JOURNAL OF VIROLOGY, June 1988, p. 1956–1962
0022-538X/88/061956-07$02.00/0

Vol. 62, No. 6

A Multigene Family Encodes the Human Cytomegalovirus Glycoprotein Complex gcII (gp47-52 Complex)

DAVID R. GRETCH,[1] BRUCE KARI,[2] RICHARD C. GEHRZ,[2] AND MARK F. STINSKI[1]*

Department of Microbiology, University of Iowa, Iowa City, Iowa 52242,[1] and St. Paul Children's Hospital, St. Paul, Minnesota 55102[2]

Received 14 December 1987/Accepted 18 February 1988

The HXLF (*Hin*dIII-X left reading frame) gene family is a group of five genes that share one or two regions of homology and are arranged in tandem within the short unique component of the human cytomegalovirus genome (K. Weston and B. G. Barrell, J. Mol. Biol. 192:177–208, 1986). These genes were cloned into an SP6 expression vector in both the sense and antisense orientations. An abundant 1.62-kilobase (kb) bicistronic mRNA, predicted to originate from HXLF1 and HXLF2, was detected in the cytoplasm of infected human fibroblast cells by Northern (RNA) blot analysis. Less abundant RNAs of 1.0 and 0.8 kb, predicted to originate from the HXLF5 and HXLF2 genes, respectively, were also detected. Monocistronic, bicistronic, and polycistronic RNAs synthesized in vitro by using SP6 polymerase were translated in rabbit reticulocyte lysates with or without canine pancreatic microsomal membranes. The HXLF1 or the HXLF1 and HXLF2 translation products were detected when the above mRNAs were used. The HXLF3, HXLF4, and HXLF5 gene products were not detected by in vitro translation of the SP6-derived polycistronic mRNA. Nonglycosylated or glycosylated HXLF1 and HXLF2 gene products were immunoprecipitated by monoclonal antibody 9E10, which is specific for a virion envelope glycoprotein complex designated gcII (gp47-52 complex). In addition, the monoclonal antibody 9E10 immunoprecipitated a diffuse glycoprotein band, designated gp47-52, from HCMV-infected cell lysates. The amino acid composition of gp47-52 purified from virion envelopes has the highest similarity to the predicted amino acid composition of the HXLF1 plus HXLF2 open reading frames, but it is more similar to HXLF2 than to HXLF1. The Northern blot results imply that gp47-52 is synthesized predominantly from the abundant 1.62-kb bicistronic mRNA encoded by the HXLF1 and HXLF2 genes. However, the glycoprotein could also be synthesized by the monocistronic 0.8-kb mRNA encoded by the HXLF2 gene as well as by the mRNAs predicted from the other HXLF genes.

Human cytomegalovirus (HCMV) is an opportunistic pathogen which causes severe and widespread disease in immunocompromised hosts including patients with acquired immunodeficiency syndrome. In addition, HCMV is a frequent cause of birth defects in humans. However, this ubiquitous member of the herpesvirus group rarely causes disease in immunocompetent hosts, even though a vast majority of the population becomes infected by adulthood (for reviews, see references 6 and 14).

The mechanisms by which the immune system controls HCMV infection are poorly understood. For related herpesviruses, some virion glycoproteins are key determinants in the host immune response to infection (1, 5, 13, 18, 23). Therefore, interest lies in identifying the HCMV glycoprotein genes and gene products. We recently described three immunologically distinct families of glycoprotein complexes associated with the envelopes of virions; they were designated glycoprotein complex I (gcI) (gp55 and gp93-130), gcII (gp47-52), and gcIII (gp86 and gp145) (3). The gene of a glycoprotein component of gcI has been mapped and sequenced (2, 11). The gene has homology with the herpes simplex virus glycoprotein B gene and is located in the long unique component.

Recent sequencing of the short unique region of the HCMV genome (AD169 strain) revealed at least 38 open reading frames (ORFs) (26). Several gene families with homologous ORFs were identified. One family, designated HXLF (*Hin*dIII-X left reading frame), consists of five ORFs that lie in tandem with various degrees of homology. The gene products were predicted to have potential glycosylation sites, signal sequences for membrane insertion, and C-terminal hydrophobic domains for anchoring in cell membranes (26).

In this report we describe the expression of the HXLF genes. We used molecular genetics, monoclonal antibodies, and biochemical analysis to demonstrate that a component of the virion envelope gcII family, designated gp47-52 (3, 9), is a product of the HXLF genes. We also investigated the transcription of the HXLF gene family and the synthesis of gp47-52 in virus-infected cells.

MATERIALS AND METHODS

Virus and cells. The culturing of human foreskin fibroblasts and the propagation of HCMV Towne strain have been described previously (20). Use of the viral DNA polymerase inhibitor phosphonoacetic acid has been described (25).

In vitro transcription and translation. A 4.5-kilobase (kb) *Xba*I-to-*Hin*dIII fragment spanning 0.857 to 0.879 map units of the Towne strain genome was subcloned into SP6 expression vectors SP64 and SP65 (Amersham Corp., Arlington Heights, Ill.) as described previously (22). RNA synthesis from linearized templates was as described by Krieg and Melton (10). SP6 polymerase was purchased from Boehringer-Mannheim Biochemicals, Indianapolis, Ind.

The in vitro translation of RNA in rabbit reticulocyte lysates (Promega Biotec, Madison, Wis.) has been described (21). Translation products were radiolabeled with [^{35}S]methionine (>1,000 Ci/mmol; Amersham). For some experiments, translation was supplemented with canine pancreatic

* Corresponding author.

microsomal membranes as recommended by the manufacturer (Amersham).

