellular function and normal ligand for the 92.5 kDa protein are currently unknown. Recently, several genomic clones for the gH receptor have been isolated in our laboratory. Analysis of these clones will undoubtedly provide critical insight into the structure and function of this cell membrane protein.

We wish to thank Dr William Britt for antibody 11-1-1 and VV-gH, and Dr Lucy Rasmussen for antibody 1G6. We also thank Dr Koji Hagiwara for helpful discussions and the National Cancer Institute for providing computing time and staff support at the Frederick Biomedical Supercomputing Center of the Frederick Cancer Research and Development Center. This work was supported by Merit Review grant support from the Veterans Administration.

References


HCMV gH receptor cloning and epitope mapping

on human cytomegalovirus glycoprotein H have conformationally distinct binding sites. Journal of Virology 67, 489–496.


Yurochko, A. D., Hwang, E.-S., Rasmussen, L., Keay, S., Pereira, L. & Huang, E.-S. (1997). The human cytomegalovirus UL55 (gB) and UL75 (gH) glycoprotein ligands initiate the rapid activation of Sp1 and NF-κB during infection. Journal of Virology 71, 5051–5059.


Received 21 October 1998; Accepted 3 September 1999