Immunoprecipitation and SDS-PAGE. Infected or mock-infected cells were pulse-labeled at various times after infection with 40 μCi of [^{3}H]glucosamine (20 to 40 Ci/mmol; Amersham) per ml as described previously (20). For some experiments, cells were pretreated for 4 h with 1.5 μM monensin (a gift from C. Grose, University of Iowa) before being pulsed in the presence of monensin. Antigens were solubilized in RIPA buffer (phosphate-buffered saline [pH 7.4] containing 0.1% sodium dodecyl sulfate [SDS], 1% Nonidet P-40, 0.5% sodium deoxycholate, 100 μg of phenylmethylsulfonyl fluoride per ml, and 0.02% sodium azide) and immunoprecipitated with monoclonal or polyclonal antibodies plus protein A-Sepharose (Pharmacia, Uppsala, Sweden) as described previously (4). The analysis of radiolabeled antigens by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography has been described (20). ^{14}C-labeled protein molecular weight standards were purchased from Bethesda Research Laboratories, Inc., Gaithersburg, Md.

The production and characterization of monoclonal antibodies 41C2 and 9E10 were described elsewhere (9); monoclonal antibody 1G6 was a gift from L. Rasmussen, Stanford University. Human convalescent-phase serum (no. 218) was a gift from L. Frankel, University of Cincinnati.

Northern blot analysis. The purification of cytoplasmic RNA from HCMV-infected or mock-infected cells and Northern (RNA) blotting conditions were described previously (25). *Escherichia coli* rRNA standards were purchased from Promega. Antisense RNA probes were synthesized from linearized pSP641T templates as directed by the supplier (Amersham), except that 150 μCi of [α-^{32}P]UTP (800 Ci/mol; Amersham) was supplemented with cold UTP to a final concentration of 50 μM to ensure full-length probe synthesis.

Amino acid composition analysis. gp47-52 was purified from virion and dense-body envelopes by using ion-exchange and gel exclusion high-performance liquid chromatography as described previously (9). After strong-acid hydrolysis, amino acid compositions were determined by reverse-phase high-performance liquid chromatography on a C-18 column (E.M. Science, Elmsford, N.Y.) as described by Scholze (17).

RESULTS

Northern blot analysis of the HXLF steady-state mRNA in infected cells. The HXLF gene family, which consists of five homologous ORFs in tandem (26), was cloned into SP6 expression vectors in sense and antisense orientations as outlined in Fig. 1. Four different mRNA transcripts were predicted from the HXLF gene family on the basis of the locations of TATA boxes and polyadenylation signals relative to the ORFs (Fig. 1B). A 1.6-kb bicistronic RNA was predicted from the HXLF1 and HXLF2 genes, whereas a 2.7-kb tricistronic RNA was predicted from the HXLF3 through HXLF5 genes. In addition, monocistronic transcripts of 0.8 and 1.0 kb genes were predicted from the HXLF2 and HXLF5 genes, respectively.

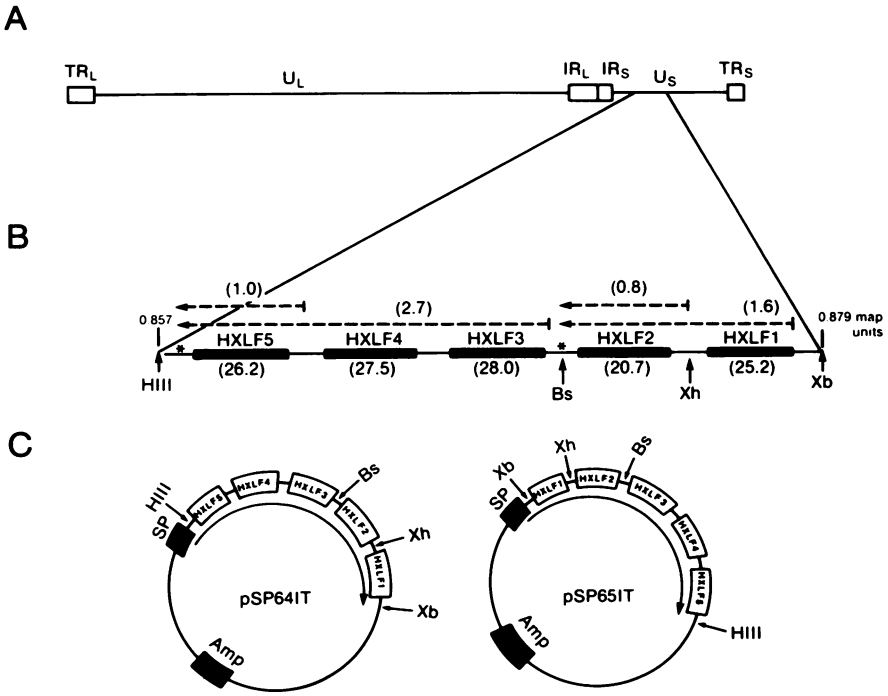

FIG. 1. Map location of the HXLF homologous gene family of HCMV and cloning into an SP6 expression vector. (A) Prototype arrangement of the human cytomegalovirus (HCMV) genome, showing large unique (U_L), short unique (U_S), terminal repetitive (TR), and internal repetitive (IR) regions. (B) The *Hin*dIII-*Xba*I fragment from the U_S region and the map units of the viral genome are designated. Symbols ——, HXLF family of homologous ORFs;- - →, predicted mRNA transcripts with approximate sizes in kilobases based on the locations of TATA boxes and polyadenylation signals (*) relative to the ORFs; ↑, restriction endonuclease sites. Beneath each bar representing an ORF is the molecular weight (in thousands) of a protein product predicted by the respective ORF. (C) The HXLF gene family was cloned into an SP6 expression vector in both the sense (SP651T) and antisense (SP641T) orientations. Abbreviations: Bs, *Bst*EII; HIII, *Hin*dIII; Xb, *Xba*I; Xh, *Xho*I; SP, SP6 promoter; Amp, ampicillin resistance gene.

Northern blot analysis was performed on steady-state cytoplasmic RNA harvested from mock-infected or HCMV-infected cells at 24 and 72 h postinfection (p.i.). In addition, RNA was harvested from infected cells treated for 24 h with phosphonoacetic acid, which inhibits the replication of HCMV DNA and, consequently, blocks the early-to-late transition in the viral replicative cycle. In addition, phosphonoacetic acid treatment increases the steady-state level of viral RNA. A 4.5-kb antisense RNA probe which covered all five HXLF genes was synthesized from pSP641T by linearizing the antisense template with *Xba*I (Fig. 1C). A shorter, 3.1-kb probe, which covered HXLF3, HXLF4, and HXLF5 genes only, was synthesized from a *Bst*EII-linearized template. Both the full-length probe (Fig. 2) and the truncated probe (data not shown) detected a predominant 1.62-kb transcript at 24 h p.i. (Fig. 2, lane 3) and at 24 h p.i. when phosphonoacetic acid was present (Fig. 2, lane 4). Reduced levels of the 1.62-kb RNA were detected by both probes at 72 h p.i. in the absence of phosphonoacetic acid (Fig. 2, lane 5). The 1.62-kb transcript is presumably the bicistronic mRNA predicted from the HXLF1 and HXLF2 genes. Both probes would be expected to hybridize to the 1.62-kb mRNA given the relative homology between the various HXLF genes. The full-length probe detected minor mRNAs of 0.8 and 1.0 kb, which correspond to the monocistronic RNAs predicted from the HXLF2 and HXLF5 genes, respectively (Fig. 2, lanes 3, 4, and 5). Neither probe detected the 2.7-kb polycistronic transcript predicted from the HXLF3, HXLF4, and HXLF5 genes. Thus, the predominant mRNA observed was a 1.62-kb mRNA predicted to be encoded by the HXLF1 and HXLF2 genes which accumulated in the cytoplasm of virus-infected cells at early times after infection. The level of this transcript relative to total cellular and viral RNA was decreased at late times after infection.

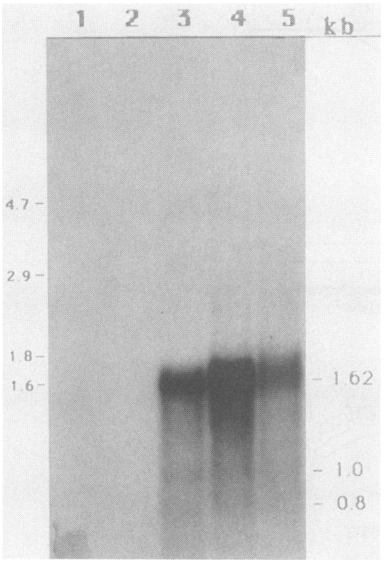

FIG. 2. Northern blot analysis of the HXLF steady-state RNA at various times after infection. Cytoplasmic RNA was isolated from mock-infected or infected cells at various times after infection, fractionated in a 1.6% agarose-formaldehyde gel, and transferred to nylon sheets. The blot was probed with ^{32}P-labeled antisense RNA derived from plasmid pSP64IT (Fig. 1) as described in Materials and Methods. The blot was probed with a 4.5-kb *Hin*dIII-to-*Xba*I probe. Lanes: 1, eucaryotic and *E. coli* rRNA; 2, mock-infected cell; 3, 24 h p.i.; 4, 24 h p.i. in the presence of 200 μg of phosphonoacetic acid per ml; 5, 72 h p.i. The sizes of the RNA transcripts detected and rRNA standards are given in kilobases.

In vitro synthesis of the HXLF gene products. To allow for the in vitro synthesis of mRNA from the first ORF (i.e., HXLF1), the first two ORFs together (HXLF1 plus HXLF2), or all five ORFs (HXLF1 through HXLF5), the sense plasmid pSP651T was completely linearized with *Xho*I, *Bst*EII, or *Hin*dIII, respectively (Fig. 1C). The approximate sizes of the in vitro-synthesized mRNAs were determined by agarose-formaldehyde gel electrophoresis (data not shown), and the RNAs were translated in rabbit reticulocyte lysates in the presence or absence of canine pancreas membranes (CPM). CPM allow for core glycosylation with *N*-acetylglucosamine and mannose sugars during glycoprotein translation in vitro (16). The translation products were either analyzed by SDS-PAGE or solubilized for immunoprecipitation as described in Materials and Methods.

After translation of mRNA from the HXLF1 gene in the absence of CPM, a diffuse band ranging in molecular mass from 21 to 25 kilodaltons (kDa) (p21–25) was detected (Fig. 3B). After translation of mRNA from the HXLF1-plus-HXLF2 genes in the absence of CPM, the diffuse p21–25 plus an additional nondiffuse 20-kDa polypeptide (p20) were detected (Fig. 3C). The apparent molecular weights of these in vitro translation products are similar to the predicted protein molecular weights from the HXLF1 and HXLF2 ORFs (Fig. 1B). The diffuse nature of p21–25 is characteristic of the HXLF1 gene only and may be due to internal initiation or premature termination during translation of the HXLF1 mRNA. Alternatively, the diffuse nature of p21–25 may be due to the higher proline content predicted to be present in HXLF1 (Table 1). Proline-rich regions can disrupt part of the alpha-helical structure of the protein, leading to anomolous mobilities in denaturing polyacrylamide gels.

After translation of mRNA from all five ORFs, a protein profile consistent with translation of mRNA from HXLF1 and HXLF2 was detected (Fig. 3D). The HXLF3, HXLF4, and HXLF5 ORFs were predicted to make proteins of 28, 27.5, and 26.2 kDa, respectively (26; Fig. 1B). Thus, the products of the downstream HXLF ORFs were apparently not expressed in vitro, even though full-length RNA was detected by agarose-formaldehyde gel electrophoresis. It is unlikely that the protein products migrated in gels in the same way as the HXLF1 and HXLF2 products did.

The HXLF ORFs were predicted to have sites for N- as well as O-glycosylation (26). Therefore, the in vitro-synthesized mRNAs were also translated in the presence of DPM to allow for core glycosylation of the nascent polypeptides (16). After translation of mRNA from the HXLF1 gene in the presence of CPM, a 30-kDa glycoprotein (gp30) was detected (Fig. 3B). When mRNA from the HXLF1 plus HXLF2 genes was translated in the presence of CPM, a major 30-kDa glycoprotein band was again detected (Fig. 3C). It is possible that the HXLF1 and HXLF2 proteins were glycosylated to approximately the same apparent molecular mass, the CPM suppressed translation of the HXLF2 mRNA, or the HXLF2 glycosylated protein is present in small amounts. A similar result was obtained when mRNA from all five genes (HXLF1 through HXLF5) was translated in the presence of CPM (Fig. 3D).

Immunoprecipitation of the HXLF gene products with an HCMV-specific monoclonal antibody or with human convalescent-phase serum. To determine whether the HXLF genes specified bona fide HCMV envelope glycoproteins, monoclonal antibodies that react with envelope glycoprotein complexes gcI (gp55 and gp93-130), gcII (gp47-52), and gcIII

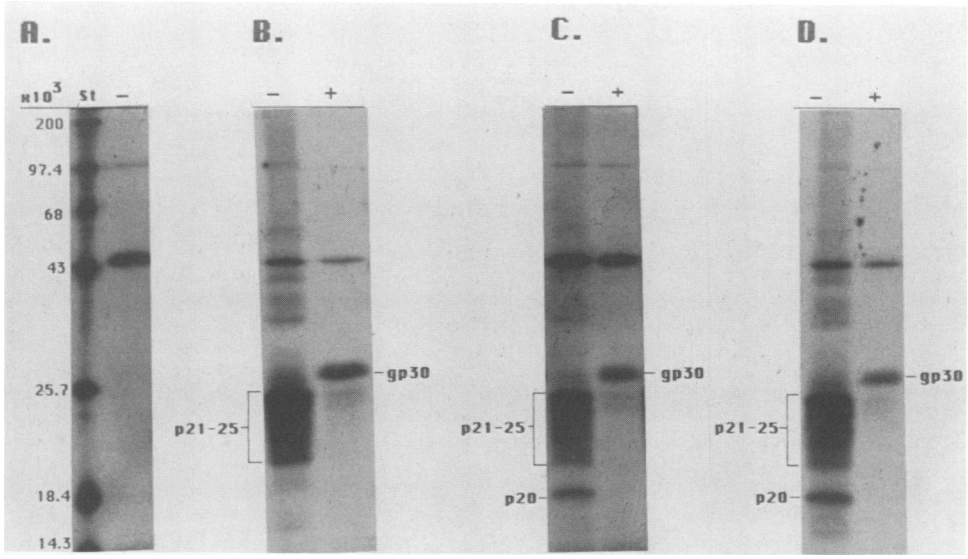

FIG. 3. In vitro translation and glycosylation of the HXLF1 and HXLF2 gene products. Plasmid pSP65IT (Fig. 1C) was linearized with *Xho*I, *Bst*EII, or *Hin*dIII, and RNA was synthesized in vitro. The RNAs were translated in vitro by using rabbit reticulocyte lysates in the absence (lanes −) or presence (lanes +) of CPM, and the [^{35}S]methionine-labeled products were analyzed by SDS-PAGE as described in Materials and Methods. (A) No added RNA; (B) RNA from HXLF1; (C) RNA from HXLF1 and HXLF2; (D) RNA from HXLF1 through HXLF5. The molecular weights of the protein (p) and glycoprotein (gp) translation products, as well as ^{14}C-labeled protein standards (lane St), are designated.

(gp86 and gp145) (3) were used for immunoprecipitation. Monoclonal antibody 9E10, which recognizes a major glycoprotein component of the gcII family (3, 9), reacted strongly with the diffuse p21–25 translated from the HXLF1 mRNA (Fig. 4A, lane 1). In addition, monoclonal antibody 9E10 immunoprecipitated both the diffuse p21–25 and the nondiffuse p20 specified by mRNA from HXLF1 and HXLF2 (Fig. 4A, lane 2). When mRNA from HXLF1 through HXLF5 was translated, monoclonal antibody 9E10 again immunoprecipitated p21–25 and p20 (Fig. 4A, lane 3). However, neither monoclonal antibody 41C2 (Fig. 4A, lane 4), which reacts with the gcI family (9), nor monoclonal antibody 1G6 (data not shown), which reacts with the gcIII family (3, 15), immunoprecipitated the HXLF mRNA translation products. Thus, p21–25 from the HXLF1 ORF is gcII specific as recognized by monoclonal antibody 9E10, and immunoprecipitation detects the entire diffuse band p21–25. The p20 product of the HXLF2 ORF may also be recognized by monoclonal antibody 9E10, even though there is only a small percent homology shared between these two ORFs (26). Immunoprecipitation of p20 via disulfide linkage with p21–25 is also a possibility. Monoclonal antibody 9E10 failed to immunoprecipitate the HCMV gB homolog gene product synthesized in vitro (data not shown).

TABLE 1. Comparison of the observed amino acid composition of gp47-52 with the predicted amino acid composition of the HXLF1 and HXLF2 ORFs

Amino acid	Amino acid composition[a]				
	Predicted from ORF[b]				Mean of observed gp47-52[c]
	HXLF1	HXLF2	Mean of HXLF1 and HXLF2	Mean of HXLF1 through HXLF5	
Lys	0.2	0.3	0.2	0.4	0.2
His	0.2	0.4	0.3	0.5	0.2
Asp	1.0	0.3	0.5	0.6	0.4
Met	0.2	0.1	0.1	0.2	0.3
Ile	0.2	0.3	0.2	0.4	0.4
Gly	0.7	0.7	0.7	0.9	0.5
Ala	1.0	1.0	1.0	1.0	1.0
Pro	1.8	0.8	1.2	1.2	1.3
Glu	2.0	0.8	1.2	1.0	1.3
Thr	1.2	1.2	1.2	1.0	1.0
Val	2.3	0.8	1.4	1.7	1.2
Leu	2.8	1.4	1.9	1.9	2.1
Tyr	1.8	0.6	1.1	1.0	0.7
Arg	2.3	0.7	1.3	1.2	0.6
Phe	0.5	0.3	0.4	0.5	0.9
Ser	1.7	0.6	1.0	1.4	0.5

[a] Compositions are given relative to Ala = 1.0 in all cases.
[b] Based on DNA sequence by Weston and Barrell (26).
[c] Based on the average of two separate analyses of purified gp47-52.

For further analysis of the HXLF gene products, mRNA from all five ORFs was translated in the presence of CPM and the products were immunoprecipitated with either monoclonal antibody 9E10 or human convalescent-phase serum 218. Both reacted with the 30-kDA product of the HXLF1 ORF (Fig. 4B, lanes 2 and 4). In addition, both immunoprecipitated a second 25-kDa species which may represent either unglycosylated HXLF1 ORF product or glycosylated product from the HXLF2 ORF. Monoclonal antibodies to gcI (Fig. 4B, lane 3) or gcIII (1G6; data not shown), as well as HCMV-negative human serum (data not shown), reacted weakly or failed to react with these translation products. The weak activity with monoclonal antibody 41C2 could be prevented by adding 1 mg of ovalbumin per ml (data not shown).

These results demonstrate that monoclonal antibody 9E10, which is specific for the envelope glycoprotein gp47-52, immunoprecipitates gene products from the HXLF1 and HXLF2 genes. In addition, human convalescent-phase serum contains antibodies which react with the HXLF gene products. It remains to be determined whether the putative HXLF3, HXLF4, or HXLF5 gene products can also be

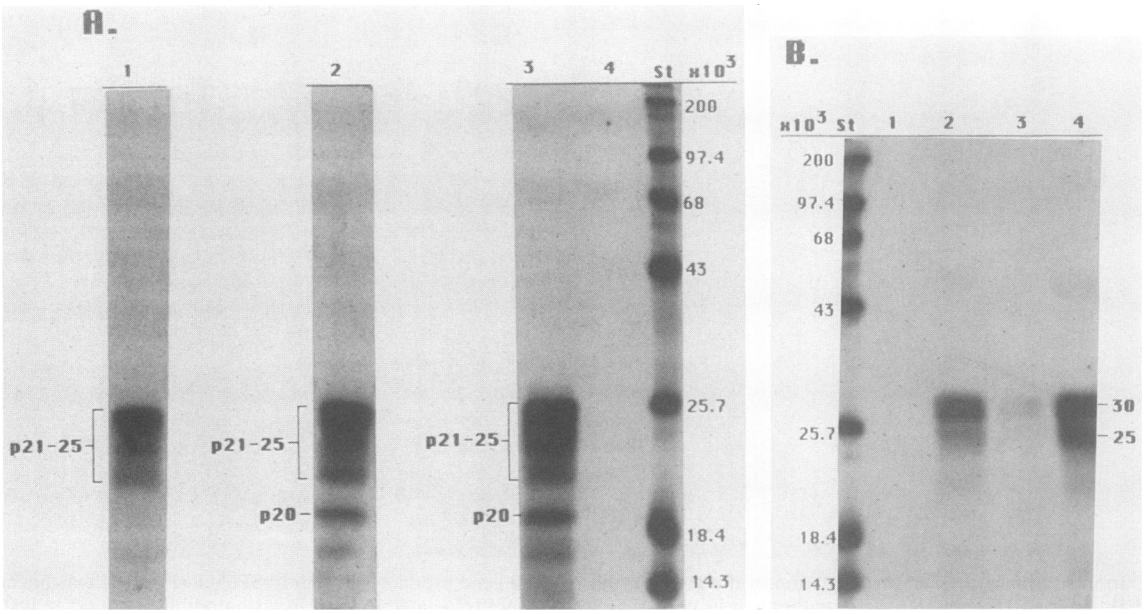

FIG. 4. Immunoprecipitation of the HXLF1 and HXLF2 gene products with an HCMV-specific monoclonal antibody or with human convalescent serum. The mRNA of the HXLF homologous gene family was synthesized in vitro by using a SP6 transcription system and translated in vitro by using a rabbit reticulocyte lysate in the presence or absence of CPM as described for Fig. 2. The [^{35}S]methionine-labeled translation products were subjected to immunoprecipitation with either monoclonal antibody 9E10 or 41C2, which are specific for HCMV glycoproteins gp47-52 and gp55, respectively, as well as with anti-HCMV human convalescent-phase serum 218. The immunoprecipitates were analyzed by SDS-PAGE as described in Materials and Methods. (A) mRNAs derived from HXLF1 alone, HXLF1 plus HXLF2, and HXLF1 through HXLF5 were translated in the absence of CPM and the viral antigens were immunoprecipitated with monoclonal antibodies 9E10 or 41C2. Lanes: 1, HXLF1 plus 9E10; 2, HXLF1 and HXLF2 plus 9E10; 3, HXLF1 through HXLF5 plus 9E10; 4, HXLF1 through HXLF5 plus 41C2. (B) mRNA derived from HXLF1 through HXLF5 translated in the presence of CPM. Viral antigens were immunoprecipitated with monoclonal antibodies 9E10 or 41C2, as well as with human convalescent-phase serum 218. Lanes: 1, mock translation plus 9E10; 2, sense RNA translation plus 9E10; 3, sense RNA translation plus 41C2; 4, sense RNA translation plus human serum 218. The molecular weights of the immunoprecipitated proteins (p) and glycoproteins (gp), as well as ^{14}C-labeled protein standards (St), are designated.

expressed in vitro and be recognized by monoclonal antibodies or human convalescent-phase serum.

Synthesis of gp47-52 in infected cells. The immunoprecipitation studies presented above suggested that gp47-52 is a product of at least the HXLF1 and HXLF2 genes. Previous studies have demonstrated that gp47-52 resides in virion envelopes as a family of disulfide-linked glycoprotein complexes collectively designated gcII (3, 9). Biochemical analysis of reduced gp47-52 demonstrated high levels of O-linked sugars, lower levels of N-linked sugars, and multiple polypeptides (B. Kari and R. Gehrz, Arch. Virol., in press). Therefore, we investigated the synthesis of gp47-52 in infected human fibroblasts cells in the presence or absence of the ionophore monensin, which inhibits Golgi transport (7) and allows the addition of high-mannose N-linked but not O-linked oligosaccharides (8).

Infected cells were radiolabeled with [^{3}H]glucosamine at various times after infection and in the presence of monensin, and antigen was solubilized and immunoprecipitated with 9E10 as described in Materials and Methods. A characteristically diffuse glycoprotein band (gp47-52) was detected at 48 and 72 h p.i. (Fig. 5A, lanes 2 and 3), but not at 24 h p.i. (Fig. 5A, lane 1) or in uninfected cells (not shown). In the presence of monensin, glycoproteins designated gp32 and gp25 were immunoprecipitated by 9E10 (Fig. 5B, lane 2). These glycoproteins are most probably simple N-linked precursors of gp47-52, since monensin blocks the processing of simple N-linked sugars as well as the addition of O-linked oligosaccharide chains (7, 8). It is unlikely that either gp32 or gp25 represents Golgi-mediated processing intermediates with different degrees of glycosylation, since the concentration of monensin used completely blocked processing of other herpesvirus glycoproteins (8), as well as the HCMV gB homolog precursor glycoprotein (data not shown). These results are consistent with the immunoprecipitation of 30- and 25-kDa HXLF gene products from in vitro translations supplemented with CPM (Fig. 4B, lanes 2 and 4), since CPM allows simple N-glycosylation in vitro (16). In addition, the identification of multiple precursors in the presence of monensin implies that gp47-52 is encoded by multiple HXLF genes.

Comparison of the amino acid composition of gp47-52 with the predicted amino acid compositions of the HXLF gene products. In a further demonstration that gp47-52 is a product of the HXLF genes, this glycoprotein was purified from virion envelopes by a combination of ion-exchange and gel exclusion high performance liquid chromatography followed by immunoprecipitation as described elsewhere (9). The reduced and alkylated gp47-52 was subjected to strong-acid hydrolysis, and its amino acid composition was determined by reverse-phase high-performance liquid chromatography as described by Scholze (17). The observed amino acid composition of gp47-52 was then compared with the predicted amino acid compositions of the HXLF genes (Table 1). The observed mean amino acid composition of gp47-52 is more similar to the predicted composition of HXLF1 plus HXLF2 than to the individual compositions. However, the observed mean composition had more similarities to the

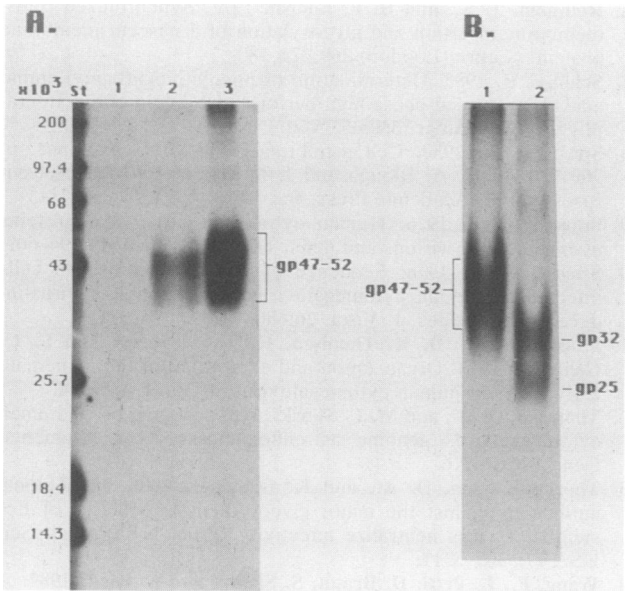

FIG. 5. Synthesis of gp47-52 in the infected cell and immunoprecipitation with monoclonal antibody 9E10. (A) HCMV-infected human fibroblast cells were pulse-labeled for 12 h with 200 μCi of [^{3}H]glucosamine at various times after infection. (B) Infected cells were pulse-labeled for 8 h with 200 μCi of [^{3}H]glucosamine at 72 h p.i. in the presence or absence of 1.5 μM monensin. Radiolabeled antigens were solubilized in RIPA buffer, immunoprecipitated with monoclonal antibody 9E10, and analyzed by SDS-PAGE as described in Materials and Methods. (A) Lanes: 1, 24 h p.i.; 2, 48 h p.i.; 3, 72 h p.i. (B) Lanes: 1, 72 h p.i. without monensin; 2, 72 h p.i. with monensin. The molecular weights of the glycoproteins (gp) as well as ^{14}C-labeled protein standards (St) are designated.

predicted composition of HXLF2 than to those of HXLF1, HXLF3, HXLF4, and HXLF5.

Although some of the variance may reflect differences between the Towne strain used in this study and the sequenced AD169 strain, the presence of the HXLF3, HXLF4, and HXLF5 gene products, or experimental error, it is likely that gp47-52 is composed of multiple HXLF gene products. The heterogeneous nature of gp47-52 and the different mRNA size classes detected by Northern blot analysis support this possibility. We conclude that gp47-52, which is a major component of the virion complex gcII, is specified by the HXLF gene family between map units 0.857 and 0.879 in the short unique component of the viral genome.

DISCUSSION

Our results demonstrate that the virion envelope glycoprotein gp47-52 is a product of the HXLF gene family of HCMV. The evidence for this is as follows. (i) Monoclonal antibody 9E10, which is specific for gp47-52, immunoprecipitated the in vitro-synthesized HXLF1 and HXLF2 gene products. (ii) The mean amino acid composition of gp47-52 is most similar to the predicted composition of the HXLF1 plus HXLF2 genes. (iii) Carbohydrate analysis of gp47-52 has revealed high levels of O-linked sugars and low levels of N-linked sugars (Kari and Gehrz, in press). This is consistent with the hydroxyamino acid content and number of N-glycosylation sites predicted for the HXLF gene products (26). (iv) Monoclonal antibody 9E10 immunoprecipitated two precursor glycoproteins of 25 and 32 kDa from monensin-treated infected cells and two HXLF glycoprotein gene products of 25 and 30 kDa from in vitro translations supplemented with CPM. Taken together, these data indicate that gp47-52 is a product of the HXLF genes.

The predominant mRNA detected from the HXLF genes by highly sensitive antisense RNA probes was a 1.62-kb transcript. A 1.6-kb bicistronic RNA is predicted from the HXLF1 and HXLF2 genes on the basis of sequence analysis. Minor RNAs of 0.8 and 1.0 kb that are predicted from the HXLF2 and HXLF5 ORFs, respectively, were also detected. The detection of these mRNAs supports the possibility that gp47-52 is a product of multiple HXLF genes. Since we were able to efficiently translate the HXLF1 and HXLF2 gene products from a bicistronic mRNA in vitro, it is possible that gp47-52 is synthesized from a bicistronic mRNA in vivo. A similar situation of bicistronic mRNA translation exists for the Epstein-Barr virus nuclear antigens (24). The lack of translation of the HXLF3, HXLF4, and HXLF5 ORFs in vitro may be due to their downstream position in the polycistronic mRNAs tested. Therefore, further subcloning and analysis of these genes are required to properly assess their expression.

The short unique region of the HCMV genome is larger and apparently more complex than analogous regions of other herpesviruses. The origin and nature of the five homologous gene families within the short unique region are intriguing from an evolutionary standpoint because it is likely that these homologous ORFs represent gene duplications and expansions of the short unique component. The remarkable preservation of regulatory sequences within the HXLF gene family such as TATA boxes and polyadenylation signals (26) suggests that the heterogeneous properties of the gcII family may have functional importance to the biology of the virus. For example, the different HXLF gene products may be essential for infection of different cell types.

ACKNOWLEDGMENTS

We thank C. Grose, S. Perlman, and M. Stoltzfus for a critical review of the manuscript.

This work was supported by Public Health Service grants HD-19937 and AI-13562 from the National Institutes of Health. D.R.G. is a recipient of a March of Dimes Birth Defects Foundation Predoctoral Graduate Research Training Fellowship.

LITERATURE CITED

1. **Cohen, G. H., M. Katz, C. Hydrean-Stern, and R. J. Eisenberg.** 1978. Type common Gp-1 antigen of herpes virus is associated with a 59,000-molecular-weight envelope glycoprotein. J. Virol. **27**:172–181.
2. **Cranage, M. P., T. Kouzarides, A. T. Bankier, S. Stachwell, K. Weston, P. Tomlinson, B. Barrell, H. Hart, S. E. Bell, A. C. Minson, and G. L. Smith.** 1986. Identification of the human cytomegalovirus glycoprotein B and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. EMBO J. **5**:3057–3063.
3. **Gretch, D. R., B. Kari, L. Rasmussen, R. Gehrz, and M. F. Stinski.** 1988. Identification and characterization of three distinct families of glycoprotein complexes in the envelopes of human cytomegalovirus. J. Virol. **62**:875–881.
4. **Gretch, D. R., M. Suter, and M. F. Stinski.** 1987. The use of biotinylated monoclonal antibodies and streptavidin affinity chromatography to isolate herpesvirus hydrophobic proteins or glycoproteins. Anal. Biochem. **163**:270–277.
5. **Grose, C., D. P. Edwards, K. A. Weigle, W. E. Friedrichs, and W. L. McGuire.** 1984. Varicella-zoster virus specific gp140: a highly immunogenic and disulfide-linked structural glycoprotein. Virology **132**:138–146.
6. **Ho, M.** 1982. Human cytomegalovirus infections in immunosup-

pressed patients, p. 171–204. *In* W. B. Greenough and T. C. Merigan (ed.), Cytomegalovirus biology and infection. Plenum Publishing Corp., New York.
7. **Johnson, D. C., and P. G. Spear.** 1982. Monensin inhibits the processing of herpes simplex virus glycoproteins, their transport to the cell surface, and the egress of virions from infected cells. J. Virol. **43**:1102–1112.
8. **Johnson, D. C., and P. G. Spear.** 1983. O-linked oligosaccharides are acquired by herpes simplex virus glycoproteins in the golgi apparatus. Cell **32**:987–997.
9. **Kari, B., N. Lussenhop, R. Goertz, M. Wabuke-Burot, M. Radeke, and R. Gehrz.** 1986. Characterization of monoclonal antibodies reactive to several biochemically distinct human cytomegalovirus glycoprotein complexes. J. Virol. **60**:345–352.
10. **Krieg, P. A., and D. A. Melton.** 1984. Functional messenger RNAs are produced by SP6 *in vitro* transcription of cDNAs. Nucleic Acids Res. **12**:7057–7070.
11. **Mach, M., U. Utz, and B. Fleckenstein.** 1986. Mapping of the major glycoprotein gene of human cytomegalovirus. J. Gen. Virol. **67**:1461–1467.
12. **McDonough, S. H., and D. H. Spector.** 1983. Transcription in human fibroblasts permissively infected by human cytomegalovirus strain AD169. Virology **125**:31–46.
13. **North, J. R., A. J. Morgan, J. L. Thompson, and M. A. Epstein.** 1982. Purified EB virus gp340 induces potent neutralizing antibodies when incorporated into liposomes. Proc. Natl. Acad. Sci. USA **79**:7504–7508.
14. **Rapp, F.** 1980. Persistence and transmission of cytomegalovirus, p. 193–232. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 4. Plenum Publishing Corp., New York.
15. **Rasmussen, L. R., D. Nelson, and T. C. Merigan.** 1984. Murine monoclonal antibody to a single protein neutralizes the infectivity of human cytomegalovirus. Proc. Natl. Acad. Sci. USA **81**: 876–880.
16. **Rothman, J. E., and H. F. Lodish.** 1977. Synchronized transmembrane insertion and glycosylation of a nascent membrane protein. Nature (London) **269**:775–780.
17. **Scholze, H.** 1985. Determination of phenylthiocarbamyl amino acids by reversed-phase high performance liquid chromatography. J. Chromatogr. **350**:453–460.
18. **Spear, P. G.** 1980. Cell membranes and viral envelopes, p. 709–750. *In* H. A. Blough and J. M. Tiffany (ed.), Herpesviruses, vol. 2. Academic Press, Inc., New York.
19. **Stinski, M. F.** 1976. Human cytomegalovirus: glycoproteins associated with virions and dense bodies. J. Virol. **19**:594–609.
20. **Stinski, M. F.** 1978. Sequence of protein synthesis in cells infected by human cytomegalovirus: early and late virus-induced polypeptides. J. Virol. **26**:686–701.
21. **Stinski, M. F., D. R. Thomsen, R. M. Stenberg, and L. C. Goldstein.** 1983. Organization and expression of the immediate early genes of human cytomegalovirus. J. Virol. **46**:1–14.
22. **Thomsen, D. R., and M. F. Stinski.** 1981. Cloning of the human cytomegalovirus genome as endonuclease XbaI fragments. Gene **16**:207–216.
23. **Thorley-Lawson, D. A., and K. Geilinger.** 1980. Monoclonal antibodies against the major glycoprotein (gp350/220) of Epstein-Barr virus neutralize infectivity. Proc. Natl. Acad. Sci. USA **77**:5307–5311.
24. **Wang, F., L. Petti, D. Braun, S. Seung, and E. Kieff.** 1987. A bicistronic Epstein-Barr virus mRNA encodes two nuclear proteins in latently infected, growth-transformed lymphocytes. J. Virol. **61**:945–954.
25. **Wathen, M. W., and M. F. Stinski.** 1982. Temporal patterns of human cytomegalovirus transcription: mapping the viral RNAs synthesized at immediate early, early, and late times after infection. J. Virol. **41**:462–477.
26. **Weston, K., and B. G. Barrell.** 1986. Sequence of the short unique region, short repeats, and part of the long repeats of human cytomegalovirus. J. Mol. Biol. **192**:177–208.